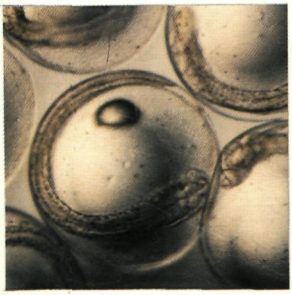
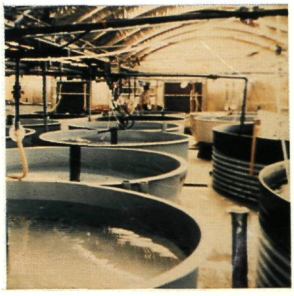
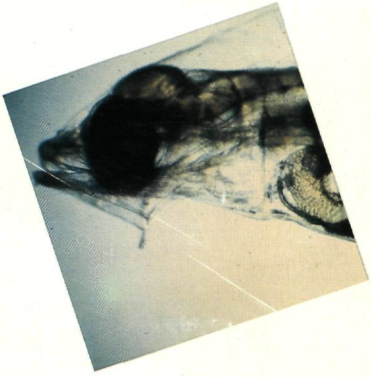
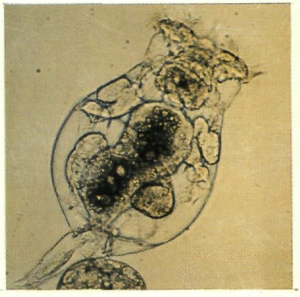
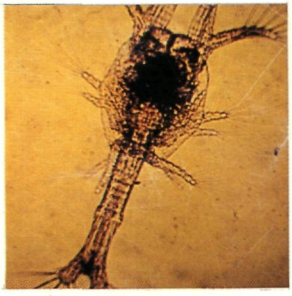
larvi '91

fish & crustacean larviculture symposium

august 27-30, 1991
gent, belgium

short communications & abstracts


editors

p. lavens
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f. ollevier

european aquaculture society

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larvi '91

Short communications and abstracts of contributions
presented at the international

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Gent, Belgium, August 27-30, 1991

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P. Lavens, P. Sorgeloos, E. Jaspers, F. Ollevier

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Editorial

The purpose of publishing this book of short communications and abstracts, is to better serve the participants and the contributors to this first international Fish and Crustacean Larviculture Symposium, LARVI '91.

As the short communications should contain all the essential information on the research presented, the participants will be in a better position to study the paper prior to its presentation or discussion, and will have a useful reference document for consultation afterwards. The contributors also benefit by the quick publication of their latest scientific results, albeit in a condensed form, in a scientific book that can also be consulted by those not attending the symposium.

The papers that the Scientific committee retained for presentation at LARVI '91 have not been peer-reviewed. We have, however, tried to edit the manuscripts as much as possible in the short time period available (more than 140 contributions were submitted).

Invaluable help was received from the following scientists who volunteered to assist with the scientific evaluation of the papers: David Bengtson, Niall Bromage, Konrad Dabrowski, Beverly Dixon, Thomas Hecht, Joan Holt, David Jones, Elin Kjørsvik, Syd Kraul, Philippe Léger, Helmut Segner, John Sweetman, Wim Tackaert, Amos Tandler, Johan Verreth, and Filip Volckaert. We would like to express our sincere thanks to them.

We acknowledge the professional and dedicated assistance of the secretarial staff of the Institute for Marine Scientific Research (IZWO): Ingrid Dobbelaere, Nora Roelandt; the Laboratory of Aquaculture and Artemia Reference Center: Marleen De Smul, Anita D'Haese, Magda Vanhooren, Brigitte Van Moffaert, and Marc Verschraeghen; the European Aquaculture Society: Linda Aspeslagh and Hilde Joncheere. They all spent extremely long working days to complete this Special Publication and meet the printer's deadline.

Gent, August 1, 1991

the Editors

Preface

During the past decade, larviculture of various species of coldwater and tropical fish as well as shrimps and prawns, has evolved from the R&D phase into succesful industrial applications. As a result, several organisms with good aquaculture potential could be added to the list of mass-cultured species. On an annual basis, billions of fish and crustacean fry are produced in more or less sophisticated larviculture systems. For many species, however, hatchery outputs need to be further improved as to alleviate the present bottle-neck situation with regard to unpredictable fry availability and quality, in order to make this industry more reliable, cost-effective, and cost-competitive.

LARVI '91 was convened with the purpose of evaluating the recent progress and identifying the future needs for the most important topics that condition successful hatchery outputs, *e.g.* dietary requirements, live *versus* artificial diets, egg and larval quality, larviculture process technology, pathology and disease contol.

Rather than having a species-oriented conference, it was the intention to promote contacts between research and production groups, and to identify similarities in problems and solutions, and to study approaches in freshwater as well as marine fish and crustacean larviculture.

The timely interest in larviculture in relation to aquaculture development was highlighted by the attendance of more than 300 participants from more than 60 countries.

It is hoped, that the outcome of this meeting will stimulate joint research efforts in the field of larviculture, and will result in further progress of fish and crustacean aquaculture.

Gent, August 1, 1991

Patrick Lavens
Editor-in-chief

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European Aquaculture Society, Special Publication No.15, Gent, Belgium. 1991.

STATE OF THE ART IN LARVICULTURE OF FISH AND SHELLFISH

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Review

During the past decade aquaculture, more particularly the controlled production of fish and shellfish, has evolved from an artisanal or experimental activity into a successful bioindustry. In various countries in Europe, SE Asia and Latin America, aquaculture products already represent a significant export commodity and several hundred thousand new jobs were created. In 1989 total aquaculture production amounted to over 11 million metric tons, representing an increase of 70% over the past 5 years. In comparison capture fisheries in the same time period increased by 14% only. For some species such as penaeid shrimp and salmon, 25% of the annual world consumption is generated through aquaculture production, *i.e.* about 500 000 metric tons of cultured shrimp and 275 000 metric tons of salmon.

Dependable availability of seed (also called fry, fingerlings or postlarvae) to stock the grow-out ponds or cages is one of the most critical factors in the commercial success of industrial production of fish and crustaceans. A breakthrough was realized only in recent years by the domestication of species, involving the development of appropriate techniques for controlled reproduction in captivity and for larviculture of the very sensitive stages.

Larviculture nutrition, more particularly start feeding in the early larval stages, appears to be a major bottleneck, not the least at industrial upscaling. For a few selected species such as salmon, minimal problems had to be overcome because their larvae at hatching carry a big yolk sac with enough food reserves for the first 3 weeks of their development. Once the yolk is consumed and exogenous feeding is starting, fingerlings are already sufficiently developed and readily accept formulated feeds. Most marine fish with aquaculture potential have very limited yolk reserves at hatching lasting for not more than 1 or 2 days. At first feeding they still have small mouths, often with an opening size of less than 0.1mm as well as a very primitive digestive system. In shrimp larvae, the feed size is not the only problem because these larvae develop through different larval stages, eventually changing from herbivorous filter-feeding behaviour to carnivorous hunters.

Over the past two decades trial and error approaches have resulted in the selection and improvement of the following larviculture diets which are today applied worldwide in experimental and commercial larviculture of fish and shellfish:
- different species of unicellular microalgae
- the rotifer *Brachionus plicatilis*
- the brine shrimp *Artemia.*

Monocultures of selected species of microalgae are costly to produce and the harvested algae do not have a consistent quality. This provided the rational to look for alternatives or supplements to live microalgae. Different approaches and formulations are already used in commercial enterprises, and many new developments in producing more cost-effective products are to be expected, *e.g.* heterotrophically-produced microalgae, manipulated yeasts, microencapsulated feeds and different kinds of microparticulated diets.

Although rotifer culture appears to be simple, many fish hatcheries experience problems in maintaining large cultures and producing, on a predictable basis, the massive numbers of *Brachionus* of a suitable food value, that are required to feed the hundred thousands to millions of baby fish they have in culture. Different microparticulated, yeast-based single cell proteins and emulsified formulations have been developed to manipulate the biochemical composition of the rotifers in order to better suit the dietary requirements in essential fatty acids and other nutrients of the fish larvae.

Among the live diets used in larviculture, brine shrimp *Artemia* nauplii constitute the most widely used food item; *i.e.* annually over 700 metric tons of dry *Artemia* cysts are marketed worldwide for on-site hatching into 0.4mm nauplii. Although the use of these cysts appears to be most simple, considerable progress has been made in the past decade in improving and increasing its value as a larval diet, *e.g.* selection of the most appropriate strains and batches, new techniques for cyst disinfection and decapsulation, nauplius hatching, enrichment and cold storage. Using particulate or emulsified products, rich in highly unsaturated fatty acids (n-3) HUFAs, the nutritional quality of the *Artemia* can be further tailored to suit the predators' requirements by bioencapsulating specific amounts of these products in the *Artemia* metanauplii. Application of this method of bioencapsulation, also called *Artemia* enrichment or boosting, has had a major impact on improved larviculture outputs, not only in terms of survival, growth, and success of metamorphosis of the fish and crustacean larvae, but also with regard to their quality, *e.g.* reduced malformations, improved pigmentation and stress resistance. Nonetheless, in many species survival rates are still under the 20% level. For several marine fish species the optimal dietary levels of (n-3) HUFAs have not yet been met by *Brachionus* and/or *Artemia.* While (n-3) HUFAs have proven to be most critical, it is very likely that other nutrients (*e.g.* other lipid classes, vitamins, free amino acids) might appear equally important and in some species even having more impact. Also egg quality, which to a large extent might be influenced by broodstock nutrition, needs to be evaluated as it may alter the quantitative dietary requirements in the larval stages.

Off-the-shelf dry substitution products for live *Brachionus* and *Artemia* are being developed and commercialized as a much more user-friendly application for the farmer.

With some species such as penaeid shrimp, the moment is approaching when the use of live food in the hatchery operation may be virtually eliminated. Fish impose much more constraints, not only in terms of nutritional requirements, but also with regard to digestibility and physical properties of the feed, *e.g.* water stability, buoyancy, and palatability. Appropriate processing technologies will most probably be developed soon, but their commercial applicability and price competitiveness might not be adequate.

The intensification of hatchery operations, from experimental facilities to industrial complexes, producing millions of fingerlings per month, brought about several zootechnical and disease problems. The introduction and adoption of new equipment, materials, and procedures resulted in more predictable outputs. There is, however, still much room for improvement not the least by a better identification of the microbial environment and elucidation of the disease problems. Oral administration of antibiotics through bioencapsulation in *Artemia* and *Brachionus* might be considered as a more effective transfer of therapeutics.

Industrial hatchery enterprises vary from large units with nominal capacities of a few million fish fry and up to fifty million shrimp postlarvae per month, to small backyard hatcheries. During recent years the latter have been mushrooming to thousands in some SE Asian countries. The use of modular systems, which allow to operate and/or disinfect parallel units, is gaining more and more interest. Several factors such as profit margins, predictability of outputs, quality of seed produced, *etc.* will ultimately determine which systems will prevail. Because of species differences and geographical discrepancies, fish and shellfish hatcheries will probably never turn into standardized blueprint methods, which can adequately be used worldwide.

Acknowledgements

Research on larviculture at the Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, is supported by the Belgian National Science Foundation (NFWO-FKFO), the Belgian Administration for Development Cooperation (ABOS), the Belgian Ministry of Science Policy (OOA), the European Community, SINTEF-Norway, and the private company Artemia Systems SA, Gent, Belgium.

NUTRIENT MANIPULATION AND REQUIREMENTS

General aspects

LARVI '91 - FISH & CRUSTACEAN LARVICULTURE SYMPOSIUM
P. Lavens, P. Sorgeloos, E. Jaspers, and F. Ollevier (Eds)
European Aquaculture Society, Special Publication No.15, Gent, Belgium. 1991.

DIETARY REQUIREMENTS FOR FRESHWATER FISH LARVAE - IN SEARCH OF A COMMON THREAD

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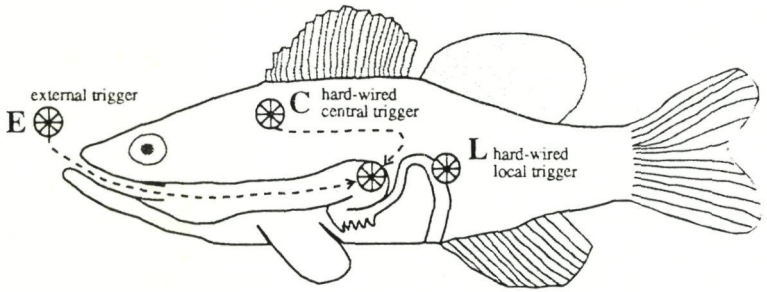
Abstract

During the first days/weeks of fish life, inescapable developmental changes occur in the structure and functions of tissues and organs. The coordination of these morpho-physiological events suggests the presence of an overriding factor that regulates pre-programmed cells. There is evidence that several factors, intrinsic or genetic can be modified so that cells can be triggered to differentiate by a specific signal(s). The importance of the ontogenic timing will be reflected in nutrient requirements of the fish. Thus the following general concept might help to recognize problems which need to be solved. I propose that the morphologic and enzymic differentation occurring during exogenous feeding of larval fish can be distinguished into external (E), central (C), and local (L) factors (Fig. 1).



E External: not programmed but triggered by external agent

C Central: pre-programmed by a centrally released substance (endocrine gland)

L Local: pre-programmed by a local timer in digestive tract

Fig. 1. Metamorphosis of digestive tract and digestive enzyme expression (modified after Diamond, 1986).

(E) An increased feeding rate (hyperphagia) is characteristic for larval fish and important for trophic control of intestinal absorptive cells. Factors involved might stimulate mucosa on the basis of substrate-enzyme interaction (Diamond, 1991). Live food might contain "metamorphosis factors" of Rembold and Fluchter (1988). The evidence exists that fish zymogens (trypsinogens) are activated by invertebrate enzymes
(C) Pituitary, thyroid and adrenal hormones modulate development of intestinal enzyme expression and trigger differentiation of enterocytes. Thyroid hormones are potent factors in fish metamorphosis (Miwa and Inui, 1987).
(L) Specific larval pancreatic enzymes are expressed during fish metamorphosis (Lauff and Hofer, 1984) and specific affinity to substrates might change dramatically during ontogeny.

These normal anatomical and physiological gradients (phases) parallel nutrient requirements in fish early ontogeny. Since fish body growth is controlled by protein deposition (synthesis minus degradation) the efficacies of fish growth should be studied by the availability of dietary amino acids, their transport through intestinal mucosa (Diamond, 1991) and their availability at synthesis sites.

Trophic responses of larval fish to a nutrient gradient cannot be accomplished with classical growth studies. In the case of the determination of amino acid requirements, some biochemical parameters can be utilized such as tissue amino acid concentration and/or oxidation of infused ^{14}C-amino acids (Walton *et al.*, 1986). In the case of the vitamin requirement, enzymes which initiate vitamin catabolism can be examined and used as biochemical indicators of vitamin status.

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ANALYSIS OF THE NUTRITIONAL CONDITION OF FISH LARVAE: STUDIES WITH *CLARIAS GARIEPINUS*, *COREGONUS LAVARETUS* AND *SCOPHTHALMUS MAXIMUS*

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Abstract

Among the environmental variables influencing the performance of fish larvae, the quantity and quality of food belong to the more important ones. Alterations of nutritional condition affect, apart from changing growth and survival, a number of structural and molecular parameters of the larvae. Particularly the absence of food has pronounced effects on histological features of, *e.g.* the liver and the intestine, on activities of digestive proteolytic enzymes, as well as on RNA/DNA ratios. The starvation-related changes are similar for the larvae of totally different fish species, *e.g. Clarias gariepinus*, a tropical freshwater species, the whitefish *Coregonus lavaretus*, from temperate freshwaters, or the turbot *Scophthalmus maximus*, a temperate seawater fish.

Structural and molecular indicator parameters are employed by fishery biologists as sensitive diagnostic tools to estimate the percentage of starving fish larvae in the field. For the aquaculturist, on the other hand, the major problem is not the diagnosis of starvation in cultured larvae, but the detection of nutritional pathologies in relation to various dietary regimes. In addition, possible reasons for diet-induced differences of larval growth have to be evaluated. Studies with *Clarias gariepinus* and *Coregonus lavaretus* show that the methods which are valid to indicate food deprivation, are basically also suited to indicate malnutrition. However, further approaches are necessary in order to identify the factors responsible for larval malnutrition. A particular need exists for detailed knowledge of larval physiology, larval energetic and intermediary metabolism, larval nutritional requirements and adaptive plasticity of the larvae.

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FEEDING STRATEGIES AND NUTRITIONAL PHYSIOLOGY IN EARLY LIFE OF CLARIID CATFISHES

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Abstract

In many countries of Africa and Asia, catfishes belonging to the genus *Clarias*, are commercially important. Also, in Europe, *Clarias* farming is developing. Together with the increasing interest in the culture of these species, the need for reliable and precise hatchery techniques is strongly growing. This has led to a wide array of both extensive and intensive techniques for larval rearing. The present paper attempts to correlate the applied feeding strategies with information on the basic biology of the respective species.

Catfishes of the genus *Clarias* (*e.g. C. gariepinus, C. batrachus, C. macrocephalus*) pass through a larval period during which several organ systems are still developing into adult organs. This situation imposes strict limitations on the type of food during the early larval period.

The African catfish, *C. gariepinus*, has been extensively studied with regard to biology and nutritional physiology during the larval stage. At the start of exogenous feeding, the larvae have an advanced digestive system with a functional pancreas, liver and nutrient absorption, but without a functional stomach. This is further exemplified by concurrent changes in the development of the enzymatic complex. Probably because of this advanced stage of development, feeding live food organisms is mostly practised for a few days only and is soon replaced by wet and/or dry diets.

The rearing and feeding strategies applied in commercial farms reflect the knowledge of the basic biology of the species under concern. In Africa, extensive and semi-extensive systems prevail for the culture of *C. gariepinus* and *C. senegalensis*, with larvae being raised in ponds and at the most with the natural food supplemented with trash fish and dry feed ingredients. In Asia, similar semi-extensive systems are applied in the culture of *C. batrachus* and *C. macrocephalus*, however, using more intensive nursing systems during the early larval period. Semi-intensive and intensive culture systems prevail in

Indonesia, South Africa and Europe, with feeding *C. batrachus* and *C. gariepinus* with dry diets and supplements of *Artemia*. The periods during which these strategies are applied and the growth rates attained vary according to the species and the region.

A comparative study of basic biological data on the development of *C. gariepinus* and *C. batrachus*, revealed that in spite of strong differences in egg and larval size, the growth rate was quite similar for both species. Further comparison between these species indicates that differences in growth results between different species and regions may be strongly correlated with the applied feeding strategy, which may have a stronger impact on the results than species related differences.

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GROWTH HORMONES FOR FISH LARVAE: CAN BIOACTIVE PEPTIDES BE DELIVERED TO GOOD EFFECT?

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Introduction

As in mammalian livestock production, growth promotants can improve total weight gain and food conversion efficiency in farmed fish. If fish larvae respond in a similar fashion, the batch time could be significantly reduced in the hatchery phase, increasing production and reducing time of exposure to a high mortality phase of culture.

Peptide delivery to larvae

Unlike steroids, large polypeptide hormones such as insulin and somatotrophin are water soluble. These proteins are subject to enzymatic and chemical degradation during digestion, prompting initial assays with somatotrophins to follow a protocol involving serial intraperitoneal injections. Recent evidence of intact protein uptake by juvenile fish via either the gastrointestinal tract (McLean *et al.*, 1990) or the gills (Moriyama and Kawauchi, 1990) suggests that it may be possible to deliver peptide-growth factors to fish larvae and accelerate development. Govoni *et al.* (1986) pointed out that fish larvae in general lack a secretory stomach and noted effective pinocytotic uptake of proteins by the hindgut, suggesting a route of entry for orally delivered bioactive peptides.

Using post-hatching zebrafish, *Brachydanio rerio*, as a model, a preliminary study is underway to test the activity of recombinant porcine somatotrophin (PST) delivered either by immersion or orally via live brine shrimp *Artemia*. Radioimmune assays confirm that immunologically active PST, mixed first with fish oil then emulsified, can be encapsulated in large amounts by *Artemia* which are then fed to the fish. Evidence for biological activity of PST in this species and implications for other larvae are discussed.

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PROTEIN TURNOVER IN FISH LARVAE

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Abstract

There are a number of components of protein metabolism in larval fish for which there is little data. Of prime interest is the relationship between the amount of protein that is synthesised and that which is retained as growth, *i.e.* the extent of protein turnover. In large fish this efficiency of retention of synthesised protein may approach 40%. In view of the apparently high cost of protein synthesis in large fish it is also of interest to know whether protein synthesis costs in small fish are also high and whether there is high efficiency of retention of synthesised proteins.

In order to answer these question a method has been developed to estimate protein synthesis rates of larval/juvenile fish. Protein synthesis rates of juveniles of *Tilapia* were determined at 26°C by incubating the animals in water containing radiolabelled phenylalanine together with a high concentration of non-labelled phenylalanine. This results, after a period of a few hours, in an elevation of the fishes phenylalanine free-pool specific radioactivity and linear incorporation of the radiolabel into proteins. Protein synthesis measurements were combined with oxygen consumption determinations.

Oxygen consumption was found to cycle during 24h with low values at night. Oxygen consumption and protein synthesis increased rapidly after a meal. Inhibition of protein synthesis indicated that this process accounted for approximately 30% of the oxygen consumption and estimates of energetic costs of protein synthesis indicate that they are close to the theoretical minimum. On a weight specific basis, protein growth, protein synthesis and protein degradation declined as body size increased. In small fish (20mg live weight) efficiencies of retention may be as high as 68%.

Similar experiments were conducted with larval herring at 8°C where inhibition of protein synthesis also resulted in a 30% reduction in oxygen consumption and energy costs of protein synthesis were low.

It is concluded that at high growth rates in juvenile fish the energy cost of protein synthesis is at a minimum and that efficiencies of retention of synthesised proteins are high but that there is considerable protein turnover.

Lipid nutrition

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IMPORTANCE OF DOCOSAHEXAENOIC ACID IN MARINE LARVAL FISH

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Abstract

Recent investigations on essential fatty acids (EFA) for fish have demonstrated that marine species of finfish require (n-3) HUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as EPA for normal growth. However, these requirements were all determined using lipids or methyl esters containing both EPA and DHA as (n-3) HUFA, providing no information as to which fatty acid, EPA or DHA, is more important for various physiological functions. In freshwater species in which linolenic acid is converted to DHA via EPA, EPA may not vary so much from DHA in EFA efficiency contrary to marine species in which the conversion ability from EPA to DHA is very limited. This is also supported by the fact that DHA, which is usually present in high amounts in the eggs of marine species, is quickly reduced during larval development. It is unknown, however, whether or not DHA is utilized as an energy source during development or converted to other physiologically important substances such as prostaglandins.

The recent studies on EFA of marine fish have shown that DHA is superior to EPA as EFA for larval fish. In case of red seabream, feeding of rotifers low in (n-3) HUFA resulted in low growth and survival rates along with a high incidence of fish with so-called hydrops. These conditions were effectively improved by incorporation of EPA and DHA or a (n-3) HUFA mixture into rotifers. However, the incidence of hydrops was not completely inhibited by EPA, but almost totally prevented by DHA. The growth and the survival rate in an activity test were also highest in the larvae fed the DHA-rotifer. The same kind of results were also obtained in larval striped knifejaw, striped jack, yellowtail, flounder etc. These results suggest that the physiological functions of EPA differ from those of DHA.

In this paper this new information on DHA is reviewed.

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NUTRITIONAL MECHANISMS CAUSING ABNORMAL PIGMENTATION IN CULTURED MARBLED SOLE LARVAE, *LIMANDA YOKOHAMAE* (HETEROSOMATA)

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Introduction

Lack of pigmentation (albinism) at the ocular side of flatfish, which results from a deficiency of pigments, has widely occurred in the process of larvae production. Although many researchers have investigated the mechanisms of this albinism, little is known in this field (*e.g.* Seikai *et al.*, 1987; Miki *et al.*, 1989).

The author found that albinism resulted from nutritional problems when 10-day-old larvae were fed microparticulate test diets. Black pigment (melanin) formation was hindered when the diet was deficient in the (n-3) highly unsaturated fatty acid, docosahexaenoic acid (22:6n-3), phospholipid, and vitamin A were deficient in the diet.

This paper reports the relationship between docosahexaenoic acid, phospholipid, and vitamin A in the nutritional mechanisms that cause abnormal pigmentation in marbled sole, *Limanda yokohamae*.

Materials and methods

Eggs of the flatfish were obtained from the Fish Culture Centre of the Oita Prefecture, transported to and hatched at the Komoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. The newly-hatched larvae were fed with the rotifer, *Brachionus plicatilis*, for 7 days, then divided into experimental groups, and reared with the microparticulate test diets under the conditions listed in Table I.

The microparticulate diet consisted of vitamin-free casein, defatted squid meal, dextrin, lipids, mineral mixture, and vitamin mixture. Zein was used as a binder. The preparation method for microparticulate test diet has been described previously (Kanazawa *et al.*, 1982).

The following experimental groups were tested: diet 1 was deficient in vitamin A palmitate (VA), soybean lecithin (phospholipid, PL) and docosahexaenoic acid (DHA); diet 2: a VA and PL deficient diet; diet 3: a VA and DHA deficient diet; diet 4: a PL and

DHA deficient diet; diet 5: a DHA deficient diet; diet 6: a PL deficient diet; diet 7: a VA deficient diet; diet 8: a complete diet; diet 9: a live food (*Brachionus* and *Artemia*).

Table I. Fish used and rearing method

Fish used	*Limanda yokohamae*
Age	8 days after hatching
Total length	5.99±0.34mm
Rearing and feeding methods	
Experimental period	50 days
Number of fish	700/tank
Tank	100 l
Water temperature	15.6±0.9°C
pH of seawater	8.2±0.1
Flow rate	350-700 $ml.min^{-1}$
Feeding frequency	10 times/day
Type of diet	Micro-bound diet
Size of diet	125-350μm

Results

The occurrence of albinism at the ocular side of *L. yokohamae* was determined 50 days after feeding the various test diets (Fig. 1). The occurrence rate of albinism (*i.e.* completely abnormal and partially abnormal) was 32.7% in the group receiving the vitamin A deficient diet. Among the three compounds (DHA, VA, and PL) the occurrence of albinism in the fish fed diets deficient in two or three compounds was higher than that fed a diet deficient in only one of them. These results suggest that DHA, VA, and PL are essential in the reduction of albinism in hatchery-reared marbled sole, *L. yokohamae*.

Discussion

The critical stage to induce albinism in flatfish larvae is at a total length of 8mm coinciding with the time when the eye retina is formed (Kawamura and Ishida, 1985). The rhodopsin in the rod cell, which conducts the vision in the dark, is composed of opsin (protein), retinol (vitamin A) and phospholipid including DHA. We assume that the rhopsodin formation of the retina is interrupted when DHA, vitamin A, and phospholipid are deficient in the initial foods offered just after hatching of the larvae. For this reason, the visual information from the retina is not delivered to the nerve center, the melanocyte-stimulating hormone is not secreted by the internal gland, eventually resulting in the interruption of black pigment formation.

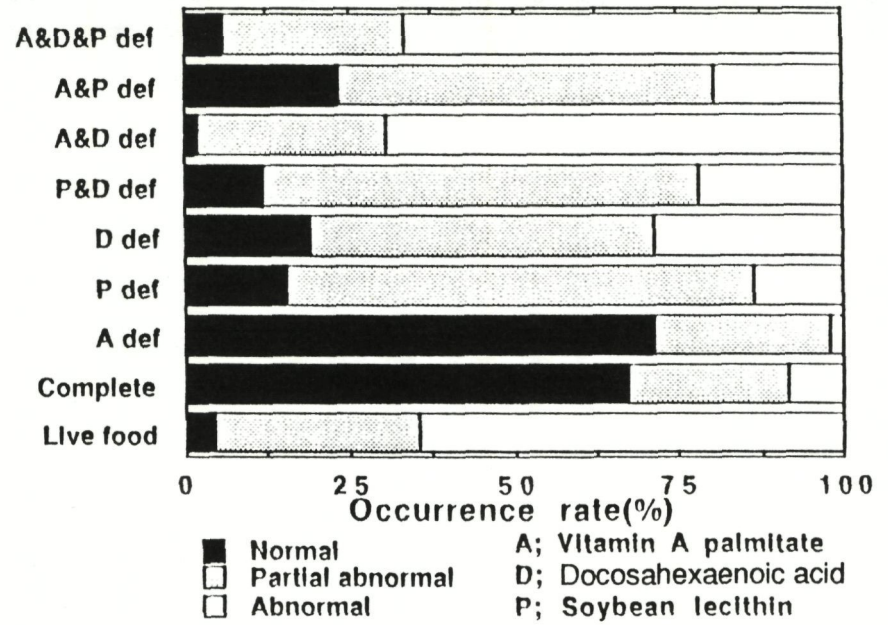

Fig. 1. Occurrence rate of albinism (coloured-type individuals) in marbled sole, *Limanda yokohamae*, fed diets deficient in vitamin A, docosahexaenoic acid, and soybean lecithin.

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PATTERN OF FATTY ACID LOSS IN SEVERAL WARMWATER FISH SPECIES DURING EARLY DEVELOPMENT

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Introduction

The importance of (n-3) HUFAs in the nutrition of marine larvae is well recognized. We studied three marine species, mahimahi (*Coryphaena hippurus*), moi (*Polydactylus sexfilis*), and milkfish (*Chanos chanos*). We wondered whether the pattern of fatty acid loss would illuminate the role of (n-3) HUFAs. We wondered further about which fatty acids would accumulate in fed larvae and how accumulation of various fatty acids related to growth, survival, and stress resistance. It will be shown that DHA (22:6n-3) is strongly conserved among starved fish and that higher feed DHA leads to higher tissue DHA. Higher DHA levels were correlated to greater stress resistance in mahimahi.

Materials and methods

In feeding studies, mahimahi were fed rotifers (*B. plicatilis*) grown on *T. chuii* between days 2-6, copepods (*E. acutifrons*) between days 6-14, and either of two kinds of *A. franciscana* thereafter until day 23. Fed milkfish were maintained on rotifers grown on baker's yeast, yeast + *N. occulata* (1:1), or *N. occulata* alone until day 30. Growth and survival were measured. Stress tests were done by netting fish, holding them out of the water for 1min and measuring survival 25min after re-immersion into the water.

Samples were freeze dried, soxhlet extracted, saponified, methylated, and quantified by gas chromatography except for a set of 22 samples that were also cold extracted to compare extraction methodologies.

Results

Fatty acids profiles were similar to one another using either cold or hot extraction procedures if "clean up" procedures remained the same.

Relative to most fatty acids, DHA and ARA (20:4n-6) seemed to be conserved during starvation in mahimahi and moi, two species with oil droplets in their eggs (Table I). Only ARA followed this pattern for awa, a species with no oil droplet.

Table II shows profiles of *Artemia* enriched with Super Selco (hiEPA/medDHA) and *Artemia* prepared from high HUFA cysts (hiEPA/loDHA). It may be seen that the former has a higher DHA level while the latter has a higher total fat level. There was no significant difference in growth rate or survival between mahimahi eating the different *Artemia*. However, mahimahi fed the *Artemia* with the higher DHA level were significantly more stress resistant (11%$\pm$12 *versus* 34%$\pm$10 mortality). Table II also shows carcass fatty acid profiles of mahimahi fed on these. DHA was significantly higher in the larvae fed the higher DHA *Artemia*. Whereas most fatty acids seemed lower in tissues than feeds, DHA seemed to be exceptional.

Table I. Several fatty acids in unfed mahimahi (% of total fatty acids; means and standard deviations)

Fatty acids	Eggs	Hatch	4-day-old
16	20.8(0.48)	20.4(0.95)	18.4(0.26)
16:1n-17	2.21(0.24)	2.11(0.77)	0.82(0.68)
18:1n-9	16.4(1.0)	17.2(1.4)	11.6(0.64)
18:2n-6	9.18(0.36)	9.08(0.35)	4.71(0.34)
20:4n-6	6.95(0.31)	8.21(0.39)	11.4(0.31)
20:5n-3	7.85(0.11)	7.49(0.34)	3.95(0.10)
22:6n-3	21.2(0.60)	19.9(2.8)	32.0(0.91)

Table II. Several fatty acids in two *Artemia* and mahimahi (mg.100mg^{-1} dry, means and standard deviations)

Fatty acids	*Artemia*		Mahimahi	
	hiEPA/medDHA	hiEPA/loDHA	hiEPA/medDHA	hiEPA/loDHA
18:2n-6	0.51(0.20)	0.73(0.07)	0.30(0.03)	0.26(0.03)
18:3n-3	0.68(0.46)	2.45(0.02)	0.66(0.04)	0.45(0.02)
20:4n-6	0.26(0.06)	0.43(0.01)	0.16(0.01)	0.28(0.01)
20:5n-3	2.05(0.12)	2.06(0.02)	0.54(0.05)	0.65(0.05)
22:6n-3	0.69(0.04)	0.04(0.01)	0.67(0.07)	0.12(0.01)
Total	10.58(1.36)	15.00(0.01)	5.80(0.03)	5.31(0.28)

Milkfish tissues showed the same pattern of increasing DHA levels when the fish were fed diets with increasing DHA levels. There was no significant difference in growth and survival but stress tests were not carried out.

Discussion

We wondered whether the pattern of fatty acid loss during starvation would illuminate the role of HUFAs in early larval life. We found that DHA (but not EPA) was

disproportionately conserved in unfed larvae of species containing egg oil droplets. This brought to mind the unusually high DHA content of yolk sac mahimahi and their efficacy as feeds for older larvae. We therefore tested *Artemia* containing medium and low DHA levels as feeds for 14 to 23-day-old mahimahi larvae. We found that the higher DHA feed led to higher tissue DHA levels in the larvae and greater stress resistance. The importance of DHA is consistent with the excellent feed properties of copepods compared to packed *Artemia* reported in another paper in these proceedings. The present data also raise a question as to whether EPA (20:5n-3) should be considered functionally identical to DHA in larval feeds.

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FATTY ACID AND LIPID UTILIZATION IN THE YOLK-SAC STAGE OF MARINE FISH LARVAE

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Introduction

The yolk-sac stage represents an important developmental period for all fish larvae. At this stage significant changes in the larval body take place before first feeding. The yolk-sac period can be as long as 55 days at 5°C in Atlantic halibut (*Hippoglossus hippoglossus*) or as short as 5 days at 10°C and 5 days at 8°C, in plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) respectively.
The energy in the yolk should be used for growth, development, and activity. Lipids (Tocher and Sargent, 1984) and free amino acids (Fyhn, 1989) have been regarded as substrates of aerobic energy production in marine fishes during embryonic and early larval development.
In the present study we show data of lipid utilization in the yolk-sac stage, in the period from hatching until first feeding.

Materials and methods

Eggs of halibut *Hippoglossus hippoglossus* (L.) were fertilized after stripping of females and males, incubated at 5°C and transported to the laboratory 12 days (*i.e.* 60 degree-days, 60D°) after fertilization. Thereafter, three egg groups were transferred to 50 l vessels in up-streaming water in darkness at 5°C. After hatching all three groups of larvae were transferred from the egg incubators to 3 l bowls with stagnant water, 25ppm oxytetracycline was added and the water maintained at 4.7±0.8°C, the density of the larvae was close to, but below, 100 larvae/l. Samples for lipid analysis were taken at hatching and at different stages of development, before feeding started: H1: 33 days after hatching (155°D); H2: 35 days after hatching (165°D) and H3: 48 days after hatching (226°D).
Artificially fertilized eggs of plaice (*Pleuronectes platessa*) were incubated and held at a constant temperature 7°C, 33ppt salinity and darkness. One day before hatching the eggs were transferred to the first feeding tanks and held at a constant temperature of 10°C and light rhythm of 0.2 to 80lux with a water exchange twice a day. Cod was incubated in similar conditions except that the temperature in the first feeding tank was 8°C. Three groups of samples of plaice and cod were taken at hatching and 5 days after hatching.

The lipids were extracted using a modified method described by Bligh and Dyer (1959). An internal standard (21:0 methyl esters) was added to the samples before extraction. The method used to prepare the fatty acid methyl esters (FAMEs) was derived from the one reported by Metcalfe *et al.* (1966). The FAMEs were determined quantitatively by a capillary gas chromatograph.

Lipids were separated by Iastroscan thin layer chromatography-flame ionization detector system (TLC) and the method was based on the one described by Fraser *et al.* (1985).

Results and discussion

Total fatty acids decreased in all the groups of the three species (Table I). In halibut the fatty acid utilization of H3 was approximately 50%, whereas in the other two series around 30%. In general all the fatty acids except 18:1 and 22:1 were not utilized in two of the groups. In cod an even consumption of total fatty acids in all the groups was shown, although it was uneven for the individual fatty acids, except 22:6n-3. In general, the decrease in total fatty acids for the three species was reflected in the utilization of (n-3) HUFA since these fatty acids constituted approximately 50% of total fatty acids in all the three species (data not shown here).

Table I. Fatty acid utilization (expressed in % reduction from hatching until just before first feeding) in halibut, plaice and cod. TFA: total fatty acids; H1: halibut larvae 33 days after hatching; H2: 35 days after hatching; H3: 48 days after hatching

	TFA	16:0	18:1	20:1	22:1	20:5n-3	22:6n-3	(n-3) HUFA
Halibut								
H1	30.1	28.5	46.1	29.2	44.6	33.9	31.1	31.9
H2	29.6	13.4	59.3	37.5	62.7	24.1	20.5	22.0
H3	50.8	40.9	30.0	64.2	63.5	50.1	42.0	45.5
Plaice								
P1	53.8	30.8	55.6	71.0	55.6	55.6	30.4	36.8
P2	11.9	26.1	11.8	0	0	9.5	12.7	12.7
P3	35.0	36.4	22.2	0	0	45.8	33.7	37.1
Cod								
C1	15.3	36.8	19.3	21.4	2.3	2.3	10.7	8.8
C2	15.6	37.5	15.7	44.4	50.0	0	11.6	8.0
C3	15.9	8.8	24.7	38.5	33.3	24.2	12.8	16.5

Dry weight levels of newly-hatched larvae of halibut were much higher (1.3-1.5mg/larva) than those of plaice (0.13-0.18mg/larva) and cod (0.11-0.13mg/larva). Thus, the quantitative content of lipid was higher in halibut, but not the relative amounts which were higher in plaice (19% DW) than in cod (9% DW) and halibut (13% DW) (data not shown here).

Relative amounts of neutral lipids and phospholipids were quite similar for the three species accounting for approximately 25% and 75% of total lipids respectively, and were constant during the yolk-sac stage (data not shown).

The utilization in % is shown in absolute terms (Table II). The dry weight decreased relatively more in cod than in halibut and plaice. In general the lipid utilization is quite irregular, in relative terms (%) the utilization of neutral lipids in plaice is higher than that of phospholipids. In cod and halibut the utilization was almost even except in H3, this implies (in H3) a higher utilization of phospholipids at a late stage of the yolk-sac phase most likely due to a higher rate of biomembrane formation during this period.

Table II. Lipid class utilization expressed as reduction in % from hatching until just before first feeding, in halibut, plaice and cod. DW: Dry weight; TL: Total lipid; NLs: Neutral lipids; CE: cholesteryl esters; TG: Triglycerides; STE: Sterols; PLs: Phospholipids; PE: Phosphatidylethanolamine; PC: Phosphatidylcholine; PS: Phosphatidylserine; SM: Sphingomyelin

	Halibut			Plaice			Cod		
	H1	H2	H3	P1	P2	P3	C1	C2	C3
DW	17.3	14.6	23.3	15.0	7.7	5.6	38.5	45.5	36.4
TL	30.1	26.6	23.3	16.2	25.6	14.4	15.2	42.2	16.0
NLs	35.8	31.5	42.9	24.8	36.6	16.3	15.8	40.0	16.3
CE	27.7	+67.4	19.1	43.8	56.8	58.7	63.6	21.9	28.9
TG	52.9	54.0	38.4	35.8	57.5	7.3	69.4	37.9	76.2
FFA	+77.	+109.6	68.8	69.4	44.7	8.4	+56.5	62.5	18.8
STE	6	26.8	47.3	+9.7	13.5	4.8	+7.1	24.5	+17.6
	24.1								
PLs	27.9	29.0	53.6	13.5	25.7	15.7	14.9	43.1	16.9
PE	+18.	+38.9	+25.8	+47.7	29.4	+27.3	+38.8	50.9	+14.1
PC	3	33.4	59.5	25.4	25.0	25.7	29.3	44.4	39.0
PS	30.6								
SM		86.1	90.7				+67.9	+40.0	73.0
	86.2								

+: increase.

Triglycerides (TG) decreased in all the groups whatever the species; the other neutral lipids seem to have an irregular catabolism as is shown for free fatty acids which are formed in H1 and H2. Phosphatidylcholine (PC) which was the major phospholipid in all the species, was significantly utilized in all the groups, meanwhile phosphatidylethanolamine (PE) seems to be biosynthesized. PC has been implicated in nutritional, biochemical, and physiological roles in eggs and larvae of cod (Fraser *et al.*, 1988) whereas PE has been considered important for regulation of localized membrane structure and fluidity in neural tissues. The results suggest that, besides its structural role,

PC also serves as a source of metabolic energy in the yolk-sac stage. Probably PE biosynthesis is derived from the catabolism of PC.

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LIPASE ACTIVITY AND TOTAL LIPID CONTENT DURING EARLY DEVELOPMENT OF RED DRUM *SCIAENOPS OCELLATUS*

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Introduction

The development of artificial diets to replace live zooplankton for marine fish larvae has thus far not been successful. Absence of a well developed digestion system in first feeding larvae has been suggested as a reason for this lack of success. Larval red drum have been reared on artificial diets, but growth rates and survival to metamorphosis were not acceptable. A study of changes in the digestive capacity of red drum larvae was carried out to gain information for increasing the efficiency of artificial diets. Lipids are important components of larval fish nutrition. In red drum eggs, lipid is the most important energy reserve supplying virtually all the catabolic needs for development (Vetter *et al.*, 1983). And the natural food of larvae, marine zooplankton contains 30 to 50% (dry weight) lipids (Sargent *et al.*, 1989). There is no established information on lipid content or lipase activity in larval red drum. A study was carried out to (1) identify changes in total lipids and lipase activity during development and (2) to evaluate shifts in these values in response to pulsed starvation or artificial diets.

Materials and methods

Red drum eggs were collected from natural spawns of laboratory broodstock, induced by photoperiod-temperature manipulation (Arnold *et al.*, 1978). Eggs (10 000 per tank) were placed in nine 150 l, conical tanks with internal biological filters. Temperature was maintained at 28°C, salinity at 31ppt and photoperiod was 12:12 throughout the study. Three feeding groups were established (1) control larvae fed rotifers beginning on the third day posthatch and *Artemia* nauplii on day 12, (2) larvae fed artificial diets, Kyowa A-250 beginning on the third day and Kyowa A-400 on day 12, and (3) periodically starved larvae, fed live food on the forth day posthatch then alternately starved 2 days and fed 2 days. Three samples of eggs and larvae were taken from each tank regularly until day 25 for measurements of total lipid, lipase activity and notochord or standard length.

Total lipids were extracted and analyzed using methods of Heath and Barnes (1970), Gallager *et al.* (1986) and Häkanson (1989). Fifty to 500 larvae, depending on size, were

transferred into covered glass tubes containing 3ml chloroform/methanol (2:1, v/v) and extracted 24h. Samples were centrifuged at 5 000rpm for 20min at 4°C, the residues washed with 2ml chloroform/methanol (1:1) and recentrifuged. Supernatants from both centrifugations were combined and evaporated in a 60°C water bath. The residue was weighed for the total lipid content.

Samples for lipase were homogenized according to Brahimi-Horn *et al.* (1989), then centrifuged at 8 000*g* for 45min at 0°C. The floating lipid pad was removed and the supernatants used for analyzing lipase activity (Sigma Procedure No. 800). Results were expressed as activity per weight of protein. A modified Lowery method for protein (Hartree, 1972) was used with bovine serum albumin as the standard. All analyses were run in triplicate and mean values used for calculations.

Results

The growth of all larvae was slow and not different among treatments for the first week, but there after the controls grew significantly better (Fig. 1). The periodically starved larvae all died after 2 weeks. There was also high mortality in the artificial food group and they were significantly smaller than the control larvae at the end of the experiment.

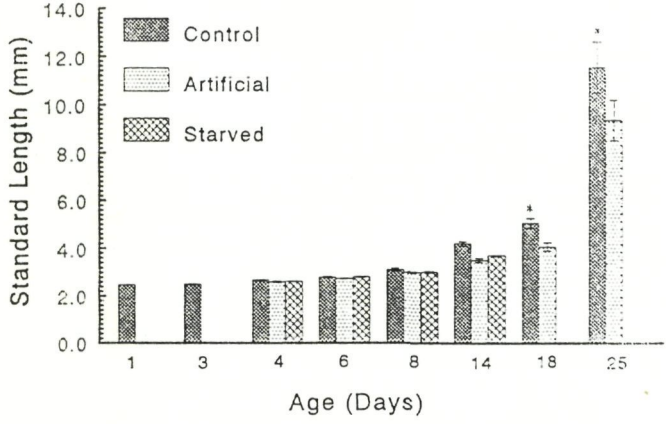

Fig. 1. Growth of red drum larvae on test diets.

Total lipid content was highest in the eggs (Fig. 2) with values equal to approximately 50% of the egg dry weight. A large yolk sac and oil globule persists on day 1; by day 3 when the yolk sac is absorbed, the total lipid was decreased to less than ¼ the egg value. Low levels of lipid persisted until day 8 in the control larvae and then increased with age and growth. Total lipid in starved fish remained low and virtually unchanged until they died, while the levels in the artificial food group increased from day 14 and reached levels similar to the controls by the end of the study.

Measurable lipase activity present in 1-day yolk-sac larvae doubled after first feeding, then remained relatively unchanged until it began to increase on day 14 (Fig. 3). Lipase activity at the end of the study was increased by an order of magnitude over values of yolk sac larvae. All three feeding groups showed similar patterns of lipase activity except for a significantly higher value in starved larvae on the final day before they all

died. Variability in lipase activity between the two control tanks masked any difference between the feeding treatments on day 25.

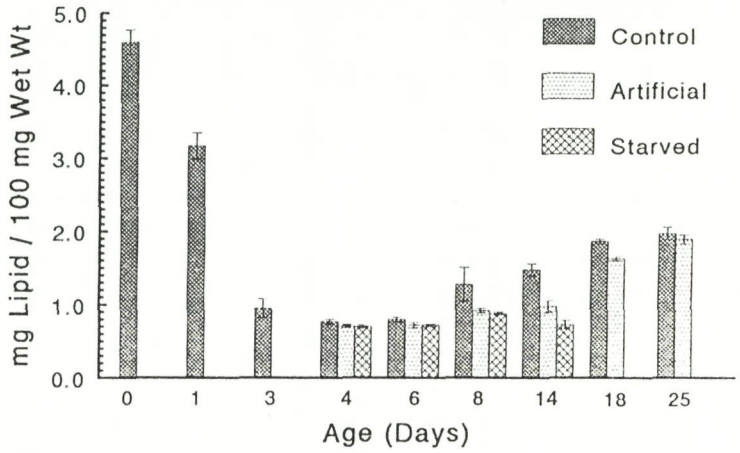

Fig. 2. Lipid content in red drum larvae on test diets.

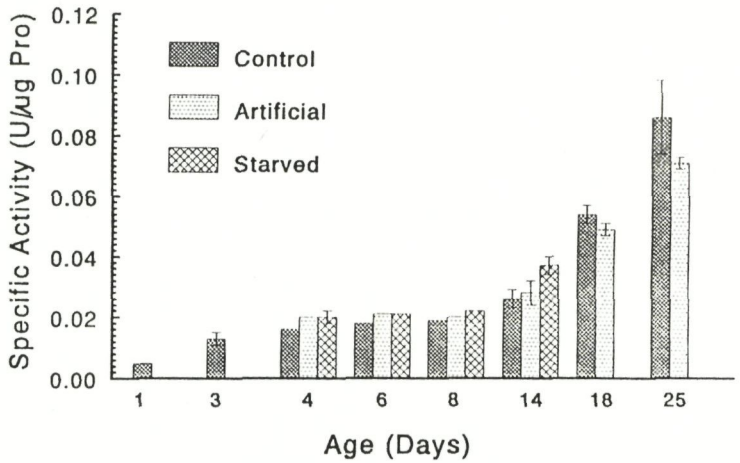

Fig. 3. Lipase activity in red drum larvae on test diets.

Discussion

High lipid levels in red drum eggs have been reported by Vetter *et al.* (1983) and similar high levels reported for eggs of other marine fish (Sargent *et al.*, 1989). The significant reduction in total lipid during the yolk-sac stage signifies the important role of lipids in supplying the energy requirements of pre-feeding larvae. Slow assimilation of exogenous lipids during the first few days of feeding corresponds to a lack of increase in biomass during this period. Total lipid values never exceeded 1% of the wet weight in starved larvae - a value that could be used as an indicator of poor nutritional status in larvae over 6 days. Lipase activity, as well as a gall bladder with the potential for production of bile salts to stimulate digestive lipases, is present at first feeding in red drum. After the first week both total lipid and lipase activity showed a consistent increase in activity with

increasing age. Lipase activity level was not a good indicator of feeding and nutritional condition in red drum larvae.

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FATTY ACID COMPOSITION OF UNFED AND GROWING COD LARVAE, *GADUS MORHUA* L., FEEDING ON NATURAL PLANKTON IN LARGE ENCLOSURES

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Abstract

Three different cohorts (1985, 1987, and 1988) of cod larvae (*Gadus morhua* L.), feeding on natural plankton in two large enclosures (20 000 m^3 and 60 000 m^3), were analyzed for fatty acid composition through development. Unfed control groups were also included in the analyses. Furthermore, different size-fractions of plankton were collected for fatty acid analysis (1987 and 1988) by sieving water from the enclosures through submerged tubes with end-mounted plankton nets of different meshsizes (80µm, 40µm, and 20µm). In the 1988 experiment, it was also possible to concentrate high amounts of 80-350µm zooplankton (mostly nauplii and early copepodites of the copepod *Eurytemora affinis*) for fatty acid analysis. Fatty acids (fatty acid methyl esters or FAME) were determined from total lipids of both cod larvae and plankton fractions by gas chromatography. In the 1988 experiment, total lipids of cod larvae were splitted in a polar and a neutral component.

Daily specific growth rates of the cod through the early larval stages were estimated to 10.2% and 12.0% in the 1985 and 1987 experiments respectively, and 20.2% in the 1988 experiment. Survival was between 16% and 37% within the first 3 to 4 weeks after release in the enclosures.

Specific content of FAME in the three experiments was between 14.7% and 6.6% of the larval dry weight (DW). The fatty acid composition of FAME in the cod larvae from all the experiments changed in a similar pattern through larval development. The changes in fatty acid profile could be separated into three developmental periods (DW<100µg, 100-400µg, and >400µg). In the first developmental period, a decrease in relative abundance of (n-9) monounsaturates together with 16:0 and 18:2n-6 occurred. An increase in relative amounts of 14:0, 16:1n-7, and (n-3) polyunsaturated fatty acids (PUFAs) was also observed. The highest increase in absolute amounts of fatty acids was found in 20:5 and 22:6, while 18:4 showed the highest relative increase. Most of the changes happened within the neutral lipid fraction. In the second developmental period, the fatty acid profiles were quite stable (non-systematic variation), and in the third period relative levels

of 18:1n-9, 18:2n-6, and 22:6n-3 decreased. In this period, 22:1n-11 was found to increase only in the 1985 experiment. In unfed cod larvae, a consistency in fatty acid profile was found with prolonged starvation. All fatty acids contributed to the reduction in total FAME, but 22:6n-3 and 18:0 seemed to be conserved.

Diatoms (*Skeletonema costatum* and *Rhizosolenia fragilissima*), together with unidentified flagellates and monades dominated the species composition. From multivariate statistics (SIMCA-classification) the fatty acid composition of the plankton fractions in 1987 and 1988 were found to be significantly different. Short-chain saturates (14:0 and 16:0), together with 16:1n-7 and (n-3) PUFAs (20:5 and 22:6) were most abundant in all the plankton fractions. The 18:4n-3 acid was most abundant in the smallest plankton fraction (2.4% to 6.8%). The zooplankton samples were closer to larval fatty acid profile than to the profiles found in the smaller plankton fractions (mostly containing algae).

The consistency in the development of the larval fatty acid profile suggests a development in both lipid absorption and lipid metabolism with larval development. The change in profile pattern at about 100µg DW corresponds with observed changes in lipid absorption in the gut epithelium of cod larvae at this moment. However, a dietary origin for the changes is also evident. Studies of the feeding ecology of cod larvae in the enclosure systems have shown that cod larvae feed directly on algae and obtain a "green gut" very short after release in the system. Especially 18:4n-3 which is known to have its origin in algae, increases in this first developmental period. The proportion of zooplankton (tintinnids, rotifers, and nauplii) in the ingested material increases with age, and continues with nauplii and juvenile stages of copepods as principal food in the suggested second and third developmental period.

More detailed information about lipid uptake and metabolism is needed throughout the larval development to better understand and evaluate the relative importance of diet and development on the resulting composition of fatty acids within the larvae.

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BIOCHEMICAL COMPOSITION OF ENRICHED AND STARVED ROTIFERS

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Introduction

Although fish larvae are usually reared on rotifers as a first food, problems related with low growth rates and high mortalities are often related with a deficiency in PUFAs in the rotifers. In recent years many attemps to improve the nutritional quality of the rotifers were made. In this experiment the biochemical composition of *Brachionus plicatilis* after 3 and 6h of enrichment, using three commercial products from Artemia Systems SA, Gent, Belgium, were studied.

Materials and methods

The rotifer *Brachionus plicatilis,* was enriched for 6h with three commercial products from Artemia Systems SA named Protein Selco and Dry Selco (both microparticulated enrichment diets) and Super Selco (an emulsion containing high levels of (n-3) HUFA). The biochemical composition (proteins, carbohydrates, glycogen, reducing components, total lipids, fatty acids and lipid classes) of the enriched rotifers was determined. The changes in the biochemical composition of enriched rotifers, kept for 6h in larval-culture tanks with *Isochrysis galbana* or seawater only, (desenrichment) were also studied.

Results

All the components studied increased when the enrichment was done with Protein Selco, while in the case of Dry Selco and Super Selco, proteins, carbohydrates and glycogen decreased after 6h of enrichment. Reducing components and lipids increased using the three products. The highest values were always obtained with Protein Selco. After 6h the dry weight of the rotifers increased when Protein or Dry Selco were used but decreased with Super Selco (Table I).

In all cases the main fatty acids increased during the enrichment (Fig. 1). Saturated and unsaturated fatty acids levels were the highest in Protein Selco-enriched rotifers while polyunsaturated fatty acids (including (n-3) HUFA) were higher for rotifers enriched with Super Selco, followed by Protein and Dry Selco. In the rotifers' phospholipids(1) are the major lipid classes followed by sterol esters+waxes(2), triacylglycerol(3), free fatty acids(4) and sterols(5). All lipid classes (except sterols) increased during enrichment.

Phopholipids and triacylglycerols values were higher in Protein Selco enriched rotifers while fatty acids and sterol+waxes were higher in rotifers enriched with Super Selco (Fig. 2).

After 6h of desenrichment the values of all the parameters studied increased for Protein Selco-enriched rotifers, and did not change or decrease for Dry Selco- or Super-Selco enriched rotifers.

Table I. Changes in dry and biochemical composition of rotifers enriched with Protein Selco, Dry Selco or Super Selco (ng/ind.)

	Dry wt	Proteins	Carbo-hydrates	Glyco-gen	Red. Comp.	Lipids
0 hours	370.00	133.00	61.54	17.83	26.11	37.00
3 hours						
Protein Selco	420.00	144.78	66.86	17.96	50.11	60.50
Dry Selco	400.00	137.02	61.06	14.95	44.38	57.00
Super Selco	331.00	139.73	59.59	12.17	43.74	46.50
6 hours						
Protein Selco	454.00	172.78	70.64	18.22	57.01	81.00
Dry Selco	386.00	120.82	46.56	11.18	37.38	61.50
Super Selco	317.00	112.12	37.18	8.97	31.34	60.00

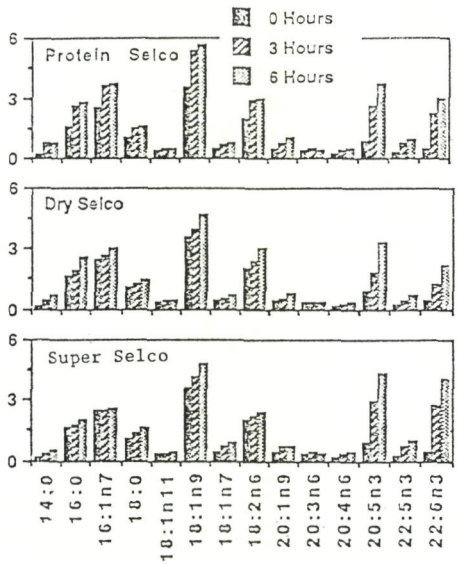

Fig. 1. Fatty acid composition of unenriched rotifers (0h) and rotifers enriched during 3h and 6h with Protein Selco, Dry Selco or Super Selco.

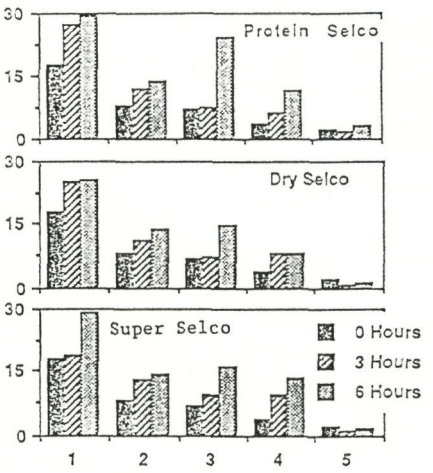

Fig. 2. Lipid classes composition in unenriched rotifers (0h) and rotifers enriched during 3h and 6h using Protein Selco, Dry Selco or Super Selco (ng/ind.). (1) phospholipids; (2) esters + waxes; (3) triacyl- glycerol; (4) free fatty acids; (5) sterols.

Discussion

Polyunsaturated fatty acids, phospholipids, and other lipid classes *versus* free amino acids are essential for larval fish (Kanazawa, 1985; Stottrup, 1989). The present results show that after enrichment the content in PUFAs and all lipid classes (except sterols) increase during the enrichment and reach in all cases the levels required for fish larvae. Protein, carbohydrates and glycogen levels increase in rotifers only when enriched with Protein Selco. It is noteworthy that enrichment levels remain adequate for 6h after transfer of the organisms to the larval-rearing tanks.

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EFFECT OF VARIOUS LIPID ENRICHMENTS IN ROTIFERS ON THE DEVELOPMENT OF EARLY STAGES IN TURBOT

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Abstract

The effect of lipid level and (n-3) HUFA level in live feed was tested on growth and development of turbot (*Scophthalmus maximus*) larvae ready for first feeding. The turbot larvae were offered cultivated rotifers (*Brachionus plicatilis*). The qualitative composition of fatty acids was obtained by long term-cultivation with either soybean oil (SO) or Super Selco, an oil rich in long chain (n-3) fatty acids (SS). A high lipid content was obtained by short-term enrichment of SS-rotifers with Super Selco (ESS). The total lipid content was ±13% in the SO- and SS-rotifers, and ±27% in ESS-rotifers. The content of (n-3) HUFA was 50% of the fatty acids in SS and ESS, and <5% in SO.

The turbot larvae were kept at 18°C and no water exchange was applied until 6 days after hatching. Two days after hatching, rotifers were added in a concentration of 5 000 ind./l. This concentration was kept throughout the experimental period (2 weeks after hatching). Growth rates were correlated with the initial lipid content of the rotifers, if no algae were added to the larval tanks. The relatively poor growth in these groups (5 to 10%/day) was probably due to an energy shortage during the period of stagnant water, as the nutritional value of the rotifers decreased. With algae (*Tetraselmis* spp.) present in the tanks, larval growth rates ranged between 10 and 20%/day, but no significant differences were found between the groups.

Histomorphological studies by electronmicroscopy revealed a substantial lipid absorption in the gut epithelial cells of ESS larvae. Small lipid-like particles were observed between the cells and in the blood vessels, which implies an intracellular digestion and a further distribution of lipids via the blood. However, the continuous supply of very high doses of lipids seemed to overload the digestive capacity of the larvae. The very high lipid level in the gut of ESS larvae also seemed to affect the protein absorption in the hindgut. In larvae fed rotifers cultivated on soyabean oil (SO), a similar large lipid absorption was obvious in the gut epithelium. However, no lipid particles were observed between gut epithelial cells or in the blood vessels surrounding the gut, thus indicating that no (or very little) intracellular digestion of these lipids took place in the larval gut.

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GROWTH AND SWIM BLADDER INFLATION IN *SPARUS AURATA* L. LARVAE

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Introduction

The purpose of the following investigation was to determine the effect of feeding regimes on growth and swim bladder inflation in *Sparus aurata* larvae.

Materials and methods

The feeding regimes used consisted of *Brachionus plicatilis* (Br) supplemented with one of the following; (A) Algal rotifero (trophic), (B) emulsion media (with cod liver oil), (C) *Chlorella* sp. (2/3) and *Isochrysis galbana* (Tiso) (1/3), (D) Frippak Booster, (E) *Chlorella* sp. and (F) Protein Selco. Four days after hatching, when the yolk sac was resorbed, the regimes (which had been washed to remove free fat) were introduced. Larvae were fed in the morning with the regimes at a level of 5-6 Br/ml.

Larvae were cultured in white cylindro-conical tanks (200 l) with a continuous through flow of aerated seawater. Light conditions followed a cycle of 12h light, 12h dark and the ambient water temperature during the study was 20±1°C. The water surface was continuously cleaned in order to remove oil which prevents swim bladder inflation (Kitajima *et al.*, 1981; Chatain and Ounais-Guscheman, 1990).

Results

The general survival rate during the experiment was 15 to 20%. None of the groups of larvae studied showed significant growth from day 4 to day 8. In all groups of larvae, growth was only observed after swim bladder inflation had occurred (day 8). Larvae fed regime C and D showed a high growth rate, but only group D where significantly higher than the other group ($P<0.05$ (1.4) = 96.49). Swim bladder inflation was not significantly different for diet A, C, D, E, and F (85-98%). However, only 45% of the larvae fed on diet B succesfully inflated their swim bladder (Fig. 1).

Conclusions

Larvae supplemented with Frippak Booster showed the greatest growth. All diets except B allowed a 85 to 98% succesful swim bladder inflation. Larvae fed regime B showed a similar growth to other groups. However, it only allowed a 45% successful swim bladder inflation. This may be due to high surface concentrations of oil due to inadequate emulsion preparation.

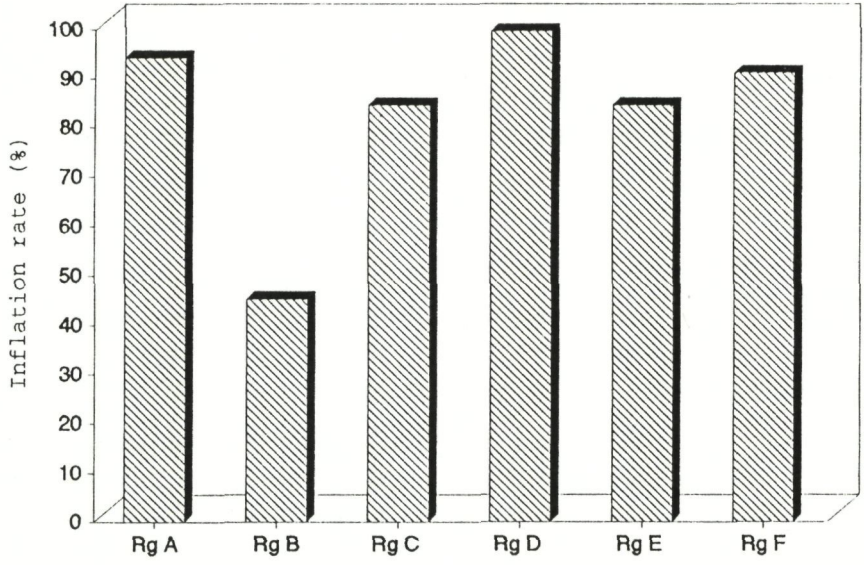

Fig. 1. Swim bladder inflation rate for *Sparus aurata* larvae fed on different diets (abbreviations of the diets are given in the text).

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GROWTH AND SURVIVAL OF LARVAL MILKFISH (*CHANOS CHANOS*) AND STRIPED MULLET (*MUGIL CEPHALUS*) GROWN ON ROTIFERS FED COMBINATIONS OF BAKER'S YEAST AND/OR *NANNOCHLOROPSIS OCULATA*

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Abstract

The rotifer, *Brachionus plicatilis* is currently viewed as an indispensable component for initial rearing of larval marine fish species. One of the reasons is that its fatty acid profiles can be altered to meet the requirements of the fish species targeted for culture. Rotifers were cultured on baker's yeast alone, *N. oculata* alone and a combination of yeast and *N. oculata* (1:1). The fatty acid profiles of the rotifers were significantly altered with the increase in the amount of yeast in the rotifers' diet. The results are given in Table I and II.

Table I. Summary of fatty acids from rotifers that exhibit significant differences when cultured on various combinations of yeast and/or phytoplankton

Fatty acid	Yeast	Yeast/*N. oculata*	*N. oculata*
14	0.17±0.04a	0.23±0.08a	0.34±0.01b
16	0.49±0.10a	1.15±0.53ab	1.33±0.11b
20:4n-6	0.11±0.05a	0.30±0.17ab	0.43±0.04b
20:5n-3	0.09±0.03a	0.68±0.51a	1.25±0.30b
22:6n-3	0.11±0.04a	0.45±0.31a	0.51±0.06b

Table II. Rearing results of larval milkfish and striped mullet

Rotifer feed	Trials	Harvest density (ind./l)	Survival (%)	Standard length (mm)
Milkfish				
N. oculata	3	8.6±4.3	41.9±14.3	12.6±1.1
Combination	3	7.3±2.5	48.9±6.5	11.9±0.7
Yeast	3	6.0±2.6	41.4±12.8	11.8±0.7
Mullet				
N. oculata	4	11.6±1.7	52.9±8.0	13.4±0.9
Combination	4	13.9±1.5	65.7±2.0	12.0±0.5
Yeast	4	6.2±1.9[1]	32.7±12.4[1]	13.9±0.5

[1] Significantly different ($P<0.01$).

The minimum fatty acid requirements are met for larval milkfish and mullet by rotifers cultured on yeast and yeast/*N. oculata*, respectively.

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COMPARISON OF COPEPODS AND ENRICHED *ARTEMIA* AS FEEDS FOR LARVAL MAHIMAHI, *CORYPHAENA HIPPURUS*

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Introduction

The first dependable methods to raise mahimahi (*Coryphaena hippurus*) from eggs required using copepods (Kraul *et al.*, 1989). Watanabe *et al.* (1978) suggested long ago that copepods' high HUFA levels are responsible for better larval survival of marine fishes. Recent improvements in enriching *Artemia* with HUFAs (Léger *et al.*, 1989) make mahi culture more practical without using copepods. We still find that mahimahi survive better when cultured copepods (*Euterpina acutifrons*) are used, especially when the larvae are under stresses such as high stocking density, disease outbreak, cold weather, or the rigors of metamorphosis (Kraul *et al.*, in review). Using copepods increases their degree of stress resistance. In this study, we find that stress resistance is a matter of degree, and is increased by increasing the concentration of docosahexaenoic acid (DHA), while eicosapentaenoic acid (EPA) does not appear limiting in the foods tested.

Materials and methods

Stress resistance was tested by netting six random samples of 10-30 postlarvae, blotting with a paper towel, and holding them out of the water for 60sec (HH *vs* SS *Artemia*) or 120sec (SS *Artemia vs* copepods). This stress was sufficient to shock 100% of the fry to immobility when placed in a bucket of tank water. After 25min, dead and live fish were counted.

The three diets tested were newly hatched high-HUFA *Artemia* (HH), *Artemia* enriched for 24h with 300ppm Super-Selco (SS), and copepods taken from a 500 l culture (50g standing crop, 20-70/ml) where they were fed the phytoplankton *Chaetoceros gracilis* and *Tetraselmis chuii*.

Incorporation efficiencies of DHA and EPA were assayed with 500-1 000 fry from 4 000 l tanks. Mahi were fed HH- or SS-*Artemia* from day 15 to day 23. In a subsequent test, copepods and SS-*Artemia* were fed to mahi from day 8 to day 18.

Results

Growth and survival differences were not statistically significant in these short tests, even though larvae gained more than five times their initial weight. However, stress resistance was significantly higher when dietary DHA was higher (Table I). Copepods have three times more DHA than Super-Selco enriched (SS) *Artemia*, which has 17 times more DHA than "High HUFA" (HH) newly hatched *Artemia*.

Table I. Stress mortality of mahimahi, *Coryphaena hippurus* in relation to wet weight HUFA concentrations in the fish and their respective feeds (means, and 95% confidence intervals in parenthesis)

	Wet HUFA concentrations		Stress mortality	
Feed or tissue	EPA ($mg.g^{-1}$)	DHA ($mg.g^{-1}$)	60sec	120sec
Artemia (HH)	2.06(0.05)	0.04(0.03)		
Artemia (SS)#1	2.05(0.17)	0.69(0.05)		
Artemia (SS)#2	1.28(0.19)	0.74(0.10)		
Copepod	2.36(0.01)	2.22(0.20)		
Mahi, fed HH-*Art.*	0.97(0.13)	0.18(0.03)	34(10)	
Mahi, fed SS-*Art*#1	0.83(0.13)	1.03(0.19)	11(12)	
Mahi, fed SS-*Art*#2	1.74(0.18)	1.61(0.13)		64(13)
Mahi, fed copepods	0.23(0.07)	1.11(0.32)		25(5)

Discussion

The copepod-fed mahi were suffering 10-20% daily mortality due to bacterial disease for 2 days prior to the stress test and assay, and their total fatty acids were only $5.55mg.g^{-1}$ ($\pm$ 0.24) wet weight compared to $12.88mg.g^{-1}$ ($\pm$ 1.14) for SS-*Artemia*-fed mahi. In spite of this, the resistance to net-stress was much higher in the copepod-fed group. The copepod-fed larvae had a significantly higher % of total fatty acids as DHA (19.9% of total FAs) compared to SS-*Artemia* (12.6% of total FAs). Copepods are also richer in essential amino acids than enriched *Artemia*.

Percent dry weight (95% confidence interval) for each tissue was: *Artemia*=10.03(0.47), copepods=13.53(0.28), mahimahi fry= 14.9 (HH), 15.4 (SS#1), 16.3 (SS#2), 13.5 (copepod-fed). Wet weight analysis was used, since mahi quantify their intake on a wet basis.

Mahi feeds with 0.04mg.g^{-1} wet DHA support good postlarval survival when stresses are low. Apparently 0.18mg.g^{-1} DHA in mahi tissue is not fatal. High EPA does not confer stress resistance when DHA is limiting.

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NUTRITIONAL QUALITY OF *ARTEMIA* DURING ENRICHMENT AND STARVATION

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Introduction

According to its origin *Artemia* nauplii as a food source for marine larvae may have nutritional deficiencies. One way of reducing the dependence on the natural quality of the nauplii could be by using enrichment diets for enhancing their nutritional value.
In this experiment we studied the chemical composition of *Artemia* nauplii enriched during 24 and 48h with emulsions containing different polyunsaturated fatty acid levels. The changes produced in the chemical composition of newly hatched nauplii and metanauplii enriched for 24h and kept for 12h in seawater with and without *Isochrysis galbana* added were also examined.

Materials and methods

Newly hatched *Artemia* (San Francisco Bay Brand) nauplii were enriched for 48h with three emulsions containing low, medium and high (n-3) HUFA levels. These emulsions were provided by the Artemia Reference Center (Gent, Belgium) in the framework of an ICES study on (n-3) HUFA requirements. Proteins, carbohydrates, glycogen, total lipids, fatty acids and neutral lipid classes were determined. To study the conservation of these components under different conditions, newly hatched nauplii and metanauplii enriched for 24h with *I. galbana* were incubated in seawater during 12h at 20°C. Their biochemical composition was compared with that of nauplii kept in seawater only.

Results

Table I gives the percentual increase or decrease of the components studied in enriched *Artemia* compared to their level in newly hatched nauplii. In all cases, a percentual drop of protein, carbohydrates and glycogen was detected during enrichment, the strongest being after 48h. A percentual increase in lipid content was observed in *Artemia* enriched with the three oils. The highest lipid levels were obtained in metanauplii enriched during 48h with "medium" oil. For dry weight, the greatest increase appeared in the metanauplii enriched for 24h with the "medium" and "high" oils .

Table I. Procentual change of dry weight, proteins, carbohydrates, glycogen and total lipids in the 24h and 48h enriched *Artemia* (using the 'high', 'medium', and 'low' emulsion) *versus* freshly hatched nauplii

Enrichment	Dry wt	Proteins	Carbohydrates	Glycogen	Lipids
24h					
High	11.63	-6.27	-52.73	-30.25	55.91
Medium	36.37	-6.09	-45.25	-1.68	64.86
Low	1.31	-27.95	-45.66	-57.14	36.10
48h					
High	-11.59	-23.29	-71.31	-35.29	32.59
Medium	6.17	-47.05	-68.69	-50.42	109.50
Low	6.73	-55.32	-63.64	-52.94	31.95

The pattern of fatty acids varied depending on the type of oil used. The content of saturated fatty acids (12:0; 14:0; 16:0 and 18:0)in *Artemia* increased when "low" oil was used for enrichment while those of polyunsaturated fatty acids (20:4n-6; 20:5n-3 and 22:6n-3) did so with "medium" and "high" oils. With the three emulsions the level of 18:1n-9 increased. The 18:3n-3 remained unchanged at 24h and dropped at 48h (Fig. 1). Most of the neutral lipids detected in newly hatched nauplii were triacylglycerol (2) followed by free fatty acids (3), sterol esters + waxes (1) and sterols (4). Levels of triacylglycerol increased after enrichment while free fatty acids and sterol esters + waxes decreased. An obvious decrease in sterol levels was detected with "medium" and "high" oil but not with the "low"one. With regard to triacylglycerol, the highest deposition occurred after 48h enrichment with "medium" oil (Fig. 2).

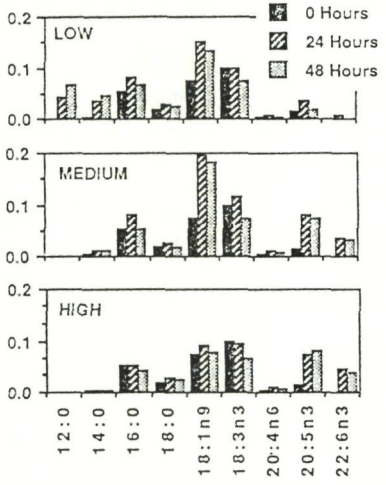

Fig. 1. Fatty acid composition of freshly hatched (■ 0h) and enriched (▨ 24h, ⊡ 48h) *Artemia* nauplii using the 'low', 'medium', and 'high' (n-3) HUFA emulsions.

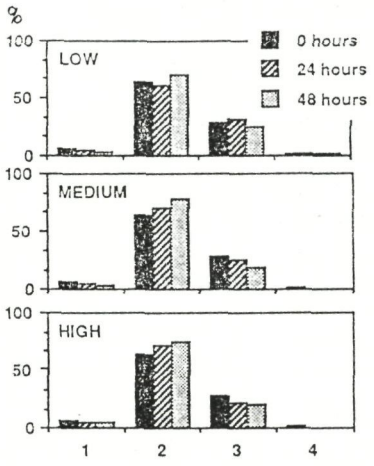

Fig. 2. Percentual composition of the neutral lipids of *Artemia* nauplii enriched with three emulsions containing 'low', 'medium', and 'high' (n-3) HUFA levels: 1= sterol esters and waxes; 2= triacylglycerol; 3= free fatty acids; 4= sterols.

Incubation of newly hatched nauplii or 24h enriched *Artemia* in *I. galbana* had no effect on their total lipid and essential fatty acid levels. In contrast, dry weight, protein, carbohydrate and triacylglycerol levels decreased (about 10%) in the absence of *Isochrysis*.

Discussion

Taking into account the (n-3) HUFA requirements and the values of the 20:5n-3/22:6n-3 ratio reported in the literature, the metanauplii enriched for 24h with the "high" and "medium" emulsions could be considered as an adequate diet for turbot larvae (Le Milinaire *et al.*, 1983). However, Gatesoupe and Le Milinaire (1984) stated that *Artemia* containing 20:5n-3 and 22:6n-3 levels of respectively 1.6 and 1.2% on a dry weight basis, are inadequate diets for these larvae. Léger *et al.* (1979), studying the requirements for turbot larvae of other fatty acids (18:3n-3 and 20:4n-6) established the nutritional requirement for 18:3n-3 of about 4% on a dry weight basis. In view of this, further research is being carried out for verifying the effectiveness of these diets in larval culture.

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DEVELOPMENT OF A LIPID-ENRICHMENT TECHNIQUE FOR *ARTEMIA* JUVENILES PRODUCED IN AN INTENSIVE SYSTEM FOR USE IN MARINE LARVICULTURE

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Introduction

Although there exists an obvious inclination to replace live larval food by formulated feeds, *Artemia* nauplii are still essential in the larviculture of marine fish species. So far, on-grown *Artemia* are rarely used in larviculture although they offer several advantages over *Artemia* nauplii: *e.g.* they contain a higher individual protein and energy content thus improving the fish larvae's energy budget; an equal amount of live food is reached with far less individuals thus a considerable saving of *Artemia* cysts; their composition of (n-3) highly unsaturated fatty acids (HUFA) may easily be improved by applying enrichment techniques (Lavens and Sorgeloos, 1991).
This study deals with the development of lipid-enrichment techniques adapted to the intensive culture of on-grown *Artemia*.

Materials and methods

Culture

Great Salt Lake (Utah, USA) *Artemia* were cultured for 7 days at 10 animals/ml on a dry feed (YM20). The water renewal was not renewed. Details will be published separately (Dhont *et al.*, in prep.).

Enrichment

Seven-day-old *Artemia* juveniles were harvested, rinsed and transferred to a cylindro-conical tank at ±50 animals/ml. To the tank 0.6g.l^{-1} HUFA enrichment emulsion (Selco) was added in one ratio (Léger *et al.*, 1987). This method was simplified by adding 0.6g.l^{-1} Super Selco (SS) directly to the culture medium, skipping harvest and transfer. YM20, Selco, Super Selco, and Dry Selco are products of Artemia Systems SA, Gent, Belgium.

In later experiments, the three main aspects of this method were screened in search of an optimal enrichment procedure:

a. Enrichment product: SS (an emulsion containing ± 450mg HUFA.g^{-1}) *versus* Dry Selco (DS: a dry powder containing ± 150mg HUFA.g^{-1});
b. distribution of enrichment product: classical (CLA) enrichment (Léger *et al.*, 1987) *versus* daily constant dose (DCD): every day an equal fraction of the total dose is added to the culture, *versus* daily increasing dose (DID): the first doses are smaller than in DCD but are increased daily as to reach the same total amount;
c. concentration of enrichment product: the standard concentration (0.6g.l^{-1}, as indicated in the guidelines of the enrichment products) *versus* higher concentrations; lower concentrations were applied in cases where water quality appeared to affect survival.

Low temperature storage

The administration of 6-day-old *Artemia* to cold-water fish larvae was simulated by transferring them to tanks filled with seawater at 12°C (halibut), 18°C (turbot) and 25°C (control). Survival and HUFA levels were monitored during 48h after transfer.

Enrichment of *Artemia* of various age and size

In fish larviculture it may be advantageous to offer gradually larger live prey. To cover prey sizes from nauplii to 7-day-old *Artemia*, the distribution of the enrichment medium was spread over 5, 4, 3, and 2 days, respectively, starting from day 0, but the total enrichment dose was kept at 0.6g.l^{-1} by increasing the daily doses accordingly (Fig. 2A). All treatments were maintained for 5 days and analysed for HUFA content.

Results and discussion

Enrichment

The enrichment of *Artemia* juveniles with SS allows to build up similar HUFA levels as obtained with nauplii (Table I). These levels are, however, reached in a much shorter time span, thanks to the better developed filter-feeding apparatus of the juveniles. There was no difference in HUFA levels between juveniles that were harvested and rinsed prior to enrichment, and animals that were enriched directly in the culture tanks. The latter considerably simplifies the enrichment procedure.

HUFA levels showed considerable differences from one experiment to another (see standard errors in Table II). Apparently food uptake is strongly affected by water quality and animal condition. Gradual enrichment over a longer period (both DCD and DID) yielded much higher HUFA levels than the classical enrichment because *Artemia* could also accumulate lipids in its body tissue in addition to the lipids stuffed in its gut. DS and SS gave equal enrichment results but the failure rate was much higher (>60%) when using SS. The culture stability improved at 0.2g SS.l^{-1} but at the expense of the HUFA level in the enriched *Artemia*. In all but one experiment, DID yielded higher levels than DCD. Final HUFA levels increased with increasing enrichment doses, at the highest dose the survival on day 7 dropped, however, to 30%. Based on these results 0.6g DS.l^{-1} distributed as daily increasing doses was adopted as the standard enrichment procedure.

Table I. Comparison of enrichment yields in *Artemia* juveniles (own results) and nauplii (from Léger *et al.*, 1987)

Artemia	Emulsion	Treatment before enrichment	Duration	20:5n-3	22:6n-3	Σ (n-3) HUFA
			(h)	(mg.g^{-1} DW)	(mg.g^{-1} DW)	(mg.g^{-1} DW)
Nauplii	S	Harvest & rinsing	12	7.9	4.4	14.4
juveniles	S	Harvest & rinsing	4	5.8	4.4	14.2
	SS	Harvest & rinsing	2	5.2	3.4	9.1
	SS	None	2	5.2	4.2	10.0

Table II. Enrichment results with different enrichment strategies, concentrations and products

Product	Method	Conc.	20:5n-3		22:6n-3		Σ(n-3) HUFA		Trials
		g.l^{-1}	mg.g^{-1} DW	SE	mg.g^{-1} DW	SE	mg.g^{-1} DW	SE	
DS	CLA	0.1	9.4	3.5	5.0	1.7	16.1	3.9	2
	DCD	0.3	10.0	-	1.4	-	11.7	-	1
		0.6	34.9	15.8	11.8	7.5	49.7	24.8	11
		1.8	38.5	-	17.1	-	59.5	-	1
	DID	0.6	44.2	13.5	16.5	7.0	64.3	21.3	8
SS	CLA	0.1	5.2	0.0	3.3	1.0	9.3	0.6	3
		0.6	8.7	4.9	4.6	2.9	14.3	7.5	2
		1.0	5.1	-	2.4	-	8.4	-	1
	DCD	0.2	9.4	2.2	1.1	0.6	10.8	3.0	8
		0.6	22.8	5.9	6.7	0.7	31.3	6.9	2
	DID	0.2	12.1	1.8	2.1	0.9	14.7	2.8	8
		0.6	36.0	-	14.0	-	53.0	-	1

Low temperature storage

HUFA levels dropped as the ambient temperature increased (Fig. 1). Apparently, starved *Artemia* thrive on their lipid reserves and metabolize less when the ambient temperature is lower. This implies that the feeding frequency is more critical in, *e.g.* turbot larviculture than halibut larviculture since preys that are not ingested within the first

hours after feeding will loose up to 25% of their HUFA. It is expected that cold storage of enriched *Artemia* juveniles (at 5°C) will allow to maintain initial HUFA levels at their maximum as was proven for nauplii for periods up to 48h by Léger *et al.* (1983).

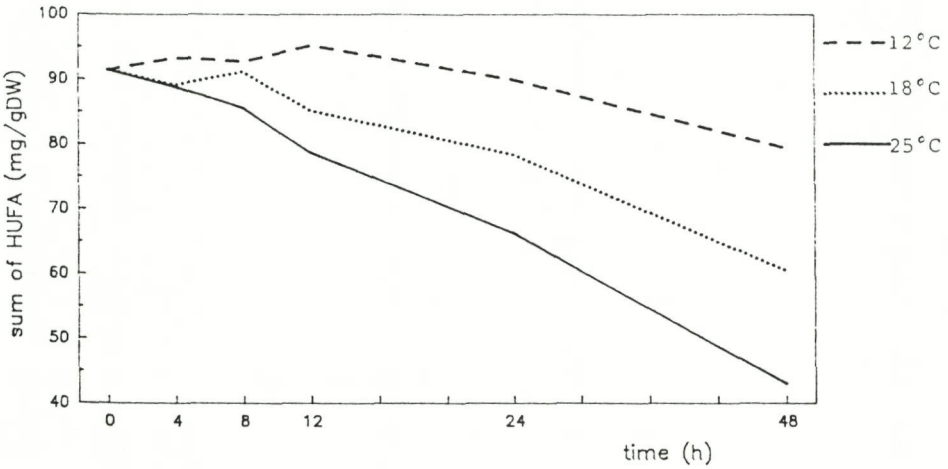

Fig. 1. Change in HUFA level of enriched *Artemia* stored at different temperatures.

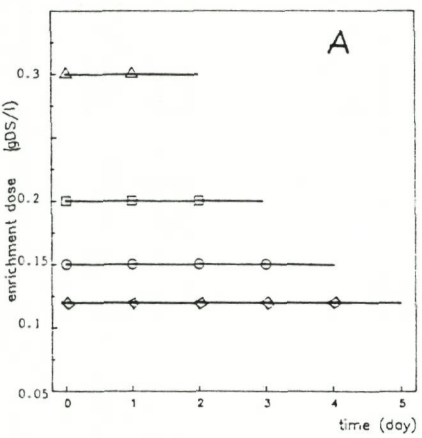

Fig. 2A. Enrichment doses of the different enrichment strategies; total enrichment dose is 0.6gDS/l for all treatments.

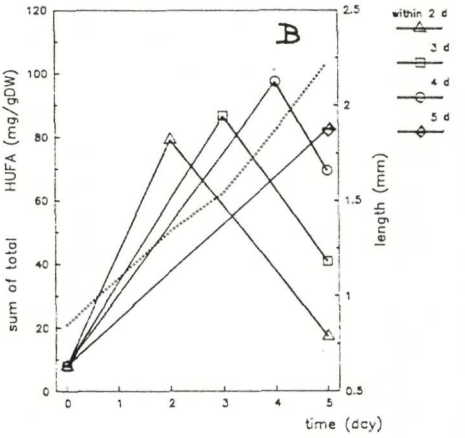

Fig. 2B. HUFA level in *Artemia* enriched within 2, 3, 4, and 5 days; the dotted line indicates animal size.

Enrichment of *Artemia* of various age and size

Artemia that received the total enrichment dose during days 0 to 2, accumulated HUFAs in their body tissue at levels comparable to those of gradually enriched 7-day-old *Artemia* (Fig. 2B). On the other hand, final HUFA levels still increased when the enrichment was spread over an extended period. Fig. 2 further shows that HUFA levels dropped drastically as soon as the daily addition of the enrichment medium is ended. This implies that part of the assimilated HUFAs is directly metabolized. As a consequence, an increase in HUFA content will only be realized when the daily HUFA uptake exceeds its decrease through metabolism. This equilibrium determines the minimal effective daily enrichment dose, while its maximum is limited by its negative effect on water quality.

Acknowledgements

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EFFECT OF DIETARY (n-3) HUFA ENRICHMENT ON SURVIVAL AND GROWTH OF SUMMER FLOUNDER, *PARALICHTHYS DENTATUS*, LARVAE

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Introduction

Investigations into the fatty acid requirements of larval and juvenile marine fish are being conducted under the auspices of the International Council for the Exploration of the Sea, Mariculture Committee, Working Group on Mass Rearing of Juvenile Marine Fish (ICES-WG) (Léger *et al.*, submitt.). Three experimental emulsions containing low, medium, and high levels of highly unsaturated fatty acids (HUFAs) have been distributed by ICES-WG to investigators for enrichment of rotifers and/or *Artemia* fed to larvae of several species of marine fish (*e.g.* turbot, seabass, red drum, and striped bass). In this study, we enriched *Artemia* of the Great Salt Lake, UT-USA (GSL) strain with the three emulsions, fed them to larvae of *Paralichthys dentatus* for 45 days, and determined survival and growth of the fish in each treatment.

Materials and methods

Larval *P. dentatus* were obtained from captive broodstock after injection with carp pituitary. The larvae were raised in 37 l glass aquaria and were fed *Brachionus plicatilis* (cultured with *Tetraselmis suecica*) and GSL *Artemia* prior to the experiment. To begin the experiment, 600 29-day-old larvae were randomly distributed into 12 black plastic rearing pans, described by Klein-MacPhee (1981), each containing 6 l of 30ppt filtered, UV- treated seawater (*i.e.*, 50 larvae/pan). The experiment consisted of three treatments (with four replicates each): *Artemia* enriched with: a) low-HUFA (no 20:5n-3 or 22:6n-3); b) medium HUFA (high 20:5n-3, low 22:6n-3); c) high HUFA (high 20:5n-3, high 22:6n-3) emulsions. On a daily basis, faeces and detritus were siphoned from the pan bottoms, a partial water change was effected, and mortalities were counted and removed. Instar II *Artemia* nauplii were enriched for 12h in an emulsion before they were fed to the larvae in the appropriate treatment, also on a daily basis. After 45 days, all fish were measured, weighed, and examined for abnormalities.

Results and discussion

Survival of larvae in the three treatments was not significantly different. Fish in the medium- and high-HUFA treatments had significantly larger lengths and weights than did fish in the low- HUFA treatment and fish in the medium-HUFA treatment had significantly larger weights than did fish in the high-HUFA treatment (Table I). Most of the fish had nearly or fully completed metamorphosis during the experiment, but some abnormalities were noted. In some fish in each treatment, the eye had not completed its migration and was still on the midline of the body; this phenomenon was much more prevalent in the low-HUFA treatment (41% of the fish) than it was in the medium-HUFA (4%) or high-HUFA (2%) treatments. Similarly, the incidence of albinism was higher in the low-HUFA treatment (33%) than in the medium-HUFA (6%) or high-HUFA (3%) treatments. Also, 14% of the low-HUFA fish were lying with the blind side up (but still alive) at the end of the experiment, whereas that behavior was not observed in the other treatments.

Table I. Survival, final standard length, and final dry weight for *Paralichthys dentatus* larvae raised on *Artemia* enriched with low-HUFA, medium-HUFA, or high-HUFA emulsions. Data are given as mean + standard deviation. Within a column, values followed by the same letter are not significantly different

	Survival (%)	Standard length (mm)	Dry weight (mg)
Low-HUFA	40+13A	13.6+0.3A	7.9+0.6A
Medium-HUFA	41+16A	18.2+0.6B	23.8+4.2A
High-HUFA	54+ 8A	16.9+1.0B	17.9+2.3C

The results indicate that the fatty acid requirements of the *P. dentatus* larvae were met by the medium-HUFA enrichment and that the higher levels of 22:6n-3 in the high-HUFA enrichment were not beneficial to the larvae.

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EFFECT OF DIETARY ESSENTIAL FATTY ACIDS ON EGG QUALITY AND LARVICULTURE SUCCESS OF THE GREASY GROUPER (*EPINEPHELUS TAUVINA*, F.): PRELIMINARY RESULTS

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Introduction

In spite of its aquaculture potential, the larval rearing of greasy grouper (*Epinephelus tauvina*) remains problematic and is not yet commercially feasible. The low and unpredictable larval survival is due to difficulties encountered in spawning the broodstock, the small size of the larvae at first feeding, and the high sensitivity of the larvae until day 45 (Lim, 1991). The nutritional requirements for the broodstock as well as for the young larvae are still enigmatical. In this presentation the impact of supplementing the highly unsaturated fatty acids (HUFA), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA) to the broodstock and larval diets is illustrated.

Materials and methods

The effect of dietary essential fatty acids (EFA) on egg quality was investigated on *E. tauvina* broodstock kept in two cages in Singapore udner identical conditions. Both groups of six female spawners were fed trash fish, at 2% of the broodstock's body weight per day during 3 months, but for the second group this diet was previously injected with the emulsified enrichment diet Marila (Artemia Systems SA, Gent, Belgium), which contained respectively 100, 80, and 195mg.g^{-1} DW of EPA, DHA and ΣHUFA's ≥ 20:3n-3. The dosage amounted to 2.5% of the body weight of the trash fish and was injected in the abdominal cavity of the fish. The fish eggs were obtained through induced hormonal spawning. They were analyzed for their biometrical and chemical characteristics. Larval survival was detected after a 7 days standard culture period (Lim, 1991).

Fatty acid requirements of *E. tauvina* larvae were determined in two feeding experiments. In the first experiment, the control diet consisted of *Brachionus*, cultured on *Chlorella*, fed from day 2 to day 18, and freshly-hatched Great Salt Lake *Artemia* nauplii (fed from 13 onwards). In the two other treatments the live food was enriched following the

bioencapsulation technique of Léger *et al.* (1987) with either Dry Selco (Artemia Systems SA) (= DS), or an experimental emulsion rich in DHA, EFA levels of the various live food diets are shown in Table I.

Table I. (n-3) HUFA-composition (in $mg.g^{-1}$ DW) of *Brachionus* and *Artemia* used in the feeding trials

	EPA 20:5n-3	DHA 22:6n-3	ΣHUFAs ≥20:3n-3
Brachionus	2.8	0.3	4.7
Brachionus + DS	21.0	16.0	40.9
Brachionus + DHA	8.9	25.0	39.2
Artemia	2.5	0.4	3.5
Artemia + DS	14.0	6.1	20.4
Artemia + DHA	16.8	24.9	45.8

Brachionus, enriched *Brachionus*, *Artemia* and enriched *Artemia* were offered from day 2, 5, 13 and 18 on, respectively. In the second set of experiments all groups were fed the control diet for at leat 18 days; DHA-enriched *Artemia* was offered at different stages from day 18. Fig. 1 and 2 illustrate the changes in the feeding strategy during the larval rearing.

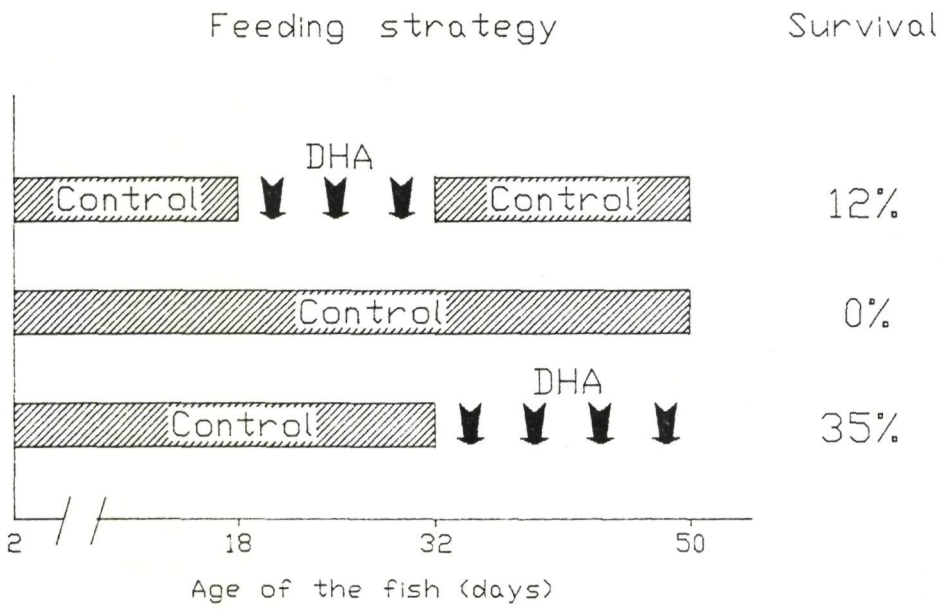

Fig. 1. Survival of grouper larvae (day 32-50) under different feeding strategies.

Results and discussion

The results given in Table II demonstrate a clear effect of the broodstock diet on egg characteristics and larval quality. The total lipid content of the eggs, for instance, increased by 21% when Marila-enriched trash fish was fed, and the size of the oil globule presented a significantly larger diameter. Although the broodstock enrichment diet had an initial EPA/DHA ratio of 1.3, the gain in the EPA component was more pronounced (46% increase compared to 9% for DHA). The better survival of the larvae originating from the Marila treatment in the 7-days-culturing experiment might indicate an improved egg quality due to a higher content of EFA. This, however, remains speculative as other components included in the Marila *e.g.* vitamin C or E, may influence the egg quality.

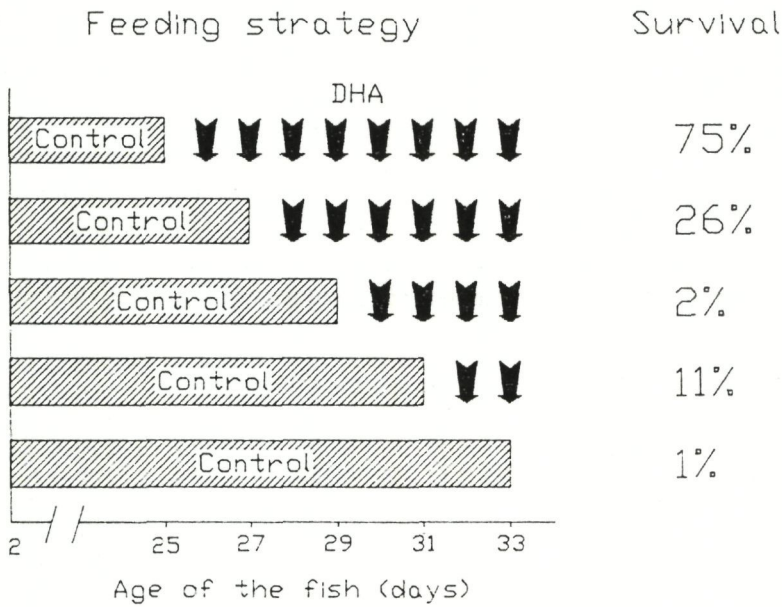

Fig. 2. Survival of grouper larvae exposed to a delayed DHA feeding.

Table II. Egg-quality characteristics of the control and Marila-fed broodstock

	Control	Marila
Egg diameter in (µm)	792±5	801±23
Oil globule diameter in (µm)	179±7	189±6[1]
Total lipids (%)	20.2±2.2	24.4±2.4[1]
EPA ($mg.g^{-1}$)	4.6±0.4	6.7±0.5[1]
DHA ($mg.g^{-1}$)	23.5±2.2	25.7±2.0[1]
Σ(n-3) ($mg.g^{-1}$)	30.7±2.4	35.9±1.9[1]
Larval survival at day 7 (%)	0	5±6[1]

[1] Significantly different at the $P<0.05$ level with the control.

During larval development the quantitative and qualitative lipid composition of the diet clearly determines the survival chances of the larvae. Table III illustrates that initially the control diet performed best. However, a deficiency in (n-3) HUFAs resulted in high mortalities in the second and third stage of the larval period. The fatty acid fortified diets did not enhance the survival during the first stage, but the DHA treatment slowed down the mortality in the following stages; there was no effect on growth.

Table III. Larval response to EPA and DHA enrichment

	Treatment	Day 12	Day 24	Day 31
Survival (%)	Control	12	5	<1
	DS	7	0	-
	DHA	7	6	2
Length (mm)	Control	3.6±1.0	8.1±0.9	8.9±1.5
	DS	3.4±0.6	-	-
	DHA	3.0±0.6	8.2±0.9	8.8±0.6

A verification run was carried out in which the DHA treatment was delayed until day 18. The results of the experiment revealed a better survival and growth of the animals in the DHA treatment (Table IV).

Table IV. Larval response to delayed DHA enrichment

Treatment	Survival (%) on day 32	TL (mm)
Control	0%	9.4±2.2
DHA-day 18	2-4%	10.8±2.4

Delaying the start of DHA-administration to day 18 or even day 32 resulted in a better survival of the larvae during the period day 38-day 50 (Fig. 1).

In order to detect until when exactly the administration of DHA can be postponed without detrimental effects on the survival of the larvae, the DHA administration was gradually delayed by 2 days, starting from day 18 (Fig. 2). It appeared that mortality due to nutritional deficiency became significant after day 25.

From these preliminary results it may be hypothesized that high DHA/EPA levels are essential in the early larval development of *E. tauvina*.

Acknowledgements

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THE NUTRITIONAL VALUE OF *ARTEMIA* NAUPLII FOR LARVAL SOLE, *SOLEA SOLEA* (L.), WITH RESPECT TO THEIR (n-3) HUFA CONTENT

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Introduction

It is common for high survival of sole larvae to be achieved on a diet of *Artemia* nauplii (*e.g.* Howell, 1973), even when fed on batches which fail to support survival of larval turbot (*Scophthalmus maximus* L.). This suggests that *Artemia* nauplii may more fully meet the nutritional requirements of the sole than those of other fish species. The problems of rearing turbot were ultimately attributed to a lack of (n-3) PUFAs in the food organisms (Scott and Middleton, 1979). The experiments reported in this paper were consequently undertaken to provide a preliminary assessment of the requirements of larval sole for these fatty acids.

Materials and methods

Two experiments were carried out in which larval sole were fed on *Artemia* originating from either Brazil or Utah (USA). Duplicate groups of larvae were offered *Artemia* either freshly-hatched or enriched with either *Isochrysis* sp. or a crustacean algal replacement diet (CAR, Frippak Feeds). The total lipid (% dry weight) and (n-3) PUFA content, as % fatty acid methyl esters (FAME), of the *Artemia* at the time they were offered to the larvae are given in Table I.

In the first experiment, 3 l glass beakers were each stocked with 30 stage 3 sole larvae and reared at a mean temperature of 14.2°C until the completion of metamorphosis (18 days). In the second experiment groups of 160 stage 2a larvae held in 10 l black polythene tank were stocked at a mean temperature of 16.6°C until the completion of metamorphosis (20 days). Each day a 75% batch water change effectively removed the majority of uneaten food before fresh food was added. All food types were tested in the second experiment, but in the first experiment the CAR enriched *Artemia* were excluded.

Table I. Total lipid and (n-3) PUFA content of freshly-hatched Brazilian (B) and Utah (U) nauplii and after enrichment with *Isochrysis* (Iso) and a crustacean algal replacement (CAR). (nd=not detectable)

FAME	B	B+Iso	B+CAR	U	U+Iso	U+Car
18:3	4.1	4.6	0.4	27.4	22.1	2.4
18:4	0.8	1.1	0.7	5.8	5.9	0.8
20:5	12.6	12.7	6.4	0.5	1.7	3.1
22:5	nd	nd	nd	nd	nd	nd
22:6	0.1	0.4	0.5	nd	nd	1.3
Total length (mm)	15.3	7.0	10.4	8.72	5.8	9.4

Results

Survival, total length and the incidence of abnormal pigmentation of duplicate groups were in close agreement ($P>0.05$) at the end of the experiments. Pooled means are therefore presented in Table II.

Table II. Survivals (S,%), mean total lengths (TL, mm) and the incidence of abnormality pigmented fish (AP,%) at the end of the experiments. Abbreviations as for Table I. (a b c indicate the significance of differences between mean lengths for each experiment. Same letter = no sig. diff. ($P>0.05$; different letter = sig. diff. ($P<0.05$))

Diet	Experiment 1					Experiment 2				
	S	TL	SD	n	AP	S	TL	SD	n	AP
B	10	15.1^a	1.8	60	30	59	15.1^a	1.4	60	70
B+ISO	95	15.6^a	2.1	57	11	56	14.7^a	1.7	60	42
B+CAR	-	-	-	-	-	58	13.6^b	1.4	60	53
U	97	13.5^b	2.0	58	20	26	11.1^c	1.3	60	42
U+Iso	95	13.5^b	2.1	57	13	43	11.8^c	1.2	60	14
U+CAR	-	-	-	-	-	45	11.6^c	1.4	60	16

During the second experiment there were high mortalities in all groups within a few days of stocking but an additional period of mortality occurred among those larvae fed on Utah *Artemia* shortly before metamorphosis. The first experiment was stocked with comparatively advanced (stage 3) larvae and this difference did not develop.

In both experiments freshly-hatched Brazilian nauplii supported the highest growth rates. Enrichment of these nauplii with *Isochrysis* had no significant effect ($P>0.05$) on larval growth and survival rates despite a 50% reduction in total lipid content. Both foods were comparatively rich in 20:5n-3 though they contained less than 1% 22:6n-3. Enrichment with CAR did result in a small but significant ($P<0.05$) decrease in growth rate, though not in survival. All these diets, however, supported higher growth and survival

(experiment 2 only) rates than either enriched or unenriched Utah *Artemia* all of which contained appreciably lower proportions of 20:5n-3 and little or no 22:6n-3.

It is also worthy of note that the percentage of abnormally pigmented fish was higher among groups fed Brazilian nauplii than those fed Utah nauplii. Enrichment of both strains, however, reduced their incidence.

Conclusions

These results suggest that sole larvae have a dietary requirement for 20:5n-3 but that high growth and survival rates may be achieved when fed diets almost deficient in 22:6n-3. This may explain the consistently higher survival rates achieved with larval sole than those of many other species.

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CONTENT OF (n-3) FATTY ACIDS IN LARVAE OF GILTHEAD SEABREAM (*SPARUS AURATA* L.) AND EUROPEAN SEABASS (*DICENTRARCHUS LABRAX* L.) FED DIFFERENT NATURAL DIETS

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Introduction

Several reports have demonstrated that marine fish need essential polyunsatured fatty acids (PUFAs) of (n-3) series, that are normally not required by cultured freshwater fish. The latter are capable of synthesizing the fatty acids 20:5n-3 and 22:6n-3 from 18:2n-6 and 18:3n-3 whereas marine fish, especially if carnivorous, lack the enzymes for the desaturation and the elongation of these precursors (Sargent *et al.*, 1989).

The rotifer, *Brachionus plicatilis*, can store small amounts of (n-3) PUFAs (Ben-Amotz *et al.*, 1987) while the concentrations of these compounds are extremely variable in eggs and nauplii of the brine shrimp, *Artemia salina* (Fujita *et al.*, 1980).

In the present study, we have determined the level of PUFA-enrichment in rotifers and brine shrimp, *Artemia* nauplii following administration of different enrichment media. We have also measured the original PUFA content of seabream and seabass eggs and larvae and monitored its enhancement in larvae when fed the enriched live food.

Materials and methods

Fatty acid profiles were analyzed by gaschromatography after lipid extraction with chloroform-methanol (2:1) according to the method described by Folch *et al.* (1956) as modified by Bligh and Dyer (1959) and fatty acid methylation with 5% HCl in methanol at 100-105°C for 1h. As enrichment media, we used Superselco (Artemia Systems SA, Gent, Belgium) for the maximal (n-3) PUFA content (A), Frippack Booster (Sanofi, France) for the combined (n-3) PUFA and protein enrichment (B) and Selco (Artemia Systems SA) for its intermediate composition with respect to A (C). The data were submitted to ANOVA, and significant differences among means established by the Student-Newman-Keuls test.

Results and discussion

Fatty acid analyses have revealed unsatisfactory levels of 20:5n-3 and 22:6n-3 in unenriched rotifers (only traces) and *Artemia* nauplii from different commercial origin (2.8 to 8.2% 20:5n-3 and no 22:6n-3).

It should be noted that the Brazilian *Artemia* strain, assumed to represent the "marine type" for its low quantity of 18:3n-3 and good level of 20:5n-3 (Watanabe *et al.*, 1980), actually lacks 22:6n-3. For the levels of fatty acids present in rotifers and *Artemia* nauplii before and after the administration of the three enrichment media, we refer to Navarro *et al.* (1988). In seabass eggs and post-hatch larvae respectively 8.8% and 1.8% of 20:5n-3, and 22.1% and 7.8% of 22:6n-3 were detected. Seabream eggs and post-hatch larvae showed 6.5% and 8.1% of 20:5n-3, and 28.3% and 31.5% of 22:6n-3, respectively. In 30-day-old seabass larvae fed the Artemia Systems *Artemia* strain enriched with A, the 20:5n-3 increased to 14.2%, but the 22:6n-3 remained constant (9.3%). When feeding the Argent strain *Artemia* + A, the 20:5n-3 increased only slightly (4.1%) and the 22:6n-3 did not change (7.2%). In seabream larvae, the Artemia Systems strain + A-diet could not prevent a dramatic fall in the percentage of the 22:6n-3 (8.0%), while the 20:5n-3 showed a minor increase.

In conclusion it can be stated that the PUFA concentration in eggs and larvae was lower in seabass than in seabream. However, amelioration of the PUFA profile following feeding an enriched *Artemia* was more pronounced in seabass than in seabream.

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THE BIOSYNTHESIS CAPACITY OF DOCOSAHEXAENOIC ACID (22:6n-3) IN CULTURED *SPARUS AURATA*: A HYPOTHESIS

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Introduction

The determination of the biochemical composition of fish larvae is a current practice to evaluate its nutritional requirements under intensive rearing conditions. The present work intends to determine the effects of the bioencapsulation of *Artemia* spp. metanauplii with highly unsaturated fatty acids (HUFA) on larviculture of gilthead seabream, *Sparus aurata.*

Materials and methods

The larvae were reared in a semi-closed circuit as described by Pousao-Ferreira and Silva (1989), and fed with *Brachionus plicatilis* bioencapsulated with *Chlorella* sp. and *Isochrysis galbana* from day 3 to day 15. The actual feeding experiments lasted from day 15 to day 44 when the larvae were fed with bioencapsulated *Artemia* metanauplii. Three treatments were tested: two commercially available enrichment products, Troffic (Troffic SA, Spain) and Selco (*Artemia* Systems SA, Gent, Belgium), and *Chlorella* sp.

At day 45 (after 1 day of starvation) the larvae were collected for chemical analysis and compared with the bioencapsulated *Artemia*. The HUFA were determined by the methods described by Bligh and Dyer (1959) and Metcalfe and Schmitz (1961) using liquid-gas chromatography. The fatty acid methyl esters (FAME) were injected in a capillary column (30m fused silica, 0.32 ID) installed in a Varian 3300 gas-liquid chromatograph. Helium was used as carrier gas at a flow rate of 1 $ml.min^{-1}$; oven temperature was 180°C for 7min then 200°C (with 4°C.min^{-1} temperature increase) over a period of 71min. Both the injector and FID detector were set at 250°C. Peak quantification was done with a Varian integrator 4290.

The experiments were carried out in three replicates and results analyzed by a one-way ANOVA. Data were normalized by an arc-sine$\sqrt{p}$ transformation (Sokal and Rohlf, 1981) and significant differences determined by a Tukey multiple comparison test, using statistic package computer Systat (Wilkinson, 1989).

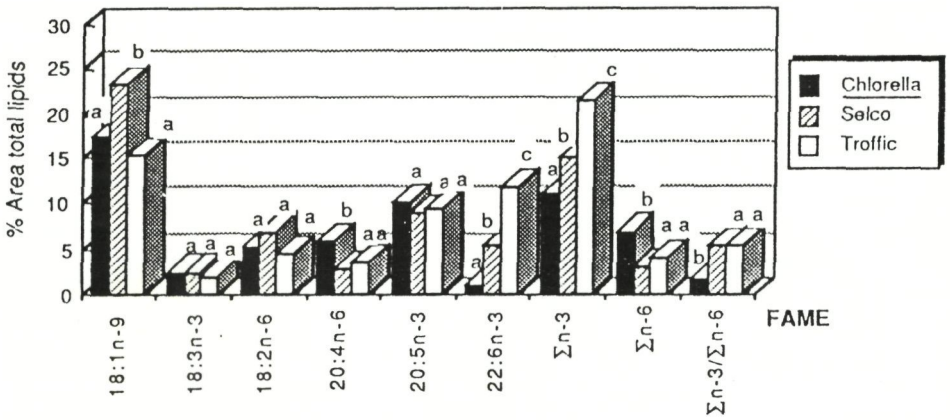

Fig. 1. The effect of *Artemia* HUFA on the fatty acid composition of *Sparus aurata* larvae aged 45 days. All values are the average of two separated analyses. Treatments having the same letter(s) are not significantly different ($P<0.05$).

Results and discussion

The results show that the total amounts of (n-3) long chain fatty acids (C>20) were significantly different in all treatments mainly due to the docosahexaenoic acid (22:6n-3) levels (Fig.1 and Table I). The ration (n-3)/(n-6) was significantly different, with a higher level of (n-6) HUFA, in the batch of larvae fed the *Chlorella*-enriched treatment.

Table I. Results of the analysis of some fatty acids methyl esters (FAME) in both enrichment products and in the enriched *Artemia* metanauplii (A2) expressed in area % of total lipids. Control was obtained with unenriched metanauplii. Values are the average of two separated analyses

FAME	Chlorella		Selco		Troffic		Control
	Product	A2	Product	A2	Product	A2	A2
18:1n-9	2.273	18.814	26.43	27.622	11.477	19.217	18.842
18:2n-6	3.798	6.014	7.245	8.417	2.610	5.902	6.071
18:3n-3	0.140	4.604	2.550	3.889	1.100	3.976	4.576
20:4n-6	4.217	2.730	0.979	1.345	1.158	1.722	2.884
20:5n-3	21.778	8.560	10.832	10.085	10.613	8.436	8.655
22:6n-3	-	-	8.488	4.369	19.303	2.233	-

According to New (1986), the (n-3)/(n-6) ratio should be considered as the critical component in a diet, rather than each isolated HUFA level, therefore it can be used as an indicator of possible HUFA deficiencies of the feeds.

The presence of 22:6n-3 in larvae fed the *Chlorella*-enriched *Artemia* seems to indicate that *Sparus aurata* larvae, contrary to the majority of the marine fish, have the capacity to bioconvert the 18:3n-3 acid from 18:1n-9 since their feed, *Artemia* metanauplii

bioencapsulated with *Chlorella*, had no 22:6n-3. The 18:1n-9 levels also decreased in larvae as compared to the levels in *Artemia*. The larvae could utilize the 18:1n-9 as a precursor of the 18:2n-6 and 18:3n-3. Likewise, the larvae showed an increase of 20:4n-6 levels as compared to their diet composition.

Conclusions

Seabream larvae seem to have the capacity to compensate HUFA deficiencies in their diet, especially 22:6n-3, by the bioconversion of precursor fatty acids; however, the low 22:6n-3 percentages found in the larvae do not allow for an affirmative conclusion about their bioconversion capacity. Further studies on the quantification of specific needs in (n-3) HUFA of *Sparus aurata* during its larval development, using radioactive labelling methods, are therefore required.

Acknowledgements

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EFFECT OF DIET QUALITY ON GROWTH AND RNA:DNA RATIO OF POSTLARVAL LOBSTERS (*HOMARUS AMERICANUS*)

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Introduction

The ratio of RNA:DNA is used as a biochemical indicator of growth rate in larval marine fish sampled from the sea. In summer, 1989, we found that postlarval lobsters reared in the laboratory on *ad libitum* feedings of frozen adult *Artemia* had significantly lower RNA:DNA ratios than those collected from the ocean. To test the hypothesis that the lower ratios may have resulted from inadequate fatty acid profiles in the frozen *Artemia*, an experiment was conducted in the summer of 1990, in which postlarval lobsters were fed adult *Artemia* that had been reared on algae with various fatty acid profiles.

Materials and methods

Female lobsters bearing eggs were collected from the ocean and transferred to the laboratory, where they were held until their larvae hatched. Larvae were fed frozen adult *Artemia* until they reached stage 4 (postlarvae), then they were transferred to individual growing chambers. Equal numbers of postlarvae were placed in the chambers, in each of four trays receiving flow-through filtered seawater (18±1°C). The larvae in one tray were fed *Artemia* that had been reared on *Dunaliella tertiolecta*, those in the second tray *Artemia* were fed with *Tetraselmis suecica*, those in the third were fed *Artemia* reared on *Isochrysis galbana*, and those in the fourth tray were cultured on commercially available frozen brine shrimp. Every 3 days, larvae were subsampled from each treatment to measure dry weight, total protein, RNA and DNA, and to determine the molt stage. Because most of the postlarvae molted to stage 5 between days 12-15 after reaching stage 4, the subsampling was conducted only on days 3, 6, 9, and 12. Analysis of variance followed by the Student-Newman-Keuls test, was used to determine the significance of differences among treatments, days of sampling, and batches of larvae.

Results and discussion

The *Artemia* fed to the lobsters had different fatty acid profiles as indicated in Table I, resulting from the various algae they were fed. As expected, *Artemia* fed *Dunaliella* (*Dun-Artemia*) were HUFA-deficient, whereas those fed *Tetraselmis* (*Tet.-Artemia*) and *Isochrysis* (*Iso.-Artemia*) had incorporated some HUFA.

Table I. Levels of the important fatty acids (as fatty acid methyl esters) in the three *Artemia*-fed algal species

Fatty acid	Percent composition in		
	Dun.-Artemia	*Tet.-Artemia*	*Iso-Artemia*
18:3n-3	26.7	9.2	6.8
20:5n-3	-	3.3	3.2
22:6n-3	-	-	1.4

The dry weight of the lobster postlarvae varied significantly with the treatment, the day of sampling, and the batch of larvae. Closer examination of the data (Table II) shows that differences in dry weight among the diet treatments occurred only on days 9 and 12. Lobsters fed *Tet.-Artemia* were significantly heavier than those fed *Tet.-Artemia*, but there were no differences between lobsters fed *Tet.-Artemia* and those fed frozen brine shrimp. The RNA:DNA ratio of the postlarval lobster varied only with the days, not with the diet treatment (Table III). The ratio increased significantly with time within each diet treatment, but on each sampling day, there were no significant differences among diet treatments.

Our results demonstrate that the commercially-available frozen *Artemia* used, yielded growth of lobsters that was indistinguishable from that obtained with live *Artemia* and that significantly poorer growth was obtained only on live *Artemia*-fed *Isochrysis* or *Dunaliella*. The RNA:DNA ratio was not usable to distinguish differences among diet treatments.

Table II. Mean dry weights (mg) of postlarval lobster reared on *Artemia*-fed various algae or frozen *Artemia*, on days 3, 6, 9, and 12 after molting to stage 4. Values in a column followed by the same letter are not significantly different

Diet	Day			
	3	6	9	12
Tet.-Artemia	12.3A	13.6A	16.1A	16.9A
Frozen *Artemia*	10.2A	13.8A	14.6A,B	15.2A,B
Iso-Artemia	11.2A	11.6A	12.7A,B	13.6B
Dun.-Artemia	9.4A	11.9A	11.4B	12.6B

Table III. RNA:DNA ratios of postlarval lobster reared on *Artemia* fed various algae or frozen *Artemia*, on days 3, 6, 9, and 12 after molting to stage 4. Values in a column followed by the same letter are not significantly different

Diet	Day			
	3	6	9	12
Tet.-Artemia	2.0A	2.5A	2.8A	3.3A
Frozen *Artemia*	2.5A	2.6A	3.0A	2.9A
Iso-Artemia	2.1A	2.5A	3.2A	2.7A
Dun.-Artemia	1.6A	2.0A	2.2A	3.1A

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GROWTH AND SURVIVAL OF TIGER SHRIMP, *PENAEUS MONODON* POSTLARVAE FED WITH *ARTEMIA* NAUPLII ENRICHED WITH (n-3) HIGHLY UNSATURATED FATTY ACIDS

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Introduction

According to Watanabe *et al.* (1978) and Léger *et al.* (1985) the presence of (n-3) highly unsaturated fatty acids (HUFAs) determines the nutritional value of *Artemia* nauplii for the larvae of marine fish and shrimp species, respectively. When using *Artemia* cyst batches with low levels of (n-3) HUFAs the lipid composition of the *Artemia* nauplii can be manipulated by applying the enrichment technique of the hatching medium with a marine fish oil emulsion.

Materials and methods

The enrichment diet for the *Artemia* nauplii was prepared by emulsifying fish oil with raw egg yolk and distilled water (7:1:2) using a regulated speed homogenizer. Enriched *Artemia* nauplii were prepared by rearing 300 000 freshly-hatched *Artemia* nauplii in 1 l seawater containing fish oil emulsion at different levels (0.0, 0.5, and 1.0g.l^{-1} seawater) and at different enrichment duration (12, 18, and 24h). After each desired duration, the *Artemia* nauplii were harvested and rinsed with clean seawater before feeding to the shrimp larvae. *Artemia* were distributed to the shrimp twice a day (in the morning and evening). The shrimp post larvae were reared from PL1 to PL15 in aquaria containing 60 l of seawater.

Results and discussion

Fatty acid analyses revealed that changes in the 20:5n-3 content of the *Artemia* nauplii depended on the duration of enrichment and level of fish oil emulsion used. The 20:5n-3 content of *Artemia* nauplii increased after 18h of enrichment, but decreased after 24h of enrichment at both 0.5 and 1.0g level. The growth response of *P. monodon* postlarvae fed with *Artemia* nauplii for a period of 15 days is shown in Table I.

Table I. Individual wet weight of *Penaeus monodon* postlarvae (PL15) fed with *Artemia* nauplii enriched for various periods with different levels of fish oil emulsion

Level of fish oil emulsion ($g.l^{-1}$)	Duration of enrichment (h)	Mean wet weight (mg)
0.0	12	11.45
	18	11.55
	24	9.70
0.5	12	11.66
	18	16.58
	24	10.88
1.0	12	15.53
	18	17.04
	24	13.22

The survival rates obtained ranged from 70 to 80%. Duration of enrichment and level of fish oil emulsion did not have a significant effect on the survival rate of the *P. monodon* postlarvae. Best results in postlarval weight were obtained for the 18h-enrichment period and with the highest levels of fish oil emulsion. These results appear to confirm the findings of Léger *et al.* (1985) with *Penaeus stylirostris* that increased (n-3) HUFA levels in the *Artemia* diet ensure faster growth in shrimp postlarvae.

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THE EFFECT OF DIETARY PHOSPHATIDYLCHOLINE IN POSTLARVAL PENAEID SHRIMP. I. DIET PREPARATION

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Introduction

The development of a suitable reference diet which is well accepted, digested and assimilated by larval organisms, and which can be used in larval nutrition studies has been the subject of several investigations (Ricardez, 1985).
Although semi-purified diets allow for a precise definition and dosage of nutritional components, they have generally been found less effective in supporting larval survival and growth (Teshima *et al.*, 1982). An alternative approach consists of enriching live foods such as *Artemia* nauplii with particular nutrients. The technique of bioencapsulation with lipid emulsions has been successfully applied to increase the levels of highly unsaturated fatty acids in live diets, and for transferring various therapeutics to predator larvae (Léger *et al.*, 1986; Verpraet *et al.*, in press).
The aim of this study was to verify if phospholipids (PL) and more particularly phosphatidylcholine (PC) could be accumulated in *Artemia* nauplii using the bioencapsulation technique and if such enriched diet could be used for qualitative and quantitative evaluation of the dietary effect of PL in larviculture of marine organisms.

Preparation of enrichment diets

Various self-emulsifying concentrates containing 60% of purified PC (Epikuron 200, Lucas Meyer GmbH, Hamburg, Germany) and 40% of methylesters in the lipid phase were prepared by *Artemia* Systems SA (Gent, Belgium) using various types/concentrations of synthetic co-emulsifiers, different ratios of the volumes of the two phases oil and water, and different techniques for pre-dissolving PC in the lipid phase. Any of those techniques, however, failed to produce a sufficiently stable emulsion which could be used for extended enrichment periods of at least 24h. Hence it was decided to produce a PC enrichment diet under the form of a dispersion in seawater. For this, Epikuron 200 was vigorously blended in seawater with an ultraturrax for at least 5min. The resulting dispersion had a particle size smaller than 10µm and proved to be stable for more than 24h under practical enrichment conditions.

Enrichment of *Artemia* nauplii

In a preliminary experiment Sudan Black-stained Epikuron 200 was used to visualise the uptake of the dispersed enrichment diet in *Artemia* nauplii. Microscopic evaluation revealed that the dispersion was readily ingested by the *Artemia* nauplii. Optimal results (*i.e.* maximal filling of the digestive tract within 1h) were obtained when using 12-h-old *Artemia* that were offered the dispersion at a concentration of $1g.l^{-1}$ of seawater (35ppt; 28°C). Three enriched-*Artemia* diets were prepared by feeding 12-h-old *Artemia* nauplii with a seawater dispersion of $1g.l^{-1}$ of Epikuron 200 for a period of 1h (treatment 1), 12h (treatment 2), and again 12h but followed by 12-h-old starvation in filtrated seawater (treatment 3).
Treatment 2 and 3 were produced to verify if the PC was further assimilated in the nauplii once the digestive tract had been filled and if assimilated PC was accumulated/preserved as intact PC or digested/metabolized into other products. A fourth treatment used 12-h-old *Artemia* fed a PC-deficient self-emulsifying concentrate containing refined soya-oil and served as a control.

Analyses of phospholipids in enriched *Artemia*

Enriched *Artemia* nauplii of the four treatments were freeze-dried and sent to the analytical laboratory of Lucas Meyer GmbH for PL analyses. PL were separated by thin layer chromatography (TLC) on silica gel Si 60 with the solvents chloroform/methanol/water (65:25:4). The separated PL were identified by selective spray reagents. Quantitative determination of PC was performed by high performance liquid chromatography (HPLC). From the results presented in Fig. 1 (TLC analysis) and Table I (HPLC analysis) it appears that all *Artemia* diets (including the soya-enriched control) contain significant amounts of other natural PL, in addition to PC. Furthermore, the levels of these PL remain relatively constant, *e.g.* dietary enrichment with PC does not enhance the PC content of the nauplii. This could indicate that the content of PL in *Artemia* is largely a function of the amount of membrane and other structural requirements, rather than the concentration of dietary PL fed to the nauplii. Unlike triglycerides which are easily bio-accumulated in *Artemia*, excess dietary PL are probably digested by *Artemia*.

Table I. Content of total lipids, phosphatidylcholine (PC) and lyso-phosphatidylcholine (LPC) in *Artemia* nauplii prepared by various enrichment treatments

Treatment	Total lipid (%)	PC (%)	LPC (%)
1. Epikuron 200 (1h; $1g.l^{-1}$)	16.6	1.4	0.8
2. Epikuron 200 (12h $1g.l^{-1}$)	16.5	1.6	0.8
3. Epikuron 200 (12h; $1g.l^{-1}$) followed by 12-h starvation			
4. Soya-oil emulsion (12h; $0.3g.l^{-1}$)	19.5	3.0	-
	16.5	2.0	-

Fig. 1. Distribution of phospholipids in *Artemia* nauplii prepared by various enrichment treatments: (1) Epikuron 200 (1h; 1g.l^{-1}); (2) Epikuron 200 (12h; 1g.l^{-1}); Epikuron 200 (12h; 1g.l^{-1}) followed by 12h starvation in filtrated seawater; (4) soya-oil emulsion (12h; 0.3g.l^{-1}). (PE: phosphatidylethanolamine; PA: phospatidic acid; PC: phosphatidylcholine; PI: phosphatidylinositol; LPC: lyso-phosphatidylcholine).

Conclusion

From the present study it appears that the bioencapsulation technique cannot be used to accumulate dietary PL in *Artemia*. It is concluded that *Artemia* is not a suitable test diet to elucidate the dietary effect of different levels/types of purified PL in marine fish and shrimp.

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THE EFFECT OF DIETARY PHOSPHATIDYLCHOLINE IN POSTLARVAL PENAEID SHRIMP. II. PRELIMINARY CULTURE RESULTS

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Introduction

Although dietary phospholipids (PL) and particularly phosphatidylcholine (PC) have been found to enhance growth and survival in penaeid shrimp (Kanazawa *et al.*, 1985), precise requirements have yet to be established. Substantially variable responses of feeding PL have been noticed in crustaceans in relation to the growth stage of the animals and the composition of the other dietary ingredients. In several shrimp feeding trials phospholipids of unspecified composition, have been used, such as crude lecithin, as a result of which it is difficult to draw conclusions with regard to the optimal levels/types of dietary PL required by penaeid shrimp.

This paper is part of a broad study which aims to assess the dietary effect of different levels/types of purified PL in marine fish and penaeid shrimp and reports on the results of a preliminar feeding trial with postlarvae of *Penaeus japonicus* using purified soya-PC in a semi-purified diet.

Materials and methods

Postlarvae of *P. japonicus* (1.3mg dry weight, 10mm total length) were reared in two successive phases of 17 and 14 days, respectively in a series of 20 l aquaria. Temperature was maintained at 25±1°C. Initial stocking density was 10 shrimp/l. At the start of the second phase, shrimp density was reduced to 4 ind./l. Further details with regard to culture set up and rearing conditions can be found in Abelin *et al.* (1989).
Two casein based microbound diets (Teshima *et al.*, 1982) of similar composition, except for the content of PC were tested (Table I). In the first diet (PC), 3% purified soya-PC (Epikuron 200, Lucas Meyer GmbH, Germany; composition: 95% PC, 3% lyso-PC, 1% other phospholipids) was included. In the second (control) diet (SO), Epikuron 200 was substituted for 3% of refined soya-oil in order to produce a PL-free diet. Shrimp were fed to excess twice a day (0900 and 1700h). Five replicate aquaria were used for both feeding treatments.
Dry weight, survival and stress resistance were recorded after the first and second rearing phase. Stress resistance was assessed by checking the survival of shrimp after a 30min exposure to deionised water followed by a 1h recovery period in 30ppt sea water

(Tackaert *et al.*, 1990). Mean dry weight and survival associated with the dietary treatments were compared by a Student's t-test.

Table I. Ingredient composition of phosphatidyl-supplemented (PC) and phospholipid-deficient (SO) semi-purified diets

Ingredient (%)	PC	SO
Casein[1]	45.0	45.0
Sucrose[1]	10.0	10.0
Wheat starch[1]	10.0	10.0
Methylesters[2]	8.5	8.5
Soya oil[3]	-	3.0
Phosphatidylcholine[4]	3.0	-
Cholesterol[5]	0.5	0.5
Mineral mix[6]	10.0	10.0
Vitamin mix[2]	2.0	2.0
Vit C-MAP[7]	1.0	1.0
Cellulose[1]	6.0	6.0
K-carrageenan[1]	4.0	4.0

[1] Sigma, St. Louis, Missouri, USA; [2]Artemia Systems SA, Gent, Belgium; [3]Vandemoortele NV/SA, Izegem, Belgium; [4]Lucas Meyer GmbH & Co, Hamburg, Germany; [5]Duphar, Weesp, the Netherlands; [6]Teshima et al. (1982); [7]Showa Denko KK., Tokyo, Japan.

Results and discussion

Growth, rearing survival and stress resistance of postlarvae of *P. japonicus* after the first (17 days) and the second phase (31 days) are presented in Table II.

Table II. Mean dry weight, stress resistance and rearing survival of postlarvae of *P. japonicus* fed either a 3% phosphatidylcholine-supplemented (PC) or phospholipid deficient (SO) semi-purified diet, after 17 (first phase) and 31d (second phase) of growth

Diet	Dry weight (mg)		Stress resistance (% survival)		Rearing survival (%)	
	17d	31d	17d	31d	17d	31d
PC	2.97	5.28	90.0	43.3	48.8	65.2
SO	1.79 (t=7.67**)	6.60 (t=2.38*)	76.7 (t=-2)	86.7 (t=-6.5**)	54.0	66.0

* $0.05 > P > 0.01$.
** $0.01 > P > 0.001$.

At the end of the first culture phase, the mean dry weight of the treatment group fed the PC-diet was significantly better than this of the group fed the PL-deficient control diet. Although not significant, a beneficial effect of PC was also noted with stress resistance; shrimp fed the PC-supplemented diet exhibited a better survival (90%) than the ones receiving the control diet (77%).

By the end of the second phase, a completely different picture was obtained. Best growth and stress survival were attained on the PL-deficient diet. This drastic change in the nutritional response of the postlarvae over a period of 2 weeks only is still unclear. While it is most likely that the younger stages of crustaceans require relatively higher levels of dietary PL than the later growth stages, there are no reports indicating a negative effect associated with supplemental PL for any growth stage/species of crustaceans. On the contrary, the beneficial effect of PL has been observed not only in crustacean larvae, but also in juveniles (Pascual, 1986; Teshima *et al.*, 1986). Several investigations suggested that the PL-requirements in crustaceans may vary according to the diet composition. Kanazawa *et al.* (1985) found a lower PL-requirement in *P. japonicus* larvae when the dietary lipid source contained high levels of (n-3) highly unsaturated fatty acids (HUFA). The inclusion of 8.5% of methylesters, containing 60% of (n-3) HUFA, as the basal lipid source in the present diets, certainly supplied the shrimp with relatively high levels of (n-3) HUFA. While this may have influenced the dietary requirements for PL to some extent, it is not believed to explain the significantly poorer performance of the PC-supplemented diet relative to the PL-deficient diet, observed during the second phase of the experiment.

Further studies are now in progress to clarify the quantitative and qualitative PL-requirements for different growth stages of penaeid shrimp.

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LIVE FOOD

Production

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TECHNICAL AND BIOLOGICAL ASPECTS OF CONTINUOUS MICROALGAE CULTIVATION

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Introduction

Continuous culture of microorganisms refers to a continuous or intermittent addition of growth medium to a fixed-volume culture, which results in a constant removal of cell suspension at the same rate. At steady state, the growth exactly balances the removal and all culture properties are constant over time.

As the propagation of small test tube cultures to large volume starter cultures is omitted, continuous cultivation is time saving when properly managed. There may also be a hygienic benefit as the cultures are isolated from the hatchery environment and treated aseptically. Continuous cultivation also offers an opportunity to control the physiological state of the microalgae by adjusting the growth rate. Adjusting the growth rate is equivalent to changing the dilution rate, and thus the protein content (Taub, 1980), the N:C ratio (Caperon and Meyer, 1972) and the content of storage products such as starch (Pirt and Pirt, 1977) or lipids (Fernández-Reiriz *et al.*, 1989) in the product may be manipulated.

Also the size of the microalgae is growth dependent. This may be of importance when assessing data based on cell counts. In algae which are incapable of inorganic nitrogen storage, cellular nitrogen consists of protein and free amino acids for algae, incapable of inorganic nitrogen storage. To investigate the effect of dilution rate on this cellular nitrogen and on cell size steady state cultures of the haptophyte, *Pavlova lutheri*, grown at different dilution rates were analyzed. The adaptation from one dilution rate to another was also studied.

To date, the practical use of continuous microalgae cultures was limited by the growth of bacterial-algal films on the inner surfaces of the cultivation vessels. Theoretically, these problems would be eliminated in axenic cultures, but at the production-scale level, truly axenic cultures are not feasible. Therefore, we here propose a simple solution to this problem, namely to co-culture a surface grazing organism. The stability of such a system

would depend on the capability of the grazer to adjust its population size to a given food level, and this in turn was expected to be related to the total area available and to the adhesion rate of the algae.

Materials and methods

For the physiological experiments continuous 750ml nitrogen-limited cultures of *Pavlova lutheri* were used. The cultures were axenic and grown under constant illumination at 10klux. Internal nitrogen was calculated according to mass balances.

At production scale *Rhodomonas baltica* and *Isochrysis galbana* were grown in 60 or 180 l cylinders in unialgal but not axenic cultures. Constant illumination was provided by four light panels each supplied with two 36W fluorescent tubes. The panels were placed 10cm from the outer surface of the cultivation vessel. Counting and measuring size distribution was done with a Coulter counter. As a surface grazer, the harpacticoid copepod *Tisbe holothuriae* was used in cultures of *I. galbana*, *R. baltica*, *P. lutheri*, and *Platymonas suecica*. Copepod production was recorded as total individual numbers and normalized according to the sampling period.

Results

In Fig. 1 the internal nitrogen in micromoles N/10^9 cells of a nitrogen limited chemostat culture of *Pavlova lutheri* is depicted against instantaneous growth rate (day 1). To convert to protein expressed as mg/10^9 cells, multiply with 0.0875.

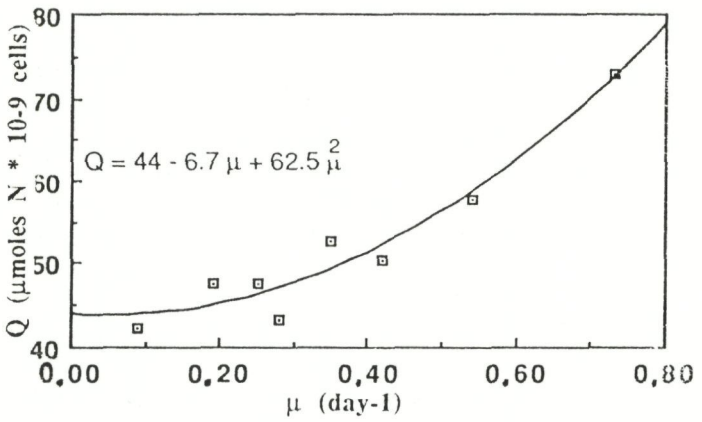

Fig. 1.

Over the observed range of growth rate, the cellular nitrogen almost doubled. It may be assumed, that cellular nitrogen is present as protein or free amino acids, as *Pavlova* has a very limited capacity for storing inorganic nitrogen.

Fig. 2 shows cell density against long-term steady-state growth rate. The data indicate an inversely proportional relationship. Fig. 3 shows the cell density change during the adaptation from a growth rate of 0.2 to 0.5/day. The average cell volume (ACV) is also shown. These data indicate that algae grown at high growth rates are characterized by

higher cellular nitrogen contents, part of which simply represents the fact that fast growing cells are bigger than slowly growing ones.

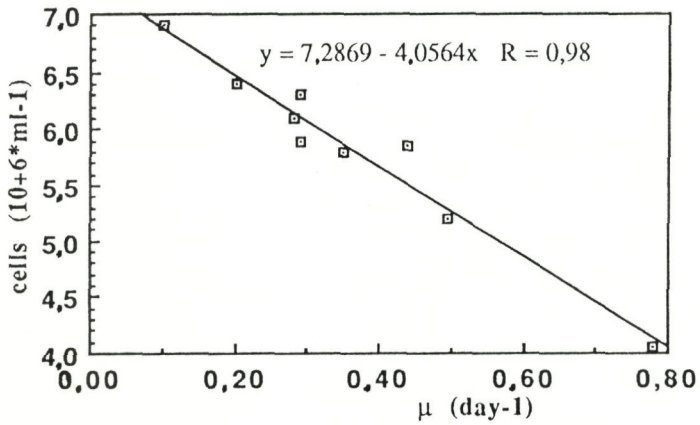

Fig. 2.

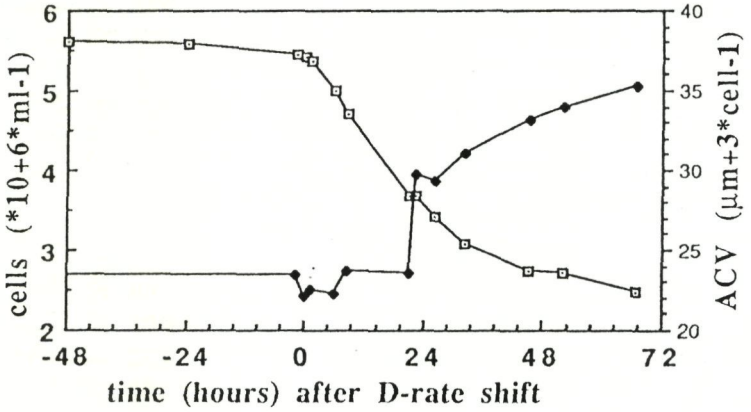

Fig. 3.

Continuous cultivation periods for more than 1 year have been attained in production-scale cultures during which *Tisbe* kept the surfaces clean (16 months in a 60 l reactor). The copepod density in the reactor depends on the algal species cultivated; *Rhodomonas* giving the highest densities and *Isochrysis* the lowest. In Fig. 4 the daily production of *Tisbe holothuriae* from a 180 l culture of *Isochrysis* and *Rhodomonas* are shown during a 20 day sampling period. The total number of individuals are given, not discriminating between stages. The production - and hence probably also the population - of *Tisbe* in the *Rhodomonas* culture is about 10 times larger than that of the *Isochrysis* culture.

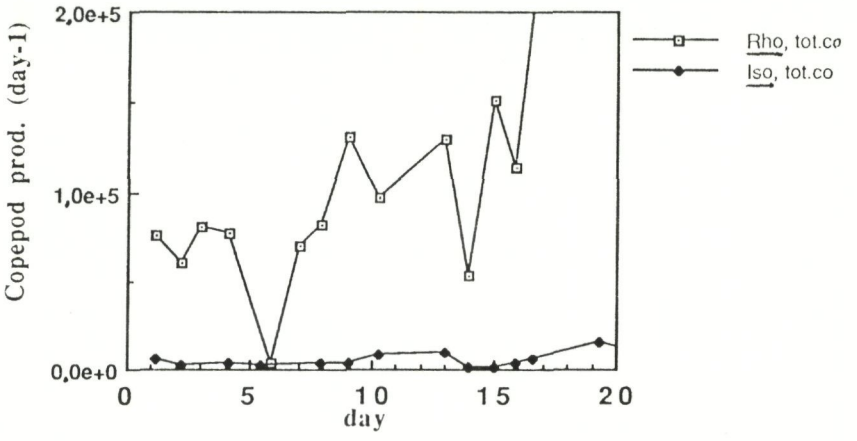

Fig. 4.

Discussion and conclusions

Continuous cultivation allows the specific growth rate to be controlled and through this parameter, also physiological properties as cell size and cellular nitrogen (protein) content. A part of the cellular nitrogen ration may be directly related to the cell size, while another part is attributable to other factors. Fast growing cells probably need a larger enzyme pool. Production-scale cultures, from 60 to 600 l, have been kept stable for extended periods using the harpacticoid copepod, *Tisbe holothuriae* for cleaning the inner surfaces.

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MASS CULTIVATION OF MARINE MICROALGAE AS FOOD FOR LARVAE OF OYSTER AND MARINE FISH

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Introduction

Cultivation of microalgae adequate to meet the requirements of mariculture species is essential for oyster and marine fish farms. Aquaculturists require knowledge of conditions that ensure a biomass yield rich in protein or lipids and with a high HUFA ratio. In this paper the chemical composition of microalgal biomass changes in relation to season and nitrogen nutrition are discussed.

Materials and methods

Two species of marine microalgae *Monochrysis lutheri* and *Phaeodactylum tricornutum* were used for rearing oyster larvae (*Ostrea edulis* L.) and marine fishes (mullets and flatfishes). Algae were cultivated under normal conditions at a fish farm at the Black Sea, in two closed pilot tube installations in the open air. Each system had a volume of 160 l. The culture system was operated as a batch cultivator for the first 8 days, then as a semi-continuous one with 1/3 to 1/5 of the suspension harvested each day. Nutrient concentrations were maintained at 230-250mg $N.l^{-1}$, 50-70mg $P.l^{-1}$, at a pH 7.0-7.7. Optimal temperatures for our strains of *M. lutheri* was 28°C, and for *P. tricornutum* 18-20°C. *M. lutheri* was cultivated in July (the stage of active growth at optimal temperature) and October (slow growth at 18-21°C). *P. tricornutum* was grown in May at optimal temperature and in July (slow growth due to increased temperature to 24-27°C). The daily radiant energy averaged $132W.m^{-2}$ in May, $171W.m^{-2}$ in July and $80W.m^{-2}$ in October.

Results

In Fig. 1 and 2 the changes in the chemical composition of *M. lutheri*, respectively *P. tricornutum* are shown.

It should be noted that under unfavourable culture conditions, the algal cells increased in size and individual weight, *i.e.* from 3.2±0.8ng to 6.9±1.3ng for *M. lutheri* and from 18.1±3.0ng to 29.4±3ng for *P. tricornutum*. The highest percentage of 20:5n-3 was registered in July (17.19-19.33% of total fatty acids), in October the content of this fatty

acid dropped to 2.53-3.97%. In *P. tricornutum* the ratio of 20:5n-3 decreased from 16.61-18.38 in May to 4.46% in July. The chemical composition of the algal biomass changed with the mineral nutritional conditions, *i.e.* a decrease in nitrogen concentration from 250 to 120mg $N.l^{-1}$ in the medium caused a decrease in protein content (Table I).

Table I. Chemical composition of *M. lutheri* and P. *tricornutum* in relation to conditions of nitrogen (% of dry weight)

Nitrogen source	Proteins	Lipids	Carbohydrates	Sum of amino acids ($g.100g^{-1}$)	20:5n-3 (% of total fatty acids)
NH_4^+ + urea 250mg $N.l^{-1}$	46.5[a] / 40.8[b]	24.5 / 18.8	15.8 / 18.4	46.47 / 55.23	19.96 / 17.59
NH_4^+ + urea 120mg $N.l^{-1}$	42.6[a] / -[b]	27.2 / -	19.3 / -	44.13 / -	18.34 / -
NO_3^- 250mg $N.l^{-1}$	42.2[a] / 39.4[b]	25.1 / 17.7	21.2 / 17.6	42.06 / 50.27	8.44 / 14.12

[a] Data for *M. lutheri*; [b] Data for *P. tricornutum*.

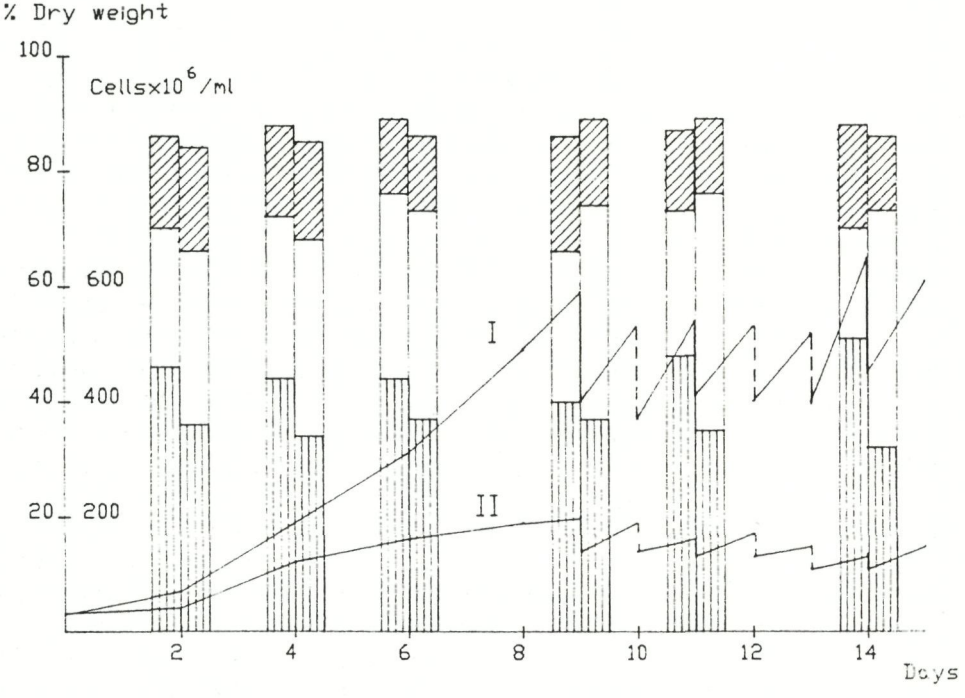

Fig. 1. Content of protein (▯), lipids (▯), carbohydrates (▨) in dry biomass of *M. lutheri* in July (left column) and October (right column). I. Growth curve for July; II. for October. Ash content 9-15%.

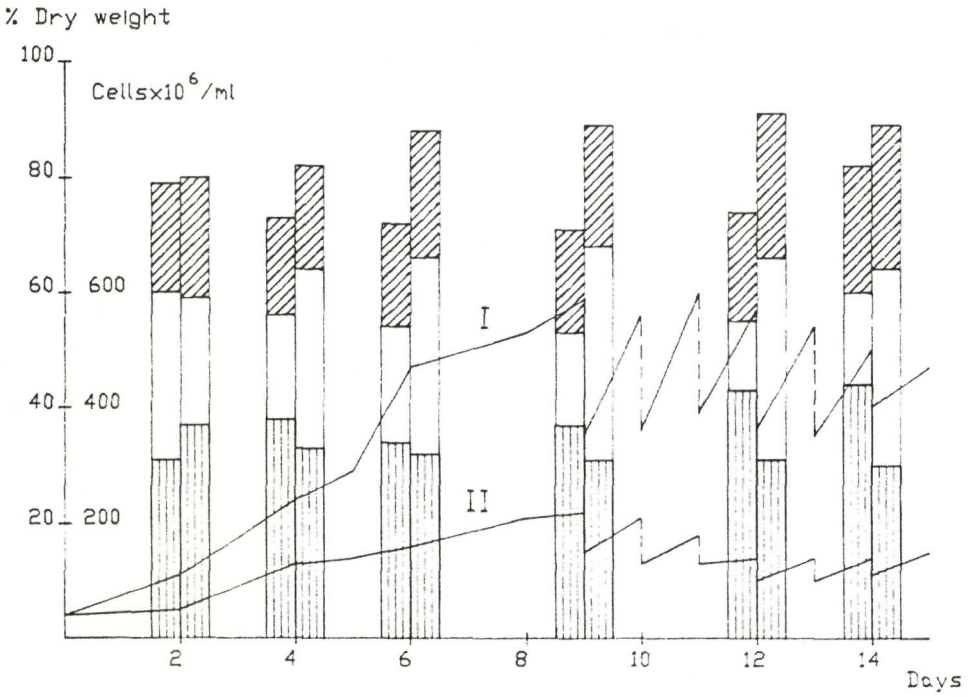

Fig. 2. Content of protein (□), lipids (□), carbohydrates (⊡) in dry biomass of *P. tricornutum* in May (left column) and July (right column). I. Growth cuve for May; II. for July. Ash content in May 17-27%, 8-18% in July.

The changing of the source of nitrogen caused no changes in growth characteristics, but affected the amino- and fatty acid content.

Discussion

Both species selected for mass cultivation changed chemical composition rather easily in response to cultivation conditions. Under unfavourable conditions they produced higher concentrations of lipids (up to 41% for *M. lutheri* and 37% for *P. tricornutum*). The highest output of protein per suspension volume unit was observed during active growth of the culture when a part of the suspension was harvested each day and urea was used as the nitrogen source. Under these conditions the total amino acid content of the biomass was also the highest. An increase in protein content in algal biomass with urea was also found in *Thalassiosira pseudonana* (Harrison *et al.*, 1990). Lipid-rich biomass is obtainable under unfavourable conditions (October for *M. lutheri* and July for *P. tricornutum*), however, Thomas *et al.* (1984) also found that culture performance decreased when lower nitrogen levels occurred. The capacity of cells to increase in size under unfavourable conditions should be taken into account when calculating feeding regimes for filtering organisms consuming a fixed number of cells. A combination of *M. lutheri* and *P. tricornutum* biomass provided valuable food for *O. edulis* and *Brachionus plicatilis* larvae at different moments of the year.

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USE OF HYPOCHLORITE TO CONTROL PROTOZOA IN *CHLORELLA* CULTURE

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Introduction

Mass culture of *Chlorella* is usually contaminated with Protozoa which often cause the failure of the *Chlorella* culture. Therefore, controlling Protozoa is necessary when culturing this alga. In Thailand, $Ca(OCl)_2$ and NaOCl have been widely used as water treatments in hatcheries (Duangsawasdi and Somsiri, 1985). The objective of the experiment was to determine the effect of $Ca(OCl)_2$ and NaOCl in controlling the Protozoa population in *Chlorella* cultures.

Materials and methods

The experiment was carried out in 100 l tanks at the outdoor laboratory of the National Institute of Coastal Aquaculture, Thailand. $Ca(OCl)_2$ and NaOCl of various concentrations were added to the stock 1h before culturing started. One, 3, 5 and 7ppm $Ca(OCl)_2$ and 7, 20, 35 and 50ppm NaOCl were added to *Chlorella* stock culture, and 1ppm of $Ca(OCl)_2$ and 7ppm of NaOCl were added to the culture. In the control treatment no hypochlorite was added. Daily, the density of *Chlorella* sp. was determined using a haemocytometer. The presence of Protozoa in the culture, was designated as "high" when the number of Protozoa exceeded the initial number of 10^4 cells/ml and "low" when lower than that of the initial number. Throughout the experiment, the water temperature was 26.5-32°C, the salinity ranged from 22 to 29ppt and the pH was 7.2 to 9.5.

Results

In the control treatment, Protozoa were found on the second day and increased in number thereafter, whereas all $Ca(OCl)_2$ treatments showed a low number of Protozoa on the fourth day until the end of the experiment. Change in the density of *Chlorella* after adding $Ca(OCl)_2$ is shown in Fig. 1. The *Chlorella* density of the control decreased after the second day until the end of the experiment. In the 5 and 7ppm of $Ca(OCl)_2$ stock treatment, the *Chlorella* density decreased on the second day, then rose abruptly. In contrast, in the 1ppm $Ca(OCl)_2$ stock treatment, the *Chlorella* density slightly increased till the third day and dropped thereafter. A sharp increase and peaking on the fifth day occurred in the 3ppm $Ca(OCl)_2$ stock treatment and the 1ppm $Ca(OCl)_2$ culture treatment. In control treatments Protozoa were observed at 1h and increased in number thereafter,

while in the NaOCl treatments, the number of Protozoa was low till the end of the experiment. In Fig. 2, it is shown that the *Chlorella* density of the control sharply declined on the third day and continued dropping throughout the experiment. The *Chlorella* density in the 35 and 50ppm NaOCl stock treatments declined on the second day then increased while the *Chlorella* density in the others increased, averaging $21.5x10^6$ cells/ml.

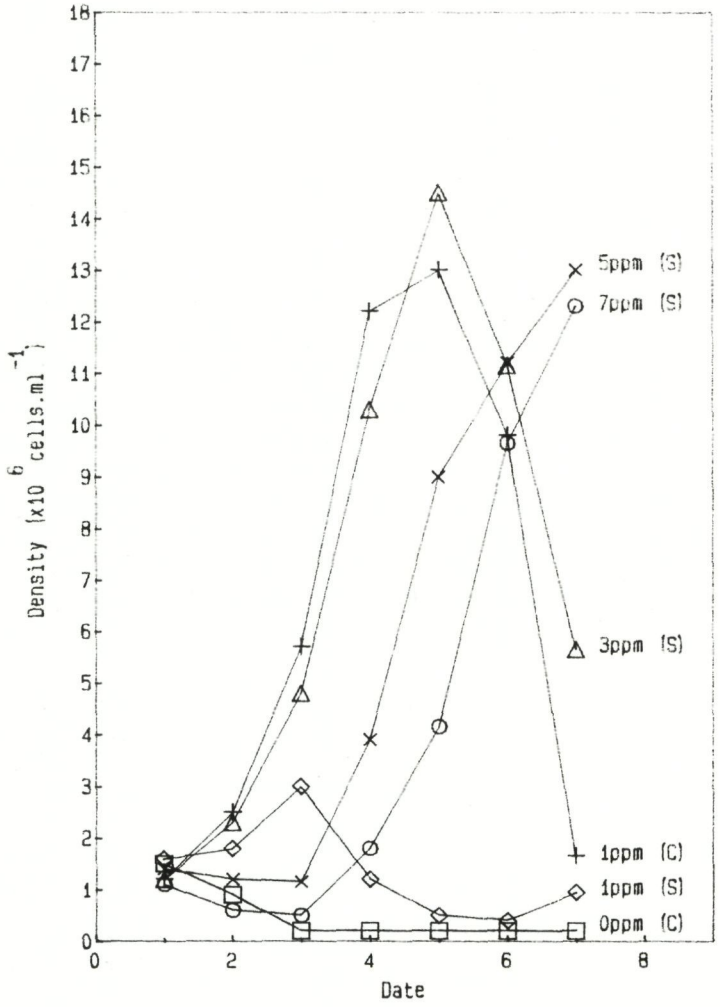

Fig. 1. *Chlorella* density after adding 0 and 1ppm $Ca(OCl_2)$ to the culture (C), and 1, 3, 5, 7ppm to the stock culture (S), 1h before culturing started.

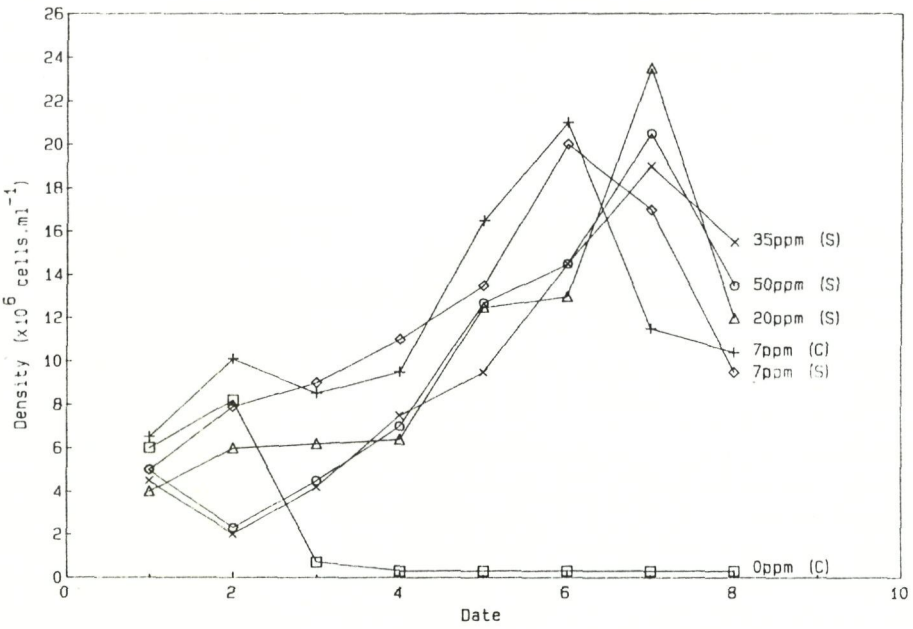

Fig. 2. *Chlorella* density after adding 0 and 7ppm to the culture (C), and 7, 20, 35, 50ppm to the stock culture (S), 1h before culturing started.

Discussion

The present study shows that hypochlorite can be used in controlling the contamination of Protozoa in *Chlorella* cultures. The results indicate that following treatment, low numbers of Protozoa, and increased *Chlorella* density were observed. Low-dose treatments did not affect the density of *Chlorella*; this may be due to the thickness of the cell wall (Wongratana, 1988). However, some *Chlorella* died in the early phase of the treatment but the remainder recovered in high-dose treatments. The present study indicates that adding 1ppm of $Ca(OCl)_2$ to the culture or 3ppm of $Ca(OCl)_2$ to the stock, or adding 7ppm NaOCl to the culture or 20ppm of NaOCl to the stock are effective doses to control Protozoa. Following application of these methods, the cultures should be checked for residual hypochlorite.

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PROGRESS IN MASS CULTIVATION OF HARPACTICOID COPEPODS OF THE GENUS *TISBE*

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Introduction

The harpacticoid copepod *Tisbe holothuriae* has been cultured in our laboratory for more than 25 years. This species and, especially, their larval stages have been found to be suitable living food organisms for mariculture purposes (Uhlig, 1981, 1984). Various studies have been made to optimize growth in mass cultures of *Tisbe holothuriae*, in terms of temperature, salinity and nutrition conditions (Schwenzer, 1985). The present study deals with effects of crowding on the larval development and the productivity of females of *Tisbe holothuriae* under laboratory conditions.

Materials and methods

The organisms derived from stocks of our mass cultures were cultivated in Millipore-filtered and pasteurized (90°C) seawater of 28ppt salinity, fed excessively on a mixed diet of *Dunaliella*, *Skeletonema* and grains of dehydrated tissue of *Mytilus edulis*. The experiments were carried out in a temperature-controlled room (20±1°C). Nauplii were reared in glass bowls (diameter = 11.5cm) at four different densities (20, 60, 180, 540ind./cm^2). To study the crowding effect on mating period, copepodites were kept in four densities (10, 30, 90 and 270 ind./cm^2) up to the moment when the first egg sacs became apparent. Then 11 ovigerous females from each density were reared individually in small glass bowls (20ml seawater) and the number of nauplii per egg sac was recorded. In the productivity experiment females were kept at 3, 9, 40, and 130 females/cm^2, respectively. At each density: 1) the percentage of females without egg sac was determined, 2) 12 ovigerous females were isolated and the number of nauplii per egg sac was counted. The T-test was used for statistical analyses.

Results

There are significant differences ($P<0.01$)in larval mortality with increasing density (20, 28, 44, and 64%, respectively) except for the two lower levels 20 and 60 ind./cm^2 ($P>0.05$). The same is true for the developmental time (from nauplii to fertile adult). It increases from 11 days at low densities (20 and 60 ind./cm^2) to 16 days in the highest

density (540 ind./cm^2). However, no differences of the sex-ratio (females-males) was found among all the densities tested (P>0.05). It remained constant at about 40:60.

The number of nauplii of the first four egg sacs depends on the density of females during the mating period; the mean number of nauplii per egg sac decreased with increasing density. There are linear relationships between the number of nauplii produced and the logarithm of density for the first four egg sacs; however, the regression slopes decline from the first to the fourth egg sac (Table I).

Table I. Mean number of nauplii per female for the first four egg sacs (Y) at different densities (s=standard deviations; r=coefficient of correlation; y=number of nauplii; x=log density)

Egg sac no.	Density (ind./cm^2)	No. ovigerous females	Nauplii/female		Linear regression
			Y	s	
E1	10	11	66.9	6.7	y=85.49-8.36x
	30	11	57.8	4.9	P<0.05
	90	11	44.4	7.1	r=0.98
	270	11	40.7	8.7	
E2	10	11	78.5	5.9	y=96.75-7.95x
	30	11	70.1	3.7	P<0.05
	90	11	56.0	8.6	r=0.96
	270	11	54.7	7.0	
E3	10	11	81.1	6.1	y=92.63-5.74x
	30	11	71.9	6.0	P<0.05
	90	11	64.3	9.5	r=0.96
	270	11	62.6	7.2	
E4	10	11	76.9	6.4	y=80.35-1.67x
	30	11	74.6	5.7	P<0.05
	90	11	72.0	8.3	r=0.97
	270	11	71.6	8.5	

Mortality of females increased significantly (P<0.01) from 20 to 60% with increasing density though no significant differences (P>0.05) between the two low densities (3 and 9 ind./cm^2) were found. On the first day of the experiment, the daily nauplii production per female was independent of density; however, the differences became greater as the experiment progressed. On the 5th day the highest number of nauplii per female (38 nauplii/female/d) was observed at the lowest density (3 ind./cm^2). With regard to the total number of nauplii produced per female, there were major differences between the low and high densities, *i.e.* 401, 333, 212, 95 nauplii/female from low to high densities, respectively. The percentage of the non-ovigerous females increased from 22 to 55%, and the number of nauplii per egg sac decreased from 72 to 36% with increasing density.

Discussion

The results of the experiments confirm that crowding plays a very important role not only in larval development, but also in the nauplii production of females. Brand (1985) found that larval crowding affects their behaviour, especially due to reduced food contact. Similarly, larvae at high densities tend to swim in the water, but those at low densities always stay on the bottom. It seems that close encounters among copepods may lead to a situation which may affect larval development and survival.

The nauplii production depends on the density of females during both the reproductive and copulative period. Fava and Crotti (1979) found that the number of nauplii hatched from the first egg sac depends on the density during the mating period. We obtained similar results for the first to the fourth egg sac. The regression slopes reflect the degree of density dependence of the number of nauplii per egg sac. It is highest in the 1st egg sac, and becomes lower and lower when the ovigerous females are removed from the crowding situation and are reared individually. It indicates that the crowding effect on the mating period may be diminished when the females are transferred to better conditions.

The high percentage of non-ovigerous females in the high densities indicates that the interval between the releases of successive egg sacs are extended. The same was observed in *Amphiascoides* sp. (Walker, 1979). Furthermore, crowding affected the productivity of females by reducing the number of nauplii hatched from one egg sac.

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VITAMIN B_{12} CONTENT AS A LIMITING FACTOR FOR MASS PRODUCTION OF THE ROTIFER *BRACHIONUS PLICATILIS*

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Abstract

The rotifer *Brachionus plicatilis* requires vitamin B_{12}. Baker's yeast or freshwater *Chlorella*, either of which does not contain vitamin B_{12}, cannot support rotifer growth under bacteria-free conditions. However, baker's yeast and freshwater *Chlorella* strengthened with the vitamin can support rotifer growth. Why is mass-culture of the rotifer sometimes successful even when fed only with baker's yeast? Bacteria propagating in the mass culture tanks support rotifer growth by acting as a source of essential nutrients such as vitamin B_{12}. However, to attain stable mass production of the rotifer, it is desirable to develop a food that can completely support rotifer growth. In this paper, we describe the process of developing condensed *Chlorella* containing vitamin B_{12} in its cells. We also tested its nutritional value as food for the rotifer.

Under almost bacteria-free condition, we cultured the rotifer in freshwater *Chlorella vulgaris* suspensions with and without supplementation of vitamin B_{12}. Supplementation of the vitamin, either by addition directly of the rotifer culture water or by incorporation in the *Chlorella* cells, was effective in promoting rotifer growth. However, the former is not practical for mass culture because it may also allow growth of bacteria requiring the vitamin and hence the depletion of the vitamin in the tank. The group of *Chlorella* which can uptake vitamin B_{12} from the medium and store it in its cells is that one which cannot produce secondary carotenoids.

Chlorella, containing varying amounts of vitamin B_{12} in its cells, were suspended in containers with 20ml of culture water. Then the rotifer was cultured in the prepared suspensions. A higher rotifer yield was obtained from the group cultured with *Chlorella* containing more vitamin B_{12} in its cells. For mass production of the rotifer, 200μg.100mg^{-1} dry weight of *Chlorella* is considered a suitable amount of vitamin B_{12}. The amount of vitamin B_{12} necessary to produce one individual rotifer is calculated at 1.32pg. Mass production of the rotifer was achieved with baker's yeast and refrigerated condensed *Chlorella* containing vitamin B_{12}. Rotifer culture was more stable and showed a 1.3 times higher production than with the usual *Chlorella*.

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SUCCESSFUL APPLICATION OF A NEW COMBINED CULTURE AND ENRICHMENT DIET FOR THE MASS CULTIVATION OF THE ROTIFER *BRACHIONUS PLICATILIS* AT COMMERCIAL HATCHERY SCALE IN MONACO, YUGOSLAVIA, FRANCE, AND THAILAND

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Abstract

Culture Selco[1], a combined culture and enrichment diet for rotifers, was used in commercial-scale culture trials to compare with the conventional applied culture diets such as baker's yeast and algae (*Chlorella* sp., *Isochrysis* sp., *Tetraselmis* sp.). Culture yields, economics and the nutritional value of *B. plicatilis* were studied.

Culture Selco is a dry and complete rotifer diet that does not require the use of algae and allows to produce high quality rotifers rich in (n-3) HUFAs.

Culture trials were carried out on a commercial scale in four different hatcheries using tanks of 600 l (Siam Aquaculture Company, Thailand), 1 000 l (CENMAR, Yugoslavia), 1 500 l (Ferme Marine de Douhet, France), 800 l and 1 500 l (P2M hatchery, Monaco). A batch culture system was compared with continuous culture procedures.

The average daily production using Culture Selco as the sole culture diet ranged consistently from 45 to 60% of the initial rotifer density, while baker's yeast and algae yielded from 19 to 33%.

The consistency of the rotifer reproduction was demonstrated in the P2M hatchery using four tanks of 800 l for a period of approximately 2 months. The culture procedure used was a batch-culture of 3 days, *i.e.* in total 21 cultures were run with four tanks. The total

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1 Culture Selco, Selco, Protein Selco, and Super Selco are live-food enrichment diets manufactured b Systems SA, Gent, Belgium.

production approximated 23 000 million rotifers, with an average daily production of 51% per tank.

These Culture Selco-grown rotifers were fed to seabass (*D. labrax*) larvae without an additional enrichment achieving equal results as when Selco[1] enriched rotifers were used. The fatty acid profiles of the cultured rotifers show that using the conventional diets only 0.4mg (n-3) HUFA/g DW of which 0.2mg 22:6n-3/g DW is obtained, while using Culture Selco 11mg.g^{-1} DW of which 3.5mg 22:6n-3/g DW can be reached. A subsequent enrichment with Protein Selco[1] increases the HUFA content up to 26mg.g^{-1} DW of which 10mg 22:6n-3/g DW. For species that require still higher (n-3) HUFA levels enrichment with Super Selco[1] should be considered (Léger *et al.*, 1989).

These results clearly demonstrate that Culture Selco can be used as a combined culture and enrichment diet for *B. plicatilis* not requiring the use of algae. Based on manpower savings and reduced infrastructure Culture Selco-grown rotifers furthermore proved more cost effective than the conventional procedures.

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MANAGEMENT, PRODUCTION AND DISEASE INTERACTION IN ROTIFER CULTURE

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Introduction

The rotifer *Brachionus plicatilis* is used in aquaculture as the sole food for many marine fish larvae during their first and second week of feeding. Since no equally successful replacement, alive or artificial, for the rotifers is yet available, much effort has been made to guarantee a predictable and stable supply of this live food. Many types of production units have been established at different places, ranging from extensive to intensive cultures, using different diets and applying different harvesting regimes.

Materials and methods

At NCM, two strains of *Brachionus plicatilis* were routinely raised as a mixed population in the food-chain facilities, a local L - strain, 350µm long and a Japanese S-strain, 200µm long. Rotifers were cultured in four $30m^3$ tanks with salinity kept at 24-26 ppt. The temperature ranged between 17 and 26°C, according to the season and day - night fluctuations. Heavy aeration maintained oxygen levels above 4ppm. A total of 20kg of fresh baker's yeast (35% dw) and approximately 70g (dw) of cultured microalgae were given daily. A 800 l sedimentation tank was connected to each culture tank and the culture was continuously circulated ($2m^3.h^{-1}$) through the two tanks with the help of an air lift. Every day, samples of the cultures were examined for rotifer density and activity, egg number and general cleanliness. Following the examination the selected tank was partly harvested according to hatchery demand and then refilled. Tanks were completely emptied, cleaned and restocked every 2-12 weeks.

Results

For over 2 years the above production unit functioned with a reasonable level of stability. The cultures consisted of 99% and less than 50% of the S-strain in summer and winter respectively. Densities at harvesting fluctuated from 120 to 300 ind./ml. The daily harvest was $0.7\text{-}1.5x10^9$ rotifers or 3-15% of the standing stock, annual average was 5% / day. At the beginning of 1990 the rotifer culture density decreased severely. The reproductive rate was very low and rotifers were generally less active. Separate small laboratory

cultures fed only baker's yeast died off slowly. Microscopic examination revealed that in some cases 80% of the rotifers were infected in their foot and body cavity with large numbers of ellipsoidal 3-4μm cells with elongated buds and with 15-20μm long boomerang shaped rods. Attempts to isolate these yeast-like microorganisms were unsuccessful although various types of yeast were isolated from mashed infected rotifers in addition to *Saccharomyces cerevisiae*. On a small scale, microalgal diet helped the rotifer population to recover. The new offspring were infection free, however the previous generation remained infected. The immediate solution was mass production of algae in two $50m^2$, pH controlled, raceway shaped ponds, which produced high densities of *Nannochloropsis* sp. Daily production of 1.5kg (dw) algae was pumped directly into the rotifer tanks in addition to 24kg of baker's yeast. This method allowed us a daily harvest of $2\text{-}4x10^9$ of rotifers or 10 -15% of the population. The infection, however, was not completely eliminated.

Discussion and conclusions

The rearing of rotifers in an outdoor system may be influenced by environmental and ecological changes. Baker's yeast has not only proved to be a poor diet for rotifer in terms of production rate, but may also result in a poor physiological state which causes low resistance to pathogens. The interaction between the rotifers, baker's yeast and the new pathogenic microorganism is not yet clear. A correlation between the presence of the infection and a low reproductive rate of rotifers could not always be established, but non-infected rotifers clearly performed better. The regime of a high daily rate of harvesting regardless of hatchery demand, kept the cultures "fresher". This regime in addition to feeding large quantities of algae substantially increased and stabilized the production of rotifers. As a first attempt to eradicate the disease by cleaning and disinfecting the facilities failed, the possibility that either the baker's yeast or the sea is the source of the infection is presently under investigation. Further study to understand and control this new phenomenon is in order.

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THE EFFECTS OF FEEDING FRESHWATER *CHLORELLA*, BAKER'S YEAST AND CULTURE SELCO ON THE CULTURE OF ROTIFERS (*BRACHIONUS* SP.)

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Introduction

Together with the brine shrimp *Artemia*, rotifers (*Brachionus* sp.) are widely used as first food-organisms in the mass culture of larval fish and crustaceans.
Great progress has been made in the area of fry production technology of marine finfish since the introduction of *Brachionus plicatilis*, as a cultured food organism (Fukusho, 1989ab). Different live and inert diets have been tried in the culture of rotifers, however, most hatcheries have continued to rely on the marine chlorella (*Nannochloropsis oculata*), baker's yeast and ω-yeast. A stable *Chlorella* supply is difficult to obtain in terms of quantity and punctuality, especially under mass culture conditions. ω-yeast is expensive to produce and presents problems of water quality control while baker's yeast presents problems of water quality and is low in essential fatty acids (HUFAs) (Fukusho, 1983; Lubzens, 1987). In the tropics (at SEAFDEC) freshwater *Chlorella* has been successfully mass cultured and acclimated before feeding (De Pauw and Pruder, 1986). To establish a rotifer mass culture at KMFRI, the effects of feeding freshwater *Chlorella*, baker's yeast, and Culture Selco (a HUFA-enriched diet obtained from Artemia Systems SA, Belgium) were tested on rotifers obtained from a salina (Fundisha saltworks) in Kenya.

Materials and methods

Rotifers obtained from Fundisha saltworks and maintained on algae (at KMFRI) were used in the experiment. Baker's yeast and Culture Selco were used as inert diets. Due to failure of marine *Chlorella* cultures, freshwater algae were used. The experiments were replicated three times for each feed in 1 l cylindro-conical glass containers. During the experiments temperatures ranged between 27.5 and 28.5°C. Three different salinities (7.5, 15, and 25ppt) were tested. Initial stocking rates of the rotifers were 20/ml. Baker's yeast was fed at the rate of 1g/million individuals. The initial feeding rate with Culture Selco was 25mg.l^{-1} and was increased to 50mg.l^{-1} on attaining densities above 50 ind./ml. *Chlorella* was fed on a saturation basis determined by colour, where deep green indicated food abundance. Daily rotifer counts were made from which density and growth rates in various salinities using the different feeds were determined.

Results

Fig. 1(A,B) and Fig. 2. show the growth patterns of the rotifers fed on the different diets. The three salinities had similar growth patterns for the various diets. During the first 3 days of culture, there was no significant difference in rotifer densities in the various salinities. Thereafter large increases were noted in population densities of rotifers fed on freshwater algae. The maximum densities varied from 206 to 381 ind./ml (mean=316.6±94.6 ind./ml) in 7.5ppt after 8 days of culture. Similar increases were observed in 15ppt and 25ppt (251 to 318 ind./ml, mean=284±31.9 ind./ml and 153 to 211 ind./ml (mean=177.6±30.0 ind./ml). Cultures fed on Culture Selco exhibited gradual, but steady growth in all salinities. In 7.5ppt the maximum density range was 196 to 238, mean=221.6 ind./ml while in 15ppt and 25ppt density ranges of 216 to 232, mean=225.0±8.1 ind./ml and 107-140±19.1/ml respectively, were attained. Growth of rotifers fed baker's yeast was poor. While in 7.5ppt a decline occurred after 5 days, 15ppt supported a maximal density range of 36 to 46±5.2 ind./ml. There was no growth at 25ppt. The mean growth rate varied for the three feeds. Growth rates were significantly different ($P<0.05$) for the three feeds at 7.5ppt and 15ppt but at 25ppt baker's yeast gave extremely low rates as compared to the other diets. A maximum growth rate of 0.37±0.02 was realized at 15ppt for freshwater *Chlorella* over an 8-day period. Culture Selco gave a mean growth rate of 0.21±0.01 over a 12-day period and baker's yeast 0.09±0.02 (Table I).

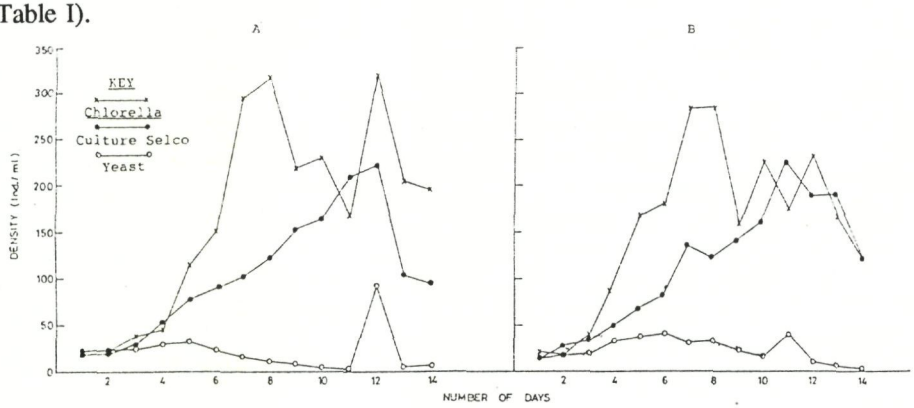

Fig. 1(AB). Population density of rotifers fed freshwater *Chlorella*, baker's yeast, and Culture Selco at 7.5ppt and 15ppt.

Table I. Mean growth rates of rotifers fed on freswater *Chlorella*, baker's yeast and Culture Selco

Diet	Salinity (ppt)		
	7.5	15	25
Chlorella	0.33±0.03	0.37±0.03	0.28±0.01
Baker's yeast	0.09±0.01	0.09±0.02	0
Culture Selco	0.23±0.01	0.21±0.01	0.26±0.01

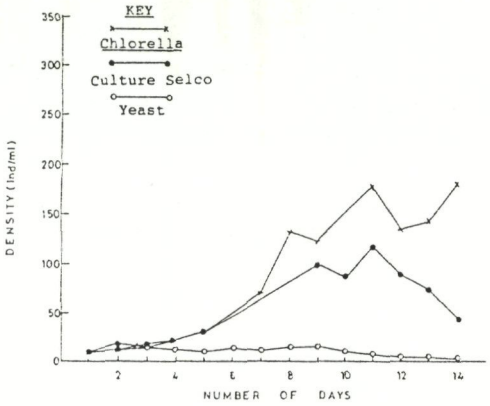

Fig. 2. Population density of rotifers fed freshwater *Chlorella*, baker's yeast and Culture Selco at 25ppt.

Discussion and conclusion

Baker's yeast-fed rotifers exhibit poor growth characteristics and are difficult to handle in mass culture. They are also lacking in (n-3) HUFAs essential for marine fish larvae (Watanabe *et al.*, 1983; Fukusho, 1989). From Fig. 1 and Table I it is evident that Culture Selco supported the best growth in view of its stable cultures especially at the optimum salinity of 15ppt. In addition, Culture Selco contains the (n-3) HUFAs lacking in yeast. Culture Selco, therefore, can do well as a replacement for baker's yeast in rotifer culture. Due to high production costs of marine *Chlorella* and since marine *Chlorella* cultures tend to decline with increasing temperature (Fukusho, 1983), freshwater *Chlorella* can be used for rotifer production in the tropics where high temperatures favour its mass culture. The food quality of rotifers fed freshwater *Chlorella* is yet to be assessed, but Culture Selco may be used for enriching rotifers in a secondary culture in cases of (n-3) HUFA deficiency or in the production of large numbers of fish larvae in order to cut down on the costs (of Culture Selco). Medium salinities supported the best growth. The same finding was reported by Gatesoupe and Robin (1981).

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Utilisation

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MASS CULTURE AND NUTRITIONAL QUALITY OF THE FRESHWATER ROTIFER (*BRACHIONUS CALYCIFLORUS* P.) FOR GUDGEON (*GOBIO GOBIO* L.) AND PERCH (*PERCA FLUVIATILIS* L.) LARVAE

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Introduction

Rotifers are an excellent food source for the first stages of small fish larvae (Watanabe *et al.*, 1983). Contrary to the established mass production of the marine rotifer (*Brachionus plicatilis*) the cultivation of freshwater rotifers has not yet gained practical importance (Shlüter *et al.*, 1987). Their use as a starter food for rearing fish fry is less developed. In this paper, the production dynamics and changes in fatty acids, carbon (C) and nitrogen (N) contents were investigated in the freshwater rotifer (*Brachionus calyciflorus*) fed on green algae (*Dictyosphaerium chlorelloides*). The suitability of rotifers for gudgeon (*Gobio gobio*) and perch (*Perca fluviatilis*) larvae during their early feeding stage was examined and compared with a microencapsulated dry feed diet.

Materials and methods

The mass culture of rotifers was carried out using a batch culture system. The algae were mass produced in 15 and 40 l polyethylene bags at a constant temperature of 25±1°C and under continuous illumination. Amictic females of the rotifer, acclimated to algae as food source, were inoculated into the culture at an initial density of about 2 ind/ml^{-1}. The larval feeding experiments were carried out in 10 l PVC aquaria incorporated in a recirculation system using a 150 l rectangular tank. The water temperature was maintained at 20±0.5°C. The flow-through in each aquarium was similar and constant (0.5 l.min^{-1}).

Larvae of gudgeon and perch were obtained respectively by artificial reproduction and natural reproduction in a tank. On the final day of yolk resorption, the average weight of the larvae was 0.5±0.05mg for gudgeon and 0.76±0.26mg for perch. Either rotifers or a microencapsulated dry food (Nippai Shrimp Feed) was supplied to the larvae during the first 2 weeks. The maximal daily consumption of rotifers by fish larvae was adjusted every 3 days, according to Kamler *et al.* (1986).

Several parameters such as growth rate, food conversion rate, growth efficiency (dry weight of food/dry weight gain of fry), protein efficiency ratio (wet weight gain of fry/weight of dietary protein), survival and biochemical composition were used to compare the effects of providing either rotifers or artificial food to fry during the early feeding stage. To evaluate nutritional quality, rotifers and larvae were analyzed by a GC Hewlett-Packard 58-90 gas chromatograph to determine the fatty acids. Carbon and nitrogen contents in rotifers were analyzed with a nitrogen/carbon analyser (Carlo Erba NA 1500).

Results

After a 12 day experiment, a mean daily production of 57.4±10.4mg of rotifers (wet weight)/l (wet weight) was recorded with initial densities of about $10x10^6$ cells/ml of *D. chlorelloides*. The mean growth rate (μ) of rotifer population ranged from 0.022 to 0.027 h^{-1} (μ_{max} from 0.043 to 0.050 h^{-1}) and the mean doubling time was 28.2±2.6 h.

The total (n-6) and (n-3) highly unsaturated fatty acids (HUFAs) increased with increasing residence time of rotifers in the *D. chlorelloides* culture. The essential fatty acids (EFAs) methyl linolenate (linolenic acid 18:3n-3) and linoleic acid (18:2n-6) showed a similar pattern. Linoleic acid was more abundant in rotifers (42-45% of the total fatty acids). The HUFA/monounsaturated ratio ranged from 2.8 at day 1 to 4.3 at day 10. The protein (Nx6.25), carbon and nitrogen contents of the rotifers were respectively 165.3, 114.8 and 26.4ng/ind. No significant fluctuation in C, N or protein was recorded between day 5 and day 10 in the mass culture.

The survival rate of gudgeon and perch larvae fed with rotifers were high during the 10 day experiment (Table I). In both cases, fry fed with rotifers during their first days of feeding grew significantly faster than fry fed with microencapsulated dry food (ANOVA $P<0.001$). The best food utilization, in terms of food conversion rate, protein efficiency ration, and growth efficiency, was obtained with the fry fed with rotifers. Combining rotifers with dry food provided a higher growth rate and food utilization than the microencapsulated dry food alone. Feeding rotifers to larvae increased their total (n-6) fatty acids. When dry food was, however, combined with rotifers, the total (n-9) and (n-3) fatty acids were significantly improved in perch larvae (Table II).

Discussion

This study confirmed the suitability of rotifers as starting food for freshwater fish. The rotifer (*B. calyciflorus*) can be mass cultured with *D. chlorelloides*. The presence of highly unsaturated (n-6) fatty acids (mainly 18:2n-6) in rotifers, which is essential for rearing freshwater fish larvae (Watanabe, 1979), proved its importance as live feed in aquaculture. In contrast to *Artemia* nauplii, which are too large for the first stage of rearing (Kestemont, pers. commun.); feeding with freshwater rotifer *B. calyciflorus* gave satisfactory growth and survival in perch larvae since the size of the rotifer was compatible with the small mouth of the larvae. According to Walton and Cowey (1982), the fatty acid composition of many fish reflects that of their food; gudgeon and perch larvae fed with rotifers follow a similar pattern.

Table I. Growth parameters of gudgeon (G) and perch (P) larvae fed with rotifers (G-r; P-r), microencapsulated dry food (G-df; P-df) or both [P-(r+df)]

	Diet				
	G-r	P-r	G-df	P-df	P-(r+df)
Growth rate (%.d^{-1})	19.60±0.70	19.30±0.60	17.8±0.50	1.17±1.08	17.2±1.07
Food conversion rate	1.31±0.03	0.80±0.01	2.03±0.01	56.9±18.6	1.22±0.05
Protein efficiency ratio	1.34±0.03	2.21±0.04	1.19±0.07	<0.1	1.71±0.07
Growth efficiency	6.57±0.17	3.80±0.06	10.2±0.05	-	5.82±0.23
Survival rate	97.5±0.71	83.5±6.36	84.5±2.12	4.00	42.5±2.12

Table II. Procentual fatty acid composition of gudgeon (G) and perch (P) larvae according to the diets used. Go and Po are larvae at the final day of yolk resorption; r= rotifers; for abbreviations of diets, see Table I

	Diet							
	Go	Po	G-r	P-r	G-df	P-(r+df)	Rotifer	df
ΣSaturated	11.98	6.64	11.21	6.40	10.54	7.90	3.50	3.18
Σ(n-9)	11.94	8.37	11.13	9.32	14.68	11.67	0.95	3.85
Σ(n-6)	1.26	1.04	4.96	3.19	5.82	3.36	6.76	1.40
Σ(n-3)	6.23	4.84	6.03	4.08	6.34	5.72	0.55	0.11

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THE IMPORTANCE OF PREY SEQUENCING DURING EARLY GROWTH OF THE GILTHEAD SEABREAM *SPARUS AURATA* L.

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Introduction

The usual feeding regime during the larval rearing of *Sparus aurata* consists of the rotifer *Brachionus plicatilis* from the 3rd-4th day posthatching and of *Artemia* nauplii from about the 3rd week on. In a previous study (Polo, 1991), we have determined the importance of small sized *Brachionus* strains when feeding commences to improve larval growth. Larvae below 45µg prefer small rotifers and larvae above 90µg prefer larges ones. The objective of the present study is to determine the best sequencing of different types of prey (small rotifers, large rotifers and *Artemia* nauplii) during the early feeding of *Sparus aurata*.

Materials and methods

Experiments were carried out in 300 l tanks, at 33ppt salinity and constant illumination. 15-20% of the water volume was renewed daily from day 3 on. Strains Bs (small) and S-1 (large) of *B. plicatilis* (Yúfera, 1982) were used as food. Four feeding regimes were tested: A) large rotifers from day 3 to day 15; B) small rotifers from day 3 on and large rotifers from day 8 on; C) small rotifers from day 3 on and large rotifers from day 12 on; and D) small rotifers from day 3 to day 15. Survival was estimated during the feeding period (days 3 to 15). The ability of larvae to ingest *Artemia* nauplii was determined for different larval sizes by microscopic examination of the guts. The dry weight was plotted against degree-days in order to compare growth curves obtained at different temperatures (between 18.5 and 20.5°C) in experiments carried out between 1988 and 1991.

Results and conclusions

Growth curves for the different feeding regimes are shown in Fig. 1 and Table I. All regimes which included small rotifers in the diet (regimes B, C, and D) supported similar larval growth and survival, while the regime with large rotifer (strain S-1) alone (regime A) shows lower values as expected. Ingestion of *Artemia* nauplii commences in some larvae above 40µg mean dry weight, while above 80µg more than 50% of the larvae eat nauplii (Fig. 2). Therefore, the rotifer replacement by *Artemia* nauplii may begin at about

100 degree-days (12.5 days at 20°C) if the diet contains small rotifers and at about 118 degree-days (15 days at 20°C) if only large rotifers are supplied as food. The results show that small rotifers (strain Bs) alone are enough to support larval growth in the best conditions before the addition of *Artemia* nauplii.

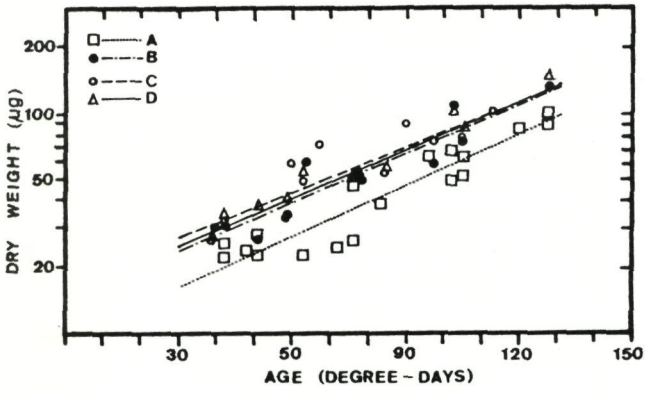

Fig. 1. Growth curves at the different feeding regimes (A, B, C, and D). 120 degree days correspond to 15 days at 20°C.

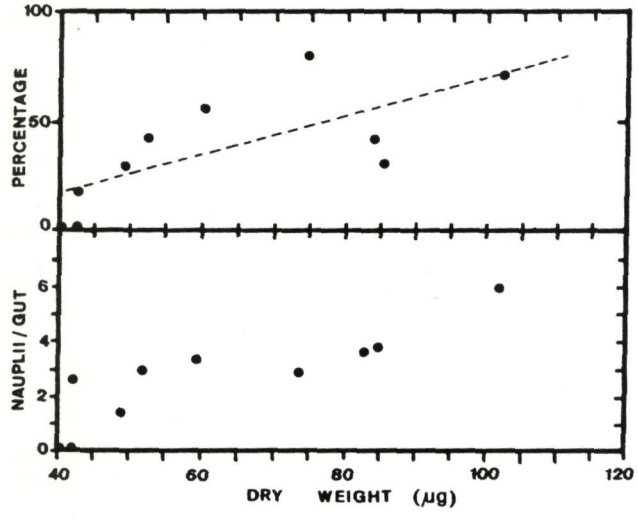

Fig. 2. (A) Percentage of larvae able to ingest *Artemia* nauplii and (B) mean number of nauplii per gut in relation to mean larval weight of the population.

Table I. Parameters of the growth curves (dry weight = ae^{bt}, t = degree-days) and estimated survival (S) for the different feeding regimes

Feeding regime	a	b	S(sd)
A	9.73	0.0177	10 (3)
B	14.04	0.0169	36 (10)
C	16.98	0.0157	39 (9)
D	15.09	0.0168	42 (12)

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DAILY RATION OF TURBOT LARVAE, *SCOPHTHALMUS MAXIMUS* IN INTENSIVE CULTURE

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Introduction

The knowledge of the daily ration for larvae is useful in aquaculture. It allows an aproximate estimation of daily live food production requirements and dozes, as well as to study metabolic changes during growth. In the present study, the daily ration for 6-day-old turbot larvae (*Scophthalmus maximus*) has been estimated using the model of Elliott and Persson (1978), based on estimates of the exponential evacuation rate (R) and on analyses of stomach contents over a period of 24h. The daily ration has been estimated as 44% of larvae's body weight.

Materials and methods

Turbot larvae, *Scophthalmus maximus*, were reared at the facilities of the Centro Experimental de Vilaxoán (CEV), in 350 l tanks with 1µm filtered seawater, at a constant temperature range of 18±1°C, a salinity of 33-35ppt and constant artificial illumination. They were fed a rotifer diet at a density of 11.5 and 15 rot./ml at day 4 and 6, respectively.

Twenty one samples (n=147) of 6-day-old larvae were taken over a 24h period to determine the daily pattern of food ingestion.

Simultaneously, approximately 120 larvae were carefully transferred to a 40 l cylindric tank filled with seawater with identical conditions as the original 250 l tank. A suction pump with a 50µm filter recirculated the water for 25min until no rotifers remained in the tank. A first sample was taken and *Artemia* nauplii were added to the tank at a concentration of 1ind./ml, only to avoid stress conditions due to lack of food. One hundred and three larvae were sampled at eight sequential 30min intervals (mean=12.9 larvae/sample), based on our previous estimations. Larvae were anaesthetized with MS222 (tricaine methanesulphonate), measured (total length ± 0.05mm), transferred into a 7% formaldehyde solution for 24h and finally in a 70% isopropyl alcohol. The larval dry weight (75.7-87.9µg) was back calculated using regression analysis with total length.

Rotifer and rotifer egg dry weight were estimated as 0.25 and 0.05µg, respectively. The stomachs were opened and the contents excluding *Artemia* nauplii counted and expressed as percentage of the larvae body weight (%BW).

Elliott and Persson's (1978) model was used to calculate the daily ration (DR). The model requires data on food intake (C_t) over the time interval from t_0 to t_t and it assumes an exponential, temperature-dependent gastric evacuation rate (R):

$$C_t=(S_t-S_0e^{-Rt})Rt/1-e^{-Rt} \quad (1)$$

where S_t and S_0 are the stomach contents weight at times t_t and t_0, respectively. DR results from summing the estimates of C_t for all the time intervals sampled during the 24h period. DR can also be estimated from

$$C_t=24SR \quad (2)$$

where S equals the mean stomach-content weight over the 24h period.

Results

For turbot larvae fed following a 24h cycle, the minimum stomach fulness (as %BW) occurred around midday (Fig. 1). The mean weight (± SE) of stomach contents over the 24h period was S=4.6074±0.2144%BW. There was a significant difference among the adjusted means of stomach content weight over the 24h period (ANCOVA), ($F_{7,126}$=3.24; P<0.01; data regrouped in eight 3h periods for analysis).

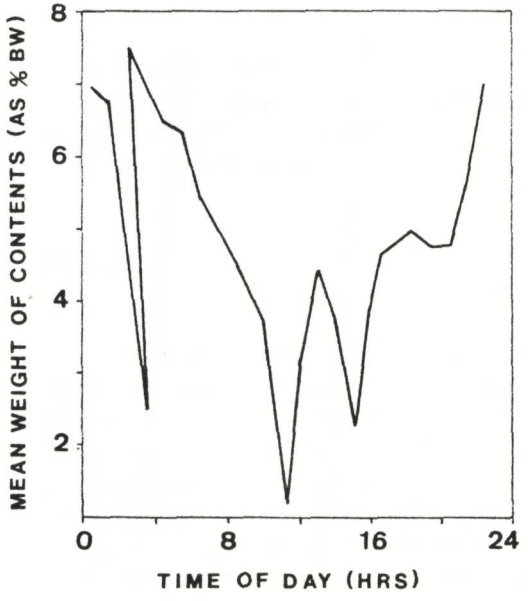

Fig. 1. Mean weight of gut contents of *Scophthalmus maximus* at different times of the day.

The relationship between dry weight of the stomach contents (as %BW) and the time period in which no *Brachionus* was fed anymore, was best fitted by a curvilinear function rather than by a linear one. A natural logarithmic model was then fitted to the data. The equation described by Elliott and Persson (1978):

$S_t=S_0e^{-Rt}$, can be linearized to:
$LnS_t=LnS_0$ - Rt, where R is the gastric evacuation rate. R can be calculated from regression analysis between LnS_t and time (t). From the present data:
$LnS_t=1.088855$ -0.3975t (with r=0.5843) or $S_t=2.971\ e^{-0.39756t}$. From (1) the daily ration is DR=24*4.6074*0.67195=74.3%BW.

Between day 6 and 7 the instantaneous growth rate of turbot larvae was 0.15. The food assimilation for growth was 0.207 on a dry weight basis.

Discussion

The value of R(-0.67195 %BW/h), is very high, as has generally been for fish larvae. The DR was estimated, to be 74.3% of BW corresponding to an ingestion of about 236 rot./6-day-old larva. There is no previous estimation of the daily ration of turbot larvae using a theoretical model, but Person-Le Ruyet (1989) and Girin (1974) reported a feeding schedule of offering 120-200 rot./6-day-old larva. Recently we reported good larval survival rates at day 25 (10-35%) using higher food concentrations than the farmer anthas (Coo *et al.*, 1990)

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FEEDING BEHAVIOR DURING DEVELOPMENT OF THE PENAEID SHRIMP *METAPENAEUS ENSIS*

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Introduction

Successful rearing of a species requires information on its feeding habit. *Metapenaeus ensis* is an important commercial species in southern China. Like other penaeids, it exhibits important morphological and dietary changes during larval development. Starting from non-feeding nauplii, the feeding behavior of larvae develops progressively from herbivorous to omnivorous. In this study, dietary changes that accompany the development of *M. ensis* are investigated using the diatom *Chaetoceros gracilis* and *Artemia* nauplii as foods.

Materials and methods

Larvae were obtained from wild spawners bought at local fish markets and reared in 500 l indoor tanks. Feeding experiments were conducted in 1 175ml glass bottles containing 0.45µm filtered seawater. For grazing experiments, various stages of each 100 shrimps were allowed to feed for 16h in bottles containing *C. gracilis* at a density of 2.5×10^5 cells/ml. Cell concentrations before and after grazing were determined with a Model ZB1 Coulter Counter. Clearance rates were calculated according to Frost (1972). For predation experiments, 20 shrimps were added to bottles containing 100 *Artemia.* Predation rates were determined by counting the number of missing preys after 4h. Two types of combined feeding experiments were conducted. First, the predation at different densities of *Artemia* was studied in the presence (10^5 cells/ml) and in the absence of *C. gracilis*. Second, grazing at different concentrations of *C. gracilis* was studied in the presence (200 ind./bottle) and the absence of *Artemia.* The amount of phytoplankton pigment in the gut of larvae was measured by the gut fluorescence method (Dagg, 1983). The effects of combined feeding were determined by a nonparametric two-way ANOVA. All experiments were carried out in darkness at 27±1°C.

Results

Clearance rate on algae increased markedly from the protozoeal (PZ) to the early mysid (M) stages, peaked at M2 and M3, and then decreased at the postlarval (PL) stages (Fig.

1). No measurable predation rate on *Artemia* was recorded before PZ3 (Fig. 2). From M1 on, the predation rate increased with age.

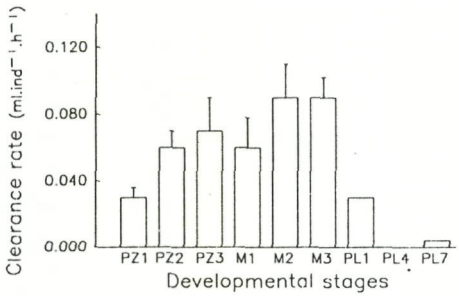

Fig. 1. Clearance rate (mean ± SE) by *M. ensis* at various developmental stages fed *C. gracilis*.

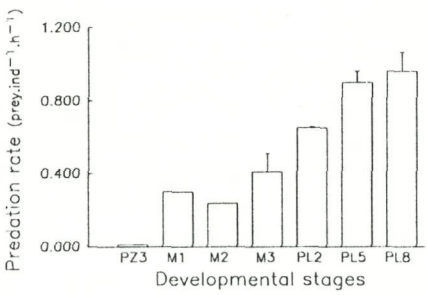

Fig. 2. Predation rate (mean ± SE) of *M. ensis* at various developmental stages fed *Artemia*.

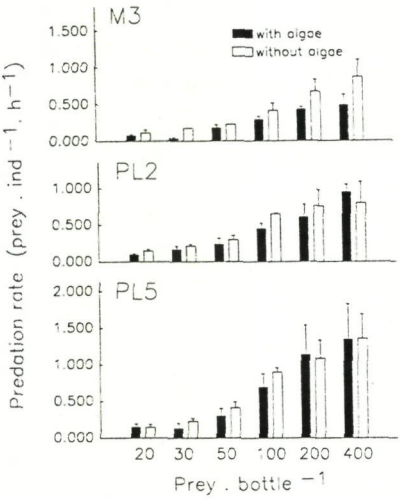

Fig. 3. Predation rate (mean ± SE) of *M. ensis* on *Artemia* in the presence and absence of *C. gracilis*.

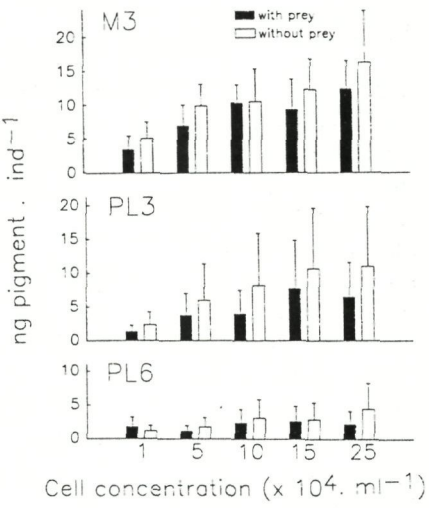

Fig. 4. Gut pigment content (mean ± SE) of *M. ensis* fed *C. gracilis* in the presence and absence of *Artemia*.

The predation rate on *Artemia* (Fig. 3) by M3 ($P<0.005$), PL2 ($P< 0.001$) and PL5 ($P<0.001$) was strongly affected by prey density; no effect was found for the presence or absence of algae ($P>0.1$). The amount of *C. gracilis* pigment in the guts of M3, PL3 and PL6 (Fig. 4) was not affected by cell concentration nor the presence of *Artemia* ($P>0.25$).

Conclusions

The results show that *M. ensis* is an obligate herbivore in the protozoeal stages. The feeding rate on algae peaks during the mysid stage, then drops as the larvae develop mechanisms for carnivorous feeding. *M. ensis* becomes more carnivorous in the late postlarval stages. Transition from herbivory to omnivory occurs at about M2 and M3. Similarly, *Penaeus indicus* has been found to shift to omnivorous feeding at about M3 (Emmerson, 1984). The presence of *Artemia* does not significantly affect herbivorous feeding in mysid and postlarvae, but animals exposed to *Artemia* generally contain fewer gut pigments. Similarly, although a significant relationship was not found, predation on *Artemia* is usually lower in the presence of algae. Future dietary studies should evaluate the energetic consequences of switching food habit.

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ENSILED *ARTEMIA* BIOMASS: A PROMISING AND PRACTICAL FEED FOR PENAEID SHRIMP POSTLARVAE

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Introduction

Unlike *Artemia* nauplii which are used extensively as convenient live feed in aquaculture, large-scale utilization of *Artemia* juveniles and adults (biomass) is still very limited and in most cases restricted to those operations which are located near an *Artemia* production site. Currently applied techniques for preservation of *Artemia* biomass (*e.g.* blast-freezing or drying) are often cost prohibitive and in several cases not applicable at the remote and scattered sites where the *Artemia* biomass is available. An inexpensive technique which can be readily applied under field conditions and allowing to stabilize and retain the nutritional quality of *Artemia* at ambient temperatures, would greatly enhance the exploitation of this resource for aquaculture. The present study reports on the use of acid-ensiled *Artemia* as a direct food source or as dietary ingredient for postlarvae of penaeid shrimp.

Materials and methods

In the first feeding trial, postlarvae of *Penaeus monodon* (0.89mg dry weight; 11.05mm total length) were reared for 2 weeks on a diet of ensiled or frozen biomass at three different feeding frequencies. The *Artemia* silage was prepared by adding 3% HCl (v/w) in order to maintain the pH<1.0, and stored for 3 weeks at ambient temperatures prior to being used as a direct feed source. Preliminary experiments have indicated that a 3% HCl treatment not only prevents microbial spoilage for extended periods (at least 10 months), but also inhibits liquefaction and associated loss of physical integrity of the animals, thus minimizing the leaching of essential nutrients from the biomass.

In the second trial, postlarvae of *P. vannamei* (0.52mg DW; 7.49mm) were reared for 2 weeks and fed formulated microbound diets containing about 9% (dry weight basis) of the following preparations of *Artemia* biomass: 1) ensiled for 4 weeks with 3% HCl; 2) ensiled for 4 weeks with propionic acid + HCl until pH 3.0; and 3) frozen. The second treatment was used because, unlike the first treatment, it produces a liquefied product brought about by the remaining activity of endogenous proteolytic enzymes in the biomass.

Rearing conditions and culture set up for both experiments were the same as described in Abelin *et al.* (1989). One-way analysis of variance and Duncan's multiple range test were performed to detect differences in mean dry weight, length, and survival among individual treatments. Survival data were normalized through an ^{1}arcsin transformation.

Results and discussion

Growth and survival results of postlarvae of *P. monodon* and *P. vannamei* are presented in Table I and Table II, respectively. This study reveals that HCl (3%) ensiled biomass can be used as an appropriate food source for postlarvae of penaeid shrimp. In the rearing trial with *P. monodon*, using *Artemia* biomass as a direct feed, the treatment fed the HCl-ensiled biomass had better growth and survival than shrimp fed frozen biomass at the same feeding frequency. Best results were obtained when the ensiled product was offered in at least three feeding rations/day. Similarly, superior growth was obtained when postlarvae of *P. vannamei* were fed the HCl-ensiled biomass as a dietary ingredient. Poor results with the liquefied propionic acid-ensiled treatment may be due to the enzymatic hydrolysis of the *Artemia* protein resulting in higher levels of amino acids in the free form which may be used less efficiently for tissue synthesis (Tacon, 1987) and may be leached out more easily from the diet as well.

Table I. Growth and survival of *P. monodon* postlarvae fed ensiled (HCl 3%) or frozen *Artemia* biomass at different feeding frequencies

Form of *Artemia*	Feeding frequency (per day)	Final weight (mg DW)		Final length (mm)		Survival rate (%)	
		Mean	SD	Mean	SD	Mean	SD
Ensiled	2	1.11^{b}	± 0.20	13.68^{b}	± 0.05	57.7	± 4.99
Ensiled	3	1.62^{a}	± 0.29	$14.81^{a,b}$	± 0.79	61.0	± 3.56
Ensiled	4	$1.66^{a,b}$	± 0.93	14.35^{a}	± 0.53	65.7	± 4.78
Frozen	2	0.93^{b}	± 0.17	12.26^{c}	± 0.63	43.0	± 5.10
Frozen	3	1.28^{b}	± 0.39	$13.35^{b,c}$	± 1.13	37.3	± 15.92
Frozen	4	1.24^{b}	± 0.12	13.66^{b}	± 0.39	43.0	± 12.03

Values in the same column with the same superscript are not significantly different ($P<0.05$).

Table II. Growth and survival of *P. vannamei* postlarvae fed experimental diets containing frozen or ensiled *Artemia* biomass as a dietary ingredient

Form of *Artemia*	Final weight (mg DW)		Final length (mm)		Survival rate (%)	
	Mean	SD	Mean	SD	Mean	SD
HCl ensiled	4.13^{a}	± 0.67	13.53^{a}	± 1.07	80.3	± 8.25
HCl + Propionic acid ensiled	2.83^{b}	± 0.60	12.08^{b}	± 1.07	74.7	± 5.97
Frozen	$3.93^{a,b}$	± 1.76	$12.70^{a,b}$	± 1.64	79.8	± 7.85

Values in the same column with the same superscript are not significantly different ($P<0.05$).

Conclusions

The technique of ensilage presents an attractive alternative to freezing for the preservation of *Artemia* biomass and could greatly advance its use in aquaculture. The technique is readily applicable under field conditions and certainly much more cost-effective than other processing techniques. No investments in processing facilities or energy, both for production or storage, are required. In fact, the only extra cost involved is the purchase of the chemical, *i.e.* approximately US$ 6 for the production of 1 ton of HCl (3%) ensiled *Artemia* biomass.

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A COMPARISON OF THE USE OF DECAPSULATED *ARTEMIA* CYSTS AND NAUPLII AS FOOD FOR *PENAEUS VANNAMEI* LARVAE

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Introduction

Use of decapsulated *Artemia* cysts (cysts from which the protective shell has been chemically removed) offers larviculturists an alternative to live nauplii as food source with important practical advantages: they can be handled as an inert diet, their size is considerably smaller (Vanhaecke and Sorgeloos, 1980) while their individual dry weight and energy content is, on average, 30 to 40% higher than freshly-hatched nauplii (Vanhaecke *et al.*, 1983). Considering the normal presence of non-hatching cysts, the use of decapsulated cysts eliminates wastage of *Artemia* product. While successful use of decapsulated cysts as direct food has been reported for larvae of various aquatic species such as milkfish (de los Santos *et al.*, 1980) and carp (Vanhaecke *et al.*, 1990), the incorporation of a new feed into commercial applications requires an understanding of the quantitative aspects of its utilization. With this aim, the present work was designed to compare the gross growth efficiency, ingestion and growth rates of the larvae of a commercially important shrimp species, *Penaeus vannamei*, fed either live nauplii or decapsulated cysts.

Materials and methods

Laboratory hatched *P. vannamei* protozoea-III larvae (L) were reared individually to 10-day-old postlarvae (PL10) at 29-31°C in 32-33ppt seawater treated with 0.5ppm Na-EDTA and 0.01ppm Treflan. They were fed Great Salt Lake (Biomarine brand) *Artemia franciscana* as either decapsulated (Bruggeman *et al.*, 1980), acetone-treated and dried cysts (DC) or newly-hatched nauplii (N). Three food concentrations (0.5, 1, 2 and 4 N, or DC/ml) and five ceiling rations (20, 40, 80, 160 and 320 N or DC/L/day) were assayed in all possible combinations with four replicates per treatment together with unfed controls. Culture volumes were adjusted in each treatment to obtain the desired ration at each food concentration. The smallest volume used was 5ml/L and largest was 640ml/L. The total length of the larvae was measured daily after immobilizing them by lowering the temperature to 22°C. Ingestion rates were evaluated from counts of uneaten *Artemia*. Media and food rations were replaced daily. All treatments began with a common 20 N or DC/L/day ration which was raised according to increased ingestion capacities until the

protocol "ceiling" rations were reached for each. Individual dry weights for GSL *Artemia* were taken from Vanhaecke *et al.* (1983) as being 2.42 and 3.23µg for instar I nauplii and decapsulated cysts, respectively. Individual biomass estimates of shrimp larvae were calculated from the relationship between total body length (tl) and dry weight (dw) found for *P. stylirostris* (Abreu-Grobois *et al.*, 1991): dw (µg) = $3.4*(tl)^{2.441}$ (mm).

Results and discussion

Final survival values varied considerably among treatments (25-100%); they were, however, not correlated with either the food concentration or ration. As growth was not affected by the concentrations administered, the results of ration treatments were pooled for analysis. Overall, growth was significantly slower for rations below 20 DC or 40 N/L/d, particularly after metamorphosis to PL. Maximal final sizes in PL10 (*ca.* 5.5mm) were obtained at rations above 40 DC or 80 N/L/d. The overall effect of increasing rations on growth exhibits an asymptotic curve with maximal average daily growth (as %/day) reached with lower DC rations (equivalent of about 100/L/d) than with N (about 160/L/d) (Fig. 1). For N-fed larvae maximal (*ad libitum*) ingestion rates (about 300 N/L/d) occurred in treatments with rations of 320 N, as expected. However, satiation levels for DC-fed larvae appeared in treatments above 80 DC/L/d with maximal observed ingestion rates of 105-144 DC/L/d. Specific ingestion rates increased drastically following increases in rations (arrows Fig. 2). However, they were consistently higher in N-fed larvae than in individuals fed with DC at the same rates. Overall gross growth efficiencies (K_1, increase in dry weight during experiment/cumulative dry weight ingested) decreased with increase in rations (particularly above about 100 DC or N/L/d), and were consistently higher for DC-fed than for N-fed larvae (Fig. 1) in spite of lower specific ingestion rates in the former at the higher rations (Fig. 2).

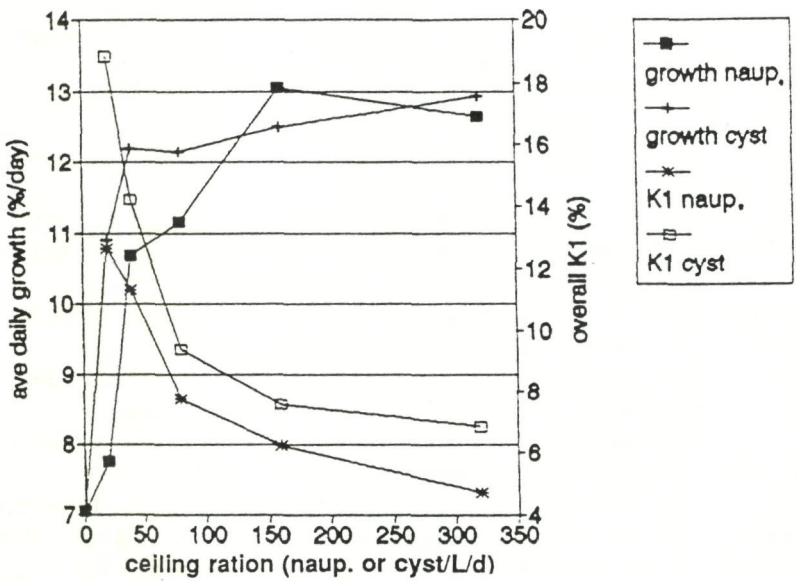

Fig. 1. Overall K_1 and average daily growth for *Penaeus vannamei* larvae cultured on various rations of *Artemia* fed as either decapsulated cysts or nauplii.

These results provide a basis for the estimation of the daily consumption rates by *P. vannamei* larvae for both DC and N. Not only do DC appear to be an effective food, they also promote growth levels equivalent to those obtained with N and at lower specific ingestion rates. The economic implications of these findings are interesting and pilot scale rearing of *P. vannamei* larvae on DC is currently being considered to evaluate the results of this research under hatchery conditions.

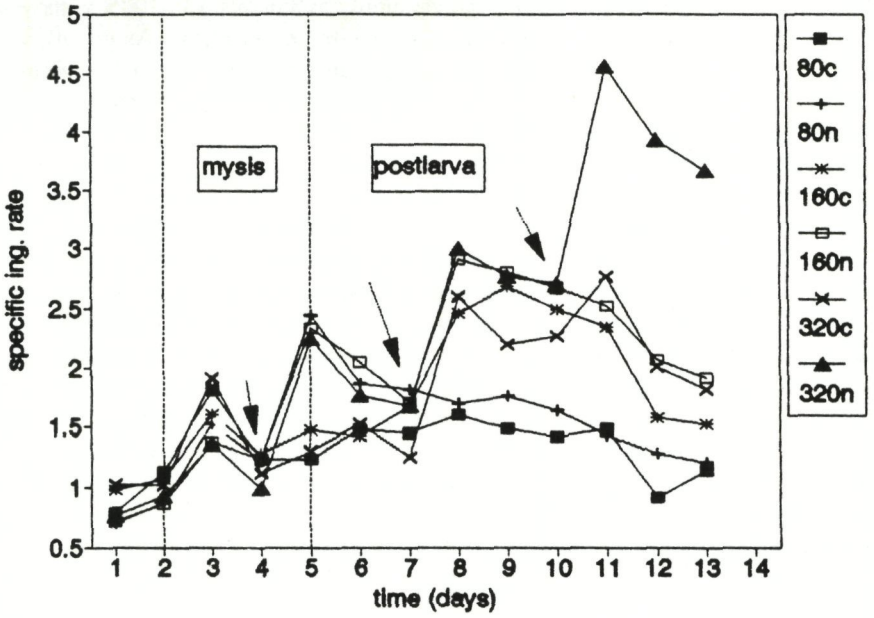

Fig. 2. Specific ingestion rate (µg *Artemia* ingested/µg individual) of *P. vannamei* larvae cultured on various rations of either decapsulated cysts or nauplii (box indicates ceiling rations and the feed: c=decapsulated cysts, n=nauplii).

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GROWTH AND GROSS GROWTH EFFICIENCIES OF *PENAEUS STYLIROSTRIS* FED CONTROLLED RATIONS OF *ARTEMIA* NAUPLII

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Introduction

Because successful commercial hatchery operations of penaeid larvae requires efficient use of its food sources much recent interest in shrimp aquaculture research has focused on the feeding behaviour of crustacean larvae (Anger and Dietrich, 1984; Chu and Shing, 1986; Emmerson, 1984) and their energy budget (Kurmaly *et al.*, 1989). However, the relationship between the amount of food consumed by the organisms and their growth has remained insufficiently studied. In this paper, the effects of controlled and *ad libitum* feeding of *Artemia* nauplii, both on gain in body size and on gross growth efficiency (K_1; Conover, 1964) of *Penaeus stylirostris* larvae were studied.

Materials and methods

Laboratory hatched *P. stylirostris* protozoea-III larvae (L) were cultured individually to postlarva-9 (PL9) at 28°C and 35ppt seawater treated with 0.5ppm Na-EDTA and 0.01ppm Treflan. They were fed controlled rations of freshly hatched San Francisco Bay Brand *Artemia franciscana* nauplii (N) with ceiling rations of 15, 30, 60 and 240 N/L/day with 10 replicates per treatment. Dry weights of individual nauplii were considered to be 1.63µg (Vanhaecke and Sorgeloos, 1980) so log-transformed ceiling rations corresponded to 0.35, 1.65, 1.95 and 2.5µg N/L/day, respectively. To avoid detrimental effects of excess food in cultures, feeding rates were raised gradually according to increasing ingestion capacity until the predetermined ration levels were reached in each treatment. Larvae in the treatment with the highest ration always received food in excess of their ingestion capacities and thus were considered to feed *ad libitum*. Daily, food and culture media were replaced and ingestion rates were determined from evaluations of remaining nauplii. Daily initial nauplii concentrations were always 5 N/ml while volumes of each treatment were adjusted to obtain the desired level of food rations. Daily length data up to mysis III were derived from previous experiments conducted under similar conditions. For later stages, length data were obtained from direct body measurements.

The relationship between total body length (lt) and dry weight (dw) in cultured *P. stylirostris* was derived from a first stage of the study through regression analysis of linearized data obtaining: $dw\ (\mu g) = 3.4*(tl)^{2.441}$ (mm) ($r=0.93$, $N=58$). This equation was

used for converting size measurements into dw in the estimation of daily percentage K_1 which was calculated from: (daily increase in dw/dw equivalent of N ingested) x 100.

Results and discussion

Final average sizes in the four treatments (Fig. 1) were all significantly different ($F=124$, $P<0.001$) as were the slopes of the growth curves ($F=132$, $P<0.001$) confirming a direct relationship between feeding levels and growth. Changes in observed daily gross growth efficiencies (K_1) with time could be explained by a combination of size (dry weight, DW) and amounts of food ingested (ingestion rate, IR) as demonstrated by 3rd-degree polynomial regression of log-transformed parameters on K_1 (K_1= 727.2+ 454.7*DW+748.5*IR-641.2*DW*IR+58.3*IR^2-55.4*DW^2+6.6*$IR^3$73.6*DW^3+ 228.6*DW^2*IR-51.2*DW*IR^2; $N=68$, $R^2=0.755$, d.f.=67, $F=19.84$, $P<0.001$) which indicates significant contributions both of single terms and their interactions. The derived coefficients from this analysis were used to generate a response surface (Fig. 2) for K_1 as a function of the log-transformed dry weights and (daily) ingestion rates. The trend surface illustrates the changes occurring in K_1 in developing larvae: (1) K_1 values near the beginning of the experiment (approx. 1.34 log(DW) units) where IR is relatively poor, are low, and tend to peak (K_{1max}) around 1.6 log(DW) units in all treatments; (2) the K_{1max} values tend to fall under the higher feeding regimes above 0.8 log(IR) units; (3) tendencies towards no growth occur in feeding regimes with log(IR) values (< *ca.* 2.4), at sizes defined by the contour line for $K_1=0$ on the trend surface; and, (4) continuous growth ($K_1>0$) is only promoted by ingestion rates > *ca.* 2.4 log(IR) units in the last growth stages studied.

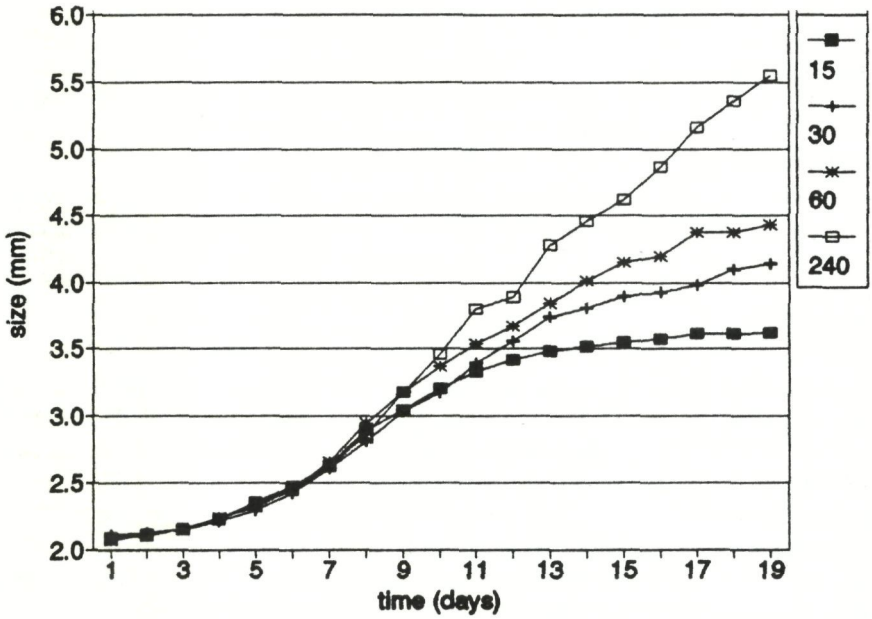

Fig. 1. Growth of *P. stylirostris* larvae reared on different rations of *Artemia* nauplii (box on right denotes the ceiling rations used in the treatments).

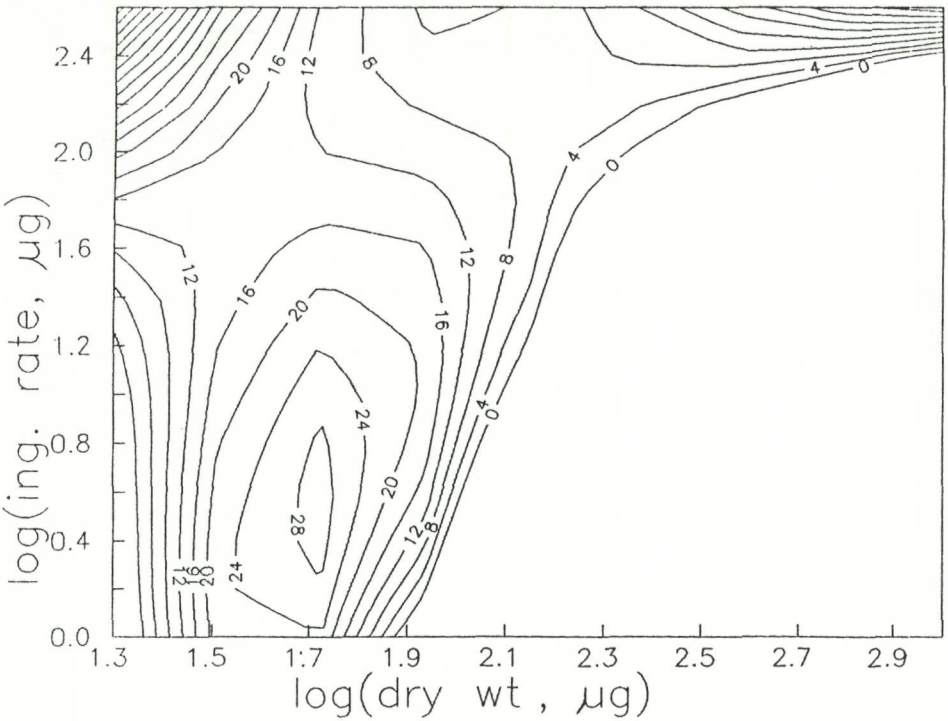

Fig. 2. Response surface for daily gross growth efficiencies (K_1) in *P. stylirostris* larval growth as a function of log-transformed dry weights and ingestion rates.

Feeding strategies can be formulated, based on present analysis, to either optimize for maximal growth efficiency (by feeding amounts corresponding to ridges of maximal K_1 values in Fig. 2 for each growth stage) or for maximam growth rate (by feeding *ad libitum*). Derivation of optimal feeding strategies in commercial conditions could be obtained by applying the analysis described here, and incorporating data on the relative costs of *Artemia* and general culture operations as well. Nonetheless the results are suggestive that more efficient food utilization is possible with feeding regimes considerably lower than the *ad libitum* practices commonly employed.

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CULTURE OF *PENAEUS PAULENSIS* WITH FIVE DIFFERENT SOURCES OF *ARTEMIA* CYSTS

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Introduction

Shrimp larviculture can be considered as one of the main markets for *Artemia* cysts. Although a large number of commercial *Artemia* cyst sources have become available in recent years, the selection of a suitable cyst batch for penaeid larviculture has often remained difficult due to the lack of knowledge on the nutritional quality of these sources. The objective of this study is to compare different sources of *Artemia* cysts as food for the larvae of *Penaeus paulensis*, which is commercially cultured in southern Brazil.

Materials and methods

The cyst sources used in this study are given in Table I.

Table I. Brands and strains of *Artemia* cysts used in the experiment

Strain	Abbreviation used	Year
Great Salt Lake, UT-USA	GSL-A	1990
San Francisco Bay, CA-USA	GSL-B	1989
San Francisco Bay, CA-USA	SFB	1989
Macau-RN-Brazil	MAC	1990
Virrila-Peru	VIR	1986

Larvae of *P. paulensis* were obtained from different spawnings and reared for 7 days in cylindro-conical fiberglass containers filled with 30 l of filtered seawater. Larvae were stocked at 50 individuals/l. The temperature was maintained at 27°C and water was changed at a rate of 80%/day. Three replicate containers were used per treatment group and all shrimp larvae were fed the same cyst source. *Artemia* nauplii of different sources were fed from protozoea III onwards at a concentration of 0.1 ind./ml. The feeding level of *Artemia* was gradually increased up to 1 nauplius/ml for postlarval stages. The diatom *Chaetoceros gracilis* was fed at a concentration of 50 000 cells/ml. After a period of 7 days, in which most of the animals reached the postlarval stage, samples were taken and

microscopic observations made in order to evaluate the survival, rate of metamorphosis, length, and dry weight of the animals. A one-way ANOVA with F-test and Duncan's test was conducted to determine if significant differences existed among treatment groups fed with the same cyst source.

Results and discussion

The results of the experiments are given in Table II.

Table II. Survival and metamorphosis rates, length and dry weight of *Penaeus paulensis* postlarvae fed with five *Artemia* cyst brands

Artemia source[1]	Survival (%)	Metamorphosis (%)	Length (mm)	Dry weight (µg)
VIR	82 A[2]	100 A	4.96 A	128A
MAC	74 B	80 C	4.85 B	121A
GSL-B	63 C	86 BC	4.81 C	118AB
GSL-A	62 C	86 B	4.96 A	97B
SFB	42 D	66 D	4.61 D	118AB

[1] For abbreviations see Table I.

[2] Results followed by the same letter are not significantly different at the $P<0.05$ level.

Discussion

Sorgeloos *et al.* (1986) indicated that *Artemia* has a high digestibility and covers most of the micro- and macro-nutrient requirements of penaeid larvae. Feeding tests with various strains of *Artemia* have, however, demonstrated that for several species a large variability in the nutritional effectiveness of *Artemia* exists. Soejima *et al.* (1980), Seidel *et al.* (1982), and Léger *et al.* (1985) found that poor culture results in marine species are to a large extent attributed to low contents of (n-3) highly unsaturated fatty acids (HUFAs), particularly eicosapentanoic acid, 20:5n-3 in the live food organism *Artemia*.

The present results confirm that the various cyst sources used in this study produce a different nutritional response in larvae of *P. paulensis*. The superior growth and survival obtained with cysts from Virrila and Macau could possibly be related to a higher content of 20:5n-3 in these *Artemia* strains.

Nevertheless, it should be indicated that, aside from nutritional quality, the selection of a suitable cyst source for penaeid larviculture should also be based on criteria such as hatching quality and costs.

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FORMULATED FEEDS

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THE POTENTIAL FOR REPLACEMENT OF LIVE FEEDS IN LARVAL CULTURE

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Abstract

The current deployment of artificial feeds in bivalve, crustacean and fish larval culture is reviewed. Although microencapsulated diets have supported metamorphosis of bivalve larvae, only spray-dried algae have produced growth (*Tapes philippinarum*) equivalent to that achieved on live diets. Spray-dried algae shows similar potential for culture of bivalve spat. Partial replacement of live diets in penaeid prawn culture using microparticulate or microencapsulated diets is now routine in hatcheries; total replacement is possible at a research level, and recently cultured algae have been replaced totally with encapsulated feed in hatchery penaeid larval culture. Apart from salmonids, commercial first feeding of fish larvae still rely on live feeds, although partial replacement with encapsulated feed has been demonstrated for *Lates calcarifer*. However, considerable progress has been made with the enhancement of live feeds by prefeeding these with diets rich in (n-3) fatty acids.

Only when a water stable formulated artificial diet is accepted, ingested, digested and assimilated at rates comparable to live feeds will it be possible to investigate specific nutritional requirements of different larvae. Problems of acceptability and digestibility of artificial diets are particularly common for carnivorous crustacean and fish larvae. Data are presented demonstrating that the success of artificial diets in penaeid larval culture may be due to the high levels of enzymes produced by such herbivores normally feeding on phytoplankton which cannot contribute enzymes to assist digestion. These herbivorous larvae readily adapt to artificial diets, especially if algal extracts are incorporated to further stimulate enzyme secretion. There is now strong evidence to support the view that digestion in carnivorous larvae relies upon prey autolysis. In addition these larvae, in contrast to herbivores, demonstrate long gut retention times so that feeds must be both highly digestible and of a high energy content.

Future research priorities are the cost effective production of spray-dried algae for bivalves, optimisation of water stable feeds for penaeid culture, and improvement in acceptability and digestibility of artificial diets for carnivorous larvae.

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A COMPREHENSIVE PROGRAM FOR THE EVALUATION OF ARTIFICIAL DIETS

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Abstract

Fish larvae, especially marine species, normally feed on zooplankton (*e.g.*, copepods) in their natural environment. Aquaculturists have mostly used some combination of rotifers and *Artemia* to feed fish larvae in artificial environments. A long-term goal of aquaculture research is the development of artificial diets, for use in artificial environments, that promote survival and growth of fish larvae equivalent to that obtained with live food. Although much research has been done on artificial diets for larval fish, including microencapsulated diets, some of which are commercially available, the goal of a live-food equivalent has not been reached.

When one evaluates an artificial diet for a larval fish species, the first, practical evaluation should be an experiment to determine whether the diet does provide growth and survival equivalent to the live food. If equivalency is not obtained, an investigation into causes of the deficiency is necessary. The investigation includes three basic categories: 1) research on the diet in the water column, 2) research on the capabilities and requirements of the larvae, and 3) research on the diet inside the larvae. All research should compare the diet to live food.

As the diet is dispersed in the water, it should be 1) available and 2) palatable to the larvae without 3) leaching significant amounts of nutrients. The assumption is made here (and can be tested) that the diet has been stored and handled properly since manufacture so that nutrients have not been lost or degraded. Availability of the diet can be determined even without the larvae, by measuring the amount of time a known quantity of diet particles spends in the water column before sinking to the bottom. Palatability can be determined simply by observing the larvae to see whether (and at what rate) they reject particles which have entered their mouths. Videotaping or image analysis can be used to determine the rate of ingestion of food particles. Leaching can be determined by chemical analyses for specific nutrients in either 1) diet particles recovered from the water at various intervals, or 2) the water itself, or 3) both.

The capabilities and requirements of the fish larvae can best be obtained by a combination of biochemical, physiological, and morphological determinations. Some approaches to estimate the nutritional requirements of a species have included

biochemical analyses of : 1) the yolk material in the fish eggs, on which the larvae have depended for endogenous food, 2) the zooplankton on which the larvae naturally feed, and 3) the *Artemia* or rotifers on which the larvae feed in hatcheries. The nutritional composition of the artificial diet should probably reflect that of at least one of these foods. Determinations of the physiological capabilities of the larvae include assays for digestive enzymes and pH of the digestive tract. Morphological studies of the larvae examine development of the sensory apparatus (*e.g.*, taste buds), alimentary canal (especially mucosal epithelium), liver and pancreas, using histological and histochemical methods.

Once the larvae ingest the diet, the digestion and assimilation of the food can be studied using histological methods, fluorescence methods, and radio-labelled compounds. Important information can often be obtained from a comparison of the digestion and assimilation of live and artificial foods, especially with regard to the relative location of these processes within the digestive tract. Further research is needed on the specific digestive functions of the rectal epithelial cells, which are apparently extremely important in larval fish digestion.

The multidisciplinary nature of this comprehensive evaluation demands that disparate groups (*e.g.*, chemical engineers, food scientists, physiologists and anatomists) communicate and cooperate with one another and with aquaculturists. Funding agencies and private companies should recognize the need for such interactive efforts and support them.

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FOOD INTAKE OF LARVAE OF *COREGONUS LAVARETUS* L. DO THEY REALLY INGEST LESS DRY DIET THAN *ARTEMIA* NAUPLII?

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Abstract

Intake of dry diet and *Artemia*-nauplii was determined at 12 and 16°C using larvae of *Coregonus lavaretus* L. of 11 to 25mm body length and 1.2 to 15mg body dry weigth, respectively. Samples of 5 to 20 larvae were taken 5, 10, 15, 25, and 40min after the beginning of feeding. From each larva the stomach/intestine was prepared. From the pooled samples of fish body without intestine and with intestine and its content, the dry weight was determined. All experiments were carried out in duplicate. In parallel the dry weight of intestines of fish which were deprived of food overnight was determined. Intestine content was calculated as the weight of intestine and its content minus the weight of empty intestine of fish of the same size.

In general, *Artemia* nauplii were eaten in a higher amount than dry diet. Satiation was reached within 15min, when dry diet was given and was not yet reached within 40min, when *Artemia* nauplii were fed. The amount of food eaten increased with increasing body weight. For a given fish size the intake of *Artemia* was significantly higher than the intake of dry diet. At 16°C the food intake was significantly higher than at 12°C.

A dry diet, which gave better growth results than the standard diet, was eaten in considerable higher amounts than the standard diet. However, the amount of diet eaten remained lower than the intake of *Artemia* nauplii.

Based on the results presented it is concluded, that among some other factors, the low amount of dry diet eaten by coregonid larvae is one of the main reasons for the lower growth rate of larvae fed on dry diet compared to larvae fed on living zooplankton/*Artemia* nauplii.

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THE MASS-REARING OF *COREGONUS LAVARETUS* L. LARVAE AT HIGH DENSITIES AND TWO REARING SCALES WITH TWO DRY DIETS

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Introduction

The decrease of production costs of 1 month pre-fed coregonid larvae is essential for hatcheries, especially for those involved in large productions (several millions) devoted to the stocking of lakes. In this applied perspective, improvements in growth and survival have already been obtained by continuous illumination and feeding (Rojas Beltran and Champigneulle, in press a). The aim of the present research was to confirm the feasibility of mass-rearing *Coregonus lavaretus* larvae at unusual high densities (> 200/l) with continuous feeding (two commercial dry diets compared, one of them being cheaper) as suggested by a preliminary study (Rojas Beltran and Champigneulle, in press b) at laboratory scale.

Materials and methods

In the spring of 1991, growth and survival trials were conducted at 10.5 ± 0.5°C in 6.5 and 55 l tanks in duplicate for each experimental treatment. For each kind of tank, three initial densities of *Coregonus lavaretus* larvae (mean total length: 11.6mm; wet weight = 5.5mg, Lake Bourget origin) were tested, namely 200, 400, and 800 ind./l. The tanks were continuously illuminated (150-350lux from direct fluorescent light) and the larvae fed with dry food (particle size: 0-250µm). Two diets described by Rojas Beltran and Champigneulle (in press b) were compared: diet 1 (Tetra Az 200 - size 000) and diet 2 (equal mixture of Aqualarve 1 and 2 from Aqualim). The tanks were cleaned daily and a Chloramine T bath (5 $mg.l^{-1}$) was given on three successive days per week.

Results at 35 days (Table I)

With diet 1, the lowest survivals (32.4-40.1%) occurred in large tanks (density 800 ind./l). In all other cases the survival varied little (58.1-72.7%) according to the scale of rearing and initial density. With diet 2 in small tanks the survival was high (> 90%) at 200 ind./l and 400 ind./l, a little lower at 800 ind./l and systematically better than survival with diet 1. At 400 ind./l and 800 ind./l with diet 2, the survival was depressed by about 30% when upscaling. In large tanks survivals were similar with diet 1 and 2 at 400 ind./l with

final densities of 200 to 300 ind./l. At 200 ind./l a significantly better survival (x 2) was observed with diet 2. On the contrary, at 800 ind./l the survival was a little higher (around 10%) with diet 1. With 800 ind./l the final densities in large tanks were very high, near 500 and 400 ind./l with diet 1 and 2, respectively.

With diet 1 the mean total length did not vary with initial density for a given rearing scale. For a given diet (1 or 2) and density, the mean length was greater in small tanks than in larger tanks. With diet 2 in small tanks, the mean length decreased with increasing initial density. In 55 l tanks the mean length was equivalent for 400 ind./l and 800 ind./l, both being significantly less than at 200 ind./l. In 6.5 l tanks for a given density, the mean total length was a little, but significantly greater with diet 2 than with diet 1. In 55 l tanks the mean length was greater at 200 ind./l with diet 2 but did not differ in other cases.

Table I. Comparative survival and size of *Coregonus lavaretus* larvae after 35 days of feeding at 10.5°C with two commercial dry diets in two kinds of tanks according to three initial densities

	Tank size					
	6.5 l			55 l		
Initial density	200 ind./l	400 ind./l	800 ind./l	200 ind./l	400 ind./l	800 ind./l
Survival (%)						
Diet 1	62.0 64.0	68.4 72.7	64.0 59.5	40.1 32.4	67.1 58.7	58.1 68.4
Diet 2	91.3 91.5	92.5 90.7	78.2 81.5	92.2 82.9	70.2 52.6	52.0 50.2
Length (mm)						
Diet 1	18.3(1.8)	18.5(1.9) 18.1(2.3)	18.2(2.1) 17.2(2.1)	17.0(2.3) 17.0(2.0)	16.3(2.2) 17.6(2.6)	17.0(2.0) 17.2(1.8)
Diet 2	19.7(1.8) 20.2(1.3)	19.1(1.7) 19.0(1.8)	18.8(2.1) 18.2(2.0)	19.0(1.6) 18.6(1.9)	17.1(2.0) 17.7(1.7)	17.0(1.9) 17.2(1.4)

Discussion

Until now the first rearing of coregonid larvae was generally carried out at densities of 200 ind./l (Champigneulle, 1988). The present experiment confirms recent preliminary results (Champigneulle and Rojas Beltran, 1990 ; Rojas Beltran and Champigneulle, in press b) suggesting the feasibility of practising higher initial densities (400-800 ind./l) but with a rearing period limited to 5 weeks at 10°C. The experiment also indicates that the effect of density seems to vary according to the diet and the rearing scale. Nevertheless an important practical result was that constant good growth (size 17-19mm) and survival

(50-70%) were obtained at the highest (400-800 ind./l) densities in the largest tanks with both diets. Champigneulle and Rojas Beltran (unpubl.) obtained very good survival (near 90% at 1 month) in industrial rearing (two $1m^3$ cylindroconical tanks) at a stocking density of 400 ind./l, and diet 2. Therefore it is hoped to drastically reduce the production costs of 1 month pre-fed larvae reared at 10-11°C. Before scaling up to an industrial level can be achieved with this technique, more large-scale experiments are needed to control the larval quality and to solve pathological problems.

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SURVIVAL AND GROWTH OF ROHU (*LABEO ROHITA*) AND SINGHI (*HETEROPNEUSTES FOSSILIS*) LARVAE FED ON DRY AND LIVE FOODS

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Introduction

The nearly dependence on live food by larvae of many cultured fishes during the early stages of exogenous feeding is a well established fact (Pillay, 1990), and constitutes a bottleneck in successful larval rearing. The ability to rear larvae exclusively on artificial diets has been achieved for only a few species (Dabrowski, 1984); for many others, it is desirable to know the earliest age or the weight, called 'adaptation weight' (Bryant and Matty, 1981) at which the larvae can be switched from natural live food to artificial diets without adversely affecting growth and survival. In this study we tried to establish the adaptation weight for the larvae of two economically important fishes, rohu (*Labeo rohita*) and singhi (*Heteropneustes fossilis*).

Materials and methods

Larvae of rohu and singhi, obtained by hatching fertilized eggs under controlled laboratory conditions, were maintained separately in large plastic tubs containing filtered tap water. Starting with day 3 (the earliest day of exogenous feeding), one batch of larvae of each species was fed exclusively artificial diets (a compounded diet of rice bran and mustard-oil cake for rohu, and rice bran and fish meal for singhi). Another batch was fed only natural live food (a mixture of rotifers, cladocerans and copepods, control-A), and a third batch was reared on a mixture of natural food and artificial feed (control-B). From the lot fed natural plankton exclusively, batches of 60 larvae were transferred at regular intervals to separate containers and their food switched to artificial diet. Nearly 80% of the water was changed twice daily in all the treatments and food was provided *ad libitum* at each change. With both natural live food and artificial diets, the mean food particle size was increased progressively during the test period to meet the changing optimal prey-size requirements of the growing larvae. The performance of the larvae in all the trials was assessed by recording mortalities and by measuring total lengths and dry weights at the time of switching to artificial diets, and at the termination of the experiment (21 days).

Results

For both rohu and singhi, the best survival and growth rates were obtained in larvae fed live plankton throughout (control-A, Fig. 1 and 2). Feeding exclusive artificial diets right from first-feeding on, resulted in poor survival (16%) in rohu and complete mortality in singhi at the end of the experiment. Switching the larvae to artificial diets at progressively later ages, improved survival and growth rates, but their final performance was significantly lower than those of the control group, even when switched after 9 to 10 days of initial feeding on live plankton food (Table I).

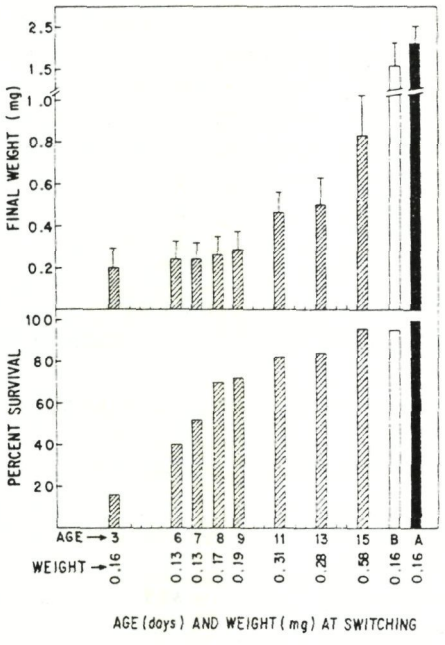

Fig. 1.Survival and growth of *Labeo rohita* larvae switched to artificial diet at different ages, compared to control A and B.

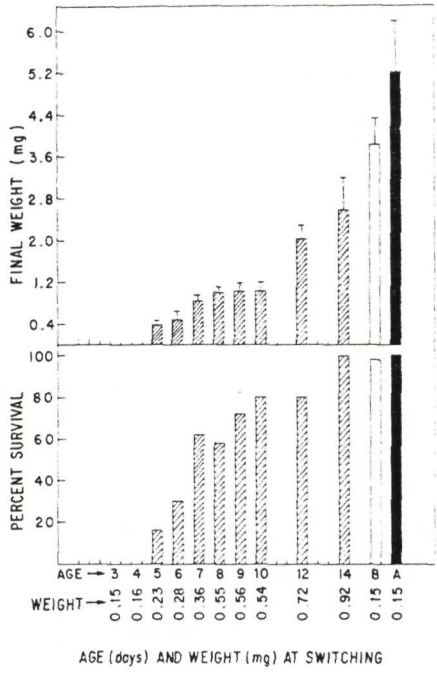

Fig. 2.Survival and growth of Heteropneustes fossilis larvae switched to artificial diet at different ages, compared to control A and B.

Table I. Survival and growth rates of fish larvae switched to artificial diets at early and later stages, compared to those shown by the control group at the end of the experiment

Fish species	Age at switching (days)	No. of days fed on live food	Performance relative to control A (%)		
			Survival	Total length	Dry weight
Labeo rohita	7	4	52	54.8	11.3
	13	10	84	65.1	23.5
Heteropneustes fossilis	7	4	62	56.8	16.1
	12	9	80	76.3	39.1

Discussion

The poor performance of both rohu and singhi larvae fed exclusively artificial diets from the start might be due to incomplete development of the alimentary canal and of the required digestive enzyme system in the early stages (Dabrowksi, 1986; Govoni *et al.* 1986). For either species, no sharply defined adaptation weight could be determined. Our earlier experiments showed that the larvae, when provided optimally-sized prey items at high concentrations, could reach these adaptation weights at a much earlier age. The satisfactory performance of control group B indicates that artificial diets could be used in the nursery ponds to supplement the natural planktonic food. Although we used ingredients traditionally used by fish seed farmers for preparing the artificial diets, the nutritional adequacy of the test diets is uncertain. Therefore it is quite possible that the survival and growth rates of dry-food-fed larvae could be substantially improved by using more scientifically formulated diets. However, the information available at present on the nutritional requirements of first-feeding larvae of these two species is scarce.

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COMPARISON OF LIVE FOOD ORGANISMS AND PREPARED DIETS AS FIRST FOOD FOR PADDLEFISH, *POLYODON SPATHULA*, FRY

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Introduction

Paddlefish, *Polyodon spathula* (Walbaum), are valued as a commercial fish for its roe and as a sport fish (Carlson and Bonislawsky, 1981). With the advent of successful spawning techniques, paddlefish are now reproduced in state and federal hatcheries. Pond-harvested live foods, such as cladocerans, are generally fed. However, collection of live food organisms is labor-intensive and unreliable. Brine shrimp nauplii, *Artemia* sp. are a convenient live food used in hatcheries to feed larvae of many species. However, variation in nutritional quality among different sources of brine shrimp create an increased need for a high quality prepared diet. The objective of this study was to evaluate growth, food consumption, and survival of newly-hatched paddlefish fry fed live *Daphnia*, two sources of brine shrimp nauplii, and three commercially prepared diets as the only food for paddlefish fry.

Materials and methods

Paddlefish fry (8-day-old) were hand-counted into each of 28, 7.5 l circular glass aquaria at a density of 80 fish/aquarium and fed one of six diets every 3h for 9 days. The diets were: live *Daphnia pulex*, Great Salt Lake (GSL) or China (CH) sources of brine shrimp nauplii, and three prepared diets, Biokyowa larval diet (Biokyowa, Inc., Cape Girardeau, Missouri, USA), Moore-Clark trout diet (Moore-Clark Co., Inc., Vancouver, BC, Canada), and Purina Trout Chow (Purina Mills, Inc., St. Louis, Missouri, USA).

Every 3 days, 10 fish were randomly sampled from each aquarium. Fish were examined under a dissecting microscope for the presence of food in the transparent digestive tract. The total length was measured to the nearest 0.10mm using a dissecting microscope and dial calipers. Each fish was blotted dry on paper towels and weighed to the nearest 1.0mg. Lipids of all diets were extracted as described by Kates (1986). Fatty acid methyl esters were analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a 30-m fused-silica capillary column and a flame-ionization detector.

Results

The fatty acid composition of the diets differed (Table I). The CH brine shrimp had a significantly higher percentage of palmitoleic acid 16:1n-7, than the other diets ($P<0.05$). The GSL brine shrimp contained a significantly higher percentage of linolenic acid, 18:3n-3, than other diets ($P<0.05$). Percentage of EPA, 20:5n-3, in all diets was greater than 1.81% of the diet except the GSL brine shrimp which had a significantly lower percentage (0.98%) of the diet as EPA ($P<0.05$). Fish fed live foods had significantly greater lengths and weights than fish fed nonliving diets after 17 days posthatch ($P<0.05$) (Table II).

Table I. Percentages (wt %) of selected fatty acids in the total lipids extracted from newly-hatched paddlefish fry and diets fed to fry[1]

Fatty acid	Diet[2]					
	MC	BK	Purina	GSL	CH	*Daphnia*
16:1n-7	4.8	3.4	10.7	3.0	16.7	6.7
18:3n-3	0.9	2.0	1.1	26.3	4.5	12.2
20:5n-3	8.1	11.6	11.6	3.3	12.4	10.3
22:6n-3	7.2	11.6	6.4	0.0	0.0	1.5

[1] Values are means of two replicates.

[2] Diets are: MC= Moore-Clark trout diet; BK= Biokyowa larval diet; Purina= Purina Trout Chow; GSL= Great Salt Lake source brine shrimp nauplii; CH= China source brine shrimp nauplii; *Daphnia*= *Daphnia pulex*.

Table II. Length, weight, specific growth rate (SGR), percentage survival and percentage of the fish consuming diets for paddlefish fry fed different living and nonliving diets[1]

Diet	Final indiv. length (mm)	Final indiv. weight (mg)	SGR	Survival (%)	Fish consuming diets (%)
Daphnia	27.5^{b}	153.4^{ab}	9.83^{a}	95.31^{a}	95.00^{a}
CH[2]	29.1^{a}	163.6^{a}	10.11^{a}	68.13^{bc}	94.17^{a}
GSL[3]	27.8^{ab}	138.6^{b}	9.36^{a}	80.00^{b}	93.33^{a}
Biokyowa	20.1^{c}	43.5^{c}	3.75^{b}	63.13^{cd}	86.67^{a}
Purina	20.0^{c}	48.5^{c}	4.28^{b}	48.44^{de}	86.09^{a}
Moore-Clark	19.5^{c}	43.4^{c}	3.72^{b}	40.63^{e}	79.00^{a}

[1] Values are means of four replicates. Means within a column with the same superscript are not significantly different ($P>0.05$).

[2] CH= China source brine shrimp nauplii.

[3] GSL= Great Salt Lake source brine shrimp nauplii.

Discussion

Growth of paddlefish fry fed prepared diets was less than that of fish fed live food organisms after 17 days posthatch indicating that prepared diets are not currently suitable for the initial feeding of paddlefish fry. Examination of the digestive tract revealed that many of the diet particles consumed did not appear to be completely digested prior to excretion. This may explain why paddlefish fed prepared diets had lower growth rates and survival than fish fed brine shrimp nauplii or *Daphnia*.

This study indicates that paddlefish fry may not require the highly unsaturated fatty acids EPA and DHA for at least 15 days posthatch. The similar growth rate of fish fed GSL brine shrimp (with a high percentage of linolenic acid and a low percentage of EPA) to fish fed CH brine shrimp (with a low percentage of linolenic acid and a high percentage of EPA) would indicate this. However, since paddlefish fry were fed for 9 days, adverse effects on growth rate may not have yet occurred. If fed for a longer time period, differences in growth might have developed since paddlefish fed CH brine shrimp nauplii had a higher individual fish weight than fish fed GSL brine shrimp nauplii after 9 days.

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THE GROWTH AND SURVIVAL OF PIKE-PERCH, *STIZOSTEDION LUCIOPERCA* L., LARVAE FED ON FORMULATED FEEDS

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Introduction

Pike-perch fry are reared in ponds with natural food supply for stocking purposes in many European countries. The production of pond-raised pike-perch fingerlings greatly increased in Finland during the 1980's. Becoming more familiar with pike-perch culture methods, Finnish fish-culturists have also become interested in the possibilities of intensive rearing of pike-perch in order to produce marketable size fish for human consumption. In Finland, state-owned hatcheries produce almost all pike-perch larvae. Intensive pike-perch rearing techniques are also used for producing broodstocks.

Some attempts to take pond-raised pike-perch fingerlings to intensive rearing have been and are still being made, but until now they have not been successful. Pike-perch fry accustomed to prey on live food, do not easily accept formulated feeds. Large-scale intensive rearing based on live food is probably becoming too expensive.

Intensive culture of pike-perch larvae and fry is possible with live food (Klein Breteler, 1989). If pike-perch larvae could be reared with formulated feeds it may hold promises for the intensive rearing of fry, juvenile and adult pike-perch.

Materials and methods

The experiment was made at the Evo State Fisheries and Aquaculture Research Station in southern Finland. Pike-perch larvae were reared in four square-shaped, grey coloured 200 l fiberglass tanks with an upwelling inlet and outflow with a 0.3mm mesh. Water from a small river (pH 6.4; colour 150mg $Pt.l^{-1}$) was filtered through a 50µm mesh, heated and aerated if necessary. The tanks have a continuous water flow of *circa* 2 l/min^{-1}. The water temperature varied between 16 and 20°C during the experiment. The tanks were exposed to continuous light with an intensity on the water surface of about 100lux.

Approximately 5 000, 4-day-old pike-perch larvae were put in each tank on June 18th. The density of larvae in the beginning of the experiment was approximately 25 ind./l.

Pike-perch larvae were given food from June 19th onwards. Three different commercial formulated feeds were used: Aquastart (Ewos), Alma Larval-Feed and Riken MF. The feeding was started with the finest grade (particle size <125µm). Belt-feeders running 30 min every hour were used to supply 2.5g feed to each tank every day.

Larvae in the fourth tank were fed with live zooplankton collected from fish ponds with a 50µm net and sieved through a 0.5mm mesh before it was offered twice a day to the larvae. Zooplankton consisted mainly of *Bosmina* sp. and rotifers which are typical food items for pike-perch larvae in ponds.

A sample of 20 larvae was taken from each of the tanks every day during a 6 day period beginning June 20 (day 1). Thereafter samples were taken on days 8, 11 and 18 from tanks in which enough larvae had survived. The length and oil globule diameter of the sampled larvae were measured and the occurrence of food in their guts was checked under a dissecting microscope.

Results and discussion

Of the formulated feeds Aquastart and Riken showed the best dispersibility estimated visually. Alma Larval-Feed did not disperse well and most of the feed never became suspended in the tank water. Percent of pike-perch larvae with food in their guts is shown in Fig. 1. Most larvae fed with Aquastart and zooplankton had food in their intestine during the entire experiment. The larvae fed with Riken feed started to feed later but from day 3 onwards, most of them had food in their guts. Most of the larvae did not accept Ama Larval-Feed. The growth of larvae (Fig. 2) was fastest in the tanks supplied with Aquastart and Riken feed.

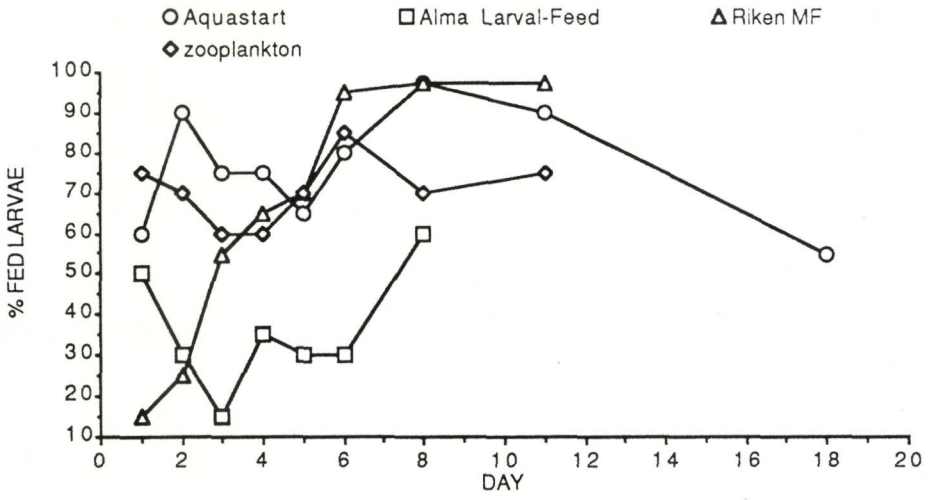

Fig. 1. Percent of pike-perch larvae with food in their guts.

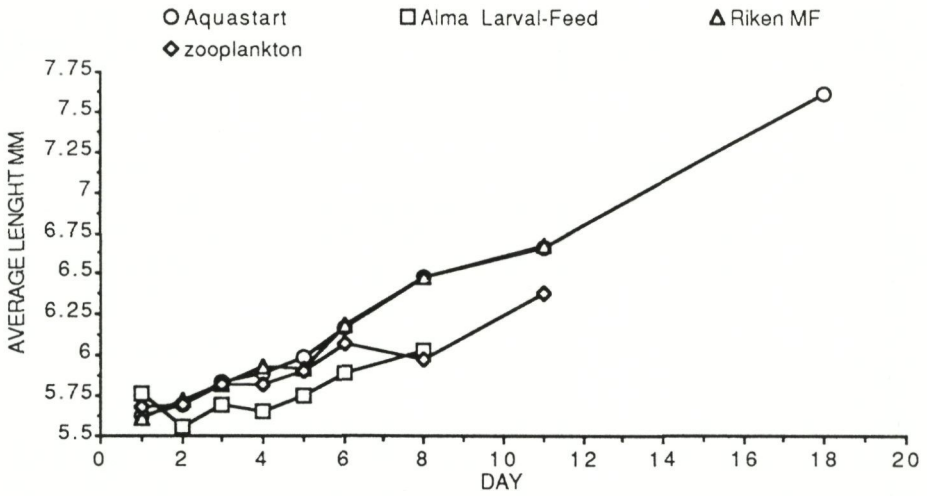

Fig. 2. Growth of pike-perch larvae.

The survival of the larvae was very poor. Larvae fed with Alma Larval-Feed all starved to death before day 10. Those fed with Aquastart and Riken feed survived better. The Riken tank accidentally ran out of water on day 14 and all the larvae died. In the Aquastart tank many larvae were dying with full guts. Some of the dead larvae showed lordosis which may indicate a lack of vitamin C in their diet. On day 16, the feed particle size of the Aquastart was raised to 125-250µm. This was done too early because most of the larvae were noticed to have empty guts and died after 2 days. The mortality of the larvae was also very high also in the control group fed with zooplankton.

This somewhat unlucky experiment showed that pike-perch larvae do accept formulated feeds. The growth and survival of the larvae were very poor compared to results obtained in intensive rearing with live food (Klein Breteler, 1989) or in pond culture.

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QUANTITATIVE FEED REQUIREMENTS OF GOLDFISH *CARASSIUS AURATUS* LARVAE FED WITH A MIXED DIET

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Introduction

Spawning of goldfish *Carassius auratus* can be achieved throughout the year by stocking the breeders under controlled temperature and photoperiod conditions (Gillet *et al.*, 1978; Kestemont *et al.*, 1990). Intensive larval rearing was carried out successfully with a mixed diet combining *Artemia* nauplii and dry food during the first weeks (Kestemont *et al.*, 1990). Considering the rapid increase in larval body weight and the decrease in specific growth rate, the aim of this study was to determine the quantitative requirements of goldfish larvae, according to their size and to the rearing temperature.

Materials and methods

The experiments were carried out in recirculating systems comprising special rearing tanks (volume 25 l) adapted from the INRA system (Charlton and Bergot, 1984), and a sedimentation and biofiltration tank (volume = 170 l each). Each rearing tank was equipped with an automatic dry food dispenser and with a PVC pipe (internal section = 1.5mm) connected to a peristaltic pump for the live food supply. Larvae with an initial body weight (W_i) of 1.15mg and 20.5mg (experiment 1 and 2, respectively) were fed with an increasing daily food ration at three temperatures (20, 24, 28°C). In experiment 1, 1 250 larvae (50 ind./l), were divided in 3x12 groups (three temperatures, five feeding levels, two replicates). In experiment 2, 625 larvae (25 ind./l) were divided in in 3x10 groups (three temperatures, five feeding levels, two replicates). Feeding levels were used to assess the optimal and maximal daily rations of each weight group. Food (*Artemia* nauplii, Ewos larvstart C20; on a dry weight basis) was dispensed automatically for 15min at 1h intervals during the daylight period (0600-2000h). The daily food ration was adjusted after respectively 4 and 5 days in experiment 1 (7 days) and experiment 2 (9 days) according to the weight of 50 larvae per tank. The survival rate was checked daily by removing the dead larvae from each rearing tank. Growth and food utilization were calculated as follows:

- specific growth rate (SGR,%) = 100 (ln W_2 - ln W_1)/Δt
- Gross or apparent food conversion ratio (FCR) = WdF/(W_2 - W_1)
- Gross or apparent protein efficiency ration (PER) = (W_2 - W_1)/$W_{prot.F}$
- Apparent net protein utilization (NPU,%) = 100($_{prot.2}$ - $W_{prot.1}$)/$W_{prot.F}$
  where $W_{1,2}$ = initial and final weight of live fish (mg), t = time (days), WdF = dry weight of dispensed food per fish (mg), $W_{prot.1,2}$ = initial and final weight of fish protein (mg), $W_{prot.F}$ = weight of protein in the dispensed food ration (mg).

Results and discussion

Survival of goldfish was generally high, except in the groups fed with a daily ration lower than the maintenance level (2.5 and 5% in experiment 1). In these groups, cannibalism was important. This phenomenon, stressed by the growth heterogeneity of the larvae (especially in the underfed groups) has been previously reported in carp by Appelbaum *et al.* (1986). Regardless of the feeding level, the best survival was obtained at 24°C (Table I). As shown in Fig. 1, the specific growth rate was largely influenced by the initial body size of the larvae, the temperature and feeding level. This was particulary clear in experiment 1, in which the larvae grew twice as fast at 28°C than at 20°C. The optimal feeding level, which corresponds to the lowest food conversion ratio, decreased markedly as the larvae grew, ranging from 20 to 35% in experiment 1, and from 2 to 4% in experiment 2, respectively at 20 and 28°C. Verreth and Den Bieman (1987) reported that in *Clarias gariepinus* larvae, the highest PER and NPU were obtained at the lower feeding level (equal or slightly higher than the maintenance level) regardless of temperature.

Table I. Effects of temperature on survival, growth and food utilization of *Carassius auratus* larvae (W_i=1.15 and 20.5mg) fed with mixed diet at the maximal feeding level

Parameters	W_i=1.15mg			W_i=20.5mg		
Temperature (°C)	20	24	28	20	24	28
Maximal feeding level (% $W_i.d^{-1}$)	35	50	70	30	40	40
Final body weight (mg)	3.03	6.02	9.03	111.0	138.3	205.5
Survival (%)	99.4	98.1	95.4	99.7	98.5	88.5
Specific growth rate (%$W.d^{-1}$)	16.1	27.6	34.3	16.9	19.1	23.1
Food conversion ratio	1.31	0.99	1.19	1.45	1.34	0.97
Protein efficiency ratio	1.39	1.85	1.54	1.26	1.36	1.88
Apparent net protein utilization (%)	13.6	19.6	15.4	16.9	17.3	25.5

W_i= initial body weight.

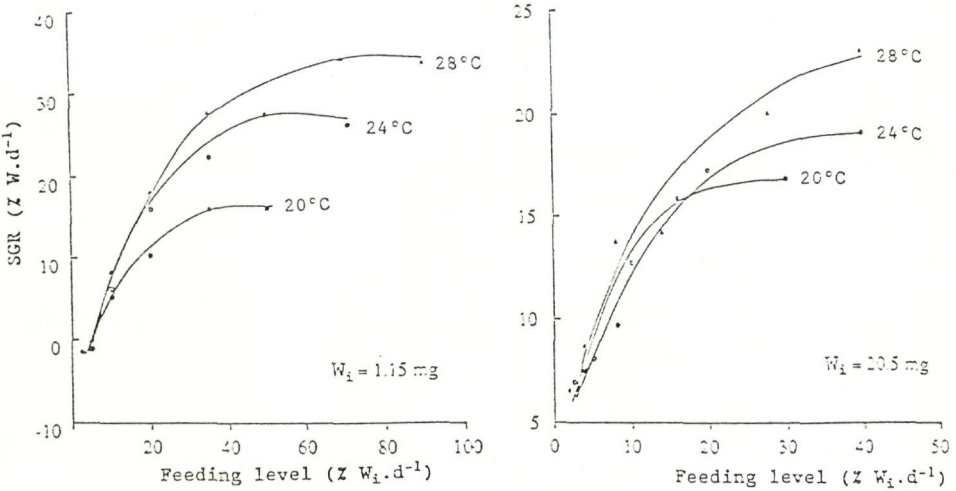

Fig. 1. Effects of temperature and feeding level (%$W_i.d^{-1}$) on specific growth rate of *Carassius auratus* larvae (W_i=1.15 and 20.5mg) fed with a mixed diet.

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THE EFFECT OF DIETARY SALT (NaCl) SUPPLEMENTATION ON THE GROWTH, SURVIVAL AND FOOD CONVERSION RATE OF AFRICAN CATFISH (*CLARIAS GARIEPINUS* B.) LARVAE

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Introduction

It has been suggested that ingestion of large quantities of inorganic ions may affect ionic and osmotic regulation of fish (Zaugg and McLain, 1969). Little effort has been made to quantify the relative importance of dietary sources of sodium and chloride in freshwater fish (Mackay, 1979). Nevertheless, freshwater fish remain dependent on an adequate supply of minerals as there is a continuous efflux of ions from the body (Cowey and Sargent, 1979).

The present experiment was designed to study the nutritional effects of dietary sodium chloride on growth, food conversion and survival of larvae of the warmwater sharptooth African catfish, *Clarias gariepinus*.

Materials and methods

Larvae were obtained by artificial reproduction of *Clarias gariepinus*. Four days after hatching they were fed decapsulated *Artemia* cysts for 1 day.

A total of 16 aquaria of 40 l capacity each were used in a recirculation culture system. Randomly 300, 5-day-old larvae were sampled and placed in each aquarium at a stocking density of 12 larvae/l. Mean weight and mean length of larvae at the starting were 2.74±0.52mg and 6.90±0.42mm respectively.

From a basic diet (containing 10% moisture, 11% ash, 2% cellulose, 55% protein, 13% fat and 0.5% NaCl) eight diets were prepared including different levels of additional NaCl supplementation: 0.0% (reference diet), 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, and 1.4%. The daily ration, calculated as a percentage dry weight of feed per wet weight of larvae, was adapted daily for each treatment according to the growth rate of the previous days. Randomly 20 larvae were sampled from each aquarium every 4 days for weight and length measurements.

The tap water entering the culture system contained 19.81mg.l^{-1} Na^+, 130.00mg.l^{-1} Ca^{++}, 40.69 mg.l^{-1} Cl^-, 2.75mg.l^{-1} K^+, 713.70mg.l^{-1} Si, 0.005mg.l^{-1} NH_4^+ and NO_2^-, 42.41mg.l^{-1} NO_3^-; the pH was 7.45 and the water temperature was maintained at 27±1°C.

Each treatment was run in duplicate for 16 days and the choice of the aquaria was made at random.

Results

At the end of the experiment the 21-day-old larvae fed on diets supplemented with 0.4% and 0.6% dietary NaCl (0.9% and 1.1% total NaCl) gained significantly higher mean weight than the control group fed on a non supplemented reference diet (Table I). Groups fed diets supplemented with more than 1.0% NaCl gained significantly lower mean weight than the groups fed diets supplemented with 0.4% and 0.6% NaCl. The mean weight of the larvae fed the diet supplemented with 1.4% was no significantly lower than the group fed the reference diet.

The best food conversion (1.65) was obtained with the 0.6% supplemented diet. An NaCl supplementation of more than 0.6% increased the food conversion rate up to 3.12 in the group fed a diet supplemented with 1.4% NaCl. The conversion for the reference diet was 2.55.

Larvae fed the diet supplemented with 0.6% salt had the highest specific growth rate (13.27%.d^{-1}) and larvae fed the 1.4% supplemented diet had the lowest one (9.93%.d^{-1}). The specific growth rate of the larvae fed the reference diet was 10.86%.d^{-1} (Fig. 1). Mortality and cannibalism were rather high in all the groups. The highest survival rate was recorded in the group fed a 0.6% NaCl supplement.

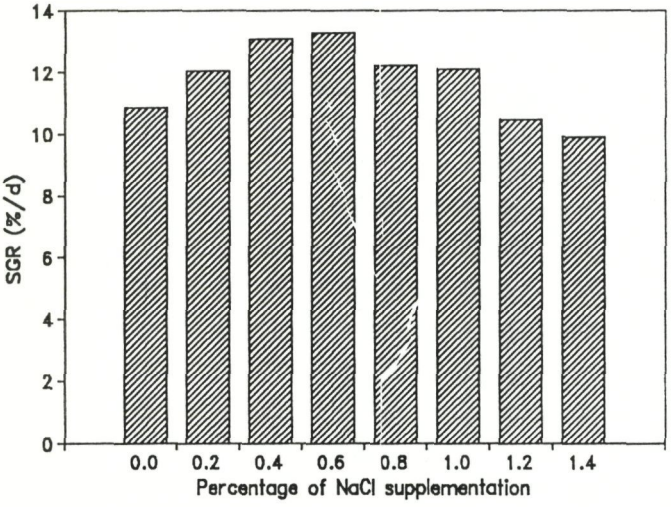

Fig. 1. Specific growth rate (SGR) of *Clarias gariepinus* larvae fed on different NaCl supplemented diets during the experiment.

Table I. Mean weight (mg) and length (mm) at day 21, food conversion (FC), survival (%) and cannibalism of *Clarias gariepinus* larvae during the experiment.

	Diet supplemented with different NaCl levels							
	0.0%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%
Weight* at day 21 (n=40)	15.57±1.50^{cd}	18.76±1.63^{abc}	22.14±1.91^{ab}	22.90±1.43^{a}	19.40±1.44^{abc}	19.00±1.67^{abc}	14.64±1.19^{de}	13.41±0.91^{de}
Length* at day 21 (n=40)	12.07±0.50	12.87±0.33	12.85±0.34	12.95±0.24	12.42±0.30	12.50±0.29	11.57±0.24	11.67±0.25
FC	2.55	2.06	1.73	1.65	2.02	2.04	2.67	3.12
Survival (%)	37.33	40.33	45.50	50.67	50.17	37.67	35.67	35.83
Cannibalism	25.00	22.17	14.33	20.00	14.83	27.00	28.83	32.50

^{abcd} Values within the same row not sharing a common superscript are significantly different ($P<0.05$).

* Mean ± SE.

Disscusion

In the experiment the highest mean weight (22.90mg) and highest specific growth rate (13.27%.d^{-1}) were recorded in the group fed a diet supplemented with 0.6% NaCl. As other freshwater species, African catfish is dependent on an adequate mineral supply from outside. The positive effect on the osmoregulatory system of these young larvae due to the supplementation with ions such as Na^+ and Cl^- present in the diet could be the reason for better growth.

Suppiementation of NaCl with more than 0.6% decreased the mean weight and overall specific growth rate. The energy demands for salt excretion might be increased by too high salt loading and the digestion or assimilation process in the alimentary tract might be negatively affected. A better knowledge of the osmotic regulatory system of *C. gariepinus* larvae could provide more information to explain these results.

Conclusions

In view of the present findings, it could be concluded that an increase in the concentration of dietary NaCl in dry feed has a positive effect on the growth and food conversion of African catfish larvae. The optimum concentration in the experiment was about 1.1% NaCl.

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GROWTH AND SURVIVAL OF FIRST-FEEDING ARCTIC CHAR, *SALVELINUS ALPINUS* FED A DRY DIET SUPPLEMENTED WITH *ARTEMIA*

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Introduction

Since Finnish fish farming is concentrated at present almost totally on rainbow trout, a number of investigations have been conducted during the last few years to find other potential species for intensive fish culture. The Arctic char, *Salvelinus alpinus*, is one of these species. The present experiment was carried out to evaluate the suitability of two commercial dry diets and the effect of *Artemia* on the growth and survival of first-feeding Arctic char.

Materials and methods

A 35 day-long growth trial was carried out at The Laukaa Aquaculture Research Station during the period May 8-June 12, 1989. A total of 300 yolk sac Arctic char fry of the Lake Inari strain (initial ww 55.2mg) were placed in nine 40 l plastic tanks. Water was heated to 12±0.8°C, filtered and aerated before entry into each tank at a flow rate of 0.6 $l.min^{-1}$. The fry (145 day-degrees old) were fed for $10h.d^{-1}$ on two commercial dry diets (Tess 0.3 and Aqua Start 0) or on these diets with 1/3 of the weight replaced with decapsulated *Artemia* cysts (Black Sea Kara-Bakas-Gol Bay strain) using belt feeders with an approximate ration of $10\%.d^{-1}$. Duplicate groups and one fasting group were included. The tanks were cleaned daily and dead fry were removed and counted. Every 5 days 10 or 20 fish were weighed (ww, dw), measured (TL), the presence of food in the stomach was analyzed visually and yolk conversion efficiency was calculated for the first 10 days (increase in body dw/ decrease in yolk dw x 100). Samples were taken for protein, fat and energy measurements at the beginning and end of the experiment.

Results

The results did not differ statistically significantly (S-N-K test) between the duplicate tanks and thus these were combined throughout. The lowest mortality was found in the groups fed on a dry diet supplemented with *Artemia* (5.3 and 7.0%) and the highest in the groups fed with dry diets only (10.5 and 27.7%) (Fig. 1). There were no statistical differences between the groups in terms of growth variables at the end of the experiment

(Table I) but the protein content of the fish was higher with *Artemia* supplementation and the fat content lower. *Artemia* supplementation also increased the yolk conversion efficiency and appeared to reduce the coefficient of variation in length.

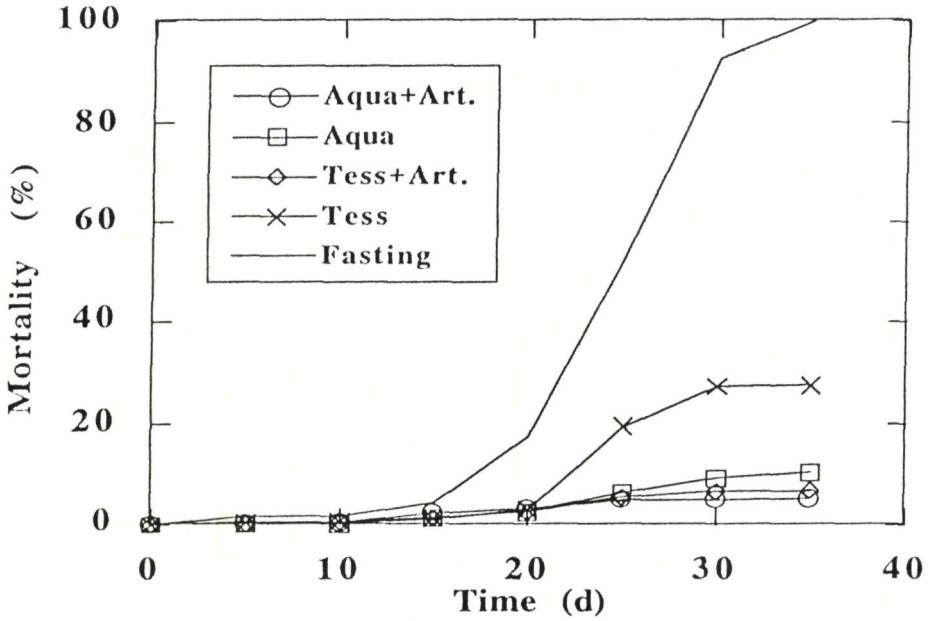

Fig. 1. Mortality during the experiment. Aqua=Aqua Start, Art=*Artemia*.

Table I. Wet weight (ww, mg) dry weight (dw, mg), total length (mm) and protein and fat content (%/ww) at the end of the experiment and yolk conversion efficiency (yolk ce, %) during the first 10 days of the experiment. Coefficient of variation (CV), Aqua = Aqua Start.

	Aqua + *Artemia*		Aqua Start		Tess + *Artemia*		Tess	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
ww	354.8	19.3	320.3	36.2	308.3	31.8	358.9	27.8
dw	61.5	14.8	56.6	21.5	53.9	37.2	63.4	30.8
Length	36.0	5.7	34.7	11.6	34.0	10.1	35.6	10.5
Protein	12.3		12.0		12.0		11.3	
Fat	3.6		3.9		2.6		3.2	
Yolk ce	381.1		169.2		135.0		67.4	

Discussion

Mortality was low with these high quality feeds, even though the temperature in this experiment was higher than the optimum defined for the Hammerfest strain of Arctic char by Wallace and Aasjord (1984). In this experiment a dry feed supplemented with *Artemia* did not seem to enhance growth, but even reduced the mean weight of the fish due to the higher survival rate among the smallest individuals (especially Tess groups). The specific growth rates achieved here (4.9-5.4%.d^{-1}) were lower than that predicted for 0.3g Arctic char (9.9%.d^{-1}) in the growth model of Jobling (1983). The difference in growth potential between the strains is probably the reason for this lower growth. The improved survival of the fry with *Artemia* cyst supplementation agrees with the observations of Drouin *et al.* (1986) that first-feeding whitefish (*Coregonus clupeaformis*) survived almost as well with trout starter supplemented with *Artemia* cysts as with live *Artemia* only. The observations on the presence of food in the stomach suggest that the groups with *Artemia* supplementation learned to eat the food provided earlier than did those fed on dry food only. This may be the main reason for their higher yolk conversion efficiency. Not only the higher nutritional quality but also the better acceptance of *Artemia* than dry feed seemed to increase survival of the fish during the first-feeding period.

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PALATABILITY ENHANCING NUTRIENTS FOR THE EUROPEAN EEL, *ANGUILLA ANGUILLA* L.

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Abstract

Feeding experiments with European glass eels *Anguilla anguilla* L., demonstrated a high degree of attractiveness for the natural diets Tubifix and cod roe. Previous experiments had indicated that incorporation of bovine spleen and/or blood into paste feeds significantly improved the growth rate and survival.

To increase feed consumption, the effect of some basic components available from the current generation of chemo-attractants were evaluated. Using a modified Omission Test Procedure, we focused on monosodiumglutamate, betaine, the nucleotides IMP and GMP, and fractions of cattle blood. These components were incorporated into non-attractive casein-based test diets and administered following standardised procedures to intensively cultured fingerling populations.

These dose-response experiments revealed that Na-glutamate, betaine, and the nucleotides IMP and GMP elicit feeding behaviour when administered separately. Only erythrocytes and the cell content of bovine blood induce an intensified feeding response. The effect of haemoglobin or organically-bound iron as attractant for eel is presently under investigation.

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FEEDING OF MARINE FISH LARVAE: MICRODIETS OR LIVE PREYS?

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Abstract

Since marine fish farming is expected to grow rapidly over the next 5 years, particularly in Europe, any diet that can reduce dependence on live prey production is of great interest. The purpose of this paper is to demonstrate that early weaning of marine fish larvae (seabass, bream, Japanese flounder, *etc.*) is technically feasible using efficient microdiets. Examples of successful results, obtained in our laboratory are given, in which different microparticulate diets (MD) of the microbounded (MBD) or the microcoated (MCD) type were used for the weaning of seabass (*Dicentrarchus labrax*) larvae.

First consistent results were obtained with alginate MBD made from raw materials in 20 to 23-day-old larvae (the usual weaning age is 35 to 45 days). In comparison to a live prey control, no difference in survival but a retardation in growth (up to 30% weight loss) and a decrease in juvenile quality were generally observed. In a second step, it was shown that MCD, made from high quality freeze-dried materials, can also be efficiently used in diets for the weaning of seabass larvae. MCD have the additional advantage that they represent some improvement in food processing. To reduce live prey utilization in seabass rearing, MD can be used either as *Artemia* substitute after a short live prey feeding or as a live prey supplement from first feeding onwards. These different feeding strategies are compared in terms of cost effectiveness.

In a second part, the reasons why MD are generally not as efficient as live prey for the very early weaning of stomachless marine fish larvae are discussed. MD tends to have a relative low acceptability and a poor digestibility. Differences between physical properties and chemical composition (HUFA and amino acid profile, vitamin content) may partly explain problems of digestion and absorption of formulated diets by larvae with a digestive sytem which is not yet completely developed.

Finally, some practical recommendations are given: 1) to make rearing techniques suitable for early weaning with respect to food availability, larvae behavior and water quality; 2) to improve MD attractiveness and stimulate larvae feeding behavior; and 3) to formulate MD well adapted to larvae in terms of digestibility and nutritional balance.

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RATES OF INGESTION AND DIGESTIBILITY AS LIMITING FACTORS IN THE SUCCESSFUL USE OF MICRODIETS IN *SPARUS AURATA* LARVAL REARING

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Introduction

Marine larvae reared exclusively on microdiets do not match the growth and survival performance of larvae fed live food organisms (Kanazawa *et al.*, 1982). Poor performance of microdiets may result from an incompletely developed digestive tract in the early stages of larval growth, with the resulting low digestive enzyme activity (Dabrowski, 1984). It has been suggested that larvae utilize the exogenous enzymes of the live prey they consume as precursors which activate zymogens in their gut to help complete the digestion process (Jancaric, 1964). The aim of this paper is to show the role of live food in improving the availability of microdiets to young gilthead seabream (*Sparus aurata*) larvae. In addition, we recently tested the direct involvement of added digestive enzymes to microdiets on the diet's digestibility and ingestion in the absence of live food.

Materials and methods

In the first experiment, live food was given in combination with a microdiet to seabream larvae reared in 400 l conical rearing tanks. The experiments were limited to 15 days (8-22 days past hatching). Three levels of rotifers were given representing 20, 50 and 100% of the concentration of live food normally offered (3, 7.5 and 15 rotifers/ml) to seabream larvae. The rotifers were offered in addition to $8g.d^{-1}$ of an NCM 50.2% protein microdiet.

The aim of the second set of experiments was to develop a ^{14}C labelled microdiet supplemented with 0.05% porcine digestive enzymes (Pancreatine, Sigma). The labelled diets made it possible to measure the microdiet intake and the effect of supplemented digestive enzymes on microdiet digestibility (protein, polar and neutral lipids). This experiment was carried out in 600ml containers in a flow through seawater system, for 36h.

Results and discussion

The use of a microdiet in the absence of live food with gilthead seabream supported reduced growth and survival as compared to live food controls (15 rotifers/ml). This suggested the following alternatives: 1) microdiet ingestion is low and/or, 2) larvae are unable to digest the ingested microdiet as a result of an incomplete digestive tract. In the following experiments we tested the ability of the larvae to make better use of the microdiet in the presence of live food. In accord with the findings for larval *Pagrus major* (Kanazawa *et al.*, 1982) we found that as concentration of rotifers, fed in combination with a microdiet, increased from 0 to 15 rotifers/ml, the 22-day-old gilthead seabream larval survival increased ($P<0.05$) from 6.3 to 34.6%. The use of a microdiet in combination with 20% (3 rotifers/ml) of the live food ration supported a growth similar to the control fed a 100% live food ration (15 rotifers/ml).

The use of a ^{14}C labelled and digestive enzyme supplemented microdiet proved that 20-day-old *Sparus aurata* larvae ingest the microdiet at a rate equal to the maintenance intake or one tenth of the intake when live feed is offered ($P<0.05$; Fig. 1). This explains the reduced larval growth and survival when fed only a microdiet. In addition, it suggests that a major obstacle to the use of microdiets in larvae is their reduced attractability.

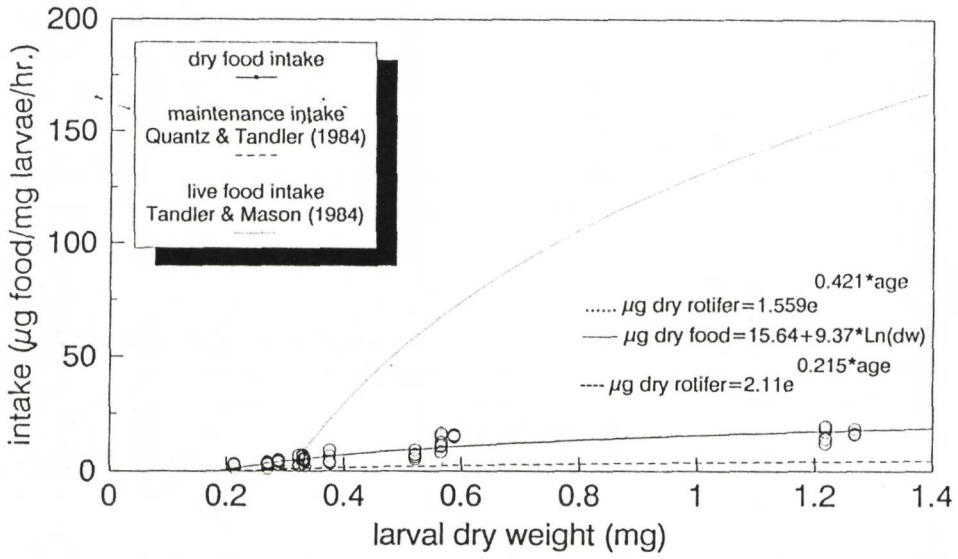

Fig. 1. Rate of intake of dry and live food in larval *Sparus aurata*.

The results also prove that supplementation of the diet with digestive enzymes improves ($P<0.05$) their digestibility by about 30%, independent of age. Moreover, they show an effect ($P<0.05$) of enzyme supplementation on protein assimilation but not on polar or neutral lipids. This was independent of larval age between 20 and 40 days after hatching (Fig. 2).

In conclusion: for better performance of microdiets in *Sparus aurata* one should consider the reduced larval digestive capability in developing larval microdiets. Improved attraction of the diet is also paramount for an increased feed intake. A combined feeding regime of microdiets with live food is recommended until more attractive and digestible diets are available.

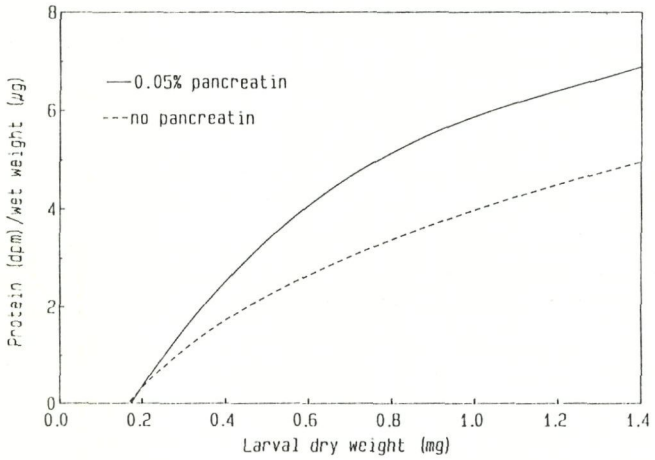

Fig. 2. Dietary enzyme effect on protein digestibility.

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LARVAL FISH NUTRITION AND FEEDING IN CLOSED, RECIRCULATION CULTURE SYSTEMS

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Abstract

Large scale production of red drum and other marine fish are currently restrained by the lack of controlled culture systems and a diet that is nutritionally adequate and readily available. Recirculating systems in which physical and chemical factors can be controlled hold the potential to diminish these limits to present yields. In order to progress in closed system production, the development of diets appropriate to each life stage is necessary. Major emphasis is now focused on determining essential nutrient requirements for larval fish and the best means for supplying those nutrients. We have developed scaled-up test tanks with a closed recirculating design to evaluate nutrients and physical dynamics of artificial diets. Early results demonstrate that larval red drum (*Sciaenops ocellatus*) require small, slightly negatively buoyant particles with a pH of 8.5, supplied at a density of five particles/ml for the first 10 days.

Our study of the digestive system shows the larval gut to be quite simple at first feeding. Development is slow, with a fully functioning stomach not present until the juvenile stage. Despite the primitive characteristics of marine larvae they require sufficient food to increase their weight one thousand fold during the larval period.

A feeding protocol was developed for red drum larvae based on a combination of live prey (rotifers) and a commercial, formulated diet (Kyowa Fry Feed) in closed, recirculation culture systems . Growth and survival were measured on larvae reared on a combination of live and formulated diets for 1-5 days and then formulated diet alone. The most satisfactory combination was feeding live food and formulated food together for the first 5 days and then completely discontinuing live prey, thus eliminating the need to feed *Artemia* to the larvae. Growth rates were as good as larvae reared on live prey with metamorphosis to the juvenile stage occurring at less than 1 month. Survival rates were a remarkable 60% from egg to the juvenile stage. Further work has led to the development of a semi-purified diet that will permit nutrient testing on larvae as young as 8 days. This will provide the framework for customizing the diet for particular species and rearing systems.

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EVALUATION OF MICROCAPSULE PERFORMANCE ON DELIVERING DIETS TO SILVERSIDE (*MENIDIA BERYLLINA*) LARVAE

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Introduction

Live foods typically produce better growth and/or survival of marine fish larvae than do inert diets. Early attempts to develop microencapsulated artificial diets for *Menidia beryllina* larvae gave poor results; therefor the artificial diet was discarded and a study of microencapsulated *Artemia* was begun (Leibovitz *et al.*, 1987). In the present study, a new microcapsule was developed and evaluated for leaching, buoyancy, and ability to support survival and growth of *M. beryllina* larvae.

Materials and methods

After preliminary trials with five microcapsule-wall-forming solutions and seven wall-hardening solutions, an albumen-alginate mixture was chosen as the microencapsulation material and a calcium chloride-ethanol-tannic acid-water mixture was chosen as the hardener. A spraying apparatus was used to produce a fine spray of droplets containing *Artemia* nauplii and wall mixture that could be directed into a vat of hardening solution.

Proximate analyses were conducted on newly-hatched *Artemia* nauplii (Reference *Artemia* II strain) and on freeze-dried microencapsulated nauplii, the latter both before and after 2h immersion in seawater. Fatty acid analyses were conducted on newly-hatched nauplii and on freeze-dried microencapsulated nauplii stored at -20°C for 60 days. The sedimentation rate was determined by placing 20mg of microcapsules in a 100ml graduated cilinder containing 30ppt seawater and counting particles on the surface, in the water column and on the bottom every 30min for 8h.

Four feeding trials (I-IV) were conducted for 14 days with larvae ranging in age from 7 to 12-days-old (initial weights in mg: I- 3.36; II- 1.17; III- 1.90; IV-0.40) in the same manner as those described by Leibovitz *et al.* (1987). Trials I, II and III compared survival and growth of larvae fed freeze-dried microencapsulated *Artemia* with those of larvae fed live nauplii, but in Trial III a separate treatment measured survival and growth of larvae fed refrigerated, fresh (*i.e.* not freeze-dried) microencapsulated nauplii. Trial IV was conducted to determine whether the frequency with which the microcapsule diet

was presented (2x, 4x, or 8x per day, but constant daily ration) affected survival and growth.

Results and discussion

Proximate analyses indicated that the compositions of live nauplii, microencapsulated nauplii, and 2h immersed microencapsulated nauplii were essentially identical, indicating that no components were preferentially leached out of the capsule. Fatty acid analyses indicated that freeze-dried microencapsulated nauplii after 60 days had the same fatty acid profile as newly-hatched nauplii. Sedimentation analyses showed that the capsules spent the first 2 to 3h at the surface, then sank relatively quickly and were mostly on the bottom after 6 to 8h.

Larval *M. beryllina* survived as well on the microencapsulated *Artemia* as they did on the live *Artemia* nauplii in all trials (Table I), but growth was always significantly poorer with the microcapsules. There were no significant differences in growth of fish fed microcapsules containing fresh *versus* freeze-dried nauplii, nor in growth of fish fed 2x, 4x, or 8x per day.

Table I. Survival (S) (%) and growth (G) (mg) of *Menidia beryllina* larvae fed live *Artemia* nauplii (LIVE), microencapsulated freeze-dried *Artemia* nauplii (MA-FD), microencapsulated fresh *Artemia* nauplii (MA-FR), or MA-FD several times per day (2x, 4x, or 8x) in four feeding trials (I-IV). Treatments within a column that are followed by different letters are significantly different

Treatment	I		II		III		IV	
	S	G	S	G	S	G	S	G
LIVE	100	46.2a	80	18.0a	100	21.7a	70	5.9a
MA-FD	98	12.5b	80	6.3b	92	8.2b		
MA-FR					88	9.5b		
MA-FD-2x							85	3.5b
MA-FD-4x							98	3.8b
MA-FD-8x							85	3.7b

The results suggest that the microencapsulated *Artemia* were biochemically similar to live nauplii and were sufficiently available to the larvae in the water column throughout the day (2x-4x-8x experiment). Larvae in Trial IV increased their weight an 8-fold during the experiment. We are therefore encouraged that the albumen-alginate capsule represents an improvement over the previous capsule.

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MICROBOUND LARVAL FEED AS SUPPLEMENT TO LIVE FOOD FOR MILKFISH (*CHANOS CHANOS* FORSSKAL) LARVAE

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Introduction

Highly variable results using *Brachionus* as food for milkfish larvae may be related to: (1) relatively low content of highly unsaturated fatty acids (HUFAs) in *Brachionus* (Villegas *et al.*, 1990) and (2) inability of first feeding larvae (day 2) to ingest rotifers in sufficient numbers. Early supplemental feeding with a microbound diet high in HUFAs may improve growth and survival of milkfish larvae.

Materials and methods

Milkfish larvae (30 larvae/l) were reared in 250 l fiberglass tanks under ambient seawater salinity (32-34ppt) and temperature (28-30°C) and subjected to the various feeding regimes. Day 2 to day 14: artificial feed (AF) (Nosan Kogyo Co. Ltd, particle size 50µm), AF + *Brachionus* (AF+Br) or *Brachionus* (Br); day 15 to day 20: all tanks were provided with *Brachionus* only, *Artemia* nauplii (3 to 4 ind./ml) were added beginning day 18. AF was fed at 100 particles/ml (350mg/tank) twice daily and *Brachionus* was maintained at 10 to 15 ind./ml.

Results and discussion

Milkfish larvae fed AF+Br were significantly larger than those fed AF or Br on day 13 and were significantly larger than Br-fed larvae on day 20 (Fig. 1). The mean wet weight of AF+Br larvae (6.47mg±0.72) was also significantly higher than that Br-fed larvae (3.88mg±0.27) on day 20. Mass mortalities occurred in AF tanks on day 14. Survival (9%) of AF+Br-fed larvae was, however, lower than Br-fed larvae (17.8%)..

A second experiment also showed better growth of larvae fed AF from first feeding on (day 2) or given 2 days before the rotifer. The survival of larvae fed AF+Br in this experiment was also enhanced. A test run in 3 000 l circular concrete tanks similarly showed higher survival of larvae initially reared on AF+Br (33%) compared to those reared on *Brachionus* only (6.67%).

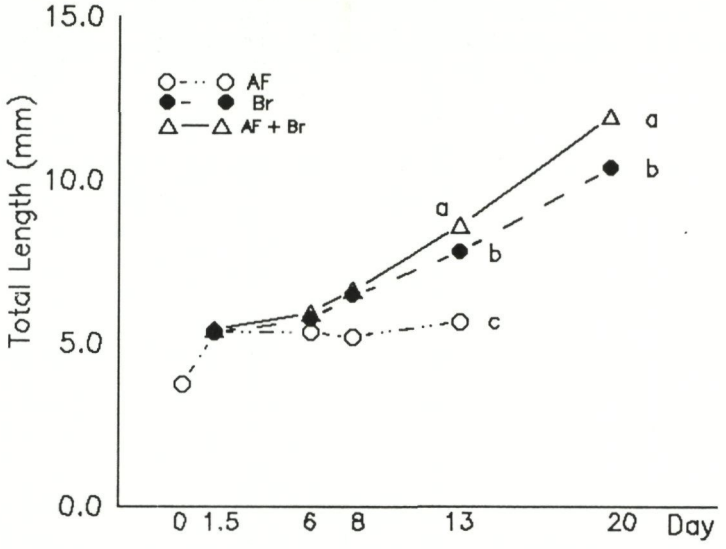

Fig. 1. Total length of milkfish larvae reared on artificial feed (AF), in combination with *Brachionus* (AF+Br), or *Brachionus* (Br) sampled at various ages.

Supplementation with microbound larval feed (AF) during the first half of the rearing period appears to have enhanced growth and survival of milkfish larvae and may have been due to increased (n-3) HUFA levels in the feed or rotifer. Dietary enrichment of rotifers and *Artemia* nauplii with microencapsulated feeds or emulsions with high HUFA levels have been reported to improve growth in seabream larvae (Koven *et al.*, 1990) and survival rates of seabass larvae reared on nutritionally deficient *Artemia* (Dhert *et al.*, 1990). The *Chlorella*-fed rotifers contained low HUFA levels (total n-3, 0.6%; Villegas *et al.*, 1990) while the artificial feeds contain high levels of HUFA (total n-3, 6.75%; n-6, 18.68%). Although the larvae were observed to ingest feed particles they may not have been able to utilize it directly. However, rotifers from the rearing tanks were observed to ingest AF and it is likely that larvae obtained nutrients from the feed through the rotifer.

Providing supplemental feed together with rotifers in the rearing tank eliminates prior incubation at high densities with lipid emulsion or microencapsulated feeds and subsequent washing. Further, early introduction of inert feed may influence later acceptability of dry feed during weaning. Although milkfish can be directly weaned to artificial diets as early as day 14, poor growth and high mortality occur during the first week when larvae are adapted to the feed (Duray and Bagarinao, 1984).

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A NEW ARTIFICIAL DIET FOR THE EARLY WEANING OF SEABASS (*DICENTRARCHUS LABRAX*) LARVAE

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Introduction

The weaning of marine fish larvae is a critical step in larval rearing. The successful transition from live food to an artificial feed depends among others on the quality of the dry feed used (*e.g.* freshness of ingredients, digestibility, quality of the formulation) and the larvae themselves (*e.g.* age, behaviour towards artificial diets, development of the digestive tract, efficiency of certain metabolic pathways). The present study shows the results obtained with a new artificial diet formulated for the weaning phase of marine fish larvae in commercial hatcheries.

Materials and methods

Two experiments were carried out in two commercial hatcheries using their rearing procedure of current practice. Main specifications were as follows for the two hatcheries respectively: tanks of 3 000 and 1 200 l, fish density 15 and 10 larvae/l, temperature 21±1°C, semi-closed recirculation system and flow-through system. Average fish weight at the start of the experiment was approximately 30mg and the larval age was 30 to 35 days. The following feeding regime (Table I) has been used but has been adapted in function of the feed consumption and the physical properties of the dry feed in the water column. The *Artemia* (Great Salt Lake, UT-USA origin) fed to the larvae during weaning were enriched with Selco emulsion (Artemia Systems SA, Gent, Belgium). The new artificial diets (Artemia Systems SA, Gent, Belgium), namely Lansy A2 (150-300µm) and Lansy W3 (300-500µm) were tested in duplicate against one or two competitive products of comparable characteristics and sizes, commonly used in European hatcheries.

Table I. Feeding regime used as a guideline during the experiment for the Lansy A2 and Lansy W3 diet. The same feeding regime was used for the competitive diets.

Age (in days)	*Artemia* $10^6/m^3$/day	Amount of dry food given		
		$g.m^{-3}.day^{-1}$	Proportion (in %)	
			Lansy A2	Lansy W3
35-37	20	20 - 30	100	-
38-41	20	30 - 40	100	-
42-45	20 -> 15	40 - 50	100	-
46-49	15 -> 10	60 - 80	70	30
50-55	10 -> 0	90 - 110	50	50
56-60	0	110 - 120	30	70
61-66	0	120 - 150	-	100
67-75	0	150 - 180	-	100
75-90	0	5-10% of biomass	-	100

Results

Experiment 1

Fish were graded on day 12 and divided in batches of large and small fishes. On day 26 the batch of large fishes was harvested and transferred to the nursery. On day 33 the batch of small fishes fed Lansy A2 and Lansy W3 diet reached an appropriate size and were harvested and transferred. The rest of the batch of small fishes fed the competitive diets needed another 5 days prior to harvesting. For both batches (small and large fishes separated after grading) the final weight obtained was higher with the diet Lansy A2 and Lansy W3 (Fig. 1), even for the batch of small fishes where the harvesting occurred five days earlier. The higher growth rate obtained with Lansy diet is also reflected after the grading as a higher proportion of large fishes. Fig. 2 shows the food efficiency for the three treatments. Using the Lansy A2/W3, 30 to 50% more fish biomass can be harvested with the same amount of food (both live and dry food).

Experiment 2

Fish were graded on day 17 and divided in batches of large and small fishes. On day 27 the batch of small fishes was graded again into batches of big (s/big) and small (s/sml) fishes. Growth results are shown in Fig. 3. The first competitive diet was tested against the product Lansy A2/W3. A higher growth rate is achieved with Lansy A2/W3 with both the batch of small and large fishes. At the end of the experiment a stress test was carried

out (Fig. 4), in which fish were transferred into 65ppt seawater and their mortality recorded every 3 min. A better resistance was noted with the fishes fed Lansy A2/W3 diet where the onset of the mortality is noted 15min later and significant differences in survival were recorded at the end of the stress test.

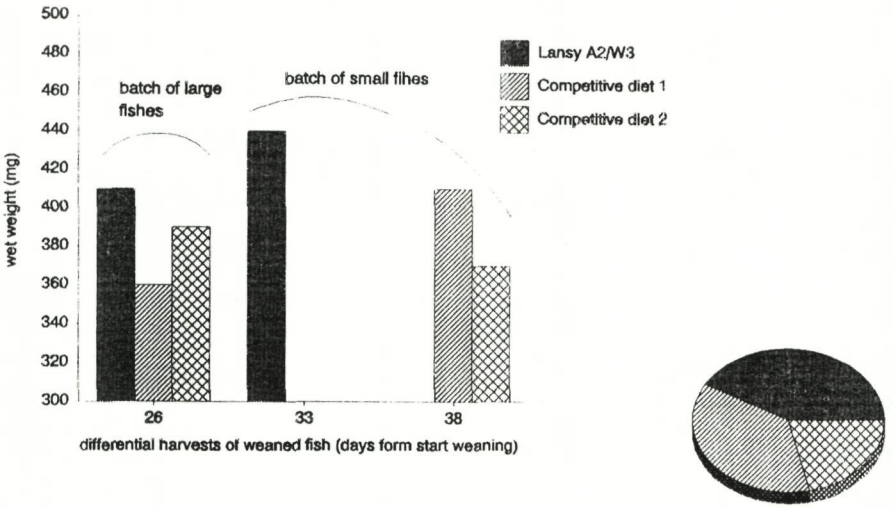

Fig. 1. Final wet weight (mg) of seabass larvae after completion of weaning for both small and large fishes. The porportion of large fishes collected after grading is also mentioned.

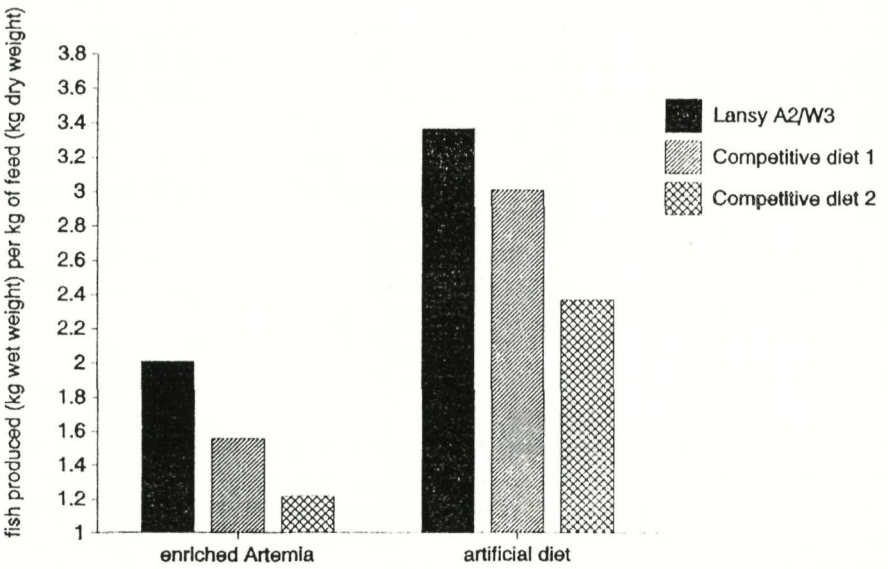

Fig. 2. Food efficiency in seabass larvae fed three different artificial diets.

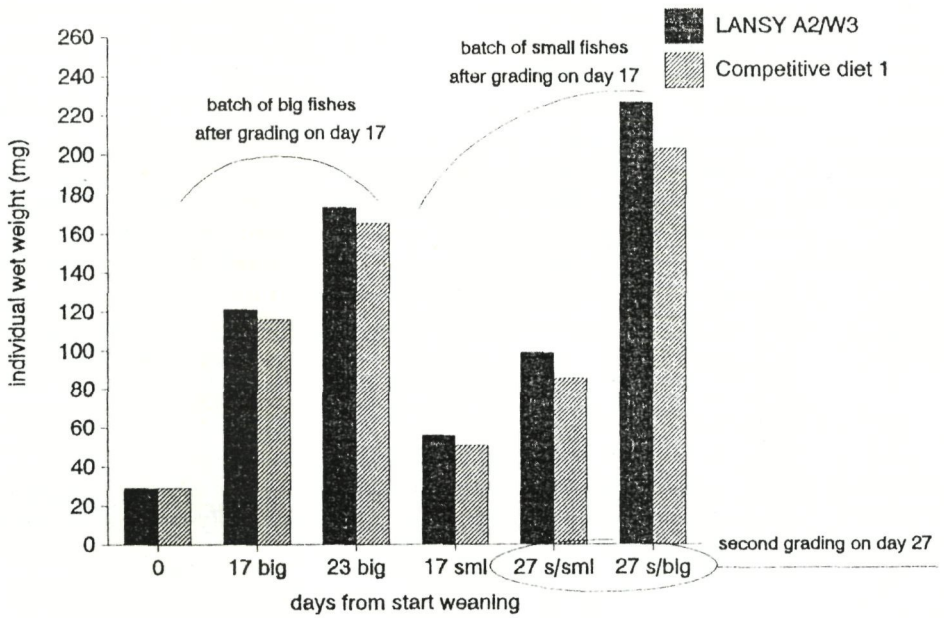

Fig. 3. Growth of seabass larvae separated in small and large larvae after the first grading on day 17, fed two different artificial diets.

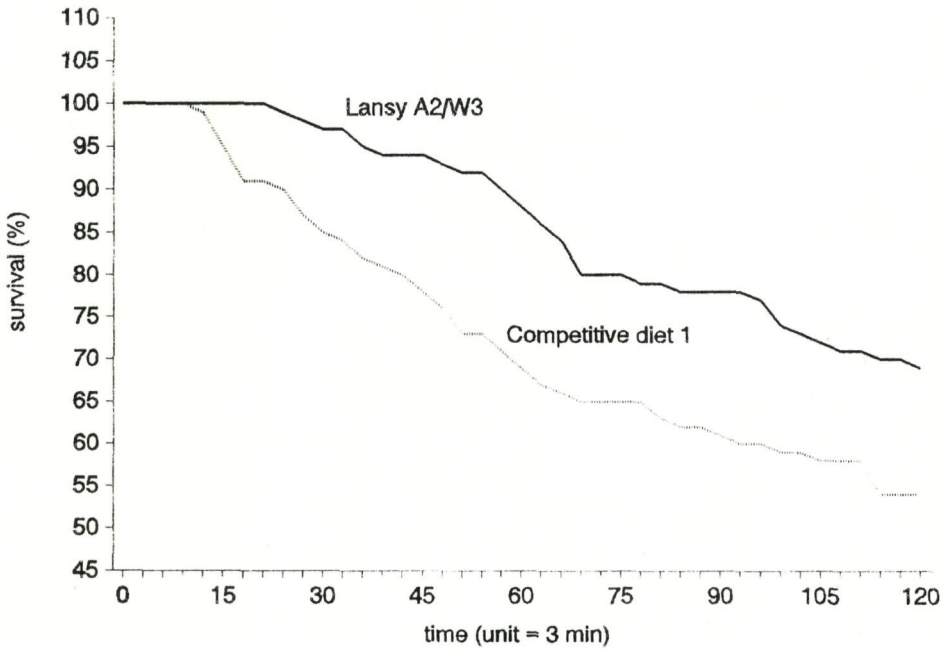

Fig. 4. Survival of weaned seabass larvae during a stress test. Mortality of the larvae is recorded each 3 min for 2h.

Conclusions

The new artificial diet Lansy A2/W3 gave superior results when applied at commercial scale conditions in all parameters investigated, *i.e.* growth rate, food efficiency, resistance to stress, uniformity of size.
A higher palatability of the diet Lansy A2/W3 and a slow sinking speed in the rearing tanks are thought to be important characteristics of the new diet. These points are important for a rational use of the diet, giving higher fish biomass with the same amount of food. The pollution within the tanks is reduced and hence the risks of possible bacterial contamination. A higher growth rate and a better resistance to stress reflect a good assimilation and utilization of the essential compounds available in the diet. The good results obtained with this product makes the culture of seabass larvae easier and more productive for the fish farmer both in terms of culture yields and economical outputs.

Acknowledgement

We would like to thank the commercial hatcheries Sepia (Gravelines, France) and Ferme Marine de Douhet (Ile d'Oléron, France) for their kind and friendly cooperation during these studies.

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WEANING OF SEABASS, *LATES CALCARIFER* B., LARVAE TO ARTIFICIAL DIET

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Abstract

Fertilized eggs from captive seabass broodstock reared in floating cages at SEAFDEC's Igang Substation were transported to the fish hatchery Laboratory at the Tigbauan Station. Newly-hatched larvae were stocked in 250 l cylindro-conical fiberglass tanks with two replicates per treatment. There were seven treatments including the control. From day 1 to 30, six experimental groups were either abruptly or gradually weaned to prepared diets while the control were fed live food consisting of rotifers and brine shrimp nauplii. The experimental group were fed prepared diets six times daily from 0700 to 1600h. The tanks were cleaned thoroughly once in the morning and the water was changed completely with flow-through for 5min between 0830 - 1030h and 1500 - 1600h. On day 30, the metamorphosed larvae (fry) were harvested and size graded. Most of these larvae were stocked into 500 l circular fiberglass tanks at 1 fry/l and reared for another 30 days by using Selco-enriched *Artemia* (Artemia Systems SA, Gent, Belgium) for the control and artificial diet for the experimental groups. Before stocking the fry, numbers were randomly assigned to the tanks corresponding to the treatments from day 1 to 30.

Results of the study indicate that seabass larvae could be weaned to artificial diet either abruptly or gradually starting as early as day 10. Better survival rates up to day 30 are obtained if larvae are fed live food or gradually weaned to artificial diet starting on day 10, 15 and 20 rather than when they are abruptly weaned to artificial diets starting on the same days. Increase in length and weight of seabass larvae up to day 30 is significantly better if they are fed live food or if they are weaned either abruptly or gradually to artificial diet starting on day 20.

The results of the study after day 30 indicate that high survival rate is obtained if seabass fry are reared on live food (Selco-enriched *Artemia*) up to day 60; cannibalism is also very much reduced. Good survival rates are obtained and a moderately low incidence of cannibalism occurs, if seabass fry, previously weaned either abruptly or gradually to artificial diets starting on day 20, are reared on artificial diet up to day 60. Low survival rates are obtained and high incidence of cannibalism occurs if seabass fry that were previously weaned to artificial diets either abruptly or gradually starting on day 10 and 15, are reared on artificial diets up to day 60.

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GROWTH AND SURVIVAL OF SEABASS (*LATES CALCARIFER*) LARVAE FED MICROENCAPSULATED DIETS ALONE OR TOGETHER WITH LIVE FOOD

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Introduction

In a study using confocal laser microscopy (Walford *et al.*, 1991), we found that seabass (*Lates calcarifer*) larvae could not digest all-protein-membrane microcapsules when these were fed alone. However, when the microcapsules were fed together with rotifers (*Brachionus plicatilis*), there was evidence that the encapsulating protein wall was broken down and absorbed in the larval gut. In the present feeding trials seabass larvae were fed all-protein-membrane microcapsules together with rotifers and their growth and survival were compared with those of larvae fed the microcapsules alone or the rotifers alone.

Materials and methods

The microencapsulated diets used were Frippak RDX 123 (experimental diet) microcapsules in three size ranges (40-90, 90-150, and 150-250µm) and Frippak #1 CAR (crustacean algal replacement) microcapsules (5-30µm). The rotifers used as live food were cultured with baker's yeast. Newly-hatched seabass larvae were stocked in larval rearing tanks containing 20 l gently aerated seawater (30ppt). Diets were offered from the second day after hatching (day 2) with two replicate rearing tanks used for each diet. The microcapsule delivery system was as described previously (Walford *et al.*, 1991). Rotifers were added daily to the tanks concerned at a density of 20-25 ind./ml until day 14 and a volume of 5 l was changed daily in all the rearing tanks. On day 14, all the rearing tanks were emptied, washed, refilled with seawater and restocked with larvae. From day 15 until the end of each trial, rotifers were added daily to the tanks concerned at a density of 25-30 ind./ml and a volume of 10 l was changed daily in all the tanks. The water temperature in the tanks was in the range 26-31°C.

The diets offered to seabass larvae in trials 1 and 2 are shown in Table I. In part 1 of trial 1 (days 2-14), larvae were stocked in four tanks at a density of 600 larvae/tank and were offered microcapsules alone or rotifers alone. All the larvae fed microcapsules alone had died by day 9. In part 2 of trial 1 (days 15-28), larvae which had been fed rotifers in the first part were restocked in six tanks at a density of 50 larvae/tank. In two of the tanks the larvae continued to be offered rotifers alone until day 28. In the other four tanks larvae were offered microcapsules together with rotifers. In two of these tanks

microcapsules and rotifers were offered until day 28, but in the other two tanks the microcapsules were offered alone for the last week (days 22-28). In trial 2, larvae were offered microencapsulated diets together with rotifers from first feeding. Eight tanks were each stocked with 660 larvae and the diets shown in Table I were offered. In part 2 of trial 2 (days 15-25), the larvae which had been fed RDX 123 microcapsules together with rotifers in the first part were restocked in four tanks at a density of 160 larvae/tank. RDX 123 microcapsules and rotifers continued to be offered to the larvae until day 25 in two of the tanks. In the other two tanks, the microcapsules were offered alone during the last week (days 18-25) of the trial. On days 14 and 28 in trial 1 and on days 14 and 25 in trial 2, the tanks were emptied, the numbers of larvae that had survived in each tank were counted, and the total lengths of 20 larvae from each tank were measured by means of a linear vernier microscope.

Table I. Diets offered to seabass (*Lates calcarifer*) larvae in trials 1 and 2

Trial 1		Trial 2	
Age of larvae	Diets and microcapsule concentrations	Age of larvae	Diets and microcapsule concentration
Day 2-14	1) Rotifers alone	Day 2-14	1) Rotifers alone
	2) RDX123 (40-90µm) alone 10/ml		2) 1 CAR (5-30µm) 450/ml and rotifers
			3) RDX 123 (40-90µm) 4/ml and rotifers, days 8-14.
			4) RDX 123 (40-90µm) 4/ml and rotifers, days 2-7; RDX 123 (90-150µm) alone 6/ml, days 8-14.
Day 15-28	1) Rotifers alone	Day 15-25	1) RDX 123 (90-150µm) 4/ml and rotifers, days 15-17; RDX 123 (90-250µm) 5/ml and rotifers, days 18-25;
	2) RDX 123 (90-150µm) 4/ml and rotifers		
	3) RDX 123 (90-150µm) 4/ml and rotifers, days 15-21; RDX 123 (90-150µm) 4/ml alone, days 22-28.		2) RDX 123 (90-250µm) 4/ml and rotifers, days 15-17; RDX 123 (90-250µm) 5/ml alone, days 18-25

Results

Growth (in terms of total length) and survival of the larvae are shown in Fig. 1. Larvae fed RDX 123 microencapsulated diet alone from first feeding in trial 1 all died by day 9. Feeding this microencapsulated diet alone to 8-day-old larvae (trial 2) as well as to 18 day-old (trial 2) and 22- day-old larvae (trial 1) produced significantly ($P<0.05$) lower growth and survival than feeding live food either alone or together with the microcapsules. When algal replacement microcapsules (5-30μm) and rotifers were co-fed to the larvae (trial 2) there was no significant ($P>0.05$) increase in growth compared to feeding rotifers alone. However, co-feeding RDX 123 microcapsules and rotifers (trials 1 and 2) produced a significant ($P<0.05$) improvement in growth in the larvae compared to feeding rotifers alone, indicating that some assimilation of the microcapsules had taken place in addition to the rotifers.

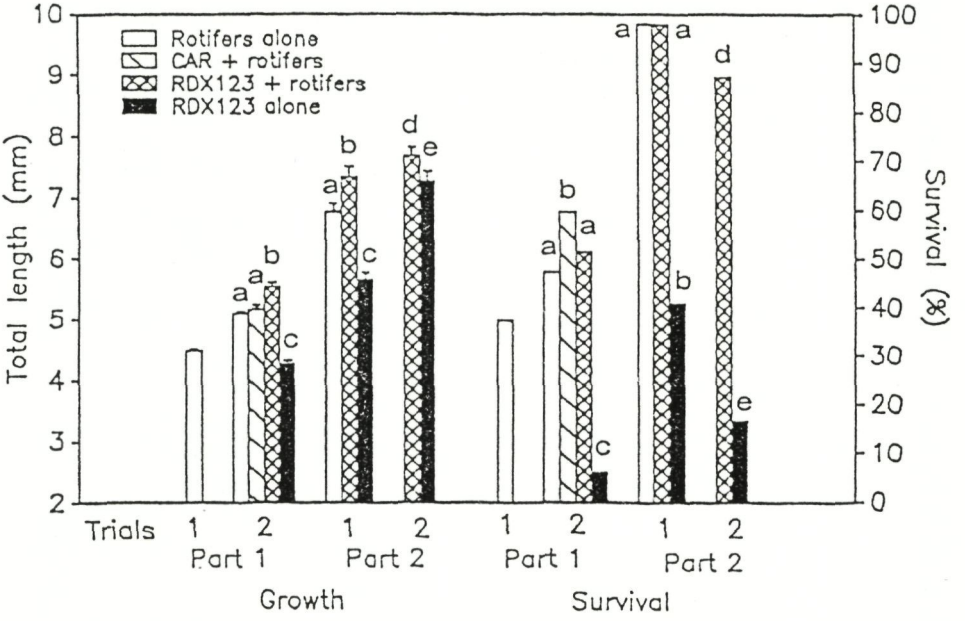

Fig. 1. Mean total length and survival of seabass (*Lates calcarifer*) larvae in trials 1 and 2 (in each trial and for each parameter, means which have the same superscript are not significantly different; error bars show SEM). Part 1: total length and survival on day 14. Part 2: total length on day 28 in trial 1 and on day 25 in trial 2 and survival from day 14 to day 28 in trial 1 and to day 25 in trial 2.

Discussion

The results support our finding by confocal laser microscopy (Walford *et al.*, 1991) that seabass larvae cannot digest the all-protein-membrane microcapsules when these are fed alone but also suggest that some assimilation of the microcapsules takes place when they are offered to the larvae together with rotifers. Thus microcapsules can be used as a

supplementary diet in this way. However, confocal microscopy also showed that first feeding seabass larvae select a narrow range of microcapsule sizes (40-60μm). Therefore, the algal replacement microcapsules (5-30μm) may have been too small for ingestion by the larvae. Since these microcapsules were small enough to be ingested by the rotifers (Walford and Lam, 1987), a further trial was conducted (results not shown) in which the microcapsule concentration was increased from 450/ml (trial 2) to 750/ml. Again there was no improvement in the growth of larvae compared to feeding rotifers alone. The particle density in the tanks may still have been too low for effective nutritional enrichment of the rotifers by the microcapsules.

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LARVAL AND POSTLARVAL NUTRITION OF PENAEID SHRIMP

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Abstract

This paper provides a summary of information on recent advances in larval or postlarval nutrition of penaeid shrimp, with a special reference to the *Penaeus monodon*. The nutritional requirements of *P. monodon* are little known despite its importance in aquaculture. Recent findings indicate a noticeable difference between some important nutrient requirements of *P. monodon* and those of *P. japonicus* which have been the most studied shrimp species. For example, the thiamin requirement for *P. monodon* was estimated to be 14mg.kg^{-1} of diet, while that reported for *P. japonicus* is 60-120mg.kg^{-1} of diet for juveniles and 40-80mg.kg^{-1} of diet for adults. Similar requirements of riboflavin (20mg.kg^{-1} for *P. monodon versus* 80mg.kg^{-1} for *P. japonicus*), niacin (10mg.kg^{-1} *versus* 400mg.kg^{-1}) and vitamin C (200-250mg.kg^{-1} *versus* 1 000mg.kg^{-1}) have been reported.

In addition to the essential knowledge on the nutritional effects and requirements for proteins, lipids, energy, vitamins and minerals, enrichments of feed or food organisms with highly unsaturated fatty acids, enzymes, amino acids and other nutritionally active components have been the major topics of attention in larval penaeid nurition research. Improved postlarval quality due to nutrient enrichment during larval feeding is of more concern than the improvements in the development and survival alone. New and effective methods allowing the incorporation of nutrients in larval or postlarval diets provide a new approach to many unsolved problems. Microencapsulation and microcoating techniques make the supplementation of unstable ingredients such as enzymes possible. Methods are also being developed to effectively deliver amino acids, which in the crystalline form cannot be assimilated by most crustaceans. This development also enables examination of the amino acid requirements of shrimp. Studies using cellulose-aceate-hydrogen-phthalate encapsulated arginine indicate that the arginine requirement of postlarval *P. monodon* is 6g.100g^{-1} protein. The application of emulsified oils and their related products have become a routine practice in many hatcheries. These new techniques allow delivery of the required nutrients through direct or indirect feeding and make research into a thorough understanding of larval crustacean nutrition possible.

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SURVIVAL AND GROWTH OF SHRIMP LARVAE FED DIFFERENT AMOUNTS OF ARTIFICIAL PLANKTON

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Introduction

Penaeus monodon is a penaeid normally commercially cultured during larval stages on diatoms such as *Skeletonema* and *Artemia* nauplii. Artificial feed such as flakes, microparticulates and microencapsules have also been used in hatcheries to replace live food (Jones *et al.*, 1979). Several questions are raised: do shrimp larvae grow well on a total replacement of *Skeletonema* by artificial diets? If shrimp larvae grow to mysis or postlarvae on artificial diets, what is the optimal feed level? Present work describes the growth of *P. monodon* larvae on different levels of artificial plankton.

Materials and methods

The nauplii were obtained from a wild-caught gravid female. A total of 100 nauplii were cultured in a 2 l vessel containing 1 l of seawater. Artificial plankton SC (Nippai Feed Inc., Yokohama, Japan) was used as a larval feed (Table I). Larvae were fed 0.75, 1.00, 1.25 and 1.50mg each meal with four meals a day (0200h, 0800h, 1400h and 2000h) for larvae at stages N6-Z1, Z1-Z2, Z2-Z3 and Z3-M1, respectively. This amount served as the standard level (B group). Other groups were fed 0.5 (A), 2.0 (C), 3.0 (D), 4.5 (G), and 6.0 (H) times the standard level (Table II). Trial I was repeated trice with five replicates, and trial II was repeated twice with five replicates. Feed was given as particulate together with water, after first weighing the feed, adding water and sieving through 117μm (A-E groups) or 59μm meshes (F-H groups). During the experiment, salinity, water temperature, and pH were maintained at 34±1ppt, 32±0.5°C and 8.2±0.03, respectively. When 75% of the larvae in any group metamorphosed to the M1 stage, survival of the larvae was counted. In total, 30 larvae were randomly sampled to determine the body length. Water parameters were analyzed. All data were subjected to analysis of ANOVA and Duncan's Multiple Range Test.

Table I. Proximate composition of artificial plankton SC and its fatty acid profiles

Moisture (%)	4.06	Fatty acid					
Protein (%)	45.00	12:0	-	18:2	10.40	20:5n-3	6.24
Lipid (%)	39.60	14:0	16.10	18:3	1.12	22:6n-3	6.59
Fiber (%)	0.81	16:0	0.34	20:0	-	other	8.91
Ash (%)	9.22	16:1	0.52	20:1	3.00		
Ca (%)	2.30	18:0	6.17	20:4	-		
Mg (%)	0.90	18:1	39.20	22:1	1.41		

Table II. Amount (mg) of artificial plankton SC for *Penaeus monodon* from the N6 through the M1 stage

Group	N6	Z1	Z2	Z3	Mesh size (µm)	Group	N6	Z1	Z2	Z3	Mesh size (µm)
Trial I						Trial II					
A	0.38	0.50	0.63	0.75	117	E	2.25	3.00	3.75	4.50	117
B	0.75	1.00	1.25	1.50	117	F	2.25	3.00	3.75	4.50	59
C	1.50	2.00	2.50	3.00	117	G	3.38	4.50	5.63	6.75	59
D	2.25	3.00	3.75	4.50	117	H	4.50	6.00	7.50	9.00	59

Results and discussion

Larvae fed at three times standard level (D group) showed the highest survival, growth, ammonia-N, and COD. The larvae fed at the standard level grew slower than the D group. However, larvae in the C group (fed two times the standard level) had the poorest survival and the highest nitrite-N. Significant statistical differences ($P<0.05$) in survival, growth, ammonia-N, and COD were found between D and A groups (Table III).

Table III. Mean (SD) survival, body length of larvae, and water parameters. Data in the same column with different letters are significantly different ($P<0.05$)

Group	Survival (%)	Ammonia-N ($\mu g.l^{-1}$)	Nitrite-N ($\mu g.l^{-1}$)	COD ($mg.l^{-1}$)	Body length (mm)	Metamorphosis (%) to MI stage
A	10.0 (5.3)c	724 (226)	342 (165)	2.8 (0.7)	2.11 (0.32)	0
B	27.0 (8.3)b	977 (369)	407 (148)	2.6 (0.4)	2.59 (0.15)	75
C	6.3 (2.3)d	1570 (785)	462 (161)	3.8 (0.6)	2.87 (0.73)	50
D	36.3 (12.5)a	1679 (550)	341 (190)	4.3 (0.8)	3.02 (0.31)	72
E	32.0 (12.2)a	1638 (242)	177 (70)	5.7 (0.7)	2.98 (0.43)	92 (0)
F	28.0 (10.1)a	1622 (369)	151 (56)	5.7 (0.4)	2.77 (0.32)	71 (13)
G	0	2188 (304)	176 (60)	6.7 (0.5)	-	-
H	0	2648 (553)	196 (136)	7.8 (1.3)	-	-

Larvae fed with 4.5 (G) and 6.0 (H) times standard level did not grow to the M1 stage. Water parameters in these two groups were worse than in the other two groups (Table III). Larvae in the E group grew significantly better ($P<0.05$) than those in the F group

(sieved through 59μm). However, no significant differences in COD, ammonia-N, and nitrite-N were shown between E and F groups. *P. monodon* larvae fed *Skeletonema* after the N2 stage and then artificial plankton SC after the Z2 stage, achieved the M1 stage with 61% survival. Artificial plankton SC may be nutritionally superior to *Skeletonema* in sustaining survival. However, it appears to be nutritionally inferior to *Skeletonema* in sustaining growth for *P. monodon* larvae. In present studies growth was higher in comparison with the data for wild *P. monodon* larvae (Motoh, 1979).

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INTRODUCTION OF A NEW *ARTEMIA* BASED LARVAL DIET

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Introduction

The continued interest in replacing live feeds, such as microalgae and zooplankton (specifically rotifers *Brachionus plicatilis* and brine shrimp *Artemia*) for larvae culture has led to the commercial development of many live feed supplements and replacements. Most of these diets are closed formulations consisting mostly of fish, shrimp or other marine sources of proteins and fats combined with terrestrial plant products and various other ingredients, including minerals and vitamins.

The wide acceptance of these types of products for larvae culture has led to the development by the authors of a larval diet based on *Artemia*. This larval feed has the nutritional aspects of freshly hatched nauplii and can be easily modified, *i.e.* specific fatty acids or other components can be incorporated to meet the requirements of various species of larvae to be cultured. This dry form of *Artemia* could reduce the operation expense associated with current zooplankton production and can be delivered to larvae in a range of particle sizes from 5µm to over 700µm.

Methods and testing

The unique production methods for which a United States patent is now pending, vary greatly from those previously described in the literature. Our production methods allow for easy modification of nutritional parameters by the addition of select fatty acids, proteins or other elements. Particle size is controlled through the production methods.

Samples of the *Artemia* Based Larval Diet (ABD) were sent to a commercial laboratory (Woodson-Tenent Laboratories, Inc., Des Moines, Iowa, USA) for proximate, vitamin and other analyses. Additional samples of 5-50µm product were sent to the Laboratory of Aquaculture & Artemia Reference Center, University of Ghent (Belgium) for pigment and fatty acid analysis.

A laboratory feeding study using *Penaeus vannamei* as a test animal was conducted by the Rangen Aquaculture Research Center which tested the ABD in the 5-50µm and the 80-225µm sizes against four commercial diets of similar particle size. Actual *P. vannamei*

production tests were performed by hatcheries in Ecuador and Mexico. Ongoing studies are currently being conducted on the larval stages of *Penaeus chinensis*, *Acipenser* spp., *Stizostedion vitreum*, *Coryphaena hippurus* and *Morone* spp..

Results and discussion

The analytical results of the proximate analysis are given in Fig. 1. In Table I, vitamin and pigment analysis and energy content are shown. The protein and fat levels are similar to those reported by Léger *et al.*(1986) for freshly-hatched nauplii. Sorgeloos (pers. commun.) reported a total lipid content of 17.8% on a dry weight basis with 20:5n-3 at 9.3% of total fatty acids and total (n-3) HUFA levels equal to 9.4%.

Table I. Vitamin and pigment analysis of ABD

Beta carotene	39mg.kg^{-1}
Canthaxanthin	227µg.g^{-1}
Biotin	4.6mg.kg^{-1}
Choline Chloride	851mg.kg^{-1}
Folic acid	16mg.kg^{-1}
Niacin	204mg.kg^{-1}
Pantothenic acid	94mg.kg^{-1}
Vitamin A	126 940IU.kg^{-1}
Vitamin B_2	32mg.kg^{-1}
Vitamin B_6	8mg.kg^{-1}
Vitamin B_{12}	6mg.kg^{-1}
Vitamin C	860mg.kg^{-1}
Vitamin E	300IU.kg^{-1}
Energy	5 302cal.g^{-1}

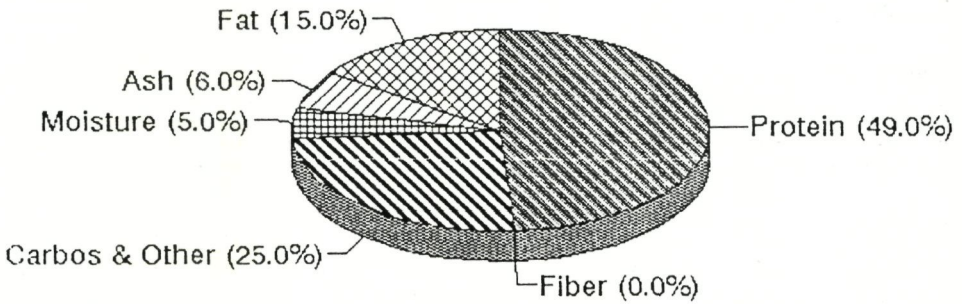

Fig. 1. Proximate analysis of ABD.

The study performed by the Rangen Aquaculture Research Center on *P. vannamei* found no significant difference in final mean weight between the two commercial diets and the ABD for the smaller particle sizes. For the larger particle size diets the ABD fed shrimp larvae had a significantly superior mean final weight than one of the commercial diets,

but equal to the other. Survival and metamorphosis rate to postlarva of the shrimp fed the ABD diet were not significantly different from the control treatment fed live food. The researchers also noticed that the larvae fed the ABD feeds had a high degree of pigmentation relative to the other feeds (Grant and Villamar, pers. commun.).

Similar results have been documented in hatchery production tests, with reports of near 90% to 100% survival and acceptable growth rates (Pavel, pers. commun.). The ongoing research on larval fish will provide the initial data required for further testing of modified forms of the *Artemia* Based Diet.

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EGG AND LARVAL QUALITY

LARVI '91 - FISH & CRUSTACEAN LARVICULTURE SYMPOSIUM
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HORMONES AND EGG/LARVAL QUALITY IN FISH

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Abstract

Recent evidence suggests that hormones are passed on to eggs by broodfish prior to spawning. This store of maternal hormones provides the necessary physiological regulation for growth, development and osmoregulation in fish larvae prior to the functional development of their own endocrine glands. Thus the hormonal levels in eggs/larvae may be an important determinant of egg/larval quality.

Such hormones include the thyroid hormones. Both thyroxine (T_4) and triiodothyronnine (T_3) are present in fish eggs and, in most cases, the levels decrease as development proceeds until a certain period after hatching when endogenous secretion comes on. Enhancement of T_4 or T_3 levels in newly hatched larvae through immersion promoted larval growth, development and survival in several species of fish. Administration of thyroid hormones to broodfish prior to spawning has also been shown to improve larval performance in the progeny. However, careful dosage control is necessary as excessive levels of thyroid hormones are detrimental. Also, in species (*e.g. Lates calcarifer*) which show early endogenous thyroid secretion, supplementation with thyroid hormones may only give a minimal positive effect, if any.

Corticosteroids are beginning to be studied similarly. Results obtained so far suggest that cortisol could also enhance larval growth, development and survival in fish, perhaps in synergism with thyroid hormones as in amphibians for the metamorphic action.

Of the pituitary hormones, prolactin has been considered. Prolactin cells appear early in development in those species studied. Whether it functions as a larval growth factor as in amphibians is still not clear, as the experiments conducted so far did not yield consistent results. However, there is evidence that prolactin accelerates hatching in fish while thyroid hormones delay it.

The paper will not only review own data, but also published results from other laboratories. Furthermore the possible involvement of other hormones will be discussed.

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CHANGES IN THYROID HORMONE LEVELS IN PLASMA, EGGS, AND YOLK SAC LARVAE OF RABBITFISH (*SIGANUS GUTTATUS*) AFTER MATERNAL THYROXINE INJECTION

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Abstract

Thyroxine (T_4) and triiodothyronine (T_3) levels in rabbitfish (*Siganus guttatus*) plasma, eggs, and yolk-sac larvae were measured before and after maternal thyroxine injection at doses of 1, 10, and 100µg T_4/g WW fish. T_4 and T_3 levels in plasma, eggs, and yolk-sac larvae were elevated following T_4 administration. Apparently, there was conversion of T_4 into T_3 in the broodfish indicating the presence of the enzyme, 5'-monodeiodinase in rabbitfish. T_4 and T_3 in maternal circulation were transferred into the oocytes and subsequently into the larvae. Elevated levels of T_4 and T_3 in eggs and larvae, however, did not significantly improve egg viability, and larval growth and survival until 7 days after hatching.

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FUNCTIONAL DEVELOPMENT OF THE ENDOCRINE SYSTEM DURING EARLY ONTOGENY OF MARINE TELEOST FISHES

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Abstract

The endocrine system is of fundamental importance in controlling development and growth of fishes. However, until fairly recently, information on the functional development during the early ontogeny of marine teleost fishes has been very scarce. However, recent advances in radioimmunoassay (RIA) and immunohistochemistry have provided a rapid and large accumulation of new data. The major aim of this paper is to review the information on the functional development of the endocrine system, particularly of studies recently conducted in our laboratory.

The following species were examined for development of the endocrine organs, such as pituitary gland, thyroid gland and interrenal tissues: rainbow trout, Pacific herring, ayu, minnow, Pacific cod, seabass, striped jack, seabream, Japanese flounder, stone flounder, marbled sole, plaice, bamboo sole, and greenlings, reared from fertilization beyond metamorphosis. Tissue concentrations of thyroid hormones (TH) and cortisol were quantified by RIA, and production of growth hormone (GH) and prolactin (PRL) were validated by immunohistochemistry.

Thyroid and pituitary glands differentiate during the final phase of yolk absorption in pelagic-eggs species, whereas in demersal-egg species they have been formed before hatching. However, in both cases, first appearance of these glands closely coincides with eye pigmentation. Interrenal tissues appear later than both glands, around 2 weeks after hatching, in Japanese flounder. Immunohistochemical techniques revealed production of GH and PRL prior to the time when the pituitary is histologically detectable. Accumulation of thyroglobulin can be histologically visible at nearly the same time as thyroid gland formation. Productivities of GH, PRL and TH appeared to be variable with larval development and environmental conditions. Tissue TH concentrations were at higher levels at fertilization, but reduced markedly with embryonic development, and finally, at the time of yolk absorption, achieved levels below one-tenth of the initial levels. They gradually increased with postlarval growth and development. TH concentration, in particular thyroxine, appreciably increased during metamorphosis (transitional phase from larva to juvenile), and especially in flatfishes, prominent surges

were commonly observed. In some flatfishes, tissue concentration of cortisol also exhibited a prominent surge, but the timing was significantly earlier than of TH.

In conclusion, the basic function of the major endocrine system has already developed at least by the final phase of embryonic development, and plays an important role in larval development and growth. Some hormones appear to be supplied from female parents and are deposited in yolk, being utilized for embryonic development when major endocrine organs have not yet developed. These findings have potential applications in the improvement of seed production techniques for marine fish larvae.

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EGG QUALITY DETERMINANTS IN FINFISH WITH SPECIAL REFERENCE TO THE TIMING OF STRIPPING AND METHODS OF FERTILIZATION IN THE ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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Abstract

One of the most important constraints on the further growth of aquacultural production, particularly of marine species, is the variable quality of the eggs which are available for growing-on to market size fish. Good quality eggs may be defined as those which exhibit low levels of mortality at fertilization, hatch and first-feeding and also those which produce the healthiest and fastest growing fry and older fish.

Many factors have been implicated as possible determinants of egg quality including the maturation, handling and nutritional status of the broodfish, the chemical and nutrient composition of the egg and its size and lastly the overripening of the egg, a deteriorative change which is determined primarily by the length of time which elapses between ovulation and the time of artificial stripping or natural oviposition.

In salmonids *e.g.* the rainbow trout maintained under commercial conditions, fertilization and hatching rates average 90% and 70% respectively with survivals of fry up to 4 months of age, of 35-40%. Within these mean survival rates, however, there is considerable variation in values of egg quality for individual fish with some batches of eggs having survivals up to 4 month-fed fry in excess of 85% whilst others experience a 100% mortality. A significant proportion of these "blanks" will almost certainly be due to overripening, inappropriate handling of broodfish and poor fertilization technique. Salmonid eggs must be stripped within 10 days of ovulation at 10°C if they are not to experience high mortalities. Dry fertilization techniques are almost universally used because of better survivals.

These quality problems are far more serious for truly marine species like the halibut, turbot, bass and bream where many more "blank" fertilizations are recorded. In halibut for example, in the Ardtoe facilities this year, 36% of all egg batches stripped had fertilization rates in excess of 70% with an average of 80% of all fertilized eggs showing

normal cell division and development. Of those batches selected for incubation approximately 77% survived to hatch and half of these reached first feeding.

Stripping eggs from individual female halibut over a 16h time-frame around ovulation, revealed a period of approximately 4-6h during which optimum fertilization rates were achieved; eggs fertilized outside this "window" were of markedly poorer quality. Wet or dry fertilization methods and the storage of eggs *in vitro* in ovarian fluid produced broadly similar egg development rates. Whether more careful consideration of the timing of stripping in relation to the ovulatory cycle and an optimisation of fertilization procedures will enable significant improvements to be made in the overall production of halibut and other marine fish larvae remains to be confirmed. The relative importance of ovulatory rhythms in relation to other possible determinants of egg quality in fish is discussed.

Acknowledgements

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TIMING OF STRIPPING RELATIVE TO SPAWNING RHYTHMS OF INDIVIDUAL FEMALES OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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Introduction

Atlantic halibut, *Hippoglossus hippoglossus*, is a batch spawner like turbot and several other marine flatfish with pelagic eggs. Distinct ovulatory rhythms have been shown in turbot (McEvoy, 1984; Howell and Scott, 1988). If turbot eggs are stripped only a few hours too early or too late compared to the correct ovulation time, very low hatching rates are obtained (McEvoy, 1984).

Similar ovulatory rhythms are also reported in Atlantic halibut (Norberg *et al.*, in press). This experiment was carried out to examine the effects of timing of stripping relative to the spawning rhythms of individual females of halibut at the research station of Akvaforsk, Norway.

Materials and methods

An individual female halibut was followed closely during several ovulatory cycles, and the mean interval between spawnings was used to predict the timing of her next spawning. This estimated correct spawning time was defined as time 0. Eggs were then stripped from the female at -6h, -3h, 0h and +3h relative to time 0. Each egg batch was then divided into smaller groups and kept in ovarian fluid until fertilization. Fertilization was carried out as outlined in Table I. The hatching rates were recorded for each group.

Table I. Fertilization of halibut eggs relative to the predicted spawning time (time 0)

Stripping time (h)	Time of fertilization relative to time 0 (hours)				
	-6	-3	0	+3	+6
-6	X	X	X	X	X
-3		X	X	X	X
0			X	X	X
+3				X	X

Results

When stripping of eggs was done 3 to 6h before the estimated correct time according to the earlier spawning rhythms, the hatching rate decreased. If these early-stripped eggs were kept in the ovarian fluid for 3 to 6h after stripping, there was an increase in the hatching rate up to the same level as for the control eggs which were stripped at the estimated correct time (Fig. 1).

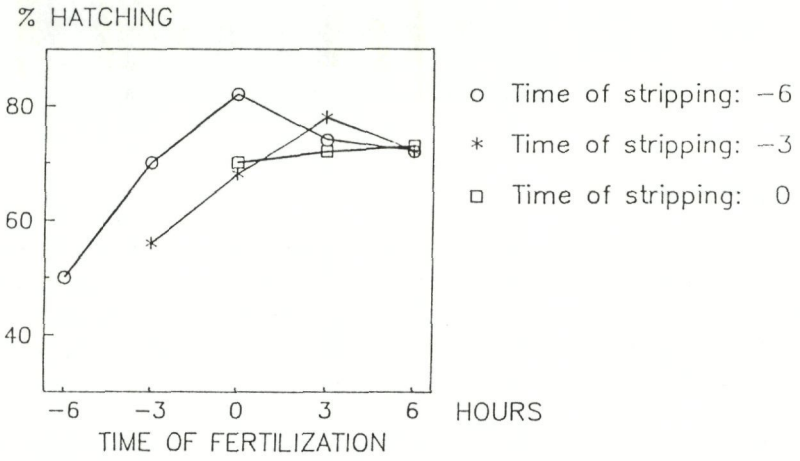

Fig. 1. Hatching rates of halibut eggs stripped and fertilized at different points of time relative to the predicted spawning time.

Discussion

The results show that very precise predictions of the spawning time will be necessary to secure an egg production of high quality when stripping halibut. If eggs are stripped too early, an improved hatching rate can be obtained when the eggs are kept in ovarian fluid for 3 to 6h before fertilization.

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VIABILITY OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) EGGS EXPOSED TO SEAWATER BEFORE FERTILIZATION

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Introduction

Atlantic halibut (*Hippoglossus hippoglossus* L.) is a batch spawner, and the egg quality is strongly influenced by the stripping time. If the eggs are stripped too early, the hatching rate is reduced. Each female fish has individual intervals between each egg release, and must be allowed to release at least three batches before an approximately correct time for stripping can be estimated. This causes a large loss of unfertilized eggs. If the unfertilized eggs could be collected and fertilized, more of the production potential could be utilized.

Materials and methods

In the experiment, six points of time for fertilization after exposure to water were tested, in three replicates for each time period. Each replicate contained approximately 100 eggs. The experiment was run in 0.5 l glass units. Eggs were stripped from one female, and milt was stripped from one male. The milt was stored on ice during the experiment, and the sperm activity was controlled microscopically before each insemination.

Approximately 100 eggs and 200ml of seawater were distributed to each glass unit. The control group was fertilized by adding eggs and sperm to seawater at the same time immediately after stripping. Superfluous milt was removed by rinsing the eggs with seawater after fertilization. The other egg groups were fertilized by adding milt to the mixture of eggs and seawater after respectively 10, 20, 30, 40, 80, 160min exposure to seawater. After rinsing, the eggs were kept in darkness at 4°C for 18h. The fertilization rates were calculated from microscopical observation at the eight cell stage (18h after fertilization).

Results

After 20min of exposure to seawater, the fertilization rate was still more than 70%, but it dropped dramatically when the eggs were exposed to seawater for 30min before fertilization (Table I).

Table I. Fertilization rates of halibut eggs exposed to seawater before fertilization

Time in water (min)	Fertilization rate (%)	Standard deviation
0	89.8	2.6
10	84.2	1.9
20	73.1	4.4
30	49.4	1.4
40	25.3	1.7
80	4.2	1.4
160	2.1	0.9

Discussion

The literature about the swelling process in halibut eggs is scarce, but the experimental results show that it is possible to obtain high fertilization rates even when the eggs have been kept in seawater for 20min before fertilization. This indicates that the micropyle does not close within 20min, and that sperm is still able to enter the egg if milt is added within this period. After 30min in seawater, there is a dramatic drop in the fertilization rate, which indicates that most of the eggs have closed their micropyle, and sperm cannot any longer penetrate the egg.

The period from contact with water to micropyle closure seems to differ among species. In rainbow trout, sperm was no longer able to fertilize the eggs after 100 to 120sec (Ginsburg, 1963). Lein and Fjalestad (1987) found that the fertilization rate for rainbow trout was close to zero when the eggs were exposed to freshwater before fertilization while Atlantic salmon eggs still had high fertilization rates after 15min.

The results of this experiment indicate the possibility of collecting unfertilized halibut eggs from broodstock ponds, and then fertilize the eggs within a period of 20min after the eggs are released from the female fish. This method to supply eggs for hatchery rearing needs a fast and gentle system to collect the eggs. If such a system could be successfully developed, larger amounts of the high production potential of the broodstock fish could be utilized.

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BIOCHEMICAL CHARACTERISTICS OF THE EGGS OF FARMED TURBOT (*SCOPHTHALMUS MAXIMUS*)

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Introduction

Overripening of mature eggs in turbot occurs rapidly and is accompanied by changes in the biochemical composition of eggs. Unfortunately, research on these changes is scarce. Presently, turbot spawning in commercial farms is frequently controlled by light and temperature manipulations. The effect of such manipulation on the biochemical composition of eggs is, however, not well understood. The current knowledge on possible indicators of egg quality has been summarized in a revision of Kjørsvik *et al.* (1990). The aim of this paper is to describe biochemical changes in turbot eggs as possible criteria of egg quality.

Material and methods

Three broodstocks of turbot from the fish farm Cultipec SA (Galicia, Spain) were followed during 1989 and 1990. The broodstocks and the spawning periods were as follows:

1989: F1 - from May 30, 89 to August 11, 89 - natural photoperiod
F3B - from June 13, 89 to July 28, 89 - photoperiod control
F3C - from July 24, 89 to July 26, 89 - photoperiod control

1990: F2 - from March 23, 90 to May 2, 90 - natural photoperiod
F3B - from May 14, 90 to August 31, 90 - photo- and thermoperiod control
F3C - from June 19, 90 to August 31, 90 - photo- and thermoperiod control

Photoperiod control was performed by progressive decrease of the light cycle from 18 to 8h/day during the recovery period and by increase up to 18h/day during the maturation period. In 1989, the temperature varied between 12.7 (January) and 18.6°C (August); in 1990 between 13.8 (February) and 16.8°C (May). Egg samples were taken for biochemical analysis (proximate biochemical composition, lipid classes and fatty acids).

Fertilization and hatching rates and the weight of eggs were also recorded. Samples of eggs were classified into three categories:

- O. EGGS: overripe eggs; fertilization not possible;
- N.B. EGGS: fraction of non-buoyant eggs from a batch submitted for fertilization;
- F. EGGS: fraction of fertilized and buoyant eggs from a batch submitted for fertilization.

Results and discussion

The biochemical composition of eggs was similar among the three categories established except for the following components:

- organic weight:	F.EGG	>	O.EGG	>	N.B.EGG
- ash:	F.EGG	<	N.B.EGG	=	O.EGG
- lipids:	F.EGG	<	N.B.EGG	=	O.EGG
- protein:	F.EGG	<	N.B.EGG	=	O.EGG
- carbohydrates:	F.EGG	>	O.EGG	>	N.B.EGG
- reducing components:	F.EGG	<	N.B.EGG	<	O.EGG
- total fatty acids:	F.EGG	>	O.EGG	>	N.B.EGG
- saturated fatty acids:	F.EGG	>	O.EGG	>	N.B.EGG
- polyunsaturated fatty acids:	F.EGG	>	O.EGG	>	N.B.EGG
- (n-3) PUFA:	F.EGG	>	O.EGG	>	N.B.EGG

Protein, reducing components, and ash content in F.EGGS from 1989 spawnings were higher than those in 1990 spawnings but the lipid content and the organic weight of F.EGGS were higher in the latter.

In the effect of spawning period on the composition of F. EGGS, the most noticeable differences observed among the photoperiods occurred as follows:

- organic weight:	F1/F2 > F3B > F3C
- lipids:	F1/F2 > F3B = F3C
- protein:	F1/F2 > F3B = F3C
- sterol esters + waxes:	F1 < F3B = F3C
- triacylglycerols:	F1 > F3B = F3C
- total fatty acids:	F2 > F3B > F3C
- saturated fatty acids:	F2 > F3B > F3C
- polyunsaturated fatty acids:	F2 > F3B > F3C
- (n-3) PUFA:	F2 > F3B > F3C
- (n-9) fatty acids:	F2 > F3B > F3C

The following significant negative correlations were obtained from a Pearson correlation analysis performed on the biochemical composition of eggs, fertilization and hatching rates and the date of spawning. The following negative significant correlations were obtained:

Fertilization	- saturated fatty acids	P<0.01	n=11
	- 16.0	P<0.01	n=11
	- 18:1n-7	P<0.01	n=11
Spawning date	- organic weight	P<0.001	n=71
	- total fatty acids	P<0.01	n=18
	- saturated fatty acids	P<0.001	n=18
	- polyunsaturated fatty acids	P<0.001	n=18
	- (n-3) PUFA	P<0.01	n=18
	- (n-9) fatty acids	P<0.01	n=18

In groups F1 and F2, spawnings were obtained as expected. In groups F3B and F3C, spawnings occurred as expected (*i.e.* 2 to 3 months later than in group F2), only in 1990. In 1989, however, spawnings started only 1 to 2 months later than F1 and the spawning period was shorter than under natural conditions.

Hatching rates negatively correlated with the carbohydrate content (% of weight) of the newly-hatched larvae (P<0.01) and a further progresive decrease in the average organic weight of the newly-hatched larvae has been observed as spawning seasons progressed.

The biochemical composition of both non-buoyant and overripen eggs was similar but significantly different from buoyant and fertilized eggs. Egg weight, carbohydrate, and total fatty acid (particularly saturates and (n-3) PUFA contents were higher in the latter but ash and relative lipid content were lower. If the spawning period was shifted, the individual weight of fertilized eggs decreased progressively. This reduction is the result of a loss in both protein and lipid content. Triacylglycerols and fatty acids, particularly (n-3) PUFA and (n-9) also decreased. It is important to point out that these lipidic components are the main energetic source during embryogenesis and the early development of larvae. The fertilization and hatching rate did not correlate well with the biochemical composition of the eggs. However, the results obtained for shifted spawnings (loss of egg weight and energetic components) could indicate a poorer egg quality. Similarly, Devauchelle *et al.* (1982) reported higher protein, lipid and PUFA levels in eggs from wild turbot than from captive fish. The biochemical composition of eggs from wild fish probably is an optimal indicator of eggs quality.

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ENERGY METABOLISM DURING EARLY ONTOGENESIS OF TURBOT (*SCOPHTHALMUS MAXIMUS*) AND THE EFFECT OF STARVATION

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Introduction

Data concerning growth and biochemical changes during early development of eggs and larvae of turbot have been previously reported (Planas *et al.*, 1989, in press; Rinnestad, 1989). However, the effect of feeding and starvation of turbot larvae has not been previously studied. In this paper the quantitative changes in the biochemical composition during early ontogenesis as well as the effect of feeding and starvation in young larvae are described.

Materials and methods

Turbot eggs from a single batch were artificially fertilized. Buoyant eggs were incubated and hatched at 15°C. The larvae were separated in two groups. One group was starved during the experiment and the other one was fed from day 3 on with rotifers enriched with Protein Selco (Artemia Systems SA, Gent, Belgium). The temperature in the rearing tanks was progressively increased up to 18°C. The experiment was terminated at day 7. Samples of eggs, larvae (days 0, 2, 5 and 7) and yolk sacs were taken for biochemical analysis.

Results and discussion

Changes occurring in the biochemical composition are given in Table I. During embryogenesis (from fertilization to prehatching), lipids and particularly triacylglycerols (TAG), were the main energetic sources. On the contrary, developing eggs showed increasing protein and phospholipid levels due to new tissue formation. A similar pattern of utilization and synthesis has been observed in yolk sac larvae during the first 2 days after hatching. During this period about 95% of the yolk sac volume has been resorbed. The yolk sac of marine fish larvae is rich in TAG. Thus, yolk sac resorption correlated with the TAG disappeared within 2 days after hatching. On the other hand, the increasing utilization of sterol esters+waxes (SE+W) corresponds with the important reduction of the oil globule volume, as pointed out by Rinnestad (1989). No information is available on the biochemical composition of oil droplets in turbot, but steryl and wax esters are the

major biochemical components in other species . From day 2 to 5, the yolk sac completely disappears and, according to Rinnestad (1989), comprises less than 15% of its original volume in eggs. During this period, the utilization of SE+W and TAG continues, especially in the former. There is a considerable reduction in the weight of the starved larvae by day 5.

Table I. Increase or decrease (in µg/ind.) of biochemical components in various developmental stages (values for starved larvae are given between brackets)

Stages	Protein	CH	Lipids	SE+W	TAG	FFA	Sterols	PL
EGG-PRE	1.27	0.38	-0.84	-0.12	-0.54	0.08	-0.11	0.71
LD0-LD2	2.24	0.12	-2.64	-0.66	-0.93	-0.02	-0.07	-0.37
LD2-LD5	5.03	1.33	-0.16	-0.84	-0.54	0.02	0.11	-0.18
	(-6.73)	(-0.42)	(-3.96)	(-0.90)	(-0.81)	(-0.19)	(-0.08)	(-2.09)
LD5-LD7	10.81	0.04	1.40	-0.27	-0.13	0.01	0.06	0.17
	(-2.45)	0.03	(-0.13)	(-0.09)	(-0.01)	(-0.03)	(-0.04)	(-0.01)

As a consequence, all the biochemical components decrease, especially those involved in structural tissue (protein, phospholipids). By day 7, fed larvae had increased their organic weight from 36 (newly-hatched larvae) up to 49.7µg, whereas the weight of starved larvae had decreased to 17.9µg. The oil droplet was still visible in 47% of starved 7-days-old larvae but only in 16% of the fed larvae. This observation correlates very well with the abrupt decrease in SE+W utilization rate in the starved larvae from day 5 to 7. Simultaneously, a preferential depletion of body protein occurs.In conclusion, starvation was strongly associated with a rapid utilization of lipids and proteins followed by an autolysis of body tissues, particularly proteins.

During embryogenesis, particularly in the latter period, and the first 2 days of larval rearing all the most important fatty acids decrease (Table II). Saturates, monoenes and polyunsaturates are depleted at a similar rate. The higher consumption rates observed correspond to the fatty acids 16:0, 22:6n-3, 18:1n-9 and 20:5n-3. After first feeding the fatty acid content of larvae progressively recuperates, but monoenes (mainly 18:1n-9 and 16:1n-7) continue to be catabolized. Starvation of larvae was especially critical from days 2 to 5. During this period more than 50% of the fatty acid reserves of the larvae disappear particularly the fatty acids of the series (n-3) PUFA, n-9 (18:1n-9) and the fatty acid 16:0. Therefore starvation changes the pattern of fatty acids utilization. Preferences of particular fatty acids for catabolism seems to be more strict in fed larvae than in starved larvae.

Table II. Increase or decrease (in µg/ind) of fatty acids in various developmental stages (values for starved larvae are given between brackets)

Stages	14:0	16:0	16:1n-7	18:0	18:1n-9	20:5n-3	22:5n-3	22:6n-3
EGG-PRE	-0.06	-0.20	-0.06	0.01	-0.08	-0.07	-0.02	-0.17
LD0-LD2	-0.08	-0.34	-0.12	-0.08	-0.26	-0.17	-0.07	-0.24
LD2-LD5	-0.01	-0.04	-0.06	0.10	-0.08	-0.10	0.01	-0.12
	(-0.07)	(-0.44)	(-0.12)	(-0.20)	(-0.34)	(-0.25)	(-0.10)	(-0.47)
LD5-LD7	-0.02	0.04	-0.01	0.08	-0.01	0.02	0.04	0.03
	(0.00)	(0.02)	(0.01)	(-0.02)	(-0.03)	(0.00)	(0.00)	(-0.01)

Stages	Sat.	Mono.	Poly.	(n-3)	(n-6)	(n-7)	(n-9)	(n-3) PUFA
EGG-PRE	-0.25	-0.21	-0.27	-0.29	-0.01	-0.07	-0.14	-0.26
LD0-LD2	-0.51	-0.47	-0.58	-0.49	-0.07	-0.17	-0.30	-0.49
LD2-LD5	0.07	-0.12	-0.04	-0.10	0.12	-0.08	-0.05	-0.20
	(-0.74)	(-0.63)	(-1.02)	(-0.88)	(-0.17)	(-0.21)	(-0.42)	(-0.83)
LD5-LD7	0.10	-0.02	0.08	0.00	-0.03	0.00	-0.02	0.11
	(0.04)	(0.09)	(0.09)	(0.05)	(0.08)	(0.03)	(0.06)	(0.00)

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ORGANOGENESIS IN TURBOT *SCOPHTHALMUS MAXIMUS*, LARVAE RELATED TO THE MAIN DEVELOPMENTAL STAGES

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Introduction

Studies on the development of turbot larvae have received increasing attention during the last few years. Rates of growth in yolk-sac stage larvae reared in the laboratory were first reported by Jones (1972). Developmental stages from hatching to the completion of metamorphosis were defined on the basis of morphological characters by Al-Maghazachi and Gibson (1984) and histological observations (mainly related to the description of the digestive tract) were reported by several authors (Cousin and Baudin-Laurencin, 1985; Cousin *et al.*, 1986). However, no literature is available on morphological characters to histological data. The aim of the present work is to study turbot organogenesis during the larval periods as defined by Al-Maghazachi and Gibson (1984).

Materials and methods

Turbot larvae were reared in 7 000 l. tanks in a flow-through system. At day 36 (post-hatching) they were transferred to weaning tanks (T = 18±3°C; salinity = 33.5-35.5ppt; dissolved O_2 = 95-100% saturation). Larvae were fed on rotifers and *Artemia* and weaned with pelleted food from day 38 to day 65. About 20 to 30 individuals were collected every 2 days until day 23 and every 3-4 days during the remaining period. Standard length (SL) was measured on live specimens after anaesthetizing them with MS-222. All larvae (n=780) were classified into one of the five main developmental stages as defined by Al-Maghazachi and Gibson (1984). Every sampling day, 10 individuals were fixed in 10% buffered formalin and embedded in 2-hydroxyethylglycol methacrylate. The histological study was based on serial sections (2-3µm) stained with Toluidine blue, Haematoxilin-Eosin and Schiff Periodic acid method.

Results and discussion

Table I summarizes the main morphological characteristics studied to classify the developmental stages during the larval periods of turbot.

Table I. Morphological characters for classification of developmental stages (Al-Maghazachi and Gibson, 1984)

Developmental stages	Main characteristics at each stage
Stage 1	Larvae symmetrical, yolk sac present
Stage 2	Larvae symmetrical, development of spines and air bladder
Stage 3	Larvae symmetrical, appearance of fin rays, notochord straight
Stage 4	Larvae asymmetrical, eye migration, notochord slanted dorsally
Stage 5	Larvae asymmetrical, completion of eye migration, spines and swimbladder resorbed

The growth curve relating the standard length to the integrated water temperature is shown in Fig. 1. The larvae grew relatively slowly throughout the first periods. The daily growth rate increased after the onset of metamorphosis (320 degree-days, 7.52mm SL, day 19). In Fig. 2 the size distribution related to developmental stage is shown. The mean larval size became markedly varied with increasing days, the largest variations corresponding to stage 4 (beginning of the metamorphosis) and 5. Similar results have been reported by other authors (Seikai *et al.*, 1986; Fukuhara *et al.*, 1986) in other species.

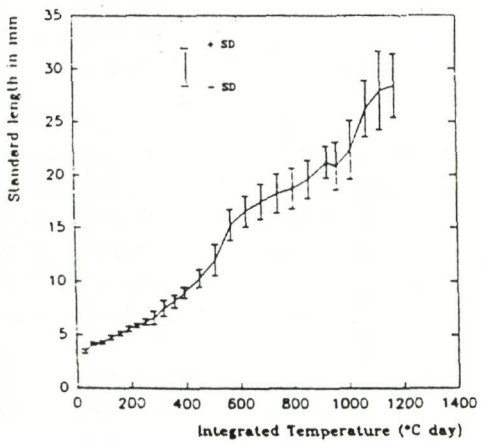

Fig. 1. The relationship between the standard length and integrated water temperature for turbot.

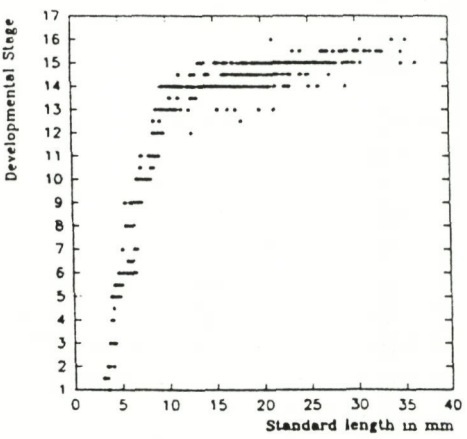

Fig. 2. Size distribution related to developmental stages of turbot larvae. Stage 1(1-4), stage 2(5-7), stage 3(8-9), stage 4(10-13) and stage 5(14-17).

Histological observations show that the different structures do not display the same pattern of development throughout the larval period. Changes in respiratory and osmoregulatory structures as well as vascular development are especially intense from stages 2 to 3. Filaments and lamellae can be observed in early stages 2 and 3, respectively. Chloride cells, in stage 1, are mainly located in the external epithelium whereas they appear in the parabranchial cavity at stage 2. The gill filament exhibits the main juvenile features at the end of stage 3. A large number of immature cells, probably immature erythrocytes, can be seen at the beginning of stage 2, some of them attached to the inner endothelium of the heart. In stage 3 most of them are mature erythrocytes. Modifications in the caudal kidney are the formation of a new tubular system (early stage 3) and glomerular structures (stage 4), giving rise to the definitive mesonephric structures.

The evolution of the digestive system has already been described by Cousin *et al.* (1986). From our observations it is, however, apparent that a large variety of morphologies of the gut (different degrees of necrosis and vacuolation in the epithelial cells) has been observed amongst larvae, mainly during stage 4. Swim bladder morphogenesis shows an erratic pattern of evolution from stage 3 to 5 (persistence of an open pneumatic duct, no inflation or retarted inflation and late degeneration of the bladder). Changes in the hepatic morphology are recorded especially at the end of stage 2. Endocrine organs display different patterns of development. The endocrine pancreas is one of the structures which appear first; the thyroid gland and Stannius corpuscles develop later, at stage 4. Thymic and splenic structures are first seen at stage 3 but complete morphological organization of the immune system is only observed after metamorphosis.

We may conclude that the main critical periods throughout the development of turbot larvae are: 1) appearance of respiratory and osmoregulatory structures (stage 2-3); and 2) changes in the morphology of the digestive tract (stage 3-4). The fact that the immune system is not completely developed at stage 5 may account for and impairement of the immune response to any infectious agent present in the environment (Gatesoupe, 1990).

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CHARACTERIZATION OF STARVED *VERSUS* FED SUMMER FLOUNDER, *PARALICHTHYS DENTATUS*, LARVAE AND JUVENILES

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Introduction

The impact of insufficient food ingestion by fish during early stages has obvious and critical effects on their subsequent growth. Therefore, the ability to identify and diagnose the starved condition in fish larvae provides a useful tool both to understand feeding cycles and to assess the suitability of artificial rearing systems. We conducted an experiment to characterize summer flounder, *Paralichthys dentatus*, larvae and juveniles using biochemical, morphometric, and histological criteria after the fish had been subjected to conditions of starvation or normal feeding. We report here the biochemical results.

Materials and methods

Adult *P. dentatus* were artificially spawned in the laboratory. The embryos were placed in a 37 l glass aquarium containing 30ppt filtered, UV-treated seawater at 19±1°C. Three days after hatching, the larvae started feeding on rotifers which were added to the tank daily. After 20 days, Reference *Artemia* III were offered for the first time, and the rotifer supply progressively reduced. Settlement to the bottom started on day 35 after hatching. On days 16 (early larvae), 33 (pre-metamorphic larvae), and 60 (post-metamorphic juveniles) after hatching, a subsample of animals was randomly removed and assigned to either of two smaller aquaria: one receiving food (control) and the other deprived of it (starved). Fish from the original aquarium were also subsampled to describe basal conditions. On at least three occasions between the time of transfer and the time of mortality due to starvation, 30 fish were sampled from each of the starved and control aquaria as follows: 10 fish for RNA, DNA, and protein determinations, 10 for morphometric and dry weight measurements, and 10 preserved in Dietrich's fixative for subsequent histological analysis.

Results

The RNA:DNA ratios and total protein values of control fish remained relatively constant compared to basal values, whereas those of starved fish decreased markedly during the starvation period (Tables I, II, III). RNA:DNA ratios in control juvenile fish were much

larger (>8) than those in control larvae (*circa* 2.5 - 3.5). These results will be correlated with histological and morphometric results, when they are available.

Table I. Standard length, individual protein content and RNA:DNA ratios of fed *versus* starved *P. dentatus* early larvae (16d at start). Values are given as mean ± standard error

	Group	Standard length (mm)	Protein (µg)	RNA:DNA
0	Basal	5.16±0.20	27.79±4.03	2.81±0.26
24h	Control	4.85±0.21	20.32±4.05	2.97±0.30
	Starved	4.61±0.18	11.95±2.84	2.49±0.19
48h	Control	5.37±0.17	22.73±5.59	2.99±0.33
	Starved	4.73±0.24	12.26±2.36	2.21±0.15
72h	Control	5.55±0.17	21.68±4.54	2.97±0.27
	Starved	5.10±0.10	9.82±2.44	1.93±0.14

Table II. Standard length, individual protein content and RNA:DNA ratios of fed *versus* starved P. dentatus pre-metamorphic larvae (33d at start). Values are given as mean ± standard error

Time of sampling	Group	Standard length (mm)	Protein (µg)	RNA:DNA
0	Basal	7.55±0.25	197.06±45.88	2.88±0.09
24h	Control	8.00±0.18	271.96±53.54	3.41±0.19
	Starved	7.35±0.22	207.65±45.56	3.26±0.20
72h	Control	8.24±0.13	252.05±49.42	2.74±0.10
	Starved	7.59±0.15	132.16±30.91	2.44±0.09
120h	Control	8.77±0.16	413.17±80.78	3.08±0.20
	Starved	7.25±0.28	140.48±42.34	2.00±0.09
192h	Control	9.26±0.36	445.15±87.45	2.94±0.20
	Starved	7.17±0.22	75.80±11.15	1.81±0.09

Table III. Standard length, individual protein content and RNA:DNA ratios of fed *versus* starved *P. dentatus* post-metamorphic juveniles (60d at start). Values are given as mean ± standard error

Time of sampling	Group	Standard length (mm)	Protein (µg)	RNA:DNA
0	Basal	12.60±0.61	872.31±110.41	8.49±0.71
72h	Control	14.46±0.65	1 591.56±340.00	8.84±0.72
	Starved	12.59±0.56	646.28±98.01	5.95±0.62
144h	Control	15.74±0.82	2 620.17±729.78	8.86±0.45
	Starved	13.24±0.56	1 020.90±231.20	5.91±0.62
216h	Control	16.63±0.63	3 051.74±847.44	8.17±0.54
	Starved	13.39±0.50	743.11±90.77	4.86±0.43

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INCUBATION OF SOLE *SOLEA SOLEA* (L.), EGGS IN ARTIFICIAL SEAWATER: A TECHNIQUE FOR EGG AND WATER QUALITY ASSESSMENTS

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Introduction

Variable and unpredictable performance is common when fish are reared through their early life history stages. It is possible that some of this variation can be attributed to changes in egg quality rather than to fluctuations in rearing conditions. It has, however, been difficult to separate the effects due to variable seawater quality from differences due to the quality of the eggs in comparative work with eggs and larvae. In this paper a technique that offers a simple method of addressing the problem by using an artificial seawater to standardize egg and larval performance, is described.

Materials, methods and results

Many of the published recipes for artificial seawater (Bidwell and Spotte, 1985) are made up according to the basic composition of seawater described by Lyman and Fleming (1940) with further additions, such as ethylene diamine tetra-acetic acid (EDTA) and sodium meta-silicate (*e.g.* Zaroogian *et al.*, 1969) in order to reduce the active concentrations of some of the more toxic elements. Comparison of the survival of sole eggs and larvae in some of these seawaters (Table I) and in natural seawater from the Conwy estuary has shown no statistically significant differences between them (Table II).

For simplicity, Formula 2 has been used routinely for incubations of eggs and larvae. It is made up at a slightly higher concentration, to a salinity 33ppt adjusted to pH 8.0 and filtered through a glass-fibre filter of 1µm particle retention size. Incubations were carried out at 12°C, in some cases larvae have been reared (feeding on *Artemia* nauplii) through to metamorphosis in volumes of 0.25 and 0.5 l. Chloramphenicol, added to the water to give a concentration of 10 $mg.l^{-1}$, has been used successfully to reduce bacterial contamination. Fifty to 100 eggs or larvae were stocked in each container and kept moving on an orbital shaker rotating at approximately one revolution/sec. Fifty percent of the water was replaced on alternate days (or daily when food had to be renewed), while any mortalities and faeces were removed daily.

Table I. Composition of the artificial seawaters used (concentrations in $g.l^{-1}$ of water)

Formula 1 (Lyman and Fleming, 1940)			
NaCl	23.5	KCl	0.66
$MgCl_2.6H_2O$	5.0	KBr	0.96
Na_2SO_4	4.0	H_3BO_3	0.03
$CaCl_2.2H_2O$	1.1	$SrCl_2.6H_2O$	0.02
$NaHCO_3$	0.2	NaF	0.003
Formula 2			
As formula 1 plus:		EDTA (Na_2)	0.001
Formula 3 (Zaroogian *et al.*, 1969)			
As formula 2 plus:		$Na_2SiO_3.9H_2O$	0.020
Formula 4			
As formula 1 plus:		$Na_2SiO_3.9H_2O$	0.020

Table II. Mean percentage survival of *Solea solea* eggs and larvae, from fertilization until first feeding, when incubated in artificial or natural seawater

	Seawater formula (see Table I)				Natural
	1	2	3	4	seawater
Survival (%)	91.9	91.5	93.2	93.8	95.8
Standard deviation	3.3	5.4	2.4	5.7	2.8
n	4	4	4	4	4

Conclusions

The technique offers a possible means of standardising conditions for performance assessments. In particular, it allows the initial water chemistry to be standardised, enabling the medium to be used for comparisons of eggs incubated at different times during a spawning season. It also offers an opportunity to compare development in a standard medium with that in various, natural seawater samples, providing a potential fish-egg or larva bioassay for assessing effective levels of pollution in coastal waters.

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VARIATION IN EGG AND LARVAL QUALITY IN VARIOUS FISH AND CRUSTACEAN SPECIES

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Abstract

It is generally accepted, that a major constraint in the further development of marine fish and crustacean aquaculture, is the variable quality of eggs and larvae used for the hatchery-production of fry. Nonetheless, valid criteria to evaluate optimal egg quality have not been identified yet. Instead, particular indicators, are used, *e.g.* the fertilization and hatching rate, overall culture success during the first rearing period, *etc.*, but these are not totally objective because hatchery-specific conditions may interfere.

The biochemical composition of the egg may be one of the possible determinants of egg quality, which can be evaluated on a more objective basis. In this respect, studies have been initiated to analyze in freshly-released eggs, the levels of two nutrients which are believed to play a critical role in the early larval development, *i.e.* (n-3) highly unsaturated fatty-acids (HUFAs) and vitamin C. Analytical data have been gathered for various species of marine fishes (*Scophthalmus maximus*, *Epinephelus tauvina* and *E. fuscoguttatus*, *Dicentrarchus labrax*, *Sparus aurata*, and *Pagrus major*) and for the freshwater prawn (*Macrobrachium rosenbergii*). Egg samples have been collected from wild females and from captive broodstock kept under various conditions at various hatcheries.

Results available so far illustrate that for some of the species tested HUFA levels and total lipid content in the eggs vary considerably among spawnings, even when coming from the same hatchery. The highest variation was noted in turbot broodstock, *i.e.* the HUFA levels and total lipid content ranged from 19 to 47mg.g^{-1} DW, and 8 to 30%, respectively. Contents of ascorbic acid in turbot eggs varied up to 100%, respectively 500% among different egg batches provided by two hatcheries.

In other studies, the effect of changing the lipid composition of the broodstock diet on the HUFA level in the eggs and the larval quality was assessed for *E. tauvina* and *S. aurata*. Preliminary results revealed significant increases in 20:5n-3, total HUFA and lipid content of respectively 47%, 18% and 21% in *E. tauvina*. In seabream eggs, only 20:5n-3

levels increased (approximately by 90%). Grouper larvae with an increased HUFA content furthermore displayed an increased survival after a 7 day standard culture period.

These results indicate that the quality of the food available to the maturing females may be one of the main causes for the noticed variability in essential nutrient composition in the eggs and, consequently, may affect egg viability. What minimum levels of these nutrients are required for normal embryonic and early larval development, is, however, not yet known and may also be species-specific.

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(n-3) HUFA COMPOSITION OF FRESHLY SPAWNED EGGS FROM EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*), SEABREAM (*SPARUS AURATA*) AND RED SEABREAM (*PAGRUS MAJOR*) COLLECTED IN DIFFERENT HATCHERIES

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Abstract

The biochemical composition of fish eggs is often used as an indicator for assessing the nutritional requirements of aquaculture species during their larval stages. Fatty acids of the (n-3) HUFA type have proven to play a critical role in providing good growth, survival and stress resistance in marine fish larvae. Hence, analyzing these fatty acids in fish eggs may provide interesting data on the requirements for those essential nutrients. By comparing the analyses of wild *versus* broodstock fish eggs differences may appear, possibly explaining differences in egg quality. Intra-spawning-season differences may further appear by following fatty acid profiles in the course of time. Finally, the influence of broodstock nutritionon egg composition may be another variable to consider in the evaluation of the above.

In this study freshly released eggs of European seabass (*D. labrax*), seabream (*S. aurata*), and red seabream (*P. major*) were collected from broodstock fish held under different conditions in various hatcheries. Temporal variations in fatty acid profiles were monitored by following a seabream broodstock during 1 month. The effect of feeding broodstock with fish-oil coated pellets on the fatty acid profiles of the eggs produced, was established for seabream.

The results indicate that for the three species the content of 22:6n-3 is considerably higher than 20:5n-3. Total (n-3) HUFA on a dry weight basis appears highest in seabass while on a relative basis (% of total fatty acids) the highest levels are found in seabream eggs and the lowest in red seabream. Differences between seabass eggs from females caught in the wild and naturally spawned broodstock fish kept in captivity, occur particularly in the lower content (half) of 22:6n-3 in the wild eggs.

Differences within the same species cultured at different locations and hence under different conditions are insignificant. The variability is in the range of 2.9 to 9.3% for the relative values and 8.6 to 18.9% for the absolute figures. The effect of changing the fat

composition of the broodstock diet was noticed within a 1 day period in the eggs produced by that fish . The increase in 20:5n-3, originating from the cod liver oil used for coating the broodstock pellets, disappeared within the second day after feeding the enriched pellets. This confirms fast metabolism and transport of lipid nutrients from the broodstock fish to the eggs.

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EGG QUALITY IN WILD AND BROODSTOCK COD (*GADUS MORHUA* L.)

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Abstract

Problems concerning egg viability (or quality) have received increasing attention, in relation to cultivation as well as to the assessment of reproduction of wild fishes. The main objectives of this paper are to discuss:

- cell morphological characteristics as indicators of egg quality in cod;
- natural mortality rates of cod eggs in the sea;
- quality of cod eggs from a broodstock;
- egg viability in wild *versus* captive fish.

Cell morphology of the earliest cells is considered as a possible criterium for determination of viability in fish eggs. The occurrence of cellular malformations in early cleavage stages of cod eggs has been correlated with later egg and larval survival, and it seems that such morphological characteristics can be used to predict normal development and mortality rates.

Natural mortality rates of fish eggs is not well understood. The studies on cod have been concentrated on egg quality in broodstock fish during a spawning season, as well as on an estimation of the egg quality of newly spawned eggs in the sea. The broodstock was kept in a spawning basin at the University of Tromsø, and field sampling was carried out in the Lofoten area, which is a major spawning ground for cod in Norway.

Results from our recent work revealed that approximately 10% (mean value) of eggs from both wild cod (planktonic samples) and from broodstock cod (naturally spawning), had abnormal cleavages at the 2-8 cell stage. Such abnormal eggs showed very poor survival and hatching rates, even though it appeared that more advanced embryonic stages proceeded to develop normally. For broodstock cod, egg viability was highest during the peak of the spawning season.

The results are discussed in relation to egg viability of other marine fishes.

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EFFECTS OF VITAMIN C IN BROODSTOCK DIETS ON EGG QUALITY OF COD (*GADUS MORHUA* L.)

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Abstract

The effects of dietary ascorbic acid on reproduction success of Atlantic cod (*Gadus morhua* L.) have been investigated. Groups of adult Atlantic cod were fed different levels of a stable monophosphate ascorbic acid incorporated in a commercial feed.
The levels of ascorbic acid ranged from zero to mega dose. The feeding was started 3 months in advance of the spawning season. Incorporation of the vitamin C in different tissues was measured at three time points before spawning for all feeding groups.

Shortly prior to spawning the broodfish were transferred to large submerged polyethylene bags. Eggs from the different feeding groups were obtained from natural spawns. The bags were supplied with an upwelling flowthrough system that enabled egg collection in separate plankton netcages mounted at the water outlet. Several egg batches were incubated in a full-scale hatchery where egg quality and larval performance were measured and monitored until completion of yolk absorption. Eggs from the separate batches were sampled from the egg incubators at four time points throughout the egg stage.

Egg quality was characterized by fertilization percentage, buoyancy, content of free amino acids, hatching percentage and egg pressure resistance. The chosen quality parameters have been described for other species elsewhere. The content of vitamin C in the egg was also measured throughout the spawning season. Good correlation between dietary and egg vitamin C was found. Larval performance was monitored as survival rates throughout the yolk-sac stage. Results from the experiments so far indicate low demands for dietary ascorbic acid in the broodstock diet with regard to egg quality.

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SPERMATOCRIT AND SPERM SWIMMING SPEED DO NOT CORRELATE WITH FERTILIZATION SUCCESS IN ATLANTIC COD (*GADUS MORHUA*)

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Introduction

The identification of sperm quality in fishes as an indicator of an individual's potential to contribute future members to a population, has received relatively little attention compared with the extent of research conducted on egg quality (Kjørsvik *et al.*, 1990). Traditionally, we perceive measures of male quality to include spermatocrit and sperm motility. Spermatocrit is simple to measure, whereas assessment of sperm motility has been restricted to coarse, qualitative scores, for example ranking the activity of sperm on a scale of 1 to 10 (Munkittrick and Moccia, 1987; Westin and Nissling, 1991). Qualitative scores of this kind cannot be easily interpreted by other investigators and do not provide the basis necessary to compare, *e.g.* sperm swimming speeds of different species. In this study, we assess these characteristics of sperm and test the hypothesis that sperm characterized by high spermatocrit and high motility will fertilize a greater percentage of eggs than sperm having lower measures of these characteristics. In addition, a new approach to assessing sperm swimming speeds using video recorded images is presented.

Materials and methods

Experimental work was conducted at the St. Andrews Biological Station using cod (*Gadus morhua*) that had been held in captivity for 3-10 years. Sperm of 12 males ranging in size from 57 to 99cm FL (2.1-10.2kg) were used to fertilize eggs of a single female 62cm FL. Gametes were stripped and fertilizations conducted on batch sizes of approximately 130 eggs. The date of stripping was based on time trends of egg observations and roughly corresponds to the mid-date of spawning for these fish. Fertilizations were carried out at sperm: seawater dilutions of 1:1 and 1:500. The latter dilution should provide an environment in which sperm potency of various individuals may be more rigorously assessed than is possible using common aquaculture techniques that employ dry fertilization. Spermatocrit was measured by spinning samples of sperm for 10min at 7 500rpm (5 500*g*). For each individual, the mean of two samples is presented (values varied by <12%).

Sperm swimming speeds were determined of individual spermatozoa at a 1:100 sperm:seawater dilution, viewed with a compound microscope at 500X and video recorded. From a subsample, the distance moved in 30sec by each sperm was measured against a graduated background (haemocytometer) from the recorded images. Video recording of each sample commenced about 30sec after the sperm had been diluted. Duration of motility was 8-10min, considerably longer than that noted for salmonids (Terner, 1986). Measurements were not taken when currents occurred under the cover slips. Fertilization success was measured by determining the percentage of eggs of each batch that was fertilized after 24h.

Results

Fertilization success for each of the 12 males was greater than 95% at the concentrated sperm:seawater dilution of 1:1, whereas fertilization success for these same males ranged from 21 to 100% at a sperm:seawater dilution of 1:500. Spermatocrit varied from 17.5 to 98.5 (% packed cells) among the 12 males. No significant correlation existed between spermatocrit and fertilization success at the 1:500 dilution (Fig. 1).

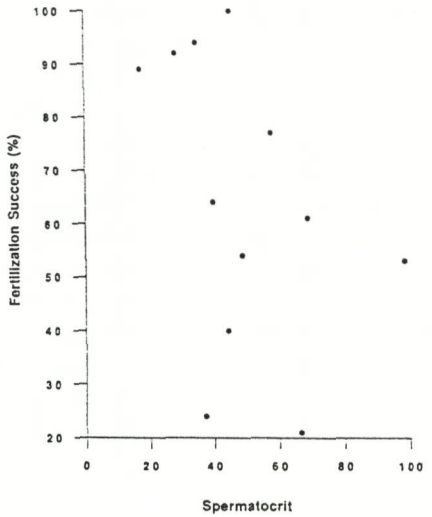

Fig. 1. Relationship between spermatocrit and fertilization success in Atlantic cod from the Scotian Shelf.

Average sperm swimming speed varied from 0 to 210μm per 30sec among the 14 males. No significant correlation existed between average sperm swimming speed and fertilization success at the 1:500 dilution (Fig. 2).

Discussion

Examination of sperm characteristics typically used by aquaculturists and population ecologists to gauge sperm potency failed to provide any insight into explaining variablity

in fertilization potential among males. Our findings caution investigators using spermatocrit and sperm motility as indicators of gamete quality. The method presented of measuring sperm swimming speeds provides an opportunity to video tape sperm of a large number of males in a single day. This permits detailed analysis of the sperm at a later date in which, in addition to sperm motility other characteristics may be assessed; such as head size, tail length, and percent occurrence of sperm in a state of vibration or non-vibration. Alternative techniques of assessing sperm quality which could assist in explaining these results might incorporate physiological and biochemical aspects of sperm.

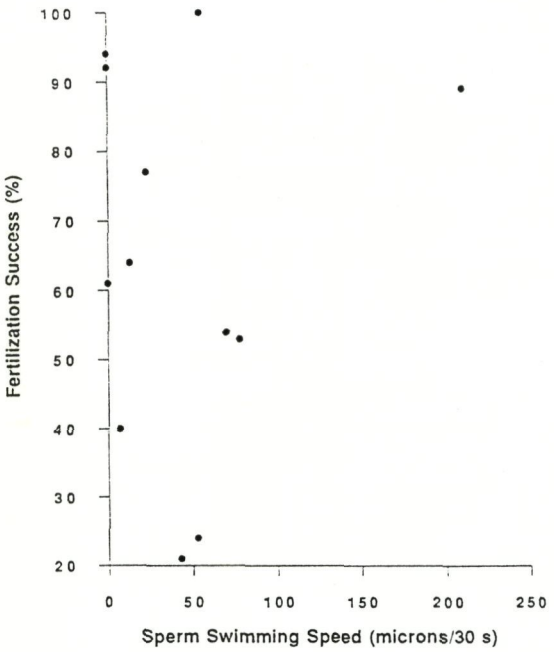

Fig. 2. Relationship of sperm swimming speed and fertilization success in Atlantic cod from the Scotian Shelf.

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EFFECTS OF INCUBATION TEMPERATURE ON EMBRYONIC DEVELOPMENT AND HATCHING OF *DICENTRARCHUS LABRAX* (L.) EGGS

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Introduction

The duration of embryonic development of bony fish is generally related to the incubation temperature. Many authors (*e.g.* Fowler, 1970; Camus and Koutsikoupoulos, 1984) described the effects of incubation temperature on the same batch of embryos with regard to both the morphology and some functional aspects of the hatched larvae. This paper examines the effects of three experimental temperatures on sea bass embryonic development, taking into account duration and asynchronies.

Materials and methods

The experiment was carried out at the Stazione Sperimentale di Acquacoltura Termica, ENEL, Torrevaldaliga (Rome, Italy). *Dicentrarchus labrax* eggs (2 blastomere stage), from a single natural spawn, were divided into 36, 1 l beakers, each containing 3g of eggs. The beakers were equally divided into three aquaria, thermoregulated respectively at 14±0.1°C, 18±0.1°C and 22±0.1°C. From the spawning temperature (13.7°C), experimental temperatures were gradually attained (2°C.h^{-1} until 18°C, afterwards 1°C.h^{-1}). Water chemistry and physical parameters were measured daily. At each temperature samples were collected every 4h and fixed in glutaraldehyde (GA) 2.5% buffered in Na- cacodylate 0.1 M pH 7.2, for SEM observations. *In vivo* examinations (n=30 for each temperature, every 1-3h until hatching) were also carried out to detect developmental stages. The data for time to each developmental stage were analyzed by stepwise multiple regression to describe embryonic development with respect to temperature and time (Colby and Brooke, 1973). Some histological observations were carried out on 3-day-old larvae, using GA-fixed specimens, dehydrated in acetone series and embedded in Agar-100 resin. Some sections have been deresinated and stained with hemalaun, alcian blue and eosin, toluidine blue and PAS.

Results and discussion

Developmental times from segmentation to hatching observed at every tested temperature are given in Table I. Regressions of developmental time on temperature were calculated from the equation log y = a+b log x, and the values for the parameters a and b are given in Table I. Low correlation coefficient values for stage 1 clearly emphasize the acclimation phase, meanwhile r-values ($P<0.05$) for stage 2 indicate the first slight temperature-induced acceleration effect. The second model applied from stage 1 to hatching for constant incubation temperatures (Colby and Brooke, 1973) assumes that developmental stage is a function of two independent variables, temperature (T, °C) and time (H, hours). The best fitting equation as determinated by the stepwise method at the 0.1% F level is:

$$y = 65.4 + 0.031\ H - 907.1\ H^{-2} + 948.8\ T^{-1} - 5065.4\ T^{-2} + 1252.2\ (T*H)^{-1} - 1.342\ (T/H)^2 + 66.03\ T*H^{-2} + 0.91\ T*H^{0.2}$$

and shows a very high correlation (0.993), with experimental data well suited to embryonic development rate of seabass. This equation should be used in interpreting estimates from future regressions which include other environmental descriptors which could affect embryonic development.

Table I. Seabass egg development rate. Time (in h) required for 50% of the surviving seabass egg to reach the successive developmental stages, from acclimatation period to hatching

Developmental stage	Developmental time (h)			Intercept	Slope	r
	14°C	18°C	22°C	(a)	(b)	
1. Morula	7.10	7.00	6.30	2.645	-0.206	0.162
2. Formation of the blastodisc Migration of germ ring past equator	23.30	14.45	10.30	5.115	-0.875	0.587
3. Embryonic streak formed. Yolk plug	31.30	19.45	14.30	7.496	-1.557	0.988
4. 5 somites. Formation of the cephalic area	36.00	22.30	16.30	8.125	-1.728	0.996
5. 8-10 somites. Cephalic area and optic cup formed Chromatophores along entire length of body	40.45	27.00	18.30	8.686	-1.853	0.981
6. 18-19 somites. Chromatophores on oil globules	51.00	34.00	24.30	8.56	-1.726	0.982
7. 26 somites. Otic capsule. Otholites Fin movements. Arhythmic heartbeat	62.30	42.30	30.30	8.317	-1.583	1
8. Olfactory placode. Rhythmic heart beat (80-90/min)	66.30	44.00	31.30	9.215	-1.87	0.986
9. Hatching (10%)	82.00	50.00	37.00	9.061	-1.769	0.997
10. Hatching (100%)	93.00	59.00	44.00	8.908	-1.662	0.998

Total length, yolk diameters, survival and anomalies at hatching, for each temperature, are given in Table II. Total length at hatch is less for larvae incubated at higher temperatures ($P<0.05$). The same was observed in many other fish (Pipe and Walker, 1987; Brooke, 1975) but not in seabass (Marangos *et al.*, 1986). Differences observed may be related to the different moment at which eggs were acclimated to experimental temperatures (3h from spawning in our study *versus* 46h). The acceleration of metabolism at higher temperatures induces the consumption of yolk sac already at hatching (Katavic, 1980).

Table II. Survival, total length, yolk consumption, and anomalies at different temperatures at hatching. One way analysis of variance one way and multiple comparison are applied to total length and yolk diameter data

Temperature (°C)	Mean incubation period (h)	Survival (%)	Total length (mm±SD)	Yolk length (mm±SD)	Anomalies
14	87	81	3.103 ± 0.12	1.383 ± 0.079	6.7
18	54	79.8	3.013 ± 0.23	1.429 ± 0.093	53.3
22	40	77.1	2.755 ± 0.14	1.28 ± 0.069	80
F			29.241	23.611	
DF			2-82	2-82	
14 *vs* 22			P<0.05	P<0.05	
18 *vs* 22			P<0.05	P<0.05	

SEM observations emphasize some asynchronies in high temperature-induced acceleration in development rate. In fact, different organs and tissues appear at different times in seabass embryos of the same stage, but reared at different temperatures. Three days after hatching, differences in developmental rates for the different organs became more evident, as emphasized by SEM and histological observations. They were represented mainly by: number of lateral line free neuromasts, appearance of pectoral fins, mouth opening, appearance of pharynx mucous cells, inner ear formation, alimentary canal differentiation, pancreas, liver, islet of Langerhans, swim bladder primordium formation. Furthermore, many differences appear in the number and type of skin mucous cells in newly-hatched larvae incubated at 18 and 22°C, but this requires a different approach not yet applied in this preliminary study. From the results obtained in this preliminary study, most (80%) developmental anomalies and functional-morphological differences seem to be related to higher incubation temperatures. Indeed different tissues and organs could react with organogenetic times to different temperatures. This causes asynchronies that influence the correct morpho-functionality of the larvae.

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CONTROL OF REPRODUCTION IN EUROPEAN SEABASS (*DICENTRACHUS LABRAX*) BY MEANS OF ECOPHYSIOLOGICAL MANIPULATION

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Introduction

The increasing demand of fingerlings of euryhaline species for intensive fish farming, valliculture and mariculture has promoted a great increase in the number of hatcheries in Italy as well as other European countries. Presently, fingerling production is still limited to the natural breeding period, because eggs and fry are only available at this season. The problem of the shortage in the supply of the most valuable species may be solved using different ecophysiological conditioning regimes to advance or delay the reproduction period of broodstocks. This research is aimed to optimize ecophysiological conditioning in European seabass, *Dicentrarchus labrax*, in order to obtain fertile eggs without hormonal treatment for a period of more than 5 months.

Materials and methods

Four cylindrical fiberglass tanks ($12m^3$) have been used for the experiments. In all tanks, fish biomass was 4 $kg.m^{-3}$ and the sex ratio 1:1. The males were aged 3 to 5 years and the females 5 year. One month before the start of the spawning season and during the trials, only fresh food was administered. In order to lengthen the breeding period, broodstocks were subjected to adapted regimes for photoperiod (plus 1h every 2 weeks for 14 weeks, then 2 weeks at the reached photoperiod and, afterwards, minus 1h every 2 weeks for 12 weeks), temperature (minus 1°C every 2 weeks for 28 weeks) and salinity (plus 0.7ppt.l^{-1} week for 28 weeks) (Fig. 1). To avoid artificial hormonal stimulation, this ecomanipulation was applied at two different times within the natural range of breeding periods of this species at different latitudes : the first was from October 1 to November 7 and the second from January 1 to February 7. Spawning started at the beginning of these periods and was approximately extended for 1 month. Twice a day, laid eggs were mechanically collected, checked, disinfected, weighed and then transferred to incubators. The most important physico-chemical parameters (dissolved oxygen, pH, ammonia, nitrites, nitrates, phosphates) of the water were monitored daily.

Results

Water parameters constantly fell within the optimal range for the species (Barnabé and Billard, 1984). In the first spawning cycle (Oct. 1 to Nov. 7) a total of 8kg of hydrated eggs was collected, which was equivalent to 12.5% of female weight while, in the second cycle (Jan. 1 to Feb. 7), 9kg were obtained, equivalent to 14% of the female weight. A small increase in egg fertility was observed in the second period.

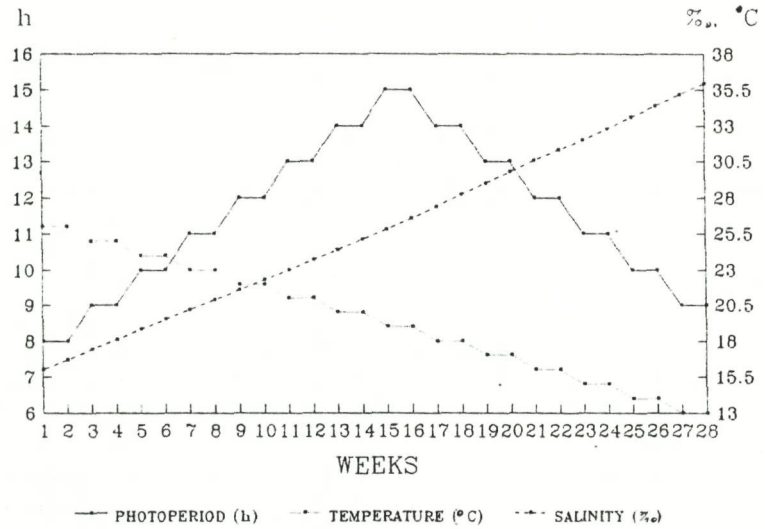

Fig. 1.*Dicentrarchus labrax*: conditioning program.

Discussion

In this preliminary experiment, we have applied ecophysiological conditioning on seabass broodstock to obtain eggs over a period of 8 weeks within the natural spawning interval and without hormonal stimulation. The quantity of laid eggs was 12 to 14% of female body weight. From our results and those by other authors (Devauchelle, 1984; Carrillo *et al.*, 1989) we expect to be able to get fertilized eggs year-round without exogenous hormonal stimulation. This could affect the future availability and market price of fingerlings due to the reduction in production costs.

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DEVELOPMENTAL ABNORMALITIES IN EGGS OF GILTHEAD SEABREAM (*SPARUS AURATA* L.) FOLLOWING SPAWNING INDUCED WITH LH-RH ANALOGUES

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Introduction

Egg quality may be regarded as the potential development capacity of the egg to produce viable fry (Kjørsvik *et al.*, 1990). In the case of the gilthead seabream, *Sparus aurata* L., egg buoyancy and lipid droplet fractionation have been empirically utilized to estimate initial egg quality.
In the present work, we have adopted these and new morphological parameters to check how egg quality might be affected by low and high dosages of the ovulation-inducing LH-RHa and by egg mishandling.

Materials and methods

We have examined the quality of eggs laid by females bearing ovarian follicles larger than 500µm and injected with either 1 (group A: wild fish; group B: domestic fish) or 10µg.kg^{-1} BW LH-RHa (group C: domestic fish). Seabream were kept unfed during a spawning period of 102 days at 18°C and 35ppt salinity. For each spawning group the daily egg production, the percentages of aborted (unsegmented or cytolysed) or coarctate eggs (with an enlarged perivitelline space enveloping not only the embryo, but also the shrunken yolk sac), the degree of fractionation of the oil droplet and its eventual disconnection from embryos with developed hearts were determined.

Results

The fish in groups B, C, and A spawned daily for 83, 101 and 102 days, respectively, losing 22 to 30% of their initial body weight. Females in groups A and C, which had mean maximal follicular diameters as follows: A= 608±7µm, C= 599±4, had total egg productions of A= 1 092 000 eggs/kg BW, C= 1 039 000, but different percentages of aborted (A= up to 23%; C= 72%) and coarctate eggs (A= up to 4%; C= 100%) during the first 2 weeks of spawning. Females in group B, which had smaller follicles (574±6µm), produced only 471 000 eggs/kg BW of which up to 81% and 15% were aborted and coarctate, respectively, during the initial 2 weeks. From the 3rd week of

spawning, these percentages remained low in all three groups, but increased again in the last 4 weeks of oviposition, especially in group C. Fractionation of the oil globule was marked (even 100%) in group C from the beginning of oviposition until day 35 and this phenomenon was again evident in all three groups in the terminal period of egg discharge. The frequency of oil globule detachment from embryos with developed hearts in all three groups was usually below 20% when eggs were collected from the spawning tank effluent within 8h of oviposition, but reached 100% when the delay was 32h.

Discussion

Injection of wild females with 1μg.kg^{-1} BW LH-RHa initiated a very prolonged spawning period with a high egg production and the lowest incidence of egg abnormalities. Although, a dosage of 10μg.kg^{-1} of the same hormone in group C induced a similar spawning performance in terms of day of oviposition and total egg number, egg quality was markedly affected, suggesting that the administered dosage was unphysiological. Similar adverse effects with the same LH-RHa dose were observed in a previous report on the same species (Colombo *et al.*, 1989). By contrast, 1μg.kg^{-1} of Lh-RHa was found to be inadequate in group B.

Deterioration of egg quality seems to be a consequence not only of unbalanced hormonal treatment of spawners, but also of the exhaustion of their metabolic reserves during fasting or following improper egg handling. Oil globule fractionation appears to be induced by both exogenous hormone and fasting, egg coarctation by fasting and mishandling and oil globule detachment from embryos with developed hearts by mishandling only. The last two abnormalities are proposed as novel morphological indicators of egg quality. Coarctation is characterized by a progressive loss of buoyancy and is probably due to water loss, firstly by the yolk sac and then by the embryo. Detachment of the oil globule prevents its covering by chromatophores, probably delays resorption of its fat content and hampers pre-larval swimming and the beginning of feeding since it moves freely within the yolk sac.

Acknowledgement

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DEVELOPMENT OF THE CHEMICAL AND LATERAL-LINE SENSE ORGANS IN *BARBUS BARBUS PLEBEJUS*

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Introduction

The development of sense organs was studied in larval barbels by means of histological and electronmicroscopical methods. This research was carried out within a programme focused on the artificial reproduction of barbels, *Barbus barbus plebejus*, using breeders from autochthonous populations, in order to restock inland waters with fry genetically homogeneous with the wild population. This study intended to gain a better understanding of larval behaviour, which is of paramount significance for the development of techniques aimed at producing juvenile fish that are well adapted to the wild. Literature on the rearing of *Barbus barbus* larvae is available, but it does not deal specifically with developmental details of larvae (Penàz, 1973; Philippart, 1982; Schmidt, 1982; Philippart *et al.*, 1987).

Materials and methods

Breeders were collected from the wild (Mignone River, Central Italy, in June 1990). Fertilized eggs were obtained by the dry method and reared in Zug bottles until hatching (temperature: 20-21°C), and then in small tanks (21 l, 2 000 larvae) at the Stabilimento Ittiogenico della Regione Lazio. The hatching rate was high, reaching 80% . Larval and juvenile stages (from 108 days) were fixed in glutaraldehyde (2.5% in 0.13M cacodylate buffer, pH 7.2), formaldehyde (4% in water) or in Duboscq-Brazil's fluid, for histological and electronmicroscopical studies. The sense organs were studied between 3 and 300 days after hatching, and their distribution was also recorded by means of camera lucida drawings.

Results and discussion

Three days after hatching: yolk sac circulation was evident in the larvae, the pectoral fins were present and the anus was open. Some superficial neuromasts could be seen in the

cephalic region, covered by small cupulae (Fig. 1). Olfactory cup-shaped placodes had already begun to deepen and ciliated cells were differentiated. In the tapetum nigrum of eye, pigmentation was scarce and pigment cell processes were absent; cones and rods were present. Otoliths could be recognized. Larvae swam upwards with uncoordinated movements, then sank to the bottom where they gathered. They showed negative phototactism.

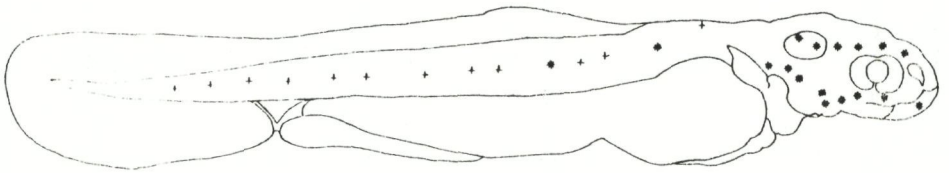

Fig. 1. Lateral line free neuromasts, completely (*) or not yet completely (+) differentiated, in a 3-day-old larva.

At 8 days: taste buds, already differentiated at 5 days, were abundant and distributed on the prenasal region, around the mouth, on the palate, on the copula joidea, in the pharyngeal region, and on the branchial arches. The olfactory cup has two lamellae. The number of cephalic free neuromasts has increased. The eye was pigmented. The larvae were more active and randomly distributed on the bottom.

At 10 days: chemical sense organs were arranged in a characteristic pattern to form a taste "mask" in the prenasal region, also rich in mucous cells. No lateral line neuromasts were comprised in this "mask". The yolk sac was completely absorbed and exogenous nutrition began. *Artemia* nauplii and egg yolk particles were taken up only when sensed at a very short distance (about 1mm). Laterial line free neuromasts formed a longitudinal medial line in the trunk region, reaching the tail peduncle, but they were not yet completely differentiated. The swim bladder's anterior and posterior chambers were activated, and the larvae became planktonic, displayed schooling and no negative reaction to light.

At 15 days: chemical sense organs were distributed in parallel rows on the edge of the mouth dorsally and ventrally. The larvae began to actively prey on plankton (copepods, cladocerans, and rotifers).

At 20 days: in the trunk region the lateral line free neuromasts were differentiated and formed a longitudinal, medial line, a horizontal one at the base of the dorsal fin, and a vertical line at the base of caudal fin. The larvae started to be benthic and feed at the bottom. They were fed powdered trout fingerling pellets, supplemented with vitamins A, D3, and E.

At 47 days: the taste buds were present in the oesophagus lining.

At 108 days: medial barbels began to form; they were rich in taste buds, which were also present in the internal gill chamber. Neuromasts began to deepen in the epidermis in the cephalic region, becoming surrounded by the lateral line tubular canal bones, forming in the opercular region. The olfactory sac opened into two nostrils, the anterior one

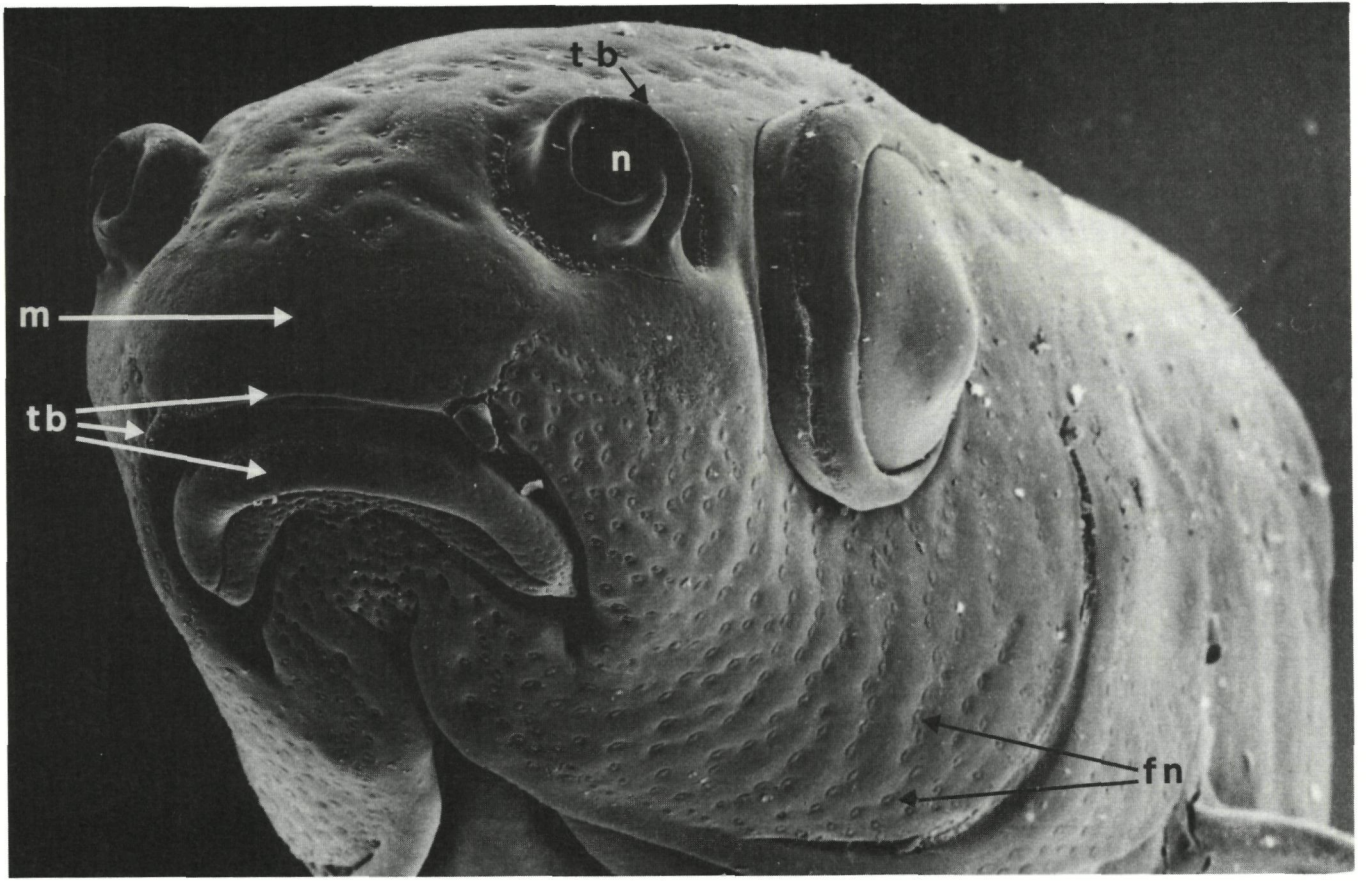

Fig. 2. SEM image of the head of a 250-days-old barbel (n = nostril; m = "mask"; fn = lateral line free neuromasts; tb = taste buds).

tube-like, the posterior one without the valve, which began to develop at about 300 days. Its floor was folded into parallel olfactory lamellae, four on each side. Presence of a complete scale covering and of pelvic fins allows to define this stage as juvenile.

At 185 days: the head lateral line free neuromasts were arranged in longitudinal rows in the ventral preopercular region. The medial lateral line of the trunk was still organized in a single row of neuromasts. Some free neuromasts appeared on the tail, and at the base of the dorsal and caudal fins.

At 300 days: at this stage, the chemical sense organs were distributed in the rostral region, under the nostrils, on the lips and in two characteristic rows on the ventral operculary folds (Fig. 2 and 3). The neuromasts on the head were contained in supra- and infraorbital lateral line canals, but were also arranged in a superficial longitudinal line above the eye, and in a system of arched parallel lines of free neuromasts on the sides, from the mouth to the operculum, reaching the ventral opercular folds. These free neuromasts had two different forms (round or diamond shaped). The trunk lateral line was now made up by free neuromasts in groups of 2, 3, or 4. At the tail there were still free neuromasts, in horizontal parallel lines. Barbels were now four in number, rich in chemical sense organs, and could be used to detect preys under the surface of the substrate.

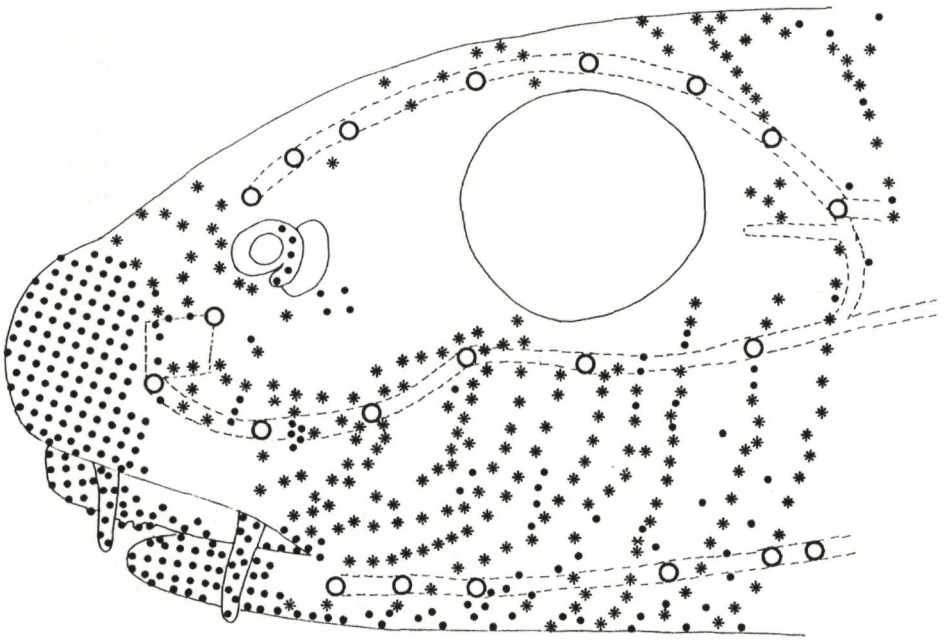

Fig. 3. Distribution of the chemical and laterial line sense organs in a 300-day-old barbel (O = lateral line canal openings; * = neuromasts, • = taste buds).

In the barbel, taste buds develop very early. In the mouth they appear 5 days after hatching, in the pharynx at the 10th day. It is also interesting to note that some taste buds, both inside the mouth, and on the skin, arranged ventrally in the rostral region and near the angle of the mouth, are of Reutter's I type, for which a possible coexistence, in the same sensorial organ, of both chemical and mechanical reception was suggested (Reutter, 1973). Barbel larvae at about 20 days change from pelagic to benthic feeders; this transformation requires an early development of sense organs able to select food particles embedded in sand or mud. The present preliminary observations on barbel larvae (at least those of the population considered) indicate that these show an "early abundance" in chemo- and mechanoreceptors that can be regarded as a character adaptive to the environment of river middle course, which is an environment more variable than upper and lower river courses. Rapid changes in turbidity may hinder the larvae to resort to eyes in prey detection, and rapid changes of water flow and turbulence must be readily perceived, in order to regulate swimming behaviour.

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EFFECT OF DIETARY α-TOCOPHEROL LEVEL ON REPRODUCTION OF *PENAEUS INDICUS*

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Introduction

Erratic quality offspring is generally produced by penaeid broodstock fed pellets, even when these pellets are suitable to ensure a good growth of juveniles. Nutritional requirements of penaeid shrimp are different for growth and reproduction. A particularly high requirement of spawners for polyunsaturated fatty acids (PUFAs) is now established. An effect of some vitamins, like α-tocopherol, acting as a biological antioxidant of membrane PUFAs can be suspected. The aim of this experiment is to determine if this vitamin is required for penaeid reproduction, as it is for fish (Watanabe *et al.*, 1984).

Materials and methods

A total of 48 tagged females (mean weight 11.4g) and 48 males (mean weight 9.0g) *P. indicus*, born at IFREMER, Centre de Brest, were randomly distributed among six tanks of $3m^3$, at the rate of eight females and eight males per tank. Three diets were tested, in two replicates per diet: a compound diet, in which no α-tocopherol was added (EL), the same diet in which 600mg of α-tocopherol acetate/kg (50%) were added (EH), and mussel as control diet (MU). The experiment was conducted over 78 days, including 30 days for diet accustoming and 48 days for experimentation. During the daily control of ovarian maturation, ready-to-spawn females were individually transferred to a spawning tank. The number of eggs and the fertilization rate were determined for each spawning. A sample of 10 000 eggs/spawning was assigned to determine the hatching rate and the larvae viability, estimated by the survival rate at zoea 1 stage. The other eggs were rinsed with freshwater, frozen at -80°C and freeze-dried for analysis. The α-tocopherol concentration in diets and eggs was analysed by chromatogaphy as described by Alvarez *et al.* (1989).

Results

The concentration of α-tocopherol in the diet was $41mg.kg^{-1}$ for EL, $346mg.kg^{-1}$ for EH, and $51mg.kg^{-1}$ for MU. The growth of both females and males was the same independent of the diet. Analysis of variance calculated on the 225 spawnings collected during the experiment, did not reveal a statistically significant difference for spawning rate and egg

number per spawning between animals fed EH and EL diets. The number of eggs obtained with shrimp fed MU was, however, significantly higher (Table I).

Table I. Reproduction of *Penaeus indicus* fed EL, EH, and MU diets

	EL	EH	MU
Final weight of males	10.8±0.2a*	10.8±0.3a	10.7±0.2a
of females	13.9±0.3a	13.5±0.3a	13.7±0.3a
Number of spawnings	70	66	89
Mean number of spawnings/female/month	3.6±0.6a	3.3±0.6a	3.9±0.6a
Mean number of eggs/spawn	45 000±1 244a	50 000±1 850a	65 500±1 982b
Mean hatching rate/spawning (%)	36±3a	50±4b	61±3b

*Mean values ± SE followed by the same letter on the same line are not significantly different (P>0.05).

The hatching rate of eggs spawned by females fed MU and EH was constant throughout the experiment at a level of 50 to 60% and 90% of the larvae were viable. The hatching rate of eggs spawned by females fed EL was decreasing with the rank of the spawning (Fig. 1) and larvae obtained from spawnings of rank >5 were abnormal and unviable.

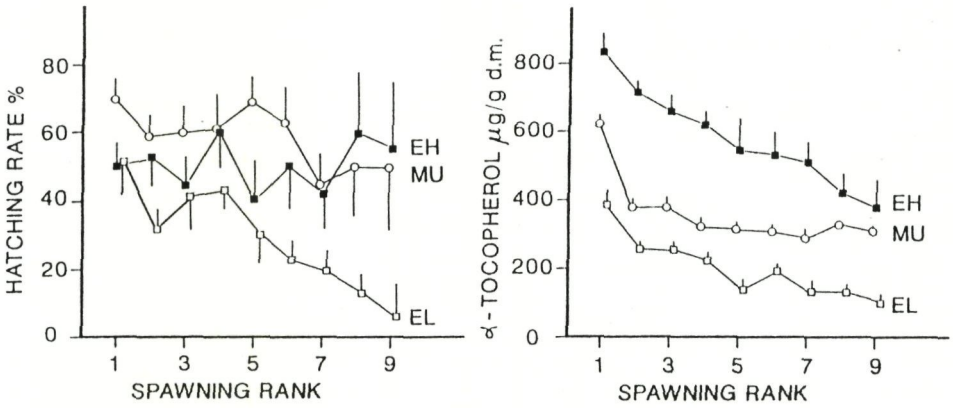

Fig. 1 and 2. Hatching rate (1) and α-tocopherol concentration (2) of eggs of successive spawnings.

The α-tocopherol concentration in eggs was decreasing with the spawning rank whatever the diet, but significant differences were maintained in this order: EH >MU >EL (Fig. 2). A linear correlation, computed with data corresponding to diets EH and EL, appeared between the hatching rate of eggs and their α-tocopherol concentration ($r = 0.32$, $n = 128$).

Discussion

The α-tocopherol concentration of eggs appeared to be related to its concentration in the broodstock diet and a minimal level appeared to be required in eggs to sustain their development. α-tocopherol is one of the components affecting the quality of penaeid eggs, in term of hatching rate and larval viability. Although the α-tocopherol concentration in mussel was low, this diet was ensuring a good deposition of eggs, suggesting that the egg α-tocopherol concentration is also depending on the concentration of other components such as PUFAs (Cahu *et al.*, 1991), and on other antioxidants such as ascorbic acid or carotenoid pigments.

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GONADAL MATURATION AND SPAWNING OF GIANT TIGER SHRIMP (*PENAEUS MONODON* FABRICIUS) IN THAILAND RELATED TO BROODSTOCK SIZE AND SOURCE

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Introduction

Thailand now produces about 5 billion *P. monodon* post-larvae (PL) annually. These PL are produced mostly from large (> 120g), gravid prawns captured from the Adaman Sea. Capture of such gravid females is costly, and is unlikely to increase. With those limitations in mind, we undertook a comparative study to evaluate the relative quality of pond-reared and wild-caught *P. monodon* broodstocks.

Materials and methods

Shrimps were collected from two sources; extensive culture ponds in a mangrove area (pond-reared), and from shallow water coastal areas in the Gulf of Thailand (wild-caught). Shrimps from each source were held separately for 60 days in two large maturation tanks at 3.4 shrimps/m^2 with a sex ratio of 1:1. Each tank contained 60 males, 30 small (< 110g) females, and 30 large (> 120g) females. All females were unilaterally eyestalk ablated at the start of the study. The tank systems were closed recirculating seawater types described by Menasveta *et al.* (in press). Shrimps were fed fresh natural and artificial feeds daily.

Results and discussion

Large shrimps, regardless of source, became gravid and had greater spawning success than small shrimps; while pond-reared and wild-caught shrimps, regardless of size had comparable gonadal maturation and successful spawning rates (Table I). Broodstock source and size was not related to latency period between eyestalk ablation and time to first spawning, which averaged 17 days. AQUACOP (1983) also reported that pond-reared *P. monodon* took 2 to 3 weeks to spawn after eyestalk ablation.

Total egg production from large shrimps was significantly greater ($P<0.05$) than from small shrimps, although the number of eggs spawned per spawner was not significantly different between the four treatment groups. Egg quality in terms of fertilization, hatching and metamorphosis varied greatly between groups (Table II). Inconsistent egg quality could be related to variable mating success. Although the present study demonstrated comparable reproductive performance between pond-reared and ocean-captured broodstock from the Gulf of Thailand, broodstock from the Adaman Sea is generally larger and gives superior performance to those tested.

Table I. Gonadal maturation and spawning success of giant tiger shrimps (*Penaeus monodon*) during study period

Shrimp groups	Number of gravid females	Number of spawning success
Small pond-reared	11	5
Large pond-reared	23	16
Small wild-caught	12	4
Large wild-caught	22	18

Table II. Egg quantity and quality of different groups of giant tiger shrimps

Egg quantity and quality	Pond-reared		Wild-caught	
	Small size	Large size	Small size	Large size
Total egg production	911 460	5 730 860	1 502 900	5 980 300
Ave. no. of eggs/spawner	182 292	636 762	500 933	498 358
Range of % fertilized eggs	17.4-76.9	0-88.6	0-94.3	0-90.6
Range of % hatching	0-37.9	0-75.9	0-25.7	0-69.3
Range of % metamorphosis from egg to protozoea	0-24.6	0-44.5	0-18.3	0-50.1

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THE EFFECT OF DIET AND EYESTALK ABLATION ON MATURATION, SPAWNING, HATCHING, AND LARVAL FITNESS OF *PENAEUS ESCULENTUS*

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Introduction

The controlled production of high quality eggs and larvae is essential for the commercial culture of penaeid prawns (=shrimp). Eyestalk ablation is commonly used to induce maturation and spawning in captive broodstock, and the efficacy of the technique for increasing spawning rate is well documented (Primavera, 1985). The effect on fecundity and egg quality is less clear. Emmerson (1980) found reduced fecundity and hatching rate for ablated *P. indicus* and a similar result for *P. monodon* was seen by Primavera and Posidas (1981). Browdy and Samocha (1985) found lower fecundity per spawn, but greater overall egg production for ablated *P. semisulcatus*; there was no difference in hatch rate or larval survival between ablated and non-ablated females. Galgani *et al.* (1989) found that diet further affected the spawning rate for ablated *P. vannamei* and *P. stylirostris*. Notwithstanding species differences, the effects of ablation and diet on broodstock production are still unclear. The current study assessed female survival, maturation rate, spawning rate, fecundity, egg hatch rate, as well as larval survival, weight and biochemical composition to quantify the effects of ablation, and natural and artificial diets on the reproductive performance and larval fitness of *P. esculentus*.

Materials and methods

Twelve female and 10 male adult *P. esculentus* from the Gulf of Carpentaria, Australia, were held in each of four circular maturation tanks (10 000 l) with controlled temperature, photoperiod and photointensity. Treatments consisted of factorial combinations of eyestalk ablation, non-ablation, natural diet, and a proprietary maturation diet. The natural diet was a combination of chopped frozen prawn (*Metapenaeus bennettae*), bivalves (*Perna canaliculus*, *Plebodonax deltoides*), squid (*Loligo* spp.) and polychaete worms (*Marphysa sanguinea*). The duration of this experiment was 155 days. Maturation and spawning rates are expressed as the mean number per 30 prawn-days (1 prawn-day = 1 day's survival by 1 prawn). Nauplii were collected to determine hatching rates and 900 were reared, without food for 62h, to assess survival to the first protozoeal stage. Total lipid of newly-hatched nauplii was determined from chloroform:methanol extracts (Folch *et al.*, 1957) and the carotenoid content determined spectroscopically. A sample of the surviving protozoeae was measured and weighed.

Table I. *Penaeus esculentus*. Summary of rates of maturation, spawning, fecundity, and larval survival along with larval weights and nutritional analysis

	Natural diet		Artificial diet	
Attribute	Non-ablated	Ablated	Non-ablated	Ablated
Reproductive performance				
Female survival (%p-d)[1,2]	76.8	73.3	49.3	31.3
Maturation rate[3]	1.16	1.85	0.86	0.93
Fecundity (eggs/spawn)	52 343	81 082	53 290	70 332
Spawning rate[3]	0.63	1.24	0.42	0.62
Hatching rate (%)	60.9	60.0	53.8	57.3
Naupliar production rate[4]	20 085	60 325	12 041	24 986
Larval fitness				
Larval survival (%)	78.3	68.4	88.4	83.6
Protozoeal production rate[5]	15 724	41 262	10 664	20 888
Naupliar lipid (µg)	0.87	0.97	0.99	1.13
Naupliar carotenoid (ng)	0.59	0.62	0.56	0.72
Protozoeal weight (µg)	1.42	1.46	1.60	1.66

[1] p-d prawn-day.
[2] (total p-d/total possible p-d) x 100.
[3] number per female per 30 p-d.
[4] mean number of nauplii produced per female per 30 p-d.
[5] egg production rate x larval survival.

Results

Survival of females in the two treatments fed the natural diet was higher (76.8 and 73.3%) than the treatments fed the artificial diet (49.3 and 31.3%) (Table I). Maturation and spawning rates followed the same trend. In both the natural diet and artificial diet treatments, ablation increased maturation rate, spawning rate and fecundity. Hatching rates were similar across all treatments. The naupliar production rate (NPR) is the cumulative effect of adult survival rate, maturation rate, fecundity, spawning rate and hatching rate. The highest NPR (60 325 nauplii per female per 30 spawn days) was in the ablated/natural diet treatment, and was three times that of the non-ablated treatment (20 085) in the same diet. The NPR of the ablated/artifical diet treatment was similar (24 986) but for different reasons. In the non-ablated/natural diet, female survival was high and fecundity was low, while in the ablated/artificial diet, female survival was low but fecundity was high. Lowest NPR (12 041) occurred in the non-ablated/artificial diet treatment due, in large part, to low adult survival and fecundity and the lowest spawning rate of all the treatments.

The survival through the lecithotropic naupliar stages to the first protozoeal stage was higher in the artificial diet treatments. However, when coupled to the NPR to produce a protozoeal production rate (PPR), the trend remains the same; the highest number of larvae were produced in the ablated/natural diet treatment and this was four times greater than the lowest PPR in the non-ablated/artificial diet. The artifical diets also produced the heaviest protozoeae. This trend was reflected in the naupliar lipids but was not statistically significant. The naupliar carotenoid content was closely related to lipid content ($r^2 = 0.684$) and again no clear differences between treatments were seen.

Discussion

The results presented here are from preliminary analyses from one of 10 maturation trials we have carried out with *Penaeus esculentus* and *P. semisulcatus*. The effect of ablation increasing reproductive output (NPR) was consistent in both diets, but the level of output was very dependent on diet. NPR values for ablated females on the natural diet were three times higher than for non-ablated females. Although ablation improved performance within the artificial diet treatments, NPR values for ablated/artificial diet females were much lower than for ablated/natural diet females, and only reached similar levels to the non-ablated/natural diet females. Larval survival was slightly lower from ablated females in both diets. Total protozoeal production from ablated/natural diet females was over 2.5 times greater than from non-ablated. Similarly, for the artificial diet, total protozoeal production of ablated females was around twice that of non-ablated females. The lower PPRs of the artificial diet were a result of comparatively low adult survival (30-50%) and lower spawning rates (0.42-0.62), compared to the natural diets (*circa* 75% and 1.2-1.8 respectively), even though fecundity and hatching rates were similar.

We have inferred larval fitness from naupliar biochemical composition, protozoeal survival and weight. It appears that the larvae from the artificial diet treatments, though lower in number, are fitter. These conclusions are very preliminary and await more detailed chemical analyses (*e.g.* detailed lipid analyses including lipid classes and fatty acid profiles) and more extensive larval bioassays beyond the non-feeding naupliar stage.

The overall conclusion from the study shows that the role of ablation cannot be examined in isolation from the nutritional environment of the prawns. This study demonstrates the positive effect of ablation on reproductive output, but more importantly, shows that in a more favorable nutritional environment non-ablated females can perform similarly to ablated females on a less favorable diet. Clearly there is a need for more detailed studies on the role of nutrition in the maturation process. Identifying components from the natural diet that enhance female survival and maturation, is a logical starting point.

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EFFECT OF EYESTALK ABLATION ON EGG PRODUCTION AND FOOD CONVERSION EFFICIENCY OF THE COMMERCIALLY IMPORTANT RIVERINE PRAWN *MACROBRACHIUM MALCOLMSONII*

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Introduction

Eyestalk ablation studies have been restricted to marine prawns and lobsters and were undertaken only with particular reference to vitellogenesis, ovulation and associated breeding activities (Lumare, 1979; Emmerson, 1980). In reptantians moulting and reproduction are antagonistic events whereas in natantians they are synergistic; hence the available energy has to be apportioned simultaneously for these events. Data on the allocation of energy for somatic growth and reproduction of crustaceans are meagre (Murugadass, 1989). The present paper reports the effect of eyestalk ablation on reproductive growth of the riverine, diedysic, iteroparous caridean prawn *Macrobrachium malcolmosonii*.

Materials and methods

Healthy individuals of *Macrobrachium malcolmsonii* (H. Milne Edwards), collected from the river Cauvery at Grand reservoir (10°50"N; 76°43"E) were acclimated (30±1°C) in a running water system. Large number of healthy freshly-moulted individuals weighing 10.1±0.227g were selected for the experiments; ten of them were treated as control and reared individually. Unilateral eyestalk ablation was done for 30 individuals; of these 20 individuals were selected for the experiment (Caillouet, 1973). Both groups were fed *ad libitum* on *Tubifex tubifex*. Quantitative data on food intake, faecal output, total growth, egg output and exuvial output were collected individually for the control and ablated prawns for a period of 240 days. Caloric contents of prawn, food, faeces, exuvium and eggs were determined in a semi-microbomb calorimeter. The bioenergetic parameters were calculated following the modified IBP formula of Petrusewicz and MacFadyen (1970).

Results

The moulting frequency was 14 in destalked females against 12 in control females. In control females only 40% of moults were berried while 60% were neuter. In destalked female 50% of moults were berried. Thus destalking not only increased moulting frequency but also the frequency of berried moults (Fig. 1). Considering the reproductive growth (egg production), the results obtained are significant. Destalked females produced 112KJ of egg (2.31×10^6 eggs) during the experimental period of 240 days while the egg production was 57KJ (1.18×10^6 eggs) in control females ($P<0.0005$). In other words, energy channelled for egg production has been doubled in destalked females compared to control females (Table I).

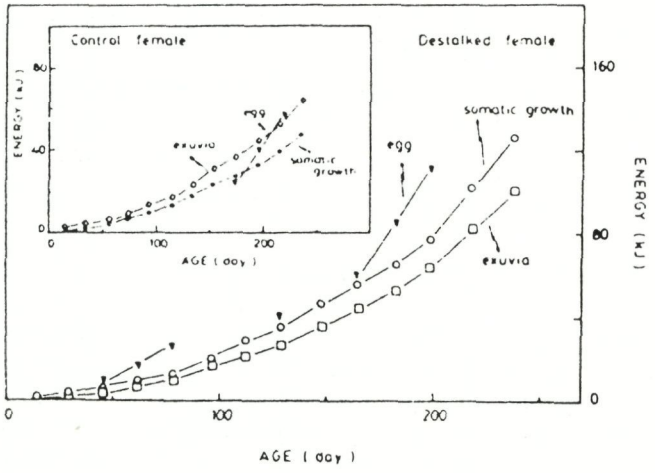

Fig. 1. Somatic growth, egg output and exuvial output observed in female destalked *Macrobrachium malcolmsonii*.

To acquire this extra energy, the ablated prawns have to be hyperphagic and/or efficient convertors. Table I shows that ablated *M. malcolmsonii* adopted both strategies to acquire extra energy to support somatic and reproductive growth acceleration. Consumption and growth increased about 1.8 and 2.1 times respectively in the ablated females. The most interesting observation in the present study is the enhanced reproductive potential of *M. malcolmsonii* after destalking. Ablated prawns undertook more frequent moults and carried more clutches than the controls. The increase in number of clutches and eggs per clutch in ablated *M. malcolmsonii* is in accordance with the results obtained for other decapods (Santiago, 1977). Egg production in the ablated (2, 30, 487) series has been doubled in comparison to the control (1, 17, 784).

Table I. Energy budget of control (male and female) destalked (male and female) *Macrobrachium malcolmsonii* during the experimental period of 240 days. All values are given in KJ per prawn and efficiencies in percentage

Parameter	Control		Destalked	
	Males	Females	Males	Females
Consumption	1 614 ± 57	1 104 ± 34	2 142 ± 61	1 939 ± 65
Faeces	110 ± 12	77 ± 9	144 ± 14	136 ± 13
Absorption	1 504 ± 53	1 027 ± 32	1 998 ± 58	1 803 ± 60
Metabolism	1 277 ± 41	858 ± 24	1 640 ± 44	1 463 ± 45
Total growth	227 ± 13	169 ± 9	358 ± 15	340 ± 16
a) somatic growth	91 ± 5	47 ± 2*	208 ± 9	127 ± 7*
b) exuvium	136 ± 8	65 ± 3	150 ± 7	101 ± 5
c) egg	-	57 + 4*	-	112 ± 6**
Absorption efficiency	93 ± 1	93 ± 1	93 ± 2	93 ± 2
K_1	14.0 ± 3	15.3 ± 3	16.7 ± 3	17.5 ± 3
K_2	15.1 ± 3	16.5 ± 3	18.0 ± 3	18.9 ± 3

* Somatic growth control female *versus* destalked male and female ($P<0.0005$)
** Egg production control female *versus* destalked female ($P<0.0005$)

Discussion

Growth acceleration, frequent moulting and increased egg output are all energy demanding processes. The increased weight gain in ablated *M. malcolmsonii* has resulted from higher consumption, higher conversion efficiency and reduction on the energy channelled to exuvia production. The same trend was noticed in *Panulirus homarus* (Vijayakumaran and Radhakrishnan, 1984). This is an interesting observation because the energy spent on exuvial production is not advantageous in the commercial point of view. The increased conversion efficiency was to meet high metabolic requirements of ablated prawn.

Kulkarni and Nagabhushanam (1980) suggested that the declining levels of the moult inhibiting hormone (MIH) and gonad inhibiting hormone (GIH) might have produced conducive conditions for the actions of the moulting hormone (MH) and gonad stimulating hormone (produced elsewhere). The first females to spawn were the ablated ones, probably due to the lower blood titre of ovary inhibitory hormone. Unablated females of *Penaeus indicus* took longer time to develop ovaries (Emmerson, 1980). The ablated female *M. malcolmsonii* took 46 days to produce first spawn whereas control female took 62 days. The ablated *M. malcolmsonii* females had undergone seven spawnings as against five spawnings in control females. The destalked females allocated

more energy for egg production than control females. It may be concluded that ablated prawns enhance egg production by saving the energy spent on exuvia production and/or by increasing the food conversion efficiency.

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INFLUENCE OF DIETARY PROTEIN ON MATURATION AND EGG PRODUCTION IN *MACROBRACHIUM NOBILII*

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Introduction

Available information on effects of different sources and concentrations of dietary protein on growth performance, survival and feed conversion of various penaeid and palaemonid species (New, 1976) points to a wide range of protein requirements during developmental stages. However, no information on the effects of dietary protein levels on maturation and egg production in *Macrobrachium* is available up to now. It is only known that restriction of ration or protein and/or ablation causes precocious moulting as well as enhancement of growth (Murugadass, 1989). The present study deals with the influence of dietary protein on sexual maturity and egg production of a riverine, diecdysic, iteroparous caridean prawn *M. nobilii*.

Materials and methods

Healthy individuals (250mg) of *Macrobrachium nobilii* (Henderson and Mathai, 1910), collected from the River Cauvery at Grand Reservoir (10°50"N;76°43"E) were acclimated (30±1°C) in the laboratory running water system; they were reared solitarily to avoid cannibalism and to aid individual observation. The freshly moulted animals were starved for a period of 24h before the experiments were started. During the experimental period, the animals were fed *ad libitum* on formulated diets for a period of 4h and the uneaten food was collected thereafter. Caloric contents of the prawns, their food, faeces, exuviae, and eggs were determined in a semi-micro bomb calorimeter (Parr Instruments, USA). Food consumption, egestion and production were estimated in terms of dry weight and then converted into energy units by using the energy density ($J.mg^{-1}$) of the respective samples. The IBP formula of Petrusewicz and MacFadyen (1970) represented as C = F+U+R+P, where P represents the sum of energy expanded on net somatic growth, exuvia and egg production of female prawns was followed in the present study.

Table I. Energy budget of *M. nobilii* fed on different dietary protein levels for a maximum experimental duration of 460 days. Values are given in kJ/prawn and efficiencies in %. Each value represents the average (X±SD) performance of 5-10 individuals

Parameters	Dietary protein level (%)								
	10	15		25		35		30	
	♀/♂	♀	♂	♀	♂	♀	♂	♀	♂
Consumption	25.53 ±0.52	82.94 ±1.23	83.28 ±1.31	84.00 ±1.31	86.06 ±1.42	83.57 ±1.43	85.86 ±1.51	86.58 ±1.42	95.36 ±1.73
Faeces + urine	3.07 ±0.23	11.72 ±0.42	11.75 ±0.52	12.42 ±0.71	12.73 ±0.53	14.44 ±0.72	14.78 ±0.53	15.68 ±0.63	17.50 ±0.54
Assimilation	22.46 ±0.42	71.22 ±1.21	71.53 ±1.13	71.58 ±1.32	73.33 ±1.43	69.13 ±1.14	71.08 ±1.22	70.90 ±1.22	77.86 ±1.23
Metabolism	21.45 ±0.33	60.63 ±1.14	61.58 ±1.24	58.89 ±0.83	60.42 ±1.22	55.71 ±0.92	57.57 ±0.84	57.56 ±0.84	64.41 ±1.24
Growth (conversion)	1.01 ±0.07	10.59 ±0.32	9.95 ±0.24	12.69 ±0.28	12.91 ±0.24	13.42 ±0.31	13.51 ±0.41	13.34 ±0.31	13.45 ±0.32
1) Somatic production	0.23 ±0.08	1.92 ±0.13	3.78 ±0.13	2.46 ±0.14	5.37 ±0.15	2.80 ±0.10	6.41 ±0.21	2.82 ±0.11	6.47 ±0.23
2) Exuvial production	0.78 ±0.05	5.10 ±0.21	6.17 ±0.21	4.96 ±0.28	7.54 ±0.17	4.83 ±0.25	7.10 ±0.25	4.62 ±0.15	6.98 ±0.21
3) Egg production	-	3.57 ±0.12	- -	5.27 ±0.13	-	5.79 ±0.20	-	5.90 ±0.21	-
Assimilation efficiency	87.98 ±3.21	85.87 ±4.31	85.89 ±4.31	85.21 ±4.43	85.20 ±4.23	82.72 ±3.14	82.79 ±3.42	81.89 ±3.21	81.65 ±3.31
Gross conversion efficiency (K_1)	3.96 ±0.53	12.77 ±2.12	11.95 ±1.22	15.11 ±2.52	15.00 ±2.54	16.06 ±3.12	15.74 ±2.23	15.41 ±2.34	14.11 ±2.23
Net conversion efficiency (K_2)	4.50 ±0.54	14.87 ±2.21	13.91 ±2.34	17.73 ±3.21	17.61 ±3.34	19.41 ±3.24	19.01 ±4.12	18.82 ±3.23	17.28 ±3.42

Results

An analysis of the data presented in Table I reveals that the fraction of energy allocated to total growth was greater in the prawns fed a protein-rich-diet (>35%). Data obtained for the growth components were related to consumption and subjected to regression analysis to assess the impact of dietary protein levels on energy allocated to somatic growth, exuvial and egg production. Egg production positively correlated with dietary protein levels ($r=0.6491$: $P<0.005$) and was 1.6 times greater in the groups receiving a protein-rich diet (>35%) than the group fed on a 15% protein diet (15%). Clearly energy allocated to egg production by high protein-fed female (43.1%) was 1.3 times higher than that allocated by low protein-fed female (33.7) (Fig. 1). However, these differences became greater, when the quantitative values were considered.

In the 10% protein group, prawns succumbed before sexual maturity: they lost 77% of the available energy on exuvia (Fig 1). Prawns in the other groups attained sexual maturity at different periods depending on their body weight. The lower the dietary protein level, the longer the time required to grow and attain the minimum body weight (600mg) for sexual maturity. The juveniles fed on 15, 25, 35, and 50% protein diet attained sexual maturity in 321, 280, 268 and 240 days, respectively; thus the higher protein diets advanced the onset of maturation (Fig. 2A).

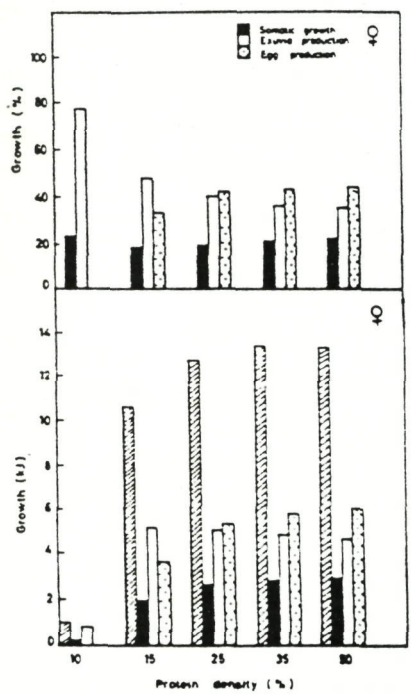

Fig. 1. Energy allocation to somatic growth exuvial and egg production (as % total growth: upper panel; in J/ind.: lower panel) of female *M. nobilii* receiving different dietary protein levels.

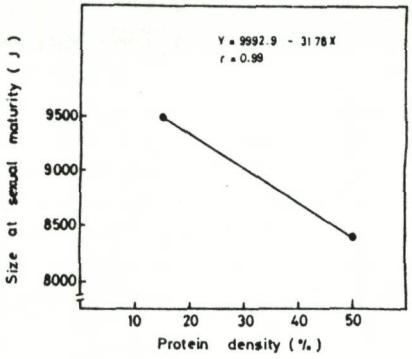

Fig. 2A. Age at maturity as a function of dietary protein level in *M. nobilii.*

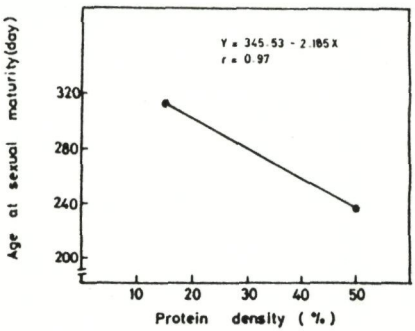

Fig. 2B. Energy content at maturity as a function of dietary protein level in *M. nobilii.*

Analysis of the data on age (*i.e.* time taken to reach 600mg body weight) at maturity showed significant differences with the dietary protein levels. Prawns fed with protein-rich diet shortened the time required for maturity by 2.2 days for every 1% increase in protein density in the diet (Fig. 2B). With the increasing protein level of the diet, energy content at maturity (J) decreased. After maturity the prawns allocated more energy to egg production than somatic growth . Interspawning period was 45, 33, 24 or 21 days, in the prawns fed with 15, 25, 35 or 50% protein diet. The high protein-fed (>35%) prawns produced not only more eggs/clutch but also larger eggs. Energy content of their eggs was 0.76 J/egg, which is significantly higher than that (0.69 J/egg) spawned by the prawn feeding on 15% protein diet (Fig. 3). For the production of more eggs with rich energy reserves, 35% protein diet is recommended for prawn farming.

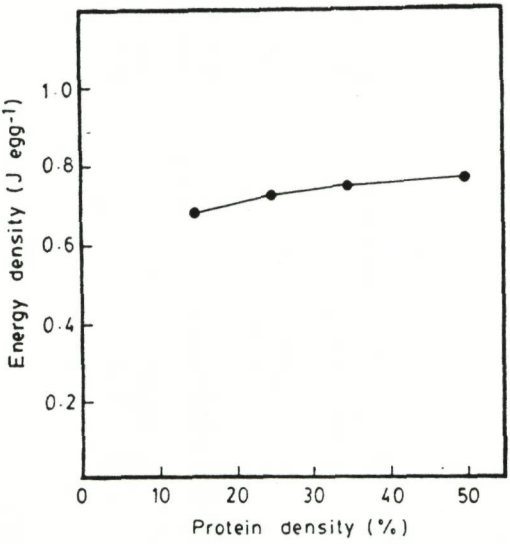

Fig. 3.Energy density (J/egg) of eggs of *M. nobilii* as a function of protein levels.

Discussion

In the present study, the low protein-fed group (10%) is unable to produce eggs. This implies that adequate protein intake is a necessity for triggering as well as ensuring the development of the ovary.

Inadequate dietary protein supply leads either to the failure of development of the ovary, or the development of an impoverished ovary, which regresses before reaching the maturity stage. At dietary protein levels of 15% and above, normal ovarian development and spawning take place. This shows that 15% dietary protein is obligatory for the successful coordination and function of the hormonal milieu. Hence, it can be hypothesized that dietary protein levels are of considerable importance in the process of egg production in crustacean decapods.

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HUFA LEVELS IN EGGS OF WILD AND CULTURED BROODSTOCK OF *MACROBRACHIUM ROSENBERGII*

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Introduction

The variation in egg quality, *i.e.* the potential of the egg to produce viable fry, is still a limiting factor in the commercial production of marine fish and crustacean fry (Kjørsvik *et al.*, 1989). Better knowledge in this field would contribute to improved and especially more consistent hatchery outputs and to the development of more suitable broodstock diets. So far, objective criteria to evaluate egg quality are scarce. However, it is very likely that the nutrient composition of the eggs may be a good indicator for egg viability as it reflects the nutrient demands of the developing embryo and larva. Since (n-3) highly unsaturated fatty acids (HUFAs) have been identified as essential nutrients for the larvae of marine fish, shrimp and prawn (Bengtson *et al.*, 1991) a case study has been initiated with the giant freshwater prawn *Macrobrachium rosenbergii* to verify variations in (n-3) HUFA contents of eggs from wild and pond-reared broodstock.

Materials and methods

Eggs were taken from different females either from wild-stock populations of the river Choa Phya (C), from various pond cultures in the Supan Buri Province (P), or from laboratory cultures (L) in Thailand during the same period, namely from March to April, 1991. The L population was a laboratory breeding of C males and P females. Female size of P and L, respectively C averaged 20g and 30g. Egg colour, developmental stage as well as percent yolk content of the eggs was observed and estimated using a microscope. Fresh samples were brought to the laboratory in Bangkok, washed and freeze-dried. Afterwards they were stored in small vials under nitrogen and kept at -25°C until transportation to Gent, Belgium. Total lipid content and fatty acids were analyzed and quantified following the procedure of Léger *et al.* (1987).

Results and discussion

The data for the different egg samples (Table I) are grouped according to yolk content (in %), and to wild stock (C) *versus* pond (P) or laboratory (L) broodstock. In general,

Table I. Characteristics of the egg samples of *Macrobrachium rosenbergii* (P=pond-reared stock; C=Choa Phaya River stock; L=Laboratory-reared stock; a=area %; b=mg.g^{-1} DW)

Sample (N°)	Yolk (%)	Egg stage	Colour of eggs	20:5n-3		22:6n-3		Σ HUFA		Total lipids (%)
				area[a]	mg[b]	area[a]	mg[b]	area[a]	mg[b]	
P2	100	yolk	orange	1.6	6.0	2.3	8.6	3.9	14.6	40.3
P8		early	orange-yellow	1.7	5.9	1.9	6.7	3.7	13.0	43.6
P14		early	orange	1.7	3.9	1.3	3.0	3.1	7.2	27.8
P17		early	orange	1.4	4.7	1.6	5.5	3.1	10.5	42.1
L3		early	yellow	2.3	8.6	3.1	11.7	5.4	20.3	47.0
L5		early	yellow	2.4	7.2	3.3	9.8	5.7	17.1	40.9
L6		early	yellow	2.1	7.0	3.3	10.7	5.6	18.2	40.0
L7		early	yellow	1.0	4.1	1.7	6.9	2.7	11.0	43.2
P7	75	early	orange-yellow	1.7	5.2	1.2	3.8	2.9	9.0	32.6
P9		early	orange-yellow	1.7	4.9	2.1	6.1	3.8	11.0	35.9
P6		early-eyed	orange-brown	1.1	3.2	1.3	4.1	2.4	7.3	34.3
P13		early-eyed	orange-brown	2.0	5.1	2.1	5.4	4.3	10.9	35.0
L1		early	orange-yellow	2.3	7.7	2.3	7.9	4.6	15.6	39.1
L2		early	orange	2.6	7.4	2.7	8.0	5.3	15.4	36.9
L4		early	orange	2.1	6.3	2.8	8.4	5.1	15.4	37.7
L8		early	orange-yellow	2.3	4.8	3.4	7.1	6.2	13.0	40.1
C7		early	orange	1.0	3.5	1.4	5.0	2.4	8.5	37.7
C1		early-eyed	orange-brown	1.9	8.1	2.1	9.3	4.0	17.4	40.6
C6		early-eyed	orange-brown	1.4	4.0	0.5	1.4	1.9	5.4	31.9
P11	50	early-eyed	orange-brown	1.1	3.2	0.9	2.5	2.0	5.7	31.7
P16		early-eyed	orange-brown	1.7	5.9	2.3	8.3	4.0	14.2	44.6
P15		eyed	orange-brown	1.6	4.0	2.0	5.0	3.6	9.0	26.6
P19		eyed	orange-brown	1.7	4.6	1.5	4.0	3.2	8.6	32.7
P4	25	early-eyed	orange-brown	1.8	5.1	2.0	5.8	3.8	10.9	38.3
P3		eyed	brown	1.6	4.2	1.2	3.2	2.8	7.4	38.3
P10		eyed	orange-brown	1.7	8.2	2.0	9.2	4.0	18.6	32.4
P12		eyed	brown	1.4	3.8	1.2	3.2	2.6	7.0	30.8
P18		eyed	brown	1.7	3.6	1.4	3.1	3.1	6.7	24.9
C2		eyed	orange	1.4	4.7	1.0	3.5	2.5	8.4	42.6
P1	0	eyed	brown	1.8	3.5	1.7	3.2	3.5	6.7	19.1
P5		eyed	brown	1.8	3.6	2.0	4.2	3.8	7.8	25.2
C3		eyed	brown	0.9	3.8	1.2	4.7	2.1	8.5	37.9

the quantitative data for the essential fatty acids 20:5n-3 (EPA) and 22:6n-3 (DHA) varied considerably among the different *Macrobrachium* egg samples, irrespective of the developmental stage or the origin (wild *versus* cultured broodstock): *i.e.* more than 100% for both EPA and DHA. Only the variation in total lipid content was lower for the eggs obtained from wild spawners or from laboratory-reared broodstock than for pond-cultured broodstock (30% *versus* 80%, respectively).

For most samples the total lipid levels were higher than those reported by Buzzi (1989) and Clarke *et al.* (1990) for *Macrobrachium rosenbergii* eggs collected from wild stock in Thailand, respectively captive stock in Scotland, UK (23-29%).

Statistical analysis revealed that the laboratory-reared population produced eggs with a significant higher content in EPA, DHA and total lipids than the eggs from the other two groups. This may be attributed to nutritional differences in the food available to the broodstock, as has been documented in fish for grouper (Dhert *et al.*, 1991) and red seabream (Watanabe *et al.*, 1985).

HUFA levels appeared to vary irrespective of % yolk content or developmental stage. This confirms the results of Clarke *et al.* (1990), who found that lipid metabolization is very limited (*i.e.* less than a 10% drop in overall energy content) during the developmental period from egg to pre-hatching nauplius.

The low content of various HUFAs together with the high ratio of EPA/DHA (=1) compared to eggs of marine fish species (Lavens and Sorgeloos, 1991) and penaeid shrimp (Cahu and Quazuguel, 1989; Millamena and Pascual, 1990) is most probably related to the absence of (n-3) HUFA series in the freshwater biotope of *Macrobrachium* broodstock. Furthermore, it may give an indication of the lower needs of especially DHA during early larval development.

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ENVIRONMENTAL FACTORS IN REARING EGGS AND LARVAE OF *LIMULUS POLYPHEMUS* L. UNDER LABORATORY CONDITIONS

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Introduction

The horseshoe crab, *Limulus polyphemus* L. lives in the coastal regions from the Yucatan peninsula (Mexico) to Massachusetts (USA) where human activity is reducing breeding and growth habitats. The natural conservation of this species requires immediate scientific attention. This investigation is part of a study on the population dynamics of *L. polyphemus* carried out at the National Autonomous University of Mexico during the past decade. The aim is to know the environmental factors which are important for rearing eggs and larvae under laboratory conditions. These experiments signify a step forward in the protection of the species.

Materials and methods

Eggs of *L. polyphemus* were collected in the Terminos lagoon in the southeastern Gulf of Mexico and transported to the laboratory in Mexico City. Eggs were reared under controlled conditions of temperature (20, 25 and 30°C), salinity (25-35ppt) and pH (7.5-8) through the first tailed stage. They were divided into two experimental groups: one without substrate and another with a sandy substrate. In a 20 l aquarium, six glass bowls (9cm diameter and 1.5cm depth) contained 50 eggs each. Some of the hatched larvae were released in the aquarium while others remained in the containers.

Results

Temperature and substrate were important factors for the development of the eggs and first "limulito" stage. The first larvae hatched at 30°C after 14 days of being sampled; the second ones hatched at 35°C after 25 days; and the third ones at 25°C after 30 days. Higher mortality occurred in all experiments with a sandy substrate. Lower mortality occurred in the one without the sandy substrate. The best results were obtained at 30°C (Fig. 1). Small eggs in earlier stages did not hatch.

Discussion

Little research has been carried out on the post-embryonic development of horseshoe crabs. This is probably a result of the absence of a method for culturing juveniles (Sekiguchi, 1988). Jegla and Costlow (1982) found that the effect of salinity on egg and larval development is not so important between 20 and 30ppt. In these experiments similar results were obtained. Jegla and Costlow (1979) also stated that according to the temperature, it might take weeks or months for the eggs to develop into trilobite larvae. Laughin (1983) reported a faster development at 30°C, which declines at 35°C, and declines even further at 20°C. The experiments carried out in Mexico City confirms this finding.

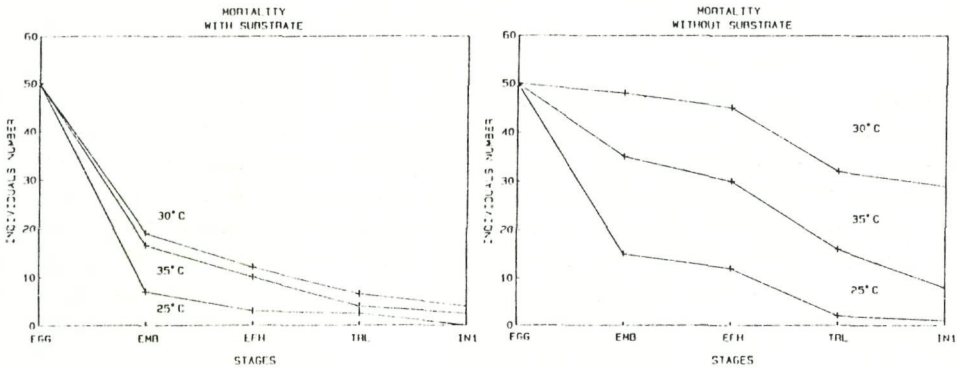

Fig. 1. Comparison of egg mortality between the experiment at 25, 30 and 35°C, with and without sandy substrate. EGG: eggs; EMB: embryo; EFH: egg for hatching; TRL: trilobite stage; IN1: instar 1 larvae.

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SECONDARY LECITHOTROPHY IN PUERULUS LARVAE OF THE WESTERN ROCK LOBSTER (*PANULIRUS CYGNUS*)

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Introduction

The western rock lobster (*Panulirus cygnus*), an endemic species of the Western Australian coast, is of considerable economic importance locally. In *P. cygnus* nine phyllosoma stages precede the puerulus stage, which forms the transitional stage between the planktonic larval stages and the benthic juvenile and adult phases in the life cycle (Phillips et al., 1980). The puerulus swims actively across the continental shelf before settling in inshore areas. Several authors (*e.g.* Kittaka and Kimura, 1989) have reported an apparent absence of feeding during the puerulus stage in various palinurids, a phenomenon which has also been observed in other crustaceans (*e.g.* Dawirs, 1981; Ikeda, 1985) and was coined "secondary lecithotrophy" by Anger (1989). Scyllarid lobsters have a similar life cycle. Here, the nisto forms the transitional stage. Their considerably shorter larval life (*e.g.* Ito and Lucas, 1990) makes them suitable for laboratory study of larval biology. Presently a study is being undertaken at the Marine Biological Laboratory (MBL) of the Zoology Department, UWA, focusing on the larval feeding biology of *P. cygnus* and three local scyllarid lobsters. Several phyllosoma stages of *P. cygnus*, *Ibacus peronii*, *Thenus orientalis* and *Scyllarus demani* have been successfully reared at the MBL and studies on metabolic rate, swimming capacities and efficiency in energy requirements of various larval stages are in progress.

The present paper focuses on the presence of secondary lecithotrophy in the puerulus of *P. cygnus*. Preliminary results from histological and morphologic observations of changes which take place during the development of the puerulus of *P. cygnus* and after the transition from puerulus to the juvenile stage are presented.

Materials and methods

Puerulus larvae were obtained by means of collectors (Caputi *et al.*, 1988) along the Western Australian coast, feeding behaviour to study both juveniles and puerulus stages. They were offered fresh mussel-meat daily. To facilitate observations, the mussel meat was stained in a 1% Congo red solution. Morphological changes in mouthparts and the digestive tract during development of the puerulus stage were studied by means of SEM

and light microscopy. Samples for SEM observations were treated as described by Felgenhauer (1987). Histological observations were made on haemotoxylin/eosin stained coupes.

Results

During repetitive observations of live pueruli and juveniles over a period of about 2 weeks, pueruli were never observed to be feeding on the mussel meat provided. In addition, the Congo red stain was absent from the intestines of the transparent pueruli while the presence of Congo red was clearly visible on the feeding appendages and in the intestines of juveniles.

Observations by SEM and dissecting microscope demonstrated that mouthparts are considerably more developed and highly setose in juveniles compared with pueruli. The endopod of the 2nd maxilliped is highly setose in juveniles but setae on the 2nd maxilliped are completely lacking during the puerulus stage. Setae on 1st and 2nd maxillae are well developed in juveniles, while setae are almost lacking in pueruli. Similar differences have been documented for other species of palinurids (Nishida *et al.*, 1990).

The foregut undergoes substantial changes during the transition from puerulus to juvenile. A deepening of the anterior section of the cephalothorax allows an increase of volume of the foregut. Gastric teeth and setae are well developed in juveniles, but are lacking in puerulus. Similar observations were described for *Jasus edwardsii* (Nishida *et al.*, 1990).

Significant changes in pigmentation during the development of the puerulus allows fairly accurate aging of puerulus collected in the field (Lewis *et al.*, 1952). During the settlement phase the majority of hepatopancreatic cells are R-cells. These cells are involved in storage of lipids. Shortly before metamorphosis to the juvenile stage, the hepatopancreatic lumen is largely surrounded by B-cells. These cells are known to be responsible for the production and storage of digestive enzymes (Gibson and Barker, 1979). This is opposite to what can be expected in feeding stages, where hepatopancreatic proteins, glycogen and lipids accumulate in preparation for moulting (Gibson and Barker, 1979). In juveniles of *P. cygnus*, the four types of hepatopancreatic cells (E, F, R and B cells) can be found, indicating active feeding.

Discussion

The above observations demonstrate that the puerulus of *P. cygnus* is a non-feeding stage, at least during its benthic phase. Absence of feeding during the puerulus phase has serious implications for the rearing of palinurid lobsters, as sufficient reserves need to be accumulated during the preceding phyllosoma stages to allow the puerulus to survive beyond the moult to the juvenile. Presently the study is focusing on changes in lipid and glycogen levels, using histological techniques and analysis of C/N ratios (Dawirs, 1981; Ikeda, 1985). Similar studies will be undertaken on phyllosoma and natant puerulus larvae which will be collected by means of plankton nets. Laboratory-reared palinurids and scyllarids will be used to complement the study. Furthermore, studies on swimming

capabilities and oxygen consumption during activity and rest will be undertaken to determine the energy requirements and efficiency.

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CULTURE ASPECTS OF VARIOUS SPECIES

Freshwater fish

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DEVELOPMENTAL PATTERNS OF LARVAL FISH OF THE CENTRAL AMAZON

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Introduction

There is a variety of early development in larval fish. It ranges from highly developed larvae at hatching with a functional mouth, pigmented eyes, rays in the caudal fin and ready exogenous feeding to a poorly developed larvae at hatching having none of these features and gradually acquiring them. Some authors suggested that part of the variation in the development styles are related to spawning strategies (Kendall *et al.*, 1983; Balon, 1985; Blaxter, 1988). Kendall *et al.* (1983) suggested that egg size also influences the stage of development of the hatching larvae. There is, however, a shortage of information on larval development patterns in quantitative terms. This omission is probably due to a lack of comparable data.

In the present paper early development patterns are shown based on the analysis of 14 species of larval fish of the three main phylogenetic groups of the Central Amazon. The developmental features were tested against egg size, egg calorific content, spawning sites, and phylogenetic influences.

Materials and methods

Thirty batches of eggs of 14 fish species (five cichlids, two siluriforms, seven characiforms) were collected in the Amazon floodplain and reared in the laboratory until their death by starvation. Larval density in the experimental system was below 2 ind/l and the water renewal rate was better than 20%/week. Dissolved oxygen and temperature varied from 80 to 100% saturation (N= 360) and 28-31°C (N= 2 500), respectively. Yolk weight at fertilization and their calorific content was measured on 5 to 20 eggs of each batch after dissecting off the chorion. Over 15 larval specimens of each batch were sampled daily for age (hours from fertilization) and larval size (dry weight) and inspected for the following developmental features: hatching, formation of pectoral bud, eye pigmentation, jaw formation, swim bladder inflation, onset of horizontal swimming, maximum size attained with exclusively endogenous feeding and the presence of pigmented blood cells. First feeding size and age was measured in a parallel experiment and defined as when 50% of the population start to have food in their stomachs. Incubation times of characiform eggs were measured *in situ* and those of the cichlids and siluriforms were obtained from the literature. Observations were made on anaesthetized

embryos/larvae with a stereomicroscope at magnification up to 160X. Weighing was carried out after dissecting off the yolk with samples preserved in 4% formalin and later corrected for fresh dry weight. The weight and age when 50% of the larvae reached the developmental features and the maximum growth rates were interpolated using a polynomial-exponential least-squares regression. The fitting of the model explained over 90% of the variability for most batches and their residuals were tested against temperature fluctuations during the rearing experiments. Additionally, the age at starvation (50% mortality) was measured compared with fed larvae, and the patterns of blood circulation, as perceived by the movements of the blood components, determined.

Results

The age and the larval weight for most developmental features were positively correlated with the yolk weight at activation, however, partial correlation analyses showed that only the latter were significant. These relationships explained the interspecific variability in the larval size at eight features (Table I). Residual analyses found no further relationship of the remaining variance with: temperature variation, calorific content, spawning sites and phylogenetic groups.

Table I. Parameters of least-squares regressions of yolk weight at fertilization and larval body weight at developmental features

Feature	*b*	SE of *b*	*a*	N	r^2
Hatching	0.565	0.058	0.065	13	0.895
Pectoral bud	0.279	0.068	0.079	14	0.586
Jaw formation	0.418	0.086	0.135	11	0.723
Eye pigmentation	0.384	0.067	0.130	13	0.749
Swimming	0.762	0.059	0.311	11	0.949
Swim bladder	0.665	0.074	0.240	11	0.913
First feeding	0.349	0.074	0.349	09	0.919
Maximum size	0.759	0.064	0.444	12	0.934

Model: larval weight= *a*; yolk= *b*; N= sample size; r^2= coefficient of determination.

The formation of caudal rays and pigmentation of blood cells lacked relationship with yolk weight. Caudal rays were not present at the characiforms until the end of the experiment. Red blood cells and a dense vascularization on the body surface were found only in the newly hatched larvae of the species that spawn in the margins. Analyses of covariance showed that the variation in age at starvation was due mainly to the maximum specific growth rate (57%) and to phylogenetic groups (25%). Larvae that grow fast starve earlier (P= 0.001) than larvae that grow slowly. Cichlids are more resistant to starvation (P= 0.008) than characiforms and siluriforms.

Discussion

The above results suggest that there are patterns in the larval development in Amazonian fish. The sizes of the larvae at several events are mainly related to the yolk original weight, and occur in a chronological sequence (Fig. 1). The variation on the formation

of caudal rays seems to have a strong phylogenetic component. Since this event is an important descriptive character it may confound the picture of possible patterns of larval development. The variation on the age at starvation and on the development of the circulatory systems are mainly related to phylogenetic differences among groups or spawning sites.

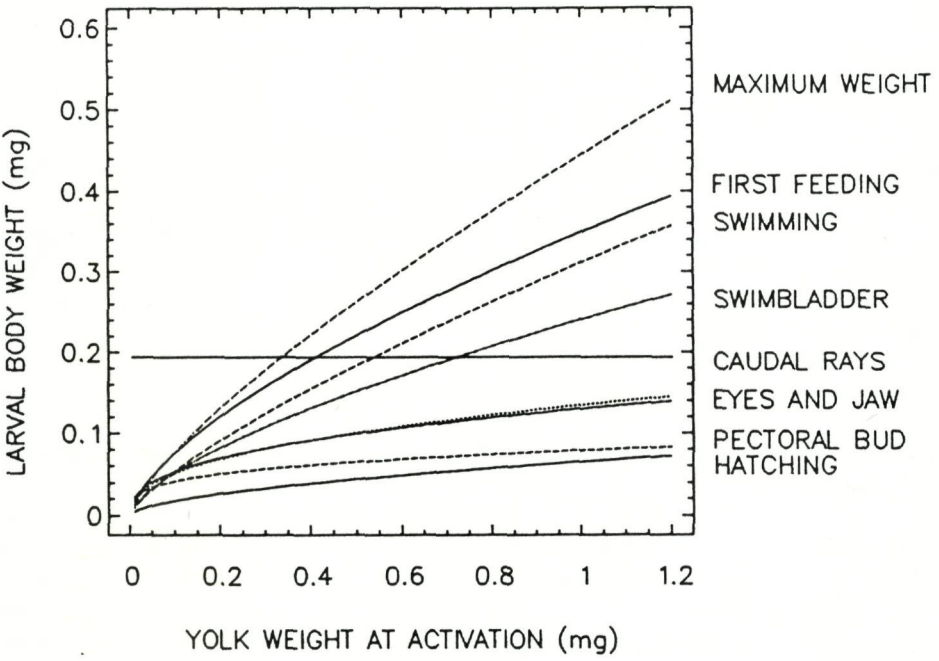

Fig. 1. Larval body weight as a function of yolk size for eight developmental features.

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THE EFFECTS OF DIFFERENT TREATMENTS ON THE DEVELOPMENT AND SURVIVAL OF PACU FISH LARVAE, *PIANACTUS MESOPOTAMICUS*

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Abstract

During 25 days the development and the survival rate of Pacu larvae, *Pianactus mesopotamicus*, was evaluated. Pacu, a native fish species of the Parana river basin, represents a great potential for aquaculture.

In this study, three different treatments were tested: (A) artificial feed; (B) dry yolk; (C) zooplankton.

Four days after hatching, 450 larvae were distributed at a density of three larvae/l in nine aquaria (1.10x0.38x0.4m) provided with constant aeration and fitted with outward filters. High rates of mortality were registered in all three treatments A, B, and C, namely 100, 95, and 70%, respectively. Under treatments A and B mortality was due to the high ammonium levels. In treatment C cannibalism occurred among the larvae due to the disparity in sizes.

At the end of the experimental period the larvae subjected to treatment C had a superior growth in comparison to those in treatment B.

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REARING TECHNIQUES OF SOME FRESHWATER PREDATOR FISH FRY AND FINGERLINGS

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Abstract

The freshwater fish fauna of Brazil is rich in those species, which are potentially good for fish culture. Today most of these species are cultured using artificial propagation and mass fry and fingerling rearing techniques. The first food of the fish larvae of these species (*Prochilodus* spp., *Colossoma* spp.) consists of zooplankton because it is small in size and can easily be caught by the unexperienced larvae, the sensory organs and coordination systems of which are not yel well developed.

The larvae of two very valuable Brazilian fish, matrincha (*Brycon lundii*) and dourado (*Salminius brasiliensis*) could hardly be nursed on zooplankton, because under natural conditions their first food are only freshly-hatched fish larvae. According to the observations, they spawn together with *Prochilodus* sp. Their floating eggs are transported together, by the current, to the flood plain of the river. The larvae of *Brycon* and *Salminius* develop very rapidly: 18-22h after hatching they have a mouth, teeth, gut and gills. On the back of their head they have an adhesive gland with which they stick to objects "facing" the open space, allowing them to catch other fish larvae which swim near their mouth. Crowded conditions in rearing tanks result in cannibalism being the only possibility for some individuals to survive. In order to rear high quantities of larvae of *Brycon* or *Salminius* they were stocked together with *Prochilodus* spp. in a ratio 1:5, and later 1:10 into the same larvae rearing tanks. Cannibalism did not occur: only *Prochilodus* larvae were swallowed. Growth and development rate of *Brycon* or *Salminius* was very good. The total length of *Brycon* and *Salminius* larvae is about 6.0±0.2mm at hatching. After 3 days feeding *ad libitum* with *Prochilodus affinis* larvae the total length increased to 1.5cm on average. They caught 1.2 larvae at once, most probably 2-3 times a day. After 8 days they reached 3.0-3.5cm in total length. Because their feeding with *Prochilodus* hatchlings became too difficult, they were stocked in earthen ponds and fed again *Prochilodus* larvae. After 30 days the survival rate was 80 to 85% on average.

For the mass propagation of fodder larvae, *Prochilodus affinis* was most suited to induce spawning. When they were injected with pituitary gland extract or with an adequate synthetic GnRH analogue spawning occurred readily and quite totally. Their eggs are relatively light and can be washed out from the spawning tank by a weak current. They

can be collected in a simple egg collector and placed into incubating jars. The spawning could be synchronized with the artificial propagation of *Brycon* or *Salminius*, by means of a combined treatment of pituitary gland extract and synthetic GnRH analogue.

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CANNIBALISM: THE HIDDEN MORTALITY FACTOR IN LARVICULTURE

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Abstract

The occurrence of cannibalism in teleosts during the larval and early juvenile stages is reviewed, whereupon a hypothesis is presented that the phenomenon is a phenotypic response to a causal mechanism. Experimental evidence is presented that cannibalism appears to be more prevalent in those species which have a more altricial life-history style than those which have adopted a more precocial course and that it is correlated to specific size- and age-related feeding strategies. Evidence is also provided that cannibalism is genetically controlled. It is submitted, on the basis of the optimal foraging theory, that the benefits of cannibalism accrue at the individual level. Victims of cannibalism are thus regarded as transitory food caches that store energy for their kin. The significance of these conclusions are considered in the light of practical hatchery management.

Results of experiments examining the causes and effects of several biotic and abiotic variables on the behaviour, and the occurrence and incidence of cannibalism in coeval sibling batches of larval and early juvenile African catfish (*Clarias gariepinus*), rainbow trout (*Oncorhynchus mykiss*), common and koi carp (*Cyprinus carpio*) are presented. Primary amongst the experimental variables were food availability, alternative live and dry feeds, size variation, population density, turbidity, photoperiod and light intensity, and the presence or absence of refuges. An experiment in which cannibalism was selected for is also described. In addition, behavioural observations on several non-cannibalistic species *e.g. Tilapia rendalli*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are discussed. These experiments suggest that cannibalistic polyphenism is proximally produced by environmental cues acting on a genotype that is sufficiently plastic to produce either normal or cannibalistic morphs.

Overall these experiments, on cannibalistic and non-cannibalistic species, in juxtaposition with their life-history styles, have elucidated ways and means in which cannibalism can be controlled under intensive larviculture conditions. A conceptual model of cannibalism is presented and its predictive capabilities are considered.

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LARVICULTURE OF THE MOST IMPORTANT CYPRINID SPECIES: ECOLOGICAL, BIOTECHNICAL AND REGIONAL ASPECTS

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Abstract

Larval culture of common carp, *Cyprinus carpio*, and three Asiatic herbivorous fish: silver carp, *Hypophthalmichthys molitrix*, bighead carp, *H. nobilis*, and grass carp, *Ctenopharyngodon idella*, is discussed. Although pond culture of common carp is relatively succesful in moderate climates, the growing period should be extended under controlled conditions to produce larger fingerlings. The culture of larval herbivorous carp is still difficult when the fish are grown outside their natural range. Survival is often low and growth rates vary widely. Because these fish develop and grow fast, the factors responsible for larval mortality are difficult to identify, due to their interactions and the dynamic relationships between fish demands and environmental conditions.
Laboratory studies show that the highest lower lethal temperature for the species in question should not exceed 16 to 17°C. Contrary to widespread opinion, tolerance to low temperature does not increase during the first 2 to 3 weeks of larval life. The upper lethal temperature (ULT) is 43 to 44°C and increases slightly with fish age (0.1-0.3°C/10 days). The optimum growth temperature is about 38°C. The lethal oxygen level decreases with growth. Resistance to starvation increases significantly in larvae heavier than 5mg. Although dry starter foods have been recently improved, natural food is still required.

Different techniques are used for larval rearing in Asia, Europe and the United States. The selection of a technique most suitable for a given region depends mainly on local climatic and social-economic conditions. Earthen ponds have been used in Asia since BC and in Europe since the Middle Ages. This technique utilizes the natural succession of fish food organisms and predators, which begins after filling the ponds. Proper timing between filling and stocking, proper stocking rates, chemical-organic fertilization, and feeding of the fish (in Asia only) are the major management methods. Because temperature conditions are often adverse during larval culture, techniques enabling temperature control have been developed. They include the use of indoor tanks followed by outdoor pond culture or the use of ponds receiving heated water effluents. Optimum thermal conditions exist in the southern US; however, ponds are not always drainable and cannot be dried completely. Under these conditions the natural succession method cannot be used as effectively. Selectively removing invertebrates with toxins combined with cage-and-pond, or tank-and-pond rearing gives the best results.

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ACCLIMATION AND SURVIVAL OF CARP (*CYPRINUS CARPIO* L.) FRY IN ALKALINE SOLUTIONS

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Introduction

Alkaline pollution is not as common in natural environments as acidic pollution. In carp ponds, however, temporary high pH levels (>10) can occur during hot summers, due to algal blooms. The purpose of the experiment was to elucidate acclimation under alkaline conditions and its effect on larval survival during acute toxicity tests in alkaline water.

Materials and methods

Carp fry (*Cyprinus carpio* L.), 18 to 20-day-old were used (offspring of one female). The fry was reared at a temperature of 20°C, in four groups; one control group (pH 7.8-8.2) and three with different alkaline conditions (pH 9.4, 9.7 and 10.3).

Chemical analyses of the water were made every second day of the experiment. Average values (in $mg.dm^{-3}$) in control and alkaline water were respectively as follows: $N\text{-}NH_4$: 0.06, 0.02; $N\text{-}NO_2$: 0.005, 0.007; Ca^{++}: 61.1, 3.0; Mg^{++}: 6.7, 3.0; Cl^-: 8.86, 8.86.

After 18 days of acclimation, acute toxicity tests (30 fishes in each test) in alkaline solutions (pH 10.6, 10.7, 10.8, 10.84 and 11.1) were carried out. Time until death of 50% of fish (LT_{50}) was determined during the tests.

Results and discussion

In Fig. 1 it is shown that the LT_{50} depends on the tested pH as well as the acclimation level.

The lowest tested level of pH differed only slightly (0.3 pH unit) from the highest acclimation level (pH 10.3). The level of pH 10.3 was not lethal; in this group, larvae developed normally under the rearing conditions. However, their growth was significantly slower, and their survival rate lower than in the control group (Korwin-Kossakowski, unpubl.).

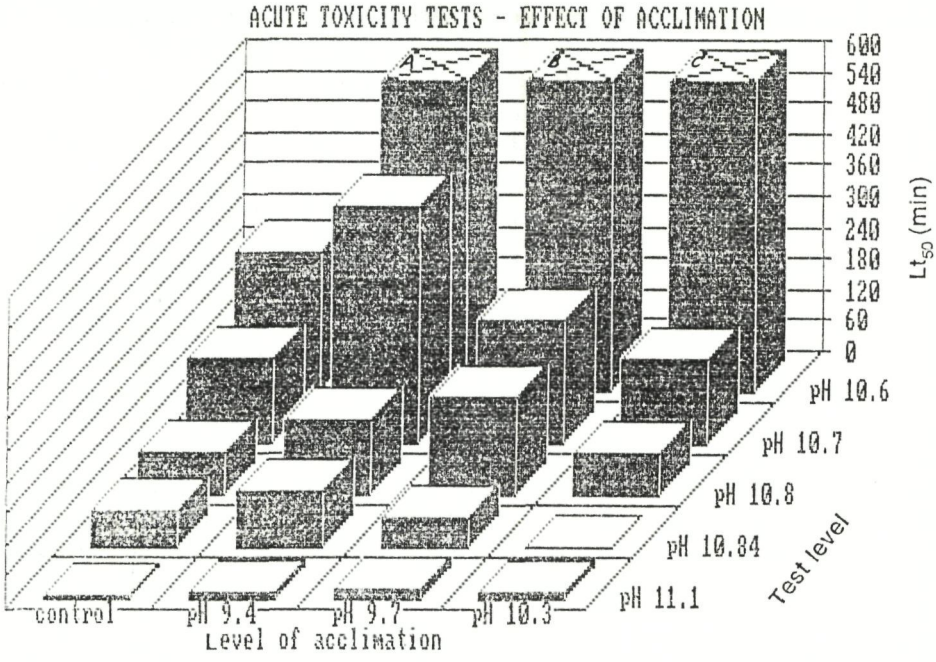

Fig. 1. Effect of acclimation on survival of carp larvae survival in alkaline acute toxicity tests. (A): <50% died during 96h; (B): <50% died during 96h; (C): 1 450min.

The toxicity level depends on many factors; individual resistance, fish condition, fish age or level of acclimation. Jezierska (1988) reported that a pH of 10.3 was lethal for carp fry acclimated to a pH of 7.0, while fry acclimated a to pH of 9.3-9.5 were slightly more resistant to this level. It is possible that the lethal alkaline level for the larvae examined in the present experiment, is between pH 10.3 and 10.6.

Unlike the acidic tests, very little is known about fish acclimation to alkaline water. However, Jezierska (1988) for carp, and Jordan and Lloyd (1964) for rainbow trout and roach, found that acclimation in alkaline water could slightly prolong the survival time in water of higher pH.

Results of the present experiment show that duration and level of acclimation were factors of a great importance for fish resistance to alkaline solutions. Carp fry was acclimated from the first day of life, during the most important period of larval development (18 days).

In Fig. 1 it is shown that the level of acclimation influenced survival time in alkaline toxicity tests. The pH 10.6 level was not lethal for larvae from groups acclimated at pH 9.4-4.7. Larvae acclimated to a pH of 10.3 were less resistant to the alkaline solutions than those from a pH of 9.4 and 9.7. This acclimation level, although not lethal, caused the worst conditions for the larvae and resulted in a higher sensitivity later on.

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IMPROVED HATCHERY OUTPUT OF A NEW SPECIES IN ORNAMENTAL FISH AQUACULTURE: THE CICHLID *PTEROPHYLLUM SCALARE*

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Introduction

The Amazonian cichlid, *Pterophyllum scalare* is a popular new aquarium species. In its natural habitat it occurs in small schools and during the reproductive season pair formation takes place. The species is a phytophilic guarder laying about 500 eggs in parallel rows, which are sequentially fertilized by the male. Offspring are cared for up to 7-8 days (Bergmann, 1971; Peters and Berns, 1982; Paepke, 1985).

An experiment with the objective of improving the hatchery output of *Pterophyllum scalare* is described. Some environmental parameters of the fertilized eggs and the fish fry were manipulated, and the effects on the hatchery output recorded. Guidelines for the controlled mass production of juveniles are described, which may also be applicable to other cichlid species.

Methods and methods

To allow for natural pair formation, a number of mature 1-year-old fish were stocked into a recirculation tank at a density of 20 fish/m^2. Once pair formation had occurred they were carefully removed to individual spawning tanks, while new fish were introduced to the holding tank.

Each spawning tank contained 50 l deionized tap water. The sides of the tanks were opaque and the front was screened. An ordinary recirculation filter provided aeration of the water. The water temperature was maintained at 26°C and 10% of the water volume was renewed each week. A single piece of submerged PVC tubing (45cm diameter), tilted at an angle of 60 to 70° was provided as a spawning substratum. The PVC tubing was detoxified with a 2% HCl solution. Selected broodstock couples were maintained for up to 1 year and fed *ad libitum* (about 1-3% of body weight/day).

Spawning and fertilization usually occurred shortly after the transfer of a couple to the spawning tank. New eggs were generally deposited every 2 weeks. Immediately after spawning and fertilization the PVC tube with the adhering eggs was transferred to the hatching chambers. These hatching chambers were 2 to 3 l cylindrical perspex incubation chambers with a conical bottom. The cylinders were immersed in thermoregulated water.

A separate gentle waterflow was maintained, through an inlet in the conical chamber bottom and an overflow outlet at the top. Synthetic freshwater may be used for this purpose, or water from a spawning tank may be transferred together with the eggs (Cairns, 1969). The volume of a hatching chamber sufficed to emerge the PVC tube with its adhering fish eggs. During hatching, the chambers were screened off from direct light.

At the time of transfer of the eggs, euflavine (acriflavine, trypaflavine) was added as a prophylactic at a concentration of $3mg.l^{-1}$. For the first 24h, the tube with the eggs was kept in the incubation cylinder at 23°C, and subsequently raised to 28°C. About 3 days after spawning, the embryos hatched. Four days later they would swim around in the cylinder. The larvae were then transferred to a grow out aquarium, the water level of which was gradually raised in the following fortnight (during which the swim bladder of the larvae developed). Feeding with *Artemia* nauplii or infusoria was started at the 7th day. Two months later the population density was kept at about 0.7 fish/l. The fry were protected against stress, and liberally fed to ensure optimal growth.

Results

The fish fry obtained a mean body length of 2cm about 2 months after fertilization. The hatchery output of the system described was approximately 8 000 fish per female per year, the mean brood being 325 fry per female every second week. More than 90% of the fertilized eggs hatched and the mortality of larvae and fry was less than 10%.

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A COMBINED REARING SYSTEM OF TANKS AND ILLUMINATED CAGES FOR COREGONID LARVAE

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Introduction

The optimization of restocking with pre-fed juveniles requires efficient and reliable techniques to rear large quantities of larvae. Recent improvement in the culturing of coregonid larvae on special dry food formulations (Champigneulle, 1988; Dabrowski and Poczyczynski, 1988; Rosch, 1988) and new studies on fish rearing in illuminated cages to attract zooplankton (Champigneulle and Rojas-Beltran, 1990; Mamcarz, 1990; Mamcarz and Kozlowski, 1990) provide new prospects to produce coregonids for restocking purposes. In the present study, an integrated rearing system in tanks and illuminated cages was used for *Coregonus lavaretus* larvae.

Materials and methods

Three week-long growth trials were conducted at Dgal Hatchery, IFI Olsztyn from March through April, 1990. Whitefish larvae were kept in two tanks with a capacity of 800 l. The water flow was 30 $l.min^{-1}$ for each tank and the temperature 17°C. Dry food was regularly supplied *ad libitum*, between 0700 and 2000h, by automatic feeders described by Mamcarz and Kozlowski (1989).The dry food used was reported by Dabrowski and Poczyczynski (1988). Fry prefed in tanks and two batches of newly-hatched larvae were kept from April to June 12, 1990 in the surface cages located in Lake Leginskie (mesotrophic, 228.3ha, 37.2m maximal depth). Each cage (volume 4.7m^3) was stocked with about 17 000 fish. After 5 weeks they were transferred to larger cages (10m^3). A nocturnal illumination was realized in each cage by an electric bulb (60W/24V) placed above the water surface. After 4 weeks supplemental dry food (Provimi Visvoeder) was added to two cages. Dry food was regularly given between 0700 and 2100h by automatic feeders. Samples of 30 to 50 fish were taken at regular time intervals for growth measurements. The knowledge of initial (w_i) and final (w_f) mean wet weight allowed to calculate the specific growth rate (SGR) on a percentage per day basis as:

$$(\log w_f - \log w_i) \times 100/(t_f - t_i)$$

Results

The survival rate of *C. lavaretus* larvae reared in tanks was 88.8 and 91.7% in tank 1 and 2 respectively (Table I). The final average length of the two batches of fish was not significantly different (Student's t-test $P>0.05$). The final average weight of fish in tank 1 was significantly ($P<0.05$) higher than in tank 2. The SGR was 4.5 and 4.9%/day.

Table I. Results of rearing coregonid larvae in tanks at 17°C (duration 19 days)

Parameter	Tank 1	Tank 2
Stocking (ind.)	35 000	43 000
Density ($n.l^{-1}$)	44	54
Catch (ind.)	32 112	38 203
Density ($n.l^{-1}$)	40	48
Survival (%)	91.7	88.8
Length (mm)	20.1(1.8)	20.0(1.5)
Weight (w_f) (mg)	43.8(13.4)	38.0(11.7)
SGR (%/day)	4.9	4.5
Final biomass ($g.l^{-1}$)	1.7	1.8

Initial weight (w^i)=5.8mg; standard deviations between brackets.

Table II. Results of cage rearing of coregonid larvae (duration 64 days)

Parameter	Cage 1	Cage 2	Cage 3	Cage 4
Stocking (ind.)	17 000	17 000	16 250	16 250
Density ($n.l^{-1}$)	3.6	3.6	3.4	3.4
Catch (ind.)	12 814	11 000	13 055	10 738
Density ($n.l^{-1}$)	1.3	1.1	1.3	1.1
Survival (%)	75.4	64.7	80.3	66.1
Length (mm)	64.4(4.7)^a	63.5(4.8)^a	45.8(4.4)^b	41.7(3.8)^c
Weight (w_f) (g)	2.0(0.6)^a	1.9(0.4)^a	0.7(0.2)^b	0.5(0.1)^c
SGR (%/day)	2.7	2.6	1.9	1.7
Final biomass ($g.l^{-1}$)	2.6	2.0	0.9	0.5

w_i=40.9mg in cages 1-2; w_i=6.9mg in cages 3-4; food: zooplankton + dry food in cages 1-3, zooplankton in cages 2-4; figures with no common superscript letter are significantly ($P<0.01$) different (Duncan's multiple range test).

The survival rate of pre-fed fishes in the cages was 64.7 and 75.4% (Table II). There were no differences ($P<0.01$) in size between pre-fed coregonids reared on zooplankton and those supplemented with dry food. Important differences in size, SGR and final biomass occurred between pre-fed larvae and those starved prior to cage stocking, within the same batch.

The present study suggests that integrated rearing (in tanks and illuminated cages) and mixed feeding with dry food and zooplankton, can be a good and fast way to produce coregonids for restocking. Fry achieve a large final size with a good survival rate and the rearing period is shortened to about 80 days.

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PRODUCTION OF PIKE-PERCH (*STIZOSTEDION LUCIOPERCA* L.) FRY, PROCEDURE AND DEVICES

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Introduction

A programme for enhancing pike-perch fry production in Finland began in 1978. We started from a combination of old Hungarian (Woynarowich, 1960) and Finnish (Hakola, 1930) production methods, which resulted in a simple but efficient procedure and technology, nowadays used to produce all pike-perch fry needed for pond culture (*circa* 20 000 000/year).

Brood fish

Spawners are captured from natural populations or from special brood-fish lakes. The latter are small lakes - usually with no native pike-perch stock, reserved for fry production. A limited stock of spawners is maintained by stocking the lakes with adult pike-perch and small prey-fish. We use fyke-nets or pound-nets to catch the spawners just before and during the spawning season on the spawning sites. An early start increases the duration of the otherwise relatively short spawning season: females, that are caught first, often spawn last, when the natural spawning season in the lake is already passed. If the fish are released after spawning, large amounts of fry can be acquired year after year from relatively small populations (Table I).

Spawning

Spawning takes place in floating net-cages anchored at the fishing sites. The cages are stocked with 1 to 3 females and 2 to 4 males per cage. If handled carefully, some 80% of the females spawn sooner or later. Average caging time before spawning has been 2 to 3 days, and the longest 30 days. Eggs attach on a nest (Fig. 1) made of bundles of rice-root fibres tied in the holes of a square aluminium plate or a piece of plastic-coated steel net. The nests are of two sizes: 65x65cm for large females (>2kg) and 45x45cm for smaller ones. One nest is enough even if there are several females in the cage. It is unlikely that two or more females spawn during the same night.

Table I. The amount of fry acquired from one natural pike-perch population and one artificial brood-fish lake in 1986-1990

	Lake Averia	Lake Pitkäniemenjärvi
Area (ha)	140	14
Max. depth (m)	6	11
Origin of stock	Introduced in 1930's, natural production	Introduced as adult fish in 1986
Fishing	Sport fishing	Fishing prohibited
Stocking	10 000 fingerlings/year	Grown-up fish 1986-1989
Size* of stock, May	1986,1987,1988,1989,1990	1986,1987,1988,1989,1990
No. of mature females	950 620 700 640 ?	0 65 65 65 ?
No. of mature males	1 280 840 640 450 ?	0 65 65 65 ?
Fishing gear	2 pound-nets, depth 4.5m	2 fyke-nets, depth 5.0m
Catch:		
No. of ripe females	100 70 75 90 75	43 37 34 20
% of mature stock	11 11 11 14 ?	66 57 52 ?
Mean size (kg)	1.4 1.3 1.3 1.5 1.7	1.7 2.0 1.9 1.9
No. of ripe males	150 120 104 140 85	30 31 30 18
Spawning facilities	40 cages, 50-80 nests	10 cages, 20 nests
No. of egg patches	90 60 59 61 65	22 24 18 16
% of females spawned	90 86 79 68 86	51 65 53 80
No. of fertilized patches	86 59 55 61 65	20 23 18 16
No. of fry, ($x10^3$)	8 000 6 250 5 165 8 500 8 245	1 235 2 031 2 697 1 722
No. of fry/patch, ($x10^3$)	93 106 95 139 127	62 88 150 108
No. of fry/fem.kg, ($x10^3$)	66 81 72 93 73	36 44 79 57

* Mark-recapture estimates.

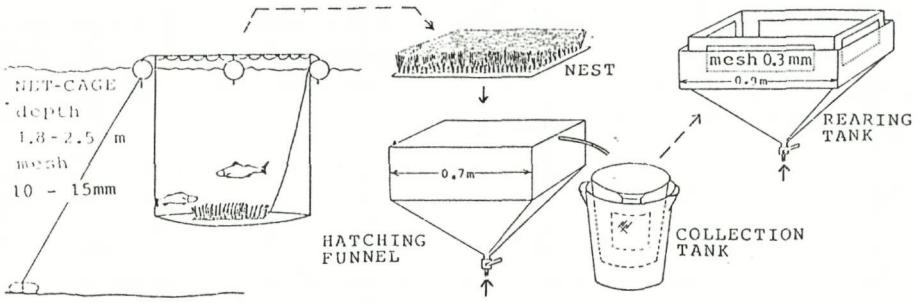

Fig. 1. Pike-perch fry production; procedure and devices.

Incubation

The nests are checked and flushed daily. After spawning, the female is released. The nest with eggs and the male, that has spawned, are removed into an empty cage, the fish still remaining in the cage receive a new, disinfected nest. The male guards the nest and keeps the eggs clean by moving water above the nest with its pectoral fins. Until 1986,

all egg-patches were incubated in a spray-chamber. Comparative tests in 1987 showed, however, that better results can be achieved if incubation takes place in the spawning cages, where *Saprolegnia* seldom infects the eggs (Table II).

Table II. Comparison of two incubation methods

Year	Spray-chamber			Net-cage		
	No. of egg-patches	Number of fry/		No. of egg-patches	Number of fry/	
		Patch	Fem.kg		Patch	Fem.kg
1986	119	83 400	55 700			
1987	35	99 400	60 000	68	115 900	85 4006
1988				164	113 400	9 300
1986-88	154	87 000	56 700	232	114 100	74 000

Hatching

The water temperature in the cages is measured daily. One day before the estimated hatching time a few eggs are placed in warm water (>18°C). If these eggs hatch in a couple of hours, it is time to bring the whole nest to the hatchery. Hatching takes place in square-shaped fiberglass funnels. Water (1-2 $l.min^{-1}$) flows in through the bottom, up through the holes of the nest plate and out through a tube near the edge of the funnel. Time and facilities needed for hatching are minimized by raising the temperature gradually to a relatively high level (18-22°C). Under these conditions hatching starts quickly and is completed in a day or two. After hatching it usually takes 2 to 5 days before the fry swim horizontally, and can be transferred to still-water ponds for further rearing. During this time the fry are kept in 200 l tanks, which are similar to the hatching funnels, but have an extra "collar" (Fig. 1). The amount of fry is estimated by taking, after careful stirring, five samples with a 50ml tube (diameter 12mm). The number of fry should not exceed 500 000 in one tank (2 500ind./l).

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NON-INFLATION OF THE GAS BLADDER OF LARVAL WALLEYE (*STIZOSTEDION VITREUM*): EXPERIMENTAL EVIDENCE FOR ALTERNATIVE HYPOTHESES OF ITS ETIOLOGY

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Introduction

Non-inflation of the gas bladder (NGB) is the dominating obstacle to the mass culture of many kinds of marine and freshwater perciform fishes. Hypotheses and experimental evidence for the etiology of the NGB problem include environmental, physiological, developmental and genetic factors. However, direct gulping and swallowing air via the pneumatic duct seems to be essential for initial gas bladder inflation in many kinds of physoclistous fishes. In walleye (*Stizostedion vitreum*), NGB is considered to be caused by a surface tension-oil film barrier that prevents the small larva from penetrating the surface film for the initial intake of atmospheric gas (Barrows *et al.* 1988; Kindschi and MacConnell 1989). Oily feed has been incriminated as the source of the problem in use of formulated feeds in the intensive culture of walleye (Colesante *et al.* 1986). To overcome this problem, Barrows *et al.* (1988) confined the feed within a feeding ring, but Kindschi and MacConnell (1989) found it to be ineffective. Friedmann and Bates (1988) reported that an oil sorbent cloth applied to the tank surface increased gas bladder inflation of larval striped bass (*Morone saxatilis*).

In the present study, experiments were undertaken to evaluate ways to improve gas bladder inflation of larval walleye reared on formulated feed in an intensive culture environment. The experiments included evaluation of practical ways to remove surface films of oil or feed from the rearing tanks, and a test of whether a rearing environment with high oxygen tension may provide a subsurface source of gas for filling the gas bladder or serve as an inducer of gas bladder inflation.

Materials and methods

Three experiments were conducted: (1) to evaluate use of a polypropylene oil-sorbent cloth to enhance gas bladder inflation; (2) to evaluate use of oxygen enrichment with pure oxygen as a stimulus to enhance gas bladder inflation; (3) to determine if a surface exit for the surface film would result in higher gas bladder inflation than fish reared in tanks without a surface outlet. The standard rearing tank used in all experiments was a flat-bottom cylinder with a surface drain; in experiment (2), however, the standard tanks

was compared with a cylindrical tank with a cone-shaped bottom described by Flowers (1989). The latter tank had a radial water inflow from a centrally located pipe, and a bottom screen and outlet. Fish in all experiments were fed entirely on formulated feed (fry feed Kyowa, BioKyowa Inc., Chesterfield, Missouri); no live feed was used.

Results

There was not a significant difference in treatment means for gas bladder inflation or survival of fish reared in tanks with and without polypropylene cloth (Table I). Fish reared in the standard tanks with a surface drain had nearly twice the bladder inflation rate of fish reared in tanks with a bottom drain and no surface outlet for surface film, but the difference between treatments was non-significant. However, there was significant difference in both survival and viability of fish reared in tanks with and without a surface drain; higher survival and viability occurred in tanks with a surface drain (Table II). The oxygenation treatment produced highly significant differences in oxygen concentration, but there was not a significant difference ($P > 0.05$) in gas bladder inflation, survival, or mean fish length between treatments measured after 23 and 41 days posthatch (Table III).

Table I. Gas bladder inflation (GBI), and survival of larval walleye reared from 2 days to 26 days posthatch (24 days in culture) in tanks with and without oil-sorbent cloth (means ± SD)

	Sorbent cloth treatment		ANOVA[a]	
Parameter	With	Without	F-Value	P-value
Survival to 26 days	4.6±2.4	7.5±2.4	1.96	0.220
GBI (%)	13.1±10.9	12.5±10.6	0.004	0.953

[a] One-way analysis of variance, five tanks equipped with sorbent cloth, two tanks without.

Table II. Survival, gas bladder inflation (GBI) and viability of walleye fry reared to 25 days posthatch in tanks without and with surface drains (F-89-1)

	Tank design		ANOVA[a]	
	Without surface drain	With surface drain	F-test	P
GBI (%) 4.6±	21.7±10.4	41.2±23.9	01.70	0.249
Survival (%)	10.1±01.7	43.4±20.2	07.72	0.039
Viability	02.1±00.9	14.3±05.0	16.41	0.010

[a] One-way analysis of variance, three replicates of the radial-flow tank and four replicates of the circular-flow tanks.

Table III. Survival, gas bladder inflation (GBI) and body lengths of larval walleye reared from 1 to 25 days posthatch and then to 41 days posthatch in tanks supplied with ambient and oxygen enriched water (F-88-2)

Parameter	Treatment category		ANOVA[a]	
	Ambient oxygen	Enriched oxygen	F-value	P-value
23 days rearing period				
GBI (%)[a]	11.7±11.5	10.0±05.0	00.05	0.830
Survival (%)	25.3±06.3	12.9±06.2	05.96	0.071
Fish length (mm)	12.1±01.4	11.8±01.2	01.48	0.226
41 days rearing period				
GBO (%)	03.3±05.8	10.0±10.0	01.00	0.374
Survival (%)	08.7±03.8	02.7±01.9	05.94	0.071
Fish length (mm)	20.4±02.8	19.3±01.7	03.44	0.069

[a] One-way analysis of variance, three tanks each treatment.

Discussion

Effective clearing of the suface is necesary because "when air is gulped from the surface and forced into the swim bladder, feed and bacteria can also enter" (Kindschi and MacConnell, 1989). An oily surface film may prevent the weak-swimming larvae from breaking through the air-water interface to gulp air for first filling their gas bladder. Equipping tanks with a polypropylene oil-sorbent cloth was reported to be a practical method to clean the water surface of an oily film, contributing to production of striped bass with a mean GBI of 96% (Friedmann and Bates, 1988). In the present study, this technique did not enhance gas bladder inflation or survival of larval walleye. Rearing tanks provided with a screened surface drain of sufficient size and large enough mesh to facilitate discharge of feed, oil or bacteria on the water surface produced a higher percentage of viable fish than tanks with a bottom drain and no surface exit. High oxygen tension did not function to induce gas bladder inflation in larval walleye.

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A NEW AUTOMATIC COUNTER FOR SMALL FISH LARVAE

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Introduction

During the larval rearing period (at experimental or production scale), it is generally advised to stock a precise number of larvae per tank to determine the rearing density, daily food ration, survival rate, *etc.* At an experimental level, the larvae are usually counted manually by siphoning or counting with a small net. If experiments require several thousands of larvae per group, this procedure becomes laborious and time-consuming. Therefore gravimetric or volumetric methods are generally used in large productions (Huet, 1970; Woynarovich and Horvath, 1981). These methods, however, only give a relatively good precision with large eggs and small freshwater or marine fish larvae. In this paper a new automatic device is described to precisely count large numbers of small fish larvae of various species. Trials were made with several freshwater species (gudgeon *Gobio gobio*, goldfish *Carassius auratus*, and African catfish *Clarias gariepinus*) at the end of the yolk resorption period or after a few days of tank rearing. Automatic counting was compared with results obtained by the gravimetric method.

Materials and methods

The counting system includes an upper stocking tank (2 l), interchangeable counting blocks with a pipe of an appropriately reduced section. The counting blocks are connected with the optical fiber set and the counter, and a lower tank to collect the larvae after counting (Fig. 1). The optical fiber set (E32-TC200F, OMRON) consists of a photoelectric switch of the light barrier type. The optical fiber can detect very small opaque objects of 0.25mm. The response time is 0.5 millisecond to switch on and off. The optical fibers are connected to an amplifying unit (E3XR-CE4, OMRON) which generates red light. When the light beam is interrupted by a small larva, the E3XR unit generates a signal output to the counter (H7CS-B, OMRON). This device allows to count up to a preset value and generates an output signal once the present value has been reached. The counting speed can be adjusted up to 10kHz. The section of the pipe must be adapted to the size of the larvae in order to allow the passing of one larva and at the same time to interrupt the light beam effectively.

This apparatus was conceived to minimalize the wounding of the fish during counting. Automatic counting of goldfish and gudgeon larvae was compared with results from manual and gravimetric counts. In the latter method exactly 500 larvae were counted and weighed. Large quantities of larvae were then weighed and the total number was deduced by analogy. Before weighing to the nearest 0.1mg, the fish were placed on a paper towel for about 10sec to remove most of the adhering water.

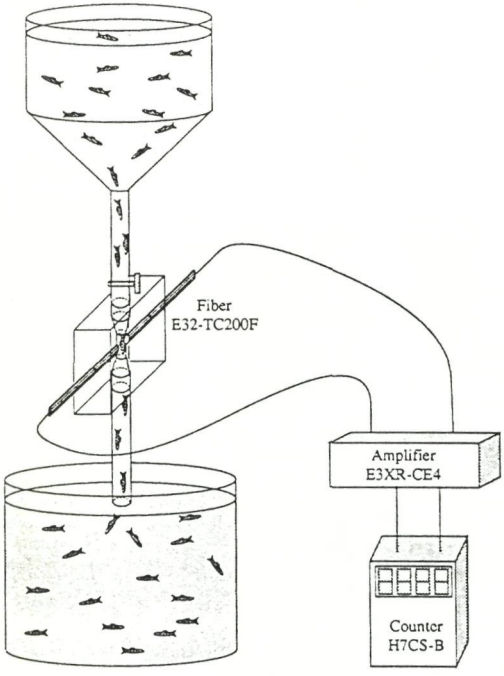

Fig. 1. Automatic device to count small fish larvae. Larvae are not drawn to scale but magnified.

Results and discussion

As shown in Table I, the automatic counting system functioned successfully, whatever the species and the size of the larvae, except for the gudgeon larvae at the onset of the swimming stage in which the error was higher than 20%. In the other trials, this error was lower than 3%. The high error percentage observed with the 2-day-old gudgeon larvae was due to their slimness and to the absence of body pigmentation before day 10 (body length = 9.5-11.5mm) (Prokes and Penaz, 1979). The best results were obtained with goldfish larvae which are well-pigmented from hatching onwards. Considering the heterogenous growth of the larvae of a same group (particulary in 25-day-old gudgeon and 9-day-old goldfish), the error percentage was not significantly reduced with the increasing size of the larvae, as could be expected. Indeed, the pipe section of the counting block must be slightly larger than the largest larva of the group (to avoid clogging of the pipe). Some of the smallest larvae were not able to interrupt the light beam. Compared to the results obtained by the gravimetric method (Table I), the precision of this automatic counter was very high and mortality was lower than 0.5%.

Table I. Performance of the automatic counting system and comparison with the gravimetric method for various freshwater fishes and sizes of larvae

Species	Age[1] (d)	Weight (mg)	Length (mm)	Actual number of larvae	Automatic counter			Gravimetric method
					Pipe section (mm)	Water flow ($l.h^{-1}$)	n^2	n^2
G. gobio	2	0.8±0.2	5.5±0.5	3 000	1.5	17	2 330±281	3 504±110
	10	5.8±1.6	9.6±1.0	2 000	2.0	28	2 020±12	2 274±76
	25	21.6±11.7	12.9±2.3	1 000	3.0	48	985±7	1 029±58
C. auratus	3	2.2±0.4	6.0±0.6	3 000	2.0	28	3 008±14	3 334±135
	9	13.1±4.5	9.9±1.3	1 000	2.5	40	990±10	1 043±41
C. gariepinus	1	3.0±0.2	7.6±0.4	200	2.0	28	196±2	-
	7	16.1±3.1	11.9±1.1	200	2.5	40	198±3	-

[1] Age of the larvae calculated at the onset of the swimming stage.
[2] Mean value and standard deviation; three replicates.

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Marine fish

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EFFECTS OF SIZE DISTRIBUTION, FEEDING AND LIGHT REGIME ON GROWTH AND SURVIVAL OF COD JUVENILES IN TANKS

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Introduction

Culture of Atlantic cod, *Gadus morhua* L., juveniles has been hampered by cannibalism and incomplete weaning during the early juvenile stage (Folkvord, in press). In the production ponds of juveniles the plankton supply will eventually become limiting (Blom *et al.*, in press). If this occurs before the juveniles can be readily weaned onto artificial diets, cannibalism will develop (Folkvord, in press). Increased size dispersion is a problem since it is not possible to grade the cod juveniles in the pond. We therefore designed two experiments to evaluate the effects of 1) initial size distribution and 2) light and feeding regime, on growth, survival and cannibalism of juvenile cod.

Materials and methods

The experiments were carried out in black 180 l circular tanks with conical bottoms. The two experiments lasted 16 and 37 days and the temperature averaged 8.5 and 9.6°C. respectively. The fish came from a juvenile production pond (Blom *et al.*, in press) and averaged 0.5 and 0.9g at the start. The stocking density was 50 fish/tank and 1mm granulate commercial dry feed was supplied in excess (every 8min, 24h per day). Five or six replicates were used per treatment. In the first experiment we used four size distribution categories: hand-graded (minimum variation), apparatus-graded, apparatus-graded plus two larger siblings (on average 50% longer) and apparatus-graded plus 10 larger siblings. In the second experiment (all apparatus-graded) we used three different feeding (F) and light periods (L) per day (16F-16L, 16F-24L or 24F-24L). In addition we used four different feeding frequencies: fish were fed every 1, 8 or 32 min or manually fed (three or four times a day).

Results

Initial size distribution had a pronounced effect on survival ($P<0.001$, one-way ANOVA, range 18-96%). About 75% of the mortality was due to cannibalism. In tanks where 10 larger siblings were added most of the remaining 40 fish were eaten during the 16 day experimental period (Fig. 1). Average growth rates were also closely related to mortality

rates because of selective removal of smaller individuals. The average daily growth rate ranged from 3.5 to 11.5% in the tanks with the lowest and the highest cannibalism rates respectively.

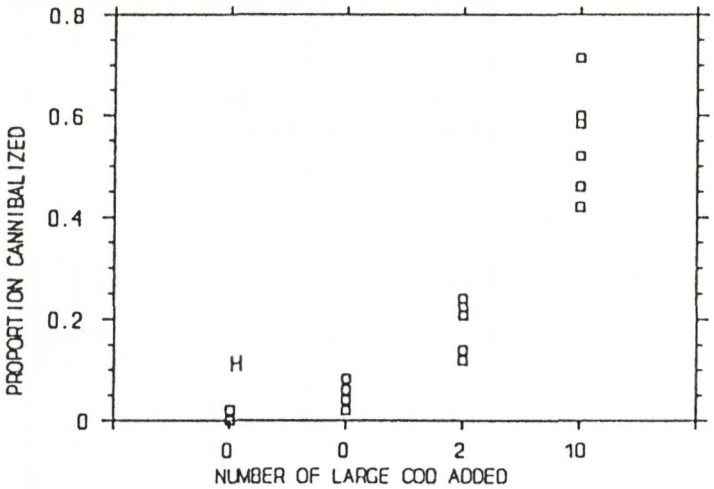

Fig. 1. Proportion cannibalized *versus* number of large cod added per tank. H indicates tanks with hand-graded fish.

In the second experiment no effects of feeding and light hours were found on survival or average growth ($P<0.05$, 1-way ANOVA). There were also no significant differences between the feeding frequencies tested ($P<0.05$, 1-way ANOVA). Overall survival was 80% (range 50-96%) and the average daily growth rate 2.7% (range 2.2 -3.4%/day). About 25% of the mortality was due to cannibalism.

Discussion

The results clearly show the dramatic effect on cannibalism and survival of size differences among equally aged cod juveniles on cannibalism and survival. We therefore strongly recommend grading of small cod juveniles to avoid problems related to size-dispersion. This implies an earlier harvest of smaller juveniles from the production ponds. The survival during weaning will be lower if it takes place at an earlier stage (Otterå and Lie, in press), but overall survival may be higher due to a shortened period of intense cannibalism.

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FOOD CONSUMPTION OF EUROPEAN SEABASS *DICENTRARCHUS LABRAX*, LARVAE REARED AT DIFFERENT WATER TEMPERATURES

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Introduction

Most fishes are extremely dependent on the ambient temperature that influences their rate of metabolism and hence their nutritional requirements. Metabolism increases with temperature up to an optimum, above which it decreases. Under rearing conditions larvae are submitted to a wide range of temperatures. Few data are available on the optimum temperature. This paper reports on intensive rearing experiments carried out to determine the effects of changing water temperature on food consumption of larval seabass, *Dicentrarchus labrax*. Consequences on larval growth and survival are also considered.

Materials and methods

Fish were reared in six cylindro-conical 500 l fiberglass tanks placed in plastic sheet enclosures. Each of them was stocked with 1-day-old larvae at a density of 50/l. Three temperatures (15, 20, and 25°C) were tested in duplicate. Experimental rearings lasted 74, 50, and 40 days for each temperature level, respectively. With a 9h photoperiod, incandescent lights provided 100lux at the water surface. Tank volumes were exchanged from 10%.h^{-1} the first days to a maximum of 36%.h^{-1} the last days. Larvae were fed *Brachionus plicatilis* from day 5 to day 16 (2 to 8 preys/ml/day) and *Artemia* nauplii and metanauplii from day 12 onwards (1 to 14 preys/ml/day). Dead fish were counted and removed every day. At the end of the experiment, the survivors were evaluated. From day 5, and every 5 days, a sample of 60 larvae was taken from each tank. Mean dry weights and mean total lengths were recorded. To estimate the food consumption, seven diurnal series of 10 to 15 samples, taken at regular intervals, were obtained at each temperature. The samples consisted of at least 30 fish and were stored in 5% formalin. Mean total lengths and mean number of preys in the stomach contents were calculated. Daily rates of food consumption were estimated from the gastric evacuation rate and the amount of food in the digestive tract during the day, using a method described by Elliott and Persson (1978). Daily meal and daily ration, the amount of food consumed per day (dry weight) and expressed as a percentage of the body weight, respectively, were calculated.

The growth efficiency coefficient (K_1), as defined by Ivlev (Ricker, 1979), equal to the increase in mass (dry weight) divided by the food ingested (dry weight), was also calculated.

Results

Data on growth (length and weight) were analyzed in a nested analysis of variance. The effect of temperature was significant ($P<0.01$). On day 40, the mean total length and mean dry weight were highest at 25°C (14.7mm and 3.9mg), and higher at 20°C (11.9mm and 1.05mg) than at 15°C (9.1mm and 0.35mg). At 15$_o$C and 20°C mean survival (51% and 40%, respectively) was significantly higher ($P<0.05$) than at 25°C (21%).
Daily meal increased with temperature and had an exponential relationship with total length (Fig. 1a). The daily ration also showed a tendency to increase with temperature and varied with the size of the fish (Fig. 1b). On the first day of feeding, the daily ration was lowest (20% to 30%) with small larvae, then it increased rapidly (80% to 165%) to finally decrease in larger fish (60% to 90%). K_1, estimated within the 0.1mg-0.6mg dry weight interval, was 8, 10.7, and 9.5% at 15, 20, and 25$_o$C, respectively.

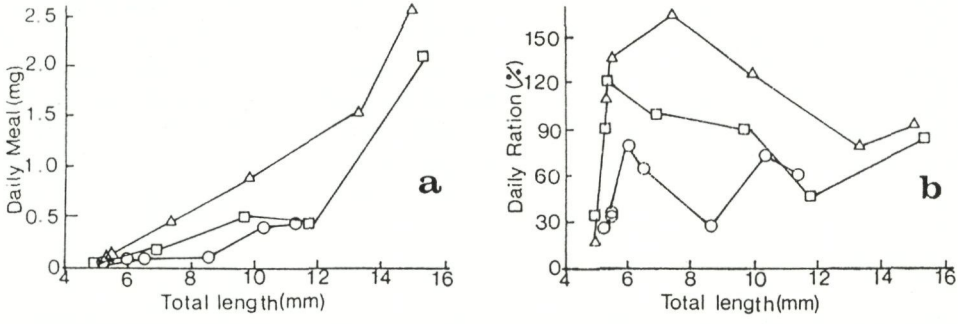

Fig. 1ab. Daily meal (a) and daily ration (b) in relation to growth (total length) of seabass, *Dicentrarchus labrax* larvae reared at 15 (circle), 20 (square), and 25°C (triangle).

Discussion

With increasing temperature, the growth of larvae improved, but a higher mortality occurred. Similar results were reported for the larvae of *Paralichthys olivaceus* (Yasunaga, 1971), *Cynoscion nebulosus* (Taniguchi, 1981), and *D. labrax* (Johnson and Katavic, 1986). Negative effects of the highest temperatures on survival were observed mainly during the first 2 weeks after hatching. Probably, small larvae were more sensitive and could not eat enough. The daily meal and daily ration were positively correlated with temperature. This increase in food consumption was more than sufficient to meet the energy requirements of the automatic increase in basal metabolism and activity (Ricker, 1979), allowing an increase in growth rate. However, this was not true for most young larvae and caused high mortality. Values found for the daily meal were higher than those already reported for *D. labrax* (Barahona-Fernandes and Conan, 1981). Decrease in daily ration with growth is in accordance with findings of other researches (Yasunaga, 1971;

Laurence, 1977). The K_1 was slightly higher at 20°C which could indicate that the temperature optimum for larval seabass in these particular rearing conditions had been reached. It would, however, be profitable to consider the range 20 to 25°C in future investigations.

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PHOTOPERIODIC EFFECTS ON THE GROWTH AND FEEDING RHYTHM OF EUROPEAN SEABASS, *DICENTRARCHUS LABRAX*, LARVAE IN INTENSIVE REARING

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Introduction

The alternation between light and darkness has a considerable impact on the normal development of living organisms. Muller (1978) considered this factor to be the most important one affecting rhythmic activities of fish. Many fish larvae are diurnal; they are reared under continuous illumination to improve growth. However, there are not sufficient experimental data for most species, and it is possible that continuous illumination is not suitable for normal fish development. This paper reports on the impacts of a variable photoperiod on the growth, survival, and swim bladder inflation of larval seabass, *Dicentrarchus labrax*. The effects on the feeding rhythm were also considered.

Materials and methods

Fish were reared in four cylindro-conical 500 l fiberglass tanks placed in plastic sheet enclosures. Each of them was stocked with 1-day-old larvae at a density of 95ind./l. Two photoperiods, 9h of light and 24h, were tested in duplicate. During the light phase, incandescent lights provided 500lux at the water surface. The water temperature increased from 16°C at the beginning to 23°C at the end of the experiment (day 30). Tank volumes were exchanged from 10%.h^{-1} on the first days to a maximum of 50%.h^{-1} on the last days. The larvae were fed *Brachionus plicatilis* from day 5 to day 13 (3 to 8 preys/ml) and *Artemia* nauplii and metanauplii from day 10 onwards (3 to 10 preys/ml). The dead fish were counted and removed every day. On day 30, the survivors were counted. From day 10, and every 5 days, a sample of 30 larvae was taken from each tank. Mean dry weights, mean total lengths, and functional swim bladder rates were determined.

On days 11 and 30 a series of 17 samples of 30 fish, taken at regular intervals, was taken for each photoperiod, and stored in 5% formalin. Mean total lengths and mean number of preys in the stomach contents were calculated.

Results

Data on longitudinal growth were analyzed in a nested analysis of variance. The effect of photoperiod was significant ($P<0.05$). An exponential relationship existed between dry weight and time; the slopes of the regressions were compared and were significantly affected by photoperiod ($P<0.01$). On day 30, the mean total length and mean dry weight were higher at 24h light (11.9mm and 1.55mg) than at 9h light (9.5mm and 0.75mg). A 9h photoperiod resulted in a mean survival of 81%, significantly higher ($P<0.02$) than the mean of 55% survival obtained with a 24h photoperiod. The same trend occurred with the number of functional swim bladders, of which the average inflation rates were 37 and 6% respectively for 9h and 24h photoperiod.

Fish reared under both conditions clearly showed a circadian feeding rhythm and being more evident with 30-day-old larvae (Fig. 1). The larvae submitted to a 24h photoperiod rarely had an empty digestive tract, but from 1200h to 0800h the number of preys was lowest and decreased to zero in many individuals. Larvae submitted to the 9h photoperiod stopped feeding in the dark and completed evacuation of the digestive tract in 4h.

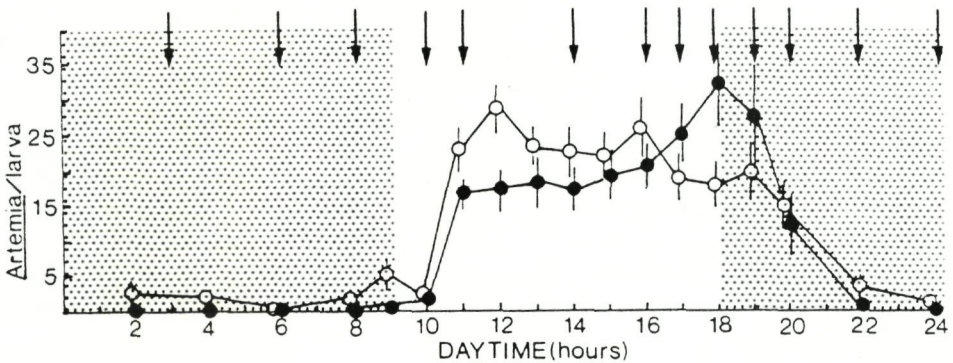

Fig. 1. Number of preys during a day in the stomach contents of 30-day-old seabass, *Dicentrarchus labrax*, larvae reared at 9h photoperiod (o) and 24h photoperiod (•). (->) prey distribution; (▒▒▒) dark phase.

Discussion

When eliminating the dark phase, larvae had a better growth and a lower survival rate than those submitted to the 9h photoperiod. Similar results were reported for the larvae of *Archosargus rhomboidalis* (Dowd and Houde, 1980) and *Mylio macrocephalus* (Kiyono and Hirano, 1981). Contrary to these and the present observations, Tandler and Helps (1985) found that 24h light was better than shorter photoperiods for the survival of *Sparus aurata*. Continuous illumination also produced the lowest number of functional swim bladder rates. This could cause serious problems afterwards (Chatain, 1987; Chatain and Dewavrin, 1989).

Fish submitted to darkness stopped feeding- and swimming activity. The highest number of preys in the stomach contents was observed at the end of the light phase (1800h). A photoperiod longer than 9h could be more suitable for seabass larvae. Fish subjected to continuous light had, on the other hand, a period (0000h to 0800h) of reduced feeding activity but with active swimming. Fish behaviour, including feeding and locomotory activities, is determined by the photoperiod (Manteifel *et al.*, 1978). According to Dowd and Houde (1980), if the amount of food that larvae can ingest in a day is limited, individuals reared under longer than optimal photoperiods expend more energy than food consumption can provide. So, less energy is available for growth and mortality can be expected. This seems to explain the differences in survival found between 9h and 24h photoperiods. Blaxter (1978) stated that a feeding rhythm may be useful in preventing overfeeding. Probably feeding has an endogenous control like many other diurnal rhythms (Muller, 1978).

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PREY CAPTURE ABILITY OF JUVENILE SILVER BREAM (*SPARUS SARBA*) UNDER DARK-ADAPTATION

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Introduction

Silver seabream (*Sparus sarba*) inhabits rocky, coastal waters and is widely distributed from Japan to Taiwan. This species is of commercial interest because of its good taste and its ease of larviculture. Studies on the morphogenetic and ontogenetic development of larvae are, however, scarce. Ontogenetic changes in larval behaviour are closely related to the morphogenetic developments of the sense organs. Vision is essential in prey-selection, predator avoidance and shoaling behaviour (Blaxter, 1968; Lasker, 1981). Therefore, it is remarkable that most pelagic marine teleosts in their early stages develop vision first and the other senses later.

The scotopic vision (dim-light vision) of most marine teleosts develops during their metamorphosis from larvae to juveniles. Although the majority of larvae are considered as diurnal feeders, the feeding regime offered is always species specific and has a decisive impact on growth (Blaxter, 1980; Houde and Schekter, 1980; Kawamura and Hara, 1980; Townsend and Winfield, 1985). The high mortality of some teleost larvae results from an inadequate or inefficient feeding response at the beginning of exogenous feeding (Hunter,1980; Lasker, 1981). Therefore, the understanding of the development of the sensory ability during the early stages becomes crucial in larviculture.

The purpose of the present study is to investigate the prey-capture ability of juveniles of silver seabream under dark adaptation in order to know their sensory functions for feeding capacity.

Materials and methods

Fertilized eggs of silver seabream (*Sparus sarba*) were obtained from the Pescadore Branch of the Taiwan Fisheries Research Institute. To compare the functionally sensory feeding mechanism, two groups of juveniles with a body length of 12 to 15mm and 30 to 40mm (respectively about 30- and 50-days old) were studied.

The juveniles were presented in respectively 1 and 3 l aquaria. *Artemia* as prey, at a concentration of 200 and 400 nauplii. The prey density was examined every 30min for 150min under dark conditions. Three replicated experiments were carried out for both stages and the Chi-square test was applied to assess the differences between active and passive feeding. The theoretical curve of passive feeding was derived from

$N_t = N_o(1- P)$ (Kawamura and Hara, 1980).

Results and discussion

The two juvenile stages of silver seabream had the different abilities of prey-capture under dark-adaptation. In Fig. 1 it is shown that the examined number of prey was identical to that of the theoretical number (*i.e.* prey captured by chance) for the smaller juveniles. A Chi-square test did not reveal significant differences. For the larger juveniles, a significant difference ($P<0.05$) in the examined and theoretically expected number of prey was, however, established by a Chi-square test (Fig. 2).
Under dark adaptation, the younger juveniles in this study could obtain preys only by passive feeding, but the older juveniles captured their preys actively or obtained it by passive feeding.

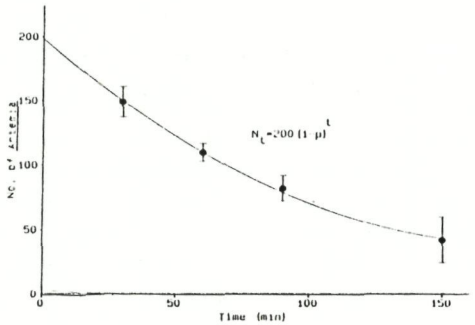

Fig. 1. Changing amount of *Artemia* nauplii fed to younger juveniles of *Sparus sarba* (TL: 12-15mm) under dark adaptation with 150min time course. The smooth curve is derived from $N_t=200(1-P)$. The solid circles and their vertical bars indicate the means and standard deviations of the exactly measured numbers at that time.

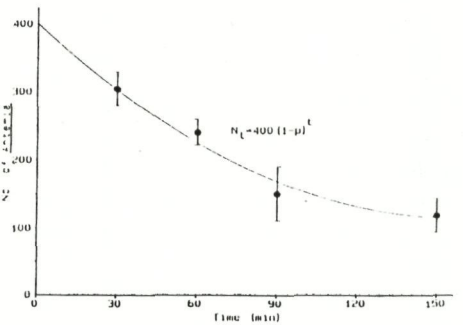

Fig. 2. Changing amount of *Artemia* nauplii fed to older juveniles of *Sparus sarba* (TL: 30-40mm) under dark-adaptation with 150min time course. The smooth curve is derived from $N_t=400(1-P)$. The solid circles and their vertical bars indicate the means and standard deviations of the exactly measured numbers at that time.

Marlivae (1981) found that the soft sculpin larvae, *Gilbertidia sigalutes*, fed visually, but their juveniles shifted from visual to "distant touch" feeding behaviour and from twilight to nocturnal feeding. In most species the adaptive functions seem to maximize feeding opportunities. The results of the present experiments showed that the younger juveniles fed passively by random food sampling under dim-light conditions, and that the older juveniles can actively feed.

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THE RELATIONSHIPS BETWEEN LIGHT AND LARVAE OF *SPARUS AURATA*

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Introduction

Among the numerous abiotic factors which regulate fish larvae activity, light plays a major role in species classified as "visual predator" such as *Sparus aurata* (Blaxter and Staines, 1971; Houde, 1973; Hunter, 1980; Tandler and Mason, 1984). Prey selection and localization by fish is highly dependent upon the light intensity and contrast existing between prey and environment (Blaxter, 1980; Kentouri, 1985). The searching time is, of course, limited by the photoperiod. Several combinations of light intensity, photoperiod and tank colour were tested in order to enhance encounter probability between prey and larvae and thus to improve the culture success of seabream larvae under intensive conditions.

Materials and methods

Sparus aurata larvae were placed in black or white cylindroconical fiberglass tanks ($0.5m^3$), immediately after hatching, at densities of about 100 ind./l. Until the age of 4 days, the larvae were kept under screened natural light (intensity 15-20lux; photoperiod 12h). On day 5, natural light was replaced by artificial incandescent light placed 1.2m above the water surface (Cold-ray bulb, PAR 38, 120 W, GE). A closed recirculating water system provided seawater at 35ppt and 20°C, with a turnover rate of $20\%.h^{-1}$ from the bottom of the tank. The aeration rate was $50ml.min^{-1}$. The food consisted of live rotifers, *Brachionus plicatilis*, starting at mouth opening (day 5, at 20°C). Prey density was adjusted twice a day to 10 rotifers/ml. The water surface was continuously cleaned by a skimmer in order to enable the larvae to inflate their swim bladder (Chatain and Ounais-Guschemann, 1990). All combinations of experimental conditions were run in duplicate. The best conditions in one experiment were selected for the following ones.

Results and discussion

Preliminary trials using combinations of two light intensities (100 and 1 500lux) and two photoperiods (12 and 24h), were run during the first 10 days of feeding. They showed that mortality was nearly total in all the tanks which were not under the highest light

intensity (1 500lux) and continuous illumination. In these tanks, only 10 to 40% of the fish fed, whereas almost all larvae (90-95%) fed in the 1 500lux - 24h photoperiod tanks.

Intensities of 150, 300, 600 and 1 300lux were tested, using black tanks under a continuous illumination regime. Growth and survival were recorded over a 30 day period and were significantly better in the 1 300 and 600lux tanks compared to the 300 or 150lux conditions. Gain in growth (length) was between 10 and 15% under high intensities compared to the two lowest intensities (Fig. 1). Survival rates were double (20% *versus* 10%). Analysis of the digestive tract's content of the larvae showed that the mean number of ingested prey per larval per 24h was two times greater under the highest light intensities. This was simply due to the fact that twice the amount of fish were feeding. The tested light intensities had no effect upon swim bladder inflation.

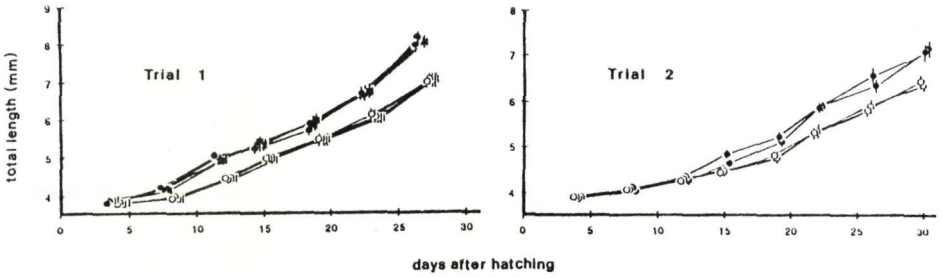

Fig. 1. Growth in length of *Sparus aurata* larvae reared under four light intensities: 1 300lux (■); 600lux (•), 300lux (□), and 150lux (○).

A 12h photoperiod was compared to continuous illumination, using a 600lux intensity and black tanks. A gain of roughly 10% in growth (Fig. 2) and a three-fold increase in survival rates (25% *versus* 8%) were observed in tanks where larvae were exposed continuously to light. Analysis of the digestive tract's content suggests that these gains result from a prey consumption two times greater under continuous light. The tested photoperiods had no effect upon swim bladder inflation.

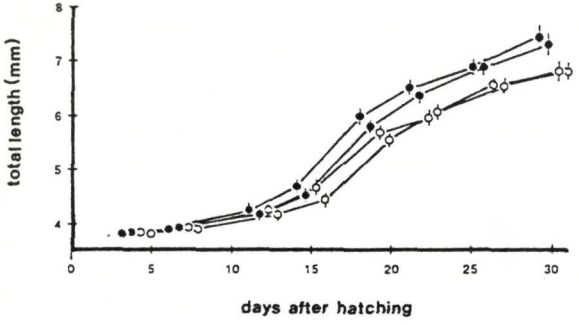

Fig. 2. Growth in length of *Sparus aurata* larvae reared under two photoperiodic regimes: 24h (•) and 12h (○).

Effects of the colour (black or white) of the wall and bottom were checked under continuous illumination of 600lux. The results show that the growth in tanks with white

walls was better, with a gain of 13% compared to that in black walled tanks (Fig. 3). On the contrary, survival rates were better in the latter (45% *versus* 21%). The colour of the bottom had no effect upon larval performance. These antagonistic results might be explained by a "density effect" : insufficient space is often a growth inhibitor. The high mortality observed in white walled tanks, just after the yolk sac resorption (day 9-12), could be related to the partial failure of the first exogenous feeding due to an insufficient contrast between the prey and the white walls of the tank.

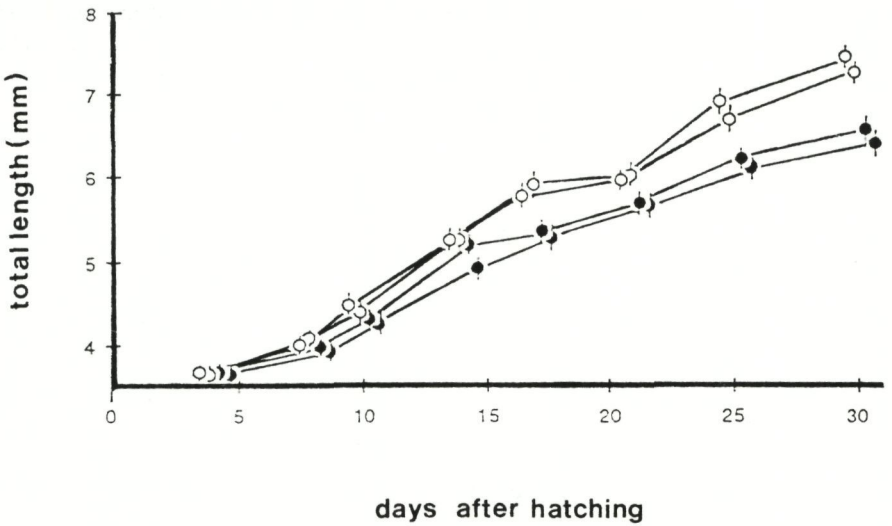

Fig. 3. Growth in length of *Sparus aurata* larvae reared in white (o) or black (•) walled tanks.

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DETERMINATION OF LARVAL MASS REARING STANDARDS IN THE SEA BREAM *SPARUS AURATA*

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Abstract

Biological and physiological aspects of the trophic life of *Sparus aurata* larvae were studied in order to define rearing standards for the mass production of 30-day-old postlarvae. The model was established using 500 l cylindroconical tanks and initial densities of 100 larvae/l. The larval cycle is achieved in totally recycled seawater at 20°C and 35ppt.

The renewal of water and air through the bottom of the tank, at respective rates of 20%.h^{-1} and 50ml.min^{-1}, associated with a 600lux continuous illumination, optimize the predatory activity at the beginning of the trophic life. Below 100-150lux, the hunting activity is negatively affected. The feeding rhythm, which is set by the alternation of darkness and light, disappears under continuous light. Tanks with black walls facilitate prey localization and double the survival rate, independently of the colour of the bottom (black or white) (Chatain and Ounais-Guschemann, 1991). The "wall syndrome", which is systematically observed in 4mm larvae (5 days), does not alter the predatory activity. It is not related to the presence of prey and occurs at light intensities as low as 150lux.

The rotifer *Brachionus plicatilis*, at sizes between 100 and 200µm, represents an adequate initial prey. Below 5 rotifers/ml, the food ingestion is reduced for 4-5mm larvae (15 days). There is no such threshold for 6-7mm larvae (22 days). The free amino acid content of prey plays a prominent role in larval growth, whereas essential fatty acids are important for growth and survival. For the latter, the amount of docosahexaenoic acid (22:6n-3) could be determining. The supply of these compounds can be provided by the enrichment of rotifers in lipid and protein. If the rotifers are only enriched with protein, the switch to *Artemia* is only possible with 7 to 8mm larvae (30 days).

The development of the larvae's swim bladder requires air gulping at the surface. It can be facilitated by continuous elimination of the superficial oily film by a blower combined with a floating trap.

All of these standards allow, for the first 30 days of rearing, a growth rate of approximately 0.2mm/day and result in a survival rate between 10 and 40% with a mean of 25%.

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LORDOTIC DEFORMATION AND ABNORMAL DEVELOPMENT OF SWIM BLADDER IN SOME HATCHERY-BRED MARINE PHYSOCLISTOUS FISHES

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Abstract

Lordosis has occurred at a high incidence and caused serious problems in some marine physoclistous juveniles which are bred at many hatcheries in Japan, such as red sea bream *Pagrus major*, Japanese seabass *Lateolabrax japonicus*, amberjack *Seriola aureovittata*, *etc*. Extensive studies have been initiated since 1975 to elucidate the causes of this disease and to propose countermeasures.

Lordosis is found in individuals which have an abnormally developed swim bladder at the larval stage. In red sea bream and many physioclists, a pneumatic duct exists for only a short period during early life. During this period, the larvae gulp air at the water surface to fill their swim bladder with air. This is essential for the normal development of the swim bladder, growing from the physostomous stage to the physoclistous stage. Individuals which fail to gulp air at the physostomous stage always swim upward in an oblique position with rapid fin strokes. The lordosis seems to be induced by the continual swimming with an oblique direction of the body axis, in order to maintain a position in the water column.

Three causes have been proposed to explain the inhibition of air-intake by the larvae of physostomous stage, *i.e.*, 1) a strong water current by too vigorous aeration; 2) a weak swimming ability of the larvae due to feeding on rotifers with a low content of n-3 HUFA; and 3) the formation of an oil film on the water surface. In addition, low egg-qualities inherited from the broodstock, inadequate diets, and bad environmental conditions of the broodstock are also presumed to be congenital factors.

All species studied until now for the incidence of lordosis in individuals with an uninflated swim bladder belong to the order of Perciformes. Accordingly, lordosis can be expected in other species belonging to the Perciformes. Presently it is, however, not clear whether or not the occurrence of the malformation is limited to this order. More investigation is necessary to elucidate the phenomenon.

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LARVAL REARING OF THE MULLET *MUGIL SO-IYU*

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Introduction

Larvae of *Mugil so-iyu* were reared under artificial conditions in the Northern Azov Sea region from 1984 to 1990. The larvae were produced from hormone-treated breeders. Larval rearing was performed in $4m^3$ fiberglass tanks. The following live feeds were grown in 300-400 l culture tanks enriched with pepton, yeasts, dry *Chlorella*, fish oil, vegetable oils, vitamins and microelements: ciliates *(Euplotes)*, rotifers, copepods and *Artemia* nauplii. The density of live feeds in the culture tanks was as follows:
ciliates 20 ind./ml, rotifers 3-5 ind./ml, copepods and *Artemia* nauplii 0.5-1 ind./ml. By observing the mullet larvae under the microscope the percentage of ingested feed at different larval development stages was determined. Experiments were conducted to determine the rate of oxygen consumption by larvae during their growth on different live feeds. The experiments were performed following the Vinberg technique (1956) in picknometers. Knowledge of the exchange amounts allowed to calculate the feeding requirements of the larvae. Two to 30-day-old larvae were grown at 12-14ppt salinity and 18-23°C.

Results

The ciliate *Euplotes* was used as starter feed, *i.e.* active feeding was noted in 90 to 100% of the larvae on day 3 post hatching, when they reached a length of 3.2mm. As of the following day rotifers were offered and ingested by more than half of the fish larvae. On day 6, larvae reached 3.7mm and 70-80% of them consumed rotifers. On day 8 those larvae that reached 3.9mm could swallow both adult copepods and *Artemia* nauplii. The feeding spectrum of the larvae was amplified with organisms caught from the wild, *e.g.* the rotifer *Synchaeta*, larvae of molluscs, and ostracods (Table I).

Table I. The feeding spectrum (% of live food items ingested) of mullet larvae

Food source	Age of the larvae (days)									
	3	4	5	7	10	15	19	23	26	29
Infusoria	100	93								
Rotifers	-	6	60.2	52	22	8	6			
Copepods	-	0.2	36.3	42	32	35	40.9	41	49	51
Artemia nauplii	-	-	-	-	39	55	42	44	49	47
Young shells	-	-	0.25	2.3	5.0	-	4.0	9.0	1.0	1.2
Synhaeta	-	0.25	0.25	3.0	0.7	1.0	1.1	3.0	0.8	0.5
Balanus larvae	-	0.25	-	0.7	0.2	0.3	5.1	0.9	0.02	0.2
Ostracods		0.3	3.0	-	0.1	0.7	0.9	2.1	0.18	0.1

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FEEDING PERIODICITY OF YOUNG TIGERFISH (*TERAPON JARBUA*)

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Introduction

Generally, teleosts are most active in their physiological functions, such as shoaling, locomotion and feeding activity at dawn and dusk. Although the timing of circadian rhythms varies among species and habitat, the ability of a teleost to integrate habitat information in adjusting its own circadian rhythm would be adaptive, and would show the animal to regulate a more flexible circadian activity to successfully meet environmental variations (Delahunty and Vlaming, 1980).

Tigerfish, *Terapon jarbua* (Forskal) is a medium-sized food fish, with wide occurrence in the inshore and brackish waters of Taiwan. Since it is easy to cultivate in ponds, together with its good taste, the tigerfish has been recognized as a candidate species for mariculture in Taiwan and the Pescadores (Liao, 1988). This species produces a sound and studies of the circadian oxygen consumption, revealed that it is a twilight-active species (Huang and Chang, 1991).

In many instances feeding periodicity is a species-specific characteristic and an important factor in determining growth effects (Blaxter, 1980; Townsend and Winfield, 1985). The high mortality of some teleost larvae has been ascribed to inadequate or inefficient feeding responses at the beginning of exogenous feeding (Hunter, 1980; Lasker, 1981). Therefore, the feeding periodicity of tigerfish during their young stage becomes crucial for successful larviculture.

Materials and methods

Tigerfish (*Terapon jarbua*) were obtained from the east coast of southern Taiwan. They were maintained in an aquarium (195x120x105cm) under recirculating conditions in well-aerated seawater (salinity: 3.4ppt) under natural photoperiod and water temperature. During the acclimation period, food was presented once daily at random timing.

Four healthy (6.1-6.4cm total length) fish were selected and transferred into four 10 l opaque plastic tanks for a further overnight acclimation period. Floating food pellets were supplied into each experimental tank at 4h intervals. After 20min of feeding, the number

of consumed pellets were calculated by substracting the numbers of food pellets left in the tank. The weight of the consumed food could then be calculated.

Results and discussion

The results obtained from the four replicates show some considerable variation in feeding activities, feeding peaks more or less coinciding with the night period.
In the early stages most pelagic marine teleosts are primarily visual feeders, and the other sensory organs develop only later. Townsend and Winfield (1985) contended that if the foraging performance of a predator is influenced by ambient light, the predation pressure will decrease during the hours of darkness. Therefore, fish with dim-light vision could solve the conflict between effective prey capture and minimizing the risk of detection by visual predators.

The present results of the feeding periodicity trials demontrate that young tigerfish probably choose nocturnal feeding as a compromise between prey availability and predator avoidance (Blaxter, 1980; Huang, 1990).

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LARVICULTURE OF THE GREASY GROUPER (*EPINEPHELUS TAUVINA* F.) AND BROWN-MARBLED GROUPER (*E. FUSCOGUTTATUS* F.) IN SINGAPORE

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Abstract

Groupers are a group of epinepheline serranid species of high aquaculture potential in tropical and semitropical waters. The shortage of fry, high market value and scarcity of the grouper from the wild have prompted many countries in Asia to embark on research programmes into its fry production technology. Although there have been some reports on spawning success in several grouper species, the larval survival rates from hatchlings to metamorphosis in almost all cases are either low or inconsistent. This paper reports the larviculture of two species of grouper, the greasy grouper (*Epinephelus tauvina*) and the brown-marbled grouper (*E. fuscoguttatus*), in Singapore and examines the technical feasibility of breeding the fish.

Broodstock of the two grouper species are raised in floating netcages. As natural males could not be found among captive stock of the greasy grouper, spawning of the fish was resorted to males sexually transformed from females treated with methyltestosterone. Attempts to condition the brooders for natural spawning were not successful as the transformed males could not fertilise eggs spawned by females. Fertilised eggs of greasy grouper for larviculture were therefore obtained by induced spawning through multiple hormonal treatments of the female brooders, followed by artificial fertilization. The quality of eggs obtained by this method was, however, inconsistent. For the brown-marbled grouper, the brooders spawned naturally in netcages almost throughout the year and they exhibited a well defined lunar spawning rhythm. Egg production was high (2.5 million eggs per female per spawning) and eggs were of good quality, with a fertilization rate of 82 to 98% and a percentage of buoyant eggs between 82 and 91.

Larval development of both grouper species was characterized by the appearance of long dorsal and ventral spines during their early larval stage (day 9-10 in greasy grouper and day 6-7 in brown-marbled grouper) and long larval periods (40-50 days or more in greasy grouper and 35-40 days in brown-marbled grouper). For both species, larviculture was divided into three operational stages, *i.e.* stage 1 (day 0-12), stage 2 (day 12-24) and stage 3 (day 24 to metamorphosis). Due to the small mouth size, the greasy grouper larvae could not feed on S-type rotifer directly. Mussel eggs and trochophores were

produced by induced spawning and used as initial feeds for the larvae. Larvae of brown-marbled grouper are able to feed directly on rotifers. Total mortality on day 5-8 was common in the early trials of greasy grouper. This was probably due to unreliable egg quality and poor water quality resulting from feeding mussel trochophores to the larvae. In recent years, several modifications have been made to improve this situation. These include the production of rotifer young for the fish larvae through stocking of egg-bearing rotifers and *Nannochloropsis* in larval tanks from day 1 onwards, intensive feeding of the rotifers with *Nannochloropsis* for at least 2h before feeding them to the fish larvae and use of a skimmer to remove the oil film from the water surface.

High cannibalism was a common problem in both species during stage 3. The high size variations among the larvae caused serious cannibalism from day 35 onwards in greasy grouper and from day 30 onwards in brown-marbled grouper. The shock syndrome exhibited by both species during this period, made culling of the fish impossible. In addition, high mortality was also observed in greasy grouper from day 25 onwards. Mortality and the shock syndrome are likely to be associated with nutritional deficiency in the larvae. It is suggested that future grouper larviculture research should focus on establishing egg quality criteria to enable selection of good quality eggs for fry production, improving egg quality in brooders through nutritional and hormonal manipulation, refining zootechnical aspects through determination of the optimal conditions for the larvae, and enhancing larval quality through feeding of enriched diets.

Based on spawning and larval characteristics, brown-marbled grouper is considered a better potential species for large scale fry production than greasy grouper and other grouper species. The male and female brooders of the grouper are easily available for spawning. They spawn readily under netcage conditions with high egg production. Supply of fertilised eggs is therefore ensured. Egg quality and larval cycle of brown-marbled grouper are also respectively better and shorter than in most other grouper species.

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SURVIVAL OF NEWLY-HATCHED LARVAE OF *EPINEPHELUS MALABARICUS* AT DIFFERENT SALINITY LEVELS

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Introduction

The development of an appropriate hatchery culture-technique for grouper *Epinephelus malabaricus* requires knowledge on the biology of this species. The environmental requirements, a critical aspect of biology, have, however, not yet been investigated. This study specifically deals with the influence of salinity on the survival of newly-hatched larvae.

Materials and methods

Newly-hatched (day 0) larvae of the grouper *E. malabaricus*, spawned and hatched in seawater with a salinity of 32ppt, were abruptly exposed to test salinities of 0, 4, 8, 16, 24, 32, 40, 48, and 56ppt. A total of 15 larvae were stocked in each 3 l container. Tanks were provided with aeration and the temperature was maintained at 29±0.5°C. The salinity was monitored daily. There was no water replacement nor feeding during the test period.

Mortalities in each tank at 0.5, 1, 2, 3, and 6h after stocking, and every 6h thereafter until 96h were recorded. Larvae were considered dead when they no longer responded to mechanical stimulation. Dead larvae were removed at every observation period. A regression line was fitted for each replicate using the probit transformation of the percentage mortality at each observation as the dependent, and the log transformation of the time (h) from stocking as the independent variables. The values of X when Y=5 (log median lethal time or LT50) were estimated using inverse prediction and compared using Duncan's new multiple range test (Sokal and Rohlf, 1969). Each treatment was replicated six times.

Results and discussion

E. malabaricus larvae exposed abruptly to 56ppt died within the shortest time (mean LT50=1.32h). Those that were stocked in salinities of 0 and 48ppt died next and exhibited similar LT50's (5.07 and 5.55h, respectively) as shown in Fig. 1. Salinities of

4 (LT50=26.68h), 40 (LT50=22.80h), and 32ppt (LT50=30.62h) gave higher LT50's than the former treatments. Best performance or highest LT50's were achieved in test salinities of 8, 16, and 24ppt (LT50=69.77, 66.48, and 42.02 respectively).

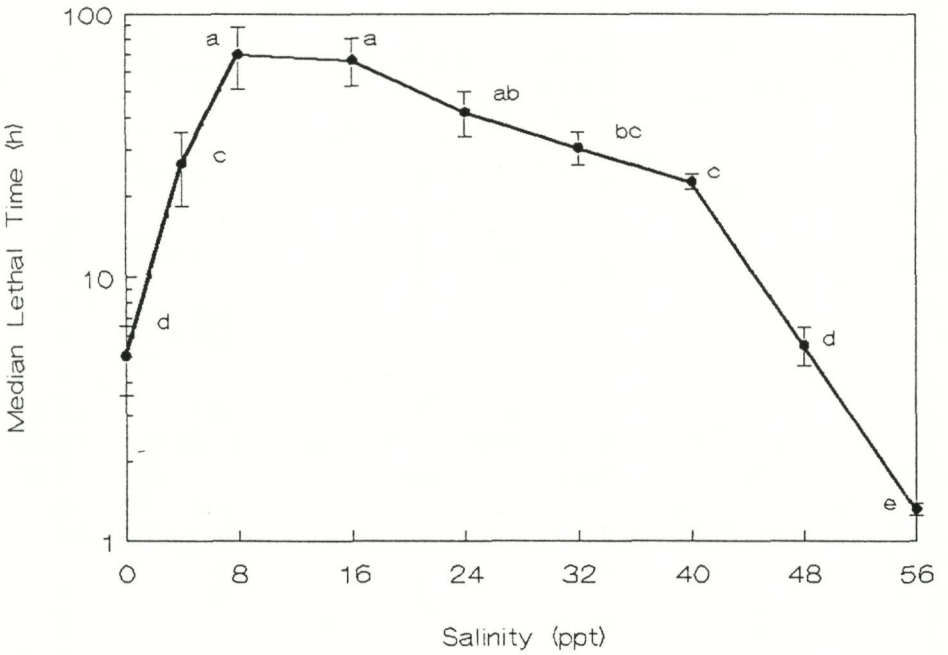

Fig. 1. Mean median lethal time at different salinities. Bars show the SEM. Points with different letters are significantly different (P<0.05).

These results suggest that *E. malabaricus* larvae can better withstand abrupt decrease rather than increase in salinity as depicted by the significantly higher LT50 obtained in 8 than in 56ppt (salinity difference = 24ppt) or in 24 than in 40ppt (salinity difference = 8ppt). Comparison of the median lethal time values also showed that newly-hatched *E. malabaricus* could best adapt to brackish water, particularly to salinities of 8 to 24ppt. These results also agree with those obtained by Akatsu *et al.* (1983) with *E. tauvina*.

Conclusions

Based on the results of this study, present larval rearing techniques which employ full-strength seawater during the hatchery rearing of this species (Maneewong *et al.*, 1986) may have to be modified. Better performance of the larvae in salinities lower than seawater also reveals valuable information regarding the natural habitat of the newly-hatched larvae of *E. malabaricus*. However, further studies using older animals should be conducted to determine the salinity tolerance of the other larval stages in the hatchery phase.

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SURVIVAL OF YOLK-SAC LARVAE OF GROUPER (*EPINEPHELUS SUILLUS*) UNDER SIMULATED TRANSPORT CONDITIONS

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Introduction

Transport of live fish is a routine activity in aquaculture. Various transport techniques have been reported by Berka (1986).

Transport of grouper eggs (*Epinephelus suillus*) between fisheries stations in Thailand is being conducted (Maneewong *et al.*, 1986). However, no report has been made on transport of yolk-sac larvae of this fish. In the Philippines transport of eggs poses difficulties, particularly for hatcheries which are distant from the broodstock rearing site since spawning occurs between 1600 and 1800h (Toledo *et al.*, 1990). Eggs can only be collected the following morning and hatching starts around 1200-1300h (20h after fertilization). Thus it seems advisable to transport yolk-sac larvae rather than eggs of grouper under such conditions. This study was conducted to determine the feasibility and optimum loading density of transporting grouper yolk-sac larvae for about 2h.

Materials and methods

Zero-day-old yolk-sac larvae of the grouper, *E. suillus* were hatched from eggs previously collected from a 50 ton broodstock tank at SEAFDEC/AQD. The larvae were packed at loading densities of 8 000, 16 000, 32 000, and 64 000 ind./l in 10 x 24cm double-lined plastic bags with 100ml seawater. Each bag was inflated with oxygen (about 1:2, water to gas ratio) and sealed tightly. To simulate motion during transport, the bags were shaken at 100rpm on a table-top laboratory orbit shaker for 2h at room temperature (31°C). Another set of bags were not subjected to the shaking motion. Then, the water and larvae in each bag were transferred into a beaker and aerated. The survival was counted after 2h. Three replicates were made of each treatment. The initial water temperature and salinity were 27°C and 33ppt, respectively. Due to the limited water volume in each bag, two bags for each treatment were kept for water parameter measurements. Final water parameters were measured immediately after shaking.

Results

Survival of grouper yolk-sac larvae at 8 000, 16 000, and 32 000 ind./l after 2h was not significantly different (P>0.05) in both shaken and unshaken bags (Fig. 1). However, survival at a density of 64 000 ind./l was significantly lower (P>0.05) than for the other densities (7.7-15.2 % for shaken bags and 0-0.44 % for unshaken bags).

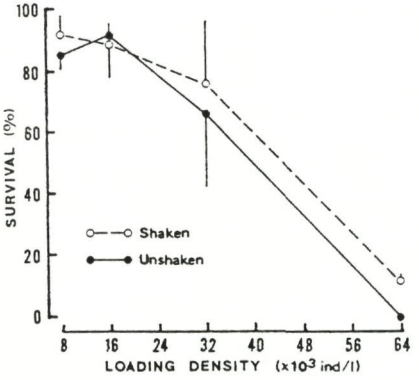

Fig. 1. Survival after 2h of simulated transport at various loading densities. Vertical bars represent SEM.

Water parameters measured after 2h in the different treatments after 2h were not significantly different (P>0.05). Ranges in water temperature, pH, nitrite, and ammonia were 29.5-30.3°C, 7.9-8.1, 0pmm, and 0.13-0.23ppm, respectively. Salinity remained constant at 33ppt.

Discussion

The results of this study indicate that a relatively high survival of grouper yolk-sac larvae may be attained even at very high loading densities of 32 000 ind./l in transport bags whether shaken or unshaken for 2h. However, the loading density may have to be less than 32 000 ind./l longer transport periods. In Thailand a loading density of 50 000 to 100 000 eggs per 6 l seawater (8 333-16 667 eggs/l) is being used when transporting grouper eggs for 3h (Maneewong *et al.*, 1986). For grouper yolk-sac larvae, the loading density of 16 000 ind./l may be optimal when transport takes more than 2h. Studies on the effect of longer shipping periods and optimal water temperature during transport of grouper yolk-sac larvae are needed.

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MECHANISMS OF ALBINISM IN FLATFISH WITH REGARD TO PIGMENT CELLS AND SKIN DIFFERENTIATION

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Introduction

Hatchery-reared flatfish may show a pigment disorder like hypomelanosis on the ocular side (albinism) and hypermelanosis on the blind side (ambicoloration). A high percentage appearance of albinism is a serious problem in flatfish seed production. Because of lower survival rates of malpigmented larvae after release in the environment, and the lower market price of the cultured product. Little is known about pigment cells which play a major role in pigmentation. The objective of this study is to analyze the mechanism of asymmetric pigmentation and albinism in the Japanese flounder, *Paralichthys olivaceus*, with regard to pigment cells and skin differentiation.

Materials and methods

Two groups of Japanese flounders were reared from hatching to climax metamorphosis. 10-day-old larvae which had been fed on the rotifer *Brachionus plicatilis* were divided into two groups. The larvae of the first group were fed with wild zooplankton. All surviving individuals of this group showed normal pigmentation. The larvae of the second group were fed a Brazilian strain of *Artemia* nauplii and rotifers, a feeding regime which induces a high percentage of albinism (Seikai, 1985). The larvae and juveniles of the two groups were studied by histological and immunocytochemical techniques to examine pigment cells and skin differentiation. In order to compare pigment cell expression, pieces of trunk with skin of larvae at mid-metamorphosis were incubated for 21 days in Leibovitz-15 tissue culture. The medium was supplemented with 15% fetal bovine serum or with 2 $IU.ml^{-1}$ ACTH or with MSH. Additionally, late neurula cells from embryos were cultured for more than 1 month in an identical medium to trace the differentiation of pigment cells.

Results

Normal development of pigment cells with the progress of metamorphosis

The larvae showed a complete bisymmetry of the integumental pigmentation until the onset of metamorphosis. With the completion of metamorphosis, numerous melanophores and xanthophores appeared but only on the skin of the left side. The iridophores became restricted to that side of the body. Histological DOPA (dopamine) assays before metamorphosis detected DOPA-positive cells (melanoblasts) is almost evenly distributed on the larvae's skin on both sides. Identical findings were made by means of immunohistochemistry using HNK-1 antibody which cross-reacts with neural crest cells.

Development of skin structure

The epidermis of the skin of both sides consisted of an epidermal layer of one or two cells. The dermis had undeveloped collagenous lamella at the pre-metamorphosis stage. At that time, no difference in structure was observed between the skin of the left and right sides. At the onset of metamorphosis, the collagenous lamella became densely packed. After climax metamorphosis, the skin became thicker. The mucus cells are most frequent on the ocular side of adult fish (Roberts *et al.*, 1972). The increase of the mucus cell ratio (left side/right side) from the onset of metamorphosis in normally pigmented larvae clearly suggests that asymmetrical differentiation of skin structure had begun from that stage on.

Pigment cell and skin differentiation relating to albinism

Adult type pigment cells did not appear on the skin of the left side of the albinic fish group after the climax metamorphosis. Histological DOPA assays detected DOPA-positive cells which were in the process of cytolysis (cell death) on the skin of both sides at that stage.

Chromatoblasts differentiation in vitro

Iridophores and melanophores appeared only in the trunk skin of the left side of larvae cultured for 21 days. They did not appear in the trunk skin of the right side nor in the skin of either side of albinic larvae. A number of large iridophores made their first appearance within 3 to 5 days after starting the culture. After 1 month a tremendous number of melanophores and iridophores, smaller in size, emerged among preexisting iridophores. The morphological traits were in good accordance with those of the adult type melanophores. An addition of ACTH or MSH to the culture medium enhanced the differentiation of these melanophores.

Discussion

The present study suggests that there are at least three factors which control melanophore differentiation: 1) a built-in clock; 2) tissue environment; and 3) external factors. The implication of a built-in clock was suggested by the appearance of the adult type melanophores with a definite timing in tissue and cell cultures. *In vivo* the chromatoblasts

were delivered evenly to the skin of both body sides in a symmetric fashion during the early stages of the development. With the progress of metamorphosis, these chromatoblasts could be exposed to tissue environmental cues which appeared to be different in either side of the body. This was translated in a higher ratio of mucus cell density (left side/right side) from the onset of metamorphosis. Though little is known about the exact nature of such cues in fish, they may result in selective differentiation of pigment cells. External factors would exert their effect in association with the structural body organization.

The substitution of skin appendices in the albinic ocular side with those of the prevalent type to the blind side would imply that the tissue environment for pigment cells is altered in nature. Morphological observations on hatchery-reared juveniles of flatfish disclosed that the characteristics of the blind side occurred also on the skin of the albinic ocular side (Seikai, 1980). The above mentioned ratio of mucus cells showed no change during metamorphosis in the albinic group. These findings indicate that albinism in flatfish accompanies malformation of skin appendices, in which the structural plan is reversed from "for the ocular" to "for the blind". This could mean that albinism is evoked as a result of disruption of the mechanisms which are responsible for the establishment of asymmetric skin structures.

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AN INTENSIVE APPROACH TO ATLANTIC HALIBUT FRY PRODUCTION

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Abstract

Atlantic halibut, is regarded as a promising cold water aquaculture species because of its high market potential, high growth rate in cold water and its large size. In this paper the results from a 3 year period (1988-90) of the project "Intensive production of halibut fry" funded by The Royal Norwegian Council for Scientific and Industrial Research and FINA Exploration Norway are summarized.

Halibut eggs were obtained from captive broodstock by stripping or after natural spawning. Light manipulation was used to control the time of spawning and increase the total spawning period. Live feed research has documented that both rotifers (*Brachionus* sp.) and *Artemia* can be enriched with very high levels of highly unsaturated (n-3) fatty acids [(n-3)HUFA]. As for most marine species, the larval stage of halibut is regarded to be the main bottleneck of the production process. Yolk sac larvae can be produced both in small stagnant units (down to 30ml) and in large flow-through systems (up to 15 000 l). A major requirement in both systems is to avoid any stress on the larvae, *e.g.* high temperature, mechanical stress, high microbial load, and high ammonia levels.

Experiments showed that larvae began to ingest algae earlier than zooplankton such as rotifers and *Artemia*. Algal addition to the first feeding-tanks has enhanced both the survival and growth rate of halibut larvae. Different enrichment procedures of the live feed has been tested in first-feeding experiments. Enriched live feed containing high levels of (n-3) HUFAs, and also high levels of total lipids, improve both the survival and growth of larvae. If algae are added to the tank water, the requirement for high enrichment levels seems to be reduced. The highest growth rates were recorded with collected zooplankton and addition of algae. Enriched cultivated feed combined with algae resulted, however, in comparable growth rates.

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[1] Project coordinator, presentation on behalf of:
- AKVAFORSK, Institute of Aquaculture Research
- Laboratory of Aquaculture and Artemia Reference Center
- Institute of Marine Research
- SINTEF, Centre of Aquaculture
- SINTEF, NHL

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INGESTION AND ASSIMILATION OF MICROALGAE IN YOLK SAC LARVAE OF HALIBUT, *HIPPOGLOSSUS HIPPOGLOSSUS* (L.)

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Introduction

It has been suggested that larvae of Atlantic halibut are able to feed and assimilate food from day 28 to 35 after hatching (5°C) (Blaxter *et al.*, 1983). Jaws are visible at day 15-20, and peristaltic contractions in the intestine are observed at day 35 (7°C) (Pittman *et al.*, 1990). Experiments have also revealed that the guts of the larvae become green when microalgae are made available in the last part of the yolk sac stage. The uptake may be a result of drinking activity or another harvesting process. The aim of this investigation was to quantify algal ingestion and assimilation in halibut yolk sac larvae and to identify the uptake mechanism.

Materials and methods

The larvae were incubated (3 l glass bowls, 5°C, darkness) in filtered seawater (0.2µm, 34ppt salinity) with 25ppm oxytetracycline. The algal uptake and assimilation of ^{14}C-labelled *Tetraselmis* sp. was measured from day 24 to day 55 after hatching (Nielsen and Olsen, 1989). Twenty larvae were transferred into small beakers (100ml) containing 5 mgC.l^{-1} of ^{14}C-labelled *Tetraselmis* sp. culture. After incubation (30min), 10 larvae were washed and transferred to individual scintillation vials. The remaining 10 larvae were washed, transferred to and kept in cold *Tetraselmis* sp. culture for 3h to allow gut evacuation of radioactive algae. Thereafter the larvae were processed as explained above. Three larval acclimation procedures were tested; no acclimation before uptake measurement, acclimation from day 30, and acclimation from day 40. The drinking rate was measured by exposure to ^{3}H-labelled dextran for 3 to 5h. Values for zero controls were subtracted. One larval group was tested at day 28 after hatching, and another was followed all through the yolk sac period.

Results

The ingestion rate of *Tetraselmis* sp. by halibut larvae (Fig. 1) was very low at day 24 [120 day-degrees (d°)] after hatching (<10^{-6}/day of larval biomass). The ingestion rate increased thereafter and reached maximum at day 43 (220 d°) where the daily algal

uptake was 4.6% of larval carbon biomass. The ingestion rate decreased after day 43, reaching 0.4% of larval biomass from day 48 onwards.

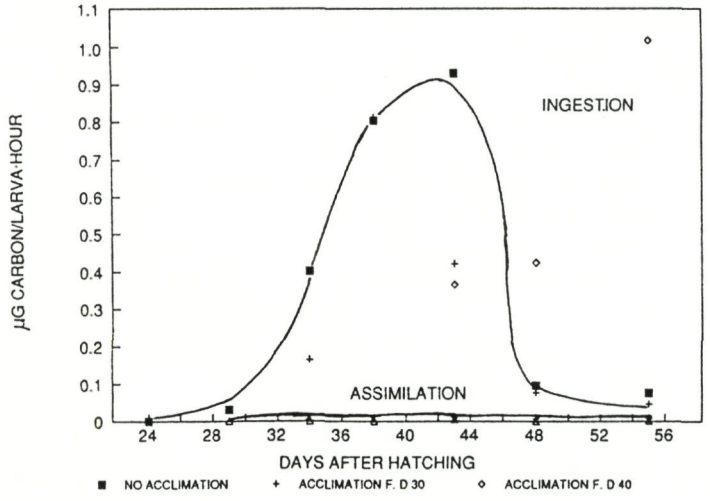

Fig. 1. Ingestion and assimilation rate of *Tetraselmis* sp. in yolk sac larvae of halibut.

The assimilation efficiency was <1% of ingested algae all through the yolk sac period. This indicates that the larvae did not assimilate *Tetraselmis* sp. to any extent. Larval clearance rate of algae (µl/h/larvae, Fig. 2) was highest at day 43 after hatching (220 d°) and decreased thereafter. The clearance rate was 100-3 000 times higher than the drinking rate of water (*i.e.* dissolved ^{3}H-dextran).

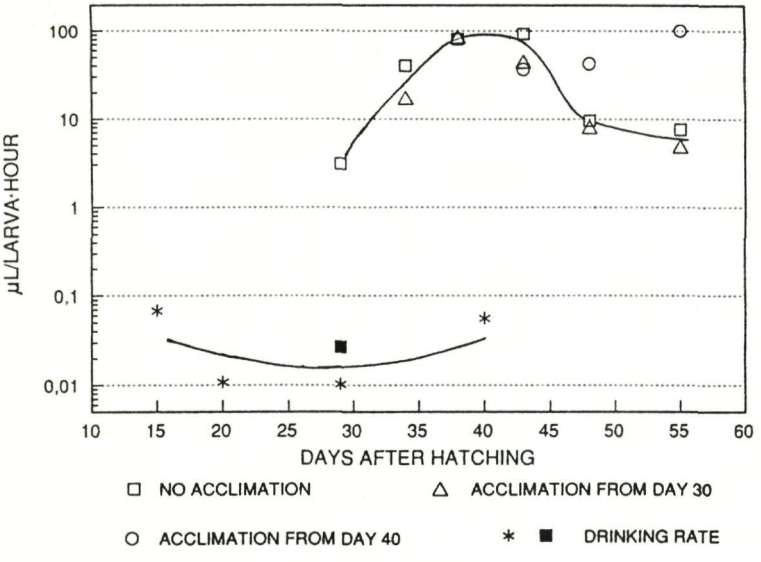

Fig. 2.Clearance rate of algae and drinking rate of water in halibut yolk sac larvae.

Discussion

The uptake of microalgae increased from the time when the mouth is expected to be functional (170 d°, Pittman *et al.*, 1990). The algal intake was low, and the cells were inefficiently assimilated. This may be because of the poorly developed digestive system and rigid cellular walls in *Tetraselmis* sp.

The clearance rate of algae was much higher than the drinking rate of water. This indicates that the larvae must take up the algae (8-10µm diameter) through active filter feeding, at least from day 33 to 48 (170-240 d°). The reduced algal uptake after day 48 may illustrate a behavioral shift to more selective feeding on large visible prey items, or it may be a result of larval exhaustion because of unfavourable conditions. If the hypothesis on filter feeding can be verified, this will have an impact on our attempts to culture halibut larvae. Similar results have been obtained for Atlantic cod (van der Meeren, 1991).

Acknowledgements

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ENHANCED FIRST FEEDING OF HALIBUT LARVAE (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) IN GREEN WATER

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Abstract

The "green water" technique, with the use of an algal suspension in tanks for first feeding marine fish larvae, is favoured by many aquaculturists. In the extensive production concept one has focused on the positive influence of planktonic algae. However, experimental studies on the effects of algae are scarce and the algae have either been identified as direct nutrition or as water quality stabilizers.

A first feeding experiment on Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) was performed in nine 1.5m diameter outdoor tanks. Feeding incidence, growth and survival was tested during the 21 days since first feeding in a suspension of natural phytoplankton (green water) *versus* in filtrated water. The larvae were fed non-enriched *Artemia* instar II. Feeding incidence at day 3 was 47% in green water and 0% in filtrated water. Both growth and survival was higher in green water compared to filtrated water. Out of a total of approximately 2 250 halibut larvae in the green water tanks, 684 larvae were found alive at the end of the experiment (day 21). Corresponding numbers for the filtrated water tanks were 57 out of 4 500. The results indicate no direct or indirect nutritional effect of the algae, and no improved growth could be related to any improved water quality in the green water tanks.

By using algae in the tanks for first feeding, the halibut larvae showed substantial survival and growth compared to larvae in filtrated water. The role of algae is not yet fully understood. However, a nutritional effect of algae seems to be of minor importance compared to changing environmental parameters (*e.g.* light regime) influencing the food uptake.

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EFFECT OF LIGHT INTENSITY ON FEEDING SUCCESS OF ATLANTIC HALIBUT LARVAE

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Introduction

To date there has only been moderate success in rearing Atlantic halibut larvae (*Hippoglossus hippoglossus*) beyond first feeding in captivity. According to Fyhn (1988) and Lein (1990), the problem may involve nutritional aspects. There has, however, been little consideration on how the physical environment could influence feeding behavior and ingestion. Fish larvae may maximise their rates of ingestion and growth within certain ranges of light intensity (Ivlev, 1961; Brett and Groot, 1963; Blaxter, 1966; Werner, 1969; Suffern, 1973). It is, therefore, conceivable that larval halibut, if held at suboptimal light intensities, has been unable to ingest sufficient quantities of food to ensure adequate growth and survival.

The objective of this research was to establish whether or not different intensities of light will produce a corresponding change in feeding success, and if possible, to indicate what the optimal values are. Accordingly, two experiments were designed in order to record the feeding success between 0.5 and 1 000lux.

Materials and methods

Eggs were obtained from two broodstock fish and held in 25 l incubators until hatching. Larvae were maintained in darkness in 3 l stationary water systems at 4-5°C for 257 d° (day degrees) prior to use in the feeding experiments. The experimental units consisted of five conically bottomed 60 l tanks with diffused light (white) coming in from the top. The larvae were transferred to the units and fed rotifers (*Brachionus plicatilis*) at a density of 12ind./ml. A minimum of 50 larvae were exposed to each experimental treatment and individual larvae were used only once.

Subsequently the larvae were examined under a microscope and the rotifer count made directly through the transparent gut. A t-test was run on the average numbers of rotifers ingested within each of the two experiments.

Results

As indicated in Fig. 1A, halibut larvae have a higher feeding success at 0.5lux and feeding declined rapidly as intensity increased to 50lux ($P<0.05$). There was a slight, but non significant trend ($P>0.05$) towards an increase between 50 and 1 000lux. The relation of feeders to non feeders (feeding incidence) shows a similar pattern; a drop from 0.5 to 50lux, followed by either no response or a slight increase at higher levels (Fig. 1B).

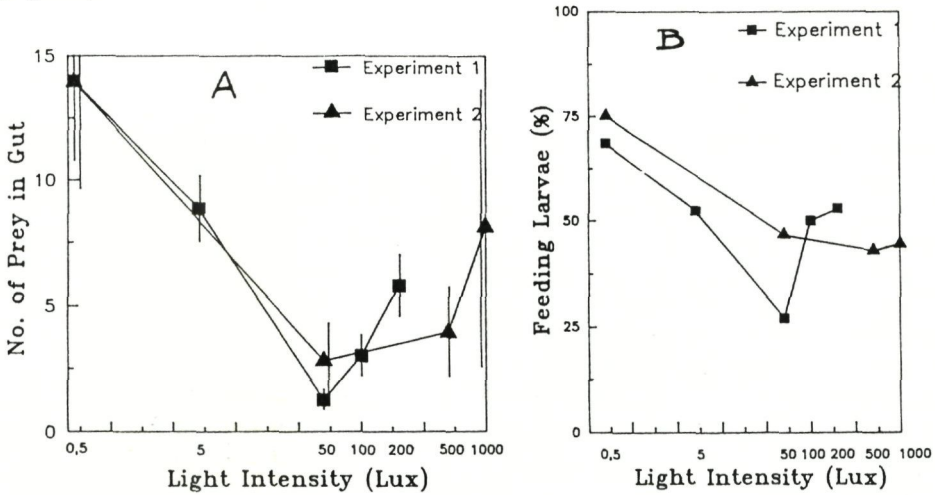

Fig. 1. A semilog representation of the feeding success (A) and feeding incidence (B) of halibut larvae at seven different light intensities.

Discussion

Clearly, the results indicate that feeding is correlated to the intensity of incoming light. Nonetheless, the majority of studies conclude that the feeding efficiency of piscine planktivores, sensitive to light, drops off rapidly as light levels decrease. The findings pertain to: the north Atlantic herring, *Clupea harengus* (Blaxter, 1966); the Pacific salmon, *Oncorhynchus* spp. (Brett and Groot, 1963); the bleak, *Alburnus alburnus* (Ivlev, 1961); the bluegill, *Lepomis macrochirus* (Werner, 1969); and the golden shiner, *Notemigonus chrysoleucas* (Suffern, 1973). Aksnes and Giske (1990) proposed a model for visual piscine predators in general which predicts that below a threshold intensity, feeding will increase when light intensities increase.

The present findings contradict the general notion that feeding drops with decreasing light intensities, although there are no previous reports about halibut in the investigated area. Theoretical evidence could explain the results as an adaptational response to conflicting demands between feeding and predation risk (Clark and Levy, 1988; Aksnes and Giske, 1990). Feeding at higher levels of light could produce a higher feed intake on account of the improved visibility, but very likely also add a cost by increasing the vigilance of the larvae.

The present findings could be important for future halibut cultivation. Increasing the larvae's feed intake by orders of magnitude would no doubt improve their chances of survival and possibly also increase their growth rate.

Acknowledgements

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A RECIRCULATION SYSTEM FOR THE EXPERIMENTAL HATCHERY-REARING OF TURBOT (*SCOPHTHALMUS MAXIMUS*) LARVAE

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Introduction

A new pilot hatchery was constructed with the aim to develop an operational system for larval nutrition studies with various fish species. Special attention was paid to develop independent rearing units, including a separate biofilter section, similar to the setups used for crustacean larviculture (Ferraz de Queiroz, 1989). The idea was to create optimal and reproducible rearing conditions on a laboratory scale, providing the possibility to test various nutritional treatments simultaneously.

Materials and methods

Since the rearing requirements largely fluctuate among cultured species and vary along larval development, physical parameters, *e.g.* illumination conditions and temperature have to be adjusted from 0 to 2000lux and 10 to 30°C respectively. The photoperiod can be regulated by a timer. The larval rearing tanks have a cylindro-conical shape and a capacity of 100 l and are made of untransparent grey polyethylene (Fig. 1).

The water in each of the rearing tanks (1) is circulated over a biofilter (2) and is returned to the bottom of the conical part of the tank. The fish are retained in the tank by a removable filter (3) with a mesh size of 150, 250, or 500µm, depending on the application. The effluent water is pumped into the biofilter at an adjustable flow rate (4) (bypass regulation preventing overheating of the suction pump at low flow rates). In the biofilter the water is aerated and mixed by two air-water lifts. After nitrification the water is drained from the bottom of the biofilter into a separate aeration chamber (5). Oxygen-saturated water is drained to the bottom of the rearing tank where it creates an upflow of oxygenated water.

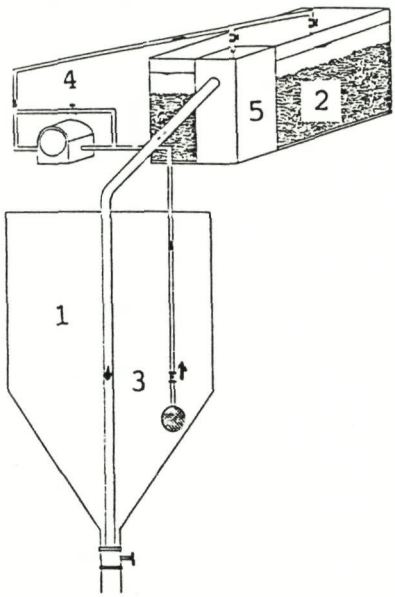

Fig. 1. Schematic drawing of the larval rearing unit. (1) Conical rearing tank (100 l); (2) submerged rock biofilter (15 l); (3) removable filter; (4) suction pump; (5) aeration chamber.

Results and discussion

In a first set of experiments, the loading capacity of the submerged rock biofilters was tested. The large size of the pebbles (± 10mm diameter) allowed the unharmed passage of *Artemia* through the biofilter system. This solved the problem of clogging of the strainer (2) when a smaller mesh size had to be used to keep the brine shrimp nauplii in the rearing tank. Rotifers, on the other hand clumped, together after passing through the pump and the biofilter and consequently were unsuitable for uptake. However, since *Artemia* can already be ingested by turbot larvae after only 5 days of culture, a short batch-rearing period was applied during the period of rotifer-feeding.

The biofilters were inoculated with 10% (volume %) pebbles taken from an operational filter. The activity of the nitrifying microbiota was activated by adding 10ppm ammonium chloride after inoculation of the filters. The ammonia was converted to nitrite and finally transformed to nitrate. Once the nitrite disappeared, 10ppm ammonium chloride was again added.

Once the nitrite was completely converted, the culture tanks were ready to be used. The turbot larvae were stocked at an initial stocking density of 100 ind./l in a volume of 60 l. During the first week of larval rearing, the fish larvae were held in a batch system to which an extra 5% of filtered seawater was added daily until a volume of approximately 80 l was reached. At that time the turbot larvae were actively swimming and could avoid

the suction area around the strainer. During the first period of batch culture a mild aeration was used in order to avoid stress to the animals and allow them to feed on the rotifers. Starting from day 5 *Artemia* could be fed for the first time. The effect of the biofilter on the water quality in the rearing tank is illustrated in Fig. 2.

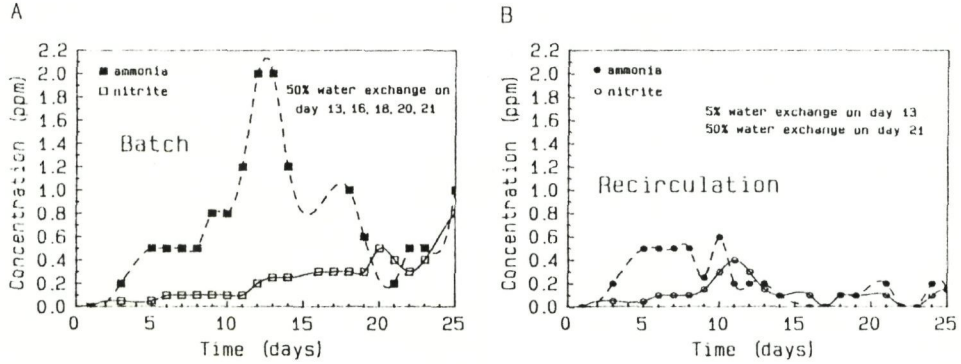

Fig. 2. Changes in ammonia and nitrite concentration under batch (A) and recirculation (B) conditions.

It is clear that ammonia was effectively removed after day 10 in the treatments with recirculation. At that time the ammonia in the batch cultures started to accumulate and the problem could only be solved by frequent water renewal. Nonetheless, stress tests, performed on day 15 did not confirm the better condition of the larvae in the recirculation system (Table I).

Table I. Difference in stress sensitivity and growth of turbot larvae reared under batch and recirculation conditions

Age of the fish	Batch treatment		Recirculation system	
	Stress sensitivity	Total length (mm)	Stress sensitivity	Total length (mm)
Day 15	126*	7.2 ± 0.8	143*	6.3 ± 0.5
Day 22	57**	10.6 ± 0.9	37**	12.7 ± 0.4
Day 27	73**	17.1 ± 2.1	64**	17.6 ± 0.5

* Salinity stress test at 55ppt.
** Salinity stress test at 70ppt.

This might be explained by the abrupt raise in the nitrite level which occurred shortly after operating the recirculation. It was probably due to the fast oxidation of the ammonia to nitrite, and the insufficient colonization of *Nitrobacter* which sustains further oxidation of nitrite to nitrate. After day 15, however, the conversion of nitrite was optimal and

acceptable nitrite levels were reached. This resulted in a far better condition of the fish which was reflected in the results of the stress tests on day 22 and 27.

In conclusion it can be stated that the recirculation system is working efficiently but the filtering capacity of the biofilter could be enhanced by a better conditioning. This could be achieved by twice adding 10ppm of sodium nitrite after the ammonium chloride treatment. The higher affinity of the filter for nitrite oxidation is illustrated in Fig. 3.

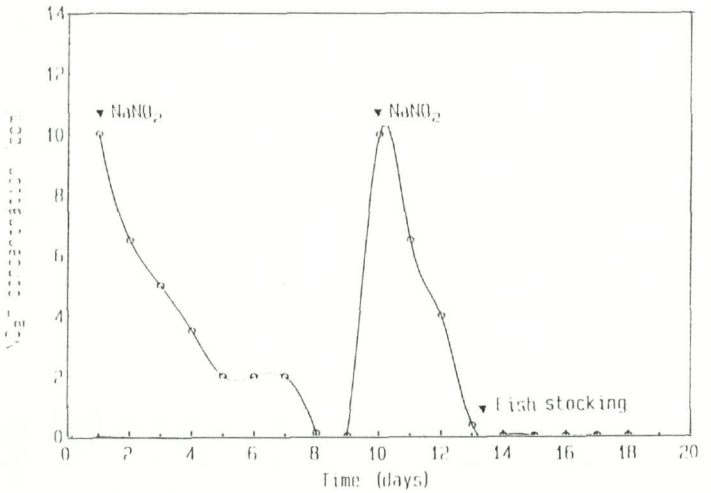

Fig. 3. Changes in nitrite concentration after adding $NaNo_2$ and stocking the fish.

After the total oxidation of nitrite, 30-day-old turbot larvae were stocked in the recirculation system and nitrite accumulation did no longer take place. Preliminary culture trials yielded about 5% survival from hatching to day 40. Reproducibility of the system will now be evaluated under various feeding regimes.

Acknowledgements

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SURVIVAL ABILITY OF TURBOT LARVAE (*SCOPHTHALMUS MAXIMUS*) TO DIFFERENT LEVELS OF DISSOLVED OXYGEN IN CONNECTION WITH THE LARVAL DEVELOPMENT DEGREE AND THEIR CONTENT OF THE POLYUNSATURATED FATTY ACID 22:6n-3

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Introduction

Many marine fish species, either wild or intensively reared, undergo a critical period (CP) during their early life, associated with high mortality (May, 1974). Several authors tried to explain this phenomenon as a minimum ratio of respiratory surface to body volume (Iwai and Hughes, 1977; Arnaiz *et al.*, 1990a; Coo *et al.*, 1990), lack of gills (Blaxter, 1969), or light development of secondary lamellae (Arnaiz *et al.*, 1990a; Padros *et al.*, 1990). Another correlated factor is the high level of unsaturated fatty acids in the larvae (Watanabe *et al.*, 1980; Arnaiz *et al.*, 1990b). In this study turbot larvae *Scophthalmus maximus* L., of different stages were subjected to various oxygen levels to test the relationship between body mass, the 22:6n-3 level and respiratory problems.

Materials and methods

Turbot larvae were reared at the facilities of the Centro Experimental de Vilaxoán (CEV), in 300 l tanks at an initial density of 20 larvae/l, constant water temperature of 18±1°C, salinity of 33-35ppt and constant illumination. Rotifers, *Brachionus plicatilis*, with a 22:6n-3 content of 9ppt on a dry weight basis, were fed to larvae between days 2 and 10. *Artemia* nauplii, with a 22:6n-3 content of 0.2ppt on a dry weight basis, were fed to larvae between day 6 and the end of the experiment. The critical period started at day 7, when daily mortality reached 5%. At days 5, 8, 9, 12 and 18, 60 to 100 larvae were transferred to a 5.5 l experimental closed system at 18°C and 100% oxygen saturation. During 4 to 6h, $3x10^5$ *Artemia* nauplii, which were incubated in a flask separated from the experimental system by a 150µm screen filter, diminished the oxygen level at a constant rate of 0.8-1.0mg $O_2.h^{-1}$, to 40% saturation. *Artemia* nauplii were chosen for their constant oxygen consumption rate between 100 and 20% of saturation (Arnaiz, unpubl.). Larvae which died due to a lack of oxygen were picked up from the bottom, washed with distilled water, liophilized and individually weighed (dry weight ± 1µg). Larvae were pooled by oxygen depletion resistance intervals for fatty acids analysis by GLC.

Results

We found an inverse relation between 22:6n-3 larval content and its body dry weight (as ppt), described by the linear function ($P<0.01$; $r^2=0.8188$; $n=10$):

$$Y\ (22:6n\text{-}3) = 13.64 - 0.0218 \times (\text{larval dry weight})$$

Moreover, we found an inverse relation between larval dry weight and resistance to oxygen level depletion (Table I). Prior to the critical period, with complete resorption of the vitelline sac, 5-day-old larvae resisted oxygen levels as low as 50% saturation. During the critical period most larvae did not support small decreases in oxygen level, although they had high levels of 22:6n-3.

Table I. Stress test of turbot larvae. O_2: oxygen saturation interval (as %); DW±SE: mean dry weight (µg) ± standard error of larvae

Day	O_2	DW ± SE	22:6n-3 (ppt)*
5	100-40	58.25 ± 2.53	14.14
	<40	35.94 ± 2.47	15.08
8	100-95	158.86 ± 10.89	8.91
	95-80	124.83 ± 13.47	12.01
	80-70	63.0 (n=1)	-
9	95-80	168.16 ± 11.60	9.41
	60-50	106.71 ± 8.07	11.07
12	100-95	352.70 ± 31.71	4.97
	95-80	247.25 ± 50.68	5.45
18	100-80	639.56 ± 66.53	2.52
	80-70	373.75 ± 68.15	3.45

* On a DW basis.

Discussion

Coo *et al.* (1990), found that for turbot larvae the critical mortality period starts when they reach a threshold body dry weight of about 150 to 220µg. In our experiments, 5-day-old larvae, with a mean body dry weight between 36 and 60µg (below Coo's threshold), and with a 22:6n-3 content of 15ppt, resisted low oxygen levels (Table I). Moreover, 9-day-old larvae, with 106.71µg of mean dry weight, resisted oxygen levels of 60 to 50%, and had high 22:6n-3 levels (11.6ppt on a dry weight basis); conversely, those 9-day-old larvae with a higher mean dry weight (168.16µg) died when oxygen saturation decreased to 80% (Table I). Watanabe *et al.* (1980), found that juveniles of *Pagrus major* showed better resistance to an activity test or stress test, when their HUFA content was higher.

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Crustaceans

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LARVAL CULTURE OF SOUTHERN KING CRAB *LITHODES SANTOLLA* AND FALSE KING CRAB *PARALOMIS GRANULOSA* UNDER LABORATORY CONDITIONS

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Introduction

The southern king crab, *Lithodes santolla* (Molina), and the false king crab, *Paralomis granulosa* Jacquinot, are the most important commercial shellfishes in Tierra del Fuego, Argentina and in the XII Region, Chile. Previous studies on both species have been made, but only on morphological descriptions (Campodónico and Guzmán,1981) and on the effects of temperature and salinity on larval stages (Vinuesa *et al.*, 1985, 1989).

The present study attempts to determine the effects of different diets on larval development of both species and to determine feeding rates in *L. santolla* larvae.

Materials and methods

Larvae for the experiments were obtained from ovigerous females collected in the Beagle Channel by commercial crab pots in early October, 1986 and 1988. Zoeae hatched during the night were collected and transferred into glass jars, after elimination of deformed or damaged ones.

The first experiment was designed to evaluate the effects of different diets on the larval development of both species. The diets used were: 1) *Artemia* nauplii; 2) nauplii and metanauplii of copepods and barnacles, echinoderm larvae and small copepods - the most important organisms in the spring zooplankton; and 3) starvation conditions as control. The larvae were reared in 350ml beakers, at concentrations of 15 zoeae per beaker, with five replicates for each trial. The beakers were examined daily for exuviae and mortality and the zoeae transferred to fresh seawater with the corresponding food.

The second experiment was carried out to evaluate the daily ingestion rates in *L. santolla*, using *Artemia* nauplii as food. Five larvae were reared in each of 20, 150 ml beakers: 10 beakers with 20 *Artemia* nauplii, and 10 beakers without *Artemia* nauplii (starvation conditions). Zoeae were transferred every day into new beakers with fresh food and seawater. The remaining *Artemia* nauplii were filtered and counted.

Results

A higher percentage of the samples of *Lithodes santolla* zoeae fed plankton reached the second stage. But survival to zoea III and glaucothoe was higher in starvation conditions. No glaucothoe was obtained with *Artemia* as food (Fig. 1).

The accumulative mortality of king crab was 93.3% in controls, 98.7% in plankton-fed cultures and 100% in *Artemia* food cultures. With *Paralomis granulosa* moulting to zoea II, the most successful results were obtained in starved conditions and no glaucothoe were obtained with *Artemia* food cultures (Fig. 1). The accumulative mortality was 92% in controls, 100% in *Artemia*-fed cultures and 94.66% in plankton-fed cultures. The mean duration of the first stage showed no significant differences among the three rearing conditions for both species (ANOVA).

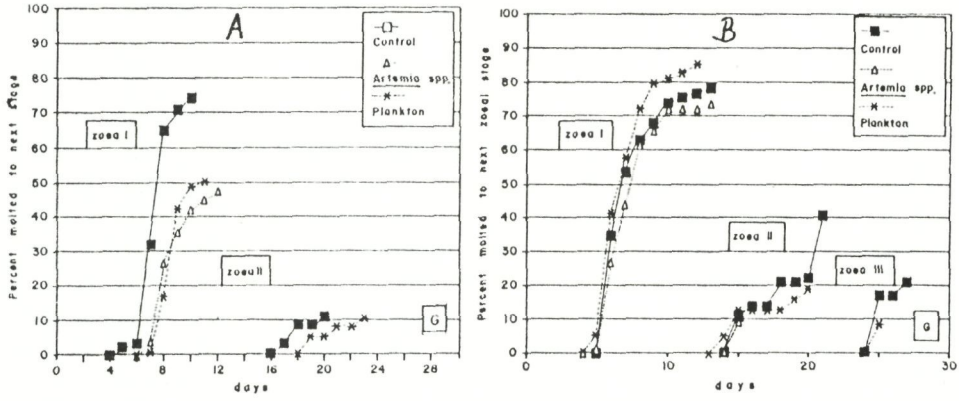

Fig. 1. Effect of different diets on molting of zoeae beginning with first zoeal smolt. Molting success for each stage is the percentage of the original number of zoeae completing the molt. (A) *Lithodes santolla*; (B) *Paralomis granulosa*.

For the second experiment no significant differences were observed in cumulative mortality between fed and starved larvae (Kolmogorov Smirnov, significance level= 0.9988). Mortality increased during molting to the third zoeal stage. The feeding rate varied greatly not only between larval instars, but also among replicate experiments and from day to day. An increase in the ingestion rate occurred during larval development. Maximum ingestion was found in zoea III, but it still varied from 0.5 to 10.5 nauplii/ind./day.

Conclusions

High mortality rates occurred in both species, reaching a critical level at moulting to the third zoeal stage for *Lithodes santolla* and to glaucothoe for *Paralomis granulosa*. *L. santolla* larvae consumed *Artemia* nauplii under laboratory conditions, although the survival rate was similar in starved conditions. It is probable that prey population densities necessary for zoeae to successfully develop, are higher than those used in these experiments. This finding corroborates the results of other laboratory studies which have demonstrated that daily feeding and high prey concentrations, 600 to 1 600 nauplii/l, result in increased survival rates for cultured zoeae. The nutritional adequacy of small

crustaceans as sole source of food for the decapod larvae examined is unknown (Paul *et al.*, 1979).

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UPDATE ON LARVICULTURE PRACTICES AND PRODUCTION FOR PENAEID SPECIES IN BRAZIL

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Introduction

After initial slow progress with a variety of marine shrimp species, Brazilian hatcheries have finally selected fewer target species mostly represented by indigeneous penaeids, and started to report better results, leading to optimistic prospects about this emerging industry in South America.

The current practices and production results in Brazilian marine shrimp hatcheries are evaluated and discussed.

Data collection

The data presented were collected from academic, private, and governmental sources as well as from a few papers available on the subject (Camara, 1990; Rocha *et al.*, 1990; Weidner, 1990).

Current practices

The development of the marine shrimp industry in Brazil was initially based on both imported (*Penaeus japonicus*, *Penaeus stylirostris*, *Penaeus vannamei*, *Penaeus penicillatus*), and indigenous species (*Penaeus brasiliensis*, *Penaeus paulensis*, *Penaeus schmitti*, and *Penaeus subtilis*). Later this approach was found to be inadequate, and recent emphasis on native *Penaeus schmitti* and *Penaeus subtilis*, and exotic *Penaeus vannamei* has brought about increasingly better results.

Current hatching practices involve the use of tank-reared (S= 35ppt; T=28°C; 100-150% daily water exchange; 200g of shrimp biomass/m^2; 1:1 sex ratio; natural photoperiod) and unilaterally ablated *P. schmitti* and *P. vannamei* females or pond matured (1 ind./m^2) unablated *P. subtilis*. Both sources of broodstock animals are usually fed at 5% of total shrimp biomass with a 35 to 40% crude protein artificial diet supplemented with frozen

adult *Artemia* (harvested from local salt ponds), shrimp heads, trash fish, mussels, and oysters (Bueno, 1990; Rocha *et al.*, 1990). Average fecundity ranges from 55 000 nauplii/spawn for *P. subtilis* to 80 000 nauplii/spawn for *P. schmitti* and *P. vannamei*. Larvae are stocked at 40 to 50 ind./l in 8 to 10m^3 clear-water rearing tanks. Final survival rates at postlarvae 10 for these three species are about 40 to 50%. Larviculture tanks are usually rectangular and made of concrete with epoxy-coated walls and bottom. Water treatment consists of decantation, and sand filtration. Larval feeding is based on microalgae (*Chaetoceros gracilis*, *Skeletonema costatum*, *Tetraselmis chuii*), and *Artemia* nauplii produced from locally collected cysts. Early postlarvae feeding with *Artemia* biomass (live or frozen) and minced mollusc flesh is also applied. The use of artificial diets in local hatcheries is still very limited due to strict import regulations for these products. Although these larval procedures have been generally successful, recently *Baculovirus penaei* infections occurred in *P. schmitti* and *P. subtilis* larvae (Bueno *et al.*, 1990). This is a worrisome fact, and the impact of these diseases on hatchery-cultured species should be fully assessed.

Results and conclusions

From the 24 major hatcheries registered in 1985-86, only eight have remained in full operation. Most of these hatcheries are located in the northeastern states (Bahia, Paraiba, Piaui, and Rio Grande do Norte) and consist of small to medium-scale facilities with a production capacity ranging from 50 to 200 million postlarvae/year. Current annual production amounts to 390 millions postlarvae, a substantial increase when compared to the 1983-1985 production figures of 50 to 100 millions postlarvae/year (Fig. 1).

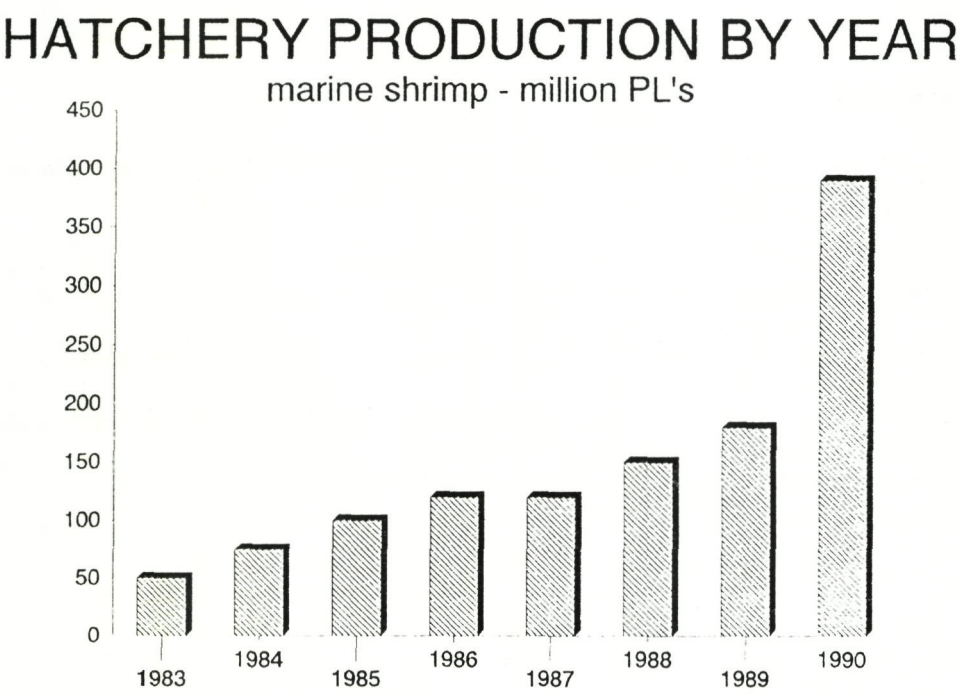

Fig. 1. Brazilian hatchery production of marine shrimp by year (in million PL's).

P. schmitti (40%), *P. subtilis* (31%), and *P. vannamei* (23%) represent the bulk of this production (Fig. 2). The increase in hachery-produced indigenous species in Brazil suggests that larviculture techniques for *P. schmitti* and *P. subtilis* are finally being mastered. This recent development of using Atlantic shrimp species will play a very important role on the just revised Brazilian governmental plan to have 30 000ha of marine shrimp ponds implanted by 1996. This target represents a substantial increase over the current production area of 2 700ha.

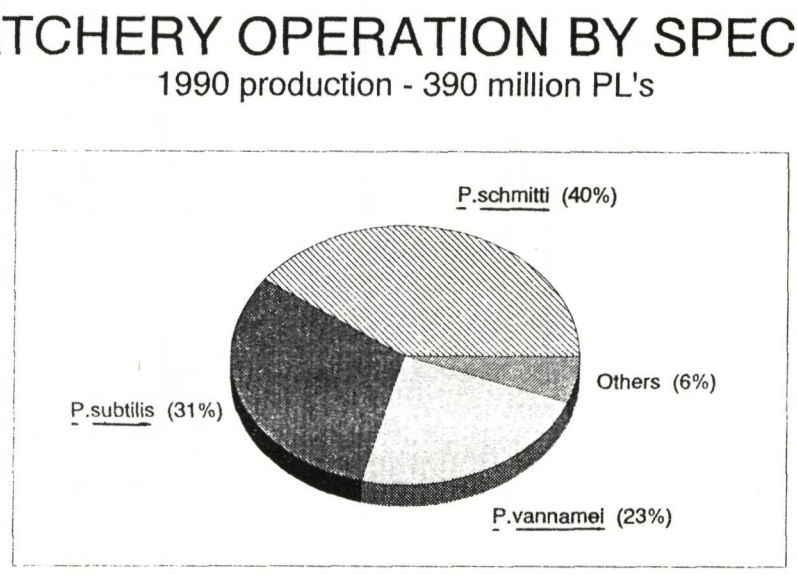

Fig. 2. Brazilian hatchery production by species in 1990.

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LARVICULTURE OF CHINESE WHITE SHRIMP *PENAEUS ORIENTALIS* IN THE PEOPLE'S REPUBLIC OF CHINA

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Introduction

In recent years, China has become the world leader in the production of farm-raised shrimp. In 1988, China produced about 100 billion of *Penaeus orientalis* postlarvae in 700 000m^3 of hatchery capacity and harvested 200 000 metric tons of live shrimp in 163 000ha of grow-out ponds. In Table I the development of the Chinese white shrimp larviculture industry is shown.

Table I. Yearly production in million of postlarvae of Penaeus orientalis in the PR China

Year	1960's	mid-1970's	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
PL production	1	10	40	350	1 600	2 830	4 240	7 770	18 900	31 400	66 900	97 000

Spawning and larval rearing

Both captive broodstock and wild spawners are used for nauplii production. Broodstock animals are overwintered in the hatchery tanks at 8-10°C. By the end of March, the water temperature is raised to 13-14°C and shrimp are given a diet of fresh, crushed bivalves and polychaetes at 8% of the shrimps' biomass/day. Two to 3 days later, mated females are restocked at a density of 20 ind./m^{-2} for spawning. Wild spawners are caught during their migration in the Bohai Gulf from the end of March to April. Wild spawners are kept at 15-17°C and fed the same diet as captive broodstock. Temperatures over 18°C may cause molting of spawners and subsequent loss of the spermatophores. For both captive and wild spawners, the seawater is treated with 5ppm of EDTA-Na and the light intensity is kept below 100lux by covering the transparant roof of the hatchery.

One of the most favorable biological characteristics of *P. orientalis* is that they can mature and spawn in captivity without eyestalk ablation or any other special treatment. *P. orientalis* is a multispawner. In the hatchery, females usually produce two to three spawns. The interval between successive spawnings is about 7 to 10 days. Spawning always takes place at night. The mean fecundity is approximately 400 000 to 500 000 eggs of which 70 to 80% (sometimes 30 to 40%) are well developed. No obvious differences between wild and captive females have been found for the hatching rate of

eggs or growth and survival of larvae produced. In 1988, the percentage of larvae produced from captive females was about 15%. In the future, larvae production will have to mainly rely on captive females, because the Chinese government is imposing stricter regulations on fishing of wild spawners. Eggs are collected during the following morning and transferred to rectangular concrete rearing tanks of 20 to 100m^3 in densities up to 500 000 eggs/m^3.

Embryonic and larval development

The larval phase of *P. orientalis* consists of six nauplius (N), three zoea (Z), three mysis (M) and several postlarval (PL) stages. Rearing temperatures and time required for hatching of eggs (E) and subsequent larval stages are indicated in Table II.

Table II. Rearing temperatures and time intervals for larval development of *P. orientalis*

Stages	E-N	N-Z	Z-M	M-PL
Temperature (°C)	21-22	22-23	23-24	24-25
Intervals (days)	1	3	7	4

Water quality

Seawater is taken from the Bohai Bay which in April usually has a salinity of 16 to 28ppt and a pH of 8.2-8.3. The pH is critical for larviculture with recommended values of 7.8-8.6 for all stages. The pH is controlled by changing the water in the seawater storage tanks every 5 to 6 days and by adding $NaHCO_3$. The latter is common practice in hatcheries which are located far from the sea and where the pH of the intake water usually exceeds 8.6.
Dissolved oxygen levels in the culture tanks are always kept above 5ppm, while the total ammonium-N is maintained below 0.1ppm. The light intensity is kept below 3 000 lux for N-Z stages and 10 000lux for M-PL stages.

Filtrated (50µm silk) seawater in the rearing tanks is usually treated with 5ppm of EDTA-Na. Chloromycetin (1-2ppm) is added to control bacterial development. Three to 4 days after hatching of the eggs, the initial water levels of 0.5m in the culture tanks are gradually increased by adding about 20cm of fresh seawater per day. Later on, daily the water exchange rates are gradually increased from 10 to 100%.

Feeds

Feeds used in larval rearing are indicated in Table III.

Until recently, most hatcheries used locally produced *Artemia* cysts. However, due to the low hatchability of this product, several hatcheries are now using imported (Great Salt Lake or San Francisco Bay strain) cysts. *Artemia* biomass is harvested from local salt ponds and purchased at a price of 1 RMB yuan/kg (± US $ 0.20). The use of live feed rotifers is a recent practice in *P. orientalis* hatcheries and has shown to produce better

survival rates. Rotifers are produced in outdoor earthen ponds. In a first step green algae are produced in separate 500m^2 and 0.8m deep brackish water ponds which are heated by geothermal energy and to which bean cake fermentation water and/or fecal sewage and/or inorganic fertilizers are added. High eutrophication rates effectively inhibit the development of copepods, while densities of green algae rapidly increase to 0.5-1.5 x 10^6 cells/ml. After the desired species of green algae become dominant, the initial water temperatures of 5-10°C are gradualy increased to 15-20°C. At higher temperatures (23-25°C) *Oxyrrhis marina* (Mastyophora) may appear in the ponds and the algal culture will fail.

Table III. Food items and feeding regimes used in larviculture of *P. orientalis*

Stage	Z_1	Z_2	Z_3	M_1	M_2	M_3	PL
Green algae	+	+					
Boiled egg yolk	+++	+					
Yeast	++	+					
Rotifers		+++	+++	++	+		
Artemia nauplii			+	++	+++	++	+
adults						++	+++

+ low concentrations; ++ moderate concentrations; +++ high concentrations.

In a second step, rotifers collected from a natural brackish water environment are inoculated in the algae ponds at a density of 1 to 5ind./ml. About 10 to 15 days later, the rotifers attain a density of 100 000-300 000 ind./ml. One pond can yield daily harvests of 10 billion rotifers for 15 to 20 days by constantly adding green water from separate algae production and/or bean cake fermentation water rich in bacteria and yeasts.

Production

Postlarval shrimp are harvested after about 40 days of culture at a size of 7 to 10 mm. Survival up to this stage is usually around 30%. The postlarvae are sold at a price of US$ 10 per 10 000.

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STRESS-TESTS: A PRACTICAL TOOL TO CONTROL POSTLARVAL SHRIMP QUALITY

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Introduction

In recent years the problem of finding an easy procedure to evaluate the quality of postlarvae produced in commercial hatcheries, has led to the development of stress tests, most of them using salinity (Errol, 1989; Soliz *et al.*, 1989; Tackaert *et al.*, 1989) or pH shocks (Arellano *et al.*, 1989; Arellano, 1990). Those bioassays allow the identification of healthy *versus* weak postlarvae, which is not always evident otherwise (Tackaert *et al.*, 1989; Arellano, 1990).

Experimental tests have been performed with American species (*Penaeus vannamei*) and an Asiatic species (*P. monodon*). However, because the kuruma prawn (*P. japonicus*) is the only species commercially cultured in Europe, we decided to try to extend those stress methods to this species, considering the fact that the weight (or age) of the PL's could have some effect on their resistance to salinity shocks.

Materials and methods

Different salinity conditions were prepared by diluting filtered natural seawater with distilled water at concentrations ranging from 0 to 40%. Triplicate tests were performed for each weight class (5, 9, 17, 22mg), using 50 PL's per test. The animals were placed into 250ml glass beakers, and the survival rate was monitored after 2h.

The test organisms were cultured during their larval stages under conditions of 40ppt salinity and 25-28°C temperature, and fed diatoms (*Chaetoceros, Skeletonema*) and *Artemia* (GSL strain).

Three biological criteria were used to distinguish dead and live animals:
- heart beat
- branchial movements
- thoracopod and exopod movements.

Results

The results obtained in the different experiments are shown in Table I, from which we conclude that the mortality was almost 100% at the lowest salinity concentration (0ppt = freshwater).

There was a significant difference in survival among the weight classes at the different salinity concentrations ($P>0.05$). This may suggest that the older the PL's are, the higher their resistance to salinity shocks is. The survival rate was highest at salinity concentrations above 8ppt.

Table I. Survival rate (%) of different weight classes of postlarval *Penaeus japonicus* after 2h stress test under several salinity conditions

Salinity (ppt)	5mg	9mg	17mg	22mg
20	99	100	100	100
16	86	100	100	99
12	67	100	100	100
8	33	97	90	93
6	-	94	93	-
4	1	76	82	-
2	0	23	47	-
0	0	0	4	16

Discussion

Various authors have pointed out the close relationship existing between diet composition (especially the HUFA content) and the animal's resistance to osmotic shocks (Soliz *et al.*, 1989; Tackaert *et al.*, 1989; Arellano, 1990). Comparing our results with those published for other species, we conclude that the feeding regime used during the larval culture period guarantees healthy PL's. On the other hand, we can also conclude that the strong resistance of *P. japonicus* PL's to drastic salinity changes may be a security factor for their seeding in the grow-out ponds.

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IMPLEMENTATION OF BACKYARD SHRIMP HATCHERY IN INDONESIA: A NEW PROFITABLE BUSINESS FOR INCREASING FAMILY INCOME

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Introduction

Backyard shrimp hatchery technology has been tested and developed at the Brackishwater Aquaculture Development Center, Jepara, Indonesia since 1980. However, this technology has only been widely adopted by local families during the past 2 years. At present more than 100 units of backyard shrimp hatcheries are established in the vicinity of the Jepara district. Several units have also been established in West Java and East Java Provinces. The development of backyard shrimp hatchery is providing new business opportunities, jobs and supports the development of the local shrimp industry.

Facilities and methods

A backyard shrimp hatchery is usually owned by one family and generally consists of: 1) not more than three larval rearing tanks made of concrete or bricks reinforced by several concrete columns, and provided with a draining tank which also serves as a harvesting chamber, 2) two to three algae culture tanks, and 3) several tanks for hatching of *Artemia* cysts. The capacity of each larval rearing tank and algae culture tank are 10-15 tons and 2-3 tons, respectively. Each larval rearing tank of 10 ton capacity is aerated by a small air blower of 40W.

Before stocking of shrimp nauplii, larval rearing tanks are washed and brushed, and left to be sundried for about 2 days. Seawater of 28-33ppt used for shrimp larval rearing is pumped directly from the sea and filtrated. Seawater is treated with 10ppm calcium hypochlorite, aerated strongly for 2 days, and then neutralized with 3ppm sodium thiosulphate. Shrimp nauplii for stocking are obtained either by onsite spawning of gravid females or are purchased from private big hatcheries. The number of shrimp nauplii stocked is about 1 million per 10 tons of seawater. During larval rearing, tanks are covered with dark colored plastic sheet. Typically, no water exchange is practised during larval rearing, except during unfavorable conditions. In certain conditions, excess feed and other debris are syphoned from the bottom of the tank to maintain a good water quality. During zoea and mysis stages shrimp larvae are fed two times a day with algae (*Tetraselmis chuii* and *Skeletonema* sp.), and eight times a day with commercial artificial plankton at a ratio of 1 to 2g.ton^{-1} of rearing medium. Postlarvae are fed two times a day

with 30-40 million *Artemia* nauplii/ton of culture medium, and eight times a day with a flake diet at a ration of 1 to 2g.ton^{-1} of culture medium. If larvae are infected by parasites and bacteria, antibiotics such as chloramphenicol, furazolidone and erythromycine are added to the culture medium at a dose of 1-2ppm. Shrimps are generally harvested at the PL15 stage which is considered a suitable size for stocking the grow-out ponds.

Production results

After 2 years of experience with backyard shrimp hatcheries in the vicinity of Jepara, it has become clear that the production results of tiger shrimp (*Penaeus monodon*) fry is still fairly inconsistent. Large variations in culture success have been recorded, not only among different hatcheries but also within the same hatchery. Many factors appear to be influencing the shrimp fry production. Among others these are disease problems, water quality of rearing medium, feed quality and feeding management. Table I illustrates production results obtained during 1 year experience in a backyard shrimp hatchery operation belonging to the writer.

Table I. Tiger shrimp (*P. monodon*) fry production in a private backyard hatchery with a capacity of 20 tons of larval rearing tanks

Month/year	Number of nauplii stocked	Number of PL harvested	Survival rate (%)
November 1989	2 000 000	461 500	23.1
December 1989	2 500 000	213 300	14.2
February 1990	1 300 000	150 200	11.6
March 1990	1 500 000	400 000	26.7
April 1990	2 000 000	349 800	23.3
May 1990	2 000 000	623 400	31.2
June 1990	1 000 000	199 800	20.0
July 1990	1 250 000	486 000	38.9
August 1990	1 500 000	482 000	32.1
September 1990	2 000 000	890 850	44.5
October 1990	1 500 000	289 000	14.5
November 1990	3 500 000	370 000	14.8
December 1990	2 500 000	52 000	0.02

These data show that the shrimp fry production during the months of December to February was particulary low. This was probably due to the heavy rains during this period which brought about poor quality of seawater and low water temperatures. As a result, pathogenic organisms were proliferating rapidly and infecting shrimp larvae. Shrimp larvae did not molt well, causing high mortalities. Based on the writer's experience, the BEP value of a backyard shrimp hatchery business is about 100 000 shrimp PL produced from 10 tons of tank capacity at the price of Rp. 8.00 per postlarva. This means that a backyard shrimp hatchery is a profitable business allowing to increase family income.

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USE OF SALT-PAN BRINE FOR SHRIMP AND PRAWN LARVICULTURE IN THAILAND

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Abstract

Culture of giant freshwater prawn (*Macrobrachium rosenbergii* de Man) and giant tiger prawn (*Penaeus monodon* Fabricus) in Thailand has expanded greatly during the past decade. This development was made possibly by, and/or resulted in the development of many industrial enterprises such as prawn hatcheries, feed mills, processing plants and cold storage facilities. At present, there are about 30 large hatcheries and not less than 1 000 small-scale (backyard) hatcheries in Thailand. Most of the latter are located in Chachongsoa and Cholburi Provinces, Central Thailand.

Prior to 1986, most backyard hatcheries produced only freshwater prawn postlarvae (PL). Thereafter, due to over-production of freshwater prawn PLs, and to an increased demand and high prices for tiger shrimps, many backyard hatcheries switched to tiger shrimp PL production.

Most backyard hatcheries are located inland, without direct access to seawater. Consequently, these hatcheries must bring either seawater or salt-pan brine to their facilities. The advantage of brine, with a salinity range of 40 to 100ppt is that transport costs are less because it can be diluted with freshwater at the hatchery. It is, however, questionable whether diluted brine provides an adequate water quality for prawn larviculture as does undiluted seawater. There are some chemical differences between the two. The current paper reviews the use of salt-pan brine for prawn larviculture in static water, closed recirculating-, and open-water culture systems.

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LABORATORY REARING TECHNIQUE FOR LARVAL PRODUCTION OF *MACROBRACHIUM NOBILII*

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Introduction

The increasing demand for prawn together with supply shortage has initiated attempts to raise prawn under controlled conditions. Since the major break-through by Ling (1969), when he discovered that salinity was an important basic requirement for the survival and development of prawn larvae through their early life stages, various techniques for raising prawn larvae have been developed in different parts of the world. Larval history of *M. nobilii* has been described by Murugadass (1989); however, apart from the brief reports of Balasundram (1980), nothing is known about mass rearing of *M. nobilii* larvae.

Materials and methods

The *M. nobilii* broodstock used in these trials was collected from the River Cauvery (South India). For the rearing trials, the berried females were selected from the broodstock holding tanks and the eggs were hatched in the *in vitro* incubator designed by Mathavan and Murugadass (1988). Upon hatching, the larvae were removed and placed in plastic troughs for counting. Larvae in each hatch were enumerated by counting them in 100ml aliquot samples. A known volume of water containing the desired number of larvae was transferred to the rearing tank.

Larval rearing system

Two kinds of techniques were tested for this study. A static system with complete water change daily. A rectangular fibreglass tank (dimensions 60x45x30cm) was used for rearing the prawn larvae. During the course of larval rearing, brackish water with a salinity of 14 to 24ppt was maintained at 20cm depth. Water aeration and light from a fluorescent lamp were maintained throughout rearing.

In the second technique a static system, containing a biological sand filter, with fortnightly water change, was used. This system consists of a rectangular fibreglass tank

(dimensions 60x45x30cm) and a submerged biological sand filter at its bottom. The thickness of the sand layer is 5cm. Salinity, aeration, light source, and water depth were similar as those in the first system. The two systems were operated indoors.

Rearing of larvae

This study aimed at determining the effect of the stocking density (10 to 100 larvae/l) on survival in each rearing system. Ten rearing trials were set up with each culture system. After the prawn larvae had been transferred from the hatching device to the respective rearing systems, they were fed with newly-hatched *Artemia* nauplii at a density of 5 ind./ml. Uneaten food and larval wastes at the tank bottom were removed daily by siphoning.

Results

The results obtained with the static system with complete water change are summarized in Table I. All rearing trials exhibited good larval survival during the first 20 days or during larval stages 1 to 5. However, the mortality rate increased drastically thereafter. The first postlarva was seen after 48 to 52 days of rearing and the majority of the larvae metamorphosed by day 54-58. The survival ranged from 3.7 to 33.5%. The yield of postlarval prawns/l water ranged from 3.4 to 6.9.

Table I. Data on larval rearing in the static system with daily total water change

Trial No.	Larvae per		Postlarvae			Day of metamorphosis	
	Aquarium	Litre	Yield No.	Survival (%)	No./l	First	Last
1	600	10	201	33.5	3.4	48	52
2	1 200	20	278	23.2	4.6	48	54
3	1 800	30	337	18.7	5.6	49	58
4	2 400	40	396	16.1	6.4	51	58
5	3 000	50	414	13.8	6.9	49	56
6	3 600	60	335	9.3	5.6	48	56
7	4 200	70	302	7.2	5.0	51	58
8	4 800	80	283	5.9	4.7	52	54
9	5 400	90	243	4.5	4.0	52	56
10	6 000	100	222	3.7	3.7	51	58

Most of the rearing trials with the static system with a biological sand filter exhibited the same survival patterns as in the static system with complete water change (Table II).

Regression analysis of the results of these systems showed that the survival rate was not constant, and it decreased at the higher stocking densities (Fig. 1). The stocking density of these trials showed a linear correlation with survival *i.e.*, the lower the stocking density the higher the survival.

Table II. Data on larval rearing in the static system containing a biological sand filter unit and with fortnightly partial water change

Trial No.	Larvae per		Postlarvae			Day of metamorphosis	
	Aquarium	Litre	Yield No.	Survival (%)	No./l	First	Last
1	600	10	172	28.6	2.9	49	54
2	1 200	20	267	22.3	4.5	48	52
3	1 800	30	292	16.2	4.9	50	54
4	2 400	40	271	11.3	4.5	49	55
5	3 000	50	282	9.4	4.7	52	56
6	3 600	60	274	7.6	4.6	54	62
7	4 200	70	248	5.9	4.1	53	59
8	4 800	80	221	4.6	3.7	54	61
9	5 400	90	174	3.4	3.1	54	61
10	6 000	100	162	2.7	2.7	54	64

Discussion

There was not much difference in the production of metamorphosed prawns between the two systems. During the course of the trials in the static system with complete water change, the ammonia level was one of the difficult water quality parameters to control. The ammonia level could be minimized by changing the water more frequently. However, this is uneconomical in an area located inland far from the sea.

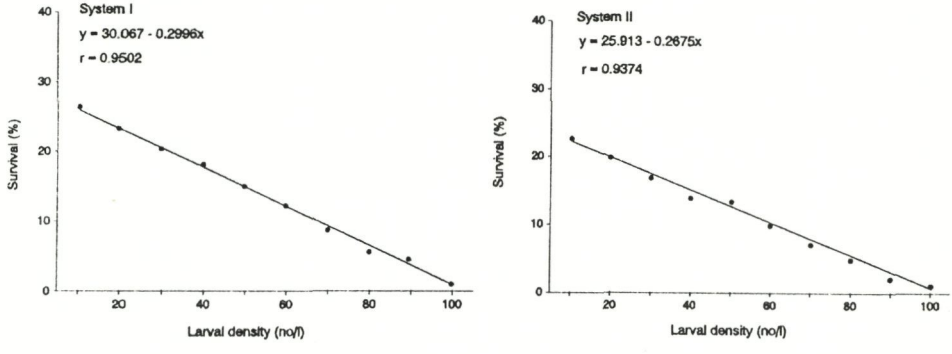

Fig. 1. Relationship between stocking density and survival of *M. nobilii* larvae in two rearing systems. System I: static system with total water change. System II: static system comprising a biological sand filter, with partial water change.

Conclusions

From these results we may conclude that the most economical way of producing *Macrobrachium nobilii* postlarvae is by using a static system with a biological sand filter. Larval densities should be maintained at not more than 20 ind./l in order to ensure reasonable survival rates.

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LARVICULTURE TECHNIQUES AND ECONOMICS OF SMALL-SCALE *MACROBRACHIUM ROSENBERGII* HATCHERIES IN THE MEKONG DELTA, VIETNAM

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Introduction

The aim of the present study was to improve outputs and cost effectiveness of *Macrobrachium rosenbergii* larviculture at two small-scale hatcheries in South Vietnam, one located at the coast (Vinh Chau) the other inland (Can Tho). Improved management and feeding strategies, using *Artemia* biomass available from local salt ponds were tested.

Materials and methods

Gravid females of *Macrobrachium rosenbergii* were disinfected with formalin (25ppm) or with malachite green for respectively 30 and 15min. Larvae were treated with malachite green (5ppm) for 15min.

The so-called clear-water culture was adopted. Rearing water of 12ppt was prepared by mixing highly saline water (varying from 60 to 110ppt and obtained from local salt ponds) with well water or tapwater. Further details regarding the water treatment are given in Fig. 1.

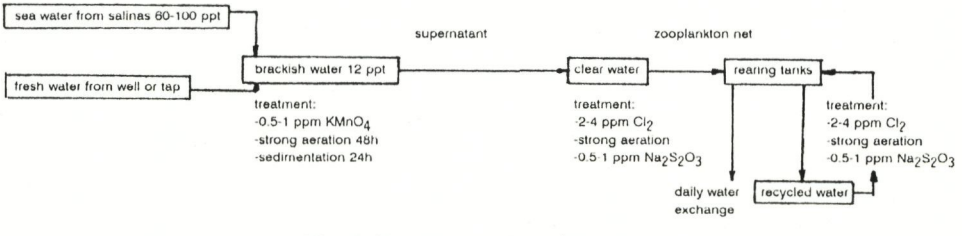

Fig. 1. Preparation of rearing water.

Before each production run, tanks and culture materials were treated with a 200ppm chlorine or formalin solution for 24h after which they were sun-dried for 2 to 3 days. During rearing all materials, after being used, were dipped in a 200ppm chlorine solution over night and cleaned with fresh water before reuse. Air stones and air pipes were replaced weekly during the experiments. The daily percentage of water exchange increased from 10 to 100% according to the growth of the larvae. Every 3 to 4 days, the rearing tanks were siphoned off before water exchange and 5ppm streptomycin was added as a prophylactic measure.

Food items, feeding regime and composition of artificial diets are detailed in Table I. The artificial diet was prepared by boiling blended ingredients for 15min. The resulting custard was screened to particle sizes of 300 to 1 000µm which were found to be appropriate for the different growth stages of the larvae.

Table I. Rearing conditions and metamorphosis rates (MR) at the end of the experiment for the different experimental runs

Hatchery	Production run	Larval density per l	Tank capacity (in l)	Use of recycled water	Feeding regime	MR (in%)
Vinh Chau	1	50	1 000	100% newly prepared water	Nauplii + live biomass, supplement of artificial diet (1)	74
Vinh Chau	2	40	1 000	100% newly prepared water	Nauplii + live biomass, supplement of artificial diet (1)	75
Can Tho	1	50	2 000	25% recycled water	Mainly artificial diet, supplement of live biomass or nauplii (2)	60
Can Tho	2	80	2 000	94% recycled water	Mainly artificial diet, supplement of frozen biomass or nauplii (2)	60
Vung Tau (3)	1	77	7 000	100% newly prepared water	*Artemia* nauplii and artificial feeds	40

(1) Stage 2 to 6: *Artemia* nauplii;
Stage 6 to postlarvae: live *Artemia* biomass at a ration of 100 to 500g/day/tank depending on the growth stage of the larvae; artificial diet at ration of 100g/day/tank; composition: chicken egg 90%, water 10%.

(2) Stage 2 to 7: *Artemia* nauplii;
Stage 7 to postlarvae: artificial diet at a ration of 100g to 400g/day/tank dependent on growth stage of the larvae; composition: fresh squid 45%, milk powder 15%, chicken eggs 30% and water 10%; supplement of 300g *Artemia* biomass/day/tank (live biomass for tank 1 and 2, frozen biomass for tank 3). If not available biomass was replaced by *Artemia* nauplii (50g cysts).

(3) Ministry of Aquaculture of the Socialist Republic of Vietnam, 1990. Shrimp Culture Conference, Vung Tau southern Province of Vietnam, June 14-15, 1990.

Results and discussion

Ammonium, nitrite and nitrate levels of the rearing water never exceeded the levels reported to be critical at the Shrimp Culture Conference in Vung Tau (Table II). Data for percent of metamorphosed larvae (number of postlarvae obtained/number of larvae stocked) obtained at day 26 are given in Table I. Metamorphosis rates in 25% water recycling (Can Tho run 1) are identical to those in 94% water recycling (Can Tho run 2) even though stocking densities were lower for the first run. At Vinh Chau and Can Tho the use of *Artemia* biomass as a main food source or as a supplement together with proper sanitation measures resulted in high metamorphosis rates when compared to those obtained at the Vung Tau hatchery.

Table II. Critical values for ammonium, nitrite and nitrate (in ppm) for different larval stages of *Macrobrachium rosenbergii* (Ministry of Aquaculture of the Socialist Republic of Vietnam, 1990 - Shrimp Culture Conference, Vung Tau, southern Province of Vietnam, June 14-15, 1990)

	Larvae 4-12 days old	Larvae 12-20 days old	Postlarvae
N (NH_3+NH_4^+)	0.05-0.25	0.25-0.9	max. 1.0-1.2
N-NO_2^-	0.01-0.15	0.15-0.28	max. 0.3-0.4
N-NO_3^-	2.8	15.20	max. 30-50

Production costs per postlarva were lower at the coastal hatchery of Vinh Chau than at Can Tho (Table III). This is mainly attributed to the use and availability of cheap *Artemia* biomass at the first location. Production costs for the inland hatchery of Can Tho are higher mainly due to extra transportation costs of the brine. However, the operating costs could be significantly reduced by applying recirculation.

Table III. Operational costs per postlarva produced for the different hatcheries

Hatchery	Production run	Operational cost per PL in Vietnamese Dong (1)
Vinh Chau	1	3.78
Can Tho	1	7.80
Can Tho	2	5.60
Vung Tau (2)	1	11.00

(1) 5 500 Vietnamese Dong - US$ 1 (June, 1990).

(2) Ministry of Aquaculture of the Socialist Republic of Vietnam. Shrimp Culture Conference in Vung Tau, southern province of Vietnam, June 14-15, 1990.

Conclusions

Previously inconsistent larviculture outputs of *Macrobrachium rosenbergii* at small-scale hatcheries in Vietnam have been improved through adequate sanitation and the use of *Artemia* biomass as a main or supplementary food source. The operational costs per PL were reduced considerably through the use of recycled rearing water in the inland hatchery and the use of *Artemia* biomass in the coastal hatchery.

Acknowledgements

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PHYSICAL PERFORMANCE OF A SINGLE *VERSUS* A DUAL INFLOW REARING TANK FOR FISH AND CRUSTACEAN LARVAE

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Introduction

Homogeneous and complete water circulation are regarded as important features in the rearing of crustacean larvae since equal mixing prevents stagnation, clumping and cannibalism; in the case of fish larvae, one can add low current conditions allowing the larvae to aim and snap at live prey items (Rosenthal and Hempel, 1970). Hughes *et al.* (1974) described a 50 l larvae rearing tank designed towards realizing such conditions. Critical features of the tank were the central bottom water circulator, which introduced water through obliquely-oriented slits along a semi-spherical bottom, and an overflow from the top of a central standpipe.
Very good rearing success was obtained with lobster and herring larvae. However, the continued use of the tank has disclosed some inadequacies. Homogeneous current conditions could not be realized in all the available volumes at low flow rates. The situation was worsened at high positive air-water temperature differences which led to a gradient, limiting circulation to the bottom third of the tank.
The present study aimed at devising, building, and testing a new tank originating from the Hughes *et al.* (1974) model but with new features that would insure a more complete water circulation and minimize temperature stratification in conditions of high air to water temperature differential and low flow rates.

Description of the tank

The general shape of the Hughes *et al.* (1974) tank has been altered slightly by enlarging the diameter and reducing the height: the new tank (Fig. 1) is made of a 25cm high and 25cm internal radius cylindrical upper part, over a flattened 15cm high semi-spherical bottom. Thermal insulation has been added in the form of a 3cm layer of urethane injected between the inner wall and a new external 0.5cm fiberglass layer. Inside and outside fiberglass layers are folded together at the top in an inclined collar and connected at the bottom by a fiberglass ring 3cm in diameter. The inside of the tank is coated with sanitary quality black gelcoat. Central bottom water injection is similar but for some measurements to the Hughes *et al.* (1974) model: an excentric standpipe connects to a bottom cylindrical chamber where water is injected along the bottom of the tank through

24 slits of 1.4x1.4mm square-cut across the 8mm thick base, oriented 30° to the tangent, clockwise from above. Coming back from the tank, water goes through a removable cylindrical nylon screen with 2x4mm oval openings, installed on the bottom chamber, up to the top of a central overflow pipe running through the chamber and the bottom of the tank. Depending on predator and prey sizes, finer mesh size nylon material (nitex) can be fitted over the screen. A new water circulator has been added around the top in the form of an internal 6cm-high circular chamber of fiberglass where water is introduced from a 1.75cmx1.75cm inlet through the top fiberglass layer. Water is injected into the tank through 24 perforations of 1.6mm-diameter oriented horizontally 30° to the tangent in a clockwise direction as seen from above. The water level is set 1cm above the peripheral perforations by adjusting the height of the central drain. The total water volume is 61.6 l. The general water circulation is clockwise on the left side of a vertical cut of the tank and clockwise also in the horizontal plane as seen from above: a water-borne particle is expected to follow a helicoidal pattern.

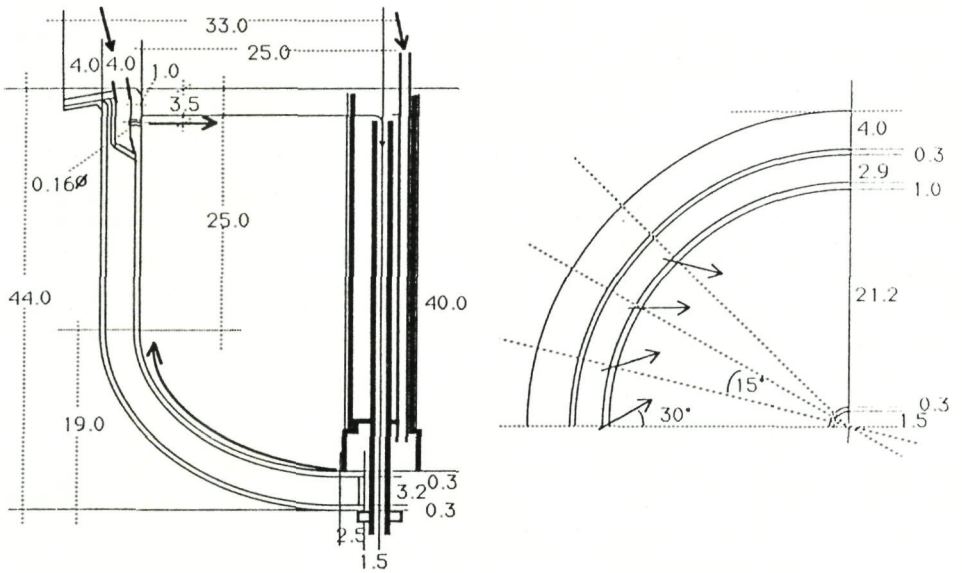

Fig. 1. Vertical cut of half of the tank plus the complete central water-circulator and screen; also horizontal quarter cut of the upper part of the tank without circulator. Dimensions in cm and water paths as arrows.

Test procedures

Temperature distribution was assessed under different flow rates at the top and bottom inlets using water at 12.0±1.0°C and 2.0±1.0°C with air temperature being maintained constant at 21.0±1.0°C. Flow rates were set and expressed in units of percent tank volume/min (1%tvm=0.61 l.min^{-1}) and were measured with a precision of 0.05 l.min^{-1} in the range 0.1-1.0 l.min^{-1}. The temperature was measured at 5cm intervals (±0.1°C). The tank was set as a flow-through system.

Artemia cysts (0.2 to 0.3mm diameter) were used to follow the water circulation in the tank. The cysts were selected from a set of 25 left in seawater for a few hours and tested for effective neutral buoyancy before and after the experiment. White 0.3mm nylon strings running from the center screen to the border provided a grid of eight horizontal directions and seven vertical levels; the distance from the center was marked every 5cm on the strings. The cysts were introduced with a pipette in the center of the tank and the particle was followed by eye using a handlight in a dark room, its position being noted every 30sec for 20min. Two sets of experiments were conducted, both at 1% tvm total flow. First, with a vertical stratification in temperature, 3.2 to 9.8°C or 5.5 to 12.8°C, created by a 100% or 67% bottom inflow; and second, without gradient, 15.8 to 16.0°C, with 50% of the water introduced from the top.

Results, discussion and conclusions

The effect of introducing the water from the top in order to control the vertical gradient can be seen in Fig. 2A where at an air to water differential of 19°C and constant total flow rate, increasing the proportion of water flow from the top, leads to the progressive disappearance of the vertical gradient in the tank. The same is true for the experiments done at 12°C initial water temperature (Fig. 2B) where at a same flow rate of 0.8% tvm, changing introduction from bottom to top changes the distorted vertical temperature pattern to a straight line. Moreover, it is suggested by the average temperature associated with 67% top flow in Fig. 2A and especially with 100% top flow in Fig. 2B, that a major proportion of the water coming from the upper periphery of the tank might help maintain a lower average temperature than at least some lower proportions of top flow. Finally, the insulating properties of the tank in a no-gradient condition at a low flow rate of 1.0% tvm and 50% inflow from the top, appear very good since temperature rose by only 5.5°C at an air-water differential of 19°C (Fig. 2A) and by 4.0°C at an air-water differential of 9°C (Fig. 2B).

Five 20min paths were monitored for each condition, *i.e.* with and without vertical gradient. In all cases, the cysts moved through a larger volume of dispersion in the absence (*e.g.* no. 5; in Fig. 3) than in the presence of a temperature gradient (*e.g.* no. g3; Fig. 3). The number of different positions occupied averaged 35 (SE=3), out of a possibility of 40, in the five tests without a vertical gradient and 18 (SE=8) with a vertical gradient. Contingency tables for the two axes in the horizontal and vertical planes produced the following average chi-square p values: 0.283 and 0.041 in the no-gradient situation and 0.000 and 0.008 in the gradient one. This indicates high frequencies of occurrences for only a few of the visited points in the gradient situation. From the presence of upper or lower paths followed by the particle, it also became apparent in the vertical stratification situation, that two separate circulation zones existed that reflected the depth of introduction of the cyst.

In conditions of positive air to water temperature differential, adding a peripheral near-surface water circulator to a bottom central one, has considerably increased the capacity of the tank to maintain at low flow rates a complete water or particle circuit by providing the possibility of eliminating the vertical temperature gradient. Insulating the walls has eased the process of reducing vertical temperature gradients. Reducing the

height to diameter ratio meant a longer water jet path before obstruction and dispersion from the walls or central screen, possibly providing a better defined helicoidal movement.

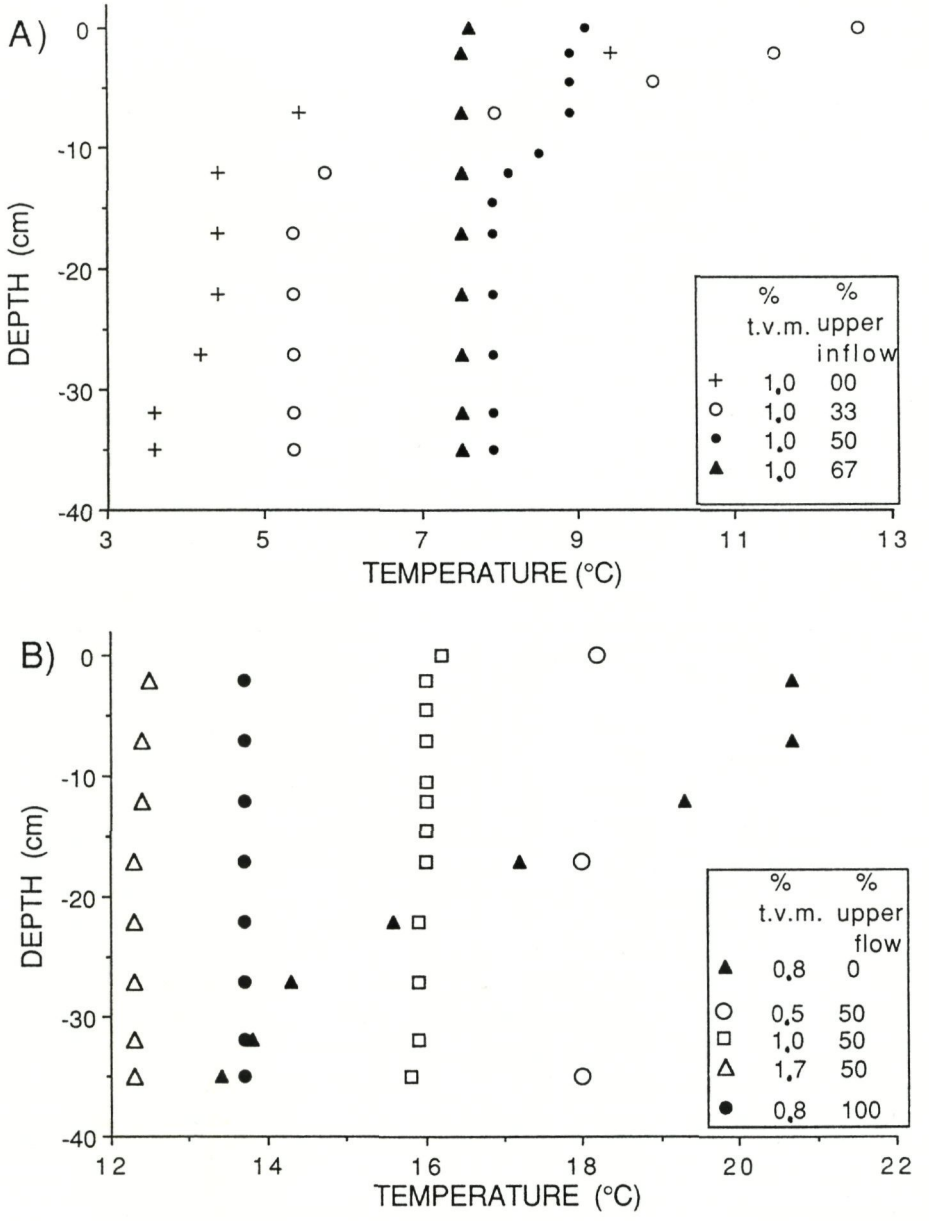

Fig. 2. Temperature distribution *versus* depth following different proportions of water intake from the upper circulator. (A) at 2°C initial water temperature; (B) at 12°C initial water temperature.

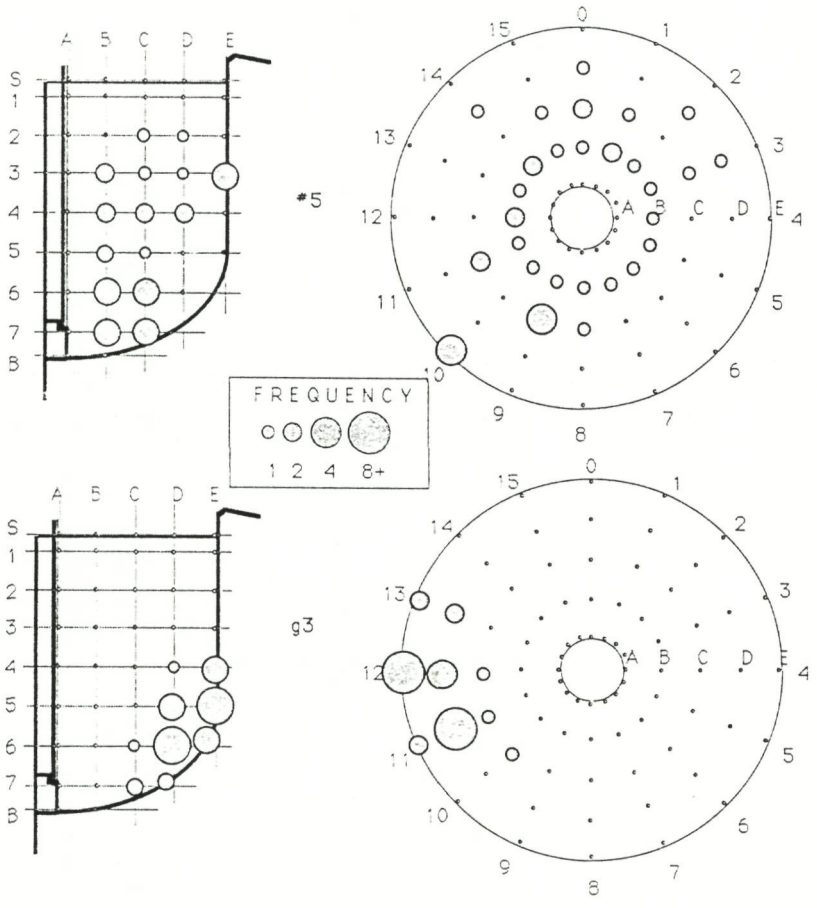

Fig. 3. Frequency of occurrence at the grid coordinates by a neutral buoyant 0.02mm particle circulating in the tank for 20min and recorded every 30sec: test no. 5 without a vertical temperature gradient and test g3 with a vertical temperature gradient of 2.5°C.

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CULTURE METHODS AND ECONOMICS OF MARINE FINFISH HATCHERIES

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MARINE AQUACULTURE IN ISRAEL WITH SPECIAL EMPHASIS ON LARVAL REARING

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Abstract

The goal of mariculture research in Israel is the development of an industry, based on available seawater, in the arid Arava, in the gulf of Eilat and along the Mediterranean coast of the country. Urgent progress is important due to the shortage of freshwater for agriculture. Highly priced euryhaline species like *Sparus aurata* and *Dicentrarchus labrax* are the obvious choice for development along with mullets (*Mugil cephalus)* and other marine species which are under investigation. The bottleneck to massive marine aquaculture of a ready supply of fingerlings is about to be alleviated by a new commercial hatchery (owned by the Kibbutzim in the southern Arava region; $4x10^6$ fingerlings/year) which goes into operation this summer. This hatchery is based on a decade of R&D at the NCM.

The aim of this paper is to present the research and zootechnical state of the art in *Sparus aurata* and *Dicentrarchus labrax* larval rearing at NCM. Egg production in both species uses the slow release of GnRH from specifically designed implants, so that about 90% of the seabream and 60% of the seabass females spawn at will. Photoperiod and temperature control ensures year round egg production of $360x10^6$ *Sparus aurata* and $15x10^6$ *Dicentrarchus labrax* eggs per year. Broodstock is fed a special diet of squid and the IOLR Standard Ration grow out diet. An extensive study of the interaction between brood stock nutrition and egg quality is presently under way.

Fertilized eggs are stocked in 600 l tanks and hatching success estimated from aliquot counts. Rearing in these tanks continues for 32 d. These tanks require only one cleaning in the course of this period and fish are easily removed with minimum mortality. Under normal rearing conditions these tanks produce 15 to 35 32d old (10mg WW) *Sparus aurata* larvae/l (20-40% survival). The variability in production of these tanks is 31% (CV). Average seabass larval survival to 80mg (40d) is 35%. Under our conditions tanks of a larger capacity do not perform as well. Average survival in 1 800 l are 5 to 12 32d seabream larvae/l. Tanks are continuously supplied with filtered (10µm), temperature and salinity controlled seawater and continuously supplied with freshly enriched food organisms through a special delivery system. Live food enrichment regimes for the two

species were developed to maximize their growth and survival. A microdiet is presently being used for 20d larvae. The problems involved in the use of dry diets with larvae are presently under investigation. Swim bladder presence of both species is around 90% as a result of a salinity reduction from the locally prevailing salinity of 40ppt. Special attention is given to the size structure of the population in order to minimize loss due to cannibalism. Once seabream end their 32d and seabass their 40d period they are graded with special graders to 2 and 3 size groups, respectively. The graded larvae are individually counted with a locally developed Computer Aided FIsh Counter (CAFIC). Finally, a special automated system is being developed to follow and control the rearing process. It will monitor environmental parameters and concentration of food organisms on an hourly basis, and adjust them according to the rearing protocol.

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MASS LARVAL REARING OF MARINE FINFISH IN JAPAN

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Abstract

The techniques for mass larval rearing of marine finfish have developed markedly in the last 20 years in Japan. In the western part of Japan, each ocean ranching or fish farming center produces every year, during a hatchery season of 3 months only, more than one million juveniles of red seabream *Pagrus major*. Juveniles of red seabream produced by the National and Prefectural Ocean Ranching Centers are stocked into the sea to increase coastal resources, while those produced in private hatcheries are used as seeds for net-cage culture.

The present paper reviews the present Japanese status of mass production technology of red seabream juveniles as a representative species for marine finfish. The developmental stages are also discussed and can be categorized into the 10 following phases: 1) the use of the rotifer *Brachionus plicatilis* as initial live food and establishment of its mass production technology; 2) rearing methods for natural spawning in tanks and evaluation for broodstock diets; 3) nutritional evaluation and improvement of food organism (live foods); 4) lordotic deformity and its solution; 5) environmental management in larval rearing systems; 6) mechanization and automation in larval rearing systems; 7) development of formulated microfeeds; 8) new rearing techniques in order to acclimate juveniles to environmental field conditions prior to stocking; 9) introduction of exotic strains; 10) genetic breeding (selection, cross breeding, all female production by gynogenesis, and triploid production).

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LATEST IMPROVEMENTS IN INTENSIVE SEABASS AND SEABREAM FRY PRODUCTION : THE FRENCH TECHNIQUE

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Abstract

Over the last 10 years, a most spectacular breakthrough in French marine finfish production was obtained through significant improvements in larval rearing.

The techniques progressively evolved from large tanks (>10m^3) with open water systems and low fry densities (<50 larvae/l) in green waters to small units (<4m^3) with closed recirculating systems, high densities (>100 larvae/l) and clear waters.
New findings on tank colour, optimal lighting and a simplified feeding sequence have also considerally modified the initial techniques. By technical and veterinary methods some of the major pathological problems such as swimbladder abnormalities and vibriosis, were overcome.

Simplification and reproducibility resulted in an overall increase in mean survival rates of fry of 1 to 2g from less than 5% in 1982 to an average of 40% in 1991.

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AUTOMATION IN MARINE HATCHERIES

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Introduction

The majority of biological constraints to the production of marine fish fry in the Mediterranean countries have been overcome in the past few years. This has led to an increase in the number and particularly in the output of individual hatcheries. The supply of fish fry is beginning to exceed demand in some regions, with a repercussion on market prices. Competition between producers is increasing as more rational and cost-effective production methods are implemented.

At present, most phases in the production process rely on both manual labor and expert supervision. This is due to the small size and vulnerability of the organisms that are cultured, and the requirement of ensuring optimum rearing conditions. Automation can nevertheless be introduced in several fields to reduce the work load and take over many control tasks in the every day hatchery management.

Hatchery production flow chart

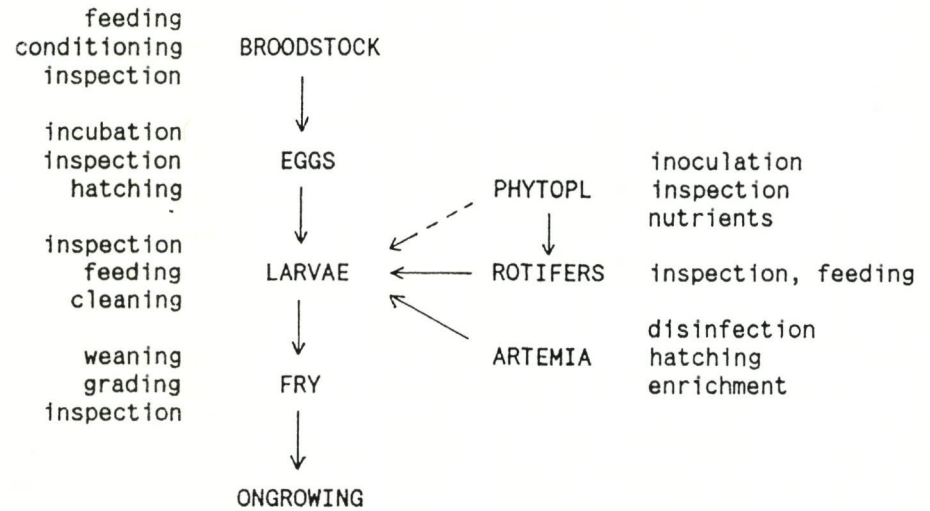

Fig. 1. Flow chart for marine fish fry production.

Fig. 1 is a very simplified model of various phases in marine fry production. Common tasks in all phases include water quality management, some form of feeding, inspection and transfer of biological materials. The degree and type of automation that can profitably be applied to fry production depends on:

- hatchery size and output capacity;
- number and size of individual rearing units;
- configuration (arrangement) of production facilities;
- availability and cost of labour;
- level of local technical know-how.

A well-planned and managed facility operates so as to fully exploit the available infrastructure and installed equipment, while minimizing the risks. In order to achieve this, it is important to load the various components of the facility as evenly as possible, and to incorporate appropriate regulation and alarm systems. This is most efficiently done in the initial design phase. Where existing facilities are concerned, the construction of a detailed flow chart of all phases in the production process will indicate the areas where automation can be efficiently applied. This is even more effective when a quantification of personnel requirements can be included, together with an assessment of the risks involved in improper application of production technology.

Examples

Among the many solutions which can be applied in particular circumstances, we will look more in detail at two which can be almost universally applied.

One is the utilization of integrated systems for the monitoring of dissolved oxygen levels in rearing basins, linked to automatic systems for the addition of oxygen or aeration systems in order to maintain predetermined levels of oxygen in the water. The components of such a system are shown in Fig. 2. Typical applications for this is in areas of high oxygen consumption, like fry-rearing tanks and *Artemia* enrichment tanks.

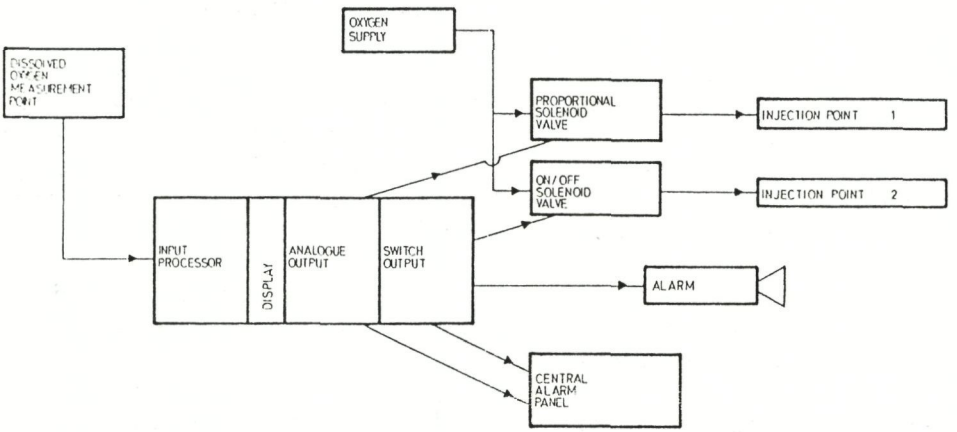

Fig. 2. Oxygen monitoring, regulation and alarm system components.

Another is the utilization of pumps for the transfer of fish from tank to tank, and during grading operations. This can be incorporated with a complete fish grading and counting station, to enable an accurate and even distribution of fry biomass in the available facilities.

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MANAGERIAL PROCEDURES AND COST EFFECTIVENESS IN LARVAL REARING

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Abstract

This paper describes the managerial, zootechnical procedures and comparative costs of seabream (*Sparus aurata*) production over the first 60 days in the larval and weaning systems at Cephalonian Fisheries SA.

The results of the August 1990 production of seabream are examined and compared with the February 1991 production run of the same species. The causes of the differences between these two production runs are discussed and the advantages and disadvantages outlined.

At Cephalonian Fisheries, in a single production run, a variation of larval rearing techniques are employed with the objective of continually upgrading the standard larval rearing method both in terms of increasing production and decreasing cost.

A significant advantage in the August 1990 production was the use of large outside tanks for the mass production of phytoplankton and zooplankton. This not only reduced the unit cost of these products through economy of scale, manpower and energy costs but by producing it in a multi purpose tank, the capital cost to the live food production was negligible. It also enabled a production of such scale that the maximum output from one rearing run rose from 800 000 to nearly 2 000 000 larvae.

Detailed operational procedures and feeding tables are presented, comparing the variations in zootechnical parameters and the influence of these variations on survival and production costs within each production run.

With the increasing demand and the availability of quality live-food replacement and early-weaning diets, significant improvement in larval survival and reduced mortalities due to cannibalism at weaning are achieved. A schedule for the rigorous grading at and after weaning is essential if losses through competitive feeding and cannibalism are to be avoided.

PATHOLOGY AND DISEASE CONTROL

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BACTERIAL DISEASES OF EGGS AND YOLK SAC LARVAE OF HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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Introduction

Halibut is an attractive species for marine cold-water aquaculture. High mortality at early life stages has, however, so far hindered commercial success. Infections by bacteria are believed to be a major cause (Bolinches and Egidius, 1987; Hansen and Olafsen, 1989; Pittman *et al.*, 1990; Opstad and Bergh, in press). Here we present studies considering experimental infection, treatment and characterization of the morphological, ultrastructural and behavioural consequences of infections at early life stages.

Materials and methods

Eggs and larvae were reared in 10ml autoclaved seawater in polystyrene multiwell dishes at 5°C in darkness. Eggs were experimentally infected 4 days before hatching by a *Flexibacter* sp. isolated from halibut eggs, *Vibrio anguillarum*, or other *Vibrio* spp. Mortality was recorded until day 37 when larvae were fixed for ultrastructural studies by transmission electron microscopy. In addition, eggs were fixed for investigation by scanning electron microscopy. In an otherwise identical infection experiment, larvae were filmed by a video camera for behavioural studies; and transferred to a buoyancy column for larval buoyancy measurements every 3rd or 4th day.

In disinfection experiments, the eggs were treated with Buffodine (Evans Vanodine, Preston, UK) for 10min at different concentrations 24h before hatching, before transfer to multiwell dishes. Mortality was recorded throughout the experiment. At day 37 after hatching, the larvae were examined by microscope for developmental disorders.

Results

Cumulative mortality in the infection experiments is shown in Table I. The *Flexibacter*-infected individuals showed high mortality at the egg stage, at hatching and early yolk-sac stage, whereas larvae infected by *Vibrio* spp. had low mortality at these early stages,

followed by a high mortality throughout the yolk-sac stage. In the uninfected control group, only 5 to 60 larvae died during the experiment. Examination by scanning electron microscopy in *Flexibacter*-infected eggs showed wounds colonized by large amounts of bacteria. The chorion was penetrated and the zona radiata was severely damaged. Transmission electron microscopy of larvae infected with *Vibrio* spp. revealed bacteria present in large amounts between gill arches, in the heart region and in the blood vessels. Uninfected control larvae appeared healthy, both on cellular and subcellular level, and no deformities or developmental disorders were observed. Research is in progress characterizing the infection-induced changes in behaviour and buoyancy.

Table I. Cumulative mortality in the disinfection experiments

Bacterium	Hatching (%)	Day 37 (%)
Flexibacter sp.	74	-
V. anguillarum	7	95
Control	0	8

As shown in Table II, surface-disinfection caused significantly increased survival as well as an increased percentage of larvae without developmental disorders of any of the kinds that could be identified.

Table II. Mortality and percentage of larvae with deformities in the disinfection experiments

		Mortality (day 37, %)	Deformed (day 37, %)
Buffodine concentration (%)	0.5	13	10
	0.05	15	32
	0.005	41	61
Control		33	59

Discussion

The infection experiment showed that the bacteria that were tested could be pathogenic to halibut eggs or larvae. The *Flexibacter* sp. is able to penetrate the chorion, and probably the zona radiata as well. This bacterium, which has been shown to be abundant on halibut eggs and larvae, as well as in the water in the incubator tanks, has not previously been described (Hansen *et al.*, in prep.).

The disinfection experiments prove that surface disinfection is an adequate way to increase survival and developmental success in halibut aquaculture. Further work is

neccessary to determine optimal concentrations and application procedures. The results presented emphasize the importance of the bacterial microflora of eggs and larvae on the survival and developmental success.

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BACTERIA ASSOCIATED WITH HATCHERY CULTIVATED TURBOT: ARE THEY IMPLICATED IN REARING SUCCESS?

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Introduction

Several studies have been carried out to determine the interaction of the bacterial flora with the cultivation of turbot larvae (Perez Benavente and Gatesoupe, 1988; Nicolas *et al.*, 1989). The intestinal flora of many larvae has been studied to evaluate its relationship with the environmental flora and the bacterial species causing mortalities (Tanasomwang and Muroga, 1988). The bacteria associated with the culturing systems of turbot has been reported as one of the destabilizing factors in its production (Perez Benavente and Gatesoupe, 1988). This study is focused on determining the environmental bacterial flora associated with turbot and water during the first weeks of culture. We measured the level of the total bacteria and total *Vibrio* associated with the larvae, the water, the rotifers and the *Artemia*. The modification of the bacterial flora produced by UV-treatment of water or antibiotic treatments in the fish tanks was also studied.

Materials and methods

Larval and juvenile turbot were obtained from the production unit of a turbot hatchery (Tinamenor SA). Fish were surface washed with 0.1% benzalkonium chloride and homogenized. When the sample had been enriched, the homogenate was inoculated on TSB 2% NaCl and alkaline peptone water (pH 9.4). Isolation was performed on TSA2%, TCBS and SGAP (Huguet and Ribas, 1991) after incubation at 21°C for 48h. Water samples were taken simultaneously with the sampling of the larvae using a similar procedure. The bacterial flora associated with *Artemia* and *Brachionus* were also studied at several stages of their cultures by homogenized aliquots. Biochemical classification of isolates was carried out using API 20E strips and additional tests. A similarity computing study was performed with the biochemical results. Statistical analysis was evaluated in a simple matching and a single linkage to define taxonomical phena.

Results

In the course of the study 564 bacterial isolates were obtained from the larvae, juveniles, tank water, and live food. In total, 95% of all isolates belonged to *Vibrio* spp. The total

bacterial counts of fish ranged from 10 to 10^3 CFU/TL^3 (cubic total length) and the total bacterial counts in the tank water ranged from 10 to 10^3 CFU.ml^{-1}. The number of bacteria associated with larvae and tank water peaked around days 10 and 23 post-hatch in the four groups studied, attaining values one or two logarithms higher than the normal range of values. Bacterial counts in live food were 10^3 CFU/rotifer and $4x10^3$ CFU/*Artemia*. A large percentage of the isolates on TSA 2% (total values) corresponded to *Vibrio* spp. as characterized by growing on TCBS and later on classified. The similarity matrix distinguished between five main phena, within which the index of similarity was ≥ 0.96. They were denoted as *Vibrio* I, *Vibrio* II, *Vibrio* III, *Vibrio* IV, and *Pseudomonas*-like. The most frequent isolates *Vibrio pelagius*, *Vibrio splendidus*, *Vibrio alginolyticus*, *Vibrio anguillarum*-like and *Pseudomonas*-like respectively. No differences were observed with respect to these phena, when comparing larvae grown with or without antibiotics, either when comparing groups grown with or without UV water treatment. In rotifers most isolates were clustered into the phena *Vibrio* II, *Vibrio* III, *Vibrio* IV and *Pseudomonas*-like, while in *Artemia* the clustering was to phena *Vibrio* I, *Vibrio* II, *Vibrio* III and *Pseudomonas*-like. Generally, there was no clear pattern of prevalence of either of the phena throughout the study. However an increase in *Vibrio* I was noted once the weaning of larvae to inert diets was initiated. Furthermore periods of high larval mortality were characterized by an increase in other phena such as *Vibrio* III.

Discussion

The prevalent bacteria associated with larvae and juveniles of turbot belong to the Vibrionaceae. This conforms with the bacterial patterns evaluated for other marine fishes (Campbell and Buswell, 1983; Tanasomwang and Muroga, 1988). A clear relationship was observed between the bacteria associated with the larvae, the live food, and the rearing tank water. The main source of bacteria into the rearing system seems to be accounted for by the live food. This relationship has practical implications in monitoring purposes: the larval tank water can be sampled for evaluating the bacteria associated with the larvae. Bacterial microflora of the alimentary tract might play an important role in the resistance of fish to bacterial infections. This associated microflora was found to be changing constantly during the initial weeks of hatching with regard to the phena characterized in this study. Following the settlement and the weaning of the fish, *Vibrio* I becomes the dominant phenon of the intestinal microflora. The significance of these findings needs to be evaluated in relation to the growth and survival of fish under intensive culture conditions.

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VIBRIOSIS OF THE SWIMMING CRAB *PORTUNUS TRITUBERCULATUS* IN LARVICULTURE

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Introduction

With the recent progress in larviculture of marine fishes in Japan, various disease problems have occurred (Muroga, in press). Seed production of the swimming crab (*Portunus trituberculatus*) is no exception. At the Tamano Station of the Japanese Sea-Farming Association, a leading hatchery for juvenile crab production, seed production of the crab was successful for more than 10 years until 1985. Since 1985, however, mass mortalities due to a vibriosis occurred frequently in zoeal larvae reared in the station. Characteristics of the pathogen (*Vibrio* sp. *Zoea*) are investigated in the present study.

Materials and methods

Six bacterial strains isolated from diseased larvae from 1985 to 1988 were examined for morphological, physiological, biochemical, serological, genetic, and pathological characteristics. Using representative strains, sensitivity to disinfectants and ultraviolet irradiation, and experimental host range were determined.

Results

Disease occurrence

The present vibriosis occurred in 1985, 1987 and 1988 producing significant losses of larvae. It occurred in zoeal larvae reared at 22-26°C but not in megalopa or juvenile crab. Infected larvae became lethargic and motile bacteria were observed microscopically inside the carapace. The bacteria were isolated on ZoBell's 2216E agar at 25°C.

Taxonomical characteristics of the pathogen

The six strains showed almost the same characteristics. The bacterium was gramnegative short rods with a single polar flagellum. Glucose was fermented without gas production. Arginine was not decomposed but lysine and ornithine were decarboxylated. Indole,

nitrate and gelatine tests were positive. Acid was produced from sucrose, mannose, galactose and some other sugars. GC value of DNA was 47.2±0.9 mol% (Muroga *et al.*, 1989). There were at least three serotypes (O-serotypes) among the examined six strains.

Physiological characteristics and disinfectant sensitivity

It grew within the range of 0.5-6% NaCl (optimum 2%), pH 6-9(7-8), and 20-35°C (30-35°C). The bacterium was killed in an *in vitro* test by a 10-min exposure to sodium hypochloride at 0.3ppm(Cl), povidone-iodine at 3ppm, benzalkonium chloride at 10ppm and formalin at 0.3%(formaldehyde). It was also inactivated by ultraviolet irradiation at 3 (on agar plate) or 6 (in seawater) x 10^3μW sec.cm^{-2}.

Pathogenicity

The isolated bacterium killed swimming crab of zoeal stages by addition (105CFU.ml^{-1}) or oral challenge with bacteria-loaded rotifers. The sensitivity to the pathogen was highest in the first stage of zoea and it became lower with the progression of the developmental stage. Megalopa and juvenile crab were no longer susceptible. According to the result of experiments on host range, crustaceans like brine shrimp (*Artemia*) and shore crab (*Hemigrapus sanguineus*) were sensitive to the pathogen but fin fishes were not.

Discussion

Fifty million juvenile swimming crab are produced annually in Japan. However, the survival rate from hatched larvae to juvenile crab at each hatchery is not stable due to unpredictable mass mortalities. Various factors, such as deteriorated environmental conditions, malnutrition and infections have been suspected, but very few studies have been made. Bacterial infections were observed in some hatcheries, but the causative agent was not isolated except for the present cases in Tamano Station. The bacterium isolated in Tamano Station was confirmed as the causative agent of the disease by a challenge test in larval swimming crab. Based on morphological/biochemical characteristics and GC value, the pathogen was classified in the genus *Vibrio*. It appeared to be closely related to *V. parahaemolyticus*, but could be differentiated from it on the basis of physiological and biochemical properties. Since genomic DNA of the present species has not been compared with related species, it has been tentatively called *Vibrio* sp. *Zoea* (Muroga *et al.*, 1989). Initially the rearing waters or live diets (rotifer and brine shrimp) were considered as the source for the pathogen, but the bacterium was not recovered from them (Suzuki *et al.*, 1990). Although the disease was reproduced by addition and oral challenges, the mode of transmission of this infection is the subject for a future study. Based on the experimental host range, the present bacterium seems to be a pathogen specific to crustaceans. There was a relationship between virulence and activity in chitin hydrolysis among the tested strains. The extracellular products of the pathogen was toxic to shore crab, but not to carp. The virulence factor(s) determining the host specificity of this organism is another interesting subject.

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THE MICROBIAL ENVIRONMENT OF ROTIFER (*BRACHIONUS PLICATILIS*) AND *ARTEMIA* PRODUCTION SYSTEMS

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Abstract

In many cases, disease problems in larval rearing seem to be related to bacterial infections. As the live food is suspected to be a source of contamination, it was our aim to look into the microbial population in algae, rotifer and *Artemia* production systems. Samplings were organized in three marine fish hatcheries. Samples were taken at different steps in the production process of the live feeds.

Bacterial numbers were determined on marine agar, BTB, and TCBS media. They ranged from 10^3 to 10^7 in the live feeds, depending on the sampling site and sampling time. During hatching of the *Artemia* cysts, bacterial numbers increased a 10^3 to a 10^5 fold compared to the initial population before the breaking of the cysts. This bacterial population remained well established and could not be removed from the nauplii by rinsing with seawater and freshwater. During the nutritional enrichment of the rotifers and the *Artemia*, the number of bacteria did not increase.

Some 300 dominant colony types were isolated for further characterization. The isolates were compared to each other and to reference strains of *Listonella*, *Photobacterium*, *Vibrio* and *Alteromonas*, using fatty acid and API20E profiles. Fatty acid profiles of the isolates were grouped using principal component analysis. The isolates fell into at least ten major groups, of which two respectively correspond to the genera *Vibrio - Listonella* and to *Alteromonas*. Some groups seem to be restricted to one type of live feeds or to one isolation site. Although 43% of the isolates belong to *Vibrio - Listonella*, only a minority of them could be readily assigned to known species.

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DETERMINATION OF THE BACTERIAL CONTAMINATION IN LIVE FOOD PRODUCTION SYSTEMS IN MARINE FISH HATCHERIES IN SOUTHERN EUROPE

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Introduction

Due to the rapid expansion of fish farming, the increasing production of larvae leads to the intensification of production techniques. As a consequence more disease problems arise in larval rearing. Most of them seem to be related to bacterial infections. As often the live food is suspected to be a source of contamination (Tanasomwang and Muroga, 1990), a sampling campaign was organized in three different marine hatcheries in southern Europe. The aim was to study the microbiological environment quantitatively in the live food production units of the hatcheries and to compare the levels of contamination in various hatcheries, using different live food production techniques.

Materials and methods

Samples of *Artemia* and rotifers were taken at different stages of culturing and kept refrigerated for maximum 2h before freezing. The *Artemia*/rotifer samples were separated from the tank water by filtering through a sterile nylon filter. The samples were quickly frozen (CO_2/methanol or liquid nitrogen), using glycerol as a cryoprotectant, and transported to the laboratory in dry ice or liquid nitrogen. In the laboratory, the tank water samples were plated directly and also in a 1:500 dilution onto the media. Excessive seawater was removed from the *Artemia*/rotifer samples, a 1% suspension in seawater was made and shaken for 10min at 28°C. The organisms were removed from the wash water by filtration through a sterile nylon filter. The filtrate was plated directly in 1:500 dilutions onto the media. The samples were plated on Marine agar (MA, Difco), BTB and TCBS (Oxoid) medium using a spiral plater.

Hatcheries A and B both use a flow-through water circulation system, while hatchery C applies a recirculation water system.

Results

The results on *Artemia* hatching and enrichment are given in Tables I and II. The hatching started with low bacterial numbers on all media; decapsulated or disinfected cysts contained a maximum of 10^3 bacteria/g. The microbiological quality of the seawater in all hatcheries was satisfactory ($<10^2$-10^3.ml^{-1} on MA). During the hatching process (*i.e.* about 14h after incubation) an important bacterial flora developed, and remained more or less constant throughout the rinsing and enrichment steps. The total count in the hatching water after 24h hatching (t_{24}) ranged from 10^6 to 10^8.ml^{-1} on MA and 10^4 to 10^6.ml^{-1} on BTB and TCBS. From hatched as well as from enriched nauplii similar numbers were recovered, *i.e.* 10^6 to 10^7.g^{-1} on MA, 10^5 to 10^7.g^{-1} on BTB and 10^4 to 10^5.g^{-1} on TCBS, by Tanasomwang and Muroga (1990).

Rinsing had no effect on removing this flora from the nauplii. Rinsing had only a diluting effect on the water surrounding the nauplii as numbers on BTB decreased a 10^4- and 10^5-fold on TCBS just after rinsing. In hatchery A, the overall number of bacteria recovered was 10 to 100 times higher when unfiltered, instead of UV-treated seawater was used.

The results on the culturing of rotifers are presented in Table III. The numbers of Vibrionaceae recovered from the algal cultures were generally low ($<10^2$-10^3.ml^{-1}), whereas total counts on MA ranged between 10^4 and 10^6.ml^{-1}.

The bacterial numbers in semi-continuous rotifer culture tanks were very stable, regardless of the rotifers' age and density in the tanks (10^4.ml^{-1} on MA, 10^2.ml^{-1} on BTB, and 10-10^2.ml^{-1} on TCBS). In the batch-production culture tanks (2m^3), the overall contamination was 10 times higher as compared to the semi-continuous cultures. The bacterial numbers isolated from the rotifers were in the same range for both culture techniques: 10^7-10^8.g^{-1} rotifers (wet weight) on MA, 10^5.g^{-1} on BTB and 10^4.g^{-1} on TCBS. Rinsing did not seem to have an effect on bacterial numbers.

Conclusions

The bacterial contamination levels found in this broad survey are similar to those found in the literature. In *Artemia* production, the bacterial blooms are associated with the breaking of the cysts during hatching. The flora developed remains rather constant during the following steps. Rinsing has no significant effect on removing this flora. Batch culturing and semi-continuous rotifer culturing result in comparable bacterial loads on the rotifers.

In order to evaluate the importance of the contaminating microflora in the live food production process it is necessary to proceed to its identification. This is the subject of an ongoing parallel study (Verdonck *et al.*, 1991).

Table I. Microbiological contamination in *Artemia* hatching and enrichment. Number of CFU.ml^{-1} medium or .g^{-1} *Artemia* nauplii on MA, BTB, TCBS after 2 to 4 days incubation at 28°C. (t_x=moment at which samples are taken, x=hours after hatching)

Sample		CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Cysts*		$<10^2$-10^3	$<10^2$	$<10^2$-10^2
Hatching water at t_{24}				
	hatchery A**	10^5-10^7	10^5-10^7	10^4-10^7
	hatchery B	10^6	10^5	10^4
	hatchery C	10^8	10^5	10^5
Hatched nauplii in A, B, C		10^6-10^7	10^4-10^6	10^4-10^5
Enrichment tank water t_{20}-t_{24}:	hatchery A**	10^4-10^7	10^3-10^6	$<10^2$-10^6
	hatchery B	10^7	10^6	10^4
	hatchery C	10^8	10^6	10^4
Enriched nauplii in A, B, C		10^6-10^7	10^5-10^7	10^4-10^5

* Desinfected or decapsulated cysts.

** The *Artemia* section of hatchery A was sampled at two different periods. The numbers vary due to the difference in water quality (UV *versus* unfiltered seawater).

Table II. The microbiological contamination during the hatching process at different time intervals (t_x; x=hours after hatching) in hatchery A and the effect of rinsing on the flora present

Sample	CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Hatching water t_0	10^3	10^2	$<10^2$
t^7	10^4	10^3	10^2
t_{20}	10^7	10^7	10^7
t_{24}	10^7	10^7	10^7
Hatched naupli			
before rinsing	10^7	10^6	10^5
after rinsing	10^6	10^5	10^5
Water surrounding rinsed nauplii	10^5	10^3	$<10^2$

Table III. Microbiological contamination in rotifer culture. Number of CFU.ml^{-1} medium or .g^{-1} rotifers (wet weight) on MA, BTB TCBS after 2 days incubation at 28°C. Comparison between semi-continuous and batch culturing

Sample	Semi-continuous culture		
	CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Rotifer culture water			
1-day-old (1 600l)	$4x10^4$	$2x10^2$	20
5-day-old (2 500l)	$4x10^4$	$4x10^2$	40
Rotifers			
before rinsing	$2x10^7$	$2x10^5$	$2x10^4$
5' freshwater rinsing	$5x10^6$	$2x10^5$	
seawater rinsing	$2x10^7$	$1x10^6$	$2x10^5$
	Batch-culture		
Master-culture water	10^5-10^7	ND	$<10^2$
10 l culture water	10^5	10^3-10^4	10^2-10^3
2m^3 culture water	10^4-10^5	10^3	10^2
Rotifers			
before rinsing	10^8	10^5	10^4
after rinsing	10^8	10^6	10^5

ND: Not determined

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MICROFLORA AND ANTIBACTERIAL TREATMENTS OF ROTIFERS AND *ARTEMIA*

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Introduction

Cultured rotifers (*Brachionus plicatilis*) and *Artemia* carry a large bacterial load that may be transferred from live preys into the tanks of fish larvae. Bacterial concentrations increase during enrichment of live preys. Some bacteria species have been reported to be the source of infections and high mortalities in fish larvae, and live preys are thought to be responsible (Muroga *et al.*, 1987; Nicolas *et al.*, 1989). Therefore, rotifers and *Artemia* are often treated in order to diminish the bacteria associated prior to feeding them to the larvae. The present study considers from a practical point of view the bacterial load of rotifers and *Artemia* and the use of antibacterial treatments in order to reduce the bacterial contamination of live preys.

Materials and methods

Experiment 1: baker's yeast fed rotifers were collected from mass production tanks (23ppt salinity). Then subsamples were placed into freshwater for 1, 2 and 5min. Rotifers were collected, passed into seawater and mortalities were recorded 20min later. The bacterial load was recorded after inoculation and cultivation (24h at 25°C) of rinsed and crushed rotifers on TSA and TCBS medium.

Experiment 2: rotifers were taken and enriched (6h) with *Tetraselmis* and Protein Selco (Artemia Systems SA, Gent, Belgium). After enrichment rotifers were divided into two groups. One received a treatment (30min) of 10ppm Furazolidone while the other group was placed into freshwater for 2min. The bacterial load of rotifers was recorded as previously explained.

Experiment 3: *Artemia* nauplii were hatched at 27°C from decapsulated eggs and enriched for 24 and 48h on Protein-Selco (Artemia Systems SA). Seawater for nauplii hatching was previously disinfected by hypochlorite treatment. Nauplii and metanauplii of *Artemia* were divided into two groups. One group received a treatment of 10ppm oxolinic acid for

1h while the other one was placed into freshwater for 30min. The bacterial load of *Artemia* was recorded as previously explained.

Experiments 4 and 5: the procedure was the same as in experiment 3. The seawater hatching was filtered (1µm) and UV treated, but not disinfected. In experiment 5, 10ppm Furazolidone was used instead of oxolinic acid and the bacterial load recorded.

Results and discussion

Freshwater baths have been used in this paper as a bacterial reducing treatment for both rotifers and *Artemia*. Experiment I was carried out in order to establish the rotifers' resistance to freshwater (Table I). A 2min bath proved to be an adequate treatment; mortality was low and the bacteria (total and *Vibrio* spp.) were reduced by more than 50%.

Table I. Effect of freshwater baths on the numbers of bacteria (CFU: colony forming units) per rotifer and mortality of rotifers (n=2)

Freshwater bath	TCBS (CFU±SD)	TSA (CFU±SD)	Mortality (%)
0min	1.8±0.9	298±14	0
1min	1.2±0.7	134±4	3
2min	0.9±0.5	123±5	12
5min	0.6±0.5	70±6	25

In Table II the results obtained in experiment 2 are given. The bacterial load during enrichment rapidly increased, particularly *of Vibrio* spp. Bacterial numbers from Protein Selco enriched rotifers were higher than those of microalgae-enriched rotifers. The effect of antibacterial treatments seemed to be more drastic with Furazolidone-treated rotifers, but the bacterial reduction in freshwater-treated rotifers was also evident. The effect of both treatments appeared to be different depending on the enrichment used. Qualitative changes in the bacterial flora associated with rotifers might be responsible.

Table II. Effect of enrichment and antibacterial treatments on the numbers of bacteria (CFU) per rotifer

	Tetraselmis		Protein Selco	
	TSA	TCBS	TSA	TCBS
Before enrichment	116	14	116	14
After enrichment (6h)	160	66	190	90
- after freshwater bath	80	50	150	54
- after Furazolidone bath	56	13	78	45

Bacterial counts in *Artemia* increased rapidly during enrichment (Table III). Sterilization of seawater for hatching (Experiment 3) yielded a significant predominance of *Vibrio* spp.

in metanauplii. The bacterial loads per metanauplii in experiments 4 and 5 (untreated seawater) were significantly higher than in experiment 3 (treated seawater), but the relative contribution of *Vibrio* spp. was lower. We have no explanation for the high value found in metanauplii (24h) in experiment 5. Contamination of the sample by the enricher might be responsible. However, qualitative changes in the bacterial flora associated with *Artemia* might also be the cause. With respect to antibacterial treatments, the results showed the effectiveness of freshwater in the reduction of bacterial loads in *Artemia*.

Table III. Numbers of bacteria (thousands of CFU) per *Artemia* nauplii and metanauplii on TSA medium (values obtained on TCBS medium are given between brackets)

Treatment	Nauplii	Metanauplii (24h)	Metanauplii (48h)
Treated seawater (Exp.3)			
Initial	4.1 (1.3)	10.5 (9.5)	12.2 (9.5)
After oxolinic bath	1.8 (1.1)	9.3 (8.4)	9.8 (8.1)
After freshwater bath	0.3 (0.2)	2.4 (1.2)	6.1 (3.7)
Untreated seawater (Exp.4)			
Initial	2.6 (0.9)	12.5 (3.2)	36.6 (3.7)
After oxolinic bath	1.8 (0.5)	6.0 (0.8)	5.4 (1.6)
After freshwater bath	1.4 (0.4)	4.9 (0.6)	1.5 (0.7)
Untreated seawater (Exp.5)			
Initial	2.1 (1.6)	43.5 (6.8)	33.3 (14.7)
After Furazolidone bath	0.8 (0.4)	14.3 (2.4)	12.8 (1.5)
After freshwater bath	0.5 (0.2)	6.7 (1.4)	7.7 (0.7)

The resistance of rotifers and *Artemia* to freshwater was encouraging. The effect of this treatment was demostrated by the reduction of the bacterial counts measured on both TSA and TCBS media. In conclusion, it has been shown that freshwater baths are a useful tool to control the bacterial load in live preys.

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EVALUATION OF FOUR CHEMICALS FOR SURFACE-DISINFECTION OF MARINE FISH EGGS[1]

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Introduction

High mortality of marine fish eggs and larvae under intensive rearing is due to a wide range of causes, including high bacterial densities. In addition fish eggs are considered as a possible vector for transmission of diseases, because pathogens may be included in the microflora (Bergh *et al.*, 1990). In this study four chemicals were tested for their ability to reduce the bacterial load on the egg surface without, having toxic effects on the embryo.

Materials and methods

Four chemicals were tested: Buffodine (iodophor: 1.06% free iodine), buffered glutar-aldehyde, chloramine-T and sodium-hypochlorite (5% free chlorine). All solutions were prepared with sterile, filtered (0.2µm) seawater. Concentrations of Buffodine and sodium-hypochlorite refer to the amount of free iodine and chlorine added to seawater. Eggs of plaice (*Pleuronectes platessa* L.), at the stage were the embryo surrounds approximately 180° of the yolk mass, were washed with sterile, filtered seawater before and after treatment to remove organic materials and residual disinfectants. Disinfection was carried out at 5°C for 10min. Single eggs (N = 60) were incubated at 10°C in M-65 seawater agar (1% agar). The amount of eggs with bacterial growth was recorded after 7 days. Egg samples were kept in darkness at 5-6°C in sterile, filtered seawater to observe mortality and hatchability. At the end of the yolk sac period, 14 to 23-day-old larvae were placed in small volumes of concentrated seawater (N = 3) with a salinity of approximately 70.0ppt and the cumulative mortality was recorded over time. The results were analyzed using a chi-square (χ^2) test.

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Results

Disinfection with 400ppm Buffodine reduced the bacterial load to some extent (Fig.1), but concentrations of 100 to 200ppm free iodine had no effect. This was confirmed in other experiments. Contrarily, all groups treated with glutaraldehyde, chloramine-T and hypochlorite showed an approximately 90% reduction in the number of eggs with bacterial growth. Replicate experiments with a standard disinfection procedure revealed a clear correlation between the bactericidal effect and the initial bacterial load on the egg (data not shown).

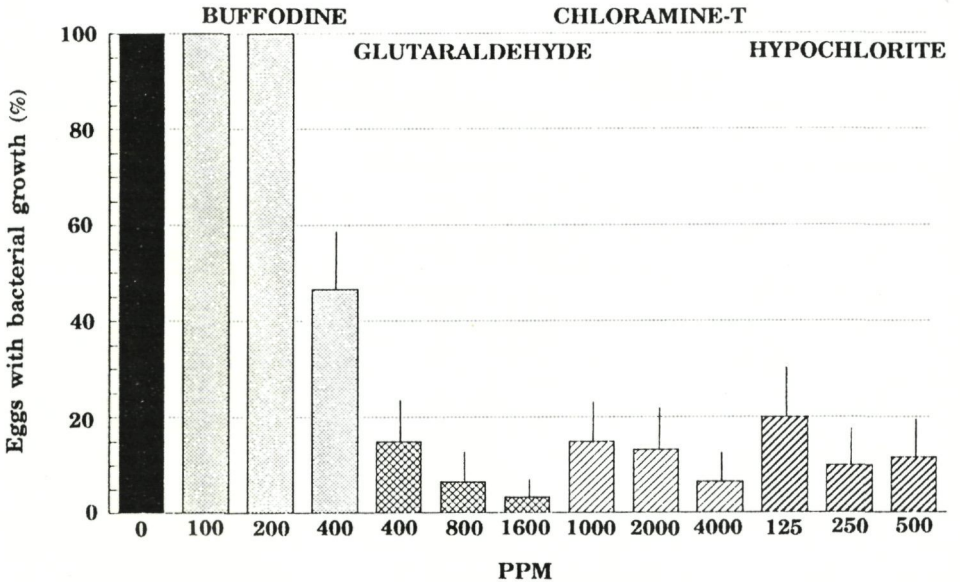

Fig. 1. Reduction in number of eggs with bacterial growth (± 95% confidence limits).

The dose of 400ppm Buffodine was highly toxic (data not shown) having a level exceeding 250ppm free iodine, which in other experiments caused 100% mortality. Treatment for 5 min treatment with 400ppm Buffodine did, however, not result in acute mortality. A significantly higher mortality ($P<0.01$) at hatching and a significant reduction in the number of eggs that hatched ($P<0.05$) was observed for groups treated with 200ppm free iodine. Eggs disinfected with concentrations of hypochlorite did not hatch and died after a week. Disinfection with doses of glutaraldehyde and chloramine-T did not have any negative effect on survival during incubation or on hatchability. This trend was confirmed for all chemicals in other experiments.

Larvae that hatched from eggs disinfected with concentrations ≥ 800ppm glutaraldehyde ($P<0.01$) and ≥ 2 000ppm chloramine-T ($P<0.001$) had a significantly higher mortality after 3h in concentrated seawater than the untreated groups (Fig. 2). Neither Buffodine nor the lowest doses of glutaraldehyde and chloramine-T influenced the tolerance of the larvae to salinity-stress. These larvae also survived for a longer period of time than the rest of the groups (≥ 54 *versus* 20-24h).

Conclusions

Doses of Buffodine that reduced the bacterial load were above the toxic level. The interval between toxic and non-toxic dose appears to be very narrow. A large number of surface-sterile eggs was observed for all doses of hypochlorite, but hatching was inhibited and all eggs eventually died. Doses of glutaraldehyde and chloramine-T had good bactericidal effects and did not affect survival of incubating eggs or hatchability. Some groups had a higher cumulative mortality at hatching or a lower hatchability, but no relation to doses was found. The lowest doses of these two disinfectants did not reduce the salt tolerance of the larvae.

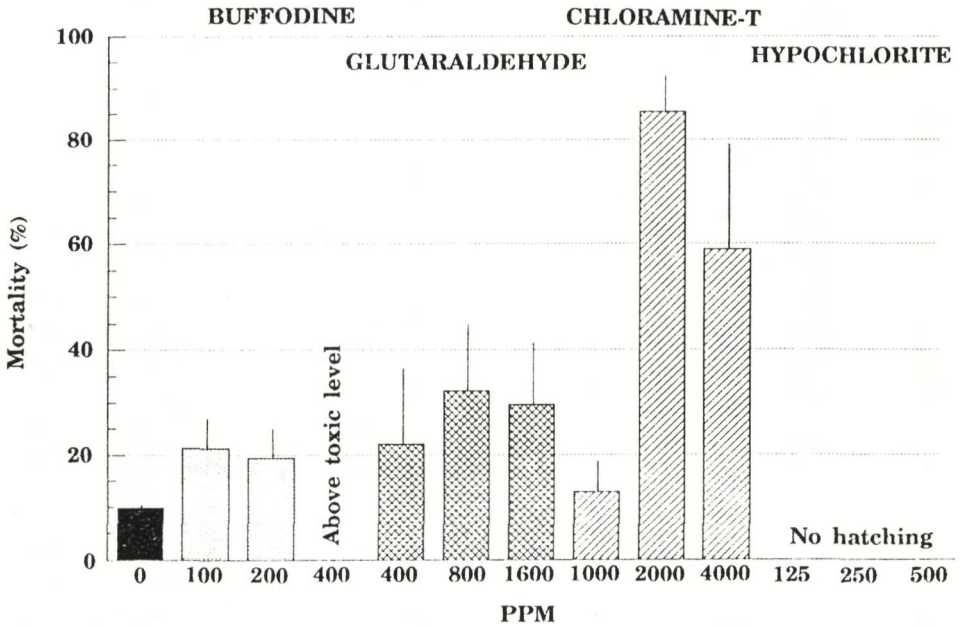

Fig. 2. Cumulative mortality of larvae (± standard error) after 3h in seawater of approximately 70ppt.

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BACILLUS SP. SPORES: A NEW TOOL AGAINST EARLY BACTERIAL INFECTION IN TURBOT LARVAE, *SCOPHTHALMUS MAXIMUS*

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Introduction

An opportunistic strain of *Vibrio* spp. was reported to cause mass mortality of turbot larvae, *Scophthalmus maximus*, during the period of feeding on rotifers (Gatesoupe, 1990). The treatment of rotifers with antibiotics or feeding them with probiotics (lactic bacteria and spores of *Bacillus toyoi*, Gatesoupe, 1989) was proposed. Although the feeding of probiotics did not improve the survival rate of turbot, their mean weight increased (Gatesoupe, 1990). In the present experiments, spores of *Bacillus* sp. were tested for probiotic efficacy.

Feeding rotifers with the spores

The rotifers were cultured according to the method described by Gatesoupe (1989). The food additive "Paciflor 9", containing spores of the *Bacillus* strain IP 5832 (Institut Pasteur), was added at the rate of 8 $mg.l^{-1}.d^{-1}$ to the experimental diet. The viable spores and bacilli were counted on Plate Count Agar with chloramphenicol and polymixin B, according to the method of Nguyen *et al.* (1988). About 4 000 colony forming units $(CFU).ml^{-1}.d^{-1}$ were fed to the experimental rotifers.

90% of the *Bacillus* were removed by filtering during the first hour, and digested during the second hour, following the first distribution of a quarter of the daily food amount (Fig. 1). After 5 days of feeding, bacteria were isolated from the rotifers as described in previous experiments (Gatesoupe, 1990). The proportion of the dominant *Vibrio* spp. was significantly decreased in the spore-fed rotifers, in comparison with the control group (13 *versus* 59% of the total bacteria counted in rotifers, respectively).

Effect on the larval rearing of turbot

In the course of three experiments, turbot larvae were reared in 150 l cylindrical tanks with conical bottom, according to the method already described by Gatesoupe (1990). The dietary value of spore-fed rotifers was compared to that of the control group (6, 9 and 6 replicates per treatment in experiments 1, 2 and 3, respectively).

At day 10 after hatching, the mean weight of turbot was significantly improved with the spore-fed rotifers (Table I). In experiment 1, the survival rate was good in all tanks, but the opportunistic strain of *Vibrio* spp. was encountered in the control groups during the two following experiments in the control groups. The survival rate improved with the spore-fed rotifers in experiments 2 and 3.

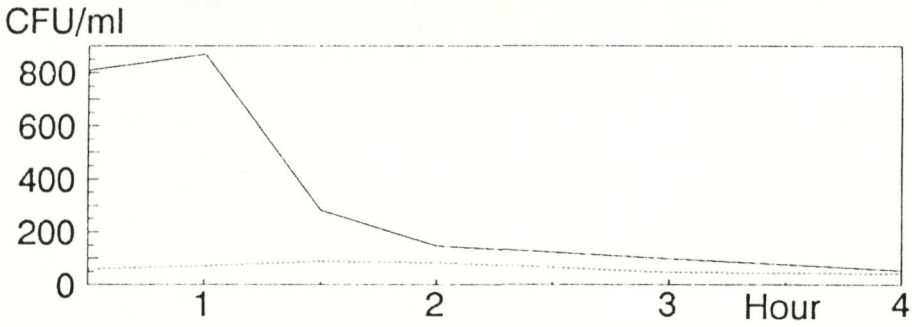

Fig. 1. Concentration of *Bacillus* sp. ingested by the rotifers (continuous line) or free in the medium (dotted line).

Table I. Effect of spore-fed rotifers on turbot at day 10 (superscripts [a] and [b] indicate the significant differences observed by analysis of variance)

Experiment	Mean weight (mg)		Survival rate (%)	
	Control	+ Spores	Control	+ Spores
1	0.43[b]	0.47[a]	55	48
2	0.57[b]	0.62a	4[b]	15[a]
3	0.42[b]	0.56[a]	29[b]	59[a]

Discussion and conclusions

Many species of *Bacillus* are known to produce antibiotics. A likely hypothesis is that such a substance inhibited the growth of both the dominant and the opportunistic strain of *Vibrio* spp. If so, the risk of selecting resistant strains may arise, indicating that the spores should be used with discernment. However, this treatment is more advisable than those in which antibiotics are dissolved in the medium at a concentration of 10ppm of pure chemical. The spore-containing additive was used at 8ppm, which corresponds to about 20 CFU/rotifer. This low level is sufficient due to the active filtration of spores by the rotifers, without affecting the medium by drugs.

Acknowledgements

Bacillus IP 5832 was put at our disposal by Prodeta (F-56038 Vannes, France).

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LIVE-FOOD MEDIATED DRUG DELIVERY AS A TOOL FOR DISEASE TREATMENT IN LARVICULTURE. II. A CASE STUDY WITH EUROPEAN SEABASS

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Introduction

The purpose of this study is to investigate the possibility of oral delivery of the antibiotics Trimethoprim (TMP) and Sulfamethoxazole (SMX) to marine fish larvae through incorporation of these antibiotics into the live food organism *Artemia*. SMX and TMP were selected for their wide range action against gram negative pathogenic bacteria (Muir and Roberts, 1988) and because of their limited solubility in seawater. Ormetoprim-Sulfadimethoxine (brand name Romet-30) is a new combination of a similar antimicrobial drug used in aquaculture (Brooks, 1989). Diseased penaeid larvae fed Romet-30 through the food chain exhibited greater survival than the untreated ones (Mohney *et al.*, 1990). In an earlier study Verpraet *et al.* (in press) have described a technique for bioencapsulating high doses of more than 500µg TMP+SMX per gram dry weight (DW) *Artemia* nauplii. In this study we have used 2-month-old European seabass (*Dicentrarchus labrax*) larvae that were fed with TMP+SMX-bioencapsulated *Artemia* nauplii.

Materials and methods

Artemia was enriched with the lipid emulsion Selco (Artemia Systems SA, Gent, Belgium) into which a 40% mixture of TMP-SMX was incorporated at a ratio of 1 to 5. *Artemia* enrichment was done following the procedure outlined by Léger *et al.* (1987). Seabass larvae were kept at three fish/l in aerated seawater at 21°C. Fish larvae were fed once with antibiotic-enriched *Artemia* nauplii offered at a density of 15 nauplii/ml. Fish sampling proceeded at t_1, t_1+1h, t_1+2h, t_1+3h, t_1+21h and t_1+43h, with t_1 corresponding to 1h after feeding.
A liquid chromatographic method was applied for the quantification of TMP and SMX in *Artemia* (Nelis *et al.*, submitt.). The determination and quantification of fish tissue antibiotics was done using a slightly modified method.

Results

Total content of antibiotics in *Artemia* amounted to 876.28µg.g^{-1}DW, *i.e.* 281.01µg TMP and 595.27µg SMX. The ratio TMP:SMX was 1:2.13, compared to a ratio of 1:2.73 as reported in Verpraet *et al.* (in press).

Table I. Accumulation of TMP and SMX in *Dicentrarchus labrax* larvae fed once with 40% antibiotics-enriched *Artemia* at a density of 15 nauplii/ml (concentrations are in µg.g^{-1}DW)

Time[1] (h)	TMP	SMX	N-ac-SMX*	Ratio TMP:SMX
1	10.41	10.44	11.69	0.99
2	8.05	4.19	8.63	1.92
3	9.69	12.95	20.20	0.75
4	8.64	6.48	9.61	1.33
22	4.47	1.08	0.56	4.14
44	2.74	0.00	0.00	-

[1] Time since feeding.
* N-ac-SMX= N-acetyl-sulfamethoxazole.

Table I shows that high levels of TMP and SMX were reached in fish. A maximal concentration of 22.64µg antibiotics/g DW fish tissue *i.e.* 9.69µg TMP and 12.95µg SMX, was reached 3h after feeding (Fig. 1). The ratio TMP:SMX increased with time from a value of 0.99 1h after feeding to a value of 4.14 24h after feeding. Already 1h after feeding N-acetyl-sulfamethoxazole, a metabolite of SMX, is found in high concentrations.

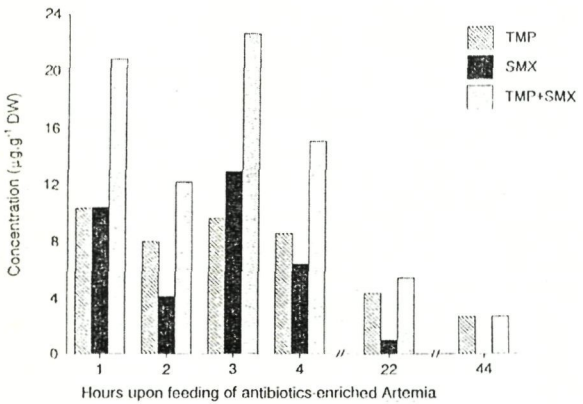

Fig. 1. Accumulation rate (in µg.g^{-1} DW) of Trimethoprim (TMP) and Sulfamethoxazole (SMX) in larval tissue of *Dicentrarchus labrax* fed one ration of antibiotics-enriched *Artemia* nauplii.

Discussion

Even though high theraupetical levels of the antibiotics TMP and SMX were reached in the fish tissue, the fish seem to be cleared less rapidly from TMP than from SMX, and hence the half life of the latter is shorter than the first. In fact the TMP:SMX ratio in *Artemia* becomes 1:1 (0.99) in fish 1h after feeding, and increases to 1:0.24 (4.14) 22h after feeding. No more SMX is found in fish tissue 44h after feeding, when the content of the antibiotics in the fish larvae is constituted solely of TMP. The early presence of N-ac-SMX in high concentrations is expected in the early life stages of fish when metabolism is highest (Johnson, 1989).

The frequency of distribution of medicated *Artemia* and/or the content of antibiotics in the *Artemia* could be modified in order to reach a targeted therapeutical concentration of antibiotics in the fish tissue. Moreover, the ratio of the two drugs in the enrichment diet could also be adapted taking into account the kinetics of the two drugs TMP and SMX in the fish tissue.

Acknowledgement

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OPTIMIZING BIOENCAPSULATION OF THE ANTIBIOTICS TRIMETHOPRIM AND SULFAMETHOXAZOLE IN *ARTEMIA* NAUPLII

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Introduction

Live *Artemia* nauplii are a primary food source to rear larvae of most cultured aquatic animal species (Sorgeloos, 1980), while they can act as potent carriers of essential nutrients which can then be transferred to the consumer organisms (Léger *et al.*, 1987). One of the major problems in fish larviculture is the development of microbial diseases (Trust, 1986), which implies serious financial losses. The treatment of these diseases by means of the currently applied methods is rather difficult, not particularly effective, costly, and might involve environmental hazards. A possible method to confront this problem might be the oral administration of antibiotics to the fish larvae through the food chain, using the bioencapsulation technique as described by Verpraet *et al.* (in press). We have verified the applicability of the latter method and report in this paper how the bioencapsulation conditions can be optimized. The aim of this study is to determine the optimal conditions for bioencapsulation of antibiotics in *Artemia* nauplii

Materials and methods

The two antibiotics used were Trimethoprim (TMP) and Sulfamethoxazole (SMX). *Artemia* cysts of the GSL strain (Great Salt Lake, USA) obtained from the *Artemia* Reference Center, Belgium, were hatched in filtered artificial seawater 35ppt, at 28±1°C, under continuous aeration and illumination (2 000lux) for 24h, when instar I nauplii were harvested. Then the enrichment medium (Selco, Artemia Systems SA, Gent, Belgium) : emulsion containing 10% (w/v oil phase) of the antibiotics TMP and SMX in a ratio of 1:5 was administered to the nauplii at t_0 and t_0+6h (t_0= onset of enrichment period) at a concentration of 0.3, 0.6, and 1.2g Selco/l incubation medium and the nauplii were harvested at t_0+8, 16, 24 or 32h. Nauplii enriched for 24h were transferred to new seawater without any enrichment medium and after 4, 8, 16, 32 or 48h; both the nauplii and the incubation medium were collected. The two antibiotics were extracted from the samples according to the methods described by Nelis *et al.* (submitt.), which included homogenization in methanol, hexane extraction and elution from a C18 column. Then the samples were injected on a Hypersil ODS C18 column, the antibiotics were eluted

with acetonitrile-0.15M phosphate buffer (15:85 v/v) containing 0.12% triethylamine at pH 4.85, and a flow rate of 1ml.min^{-1}. The analysis was performed on an HPLC chromatograph, consisting of a Gilson M305 isocratic pump, a Rheodyne 7125 valve injector with a 100µl loop, a Gilson 112 UV/Vis detector set at 254nm and a Varian 4290 integrator.

Results

An increase of the antibiotic contents of the nauplii was observed when either the concentration of the enrichment medium (Fig. 1) or the duration of the enrichment period (Fig. 2) increased. The ratio TMP:SMX which was 1:5 in the enrichment emulsion increased in the nauplii when the concentration of the enrichment medium increased, but decreased when the enrichment period lasted longer. After the transfer of the enriched nauplii to new seawater without any enrichment medium, the concentration of the two antibiotics decreased in the nauplii (Fig. 3A) and increased in the incubation medium (Fig. 3B). The ratio TMP:SMX in the nauplii decreased during the first 8h of the experiment and then increased reaching a value of 1:2.2 after 48h (Fig. 3A).

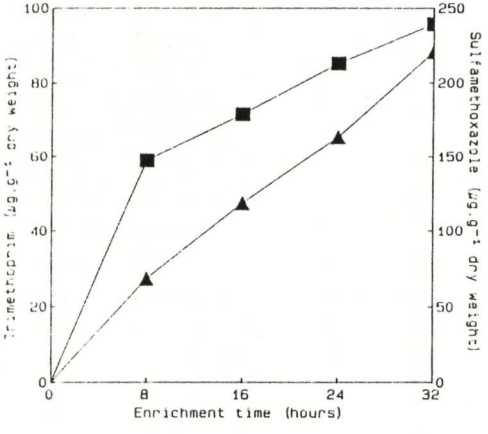

Fig. 1. Effect of the concentration of the enrichment medium on TMP (■) and SMX (▲) concentration in the nauplii.

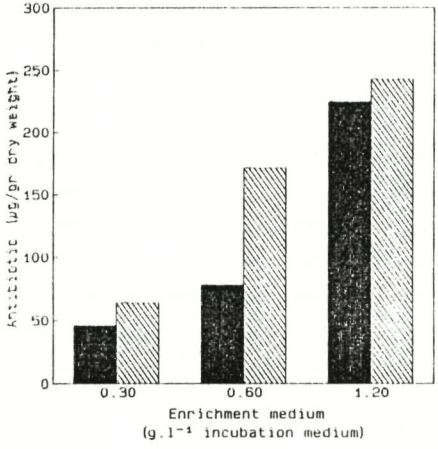

Fig. 2. Effect of the duration of the enrichment period (application of two doses of 0.3g.l^{-1}) upon the antibiotic TMP (■) and SMX (□) content of the nauplii.

Discussion

The results presented in this study show that TMP can be more easily bioencapsulated in the nauplii than SMX, which is in agreement with the results of Verpraet *et al.* (in press) and that SMX accumulates at a slower rate than TMP. Both the antibiotics are discharged from the nauplii in their incubation medium and the discharge is more obvious 8h after the end of the enrichment period. The fact that the amount of the antibiotics in the incubation medium does not compensate for the decrease of their concentration in the nauplii, together with the existence of peaks of unknown identity in the chromatograms

of the discharge experiments only, suggest that a metabolic alteration of the therapeutics by the nauplii might be possible. *Artemia* nauplii can act as potent carriers of the therapeutics TMP and SMX for use in fish larviculture. The time allowed to the fish larvae to consume the enriched nauplii should be less than 8h. Research is in progress to determine the efficiency of the proposed method and its possible effects on other parameters involved in the development of the cultured organism.

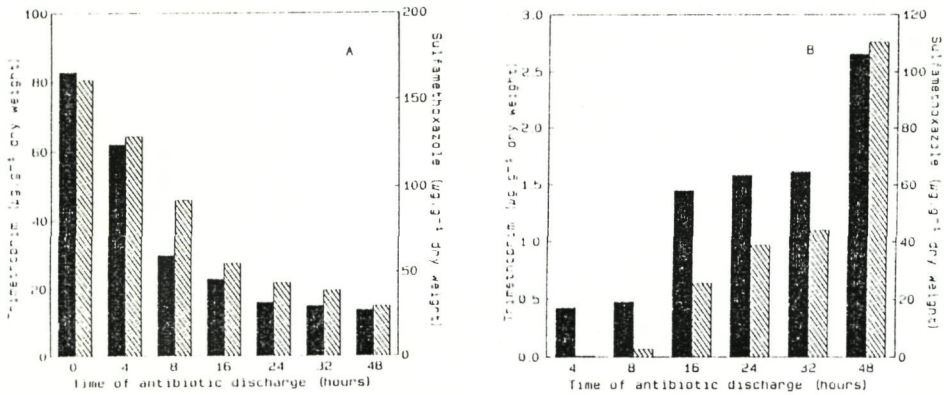

Fig. 3. Alteration in the concentration of the antibiotics TMP (■) and SMX (□) in the nauplii (A) and the medium (B) (vacuum dried to express the results per mg DW medium).

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Acknowledgements

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ANTIBIOTIC RESISTANCE OF BACTERIAL FISH PATHOGENS

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Abstract

Bacterial infections are responsible for heavy losses in intensive fish culture, ranging from the farm level to the hobbyist tank. Over the years, nearly two dozen bacterial species have been isolated and identified from infected fish. Some of these, such as *Clostridium* and *Eubacterium*, are considered rare and their significance as pathogens is not well understood. Other bacteria are associated with certain groups of fish, such as *Renibacterium* that produce chronic kidney disease in salmonids and *Edwardsiella* that produce enteric septicemia in channel catfish. Still others, such as *Aeromonas* and *Vibrio* are more ubiquitous in their range. *Aeromonas hydrophila*, for example, has been identified from a wide variety of freshwater fish including wild gamefish, foodfish, baitfish and ornamental petfish.

Generally, it is considered that bacterial infections in fish are secondary problems relating to the stresses of intensive culture. Stresses such as temperature change, handling, breeding, poor water quality, parasite load, shipping, and even chemotherapeutic treatment, are correlated with bacterial outbreaks. Stress is known to interfere with the immune response, resulting in decreased efficacy in both the humoral and cellular responses and phagocytosis. Attempts to minimize stress have been coupled with use, and often abuse, of available antibacterial chemotherapy. Antibiotics used in both human and various areas of veterinary medicine, have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery, and governmental restrictions have limited the available antibiotics to a select few, especially in foodfish culture.

Increasingly, antibiotic resistance of bacterial fish pathogens is reported from all areas of aquaculture, ranging from warmwater to coldwater, and marine to freshwater. Decreased efficacy has been documented in antibiotics regardless of their mechanism of action. For example, resistance has been evidenced in different classes of antibiotics including those affecting protein synthesis such as tetracycline and erythromycin, aminoglycosides like neomycin, antimetabolites such as the sulfa drugs and potentiated sulfonamides, and quinolones like oxolinic acid. In this paper current data on the antibiotic resistance problem, and the development of new alternatives are reviewed.

AUTHOR INDEX

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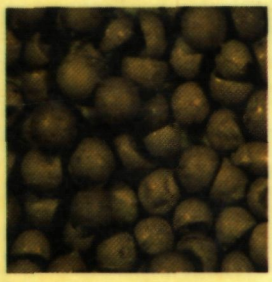

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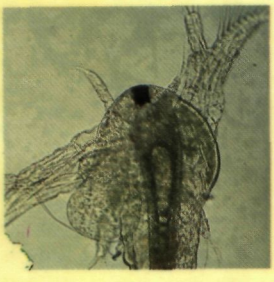

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