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The nutritional condition of larvae of anchovy (*Engraulis encrasicolus* L.) in the outflow of the River Po (Northern Adriatic)

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Larvae of anchovy, Engraulis encrasicolus, *were sampled in August 1995 along a transect across the outflow of the River Po. Their nutritional condition was determined by analysis of otolith daily growth rings and histological grading of tissues of the midgut, liver and trunk muscle. The growth rate of larvae 4-8mm in length was higher for those sampled inshore (0.515mm/day) compared with larvae from further offshore (0.417mm/day). These data are supported by results from the histological assessment of nutritional status which showed larvae to be in poorer condition further offshore, where food availability was lower, than at the inshore sampling site where food availability was higher. Overall, an estimated 6% of inshore larvae were classified as starving compared with 22% from offshore. Nutritional condition also reflected the diel feeding rhythm of the larvae, with liver storage reserves being laid down following day-time feeding and then being utilised during the night-time period of non-exogenous feeding.*

INTRODUCTION

The causes of recruitment variability in marine fish remains obscure and a subject of considerable debate (BAILEY and HOUDE, 1989; CUSHING, 1990; LEGGETT and DEBLOIS, 1994). Nevertheless, it is well established that mortality during the early life-stages is one important determinant of recruitment strength (MAY, 1974; SINCLAIR and TREMBLAY, 1984). Two groups of hypotheses have been suggested to explain variations in larval mortality: those based on food availability and predation as the principal causative agents (LASKER, 1975; MULLIN *et al.*, 1985; CUSHING, 1990; LEGGETT and DEBLOIS, 1994) and others in which physical factors such as small-scale turbulence and advection are dominant (WROBLEWSKI, 1984; ROTHSCHILD and OSBORN, 1988). However, it is recognized that it is a combination of both physical and biological influences that govern the complex processes determining larval survival. This approach has been incorporated in a number of studies in which physical processes (e.g. upwelling - CURY and ROY, 1988; frontal systems - BUCKLEY and LOUGH, 1987; vertical stratification processes - COOMBS *et al.*, 1983; and wind-stress events - PETERMAN and BRADFORD, 1987) influence plankton trophodynamics, larval retention and subsequent larval recruitment (LASKER, 1981; ROTHSCHILD and OSBORN, 1988).

As part of an investigation of the above processes, a joint study was initiated between the Istituto Centrale per la Ricerca scientifica e tecnologica Applicata al Mare (ICRAM), Chioggia, and the Plymouth Marine Laboratory (PML), to study the effects of wind mixing on food availability and survival of anchovy larvae. An initial pilot cruise was designed to refine multi-disciplinary methods for measuring hydrobiological conditions, including food availability for anchovy larvae, and to sample larvae for measures of their nutritional condition. At the same time a complimentary laboratory based experimental programme for development and validation of the indices of nutritional condition was being carried out. Results from the field work describing the environmental conditions, including food availability, are given in COOMBS *et al.*, 1997. In the present paper, the results from histological and otolith daily growth ring analyses of the condition of the field-sampled anchovy larvae are described.

Measures of larval nutritional condition integrate feeding success over time and can be used to give an indication of starvation and potential survival. Various techniques are available for the analyses of nutritional condition of fish larvae (e.g. elemental composition - EHRLICH, 1974; histology - McFADZEN *et al.*, in press; lipids - HÅKANSON, 1989; morphometry - FRANK and McRUER, 1989; RNA:DNA - CLEMMESEN, 1987; otolith growth rate - CAMPANA and NEILSON, 1985). In an appraisal of condition measures, FERRON and LEGGETT (1994) considered histology to be one of the more favourable techniques. Advantages of the histological approach are that i) it reliably detects changes in nutritional condition, ii) its ability to discriminate nutritional stress from other sources, iii) sensitivity, and iv) wide applicability to various species and ages. Previous reports on the histological condition of anchovy larvae have been given for *Engraulis mordax* from Californian coastal waters (O'CONNELL, 1976; THEILACKER and WATANABE, 1989), for *E. anchoita* from the southwest Atlantic (SIEG, 1993), and for *E. encrasicolus* in the Mediterranean Sea (KHODJA, 1979).

Equally, analysis of the rhythmic depositional patterns in otoliths is now a recognised tool for the study of larval growth and provides data that can be related to individual variation in growth and survival and the effects of environmental factors (temperature, photoperiod, food availability and meteorological events; CAMPANA and NEILSON, 1985; JONES, 1986; GEFFEN, 1987; CAMPANA and JONES, 1992), as well as life-history patterns (RADTKE and MORALES-NIN, 1989) and stock identification (LEVI *et al.*, 1994). Daily growth rates of anchovy larvae from the Adriatic have been given by REGNER (1985), REGNER and DULČIĆ (1990) and DULČIĆ and KRALJEVIĆ (1996). Elsewhere, WALLINE (1987) and PALOMERA *et al.* (1988) have presented results from samples taken in the Mediterranean, RÉ (1987) from sampling in the Mira estuary of Portugal and OWEN *et al.* (1989) for *Engraulis mordax* off southern California.

MATERIALS AND METHODS

Sampling was carried out on 12 and 13 August 1995 from the research vessel Salvatore Lo Bianco along a 20 mile transect at 44° 52.5'N in the outflow of the River Po (Fig. 1). An initial series of stations were worked along the transect at five mile intervals (stations 1-5, Fig. 1) followed by selection of an Inshore station and contrasting Offshore station for more detailed day and night sampling. Full details of the methods and sampling scheme are given in COOMBS *et al.* (in prep.).

Plankton sampling was carried out at each of the five station positions along the transect (Fig. 1) by oblique slow speed (3 knots) tows using a 20cm diameter BONGO net fitted with non-filtering cod-ends to minimise damage to

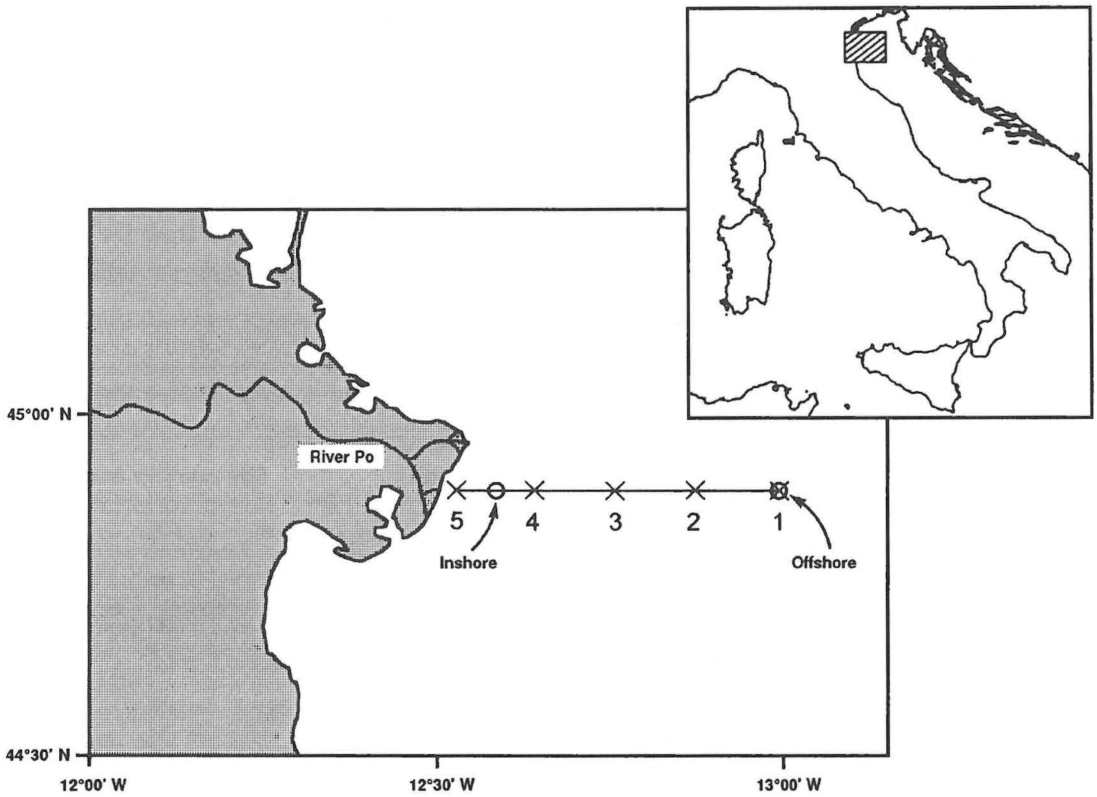

Fig. 1. Sampling area showing the five station positions along the transect and the selected Inshore and Offshore sampling sites

the larvae. A 250µm mesh aperture net was fitted to one side of the BONGO frame and a 333µm net to the other side. On completion of each BONGO haul (<10 minutes duration), the nets were gently rinsed before removal of the cod-ends. Samples from each net were preserved separately in borax buffered 4% formaldehyde solution, those from the 333µm net being subsequently used for analysis of the otoliths from larval anchovy. Similar tows were made at the selected Inshore and Offshore stations (Fig. 1) using a 30cm BONGO net system fitted with 280µm mesh aperture nets; anchovy larvae for histological analysis were carefully sorted from these samples and placed in cold (4°C) BAKER's formol calcium; this being completed within approximately 10 minutes of haul recovery in order to minimise potential post-mortem deterioration.

Otolith analysis

On return to the laboratory, preserved anchovy larvae were measured to the nearest 0.01mm (standard length) under a dissecting microscope. Both sagittal otoliths were then removed from each larva using fine dissecting needles. The otoliths were then mounted on a glass slide and fitted with a coverslip using Permount (Table 1).

Table 1. Number of larvae analysed for otolith daily growth rings

Station	Size category (mm)			
	2-4	4-6	6-8	8-10
1	10	7	8	4
2	8	11	9	3
3	8	8	6	-
4	7	10	6	-
5	9	8	6	1

Readings of growth increments were made under a light microscope at a magnification of X1250 using oil immersion. Phase contrast and polarising filters were also used to enhance increment definition (MORALES-NIN, 1992). Identification of the growth rings, these being a light zone (incremental unit) followed by a dark zone (discontinuous unit), were made following the method outlined by PALOMERA *et al.* (1988). Because the first ring surrounding the nucleus (core) is considered to be laid down following yolk-sac utilisation and at the beginning of exogenous feeding (RÉ, 1994), counting of the growth rings started from the core boundary which is marked by a distinct discontinuity or check zone.

A LAIRD-GOMPERTZ growth function (ZWEIFEL and LASKER, 1976) was used to describe the overall relationship between larval length and number of otolith daily growth rings. Larvae with zero ring counts were excluded from this analysis, since such larvae were essentially of indeterminate age and could be of any age prior to deposition of the first growth ring. The LAIRD-GOMPERTZ model of growth has been shown to be particularly suitable for summarising the growth trajectories of fish larvae and has been applied by REGNER and DULČIĆ (1990) and DULČIĆ and KRALJEVIĆ (1996) to anchovy larvae from the Adriatic and by PALOMERA *et al.* (1988) for larvae from the western Mediterranean. Due to the relatively small number of specimens and the sensitivity of the LAIRD-GOMPERTZ model, it was not appropriate to use it for comparisons between stations; for this purpose we used linear regressions which are adequate descriptors (e.g. see RÉ, 1994) for the relatively short length range of larvae in the present study. The comparison between results from the otolith and histological studies was restricted to larval length classes of 4-8mm for which there were sufficient larvae common to both methods of analysis.

Histological analysis

On return to the laboratory, preserved larvae were measured to the nearest 0.01mm (standard length) under a dissecting microscope and grouped into three size classes (Table 2). All larvae used for histological analysis were post yolk-sac and without disease or external parasitic infection.

Table 2. Number of larvae analysed histologically

Station	Size category (mm)		
	4-6	6-8	8-10
Inshore day	31	18	9
Inshore night	30	18	9
Offshore day	30	18	9
Offshore night	31	18	9

Larvae were processed for methacrylate embedding as described by McFADZEN *et al.* (1994) and serially sectioned at 2μm intervals in the sagittal plane, using disposable RALPH glass knives. Sections for tissue grading were stained in LEE's methylene blue/basic fuchsin (BENNETT *et al.*, 1976). In addition, sections were stained with periodic acid SHIFF's (PAS) for the detection of carbohydrates. A supplementary amylase digestion was included as a control for glycogen detection (BANCROFT, 1967).

Histological grading

Histological grading systems were based on the cellular condition of selected tissues, following criteria used by O'CONNELL (1976) for larvae of anchovy *Engraulis mordax* and sardine *Sardina pilchardus* (McFADZEN *et al.*, in press.). A number of studies (e.g. O'CONNELL, 1976; MARGULIES, 1993; McFADZEN *et al.*, in press) have shown that degenerative cellular responses to low food availability are common to many teleost larvae. In the present study three tissues were selected: the relatively labile digestive tract and liver, both with relatively rapid response times (6-12 h) to food deprivation (MARGULIES, 1993; McFADZEN *et al.*, 1994) and the more resilient tissue of the trunk muscle (O'CONNELL, 1976); laboratory studies on teleost larvae having demonstrated that severe damage to the trunk muscle occurs only after prolonged periods of food deprivation (THEILACKER, 1986; McFADZEN *et al.*, 1994).

Initial inspection of the tissues was carried out to reject specimens with evidence of autolytic degradation (HIBIYA, 1982). Examination of the remaining larvae was carried out with a WILD M20 microscope using standard KHÖLER illumination. Before assigning the nutritional grade to a particular tissue, serial sections taken throughout each larva were examined for a representative assessment of the overall degree of tissue condition within the specimen. The classification of a tissue grade was based on the poorest observed status of each specific tissue parameter. It was not unusual for a specimen to have a superficially healthy tissue condition, but when additional sections of the same tissue were viewed, they were found to have regions of degenerative necrosis. Such foci of necrosis are irreversible and hence indicative of poorest possible condition. The degree of tissue alteration throughout the specimen was determined and a grade assigned (Table 3), from good (scoring 1) to degraded (scoring 3). The composite nutritional score (the sum of the three tissue grades) was classified as Healthy (total score 3 to 5), Intermediate (total score of 6) or Starving (total score 7 to 9); results were used only for those larvae in which all tissues could be assessed.

Table 3. Histological criteria for tissue condition

TISSUE	GRADE		
	1 (good)	**2 (intermediate)**	**3 (degraded)**
Midgut mocosa	Entercytes intact; villi with deep longitudinal folds; cytoplasm homogenous; no vacuolation; microvilli intact.	Separation of entercytes in basal region; coarse dark cytoplasm; frequent areas of microvilli degeneration.	Enterocytes small dark and separated; extensive vacuolation; sloughed cells may be present; microvilli often indistinct.
Trunk muscle	Parallel fibres, myofibrids striated; abundant basophilic interfibrillar tissue.	Slight separation of fibres; loss of myofibrillar striation; variable nuclear staining; reduced basophilic tissue.	Pronounced fibre separation; highly reduced myofibrillar striations; dark indistinct nuclei.
Liver hepatocyte	Nuclei light granular, small and indistinct; cytoplasm varied, scattered granules; large and numerous cytoplasmic vacuoles following cell boundary and encroaching on the nucles	Slight separation of fibres; loss of myofibrillar striation; variable nuclear staining; reduced basophilic tissue.	Nuclei small dark and pyknotic; cytoplasm lacking texture and often separated from the cell boundary; cell devoid of PAS positiv vacuoles; often large sinusoid spaces.

Digestive tract

The mid-gut region of the digestive tract was assessed for cellular condition, this being the principal area concerned with nutritional absorption. The midgut mucosa of anchovy larvae is characterised by a relatively thick, highly convoluted columnar epithelium with distinct basal nuclei supporting a continuous microvillous border. The midgut epithelium often forms deep folds, especially ventrally, with occasional PAS positive mucus cells being present which increase in number caudally. Cellular integrity and the degree of intracellular vacuolation was variable and graded according to the criteria described in Table 3.

Liver

The liver of the European anchovy, similar to that of other engraulids, is composed of densely packed tubules of pyramidal hepatocytes, containing pale staining regular nuclei. Hepatic condition was graded according to the criteria given in Table 3.

Muscle

Trunk muscle was examined from the main body, caudal to the cranial arch and was graded according to the criteria outlined in Table 3.

RESULTS

Otolith analysis

The fitted LAIRD-GOMPERTZ growth curve for larvae taken at all stations along the transect is shown in Fig. 2. with parameter values for the regression equation given in Table 4. In order to facilitate comparison with previously published LAIRD-GOMPERTZ growth curves, some of which have incorporated various correction factors, values are also given in Table 4. for the growth curve fitted to larvae from the present study which have had similar factors applied. In all cases the LAIRD-GOMPERTZ growth model is a good fit (r^2 values all >0.95; Table 4).

The parameter values for the linear regressions used to compare the growth rates of larvae in the size range 4-8mm showed lower growth rates (slope) at the two offshore stations (stations 1 and 2) compared with the more inshore sampling sites (stations 3-5; Table 5). The lowest growth rate was at the most offshore station 1 (0.417mm/day) and the highest at station 4 (0.540mm/day) which is near the inshore end of the transect. However, the only significant difference (at $p = 0.05$) was between the slopes of the most offshore and inshore stations (stations 1 and 5 respectively). The estimated lengths at deposition of the first ring (intercept) are quite variable, these being from 3.08mm to 4.10mm (Table 5), to some extent reflecting the limited data on which these regressions are based (see Table 1).

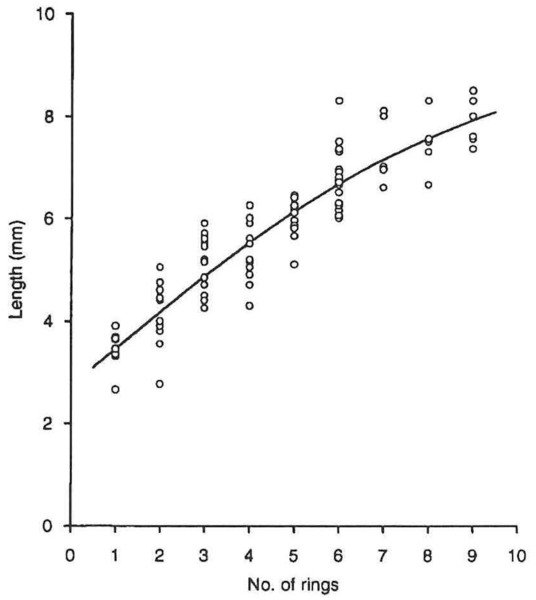

Fig.2. Fitted LAIRD-GOMPERTZ growth curve for larvae from all stations analysed for daily growth rings

Table 4. Parameter values for fitted LAIRD-GOMPERTZ growth curves

Source	L_0	A_0	α	r^2
Present paper	2.75	0.2545	0.2008	0.99
Present paper*	3.67	0.2226	0.1921	0.99
DULČIĆ and KRALJEVIĆ, 1996	2.63	0.21	0.08	0.99
REGNER and DULČIĆ, 1990**	3.51	0.1829	0.0891	0.96
PALOMERA *et al.*, 1988*	3.40	0.1868	0.0781	-
PALOMERA *et al.*, 1988*	3.76	0.1560	0.0589	-

* Shrinkage allowance for capture and preservation according to THEILACKER (1980).
** Shrinkage allowance for preservation only, according to REGNER (1985).

Table 5. Parameter values for fitted linear regressions

	Station				
	5	4	3	2	1
Slope	0.515	0.540	0.519	0.474	0.417
Intercept	3.08	3.45	3.15	3.74	4.10
r^2	0.80	0.91	0.90	0.74	0.68

Histological analysis by tissue type

Digestive tract

In all larvae, regardless of their nutritional classification, there was evidence of supranuclear inclusion bodies in the hindgut which is indicative of a past feeding history (O'CONNELL, 1976) showing that the larvae have progressed from yolk-derived nutrition, to exogenous feeding. The condition of the midgut varied considerably in larvae sampled at both the Inshore and Offshore stations. Intracellular vacuolation of the midgut was also highly variable, ranging from healthy intact columnar cells with no vacuolation, to extensive vacuolation and sloughing of cells into the enlarged lumen. Severe vacuolation was invariably identified together with sloughed cells, areas of extensive necrosis and reduction of cell height and villi projection. Poor condition midgut tissue was most often associated with larvae sampled at the Offshore station. In general, larvae sampled at night showed an increased level of columnar cell atrophy, compared to specimens sampled in the day; such a reversible decline in tissue integrity resulting in a reduction in the degree of intestinal folding.

Liver

The overall condition of the liver was variable between individuals. Hepatic sinusoid dilation ranged from prominent enlarged tubules to those that were compacted and narrow. Both nuclear and cytoplasmic appearance was variable between larvae, although generally homogeneous within an individual. The extent of PAS positive intracellular vacuolation was not constant, ranging from a complete absence in some small larvae, to numerous large vacuoles often encroaching on the nucleus in the larger specimens. Healthy hepatocytes had pale staining nuclei being laterally displaced by PAS positive intracellular vacuoles (glycogen rich). Hepatocytes lacking glycogen reserves (PAS negative cells, highly reduced vacuoles) had dark staining collapsed nuclei (often pyknotic) and enlarged sinusoids; larvae in such condition were predominantly less than 8mm in length and in samples collected at night. A greater proportion of larvae sampled at the Offshore station stained negatively with the PAS histochemical reaction than those sampled at the Inshore station, indicating that hepatic storage reserves had been utilised.

Trunk muscle

Trunk muscle showed the least variation in cellular condition in all specimens from both stations sampled. Most larvae <8mm in length were graded as intermediate for muscle condition.

Histological condition by size class and diel variation

Results from the histological assessment of nutritional status showed that, overall, a higher proportion of larvae from the Offshore station were in poorer condition (p = <0.005) than at the Inshore station. Following the determination of tissue grades and calculation of the composite nutritional scores, an estimated 6% of Inshore larvae were classified as starving compared with 22% of Offshore larvae.

Larvae in all size classes were in generally better nutritional condition from sampling during the day than when sampled at night (Fig. 3; Table 6); they were also in generally better condition at the Inshore station compared with the Offshore station (Fig. 4; Table 6).

For the smallest size category of larvae at 4-6mm in length, the most extreme value for the proportion of starving larvae was for specimens sampled from the Offshore station at night; these larvae were in significantly poorer condition (p = <0.005) than those sampled either at the same station during the day, or from day or night sampling at the Inshore station (Table 7). Intermediate sized larvae, at 6-8mm in length, were significantly healthier during the day than at night at both Inshore and Offshore stations (p values of <0.001 and <0.1 respectively; Table 7). Results for the largest size category of larvae, at 8-10mm in length, were somewhat contradictory, possibly due, in part, to the relatively low numbers taken

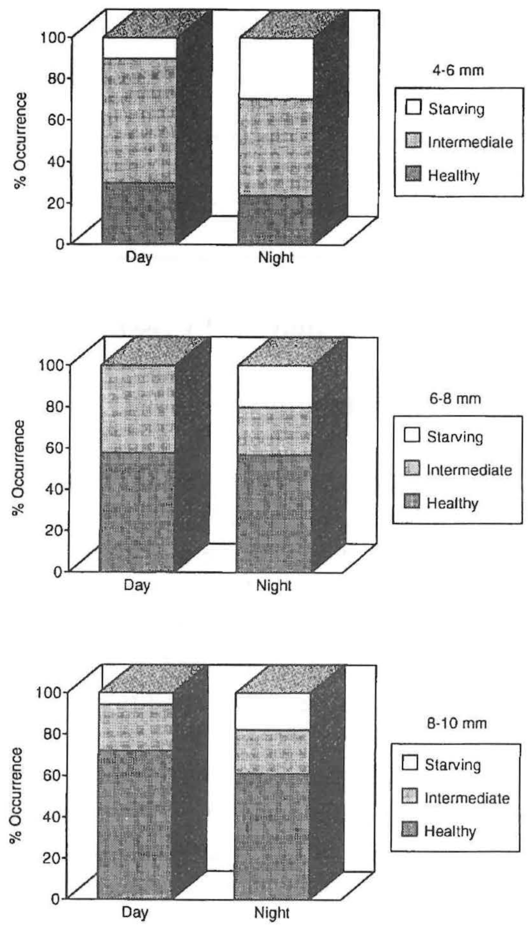

Fig.3. Diel variation in histological condition by size class for larvae sampled at both the Inshore and Offshore stations

(Table 2). By day, these larger larvae sampled at the Offshore station were in better condition than those at the Inshore station (p = <0.05), whilst at night the larvae from the Offshore station were in a significantly poorer condition (p = <0.001; Table 5). At the Inshore station by day, larvae at 8-10mm in length were in significantly poorer condition than at the same station at night (p = <0.005), whereas the opposite was found at the Offshore station, with the poorest condition larvae (p = <0.001) occurring at night (Table 7).

Table 6. Histological assessment of the percentage of starving anchovy larvae

Size category	**% Starving**			
	Inshore Day	**Inshore Night**	**Offshore Day**	**Offshore Night**
4-6 mm	5.9	17.6	14.3	41.7
6-8 mm	0	0	11.8	28.5
8-10 mm	11.2	0	0	35.5

Table 7. Statistical comparison of mean histological grades: paired t-test matrix and probabilities (p=<0.1, ** p=<0.05, *** p=<0.01, **** p=<0.005, ***** p=<0.001).*

	Inshore Day		**Inshore Night**		**Offshore Day**		**Offshore Night**
4-6 mm	df		df		df		
Inshore Day		0.000					
Inshore Night	62	- 0.941		0.000			
Offshore Day	66	- 1.18	62	- 0.296		0.000	
Offshore Night	56	- 4.44*****	52	- 3.39*****	56	- 2.34****	0.000
6-8 mm							
Inshore Day		0.000					
Inshore Night	18	- 4.73*****		0.000			
Offshore Day	26	- 1.95**	23	1.38*		0.000	
Offshore Night	24	- 3.67***	21	-0.14	30	- 1.65*	0.000
8-10 mm							
Inshore Day		0.000					
Inshore Night	16	- 2.96****		0.000			
Offshore Day	16	- 2.54**	16	- 0.178		0.000	
Offshore Night	38	- 2.25**	38	- 5.79*****	38	- 5.039*****	0.000

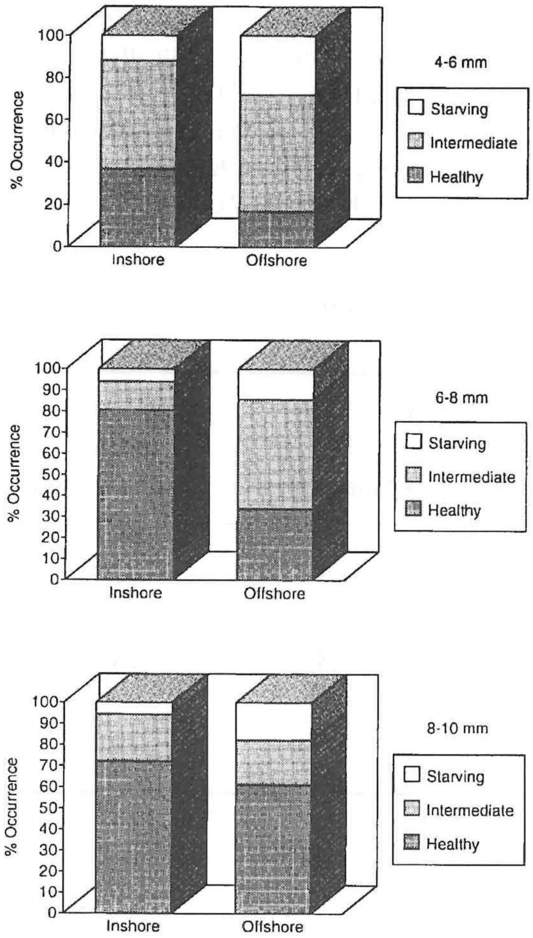

Fig.4. Percentage occurrence by size class of larvae which were classified as being in Healthy, Intermediate or Starving condition, as classified from histological grading; larvae are included from both day and night sampling

DISCUSSION

For recruitment studies, one of the main requirements of condition indices is that they should be capable of providing reliable estimates of the potential mortality of fish larvae due to starvation (FERRON and LEGGETT, 1994). Thus, such indices are most usefully based on processes operating on longer time-scales than the diel feeding rhythmicity of the larvae (COOMBS and HÅKANSON, 1991). In their appraisal of measurements of condition, FERRON and LEGGETT (1994) stated that no single index was ideal, although most condition indices did, at least, register a change shortly after the onset of starvation. However, only histological scores and protein measurements (biochemical indices) continued to change beyond the point of irreversible starvation, a requirement for quantification of the proportion of larvae in a sample destined to die regardless of future feeding conditions (FERRON and LEGGETT, 1994).

The particular benefit of growth studies based on otolith ring deposition, is the potential to follow the previous growth history of individual specimens and then to relate changes in growth to environmental conditions; growth being taken as an inverse measure of survival, as supported by a number of observational and theoretical studies (BROTHERS and McFARLAND, 1981; CAMPANA and NEILSON, 1982; CAMPANA, 1984; CAMPANA and NEILSON, 1985; JONES, 1986; RADTKE and MORALES-NIN, 1989). Similarly, the advantages of histological methods of condition assessment are that a range of tissues responsive to both short-term and long-term feeding history can be examined and some assessment can be made of the aetiology of the stressor, i.e. the source of tissue alteration ascribed to food deprivation (THEILACKER, 1986; THEILACKER and WATANABE, 1989; MARGULIES, 1993) contaminant exposure (WESTER and CANTON, 1991; JOHNSON and BERGMAN, 1984), or parasitic/disease effects (LOM, 1970).

The growth rate parameters derived in the present work (Tables 4 and 5) were comparable with previously published information on anchovy larvae. However, any detailed comparison of growth parameters is complicated by a number of factors which affect the precision of the various models. Among others, these include the sensitivity in estimation of the LAIRD-GOMPERTZ equation parameters, as highlighted by PALOMERA *et al.* (1988), the influence of using various correction factors for shrinkage (Table 4) and the different length ranges of larvae in the various studies.

In the non-linear LAIRD-GOMPERTZ equation, the parameter estimate of L_0, (the predicted length of larvae at deposition of the first growth ring), calculated in the present study was in the same range as in other reports, allowing for differences due to corrections for shrinkage caused by capture and preservation (Table 4). Deposition of the first growth ring in anchovy larvae is related to the onset of active feeding (BROTHERS et al., 1976 ; RÉ, 1994), the estimated value of L_0 (3.67mm, including correction for shrinkage; Table 4) falling within the length range (3.23 - 3.90mm) in which REGNER (1985) observed formation of a functional mouth and appearance of eye pigmentation in anchovy larvae. The specific growth rate (A_0) at the larval length of first increment deposition (L_0; where growth rate, in units of mm/ring, equivalent to mm/day, is given by $A_0.L_0$ at time t_0) is somewhat higher than in other studies, implying an initially faster growth rate; conversely α, a term describing the progressive reduction in growth rate with length of larvae, is noticeably higher in the present studies than in others (Table 4). Thus, the larvae sampled in 1995 had a relatively high initial growth rate but one which declined rather more rapidly than in the other studies. Considering the overall linear growth rates for larvae in the length range 4-8mm, values in the present study (0.417 - 0.540mm/day) are comparable with results reported by WALLINE (1987) for anchovies sampled in coastal waters of Israel (0.55 mm/day) but somewhat higher than those given by RÉ (1994) in studies of anchovy spawning in a Portuguese estuary (0.25 - 0.41 mm/day).

Histological analyses indicated midgut, liver and muscle pathologies in anchovy similar to those reported for other species in field surveys (O'CONNELL, 1976; THEILACKER, 1986; MARGULIES, 1993; McFADZEN *et al.*, in press). In the present study the condition of tissue of both the digestive tract and liver reflected the diel feeding rhythm of the larvae, with liver storage reserves being laid down following intensive day-time feeding. Trunk muscle has the longest response time to past feeding history among the graded tissues (McFADZEN *et al.*, 1994) and was generally found to be healthy or in intermediate condition, irrespective of location or sampling time. It is, perhaps, not unexpected that long-term indices such as muscle condition give a consistent indication of larvae in good condition, since individuals with any accumulation of poor short-term condition indices (reflected in the liver and midgut) might be expected to have been removed by some combination of starvation and predation. Similar observations of the generally healthy status of field-caught larvae have been reported elsewhere (O'CONNELL, 1976; PITCHER and HART, 1982; THEILACKER, 1986; OWEN *et al.*, 1989; McFADZEN *et al.*, in press).

Considering the geographical distribution of larval condition, there was substantial agreement between otolith and histological assessments of larval condition. The otolith results showed a general increase in growth rate from offshore to inshore while, similarly, the histological grading estimated 22% of larvae at the Offshore station as being in starving condition compared with 6% of larvae at the Inshore station.

The above observations imply that food availability was better at the inshore sites than further offshore, as was actually observed (COOMBS *et al.*, 1997). Suitable food items for larval anchovy were in general around 2 x more abundant at the Inshore station than at the Offshore; more particularly, the most marked difference in food availability was for the smaller larvae (<8mm in length), these feeding predominantly on copepod nauplii which declined from a sub-surface peak of abundance of 56 nauplii/litre at the Inshore station to 19/litre at the Offshore station. An equivalent increase in the relative proportion of smaller larvae in healthy condition compared with larger larvae was seen at the Inshore station compared with the Offshore station (Fig. 4).

Food abundance along the transect was related by COOMBS *et al.* (1997) to oceanographic conditions that showed a typical pattern for a river outflow plume into a seasonally stratified sea (GRIMES and KINGSFORD, 1996). Although there was considerably more stratification at the inshore end of the transect, there was relatively little difference between the inshore and offshore ends of the transect in the temperature of the upper 10m of the water column, in which most of the anchovy larvae were found (COOMBS *et al.*, 1997). Thus, the changes in growth rate along the transect are less likely to be due primarily to temperature differences than to some other environmental agent; however, within the limitations of the restricted data sets, the observed variations in both growth rate and histological condition are, at least, consistent with changes in food availability along the transect.

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Stanje ishranjenosti larvi brgljuna (*Engraulis encrasicolus* L.) u estuariju rijeke Po (sjeverni Jadran)

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KRATKI SADRŽAJ

Smatra se da smrtnost ranih stadija morskih riba znatno utječe na uspješno obnavljanje. Čimbenici koji utječu na promjene smrtnosti su biološke i fizikalne prirode. U svrhu istraživanja tih procesa izvršeno je uzrokovanje uzduž transekta 44°52.5'N u estuariju rijeke Po tijekom kolovoza 1995. da bi se odredio utjecaj hidrobioloških uvjeta i raspoložive hrane na preživljavanje larvi papaline (*Engraulis encrasicolus*). Stanje ishranjenosti larvi je shvaćeno kao indeks preživljavanja a određeno je pomoću dnevnog rasta prstenova otolita i histološkog stupnjevanja tkiva probavila, jetre i mišića.

Rast larvi je opisan pomoću LAIRD-GOMPERTZ-ove funkcije da bi se procijenili parametri Lo, Ao i L, čije vrijednosti su sukladne ranije publiciranim procjenama. Dužina pri vađenju prvog prstena rasta je ocijenjena na 3.67 mm ili 2.75 mm, što odgovara vrijednostima sa ili bez odbitka zbog stiskanja larvi pri ulovu i konzerviranju. Linearna regresija između starosti i dužine za larve od 4-8 mm ukazuje na znatno višu (kod $p=0.05$) stopu rasta za larve uhvaćene bliže obali (0.515 mm/dan), gdje ima više raspoložive hrane, nego za larve uhvaćene dalje od obale (0.417 mm/dan) gdje ima manje hrane na raspolaganju. Ovi rezultati su potvrđeni histološkim opažanjem stanja ishranjenosti larvi. Loše stanje tkiva probavila i jetre je češće u uzorcima sabranim dalje od obale. Mišićno tkivo pokazuje manje razlike u priobalju i dalje od obale. Procijenjeno je da se oko 6% larvi u priobalju može smatrati nedovoljno ishranjenima, a na vanjskim postajama gladuje 22% larvi. Raspoloživa hrana utječe i na dnevni ritam ishrane, što se vidi na zalihama u jetri, koje se koriste noću kada nema vanjskog hranjenja.

Može se zaključiti da je određivanje stanja larvi pomoću dnevnog rasta otolitskih prstenova kao i pomoću analize stanja tkiva pokazalo vidljiv odnos s količinom raspoložive hrane i hidrografskim svojstvima uzduž estuarija.