

Anchovy spawning in the Mira estuary (southwestern Portugal)

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SUMMARY: The aim of the present paper is to review current knowledge concerning anchovy spawning within the Mira estuary (1985/1992): (i) temporal and spatial dynamics of the planktonic phase (abundance, distribution, annual variability of spawning intensity); (ii) larval ecology (feeding, growth, swim bladder inflation) and (iii) factors controlling egg and larval mortality and recruitment variability.

Key words: Anchoa, *Engraulis encrasicolus*, eggs and larvae, spawning, estuaries, North Atlantic.

RESUMEN: LA PUESTA DE LA ANCHOA EN EL ESTUARIO DEL MIRA (SUROESTE DE PORTUGAL). – El propósito de este trabajo es revisar los conocimientos actuales relativos a la puesta de la anchoa en el estuario del Mira (1985/1992): (i) dinámica temporal y espacial de las fases planctónicas (abundancia, distribución, variabilidad anual de la intensidad de puesta); (ii) ecología larvaria (alimentación, crecimiento, inflación de la vejiga natatoria) y (iii) factores que controlan la mortalidad de huevos y larvas y la variabilidad del reclutamiento. (Traducido por los Editores).

Palabras clave: Anchoa, *Engraulis encrasicolus*, huevos y larvas, puesta, estuarios, Atlántico norte.

INTRODUCTION

Engraulis encrasicolus (Linnaeus, 1758), is the only representative of the family Engraulidae in the northeastern Atlantic and in the Mediterranean. Within its distribution area this species tolerates salinity and temperature ranges of 5 to 41.55‰ and 6 to 29°C respectively. The eggs and larval stages are generally not found in waters with temperatures below 13°C and usually are not collected before March or after November in the Mediterranean and adjacent seas. Peak spawning activity is normally restricted to spring-summer months (Demir, 1965, 1974).

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Anchovy eggs and larvae are known to be major components of estuarine ichthyoplankton in the Iberian Peninsula (Chícharo and Teodósio, 1991a, 1991b, Duarte, 1987, Ferreiro and Labarta, 1984, 1988a, 1988b, López-Jamar, 1977, Ré, 1984a, 1984b, 1986a, 1987, 1990a, 1990b, 1991a, 1991b, 1994, Ré and Gonçalves, 1993b, Ré *et al.*, 1983, Ribeiro, 1989, 1991a, 1991b). Anchovy is also a regular component of Portuguese mid-shelf ichthyoplankton during spring and summer (John and Ré, 1993, Ré, 1979, Sobral, 1975).

As discussed by Dando (1984) estuarine spawning by species producing planktonic eggs is uncommon since larvae can be rapidly flushed out of the estuary and dispersed by the net seaward transport of the surface layers. The majority of species that

spawn in estuaries deposit demersal eggs. Anchovy seems to be an exception to this rule. Together with anchovy, Gobiids (*Pomatoschistus* spp.) represent the great majority of the Portuguese estuarine ichthyoplankton (see Ré, in press, and Ribeiro, 1991a, for reviews).

The aim of the present paper is to review current knowledge concerning anchovy spawning within the Mira estuary. Previous papers dealt with the ecology of the planktonic phase of the anchovy (Ré, 1987), with tidal transport and retention of eggs and larvae (Ré, 1990a) in this small tidal estuary and with the feeding ecology of larval anchovy (Ferreira and Ré, in press).

MATERIAL AND METHODS

Study area

Mira estuary (37°40'lat.N. 8°45'long.W) is a relatively small tidal estuary located on the southwestern coast of Portugal. The estuary is approximately 40 kilometres in length with a maximum width of about 400 meters near the mouth. The depth varies from 5 to 10 meters in the lower and middle reaches to less than 3 meters at the upper limit of the tidal influence. The mouth (approximately 100 meters wide) is permanently open to the sea and the estuary may be described as channel-like along its entire length. In spring and summer the water varies from well mixed to slightly stratified during neap and spring tides situations respectively. The general characterization of the Mira estuary concerning bathymetry, tidal period and tidal flow, water circulation and structure of benthic communities is presented by Andrade (1986).

Sampling

Anchovy eggs and larval stages were collected within Mira estuary throughout a period of eight years (1985/1992) using different sampling designs.

During 1985 plankton tows were taken monthly from January through December at 16 stations (Fig. 1). Ichthyoplankton was sampled with a conical plankton net (40 cm aperture, 150 cm length, 500 μ m mesh aperture) equipped with a Hydro-Bios digital flowmeter. A 3.5 meter boat, cruising at about 2 knots (1m/s) powered by a 7.5-hp outboard motor, was used to haul the net in an horizontal tow at a depth of 1 meter at all stations, during a period of 10

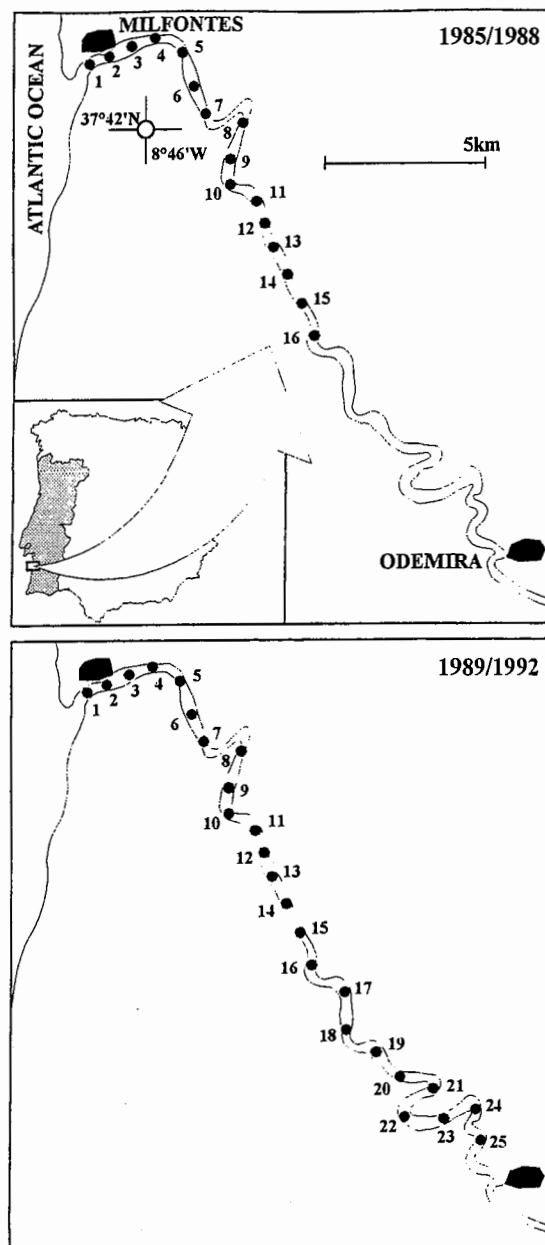


FIG. 1. – Grid of stations sampled (full circles) in Mira estuary in 1985/1988 and 1989/1992.

to 15 minutes. Sampling was carried out only during daylight hours and all tows were made at new moon ebb tides. A different number of stations were visited on each cruise. During summer months, due mainly to the occurrence of great concentrations of the Hydromedusa *Blackfordia virginica* Mayer as well as an undetermined Scyphomedusa at interior stations, it was not possible to adequately sample the ichthyoplankton. Surface water temperature and salinity were measured at each station using a Kent Industrial Instruments model 5005 salinity/temperature meter.

In 1986 and 1987, a different sampling design was used. Samples were taken at a fixed station (#13, cf. Fig. 1) occupied continuously for 12 hours on 25-26 April 1986 and 14-15 April 1987 in order to cover two complete tidal cycles during peak spawning activity. At 30 minute intervals the plankton net was hauled horizontally at a depth of 1 meter during a period of 5 minutes at a speed of 2 knots. All samples were collected during spring tide periods (full moon) covering all the dark phase, dusk and dawn. Sampling started at dusk near the time of high tide (indicated by maximum surface salinity) and continued at 30 minute intervals so that a complete ebb and flood cycle during the dark period was sampled. At 30 minute intervals, surface and bottom temperature and salinity were recorded.

In April 1988 anchovy eggs and larvae were sampled throughout a grid of 20 stations spread 1-2 km apart, within the Mira estuary during spring and neap tide situations (new and first quarter moons) (Fig. 1). Sampling was carried out during consecutive high and low tides at a grid of 20 stations. Ichthyoplankton was sampled with the same plankton net described above. The net was hauled during 5 minutes in an horizontal tow at a depth of 1 meter at all stations. Towed ichthyoplankton samples were taken only during the periods of slack tide at all stations. The tidal stage during sampling at each station was approximately the same since there is a lag of about 2h between the same tidal stage in the upper estuary in relation to the lower estuary. Salinity and temperature were measured near the surface at each station using a Kent Industrial Instruments model 5005 S-T meter.

This sampling procedure (1988) was complemented with fixed station studies. At the fixed station (#13 Fig. 1) samples were performed at 60 minute intervals during a period of 24 hours in order to cover two complete tidal cycles (spring and neap tide situations). The same plankton net was hauled horizontally during 5 minutes at a depth of 1 meter and during 3 minutes near the bottom. The bottom samples were performed with the net mounted on an epibenthic sled. Each net was equipped with a Hydro-Bios digital flowmeter. Although the epibenthic sled was not of the opening/closing type, vertical contamination was controlled by lowering the net rapidly enough to avoid filtering water during deployment and backing down on the net with the towing boat to minimize filtering water during retrieval. Temperature and salinity was determined hourly at the surface and near the bottom using a Kent Industrial Instruments model 5005 S-T meter.

From 1989 to 1992 plankton samples were taken in a different grid of stations spread 1km apart (Fig. 1). Several sampling schemes were used: (i) horizontal sampling surveys; (ii) fixed station studies performed at different locations (upper, middle and lower estuary) and (iii) drift studies. The methods and plankton nets used were identical to those described above. During some horizontal surveys small bongo nets (25 cm mouth aperture, 150 cm length, 500/300 μ m mesh aperture) and neuston nets (60x15 cm mouth aperture, 200 cm length, 500 μ m mesh aperture) were also used. During 1991 and 1992 drift studies were performed after identifying patches of eggs and/or larvae and following drogues or buoys for a period of 3 to 6 hours.

The 1985 sampling methodology was mainly used to establish the seasonal occurrence and spatial distribution of anchovy eggs and larval stages. Chronological time series of eggs and larval fish abundance obtained in 1986 and 1987 were used primarily to study the extent and direction of the longitudinal transport considered in conjunction with the tidal flow as well as to study certain diel rhythmic activities of the larval stages. The horizontal sampling scheme (1988) was used to monitor the abundance and horizontal distribution of anchovy eggs and larval stages, as well as to study the extent of its longitudinal transport during spring and neap tide cycles. Sampling performed from 1989 to 1992 was used mainly to study particular aspects of the ecology of the planktonic phase of the anchovy within Mira estuary (namely larval feeding and daily growth).

All plankton samples were fixed immediately after collection with 5% borate-buffered formalin (pH 8.5-9). All anchovy eggs and larval stages were sorted and enumerated. Samples containing excessive numbers of eggs and/or larval stages were subsampled with the aid of a Folsom plankton splitter. With this technique a coefficient of variation of 5-15% can be expected for total abundance estimates (Van Guelpen *et al.*, 1982). Final preservation of eggs and larvae was also made with 5% buffered formalin. Abundance estimates were expressed in numbers per 1 and 10 cubic meters.

Laboratory procedures

All measurements were made on preserved material less than one month old. Eggs were measured with the aid of a calibrated micrometer eyepiece and a stereoscopic binocular microscope to the nearest

0.01 mm (only subsamples of non disintegrated eggs were measured for major and minor axis). Total lengths of the larval stages were measured also with a binocular microscope to the nearest 0.5mm by placing the larva on a transparent grid marked in millimeters and illuminated from below. For large numbers of specimens a subsample of at least 50 randomly chosen individuals was measured. No shrinkage correction was applied to the lengths of the larvae.

Diel Spawning time was deduced from the presence of early stage eggs in the plankton trawls. The formalin fixed eggs were graded into the series of stages described by Moser and Ahlstrom (1985) for *Engraulis mordax* corresponding essentially to the stages of *Engraulis encrasicolus* eggs.

A total of 545 anchovy larvae (length classes 3 to 7 mm) belonging to 16 different samples were examined to study larval feeding ecology. Sampling consisted at two horizontal surveys conducted during a low neap tide (20 April 1991) and a low spring tide (25 April 1991). Their digestive tracts were dissected in glycerin that, according to Arthur (1976), presents three advantages over water, namely: i) its clearing qualities facilitate the detection of food particles in the gut; ii) the greater viscosity of this medium dampens the movement of the particles during dissection and iii) larvae in glycerin seem to be more pliable and the intestinal walls do not tend to fragment so easily. The dissection of the digestive tract was performed at the binocular microscope using two sharp needles. Considering the semi-digested state of most organisms located in the gut, the food particles were identified at the microscope to the lowest possible taxon. The length and width of food organisms were measured under the microscope to the nearest 0.001 mm. When appropriate (in the case of copepod nauplii and copepodites), width was measured with (maximum width) and without appendages. Larval feeding incidence was defined as the percentage of larvae containing at least one food item in the gut for a given sample (Arthur, 1976). This index refers to presence of food in the gut and does not necessarily reflect recent feeding activity.

The diel rhythm of swim bladder inflation was also determined by plotting the percentage of larvae having gas in their bladders against time of day. Swim bladder volumes were not evaluated.

Anchovy larvae were aged using daily microgrowth increments in sagittal otoliths. The methods used for obtaining, mounting and observ-

ing the sagittae were described in Ré (1983). The daily nature of otolith microgrowth increments in field-captured larvae was corroborated using the method (marginal increment technique) outlined by Ré (1984c). Growth of anchovy larvae was estimated from the relation between body size and number of daily increments enumerated from the otoliths. Growth up to an age of 30 days was adequately described using linear regression analysis. A Video-Microscope-IBM/PC image analysis system (IAS) was used for the study larval otolith microstructure. The IAS used consisted of an IBM compatible microcomputer, a Sony video camera, a video monitor, image analysis software (Image-Pro Plus software) and hardware (digitizer board PCVISION-plus) interfaced with a compound optical microscope. This IAS was used mainly to measure the widths of daily growth increments and to study otolith microstructure using its image processing capabilities (Ré and Gonçalves, 1993a).

Experimental studies under controlled laboratory conditions were pursued mainly to study larval development, ontogeny of larval behaviour and to validate some methods used for determining larval condition (mainly otolith microstructure). Anchovy eggs were sampled within Mira estuary (using the 40cm ring trawl described above) during peak spawning activity in 1991 and 1992, and immediately transported to the laboratory. The plankton net was hauled for a period not more than to 3 min and the eggs were transferred to a large aerated container used for transport. Anchovy larvae were reared during a period of approximately 30 days in the laboratory. The different tanks were maintained under three different experimental conditions: (1) larvae fed daily *ad libitum*, photoperiod of 12 hours of light/12 hours of darkness, constant temperature (LD 12:12 F/24h); (2) larvae fed *ad libitum* during the first week after yolk sac absorption, and under restricted feeding conditions onwards (once a week), photoperiod of 12 hours of light/12 hours of darkness, constant temperature (LD 12:12 F/week); (3) larvae not fed, photoperiod of 12 hours of light/12 hours of darkness, constant temperature (LD 12:12 F/0). The larvae were fed daily on *Brachionus plicatilis* from day 2 to day 15 after hatching and on *Artemia* sp. nauplii from day 12 onwards. Three to eight individuals from each experiment were sacrificed every two days until the end of the experiment. Live specimens (anaesthetized with MS222) were filmed daily using a Sony video camera adapted to a compound light microscope.

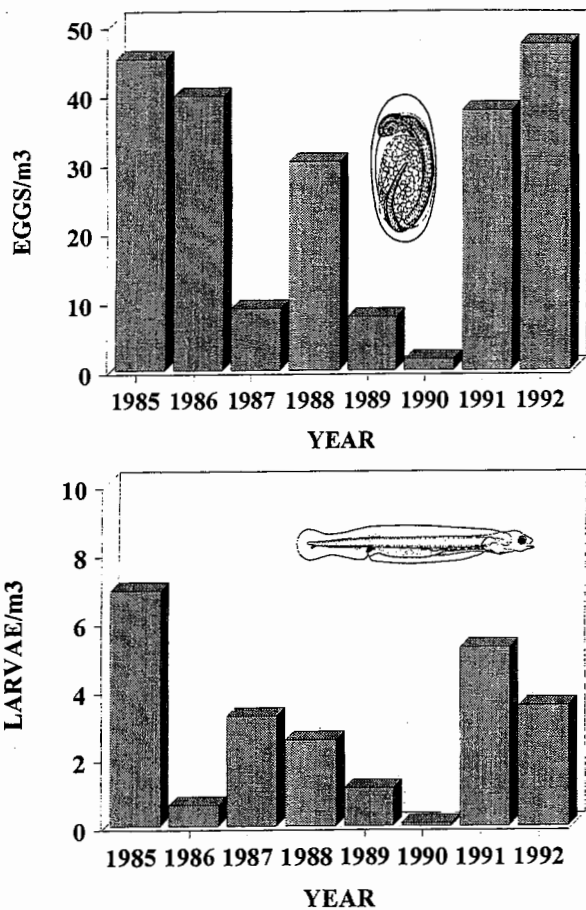


FIG. 2. - Average number of anchovy eggs and larvae sampled in April during spring tide situations (1985/1992) (Mira estuary).

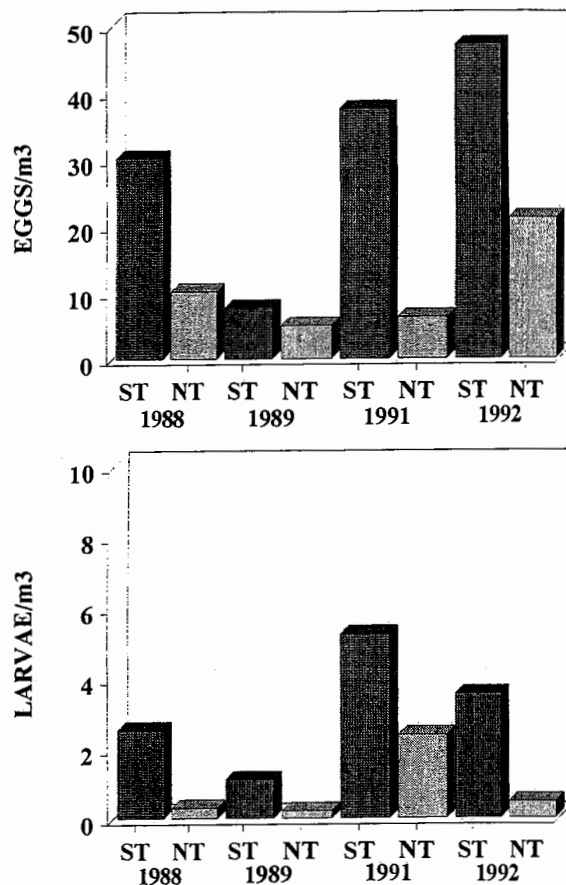


FIG. 3. - Average number of anchovy eggs and larvae sampled in April during spring (ST) and neap (NT) tide situations (1985/1992) (Mira estuary).

RESULTS AND DISCUSSION

Seasonal occurrence and spatial distribution of eggs and larvae (Annual variability of spawning intensity)

Spawning activity (egg and larval abundance) was always highest in April during the surveyed period (1985/1992). Anchovy eggs and larvae were sampled from February to August within Mira estuary. The eggs were abundant from March to May and the larval stages were only adequately sampled in April: their occurrence was occasional in other months. The eggs and larval stages had completely different patterns of horizontal distribution: eggs were dominant in the middle reaches while larvae were sampled predominantly in the upper estuary.

The average number of anchovy eggs and larvae sampled in April (1985/1992) during spring tide situations (horizontal and fixed stations studies) is pre-

sented in figure 2. The average number of eggs and larvae captured in April during the same period in different tidal situations (spring and neap tides) is given in figure 3.

There is an evident inter-annual variability of spawning intensity. The eggs were collected in greatest numbers during 1985, 1986, 1988, 1991 and 1992. Larvae were more abundant during 1985, 1991 and 1992. The eggs and larval stages were always captured in highest concentrations during spring tides.

Anchovy eggs and larvae were sampled within Mira estuary when the surface water temperatures ranged from 13 to 26°C. Spawning activity was highest through a water temperature range of 15.5 to 19.5°C. Anchovy spawning within Mira, Sado and Tejo estuaries occurs earlier in the year in relation to other Portuguese estuaries, lagoons and 'rias' along the Iberian Peninsula. While in the former spawning activity is highest during spring months (mainly April) in the latter (*e.g.* Mondego,

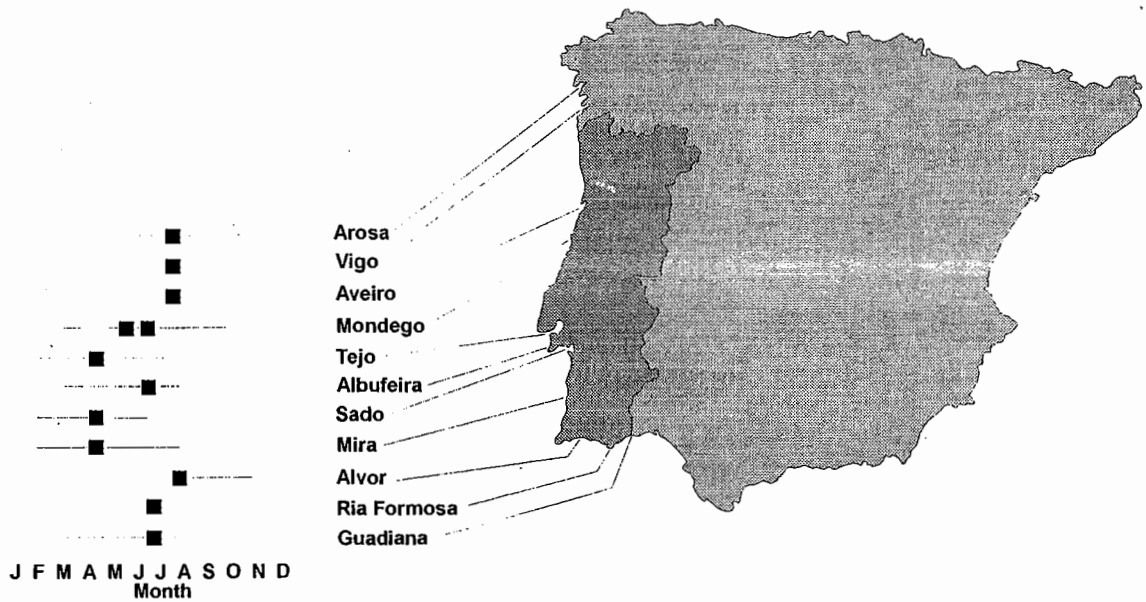


FIG. 4. - Anchovy spawning in estuaries, lagoons and 'rias' along the Iberian Peninsula. Lines indicate the occurrence of anchovy eggs and larval stages, and squares indicate the peak spawning periods.

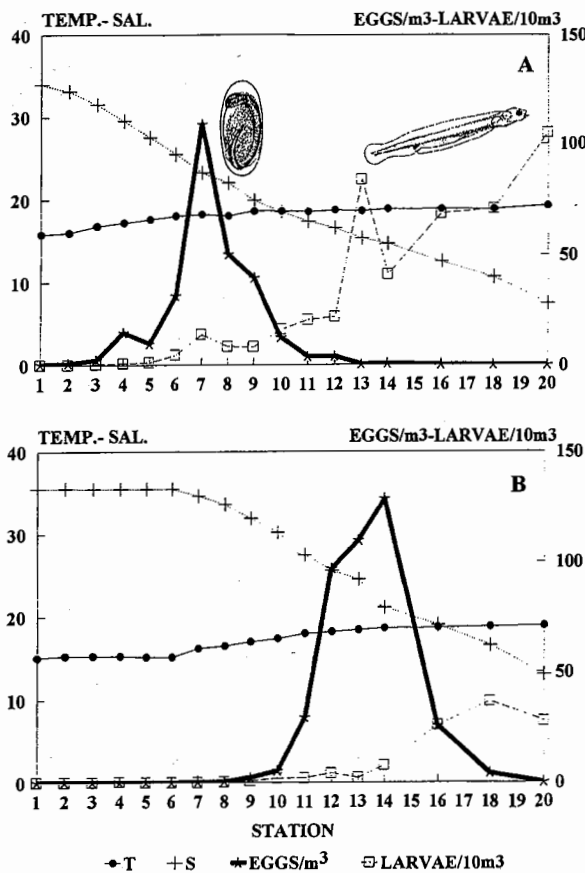


FIG. 5. - Results of a spring tide horizontal sampling survey (A- low tide, B- high tide) (16.04.1988) (Mira estuary).

Guadiana) the eggs and larval stages are mainly sampled during summer months (mainly June/July) (Ribeiro 1991a) (Fig. 4).

The horizontal distribution of the eggs can be indicative of the extent of the longitudinal transport. Taking this into account the longitudinal transport was 7 and 4 km, during spring and neap tide situations respectively (Figs. 5 and 6). Larvae of higher length-classes were always found in the upper estuary. This strongly suggested the existence of some kind of retention mechanism exhibited by anchovy larvae in order to maintain their position within the estuary.

Data obtained during fixed station studies showed that egg and larval concentrations changed more or less in phase with the salinity cycle (Fig. 7). These variations in ichthyoplankton abundance are obviously related to the extent of the longitudinal transport considered in conjunction with the general trend of water circulation within the estuary. The association between egg concentration and salinity is a measure of the position of the spawning ground. It is obvious from the chronological time series obtained that the eggs have a more agglomerative contagious distribution than the larvae. This different horizontal distribution of eggs and larvae is another indication that larval stages are retained within the estuary (see below).

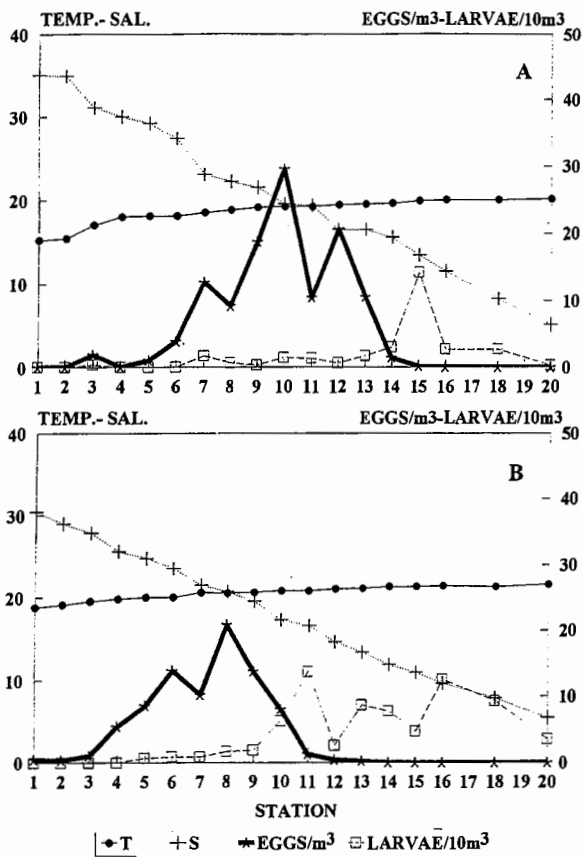


FIG. 6. - Results of a neap tide horizontal sampling survey (A- high tide, B- low tide) (23.04.1988) (Mira estuary).

Sampling efficiency

The evaluation of sampling efficiency is a fundamental need in quantitative plankton research. Many different plankton gears have been developed to increase the efficiency of plankton sampling. High speed samplers have been designed to catch active plankton organisms like fish larvae therefore reducing avoidance. The problem of extrusion and clogging arises however in these type of samplers. The majority of plankton samples taken within Mira estuary were performed using small ring trawls (40 cm aperture, 150 cm length, 500 μm mesh aperture) hauled horizontally near the surface (-1m) at a speed of 2 knots. Anchovy egg and larval extrusion was evaluated using a small bongo net (25 cm mouth aperture, 150 cm length, 500/300 μm mesh aperture) during several horizontal sampling surveys. The results of one of these studies is presented in figure 8. From these data it is evident that eggs were adequately sampled with both nets (500 and 300 μm) (Wilcoxon matched pairs test, $W=0.408$, $p>0.05$, $n=14$) while larvae were only adequately captured with the 300 μm net ($W=2.521$, $p<0.05$, $n=14$). Results from other horizontal sampling surveys (Ré, unpublished data) suggest however that a sampling gear with a mesh aperture of 300 μm is a more effi-

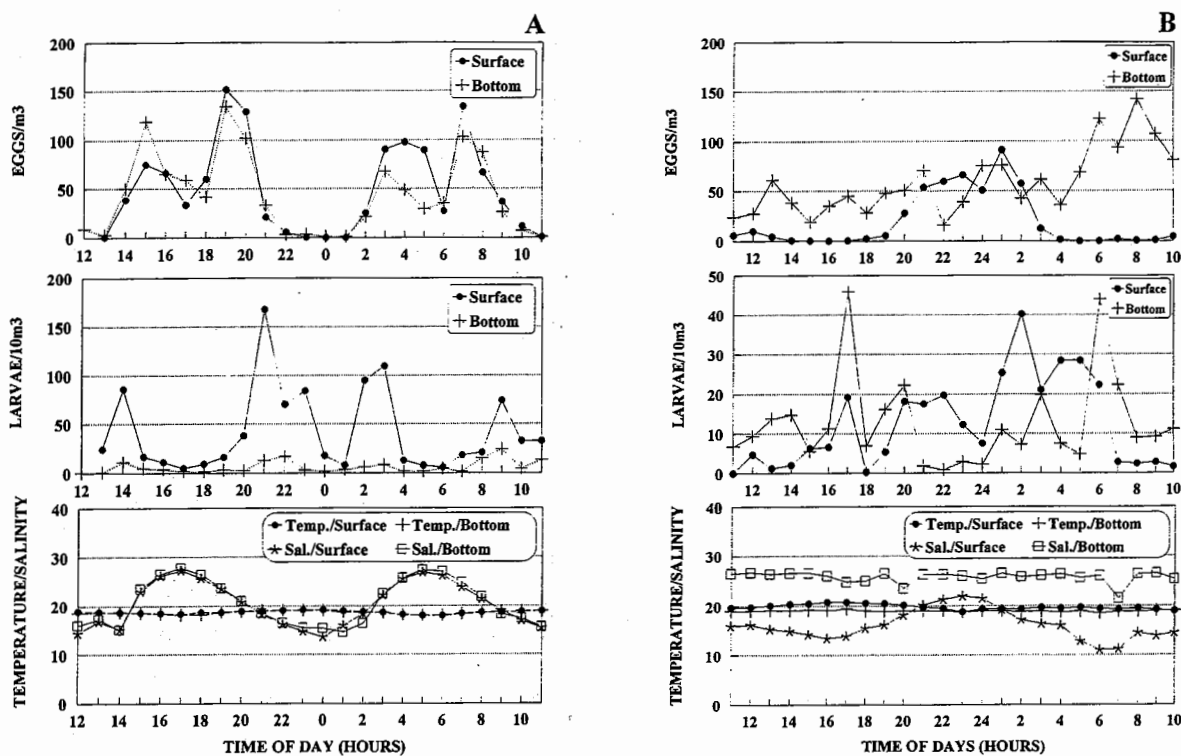


FIG. 7. - Results of two fixed station studies performed during a spring tide situation (A) (#13, 17/18.04.1988) and a neap tide situation (B) (#13 24/25.04.1988) (Mira estuary).

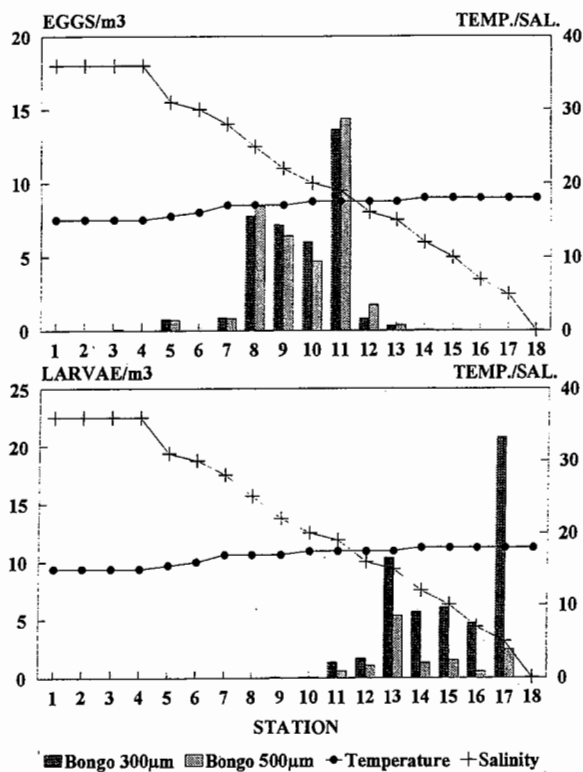


FIG. 8. — Results of a spring tide horizontal sampling survey performed with a bongo net for the evaluation of sampling efficiency (1991.04.21) (Mira estuary).

cient sampler for anchovy eggs and larvae. These results indicate an underestimation of the number of both eggs and larvae sampled during the eight year study in the Mira estuary.

Egg size

The seasonal change in egg size appears to be a common pattern among marine teleosts, particularly Clupeoids. The trend is similar among clupeoids in both hemispheres, with the largest eggs being spawned in the local winter and the smallest in the local summer. In multiple spawners the seasonal decline in egg size can be attributed to: (i) a reduction in energy reserves over the spawning season; (ii) a change in the partitioning of energy between growth and reproduction and (iii) a seasonal change in the age structure of the spawners among other factors (Blaxter and Hunter, 1982).

The size of anchovy eggs collected within Mira estuary decreased significantly as the spawning season progressed (see Ré, 1987). A similar situation is referred to by Ribeiro (1991a) for the Mondego estuary. This decrease in egg dimensions can probably be related to the partial spawning of the species

and/or the age structure of the spawners. It could be assumed that this phenomenon is related to the spawning of a different group of ovarian eggs that would reach maturity somewhat later. It might also be possible that, as the reproductive season progresses, spawning is assured primarily by individuals that reach first sexual maturity and naturally produce smaller eggs.

Diel spawning time

The time of spawning can be deduced from the presence of early stage eggs in the plankton. Nocturnal or crepuscular spawning seems to be a rather common practice among clupeoids (Blaxter and Hunter, 1982).

Fixed station studies performed during peak spawning activity of the anchovy were in part designed to evaluate the diel spawning time. Early stage eggs were only sampled at dusk and during the first hours of darkness. Stage 1 eggs were only collected between 18:30-21:00 and stage 2 were sampled between 18:30- 22:00 and between 02:30-06:30 in 1986.04.25/26 (local time T.U.C.+1h). From these data it seems probable that most spawning occurs before midnight and during the first hours of the dark period. Ré (1984b, 1986a) found a similar situation within Tejo estuary. In this estuary early stage eggs (stage 1 and 2) were sampled between 18:00 and 00:30 (local time). Ribeiro (1991a) also found early stage anchovy eggs in Mondego estuary between 18:00 and 03:00 (local time). This nocturnal spawning might be viewed as an adaptation in order to reduce selective predation by diurnal planktivores on pelagic eggs at the time of spawning when eggs have a more contagious distribution (Blaxter and Hunter, 1982).

Larval feeding

Knowledge of the food and feeding habits of fish larvae is important for the understanding of their role in the plankton. The availability of suitable food for the larvae is usually considered to be a key factor in determining the size of the subsequent year-class strength. One of the problems of this kind of study is the low incidence of field-captured clupeoid larvae with food in their guts (larvae generally void their gut contents during the shock created by capture and subsequent fixation). This might constitute a serious source of error in larvae with straight guts. Nevertheless it seems perfectly valid to make esti-

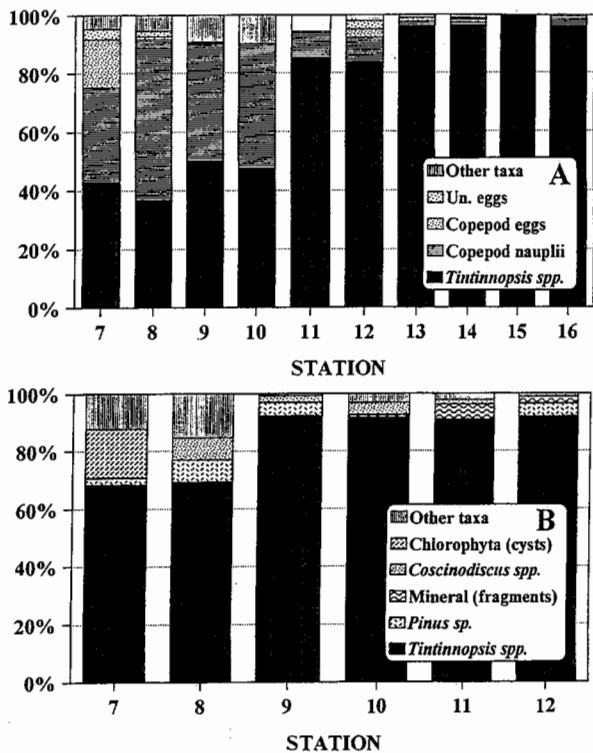


FIG. 9. - Percentage occurrence of main food organisms in each station (A- neap tide, 20.04.1991, B- spring tide, 25.04.1991) (Mira estuary).

mates of feeding incidence over the circadian period using the same sampling methodology. Several authors have reported a diel rhythm in feeding activity indicating that clupeoid larvae feed mainly if not exclusively during the day (see Blaxter, 1965 and Blaxter and Hunter, 1982 for a review).

The main prey items observed within the gut of anchovy larvae in each sampled station are represented in figures 9 and 10. The two main identified taxonomic groups, *Tintinnopsis* spp. and copepod nauplii, showed an inverse trend in the percentage occurrence throughout the estuary during neap tide.

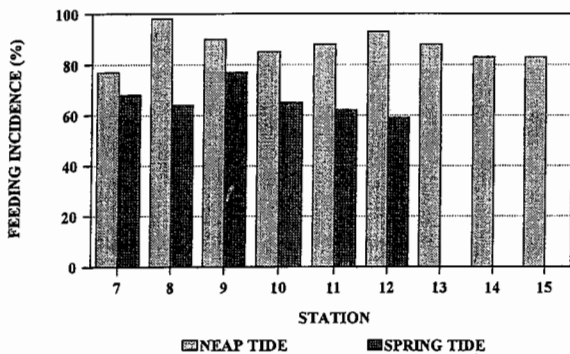


FIG. 10. - Feeding incidence throughout the Mira estuary during neap and spring tide situations (20.04.1991 and 25.04.1991).

Tintinnopsis spp. represented the main food organisms in all sampled stations during spring tide. Phytoplankton (*Coscinodiscus* sp., and cysts of Chlorophyta) was not a main food item. Copepod nauplii were ingested "head" first with antennae folded back along the body.

The variation of the feeding incidence throughout the estuary is shown in figure 10. High values were found at almost all sampled stations. Although the feeding incidence is similar at neap tide and spring Tide situations, the latter is lower than the former.

Anchovy larvae exhibit a clear rhythm of feeding: feeding incidence decreases significantly with the onset of the dark period and increases at dawn to maintain high values during daylight hours (Fig. 11). In anchovy larvae and clupeoid larvae in general, food is passed after ingestion almost immediately to the anal end of the gut where digestion takes place. Digestion times can be determined (criteria being the transparency of the gut) if it is assumed that feeding activity stops at dusk to be resumed at dawn. Values of 1 to 3 hours could be evaluated during fixed station studies. Digestion times are dependent on the quantity of food taken, length of the larvae and environmental temperature among other factors.

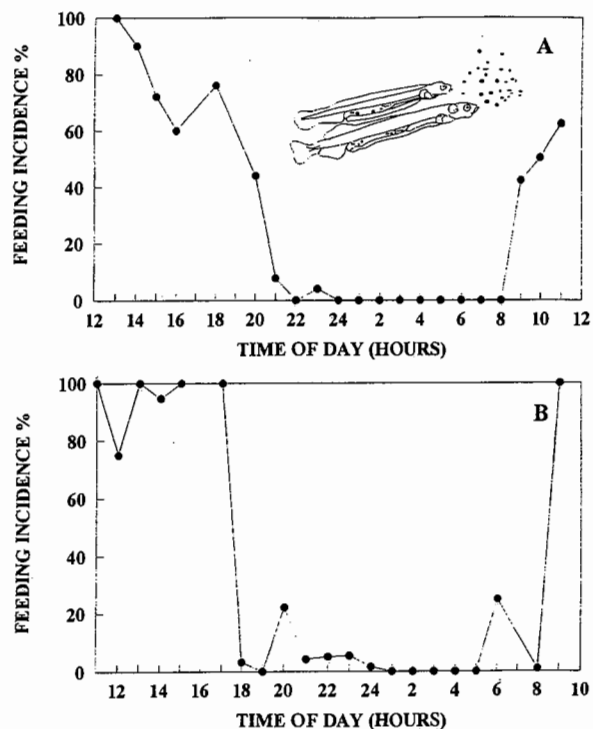


FIG. 11. - Feeding incidence in relation to time of day (fixed station study #13, A- spring tide, 17/18.04.1988, B- neap tide, 24/25.04.1988) (Mira estuary).

Swim bladder inflation/deflation

Hunter and Sánchez (1976) proposed that an observed diurnal swim bladder inflation/deflation rhythm *Engraulis mordax* larvae might be viewed as an energy-sparing mechanism providing the necessary buoyancy that the larvae need in order to stay inactive near the surface during the night. These authors also suggested that anchovy larvae fill their gas bladders by gulping air at the water surface, which might constitute a universal procedure for fish larvae exhibiting a circadian rhythm of swim bladder inflation/deflation (Ré *et al.*, 1985). This diel rhythm of inflation/ deflation of the swim bladder has been reported for other clupeoid larvae by several authors (Uotani, 1973, Hoss and Phonlor, 1984, Ré, 1984a, 1986b).

In anchovy larvae the swim bladder becomes noticeable as a small vesicle when the individuals have a total length of 7 mm (around 13 days old). Diel rhythms of swim bladder inflation begin when the larvae have total lengths equal to or greater than 10 mm (Ré, 1986c).

Larvae sampled during the fixed station studies covering a complete circadian period exhibited a clear diel rhythm of gas bladder inflation/deflation. Without exception, individuals (total lengths 10 mm) sampled during the day had deflated bladders, while almost all larvae captured after the onset of the dark period had inflated gas bladders (Fig. 12). The filling of the swim bladders is performed only during the twilight period probably by gulping air at the water surface. The number of larvae examined per sample was small, due to the difficulty of collecting large individuals; nevertheless net avoidance is usually less intense during the dark period.

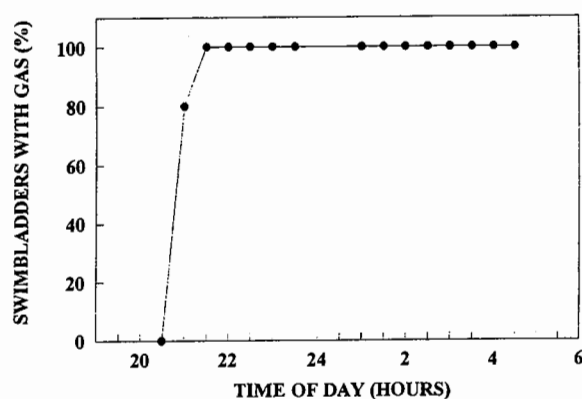


FIG. 12. - Percentage of anchovy larvae with gas in their swim bladders in relation to time of day (fixed station study #13, 25/26.04.1986) (Mira estuary).

The adaptative advantages of this procedure seem to be the reduction of swimming activity during nonfeeding periods and the maintenance of a relatively fixed position in the water column. The reduction of swimming activity during the dark period may also result in a reduction of predation since some predators of larval fish detect their prey by the water movements (*e.g.* chaetognaths) (Hunter and Sánchez, 1976).

Larval growth

Otolith microstructure examination is now a preferred tool for the study of young fish providing a wealth of information to larval fish ecologists. Applications of information derived from otolith microstructure are numerous: (i) age determination; (ii) daily growth rate estimations; (iii) mortality; (iv) migratory and environmental history; (v) competition; (vi) abundance; (vii) condition and (viii) taxonomy, among others (Stevenson and Campana, 1992). The quantification of some of these life history parameters is essential for the evaluation of the causes underlying recruitment variability, especially as described by Hjort's (1914) critical period concept, Lasker's (1981) stable ocean hypothesis and Sinclair's (1988) member/vagrant hypothesis.

The time of completion of a microgrowth increment was determined following the change of the index of completion for current increment in relation to the time of the day (marginal increment technique). This index varied from 63.6 to 100% between 18:30 and 00:30 and from 6.6 to 16% between 01:30 and 04:30 (local time T.U.C.+1h) (Ré, 1987). The discontinuous zone of each increment was formed during the first hours after the onset of the dark period. The initiation of a new microgrowth increment began around 01:00. These data are consistent with the deposition of one microgrowth increment per day.

Microgrowth increments in anchovy larval otoliths seem to be deposited only after yolk-sac absorption; yolk-sac larvae always have otoliths without growth increments. Sagittae have a clear nucleus and daily growth increments are only deposited after the onset of exogenous feeding. Each increment is composed of an incremental (white) and discontinuous (dark) zones when viewed under a light microscope with transmitted light. The age of the larvae can thus be determined by adding 1 to 5 days (yolk-sac period, temperature

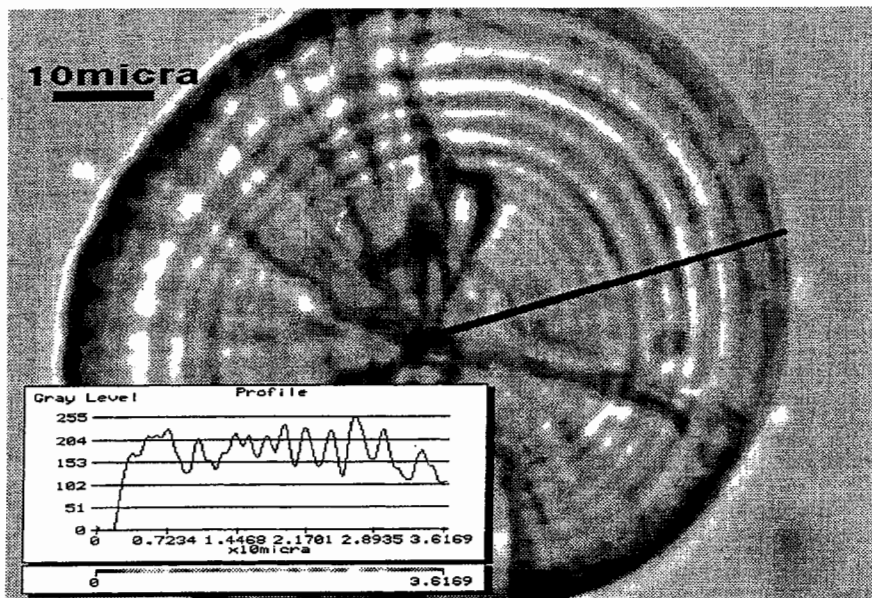


FIG. 13. – Anchovy larval otolith (processed image). Standard length of the larva- 10 mm. The gray scale index at each pixel along the indicated line is shown (dicontinuous-black and incremental-white zones of each daily growth increment are apparent, 0=black, 255=white).

dependent, 25/16°C, Regner, 1985) to the number of daily microgrowth increments enumerated from the sagittae.

The anchovy larval otolith was studied using a video-Microscope-IBM/PC image analysis system. Several transitions were identified in the microstructure of the sagittae (Fig. 13). A clear nucleus is apparent and daily growth increments vary both in width and intensity. The nucleus corresponds to the yolk-sac period and the first microgrowth increment is formed after the onset of exogenous feeding. The first 8 to 10 microgrowth units are comparatively less intense, increments became sharper in most of the studied otoliths from ring 12 onwards. This second transition might be related with the initiation of diel rhythms of swim bladder inflation/deflation, in conjunction with other rhythms of activity (mainly feeding and vertical migration).

Larval daily growth rates were estimated only from the relation between age and body size (integrated daily growth rates). Growth was adequately described, up to an age of about 30 days, using linear regression analysis. Larval anchovy growth rates based on length *versus* age were estimated from the slope of the regression. Regression parameters obtained during the sampling period are referred to in Table I. Larval growth parameters are presented in figure 14. Integrated daily growth rates varied in Mira estuary from 0.25 to 0.41 mm/day. Similar values were found by Ribeiro (1991a) in the Mondego estuary (0.51 mm/day). Higher daily growth rates found by other authors (*e.g.* Palomera *et al.*, 1988, 0.9/1.0 mm/day; Regner, 1985, 0.8 mm/day; Regner and Dulcic, 1990, 0.9 mm/day; Walline, 1987, 0.6 mm/day) are probably related to two main factors: temperature and feeding availability.

TABLE 1. – Larval growth: linear regression parameters ($y=a+bx$). SL- Standard length (mm), DGI- Daily growth increments, r^2 - Correlation coefficient squared, n- Number of data points, Range- SL range of studied larvae.

| Date | y | x | a | b | r^2 | n | Range |
|---------------|----|-----|-------|-------|-------|----|---------|
| 19.04.1985 | SL | DGI | 4.068 | 0.343 | 0.877 | 53 | 4/9 mm |
| 25/26.04.1986 | SL | DGI | 3.116 | 0.387 | 0.863 | 61 | 6/14 mm |
| 14/15.04.1987 | SL | DGI | 2.547 | 0.405 | 0.891 | 58 | 4/16 |
| 07.04.1989 | SL | DGI | 4.217 | 0.264 | 0.709 | 41 | 5/9 |
| 04.11.1990 | SL | DGI | 4.215 | 0.248 | 0.724 | 42 | 5/10mm |
| 23.11.1990 | SL | DGI | 3.832 | 0.413 | 0.959 | 67 | 4/16mm |

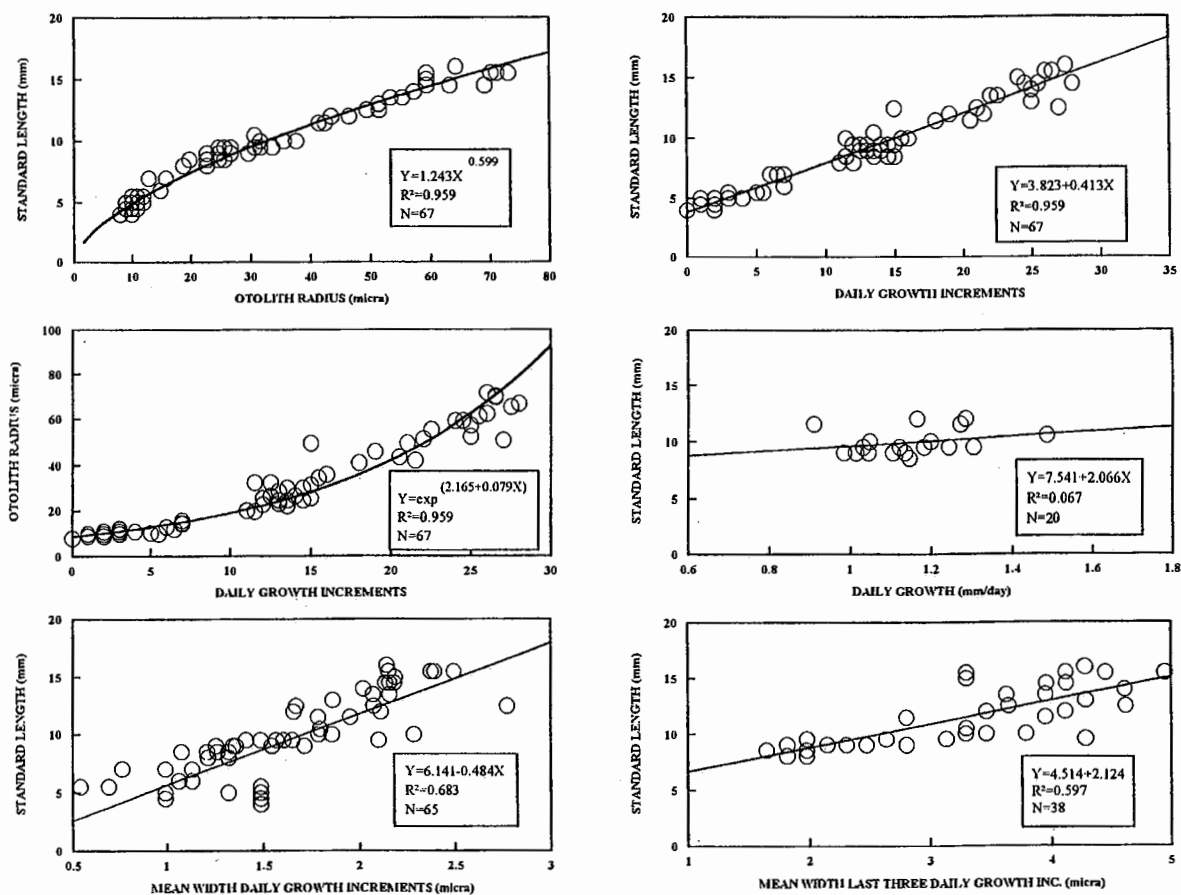


FIG. 14. - Growth parameters of anchovy larvae (fixed station study 23.04.1992) (Mira estuary).

Larval growth was also studied in the laboratory under controlled conditions. The main early life history stages and transitions were identified, namely: egg or embryonic stage; larval stage (including preflexion, flexion and postflexion stages) and metamorphosis (when the fish assumes the general features of the juvenile). The embryonic period (temperature dependent) varies from 2 to 3 days (19/20°C). Prior to hatching the embryo exhibit typical movements (intermittent shaking). The newly hatched larva has the typical clupeid form (Standard length-SL 3.5/4 mm). The yolk sac is absorbed after two days (19/20°C) and the eyes became pigmented at the same time that the mouth and digestive tract become functional (SL approximately 5 mm, chronological age three days). The swim bladder is formed at an age of 8 days and the dorsal, anal and caudal fins begin its development soon after (age 10/11 days, SL 6/7 mm). Flexion of the notochord begins at an age of 13 days (SL 9/10 mm) and metamorphosis occurs at an age of approximately 60 days (SL 35/40 mm).

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