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COMPARISON OF BIOCHEMICAL AND HISTOLOGICAL METHODS FOR THE EVALUATION OF THE IN SITU NUTRITIONAL CONDITION OF MARINE FISH LARVAE

by

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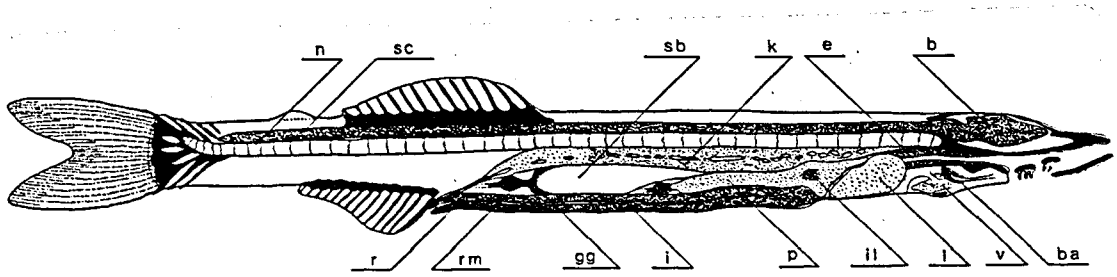

Vinciguerria spec.

ABSTRACT

To estimate the importance of starvation induced mortality for recruitment of marine fish larvae three distinct methods were applied to determine the nutritional condition of fish larvae in situ. In addition to highly sensitive fluorescence techniques for analysing RNA/DNA ratios and tryptic enzyme activities histological standard methods were used to compare the nutritional status of fish larvae of the genus Vinciguerria (Photichthyidae) caught in two ecologically different areas of the Indian Ocean: In the central Arabian Sea and on the continental shelf of Pakistan. A comparison of the results elaborated by the distinct methods shows a trend towards better nutritional conditions for fish larvae from the offshore region.

INTRODUCTION

Understanding the variability in recruitment is essential to the evaluation of the dynamics of fish populations (see summary, MAY 1974). It is assumed that predation and/ or starvation may be factors causing high mortality during the larval phases (BLAXTER 1969, HUNTER 1984, NELLEN 1986). Following HJORT's (1914) hypothesis postulating the existence of a food limited critical period for marine fishes during their larval development many workers have pursued studies with the objective of defining criteria to determine the nutritional condition of fish larvae.

The present study attempts to estimate the starvation induced mortality in situ by analysing nutritional conditions of marine fish larvae applying three different analytical methods on the same larval material. In addition to histological standard methods for evaluating organ and tissue conditions of fish larvae (O'CONNELL 1976, THEILACKER 1978, SIEG 1989) highly sensitive fluorescence techniques were used for measuring RNA/DNA ratios (CLEMMESEN 1987, 1988) and tryptic enzyme activities (UEBERSCHÄR 1988). Histological analysis is a time consuming and very extensive methode, which includes a lot of

diagnostic criteria (alterations in liver, pancreas, intestine, etc.). Both biochemical techniques result in relatively fast and defined measurements and determine one distinct parameter. Both techniques, as shown in laboratory experiments on herring larvae (UEBERSCHÄR 1985, CLEMMESEN 1987), are useful for the determination of the nutritional condition. The DNA content per cell is approximately constant and the amount of RNA in the cell is proportional to the amount of protein synthesis occurring, starved larvae have lower RNA/DNA ratios than fed ones. The measurement of tryptic enzyme activity takes into account that for fish larvae, its activity level is dependent on the feeding activity, because trypsin is a proteolytic enzyme and protein is the major component of their diet. Therefore fish larvae show lower enzyme activities under starving conditions than fed ones .

The advantage of the three used analytical methods is their ability to analyse each single fish larvae individually - and this could be essential for detecting starving individuals and allows for the determination of the variability in situ. The increase in sensitivity by applying highly sensitve fluorescence techniques for measuring biochemical parameters (CLEMMESEN 1988, UEBERSCHÄR 1988) now allows a simultaneous comparison with results from histological investigations. Therefore the applicability of the three methods for assessing nutritional conditions of marine fish larvae in situ was tested on larvae of the mesopelagic genus Vinciguerria (Photichthyidae). Samples were taken at two ecologically different sites during the Indian Ocean cruise of R. V. "Meteor" in spring 1987.

MATERIALS & METHODS

Fish larvae were caught in 44 hauls using the MOCNESS equipment (WIEBE et al. 1976) with 350 µm mesh size. The hauls were distributed in two distinct areas, which were estimated as ecologically different. Fig. 1 gives the areas sampled during the Indian ocean cruise of R.V. "Meteor" in spring 1987: the central Arabian Sea (leg 3b, Fig.1) and the shelf of Pakistan

(leg 3c, Fig.1). Most hauls were taken at night, because the abundance of fish larvae in the upper water layers was much higher during darkness than during daylight. The samples were limited to a maximum depth of 75 m and an overall duration of 15 minutes to avoid damaging and shrinkage of caught larvae during collection (HEWITT et al. 1985, THEILACKER 1986).

Immediately after catching the samples were transferred to buckets, ice cubes were added to reduce the influence of temperature on the degradation processes and the larvae were sorted out.

For the histological evaluation the fish larvae were fixed in a 4% glutaraldehyde-phosphate buffer-solution and were stored aboard in a 6% formaldehyde-seawater-solution at 4°C. At the institute the coded larvae were measured (standard lenght), dehydrated in increasing alcohol concentrations and after staying in benzylen benzoate for about one hour they were embedded in paraffine. The serial sections, made at 4-8µm in the sagittal plane, were mounted and stained using the hematoxyline/eosine-, the azan-, or a simultaneous HE/PAS-technique (SIEG 1989).

Since older fish larvae are more resistant to insufficient nutritional conditions than younger ones (LOVE 1980, POWELL and CHESTER 1985, RICE et al. 1987) and the composition of tissues and organs may change during ontogenesis (replacement of storage substances in liver for instance) an approximate correct interpretation of tissue and organ conditions due to starvation depends on the knowledge of the ontogenetic age of the analysed fish larvae (SIEG 1989). Therefore the ontogenetic age of each single Vinciguerria larvae was determined by comparing the levels of organic development prior to the evaluation of the nutritional condition using histological methods. This led to five defined age classes and the comparison of tissues and the investigation of organic alterations took place in each class separately.

For interpreting several states of nutrition the liver content of PAS-positive substances and the tissue conditions of

pancreas and intestinal mucosa were investigated qualitatively (SIEG 1989). Finally the analysed Vinciguerria larvae were decoded and classified according to their origin. To estimate, whether the two investigated sites differed significantly in their nutritional qualities for fish larvae, a non-parametric two tailed probability test was applied (WILCOXON, MANN and WHITNEY).

For the biochemical investigation the Vinciguerria larvae were transferred into microtubes and were stored at -20 °C not longer than three weeks. Immediately before analysis the larvae were examined with a binocular, checked for possible damages and total lenght was measured. The determination of the nucleic acids content followed a procedure given by CLEMMESEN (1988) Nucleic acid solutions are extracted and purified from larval homogenates and the RNA and DNA content is fluorimetrically determined using nucleic acid specific dyes (ethidiumbromid, bisbenzimidazole). The tryptic enzyme activity was measured as described by UEBERSCHÄR (1988). The fluorescence measurement gives an increase in emission per time interval which is directly proportional to the tryptic enzyme concentration in the fish larva. For comparison between lenght classes and for eliminating the effect of larval size the tryptic enzyme activities were divided by the total lenght of the corresponding larvae. Enzyme activity classes were constituted and the percentage of larvae in each class is given in the results as relative frequencies.

RESULTS

Fig. 2 gives the frequency histogram of the length distribution of the larvae used for the histological determination of the nutritional condition. A higher number of larger larvae was analysed from leg 3b (central Arabian Sea) compared to leg 3c (continental shelf of Pakistan). Fig. 3 shows the results of the analysis of the nutritional condition of larvae determined with histological methods divided into five defined nutritional classes. Larvae from class 1 have the best nutritional condition. It can be seen, that Vinciguerria larvae from the

offshore area show a trend towards better nutritional conditions compared to individuals caught inshore. (WILCOXON, MANN & WHITNEY, $p<0.05$).

Fig. 4 and 6 show the frequency histograms of the lenght distributions of the fish larvae used for the biochemical analyses. As in the histological evaluation a higher percentage of large-sized Vinciguerria larvae was sampled on leg 3b.

For interpreting the analysed RNA/DNA ratios the values were divided into classes with a step size of 0.5. The corresponding frequency histogram is given in Fig. 5. The peak in the ratios of larvae sampled on both legs is in the range of 1.0 - 2.0. When comparing the medians of the RNA/DNA ratios of Vinciguerria larvae from the two locations no significant differences could be found ($p>0.05$, WILCOXON, MANN & WHITNEY) But a trend towards higher RNA/DNA ratios for larvae caught offshore (leg 3b, Fig. 1) can be observed. Fig. 7 shows the distribution and frequencies of tryptic enzyme activity of Vinciguerria. The distribution of the relative frequencies is significantly different ($p<0.05$, WILCOXON, MANN & WHITNEY). Larvae from the open water area were in a better condition than larvae on the shelf.

DISCUSSION

To estimate the in situ applicability of three different analytical methods for investigating the nutritional condition in fish larvae, samples of the mesopelagic genus Vinciguerria (Photichthyidae) were taken at two sites in the Indian ocean, which were assumed as ecologically different. It was supposed that the inshore area on the continental shelf of Pakistan (leg 3c, Fig. 1) had higher values of primary production and therefore better nutritional conditions for fish larvae than the offshore area in the central Arabian Sea, especially because of the nearby Indus estuary. The results of the three applied methods contradict the hypothesis. Sampled fish larvae of leg 3b (offshore area) showed better nutritional conditions than those from leg 3c. It can be assumed that feeding conditions in

the offshore area were more advantageous at time of catching. Measurements of primary production on both legs support this assumption: On the offshore leg 3b 700 mg C/m²/d were measured, whereas on leg 3c only 300-500 mg C/m²/d were found, each value integrated over the water column (POLLEHNE, pers. comm.). If these diverging primary productions were followed by different zooplankton densities (PARSONS, TAKAHASHI and HARGRAVE, 1984) this could result in differences in the frequencies of the analysed parameters to determine the nutritional conditions of Vinciguerria larvae. At the moment the results of the corresponding analysis of zooplankton densities are not available.

The application of the three different methods for the determination of the nutritional condition on larval material sampled at the same locations results in the same tendencies (Fig. 3, 5 & 7). Vinciguerria larvae caught in the offshore area had a generally better nutritional condition compared to larvae from the shelf of Pakistan. Therefore it can be concluded that the three applied methods are valid for the determination of the individual physiological condition of field-caught larvae. At the moment only a qualitative comparison of the three method seems justified. Both biochemical and the histological method possess particular critical levels below which a fish larva passes from normal nutritional condition into starvation. Up to now no simultaneous calibration experiments have been performed in the laboratory. Therefore it is possible that individuals were assumed as starving by measuring their RNA/DNA ratios and tryptic enzyme activities, whereas histological analyses would have estimated them being in normal nutritional condition.

The length frequency distributions of Vinciguerria larvae used for the three methods were shifted towards larger larvae on leg 3b as shown in Fig. 2, 4 & 6. Because older fish larvae are less susceptible to insufficient feeding conditions than younger ones (LOVE 1980, POWELL and CHESTER 1985, RICE et al. 1987) the different lenght frequency distributions could be an explanation for the different analysed nutritional conditions found in the two investigated areas. The results from the

histological investigation are contrarily to that assumption. Larvae belonging to the nutritional classes 1-3 are determined as not starving, whereas larvae from the classes 4-5 show starvation symptoms .Based on this grading system 100% of the larvae from leg 3b would be determined as not starving and 13.9% of the larvae caught on leg 3c would be starving (SIEG 1989). Since most of the larvae used in the histological analyses came from a smaller length class compared to the larvae from the biochemical analyses the results contradict the assumption that the analysed Vinciguerria larvae of the smaller length classes are more susceptible to food deprivation than older larvae.

A defined nutritional condition cannot be given at the moment , because Vinciguerria larvae have not be reared in the laboratory under defined feeding conditions. Therefore no values from larvae of defined states of nutrition exist. It should be attempted to apply calibration data for different nutritional conditions taken from other species to data from Vinciguerria larvae. Only calibration data from herring larvae are available to the authors at present. Studies on laboratory reared herring larvae indicate that a critical RNA/DNA ratio, below which starvation symptoms can be observed, is 1.5 in young and 2.5 in older herring larvae (CLEMMESEN in prep.). The peak in the RNA/DNA frequency histogram (Fig. 5) is in the range of 1.0-2.0. It is not likely to assume that all larvae below the critical level determined for herring larvae are suffering starvation effects. It can be expected that the metabolic activity of herring compared to tropical fish larvae is different due to the enviromental conditions experienced during their larval phases. Therefore the RNA/DNA ratios determined on laboratory reared herring larvae cannot be applied to fish larvae from the tropical areas without compensating for temperature effects. Theilacker (1986) detected divergences of histological reactions due to starvation between rearing experiments and field studies in Trachurus symmetricus larvae. One cause might be that in situ no total deprivation of food exists and that insufficient feeding condition in situ create other tissue reactions than in the laboratory.

It can be concluded that all three introduced methods for estimating nutritional conditions of fish larvae are suitable for qualitative in situ evaluations. Quantitative results can only be produced by temporal and spatial small-scaled investigations, or by further laboratory calibration experiments dealing with larvae from different species and ages. Further temperature calibration studies have to be performed. The results might yield a general model which would be applicable to tropical fish larvae like Vinciguerria and should present a tool for the determination of starvation induced mortality independent of species, age and environmental conditions.

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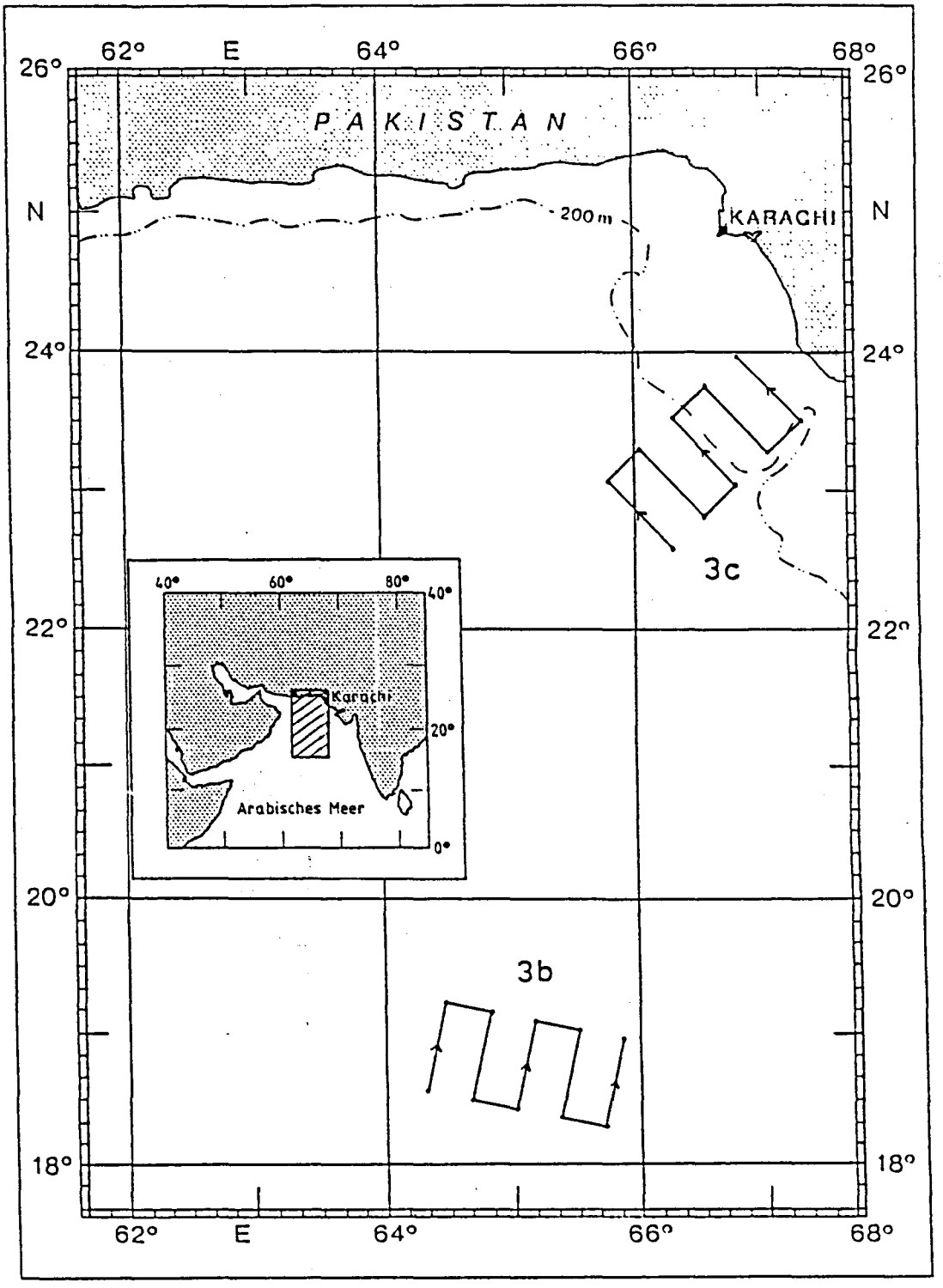

Fig.1: Position of sampling sites where the *Vinciguerria* larvae were caught.

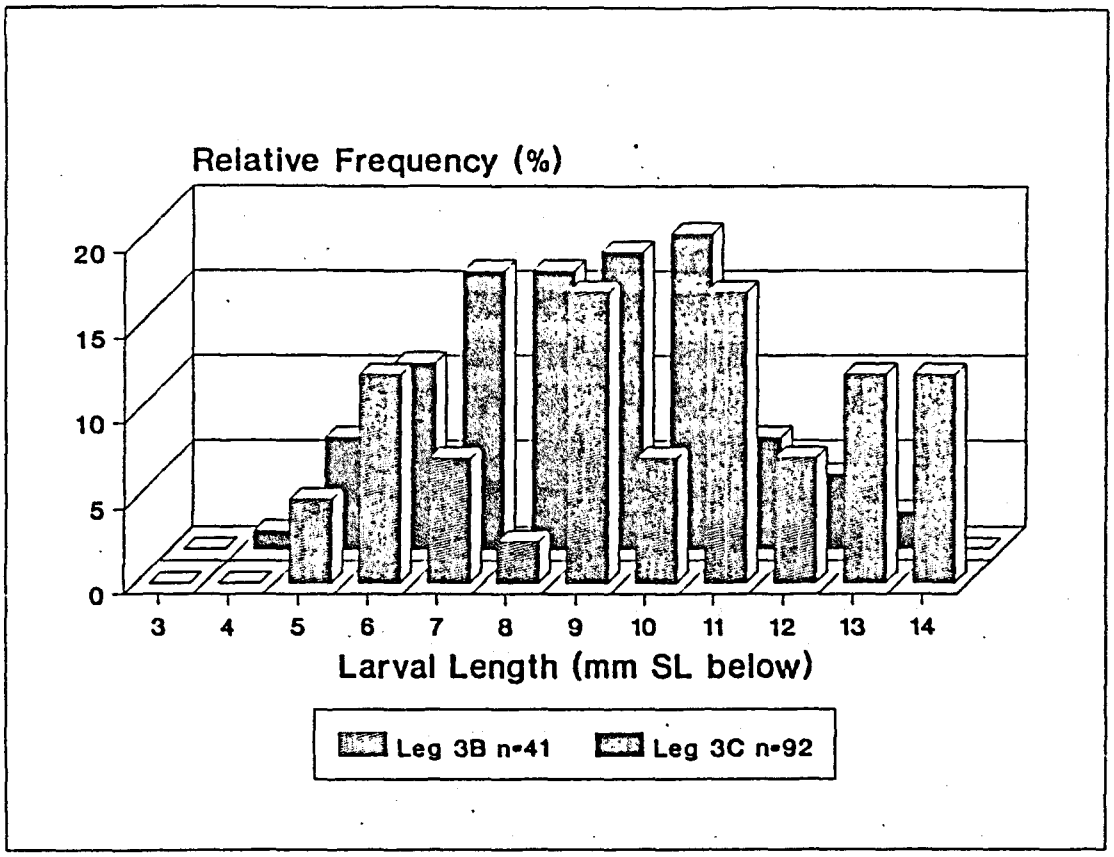

Fig.2: Relative standard length frequencies of histological analyzed *Vinciguerria* larvae.

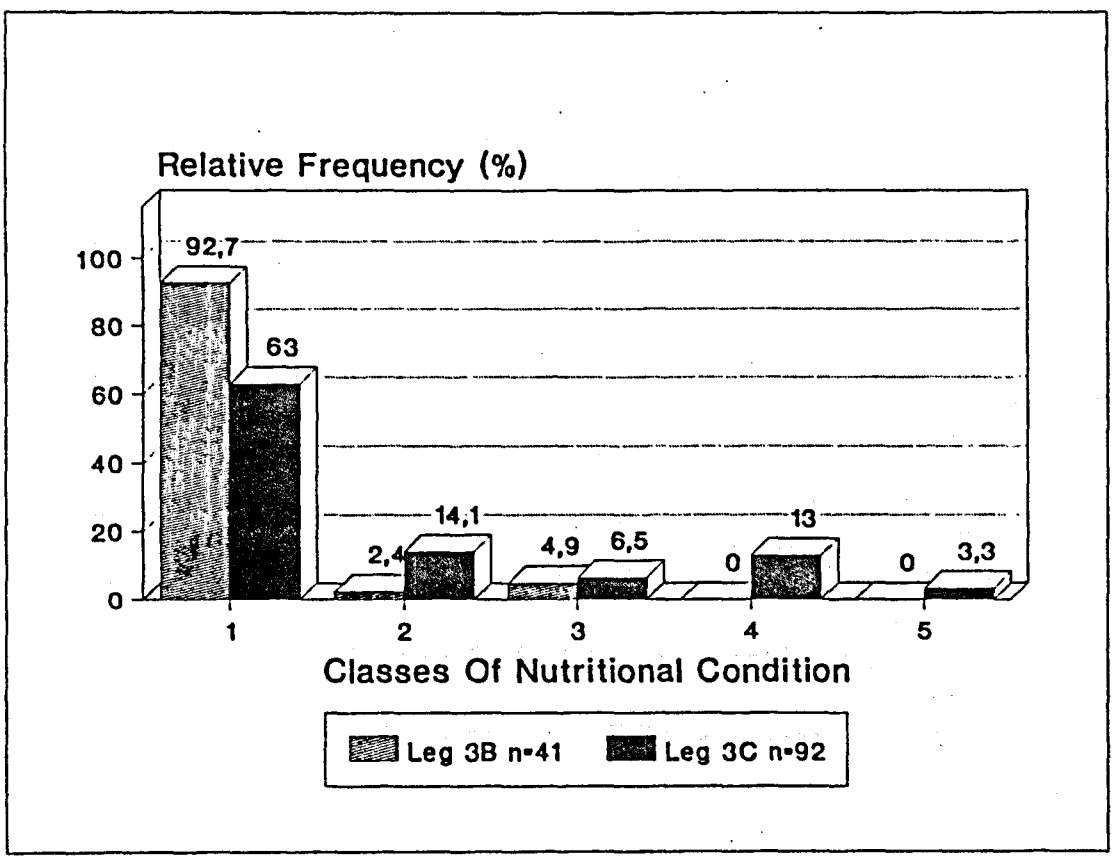

Fig.3: Relative frequencies of five different nutritional classes based on the histological evaluation of *Vinciguerria* larvae from two different sampling sites.

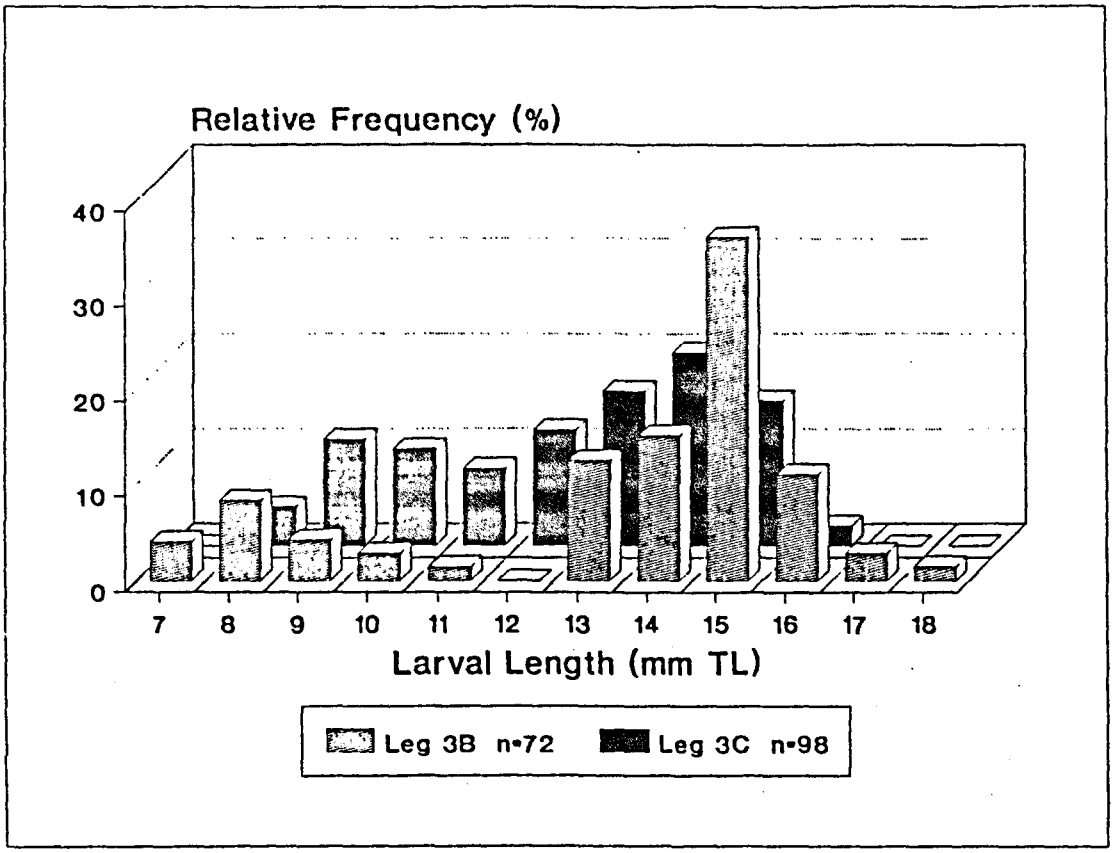

Fig.4: Relative total length frequencies of *Vinciguerria* larvae whose RNA/DNA ratios are given in Fig. 5.

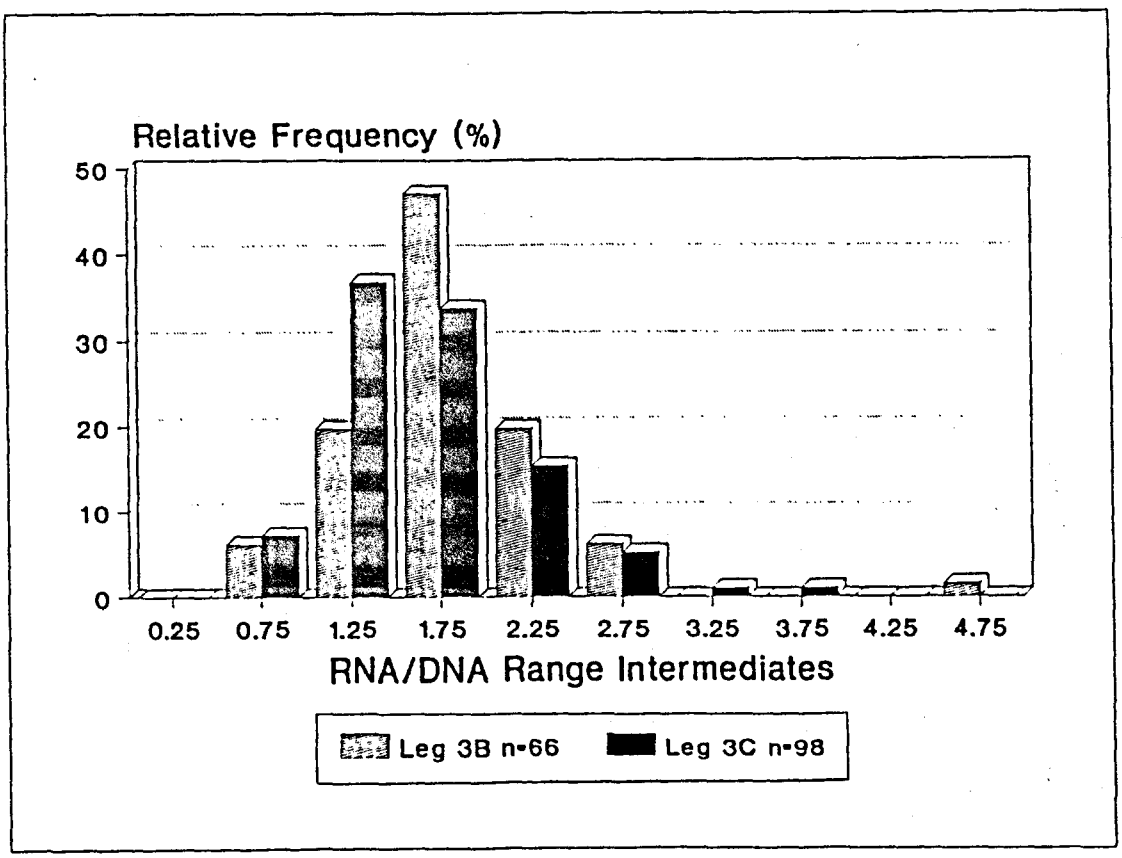

Fig.5: Distribution and relative frequencies (intermediates of 0.5 RNA/DNA step sizes) of RNA/DNA ratios of individual *Vinciguerria* larvae from two different ichthyoplankton communities in the Indian Ocean.

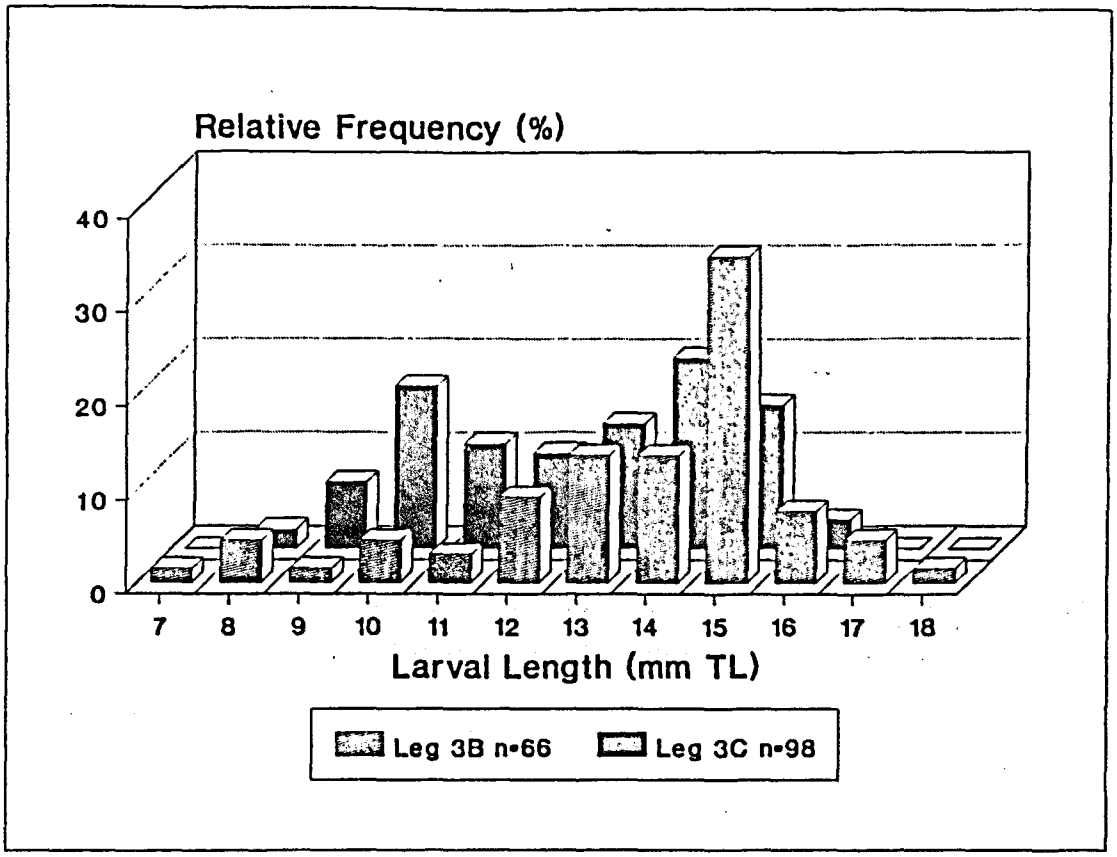

Fig.6: Relative total length frequencies of *Vinciguerria* larvae whose enzyme activity are given in Fig.7.

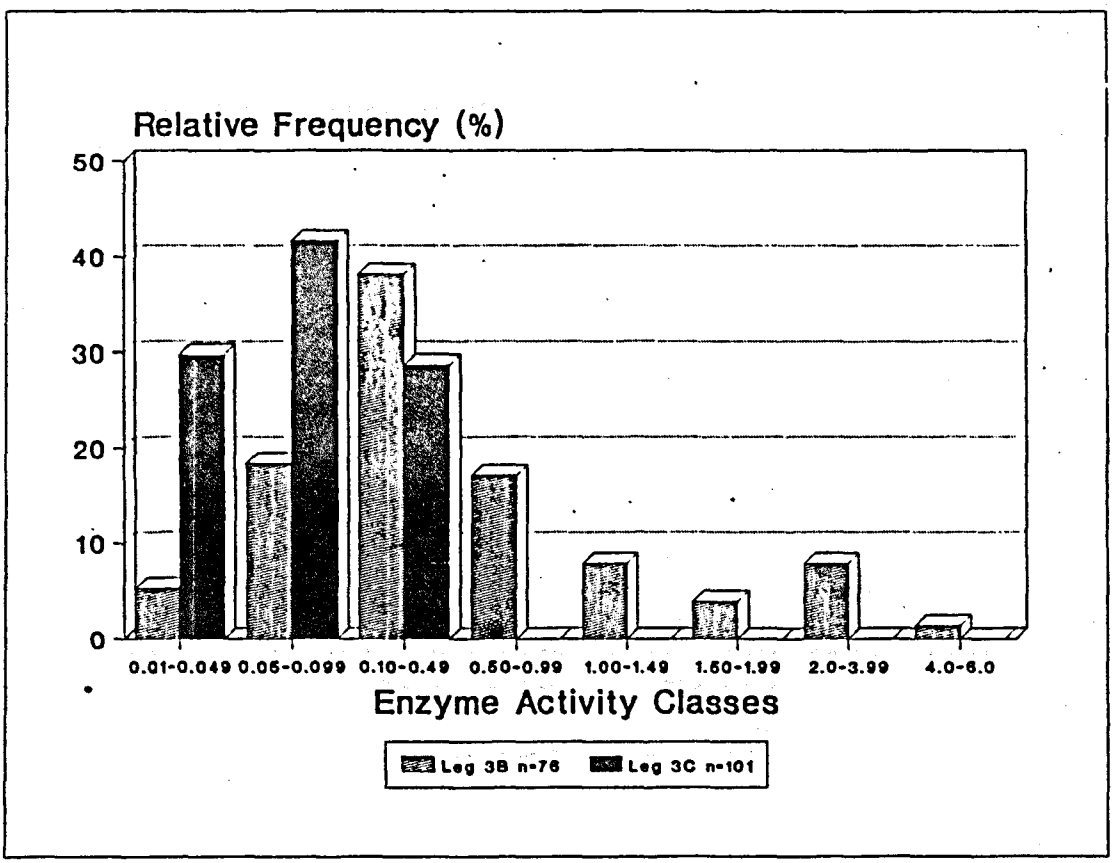

Fig.7: Distribution and relative frequencies (enzyme activity classes with different step sizes) of tryptic enzyme activity in individual *Vinciguerria* larvae from two different ichthyoplankton communities in the Indian Ocean.