

**HISTOLOGICAL STUDY ON LARVAL NUTRITIONAL CONDITION OF THE  
 SOUTHWEST ATLANTIC ANCHOVY, *ENGRAULIS ANCHOITA* (HUBBS AND  
 MARINI 1935), CAUGHT IN THREE HYDROGRAPHICALLY DIFFERING  
 FRONTAL SYSTEMS OF THE SOUTHWEST-ATLANTIC**

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**ABSTRACT**

During the 11<sup>th</sup> cruise of R.V. METEOR in autumn 1989, larvae of the Southwest Atlantic anchovy, *Engraulis anchoita* (HUBBS & MARINI 1935), were caught in three areas of high oceanographic diversity in the Southwest Atlantic. One objective was to compare the amount of well or insufficiently fed fish larvae, when they have grown up in hydrographically differing environments. The samples were taken in subtropical waters on the Brazilian shelf characterised by upwelling events, in a region off Uruguay which is influenced by the freshwater outflow of the Rio de la Plata, and in a tidal mixing front on the Argentine shelf around the Valdez peninsula which is influenced by the cold Malvinas current. Hauls were carried out with a modified MOCNESS equipment, and about 1000 larvae were selected and prepared using standard histological techniques. The histological appearance of gut, liver, pancreas and of musculature were selected as condition criteria, and the height of the midgut mucosa was measured. A simultaneous classification of organ developmental stages allowed a stage related comparison of the individual larval nutritional condition. The histological analysis classified more larvae in insufficient nutritional conditions caught off Brazil, than off Uruguay and Argentina.

## INTRODUCTION

One task of the 11<sup>th</sup> cruise of R.V. METEOR in 1989 was to investigate the biological-oceanographic conditions, which are assumed to affect variability in recruitment of the Southwest-Atlantic Anchovy, *Engraulis anchoita* (HUBBS & MARINI 1935). Presuming fish stock recruitment to depend on the survival of the early life stages (reviewed by MAY 1974), which is controlled by development and density of larval food and on the frequency of predators (BLAXTER 1969, HOUDE 1987, HUNTER 1984, NELLEN 1986), main attention was directed towards those environmental conditions which should affect mortality during the early life stages. As part of the *Ocean Science In Relation To Living Resources* (OSLR/SARP) program, the investigation had to be carried out in a marine area of high oceanographic diversity in order to check its effect on quality and density of phyto- and zooplankton, the nutritional condition of the fish larvae, and the small-scale distribution of fish eggs and larvae and their predators. The Southwest-Atlantic anchovy was particularly suitable for this kind of study because of its long spawning season and its extended spawning area, covering the shelf from South Brazil down to Patagonia. The research area was subdivided into sections off Brazil, Uruguay and Argentina regarding the different hydrographical situations (Fig. 1). As depicted in Figure 2, the Brazilian area is characterised by upwelling events in the subtropical waters on the shelf off Santa Marta (Area B, Fig. 1), while the Uruguayan section (Area U, Fig. 1) is affected by the freshwater outflow from the Rio de la Plata, by cold Subantarctic coastal waters, and by the tropical Brazil current, which result in water bodies with different salinities. The area of the Argentinean shelf around the Valdez peninsula (Area A, Fig. 1) is influenced by tidal mixing fronts and the cold Malvinas Current.

Among others, it was intended to classify well or insufficiently fed fish larvae employing biochemical (RNA/DNA ratios (CLEMMESSEN 1989) and trypsin activities (UEBERSCHÄR 1988)) and histological standard techniques, and to compare their frequency according to the three hydrographically differing environments. For the histological approach, the appearance of gut, liver, pancreas and of musculature should be taken as condition criteria, and the midgut cell height should be measured simultaneously.

Several authors discussed developed fish larvae to be less susceptible to short-time insufficiency of food supply (BLAXTER & EHRLICH 1974, RICE *et al.* 1987) due to enhanced energy reserves (POWELL & CHESTER 1985, YIN & BLAXTER 1987). Consequently, samples of developed larvae should be composed of less individuals in starving conditions than those with many younger ones, which have been exposed to the same insufficient food supply. Storage ability should be more linked to organ than to somatic development or age. Several studies have pointed out that different temperature can result in different organ developmental stages at the same length (GORBUNOVA 1981, JOHNSON

1983, JOHNSON & FELTES 1984, SIEG 1992a, 1992b). For that reason, measurements of length do not seem to be reliable enough to estimate potential energy reserves. Both might be essential for a *surplus* of energy, enough food to digest and enough organic ability. The larval organ system develops ability and quality of food digestion during ontogenesis, at least with a functional stomach. Hence, a histological classification of organ development should allow to estimate on the potency of a fish larva to store energy, although this method does not give clear information about the fat reserves. Following these ideas, organ developmental stages of larval *Engraulis anchoita* were classified prior to the definition of the nutritional condition.

Moreover, it is essential to consider organ development in order to compare larval nutritional condition classified by measurements of the midgut cell height. In this study, the attempt was made to employ the method described by THEILACKER & WATANABE in 1989 also for larvae which had their midgut epithelium already folded. However, during the development from a stretched to a folded structure, gut epithelium height is markedly decreased, and later increases again. Consequently, midgut cell height as criterion of nutritional condition can only be compared within a defined developmental stage.

This paper concentrates on the presentation of preliminary results of the histological investigation of larval *Engraulis anchoita*, since the analyses of zooplankton densities and biochemical conditions are still in progress.

## MATERIAL & METHODS

Three sections/boxes were studied along the east coast of South America off Brazil, Uruguay and Argentina (Fig. 1). The area off Uruguay (Box U) was visited twice because of unfavourable weather conditions during the first visit. All sections were subdivided into transects running from near coast to off coast. Positions of the transects were selected for biological sampling according to the abundance of anchovy eggs and/or larvae and to different water bodies. Biological sampling was repeated twice to four times on each transect in order to compare samples from different times of the day. The fish larvae were caught using a modified MOCNESS equipment (WIEBE *et al.* 1976, 1985, NELLEN *et al.* 1988). Since larval *Engraulis anchoita* were supposed to be concentrated in the upper 50 m of the water column (CIECHOMSKI *et al.* 1986), the gear was lowered to a depth of 50 m at maximum, and the overall time of the tows was 12 to 15 minutes. The larvae were immediately fixed in a buffered 6% formaldehyde seawater solution. All specimen were individually checked for sampling damage under a microscope, and up to 45 specimen per sample were transferred and stored in new fixative at 4°C until further preparation. Larvae were coded to exclude arbitrary

evaluations by knowing the individual's identity. Standard length of the fixed larvae was determined to the next millimetre below. About 1000 larvae were dehydrated in alcohol series, immersed in benzyl benzoate and finally embedded in paraffin. Sagittal sections of 5  $\mu$ m were mounted and stained using a mixed PAS (Periodic Acid *Schiff's*) - HE (Hematoxylin-Eosin) technique.

The histological grading covered the following organs/tissues:

Table 1:

- |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>- <b>Liver/hepatocytes:</b> Glycogen content, cytoplasm, nuclei, cellular shrinkage/separations</li> <li>- <b>Pancreas:</b> Cellular ratio zymogen/cytoplasm</li> <li>- <b>Foregut:</b> Cell height</li> <li>- <b>Midgut:</b> Intracellular vacuoles, mikrovilli, cytoplasm, cell separations</li> <li>- <b>Hindgut:</b> Pinocytotic vacuoles</li> <li>- <b>Gut content:</b> Distinguished between food in the fore- and in the mid-/hindgut</li> <li>- <b>Musculature:</b> Fibre separation, fibrils visible, presence/absence of the connective tissue</li> <li>- <b>Cartilage of the jaws and the branchial elements:</b> Shrunk chondrocytes</li> </ul> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Tissues/cells were graded according to the histological classification by O'CONNELL (1976 & 1980) for larval *Engraulis mordax*, but in the present study, the histological indices given above were graded into five classes ranging from 1 (best) to 5 (worst condition). In addition to the histological interpretations, midgut cell height was measured following the method of THEILACKER & WATANABE presented in 1989. It was attempted to employ the method also for larvae which had the midgut epithelia already folded. These midgut folds are usually composed of cells of different height: the cells on the top of the folds are stretched and columnar, those at the bottom between the folds are lower and cubic. Therefore, midgut cells were chosen for analysis which were located in the middle of the height in each fold. Several measurements were taken of the ventral row in the posterior region of the midgut, the range was recorded, and the mean value was calculated to be connected with the histological score.



## RESULTS & DISCUSSION

### Length Frequencies & Developmental Stages

Standard length frequency of selected and fixed larval *Engraulis anchoita* ranged from 3 to 12 mm, determined to the next mm below (Figs. 3-6). Selection of larvae for histological analysis concentrated on the smaller individuals because of their higher susceptibility to starvation. Consequently, the length frequencies are biased by this selection and by the fact that not all caught larvae were prepared for histology. Nevertheless, since no smaller larvae could be sampled in the Brazilian section (Box B), a comparison between Box B and Box A (Argentina) indicates that the population off Brazil was further developed than the individuals off the Valdez peninsula.

Higher temperatures influence larval metabolism, velocity of growth and organ development, as well as the relation between organ developmental status and length. Measuring larval length provides only restrictive information about the developmental stage, which is essential to know in order to estimate the development-dependent susceptibility to starvation, and to allow comparisons of midgut cell height, in general. Six developmental stages of larval *Engraulis anchoita* were defined (Table 2).

Data were calculated for each area/box separately in order to check how different water temperature influences developmental stage (DS) at standard length (SL) (Figs. 7-10). Individuals off Brazil (Fig. 7; T (C°) at the surface = 23.5-21.9) measured 4 mm below ranged from DS III to IV (Arabian numbering in the graphs) with a median at DS III, while at the same standard length, larvae of the colder waters off Patagonia (Fig. 10; T (C°) at the surface = 16.4-12.7) fluctuated between DS II and III with a median at DS II. DS VI was first classified at a SL of 11 mm off Argentina. Some larvae caught off Brazil reached this stage at a SL of 6 mm already. At these orders of magnitude, bias due to the length measurement to the next millimetre below can be regarded to be neglectable. Since the slope of the regression calculated for the medians decreased during growth, it can be assumed that this phenomenon is mainly restricted to the very early larval development: either the Brazilian larvae had a smaller hatching length, or they had a higher velocity of organ development during their early lifetime.

Figures 11 to 14 depict the frequencies of developmental stages determined from the prepared material of each area/box. According to the larger standard length measured off Brazil (Fig. 3), organ development was enhanced, too (Fig. 11). Both length and DS frequencies in the Uruguayan area (Figs. 4 & 5, 12 & 13; Box U1 & U2) showed a trend towards higher values

Table 2:

**DS I: *Jolk Sac Stage*** - jolk mass clearly detectable or remnants anterior to the developing liver

**DS II:** No jolk detectable - first small swim bladder bulge - straight digestive tract - ventral jaw with a single layer of chondrocytes

**DS III:** Swim bladder anlage with multiplied cell layers - pancreas starts cranial extension - esophagus epithelium height increased - gut epithelium still unfolded - ventral jaw possesses several layers of chondrocytes

**DS IV:** Swim bladder further increased, shows small cavity and firstly a cranial tubular branch - ventral midgut mucosa folded, dorsal row starts folding - hindgut still unfolded

**DS V:** Swim bladder with different types of cell layers - ventral and dorsal midgut mucosa folded - hindgut starts folding

**DS VI:** Swim bladder either with a very thickened wall or strongly extended because of first filling - esophagus cell height further increased, now with several cell layers - pancreas extends cranial beneath the glomerulum and caudal above the anterior part of the midgut - hindgut folding completed

during the second biological sampling (U2), which can be attributed to the time interval of 10 days between both visits. Anyway, when determining numbers of well fed and starving larvae according to the sampled areas/boxes, these data have to be considered because of the bigger and older larvae caught off Brazil, which have had more time to store energy reserves. Otherwise, insufficient feeding conditions off Brazil could be underestimated.

#### Midgut Cell Height In Relation To Histological Grading

Different cell indices were cited in order to check the relation between histological scoring and midgut cell height. Best histological appearance should co-occur with high midgut epithelium, whereas a shrunken mucosa is to be expected when cellular breakdown can be observed. DS I was not considered, since jolk sac larvae do not starve. All individuals were taken for the first comparison, which hepatocytes were filled with glycogen (PAS-positive reacting substances in the liver were defined as glycogen according to O'CONNELL & PALOMA (1981)). These larvae were in optimal condition (Class 1), since glycogen is regarded to be an energy reserve (LOVE 1974, O'CONNELL & PALOMA 1981, POWELL & CHESTER 1985), which can be completely exhausted within a single day during food deprivation (WATANABE 1985). This was true for the next comparison, when liver glycogen was classified as "5": cell inclusions

were completely absent, but the individuals of this group still possessed hepatocytes as well as mid- and hindgut cells in good condition (refer to Table 1). The next group included those larvae, which showed midgut cell separations and dark cytoplasm, and for the last check all larvae were cited, which scores given for hepatocyte cytoplasm and close cell contact summed up to 8 or higher, which midgut cells showed the conditions of the previous comparison, and which hindgut cells lacked any cell inclusions (refer to Table 1). The results of these comparisons are summarised in Figures 15 to 19. Other organs/tissues, as there were cartilage, foregut, musculature, and pancreas, did not show such as clear histological alterations as reported by O'CONNELL 1976 & 1980. Individuals without glycogen can not be classified as having been in emaciated condition. According to O'CONNELL & PALOMA (1981), this can be due to normal daily periods of feeding and nonfeeding. In contrast, the histological pictures cited for the last comparison (very right columns in the Figs. 15-19) are characteristic for starving larvae with irreversible cell damages (comp. O'CONNELL 1976, 1980, THEILACKER 1978). No larvae of DS II and III of all transects were found in such bad condition (refer to Figs. 15 & 16, both right columns). Consequently, no information is available about the respective decrease of midgut cell height, when *Engraulis anchoita* larvae pass into starvation.

There are many overlappings in the frequencies of midgut cell height and the different histological classification when comparing DS IV to VI (Figs. 17-19). For example, in Figure 17 (DS IV), it is not possible to relate a larva with a midgut cell height of 12,5  $\mu\text{m}$  to a histological condition. Only DS V (Fig. 18) showed an obvious deviation in midgut cell height between well fed individuals, and the starving ones of the very right column.

There are two explanations for this finding: either larvae attached to bad histological condition had not starved long enough to give clearer deviations from "normal" midgut cell heights, or they had starved until cellular damage, but the midgut cell heights did not vary more than those of fed individuals of the same developmental stage. Both could be true: THEILACKER & WATANABE (1989) presented measurements of midgut cell height in larval *Engraulis mordax*, ranging from about 18.7 down to 7.3  $\mu\text{m}$  (average=11.8  $\mu\text{m}$ ) for individuals of SL<4 mm, which had starved for three days at 15.5 °C. All larvae had a straight, unfolded gut epithelium. Referring to the material of this study (Figs. 15 & 16), when citing liver glycogen classified as "1" (comp. temp. range, same DS, same SL), the larvae between the lower and upper quartiles (indicated by the frame) were out of the range of the average value reported by the authors. Consequently, even starved *Engraulis anchoita* larvae of these stages can be expected to have a lower mucosa cell height. On the other hand, the histological features taken for both right comparisons in the graphs of Figures 15 to 19 are based on O'CONNELL's *in situ* study on *Engraulis mordax* in 1980, in which he attributed these findings to emaciated larvae. From this reference, the respective larvae of this study should have starved, but

measurements of their midgut cell height did not yield in frequency distributions, which are sufficiently divergent from those of fed individuals. In conclusion, further calibration work is needed in order to obtain more reliable information on the definite relation between midgut cell height and larval histology at different temperatures, levels of starvation and developmental stage.

#### Nutritional Conditions On The Transects Sampled

Because of the findings given above, the measurements of midgut cell height were not considered for the classification of different nutritional conditions on the transects sampled. Figures 20 to 28 depict the frequencies of larval *Engraulis anchoita* on each transect according to their different nutritional condition. Larvae of Grade 1 had food in the mid- or hindgut. Some individuals had food in the esophagus, however, this was ignored in the evaluation, because it could result from both normal feeding as well as stress-induced food intake during net time. Grade 2 in these graphs includes all larvae who had no food in the midgut, but which hepatocytes were full of glycogen (defined as Class 1, refer to Chap. *Material & Methods*) or which had glycogen remnants (defined as Classes 2-4). Individuals were classified to the worst Grade 4, when their organs/tissues showed alterations as described for a starving condition in the graphs of the Figures 15-19: the scores of hepatocyte cytoplasm and close cell contact summed up to 8 or higher, the midgut cells showed dark cytoplasm and cell separations, and the hindgut cells lacked any cell inclusions (refer to Table 1). All larvae who had better histological conditions than the individuals of Grade 4 but did not have cell inclusions in the liver were attached to Grade 3.

In general, most of the *Engraulis anchoita* larvae seemed to prefer feeding during daylight, as indicated by the empty rectangles above the columns including larvae graded "1". This is in accordance with data of SÁNCHEZ *et al* (1991), who described a daily feeding pattern over a 14 h period started after sunrise. Glycogen was present in the liver during day and night. According to WATANABE (1985), who found at 15-20 °C a decrease of liver glycogen in goby larvae already 5h, and a total reduce one day after food deprivation, larval *Engraulis anchoita* should show a decreased glycogen level at dawn due to non-feeding during night. This would be in line with O'CONNELL & PALOMA 1981, who found daily circles in the hepatocyte's glycogen quality. Grade 2 in the Figures 20 to 28 includes larval livers which glycogen contents scored from Class 1 to 4, and, therefore, it includes not only the best glycogen class "1". Nevertheless, many larvae caught in the early morning had empty guts, but best glycogen level. In conclusion, in spite of daily fluctuations in the hepatocyte glycogen content, a complete consumption should not occur when exposed to normal food conditions.



First measurements of RNA/DNA ratios of *Engraulis anchoita* larvae caught at station 995 off Brazil revealed about 80 % of the sampled individuals with SL 5-10 mm to be in bad nutritional condition (CLEMMESSEN-BOCKELMANN, pers. comment). This seems to match the histological findings, which classified 87% of the respective larvae (SL=4-12 mm) in a suboptimal condition (refer to Fig. 21). This percentage consists of individuals with reversible suboptimal cell conditions (Grade 3) and of those without having had the opportunity to survive due to cellular collapse (Grade 4). From these findings it can be concluded that determining RNA/DNA ratios is not reliable enough without further calibration work to detect fish larvae with irreversible cell damages. The latter have passed the "point of no return", and contribute to larval mortality rates due to starvation.

Figures 20 to 28 were based on individuals of all developmental stages (II-VI) to give a first general survey of the feeding situation on the transects sampled. In the future, it is intended to evaluate the histological data in relation to developmental stages to allow comparisons of the frequencies of different nutritional condition at each station, on each transect, and finally in each area investigated. Unfortunately, no larvae in early stages were caught off Brazil, where the food supply seems to have been suboptimal. They would have made possible a comparison of the nutritional condition of early post yolk sac stages, which are assumed to be most susceptible to starvation (comp. RICE *et al.* 1987). However, THORISSON (1992) discussed metamorphosis as another critical stage to affect the energy budget in the life history of marine fish larvae. This idea and the histological appearance of the developed larvae of the Brazilian section stress the need of studies on nutritional condition also for later stages than those of the early post yolk sac period.

HUBOLD (1980) measured lower values for chlorophyll-a concentrations and zooplankton densities in those areas off south Brazil and the Rio de la Plata estuary, which were under dominant influence of the tropical waters. In contrast, higher concentrations of both parameters were found in the Subantarctic water. This may indicate a lower primary production off Brazil as compared to the Argentine section. ALHEIT *et al.* (1991), however, presented data of the chlorophyll-a measurements determined on this cruise of R.V. METEOR, which showed quite similar values off Brazil, Uruguay and Argentina, fluctuating between 1.5 to 5 mg/m<sup>3</sup>. Since the zooplankton analysis and the measurement of RNA/DNA ratios are still in progress, a final valuation of the ecological situation at time of the cruise can be given not before the end of this year.

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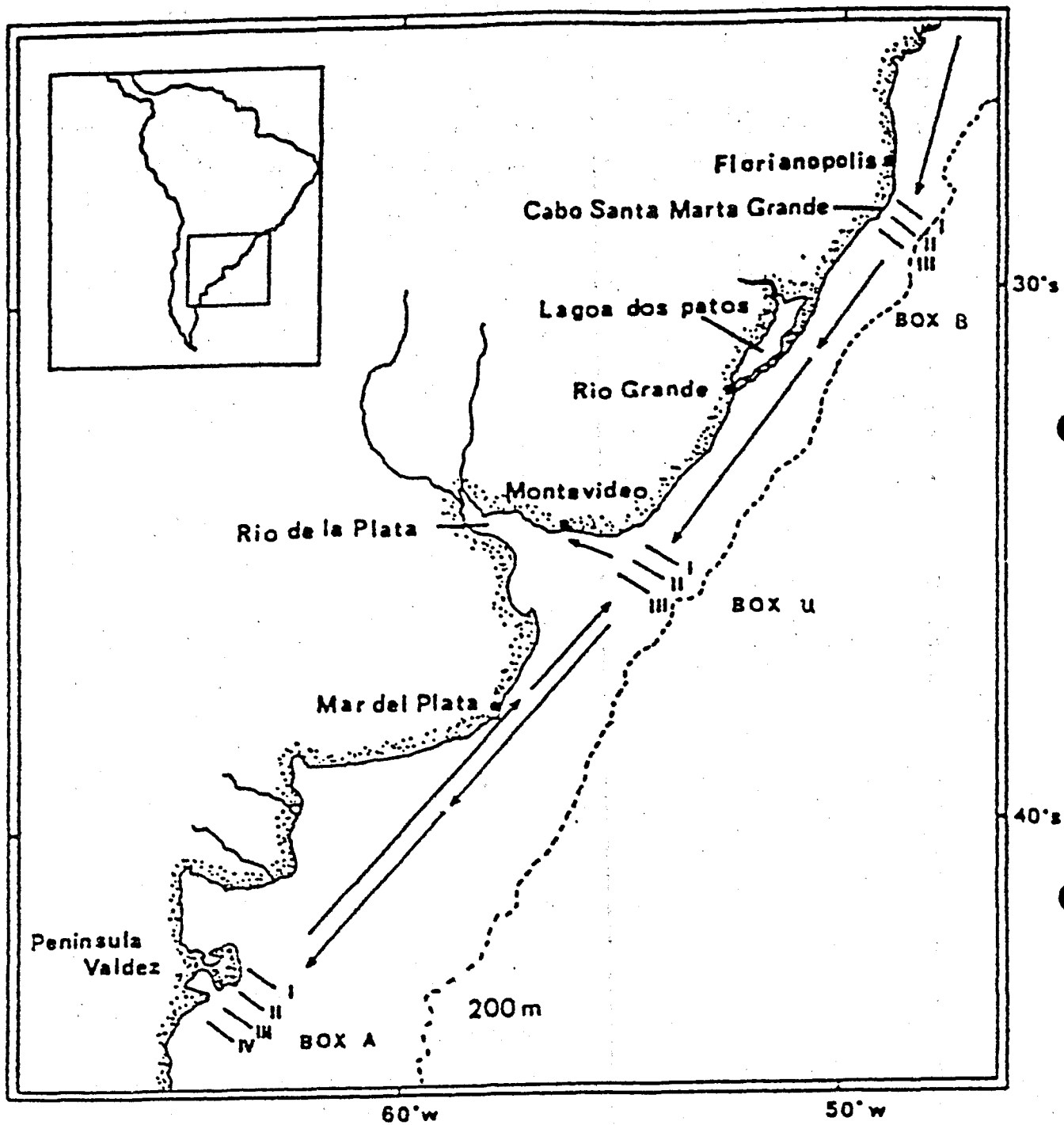


Fig. 1: Location of sampling areas/boxes and transects during METEOR cruise 11/3. Box B = Box off Brazil, Box U = Box off Uruguay, Box A = Box of Argentina.



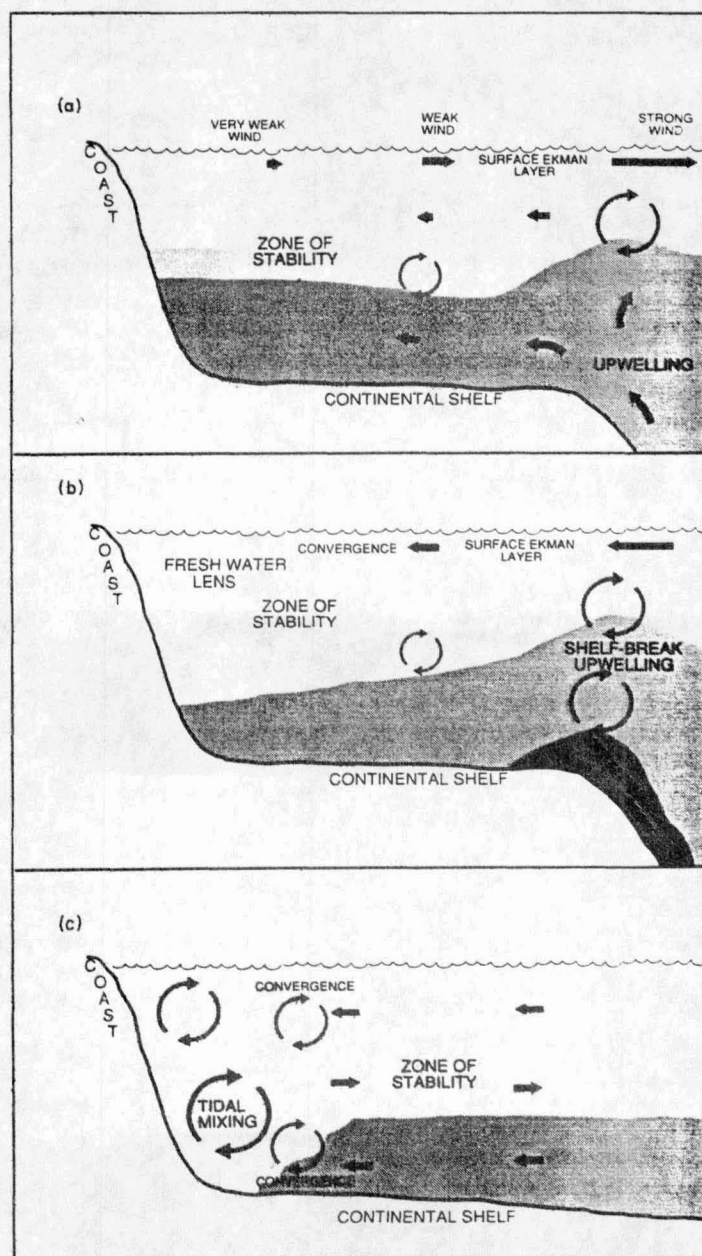


Fig. 2: Schematic diagrammes on environmental situations in the three different areas sampled during METEOR cruise 11/3: a) The wind-driven coastal upwelling regime off Cape Santa Marta Grande, Brazil (Box B); b) The estuarine and shelf-break fronts off Uruguay (Box U); c) The tidal mixing front off Peninsula Valdez, Argentina (Box A). Reproduced from BAKUN & Parrish. 1991. ICES J. mar. Sci. 48.

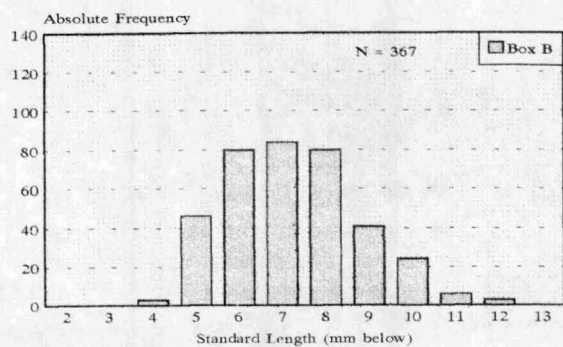


Fig. 3: Length frequency of prepared *Engraulis anchoita* larvae caught in the Brazilian area.

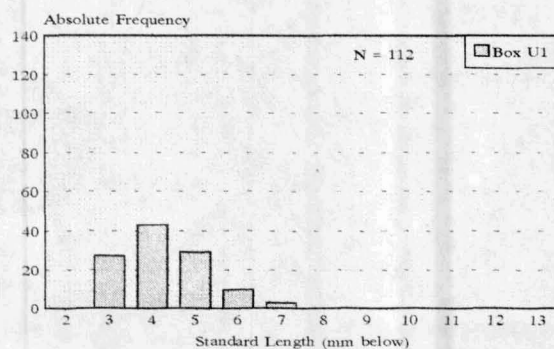


Fig. 4: Length frequency of prepared *Engraulis anchoita* larvae caught in the Uruguayan area (1. run).

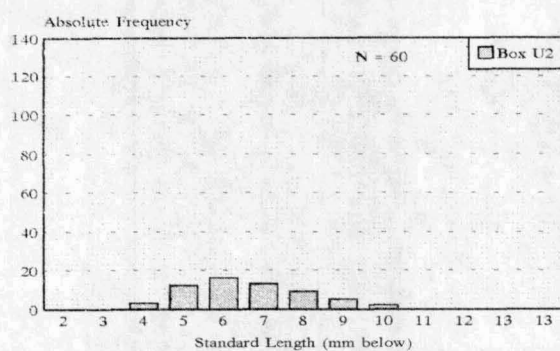


Fig. 5: Length frequency of prepared *Engraulis anchoita* larvae caught in the Uruguayan area (2. run).

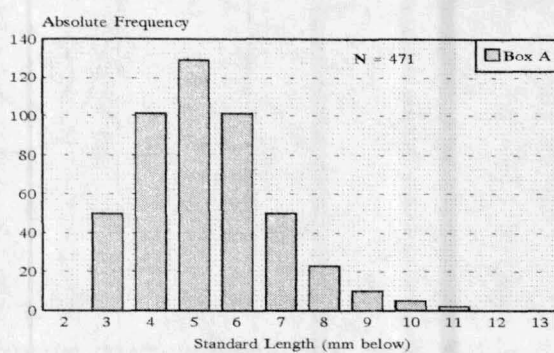


Fig. 6: Length frequency of prepared *Engraulis anchoita* larvae caught in the Argentine area.

# Sampling Area Off Brazil (Surface Temp. Range: 23.5 - 21.9 °C)

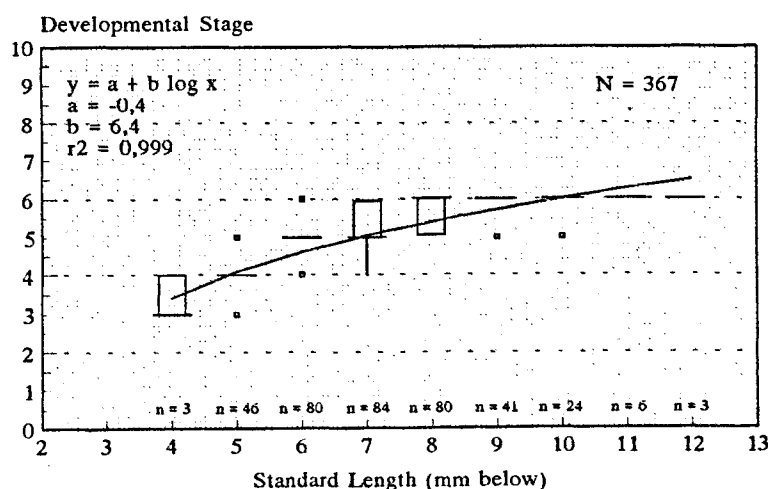


Fig. 7: Sampling area off Brazil: Frequency distributions of different developmental stages within the standard length classes. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis. The logarithmic regression was calculated for the medians.

## 1. Sampling Area Off Uruguay (Surface Temp. Range: 19.1 - 18.0 °C)

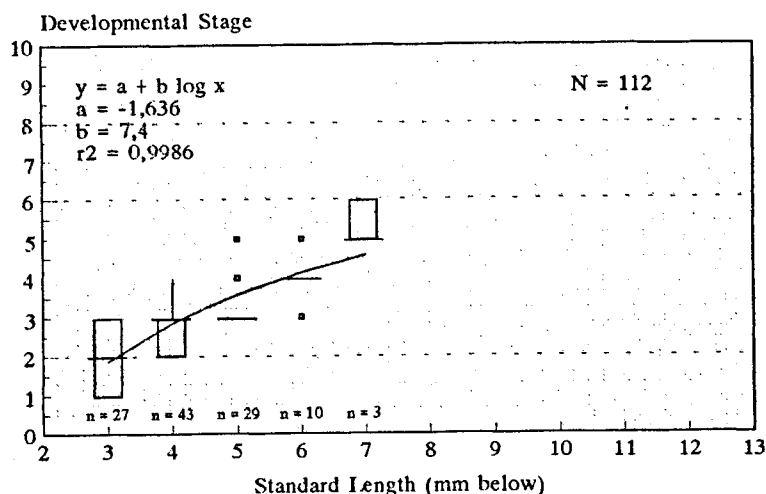


Fig. 8: Sampling area off Uruguay - 1. run: Frequency distributions of different developmental stages within the standard length classes. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis. The logarithmic regression was calculated for the medians.

## 2. Sampling Area Off Uruguay (Surface Temp. Range: 20.6 - 18.5 °C)

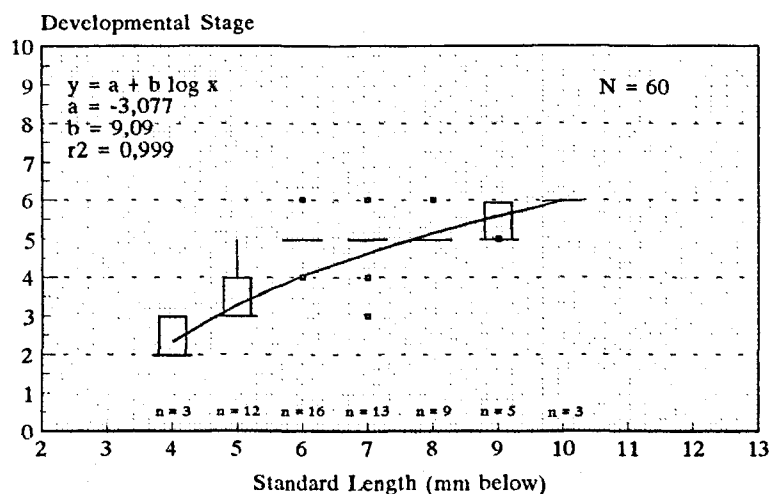


Fig. 9: Sampling area off Uruguay - 2. run: Frequency distributions of different developmental stages within the standard length classes. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis. The logarithmic regression was calculated for the medians.

## Sampling Area Off Argentina (Surface Temp. Range: 16.4 - 12.7 °C)

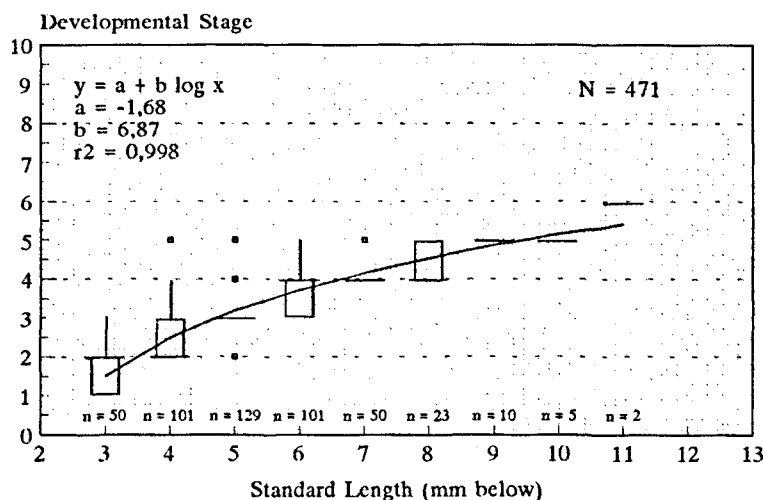


Fig. 10: Sampling area off Argentina: Frequency distributions of different developmental stages within the standard length classes. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis. The logarithmic regression was calculated for the medians.



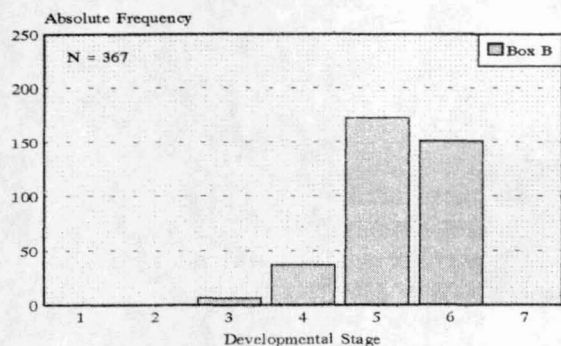


Fig. 11: Frequency of larval developmental stages of prepared *Engraulis anchoita* larvae caught in the Brazilian area.

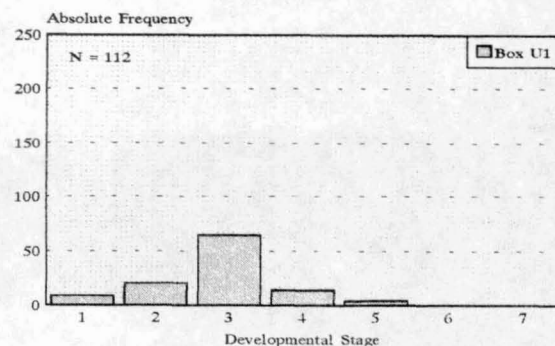


Fig. 12: Frequency of larval developmental stages of prepared *Engraulis anchoita* larvae caught in the Uruguayan area (1. run).

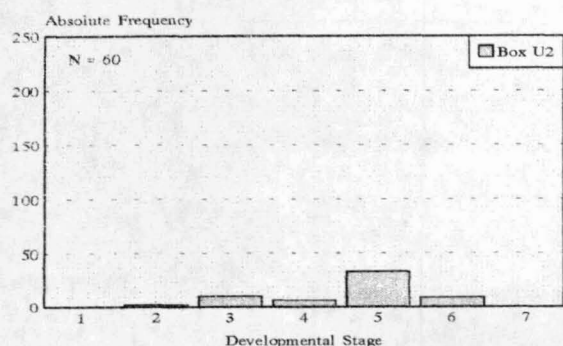


Fig. 13: Frequency of larval developmental stages of prepared *Engraulis anchoita* larvae caught in the Uruguayan area (2. run).

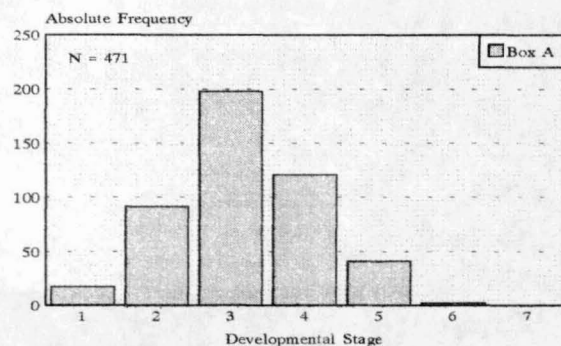


Fig. 14: Frequency of larval developmental stages of prepared *Engraulis anchoita* larvae caught in the Argentine area.

### Relation: Histological Classes - Midgut Cell Height Developmental Stage II

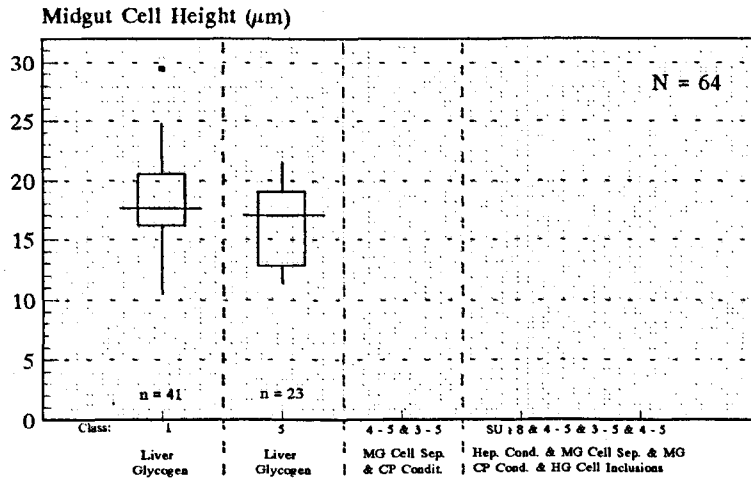


Fig. 15: Relation between the histological nutritional classes and the respective midgut cell heights for all larvae of the developmental stage II. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give unusual values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis.

### Relation: Histological Classes - Midgut Cell Height Developmental Stage III

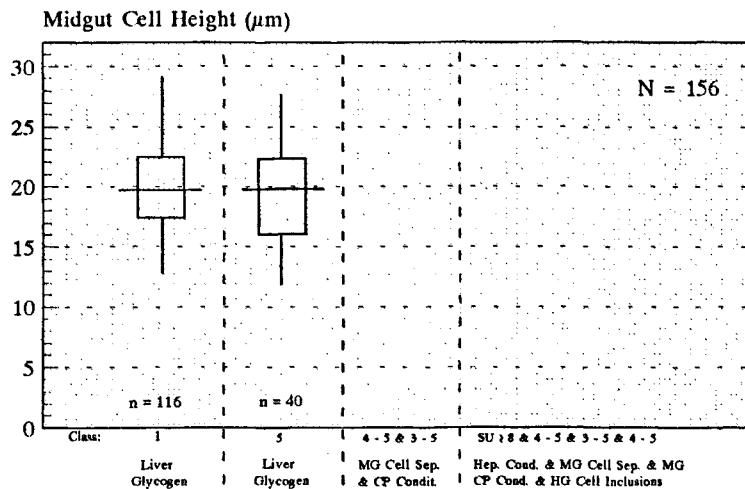


Fig. 16: Relation between the histological nutritional classes and the respective midgut cell heights for all larvae of the developmental stage III. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give unusual values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis.



**Relation: Histological Classes - Midgut Cell Height  
Developmental Stage IV**

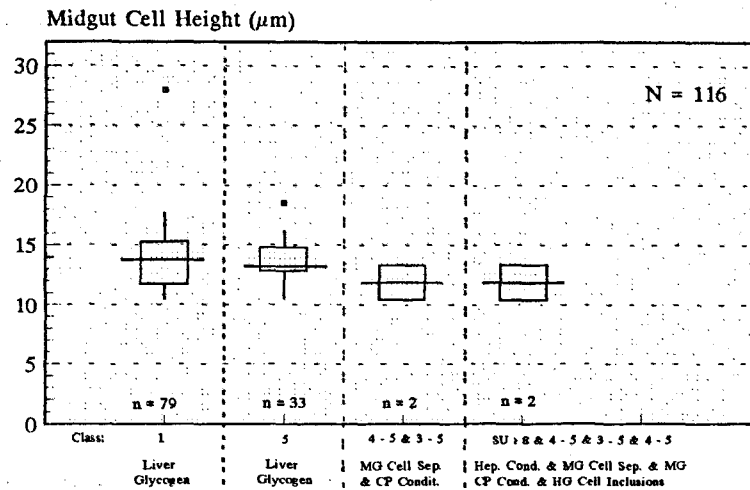


Fig. 17: Relation between the histological nutritional classes and the respective midgut cell heights for all larvae of the developmental stage IV. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give unusual values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis.

**Relation: Histological Classes - Midgut Cell Height  
Developmental Stage V**

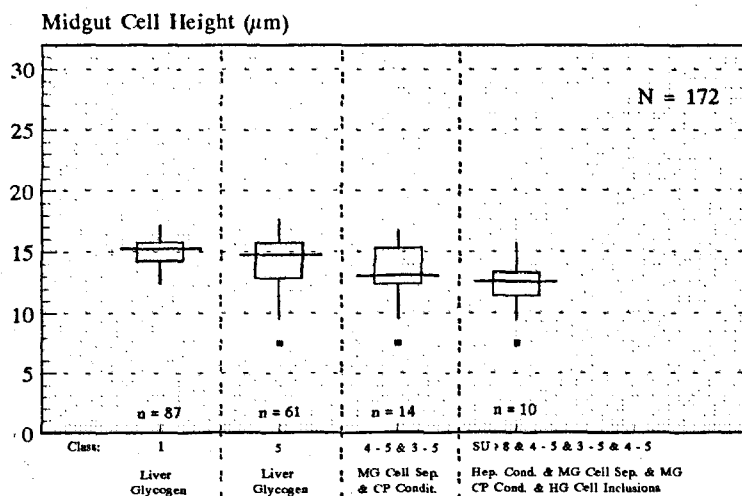


Fig. 18: Relation between the histological nutritional classes and the respective midgut cell heights for all larvae of the developmental stage V. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give unusual values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis.

# Relation: Histological Classes - Midgut Cell Height Developmental Stage VI

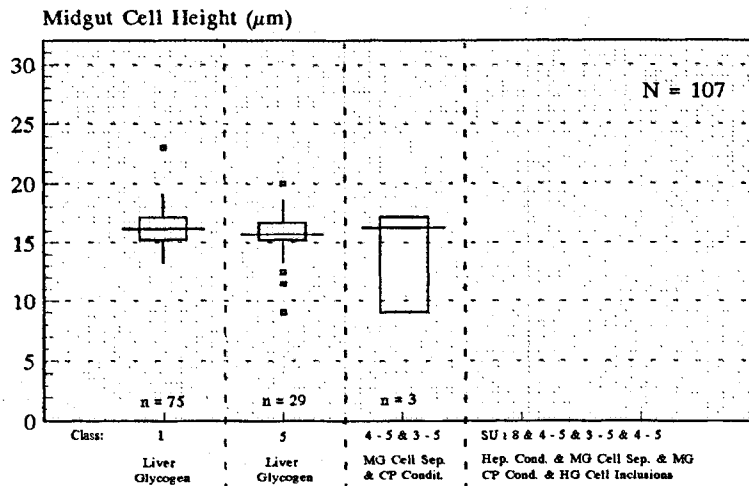


Fig. 19 Relation between the histological nutritional classes and the respective midgut cell heights for all larvae of the developmental stage VI. The horizontal line is the median, the boxes indicate the lower and upper quartiles, and the vertical lines extend out to the extremes. The dots give unusual values occurring more than 1.5 times away from the interquartile range. The respective "n" are given above the x-axis.

## Nutritional Conditions Of Larval *Engraulis anchoita* Transect Brazil 1

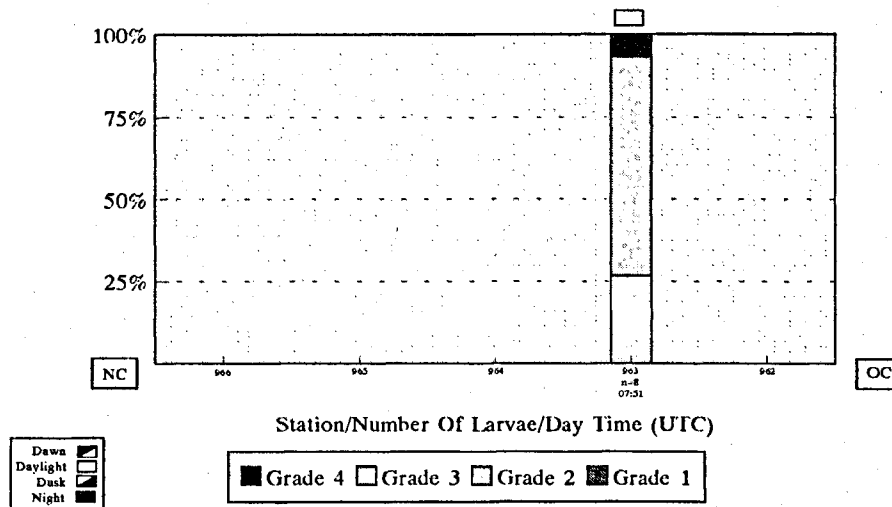


Fig. 20: Nutritional conditions of larval *Engraulis anchoita* caught on transect Brazil 1. Histological grades are defined as follows: Grade 1 = Food in the mid- or hindgut; Grade 2 = No food in the gut, but glycogen in the hepatocytes; Grade 3 = No food & no glycogen, but better histology than of Grade 4; Grade 4 = Sum of scoring of hepatocyte cytoplasm and cell contact  $\geq 8$ , midgut cells dark & separated, hindgut cells without inclusions. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Brazil 2

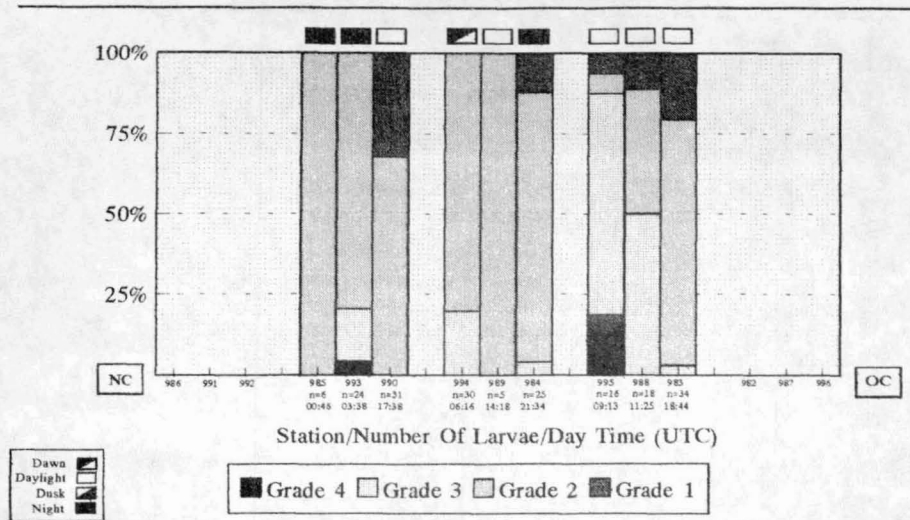


Fig. 21: Nutritional conditions of larval *Engraulis anchoita* caught on transect Brazil 2. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Brazil 3

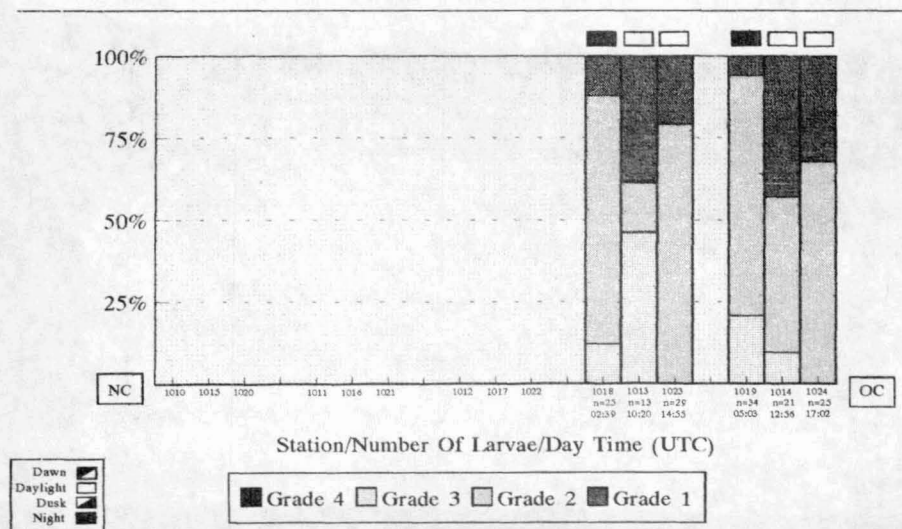


Fig. 22: Nutritional conditions of larval *Engraulis anchoita* caught on transect Brazil 3. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Uruguay 1

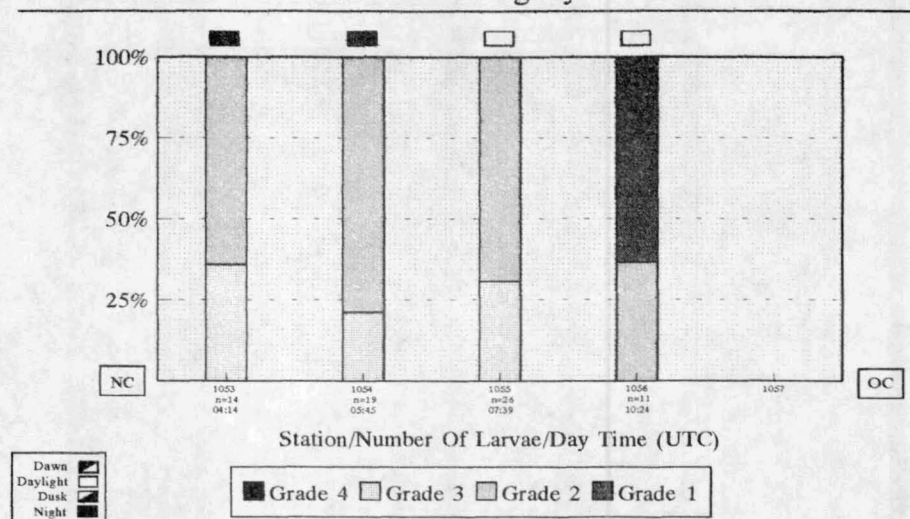


Fig. 23: Nutritional conditions of larval *Engraulis anchoita* caught on transect Uruguay 1. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Uruguay 3

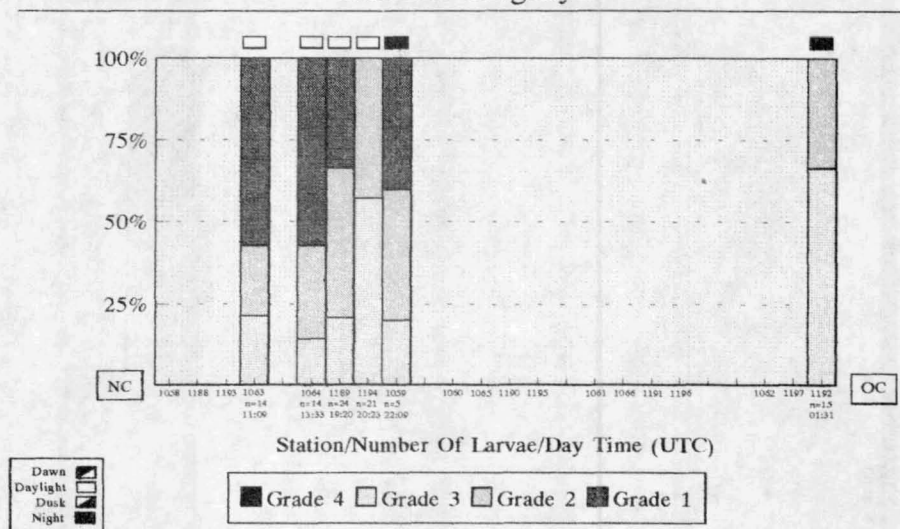


Fig. 24: Nutritional conditions of larval *Engraulis anchoita* caught on transect Uruguay 3 (both runs). Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.



### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Argentina 1

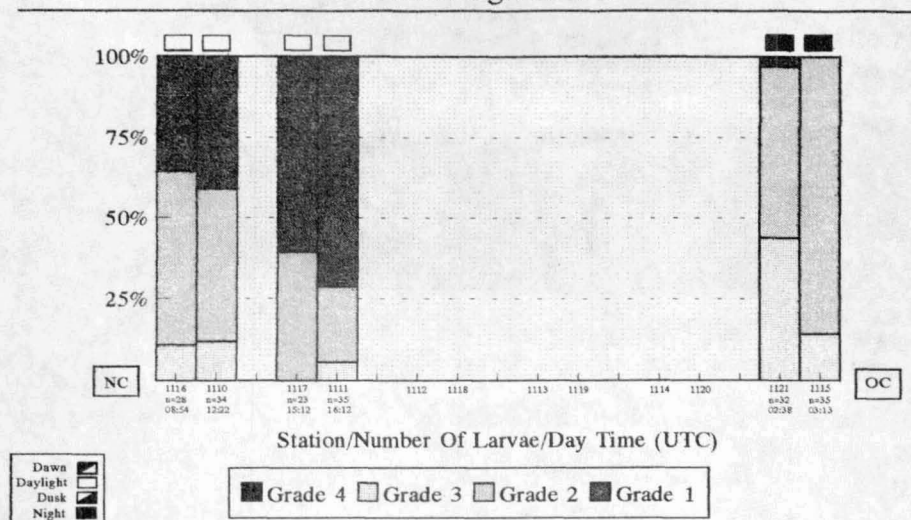


Fig. 25: Nutritional conditions of larval *Engraulis anchoita* caught on transect Argentina 1. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Argentina 2

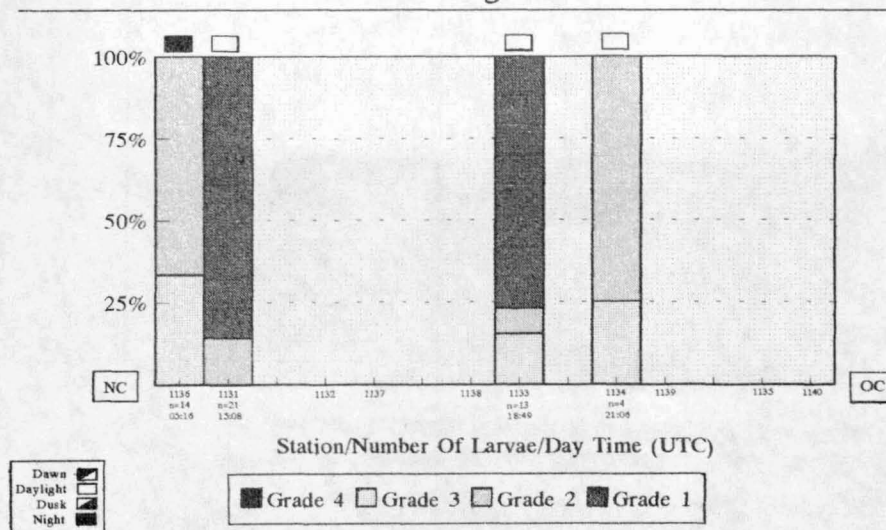


Fig. 26: Nutritional conditions of larval *Engraulis anchoita* caught on transect Argentina 2. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Argentina 3

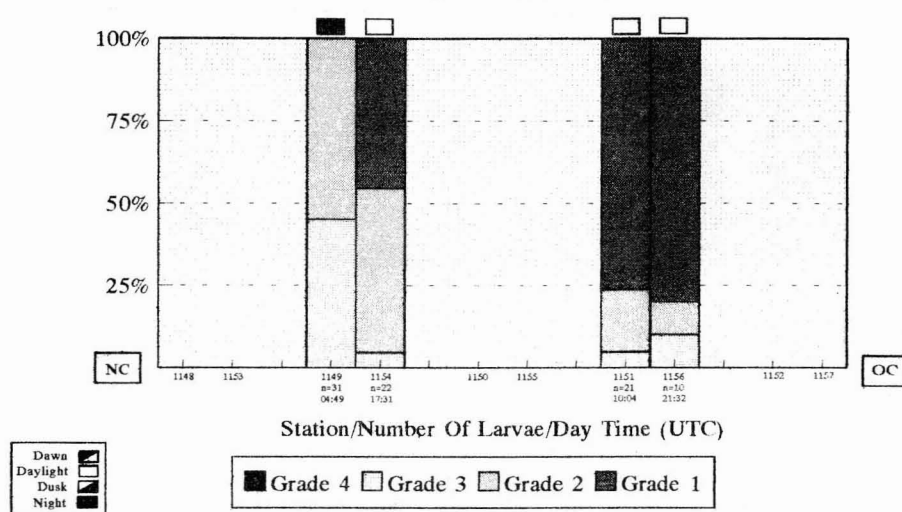


Fig. 27: Nutritional conditions of larval *Engraulis anchoita* caught on transect Argentina 3. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.

### Nutritional Conditions Of Larval *Engraulis anchoita* Transect Argentina 4

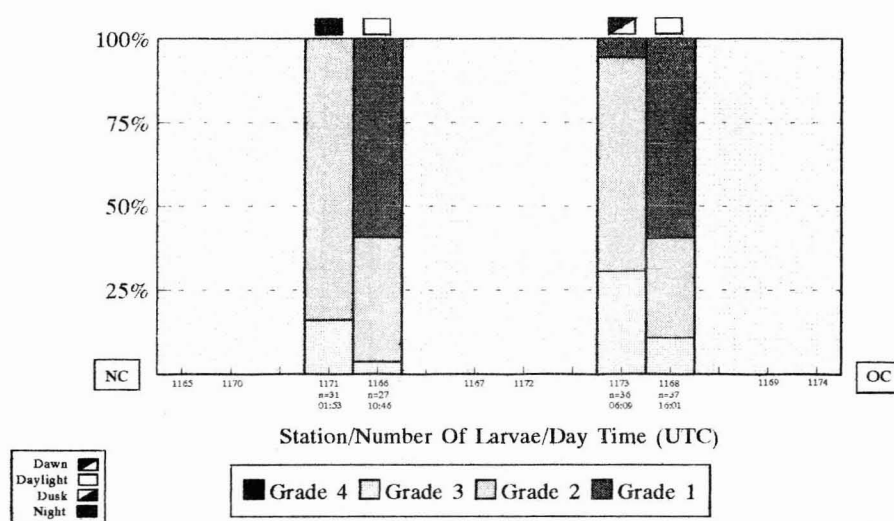


Fig. 28: Nutritional conditions of larval *Engraulis anchoita* caught on transect Argentina 4. Histological grades refer to Fig. 20. X-axis gives the station sampled, number of analysed larvae, and time of the haul. Stations were grouped according to same position and daytime. Day & night is indicated by the respective symbols above the columns. NC = near coast, OC = off coast.