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Otolith growth of European sea bass (*Dicentrarchus labrax* L.) larvae fed with constant or varying food levels

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SUMMARY: Otolith growth and the value and properties of the Recent Otolith Growth Index (ROGI) were studied in sea bass (*Dicentrarchus labrax* L.) larvae that were reared for the first month of life with four different feeding regimes: fed, non-fed, late-feeding and late two-day fast. A marking experiment using alizarin complexone was previously carried out to validate increment deposition. Daily increment deposition was observed to take place from day two after hatching (DAH). The different feeding regimes did not significantly affect the periodicity of otolith increment deposition but did affect increment width. The ROGI was used as a tool for assessing feeding-induced differences in condition. Non-fed larvae had significantly smaller otoliths than fed larvae at the same age. In the late-feeding larvae (food available from 13 DAH), increment width increased progressively once food was supplied, and reached values similar to those for fed larvae after one week of feeding. Deprivation of food for two days in post-flexion larvae (in the fourth week of larval development) was reflected in the formation of progressively narrower increments which had still not returned to normal width two days after feeding was resumed. Our results show that the width of the outermost otolith increments reflect the past feeding history and that the ROGI can be used to distinguish well fed from suboptimally nourished larvae.

Keywords: otolith, recent growth, condition, *Dicentrarchus labrax,* larvae, feeding, validation.

RESUMEN: CRECIMIENTO DEL OTOLITO EN LARVAS DE LUBINA EUROPEA (*DICENTRARCHUS LABRAX*, L.) BAJO RÉGIMEN DE ALIMENTACIÓN CONSTANTE O VARIABLE. – Se estudió el crecimiento del otolito y las propiedades del índice de crecimiento reciente de los anillos (ROGI) en otolitos de larvas de lubina (*Dicentrarchus labrax* L) criadas durante el primer mes de vida bajo cuatro regímenes alimenticios diferentes: larvas alimentadas, privadas de alimentación, con el inicio retrasado de la alimentación, o alimentadas normalmente y sometidas a un ayuno puntual tardío. Previamente se realizó un experimento de marcado con alizarina complexona para validar la frecuencia de deposición de los incrementos en el otolito. El primer incremento se observó a los dos días tras la eclosión. Los regímenes alimenticios no afectaron la frecuencia de formación de los incrementos pero sí a su grosor. El índice ROGI se utilizó como índice de condición nutricional. Las larvas no alimentadas mostraron otolitos siempre más pequeños que los de las larvas alimentadas. En las larvas a las que se les retrasó la alimentación (13 días tras la eclosión), el grosor de los anillos diarios aumentó tras el inicio de la alimentación. Una semana después de comenzar a alimentarse, el grosor medio de las bandas fue similar al de las larvas siempre alimentadas. La retirada de alimento durante dos días de la cuarta semana de vida de las larvas que siempre se alimentaron se tradujo en un decrecimiento del grosor medio de las bandas, detectable el segundo día del comienzo del ayuno. El índice ROGI se mostró útil para detectar las larvas alimentadas de forma subóptima de las alimentadas de forma correcta.

Palabras clave: otolito, crecimiento reciente, condición, lubina, *Dicentrarchus labrax*, larvas, alimentación, validación.

INTRODUCTION

Examining the otolith microstructure provides a dated record of past fish growth which has been used for age determination and as a source of information on growth rate and life history. Daily increment analysis has developed into a powerful tool used to elucidate the course of growth trajectories of individual fish based on the assumption that increment width reflects growth rate (Jenkins and Davis, 1990; Geffen, 1995).

Photoperiod, temperature, and feeding level are the most important factors that affect otolith increment deposition, and their effects are well documented in the literature (e.g. Methot and Kramer, 1979; Pannella, 1980; Campana and Neilson, 1985; Morales-Nin, 2000; Folkvord *et al*., 2004). Suboptimal feeding, through its effects on growth, is regarded as one of the main causes of mortality in marine fish larvae according to the "growth-mortality" hypothesis (Hare and Cowen, 1997), and has been found to halt daily increment formation (Methot and Kramer, 1979; Geffen, 1982). Suboptimal feeding is thus regarded as a significant environmental stressor and a potential brake to developmental success (Swaddle and Witter, 1994; González-Quirós *et al*., 2007), although some works have reported relatively extended periods of starvation to have no effect on increment deposition (e.g. Neilson and Geen, 1985; Maillet and Checkley, 1990).

As was first suggested by Taubert and Coble (1977), analysis of daily increment deposition patterns on the otoliths of early-stage larvae has shown the number of increments to be age-dependent and increment width to be growth-dependent (Karakiri, 1989; Karakiri and von Westernhagen, 1989). However, there may be high species-specificity, and growth-dependence of daily deposition rhythms has also been observed (Folkvord *et al*., 2000). An understanding of these interactions is important when otolith size and growth increment widths are to be used for estimating fish growth (Otterlei *et al*., 2002). Studies on the effects of food levels and the formation of otolith microstructure are still limited. Growth rates of fish and otoliths depend not only on the recent but also on the growing inertia due to past growth (Zhang and Runham, 1992). However, the effect of short-term episodes of food deprivation may affect the survival probabilities of early stages even in relative terms (to past growth), and therefore there are many condition indices that measure the recent physiological status of fish larvae in the larval fish literature (reviewed in Ferron and Leggett, 1994; Suthers, 1998).

The European sea bass *Dicentrarchus labrax* (L) is a highly valued fish that inhabits coastal areas of the Mediterranean and Atlantic, from Morocco to the Irish Sea, Baltic Sea and North Sea (Barnabé *et al.,* 1976). Its ontogeny, laboratory rearing requirements and growth are considered to be well-known (Barnabé *et al.,* 1976; Marangos, 1986), which makes it a suitable species for ecological studies. One requirement for using otolith growth as an indicator of environmental stress is that increment periodicity is well established, as well as the time of first increment formation. Further, the relationship between somatic growth and increment width must mainly depict the factor we are interested in testing, and the variability due to other factors (water quality, temperature etc.) must be adequately accounted for. Previous validation studies on otolith increments in sea bass larvae have proven somewhat inconclusive. Some researchers have reported daily increment deposition in laboratory conditions (Morales-Nin, 1985; Gutiérrez and Morales-Nin, 1986; Ré *et al.*, 1986), but a later study reported that microincrements were laid down every two days (Planes *et al.*, 1991). There is also a discrepancy among investigators concerning initial increment deposition. Morales-Nin (1985), Gutiérrez and Morales-Nin (1986), and Regner and Dulčić (1994) observed the start of increment deposition on day two after hatching, while Ré *et al.* (1986) and Planes *et al.* (1991) observed the first deposition to take place on days seven and three after hatching respectively. Thus, validation of the onset and daily periodicity of otolith microincrement deposition in sea bass larvae was carried out as a preliminary stage in this study.

Although there are data on the effect of feeding regimes on muscle-based condition indices of this species (e.g. Catalán *et al*., 2007), few data are available on the effects of feeding levels on the somatic-otolith growth relationship, as most studies that focus on otoliths have been conducted under *ad lib* food availability (e.g. Gutiérrez and Morales-Nin, 1986; Regner and Dulčić, 1994). The aim of this work was therefore to examine the effect of constant and varying food levels on the otolith microstructure of sea bass, and its potential to be used as a physiological condition index in an ecological context.

MATERIALS AND METHODS

Rearing system and feeding conditions

Two rearing experiments with *D. labrax* larvae were conducted for this study. Firstly, a marking experiment using alizarin complexone (ALC) was performed to validate daily increment deposition. The second experiment used different feeding regimes to establish a condition index for sea bass. Larvae from each experiment were obtained from fertilised eggs from a natural spawning by a single female of Mediterranean origin. Larvae were reared in a small-scale closed recirculation system (with a temperature of 19.5ºC ± 0.85ºC). The system consisted of 12 four-litre cylinders with meshed-ends immersed in a 310 l tank that was connected to a 220 L reservoir tank equipped with a biological filter, and an intermediate 85 L tank where mechanical filtering took place. Each cylinder had an independent water inflow and aerating system. The description of this system and its suitability for experimental work was shown in Olivar *et al.* (2000). Growth and survival of larvae fed *ad lib* in the present work was described in the article cited above and was similar to that obtained from several other culture systems at several scales. Stocking density was aprox. 130 eggs L^{-1}, and hatching success was ca. 64%.

In the marking experiment, larvae were kept without food *ad mortem*. The marking protocol followed Blom *et al.* (1994). The larvae to be marked at any given day were firstly immersed in an ALC solution (50 mg/l) for 24 h, after which they were washed and returned to the rearing tank. Marking began on the day of hatching and took place daily until day 7 after hatching (DAH). The marking procedure was always performed at 11:00 a.m. (GMT). For the marking experiment, 106 larvae ranging from 2.5 mm (3 DAH) to 4.5 mm (9 DAH) total length (TL) were analysed.

For the condition experiment, larvae were reared from fertilisation to 28 DAH. Three feeding regimes were initially established. Fed larvae (5 replicates) were supplied with food *ad libitum* from 4 DAH (one day before mouth opening). Non-fed larvae (4 replicates) were deprived of food for the entire experiment. A minimum of 3 fed and non-fed larvae were randomly sampled from each replicate and frozen for later analysis. No significant within-treatment differences in growth or survival were observed (non-significant interaction term of a general linear model, GLM, on ln-linearised dependent variables, age or length taken as covariates). From 13 DAH, one of the rearing cylinders from the non-fed groups was supplied with food in order to start the late-feeding treatment. The major developmental events were the exhaustion of maternal reserves (yolk-sac on day 7 and oil globule at 13 DAH) and notochord flexion (between 22 and 24 DAH). In addition, to assess the effect of two days of fasting on the otoliths of normally nourished larvae, one rearing cylinder belonging to the fed group was deprived of food for two days (24 and 25 DAH) and re-fed afterwards. This fourth feeding regime was designated as late two-day fast. The size of the analysed larvae ranged from 3.1 mm TL (4 DAH) to 13 mm TL (26 DAH). The larvae were fed at the same time in the morning, and the feeding regime included rotifers, *Artemia* nauplii and 1-day-old enriched metanauplii following the scheme by Barnabé (1991) (see Olivar *et al.* (2000) for further details).

Otolith preparation and analysis

Prior to otolith removal, the TL of each larva was measured to the nearest 0.1 mm. The otoliths were located by means of polarised light under a binocular dissecting microscope. Both the sagittal and lapillar otoliths were extracted, placed carefully on a microscope slide (sulcus side down), allowed to dry, and mounted in DPX. Sagittal otoliths were selected for further analyses after initial examination of the lapillar properties (see results). The location of the right and left otoliths on the slide was always noted so that they could be identified in subsequent analyses. Each pair of sagittae from the condition experiment was examined under a light microscope using polarised light at magnifications ranging from 400x to 1000x with the aid of a computer-enhanced video image-analysis system (Optimas 6.0, Optimas Corp., USA). In contrast, the otoliths from the larvae used in the marking experiment were examined under an epifluorescence microscope (Nikon Diaphot 200). Two images of each otolith were taken at 1000X with this microscope, one under fluorescent light and the other under white light. The two images were then superimposed using the image analysis software for counting the number of increments laid down after the fluorescent mark.

Counts of the increments were made blindly and randomly with regard to age, specimen, length, and feeding regime. Three counts were made for each

otolith; when counts differed by more than 10% of the mean count, the otolith was excluded from further analysis.

Sagittal otoliths were measured to the nearest 0.01 μm by means of the cited image analysis software. For the condition experiment, the following measurements were taken: core diameter, maximum otolith radius and diameter, and the increment reading radius, on the region between the rostrum and the dorso-ventral axis, which is generally the easiest region to read on these otoliths. The width of each of the last seven complete growth increments was measured along the reading transect in order to explore the late growth trajectory of the otolith. The outermost increment was deemed potentially incomplete and was therefore excluded from subsequent analysis. When one of the two sagittae was unreadable for any reason, it was excluded from the analysis and the other otolith was used. When both the left and the right otoliths were readable, the mean for the two sagittae was used in the data analysis, as long as there were no significant differences between the two otoliths (95-% confidence level comparison).

Accordingly, in the marking experiment, otoliths from 81 of the initial 106 larval specimens were usable, whereas for the condition experiment, otoliths from 161 specimens were analysed out of the initial 211, the rest were discarded as neither of the two sagittae was readable. However, for some analyses in order to compare treatments several young larvae were discarded (specified in the corresponding figures), as larvae before 14-16 DAH showed similar growth patterns (Olivar *et al*. 2000) due to the existence of oil-globule reserves.

Condition index

The Recent Otolith Growth Index (ROGI) proposed by Hovenkamp and Witte (1991) was explored with regard to its ability to describe the recent feeding history of the larvae. Increment widths cannot be compared with each other directly, as they are at least partly dependent on otolith size (Hovenkamp, 1990). The method is based on analysis of the residuals of the relationship between the sum of the widths of some of the most recent increments and the length from the core to those increments. The rationale for residual analysis is that since a residual is a measure of an individual's departure from the population, it can be viewed as an indicator of condition. According to this relationship, when increment width is larger than expected, the residuals of the regression will be positive, which means that otolith growth has been above average. When increment width is smaller than expected, the residuals will be negative, which means that otolith growth has been below average. We analysed the residuals of the relationship between the width of the last three complete growth increments and otolith radius at the time of formation of the first of these increments for the control (fed) group. This implies that we assumed the fed group to be the standard for maximum growth at the given temperature. Both variables were log transformed so that the variances would be independent of the mean.

Statistical analysis

In both experiments the number of increments observed was fit to the real age of the larvae by linear regression when possible. In addition, the differences between real age and expected age for each single day were tested using a 1-sample t-test in the marking experiment. Differences in increment numbers among feeding groups were tested through ANOVA techniques with post-hoc Tukey's HSD tests. The relationships between larval size and otolith size for the different feeding groups were fit by power equations, and slopes were compared using the aforementioned techniques on ln-linearised variables, using GLM Procedures. Differences in ROGI were also tested by ANOVA. Significance levels for all analyses were set at $\alpha = 0.05$. All statistical tests were performed using Statistica 7.0 (Statsoft Inc., USA).

RESULTS

The relationship between otolith size and larval size was initially considered both for the sagittae and for the lapilli. In the earliest days of life, both pairs of otoliths showed similar radius sizes (Fig. 1). The nucleus size was comparable for sagittae (17.67 ± 1.87 μm, n=161) and for lapilli (17.01 ± 2.02 μm, n=137). As larval growth progressed, the sagittae deposited wider increments which sped up otolith growth (Fig. 1) and facilitated their manipulation; thus, only the sagittae were analysed further.

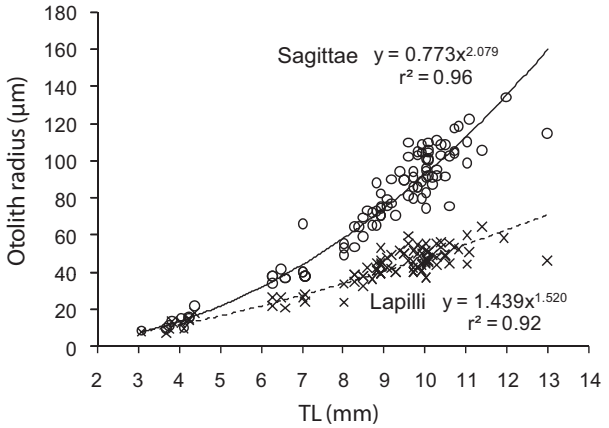

FIG. 1. – Growth of sagittal (open circles) and lapillar (crosses) otoliths (as measured along the longest radius) on total fish length (TL) for the fed larvae from both experiments up to 28 days of age (n = 97). All data points plotted are the means for the left and the right otoliths.

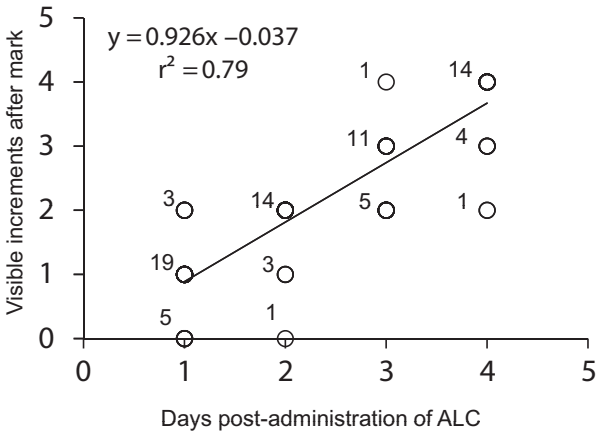

FIG. 2. – Relationship between the number of days subsequent to immersion of larvae in ALC and the number of increments visible after the fluorescent mark. The regression was fit using all the points (numbers shown close to each dot). Each observed mean marked band was only non-significantly different from its 1 band/1 day correspondence (t-tests, all p>0.05).

Validation of increment deposition

Observing the marked (with alizarine complexone) otoliths showed that there was no evidence that, on average, increments were laid down other than daily. For each day after hatching, an average of one marked increment was observed (Fig. 2). T-tests showed that bands were only non-significantly different from their corresponding real age (all t-tests with p>0.05), as the slope of the regression was close to 1 (Fig. 2). To analyse the effect of the different feeding regimes on the periodicity of increment deposition, the number of increments counted on the sagittae were fit to the known larval age (DAH) for each feeding treatment. The slopes obtained were not significantly different from unity (Table 1, t-tests, p>0.05), thereby corroborating that otolith incre-

TABLE 1. – Linear regression parameters for the relationship between observed increments (dependent variable) and real days (independent variable) for each feeding treatment for which more than 4 days were available. n is the number of observations, a is the intercept, b is the slope, r^2 is the determination coefficient and SE is the standard error. All regressions were significant at p<0.0001.

	N. days	N. individuals	a (± SE)	b (± SE)	r^2
Fed	22	108	-2.401(0.164)	1.007 (0.008)	0.99
Non-fed	5	14	-2.392 (1.821)	1.018 (0.100)	0.89
Late-feeding	6	12	-1.959 (1.968)	1.013 (0.104)	0.90

TABLE 2. – Mean maximum otolith diameter (µm), standard deviation (SD) and sample size (n) for *Dicentrarchus labrax* larvae aged 16 to 28 DAH. For a given day, different letters at the mean values indicate significant differences across feeding treatments. In the last group, numbers in bold refer to the larvae sampled during the short food-deprivation event, which were re-fed in subsequent days. Differences were tested using ANOVA (3 groups) with Tukey's HSD, or t-tests (two groups). The sample size differs from Table 1 because some otoliths could be read for diameter (and were included) but not for daily increments.

	Fed			Non-fed			Late-feeding and late 2-D feeding		
Age (DAH)	Mean Otolith diameter	SD	n	Mean Otolith diameter	SD	n	Mean Otolith diameter	SD	n
16	94.53[a]	4.88	5	59.99[b]	4.89	3	63.06[b]	8.88	4
17	106.40[a]	11.72	4	61.83[b]	6.79	3	74.88[b]	6.03	5
18	102.37[a]	29.07	3	76.34[b]	13.47	4	88.30	0	1
19	133.83[a]	21.73	2	65.25[b]	4.26	2	72.24[b]	3.78	3
20	118.82[a]	34.54	2	83.70[b]	9.41	2	66.49	0	1
21	161.19[a]	4.00	2	59.99[b]	4.89	4	130.83[c]	8.69	4
22	135.11	0	1				90.24	0	1
23	188.28	0	1				139.60	15.22	3
24	187.48[a]	4.11	3				**153.75[b]**	**16.34**	**8**
25	194.06[a]	32.20	4				**162.61[a]**	**13.46**	**3**
26	217.04[a]	22.65	12				163.94[b]	14.32	3
27	222.29[a]	31.02	15				220.54[a]	27.94	12
28	248.22[a]	14.79	17				223.18[b]	34.66	8

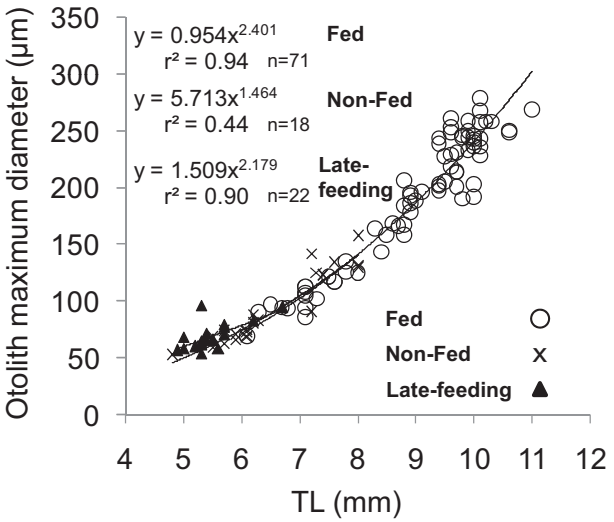

FIG. 3. – Relationships between otolith size of the condition experiment (maximum diameter) and larval body size (total length, TL) for the three main feeding treatments (the length range was too small in the fourth treatment for regression analysis). The sample size is indicated by n.

ments are deposited on a daily basis. The regression lines intersected at the x-axis at a point very close to an age of two days, which indicates that otolith increment deposition in sea bass larvae commences two days after hatching.

Otolith growth

For the three main experimental feeding regimes the relationship between otolith size (diameter) and larval size (TL) was described by a power equation (Fig. 3). The differences in otolith growth between fed and late-feeding larvae were not significant (t-test on slopes of ln-transformed variables, $p>0.05$). This indicates that length was only related to otolith diameter when it interacted with extreme feeding conditions.

However, when mean otolith size at age was analysed, appreciable differences appeared among the main feeding treatments (Table 2). Mean otolith diameter of fed larvae continuously increased until the end of the experiment. The otoliths of non-fed larvae were consistently smaller than those of fed larvae. Late-feeding larvae showed values that were not significantly different from the non-fed larvae until 20 DAH, and otoliths from both treatments were always significantly smaller than those of fed larvae until 20 DAH. On day 21 after hatching, otoliths from non-fed larvae were smaller than both the fed and late-feeding larvae, and late-feeding larvae had otoliths that were larger than those of non-fed larvae but smaller than those of fed larvae. The smaller size of otoliths of suboptimally fed larvae was directly related to lower mean somatic growth rates observed during this period. The otoliths of the late two-day fast larvae (day 26-28) tended to show lower mean otolith diameters with respect to fed larvae (Table 2).

Following verification that suboptimal feeding led to decreased otolith growth, otolith growth increment was analysed to examine how increment width responded to the different feeding regimes (Fig. 4). The width of the last complete increment was larger in the fed larvae than in larvae reared under no food or late-feeding regimes already before the last maternal reserves were exhausted on day 13 (Fig. 4). Increment width of fed larvae progressively increased from 2.55 ± 1.32 µm on 10 DAH to 5.63 ± 1.01 µm on 21 DAH. Increment width in the non-fed larvae did not vary with larval age ($r = 0.0$, $p = 0.96$) and ranged from 1.22 ± 0.20 µm (11 DAH) to 1.59 ± 0.56 µm (17 DAH).

For a given age, increment widths of non-fed larvae were significantly narrower than those of larvae that had always had access to a food supply (Multiple t-tests for all days where $n>3$ in each treatment, d.f. ranging from 4 to 22, $p<0.05$). From 13 DAH food was supplied to larvae to begin the late-feeding treatment; increment width of these larvae began to increase on the following day (Fig. 4), until, by 21 DAH, these larvae had resumed daily increment growth with respect to fed larvae. The effect of two days of food deprivation (days 24 and 25 after hatching) on increment width in post-flexion larvae

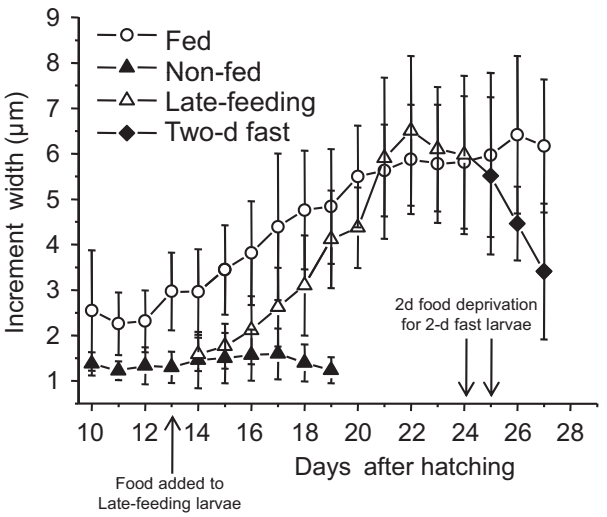

FIG. 4. – Absolute increment widths on the sagittae under all four feeding regimes.

was not noticed until two days after the fast (Fig. 4). Even two days after feeding had resumed, on day 27, the average increment width of the late two-day fast larvae was ca. 2 μm smaller than prior to the food deprivation episode (Fig. 4).

Condition index

In order to remove size-dependence, a linear regression was used to fit the ln-transformed sum of the widths of the last three complete increments vs. the ln-transformed otolith radius at the time of deposition of the first of these increments for the fed larvae, which yielded a strong, significant linear relationship (Fig. 5). When the data for the non-fed and late-feeding larvae were graphed, it was seen that non-fed larvae showed significantly lower values than the fed larvae (Fig. 5). However, some of the values for the youngest (smaller) fed larvae tended to be confused with values of the starved larvae. The late-feeding larvae showed a mixed size range, with some of them being closer to the non-fed larvae and others within the otolith growth-line of the fed treatment. The ROGI values of non-fed larvae were mostly negative (Fig. 6), and were significantly different to those of fed larvae at all ages when more than 3 larvae were available for comparison (t-test, d.f. ranging from 7 to 13, $p<0.05$). The otoliths of the late-feeding larvae also yielded negative ROGI values at first, but later the values began to increase gradually and eventually caught up with the values for the fed larvae. Overall, the residuals for the late-feeding larvae were significantly different (t-test, d.f. = 108, $p<0.05$) from those for the fed larvae.

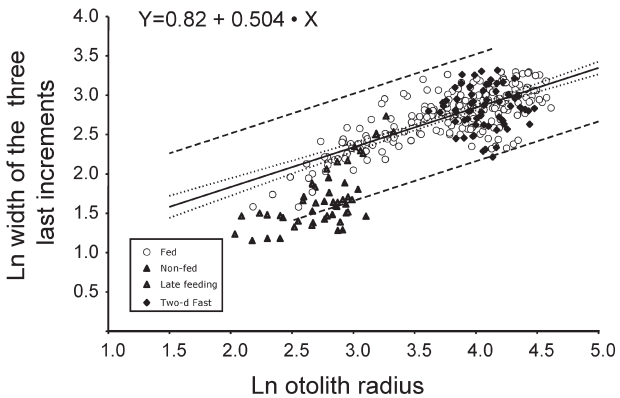

FIG. 5. – Relationship of the otolith radius (μm) vs. the width of the three outermost complete growth increments (μm) for the fed larvae (solid line, Ln-transformed units). Broken lines: 95-% confidence interval (CI) for the mean (inner CI) and observations (outer CI). The data from the other treatments are superimposed but are not used for the regression.

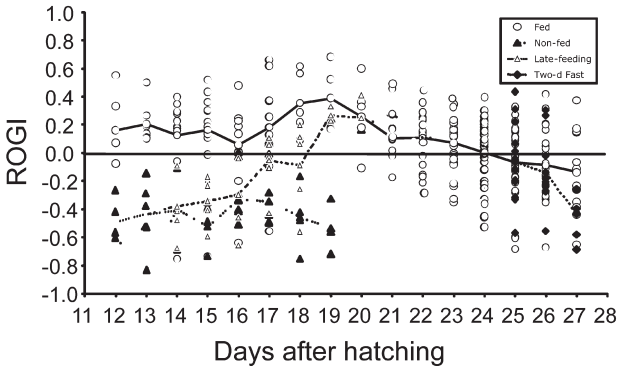

FIG. 6. – Residuals plot (Recent Otolith Growth Index, ROGI) for the four feeding regimes.

However, when the residuals were compared at age, differences were no longer significant from day 17 (t-tests, d.f. ranging from 7 to 18, $p>0.05$ for all ages from day 16), which suggests that the late-feeding larvae began to recover four days after food began to be supplied.

In the larvae that underwent a short, late two-day fast (Fig. 6), the index furnished the same information as in the cases just referred to above, and the two-day fast was observed to decrease the ROGI values by day 27 after hatching.

DISCUSSION

Validation of the increment deposition frequency is a prerequisite for otolith studies in all species, and marking using fluorescent compounds is one of the most commonly used techniques (Geffen, 1992). Since Hettler (1984) first described marking otoliths by immersing the larvae in a marking solution, this method has been improved and modified to adapt it to a range of different conditions (Dabrowski and Tsukamoto, 1986; Tsukamoto *et al.*, 1989; Secor *et al.*, 1991). Marking using alizarin complexone revealed that growth increments in *D. labrax* were laid down daily as previously described by Morales-Nin (1985), Gutiérrez and Morales-Nin (1986), and Ré *et al.* (1986). In addition, the present marking experiment showed that growth increments continue to be laid down daily on the otoliths irrespective of the larval feeding regime employed. While environmental conditions have been reported to mask or alter the periodicity of increment deposition (Campana and Neilson, 1985; Berghahn, 1989), our results indicate that even periods of prolonged starvation do not affect increment deposition periodicity. The results obtained indicate that sea bass larvae begin to lay

down growth increments on their otoliths two days after hatching, which is in agreement with the results observed by Morales-Nin (1985), Gutiérrez and Morales-Nin (1986), and Regner and Dulčić (1994).

Generally, the number of growth increments on the otoliths is a factor of age, while increment width is a factor of larval growth (Volk *et al.*, 1984). It therefore follows that growth increment width of sea bass otoliths, i.e., otolith microstructure, can be used to calculate an index that reflects the feeding conditions experienced by individual larvae (Karakiri, 1989). Otolith growth increment width has been used by different investigators in the past as an indicator of variations in certain environmental factors, including temperature, salinity, light, upwelling intensity, oxygen deficiency and feeding conditions (Gutiérrez and Morales-Nin, 1986; Morales-Nin, 1987; Koutsikopoulos *et al.,* 1989; Karakiri, 1989; Folkvord *et al*. 2004).

In the present study growth increment width of the otoliths of larval sea bass furnished information on the feeding conditions experienced by the larvae. Certain minimum resources appear to be allocated for otolith growth even under adverse conditions, and increment deposition goes on without interruption even in larvae suffering from food stress. The narrower increments in the otoliths of non-fed larvae with respect to larvae feeding at several intensities agree with previous observations on other species (Rice *et al*., 1987; Maillet and Checkley, 1990). Thus, larvae that have never been fed can be said to exhibit baseline growth, by which narrow increments are deposited to yield similarly-sized otoliths. When the larval stage of this species are cultured, a relatively common practice is to keep the larvae unfed in the dark for the first 10 days of life. By doing this, the rotifer feeding stage can be avoided. It would appear that the reserves available to the larvae during this stage are sufficient to allow normal development (McVey, 1983). Otolith size is one of the most important attributes that enable the otolith to fulfil its functions within a fish's inner ear (Morales-Nin, 2000). This probably explains why, once they began feeding, the otolith of larvae that had undergone protracted fasting (until 13 DAH) grew at a faster pace than larvae of the same age that had been well fed (Fig. 4). The same phenomenon can be seen in the growth of larval length in this experiment (see Olivar *et al.*, 2000). Therefore, there is a good agreement among food variation, somatic growth and otolith growth. Analysis of the otoliths from the larvae that underwent the late two-day fast revealed a metabolic lag time of two days between the episode of food deprivation and the corresponding imprint on the otolith, as described by Campana and Neilson (1985). Other researchers have observed the same phenomenon when other environmental factors were tested. Gutiérrez and Morales-Nin (1986) found that the effects of low temperature on sea bass larvae appeared on the otoliths after a delay of three days. Neilson and Geen (1985) observed a delay of around three weeks before changes in increment size related to feeding frequency became discernible in *Oncorhynchus tshawytscha* otoliths, and Molony and Choat (1990) reported that changes in the otoliths of *Ambassis vachelli* were not observable until 10 to 15 days after there had been a change in body growth rate.

Studying the whole otolith of wild-collected specimens integrates all past environmental variation. However, the last increments can be related to recent environmental conditions (but see below), which can be assumed to be similar to those registered at the day of sampling in young planktonic stages. Recent otolith growth analysis has been used in a number of experiments to determine the effects of variations in certain environmental factors on fish growth (Methot, 1981; Govoni *et al.*, 1985; Hovenkamp, 1990; Maillet and Checkley, 1991; Suthers and Sundby, 1993; Suthers, 1996; Morales-Nin *et al*., 2002). The number of growth increments used in these studies (ranging from one to twenty) depended on the species, the age of available specimens, and the environmental factor considered. The results obtained in the present study indicate that the condition index tested (using the three outermost complete growth increments) has high potential for evaluating the recent food status of sea bass larvae collected from the natural habitat, as previously pointed out by Powell *et al.* (1990). The ROGI is only a measure of relative condition when applied to wild samples, as residuals will depend on the reference population used for analysis. The ROGI, at least for this species, adds to other condition tools and it should be better exploited when the age of individuals is needed, as otolith processing is a requirement.

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