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Culture of sea bass larvae (*Dicentrarchus labrax*) in completely closed recirculation systems with artificial seawater

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Abstract

Sea bass larvae (*Dicentrarchus labrax*) were cultured under laboratory conditions in "completely"-closed recirculation systems with artificial seawater. Daily measurements ensured that the quality of the water was always under control. A 3-year survey of the bacterial population in this system revealed on the one hand, the permanent presence of *Vibrio alginolyticus* and *Pseudomonas* species and, on the other hand, a low bacterial concentration during the first 30 days of the rearing period. After 3 years of investigation, a larval survival of 22% was obtained at day 45. The growth rate was similar to that reported by other investigations (18.4mm at day 45). The major problems were: absence of the swimbladder, swimbladder hypertrophy, and a spinning movement syndrome or whirling disease.

KEYWORDS: Sea bass larvae, Recirculation, Bacteria, Swimbladder, Spinning movement.

Introduction

The sea bass (*Dicentrarchus labrax*) is an important fish for mariculture because of its high economic value and its ability to adapt to salinity- and temperature variations. The major obstacles to the successful mariculture of this species still remain the low survival of the larval stages and quality problems of the larvae due to the abnormal development of the swimbladder.

The first trial to culture sea bass larval stages was attempted in 1969 by Barnabé, but all the larvae died within 17 days (Barnabé, 1974). Nowadays a survival rate of 20 to 30% is obtained in most of the centers (France, Italy, Greece) although some publications mention higher survival rates (Coves, 1985a,b; Katavic, 1986; Olesen, 1986). All marine hatchery stations are close to the sea and use natural seawater. To save heating costs, all hatcheries use a semi-closed recirculation system with a 10% renewal per day.

In our laboratory, we investigated the possibility of cultivating sea bass larvae in artificial seawater. As far as we know, this has not, as yet, been attempted. Our experiments were performed using a completely-closed recirculation system. In this way, it was possible to learn whether a completely closed system was feasible and, secondly, whether such a system reduced the necessary amount of artificial sea salt and hence the associated costs.

Materials and methods

Description of the system

Fig. 1 shows the setup of the larval rearing system. The system consists of two separated "completely"-closed circuits composed of conical tanks (6 tanks of 100 l and 8 tanks of 35 l) connected with a mechanical (3) and a biological filter (7). The mechanical filter (50 l) consists of one rectangular tank filled with successive layers of active charcoal and wadding. Before entering the biological filter, the water is filtered through a woollen bag (5µm) (11). The biological filter (200 l) is built up of bio-disks (Filterpack,

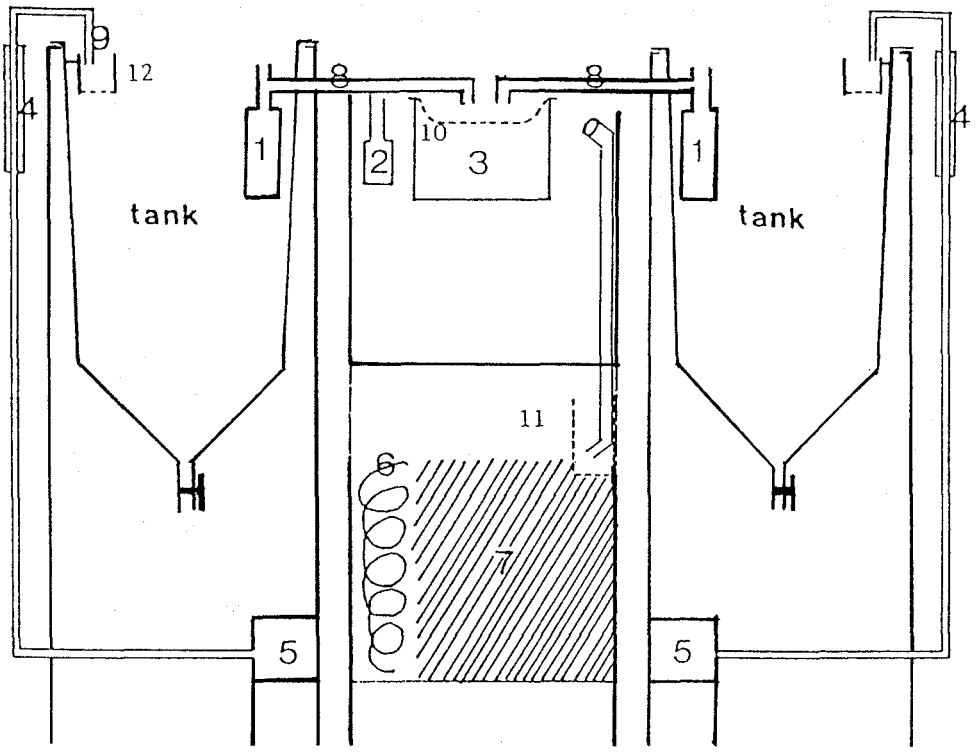

Fig. 1. Schematic diagram of the recirculation system with two rearing tanks. (1) Screen (100μm); (2) ozonificator; (3) mechanical filter; (4) UV; (5) pump; (6) cooling spiral; (7) bio-disks; (8) outlet pipe; (9) inlet; (10) woollen filterbag; (12) inlet cup.

Antwerpen, Belgium). Before the water enters the larval tanks, it is cooled (6) and then sterilized by UV-radiation (4) and ozonification (30mg.h^{-1}) (2). All the tanks are painted black since we have indications that this reduces the larvae's stress. To prevent clogging of the screen, we use two different screens during the *Brachionus* feeding period. The first screen (mesh 100μm) is located in the tank (1), and a second screen (mesh 50μm) outside the tank (10). Besides preventing clogging, this system has the advantage of ensuring that unconsumed *Brachionus* are slowly removed from the tank. In this way the fish larvae are regularly supplied with newly-enriched *Brachionus*. To avoid turbulence when the recycled water is entering the tanks, the water first flows into a wadding-filled cup hanging in the tank (12).

Rearing procedure

Fertilized eggs or yolk-sack larvae were obtained from different French centers (Deva-Sud, Palavas-les-Flots, Centre Océanologique de Bretagne, Brest). The larvae and fertilized eggs were always transported by air in polyethylene bags filled with pure oxygen. After temperature acclimatization the larvae were put into the tanks; the eggs were also incubated directly in these larval-rearing tanks. At the onset of the experiments the larval densities were 75-100 larvae.l^{-1}. The rearing procedures were the same as those described by Johnson and Katavic (1986), Coves, (1985) and Barnabé (1986) but a lower light intensity (10-50 lux) was used. Each day, the following parameters were measured: temperature, oxygen concentration,

Table I. Parameters of the water quality from January-March 1987

Larval age	Temperature (°C)	Oxygen (% sat.)	NO_2^- (ppm)	Salinity (‰)	Water flow rate (%/V/h)	Light intensity (lux)	pH
Day 0- 5	15.2-16.4	91-93	0.025	37	2.5- 3	0	8.3
Day 6-12	17.3-18	90-88	0.05 -0.05	37	2.5- 5.2	12	8.3
Day 13-22	17.9-20.5	89-89	0.025-0.05	36	5.5-12.8	18	8.3
Day 23-30	20.3-19.7	89-91	0.06 -0.05	36	12.6-11.7	15	8.3
Day 31-40	19.7-19.7	91-86	0.025-0.05	36	11.3-26.3	48	8.2
Day 41-48	19.5-19.5	84-96	0.025-0.025	36	26.3-25.5	42	8.2

Table II. Number of bacterial colony-forming units per 100 ml in two completely-closed recirculating circuits from January-March 1987

Day	Larval age (d)	Circuit A			Circuit B			Food	Enrichment
		Filter tanks	Larvae tanks	No.[1]	Filter tanks	Larvae tanks	No.[1]		
-1	3	600	420	2	70	260	2	No	No
7	10	/	15 600	5	220	19 000	8	Rotifers	*T. suecica* Selco
13	16	720	11 800	5	40	1 970	8	*Artemia* nauplii	No
27	30	/	/	5	30	1 670	1	*Artemia* metanauplii	Selco *T. suecica*
34	37	26 700	131 700	5	115 600	44 600	8	*Artemia* metanauplii	Selco
44	40	133 000	199 870	5	560	273 500	8	Frozen *Artemia* metanauplii	No

[1]No.: number of tanks analyzed.

nitrite- and ammonia concentration, water flow-rate, salinity, light intensity, pH, and prey concentration. The prey concentration always varied between 3 and 5.ml^{-1}.

Development of bacteria in the tanks

During the 3 years of experimentation, the bacterial species present in the tanks were regularly checked. Qualitative analyses were based on the API-NE system; for the quantitative analyses membrane filtration was used.

Growth and malformations

During all the experiments, the growth of the larvae was closely followed and data on malformations were collected.

Results

Water quality and physicochemical conditions in the experimental rearing tanks are listed in Table I. The bacterial species found (Table II), mostly belonged to the Pseudomonaceae, although *Vibrio alginolyticus* was also present most of the time. *V. alginolyticus* was also detected in the algal and rotifer cultures. *Pseudomonas putida* and *stutzerii* were nearly always present in the larval circuit.

Quantitative analyses (Table III) during the last experiment in January-March 1987 revealed a low bacterial concentration (100-20 000 colony-forming units.100ml^{-1}) during the first month of larval rearing. The low starting concentrations were due to the fact that during each new larval period the water of the circuits was completely renewed. After 37 days the bacterial counts were much higher (27 000 - 275 000 cfu.100 ml^{-1}).

The feeding schedule (Table IV) slightly differed from that used in other centers. We started with a small strain of *Brachionus* from Spanish origin (235±18µm) enriched with green algae (*Tetraselmis suecica*). Once the larvae were eating well, a larger Russian strain of *Brachionus* (300±37µm) was given enriched with Selco (Artemia Systems NV., Gent, Belgium). The rest of the feeding schedule was similar to that applied in all other marine centers.

During the experiments problems arose with abnormal development of the swimbladder, spinning syndrome or whirling disease, and malformations of the lower jaws and operculae (Table V). In one tank, with very low aeration (1% of normal aeration, 0.4 l.m^{-3}.min^{-1}, all larvae showed hypertrophy of the swimbladder after 13 days. The relative length of the swimbladder was

Table III. Bacterial species determinated in the closed circuits from April 1985-March 1987

	4/85	12/85	3/86	4/86	12/86	1/87	3/87
Vibrio alginolyticus			*		*	*	*
Aeromonas caviae		*					
Pseudomonas putida		*	*			*	
Pseudomonas stutzerii		*		*		*	
Pseudomonas maltophilia	*					*	
Pseudomonas putrefaciens						*	
Pseudomonas cepacia	*						
Pseudomonas aeroginosa	*						
Pseudomonas mendocinia		*					
Pseudomonas pseudomallei		*					
Pseudomonas fluorescens		*					
Pseudomonas picketti			*				
Pseudomonas vescicularis				*			
Acinetobacter calco var lwoffi		*	*				
Citrobacter freundii		*					
CDC-gr IV C-2- *Bordetella*				*			

not different from that of the fish in the other tanks, but the relative width (0.260±0.135mm versus 0.163±0.048mm) was significantly higher in the tank with low aeration ($P<0.001$).

Discussion

Physicochemical conditions indicated in Table I, show that it was possible to keep all water parameters within the adequate limits to culture the sensitive sea bass larvae. In this respect, the good water quality, the acceptable survival rate (22% at day 45) and good growth (18.4mm at day 45) prove that it is possible to culture larvae of sea bass in completely-closed systems, even with artificial seawater.

Qualitative analysis of the bacteria revealed the continual presence of *Vibrio* and *Pseudomonas* species. *Vibrio alginolyticus* is described as a pathogen that provokes mortality after extensive handling of sea bream (*Sparus aurata*) in Israel (Austin and Austin, 1987). Several *Pseudomonas* species are toxic to fish, but the sensitivity of fish to these bacteria is markedly increased when the animals are under stress. We have no evidence to suggest losses of larvae due to such bacterial infections. Quantitative analyses revealed low bacterial concentrations during the first month of larval culture (Table III). The increase of bacterial concentration after the first month is possibly related with the accumulation of faeces on the bottom of the tanks. These faeces were only removed once a week, to reduce the stress as much as possible.

The food schedule (Table IV) was slightly modified compared to that used in other centers. We started with a small strain of *Brachionus* primarily fed on baker's yeast and finally enriched with green algae (*Tetraselmis suecica*). This enrichment with green algae allows the larvae to more clearly see the normally translucent rotifers. *Brachionus* enriched with green algae are taken up sooner and in higher quantities when compared with rotifers enriched with other coloured substances (Selco, no enrichment). Twelve hours after the sea bass larvae received their first live food, 37.5% of the larvae had already taken up rotifers enriched with green algae, whereas in the non- enriched condition only 6.7% started feeding (Corneillie and Ollevier, in prep.). Similar results were obtained by Dendrinos et al. (1984) with larvae of Dover sole fed with *Artemia* nauplii. Once the larvae are eating well, a larger strain of *Brachionus* enriched with Selco was used, which is nutrionally better than algae thanks to the presence of highly poly-unsaturated fatty acids (22:6ω3 and 20:5ω3). These essential fatty acids improve the growth and the survival rate of the larvae (Jones, 1986; Franicevic et al., in prep.).

Quality problems of sea bass larvae are mostly due to the malformation of the swimbladder (absence or hypertrophy) (Weppe and Bonami, 1983; Coves, 1985a,b; Bagarinao and Kingvankij, 1986; Chatain, 1986; Katavic, 1986). As to the absence of the swimbladder no evident reason can be proposed, but hypertrophy of the swimbladder ban be caused by a too low aeration. This was also found for *Mugil cephalus*. Nash et al. (1977) demonstrated that this problem can be overcome for this species by increasing the aeration. In this way the larvae stayed below the water surface and the swimbladder subsequently formed and functioned normally.

Another problem is the spinning syndrome or whirling disease of the larvae. In December 1986, when the larvae reached the age of 30

Table IV. Food schedule for the culture of sea bass larvae

Day 0- 5:	Yolk sack
Day 5- 8:	*Brachionus plicatilis* (Spanish strain)
	Enrichment: green algae (*Tetraselmis suecica*)
Day 9- 15:	*Brachionus plicatilis* (Russian strain)
	Enrichment: Selco (Artemia Systems NV, Gent, Belgium)
Day 12- 20:	*Artemia* nauplii (San Francisco Bay, USA)
Day 21- 40:	*Artemia* metanauplii (Great Salt Lake, USA)
	Enrichment: Selco (Artemia Systems NV, Gent, Belgium)
Day 40- 45:	Frozen *Artemia* metanauplii
Day 43:	Granules, Trouvit 000 (Trouw, Gent, Belgium)

Table V. Quality problems with sea bass larvae from May 1985-March 1987

May-June 1985:	Swimbladder absence; malformation of opercula
Dec.-Jan. 85/86:	Swimbladder absence (63%)
April-May 1986:	Skeletal malformations just after hatching (25%), swimbladder absence
Nov.-Dec. 1986:	Spinning round syndrome (100%); hypertrophy of swimbladder
Jan.-Feb. 1987:	Swimbladder absence (50%); spinning round syndrome (20-25%); malformation of the lower jaw (in some tanks up to 90%)

days, all the larvae died due to this symptom. The available data concerning this spinning syndrome are very confusing. On the one hand, such a diseased condition, together with a distended swimbladder, white faecal casts, and exophthalmia in sea bass larvae was associated with a Birna virus (Bonami et al., 1983). We found a positive correlation between swimbladder absence and larvae that spin. The question whether spinning is caused by swimbladder malformation or by viral infection is still under discussion. Recently, it was shown that cultured marine fishes such as turbot and sea bass are also susceptible to VHS infection (Hill, 1986). It would be worth to investigate possible interactions.

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