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The distribution of eggs and larvae of anchovy (*Engraulis encrasicolus*) in relation to hydrography and food availability in the outflow of the river Po.

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Sampling was carried out in August 1995 along a transect across the outflow of the river Po. The river plume was clearly seen as a superficial layer of turbid low salinity water with a relatively high concentration of chlorophyll a. *Eggs of anchovy (*Engraulis encrasicolus*) were most abundant at the station close to the river plume front and in relatively low numbers at stations both further inshore and offshore from this position. Larvae of anchovy were also most abundant at the position of the front and remained relatively numerous further offshore from this station; at the most coastal station larvae were less abundant. The mean length of larvae increased with distance from the coast. Both anchovy eggs and larvae were mostly in the top 20m of the water column, the eggs in increasing numbers towards the surface whereas the larvae exhibited a sub-surface peak of abundance at a depth of 6-8m. Larvae fed during daylight hours only, their diet changing from mainly copepod nauplii at larval lengths of <6mm, to predominately early copepodite stages and small adult copepods at lengths >8mm. Abundance of potential prey items for anchovy larvae, as an integrated measure through the entire water column, showed a general decline from inshore to offshore. Highest concentrations of food particles were at similar sub-surface depths to those at which the larvae occurred. The results suggest that for smaller larvae, feeding conditions were most favourable in inshore areas adjacent to the spawning grounds where nauplii were most abundant and occurred in maximum abundance at the same depth as the larvae; larger larvae were advected offshore in the river plume to areas where the copepodite stages, which formed their main prey items, represented a higher proportion of potential food items.*

INTRODUCTION

The European anchovy (*Engraulis encrasicolus*) is widespread throughout neritic regions of the Mediterranean and the adjacent coastal margin of the North Atlantic (REID, 1967). In the central and northern Adriatic the estimated catch and biomass of anchovy has varied considerably from year to year, these changes being reflected in the fortunes of the commercial fishery (REGNER, 1990; BOMBACE, 1992; CINGOLANI *et al.*, 1996).

Spawning of anchovy is associated with areas of high production in transitional zones between different water masses, including the shelf-edge and river plume fronts (PALOMERA, 1992; MOTOS *et al.*, in press). In the Adriatic, spawning is widespread but is generally at higher levels in the more northern areas and, in particular, in the region of the outflow of the river Po (PICCINETTI *et al*, 1980). A considerable amount of information has been obtained on the distribution of eggs, mainly as a pre-requisite for estimates of the size of the spawning stock (e.g. REGNER *et al.*, 1986). There is substantially less published information on the distribution of larvae, much of the earlier data on the planktonic stages of anchovy in the Adriatic having been summarised by REGNER (1985).

Variations in the abundance of adult anchovy in the Adriatic have been ascribed to hydrobiological influences on larval survival, irrespective of fishing pressure (BOMBACE, 1992). To some extent, this is true of all small pelagic fisheries which are based on short-lived species existing in a highly variable environment (HUNTER AND ALHEIT, 1994). Hydrobiological conditions in the northern Adriatic are dominated by the cycle of seasonal thermohaline stratification enhanced by fresh water and nutrient input from the river Po and the contrasting autumn/winter period of mixing and cooling of the water column (FRANCO, 1970; BARALE *et al.*, 1986).

Wind mixing is often driven by the locally intense north-easterly katabatic airflow ("Bora"). This wind is most prevalent in the late summer and autumn but can occur for periods of up to a few days at other times of the year, with important consequences for water mass advection and production regimes (MALANOTTE-RIZZOLI and BERGAMASCO, 1983; ZORE-ARMANDA and GAČIĆ, 1987).

It was in this context that 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 (McFADZEN AND FRANCESCHINI, 1997). This preliminary work, as described here, forms the basis for subsequent more detailed field sampling in relation to changes in wind stress, together with support from laboratory rearing studies for validation of the condition indices.

MATERIAL 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.

Plankton sampling was carried out at each of the five station positions along the transect by oblique 20cm diameter BONGO net tows at a towing speed of 3 knots (Table 1). A 250µm mesh aperture net was fitted to one side of the BONGO frame and a 333µm net to the other side. A General Oceanics flowmeter was fitted in the aperture on the side of the 333µm net to

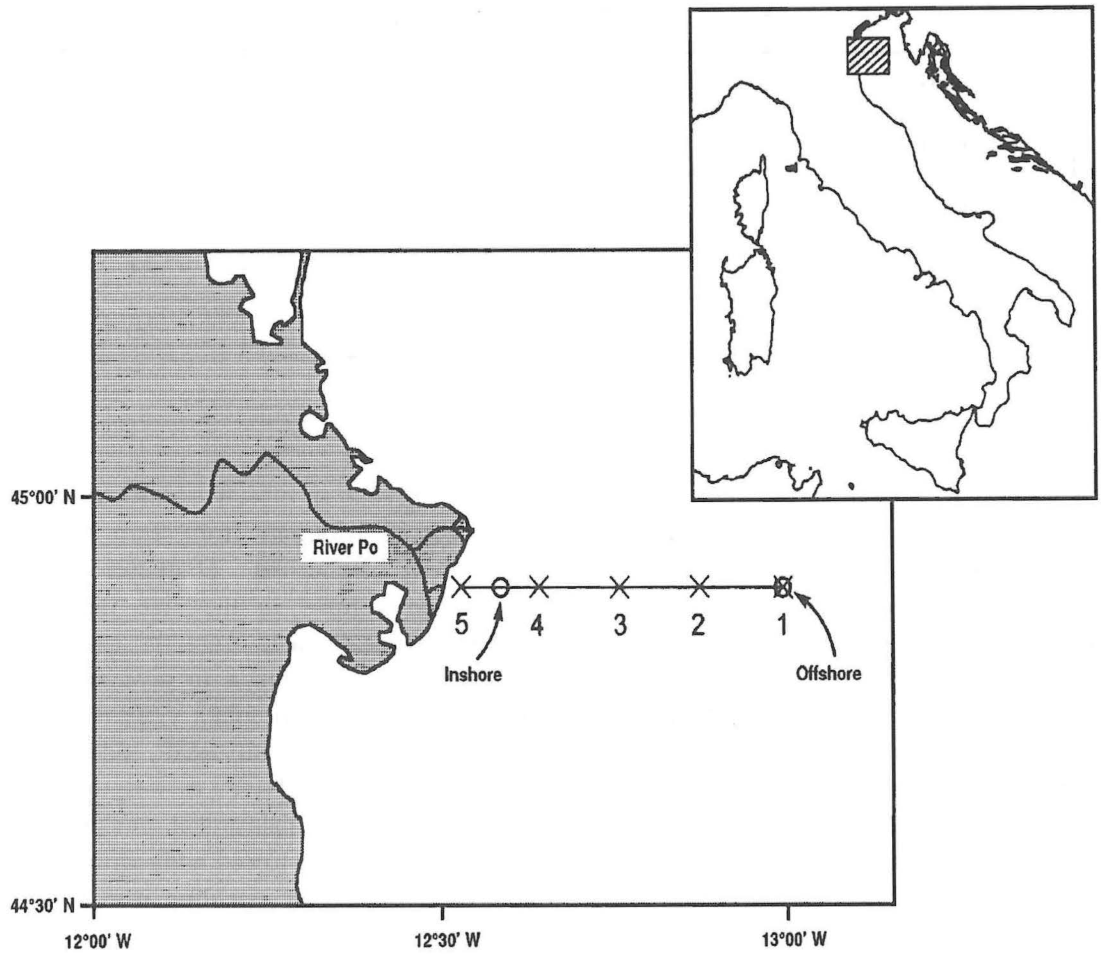

Fig. 1. Survey area showing the transect and station positions

determine the volume of water filtered. Following each haul, the samples were preserved separately in 4% formaldehyde solution buffered with borax to maintain a pH of around 8.2. Samples from the 333µm net were subsequently sorted for anchovy eggs and larvae, the larvae being measured (standard length) using a binocular microscope fitted with an eyepiece graticule. Numbers of eggs and larvae on each haul were standardised to numbers/m^2 using the computed volume of water filtered and depth sampled, the latter value being recorded by a miniature depth/temperature logger attached to the BONGO frame.

Attached to the towing wire immediately above the 20cm BONGO was a similar paired 10cm diameter BONGO net system fitted with nets of 53µm mesh aperture. Samples from these nets were preserved in 4% buffered formaldehyde following the haul, and were subsequently analysed for microzooplankton abundance as a measure of food availability for

Table 1. Concurrent 10 cm and 20 cm BONGO tows

Date	Station No.	Start time (GMT + 2)	Water depth (m)	Sampled depth (m)
12/08/95	1	0449	36	27
12/08/95	2	0540	33	24
12/08/95	3	0712	30	23
12/08/95	4	0802	26	21
12/08/95	5	0921	16	16

anchovy larvae. A General Oceanics flowmeter fitted in the inlet aperture on one side of the 10cm BONGO provided estimates of the volume of water filtered and hence allowed standardisation of the microzooplankton to numbers/litre.

Additional tows using a 30cm diameter BONGO net system were made at the selected Inshore and Offshore stations (Fig. 1) to provide further material for studies of larval feeding, growth and condition (McFADZEN AND FRANCESCHINI, 1997).

CTD profiles were obtained at the five station positions along the transect using a Sea-Bird 911 series CTD fitted with additional sensors for chlorophyll a fluorescence and light transmission. Water samples for particulate size frequency analysis were obtained concurrently with the CTD profiles by means of attached water bottles. At each station water samples were obtained from the near-surface layer above the pycnocline (samples at 1-5m depth) and from around the mid-depth of the deeper water layer below the pycnocline (samples at 10-25m depth). Particulate analysis was carried out onboard the survey vessel using a Coulter Multisizer II, each sample being pre-filtered through 100µm mesh gauze to remove larger organisms and any large detritus. A 100µm aperture tube was used for the sample analysis, giving an effective particulate size-frequency distribution from 2µm to 60µm Equivalent Spherical Diameter (ESD). Particulate volume in the size range 2.7-40.1µm was calculated from the sum of the individual size categories to give an overall measure of Total Particulate Volume (TPV).

Additional bio-physical data were obtained from an UNDULATOR tow along the transect. This instrument consists of a small hydrodynamic towed body with a servo-controlled diving fin, enabling it to follow a saw-tooth dive and climb profile while being towed by the survey vessel. Mounted inside the UNDULATOR is a solid-state logger which records sensor signals at two readings per second, including measurements of temperature, conductivity, pressure and chlorophyll *a* fluorescence.

Vertical distribution sampling for zooplankton was carried out using an LHPR sampler (LONGHURST-HARDY Plankton Recorder; WILLIAMS *et al.*, 1983; see also PIPE *et al.*, 1981) for day and night hauls at each of the selected Inshore and Offshore sampling stations (Fig 1, Table 2). The LHPR is a plankton net system for taking a sequential series of plankton samples along an oblique tow at a towing speed of around 3.5 knots. The main net system was of 200µm mesh aperture

Table 2. LHPR hauls

Date	Station	Haul No.	Start time (GMT + 2)	Water depth (m)	Max. depth sampled (m)
12/08/95	Inshore	1	0750	22	21.5
12/08/95	Offshore	2	1345	37	37.0
12/08/95	Inshore	3	2050	24	22.8
12/08/95	Offshore	4	2315	36	34.0

for sampling the ichthyoplankton while a smaller net system of 53µm mesh aperture, mounted on the same sampler frame, provided a second concurrent set of samples for the vertical distribution of microzooplankton. An electronics package on the sampler frame allowed real-time transmission of data via cored-cable for onboard PC display of system function, together with readings from sensors for depth, temperature, chlorophyll *a* and water flow into each net system.

In order to derive realistic estimates of the availability of suitable food in the plankton, the gut contents of anchovy larvae were first examined to determine the composition of their diet. A total of 456 larvae, from 2.7mm to 10.5mm in length, were examined from a selection of the preserved 20cm and 30cm BONGO net samples to give a representative series through the 24 hour period. Each specimen was measured (standard length) under a binocular microscope fitted with an eyepiece micrometer; the complete gut was then detached and opened for all organisms to be counted and identified as far as their condition would allow.

RESULTS

Hydrography and environmental parameters

The hydrographic situation along the transect was typical of a river outflow into a seasonally stratified coastal sea. The influence of the river plume and the position of the river plume front, extending about five miles offshore, are clearly shown in the contour plots of salinity and chlorophyll *a* (Fig. 2). Light transmission has a similar pattern, this parameter being essentially related to a combination of the amount of suspended solids, organic detritus and phytoplankton production associated with the fluvial input. Temperature stratification, with a change across the thermocline of around 7°C, extends along most of the transect, except in the shallowest inshore region where the thermocline deepens, due to the overlying wedge of less saline water, and abuts the shallower sea-bed. The overall pattern of stratification is indicated in the plot of water column density (Fig. 2) which combines the influences of both temperature and salinity to show a pycnocline along the entire transect.

Changes in hydrography along the transect were reflected in particulate concentrations. Highest values for Total Particulate Volume (TPV) were observed in near-surface water towards the coast (Fig. 3) where fresh water inflow and chlorophyll *a* concentration were also highest (Fig. 2). TPV was lower both further offshore and in deeper samples at all stations (Fig. 3) due to equivalent lower concentrations of phytoplankton and detritus. The size-frequency distribution of particulates was essentially monotonic in all samples except at the surface in the most inshore samples where the presence of higher concentra-

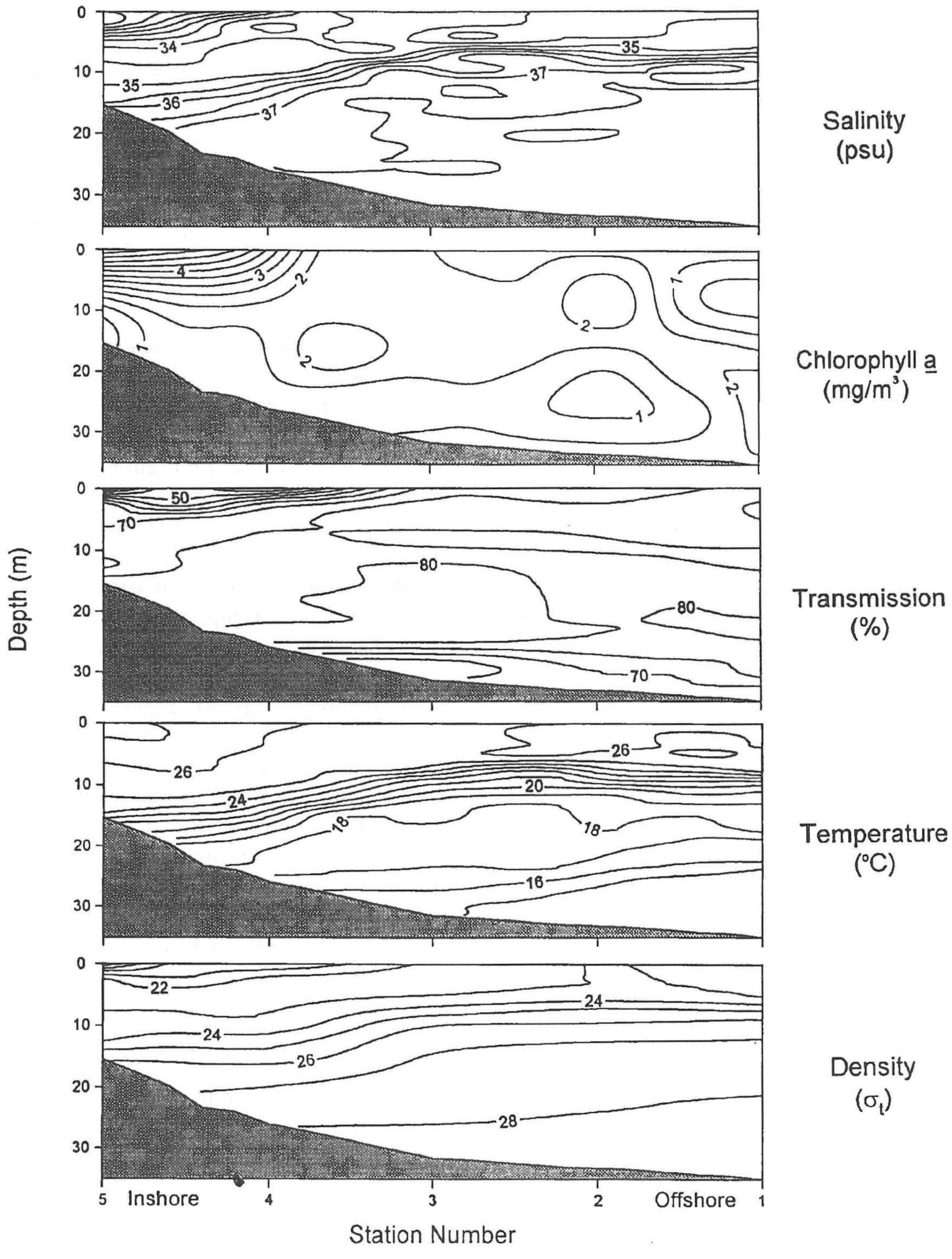

Fig. 2. Contour plots of UNDULATOR and CTD data taken along the transect shown in Fig. 1

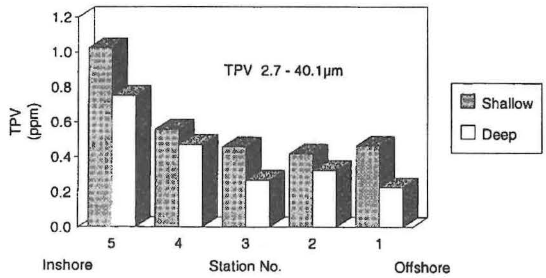

Fig.3. Total Particulate Volume (TPV) from Coulter Multisizer analysis of water samples from shallow (1-5m depth) and deep (10-25m depth) water bottle samples at each station

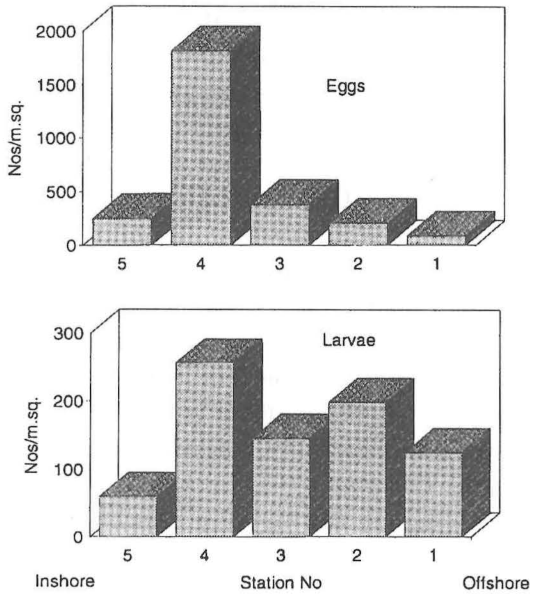

Fig. 4. The abundance of eggs and larvae of anchovy from BONGO sampling at each station

tions of phytoplankton was observed as a peak at around 6µm - 10µm ESD.

Distribution and abundance of anchovy eggs and larvae

Both eggs and larvae of anchovy were taken in highest abundance, $1817/m^2$ and $256/m^2$ respectively, at station 4 (Fig. 4), this being close to the position of the river plume front (see Fig. 2). At all other stations egg abundance was considerably lower ($<249/m^2$) and showed a progressive decrease with distance offshore from station 4 (Fig. 4). Larvae showed a similar pattern of low numbers ($60/m^2$) at the most inshore station (station 5) as seen for the eggs (Fig. 4). In contrast, there were relatively more larvae than eggs at the three more offshore stations (stations 1-3), all having larvae present in the range $124/m^2$ - $198/m^2$ (Fig. 4).

The size frequency distribution of larvae changed progressively along the transect with relatively larger larvae being found at the more offshore stations (Fig. 5; Table 1). At the most inshore sampling location (station 5), 63.8% of larvae were in the 2-4mm length category whereas at the most offshore sampling site (station 1) there were only 29.5% of larvae in this same size range. Conversely, only 6.8% of larvae were in the 6-8mm length range inshore at station 5 compared with 37.2% offshore at station 1 (Fig. 5).

Anchovy eggs were found mostly in the upper 20m of the water column (99%) and in increasing numbers towards the surface (Fig. 6).

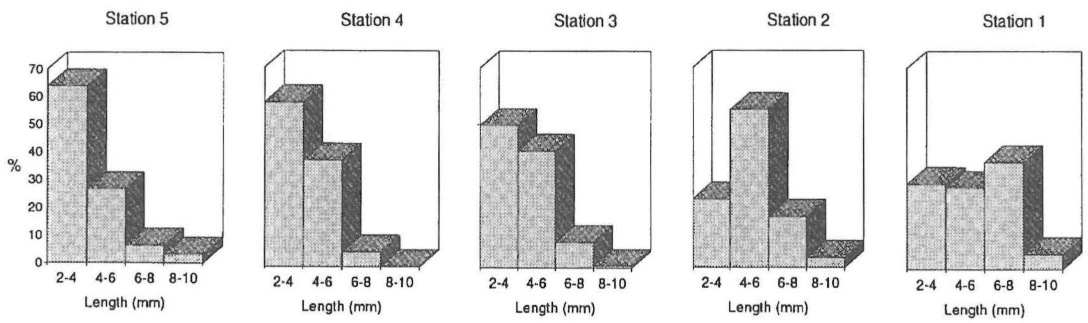

Fig. 5. The length-frequency distribution of anchovy larvae from BONGO sampling at each station

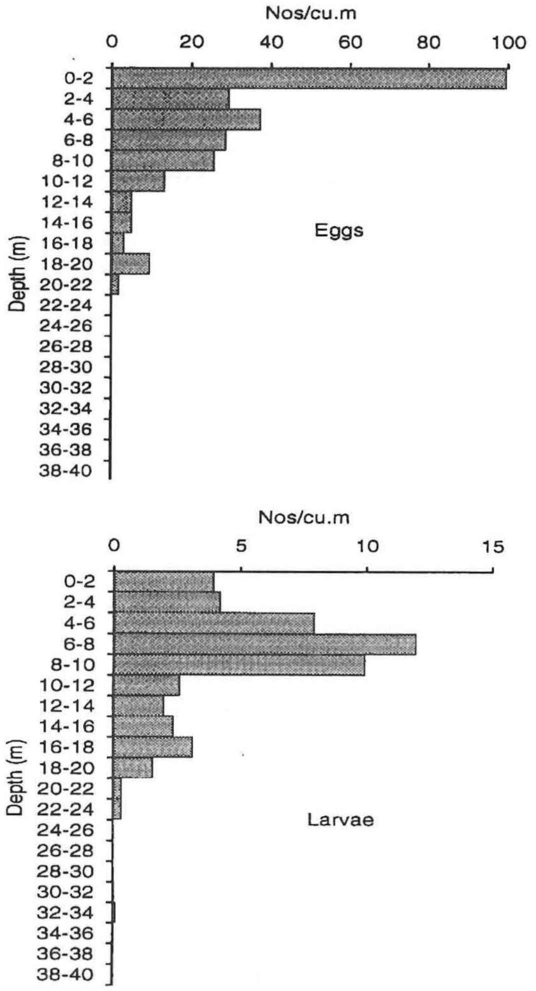

Fig. 6. The mean vertical distribution of eggs and larvae of anchovy from LHPR sampling at the Inshore and Offshore stations (see Table 2 and Fig. 1)

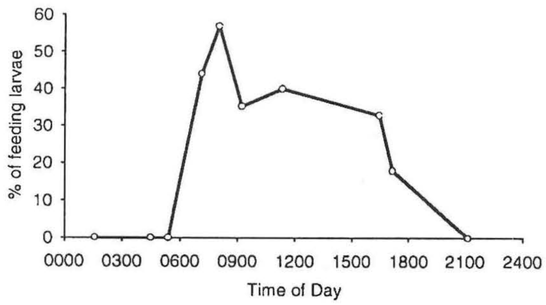

Fig. 7. The percentage occurrence of feeding anchovy larvae by time of day

There was some indication that eggs at later stages of development were deeper than those at the earlier stages.

Larvae were also distributed mostly in the upper 20m of the water column (98%) but with a sub-surface peak of abundance at a depth of 6-8m (Fig. 6). There were no clear differences between the vertical distributions of different sizes of larvae, whether sampled by night or day or between the results from the Inshore and Offshore stations. This may be due, in part, to the relatively few hauls and low numbers of larvae taken (Table 1: 4 hauls, 387 larvae). However, there was some indication of commencement of diel vertical migration for larvae at 8-10mm in length (n=62), these being present in highest concentrations at a depth of 6-8m at night and 16-18m by day.

Gut contents

Anchovy larvae contained food particles in their guts predominately during daylight hours (Fig. 7). There was a progressive change in the main dietary items from the naupliar stages of copepods for larvae <6mm in length, to early copepodite stages and adults of small copepods (mostly *Oithona* spp.) for larvae >8mm in length (Fig. 8). Larvae <8mm in length contained a small proportion of copepod eggs. Other food noted in the gut contents of intermediate sized larvae 4-7.9mm in length, included small numbers of cladocerans (*Evadne* sp.), the dinoflagellate *Peridinium* sp. and various remains which could not be identified.

Distribution and abundance of food particles

The 53µm BONGO samples taken at each station along the transect were analysed for food items identified from the gut contents of anchovy larvae. This measure of integrated food abundance down the water column showed the highest abundance of esculent par-

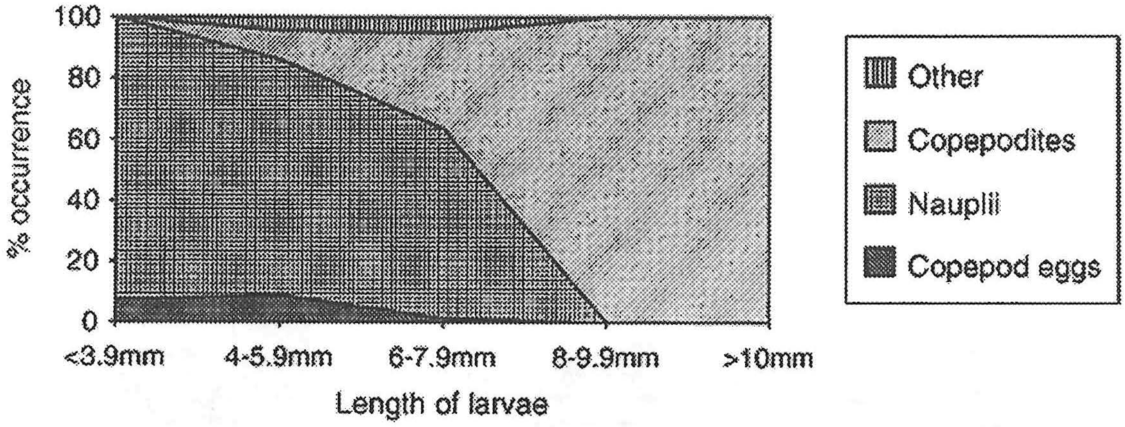

Fig. 8. The percentage composition of the diet of anchovy larvae by length

ticles at the most inshore station (21.7 particles/litre at station 5; Fig. 9) and lower values at the more offshore stations (7.2 particles/litre and 6.4/litre at stations 1 and 2 respectively). Nauplii and copepodite stages of copepods (including adult *Oithona* spp.) comprised the majority (88-94%) of identified food items, a slightly higher proportion of nauplii being observed at the more inshore stations (Fig. 9). Apart from relatively low numbers of cladocerans, which were present at all stations (6-12%), there were only negligible amounts of other food items for anchovy larvae in the 53µm BONGO samples.

The abundance of food items suitable for anchovy larvae was also determined from the LHPR vertical distribution samples. The most common items were the naupliar and copepodite stages of copepods (including adult *Oithona* spp.); a few copepod eggs and cladocerans were also noted in the samples at low abundance (a combined maximum of 1.0/litre). The relative proportions of nauplii and copepodites were similar to those found in the 53µm BONGO samples; this being a fairly equal ratio of nauplii to copepodites at the more inshore end of the transect and a rather lower proportion of nauplii further offshore (Fig. 10).

At both Inshore and Offshore stations, nauplii had relatively shallow sub-surface peaks of abundance at depths just below the

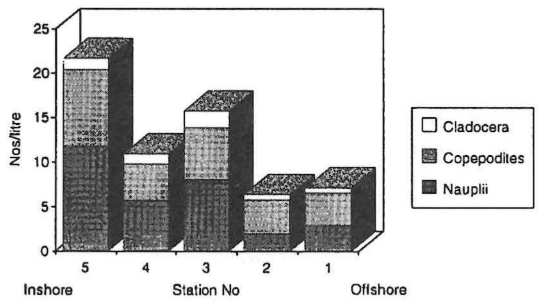

Fig. 9. The concentration of food particles suitable for anchovy larvae from BONGO sampling at each station

pycnocline (at 4-6m depth at the Inshore station and 6-8m at the Offshore station; Figs. 10 and 11). In contrast, copepodite stages had their highest abundance somewhat deeper in the water column (at 18-20m at the Inshore station and 10-12m at the Offshore station; Fig. 10) and, in general, were more evenly spread throughout the water column than the nauplii. At both sites there was little difference between day and night profiles.

The plots for the total numbers of food particles showed peak numbers of particles at 82.8/litre at 4-6m depth at the Inshore station and 43.8/litre at 10-12m depth at the Offshore station (Fig. 10). At both sites, these peak abundance levels represented 1.7 x the average abundance of food particles down the water column.

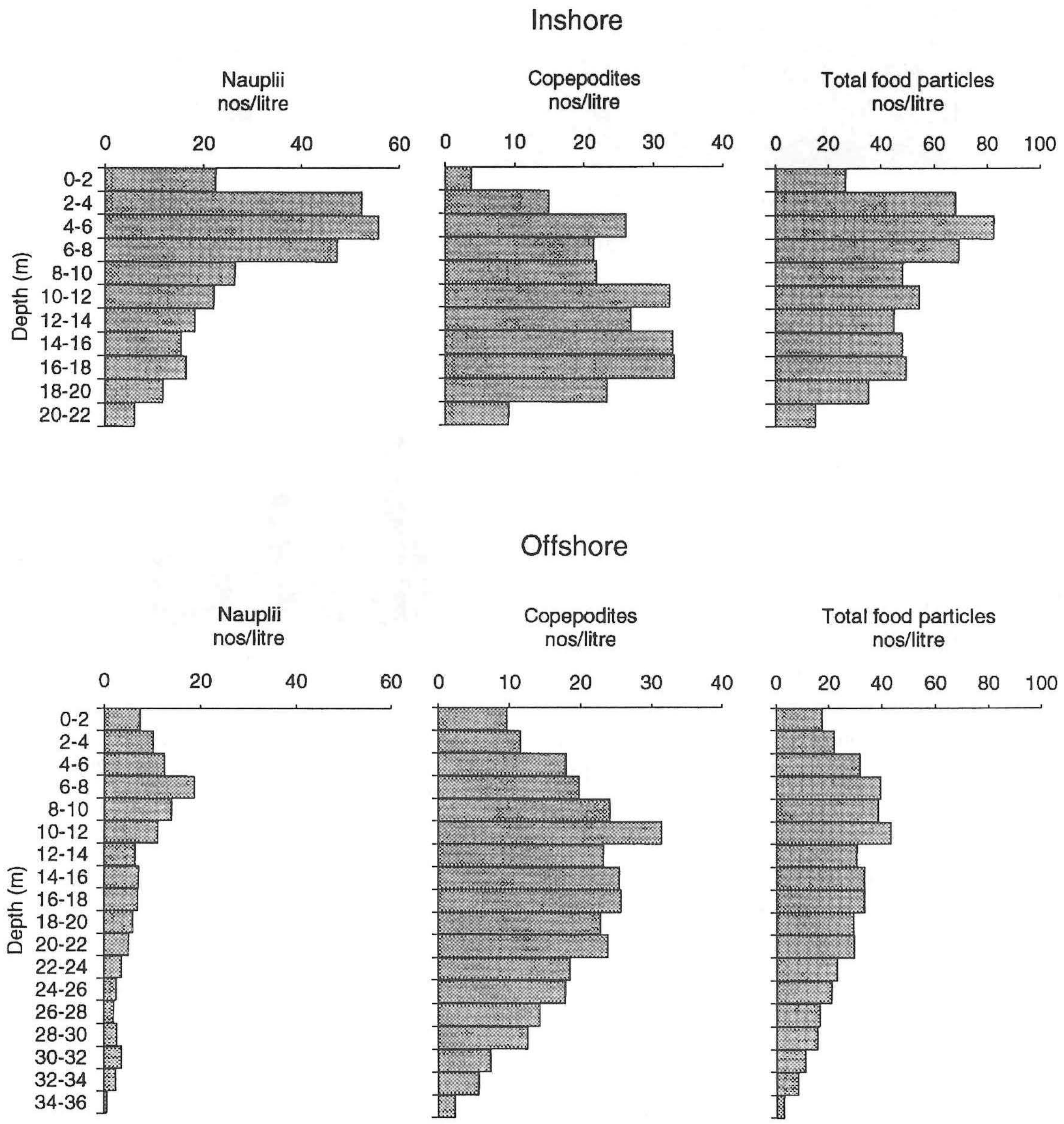

Fig.10. The mean vertical distribution of food particles suitable for anchovy larvae from LHPR sampling at the Inshore and Offshore stations (see Table 2 and Fig. 1); the plot for "Total food particles" includes nauplii, early copepodite stages, Oithona *spp. adults and small numbers of copepod eggs, cladocera and unidentifiable remains*

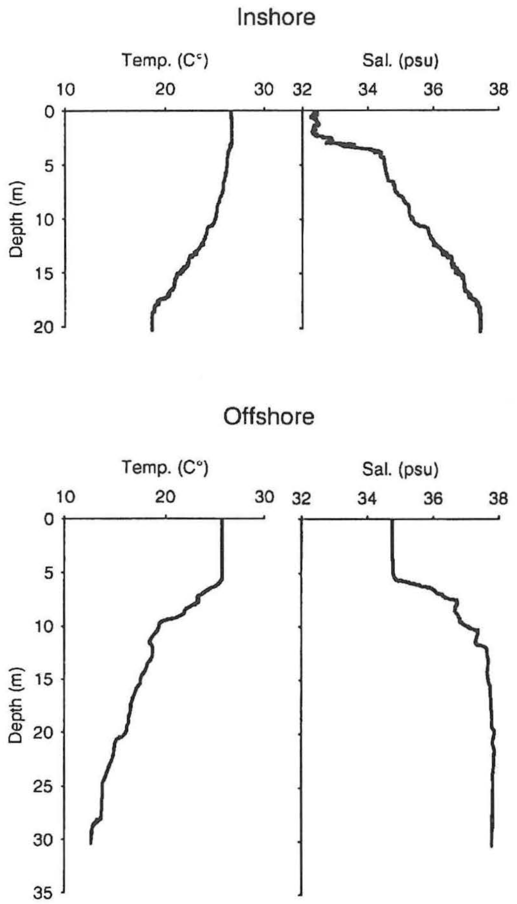

Fig.11. CTD profiles for temperature and salinity at the Inshore and Offshore stations (see Fig. 1)

DISCUSSION

An essential pre-requisite for the present study of the feeding environment of anchovy larvae was an analysis of their diet. This was seen to be predominantly the naupliar and copepodite stages of copepods, which is consistent with previous information on feeding of anchovy larvae both from the Adriatic (DUKA, 1963; REGNER, 1971), and further west in coastal and estuarine environments around the Iberian peninsular (PALOMERA, 1991; FERREIRA and RÉ, 1993; RÉ, 1994). It is also the typical diet of the young stages of other clupeids (CONWAY *et al.*, 1994) and fish larvae in general (LAST, 1980). Similarly, the observed daylight feeding habit is common to larvae of anchovy and of other clupeids (BLAXTER and HUNTER, 1982; RÉ, 1994).

Information on the dietary preferences of anchovy larvae allowed food availability in the plankton to be assessed. Results from the BONGO sampling indicated a general decrease in abundance from inshore to offshore, in parallel with the gradient of potential productivity as indicated by chlorophyll *a* fluorescence. There was not the same peak in abundance of food at the frontal zone as noted for anchovy eggs and, to some extent for larvae, although both sets of observations should be judged within the limitations of the sampling scheme.

The most relevant estimates of potential food availability were from the vertically stratified LHPR hauls. Results from this sampling showed a 1.7 x increase in abundance of food particles at discrete depths compared with the mean abundance down the water column. The resultant concentrations of around 40-80 particles/litre are comparable with estimates from elsewhere in the Adriatic (KRŠINIĆ, 1982 - maximum of 61 nauplii/litre; REGNER *et al.*, 1987 - maximum of 27 nauplii/litre) and are somewhat higher than typical measurements from temperate coastal seas (e.g. COOMBS *et al*, 1992). Such concentrations are somewhat less than are generally required for experimental rearing of either anchovy larvae (REGNER, 1985) or larvae of many other species (THEILACKER and DORSEY, 1980); although it is acknowledged that the concentrations of food used in artificial rearing are not directly comparable with those measured in the natural environment (MacKENZIE *et al.*, 1990). There is also the possibility that a finer scale of vertical sampling resolution would reveal yet higher concentrations of microzooplankton (OWEN, 1981). Inclusion of tintinnids as potential food items for anchovy larvae will also increase the estimates of prey availability (REGNER, 1990; DULČIĆ and KRALJEVIĆ, 1996). However, only negligible numbers of tintinnids were recognised in the gut contents of anchovy larvae and, due to their small size, they will make little energetic contribution, unless consumed in large numbers.

Since the BONGO hauls on which the length distributions were based, were all taken during daylight hours, and there is little evidence of significant net avoidance by anchovy larvae before about 8mm in length (PICCINETTI *et al.*, 1982), the observed length distributions can be taken as true reflections of the local populations. Thus, within the limitations of a single survey, the combined evidence of peak numbers of anchovy eggs at the near frontal station and the decline in larval abundance together with increase in the length distributions further offshore, suggests offshore advection from spawning in the frontal region. An eastwards spreading of larvae in the plume of the river Po is a likely drift scenario, both from observational and modelling studies (MALANOTTE-RIZZOLI and BERGAMASCO, 1983; BARALE *et al.*, 1986; BERGAMASCO and BARALE, 1988). A similar selection of frontal regions for anchovy spawning has been noted by PALOMERA (1992) and MOTOS *et al.* (in press). River plumes and fronts are generally favourable areas for fish spawning (GRIMES and KINGSFORD, 1996) due to enhanced production associated with increased nutrient input, as noted for the river Po outflow (e.g. FONDA-UMANI *et al.*, 1992).

The vertical distributions of food items, with sub-surface peaks in abundance of nauplii and copepodite stages, were consistent with previously published information from the Adriatic and elsewhere (REVELANTE and GILMARTIN, 1990; COOMBS *et al*, 1992). At the Inshore station, there was a close correspondence between the depth of nauplii and anchovy larvae, thereby ensuring optimum availability of the preferred prey for the smaller larvae which were more prevalent inshore. The abundance of nauplii was also higher at the inshore stations than further offshore. Conversely, as advection dispersed the larvae further offshore, the concentrations of copepodite stages, on which these larger larvae were feeding, remained high. Although the copepodite stages were somewhat deeper in the water column at the Offshore station, the development of diel migration, due to increased locomotory capacity of the larger larvae, still makes them accessible as a food resource.

The present results indicate considerable small-scale variation in the distribution of eggs and larvae of anchovy, as well as of their microzooplankton prey. This is not entirely unexpected in a dynamic inshore area with marked physical gradients. It also demonstrates the relatively subtle interactions between distributions of predator and prey in relation to growth and advection. Small shifts in the local environmental field (e.g. increased river run-off or directed wind stress) could thus have a significant effect on larval survival. For a fuller understanding of these processes a more extensive field investigation is planned in order to follow the temporal evolution of food availability and larval condition which is considered as an analogue of survival.

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Raspodjela larvi i jaja brgljuna u odnosu na hidrografiju i raspoloživu hranu u estuariju rijeke Po

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KRATKI SADRŽAJ

Uzorkovanje je provedeno 12. i 13. kolovoza 1955. uzduž transekta dugog 20 milja na paraleli 44° 52.5' N preko estuarija rijeke Po. Inicijalni niz obrađenih postaja bio je uzduž transekta na udaljenostima od 5 milja što je uz odgovarajuće priobalne postaje trebalo pridonijeti detaljnijem dnevno-noćnom uzorkovanju. Uzorkovanje jaja i larvi brgljuna (*Engraulis encrasicolus*) i raspoložive hrane za larve izvedeno je pomoću mreže BONGO, a za studij vertikalne rasprostranjenosti pomoću LONGHURST-HARDY plankton rekordera (LHPR). Hidrografska opažanja su na postajama provedena pomoću CTD uređaja i kontinuirano uzduž transekta pomoću sistema uronjenih senzora (UNDULATOR). Uzorci vode su bili analizirani pomoću Coulter Multisizer II za mjerenje koncentracije čestica.

Utjecaj rijeke je u površinskome sloju jasno vidljiv zbog mutne vode niskog saliniteta s relativno visokom koncentracijom klorofila *a*. Jaja brgljuna su bila najbrojnija (1817/m^2) na postaji bliskoj fronti između riječne i vanjske vode i na relativno malom broju postaja izvan i unutar toga lokaliteta. Larve brgljuna su bile također najbrojnije (256/ m^2) na postaji na fronti i ostale su relativno brojne prema otvorenom ; na većini obalnih postaja bile su manje brojne. Srednja dužina larvi je rasla s udaljenosti od obale. I jaja i larve brgljuna su bile najbrojnije u gornjem sloju do 20 m dubine (99% odnosno 8% u gornjih 20 m). Broj jaja je rastao prema površini, dok larve imaju podpovršinski maksimum pojavljivanja na dubini 6-8 m. Larve se hrane samo tijekom dnevnih sati. Hrane se pretežno kopepodnim nauplijima larvalne dužine manje od 6 mm do predominantnih ranih stadija kopepodita i malih odraslih kopepoda dužine veće od 8 mm. Obilje potencijalne hrane za larve brgljuna uzete za cijeli stupac vode pokazuje smanjenje idući od priobalja prema otvorenom moru. Vertikalna raspodjela hranidbenih jedinica pokazuje najveću koncentraciju (82.8/l na unutarnjoj postaji i 43.8/l na vanjskoj postaji) na sličnim podpovršinskim dubinama gdje se javljaju i larve. Rezultati sugeriraju da su za manje larve prehrambeni uvjeti bili najbolji u priobalnom području blizu mrijestilišta gdje su naupliji najbrojniji, a u maksimalnim količinama se javljaju na istim dubinama kao i larve; veće larve su odnošene prema otvorenom moru s riječnim donosom prema području gdje kopepoditni stadiji, koji su glavni i raspoloživi objekti, predstavljaju visoku proporciju u potencijalnoj hrani.