International Council for the
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REPORT OF THE WORKING GROUP ON MASS REARING OF JUVENILE MARINE FISH TO THE MARICULTURE COMMITTEE OF ICES

Gent, Belgium 31 August - 2 September, 1991.

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1. PARTICIPATION

The Working Group convened its fourth meeting at Gent, Belgium on August 31 - 2 September, 1991. Members attending the meeting were:

Belgium: P. Sorgeloos. Denmark: J. G. Støttrup. Canada: J.A. Brown, G. Goff, K. Waiwood. Faroe Islands: I. Fjallstein. France: B. Chatain. Germany: G. Quantz. Norway: D. Danielssen, I. Holmefjord, I. Huse, E. Kjørsvik, I. Lein, A. Mangor-Jensen, K. Naas, Y. Olsen, G. Rosenlund. Portugal: P. Pausao-Ferreria. Spain: J. Iglesias, I. Martinez, G. Minkoff, J.B. Peleteiro. Sweden: P.-O. Larsson. UK: B.R. Howell.

During 1990, several members attended by invitation the World Aquaculture Society annual meeting in Halifax, Canada, where a Larviculture Task Force within the WAS

chaired by Patrick Sorgeloos (Belgium) and David Bengtson (USA) was formed. Several WAS/LTF members also attended part of this meeting. Thus, apart from the ICES members, the following were present:

Belgium: T. Bosteels, Ph. Dhert, A. Komis, P. Lavens, Ph. Léger, I. Roelants, G. Van Stappen, L. Verdonck, F. Volckaert. Denmark: N.E. Poulsen. France: S. Kaushik. Germany: H. Segner. Greece: G.M. Robbins, E. Sweetman, J. Sweetman. Israel: Y. Barr, L. Samuel, A. Tandler, O. Zmora. Japan: K. Muroga, M. Tanaka. Mexico: A. Abreu Gobrois. Netherlands: J. Verreth. Norway: G. Adoff, A. Folkvord. Scotland: J. Dye, J.R. Sargent. Singapore: T.J. Lam, C.L. Lim, J. Walford. S.Africa: T. Hecht. Sweden: J. Pickova. Taiwan: H.-Y. Chien. Thailand: D. Fegan, P. Menasveta, S. Piyatiratitivorakul, J.-F. Rees. USA: H. Ako, D. Bengtson, J.G. Holt, S. Kraul, J.A. Tellock.

See Appendix I for addresses.

I. Huse, Norway, (chairman) and J.G. Støttrup, Denmark, kindly served as rapporteurs for the meeting.

2. TERMS OF REFERENCE

The ICES Working Group on Mass Rearing of Juvenile Marine Fish met to work according to the following terms of reference (ICES C.M. 1990/F:65):

a) prepare a report describing standardized procedures and conditions for experimental fry production of turbot and sea bass as model species, including criteria in addition to growth and survival, for the evaluation of the quality of eggs, larvae and juveniles;

b) describe nutritional requirements of marine fish, primarily for fatty acids and amino acids and collect information on the function of individual compounds in the organisms;

c) advice on alternative strategies to the use of antibiotics in the control of microflora in culture systems.

3. AGENDA

During the meeting, three plenary sessions and one concurrent group session were held with the following subjects covered:

A) Egg, larval and juvenile quality (E. Kjørsvik, Norway)

B) Nutrition (P. Sorgeloos, Belgium and J. Verreth, Holland)
J. Sargent: Lipid metabolism
S. Kaushik: Protein metabolism

C) Standardized procedures and conditions for experimental fry production of model species. (B. Chatain, France and J. G. Støttrup, Denmark)

D) Hygiene strategies (G. Minkoff, Spain)

In the first two plenary sessions each topic was introduced by 1-2 short presentations, followed by discussion. The nutrition session also included two invited speakers on the main topics; lipid and protein metabolism.

Each subject was discussed in more detail in smaller groups during the concurrent sessions.

The recommendations were discussed and revised during the final plenary session.

4. EGG, LARVAL AND JUVENILE QUALITY

Convener: Elin Kjørsvik
Rapporteurs: Bari Howell, Kenneth Waiwood

Considerable variability in the performance of eggs and larvae is observed during the mass rearing of marine fish. Much of this variability may be attributable to egg quality defined as *the potential of the egg at he completion of fertilization to produce viable fry*. It will consequently embrace any effects associated with the quality of sperm, a subject that has been studied little in marine fish but one that can not be ignored.

There is an urgent need among practitioners to identify characteristics of eggs and larvae that reflect their quality as well as to improve our understanding of the mechanisms involved in generating the observed variability. The Working Group consequently recognized two important aspects for future work:

I The requirement for making simple, rapid predictions of subsequent performance of eggs and larvae.

II The development of strategies for the investigation of factors and mechanisms that influence egg quality.

I. IDENTIFICATION OF QUALITY CRITERIA

Ia Eggs

The following parameters have been or may prove to be useful indicators of egg quality in several marine species:

- fertilization rate
- hatching rate
- buoyancy

- cleavage pattern (morphology)
- mortality rates (at different stages)
- relative developmental rates
- turgor pressure (egg hardness)
- chorion appearance / fertilization process
- oil globule distribution
- opacity
- 'stress' tests (eg. mechanical shock)

Of these, it was considered that fertilization and hatching rates, buoyancy and cleavage patterns offered the greatest potential for routine assessment and that these measurements should be included in all assessments of quality. Nevertheless, it was recognized that, because egg quality may vary in response to a variety of factors, there may not be a universal indicator of egg quality and that different parameters may need to be used according to species and procedures used.

Ib Larvae

It is important that larvae quality is assessed at a defined developmental stage. the onset of exogenous feeding would be an appropriate stage and is the last stage at which larval quality can realistically be attributed to maternal influence. This should not, however, preclude eventual studies of effects on later developmental stages.

The following parameters were identified as being worthy of further evaluation:

- deformities
- pigmentation
- survival
- growth
- synchrony in development
- development of stages with time
- yolk absorption rate
- disease resistance
- 'stress tests' (eg. salinity, air exposure)
- 'performance tests' (eg. behavior, response to stimuli)

II DEVELOPMENT OF RESEARCH STRATEGIES

IIa Factors affecting egg quality

The following broodstock-related factors were identified as potential determinants of egg quality:

- time within the spawning season (batch spawners)
- fish age
- nutrition
- stripping/handling/fertilization
- over-ripening

- induced ovulation /spawning (hormones)
- environmental conditions (eg. temperature, salinity, light)
- water quality (eg. O_2, NH_3)

IIb Mechanisms and manifestations of egg quality

The investigation of the following factors may provide an insight into the mechanisms involved in the determination of egg quality:

- energy charge (ATP/ADP)
- time dependent changes in, for example, hormones (eg. thyroxine), enzymes and nutrients
- cell cycles (eg. regulation of cell cleavage)
- chromosome aberrations/karyotyping of chromosomes

It was stressed that the value of 'snap-shot' analyses were often limited and that more effort should be devoted to studies of dynamic processes during development.

IIc Research priorities

The following priority areas for future research were identified:

1. Standardization of methods and criteria.
   An effort should be made to link the empirical routine criteria to the results of more advanced scientific investigations in order to more fully understand the underlying mechanisms involved in the determination of egg quality.

2. Development of methods for determining the timing of ovulation and the post-ovulatory age of eggs. Such methods may be related to:
   - rates of drinking / salt excretion
   - ultrasonic observation
   - ovulatory cycles
   - refinement of methods for natural spawning

   These studies would be facilitated by an understanding of the hormonal control mechanisms which could even lead to the development of 'fish-ovulation test kits'.

3. Sperm quality assessment which should also include effects of storage methods.

RECOMMENDATIONS

The following recommendations were proposed and accepted by the Working Group:

1. A standardized protocol for assessment of egg and larval quality should be devised.

2. The design and distribution of a proforma data sheet to record egg production methods, the characteristics of eggs at fertilization and their subsequent performance.

3. Research should be encouraged in the following areas:

   a) broodstock management procedures
   b) reproduction mechanisms of the fish
   c) ovulation/over-ripening mechanisms
   d) time dependent compositional changes during the egg and larval stages.

5. NUTRITION

Convener: Patrick Sorgeloos & Johan Verreth
Rapporterus: Patrick Sorgeloos & Dave Bengtson

There is an urgent need to develop an appropriate protocol for nutritional experiments in fish larviculture. In that respect the following proposal was worked out:

a1) A standard live diet should be used consisting of:

- ICES-reference Artemia (small nauplii with high content of n-3 HUFAs) as starter feed (to be selected and made available by the Artemia Reference Center, Belgium), followed by

- Great Salt Lake (Utah, USA) Artemia enriched by a standard protocol using:
  - ICES-standard emulsion as external standard
  - local enrichment procedure as internal standard

a2) Regarding the green-water technique and Brachionus culturing, each laboratory should use the procedure they feel appropriate for the species cultured. Applied procedures should be well documented

a3) A protocol should be developed so that nutritional tests can be performed under standardized, optimal conditions, including at least:
- removal of all remaining food at least once a day
- identification of feeding levels

a4) Experimental conditions need to be optimize in order to realize maximum growth for use as reference conditions in later dose-response studies

a5) Experimental results should be evaluated in relation to the maximum growth potential and in relation to a low growth under known diet-deficient conditions

a6) The following parameters should be considered for evaluating the experimental results:
- survival
- size of the fish, e.g. length, dry weight and any other mass-related parameter
- stress resistance
- size variation
- time and size until certain developmental stage, such as gastric pH change, metamorphosis, pigmentation, organ development
- RNA/DNA ratio

a7) The need is expressed that a literature search shoul be conducted on the technology, formulation and use of standard reference inert diets for larvae. Dr. J. Walford has accepted to take up this responsibility.

b1) The procedure for quantitative (n-3) HUFA analysis as prepared by Ph. Léger has been improved since the Palavas WG-meeting, will be further amended to exclude the use of carcinogens (benzene) and will be submitted for final evaluation to J.Sargent. The WG recommends that this method should be proposed for adoption as a standard ICES-procedure for (n-3) HUFA analysis.

b2) Regarding the (n-3) HUFA requirement study proposed a the WG meeting in Vigo (1989) experimental results are available for sea bass, sea bream, turbot, striped bass, red drum, and summer flounder. it appears that best results are obtained with the medium (n-3) HUFA emulsion. sometimes better results were obtained with high (n-3) HUFA emulsion, although analytical data show that enrichment levels obtained with medium may equal those obtained with high, which indicated that enrichment success might have been different. it is therefore essential the along with the biological tests, analysis for the enriched prey is taken into consideration. The WG recommends the the experimental results should be compiled and proposed in a final report (draft as Appendix II).

b3) Since it appears from the previous study that much variability exists in (n-3) HUFA enrichment success with Brachionus and Artemis, even when using the same enrichment emulsions, and the present standard procedures, it is important to estimate present variability in (n-3) Hufa enrichment procedures. In this respect and in order to be able to advice optimal and standard enrichment procedures, an enrichment intercalibration exercise should be performed using an ICES-reference emulsion (made available by the Artemia Reference Center, Belgium) and Great Salt Lake (Utah, USA) Artemia. Protocols for experimental and analytical procedures will be prepared by J. Sargent and P. Sorgeloos.

c) A bibliography on nutrition and histology has been compiled by D.A. Bengtson.

RECOMMENDATIONS

The following recommendations were proposed and accepted by the Working Group:

1. A protocol should be developed so that nutritional tests can be performed under standardized, optimal conditions.

2. It is recommended that a reference diet should be included in nutritional studies and this should consist of:

   - ICES-reference Artemia as starter feed (to be selected and made available by the Artemia Reference Center, Belgium), followed by

   - Great Salt Lake (Utah, USA) Artemia enriched by a standard protocol (to be developed), using:
     -ICES-standard emulsion as external standard
     -local enrichment procedures as internal standard

3. Results should be evaluated in terms of both maximal growth and some low-growth reference

4. Experimental results should be evaluated following well-defined criteria

5. To standardize (n-3) HUFA enrichment procedures, an enrichment intercalibration exercise should be performed.

6. A literature search should be conducted on the technology, formulation and use of standard reference inert diets for larvae (J. Walford).

6. STANDARDIZED PROCEDURES FOR EXPERIMENTAL FRY PRODUCTION

Conveners & rapporteurs: Beatrice Chatain & Josianne G. Støttrup

It was decided that the standardized system should serve as a reference protocol rather than a rigid experimental procedure. That is, the protocol should ascribe to each physical parameter a certain value and quality control within which effects of variation are negligible. Where actual experiments deviate from this protocol, it would then serve as a reference protocol.

The protocol could contain certain general laboratory procedures in sufficient detail to enable an operator to carry them out. It should be intended for experience biologists and laboratory staff, familiar with these types of experiments.

The following protocol was proposed and accepted by the Working Group for the standardization of procedures for experimental fry production of turbot and sea bass:

PROTOCOL

1. Introductory information

   - water quality; description of water treatment and analytical procedures
   - egg and larval quality; how to assess the egg and larval quality

2. Method

   - definitions
   - principles of rearing methods
   - replicates and controls
   - experimental design
   - description of experimental parameters
   - experimental data requirements
   - description of experimental procedures
   - conditions for judging the validity of the experiment

3. Report

   - information to be included in the report

B. Chatain, France provided information on sea bass rearing techniques as a basis for discussion and J.G. Støttrup, Denmark that on turbot. Due to the complexity of the subject and the short time available, many important details were not discussed, nor was there time to work out a comprehensive draft of the protocol at the meeting. The two reports on sea bass and turbot will therefore be compiled by each of the two conveners and sent to the WG members for further comments before the final draft can be submitted. At the earliest this may be for the ICES Statutory Meeting, 1992.

It was proposed that the final draft be submitted as an ICES Cooperative Research Report.

RECOMMENDATIONS

The following recommendations were proposed and accepted by the Working Group:

1. Research priorities should include:

   a) the elucidation of the role of algae in rearing systems.

   b) an assessment of the effects of the quality and quantity of light on larval performance (including effects of tank color/reflectivity)

   c) the development of an empirical feeding model to provide a basis for hatchery feeding strategies

2. Composition, source and shelf-life of ingredients and storage conditions when commercial products are used in nutritional studies, should be provided by the manufacturers.

3. Research reports should include starvation (100%) times in clear water under the same experimental conditions as control groups.

7. HYGIENE STRATEGIES

Convener: Gidon Minkoff
Rapporteur: Øivind Bergh

RECOMMENDATIONS

The following recommendations were proposed and accepted by the Working Group:

A. Marine finfish hatcheries are recommended to take the following measures:

1. Disinfect eggs, employing methods adapted to the species in question

2. Divide the hatchery into production zones, physically separated by infection barriers. Movement of material should be carried out down the production line. Duplicate facilities are advisable.

3. Periodic shut-down of the plant should be carried out in order to disinfect the facilities.

4. As the live food is seen as a major vector of contaminants, it is advisable to use methods such as rinsing to reduce the associated bacterial abundance.

5. For monitoring bacterial growth, florescence microscopy is recommended if possible, otherwise marine agar and TCBS.

B. There is a need for research on:

6a. Bacterial populations associated with different life stages under different conditions

6b. Interactions between larvae and associated bacteria including both pathogens and probiotics, taking into account the nutritional condition of the larvae, as well as their physical rearing conditions.

6c. Procedures to improve system stability with regards to bacterial populations

6d. The development of the immune system in early life stages.

8. NEXT MEETING

The Working Group on Mass Rearing of Juvenile Marine Fish recommends that the group should continue its work and meet in Bergen on June 25-26, 1993 with Ingvar Huse as Chairman.

The following terms of reference were suggested by the group for the Working Group meeting in 1993. The group should meet to work towards the establishment of:

a) a protocol for standardized monitoring of egg and larval quality

b) an inter-laboratory investigation of egg and larval quality

c) a protocol for hygiene procedures in rearing systems

d) a protocol for standard nutrition research taking into account data available on the performance of the proposed standard live diet and experimental procedure as well as the results of the (n-3) HUFA enrichment intercalibration exercise.

Furthermore, the group should meet to:

e) compile information on standard inert reference diets.

APPENDIX I

List of participants from ICES countries

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Appendix II

BIBLIOGRAPHY ON NUTRITION AND HISTOLOGY (Draft No. 1)
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ICES Working Group on Mass Rearing of Juvenile Marine Fish
Meeting at Ghent, Belgium, 31 August - 2 September 1991.

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Appendix III

I.C.E.S. Study on (n-3)HUFA Requirements in Marine Fish Larvae.

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Abstract

This paper summarizes the results obtained in the framework of a study launched and coordinated by I.C.E.S. (International Council for the Exploration of the Seas). This study aims at establishing requirement levels of the essential fatty acids (n-3)HUFA for the early life stages of marine aquaculture organisms. Sofar results have been reported for the following species: the giant prawn *Macrobrachium rosenbergii*, seabass *Dicentrarchus labrax*, seabream *Sparus aurata*, turbot *Scophtalmus maximus*, striped bass *Morone saxatilis*,and red drum *Sciaenops ocellatus*.

During its meeting in Vigo (1988,Spain) the I.C.E.S. working group on 'Early Life Stages of Marine Fish Larvae' recommended to '...better identify the qualitative and the quantitative (n-3)HUFA requirements in marine larvae...'. The term (n-3)HUFA refers to long chain highly unsaturated fatty acids, more particularly eicosapentaënoic acid (EPA or 20:5n-3) and docosahexaënoic acid (DHA or 22:6n-3). These fatty acids have an important structural role in the build up of biomembranes and they act on a number of biochemicals called 'eicosanoids' such as leukotriënes and prostaglandins. These molecules act as hormones involved in regulating several vital body functions such as the immune response mechanism and inflammatory processes. The essentiality of these fatty acids for young marine animals has been documented quiet extensively the last years. Several publications evidence the qualitative dietary importance of these fatty acids for the larvae of several species of marine organisms. Establishing quantitative requirement levels for commercially important species would be very beneficial for improving and optimizing their larviculture. This is however a complex issue as quantitative dietary requirement levels may differ from species to species and may furthermore be affected by larval age, larval quality, environmental conditions, etc. . Provided these conditions are determined and standardized, still then establishing dietary requirement levels of f.ex. (n-3)HUFA is very difficult as today no reference compound larval diets -with (n-3)HUFA as the only variable- is available. Even when such diet would be available it might not be suitable as the first feeding stages of most marine fish larvae do not accept compound feeds as

a sole diet. For these reasons it was suggested that for the present I.C.E.S. study the commonly used feeding regime based on the use of the live food organisms *Brachionus plicatilis* and *Artemia* nauplii would be applied in a first step. The fatty acid profile of the rotifer *Brachionus plicatilis* and the nauplii of the brine shrimp *Artemia*, especially their content of the (n-3)HUFA,can be modified by applying the technique of enrichment (Léger et al.1985, 1986; Watanabe et al. 1983). This way the content of (n-3)HUFA can significantly be increased from a few milligrams per gram dry weight to 50 milligrams and more (Léger et al. 1987). These high values are obtained by feeding the live food organisms (n-3)HUFA rich emulsions.

Materials and Methods

For the present study three emulsions have been formulated for the enrichment of the live food organisms. The emulsions had a different content of (n-3)HUFA : low, medium and high (see Table I).
Detailed instructions for rotifer and *Artemia* enrichment were provided by I.C.E.S. to the 14 participants of the study:

1. *Artemia* enrichment

- incubate the daily required amount of *Artemia* cysts (Great Salt Lake strain, 2 g per liter seawater) in natural or artificial seawater:
    - salinity = 35 ppt
    - temperature = 28°C +/- 1°C
    - pH = 8 à 9 throughout hatching; eventually add sodium bicarbonate
    - light = > 2000 lux
    - aeration = fairly strong

- harvest and rinse the *Artemia* nauplii after 24 h incubation and transfer them to the enrichment vessel filled with filtered seawater at a density of 100 individuals per ml.

- mix (with kitchenblender) 0.3 g enrichment emulsion (per liter enrichment medium) for 30 seconds in a small volume of water and add to the enrichment vessel.

- maintain a temperature of 28°C +/- 1°C.

- ensure high oxygen levels (min. 4 ppm D.O.) by applying an airstone aeration.

- no illumination is required.

- a second ration of 0.3 g enrichment emulsion (per liter enrichment medium) is prepared and added after 16 h enrichment

(between 12 and 18 h).
- after a total enrichment period of 24 h the metanauplii are harvested and thoroughly rinsed; now they are ready for feeding to the larvae.

2. Rotifer enrichment

- harvest and rinse cultured rotifers on an immersed sieve (45 à 60 µm).

- gently transfer the rinsed rotifers into filtered pre-aerated seawater of 25 ppt salinity at a density of 500 rotifers per ml.

- maintain a temperature of 27°C +/- 1°C.

- maintain a slight aeration as to keep all rotifers well suspended; avoid strong aeration in order to minimize clogging.

- the enrichment media are prepared as above for *Artemia*; instead of two times 0.3 g/l for *Artemia* enrichment two times 0.1 g/l are used for rotifer enrichment; a first ration is added when the rotifers are being transferred to the enrichment tank (t_{0h}) (start of enrichment) and the second ration is added after three hours (t_{3h}) incubation.

- after a total enrichment period of six hours (t_{6h}), gently harvest and rinse the enriched rotifers on an immersed sieve; avoid strong turbulence; now the enriched rotifers are ready for feeding.

By applying the above instructions the content of (n-3)HUFA in rotifers and *Artemia* is altered depending on the type of emulsion used: rotifers and *Artemia* enriched with the low, medium and high (n-3)HUFA emulsion contain respectively low, medium and high levels of (n-3)HUFA (example for *Artemia* see Figure 1 and 2).

Results

The detailed results obtained by the different participating institutes are being published elsewhere as individual papers. A summary of the first results are being presented hereunder.

1. *Dicentrarchus labrax*

Two different laboratories ran the requirement study on seabass (*Dicentrarchus labrax)* larvae.
One study (Corneillie et al., Zoological Institute, University of Leuven, Leuven, Belgium)was conducted on first feeding larvae which during a period of 40 days were offered rotifers and subsequently *Artemia* both enriched with the three emulsions containing

increasing levels of (n-3)HUFA.
A clear impact of the fatty acid composition of the feeds on larval survival was noted: survival after 60 days culturing was 3.3 % +/- 0.1 % in the low (n-3)HUFA group , 12.7 % +/- 0.6 % in the medium (n-3)HUFA group and 13.0 % +/- 0.1 % in the high (n-3)HUFA group. A massive mortality was noted between day 30 and day 60 in the larvae fed the low (n-3)HUFA enriched rotifers and *Artemia*. During this period many larvae in this treatment exhibited the symptoms of the so-called 'whirling disease'. No larvae from the medium and high (n-3)HUFA treatments were affected. Growth in terms of individual dry weight was also affected by the diet composition though significant differences appeared only from the third week onwards (see Fig.4). The same was observed for growth in terms of individual length. Besides survival and growth effects on the morphological development of the larvae were also studied. No relation was found between the diet composition and the occurrence of skeletal deformities, shortening of the opercula nor the degree of swimmbladder inflation. A significant interaction was detected however between the (n-3)HUFA content of the diet and the development of the gall bladder: low dietary (n-3)HUFA levels appeared to induce hypertrophy of the gall bladder.

The second study (Martinez and Alcazar, Oceanographic Institute, Mar Menor, Murcia, Spain) was carried out with 35 d old *Dicentrarchus labrax* larvae which were fed the enriched *Artemia* preparations during 29 days. During this stage of larval development noticeable effects of dietary fatty acid content on survival are only observed between the low (n-3)HUFA treatment and both others (see Fig.5). Effects on growth were not significant.

Neither of the two studies carried out fatty acid analysis on the enriched live feed used in the experiment. Hence absolute requirements seabass larvae for n-3HUFA may not be drawn from the above studies. Taking into consideration the values as illustrated in Figures 1 and 2 one could conclude that estimated requirements would be about ... to ... mg n-3HUFA per gram dry feed offered.

2.*Sparus aurata*

Sofar results with seabream larvae are limited to those obtained by Martinez and Alcazar (cfr above). Results relative to the treatments are very comparable to those obtained by the same authors for seabass *D. labrax* (see above). Figure 6 shows again a massive mortality in the low (n-3)HUFA treatment and a relatively high survival in both other treatments.
Koven *et al.* carried out a two weeks experiment with ...days old seabream larvae fed *Artemia* nauplii enriched with soybean oil (no n-3HUFA) supplemented with increasing levels of SUPER SELCO (Artemia Systems S.A., Ghent Belgium; SUPER SELCO contains an equivalent concentration of n-3HUFA as the I.C.E.S. High emolsion). The supplementation of n-3HUFA in the *Artemia* increased survival in the bream larvae significantly though a levelling off could be noted at ....mg/g (see Fig....). On the contrary however, growth

continued to improve along with the level of n-3HUFA in the diet indicating that for growth requirements are above ...mg n-3HUFA per gram dry feed offered (see Figure....).

3.*Macrobrachium rosenbergii*

Two studies have been performed evaluating the effects of feeding newly hatched *M. rosenbergii* larvae a sole diet of *Artemia* nauplii enriched with the I.C.E.S. emulsions. A first study by Buzzi *et al.*(1989) carried out at the Sterling University - Tropical Prawn Unit- indicated that best results in terms of survival, growth and metamorphosis rate were achieved when the prawn larvae were fed the highest n-3HUFA containing diet (High-emulsion). The results were significantly better than when feeding the Medium enriched nauplii. Lowest culture performance was observed in the treatment receiving Low n-3HUFA *Artemia* (see Figures....). Fatty acid profiles of the enriched *Artemia* nauplii are given in Table.... From these results the authors concluded that for meeting the n-3HUFA requirements of *M. rosenbergii* larvae the diet offered should contain at least ....mg n-3HUFA per gram dry weight.
In a second study Devresse *et al.*(1990) performed the same experiment at the Artemia Reference center (University of Ghent, Belgium). Their results indicated that best culture performance (in terms of growth, survival, vitality and metamorphsis rate) was achieved when offering *Artemia* nauplii enriched with the Medium emulsion. No further improvement was noticed when nauplii were offered enriched with the High n-3HUFA emulsion. From the fatty acid analysis (see Table...) the authors conclude that the requirements of *M. rosenbergii* larvae should be in between 5.2 and 37.5 mg per gram dry diet offered - probably toward the higher end. In this sense these results agree with those obtained by Buzzi *et al.*(1989). This further correlates with the results obtained by Sandifer and Joseph (1976) who improved the performance of a postlarval diet by increasing the n-3HUFA content to 19 mg/g. Considering n-6 fatty acids Devresse *et al.*(1990) could not confirm a requirement in *M. rosenbergii* larvae as was identified in postlarvae by Reigh and Stickney (1989).
Romdhane *et al.*(in preparation) carried out an experiment feeding newly hatched *M. rosenbergii* larvae during an increasingly shorter (delayed) period with *Artemia* enriched with SELCO (Artemia Systems S.A., Ghent, Belgium). n-3HUFA levels of SELCO enriched nauplii correspond to those obtained when using the Medium I.C.E.S. emulsion. From their results (see Figure...) the authors conclude that for optimal results (in terms of growth, survival, stress resistance and metamorphosis rate) prawn larvae should be fed n-3HUFA enriched *Artemia* nauplii preferrably from first feeding onwards.

4.*Morone saxatilis*

One study on larval striped bass (*Morone saxatilis*) has been

conducted by Lemm and Lemarie (1991, in press). Four days post-hatch Tennessee striped bass larvae were cultured during 14 days on a diet consisting of freshly hatched or enriched (I.C.E.S. emulsions) *Artemia* nauplii. The authors report on the fatty acid profiles of the emulsions, the *Artemia*, and the fish larvae (values of n-3HUFA in *Artemia* are represented in Table....).

Fatty acid	LOW HUFA		MEDIUM HUFA		HIGH HUFA		unenr
	emul	*Art.*	emul	*Art.*	emul	*Art.*	
20:5n-3	0.0	2.6	9.7	8.2	25.0	12.5	3.8
22:6n-3	0.0	0.0	7.8	3.1	36.9	9.4	0.0
Σn3HUFA	0.0	2.6	19.1	11.3	66.5	22.8	3.8

As can be noticed from Table... n-3HUFA levels in *Artemia* reflect those of the enrichment emulsions; they are however inferior to those presented in Table.... Total lipid levels as reported by the authors decrease during enrichment (about 20 %) which would indicate that the enrichment procedure was not optimal. Culture results are summarized in Table...

Artemia treatment	length	survival	swimmbladder inflation
Unenriched	9.3 +/- 1.0	5	(29)
LOW HUFA	9.8 +/- 0.9	23	26
MED. HUFA	10.3 +/- 0.8	64	33
HIGH HUFA	10.2 +/- 0.8	48	32

Growth and survival were significantly improved in the fish larvae fed the n-3HUFA enriched *Artemia*. A n-3HUFA content of 11.3 % of total fatty acids in *Artemia* appears to fulfill the requirements of first feeding striped bass larvae. Increasing the level to 22.8 % did not further improve the performance. Swimmbladder inflation was not significantly affected by the dietary n-3HUFA content.

6.*Sciaenops ocellatus*

Craigh, Holt and Arnold (in press) report on experiments assessing the effects of feeding enriched rotifers ,using the I.C.E.S. emulsions, on the growth and fatty acid composition of red drum (*Sciaenops ocellatus*) larvae. Fish were cultured during ten days and no *Artemia* was fed. The contents of n-3HUFA in the enriched rotifers are given in Table....

Fatty acid	CONTROL	LOW n-3HUFA	MEDIUM n-3HUFA	HIGH n-3HUFA
20:5n-3		1.5	8.6	10.7
22:6n-3		0.0	6.7	12.5
Σn-3HUFA		1.5	15.3	23.2

Growth rates were best in the larvae fed the n-3HUFA enriched rotifers (medium and high n-3HUFA emulsions); they were however not significantly different from the control treatment fed rotifers grown on a mixture of algae, fish oil and yeast. Poorest results were recorded in the fish fed the low n-3HUFA enriched rotifers. The authors conclude that 22:6n-3 significantly affect growth in red drum larvae. XXXXXXXXXXX--control rotifers_---XXXXXX
Ten day old red drum larvae exhibit a fairly constant fatty acid profile independant of the diet offered. An extended trial covering the subsequent culturing phase including *Artemia* would allow to verify the evolution of the fatty acid profile in red drum larvae beyond the rotifer stage. More pronounced differences between treatments could eventually show up when reserve fatty acid pools would get depleted.

Conclusions

Reference List

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7. SUMMER FLOUNDER (Paralichthys dentatus)
Lubal + Reynton (LARVI '91)

Biochemical composition enriched Artemia
Fernández-Diez et al (LARVI '91)