

A closed water recirculation system for ecological studies in marine fish larvae: growth and survival of sea bass larvae fed with live prey

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Received June 7, 1999; accepted January 12, 2000

Abstract — This paper describes the suitability of a closed recirculation system to study the development of fish larvae in a strictly controlled environment, where only feeding was varied (fed, starved, delayed and late fast treatments). The system served both as an incubator and hatchery. The time variation of physical and chemical parameters together with survival and growth of reared sea bass larvae (*Dicentrarchus labrax*) were studied over the first month of life. The recirculation design allowed for the maintenance of levels of ammonia, nitrite and nitrate below those cited as responsible for mortality or decreased feeding ability in other marine fish larvae. Almost no larval mortality occurred in the fed larvae from day 9 after hatching. The starved group of larvae showed a sharp decline in survival after 16 days of food deprivation. Larvae for which feeding was delayed until day 13 ceased dying 4 days after food was supplied. Fast feeding on days 24 and 25 had no effect on larval survival. Growth in length was similar during the first 2 weeks of larval life regardless of feeding treatment. Two days of late fast had no effect on larval growth. Growth patterns of fed larvae in this study were similar to those reported for larvae reared in flow through systems. We believe that survival and growth of the reared larvae was a direct function of diet, and that the type of rearing system did not adversely affect these parameters. The rearing design and the results obtained suggest that future nutritional studies of field-collected larvae will benefit from this kind of rearing experiment. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Closed recirculation system / nitrogen compounds / sea bass larvae / survival / growth / *Dicentrarchus labrax*

Résumé — Un système d'élevage de larves de poisson marin, en circuit fermé : croissance et survie de larves de bar nourries de proies vivantes. Cette étude décrit l'adaptation d'un système de circulation d'eau en circuit fermé pour analyser le développement de larves de poisson en milieu strictement contrôlé lorsque seule, l'alimentation varie (larves nourries, à jeun, alimentation différée, et alimentation accélérée mais tardive). Le système sert à la fois d'incubateur et d'écloserie. Les variations dans le temps des paramètres physiques et chimiques, associées au taux de survie, à la croissance des larves de bar (*Dicentrarchus labrax*) ont été étudiées au cours de leur première année. Le système de recirculation permet le maintien de niveaux d'ammoniac, nitrite et nitrate inférieurs à ceux connus pour provoquer des mortalités ou d'une baisse des capacités nutritionnelles des larves. Presqu'aucune mortalité larvaire ne s'est produite chez les larves nourries à partir du 9^e jour après l'éclosion. Le groupe de larves restées à jeun a montré une forte hausse de la mortalité après 16 jours de privation de nourriture. Les larves dont l'alimentation a été différée jusqu'au 13^e jour, cessèrent de disparaître 4 jours après qu'elles soient alimentées. Les larves nourries plus rapidement le 24^e et 25^e jour n'ont pas eu un taux de survie modifié. La croissance en longueur était similaire durant les deux premières semaines de vie larvaire quel que soit le traitement. Deux jours d'alimentation différée mais rapide n'ont pas eu d'effet sur la croissance. Le modèle de croissance des larves nourries dans cette étude était similaire à celui de larves élevées en circuit ouvert. Nous pensons que la survie et la croissance de larves en élevage est une fonction directe de l'alimentation et que ce type de système d'élevage n'affecte pas ces paramètres. Le système d'élevage et les résultats obtenus montrent que des études nutritionnelles sur des larves collectées dans le milieu naturel bénéficieront de ce type d'expérimentation. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Système en circuit fermé / composés azotés / larves de bar / survie / croissance / *Dicentrarchus labrax*

1. INTRODUCTION

Low food concentrations in marine systems may result in direct or indirect larval mortality due to both starvation and reduced growth, which, in turn, may result in increased predation [16]. However, numerous environmental factors affect both development and survival of fish larvae in the wild. Culture techniques developed in aquaculture, where most variables can be controlled, have become useful for these types of studies.

This work is part of a broad multidisciplinary study that deals with the factors influencing survival of fish larvae in the north-western Mediterranean. Accurate laboratory experiments that discriminate factors potentially affecting survival (e.g. starvation) are crucial to understand the relative importance of such phenomena in the wild. As a first step, the effect of food deprivation on larval survival and growth was studied in larvae reared under controlled conditions. In order to ensure that these parameters were only dependent on feeding regime, it was necessary to set up an experiment in which, in a strictly controlled environment (closed recirculation system), only the feeding ration varied. The sea bass, *Dicentrarchus labrax*, was chosen for the experiments because of its wide distribution around Europe.

Most studies using closed recirculation systems focus on particular aspects of the reared species, but it is difficult to find detailed descriptions for short-term experimental cultures. Previous studies reported on acceptable levels of several physical and chemical parameters that produce good levels of growth and survival for *Dicentrarchus labrax* [2, 10, 17]. None of these studies, however, described the time variation of all these parameters along with data on growth and survival at different feeding levels in a closed recirculation system.

The goal of the present work was to analyse the suitability of a short-scale integrated incubation-hatchery recirculation system to help in future studies of field-collected larvae (in this case, for calibration laboratory experiments to study nutritional condition). To this end, we analysed the results of growth and

survival of fed and starved larvae, together with the daily monitoring of the main physico-chemical parameters.

2. MATERIALS AND METHODS

The basic design was taken from Amat et al. [1] and adapted to a closed system. These changes were made to enhance the water circulation in order to maintain a uniform water quality and enhance the elimination of non-consumed food.

Figure 1 shows the main components of the recirculating system. It was composed of a 310-L rearing tank and a reservoir of 220 L. A biological filter (BF) was placed on top of the reservoir tank and a mechanical filter of 85 L (MF) was located between the two tanks. Twelve grey polyvinyl chloride (PVC) rearing cylinders of 4 L (RC) were set in the rearing tank. A 500- μm mesh size was glued to the bottom of the cylinders. A PVC ring with a 100- μm mesh glued to it was used as a lid for the bottom of the cylinders (FC, figure 1b). These lids were placed during feeding hours to prevent the escape of living prey. The total water flow through the system was kept constant at 17 L·min⁻¹ (1 020 L·h⁻¹) during the whole experiment. This meant a water renewal in the rearing tank of 4.8 h⁻¹. Renewal rate in the rearing cylinders fluctuated between 0.37 and 2 h⁻¹ (table I).

Initially, the system was filled with water that had been filtered to 0.2 μm and sterilised by UV radiation. Water loss due to evaporation was replaced by distilled water, which was added to the reservoir on a daily basis. The whole system was set in an isothermal chamber at 15 °C. Water temperature was adjusted with thermostats immersed in the reservoir (H, figure 1a). Salinity was adjusted during the different rearing periods, varying from 37 \pm 0.1 at incubation and progressively decreasing to 30 (table I). Temperature was gradually raised throughout the experiment from 16 to 20 °C (table I). Photoperiod was established at 9 h light/15 h dark.

Table I. Controlled parameters of the water quality for laboratory-reared sea bass larvae.

Age	Light (Lux)	Temperature (°C \pm SD)	Salinity (\pm SD)	Oxygen (mg·L ⁻¹)	pH (\pm SD)	N-NO ₂ (\pm SD) (mg·L ⁻¹)	N-NO ₃ (mg·L ⁻¹)	WF in cylinders (L·h ⁻¹)	Turnover (h ⁻¹)	Aeration (mL·min ⁻¹)
Eggs	11–15	16	37	9.2	8.2	0.004 \pm 0.001	< 5	4–6	1–1.5	40
L ₀ L ₂	11–15	16	37	9.6 \pm 0.2	8.0 \pm 0.1	0.004 \pm 0.001	< 5	1.5–1.6	0.37–0.40	20
L ₃ L ₁₀	15	17.5 \pm 0.6	35.6 \pm 1.3	7.9 \pm 0.7	8.1 \pm 0.1	0.02 \pm 0.019	0–5	1.5–1.6	0.37–0.40	20
L ₁₁ L ₁₈	15	20.1 \pm 0.3	30.9 \pm 1.0	6.9 \pm 0.5	7.8	0.12 \pm 0.019	10–15	1.5–1.6	0.37–0.40	20
L ₁₉ L ₂₅	40	19.7 \pm 0.2	30.1 \pm 0.3	7.3 \pm 0.2	7.9 \pm 0.1	0.181 \pm 0.025	15–40	1.5–1.6	0.37–0.40	40–60
L ₂₆ L ₂₈	40	20.5 \pm 0.9	29.9 \pm 0.3	7.3	7.9	0.216 \pm 0.013	15–40	2.4–2.6	0.60–0.65	40–60

WF, water flow. Turnover refers to the rearing tank.

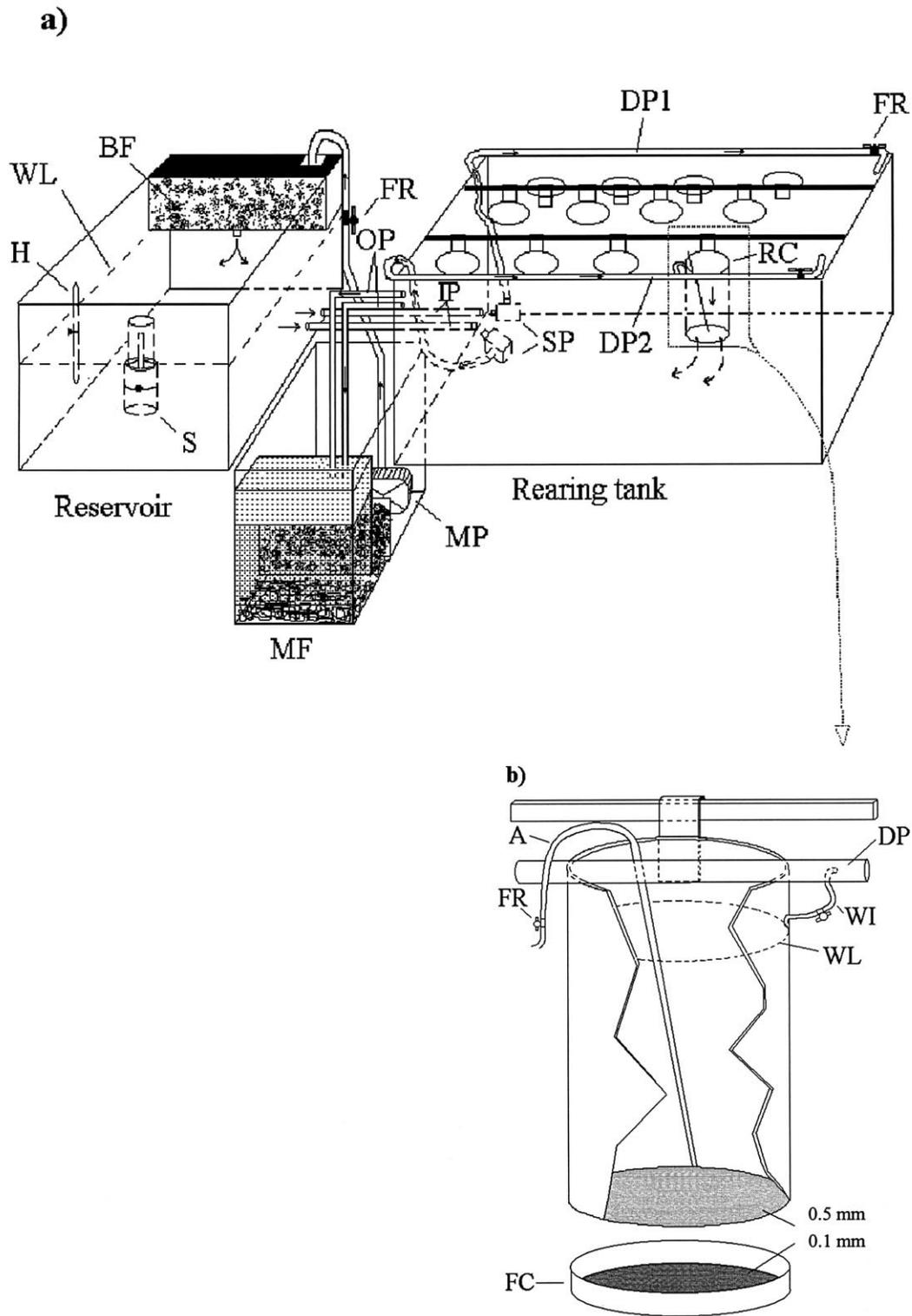


Figure 1. a) Schematic diagram of the enclosed recirculating system for rearing marine fish larvae. BF, biological filter; DP1 and DP2, distribution pipes; FR, flow regulators; H, heater; IP, inlet pipes; MF, mechanical filter; OP, outlet pipes; MP, main pump; S, skimmer; SP, secondary pumps; RC, rearing cylinder; WL, water level. b) Detail of one of the rearing cylinders. A, air pipe; DP, distribution pipe; FC, feeding cap; WI, water inlet. Arrows indicate water flow.

Eggs were placed in the cylinders 43 days after the filter was started up. Sea bass eggs in good condition have positive buoyancy at salinities over 34.5 [3]; thus, they could be separated following the procedure of Felip et al. [14] and evenly distributed in each cylinder using a Pasteur pipette. The effective number of individuals (eggs or larvae) by cylinder was obtained by adding the number of survivors at the end of the experiment to the number of dead and sampled larvae every day. The accumulated survival throughout the experiment is given as a percentage of the living larvae in each cylinder at first day of feeding.

Larvae were fed at the same hour every morning. The food consisted of live prey according to Barnabé [3] (rotifers, *Artemia* nauplii and 1-day-old enriched metanauplii). Initially, three feeding treatments were established: fed, late fast and starved, and these comprised four cylinders each. Cylinders of each treatment group were randomly placed in the rearing tank. The fed treatment group was supplied with food ad libitum from day 5 after hatching. In order to assess the effect of 2 days of fast in normally nourished larvae, feeding of four cylinders was stopped on days 24 and 25. From day 13, one of the cylinders from the starved group was supplied with food, to set the delayed treatment. We sampled three to four larvae per day and per treatment.

Larvae were preserved in 10 % phosphate-buffered formalin. Larval length used for growth analyses refers to total body length (TL), measured as the distance along the midline of the body from the tip of the snout to the end of the caudal fin. Growth was analysed by fitting a Gompertz model to the data on TL. This model has been used to describe early larval development in marine fish larvae [19, 21, 24].

Gompertz equation: $TL = a e^{-be^{-ct}}$ where TL is the total body length (mm), a is the asymptote (mm), c the instantaneous growth rate at the inflexion point (days^{-1}); t the age in days and b is a dimensionless parameter, such that $b \times c$ is the instantaneous growth rate when $t = 0$. The Hotelling's T^2 test [6] was used to screen for differences between growth curves of different treatments. Absolute growth rate at an age t (dTL) was calculated from:

$$dTL = c TL (\ln a - \ln TL)$$

3. RESULTS

3.1. Incubation and hatching

Eggs were allocated among the rearing cylinders when they already exhibited an advanced stage of development. The embryo, already differentiated, had the caudal region separated from the yolk. All the eggs hatched on the same day. During incubation time and for the following 4 days after hatching concentrations of total ammonia ($\text{N-NH}_4^+ + \text{NH}_3$ in $\text{mg}\cdot\text{L}^{-1}$), union-

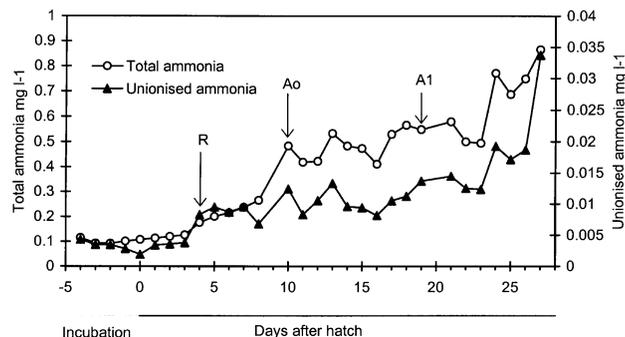


Figure 2. Concentrations of total ammonia and unionised ammonia from 5 days before hatching until the end of the feeding experiment. Arrows indicate the initiation of feeding with rotifers (R), *Artemia* nauplii (A0) and 1-day-old *Artemia* metanauplii (A1).

ised ammonia (N-NH_3), nitrite (N-NO_2^-) and nitrate (N-NO_3^-) concentrations were very low (figure 2, table I).

Despite the attempted initial even distribution of eggs among cylinders, there was some variability in the actual number of eggs per cylinder (mean = 130 eggs $\cdot\text{L}^{-1}$, SD = 30). The hatching success, calculated as number of living larvae at day four after hatching, was 64.1 % (SD 14.8 %). Daily mortality in this period was highly variable both within and among cylinders. There was no correlation between the initial egg densities and the daily mortality observed during this period.

3.2. Larval rearing

During the experimental period, total ammonia, unionised ammonia and nitrite concentrations fluctuated within a range considered as non-toxic for fish larvae [13]. However, the values tended to increase towards the end of the experiment (figure 2, table I). The first clear increase in ammonia concentration (total and unionised) occurred on the first feeding day, when rotifers were introduced into the system (4 days after hatching) (figure 2). The later introduction of *Artemia* nauplii (10 days after hatching) also produced an increase in total ammonia levels. However, concentrations of unionised ammonia could be kept below 0.014 $\text{mg}\cdot\text{L}^{-1}$ until the introduction of enriched *Artemia* nauplii (19 days after hatching). Values of these metabolites reached the highest values from days 24 to 27 (figure 2).

3.3. Survival

Larval density at first exogenous feeding was 60 larvae $\cdot\text{L}^{-1}$ (SD 20). The data for larval survival in the three treatments are shown in figure 3. Survival decreased in all treatments until day 9 after hatching. Next followed a steady period of scarce or null mortality which, for the fed larvae, continued until the end of the experiment. This phenomenon was also

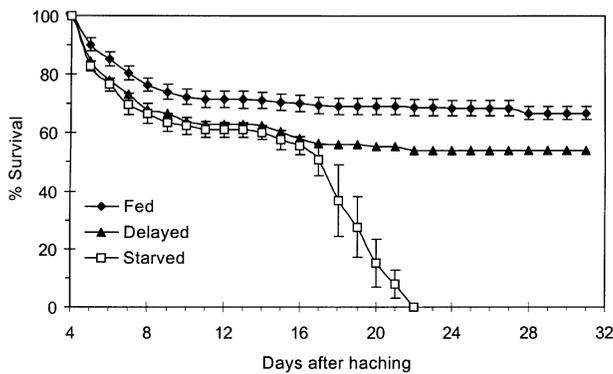


Figure 3. Survival curves for *Dicentrarchus labrax* larvae raised under different feeding conditions. Fed: continually fed; delayed: feeding was delayed until day 13 after hatching; starved: larvae were starved from hatching day. Bars indicate standard errors.

observed in the starved group until day 14, although accumulated mortality was higher. After day 14, the starved group underwent a drastic drop in the number of larvae, all of which died within the period of a week. When larvae from the delayed group were fed, their mortality rate continued dropping for 3 days (following a similar dynamics to the starved treatment larvae), until a steady-state similar to that of fed treatment larvae was attained.

Larvae deprived of food on days 24 and 25 did not show any significant increase in mortality compared to the larvae that were continually fed. Average daily number of dead larvae from the onset of this 2-day starving period until 5 days later did not show significant differences; thus both groups were pooled for the analysis of survival.

3.4. Growth

Growth was described by two-cycle Gompertz curves for both fed and delayed treatments, while the starved treatment group followed a one-cycle Gompertz curve (figure 4). Larvae treated as late fast (food deprivation on days 24 and 25) did not show any significant change in size on the subsequent days compared to fed treatment. It is apparent that all treatments underwent an initial high growth rate up until day 4. After that day, growth rate decreased to a plateau that lasted differently depending on the treatment, but which was similar for all groups until day 14. The fed treatment group showed a second increase in growth rate (GR) from this day (estimated GR on day 13 = 0.60). After urostyle flexion (days 20–22) a conspicuous decrease in growth rate was observed (estimated GR on day 22 = 0.22). Larvae for which the first feeding was delayed until day 13 did not show the second increase in growth rate until day 16 (estimated GR on day 16 = 0.43). The estimated asymptotic size was similar for the fed and delayed treatments. The second growth cycles of the fed and delayed treatment groups were compared and found to be significantly

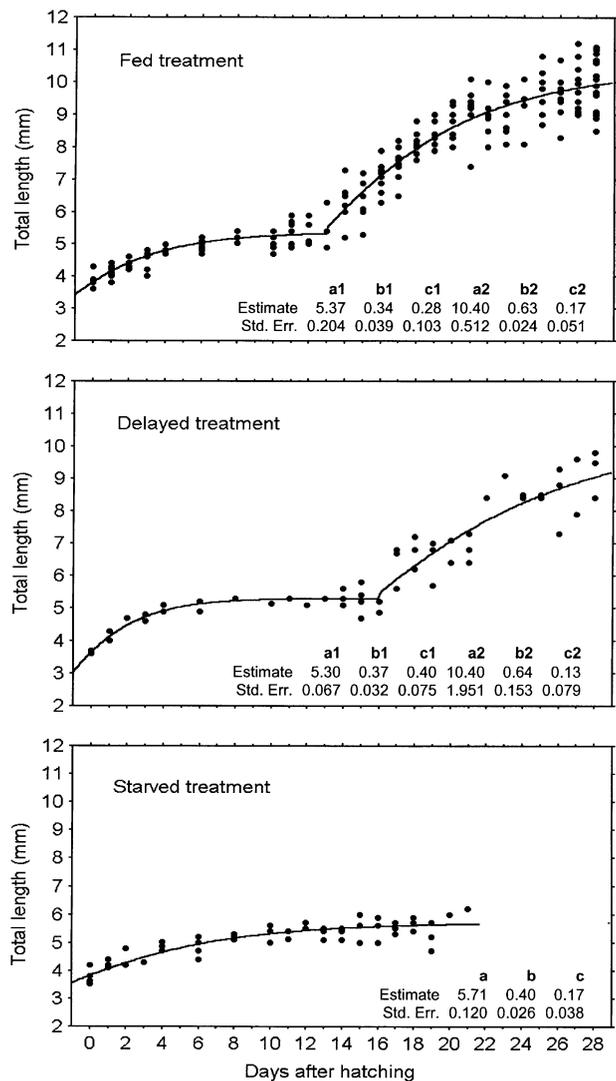


Figure 4. Gompertz fit of total length versus age of *Dicentrarchus labrax* larvae reared under different feeding conditions. Fed: larvae continually fed; delayed: feeding was delayed until day 13 after hatching; starved: larvae were starved from hatching day. Estimated growth parameters and standard errors of the Gompertz curves inside each figure.

different (Hotelling’s test: $T_2(3, 814) > T_{02}(12)$). This difference was due to the lower value of parameter c of the fitted curve in the delayed treatment.

4. DISCUSSION

The most dangerous metabolites for larval development (unionised ammonia and nitrite) are even more important in the first phases of ontogeny [15]. However, in the case of nitrite, its toxicity in salt water is much lower than in fresh water systems. Concentrations of unionised ammonia and nitrite during the present experiment were much lower than those considered dangerous for survival [8, 13, 15].

The system design permitted an easy adjustment of the most important physical and chemical parameters and allowed for the successful maintenance of low levels of ammonia, nitrite and nitrate. However, the system performance was not optimal, as an increase in nitrogen compounds was observed with time (figure 2). There are several explanations for this phenomenon. At the beginning of the exogenous feeding, fast accumulation of food waste could have exceeded the filter capacity (the filter was probably too 'young' so bacteria could not cope with the input of organic matter). Also, feeding with *Artemia* implies the introduction of aerobic bacteria into the system, which can out-compete the nitrifying bacteria [7]. The increase in metabolites towards the end of the experiment could be related to the gradual accumulation of non-captured prey into the mechanical filter.

The observed levels of unionised ammonia ($0.013 \text{ mg}\cdot\text{L}^{-1}$) and nitrite ($0.118 \text{ mg}\cdot\text{L}^{-1}$) in the present experiments, from the start of exogenous feeding and through the first month of life, were lower than those cited as responsible for mortality or decreased feeding ability in larvae and juvenile marine fish. Brownell [8] investigated the tolerance of marine fish larvae to ammonia by monitoring the decrease in first-feeding incidence following a 24-h exposure as the criterion of response. This author found concentrations of unionised ammonia $\text{N}\cdot\text{NH}_3$ between 0.03 and $0.13 \text{ mg}\cdot\text{L}^{-1}$ to be responsible for a 10 % decrease in first-feeding success. Holt and Arnold [15] observed that after 14 days of exposure to $\text{N}\cdot\text{NH}_3$ concentrations of $0.3 \text{ mg}\cdot\text{L}^{-1}$ survival of *Sciaenops ocellatus* larvae decreased.

Percentage hatching and survival in the first week of life obtained in our study lies within the range found in previous works [4, 17, 20]. The maximum larval length also agrees with the literature [9, 17, 22]. The two different growth cycles fitted in the present study show good parallelism with the growth in body length [22] and mg C [5] of larvae reared in semi-enclosed and flow through systems, respectively. In both studies a first phase of slow growth was observed followed by a sharp increase from days 12 to 14, which again decreased by the time of urostyle flexion, as found in the present work. Larval survival in feeding larvae was of the same order or even higher than in previous studies [2, 11, 17]. Therefore, it is unlikely that the obtained values for the main rearing parameters caused any stress on the larvae.

In the marine environment, variation in prey abundance is particularly important for first-feeding larvae [12]. The experimental setting of different nutritional regimes could simulate an uncoupling between larvae and its prey in the wild. The obtained results on growth and survival, which we believed were not affected by other parameters than feeding ratio, could be of value for further studies on trophic ecology in the field. Survival of feeding larvae was higher than that of starved larvae from day 6 after hatching. Nevertheless, larvae that did not receive any food showed low

mortality throughout the second week of life. Sea bass larvae are very resistant to food deprivation during the first stages [5, 17]. This is due to the large yolk sac and oil globule, which are used as energy source during first development [18]. This fact could explain the similarity in the growth curves, regardless of feeding treatment, until day 13 after hatching. Initial high growth rates in the three treatments, up until day 4 (figure 4), was probably due to the conversion of most yolk reserves directly into skeletal growth, measurable as an increment in TL. From day 4 until day 13 a decrease in growth rate was observed, which followed a plateau-shaped curve. During that time, starved and delayed treatment groups could have been using the internal reserves purely as maintenance energy [18]. For the fed larvae, this could be partly related to the inefficiency in the prey capture during the first days of feeding. However, the sudden increase in growth rate after day 13 suggests that the fed animals had been investing the energy in building up body tissues or accumulating reserves in some organs [19].

The initial decrease in larval survival for the starved treatment was observed on day 15 after hatching, which coincided with observed total resorption of the oil globule. Delayed-feeding larvae did not attain a survival stabilisation until 4 days after food was supplied. From that moment on, mortality remained near zero as in the fed treatment. Finally, the effect of 2 days of food deprivation in larvae over 20 days old that had previously been continually fed was not reflected in an increased mortality compared to the fed group.

Growth patterns of larvae for fed and delayed treatments in the second Gompertz cycle showed significant differences. The starting point for the second growth cycle was on day 13 in fed larvae and on day 16 in delayed feeding larvae. The initial growth rate was also lower for delayed feeding larvae, indicating their poorer nutritional condition, which coincided with the observations for *Engraulis mordax* [23]. In our work, differences in size and growth rates were reduced after 2 weeks of normal feeding. Larvae deprived of food for 2 days (days 24 and 25) did not show a significant size variation compared to fed treatment larvae. Further studies on the nutritional condition of these larvae could reveal an effect of these 2 days of food withdrawal.

To summarise, in this work reference values for total and unionised ammonia and nitrite are given that permit suitable rearing conditions for sea bass larvae during the first month of life. In the light of the results presented here, it is concluded that differences in growth and survival among treatments were exclusively due to feeding regime. The high values for survival and growth as well as the normal larval development prove the adequacy of the rearing system. We believe that future nutritional studies of field collected larvae will benefit from this kind of environmentally controlled experimental design.

Acknowledgements. This work was supported by the Comisión Interministerial de Ciencia y Tecnología, Spain (CICYT Projects MAR 97-0902). We are especially grateful to J.C. Navarro (Instituto de Acuicultura de Torre de la Sal) and to R. Flos, A. Juliá, F. Piferrer and R. Villanueva (Instituto de Ciencias del Mar) for their advice on the design of the culture system. E. Berdalet, Ll. Cros (ICM) and C. Hispano (Aquarium

de Barcelona) provided inoculates of algae and rotifers and helped us with the culturing techniques. J. Sánchez and M.L. Martínez instructed us in the use of the facilities for the physical and chemical analyses and G. Fuster offered her technical assistance. Thanks also go to J. Leonart for his assistance with statistical analysis and to I. Palomera and F. Piferrer for their comments on the manuscript.

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