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Changes in biochemical composition and otolith microstructure of larval common soleas, *Solea solea* (L.) under experimental starvation*

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Abstract: Experiments on starvation were carried out at 12 and 19℃ on larvae of *Solea solea* (L.), in order to investigate indices of nutritional state through their biochemical composition and a potential storage of informations thanks to modification of their otoliths.

At 19℃ the increase of dry weight and biochemical compounds during the early ontogeny of fed larvae was more speeded up than at low temperature, and protein deposition was more promoted than storage of energetic lipid. Dry weight and actual amounts of proteins, free amino acids and triglycerides quickly decreased in starved fish and could be used as nutritional indices for reared larvae. Nevertheless, as the essential to non-essential amino acid ratio, they were too dependent upon ontogenetic stages to be used in the field. Because sterols remain constant under starvation, the triglyceride to sterol ratio appears to be a very sensitive and more reliable indice, provided that age is roughly estimated.

Both temperature and starvation modified the larval and sagittal growth parameters, resulting in variable larva-otolith size relationships. Low temperature produced smaller otoliths for equal-sized larvae while, at the same temperature, otoliths were bigger for starving larvae. In early starved larvae, somatic growth was arrested, whereas deposition of low-contrast increments, as observed before first-feeding, was generated. Identified to stress marks, low-contrast increments increased variability of age estimates, although a daily rate of deposition was verified after the onset of exogeneous feeding. Observed in sea-caught larvae, they represent complementary informations on he larva story prior to capture.

1. Introduction

Among all the factors controlling fish recruitment, starvation during early life and duration of the larval and juvenile phases have been suspected for a long time to be two of the main components influencing survival (MAY, 1974). In the field, starved larvae have been detected (e.g. THEILACKER, 1986) and differences in growth rate have been pointed out (BUCKLEY and LOUGH, 1987; HOVENKAMP, 1990). But stunting larvae may be quickly eliminated by predators (BAILEY and HOUDE, 1989), and methods have to be sensitive enough for early diagnosis of starvation and as simple as possible for sea-sample use. Starvation has been known not only to lead to protracted larval life, but also to modify the deposition rate of otolith increments for larvae reared under suboptimal conditions (GEFFEN, 1982). Reliability of age estimates for slow-growing larvae has been opened to questions (RICE, 1987) and changes in otolith microstructure have been investigated under light and electron microscopy (JONES and BROTHERS, 1987; CAMPANA *et al.*, 1987).

The common sole is serial spawner and, on the French Atlantic coast, lays its eggs from late winter to spring (ARBAULT *et al.*, 1986). Seawater temperature and food availability may vary enough during the spawning season to significantly modify the sole larval condition and consequently their growth rate. Therefore, the first step of our studies was to identify the effects of starvation in early life through changes in biochemical contents and otolith microstructures in order to define biochemical indices unabling us to state, as early as possible, the quality of any larva caught from the sea, and to investigate a potential storage of information relative to fasting on the otoliths (RICE *et*

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al., 1985). Besides, we also need to appraise how microstructural alterations conflict with validation of the ageing technique for otliths of this species.

2. Materials and methods

Experimental conditions

Experiments were carried out in the aquaculture facilities of IFREMER Center, in Brest, Sole larvae were reared under two thermal regimes (19-12℃) and constant photoperiod (LD 18:6), and were fed *Artemia nauplii*. Control larvae were fed once a day as soon as their mouth opened, i.e. on day 2 and 4 at 19 and 12℃, respectively. Larvae were submitted to starvation at early ontogenetic stages; either complete starvation (19 and 12℃ experiments) or delayed first-feeding of 2 to 5 days (point of no return determination at 19℃). The sampling methodology is detailed in LAGARDERE (1989), BOULHIC and GABAUDAN (in press), and RICHARD *et al.*, (in press).

Dry weight estimations

Dry weight estimations were carried out individually for protein and amino acid analyses (RICHARD *et al.*, in press), and on pools of 50 to 5 larvae for lipid analyses. In this case, the larvae were counted, rinsed with an ammonium formate solution, and deposited onto a clean, pre-weighted glass-fiber filter. The samples were then freeze-dried and weighted on a microbalance.

Biochemical analyses

Total proteic content was determined after acid hydrolysis and derivatization with an OPA reagent on single larva homogenates through spectrofluoremetric measurement of primary amines (RICHARD *et al.*, in press). After extraction by trichloroacetic acid on the same homogenate, amino acids were derivatized either with OPA for primary amino acids (LINDROTH and MOPPER, 1979) or with FMOC-Cl for secondary ones (EINARSSON, 1985), and measured by HPLC and spectrofluorometry.

For lipid analyses, pools of 50 to 5 larvae were extracted according to FLOCH *et al.* (1957). Concentrated aliquots of the extracts were deposited onto Chromarods, and total lipids were measured with a Iatroscan TH10 (TLC-FID). Lipid classes were separated on Chromarods using different mixtures of hexane, diethylether and formic acid, and then determined on the Iatroscan (PARRISH, 1987).

Larval and otolith measurements

Larvae were staged and SL measured to the nearest 0.1 mm under a biocular microscope. The gut content was examined in order to evaluate the efficiency of feeding. Otoliths were removed from their otic capsules and were mounted whole on ultrathin cover slips, in a drop of PERMOUNT. They were observed and measured under light microscopy with a magnification of x1,250 and the use of immersion oil. The resolving power was in the 0.2-0.5 μm range. Micrographs were taken with an additional green filter (546nm). Increments were counted from micrographs of otoliths from the 12℃ reared larvae only (N=145). Some comparisons were made between those counts and the results of blind readings obtained by image analysis (N=26). This method, developed at the IFREMER Brest Center, utilizes a technique of pattern recognition where the growth dynamic is taken into account via a structuring function, θ, defined as the reciprocal growth function (H. TROADEC, unpublish. data).

3. Results

Survival and food intake

Disregarding temperature and first-feeding dates, survival varied (41-94%) but declined drastically after the point of no return PNR (0-2.6%). In the 19℃ experiment, the PNR was reached at day 7, i.e. 5d after first-feeding of the control or 2d after yolk resorption (Fig. 1B). The PNR was not determinated at 12℃ but it may be estimated as double because larvae survived 9d (19℃) and 18d (12℃) of complete starvation.

The intake of food immediately followed the *Artemia* supply, even when first feeding was delayed in the 19℃ experiment. First feeding did not start before day 6 in the 12℃ experiment, although food was provided at day 4. Low temperature had probably reduced the activity of early larvae, and

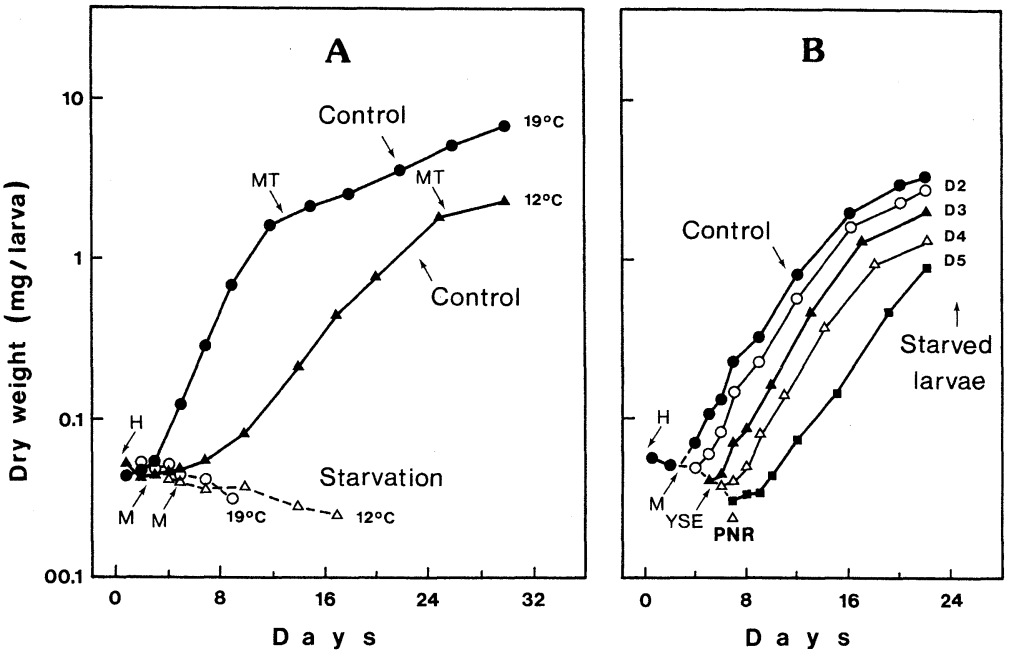

Fig. 1. Variations of individual dry weight in *Solea solea* fed and starved larvae at 12 and 19℃ (A), and effect of delayed feeding at 19℃ (B). H: hatching, M: mouth opening, YSE: Yolk-sac exhaustion, MT: metamorphosis.

thus their feeding ability on preys of large size, so that those larvae were not a good control (LAGARDERE, 1989).

Dry weight variations

The individual dry weight of control larvae increased following the onset of feeding. This increase was two fold quicker at 19℃ than at 12℃ (Fig. 1). Growth curves indicated that experiments lasted long enough to produce juveniles at 19℃. Even if soles began swimming as flatfish at 12℃, their transformation was not completed. Change in swimming behaviour occurred around days 12-13 at 19℃ and days 24-26 at 12℃. A reduction of the growth rate was then observed.

Starved larvae lost weight more rapidly at 19℃ than at 12℃. When larvae were submitted to a late first-feeding, the weight increased just after the first food intake, except for the survivors of the 5d-starved group. However, the initial delay in growth caused by starvation was not recovered, especially in the last group.

Total protein and free amino acid variations

During total starvation experiments (Fig. 2A), protein content per larva dropped till day 2 at 19℃ as vitellus was resorbed. It increased soon after mouth opening in fed larvae while it went on decreasing in starved ones. The larvae still alive on day 9 at 19℃ lost half of the protein content they had on hatching. Protein content rise was slackened upon metamorphosis in fed larvae. The same trends were noticed at 12℃, with some staggering on the longer time: proteic content was equivalent at 12 and 19℃, for a same ontogenetic stage. Relative to dry weight (not shown here), protein content was always lower in starving larvae than in fed ones after yolk sac exhaustion. During PNR experiments (Fig. 2B), protein content restoration after feeding showed that there was no compensatory effect: the batches of larvae kept the same differences all along the experiment. The recovery seemed good even for the 4d-starved larvae but not for the 5d-starved group: protein content restoration started only on day 12 and the large variability noticed thereafter occurred at the same time as high mortality.

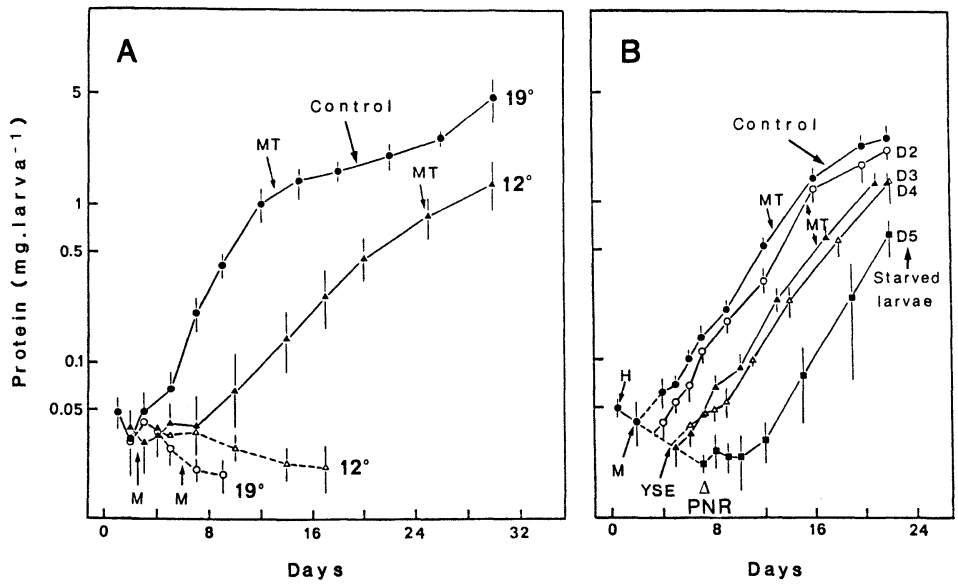

Fig. 2. Variations of total protein content in *Solea solea* fed and starved larvae at 12 and 19℃ (A), and effect of delayed feeding at 19℃ (B). Further specifications as in Fig. 1.

Total free amino acids were strongly lowered by starvation. As essential amino acids can only be taken from food, they were very sensitive to starvation and therefore the essential to non-essential amino acid ratio was also very quickly affected (Fig. 3). It decreased during starvation from 0.85 to 0.27 and to 0.35 at 19℃ and 12℃ respectively. However, it showed large variations during the normal early ontogeny of sole: at 19℃ it decreased sharply during the 5 first days, then rose and decreased again to a very low level after metamorphosis. At 12℃, the peak before metamorphosis was delayed and was higher but there was also a large decrease thereafter.

Variations in lipid composition

The triglyceride level showed a decrease linked to the utilization of yolk reserves (Fig. 4). After the onset of feeding, the level quickly increased up to a maximum value corresponding to metamorphosis. This value is much higher at 12℃ than at 19℃. When no food was ingested, the triglyceride level decreased dramatically. The minimum of the curves corresponded to the yolk-sac exhaustion. After a delayed first-feeding, 2d and

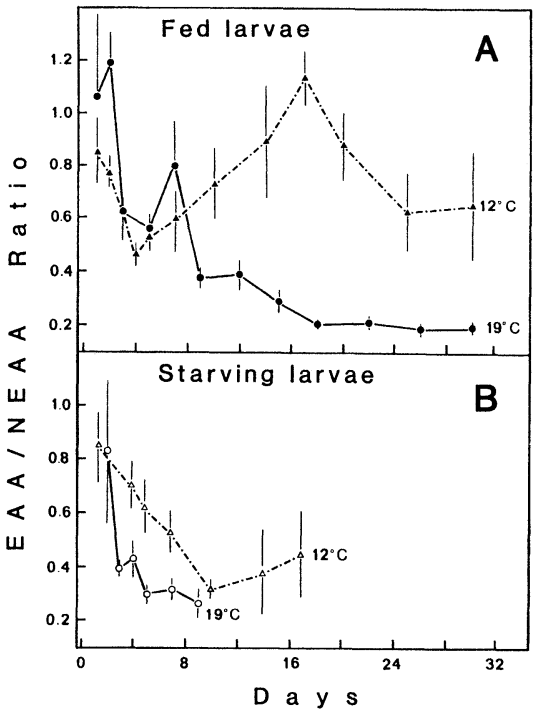

Fig. 3. Variations of essential to nonessential amino acid ratio in Solea solea larvae, fed (A) and starved (B) at 12 and 19℃.

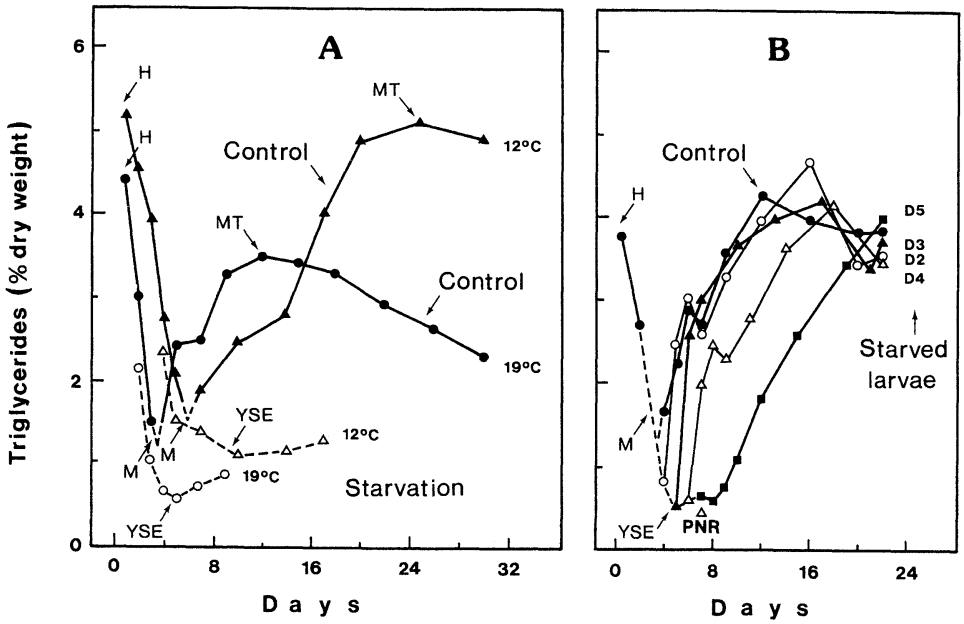

Fig. 4. Variations of triglyceride level in *Solea solea* fed and starved larvae at 12 and 19℃ (A), and influence of delayed feeding at 19℃ (B). Further specifications as in Fig. 1.

3dstarved larvae quickly recovered normal levels of triglyceride and then, showed no clear differences as compared to the control group. 4d-starved larvae had some difficulties in recovering normal values, while the survivors of 5d-starved group could not reach the level associated with metamorphosis during the experiment. Total lipid variations, not reported here, showed approximately similar trends, but they were not so accute.

Replenished larvae always showed a higher triglyceride content than the sterol one, the contrary occurring in starving larvae (Fig. 5). If the amount of triglyceride could be related to feeding or starvation, sterol content was less dependent on the larval nutritional state, and this pattern was not affected by temperature. The largest difference between triglyceride and sterol contents corresponded to the PNR. Hence, a triglyceride to sterol ratio can be calculated. Just following hatching, values higher than 2 were observed. The ratio dropped to 1 before first feeding of the control. For this group, it quickly increased up to 2.6 (19℃) or 3.1 (12℃) before metamorphosis. In starved larvae, the ratio fell dramatically to 0.6 (12℃) or even to 0.4 (19℃), when the PNR was reached.

Changes in the incremental pattern of the sagitta

Larval and sagittal growth curves allowed within-temperature comparisons between specific growth rates (b parameter, Table 1a). Subdaily rings were discriminated when increment width increased (Fig. 6A-B and 7A-B). The larva-sagitta length relationships varied significantly, according to the temperature, with a steeper regression in the 12℃ experiment (Table 1b, slopes were significantly different, t-test, $p<0.05$), i.e. smaller otoliths for equal-sized larvae. When food limitation reduced the growth rate at the same temperature (19℃ experiment of delayed first-feeding), inverse consequences arose from these relationships, slowly growing larvae (survivors of the 5d starved group) having bigger otoliths than equal-sized larvae from the control (LAGARDERE, 1989). Otolith measurements of early larvae under starvation demonstrated their continuous but decreasing daily growth until death, while somatic growth was arrested and larvae shrunk through stress.

The incremental pattern of otoliths from the 19℃ reared larvae indicated that except

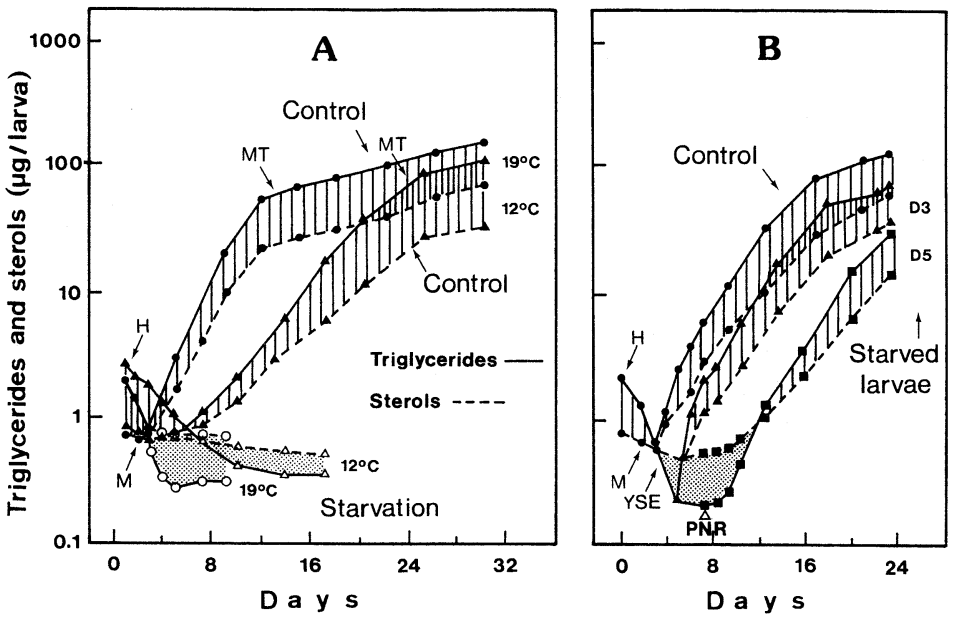

Figure 5. Variations of triglyceride and sterol amounts in Solea solea fed and starved larvae at 12 and 19℃ (A), and influence of delayed feeding at 19℃ (B). Further specifications as in Fig. 1.

for ontogenetic checks, the formation of well-defined increments followed the onset of exogeneous feeding (Fig. 6A). Only hyaline material (i.e. without clear daily increments) was deposited during embryogenesis (Fig. 6 C, band 1) and this pattern was extended during starvation as long as larvae had endogeneous supplies to use (Fig. 6C, band 2 and 3). A significant reduction in width of daily deposits interfered once the yolk-sac was exhausted (band 3). Those deposits were developed into stress bands until somatic growth resumed (Fig. 6D). They contributed to enhance checks, previously identified (LAGARDERE, 1989) at the time of hatching (h), mouth-opening (m) and yolk-sac resorption (y). Low-contrast increments were observed in otoliths of the supposed control larvae from the 12℃ experiment (Fig. 7A-B), and they were related to a delay in first-feeding of 2 to 4d.

The use of the ageing technique was validated

Table 1. Influence of rearing temperatures on the somatic and sagittal growth relationships of sole larvae.

(1a) Specific growth rates (in percent as derived from exponential growth curves of pelagically swimming control larvae (see for further details LAGARDERE, 1989)

	larval length	sagittal diameter
12℃ experiment (days 1-26):	3.45	7.25
19℃ experiment (days 0- 9):	9.23	16.63

(1b) Summary statistics of the larva-sagitta length relationships (in transformed date of control larvae from both experiments)

lnSL = a lnSAG+b: Regression parameters

	intercept	se	slope	se	N	R	R2 percent
12℃ experiment	−0.0779	0.0267	0.4753	0.0064	172	0.98	96.99
19℃ experiment	0.0091	0.0468	0.4490	0.0112	95	0.97	94.53

through counts of increments deposited in the otoliths of the 12℃ reared larvae because of their close resemblance with those of field-sampled specimens (Fig. 7 A-B and D-E). Counts were processed from the m check, whose age at formation depended upon temperature (day 4), while initiation of uninterrupted daily increment deposition varied with age at first-feeding (LAGARDERE, 1989). Reliability of age estimates (i.e. count +3) was tested because of the low contrast of unit increments, and to compare readings from micrographs or image analysis. The regression of increment counts against days was best fitted by a linear model ($y=-0.5601 + 1.0052\ x$; $N=145$ $R=0.99$). The deposition

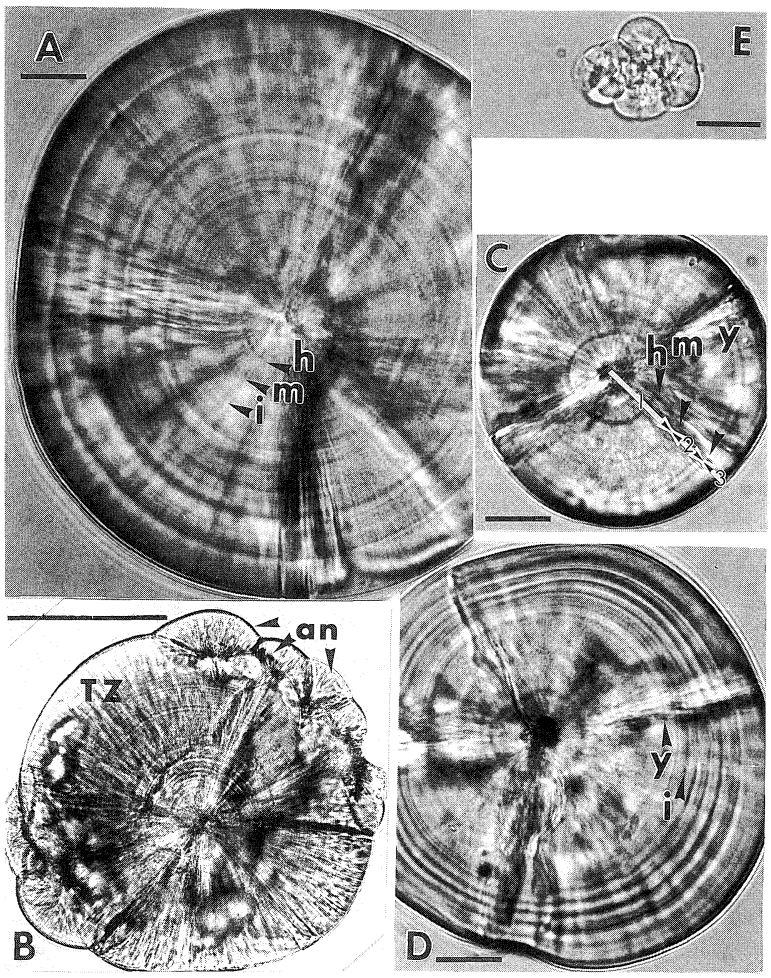

Fig. 6. Sagittal otolith of larval sole (Scale bars: ACDE = 10 μm B = 100 μm).
A-D (19℃ rearing experiment) - A: 10d old control larva, the first defined increment (i) indicates yolk absorption and day 5; B: 14d control larva; C: 10d old larva of the 5d-starved group, low-contrast bands correspond to increments deposited during embryogenesis (1), before yolk absorption (2) and before growth resumption (3); D: 15d old larva of the 5d-starved group.
E: sea-sampled larva at hatching, see multiple primordia of the nucleus coalescing.
(an:accessory nuclei; h, m, y: hatch, mouth-opening and yolk-sac exhaustionch ecks; i: first well-defined increment, N: nucleus; TZ: transition zone of metamorphosis)

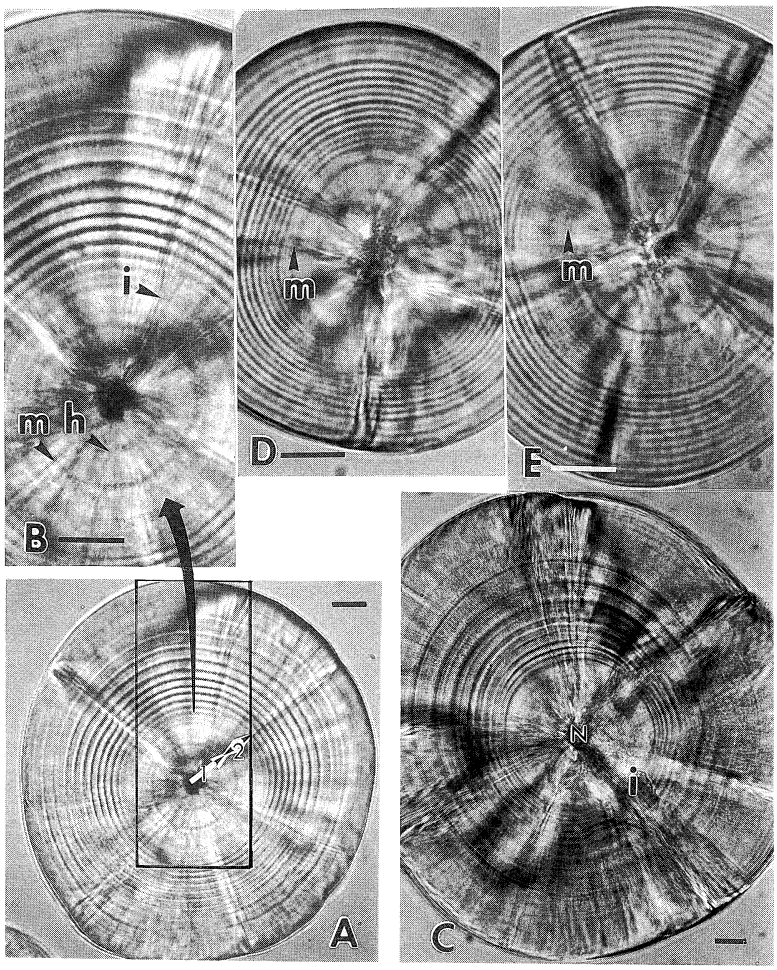

Fig 7. Sagittal otoliths of laval sole (Scale bars: 10 μm)
A-B: 22d old control larva (12℃ experiment)
C : 22d old larva from the 5d-starved group (19℃ experiment)
D-E: Sea-sampled larvae with an increasing development of faint increments deposited after the m check (see Fig. 7 for further explanations).

rate calculated from its slope was not significantly different from one increment per day (t-test: with α : 0.05, $t'=0.54$, 143 df). However, the intercept was significantly different from 0 and showed a slight but constant underestimation of age. The sources of errors were double, low-contrast increments of the first-feeding stage and subdaily rings of the transition zone. The first might include 2-4 low-contrast increments $\geq$ 1 μm in width, as calculated from differences in size of otoliths at known-ages. However, overestimation, due to subdaily rings of the transition zone, had probably reduced this tendency to underestimate ages. Comparisons between the mean of errors on ages, as derived from micrographs and from image analysis (LAGARDERE and TROADEC, unpublish. data) allowed to evaluate the reliability of estimates. They were less precise but more accurate from image analysis (0.2±3) than from micrographs readings (−0.5±2).

4. Discussion

Morphological and behavioural changes occurring during the ontogeny are also

reflected in the biochemical composition of sole larvae. Tissue reorganization, during vitellus resorption, setting up of the digestive tract or metamorphosis, leads to variable needs for the different biochemical compounds. This was clearly shown by the variations in the ratio of essential to nonessential free amino acids during the early stages of development: free amino acids, involved in many different biochemical pathways, are extremely sensitive to any metabolic change. Therefore they undergo wide variations during larval development (GATESOUPE, 1986). This was also seen but in a lesser extent in the lipid changes of sole larvae. Despite wide differences in lipid utilization during the development of marine fish, it is well established that those compounds constitute the most important energy source for the larvae in many species (SARGENT *et al.*, 1989) . Variations of triglyce rides are of particular interest, because these components play an energetic role and show simple accumulation or utilization patterns. They dramatically drop during the yolk-sac resorption, since a large part of them are used as an energy source by the larvae (e.g. herring, FRASER *et al.*, 1987). When fed, sole larvae accumulated large amounts of triglyceride as larvae of atlatntic herring do (GATTEN *et al.*, 1983). At metamorphosis, the increase of amounts in biochemical components is clearly slackened. The onset of metamorphosis seems to require a high level of triglyceride to cover the energetic needs linked to the anatomic and metabolic changes. The trigly ceride level slightly decreased after metaphosis, as also shown in plaice (EHRLICH, 1974a) and pacific herring (FUKUDA *et al.*, 1986).

Starved larvae can be distinguished from fed ones of the same batch after one or two days of food deprivation through dry weight, protein or total amino acid measurements. These biochemical compounds decrease steadily and much more quickly in the youngest larvae or at high temperature. The triglyceride content of a sole larva seems to be the most sensitive to the nutritional state. In starved larvae, triglycerides were immediately mobilized and quickly decreased to a very low value which was associated to high mortality. This pattern was also observed in other species (EHRLICH, 1974a, b; TANDLER *et al.*, 1989; HAKANSON, 1989a). The sterol content followed the increase in weight for the control larvae, but it did not change much during starvation, as also pointed out for anchovy larvae (HAKANSON, 1989a). Sterols are mainly membrane constituents and are preferentially conserved. So the ratio triglyceride/sterol appears to be a good index of the nutritional condition for field larval fish, as it would be relatively independent of changes in other biochemical compounds (FRASER *et al.*, 1987; HAKANSON 1989a, b).

Except sterols, all the components we studied reached a critical level at yolk absorption. This shows that the yolk reserve is not sufficient to ensure growth and hence confirms the critical importance of early food availability (BLAXTER and STAINES, 1971; BUCKLEY, 1980). Larvae starved 2 to 4 days after mouth-opening recovered and survival was not affected. Once on the PNR day, mortality in these batches was very high. The survivors could not recover a good growth before day 12, as also shown by otoliths. In case of severe fasting, metamorphosis was delayed if the amount of triglyceride previously accumulated was not large enough. Contrarily to the studies on other fish (DABROWSKY *et al.*, 1986; MIGLAVS and JOBLING, 1989), we did not observed any compensatory effect on weight and on biochemical components. Ontogenetic changes were delayed by a period equal to the length of the starvation interval.

Temperature affected differently the increase of each biochemical compound in fed larvae, and therefore their relative biochemical composition. The final level of percent protein was lower at 12° than at 19°, while it was just the reverse for triglyceride content relative to dry weight and for the mean ratio between essential and non-essential free amino acids. These disparities depending on the biochemical component indicated differences in the effects of temperature on their metabolism. High temperature enhanced the general metabolism, but promoted protein catabolism rather than energetic lipids storage. This temperature effect can lead to completely

different changes in starved larvae, as shown by the essential to non-essential amino acid ratio. Thus temperature influence needs to be considered in studying nutritional state (STRUSSMANN and TAKASHIMA, 1989).

It is admitted that starved larvae produce either low-contrast increments (RICE *et al.*, 1985) or no increments because of uncountable discontinuous zones. But the term "increment" implies two structures which are together measured and counted, the incremental and discontinuous zones respectively, and otoliths continue to grow even when larvae are starving at early stage. During the 19℃ experiment, otolith growth appears clearly once somatic growth resumes in the survivors of the 5d-starved group, as matarial deposited into the perinuclear area. It was made of poorly defined increments mor and more narrow, and it was identified to stress marks (sensu RICE *et al.*, 1987). Lowcontrat increments were first observed in some otoliths of sea-caught larvae (LAGARDERE and CHAUMILLON, 1988; this paper, Fig. 7 D-E). They are a classic feature of early growth (e.g. herring) and associated with slow-growing larvae at the first-feeding stage (CAMPANA *et al.*, 1987). This incremental pattern is linked to conservative properties of otoliths and shows more dependence upon metabolic activity than somatic growth (HOVENKAMP, 1990). Sagittal growth, on the form of low-contrast increments whereas somatic growth was arrested, involves significant variations between the larval-otolith size relationships (e.g. REZNICK *et al.*, 1989; SECOR and DEAN, 1989; LAGARDERE, 1989). The use of these regressions as shrinkage estimator (RADTKE, 1989), as well as backcalculation of previous length at age (CAMPANA, 1990), if done without caution, may underestimate larval size.

The low contrast of unit increments in the central part of larval otoliths causes the main difficulty in using optical microscopes and therefore image analysis, insofar as the resolving power may be limited by increment width and contrast between incremental and discontinuous zones (CAMPANA *et al.*, 1987; LAGARDERE and TROADEC, unpublish. data). Moreover, extensive use of electron microscopy for field studies is not realistic (RADTKE, 1989). In the case of the 12℃ reared larval sole, the low-contrast increment width ($\geqq 1$ μm) was not a limiting factor. The lack of definition led to a slight underestimation of ages, well-corrected by image analysis. Further improvements are needed to gain more precision, but it appears nevertheless that low-contrast increments were correctly interpreted, if not discriminated, by this image analysis methods. It would be helpful in reading larval otoliths as long as the sagittal growth is detectable. However, errors and uncertainties of ages for sea-caugth larvae are to be suspected when their when their otoliths have stress marks. There are probabilities that their age estimates will not justify the same precision level as the one of known-age larvae reared in a more favorable thermal and trophic environment.

In conclusion, most of the biochemical parameters can work well when applied as nutritional indices for *reared* larvae, when age, stage and history are known. However, some of them, as proteins, total free amino acids, total lipids and triglycerides, are closely linked to the weight (age), which is difficult to measure accurately on the youngest larvae. Moreover, in recruitment studies, larvae caught in the same water mass could have different histories. It is better to precise the variability within a population and thus preferable to analyze individuals. Use of biochemical indices, such as the triglyceride to sterol ratio, less dependent on the larval ontogeny, may be promise to development, once calibrated for sea-sampled specimens. This ratio is more reliable, very sensitive to denutrition and less dependent on ontogenetic changes. Triglyceride and sterol contents may be now estimated on a single sole larva by means of TLC-FID analysis, with satisfactory accuracity and reproducibility. It would provide an instantaneous diagnosis of the larval nutritional state, while saving the possibility of otolith examination (HAKANSON, 1989b). The conservative structure of otoliths account not only for age. Links between dated increments or dated marks and environmental factors are to investigate and may

complete the larval history with data existing before capture.

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References

ARBAULT S., P. CAMUS and C. LE BEC (1986): Estimation du stock de sole (*Solea vulgaris*, Quensel 1806) dans le golfe de Gascogne a partir de la production d'oeufs. J. appl. Ichthyol., **4**, 145–156.

BAILEY K. M. and E. D. HOUDE (1989): Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. mar. Biol., **25**, 1–83.

BLAXTER J. H. S. and M. E. STAINES (1971): Food searching potential in marine fish larvae. *In*: Proceedings 4th European Marine Biology Symposium, D. J. CRISP (ed.), Cambridge University Press, Cambridge, pp. 467–481.

BOULHIC M. and J. GABAUDAN (in press): Histological study of the organogenesis of the digestive system and gas bladder of the Dover sole *Solea solea* (Linnaeus 1758). Aquaculture.

BUCKLEY L. J. (1980): Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. Fish. bull. U.S., **77**, 703–708.

BUCKLEY L. J. and R. G. LOUGH (1987): Recent growth, biochemical composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. Can. J. Fish. Aquat. Sci., **44**, 14–25.

CAMPANA S. E. (1990): How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci., **47**, 2219–2227.

CAMPANA S. E., J. A. GAGNE and J. MUNRO (1987): Otholith microstructure of larval herring (*Clupea harengus*): image or reality? Can. J. Fish. Aquat. Sci., **44**, 1922–1929.

DABROWSKI K., F. TAKASHIMA and C. STRUSSMANN (1986): Does recovery growth occurs in larval fish? Bull. Japan. Soc. Sci. Fish., **52**, 1869.

EINARSSON S. (1985): Selective determination of secondary amino acids using precolumn derivatization with 9-fluorenylmethylchloroformate and reverse phase high-performance liquid chromatography. J. Chromatogr., **348**, 213–220.

EHRLICH K. F. (1974a): Chemical changes during growth and starvation of larval *Pleuronectes platessa*. Marine Biology, **24**, 39–48.

EHRLICH K. F. (1974b): Chemical changes during growth and starvation of herring larvae. *In*: The early life history of fish, J. H. S. Blaxter (ed.), Springer Verlag, Berlin, pp. 301–323.

FOLCH J., M. LEES and G. H. SLOANE STANLEY (1957): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., **226**, 497–509.

FRASER A. J., J. R. SARGENT, J. C. GAMBLE and P. MACLACHLAN (1987): Lipid class and fatty acid composition as indicators of the nutritional condition of larval atlantic herring. Am. Fish. Soc. Symposium, **2**, 129–143.

FUKUDA M., H. NAKANO and K. YAMAMOTO (1986): Biochemical changes in Pacific herring during early developmental stages. Bull. Fac. Fish. Hokkaido Univ., **37**, 30–37.

GATESOUPE F. J. (1986): The effect of starvation and feeding on the free amino acid composition of sea bass larvae, *Dicentrarchus labrax*. Océanis, **12**, 207–222.

GATTEN R. R., J. R. SARGENT and J. C. GAMBLE (1983): Diet-induced changes in fatty acid composition of herring larvae reared in enclosed ecosystems. J. mar. biol. Ass. U.K., **63**, 575–584.

GREEN A. J. (1982): Otolith ring deposition in relation to growth rate in herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Mar. Biol., **71**, 317–326.

HAKANSON J. L. (1989a): Analysis of lipid components for determining the condition of anchovy larvae, *Engraulis mordax*. Marine Biology, **102**, 143–151.

HAKANSON J. L. (1989b): Condition of larval anchovy (*Engraulis mordax*) in the southern California Bight, as measured through lipid analysis. Marine Biology **102**, 153–159.

HOVENKAMP F. (1990): Growth differences in larval plaice *Pleuronectes platessa* in the Southern Bight of the North Sea as indicated by otolith increments and RNA/DNA ratios. Mar. Ecol. Prog. Ser., **58**, 205–215.

JONES C. and E. B. BROTHERS (1987): Validation of the otolith increment aging technique for striped bass, *Morone saxatilis*, larvae reared under suboptimal feeding conditions. Fish. Bull., U.S., **85**, 171-178.

LAGARDERE F. (1989): Influence of feeding conditions

and temperature on the growth rate and otolith-increment deposition of larval Dover sole, (*Solea solea* (L.). Rapp. P.v. Réun. Cons. int. Explor. Mer, **191**, 390–399.

LAGARDERE F. and G. CHAUMILLON (1988): Influenc ed'une premiére alimentation différée sur la cr oissance des larves de sole, *Solea vulgaris*, et l'accroissement de leurs otolithes. C. R. Acad. Sci. Paris, t. **306**, Ser III, 601–607.

LINDROTH P. and K. MOPPER (1979): High performance liquid chromatography determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *o*-phthaldialdehyde. Anal. Chem., **51**, 1667–1674.

MAY R. C. (1974): Larval mortality in marine fishes and the critical period concept. *In*: The early life history of fish. J. H. S. Blaxter (ed.), Springer-Verlag, Berlin, pp. 3–19.

MIGLAVS I. and M. JOBLING (1989): Effects of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. J. Fish. Biol., **34**, 947–957.

PARRISH C. C. (1987): Separation of aquatic lipid classes by Chromarod thinlayer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci., **44**, 722–731.

RADTKE R. L. (1989): Larval fish age, growth, and body shrinkage: information available from otoliths. Can. J. Fish. Aquiat. Sci., **46**, 1884–1894.

REZNICK D., E. LINDBECK and H. BRYGA (1989): Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci., **46**, 108–112.

RICE J. A. (1987): Reliability of age and growth-rate estimates derived from otolith analysis. *In*: R. C. SUMMERFELT and G. E. HALL (eds)., The age and growth of fish, Ames, Iowa: The Iowa state university press, pp. 167–176.

RICE J. A., L. B. CROWDER and F. P. BINKOWSKI (1985): Evaluating otolith analysis for bloater *Coregonus hoyi*: do otoliths ring true? Trans. Am. Fish. Soc., **114**, 532–539

RICE J. A., L. B. CROWDER and M. E. HOLEY(1987): Exploration of mechanisms regulating larval survival in Lake Michigan bloater: a recruitment analysis based on characteristics of individual larvae. Trans. Am. Fish Soc., **116**, 703–718.

RICHARD P., M. BOULHIC, J. P. BERGERON, R.GALOIS and J. PERSON-LE RUYET (in press): Effect of starvation on RNA, DNA and protein content of laboratory reared larvae and juveniles of *Solea solea* (L.). Mar. Ecol. Prog. Ser.

SARGENT J., B. J. HENDERSON and D. R. TOCHER (1989): The lipids. *In*: Fish nutrition, J. E. HALVER(ed.), Academic Press, Inc., pp. 153–218.

SECOR D. H. and J. M. DEAN (1989): Somatic growth effects on the otolith-fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci., **46**, 113–121.

TANDLER A., T. WATANABE, S. SATOH and K. FUKUSHO (1989): The effect of food deprivation on the fatty acid and lipid profile of red seabream (*Pagrus major*) larvae. British J. Nutr., **62**, 349–361.

THEILACKER G. H. (1986): Starvation induced mortality of young sea-caught jack mackerel, *Trachurus symmetricus*, determined with histological and morphological methods. Fish. Full., U.S., **84**, 1–17.

STRUSSMANN C. A. and TAKASHIMA F. (1989): Effects of temperature upon survival and histological changes of starved Pejerry *Odontesthes bonariensis* larvae. Nippon Suisan Gakkaishi, **55**, 247–254.

Effets du jeune expérimental sur la composition biochimique et la microstructure des otolithes chez les larves de la sole commune, *Solea solea* (L.)

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Résumé: Des larves de sole, *Solea solea* (L.), ont été soumises à des jeûnes expérimentaux à 12 et 19℃, afin de rechercher des indices biochimiques de leur état nutritionnel et de-ventuelles marques de jeûne sur les otolithes.

L'accroissement du poids sec et l'accumulation des divers composés biochimiques pendant les premier stades ontogéniques est plus rapide à 19 qùà 12℃, avec une augmentation plus marquée du taux protéique que des lipides énergétiques à température élevée. Le poids sec et les quantitie's absolues de protéines, acides aminés libres totaux et triglycerides diminuent proportionnellement à la durée du jeûne et peuvent donc etre utilisées comme indices de l'état nutritionnel pour des larves en élevage. Cependant, leurs variations, comme cells du rapport acides aminés essentiels sur non-essentiels, sont trop dépendantes du stade de développement pour que ces paramètres puissent servir à caractériser l'état nutritionnel de larves capturées en mer. Par contre, les stérois demeurant constants au cours du jeûne, le rapport triglycérides sur stérols est un indice très sensible et plus fiable, qui demande cependant une estimation approximative de l'âge.

La température et le jeûne modifient les paramétras de croissance des larves et des otolithers, de telle sorte que les relations existant entre la taille des larves et celle des otolithes sont variables. Pour une même taille de larves, une baisse de température produit des otolithes plus petits, alors qùà température égale, les otolithes sont plus gros pour les larves qui jeunent. Quand le jeûne suit l'ouverture de la bouche, la croissance somatique s'arrete, alors qu'on observe des accroissements á faible contraste, tels qùils se forment avant la premiere alimentation. Identifiées à des marques de stress, ces structures augmentent la variabilité des estimations de l'âge, même, si le dépôt d'accroissements journaliers se vérifie après le début de l'alimentation exogéne. Observes sur des larves captureées en mer, ils fournissent des informations complementaires sur l'h istoire de la larve avant sa capture.