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DETERMINATION OF LARVAL FISH SURVIVAL FROM FEEDING AND DISTRIBUTION OBSERVATIONS

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DETERMINATION OF LARVAL FISH SURVIVAL FROM FEEDING AND DISTRIBUTION OBSERVATIONS

by

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Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award. The work here was carried out as part of the author's duties at the Plymouth Marine Laboratory (PML) as an employee of the Natural Environment Research Council (NERC). Research cruises on which the author participated are marked with an asterisk

The experimental study on digestion of natural food organisms by larval turbot (Conway *et al.*, 1993) was carried out at the PML between August 1992 and March 1993.

The experimental study on the digestion of copepod eggs by larval turbot (Conway *et al.*, 1994a) was carried out at GSP Fish Farm, Hunterston, Scotland in February 1992 and at the PML between August 1992 and December 1993.

Field sampling for the study on the feeding of larval sardine off the north coast of Spain (Conway *et al.*, 1994b) was carried out during April/May 1991, March 1992* and May 1992* from the Spanish research vessel 'Cornide de Saavedra', in May 1992 from the German research vessel 'Valdivia' and in May/June 1992* from the NERC research vessel 'Challenger'. Processing of samples to completion of the manuscript took place at the PML between June 1991 and August 1994.

Field sampling for the study on the vertical distribution of fish eggs and larvae in the Irish and North Seas (Conway *et al.*, 1997) was carried out from the NERC research vessel 'Challenger' in March/April 1987* and May June 1988*, and from the Fisheries Laboratory, Lowestoft's vessel 'Cirolana' in May/June 1987*, April 1988* and April 1989*. Laboratory processing of samples and preparation of the manuscript took place at the PML between May 1988 and January 1996.

Field sampling for the study on the carbon content of larval sardine off the north coast of Spain (Coombs *et al.*, 1994) was carried out during April/May 1991, March 1992* and May 1992* from the Spanish research vessel 'Cornide de Saavedra', in May 1992 from the German research vessel 'Valdivia' and in May/June 1992* from the NERC research vessel 'Challenger'. Carbon analysis of samples, to completion of the manuscript, took place at the PML between June 1991 and January 1999.

Field sampling for the study on the feeding of anchovy in the Adriatic was carried out from the Italian research vessel 'Thetis' in June/July 1996*. Anchovy gut contents and plankton samples were analysed and the manuscript completed at the PML between August 1996 and May 1998.

Signed---D P Conway---- Date---6th Nov 2000---

Abstract

DETERMINATION OF LARVAL FISH SURVIVAL FROM FEEDING AND DISTRIBUTION OBSERVATIONS

David Vernon Pollock Conway

The series of papers round which this thesis is based highlight the inherent problems in assessing larval fish feeding success from sampling programmes and gut contents analysis, and show through novel observations, that counting the number of food items in the gut is not necessarily a good indication of feeding success. Food type, digestibility and size, gut passage rates and the particular lipid classes the food contains, must all be considered, illustrating the difficulties in interpreting larval fish energetics from field studies. The resistance to digestion demonstrated for copeped eggs, means that a potentially rich source of energy cannot be utilised. This may have severe nutritional consequences for the early larvae of many important commercial fish species which can feed heavily on these eggs, possibly influencing ultimate recruitment levels. While much can be learned from coarse grid, depth integrated plankton sampling, interpretation of relationships between food availability and larval condition was shown to require precise sampling of the larvae in their actual feeding environment. Intense integrated and vertically stratified plankton sampling allowed the most detailed observations on food selection by fish larvae ever carried out and demonstrated their considerable foraging adaptability, even in a changing food environment. Food availability is undoubtedly an important factor in larval fish survival because of its affect on condition and growth rates, but many other factors are now known to be involved and it is accepted that recruitment in a species may be decided at almost any stage of early development. The traditional approach to understanding the environmental impact on larval survival and fish recruitment has been by observational field ecology and correlation techniques. However, more recently, advances in mathematical modelling techniques in which we are involved, are allowing the representation and simulation of both physical and biological oceanographic processes. Progress in understanding how selective forces generally shape recruitment will depend increasingly on these exciting new techniques.

List of published work round which this thesis is based

Paper I - Conway DVP, Tranter PRG, Coombs SH (1993) Digestion of natural food by larval and post larval turbot *Scophthalmus maximus*. Mar Ecol Prog Ser 100: 221-231

Paper II - Conway DVP, McFadzen IRB, Tranter PRG (1994) Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage. Mar Ecol Prog Ser 106: 303-309

Paper III - Conway DVP, Coombs SH, Fernández de Puelles M., Tranter PRG (1994) Feeding of larval sardine *Sardina pilchardus* (Walbaum) off the north coast of Spain. Bol Inst Esp Oceanogr 10: 165-175

Paper IV - Conway DVP, Coombs SH, Smith C (1997) Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES J Mar Sci 54: 136-147

Paper V - Conway DVP, Coombs SH, Smith C (1998) Feeding success of anchovy (*Engraulis encrasicolus*) larvae in the north-western Adriatic Sea in response to changing hydrobiological conditions. Mar Ecol Prog Ser 175: 35-49

Paper VI - Coombs SH, Conway DVP, Morley SA, Halliday NC (1999) Carbon content and nutritional condition of sardine (*Sardina pilchardus*) larvae off the Atlantic coast of Spain. Mar Biol 134: 367-373

Critical appraisal

DETERMINATION OF LARVAL FISH SURVIVAL FROM FEEDING AND DISTRIBUTION OBSERVATIONS

Introduction

In the late 1800's it was realised that there was considerable interannual fluctuation in adult stock numbers of the important commercial fish species, apparently independent of fishing effort, while little was known about the biology of these species (Graham 1942). Understanding recruitment has subsequently become the major component in fisheries research (Cushing 1972), studies initially centring on single factor hypotheses and on the possibility that recruitment was determined during the larval stages.

The course of these studies has been punctuated by the theories of three prominent scientists, who provided a conceptual framework from which the majority of subsequent studies have developed. Hjort (1914) proposed the 'critical period hypothesis', whereby availability of suitable amounts of food during the transition period, between absorption of the yolk sac and initiation of exogenous feeding, determined larval mortality levels. Cushing (1975) refined Hjort's theory and proposed the 'match mismatch hypothesis' which suggested that while the spawning period of fish was relatively constant each year, there was considerable interannual variation in the timing of the annual plankton production cycle and thus food availability, which resulted in variable mortality. The 'stable ocean hypothesis' was proposed by Lasker (1975), who acknowledging that plankton could be patchily distributed horizontally, suggested that to obtain sufficient food, first feeding larvae rely on concentrations of food, such as can be found at the pycnocline, the region where the density of the water rapidly increases with depth because of differences in salinity, temperature or both. If these concentrations were dispersed by mixing, starvation could potentially result. However, it was found difficult to demonstrate evidence of larval starvation in the sea and there was a major shift in research effort towards examining the possibility that predation level was the determinant of recruitment success (Bailey & Houde 1989).

It is now accepted that while highest mortality is in the larval stages, many factors and combination of factors, apart from food availability, can be involved in determining recruitment, such as parental condition, egg quality, disease, turbulence, predation, advection etc. (Reviewed by Anderson 1988), that it may be determined to varying degrees at all stages of development (Bradford & Cabana 1997) and the stage which is most vulnerable can change for a species (Bailey 2000). However, some of the latest research has still concluded that recruitment studies should continue to focus on the planktonic phase and feeding aspects (Horwood *et al.* 2000).

Over the past few decades, increasingly sophisticated oceanographic monitoring technology has been developed and dedicated intensive research programmes studying larval fish survival initiated. Some of these programmes, such as the study on larval cod on Georges Bank (Lough *et al.* 1996), or that on larval walleye pollock off Alaska (Kendall *et al.* 1996), have used multidiciplinary approaches, simultaneously examining a broad range of factors which could potentially affect recruitment. While these and other studies have been successful in relating certain aspects of recruitment to environmental factors, at the culmination of over 100 years of research, the results are still without any convincing predictive capability.

Objectives

This thesis is based round a series of 6 published papers, the slightly diverse themes of which reflect, over the years covered, changes in funding opportunities. However, the basic aim of all these papers is directed towards improving our understanding of the processes determining larval fish survival, whether it be by better understanding their vertical distribution pattern in order to improve sampling design (Paper IV), how their feeding success changes in relation to environmental factors (Papers III and V), how their condition changes in response to food availability (Paper VI) or how their feeding success may be assessed through a better understanding of how they digest particular food organisms (Papers I and II). The published literature on larval fish survival is considerable, so the focus of the following discussion is mainly restricted to feeding aspects, including suggestions where further research might be productive.

Discussion

Sampling fish larvae

To understand and test relationships between fish larvae and environmental conditions in the field, requires appropriate sampling equipment and strategies. With the exception of fish species which spawn in intense localised concentrations, such as anchovy (Moser & Pommeranz 1999), larval fish are generally sampled in low densities in the sea (Paper IV), requiring filtration of large volumes of water to sample even small numbers. Most surveys examining larval distribution are carried out using plankton nets, typically depth integrated oblique sampling over grids of stations (e.g. Papers III, V; Bailey 2000; Sabatés & Saiz 2000), using equipment such as Bongo nets (McGowan & Brown 1966) or higher speed samplers similar to the Gulf V design (Nellen & Hempel 1969) and, depending on the grid resolution, can give acceptable horizontal distributional information. However, to ensure that the whole larval vertical distribution is sampled, hauls are typically taken deeper than larvae are expected to be sampled, which results in longer duration hauls than may be necessary, using expensive ship time. This deep sampling is necessary because little detailed information is known about the vertical distributions of fish larvae, apart from a few restricted studies, usually on individual species (e.g. Palomera 1991).

Most studies on vertical distribution of fish larvae have been carried out using multiple closing nets, with restricted numbers of nets and thus coarse vertical resolution (Gray 1996; Moser & Pommeranz 1999). An exception is Paper IV, where a Longhurst Hardy Plankton Recorder (LHPR, Williams *et al.* 1983) was used. This is a high speed net which has a minimum sampling depth resolution of around 2 m and can take up to 100 consecutive samples, with associated salinity, temperature, fluorescence and depth information. Distributions were described for a variety of larval fish species in contrasting environmental condition, particularly in the Irish Sea, information which has proved useful in subsequent research (Dickey-Collas *et al.* 1997) in an area where increasing research effort is currently being concentrated.

Larval fish are generally distributed in the upper 50 metres of the water column (Papers IV, V), in the euphotic zone, where food particles are present in highest abundance. However, their distributions may be modified by turbulence, vertical migration and ontogenetic and behavioural differences in response to environmental factors (Reviewed by Neilson & Perry 1990). Even using high resolution sampling, it is still difficult to

demonstrate changes in depth and vertical migration of fish larvae in the sea (Paper IV) unless it is synchronised amongst individuals (Pearre 1979).

Diet and growth of larval fish

The diet of larval fish is well established and is typically the various developmental stages of copepods, with other organisms usually in much lower numbers (Papers III, V; Last 1980), although interspecific differences in diet can be found at the same position (Conway 1980), which may relate to correspondence in small scale depth distributions of particular species and their prey. There can also be intra- and interspecific variability in prey size of even closely related fish species (Sabatés & Saiz 2000), but while the maximum prey size increases with increase in length, large larvae still take small prey such as copepod nauplii (Papers III, V, Pepin & Penney 1997). Larger larvae feeding on small items is inefficient, since they will contribute relatively less to the biomass of food ingested than a smaller number of large particles, but this may be a strategy to optimise the utilisation of trophic resources. The importance of larger prey in the diet was demonstrated experimentally by Geffen (1997), the size range of available food determining larval fish cohort size structure and growth rate.

Not surprisingly, growth rates can be directly related experimentally to food availability (Gotceitas *et al.* 1996). Mortality rates generally decrease with increase in size (Houde 1997; Sogard 1997), so rapid growth rates means that larvae will spend less time during their most vulnerable period of development. The larger larvae outgrow individual predator fields (Cowan & Houde 1992) and are more able to detect and avoid predators.

Relating larval fish distributions to that of their food

Fish larvae and their prey can be patchily distributed, both horizontally and vertically (Paper V) and have different net avoidance potentials because of their different sizes, so the same sampling equipment may not be ideal for simultaneously catching both (Sameoto *et al.* 2000). A coarse mesh net will sample fish larvae but not the microzooplankton prey of the smaller larvae. A fine mesh net will sample the prey, but because of the poorer filtration will have to be towed slower, may clog and be avoided by larvae.

Leggett & Deblois (1994) discussed how some studies have related larval distribution and food availability using a single coarse net. While a coarse net is inadequate for sampling the prey of larval fish, the samples obtained may still be of use as an indicator of secondary production. Microzooplankton distribution may not necessarily be related to the

distribution of the mesozooplankton, but certainly in conditions of high primary production, if there are high numbers of copepods, they should be producing nauplii, the main food of larval fish (Paper V). Runge *et al.* (1999) were able to show a positive relationship between mesozooplankton abundance and mackerel recruitment.

A Bongo net grid survey using a combination of nets of fine and coarse mesh, to sample larval sardine and their food, is described in Paper III. The depth integrated samples showed some correspondence between larval and prey abundances and prey abundance and feeding success, but not consistently, because larvae and prey estimates were obtained over a large vertical and horizontal as well as temporal scale. Environmental factors such as light, temperature, salinity and food availability change more rapidly in the vertical than in the horizontal plane, so a more intimate examination of larval fish and prey vertical distribution are necessary. This was highlighted when comparisons of laboratory and *in situ* studies suggested that ingestion rates estimated for larvae from the field were often considerably higher than those observed in the laboratory (MacKenzie *et al.* 1990). This may be a result of field conditions being poorly replicated in the laboratory, or field sampling techniques which inadequately resolve the prey field. As an example of the type of questionable field sampling methodology which is not uncommon, Viñas & Ramírez (1996) carried out vertically stratified sampling for fish larvae and their microzooplankton prey using different mesh nets, but not simultaneously.

Depth integrated Bongo sampling, as described in Paper V, is limited in the interpretation it can give to the correspondence between ichthyoplankton and their immediate physical and biological environment, but gives rapid horizontal distributional information. This was carried out in association with detailed vertical sampling using a double net LHPR system at 2 metre resolution. Fish larvae and microzooplankton food were sampled simultaneously, giving probably the most accurate representation of prey field and food selection analysis from a field study ever carried out. However, this still represented approximately $10m^3$ of water filtered to collect the larvae and 0.5 m^3 for the microzooplankton, which illustrates the sampling difficulties.

Particular food organisms appear to be taken in preference to others which are there in greater abundance (Paper V). While the type of food selection analysis which was carried out is typical of other studies (e.g. Hillgruber *et al.* 1997) , it may not be a valid type of analysis using the current sampling methods. Additionally, selection analysis still does not answer why particular organisms are selected. This can only be resolved by experimental work.

It is suggested that selection may be related to features such as particular locomotory movement or visibility of the organisms (Buskey *et al.* 1993; Hillgruber *et al.* 1997). Subtle differences in locomotion between species of copepod nauplii has certainly been observed (Paffenhöfer *et al.* 1996). Accurate sampling methods to evaluate prey selectivity by fish larvae is also critical for studying the effects of predation on the zooplankton community (Luo *et al.* 1996; Dagg & Govoni 1996), as concentrations of larvae could potentially deplete their local food resources to a critical level.

Problems of interpretation of larval fish gut contents analysis

While the composition and number of food organisms in the guts of fish larvae should give information on dietary range, feeding incidence and intensity, a recognised problem which must be considered in interpretation of results, is defecation or regurgitation of food, mainly during the sampling process but perhaps also during preservation (Arthur 1976). Larvae with straight guts, such as clupeoids, are particularly vulnerable (Paper V; Dekhnik 1974; Arthur 1976), but it has also been observed during experiments with species which have coiled guts (Canino & Bailey 1995). Some dietary studies make separate notes of amount of food in different parts of the alimentary canal, but this may not be valid if food is being displaced during sampling.

Loss of food should not alter observations on the range of species in the diet or any general diel feeding patterns, but care should be taken in the interpretation of other observations such as numbers of items in the guts. There may also be differences in susceptibility to loss of food depending on the age of larvae, related to the stage of gut development (Paper V). This feature requires further experimental study, as current feeding observations, particularly on larvae with straight guts, may be misleading.

Feeding response of larvae to changing environmental conditions

Larval fish typically show a higher feeding incidence and intensity during the day than at night (Paper V; Last 1980), associated with a reliance on visual feeding (Batty 1987). A reduction in feeding success with depth in shallow water has also been observed (Paper V), which may be related to a reduction in light levels due to turbid conditions. Interestingly, at least some marine and freshwater larval fish species have been shown to feed in complete darkness (Last 1979; Mookerjii & Rao 1993). Fresh, undigested organisms have also been found in the guts of sardine larvae at night (Paper III) suggesting the ability to capture food using senses other than sight.

Different species and also different stages of individual species, differ in their response to food deprivation (Theilacker & Porter 1994). The mid-gut epithelial cells of anchovy have been experimentally shown to deteriorate to a point where they can no longer return to a functioning state, if food is withheld for only 24 hours (IRB McFadzen Pers. comm.). Research is required to assess how important the role of feeding at night might be to enhanced survival in some species.

Lasker (1975) proposed that turbulence, caused by wind or tidal and current mixing, could disperse the food concentrations which are typically found around the chlorophyll maximum, to a level where there was reduction in larval feeding success, a decrease in growth rates and an increase in mortality rates. While some of these effects are acknowledged (Maillet & Checkley 1991) current opinion is that moderate turbulence in the water column may actually accumulate concentrations of food, increase production and act to enhance feeding success in larvae, by concentrating larvae and prey and thus changing encounter rates (Sundby & Fossum 1990). However, this has to be balanced against the possibly increased energetic costs associated with feeding in turbulent waters. Turbulence is difficult to assess in the field and most studies are theoretical modelling exercises (Sundby 1995).

The effect of turbulent mixing on anchovy larvae, following a storm, was studied in the northern Adriatic (Paper V). The storm cause a 41% decrease in concentration of the preferred food items and a change in the food species composition, but larvae were still able to maintain a similar food intake, demonstrating remarkable adaptability. There was also no indication of higher mortality rates following the storm (Coombs *et al.* In press). While considerable effort is being directed towards understanding the effect of turbulence on larval feeding, the results suggest that effort should also be concentrated on understanding the effects of turbulence on plankton reproduction and production. Harpacticoid copepods were able to maintain their reproductive output during turbulent conditions (Paper V), while calanoid and cyclopoid nauplii reduced in numbers. The ability of harpacticoids to successfully feed in turbulent conditions has been demonstrated experimentally (Suderman & Thistle 1998), which is almost certainly the key to maintenance of reproductive output.

Assessing condition of fish larvae

It is difficult to sample dead or moribund larvae in the sea, because a weak larvae will be quickly preyed upon (Bailey & Houde, 1989). To assess the proportion of larvae which are

in poor condition and are unlikely to survive, larval condition can be estimated by morphometric, histological and biochemical methods (Reviewed by Ferron & Leggett 1994).

There is good evidence that food availability can directly affect larval condition and survival (Anderson, 1994; Theilacker *et al.*, 1996), but some methods such as histology and gut enzyme analysis are susceptible to post-mortem deterioration of the larvae, unless processing is rapid. Theilacker & Porter (1994) even introduced correction factors in their histological studies to allow for this deterioration.

The importance of particular lipids in the diet is well recognised (e.g. Bell & Sargent 1996), but analysis of total lipid as a measure of condition using whole larvae is confounded by lipid in the gut contents, which can account for as much as 56% of total lipid (Lochmann *et al.* 1996).

The use of carbon analysis as a measure of condition has recently seen a revival (Paper VI; Westernhagen 1998). The practical advantages of this type of elemental analysis is that it is relatively insensitive to post-mortem deterioration. However, the source of carbon utilised during starvation varies between species and developmental stage, so carbon content can be regarded only as a gross index of condition.

Carbon analysis of the total larvae will also include the gut contents, but the bias introduced during this and other processing which utilise the complete larva, may be less of a problem with clupeoids such as field-caught sardine larvae, as there are typically few organisms in their guts because of regurgitation (Paper III). Additionally, a higher proportion retained food in their guts at night compared to many other fish larvae (Last 1980). The low level of diel feeding variability was reflected in the absence of any clear day/night change in carbon content.

Changes in carbon content can be a useful indicator of condition in fish larvae (Westernhagen 1998) and larval sardine were shown to have the lowest carbon content, and to be significantly undernourished, during periods when food availability was lowest (Paper VI). However there was no consistent relationship during other periods, probably because relative fine-scale horizontal and vertical distributions of larvae and prey were not accounted for in the sampling methods.

Condition analysis using RNA/DNA techniques were carried out on larval sardine collected during the same cruise as the larvae analysed for carbon in Paper VI (Chícharo *et al.* 1998). While the DNA/RNA ratios were significantly correlated with zooplankton abundance, <1% of larvae were considered to be starving and the period when most were

starving did not correspond to the same period when carbon values were lowest (Paper VI), highlighting how different condition assessments may respond to different influences.

During the study on larval anchovy (Paper V), it was noted that larvae with the deepest distributions had significantly less food in their guts. While this may be related to visual difficulties capturing food at depth, it has been noted that fish larvae in poorest condition can be sampled deeper in the water column (Grønkjær *et al.* 1997; Sclafani *et al.* 1997). Rather than collecting larval fish material for condition studies using depth integrated sampling methods, consideration should be given to using stratified sampling and comparing condition between depths.

Digestion of natural food by larval fish

Aspects of the digestion process in fish larvae were reviewed by Govoni *et al.* (1986), who recommended further research on the changing digestive and assimilative abilities during larval development, to provide a better understanding of their enzyme systems. Very little is known about how efficiently different foods are digested, apart from the unnatural diets such as brine shrimps and formulated foods used in aquaculture (Spyridakis *et al.* 1989; Webster & Lovell 1990). The natural diet of fish larvae includes organisms with great morphological and structural diversity (Papers III, V) features which must affect digestibility and nutritional potential. However, how well individual organisms are digested cannot be assessed from the gut contents, which will have been there for varying periods of time, but only from faeces.

Govoni *et al.* (1986) stated that larval fish do not produce discrete faeces, but gave no basis for this observation. However, while feeding batches of turbot larvae (*Scophthalmus maximus*) natural plankton, preparatory to histological studies, it was observed that they produced faeces enclosed in a membrane. It was realised that by harvesting these faeces, assessment of how well different food items which had traversed the complete alimentary canal were digested could be obtained (Paper I). It was found that there was considerable interspecific difference in the degree to which different organism were digested, as measured by comparing their dry weight, carbon and nitrogen content with that of fresh individuals of the same species.

The aquaculture industry is increasingly experimenting using either pumped natural prey from local waters (Pittman 1996) or cultured natural prey in larval fish culture (Støttrup & Norsker 1997), because of problems with vitamin and amino acid deficiencies in some of the feeds which are currently used, deficiencies which can cause developmental

abnormalities in the larvae (McEvoy *et al.* 1998). Further experiments under controlled conditions using the techniques developed (Paper I), have the potential to provide information for the aquaculture industry, on how well individual natural food organisms are digested, the feeding levels necessary for healthy and efficient growth of the fish larvae and which organisms would be worth culturing as food sources. However, if the organisms were cultured on monocultures of phytoplankton, dietary deficiency problems could arise in fish larvae, as their diet would not include the wide range of natural particles which they would filter out in the sea (Kleppel & Burkart 1995). Thus they may not accumulate certain vitamins or amino acids essential to the fish larvae. The study in Paper I has already contributed towards a better understanding of topics as diverse as digestive efficiency (Johnston & Mathias 1996) and sedimentation of copepod carcasses (Genin *et al.* 1995)

It was noted in several larval fish dietary studies that copepods eggs often appeared to be undigested in the guts (e.g. Conway 1980; Nakata 1988). It was subsequently observed (Paper I) that copepod eggs appeared to pass through larval turbot guts in an apparently undigested condition. This indigestibility has important nutritional implications, as copepod eggs can form a substantial proportion of the diet of the early stages of a wide range of larval fish species.

Digestibility of copepod eggs was investigated in greater detail (Paper II), and it was shown for the first time that a substantial proportion of the eggs of a range of copepod species, including species which do not produce resting eggs, were not digested by different ages of turbot larvae, even following gut passage times of up to 6.3 hours, and that certain species could also retain their viability. These observation also have implications for copepod survival, since egg-carrying female copepods are known to be visually selected for by predators, because of their greater bulk (Bollens & Frost 1991).

The copepod eggs most commonly found in the guts of early stage fish species, including important commercial species, are the large (180μm diameter) free spawned eggs of *Calanus* spp. (Papers III, V; Last 1980). Inability of fish larvae to digest these eggs could potentially contribute to interannual or areal differences in larval fish mortality, perhaps ultimately modifying recruitment. Experimental work feeding *Calanus* spp. eggs to larvae of these commercial fish species is essential.

The knowledge that copepod eggs may not be digested during larval fish gut passage (Paper II) has led to reassessment of feeding success observations in larval fish feeding studies (e.g. Hillgruber *et al.* 1997; Fox *et al.* 1999) and in the routes by which viable copepod eggs arrive in the bottom sediments (e.g. Marcus 1995; Lindley *et al.* 1998), as

well as stimulating further studies on retention of viability in other invertebrate eggs when fed to larval fish (Saint-Jean & Pagano 1995).

The rate of food passage through the gut

In studies of energetics and food requirements of larval fish, the daily ration is based on calculations of the amount of food in the gut at different times of day, and statistical derivations of gut passage rate (e.g. Hillgruber *et al.* 1997), as there is a dearth of information on how rapidly food passes through the gut and the factors which determines passage rates. Rates are described for turbot larvae in Paper I where mixed natural prey were used. Gut passage could take between 1.2 and 6.3 hours, depending on the size of the organisms. Similar experiments were subsequently conducted by Canino & Bailey (1995) using dyed *Artemia*, who criticised the results in Paper I on the basis that passage rates could not be estimated from mixed plankton food, as size might affect rate. They suggested a single species should be used, ignoring the fact that fish larvae feed on a range of particle sizes in the wild.

Gut passage rates slow down when feeding stops (Canino & Bailey 1995), so because larvae generally stop feeding at night, food can be retained in the guts for at least a substantial part of the night (Paper III; Last 1980). During this period of relative inactivity, the larvae can digest and assimilate food very efficiently (Canino & Bailey 1995). Anchovy larvae, at least in the Adriatic region, were found to be unusual among fish larvae, in that their guts were completely emptied within 30 minutes of sunset (Paper V), suggesting a different energetic strategy from the majority of other fish larval species. This is particularly surprising in this species, as it has been found to be particularly vulnerable to food deprivation (IRB McFadzen Pers. comm.).

Future research

The earliest seminal theories on the affect of food availability on larval fish survival (Hjort 1914; Cushing 1975) suggested that adequate food during particular periods of development was critical and that mortality levels sustained during early development might determine ultimate recruitment levels. While food availability is undoubtedly an important factor in larval fish survival because of its affect on condition and growth rates (Gotceitas *et al.* 1996; Theilacker *et al.* 1996), many other factors are now known to be

involved (Anderson 1985) and it is accepted that recruitment in a species may be decided at almost any stage of early development (Sogard 1997).

It would be impossible to encompass all known factors affecting larval fish survival in one study project, without massive resources. Their interactions are potentially very complex and recent reviews (e.g. Houde, 1997) have suggested that progress in understanding how selective forces generally shape recruitment will depend increasingly on modelling.

Recent advances in mathematical modelling techniques (Heath & Gallego 1997) allow the representation and simulation of both physical and biological oceanographic processes which offer the possibility of extensive examination of a range of different scenarios. While in the past, biological processes have been poorly represented in larval fish survival models, these new models are being coupled with work we are currently involved in, developing algorithms to convert satellite derived chlorophyll measurements and Continuous Plankton Recorder (Glover 1967) plankton distributions, to estimates of regional plankton secondary production. These are exciting developments towards better understanding and predicting fish recruitment.

References

Anderson JT (1988) A review of size dependent survival during pre-recruitment stages of fishes in relation to recruitment. J Northw Atl Fish Sci 8: 55-66

Anderson JT (1994) Feeding ecology and condition of larval and juvenile redfish *Sebastes* spp. Mar Ecol Prog Ser 104: 211-226

Arthur DK (1976) Food and feeding of larvae of three fishes occurring in the California current, *Sardinops sagax*, *Engraulis mordax*, and *Trachurus symmetricus*. Fish Bull *US* 74 : 517-530

Bailey KM (2000) Shifting control of recruitment of walleye pollock *Theragra chalcogramma* after a major climatic and ecosystem change. Mar Ecol Prog Ser 198: 215-224

Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. Adv Mar Biol 25: 1-83

Batty RS (1987) Effect of light intensity on activity and food searching of larval herring *Clupea harengus*: a laboratory study. Mar Biol 94: 323-327

Bell MV, Sargent JR (1996) Lipid nutrition and fish recruitment. Mar Ecol Prog Ser 134: 315-316

Bollens SM Frost BW (1991) Ovigerity, selective predation, and variable diel vertical migration in *Euchaeta elongata* (Copepoda :Calanoida) Oecologia 87: 155-161

Bradford MJ, Cabana G (1997) Interannual variability in stage-specific survival rates and causes of recruitment variation. In: Chambers R, Trippel EA (eds) Early Life History and Recruitment in Fish Populations. Chapman & Hall, London, p 469-493

Buskey EJ, Coulter C, Strom S (1993) Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bull mar Sci 53: 29-43.

Canino MF, Bailey KM (1995) Gut evacuation of walleye pollock larvae in response to feeding conditions. J Fish Biol 46: 389-403

Chícharo MA, Chícharo L, Valdés L, López-Jamar E, Ré P (1998) Estimation of starvation and diel variation of the DNA/RNA ratios in field caught *Sardina pilchardus* larvae off the north of Spain. Mar Ecol Prog Ser 164: 273-283

Conway DVP (1980) The food of larval blue whiting, *Micromesistius poutassou* (Risso), in the Rockall area. J Fish Biol 16: 709-723

Coombs SH, Giovanardi O, Halliday N C, Franceschini G, Conway DVP, Manzueto L, Barrett CD, McFadzen IRB (In Press) Wind mixing, food availability and mortality of anchovy larvae (*Engraulis encrasicolus*) in the northern Adriatic. Mar Ecol Prog Ser

Cowan JC, Houde ED (1992) Size dependent predation on marine fish larvae by Ctenophores, Scyphomedusae, and planktivorous fish. Fish Oceanogr 1: 113-126

Cushing DH (1972) The production cycle and the numbers of marine fish Symp Zool Soc Lond 29: 213-232.

Cushing DH (1975) Marine ecology and fisheries. Cambridge University Press, Cambridge, 278 pp

Cushing DH (1990) Plankton production and year-class strength in fish populations: An update of the match-mismatch hypothesis. Adv Mar Biol 26: 250-294

Dagg MJ, Govoni JJ (1996) Is ichthyoplankton predation an important source of copepod mortality in subtropical coastal waters? Mar Freshwater Res 47: 137-144

Dekhnik TV (1974) Comments on the articles by V Ye Zaika and NA Ostrovskaya entitled "Indicators of the availability of food to fish larvae". J Ichthyol 14: 804-808 [Translation from Vopr Ikhtiol]

Dickey-Collas M, Brown J, Fernand L, Hills AE, Horsburgh KL, Garvine RW (1997) Does the western Irish Sea gyre influence the distribution of pelagic juvenile fish? J Fish Biol 51 (Suppl A): 206-229

Ferron A, Leggett WC (1994) An appraisal of condition measures for marine fish larvae. Adv Mar Biol 30: 217-303

Fox CJ, Harrop R, Wimpenny A (1999) Feeding ecology of herring (*Clupea harengus*) larvae in the turbid Blackwater Estuary. Mar Biol 134: 253-265

Geffen AJ (1996) Effect of experimental manipulation of feeding conditions on the population structure of larval cod (*Gadus morhua*) and Herring (*Clupea harengus*). Mar Freshw Res 47: 291-300

Genin A, Gal G, Haury L (1995) Copepod carcasses in the ocean 2. Near coral-reefs. Mar Ecol Prog Ser 123: 65-71

Glover RS (1967) The Continuous Plankton Recorder Survey of the North Atlantic. Symp zool Soc Lond 19: 189-210

Gotceitas V, Puvanendran V, Leader LL, Brown JA (1996) An experimental investigation of the 'match/mismatch' hypothesis using larval Atlantic cod. Mar Ecol Prog Ser 130: 29-37

Govoni JJ, Boehlert GW, Watanabe Y (1986) The physiology of digestion in fish larvae. Environ Biol Fish 16: 59-77

Graham M (1942) The fish gate. Faber and Faber, London, 196 pp

Gray CA (1996) Do thermoclines explain the vertical distributions of larval fishes in the dynamic coastal waters of south-eastern Australia? Mar Freshwater Res 47: 183-190

Grønkjær P, Clemmesen C, John St M (1997) Nutritional condition and vertical distribution of Baltic cod larvae. J Fish Biol 51 (Suppl A): 352-369

Heath M, Gallego A (1997) From the biology of the individual to the dynamics of the population: bridging the gap in fish early life study. J Fish Biol 51(Suppl A): 1-29

Hillgruber N, Kloppmann M, Wahl E, Westernhagen H von (1997) Feeding of larval blue whiting and Atlantic mackerel: a comparison of foraging strategies. J Fish Biol 51 (Supp A) : 230-249

Hjort J (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp P-V Reun Cons Int Explor Mer 19: 1-228

Horwood J, Cushing D, Wyatt T (2000) Planktonic determination of variability and sustainability of fisheries. J Plank Res 22, 7: 1419-1422

Houde ED (1997) Patterns and consequences of selective processes in teleost early life histories. In: Chambers R, Trippel EA (eds) Early life history and recruitment in fish populations. Chapman & Hall, London. p 173-195

Johnston TA, Mathias JA (1996) Gut evacuation and absorption efficiency of walleye larvae. J Fish Biol 49: 375-389

Kendall AW, Schumacher JD, Kim S (1996) Walleye pollock recruitment in Shelikof Strait: applied fisheries oceanography. Fish Oceanogr 5(Suppl 1): 4-18

Kleppel GS, Burkart CA (1995) Egg-production and the nutritional environment of Acartia tonsa - the role of food quality in copepod nutrition. ICES J Mar Sci 52: 297-304

Lasker R (1975) Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fish Bull US 73: 453-462

Last JM (1979) The food of larval turbot *Scophthalmus maximus* L. from the west central North Sea. J Cons int Explor Mer 38: 308-313

Last, J.M. 1980. The food of twenty species of fish larvae in the west-central North Sea. Fish Res Tech Rep No. 60, MAFF Directorate of Fish Res, Lowestoft

Leggett WC, Deblois E (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth J Sea Res 32: 119-134

Lindley JA, George CL, Evans SV, Donkin P (1998) Viability of calanoid copepod eggs from intertidal sediments: a comparison of three estuaries. Mar Ecol Prog Ser 162: 183-190

Lochmann SE, Maillet GL, Taggart CT, Frank KT (1996) Effect of gut contents and lipid degradation on condition measures in larval fish. Mar Ecol Prog Ser 134: 27-35

Lough RG, Caldrone EM, Rotunno TK, Broughton EA, Burns BR, Buckley LJ (1996) Vertical distribution of cod and haddock eggs and larvae, feeding and condition in stratified and mixed waters on southern Georges bank, May 1992. Deep-Sea Res 43: 1875-1904

Luo J, Brandt SB, Klebasko MJ (1996) Virtual reality of planktivores: a fish's perspective of prey size selection. Mar Ecol Prog Ser 140: 271-283

Maillet GL, Checkley DM (1991) Storm-related variation in the growth rate of otoliths of larval Atlantic menhaden *Brevoortia tyrannus*: a time series analysis of biological and physical variable and implications for larval growth and mortality. Mar Ecol Prog Ser 79: 1-16

MacKenzie BR, Leggett WC, Peters RH (1990) Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar Ecol Prog Ser 67: 209-225

Marcus NH (1995) Seasonal study of planktonic copepods and their benthic resting eggs in northern California coastal waters. Mar Biol 123: 459-465

McEvoy LA, Naess T, Bell JG, Lie Ø (1998) Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163: 237-250

McGowan JA, Brown DM (1966) A new opening-closing paired zooplankton net. *SIO Reference* 66, 23 : 56 p

Mookerjii N, Rao TR (1993) Patterns of prey selection in rohu (*Labeo rohita*) and singhi (*Heteropneustes fossilis*) larvae under light and dark conditions. Aquaculture 118: 85-104

Moser HG, Pommeranz T (1999) Vertical distribution of eggs and larvae of northern anchovy, *Engraulis mordax*, and of the larvae of associated fishes at two sites in the Southern Californian Bight. Fish Bull US 97: 920-943

Nakata K (1988) Alimentary tract contents and feeding condition of ocean-caught post-larval Japanese sardine *Sardinops melanostictus*. Bull Tokai Reg Fish Res Lab 126: 11-24

Neilson JD, Perry RI (1990) Diel vertical migration of marine fishes: an obligate or facultative process? Adv Mar Biol, 26: 115-168

Nellen W, Hempel G (1969) Versuche zur Fängigkeit des 'Hai' und des modifizierten Gulf-V-Plankton-samplers 'Nackthai'. Ber Dt Wiss Komm Meeresforsch 20: 141-154

Paffenhöfer GA, Strickler JR, Lewis KD, Richman S (1996) Motion behavior of nauplii and early coepodid stages of marine planktonic copepods. J Plank Res 18: 1699-1715

Palomera I (1991) Vertical distribution of eggs and larvae of Engraulis encrasicolus in stratified waters of the western Mediterranean. Mar Biol 111: 37-44

Pearre S (1979) Problems of detection and interpretation of vertical migration. J Plank Res 1: 29-44

Pepin P, Penney RW (1997) Patterns of prey size and taxonomoc composition in larval fish: are there there general size-dependent models? J Fish Biol 51 (Suppl A): 84-100

Pittman K (1996) Rearing halibut in Norway: present practices and challenges. Mar Freshw Res 47: 243-249

Runge JA, Castonguay M, de Lafontaine Y, Ringuette M, Beaulieu J-L (1999) Covariance in climate, zooplankton biomass and mackerel recruitment in the southern Gulf of St Lawrence. Fish Oceanogr 8: 139-149

Saint-Jean L, Pagano M (1995) Egg mortality through predation in egg-carrying zooplankters. Studies on *Heterobranchus longifilis* larvae fed on copepods, cladocerans and rotifers. J Plank Res 17: 1501-1512

Sabatés A, Saiz E (2000) Intra- and interspecific variability in prey size and niche breadth of myctophiform fish larvae. Mar Ecol prog Ser 201: 261-271.

Sameoto D, Wiebe P, Runge J, Postel L, Dunn J, Miller C, Coombs S (2000) Collecting zooplankton. In: Harris R, Wiebe P, Lenz J, Skjoldal HR, Huntley M (eds). ICES zooplankton methodology manual. Academic Press, London, p 55-81

Sclafani M, Stirling G, Leggett WC (1997) Osmoregulation, nutritional effects and buoyancy of marine larval fish: a bioassay for assessing depth changes during the earliest life-history stages. Mar Biol 129: 1-9

Sogard S (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull Mar Sci 60: 1129-1157

Spyridakis P, Metallier R, Gabaudan J, Riaza A (1989) Studies on nutrient digestibility in european sea bass (*Dicentrarchus labrax*) 1. Methodological aspects concerning faeces collecton. Aquaculture 77: 61-70

Støttrup JG, Norsker NH (1997) Production and use of copepods in marine fish larviculture. Aquaculture 155: 231-247

Suderman K, Thistle D (1998) Adult female harpacticoid copepods maintain their energy reserves by feeding while suspended during storms. Mar Ecol Prog Ser 164: 245-252

Sundby S (1995) On the dome-shaped relationship between wind-generated turbulence and larval feeding. Neth J Sea Res 34: 243-244

Sundby S, Fossum P (1990) Feeding conditions of Arcto-Norwegian cod larvae compared with the Rothschild-Osborn theory on small-scale turbulence and plankton contact rates. J Plank Res 12: 1153-1162

Theilacker GH, Porter SM (1994) Condition of larval walleye pollock, *Theragra chalcogramma*, in the western Gulf of Alaska assessed with histological and shrinkage indices. Fish Bull US 93: 333-344

Theilacker GH, Bailey KM, Canino MF, Porter SM (1996) Variations in larval walleye pollock feeding and condition: a synthesis. Fish Oceanogr 5 (suppl 1): 112-123

Viñas MD, Ramírez FC (1996) Gut analysis of first-feeding anchovy larvae from the Patagonian spawning areas in relation to food availability. Arch Fish Res 43: 231-256

Webster CD, Lovell RT (1990) Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture 90: 49-61

Westernhagen H von, Freitas C, Fürstenberg, G., Willführ-Nast, J. (1998) C/N data as an indicator of condition in marine fish larvae. Arch Fish Mar Res 46: 165 - 179

Williams R, Collins NR, Conway DVP (1983) The double LHPR system, a high-speed micro- and macroplankton sampler. Deep-Sea Res 30 : 331-342

Publications

Paper I

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Digestion of natural food by larval and post-larval turbot *Scophthalmus maximus*

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ABSTRACT: The digestion of natural, mainly crustacean zooplankton, by different age groups of turbot *Scophthalmus maximus* larvae was evaluated by comparisons of visual appearance, dry weight and carbon and nitrogen content of fresh food organisms with material recovered from faeces. Visually, the degree of digestion of food particles ranged from no discernible change of lamellibranch larvae, copepod eggs, intact copepod faecal pellets and some phytoplankton species, to varying degrees of removal of body constituents in copepods, cladocerans and decapod zoea. For crustaceans, the proportion of body constituents removed was related to the size and construction of their apparently indigestible exoskeleton. Upon defaecation larger organisms showed the greatest percentage loss in dry weight and carbon. A high percentage of nitrogen was extracted from all organisms. There was no consistent difference in digestive efficiency between different age groups of larvae.

INTRODUCTION

Starvation is suggested to be one of the primary factors influencing the high mortality experienced by the early larval stages of marine fish (Bailey & Houde 1989). Although considerable research effort has been directed towards understanding the relationship between survival of larvae and food availability, the digestibility of the various food organisms (i.e. how efficiently their constituents are removed during gut passage) has received little consideration.

Although they can consume a wide spectrum of food organisms (Turner 1984), the main food for larval fish is crustacean, especially the various developmental stages of copepods. Their diet generally reflects the species composition of the surrounding plankton (Checkley 1982, Young & Davis 1992). There can be great morphological and structural diversity and differences in chemical composition between the various food organisms, which must give equivalent variations in digestibility and nutritional potential. Furthermore, larval fish, with few reported exceptions (e.g. Rösch & Segner 1990), do not mechanically disrupt their prey during ingestion. Thus, while soft-bodied prey may be easily broken down enzymatically in the gut, organisms with apparently indigestible exoskeletons or shells, such as crustaceans and mollusc larvae, may be more difficult to digest efficiently and there may be a range of digestibility between prey types. Larval fish growth and survival could thus vary regionally and temporally due to differences in the digestibility of the most abundant available food.

Assessment of the ability of larval fish to digest particular organisms from conventional gut content investigations on preserved larvae is misleading, because as they are still within the digestive tract, digestion has not been completed. In the present study food organisms from freshly recovered faeces of different age groups of turbot *Scophthalmus maximus* larvae are examined and analysed to compare the efficiency of feeding on different items.

MATERIALS AND METHODS

Larval turbot, 17 and 34 d post-hatch were obtained from Golden Sea Produce Ltd, Hunterston, Scotland, in August 1992 and transported in insulated containers to Plymouth, England. The 34 d old turbot were undergoing metamorphosis but, for ease of description, all

ages are termed larvae. Experiments were subsequently carried out on 3 age ranges of larvae, 21 to 27 d (6.2 to 10.6 mm, ca 100 larvae), 37 to 46 d (14.4 to 20.5 mm, ca 60 larvae) and 53 to 67 d (21.6 to 39.2 mm, ca 30 larvae). While it would have been desirable to have also worked with a younger, potentially more vulnerable age range of larvae, there would have been practical difficulties in carrying out some of the procedures because of the small size of their food.

At the Plymouth Laboratory the larvae were maintained under continuous subdued lighting conditions, at temperatures of 18 to 19 °C, in small aquaria of 5 or 15 l, at a stocking density of 5 to 25 per aquarium, depending on size. The aquaria were not aerated but half the seawater (salinity approximately 34.0 psu) was replaced each day. Prior to transport to Plymouth the 17 d old larvae had been fed rotifers *Branchionus plicatilis* and *Artemia salina* nauplii, and the 34 d old larvae *A. salina* nauplii alone. These diets were replaced in the experiments with a mixture of wild zooplankton, collected regularly off Plymouth using a variety of plankton nets (50 to 200 µm). Some additional feeding experiments were carried out using the brackish water copepod *Eurytemora velox*. The range of organisms fed to the turbot larvae was, apart from *E. velox*, a similar mixture to that which they would encounter, and on which they have been observed feeding in the wild (Jones 1972, Last 1979). All experimental observations were made between 11:00 and 20:00 h GMT to reduce the influence of any diurnal changes in feeding intensity.

Feeding experiments were started at 07:00 h GMT when mixed plankton was introduced to each aquarium at a density of approximately 200 organisms l^{-1}. Plankton density was regularly maintained throughout the experiment and dead material removed. Collection of faecal material from the larvae was facilitated by turbot larvae producing faeces which are encased in a thin membrane which maintains their structural integrity. (Membranes were also observed on the faeces of *Gobius* sp. but not *Callionymus lyra* larvae, which had been collected in the plankton samples taken for food.) Over the first 4 h of each experiment, faeces were collected from the bottom of the aquaria using a wide-bore pipette and discarded. After 4 h, once the guts had been flushed out with more recently ingested food, faeces were collected at 5 min intervals. They were immediately opened in seawater under a microscope and a selection of intact individual organisms (n = 1717) removed for processing. A record was kept of the visual state of digestion of the organisms, as well as organism size (cephalothorax length for copepods, carapace length for decapod zoea, total length for cladocera and diameter for copepod eggs). They were then briefly dipped in distilled water to remove adherent salt. This procedure may lead to the loss of small amounts of organic material but is necessary in order to obtain accurate dry weights to which the other analyses are related. Organisms were then placed in solvent-cleaned (acetone and chloroform) preweighed (Cahn 25 Electrobalance) tin cups (5.3 × 3.2 mm). Depending on the weight of the organism, a variable number of specimens was placed in each cup to give a minimum of 20 µg sample weight. The open cups were then dried for 24 h at 60 °C, compacted, and stored in a desiccator. Subsequently the samples were weighed and then analysed for carbon and nitrogen with a Carlo Erba model NA 1500 Series 2 elemental analyser, using acetanylide as a calibration standard. Faecal membranes from 2 age groups of larvae were also collected and analysed.

In order to measure the dry weight and carbon and nitrogen content of undigested plankton, specimens (n = 947) of the same range of species as found in the faeces were selected from the same fresh plankton as supplied as food to the larvae. Processing of these samples was in the same way as for those extracted from the faeces.

During the experiments a restricted number of observations (n = 14) were made on the rate of passage of food particles through the intestinal tract. This was carried out by placing groups of 5 larvae in aquaria, feeding them until they were producing faeces and then introducing a different, easily recognisable marker food (e.g. *Eurytemora velox*). Feeding was then continued and faeces collected and examined at 10 min intervals until the first appearance of the marker.

As a measure of general larval condition and as a check on the functional integrity of the gut, samples of individual larvae (n = 24) were taken at intervals during the experiments and preserved in Baker's formol calcium fixative for subsequent histological examination. Following fixation, larvae were processed for methacrylate embedding, then serially sectioned at 2 µm in the sagittal plane, using Ralph glass knives, and stained in Lee's methylene blue/basic fuchsin, before mounting in Canada Balsam (McFadzen et al. 1991).

RESULTS

Feeding behaviour and rate of passage of food

Turbot larvae fed on a wide variety of prey, reflecting availability in the size range which they could ingest. From observations through the sides of the aquaria during the experiments it was noted that larger food particles were selected preferentially before smaller ones. This was especially the case for very motile and visible organisms such as decapod

zoea, although these were sometimes ejected from the mouth several times before being swallowed, probably a consequence of their spiny carapace and vigorous struggling.

The rate of passage of food through the guts of larvae was unpredictable and took between 1.2 and 6.3 h (Fig. 1). The main pulse of marker particles usually occured about 1 h after the first appearance. Food ingested concurrently did not necessarily pass through at the same rate; on 2 occasions *Eurytemora velox* eggs were observed in the faeces of 46 and 64 d old larvae, 50 min and 1 h respectively, before the adult female exoskeleton.

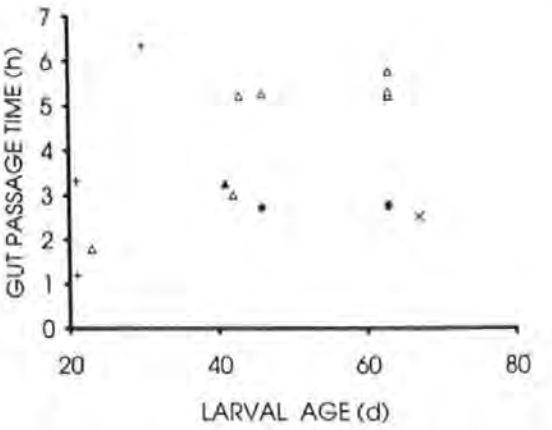

Fig. 1. *Scophthalmus maximus*. Rate of passage of a range of food items, through the intestinal tracts of larvae of different ages. Each point represents the fastest rate for a group of 5 larvae. (▵) *Eurytemora velox*; (▲) *Anomalocera patersoni*; (●) *Temora longicornia*; (×) decapod zoea; (+) mixed plankton, including cirripede nauplii and the copepods *Pseudocalanus elongatus*, *Temora longicornis* and *Oithona* spp.

Histological examination of the gut

Histological examination of the digestive tract of turbot larvae from each of the 3 age groups showed that the fore-, mid- and hindgut regions were in good condition. Deep longitudinal folds (villi) were evident throughout the gut, particularly in the ventral region, which is indicative of normal healthy development (Cousin et al. 1986). In particular, the hindgut epithelium showed normal cellular integrity, with large supranuclear inclusion bodies present which are reported to be indicative of intracellular digestion of food particles engulfed by pinocytosis (O'Connell 1976). Other tissues assessed were the liver, pancreas, kidney, gills, trunk muscle, notochord and cartilage. All tissues were found to be healthy, in accordance with descriptions of Cousin et al. (1986).

Contents of the faeces

The membrane surrounding the faeces was usually tinted orange or brown. Faeces were usually well compacted, but a large proportion of crustacean exoskeletons were intact, although sometimes crushed and distorted. The largest copepods consumed were *Anomalocera patersoni*, *Labidocera wollastoni* and *Calanus helgolandicus* (Table 1) which were often broken into 2 or 3 pieces in the faeces, suffering greater disruption to the exoskeleton than smaller copepods. Of the smaller copepods, robust compact species such as *Centropages typicus*, *Temora longicornis*, *Corycaeus anglicus*, *Euterpina acutifrons* and *Oncaea* spp. were rarely crushed, while *Acartia clausi* and the cladoceran *Evadne nordmanni*, which are less robust, were usually crushed.

All nutrient digested from the food is not necessarily absorbed and an unknown and probably variable amount of dissolved nutrient, which cannot be quantified with the present experimental procedure, may be egested with the faeces and either immediately leach out into the aquarium or be released when the faecal membrane is removed. Results obtained are therefore a measure of the amount of material which the turbot larvae can digest from a particular organism and not what is actually absorbed.

Organisms in the faeces were usually well digested, with copepods reduced to transparent exoskeletons. There were, however, some exceptions. *Corycaeus anglicus* often appeared to be largely undigested, with orange liquid contents which were ejected if the copepod was punctured; *Temora longicornis* and *Centropages typicus*, which generally have very opaque bodies, sometimes had small amounts of undigested material remaining inside the exoskeleton. In *Pseudocalanus elongatus*, a species which often has large lipid reserves, lipid globules sometimes still remained in the exoskeleton, or occasionally globules were found free inside the faecal membrane when these copepods were present. In *Acartia clausi* especially, intact undigested faecal pellets liberated from the copepods gut were commonly observed free within the otherwise transparent exoskeleton. The subitaneous (non-diapause) eggs of *A. clausi*, *Eurytemora velox*, *Euterpina acutifrons*, *Oncaea* spp. and *C. anglicus* and the thick walled diapause eggs of *Evadne nordmanni* were commonly found apparently undigested in the faeces (Conway unpubl.).

Some non-crustacean food also appeared to resist digestion. Of the mollusc larvae, most lamellibranchs did not appear to be digested, only a few were noted with open shells and no contents. A few chitinous

Table 1. *Scophthalmus maximus*. Length range, mean dry wt of fresh food organisms and mean percentage loss in weight of the same range of food organisms after digestion by 3 age groups of larvae. Number of observations (n) are shown and also the standard deviations (SD) of the means where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Length range (mm)	Mean fresh dry wt (µg)	SD	n	Mean % loss in weight after digestion					
					21-27 d	n	37-46 d	n	53-67 d	n
Copepoda										
Anomalocera patersoni 5M	1.8-1.9	129.4	-	2	-	-	87.6	2	-	-
A. patersoni 6F	1.8-2.8	253.9	27.9	4	86.8	1	84.5	6	-	-
A. patersoni 6M	2.2-2.4	185.3	12.0	5	-	-	85.6	7	82.7	1
Calanus helgolandicus 6F	1.8-2.3	107.5	7.9	6	-	-	85.1	2	85.1	2
C. helgolandicus 6M	2.0-2.2	128.0	-	1	84.4	1	79.7	1	-	-
Labidocera wollastoni 6F	1.8-2.4	132.2	-	2	-	-	89.6	3	81.1	1
L. wollastoni 6M	1.7-2.0	87.0	-	1	-	-	79.3	2	88.5	1
Centropages typicus 5F	0.8-0.9	20.4	-	2	-	-	87.7	1	60.8	1
C. typicus 6F	1.3-1.5	35.4	8.8	4	-	-	59.3	7	72.9	3
C. typicus 6M	1.1-1.3	35.8	2.9	4	-	-	72.1	2	69.0	4
Temora longicornis 5	0.7-0.8	8.6	-	1	-	-	40.7	3	44.2	2
T. longicornis 6F	0.8-0.9	22.1	0.8	4	-	-	65.2	4	69.7	19
T. longicornis 6M	0.7-0.9	14.5	-	2	-	-	-	-	60.0	21
Eurytemora velox 5F	0.6-1.0	15.5	1.4	3	80.6	5	-	-	-	-
E. velox 5M	0.6-0.8	12.0	-	1	81.7	2	-	-	-	-
E. velox 6F	0.9-1.1	16.9	1.8	11	78.7	4	-	-	79.3	2
E. velox 6F with eggs	1.0-1.1	19.3	1.5	9	-	-	-	-	-	-
E. velox 6M	0.7-1.0	14.8	1.4	9	77.0	3	-	-	70.9	2
E. velox eggs	0.09-0.11	0.3	-	2	-	-	-	-	-	-
Pseudocalanus elongatus 6F	0.7-0.08	9.0	0.3	3	-	-	75.6	1	-	-
Corycaeus anglicus 6F	0.5-0.7	8.1	0.1	3	-	-	64.2	5	54.3	4
Acartia clausi 6F	0.7-0.9	5.3	-	2	49.1	2	35.8	4	49.1	6
A. clausi 6M	0.7-0.9	8.1	1.6	3	-	-	67.9	1	72.8	2
Paracalanus parvus 5	0.6-0.7	5.3	-	1	73.6	1	-	-	-	-
P. parvus 6F	0.6-0.8	5.4	-	1	-	-	-	-	48.1	1
Euterpina acutifrons 6F	0.5-0.7	3.6	0.6	3	-	-	27.8	3	47.2	2
Cladocera										
Podon intermedius	0.7-0.9	9.7	0.7	3	-	-	80.4	3	56.7	2
Evadne nordmanni	0.4-0.5	3.1	0.7	3	45.2	3	48.4	3	77.4	1
Decapod zoea										
Necora puber	1.3-1.6	84.7	30.7	3	-	-	55.0	3	-	-
Pisidia longicornis	0.7-0.9	18.7	-	1	-	-	52.9	1	-	-
Faecal membrane										
37-46 d larvae	-	-	-	-	-	-	-	2	-	-
57-67 d larvae	-	-	-	-	-	-	-	-	-	15

chaetognath jaws were found, the only part of the organism apparently resistant to digestion. Occasionally the large diatom *Coscinodiscus concinnus* and the prasinophyte *Halosphaera* spp. occurred in the faeces, to all appearances undigested and still with green contents, although the contents were noticeably disrupted and the cells no longer viable.

The length of digested copepods was found to be slightly less than for undigested material. For example a group of *Eurytemora velox* females which were measured had a cephalothorax length range of 0.92 to 1.13 mm and a mean length of 1.01 ± 0.03 mm (n = 21) while the same stage after digestion had a cephalothorax length range of 0.81 to 1.01 mm and a mean length of 0.93 ± 0.04 mm (n = 45), a significant (from *t*-test analysis $p < 0.001$) reduction in length of 7.9%. The observed shrinkage may be due to partial collapse of the exoskeleton after loss of turgidity or to denaturation of the protein holding the exoskeleton together. The shrinkage, which is comparable to that found after fixation in formalin, is a consideration if one was trying to relate gut content measurements to fresh food measurements.

Dry weight analysis of fresh and digested food

The length range of fresh food organisms, their mean dry weight and their mean percentage loss in weight after digestion are given in Table 1. There were con-

siderable differences in mean fresh dry weight between male and female copepods of the same species and between species of the same cephalothorax length. Egg-bearing female copepods are not necessarily substantially heavier than those without eggs, as demonstrated by the similarity in mean dry weight of *Eurytemora velox* Stage 6 females with and without eggs. By weighing detached egg masses, individual egg weight was estimated to be 0.3 µg. The highest number of eggs counted in an egg mass was 29, so that the egg mass could represent up to to 57 % of the mean body dry weight (16.9 µg).

The mean percentage loss in dry weight by food after digestion varied from 27.8 to 89.6 % between different groups of food organisms. In general there was little difference in digestive weight loss of food between fish larvae of different ages. Only for the cladocerans was there any great variability, but with no consistent pattern. Weight loss was highest (>80 %) in the largest, heaviest copepods such as *Calanus helgolandicus*, *Anomalocera patersoni* and *Labidocera wollastoni*. Among the smaller copepods (*Pseudocalanus elongatus*, *Acartia clausi*, *Corycaeus anglicus*, *Paracalanus parvus* and *Euterpina acutifrons*), weight loss after digestion was more variable at between 27.8 and 75.6 %. Both species of decapod zoea had low weight loss (52.9 and 55.0 %) even though *Necora puber* had a fresh weight comparable to the larger copepods which showed a high weight loss. Within the different stages of the same copepod species percentage weight loss was generally similar.

Carbon and nitrogen analysis of fresh and digested food

The mean percentage carbon and nitrogen of the dry weight of fresh food organisms and their mean percentage loss in carbon and nitrogen following digestion, for the same group of specimens as in Table 1, are given in Tables 2 & 3. The range of percentage carbon for fresh copepods varied from 28.2 to 52.4 % and while there was no clear relationship with size, lower values found tended to be among the smaller copepods such as *Euterpina acutifrons*. On passage through the guts of turbot larvae, copepods lost 55.7 to 97.7 % of their carbon, in the majority of cases decreasing by over 80 %. Larger copepods tended to lose a greater percentage. A high proportion of carbon (80.2 to 89.5 %) was also removed from cladocerans and the decapod zoea *Necora puber*, although in the other zoea, *Pisida longicornis*, it was reduced by a lesser amount (64.8 %). There was no clear difference in digestive ability between larvae of different ages although the older turbot larvae tended to extract a greater percentage of carbon.

The proportion of the fresh dry weight of copepods represented by nitrogen ranged from 6.4 to 11.5 % (Table 3). In general the smaller copepods had the lower mean percentages. A very high proportion of nitrogen was extracted during digestion (78.7 to 100 %) and in approximately half the analyses it was not detectable in the faeces. There were no clear patterns between different organisms, stages of copepods or ages of larvae. The mean C:N ratio for fresh organisms (Table 3) varied for most species over a narrow range from 3.6 to 4.5, though the high carbon content of *Eurytemora velox* led to C:N values of up to 5.3.

When the individual determinations of percentage carbon and nitrogen of fresh and digested food are plotted against dry weight for 4 large (Fig. 2a, b) and 4 small copepod species (Fig. 3a, b) the effects of digestion are highlighted. In fresh copepods the carbon and nitrogen values fall within a restricted range. The greatest variability, especially in percentage carbon, was among copepods weighing <20 µg. The greater variability in carbon and nitrogen composition observed among smaller, lighter organisms is not a problem of analytical sensitivity because the smaller items were bulked together to give similar sample weight to larger organisms, but reflects real differences in chemical composition and differences related to structural diversity. In digested food (Figs. 2b & 3b) the spread of values is much greater than in fresh food. Greater variability was again in food remains weighing <20 µg where a large proportion of specimens had low or no detectable nitrogen.

Faecal packaging does not appear to occur in all fish larvae and whatever advantage is gained has to be balanced against energy lost in the process. The amount of carbon and nitrogen lost as a percentage of the dry weight of the faecal membrane (Tables 2 & 3) was very low (14.4 to 14.7 % and 0 to 1.1 % respectively), so the membrane has no more nutritional potential than the faecal constituents.

DISCUSSION

Larval fish have been shown, both in experimental studies and in the field, to lose condition when food is scarce (Werner & Blaxter 1980, Canino et al. 1991). In the present study turbot larvae were fed zooplankton at approximately 200 l^{-1} which is a higher concentration than found under most natural conditions (Canino et al. 1991, Coombs et al. 1992). The feeding concentration was chosen so that larvae were not constrained by lack of food and with the potential for an element of selectivity. Higher concentrations were not offered

Table 2. *Scophthalmus maximus*. Mean weight of carbon and mean percentage carbon of the dry weight of fresh food organisms and mean percentage loss in carbon of the same range of food organisms after digestion by 3 age groups of larvae. The number of observations are as for Table 1. Standard deviations (SD) of the means are shown where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Mean fresh weight of carbon (µg)	SD	Mean % carbon of fresh dry wt	SD	Mean % loss in carbon after digestion 21-27 d	37-46 d	53-67 d
Copepoda							
Anomalocera patersoni 5M	49.3	-	38.0	-	-	92.9	-
A. patersoni 6F	102.7	12.2	39.8	0.7	91.8	93.2	-
A. patersoni 6M	76.2	6.6	40.7	1.0	-	91.6	89.3
Calanus helgolandicus 6F	42.4	5.6	38.7	2.5	-	94.8	88.7
C. helgolandicus 6M	49.3	-	38.5	-	91.5	87.8	-
Labidocera wollastoni 6F	52.4	-	42.1	-	-	89.5	91.2
L. wollastoni 6M	35.5	-	40.8	-	-	90.0	90.2
Centropages typicus 5F	10.6	-	34.2	-	-	92.4	91.2
C. typicus 6F	14.4	4.3	40.3	3.4	-	81.9	84.7
C. typicus 6M	13.2	0.6	36.4	3.4	-	71.7	84.3
Temora longicornis 5	3.2	-	37.4	-	-	68.3	79.3
T. longicornis 6F	9.3	0.6	42.1	3.0	-	80.6	82.7
T. longicornis 6M	5.4	-	37.7	-	-	-	72.3
Eurytemora velox 5F	7.8	0.3	50.3	3.4	93.3	-	-
E. velox 5M	6.3	-	52.4	-	97.7	-	-
E. velox 6F	7.2	0.9	43.1	5.4	90.9	-	94.6
E. velox 6F with eggs	9.2	0.8	47.6	1.2	-	-	-
E. velox 6M	7.0	0.8	47.7	4.5	94.5	-	93.9
E. velox eggs	0.1	-	35.1	-	-	-	-
Pseudocalanus elongatus 6F	3.7	0.2	42.1	3.0	-	94.5	-
Corycaeus anglicus 6F	2.8	0.2	34.0	2.2	-	72.0	80.5
Acartia clausi 6F	2.4	-	44.7	-	74.1	64.9	84.2
A. clausi 6M	2.4	0.1	31.9	8.8	-	87.5	85.7
Paraclanus parvus 5	1.6	-	30.6	-	83.0	-	-
P. parvus 6F	1.7	-	32.0	-	-	-	75.9
Euterpina acutifrons 6F	1.0	0.2	28.2	3.0	-	55.7	74.9
Cladocera							
Podon intermedius	3.3	0.3	34.1	3.2	-	85.6	-
Evadne nordmanni	1.2	0.2	39.6	1.9	83.1	80.2	87.4
Decapod zoea							
Necora puber	26.9	8.0	33.2	3.1	-	89.5	-
Pisidia longicornis	4.7	-	25.4	-	-	64.8	-
Faecal membrane							
37-46 d larvae	-	-	14.4	-	-	-	-
57-67 d larvae	-	-	14.7	4.6	-	-	-

since an excess of food is suggested to lead to supression of digestion (Werner & Blaxter 1980). The larvae preferentially fed on the largest suitable organism available, which has also been noted in other work on turbot (Meeren 1991) and, in common with most larval fish feeding studies, food organisms appeared to be ingested without any obvious mechanical disruption.

One measure of condition of fish larvae is the state of the cells lining the intestine, as estimated from histological criteria (e.g. Oozeki et al. 1989). During starvation the cell height of the gut wall reduces, enzyme production declines and thus digestive capability diminishes (Pedersen et al. 1990) and food may be egested undigested. In the present study, histological sections of the gut and other organs of larval turbot confirmed that the larvae were in good condition. It may thus be concluded that there was no undue feeding stress on the larvae and that adequate nutrition was available.

The extent to which food organisms were digested depended on their size and structure. The larger copepods which have a strong exoskeleton were often broken into 2 or 3 pieces. Because of their bulk they are probably more easily crushed by peristaltic action as they pass through the gut, although some of the disruption could also have taken place as they passed through the anal sphincter. Among the smaller species of copepods, the degree to which they were crushed in the faeces depended on the thickness of the exoskeleton and the compactness of their body. Species which

Table 3. *Scophthalmus maximus*. Mean weight of nitrogen and mean percentage nitrogen of the dry weight and C:N ratio of fresh food organisms, and mean percentage loss in nitrogen of the same range of food organisms after digestion by 3 age groups of larvae. The number of observations are as for Table 1. Standard deviations (SD) of the means are shown where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Mean fresh weight of nitrogen (µg)	SD	Mean % nitrogen of fresh dry wt	SD	C:N ratio	Mean % loss in nitrogen after digestion 21–27 d	37–46 d	53–67 d
Copepoda								
Anomalocera patersoni 5M	13.7	–	10.6	–	3.6	–	100.0	–
A. patersoni 6F	27.2	3.3	10.5	0.2	3.8	94.5	95.1	–
A. patersoni 6M	21.0	2.0	11.2	0.5	3.6	–	95.2	93.2
Calanus helgolandicus 6F	11.5	1.5	10.5	0.7	3.7	–	100.0	97.6
C. helgolandicus 6M	12.5	–	9.8	–	3.9	100.0	92.3	–
Labidocera wollastoni 6F	14.3	–	11.5	–	3.7	–	100.0	93.0
L. wollastoni 6M	10.0	–	11.4	–	3.6	–	100.0	100.0
Centropages typicus 5F	2.4	–	7.8	–	4.4	–	100.0	100.0
C. typicus 6F	3.5	1.0	9.7	0.5	4.1	–	97.1	94.2
C. typicus 6M	3.4	0.2	9.5	1.2	3.8	–	81.8	95.1
Temora longicornis 5	0.8	–	9.8	–	3.8	–	97.0	94.9
T. longicornis 6F	2.4	0.1	10.7	0.4	3.9	–	87.6	86.7
T. longicornis 6M	1.4	–	9.7	–	3.9	–	–	80.2
Eurytemora velox 5F	1.7	0.1	10.9	0.9	4.6	100.0	–	–
E. velox 5M	1.2	–	9.9	–	5.3	100.0	–	–
E. velox 6F	1.8	0.2	10.8	1.4	4.0	100.0	–	99.0
E. velox 6F with eggs	2.1	0.2	11.2	0.3	4.2	–	–	–
E. velox 6M	1.5	0.1	10.2	0.8	4.7	100.0	–	98.9
E. velox eggs	0.027	–	8.3	–	4.2	–	–	–
Pseudocalanus elongatus 6F	0.9	0.1	10.8	1.0	3.9	–	100.0	–
Corycaeus anglicus 6F	0.6	0.1	8.1	0.6	4.2	–	78.7	95.1
Acartia clausi 6F	0.6	–	10.9	–	4.1	82.2	94.5	98.1
A. clausi 6M	0.6	0.1	8.0	1.8	3.9	–	97.2	96.9
Paracalanus parvus 5	0.4	–	7.4	–	4.1	100.0	–	–
P. parvus 6F	0.4	–	8.4	–	3.8	–	–	100.0
Euterpina acutifrons 6F	0.2	0.1	6.4	0.9	4.5	–	92.1	100.0
Cladocera								
Podon intermedius	0.7	0.1	7.6	0.7	4.5	–	100.0	–
Evadne nordmanni	0.3	0.1	10.7	1.2	3.7	100.0	100.0	100.0
Decapod zoea								
Necora puber	6.7	1.7	8.5	1.6	4.0	–	94.3	–
Pisidia longicornis	1.1	–	5.9	–	4.3	–	100.0	–
Faecal membrane								
37–46 d larvae	–	–	0.0	–	–	–	–	–
57–67 d larvae	–	–	1.1	1.2	–	–	–	–

occasionally still had small amounts of obviously undigested contents were generally those which were resistant to crushing and thus from which it may be more difficult to extract their contents. While *Corycaeus anglicus* seemed particularly resistant to digestion, this was not completely reflected in the dry weight and chemical analysis of digested specimens.

From an examination of the faeces it is clear that particular components in the diet are poorly digested by turbot larvae. Chitinous crustacean exoskeletons at least superficially appear to be unchanged by the digestion process. Few studies have analysed for chitinolytic enzyme activity in fish larvae, although chitinase has been reported in the eggs and larvae of red sea bream *Pagrus major* (Kono et al. 1987) and from the late yolk sac stage and beyond in trout *Salmo gairdneri* (Lindsay 1985). Even in adult fish which feed on crustaceans, chitin does not appear to be fully utilized (Seiderer et al. 1987). Lindsay (1984) concluded that, in adult fish, the primary function of chitinase may be for the initial chemical disruption of the exoskeleton of prey. This may also be the function in larval fish, although some nutritional gain cannot be discounted.

Lipid absorption has been demonstrated in many larval fish species (Rösch & Segner 1990, Deplano et al. 1991) and turbot larvae have been shown to possess the enzyme lipase (Cousins et al. 1987). However, in this study, turbot larvae had apparently not extracted all the storage lipid from *Pseudocalanus elongatus*.

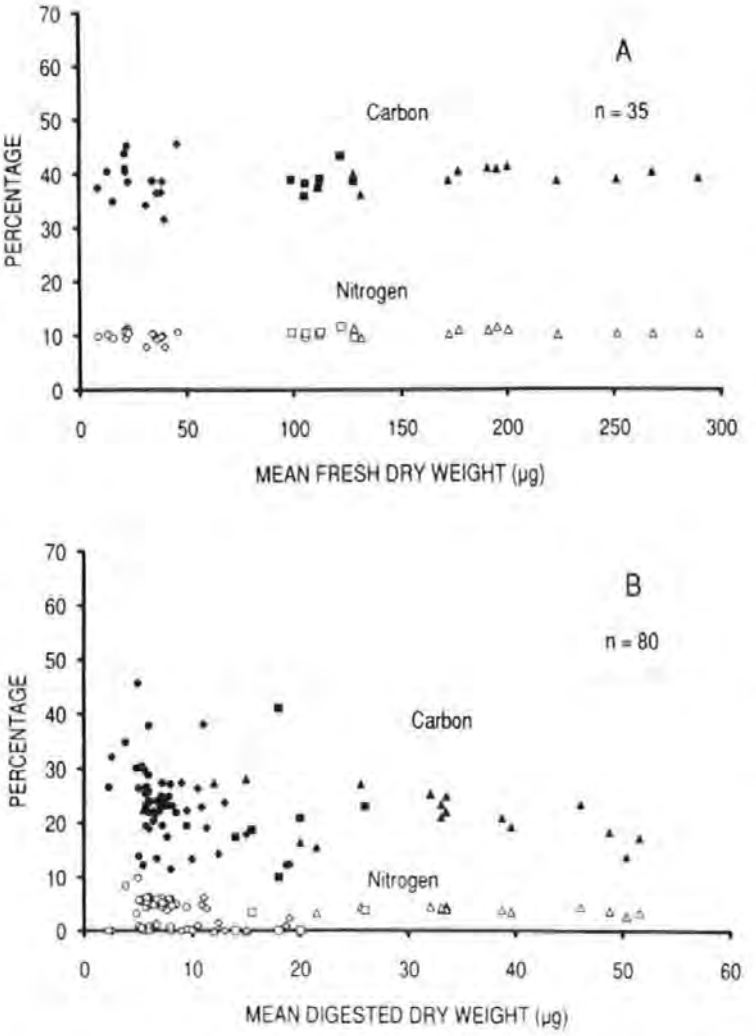

Fig. 2. *Scophthalmus maximus*. Percentage carbon and nitrogen of the individual mean dry weights of 4 of the larger copepods taken as food: (A) fresh and (B) after digestion. No. of observations (n) for each analysis are given. (▲, △) *Anomalocera patersoni*; (■, □) *Calanus helgolandicus*; (◆, ◇) *Centropages typicus*; (●, ○) *Temora longicornis*

Checkley (1982) and Pedersen (1984) also noted poor digestion of copepod storage lipid in larval herring *Clupea harengus*. Wax ester, the main storage lipid of copepods, is more difficult to digest than other lipid classes (Patton et al. 1975) so that when large quantities appear in the gut, larvae may not have the enzyme capacity to cope with it.

Certain complete organisms were almost invariably found apparently unaltered by the digestion process. These included lamellibranch larvae whose resistance to digestion by larval fish is well documented and has been noted by Bhattacharyya (1957), Spectrova et al. (1974), Checkley (1982), Kane (1984), Tilseth & Ellertsen (1984), Nakata (1988) and Viñas (1991). Lamellibranch larvae are numerous in the diet of fish larvae in particular areas (Checkley 1982, Brewer & Kleppel 1986, Nakata 1988) and there is evidence that they were actively selected by Black Sea turbot *Scophthalmus maeoticus* (Spectrova et al. 1974).

The observed resistance of phytoplankton to digestion has also been noted by Nakata (1988) for diatoms, by Checkley (1982) for prasinophytes and by Kane (1984), Nakata (1988) and Viñas (1991) for dinoflagellates. Digestion of phytoplankton in many fish larvae may be limited to species with less robust cell walls. Phytoplankton has been observed in the guts of many larvae at the early stages of development although it is commonly reported as an amorphous mass (Last 1979, Kane 1984). Copepod faecal pellets are also a source of phytoplankton material in larval fish guts (Ellertsen et al. 1980). In the present study and in that by Bhattacharyya (1957) there was no visual evidence of copepod faecal pellets being digested. The chemical composition of the pellet membrane is still controversial, but chitin is considered to be a major component (Bochdansky & Herndl 1992). Since the membrane is closed at both ends it forms an unbroken barrier against enzyme penetration. However, when the pellets have been free in the sea for some time and been exposed to bacterial and mechanical disruption (Lampitt et al. 1990), they may be more digestible.

Because of the variability of copepod morphology there were considerable dry weight differences between species and between sexes. It is clear that body dimension measurements would have to be more comprehensive than cephalothorax length alone to be able to relate them accurately to weight. Reproductive status could also affect dry weight. However the mean dry weight of Stage 6 females of *Eurytemora velox* was similar whether or not they were carrying eggs, even though the egg mass can represent up to 57% of the mean body dry weight. This is understandable because some of the females were carrying few eggs.

while some without eggs had many late development eggs already in the oviducts ready to be laid, and so were effectively carrying them internally.

There was little clear difference in digestion efficiency with increasing larval age, which is surprising since digestive enzyme complement has been demonstrated to change with age in turbot larvae (Cousin et al. 1987), suggesting that over the age range of larvae examined, the enzyme systems are similarly developed.

After digestion the heavier organisms tended to have the highest percentage weight loss (>80%). It appears that while the exoskeleton of crustaceans becomes thicker and heavier with increasing size, the increasing ratio of body volume to surface area outweighs this, so that the weight proportion of digestible internal contents increases. There are exceptions to this as in the decapod zoea; *Necora puber* has a high dry weight but lost only 55.0% on digestion, which is low compared with a copepod of the same weight, probably due to differences in exoskeleton bulk. Among the smaller crustaceans the harpacticoid copepod species *Euterpina acutifrons* had a low weight loss (27.8 to 47.2%) which may be due to their small size, particular cylindrical body shape, and thus high exoskeleton weight relative to contents.

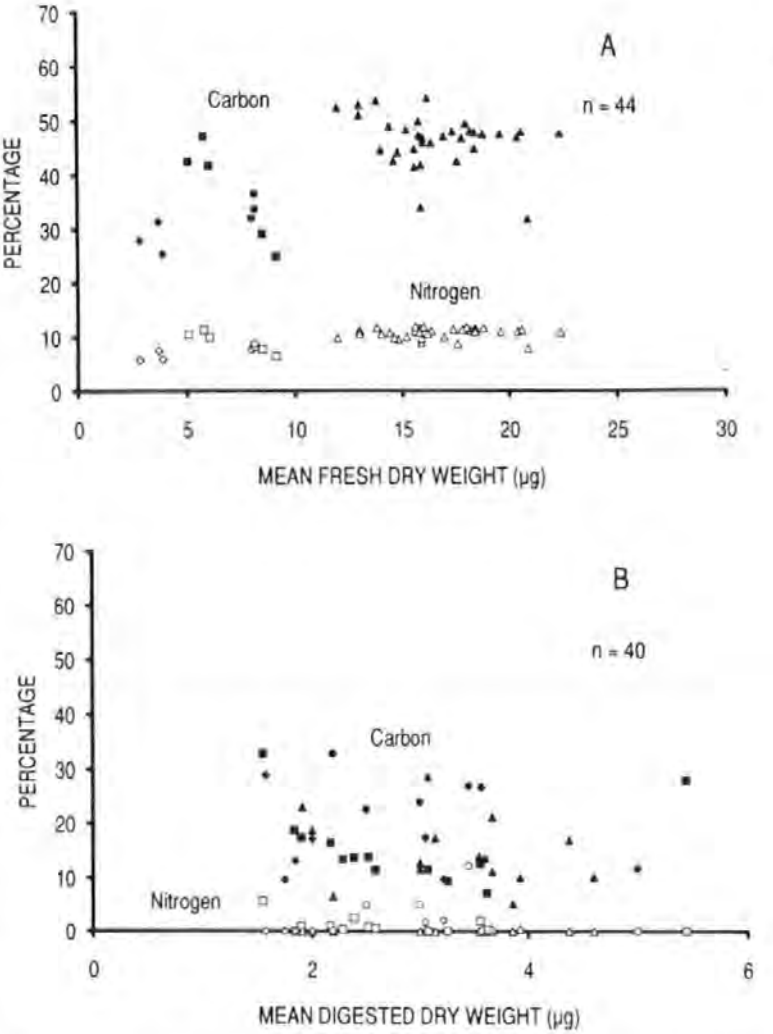

Fig. 3. *Scophthalmus maximus*. Percentage carbon and nitrogen of the individual mean dry weights of 4 of the smaller copepods taken as food: (A) fresh and (B) after digestion. No. of observations (n) for each analysis are given. (▲, △) *Eurytemora velox*; (■, □) *Acartia clausi*; (◆, ◇) *Euterpina acutifrons*; (●, ○) *Corycaeus anglicus*

Pedersen & Hjelmeland (1988) estimated that 82 to 95% of carbon was extracted from mixtures of *Acartia tonsa* nauplii and early copepodite developmental stages during digestion by larval herring. These results are similar to the present study where reduction in carbon content on digestion reflected the reduction in dry weight, being greater in the larger copepods (>85%) and less, and more variable, in small copepods and decapod zoea. Since turbot larvae were observed to preferentially select the larger food items, this represents an efficient feeding strategy. Nitrogen compounds appear to be more readily extractable components than carbon; in half the analyses nitrogen was reduced to the level where it was not detectable. Nitrogen is associated with the soft parts of the prey while the chitinous exoskeleton is high in structural carbon. Chitin contains low levels of nitrogen and this will contribute to the value measured in digested food. Zero nitrogen values suggest either that there is partial digestion of chitin or that values are below detection limits. No clear pattern of reduction in nitrogen was seen between different organisms.

Irrespective of the degree of digestibility of different food items, the actual amount of food absorbed is the important factor in the nutrition of larval fish. Rate of food passage through the gut, which can be modified by many factors (Karjalainen et al. 1991), may be an

important determinant of the efficiency of food absorption. Experimental results have indicated that passage rate can be altered by changing the food concentration, such that *Artemia salina* nauplii fed at high densities were still alive after passing through the gut of larval herring *Clupea harengus*, whereas they were digested at lower densities (Werner & Blaxter 1980). Similar observations were made on Black Sea turbot *Scophthalmus maeoticus* (Spectorova et al. 1974). Boehlert & Yoklavich (1984) found for larval Pacific herring (*C. harengus pallasi*) that, while evacuation rate increased with increasing food concentration, carbon assimilation from individual food particles decreased; however, overall, the greater food throughput gave a higher energy gain. In the present series of experiments, where food concentration was relatively high, the greatest variable possibly being the species composition of the plankton supplied as food, gut passage time was variable and unpredictable. However, food organisms were invariably well digested.

The rate of passage of different sizes of food organisms through the larval fish digestive tract is poorly understood. In larval herring *Clupea harengus*, which have straight guts, Pedersen (1984) observed that copepods did not change their position relative to one another. However, peristaltic action has been observed to alter the relative position of food in larval cod *Gadus morhua* of 7 d post-hatch, which at this age have simple straight tubular guts (Tilseth & Ellertsen 1984). In the present study, while passage rates were unpredictable, particles of different sizes, ingested at the same time, such as *Eurytemora velox* and their eggs, were observed to have different rates in the looped gut of turbot larvae. Karjalainen et al. (1991) observed that when feeding of larval vendace *Coregonus albula* was suspended, copepodites remained in the guts longer than nauplii, also suggesting slower passage rates of larger organisms. Possibly, differential retention of food by larval fish is more evident at even later stages of gut development.

The results from this study demonstrate, for larval turbot, a considerable range in digestibility of natural food both within and between prey species. However, data were collected over several weeks and factors such as changes in individual condition of larvae, differences in nutritional quality within and between food species, the species mix of food made available and the possibility that some organisms spend longer passing through the gut than others, could all interact to produce the variability observed in the results. These are all factors the larvae would be subjected to in nature and therefore the results may be representative of natural conditions.

Fish larvae feed on a variety of organisms and stages and while particular food organisms may be of lower nutritional potential than others, present results suggest that it is unlikely under conditions of high food concentration and broad species diversity that their nutritional requirements would not be largely satisfied. It is possible that if food density was at best marginal to their requirements and this was coupled with a large proportion being poorly digestible, they could suffer loss in condition, causing death through starvation or an increased vulnerability to predation. Digestibility of food may thus be a contributory factor in larval fish survival.

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LITERATURE CITED

Bailey, K. M., Houde, E. D. (1989). Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. mar. Biol. 25: 1–83

Bhattacharyya, R. N. (1957). The food and feeding habits of larval and post-larval herring in the northern North Sea. Mar. Res. No.3: 1–16

Bochdansky, A. B., Herndl, G. J. (1992). Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. V. Role of fecal pellets in marine snow. Mar. Ecol. Prog. Ser. 89: 297–303

Boehlert, G. W., Yoklavich, M. M. (1984). Carbon assimilation as a function of ingestion rate in larval Pacific herring, *Clupea harengus pallasi* Valenciennes. J. exp. mar. Biol Ecol. 79: 251–262

Brewer, G. D., Kleppel, G. S. (1986). Diel vertical distribution of fish larvae and their prey in nearshore waters of southern California. Mar. Ecol. Prog. Ser. 27: 217–226

Canino, M. F., Bailey, K. M., Incze, L. S. (1991). Temporal and geographic differences in feeding and nutritional condition of walleye pollock larvae *Theragra chalcogramma* in Shelikof Strait, Gulf of Alaska. Mar. Ecol. Prog. Ser. 79: 27–35

Checkley, D. M. Jr. (1982). Selective feeding by Atlantic herring (*Clupea harengus*) larvae on zooplankton in natural assemblages. Mar. Ecol. Prog. Ser. 9: 245–253

Coombs, S. H., Nichols, J. H., Conway, D. V. P., Milligan, S., Halliday, N. C. (1992). Food availability for sprat larvae in the Irish Sea. J. mar. biol. Ass. U.K. 72: 821–834

Cousin, J. C. B., Baloüet, G., Baudin-Laurencin, F. (1986). Altérations histologiques observées chez des larves de turbot (*Scophthalmus maximus*) en élevage intensif. Aquaculture 52: 173–189

Cousin, J. C. B., Baudin-Laurencin, F., Gabaudan, J. (1987). Ontogeny of enzymatic activities in fed and fasting turbot, *Scophthalmus maximus* L. J. Fish. Biol. 30: 15–33

Deplano, M., Diaz, J. P., Connes, R., Kentouri-Divanach, M., Cavalier, F. (1991). Appearance of lipid-absorption capacities in larvae of the sea bass *Dicentrarchus labrax*

during transition to the exotrophic phase. Mar. Biol. 108: 361–371
Ellertsen, B., Solemdal, P., Strømme, T., Tilseth, S., Westgård, T., Moksness, E., Øiestad, V. (1980). Some biological aspects of cod larvae (*Gadus morhua* L.). FiskDir. Skr. Ser. HavUnders. 17: 29–47
Jones, A. (1972). Studies on egg development and larval rearing of turbot, *Scophthalmus maximus* L., and brill, *Scophthalmus rhombus* L., in the laboratory. J. mar. biol. Ass. U.K. 52: 965–986
Kane, J. (1984). The feeding habits of co-occurring cod and haddock larvae from Georges Bank. Mar. Ecol. Prog. Ser. 16: 9–20
Karjalainen, J., Koho, J., Viljanen, M. (1991). The gastric evacuation rate of vendace (*Coregonus albula* L.) larvae predating on zooplankters in the laboratory. Aquaculture 96: 343–351
Kono, M., Furukawa, K., Satoh, H., Matsui, T., Shimizu, C. (1987). Changes in the chitinase activity at different stages of red sea bream *Pagrus major* egg, larva, and juvenile. Nippon Suis. Gak. 53: 1289–1293
Lampitt, R. S., Noji, T., von Bodungen, B. (1990). What happens to zooplankton faecal pellets? Implications for material flux. Mar. Biol. 104 : 15–23
Last, J. M. (1979). The food of larval turbot *Scophthalmus maximus* L. from the west central North Sea. J. Cons. int. Explor. Mer 38: 308–313
Lindsay, G. J. H. (1984). Distribution and function of digestive tract chitinolytic enzymes in fish. J. Fish. Biol. 24: 529–536
Lindsay, G. J. H. (1985). Development of gastric chitinase activity in trout (*Salmo gairdneri*). Aquaculture 48: 285–290
McFadzen, I. R.B., Coombs, S. H., Lowe, D. M. (1991). Histological indices of condition of larval sprat *Sprattus sprattus* L., in relation to the Sardine Anchovy Recruitment Project (SARP). Comm. Meet. int. Coun. Explor. Sea C.M.-ICES 1991/L: 48: 1–8
Meeren, T. van der (1991). Selective feeding and prediction of food consumption in turbot larvae (*Scophthalmus maximus* L.) reared on the rotifer *Brachionus plicatilis* and natural zooplankton. Aquaculture 93: 35–55
Nakata, K. (1988). Alimentary tract contents and feeding conditions of ocean-caught post-larval Japanese sardine, *Sardinops melanostictus*. Bull. Tokai Reg. Fish. Res. Lab. 126: 11–24
O'Connell, C. P. (1976). Histological criteria for diagnosing the starving condition in early post yolk sac larvae of the northern anchovy, *Engraulis mordax* Girard. J. exp. mar. Biol. Ecol. 25: 285–312
Oozeki, Y., Ishii, T., Hirano, R. (1989). Histological study of the effects of starvation on reared and wild-caught larval stone flounder, *Kareius bicoloratus*. Mar. Biol. 100: 269–275
Patton, J. S., Nevenzel, J. C., Benson, A. A. (1975). Specificity of digestive lipases in hydrolysis of wax esters and triglycerides studied in anchovy and other selected fish. Lipids 10: 575–583
Pedersen, B. H. (1984). The intestinal evacuation rates of larval herring (*Clupea harengus* L.) predating on wild plankton. Dana 3: 21–30
Pedersen, B. H., Hjelmeland, K. (1988). Fate of trypsin and assimilation efficiency in larval herring (*Clupea harengus*) following digestion of copepods. Mar. Biol. 97: 467–476
Pedersen, B. H., Ugelstad, I., Hjelmeland, K. (1990). Effects of a transitory, low food supply in the early life of larval herring (*Clupea harengus*) on mortality, growth and digestive capacity. Mar. Biol. 107: 61–66
Rösch, R., Segner, H. (1990). Development of dry food for larvae of *Coregonus lavaretus* L. 1. Growth, food digestion and fat absorbtion. Aquaculture 91: 101–115
Seiderer, L. J., Davis, C. L., Robb, F. T., Newell, R. C. (1987). Digestive enzymes of the anchovy *Engraulis capensis* in relation to diet. Mar. Ecol. Prog. Ser. 35: 15–23
Spectorova, L. V., Aronovich, T. M., Doroshev, S. I., Popova, V. P. (1974). Artificial rearing of the Black Sea turbot larvae (*Scophthalmus maeoticus*). Aquaculture 4: 329–340
Tilseth, S., Ellertsen, B. (1984). Food consumption rate and gut evacuation processes of first feeding cod larvae (*Gadus morhua* L.). In: Dahl, E., Danielssen, D. S., Moksness, E., Solemdal, P. (eds.) The propagation of cod *Gadus morhua* L. Arendal, Norway. Flødevigen rapportser 1: 167–182
Turner, J. T. (1984). The feeding ecology of some zooplankters that are important prey items of larval fish. NOAA natn. mar. Fish. Serv. tech. Rep. Circ. 7: 1–28
Viñas, M. D. (1991). Feeding ecology of *Engraulis anchoita* first feeding larvae in a tidal mixed front off the Valdes Peninsula (Argentina). Comm. Meet. int. Coun. Explor. Sea C.M.-ICES 1991/L: 83: 1–19
Werner, R. G., Blaxter, J. H. S. (1980). Growth and survival of larval herring (*Clupea harengus*) in relation to prey density. Can. J. Fish. Aquat. Sci. 37: 1063–1069
Young, J. W., Davis, T. L. O. (1992). Feeding ecology and interannual variations in diet of larval jack mackerel, *Trachurus declives* (Pisces: Carangidae), from coastal waters of eastern Tasmania. Mar. Biol. 113: 11–20

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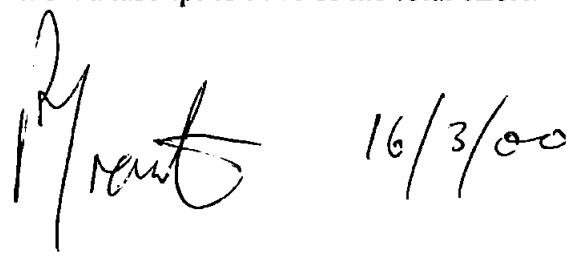
P.R.G. Tranter  16/3/00

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NOTE

Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage

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ABSTRACT: Between 20.5 and 93.6% of the subitaneous eggs of 6 species of egg-carrying copepods passed undigested through the digestive tracts of larval and early post-larval turbot *Scophthalmus maximus*. Viability of the eggs of *Eurytemora affinis*, *E. velox* and *Euterpina acutifrons* remained high on egestion (67.0 to 91.7%), *Pseudocalanus elongatus* and *Oncaea venusta* eggs had low viability (1.1 to 1.5%), while all *Corycaeus anglicus* eggs were rendered inviable. The indigestibility of the eggs denies the turbot larvae a potentially valuable food resource, while retention of high egg viability in certain species reduces the effect of predation.

KEY WORDS: *Scophthalmus maximus* larvae · Copepod eggs · Digestion · Egg viability

Organisms consumed when larval and early post-larval turbot *Scophthalmus maximus* are fed on mixed zooplankton vary in their digestibility (Conway et al. 1993). Of particular note, copepod eggs appear to remain completely undigested. This has important nutritional implications, as numerically, eggs can form a substantial proportion of the diet of larval fish (e.g. Conway 1980, Kellerman 1990). Resistance to digestion by copepod eggs has previously been indicated from gut content studies, where apparently undigested eggs, of a range of species, have been observed in the digestive tracts of the larvae of herring *Clupea harengus* (Schnack 1972), grey mullet *Mugil* spp. (Zismann et al. 1975), blue whiting *Micromesistius poutassou* (Conway 1980), Japanese sardine *Sardinops melanostictus* (Nakata 1988), rockling *Ciliata* spp. (Tully & Ó Céidigh 1989) and anchovy *Engraulis anchoita* (Viñas 1991). Since these observations were on eggs still on passage through the intestinal tract, it cannot be discounted that they had recently been ingested. However, gut content observations can indicate whether there is differential digestion between food items, and this was indeed noted during a study on feeding of larval sprat *Sprattus sprattus* (Conway et al. 1991). In that study female copepods of *Pseudocalanus elongatus* and *Oithona* sp., both egg-carrying species, were found in the hind-guts well digested with only their exoskeletons remaining, while eggs in close association, presumably from the copepods, were undigested (Conway unpubl.). A similar differential digestion of copepod females and eggs has also been noted during gut content examination of the chaetognath *Sagitta elegans* (Alvarez-Cadena 1993).

Resistance to digestion of a range of crustacean eggs has been confirmed from faecal examination of several groups. Faeces of the shore bird *Charadrius vociferus* contained a selection of freshwater crustacean (anostracan, copepod and ostracod) eggs (Proctor et al. 1967) and some were still viable. Copepod eggs, both subitaneous and diapause, were isolated from the faeces of marine benthic polychaetes and a high proportion were viable (Marcus 1984). Non-digestion of subitaneous copepod eggs has been further demonstrated by the work of Crawford & Daborn (1986), who found that when adults of a small estuarine fish *Menidia menidia* fed on egg-carrying females of the brackish water, estuarine copepod *Eurytemora herdmanni*, many intact eggs were egested. Reddern & Daborn (1991), using the same 2 species, established that 90% of egested copepod eggs were viable.

The above, largely qualitative experiments, were all carried out on adult animals from either fresh water or estuarine/coastal marine environments. The crustacean eggs fed upon were either diapause, or from species which can produce diapause eggs, so the eggs may be particularly adapted to survive environmental stress. This study is the first to investigate the ability of a larval fish species, the turbot, to digest the subitaneous eggs of a selection of egg-carrying copepod species of both estuarine/coastal and open sea distrib-

utions, including species which are only known to produce subitaneous eggs. Turbot larvae are particularly suited to this type of study as they package their faeces in a thin membrane (Conway et al. 1993), so output of faecal material can be monitored.

Material and methods. Studies were carried out at a commercial fish farm, Golden Sea Produce Ltd, Hunterston, Scotland, in February 1992 and at the Plymouth Marine Laboratory, Plymouth, England, in August 1992 and July and August 1993 using farmed turbot larvae aged 25 to 77 d post-hatch, length 6.8 to 36.3 mm. Larval turbot commence metamorphosis at approximately 35 d post-hatch, but for convenience all specimens were termed larvae. Depending on larval size, groups of 3 to 10 were placed in 5 or 15 l glass aquaria containing filtered (20 µm) seawater (33 to 34.5 psu) collected in the open sea off Plymouth. They were maintained under conditions of continuous subdued lighting and gentle aeration at 16 to 19 °C. The water of the experimental aquaria was replaced at least every second day with fresh seawater at the same temperature and salinity .

Turbot larvae had been fed on brine shrimp *Artemia salina* nauplii at the fish farm, but this diet was replaced with live zooplankton, collected locally to Plymouth approximately 3 times a week using a variety of plankton nets (50 to 200 µm). In the experiments female copepods which carry their eggs rather than species laying free-spawned eggs were used, to encourage larvae to feed on large numbers of copepod eggs in a short time period and to allow more accurate assessment of throughput of eggs.

The calanoid copepod *Pseudocalanus elongatus*, the cyclopoids *Oncaea venusta* and *Corycaeus anglicus* and the harpacticoid *Euterpina acutifrons* were obtained from the zooplankton samples collected off Plymouth in a salinity of 33 to 34 psu. The calanoid copepod *Eurytemora affinis* was collected from the upper tidal River Tamar north of Plymouth in a salinity of 3 to 4 psu and *Eurytemora velox* obtained from Golden Sea Produce Ltd, where it was being cultured in a salinity of approximately 12 psu. Females of these copepods were extracted from samples and kept in beakers until they produced eggs. For each experiment a sub-sample of the female copepods was measured (total length), the number of attached eggs counted and egg diameter measured (see Table 1).

Only in *Oncaea venusta* and *Corycaeus anglicus* were the eggs clearly carried in sacs; the eggs in *O. venusta* were compressed to a sub-circular shape within the sac. *Eurytemora affinis*, *E. velox*, *Pseudocalanus elongatus* and *Euterpina acutifrons* carry their eggs in a single mass without any obvious outer egg sac. Apart from *P. elongatus* and *O. venusta*, all other experimental species have dark egg membranes.

Experiments were started at approximately 08:00 GMT following a 14 h overnight period when no further food was added, so food density decreased. The seawater in the aquaria was first replaced with fresh filtered seawater and the larvae allowed to acclimatize for 0.5 h. All egg-carrying copepods of the species being examined were extracted from the beakers, transferred to the aquaria and the turbot larvae observed at intervals until they had ingested the copepods. Live, mainly copepod zooplankton was then introduced, so that movement of food through the digestive tract was encouraged. Plankton density was maintained at approximately 200 organisms l^{-1}. Experiments were conducted each day, so all copepod eggs consumed were aged 1 to 24 h post-laying.

Larval turbot produce faeces covered in a thin membrane, which maintains their structural integrity. To see how long it took for the membrane to break down, samples of intact faeces were incubated at 16 °C in seawater. Faeces settle out immediately and are easily identifiable on the bottom of the aquaria as the membrane is usually orange or brown in colour. They were gently collected every 15 min using a large, wide-bore pipette, placed in solid watch glasses containing filtered seawater and dissected under a microscope. The time taken for the experimental copepods and their eggs to appear in the faeces (gut passage time) was recorded. To estimate whether any eggs were being lost through complete digestion, the number of female copepods and number of eggs associated with them in the faeces was noted and compared with the mean number of eggs being carried before they were fed to the larvae.

To check the viability of eggs in the faeces from the feeding experiments above, eggs were left in the watch glasses, while all other faecal remains and as much seawater as possible were removed with a fine pipette. Fresh, filtered seawater was then introduced and the watch glasses covered with glass slides to prevent evaporation. The eggs were incubated at 16 °C in continuous subdued light. They were examined at regular intervals for up to 9 d and the appearance of nauplii used as a measure of viability. Their viability was compared with that from a control group of eggs which had not passed through turbot larvae.

The calanoid copepods *Acartia clausii* and *Temora longicornis* spawn subitaneous eggs freely into the water. During egg laying, small numbers of eggs (2 to 5) were observed to remain attached to the genital segment for some time before being dislodged by sudden movement of the female, or physical contact with other zooplankton. Eggs can thus be ingested incidentally by larval fish when feeding on the female copepod. Small numbers of their eggs were recovered from the faeces and incubated as above.

To ascertain the condition of the larvae during the experiments small numbers were removed at intervals, anaesthetised using MS-222 and preserved in Baker's formol calcium fixative for histological examination. Following fixation, smaller larvae were processed for methacrylate embedding and sectioned at 2 µm in the sagittal plane (McFadzen et al. in press). Larger larvae were processed for paraffin wax embedding, then serially sectioned at 5 µm in the sagittal plane. All methacrylate sections were stained in Lee's methylene blue/basic fuchsin (Bennett et al. 1976) and wax sections in haematoxylin and eosin prior to examination for tissue condition.

Results. Between copepod species the number of eggs carried varied considerably (Table 1). *Corycaeus anglicus* had the greatest mean number of eggs (38.0 ± 14.46), *Pseudocalanus elongatus* the fewest (8.3 ± 2.69). Because female copepods were at different states of egg laying when examined, there was considerable variation in the number of eggs carried by individuals of the same species. In *C. anglicus*, *Eurytemora affinis* and *E. velox*, eggs were strongly attached to the females, while in *P. elongatus* especially but also in *Oncaea venusta* and *Euterpina acutifrons*, eggs were more readily detached from, or discarded by, the females.

It was noted that female *Eurytemora velox* containing ripe eggs in the oviducts sometimes transferred them to the egg mass when stressed, e.g. when they were transferred to a small petri dish for microscopic examination. Eggs in the oviducts measured slightly less in diameter (73 to 82 µm) than those in the egg mass (90 to 110 µm), but were already circular in shape.

Hatching time of the fresh, control eggs was generally the value given at the lower end of the ranges in Table 1; only small numbers of eggs required the full number of days given. Viability was >84.8% for all species except *Euterpina acutifrons* (68.6%). Of all the individual batches of eggs of *Eurytemora velox*, *Corycaeus anglicus* and *Oncaea venusta* incubated, at least a proportion of each batch hatched. In *Eurytemora affinis*, *Pseudocalanus elongatus* and *E. acutifrons* there were respectively 5.7, 8.7 and 13.3% of individual copepod egg batches totally failing to hatch. The low egg viability in *E. acutifrons* resulted from a combination of high individual egg batch failure coupled with a general low hatch rate.

While food was not replenished overnight, the turbot larvae were not starved at any time, as live food was still present in the aquaria in the mornings. The initial faeces produced during the experiments were composed of food introduced the previous day. Examination of faecal material showed that while copepodites were digested to the extent that only exoskeletons remained, a proportion of the ingested eggs of all copepod species were present, apparently unaffected by digestion. The time taken for female copepods and their eggs to pass through the intestinal tract was variable (Fig. 1), ranging from 1.8 to 6.5 h (mean overall time 3.9 ± 1.2 h) and was not obviously related to larval

Table 1. *Scophthalmus maximus*. Mean lengths, egg diameter, number of eggs carried, hatching time and egg viability, fresh and after gut passage, for the 6 main copepod species fed to turbot larvae. SD given where possible

	Eurytemora affinis	*Eurytemora velox*	*Pseudocalanus elongatus*	*Oncaea venusta*	*Corycaeus anglicus*	*Euterpina acutifrons*
Range of total length (mm)	1.07–1.29	1.36–1.68	1.00–1.18	0.64–0.68	0.86–1.07	0.68–0.79
Mean length	1.19 ± 0.06	1.48 ± 0.09	1.08 ± 0.05	0.66 ± 0.02	0.96 ± 0.06	0.72 ± 0.04
No. of observations	20	18	19	13	18	6
Range egg diameter (µm)	73–98	90–110	102–122	45–57	54–60	54–73
Mean egg diameter (µm)	85.5 ± 5.50	103 ± 7.38	116 ± 7.04	53 ± 3.53	57 ± 2.02	62 ± 5.88
Range no. of eggs carried	7–44	3–23	2–14	13–34	15–57	9–20
Mean no. of eggs carried	21.8 ± 9.97	11.7 ± 4.78	8.3 ± 2.69	22.3 ± 7.34	38.0 ± 14.46	14.1 ± 2.87
No. of observations	70	21	68	18	18	27
Hatch time (d)	2–3	2–4	3–6	2–6	3–4	2–3
Viability of fresh eggs (%)	89.4	95.4	84.8	87.1	85.7	68.6
No. of females	63	11	29	18	18	27
Total no. of eggs in experiment	1370	131	224	402	701	390
Viability of egested eggs (%)	91.7	82.5	1.1	1.5	0	67
Total no. of eggs in experiment	1692	532	184	66	420	264
Mean no. of eggs female^{-1} in faeces	16.1	7.5	1.7	–	20	13.2
Eggs passing through the gut undigested (%)	73.9	64.1	20.5	–	52.6	93.6
Larval turbot age (d)	32–72	30–62	35–74	28–72	26–60	25–62

age. A small number of eggs (< 1 %) had disrupted egg membranes which may have been due to digestive action, or possibly handling damage. In *Eurytemora velox* and *Euterpina acutifrons* the eggs tended to remain in a group, closely associated with the female copepod exoskeleton. In *Eurytemora affinis*, *Pseudocalanus elongatus* and *Corycaeus anglicus*, eggs were individually scattered through the faeces (Fig. 2a, b). In *Oncaea venusta* the eggs remained within the egg sacs although separated from the females.

A comparison of the mean number of eggs carried by live copepod females, with the mean number associated with female exoskeletons of the same species in the faeces, showed that there was a reduction in number on egestion for all species (Table 1). This reduction was variable between species. The percentage of *Euterpina acutifrons* eggs recovered after passing through the intestinal tract was 93.6%, while that for *Pseudocalanus elongatus* was 20.5%. Because *Oncaea venusta* did not always have paired egg sacs and as there were so few observations on this species, it was not possible to calculate the number of eggs per female in the faeces. Their egg sacs were not broken down by digestion, so it is possible that all their eggs could be recoverable from the faeces.

Viability of the eggs of *Eurytemora affinis*, *E. velox* and *Euterpina acutifrons* from faeces was similar to the viability of their fresh eggs. In contrast, the viability of egested eggs of *Pseudocalanus elongatus* and *Oncaea venusta* was reduced to 1.1 and 1.5% respectively, while all the eggs of *Corycaeus anglicus* were inviable.

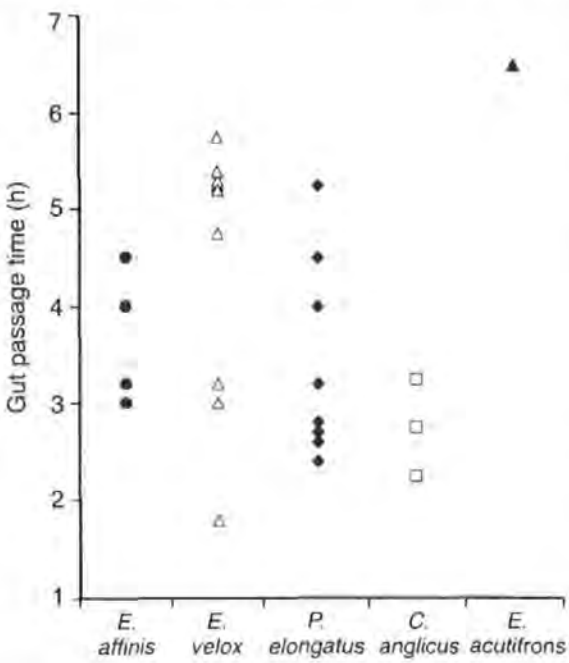

Fig. 1. *Scophthalmus maximus*. Time interval between feeding on egg-carrying copepods and time of eggs first appearing in the faeces of turbot larvae aged 23 to 76 d, for 25 individual experiments

Small numbers of eggs of free-spawning copepod species were recovered from the faeces. Of 4 *Temora longicornis* eggs (80 to 82 µm diameter) recovered, 50% hatched and of 9 *Acartia clausii* eggs (75 to 82 µm diameter), 55% hatched.

It was difficult to make internal microscopic observations on eggs with dark egg membranes. *Pseudocalanus elongatus* egg membranes are clear and on egestion eggs had changed colour from pale golden to light orange and some contained globules of oil. In most cases cells could not be distinguished internally.

Faeces were left to decompose at 16°C. After 3 d, when ciliate numbers had built up, there was evidence of the membrane starting to be broken down. The faeces break off as they are produced so the faecal membrane does not usually cover the ends of the faeces. Nauplii hatching out from eggs located close to the ends were seen to escape from the open ends. However, other nauplii hatching out further within the faeces appeared to be trapped and dying there.

There was no suggestion that digestion was at any time impaired, as copepods in the faeces were reduced to exoskeletons and larvae grew continually and well. As confirmation of these observations, histological sections of whole larvae used in the egg viability trials demonstrated that the entire digestive tract and its associated glands (liver and pancreas) were in excellent condition. Large regular villi were present in the fore- and mid-gut regions, with masses of microvilli covering the luminal surface of the epithelium. All tissues were found to be healthy, in accordance with descriptions by McFadzen et al. (in press).

Discussion. It is clear from the present study that a substantial proportion of the eggs of all copepod species examined are not digested by larval turbot. Histological observations demonstrated that the experimental turbot larvae were in good condition, so digestion resistance of copepod eggs could not be attributed to degeneration of the intestinal lining, which can develop within a few days in turbot larvae, following periods of low food intake, or starvation (McFadzen et al. in press). Resistance to digestion was not because the eggs were the thick-walled diapause type which some copepods, including *Eurytemora affinis* (Ban & Minoda 1991) and *E. velox* (Champeau 1970), produce in response to environmental stress. This was confirmed by the short hatching time of < 6 d in all species. There was also no suggestion that digestion of eggs was affected by variations in gut retention time. *Euterpina acutifrons* had the longest passage time (6.5 h), yet eggs were not digested and mean number of eggs

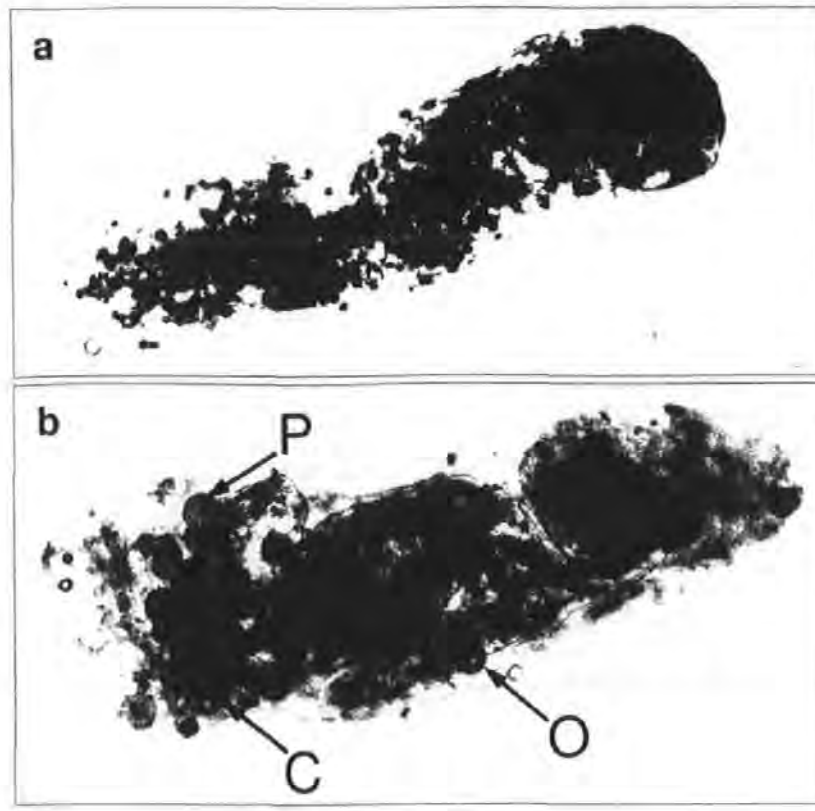

Fig. 2. *Scophthalmus maximus*. Faecal pellets of turbot larvae containing (a) *Eurytemora affinis* eggs (29 d larvae), ×20, and (b) *Pseudocalanus elongatus* eggs (P), *Corycaeus anglicus* eggs (C) and oil globules (O) probably from *P. elongatus* (29 d larvae), ×50

per female in the faeces was closer to the fresh value than for any of the other species. The wide variations observed in gut passage time of copepods may have been due to fluctuations, between collections, in the size structure of the mixed, natural zooplankton supplied as food, since larger food organisms pass through the intestinal tracts of larval fish slower than smaller ones (Karjalainen et al. 1991).

There was no evidence that the considerable discrepancy in number of eggs per female copepod in the faeces compared with the number carried by females before ingestion, for *Pseudocalanus elongatus* and to a lesser extent *Corycaeus anglicus* and other species, was due to the eggs being digested, but may arise from them being discarded or separated from females during capture and ingestion, which particularly appeared to be the case in *P. elongatus*. In addition, the faeces break off as they are produced and eggs may be lost from the open ends.

There are periods during copepod egg development when they may be especially susceptible to digestion, particularly when they are first extruded. In *Calanus helgolandicus* the egg emerges from the genital pore like a drop of fluid and within 5 min, once membranes develop, becomes round (Marshall & Orr 1955, authors' pers. obs.). A similar process has been described in *Pseudocalanus elongatus* (Corkett & McLaren 1978). *Eurytemora velox* eggs may be extruded at a more advanced stage of encapsulation compared to the species above, as when observed within the oviducts were already spherical. It has also been suggested that late development eggs may be more vulnerable to digestion due to osmotic effects of digestive fluids causing nauplii to be liberated (Redden & Daborn 1991).

Copepod egg membranes are considered to be largely composed of chitin (Marshall & Orr 1954), which is not, at least substantially, digested by larval fish (Conway et al. 1993). As the egg membranes form an unbroken barrier, enzyme penetration must depend on enzymatic or mechanical disruption. Kono et al. (1987) demonstrated chitinolytic enzyme activity in egg and larval stages of sea bream *Pagrus major* and Lindsay (1985) from the late yolk-sac stage onwards in trout *Salmo gairdneri*, although the importance of its role in digestion has yet to be established. The tough outer membrane of eggs and their spherical shape renders them relatively resistant to mechanical disruption, though some fish larvae, such as mackerel *Scomber scomber*, have a well developed set of teeth on the

jaws and develop teeth from an early age (Russell 1976), which could be used to puncture food. One of the few reports of fish larvae disrupting their prey during ingestion was in *Coregonus lavaretus* (Rösch & Segner 1990).

Eggs can form on average 29% of the dry weight biomass of adult female *Eurytemora velox* (Conway et al. 1993) and 13 to 53% for *E. herdmanni* (Crawford & Daborn 1986) so non-digestion of eggs can represent a considerable loss of potentially valuable food. Fish larvae which cannot digest the eggs of ingested egg-carrying copepods will still obtain nutrition from the copepod. However, it is of great interest to know if first feeding larvae, whose limited swimming ability and jaw gape restricts them to easily caught small prey, are able to digest eggs of copepods which are free-spawned, such as *Calanus helgolandicus* and *C. finmarchicus*. These species can dominate the copepod zooplankton biomass in the North Atlantic region (Williams & Conway 1988) and in some areas their large eggs (approximately 140 to 180 µm) can be a major primary food source in the diet of early larval fish, often of important commercial species (Bainbridge & McKay 1968, Bjørke 1978, Conway 1980, Kane 1984, Economou 1991). Survival and recruitment of these larvae could be influenced if they were unable to digest copepod eggs.

From gut content analysis of early larval (3 to 7 mm) blue whiting *Micromesistius poutassou*, the diet comprised by number 29% *Calanus finmarchicus* eggs (Conway 1980), the majority showing no evidence of digestion. In contrast there are several reports, from a range of larval fish species including cod *Gadus morhua* and haddock *Melanogrammus aeglefinus* (Kane 1984), redfish *Sebastes* spp. (Bainbridge & McKay 1968) and herring *Clupea harengus* (Bjørke 1978), detailing observations on digestion of *Calanus* spp. eggs. It is possible that only certain fish larvae can digest copepod eggs and perhaps only from particular species.

There was a range of viability of fresh eggs between copepod species. This was not large between most species (84.8 to 95.4%), apart from *Euterpina acutifrons* (68.6%). The low viability in *E. acutifrons* does not necessarily mean that the eggs had not been fertilized, since large seasonal fluctuations in individual copepod species hatching success can occur (Ianora et al. 1992). The response of eggs to digestive action, as measured by their viability on egestion, separated the copepods into 2 groups. Eggs of *E. acutifrons*, *Eurytemora affinis* and *Eurytemora velox* retained a high viability, similar to results obtained by Redden & Daborn (1991) derived from feeding experiments on *Eurytemora herdmanni*. The viabilities measured for *Pseudocalanus elongatus*, *Oncaea venusta* and *Corycaeus anglicus* eggs showed they are considerably less resistant. It was clear by the appearance of *P. elongatus* eggs on egestion that the high loss of viability in this species was because cells of most eggs were being disrupted by digestive action, while the egg membrane remained intact and the contents apparently not removed.

The reasons for the interspecific variation in egg viability following gut passage are not known, but are presumably related to a combination of differences in the structural composition of the egg membranes and osmotic tolerance of the internal cells. The eggs of the euryhaline species *Eurytemora affinis* and *E. velox* may be pre-adapted to surviving the osmotic stress of gut passage because of their wide salinity tolerance. Eggs of *Temora longicornis* and *Acartia clausi* may also be adapted to withstand environmental stress. These species have been shown to produce resting, as opposed to diapause, eggs, which remained viable within bottom sediments and still hatched after a year (Lindley 1990). Lindley (1992) found differences between formalin preserved eggs of *Acartia* and *Temora* spp. and those of *Pseudocalanus* and *Calanus* spp. Eggs of the former 2 species did not take up stain and also did not respond as conspicuously to osmotic stress as the latter 2 species, suggesting differences between them in the structure of the egg membranes.

Retention of a high viability in egested eggs of some of the copepod species has implications for copepod survival, since egg-carrying species have been shown to be selected by fish larvae, the eggs increasing their profile and hence vulnerability (Bollens & Frost 1991). However, an unknown proportion of nauplii from hatching of faecal eggs may not escape the faeces. The proportion escaping will depend on how rapidly the faecal membrane breaks down under natural conditions. Faeces probably sink very rapidly, as has been shown for adult fish faeces (Robison & Bailey 1981), and many nauplii may not survive.

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LITERATURE CITED

Alvarez-Cadena, J. N. (1993). Feeding of the chaetognath *Sagitta elegans* Verrill. Estuar. coast. Shelf Sci. 36: 195-206

Bainbridge, V., McKay, B. J. (1968). The feeding of cod and redfish larvae. Spec. Publs int. Commn NW Atlant. Fish. 7: 187-217

Ban, S., Minoda, T. (1991). The effect of temperature on the development and hatching of diapause and subitaneous eggs in *Eurytemora affinis* (Copepoda: Calanoida) in Lake Ohnuma, Hokkaido, Japan. Bull. Plankton Soc. Japan. Spec. Vol.: 299–308

Bennett, H. S., Wyrick, A. D., Lee, S. W., McNeil, J. H. (1976). Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Technol. 51: 71–97

Bjørke, H. (1978). Food and feeding of young herring larvae of Norwegian spring spawners. FiskDir. Ser. Havunders. 16: 405–421

Bollens, S. M., Frost, B. W. (1991). Ovigerity, selective predation, and variable diel vertical migration in *Euchaeta elongata* (Copepoda: Calanoida). Oecologia 87: 155–161

Champeau, A. (1970). Etude de la vie latente chez les Calanoides (Copépodes) caractéristiques des eaux temporaires de Basse-Provence. Annls Fac. Sci. Marseille 44: 155–189

Conway, D. V. P. (1980). The food of larval blue whiting, *Micromesistius poutassou* (Risso), in the Rockall area. J. Fish. Biol. 16: 709–723

Conway, D. V. P., Tranter, P. R. G., Coombs, S. H. (1993). Digestion of natural food by larval and post-larval turbot *Scophthalmus maximus*. Mar. Ecol. Prog. Ser. 100: 221–231

Conway, D. V. P., Tranter, P. R. G., Fernandez de Puelles, M. L., Coombs, S. H. (1991). Feeding of larval sprat (*Sprattus sprattus* L.) and sardine (*Sardina pilchardus* Walbaum). Comm. Meet. int. Coun. Explor. Sea C.M.-ICES 1991/L: 76: 1–15

Corkett, C. J., McLaren, I. A. (1978). The biology of *Pseudocalanus*. Adv. mar. Biol. 15: 1–231

Crawford, P., Daborn, G. R. (1986). Seasonal variations in body size and fecundity in a copepod of turbid estuaries. Estuaries 9: 133–141

Economou, A. N. (1991). Food and feeding ecology of five gadoid larvae in the northern North Sea. J. Cons. int. Explor. Mer 47: 339–351

Ianora, A. Mazzocchi, M. G., Grottoli, R. (1992). Seasonal fluctuations in fecundity and hatching success in the planktonic copepod *Centropages typicus*. J. Plankton Res. 14: 1483–1494

Kane, J. (1984). The feeding habits of co-occurring cod and haddock larvae from Georges Bank. Mar. Ecol. Prog. Ser. 16: 9–20

Karjalainen, J., Koho, J., Viljanen, M. (1991). The gastric evacuation rate of vendace (*Coregonus albula* L.) larvae predating on zooplankters in the laboratory. Aquaculture 96: 343–35

Kellerman, A. (1990). Food and feeding dynamics of the larval Antarctic fish *Notothenopsis larseni*. Mar. Biol. 106: 159–167

Kono, M., Furukawa, K., Satoh, H., Matsui, T., Shimizu, C. (1987). Changes in the chitinase activity at different stages of red sea bream *Pagrus major* egg, larva, and juvenile. Nippon Suisan Gakk. 53: 1289–1293

Lindley, J. A. (1990). Distribution of overwintering calanoid copepod eggs in sea-bed sediments around southern Britain. Mar. Biol. 104: 209–217

Lindley, J. A. (1992). Resistant eggs of the Centropagoidea (Copepoda: Calanoida): a possible preadaption to colonization of inland waters. J. Crust. Biol. 12: 368–371

Lindsay, G. J. H. (1985). Development of gastric chitinase activity in trout (*Salmo gairdneri*). Aquaculture 48: 285–290

Marcus, N. H. (1984). Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. Mar. Ecol. Prog. Ser. 15: 47–54

Marshall, S. M., Orr, A. P. (1954). Hatching in *Calanus finmarchicus* and some other copepods. J. mar. biol. Ass. U.K. 33: 393–401

Marshall, S. M., Orr, A. P. (1955). The biology of a marine copepod. Oliver & Boyd, Edinburgh

McFadzen, I. R. B., Lowe, D. M., Coombs, S. H. (in press). Histological changes in starved turbot larvae (*Scophthalmus maximus*) quantified by digital image analysis. J. Fish Biol.

Nakata, K. (1988). Alimentary tract contents and feeding conditions of ocean-caught post-larval Japanese sardine, *Sardinops melanostictus*. Bull. Tokai Reg. Fish. Res. Lab. 126: 11–24

Proctor, V. W., Malone, C. R., DeVlaming, V. L. (1967). Dispersal of aquatic organisms: viability of disseminules recovered from the intestinal tract of captive killdeer. Ecology 48: 672–676

Redden, A. M., Daborn, G. R. (1991). Viability of subitaneous copepod eggs following fish predation on egg-carrying calanoids. Mar. Ecol. Prog. Ser. 77: 307–310

Robison, B. H., Bailey, T. G. (1981). Sinking rates and dissolution of midwater fish fecal matter. Mar. Biol. 65: 135–142

Rösch, R., Segner, H. (1990). Development of dry food for larvae of *Coregonus lavaretus* L. 1. Growth, food digestion and fat absorbtion. Aquaculture 91: 101–115

Russell, F. S. (1976). The eggs and planktonic stages of British marine fishes. Academic Press, London

Schnack, D. (1972). Nahrungsökologische Untersuchungen an Heringslarven. Ber. dt. wiss. Kommn Meeresforsch. 22: 273–343

Tully, O., Ó Céidigh, P. (1989). The ichthyoneuston of Galway Bay (west of Ireland). II. Food of post-larval and juvenile neustonic and pseudoneustonic fish. Mar. Ecol. Prog. Ser. 51: 301–310

Viñas, M. D. (1991). Feeding ecology of *Engraulis anchoita* first feeding larvae in a tidal mixed front off the Valdes Peninsula (Argentina). Comm. Meet. int. Coun. Explor. Sea C.M.-ICES 1991/L: 83: 1–19

Williams, R., Conway, D. V. P. (1988). Vertical distribution and seasonal numerical abundance of the Calanidae in oceanic waters to the south-west of the British Isles. Hydrobiologia 167/168: 259–266

Zismann, L., Berdugo, V., Kimor, B. (1975). The food and feeding habits of early stages of grey mullets in the Haifa Bay region. Aquaculture 6: 59–75

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D.V.P. Conway carried out the experimental work, sample analysis, data interpretation and preparation of the manuscript to 95% of the total effort.

I.R.B. McFadzen 16/3/00

P.R.G. Tranter 16/3/00

Paper III

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Feeding of larval sardine, *Sardina pilchardus* (Walbaum), off the north coast of Spain

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ABSTRACT

In April and May 1991 and between March and June 1992 data regarding the diet of larval (4-24 mm) sardine, Sardina pilchardus *(Walbaum), in relation to food availability was gathered off the north coast of Spain as part of a study on larval conditions and mortality. Interpretation of results is compromised by the tendency of sardine larvae to defecate their gut contents during sampling. The most common food organisms in the guts (78-89 %) were the developmental stages of copepods (eggs, nauplii and copepodites). Percentage composition of copepod nauplii in the diet decreased with increasing larval size, while copepodites increased. The largest larvae still consumed a high proportion of small food particles. There was no consistent relationship between food availability and feeding success, probably because feeding conditions were generally adequate. However, in May 1992, when food availability was consistently lowest, the nutritional condition of larvae was also lowest, providing a link between these two factors.*

Key words: Sardine larvae, feeding, copepods, defecation, northern Spain.

RESUMEN

Alimentación de las larvas de sardina, *Sardina pilchardus* (Walbaum), en la costa norte de España.

Durante los meses de abril y mayo de 1991 y entre marzo y junio de 1992 se investigó la dieta de las larvas (4-24 mm) de Sardina pilchardus *(Walbaum) en la costa norte de España, y se comparó con el alimento disponible en el medio natural. El objetivo principal fue relacionar la alimentación con la condición larvaria y su mortalidad. La tendencia de las larvas a expulsar el contenido estomacal durante el muestreo ha hecho difícil sin embargo la interpretación de los resultados obtenidos. El alimento más común en el estómago (78-89 %) fue el de distintos estadios de copépodos (huevos, nauplios y copepoditos). En la dieta, el porcentaje de nauplios de copépodos disminuyó al aumentar el tamaño de las larvas, mientras que el porcentaje de copepoditos aumentó; las larvas mayores también consumieron una proporción elevada de pequeñas partículas de alimento. No se encontró relación alguna entre la disponibilidad de alimento y el éxito alimenticio, probablemente debido a que las condiciones de alimentación fueron generalmente adecuadas. En mayo de 1992, sin embargo, cuando la disponibilidad de alimento fue bastante menor, las condiciones nutricionales de las larvas también fueron peores, obteniéndose así una conexión entre estos dos factores.*

Palabras clave: Larvas de sardina, alimentación, copépodos, defecación, norte de España.

INTRODUCTION

The sardine, *Sardina pilchardus* (Walbaum), is an important commercial fish species in the coastal waters of northern Spain (Robles *et al.*, 1992); consequently, annual fluctuations in recruitment are of considerable social and economic consequence.

As part of a European Sardine Anchovy Recruitment Project (SARP), a series of research cruises were carried out in 1991 and 1992 off the northern coast of Spain (López-Jamar *et al.*, submitted) to investigate factors affecting survival of sardine larvae. One aspect of the program was an investigation of larval condition (McFadzen *et al.*, in prep.) and of how this was related to environmental variables and subsequent mortality. It is generally considered that food availability is one of the major factors affecting larval fish survival (Buckley and Lough, 1987) and it has been shown experimentally that food deprivation rapidly increases mortality in sardine larvae (Silva and Miranda, 1992). A knowledge of the diet and prey selectivity of larval sardine is thus required in order to assess food availability in the plankton.

A preliminary description of the diet of sardine larvae, from sampling on a cruise off northern Spain in 1991, has been given by Conway *et al.* (1991). The present study extends this work with results on the diet of sardine larvae and food availability from further sampling on a series of cruises in 1992.

MATERIALS AND METHODS

Sardine larvae were collected in zooplankton samples taken during five cruises off the north coast of Spain in April/May 1991 and between March and June 1992 (table I). On each cruise a grid of plankton stations was worked, based on a sample spacing of 8 nautical miles along transects out from the coast. For comparative purposes stations have been grouped in three areas, namely off La Coruña, Gijón and Santander (figure 1), based on hydrographic regimes and larval aggregations. Sampling at each station was carried out using slow speed (~ 2 knots), 40 cm or 72 cm diameter mouth aperture bongo net tows (200 or 280 µm mesh) fitted with partial-filtering style of cod-ends to minimize sample damage. Sampled depth was to approximately 100 m, or within about 5 m

Table I. Sampling and analysis details.

Vessel	Sampling dates	Sampling area	N.° stations larvae examined from	N.° larvae examined	N.° stations analyzed for zooplankton
B. O. *Cornide de Saavedra*	17 April-11 May 1991	La Coruña	16	163	4
		Gijón	17	131	4
		Santander	9	71	3
B. O. *Cornide de Saavedra*	6-18 March 1992	La Coruña	7	64	4
		Gijón	3	44	3
		Santander	10	78	4
B. O. *Cornide de Saavedra*	1-13 April 1992	La Coruña	7	51	4
		Gijón	6	56	2
		Santander	14	85	5
F. S. *Valdivia*	10-21 May 1992	La Coruña	25	232	4
		Gijón	10	79	4
		Santander	9	69	4
R. R. S. *Challenger*	26 May-6 June 1992	La Coruña	12	131	30
		Gijón	6	80	16
		Santander	10	95	23

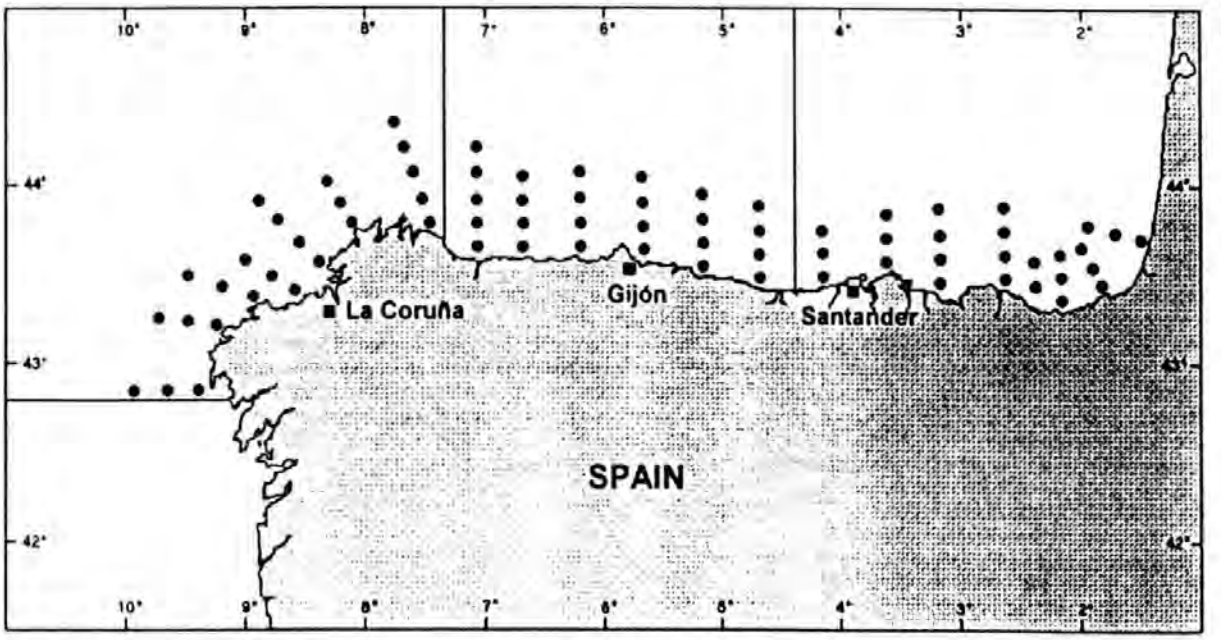

Figure 1. Sampling area off the north coast of Spain showing the three areas used for data analysis and position of standard stations for all the cruises.

off the bottom in shallower water. Water flow through the nets was measured by means of a flowmeter fitted to one side of the nets, and maximum depth sampled was recorded by a depth recorder attached to the sampler frame. Following the haul, the fresh sample was emptied into a glass tray and sardine larvae for gut contents analysis was sorted into glass vials containing 4 % borax buffered formaldehyde solution. Specimens ranged in size from 4 mm to 24 mm; all are here termed as larvae.

Subsequent analysis was based on at least 10 larvae, when available, from each station (table I). Each specimen was measured (standard length) under a dissecting microscope fitted with an eyepiece micrometer, no allowance being made for shrinkage. The complete gut was then detached, opened and all contained organisms counted, identified to the taxonomic level as allowed by their condition, and a proportion measured for length and width. Brief notes were also kept of the state of digestion of the organisms.

Data were analyzed by larval size, separated into three length ranges <10 mm, 10-15 mm and >15 mm. The number of larvae examined in each area is given in table 1 and over the 24-hour period for all cruises combined is given separately for each of the three larval size ranges in figure 2. A total of 1 429 larvae were examined, comprising 751 larvae <10 mm in length, 545 larvae 10-15 mm and 133 larvae >15 mm. Incidence of larval feeding was calculated as the percentage of larvae in which food was observed, irrespective of the quantity present. Incidence of food occurrence in the guts was calculated as the percentage of larvae in which at least one specimen of the target organism was observed.

Estimates of food availability in the plankton were obtained from microzooplankton sampled concurrently with 10 cm diameter mouth aperture bongo nets (53 µm mesh), fitted on one side with a flowmeter and attached to the towing wire above the main nets. These samples were preserved in 4 % formaldehyde solution and a representative selection from each area subsequently analysed (table I) and counted for the organisms identified from the gut contents analysis as being most important in the diet of sardine larvae (i.e. copepod eggs, nauplii and copepodites with a <0.9 mm cephalothorax length).

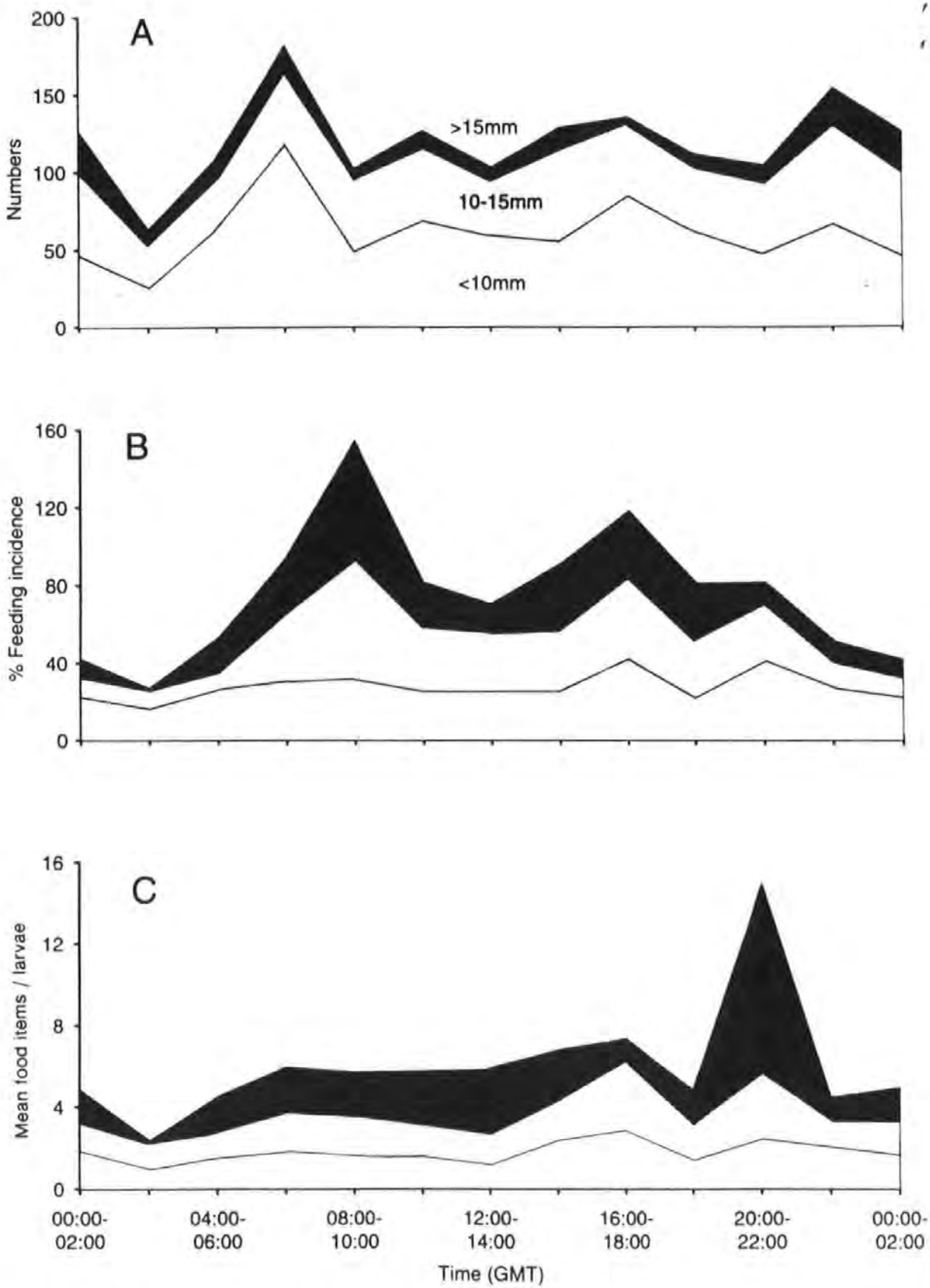

Figure 2. For all cruises combined (a): number of larvae examined, (b): percentage feeding incidence and (c): mean number of food items per feeding larva by 2 hour intervals over the 24 h period. All values are cumulative. Time of dawn was from 18:18-20:01 h GMT and dusk from 04:42-06:54 h GMT.

Table II. Percentage food composition and incidence, all cruises combined.

	% Composition			% Incidence		
	<10 mm	10-15 mm	>15 mm	<10 mm	10-15 mm	>15 mm
Calanus helgolandicus	—	1.5	2.0	—	3.2	4.2
Para/Pseudo/Clausocalanus spp.	1.4	6.2	30.0	2.8	9.6	25.0
Acartia spp.	1.2	7.7	2.0	0.9	11.5	4.2
Centropages spp.	—	0.3	—	—	0.6	—
Oithona spp.	2.2	4.1	4.0	2.8	7.6	8.3
Oncaea spp.	—	0.9	2.0	—	1.9	4.2
Microsetella sp.	0.2	—	—	0.5	—	—
Unidentified copepods	4.1	13.9	4.0	7.4	15.9	12.5
Copepod eggs (~55 µm dia.)	0.2	0.6	—	0.5	0.6	—
Copepod eggs (~73 µm dia.)	5.8	1.8	—	8.3	1.3	—
Copepod eggs (~92 µm dia.)	—	0.9	—	—	0.6	—
Copepod eggs (~110 µm dia.)	—	0.6	—	—	1.3	—
C. helgolandicus eggs (~183 µm dia.)	2.2	8.6	8.0	3.2	7.0	16.7
Copepod nauplii	60.8	42.3	30.0	60.4	50.3	45.8
Unidentified invertebrate eggs	0.7	1.2	2.0	1.4	2.5	4.2
Euphausiid calyptopis	0.2	0.3	—	0.5	0.6	—
Evadne sp.	—	—	2.0	—	—	4.2
Limacina sp.	0.2	—	—	0.5	—	—
Gastropod larvae	—	0.3	—	—	0.6	—
Lamellibranch larvae	0.2	—	—	0.5	—	—
Tintinnid	5.5	—	—	2.8	—	—
Rotifer	0.2	—	—	0.5	—	—
Peridinium sp.	0.2	—	—	0.5	—	—
Unidentified remains	14.4	8.9	14.0	28.1	18.5	25.0
Total feeding larvae				217	157	24
Mean n.° organisms/feeding larvae				1.9	2.2	2.1

RESULTS

Composition of gut contents

Food organisms were generally located at the distal end of the hind-gut, some times protruding from the anus. On two occasions only was food found in the fore-gut. The majority of food items taken by the three size ranges of larvae were the copepodite (9-44 %), nauplii 30-61 %) and egg (8-12 %) stages of copepods (table II, figure 3). Copepodites were usually observed only as their remaining exoskeletons, making it difficult to discriminate between the developmental stages of superficially similar genera such as *Paracalanus, Pseudocalanus* and *Clausocalanus*. This was the most common copepodite group in the diet, especially in larvae >15 mm in length (30 % of composition). Nauplii were numerically the most common organism in the diet, their percentage composition dropping with increased larval size. The largest and most frequently encountered copepod eggs (approximately 183 µm diameter) were of *Calanus helgolandicus*, copepodites of which were taken in low numbers (approximately 2 % of composition) from larvae >10 mm. These are freely-spawned eggs and hence were ingested individually. Other copepod eggs could be separated into discrete size groups and were probably from individual copepod species, but because of the high copepod species diversity in this region, they could not be positively identified. Eggs were usually present in the absence of female copepods, suggesting they were free-spawned rather than

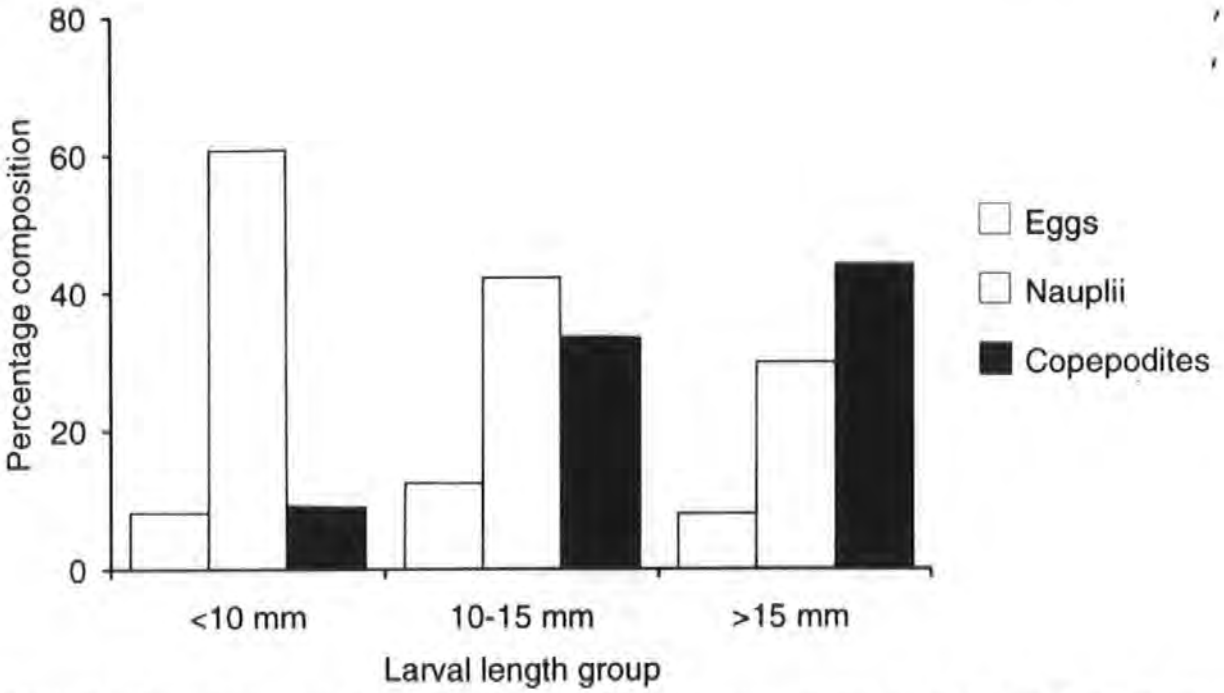

Figure 3. Percentage composition of copepod eggs, nauplii and copepodites in the diet of the three length ranges (<10 mm, 10-15 mm and >15 mm) of larvae.

carried eggs. Very rarely were the egg membranes disrupted due to digestion. The unidentified invertebrate eggs (table II) were most probably of euphausiids or chaetognaths. Tintinnids made up 5.5 % of the diet in larvae <10 mm, although this value was inflated due to a small number of larvae containing high numbers of these organisms. Other identifiable zooplankton organisms had a general low incidence in the diet (1-2 %). The only phytoplankton material which was identifiable was low

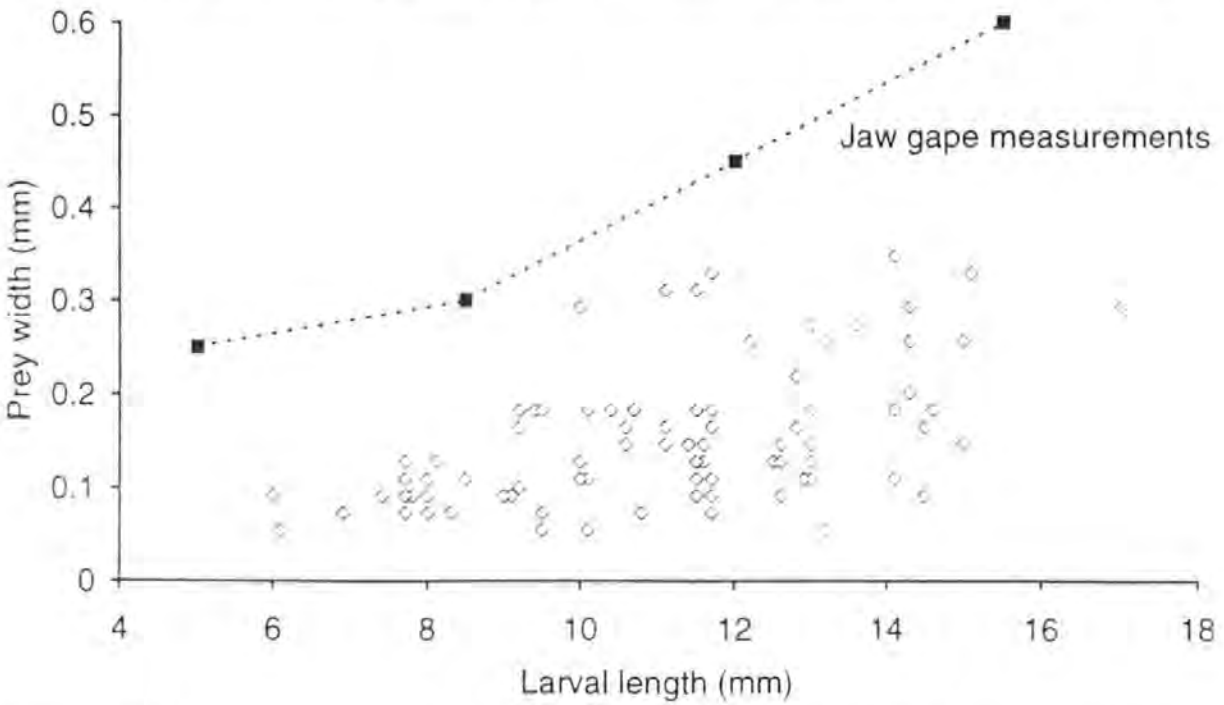

Figure 4. Width of prey consumed by *Sardina pilchardus* larvae (n=110) in May 1992. Jaw gape measurements are from Blaxter (1969).

numbers of the dinoflagellate, *Peridinium* spp. Unidentifiable remains constituted a substantial proportion (9-14 %) of the food, usually consisting of amorphous material which may have also contained some phytoplankton remains.

Incidence of food particles and prey size

A similar pattern in the incidence of food items in the diet was observed as for the composition of the diet (table II). The incidence of copepodites increased with larval size, constituting 9.1 % of the diet in larvae <10 mm in length, 34.6 % in larvae of 10-15 mm and 44 % in larvae >15 mm in length. The largest copepodites (e.g. *Calanus helgolandicus*) were restricted to the largest larvae. While incidence of nauplii remained high in the diet of larger larvae, their importance in the diet (i.e. proportion of the gut contents) was reduced, indicating that larger larvae fed on smaller numbers. In contrast, *C. helgolandicus* eggs were mostly consumed by larvae >10 mm in length and also formed a greater proportion of the diet of these larger larvae.

As an example of the change in size of food particles with increasing larval size, width measurements from 110 food items taken by sardine larvae in May 1992 are plotted in figure 4, together with sardine jaw gape measurements from Blaxter (1969). With increase in larval size the maximum width of food particles increased. There was no direct relationship between width of food consumed and larval size, due to larger larvae continuing to feed on a high proportion of small particles such as copepod eggs and nauplii.

Diurnal feeding patterns

Food was found in larvae at all times of the day, but with a clear reduction between about 00:00-04:00 hours (figure 2b). During the period of darkness, occasional completely undigested organisms were found, suggesting they had been newly ingested. There was increased feeding incidence following dawn, around 06:00-08:00 hours; and again in the afternoon, between 14:00-18:00 hours. At most times a lower percentage of larvae >15 mm had food in their guts than smaller larvae.

As an indication of the intensity of feeding at different times of the day, the mean number of food items per feeding larva is plotted in figure 2c. The mean numbers of particles per larva was mostly <2 for all larval lengths. The number of particles showed little diurnal variation in larvae <15 mm. The greater variation in larvae >15 mm may be related to the smaller number of this group examined. The prominent peak at 20:00-22:00 hours was due to an individual larva > 15 mm in length containing many *Para/Pseudo/Clausocalanus* copepodites.

Differences between areas in food availability, food selection and feeding success

Results from microzooplankton analysis (table III) showed little difference in availability of suitable food between areas during each cruise. Copepod nauplii were the most abundant organism (2.4-17.3/l), followed by copepodite stages (2.3-77.0/l). The greatest differences in food availability was between cruises. Highest numbers of *Calanus helgolandicus* eggs were sampled in April/May 1991, although they were present during all cruises in low numbers (<0.4/l). On the basis of the microzooplankton sampling, food availability was highest in May/June 1992 and lowest in April/May 1991 and May 1992.

The mean number of particles consumed per feeding larva by areas and cruises is also given in table III. Most *Calanus helgolandicus* eggs were consumed during cruises from late April to June, especially off La Coruña and Santander. In most instances, nauplii formed the highest proportion of the gut contents. In individual cruises the number of particles taken per lava tended to be lower off Santander.

Only off Santander was there any evidence of an increase in feeding incidence with increase in amount of available suitable food; in other areas the relationship

Table III. Availability of organisms suitable as food in the plankton of each area and mean number of the same organisms per feeding larva. Standard errors of the means are given, except where large numbers of zero data values invalidate this procedure. Copepodite measurements are cephalothorax length.

	C. helgolandicus eggs. Mean n.°/l	*C. helgolandicus* eggs. Mean n.°/larva	*Naupli.* Mean n.°/l	St. error	*Naupli.* Mean n.°/larva	Copepodites <0.9 mm N.°/l	St. error	Copepodites <0.9 mm Mean n.°/larva
La Coruña								
April/May-91	0.4	0.1	2.4	0.91	1.0	2.3	0.28	0.2
Mar-92	0.1	—	7.6	2.24	1.8	3.9	1.22	0.2
Apr-92	0.3	—	9.3	3.79	1.2	4.8	0.82	0.8
May-92	0.1	0.2	3.6	1.42	1.0	4.4	4.03	0.5
May/June-92	0.1	0.1	17.2	2.83	0.6	5.6	1.34	0.3
Mean	0.1	0.1	13.2	2.53	1.1	5.0	0.99	0.3
Gijón								
April/May-91	0.3	—	3.4	1.76	1.8	3.0	0.62	0.5
Mar-92	—	—	4.4	1.25	0.6	2.6	0.38	1.6
Apr-92	—	—	6.3	0.01	1.1	5.1	1.49	0.7
May-92	0.1	—	3.3	2.43	0.4	2.9	3.25	0.2
May/June-92	0.1	0.1	17.3	3.70	1.0	5.5	1.24	0.6
Mean	0.1	<0.1	11.4	3.07	1.2	4.4	0.92	0.7
Santander								
April/May-91	0.2	—	2.8	1.61	1.0	6.4	2.23	0.3
Mar-92	—	—	12.9	12.00	1.4	7.0	5.24	0.3
Apr-92	0.1	—	5.7	2.46	0.6	3.9	1.47	0.3
May-92	0.1	0.3	4.4	2.80	0.1	2.6	1.41	0.6
May/June-92	0.1	0.6	12.7	2.77	1.0	6.3	1.46	0.5
Mean	0.1	0.2	10.2	2.34	0.9	5.7	1.10	0.4

was erratic (figure 5). The percentage of larvae with food varied from 18-52 % among the three areas over the five cruises. Percentage feeding values off La Coruña and Gijón were similar within each cruise, while off Santander values were generally highest (32-52 %). In March 1992 the percentage feeding was highest in all three areas and with the values most closely grouped (44-52 %).

DISCUSSION AND CONCLUSIONS

The low proportion (27.8 %) of sardine larvae which contained food in their guts is typical both for clupeid larvae examined from preserved plankton samples and for other larval fish with straight intestinal tracts (Dheknik, 1974). Last (1980) found that 23.7 % of herring and 26.0 % of sprat larvae contained food, compared with a mean of 64.3 % for all other non-clupeid species. The tendency of clupeids to evacuate food in response to the trauma of sampling is well documented (June and Carlson, 1971; Kjilson *et al.*, 1975; Hay, 1981). Food may also be evacuated when live larvae are placed in preservative (Blaxter, 1965) but, in survey material, clupeid larvae are invariably dead when retrieved from a plankton sampler, so that it is unlikely that further food expulsion will occur on preservation. In the present study, food was generally found in the hind-gut and towards the anus, suggesting that food was being defecated rather than regurgitated. The proportion of clupeids evacuating their food may be determined by sampling methodology (Hay, 1981). Fortier and Harris (1989), using pumped samples, found that only 1.2% of sprat (*Sprattus sprattus*) larvae contained food, while 58 % of net-caught samples (Conway *et al.*, 1991) of the same species contained food.

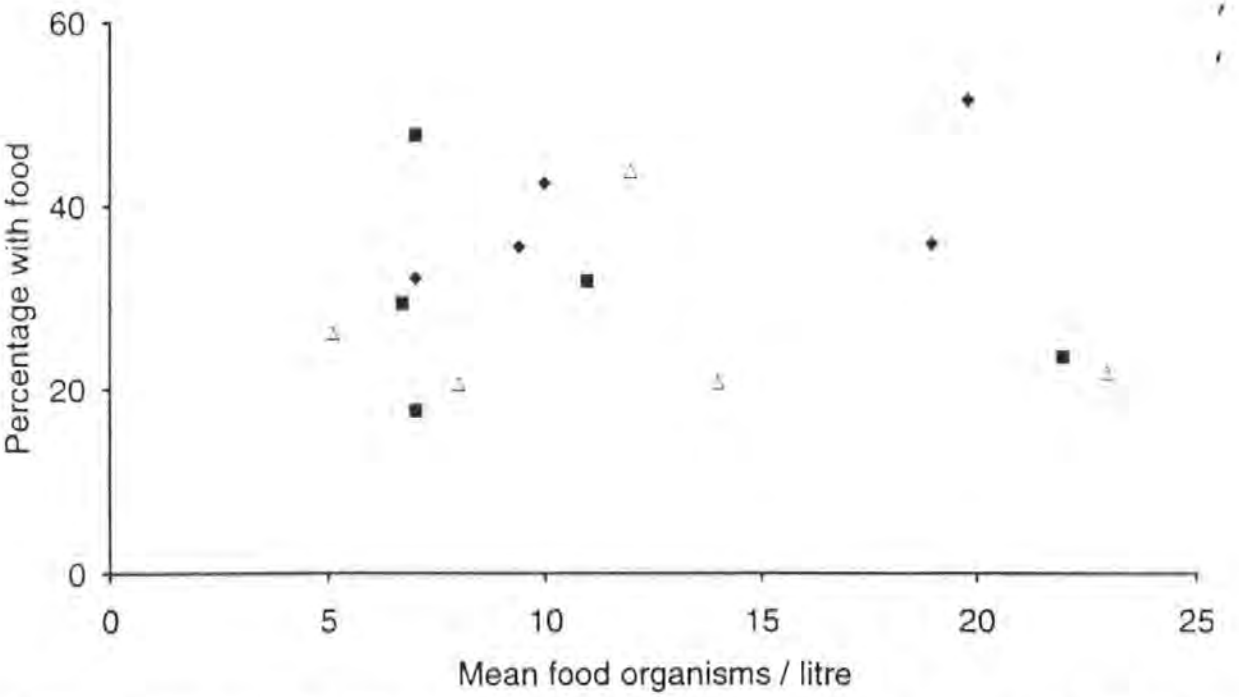

Figure 5. Percentage of *Sardina pilchardus* larvae with food in their guts (excluding larvae sampled between 00.00 and 04.00 hours, when most had little or no food) by area: La Coruña (Δ), Gijón (■), Santander (♦) for each cruise, separately for the three areas, plotted against the mean total number of esculent organisms/liter (*Calanus helgolandicus* eggs, copepod nauplii and copepod copepodites with <0.9 mm cephalothorax length).

As larval fish grow they require and consume increasingly larger prey (Hunter, 1977; Theilacker and Dorsey, 1980), and a similar observation was recorded in the present study. While there was an increase in maximum size of prey, there was little increase in the numbers of prey, which is not typical of other studies (e.g. Last, 1980). The mean number of particles per gut of feeding larvae was consistently low, generally <2, which may partially be a result of defecation related to sampling and thus not a true reflection of feeding intensity. However, in laboratory experiments the greatest number of food particles found in sardine larvae (10-14 mm in length) was only 3 (Blaxter, 1969).

A substantial proportion of small particles were consumed by larger larvae. For example, the percentage of copepod nauplii in the diet decreased from 60.8 % to 30.0 % with increase in larval size from <10 to >15 mm, and total copepod eggs remained relatively constant between 8.0 % to 12.5 %. Feeding of larger larvae on relatively small organisms such as nauplii is inefficient, since they will contribute relatively less to the food biomass than a smaller number of large particles. The situation for copepod eggs may be less significant, since a substantial proportion of the smaller eggs may have been taken incidentally as egg sacs attached to adult females. Other studies (Conway, 1980) have shown free-spawned eggs restricted to early fish larvae, possibly because they can capture these non-motile particles relatively easily, while older larvae feed on larger prey. *Calanus* spp. eggs, which are the most numerous free-spawned copepod eggs in the north Atlantic region, can be abundant in the diet of clupeid larvae (Bjørke, 1978), but in the present study *C. helgolandicus* eggs formed only 2.2 % of the diet of larvae <10 mm and surprisingly, a higher percentage (8.0 %) in larger larvae (>15 mm in length), suggesting some element of selection. However, the resistance of copepod eggs to digestion by larval fish (Conway *et al.*, 1994) may prevent any substantial contribution being made by them to larval nutrition.

In laboratory experiments with sardine larvae, Blaxter (1969) observed a feeding incidence of approximately 10 % for daylight hours, falling to 0 % at night and rising rapidly to around 30 % in the early morning. The feeding incidence values of the present study are considerably higher than these, but with a similar superimposed diurnal cycle, corresponding to observations on other clupeid larvae (Blaxter, 1965; Blaxter and Hunter, 1982). However, the occurrence of occasional, obviously freshly ingested food in the guts of sardine larvae during the dark period suggests that some feeding may occur using senses other than vision (Govoni *et al.*, 1983).

Food concentrations in the range of 5-25 particles/l, as measured in the present study, are typical of integrated values down the water column in many areas where fish larvae are abundant (e.g. in the Irish Sea, Coombs *et al.* 1992, and off the coast of North America, Arthurs, 1977); higher concentrations occur at discrete depths due to vertical stratification and aggregation in layers. Specifically, as part of the same SARP study off Spain, Fernández de Puelles, *et al.* (1993) and López-Jamar *et al.* (1991) have shown vertical heterogeneity and coincidence of microzooplankton and sardine larvae in the upper 50 m of the water column. Thus, it may be that the lack of correspondence between food availability and quantity of food in the larval guts is due, in the present study, to food abundance being generally adequate, without sufficently low levels being encountered to lead to a reduction of food intake.

The ultimate aim of the SARP study was to relate juvenile survival to oceanographic and biological conditions (e.g. food availability) during larval development. McFadzen *et al.* (in prep.) used histological criteria as an index of larval, and hence juvenile survival, and identified larvae from the May 1992 cruise as being in consistently lower nutritional condition than larvae from the other cruises. Similar results were obtained using other measures of larval condition (López-Jamar *et al.*, in press). Although there is no clear relationship between the amount of food in the guts of larval sardine and prey availability in the plankton, the present study identifies food availability as being consistently lower in the May 1992 cruise than at other times. As such, these results provide a link between the nutritional condition of fish larvae and food availability.

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REFERENCES

Arthur, D. K. 1977 Distribution, size, and the abundance of microcopepods in the Californian Current system and their possible influence on survival of marine teleost larvae. *Fish. Bull. NOAA* 75 (3): 601-611.

Bjørke, H. 1978. Food and feeding of young herring larvae of Norwegian spring spawners. *Fiskeridir. Skr. Ser. Havunders.* 16: 405-421.

Blaxter, J. H. S. 1965. The feeding of herring larvae and their ecology in relation to feeding. *CalCOFI Reports* 10: 79-88.

Blaxter, J. H. S. 1969. Experimental rearing of pilchard larvae, *Sardina pilchardus*. *J. Mar Biol. Ass. (UK)* 49 (3): 557-575.

Blaxter, J. H. S. and J. R. Hunter. 1982. The biology of the clupeoid fishes. *Adv. Mar. Biol.* 20: 1-223.

Buckley, L. J. and R. J. Lough. 1987. Recent growth, biochemical composition and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. *Can. J. Fish. Aquat. Sci.* 44 (1): 14-25.

Conway, D. V. P. 1980. The food of larval blue whiting, *Micromesistius poutassou* (Risso), in the Rockall area. *J. Fish. Biol.* 16 (6): 709-723.

Conway, D. V. P., I. R. B. McFadzen and P. R. G. Tranter. 1994. Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage. *Mar. Ecol. Prog. Ser.* 106 (3): 303-309.

Conway, D. V. P, P. R. G. Tranter, M. L. Fernández de Puelles and S. H. Coombs. 1991. Feeding of larval sprat (*Sprattus sprattus* L.) and sardine (*Sardina pilchardus* Walbaum). *ICES C. M.* 1991/L: 76.

Coombs, S. H., J. H. Nichols, D. V. P. Conway, S. Milligan and N. C. Halliday. 1992. Food availability for sprat larvae in the Irish Sea. *J. Mar. Biol. Ass. (UK)* 72 (4): 821-834.

Dekhnik, T. V. 1974. Comments on the articles by V. Ye. Zaika and N. A. Ostrovskaya entitled "Indicators of the availability of food to larval fish". *J. Icthyol. Engl. Trans. Vopr. Ikhtiol.* 14 (5): 804-808.

Fernández de Puelles, M. L., L. Valdés, M. Varela and M. T. Álvarez-Ossorio. 1993. Diel variations in vertical distribution of copepods in the north Iberian coast. *ICES C. M.* 1993/L: 51.

Fortier, L. and R. P. Harris. 1989. Optimal foraging and density-dependent competition in marine fish larvae. *Mar. Ecol. Prog. Ser.* 51 (1/2): 19-33.

Govoni, J. J., D. E. Hoss and A. C. Chester. 1983. Comparative feeding of three species of larval fishes in the Northern Gulf of Mexico: *Brevoortia patronus, Leiostomus xanthurus,* and *Micropogonias undulatus. Mar. Ecol. Prog. Ser.* 13 (2/3): 189-199.

Hay, D. E. 1981. Effects of capture and fixation on gut contents and body size of Pacific herring larvae. *Rapp. P-V Reun. Cons. Int. Explor. Mer.* 178: 395-400.

Hunter, J. R. 1977. Behaviour and survival of northern anchovy *Engraulis mordax* larvae. *CalCOFI Reports* 19: 138-146.

June, F. C. and F. T. Carlson. 1971. Food of young Atlantic menhaden, *Brevoortia tyrannus,* in relation to metamorphosis. *Fish. Bull. NOAA* 68 (3): 493-512.

Kjelson, M. A., D. S. Peters, G. W. Thayer and G. N. Johnson. 1975. The general feeding ecology of post larval fishes in the Newport River Estuary. *Fish. Bull. NOAA* 73 (1): 137-144.

Last, J. M. 1980. The food of twenty species of fish larvae in the west-central North Sea. *Fisheries Research Technical Report, MAFF, Directorate of Fisheries Research. Lowestoft* 60: 44 pp.

López-Jamar, E., S. H. Coombs, F. Alemany, J. Alonso, F. Álvarez, C. D. Barrett, J. M. Cabanas, B. Casas, G. Díaz del Río, M. L. Fernández de Puelles, C. Franco, A. García, N. C. Halliday, A. Lago de Lanzós, A. Lavin, A. Miranda, D. B. Robins, L. Valdés and M. Varela. 1991. A SARP pilot study of sardine (*Sardina pilchardus*) off north and northwest Spain in April/May 1991. *ICES C. M.* 1991/L: 69.

Robles, R., C. Porteiro and J. M. Cabanas. 1992. The stock of Atlanto-Iberian sardine: Possible causes of variability. *Rapp. P-V Reun. Cons. Int. Explor. Mer.* 195: 418-423.

Silva, A. and A. Miranda. 1992. Laboratory rearing of sardine larvae, *Sardina pilchardus* (Walb.), and early affects of starvation: a preliminary experiment. *Bol. Inst. Esp. Oceanogr.* 8 (1): 163-174.

Theilacker, G. and K. Dorsey. 1980. Larval fish diversity, a summary of laboratory and field research. *Intergovernmental Oceanographic Commission. Workshop Report* 28: 105-142.

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Authors declaration Paper III

Conway, D.V.P. Coombs, S.H., Fernández de Puelles M.L., Tranter, P.R.G. (1994). Feeding of larval sardine *Sardina pilchardus* (Walbaum) off the north coast of Spain. Boletín Instituto Español de Oceanografia, 10: 165-175.

D.V.P. Conway carried out the sample collection and analysis, data interpretation and preparation of the manuscript to 80% of the total effort.

M.L. Fernández de Puelles M.L. Fernandez de Puelles 3-3-00

P.R.G. Tranter 16/3/00.

S.H. Coombs S.H. Coombs 16/3/2000

Paper IV

Conway DVP, Coombs SH, Smith C (1997) Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES J Mar Sci 54: 136-147

ICES Journal of Marine Science, 54: 136–147, 1997

Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea

D. V. P. Conway, S. H. Coombs, and C. Smith

Conway, D. V. P., Coombs, S. H., and Smith, C. 1997. Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. – ICES Journal of Marine Science, 54: 136–147.

Fish eggs and larvae were analysed from 63 vertically stratified plankton hauls in the Irish Sea and southern North Sea. The dominant species were sprat (*Sprattus sprattus*), dragonet (*Callionymus* spp.), dab (*Limanda limanda*) and to a lesser extent rockling species, sandeel (*Ammodytes* spp.), whiting (*Merlangius merlangus*) and flounder (*Platichthys flesus*). There was little difference between species in the vertical distribution of either eggs or larvae. Most were concentrated in the upper 50 m of the water column, eggs in progressively increasing numbers towards the surface and larvae with a sub-surface peak at a depth of 10–15 m. The vertical distribution of eggs extended deeper in the water column than larvae, possibly due to some combination of eggs being spawned deeper and their passive susceptibility to turbulent mixing. There were no significant differences between day and night distributions and under mixed or isothermal conditions.

Key words: fish larvae, fish eggs, vertical distribution, Irish Sea, North Sea.

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Introduction

In temperate neritic waters, the majority of pelagic marine fish eggs are spawned at shallow depths (<50 m) and are neutrally or slightly positively buoyant (Sundby, 1991). These attributes favour hatching in the more productive surface waters. However, in European shelf waters many fish species spawn early in the year (Harding and Nichols, 1987; Nichols *et al.*, 1993), either before the establishment of a seasonal thermocline, or in areas that remain well-mixed throughout the year. This absence of water column stability may result in eggs and larvae being mixed deeper in the water column and in larvae being retained at a depth where food supply is inadequate or less suitable.

Information on the vertical distribution of fish eggs and larvae is sparse, partly due to sampling difficulties associated with their presence in generally low numbers and the specialised sampling equipment required to obtain vertical resolution (e.g. Longhurst Hardy Plankton Recorder – LHPR, Pipe *et al.*, 1981; Williams *et al.*, 1983; Multiple Opening/Closing Net and Environment Sensing System – MOCNESS, Wiebe *et al.*, 1976; pumps, Fortier and Harris, 1989). In the North Sea a few studies have provided information on the vertical distribution of eggs and larvae of individual fish species (e.g. Coombs *et al.*, 1981; Heath *et al.*, 1991; Kloppmann, 1991) or for a limited range of species (Harding and Nichols, 1987). In the Irish Sea, less sampling has been carried out for the vertical distribution of ichthyoplankton, with the descriptions being restricted to one species (Coombs *et al.*, 1992).

Between 1987 and 1990 sampling was carried out in the central Irish Sea and southern North Sea as part of a programme examining zooplankton production processes during the spring and early summer (Coombs *et al.*, 1994; Lindley *et al.*, 1994). At the same time the opportunity was taken to describe the vertical distribution of fish eggs and larvae under different environmental conditions. Results from the analysis of this material is presented here.

Methods

Zooplankton sampling

Fish eggs and larvae were sampled in the Irish Sea (Fig. 1a) and southern North Sea (Fig. 1b) using a modified

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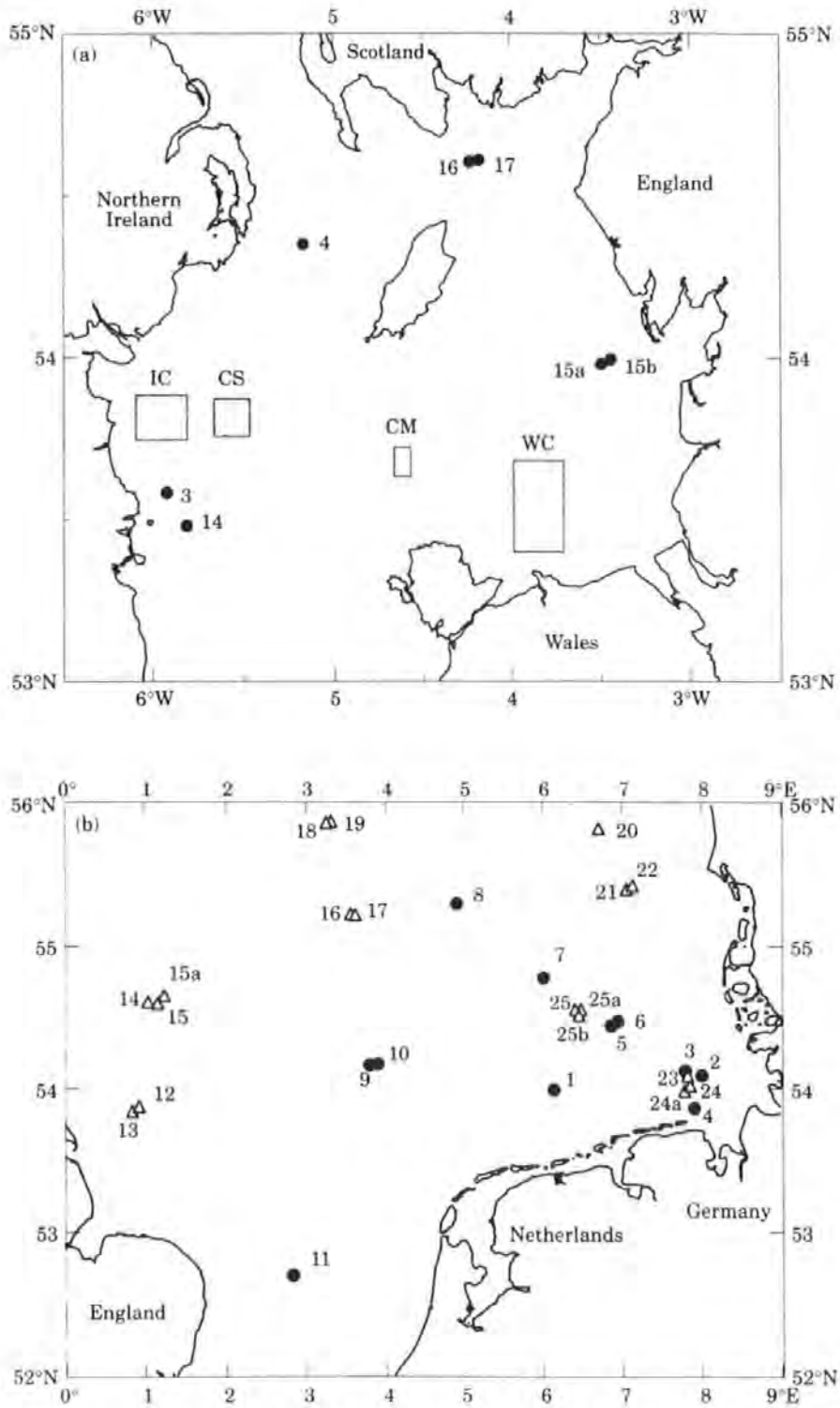

Figure 1. (a) Positions of LHPR stations in the Irish Sea; the four main sampling sites are identified as IC (Irish Coastal), CS (Central Stratifying), CM (Central Mixed) and WC (Welsh Coastal). Other haul positions are marked individually. (b) Positions of LHPR stations in the North Sea for June 1989 (●) and April 1990 (△).

LHPR sampler (Longhurst *et al.*, 1966; Williams *et al.*, 1983). This system collects plankton between two rolls of filtering gauze which are advanced at intervals inside a codend box to give a series of consecutive samples. The LHPR was towed at 3–4 knots on oblique hauls, sampling from the surface to near bottom while taking samples at intervals of 1 min, equivalent to approximately 5 m depth intervals. Mesh aperture of the collecting net and filtering gauze for all the hauls was 200 µm. Water temperature was recorded throughout the hauls and flow rate was monitored by a flowmeter mounted in the intake aperture, approximately 10 m^{-3} of water being filtered for each sample. On recovery of the sampler, the individual plankton samples were preserved in 4% borax buffered formaldehyde prepared using fresh water and subsequently sorted and analysed for fish eggs and larvae, with nomenclature following that of Russell (1976). Conductivity, temperature, depth (CTD) profiles were taken either by a Neil Brown or Guildline CTD before and after each LHPR haul.

In the Irish Sea four main sites were studied between March and June for the years 1987 to 1989 (Fig. 1a). These were: (a) a seasonally thermally stratified site of ~40 m depth close to the Irish coast, (b) a more central Irish Sea site of ~130 m depth which becomes strongly seasonally stratified, (c) a central Irish Sea, continuously mixed, isothermal site of ~70 m depth and (d) a site off the coast of north Wales of ~50 m depth, which is subject to considerable mixing. Two LHPR hauls were usually taken each time a site was visited, one at approximately midday and the other at near midnight (Table 1a). LHPR sampling was also carried out at a few additional positions in the Irish Sea as indicated in Fig. 1a.

In the southern North Sea, LHPR hauls were taken in June 1989 and April 1990 (Fig. 1b) at various times of the day and night, with some day/night pairs at the same position (Table 1b). Station positions were distributed over a wide area of the southern North Sea but with a higher concentration in the region of the German Bight; all are referred to collectively as North Sea samples. Sampling depths in the North Sea (11–67 m) were shallower than in the Irish Sea (16–125 m), reflecting differences in maximum water depth between the two areas. Further details of the environmental conditions in the two areas for the period when sampling took place are given in Coombs *et al.* (1992), Lindley *et al.* (1994) and Nichols *et al.* (1993).

Data processing

Results were used from 34 LHPR hauls (617 samples, Table 1a) in the Irish Sea and from 29 hauls in the North Sea (321 samples, Table 1b). For each individual species or group of species the vertical distribution data were processed only for those hauls on which there were a minimum of 20 eggs or 10 larvae, numbers below these levels representing sporadic occurrence only. Numbers of eggs and larvae in each haul were first standardised to give numbers m^{-3} in 5 m depth bands, using the measured volume filtered and depth range over which each sample was taken. These values were then converted to percentage depth distributions, both for comparability of presentation and to facilitate statistical analysis. Groups of hauls were combined to give overall mean depths by averaging the percentages (i.e. equal weighting being given to each haul). For comparison of depth distributions under different environmental conditions, weighted mean depth values (Roe *et al.*, 1984; Barenge, 1990) were calculated for each species or group on each haul and compared by single factor analysis of variance (ANOVA) using logarithmically transformed data where appropriate.

Results

Environmental conditions in the Irish Sea and North Sea

In the Irish Sea there is a restricted area of deep water (>100 m depth) in the west, with most other areas being <50 m in depth. There is strong tidal mixing in the shallower areas of the eastern Irish Sea (Dickson, 1987; Burkart *et al.*, 1995) and relatively low fresh water input, which is confined mainly to coastal areas in the east. These conditions were reflected in the observed hydrography. There was little thermal structure in the water column at all sampled sites in April (Fig. 2a, Table 1a). Seasonal stratification was not evident until about mid-May and had only developed to any extent by the end of May at the deep water central stratified site (0–50 m Δt of 2.5°C; Fig. 2b, Table 1a). Surface salinity at the sampled stations varied between 33.02–34.31 ppt with little observed salinity stratification (0–50 m Δs of <0.59 ppt at all stations) in any month of sampling.

The southern North Sea is largely <50 m in depth with increasing tidal mixing towards the shallower coastal areas. Relatively high levels of freshwater input can enhance stratification in coastal areas, especially in the German Bight and along the Dutch coast (Visser *et al.*, 1991). At all the sampling stations in April the water column was well mixed with negligible thermal stratification (Fig. 2c, Table 1b). During June the more coastal stations (e.g. NS1, NS3) generally showed development of a weak thermocline (0–50 m Δt of 1.2–3.9°C), while at the more offshore stations (e.g. NS6, NS8) there was a more marked development of thermal structure (0–50 m Δt of 7.2–9.6°C). On the most southerly haul (NS11), taken in the shallower water of the Southern Bight, the water column was fully mixed. Surface salinity at the North Sea sampling stations in April 1990 varied between 33.44–35.10 ppt with little salinity stratification (0–50 m Δs of <0.51 ppt at all stations). In June 1989

Table 1. LHPR haul information. The four sampling sites are IC (Irish Coast), CS (Central Stratifying), CM (Central Mixed) and WC (Welsh Coastal). Their positions are shown in Fig. 1a.
(a) LHPR haul information for the Irish Sea

Haul number	Date	Time GMT	Station position	Sampling site	Bottom depth (m)	Sampled depth (m)	Number of samples	0–50 m Δ (°C
RRS "Challenger" 29 March–19 April 1987								
IS3	2-4-87	12:28	53°35'N 05°56'W	—	52	50	22	0.1*
IS4	5-4-87	17:18	54°21'N 05°10'W	—	132	125	36	0.0
IS5	6-4-87	13:35	53°52'N 05°40'W	CS	101	100	31	0.1
IS6	9-4-87	12:38	53°53'N 05°58'W	IC	41	35	13	0.1
IS7	9-4-87	23:05	53°53'N 05°57'W	IC	41	39	13	0.1
IS8	11-4-87	12:10	53°50'N 05°28'W	CS	130	115	33	0.3*
IS9	11-4-87	23:46	53°50'N 05°28'W	CS	130	120	32	0.3*
IS11	15-4-87	12:34	53°40'N 03°59'W	WC	46	42	12	0.2
RV "Cirolana" 26 May–7 June 1987								
IS13	29-5-87	19:48	53°36'N 03°53'W	WC	42	38	12	0.6
IS14	30-5-87	15:11	53°29'N 05°49'W	—	74	73	23	1.5
IS15a	1-6-87	18:42	53°59'N 03°30'W	—	23	19	10	0.1
IS15b	1-6-87	19:00	53°59'N 03°30'W	—	23	16	10	0.2
IS16	4-6-87	13:21	54°36'N 04°14'W	—	60	47	26	0.9
IS17	4-6-87	23:42	54°36'N 04°15'W	—	56	50	20	0.9
RV "Cirolana" 9–21 April 1988								
IS18	11-4-88	23:29	53°47'N 05°49'W	IC	63	62	14	0.5
IS19	12-4-88	11:56	53°45'N 05°50'W	IC	56	50	12	0.5
IS20	14-4-88	12:09	53°51'N 05°32'W	CS	102	100	27	0.7
IS22	15-4-88	23:23	53°51'N 05°32'W	CS	108	106	24	0.3
IS23	17-4-88	12:23	53°41'N 03°59'W	WC	48	45	14	0.0
IS24	17-4-88	22:59	53°40'N 04°00'W	WC	48	40	13	0.0
RRS "Challenger" 21 May–4 June 1988								
IS25	25-5-88	00:13	53°49'N 05°33'W	CS	93	85	24	2.5
IS26	25-5-88	12:47	53°49'N 05°31'W	CS	105	100	20	2.3
IS27	26-5-88	14:51	53°24'N 03°43'W	WC	25	20	4	0.6
IS28	29-5-88	23:41	53°48'N 05°49'W	IC	47	40	12	2.2
IS29	30-5-88	12:55	53°48'N 05°56'W	IC	45	40	13	1.6
IS30	1-6-88	12:12	53°43'N 04°37'W	CM	65	60	18	0.1
RV "Cirolana" 14–29 April 1989								
IS32	19-4-89	13:05	53°29'N 03°45'W	WC	37	34	12	0.2
IS33	19-4-89	23:44	53°28'N 03°53'W	WC	38	32	14	0.1
IS34	21-4-89	12:29	53°50'N 06°06'W	IC	31	28	9	0.5
IS35	21-4-89	23:01	53°48'N 06°05'W	IC	31	28	11	0.3
IS36	22-4-89	23:09	53°45'N 05°29'W	CS	104	100	25	0.4
IS37	23-4-89	12:42	53°45'N 05°30'W	CS	107	99	22	0.4
IS39	25-4-89	12:56	53°41'N 04°35'W	CM	70	62	18	0.0
IS40	25-4-89	22:54	53°38'N 04°40'W	CM	86	85	18	0.1

*Increase in temperature towards bottom.

surface salinity was lower (30.00–33.71 ppt). Salinity stratification was greatest at the inshore German Bight stations (e.g. NS2 and NS3, 0–50 m Δs of 2.03–2.52 ppt) due to fresh water outflow. Tidal mixing at the most inshore station (NS4) prevented the establishment of any (observed) salinity stratification.

Fish eggs and larvae

The species composition and occurrence of fish eggs and larvae on LHPR hauls in the Irish Sea and North Sea are given in Table 2. In the Irish Sea, 21 species of eggs and 27 species of larvae were identified, a wider range than from the less intensive sampling in the North Sea where 15 species of eggs and 20 of larvae were recorded. Of the eggs, those of sprat (*Sprattus sprattus*), dragonet (*Callionymus* spp.) and dab (*Limanda limanda*) were the most abundant, together comprising 68% of all eggs in the Irish Sea and 92% in the North Sea. Eggs of rockling species were also relatively common in the Irish Sea (5.9% of all eggs). Larvae of *S. sprattus* (3–22 mm in length), *Callionymus* spp. (2–7 mm) and *L. limanda*

Table 1. LHPR haul information. The four sampling sites are IC (Irish Coast), CS (Central Stratifying), CM (Central Mixed) and WC (Welsh Coastal). Their positions are shown in Fig. 1a.
(b) LHPR haul information for the North Sea.

Haul number	Date	Time GMT	Station position	Bottom depth (m)	Sampled depth (m)	Number of samples	0–50 m Δ t°C
RRS "Challenger" 9–21 June 1989							
NS1	11-6-89	12:25	54°00'N 06°09'E	29	27	11	1.2
NS2	12-6-89	13:20	54°06'N 08°00'E	29	21	7	3.5
NS3	12-6-89	22:40	54°08'N 07°47'E	39	37	12	3.9
NS4	13-6-89	17:06	53°52'N 07°54'E	16	11	6	0.3
NS5	15-6-89	11:27	54°27'N 06°51'E	35	33	12	7.6
NS6	15-6-89	21:12	54°27'N 06°51'E	36	34	13	8.2
NS7	16-6-89	11:23	54°47'N 06°03'E	39	37	15	7.2
NS8	17-6-89	11:00	55°19'N 04°55'E	47	46	17	8.0
NS9	20-6-89	10:17	54°10'N 03°49'E	44	42	18	8.3
NS10	20-6-89	21:36	54°10'N 03°49'E	44	42	20	9.6
NS11	21-6-89	11:53	52°42'N 02°51'E	42	13	5	0.0
RV "Cirolana" 10–24 April 1990							
NS12	12-4-90	12:51	53°53'N 00°57'E	45	41	16	0.4
NS13	12-4-90	22:06	53°51'N 00°55'E	45	40	12	0.1
NS14	13-4-90	12:26	54°37'N 01°03'E	46	45	10	0.1
NS15	13-4-90	22:10	54°38'N 01°06'E	47	46	9	0.0
NS15a	13-4-90	22:50	54°38'N 01°06'E	43	40	9	0.2
NS16	15-4-90	12:09	55°14'N 03°34'E	26	24	6	0.0
NS17	15-4-90	22:08	55°14'N 03°37'E	27	26	6	0.0
NS18	16-4-90	12:03	55°52'N 03°16'E	69	66	19	0.3
NS19	16-4-90	21:35	55°52'N 03°17'E	68	67	16	0.3
NS20	18-4-90	22:02	55°49'N 06°43'E	39	38	11	0.1
NS21	19-4-90	11:57	55°24'N 07°03'E	30	29	8	0.1
NS22	19-4-90	21:40	55°26'N 07°06'E	30	29	8	0.1*
NS23	20-4-90	22:51	54°05'N 07°50'E	42	41	11	0.2
NS24	21-4-90	12:31	54°05'N 07°47'E	41	40	9	0.4
NS24a	21-4-90	12:45	54°05'N 07°47'E	40	34	9	0.3
NS25	22-4-90	20:14	54°33'N 06°30'E	38	37	8	0.3
NS25a	22-4-90	21:15	54°33'N 06°30'E	39	38	9	0.3
NS25b	22-4-90	21:30	54°33'N 06°30'E	39	38	9	0.2

*Increase in temperature towards bottom.

(2–17 mm) together comprised 64% of all larvae in the Irish Sea and 80% in the North Sea. Also common in the North Sea were larvae of sandeel (*Ammodytes* spp.) (6–26 mm in length; 7% of all larvae), whiting (*Merlangius merlangus*) (2–17 mm; 5.6%) and flounder (*Platichthys flesus*) (5–10 mm; 2.6%). Measurements taken make no allowance for larval shrinkage due to sample processing and preservation, but since this procedure was standardised for all hauls, shrinkage should be similar, at least within species. Typical shrinkage values for fish larvae preserved in 4% formaldehyde fall between 5 and 10% (Fox, 1996), but there are variations due to inter- and intra-specific differences and to processing routine and composition of the preservation fluid (Jennings, 1991). However, accurate length measurements were not an important component of the present study.

Vertical distribution of eggs and larvae

The variability in the mean depth distribution of the eggs between hauls was generally low, both within and between the Irish Sea and North Sea (range 13.1–22.8 m, S.E. <4.9 m; Table 3a). Exceptions were *Callionymus* spp. on stratified hauls ($\Delta t > 0.5$°C) and rockling species on night hauls (S.E.s of 13.5 m and 19.7 m respectively). There were no significant differences between mean depths due to time of day ($p = 0.531$–0.841) or temperature structure ($p = 0.104$–0.390).

Comparable information for the more abundant fish larvae is given in Table 3b. The weighted mean depths again fell within a relatively narrow range (12.3–26.2 m) as did most standard errors which were <8.7 m, except *P. flesus* (10.6 m, but based on 3 hauls only). There were also no significant differences between hauls related to temperature structure or time of day, except *L. limanda* where there was a significant difference in the distributions between mixed (mean depth 13.5 m) and stratified conditions (mean depth 21.8 m).

Due to the similarity in depth distributions between all hauls, it was justifiable to combine all hauls for each species or group to give a description of their overall

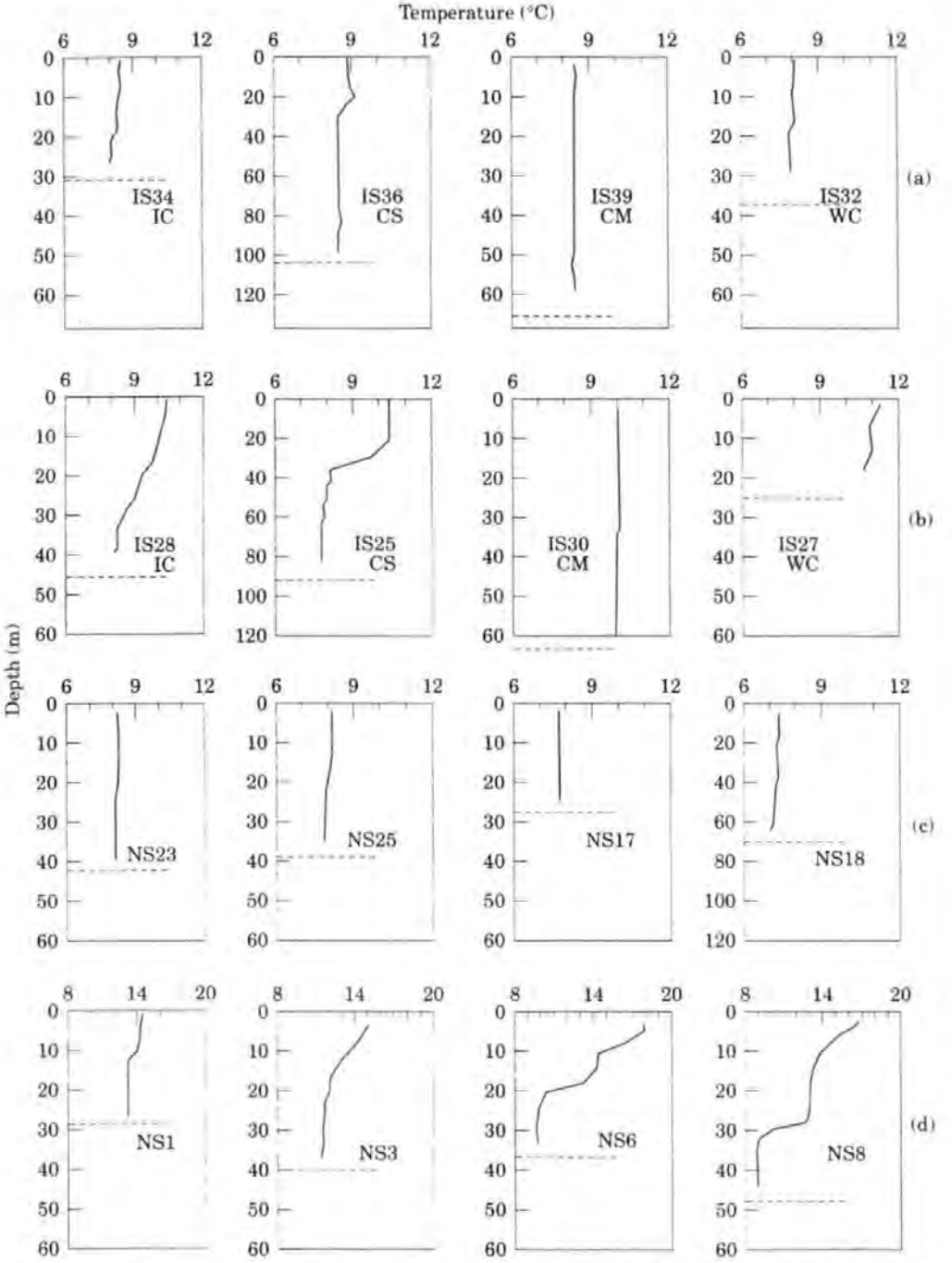

Figure 2. Representative CTD temperature profiles from the four sites, identified as IC (Irish Coastal), CS (Central Stratifying), CM (Central Mixed) and WC (Welsh Coastal), in the Irish Sea from (a) April 1989 and (b) May/June 1988 and from stations in the North Sea for (c) April 1990 and (d) June 1989. Broken lines indicate the water depth at each station.

pattern of vertical distribution. The resulting vertical distribution of eggs and larvae of *L. limanda*, *S. sprattus* and *Callionymus* spp., representing those species which were taken in higher numbers as both eggs and larvae, are plotted in Figure. 3. Their distributions were restricted mainly to the upper 60 m of the water column, with most within the top 30 m. Abundance of eggs of all three species increased towards the surface, whereas for larvae only those of *L. limanda* were most abundant at the surface, those of *S. sprattus* and *Callionymus* spp.

Table 2. Species composition and percentage occurrence of fish eggs and larvae in 34 LHPR hauls in the Irish Sea and 29 hauls in the North Sea (+ = occurrence at <0.1%).

Species	Irish Sea						North Sea					
	Positive hauls	Total eggs	% Total eggs	Positive hauls	Total larvae	% Total larvae	Positive hauls	Total eggs	% Total eggs	Positive hauls	Total larvae	% Total larvae
Agonus spp.	—	—	—	1	1	0.1	—	—	—	—	—	—
Ammodytes spp.	—	—	—	18	207	10.6	—	—	—	22	349	7.0
Arnoglossus laterna	1	6	0.1	2	19	1.0	—	—	—	—	—	—
Buglossidium luteum	2	2	+	5	13	0.7	5	46	0.3	4	11	0.2
Callionymus spp.	19	639	9.4	18	103	5.2	25	1631	10.4	12	186	3.8
Clupea harengus	—	—	—	1	2	0.1	—	—	—	1	1	+
Ctenolabrus rupestris	1	2	+	—	—	—	2	4	+	—	—	—
Gadus morhua	—	—	—	17	42	2.1	—	—	—	4	36	0.7
Glyptocephalus cynoglossus	—	—	—	3	6	0.3	—	—	—	—	—	—
Gobius spp.	—	—	—	13	35	1.8	—	—	—	5	6	0.1
Hippoglossoides platessoides	3	6	0.1	2	3	0.2	—	—	—	1	1	+
Limanda limanda	16	1738	25.5	22	284	14.5	27	8225	52.4	24	3170	63.9
Liparis spp.	—	—	—	6	18	0.9	—	—	—	—	—	—
Lumpenus lampretaeformis	—	—	—	3	13	0.7	—	—	—	—	—	—
Merlangius merlangus	—	—	—	15	99	5.0	—	—	—	15	277	5.6
Merluccius merluccius	1	1	+	—	—	—	2	3	+	—	—	—
Microchirus variegatus	1	1	+	—	—	—	—	—	—	—	—	—
Microstomus kitt	—	—	—	4	8	0.4	—	—	—	1	2	+
Molva molva	2	3	+	—	—	—	—	—	—	—	—	—
Mullus surmuletus	—	—	—	2	4	0.2	1	1	+	—	—	—
Pholis gunnellus	—	—	—	9	55	2.8	—	—	—	—	—	—
Phrynorhombus norvegicus	1	2	+	—	—	—	—	—	—	—	—	—
Platichthys flesus	4	7	0.1	2	4	0.2	—	—	—	6	128	2.6
Pleuronectes platessa	8	100	1.5	8	30	1.5	3	4	+	5	21	0.4
Pollachius pollachius	—	—	—	3	5	0.3	—	—	—	4	11	0.2
Rockling spp.	24	403	5.9	15	55	2.8	15	124	0.8	14	61	1.2
Scomber scombrus	6	155	2.3	—	—	—	8	139	0.9	6	38	0.8
Scophthalmus maximus	5	10	0.1	—	—	—	8	37	0.2	—	—	—
Scophthalmus rhombus	5	24	+	1	2	0.1	19	177	1.1	—	—	—
Solea solea	3	24	0.4	10	43	2.2	—	—	—	4	9	0.2
Sprattus sprattus	30	2234	32.7	25	873	44.5	20	4511	28.7	20	629	12.7
Taurulus bubalis	—	—	—	9	16	0.8	—	—	—	3	7	0.1
Trachurus trachurus	—	—	—	—	—	—	7	141	0.9	—	—	—
Trachinus vipera	—	—	—	—	—	—	2	71	0.5	—	—	—
Trigla spp.	13	116	1.7	1	1	0.1	13	39	0.2	1	1	+
Trisopterus spp.	2	3	+	7	21	1.1	—	—	—	6	15	0.3
Zeugopterus punctatus	3	5	0.1	—	—	—	—	—	—	—	—	—
Unidentified gadoid eggs	26	1346	19.7	—	—	—	27	544	3.5	—	—	—

Table 3a. Weighted mean depths and standard errors of the means for the more abundant fish eggs from all positive LHPR hauls in the North Sea and Irish Sea combined. The weighted mean depth range for all hauls, the number of hauls (n) and the probability (p) value from ANOVA analysis are also shown.

Species	Temperature structure/time of day	Mean depth (m)	Standard error	Range of mean depths (m)	n	p
Limanda limanda	Day	15.2	3.2	4.9–33.5	17	0.841
	Night	14.8	2.5	10.6–24.4	11	
	Isothermal	14.9	2.2	4.9–33.5	27	—
	Stratified	18.6	—	—	1	
	Total	15.1	2.1	4.9–33.5	28	
Sprattus sprattus	Day	14.1	3.2	4.4–28.4	20	0.698
	Night	15.1	2.2	9.3–19.9	9	
	Isothermal	15.2	3.0	5.4–28.5	19	0.390
	Stratified	13.1	3.6	4.4–19.1	10	
	Total	14.4	2.3	4.4–28.4	29	
Callionymus spp.	Day	18.4	4.9	4.9–50.1	18	0.546
	Night	16.4	3.0	10.1–26.9	12	
	Isothermal	16.3	2.1	11.2–31.4	24	0.213*
	Stratified	22.8	13.5	4.9–50.1	6	
	Total	17.6	3.2	4.9–50.1	30	
Rockling spp.	Day	14.1	9.1	8.0–27.6	4	0.643*
	Night	20.7	19.7	6.8–39.7	3	
	Isothermal	16.9	9.3	6.8–39.7	7	—
	Stratified	—	—	—	—	
	Total	16.9	9.3	6.8–39.7	7	

*Using logarithmically transformed data.

showing sub-surface peaks at 5–10 m and 10–15 m respectively.

The depth distributions of eggs of rockling species and larvae of *M. merlangus*, *Ammodytes* spp. and *P. flesus*, all of which were taken in moderate numbers as either eggs or larvae, are plotted in Figure 4. Eggs of rockling species, larvae of *P. flesus* and, to a lesser extent larvae of *M. merlangus* showed increased abundance towards the surface. There was some evidence of bimodality in the depth distribution of rockling eggs, possibly due to the presence of eggs of more than one species, which may also have accounted for the observed high standard error (19.7 m, Table 3a) of the mean depth. Larvae of *Ammodytes* spp. had a more even distribution down the water column, this possibly being related to the eggs being spawned demersally. Both *M. merlangus* and *P. flesus* had noticeably shallower distributions (<40 m) than other larvae taken (cf. Figs 3 and 4).

The combined distributions of all eggs and all larvae have been amalgamated in Figure 5. This figure highlights the deeper distribution of eggs (weighted mean depth of 35 m), which were taken down to the maximum depth sampled (100 m), compared to larvae (weighted mean depth of 20 m) which were restricted to depths above 55 m. Eggs were found in peak numbers at the surface while larvae have a sub-surface peak of abundance.

Discussion

There were few significant differences between the vertical distributions of different ichthyoplankton species from the Irish Sea and North Sea, either for eggs or larvae. In part, this may be due to the effects of averaging results from a number of hauls taken at different times and under different biological and hydrographic conditions.

For pelagic eggs, their vertical distribution is determined by the relationship between physical properties of the eggs, seawater density and the degree of vertical mixing of the water column (Sundby, 1991; Nissling *et al.*, 1994). The distributions observed, with increasing numbers of eggs towards the surface, is as expected for passive buoyant particles under the dominant influence of wind mixing at the surface (Sundby, 1991). The

Table 3b. Weighted mean depths and standard errors of the means for the more abundant fish larvae from all positive LHPR hauls in the North Sea and Irish Sea combined. The weighted mean depth range for all hauls, the number of hauls (n) and the probability (p) value from ANOVA analysis are also shown.

Species	Temperature structure/time of day	Mean depth (m)	Standard error	Range of mean depths (m)	n	p
Limanda limanda	Day	17.6	4.3	2.7–26.1	11	0.637
	Night	15.9	5.8	4.3–28.1	9	
	Isothermal	13.5	4.1	2.7–26.1	12	0.015
	Stratified	21.8	4.4	10.0–28.1	8	
	Total	16.8	3.5	2.7–28.1	20	
Sprattus sprattus	Day	13.7	2.7	4.9–23.2	16	0.236
	Night	16.3	3.0	9.3–24.4	10	
	Isothermal	14.4	2.4	4.9–21.2	16	0.619
	Stratified	15.4	3.9	6.7–24.4	10	
	Total	14.8	2.1	4.9–24.4	26	
Callionymus spp.	Day	14.6	4.7	5.9–24.7	7	0.350
	Night	12.3	6.7	4.1–20.2	4	
	Isothermal	13.1	8.7	4.1–24.7	4	0.789
	Stratified	14.2	3.8	5.9–20.2	7	
	Total	13.8	3.9	4.1–24.7	11	
Merlangius merlangus	Day	14.3	2.5	10.1–18.0	5	0.499
	Night	16.1	4.1	7.2–19.7	6	
	Isothermal	15.3	2.7	7.2–19.7	10	—
	Stratified	14.6	—	—	1	
	Total	15.3	2.7	7.2–19.7	11	
Ammodytes spp.	Day	19.8	3.9	11.4–26.5	8	0.143
	Night	26.2	7.2	10.2–41.8	8	
	Isothermal	23.6	4.8	10.2–41.8	14	—
	Stratified	18.4	—	15.8–21.0	2	
	Total	23.0	4.3	10.2–41.8	16	
Platichthys flesus	Day	18.7	—	—	1	—
	Night	13.7	10.6	8.2–24.3	3	
	Isothermal	14.9	7.9	8.2–24.3	4	—
	Stratified	—	—	—	—	
	Total	14.9	7.9	8.2–24.3	4	

occurrence of eggs in significant numbers in relatively deep water (>50 m depth) where wind mixing has little effect, may be attributed, in part, to tidal mixing, which is strong in many areas of the Irish Sea and possibly also to some species spawning deep in the water.

While the mean depth distributions of larvae were similar, the pattern of their distributions was variable. The vertical distribution of larvae is influenced by the same physical factors as for eggs but with the additional effects of ontogenetic and behavioural differences in response to environmental factors. There is a general feeding advantage in being distributed in the upper, more productive, layers of the water column, where food particles are present in higher abundance (e.g. Coombs *et al.*, 1992; Coombs *et al.*, 1994) and, more specifically, some studies have found the vertical distribution of larvae to be related to the depth of highest abundances of copepod nauplii (e.g. Haldorson *et al.*, 1993). Additionally, there is the influence of predation which may lead to the observed peak of larval abundance, this being the optimum balance between visual avoidance and food availability (Fortier and Harris, 1989).

The observed sub-surface peak of larval abundance may also reflect some aspect of diel migration, or net

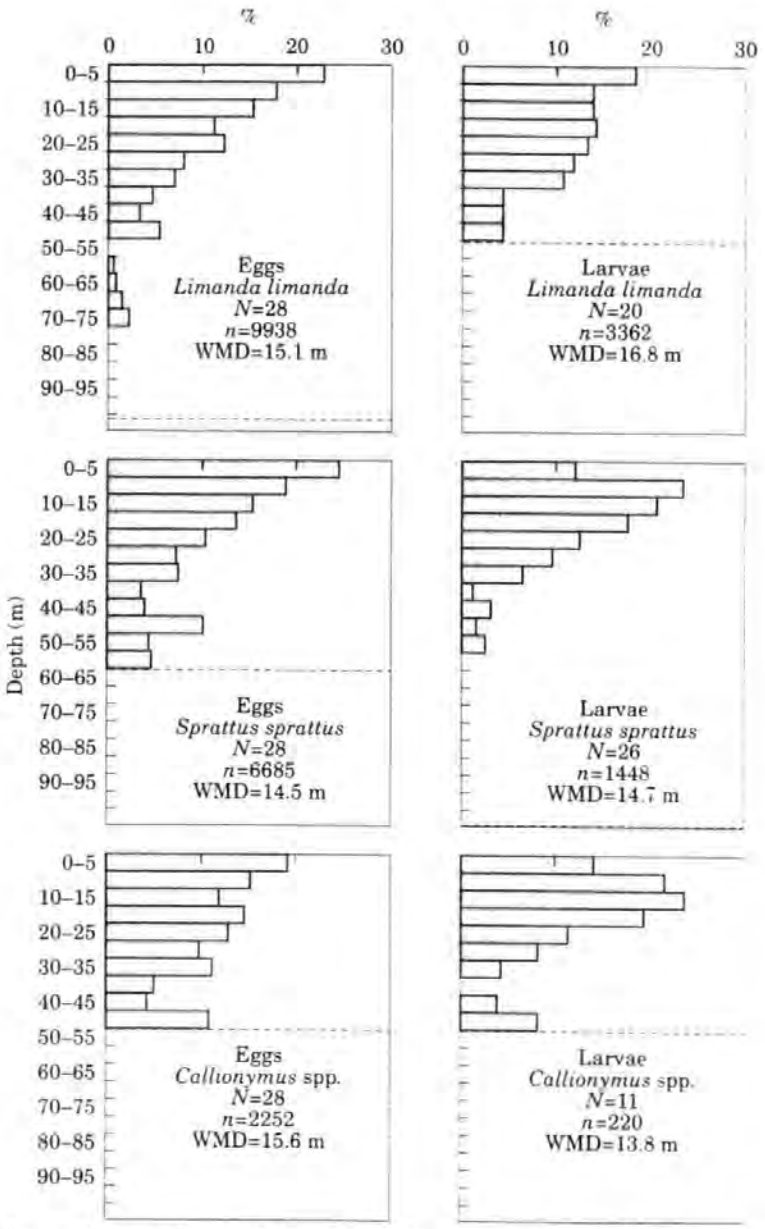

Figure 3. Vertical distribution of eggs and larvae of *Limanda limanda*, *Sprattus sprattus* and *Callionymus* spp. plotted as the mean percentage occurrence on all LHPR hauls from the Irish Sea and North Sea on each of which there were >20 eggs or >10 larvae. Also shown are the number of hauls (N) and total number of larvae (*n*) on which the distributions are based, together with the weighted mean depth (WMD) of the distributions. The maximum depth to which sampling was carried out is marked with a broken line.

avoidance in the more strongly illuminated surface layers. Although there was no direct evidence in the present study for diel migration of larvae, this can be difficult to detect unless it is synchronised amongst individuals (Pearre, 1979) and consistent over a particular size range of larvae. Röpke (1989) observed larvae of a number of species performing diel vertical migrations, but concluded that two of the species recorded in the present study (*Callionymus* spp. and *M. merlangus*) did not exhibit it. Similarly, Fortier and Harris (1989) found little difference in the diel vertical distribution of a range of fish larvae (mainly sampled within the top 40 m) except that larger larvae were sampled deeper in the water column with increasing size. Many studies have suggested that diel changes are simply a dispersal of larvae once the light stimulus disappears (Kloppmann, 1991; Leis, 1991), with the distance of dispersal being related to swimming ability. However, changes in

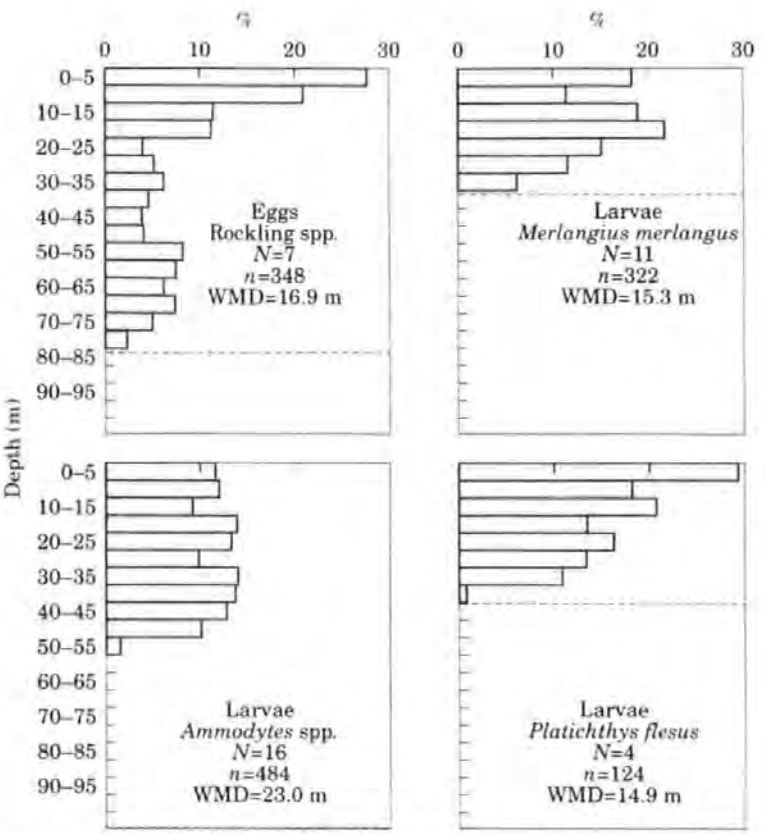

Figure 4. Vertical distribution of eggs of rockling species and larvae of *Merlangius merlangus*, *Ammodytes* spp. and *Platichthys flesus* plotted as the mean percentage occurrence on all LHPR hauls from the Irish Sea and North Sea on each of which there were >20 eggs or >10 larvae. Also shown are the number of hauls (N) and total number of larvae (*n*) on which the distributions are based, together with the weighted mean depth (WMD) of the distributions. The maximum depth to which sampling was carried out is marked with a broken line.

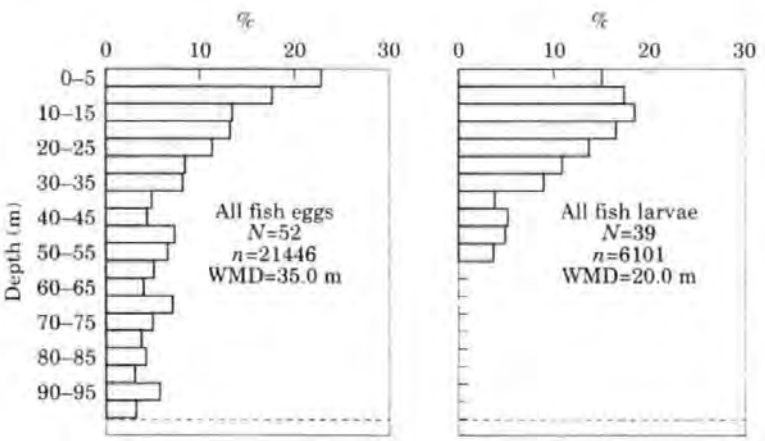

Figure 5. Vertical distribution of all eggs and larvae plotted as the mean percentage occurrence on all LHPR hauls from the Irish Sea and North Sea on each of which there were >20 eggs or >10 larvae. Also shown are the number of hauls (N) and total number of larvae (*n*) on which the distributions are based, together with the weighted mean depth (WMD) of the distributions. The maximum depth to which sampling was carried out is marked with a broken line.

vertical distribution can be difficult to demonstrate unless larvae are present in high concentrations and sampling is carried out at sufficient temporal and vertical resolution.

In the present study, there was little evidence of the influence of thermal stratification on the vertical distributions of larvae. Strong thermoclines, when present, tended to be at around 30 m depth, with the majority of larvae being above this depth irrespective of stratification. Other studies have generally shown a restriction of ichthyoplankton to the upper mixed layer (Coombs *et al.*, 1981; Boehlert *et al.*, 1985) and more rarely, under particular conditions, below the thermocline (Munk and Nielsen, 1994, for *S. sprattus* and *M. merlangus*). In the present study when a significant difference in depth distribution was noted between mixed and stratified conditions, as found for *L. limanda* larvae, the overall difference in weighted mean depth was only 8 m.

Acknowledgements

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References

Barenge, M. 1990. Vertical migration and habitat partitioning of six euphausiid species in the northern Benguela upwelling system. Journal of Plankton Research, 12: 1233–1237.

Boehlert, G. W., Gadomaski, D. M., and Mundy, B. C. 1985. The vertical distribution of ichthyoplankton off the Oregon coast in spring and summer months. Fishery Bulletin US, 83: 611–622.

Burkart, C. A., Kleppel, G. S., Brander, K., Holliday, D. V., and Pieper, R. E. 1995. Copepod and barnacle nauplius distributions in the Irish Sea: relation to springtime hydrographic variability. Journal of Plankton Research, 17: 1177–1188.

Coombs, S. H., Nichols, J. H., Conway, D. V. P., Milligan, S., and Halliday, N. C. 1992. Food availability for sprat larvae in the Irish Sea. Journal of the Marine Biological Association, United Kingdom, 72: 821–834.

Coombs, S. H., Pipe, R. K., and Mitchell, C. E. 1981. The vertical distribution of eggs and larvae of blue whiting (*Micromesistius poutassou*) and mackerel (*Scomber scombrus*) in the eastern North Atlantic and North Sea. Rapports et Procès-Verbaux des Réunions. Conseil International pour l'Exploration de la Mer, 178: 188–195.

Coombs, S. H., Robins, D. B., Conway, D. V. P., Halliday, N. C., and Pomroy, A. J. 1994. Suspended particulates in the Irish Sea and feeding conditions for fish larvae. Marine Biology, 118: 7–15.

Dickson, R. R. 1987. Irish Sea Status Report of the Marine Pollution Monitoring Management Group. MAFF Aquatic Environment Monitoring Report, 17: 83pp.

Fortier, L., and Harris, R. P. 1989. Optimal foraging and density-dependent competition in marine fish larvae. Marine Ecology Progress Series, 51: 19–33.

Fox, C. J. 1996. Length changes in herring (*Clupea harengus*) larvae: effects of capture and storage in formaldehyde and alcohol. Journal of Plankton Research, 18: 483–493.

Haldorson, L., Prichett, M., Paul, A. J., and Ziemann, D. 1993. The vertical distribution and migration of fish larvae in a Northeast Pacific bay. Marine Ecology Progress Series, 101: 67–80.

Harding, D., and Nichols, J. H. 1987. Plankton surveys off the north-east coast of England in 1976: an introductory report and summary of the results. Fisheries Research Technical Report No. 86. Directorate of Fisheries Research, Lowestoft, UK. 56pp.

Heath, M. R., Brander, K. M., Munk, P., and Rankine, P. 1991. Vertical distributions of autumn spawned larval herring (*Clupea harengus* L.) in the North Sea. Continental Shelf Research, 11: 1425–1452.

Jennings, S. 1991. The effects of capture, net retention and preservation upon lengths of larval and juvenile bass, *Dicentrarchus labrax* (L.). Journal of Fish Biology, 38: 349–357.

Kloppmann, M. 1991. Vertical distribution of sprat larvae in the German Bight. ICES CM 1991/L: 26, Biological Oceanographic Committee Theme Session V. 11pp.

Leis, J. M. (1991). The vertical distribution of fish larvae in the Great Barrier Reef Lagoon, Australia. Marine Biology, 109: 157–166.

Lindley, J. A., Williams, R., and Conway, D. V. P. 1994. Variability in dry weight and vertical distributions of decapod larvae in the Irish Sea and North Sea during the spring. Marine Biology, 120: 385–395.

Longhurst, A. R., Reith, A. D., Bower, R. E., and Seibert, D. L. R. 1966. A new system for the collection of multiple serial plankton samples. Deep Sea Research, 13: 213–222.

Munk, P., and Nielsen, T. G. 1994. Trophodynamics of the plankton community at Dogger Bank: predatory impact by fish larvae. Journal of Plankton Research, 16: 1225–1245.

Nichols, J. H., Haynes, G. M., Fox, C. J., Milligan, S. P., Brander, K. M., and Chapman, R. J. 1993. Spring plankton surveys of the Irish Sea in 1982, 1985, 1987, 1988 and 1989: Hydrography and the distribution of fish eggs and larvae. Fisheries Research Technical Report No. 95, Directorate of Fisheries Research, Lowestoft, UK. 111pp.

Nissling, A., Kryvi, H., and Vallin, L. 1994. Variation in egg buoyancy of Baltic cod *Gadus morhua* and its implications for egg survival in prevailing conditions in the Baltic Sea. Marine Ecology Progress Series, 110: 67–74.

Pearre, S. 1979. Problems of detection and interpretation of vertical migration. Journal of Plankton Research, 1: 29–44.

Pipe, R. K., Coombs, S. H., and Clarke, K. R. 1981. On the sample validity of the Longhurst-Hardy Plankton Recorder for fish eggs and larvae. Journal of Plankton Research, 3: 675–683.

Röpke, A. 1989. Small-scale vertical distribution of ichthyoplankton in the Celtic Sea in April 1986. Meeresforschung, 32: 192–203.

Roe, H. S. J., Angel, M. V., Badcock, J., Domanski, P., James, P. T., Pugh, P. R., and Thurston, M. H. 1984. The diel migrations and distributions within a mesopelagic community in the north east Atlantic. I. Introduction and sampling procedures. Progress in Oceanography, 13: 245–268.

Russell, F. S. 1976. The eggs and planktonic stages of British marine fishes. Academic Press, London. 524pp.

Sundby, S. 1991. Factors affecting the vertical distribution of eggs. ICES Marine Science Symposia, 192: 33–38.

Visser, M., Ruijtger, W. P. M. de, and Postma, L. 1991. The distribution of suspended matter in the Dutch coastal zone. Netherlands Journal of Sea Research, 27: 127–143.

Wiebe, P. H., Burt, K. H., Boyd, S. H., and Morton, A. W. 1976. A multiple opening-closing net and environmental sensing system for sampling zooplankton. Journal of Marine Research, 34: 313–326.

Williams, R., Collins, N. R., and Conway, D. V. P. 1983. The double LHPR system, a high speed micro- and macroplankton sampler. Deep-Sea Research, 30: 331–342.

Authors declaration Paper IV

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Paper V

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Feeding of anchovy *Engraulis encrasicolus* larvae in the northwestern Adriatic Sea in response to changing hydrobiological conditions

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ABSTRACT: Results from depth integrated and vertically stratified plankton sampling in the northwestern Adriatic Sea were used for comparison of gut contents of larvae of European anchovy *Engraulis encrasicolus* with composition and concentration of potential prey in the plankton. Sampling was carried out over a grid of stations both before and after a period of increased wind mixing to investigate changes in food availability and larval feeding success. All larvae had empty guts soon after dusk, indicating daytime feeding and rapid gut clearance. With increasing larval length there was a greater percentage of specimens with empty guts, despite suitable food being available in the plankton for these larger larvae; this suggests differential gut evacuation during sampling—possibly related to the degree of gut development. Larval diet was principally the various developmental stages of copepods, especially calanoid and cyclopoid nauplii, which were preferentially selected by larvae, whereas selection was against harpacticoid nauplii. Lamellibranch larvae and *Peridinium* spp. were generally abundant in the plankton, but were only present in the gut contents in any number when the preferred dietary organisms were present in the plankton at low concentrations. The number of food organisms in the gut contents increased with concentration of the preferred food organisms in the plankton up to a limit of ~50 organisms l^{-1}. Within the upper 18 m of the water column, there was a reduction in the proportion of larvae with food in their guts with increasing depth, irrespective of the vertical profile of food concentration. Following a period of wind mixing the composition of the plankton changed. This was reflected in the diet of anchovy larvae, which altered in parallel. There was also an overall 41% decrease in concentration of the preferred food particles of larvae in the plankton following the period of wind mixing, but larvae were still able to maintain their food intake. These results show that anchovy larvae can successfully adapt their diet to a changing prey field and suggest that in the conditions observed in the northern Adriatic, quite radical changes in the feeding environment were probably insufficient to affect overall larval mortality.

KEY WORDS: Anchovy larvae · Diet · Feeding success · Food selection · Wind mixing

INTRODUCTION

The recruitment strength of many fish stocks is thought to be determined within the first year of life (Bradford & Cabana 1997), and while much research has been directed towards establishing the contribution to mortality from variation in feeding success during the early larval stages, the connection is still controversial (Leggett & Deblois 1994). One problem in establishing this link has been that the results of laboratory studies on food requirements of larval fish are often at variance with field observations (MacKenzie et al. 1990). This discrepancy may be a reflection of a combination of inadequate attempts to replicate field conditions experimentally and field sampling techniques which do not accurately resolve the larval prey field. Regardless of the problems of reconciling studies on the relationship between feeding and recruitment, there is continuing evidence from field studies of a link

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between feeding success, availability of preferred food and the proportion of fish larvae which are in poor condition (Anderson 1994, Theilacker et al. 1996). Additionally, it has been shown in laboratory experiments that the mortality of larval fish can decrease with increasing food availability (Gotceitas et al. 1996) and that the size range of available food can determine growth rate and cohort size structure (Geffen 1996). Because mortality rates generally decrease with increase in larval size (Pepin & Myers 1991), feeding efficiency (quantity and assimilable content of food organisms, balanced against energy expended capturing them), in parallel with predation (Bailey & Houde 1989), must still be considered as a potentially prime biological factor in modifying recruitment.

A physical factor which has not been integral in most experimental studies, but which is considered to have a substantial effect on feeding success in larval fish, is degree of turbulence, due primarily to wind mixing, although this may have positive or negative effects (Dower et al. 1997). Moderate turbulence in the water column may act to enhance feeding success in larvae through changes in encounter rates between larvae and their prey, although this has to be balanced against the possibly increased energetic costs associated with feeding in turbulent waters. Conversely, turbulence may disrupt established thermal structure and plankton concentrations (Lagadeuc et al. 1997), resulting in a reduction in larval feeding success (Lasker 1975), decreased growth rates (Maillet & Checkley 1991) and increased mortality rates (Peterman & Bradford 1987).

Data for the present study were obtained on a cruise carried out in June/July 1996 in the northwestern Adriatic Sea in the region of the River Po outflow. This formed part of a joint programme between the Plymouth Marine Laboratory (PML) and the Istituto Centrale per la Ricerca scientifica e tecnologica Applicata al Mare (ICRAM), the overall aim of which was to measure the nutritional condition and survival of larval European anchovy *Engraulis encrasicolus* in response to changes in environmental conditions following a period of wind mixing (Coombs et al. 1997, McFadzen & Franceschini 1997).

The shallow northern Adriatic is vulnerable to periods of strong wind mixing, even during the summer months (July and August) of peak spawning of anchovy (Zore-Armanda & Gačić 1987, Regner 1996). An extensive sampling programme was carried out which gave broad-scale information on both hydrography and plankton distribution and was coupled with high resolution sampling at selected sites in order to determine the vertical distribution of anchovy larvae and their food resources. These data were utilised to give a detailed account of the diet of larval anchovy and to assess changes in diet and feeding success in response to food availability when stable hydrographic conditions were interrupted by a period of wind mixing.

METHODS

Sampling for anchovy larvae and associated plankton and concurrent environmental observations were carried out from the research vessel N/O 'Thetis' in the northwestern Adriatic Sea (Fig. 1) between 16 June and 12 July 1996.

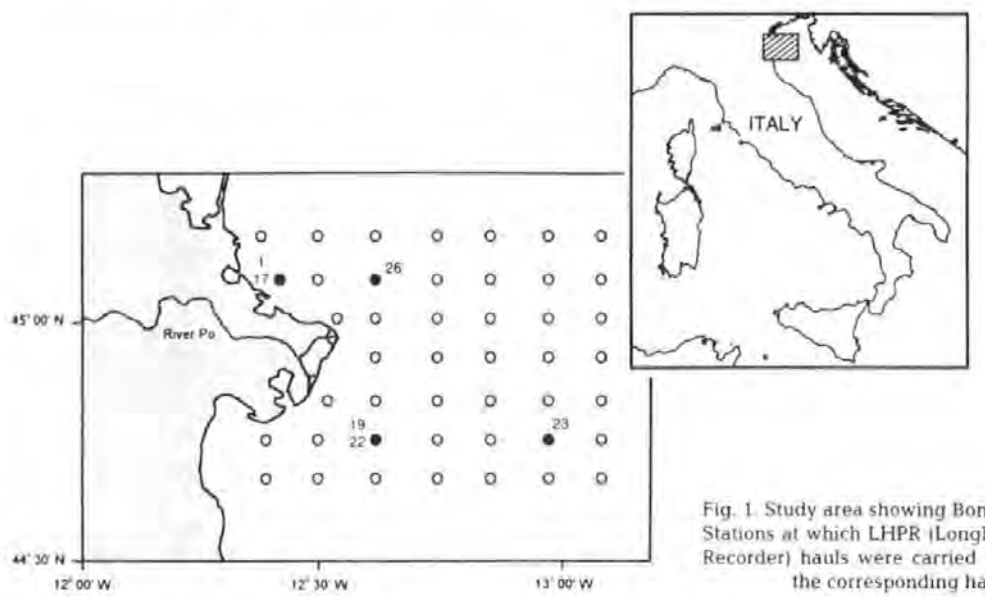

Fig. 1. Study area showing Bongo sampling stations. Stations at which LHPR (Longhurst Hardy Plankton Recorder) hauls were carried out are marked with the corresponding haul numbers

Table 1. Bongo net sampling grid information for anchovy larvae 2.4 to 10.9 mm in length

Grid	Date (1996)	Samples containing larvae	Samples containing feeding larvae	Total larvae examined	Larvae containing food
1	16–18 Jun	41	33	1011	326
2	2–4 Jul	44	28	840	176

Table 2. Dimensions and dry weight conversion factors for food organisms taken by anchovy larvae 2.4 to 10.9 mm in length

Organisms	Mean length (µm)	Mean width (µm)	Weight conversion factor (µg)
Peridinium spp.	74	37	0.10[a]
Copepod eggs	73	73	0.10
Calanoid nauplii	172	68	0.27
Cyclopoid nauplii	109	52	0.20
Harpacticoid nauplii	145	87	0.24
Oithona spp.	218	115	0.34
Other copepods	350	138	0.65
Lamellibranch larvae	74	64	0.17[a]

[a]Estimated from nauplii dimensions

Grid sampling. General features of the diet of anchovy larvae were studied from samples taken on 2 Bongo net sampling grids of 45 stations at a spacing of 5 nautical miles (Fig. 1). The first Bongo grid was sampled from 12:00 h local time (UTC + 2 h) on 16 June to 06:00 h on 18 June 1996; following an interval of 14 d, the second grid was sampled from 12:00 h on 2 July to 06:00 h on 4 July 1996. On both grids, sampling was carried out during both day and night. The coarse mesh (280 µm) Bongo sampler was 30 cm in diameter; attached below it was a 9 cm diameter fine mesh (53 µm) system. Both Bongo net systems were fitted with flowmeters to allow standardisation of the catch to unit volume filtered. The nets were towed at 2 knots from the surface to ~2 m above the sea bed in a double oblique haul. Values for real-time monitoring of sampler depth trajectory, together with sensor readings for flow, temperature and salinity, were obtained using an electronic sensor package mounted above the nets. Following each haul the plankton samples were preserved in 4% borax-buffered, fresh water formaldehyde solution (pH 8.2).

Larvae for feeding studies were subsequently sorted from the 280 µm samples. The term 'larvae' has been used generally here to refer to all specimens post-hatch. Whenever sufficient numbers of larvae were available, the complete digestive tracts of a minimum of 25 undamaged larvae were examined from each station of the 2 grids (Table 1). Larvae were measured (standard length) then the complete digestive tract was removed. Terminology for the regions of the digestive tract follows O'Connell (1976). The thin walled fore-gut is transparent and any food organisms within it could be identified and counted without dissection. The thicker walled mid- and short hind-gut were opened with dissecting needles in a petri dish containing water and examined under a binocular microscope. Sufficient larvae were obtained for analysis of results for specimens between 2.4 and 10.9 mm in length, which were separated into 1 mm length categories. Measurements taken make no allowance for shrinkage due to handling and preservation, but for clupeoids shrinkage is typically between 5 and 10% (Fox 1996).

Gut contents were identified to the lowest taxonomic level and counted. Because of the wide species range of copepod nauplii found in this region, these could only be identified to their Order, e.g. calanoid, cyclopoid or harpacticoid. Measurements of the size of food organisms were made for all measurable items. Dimensions measured were the total length and maximum width for copepod nauplii and *Peridinium* spp., cephalothorax length (not including the posterior reduced segment in *Oithona* spp.) and maximum width for copepods, maximum shell width and depth for lamellibranch larvae and diameter for copepod eggs. Using the observed mean dimensions, numbers of food organisms were converted to approximate dry weights (Table 2) utilising values from Hay et al. (1988) and equations from Thompson & Harrop (1991).

The 53 µm Bongo net samples were subsequently sieved through a 20 µm mesh, rinsed with fresh water to remove the formaldehyde and transferred to fresh water in a 300 ml graduated flask. Using a Stempel pipette, subsamples to provide approximately 150 of the organisms established from gut content analysis as the preferred dietary items of anchovy larvae were extracted and counted for estimates of regional food abundance. The counts of copepods were restricted to those <275 µm in cephalothorax width, this being the maximum width of the copepods measured in the diet.

Vertical distribution sampling. Longhurst Hardy Plankton Recorder (LHPR, Williams et al. 1983) hauls were taken at selected stations (Fig. 1) in order to study the vertical distributions of anchovy larvae and their potential food, and hence food selection by larvae at different prey concentrations down the water column. A double net LHPR system was used, this consisting of a coarse mesh net system of 280 µm or 500 µm mesh aperture (selected according to the ambient plankton

Table 3. Sampling information for anchovy larvae from Longhurst Hardy Plankton Recorder (LHPR) hauls for samples with >10 anchovy larvae 2.4 to 6.9 mm in length

Haul no.	Date (1996)	Time (local) (h)	Maximum depth sampled (m)	No. of samples	Total larvae examined	Larvae with food
1	21 Jun	11:30	20	9	283	122
17	7 Jul	13:30	18	3	110	99
19	9 Jul	14:50	26	6	225	93
22	10 Jul	10:30	28	2	32	15
23	10 Jul	15:45	36	1	22	10
26	11 Jul	16:36	26	7	125	48

concentration) for collection of the larvae and a separate 53 µm net fine mesh system for collection of the food organisms. The LHPR was towed obliquely at around 3.5 knots from the surface to ~1 m above the sea bed, simultaneously collecting coarse and fine mesh samples at a sampling interval of 1 min, with a vertical sample resolution of approximately 2 m depth. The coarse net filtered approximately 14 m^3 of water for each sample, while the fine mesh filtered approximately 200 l. Following each haul, samples were preserved in 4% borax-buffered, fresh water formaldehyde solution (pH 8.2). Values for real-time monitoring of depth and flow, together with profiles of temperature and salinity, were obtained on each haul via cored cable transmission of data from an electronic package mounted on the sampler frame. Anchovy larvae were subsequently sorted from the coarse mesh samples and analysed for gut contents in the same way as for specimens from the Bongo samples, sufficient larvae for analysis being available on 6 of the LHPR hauls (Fig. 1, Table 3). The 53 µm zooplankton samples were analysed for the concentration of food particles in the same way as for the fine mesh Bongo samples. Counts of organisms from the LHPR samples were standardised to numbers in 2 m depth strata to enable comparison between hauls.

Data analysis. The feeding incidence of the anchovy larval population was taken as the proportion of specimens containing at least 1 food particle, and the feeding intensity as the mean number of food particles in the gut contents of feeding larvae. Data were tested for normality using the Kolmogorov-Smirnov test and for homoscedasticity using Levene's test. Appropriate transformations were applied to non-normal and heteroscedastic data. Comparisons of feeding and food availability between Bongo grids were carried out using *t*-tests; where appropriate, the Mann-Whitney *U*-test was used as an alternative. The mean number and weight of food items in the guts of larvae of different lengths were compared by ANOVA and, where appropriate, the Kruskal-Wallis test. Because LHPR samples within hauls are not independent, data among depths within hauls were pooled for comparison. The relationships between food concentration and depth, the proportion of larvae containing food by depth and food concentration, and mean number of food items in the guts with depth and food concentration were tested by linear regression. Diet selectivity was assessed using the natural log transformation (ln) of the Odds Ratio method (Gabriel 1978, Hillgruber et al. 1995). This selectivity index is symmetrically distributed around a mean of zero and ranges from zero to $+\infty$ or $-\infty$ in cases of positive and negative selection, and the significance can be tested statistically.

RESULTS

Meteorology and hydrography

In the 2 wk prior to the commencement of the cruise on 16 June 1996, meteorological conditions were stable, with high atmospheric pressure resulting in low winds and a progressive increase in air and sea surface temperature. Weather conditions remained relatively stable during the first Bongo grid (16 to 18 June), with warm surface water (>24°C) over much of the sampling area. Low salinity water, originating from the River Po, extended eastwards across the sampling area, with values from <30 at the coast to >36 farther offshore. The combined effects of high surface temperature and low salinity gave relatively high stratification values over much of the sampled area (0 to 20 m $\Delta\sigma_t > 3$ kg m^{-3}), with highest values adjacent to the Po outflow area (0 to 20 m $\Delta\sigma_t > 7$ kg m^{-3}).

In the period 19 to 23 June, immediately following the first Bongo grid, the weather over the northern Adriatic was dominated by a series of depressions and associated fronts with high winds (20 to 30 knots) and rough seas. Unsettled weather continued for much of the period between the 2 grids, and markedly changed environmental conditions were recorded on the second Bongo grid (2 to 4 July), which was carried out during a break in the weather. Surface temperatures were lower (>22°C) and more uniform throughout the sampled area than on the first grid. Despite the increased wind mixing the pattern of surface salinity was similar to that of the first grid, but with the areas of lowest salinity (<32) more immediately localised around the Po outflow, reflecting the increased river run-off associated with precipitation during the period of poor

weather. Stratification was lower than on the first grid, but still appreciable over much of the survey area (0 to 20 m $\Delta\sigma_t > 2$ kg m^{-3}). Several days of unsettled weather followed the second Bongo grid, with conditions gradually improving until the end of sampling on 12 July.

Diurnal feeding pattern

Clupeoid larvae are particularly susceptible to evacuation (regurgitation or defaecation) of their gut contents during sampling and preservation (Arthur 1976). Consequently, while examination of gut contents will indicate the number of larvae containing food and the range of species consumed, it may not provide an accurate quantitative measure of food intake. Of the total anchovy larvae (2.4 to 10.9 mm in length) examined from both Bongo and LHPR sampling, 889 contained some food remains, but in only 2 larvae was there any food in the fore-gut. In all other specimens the food was in the mid- and hind-guts.

No larvae containing food were sampled from the Bongo grids between 21:34 and 04:37 h (Fig. 2). These times corresponded closely to sunset and sunrise times of ~21:00 and ~05:30 h respectively. Although there was agreement between the 2 grids in the timing of the larval feeding period, there was considerable variability in the percentage of larvae containing food at different times of the day, with no clear pattern being evident. Overall there was no significant difference between the 2 grids in the percentage of larvae containing food during daylight hours (Grid 1, 39.7 ± 2.37% and Grid 2, 38.5 ± 4.16%; Mann-Whitney U = 294, df = 47, p = 0.644).

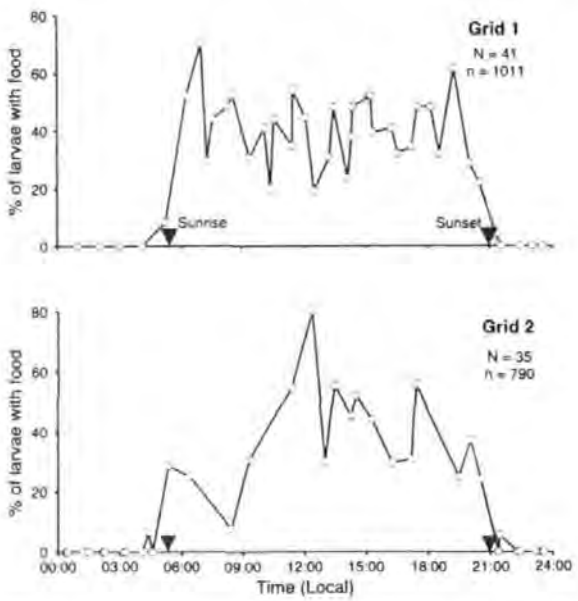

Fig. 2. *Engraulis encrasicolus*. Diurnal feeding incidence of anchovy larvae sampled from the 2 Bongo grids (Grid 1, 16 to 18 June; Grid 2, 2 to 4 July) plotted as the percentage of larvae with food in their guts for samples containing > 10 larvae. Times of sunrise and sunset are indicated. N = total number of samples, n = total number of larvae examined

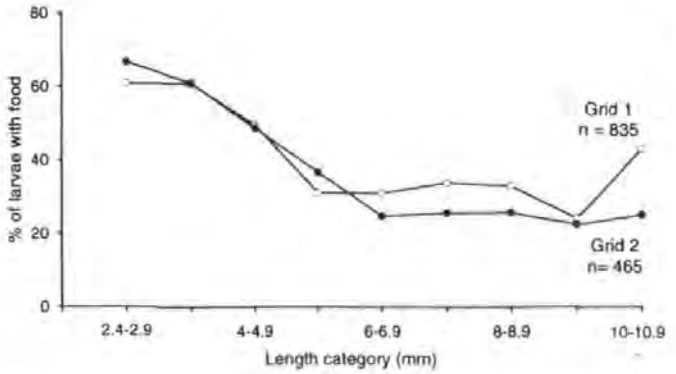

Fig. 3. *Engraulis encrasicolus*. Percentage incidence of anchovy larvae containing food in relation to larval length sampled on the 2 Bongo grids, based on day samples containing >10 larvae. n = total number of larvae on which the observations are based

Feeding incidence and intensity in relation to length

Considering samples taken during the day, feeding incidence decreased with increasing size of larvae to larval lengths of 5 to 5.9 and 6 to 6.9 mm on Bongo Grids 1 and 2, respectively (Fig. 3). Feeding incidence was then at a more constant level on both grids, although there was an increase on Grid 1 for larvae at 10 to 10.9 mm in length.

The maximum number of food organisms found in an individual larvae was 17 in a larva of 5.1 mm in length; otherwise the gut contents comprised mostly between 2 and 10 organisms, with a mean of between 1 and 4 organisms per larva containing food (Fig. 4a). The feeding intensity per larva containing food was not significantly different between the different larval length categories (ANOVA, $F_{8,493} = 1.16$, p = 0.320), but was significantly different between grids (t-test, $t_{500} = 3.26$, p = 0.001). The mean number of organisms per larva was 1.96 (± 0.089) on Grid 1 and 29% higher at 2.53 (± 0.167) on Grid 2.

As an indication of changes in the weight of gut contents in relation to larval length, numbers of food

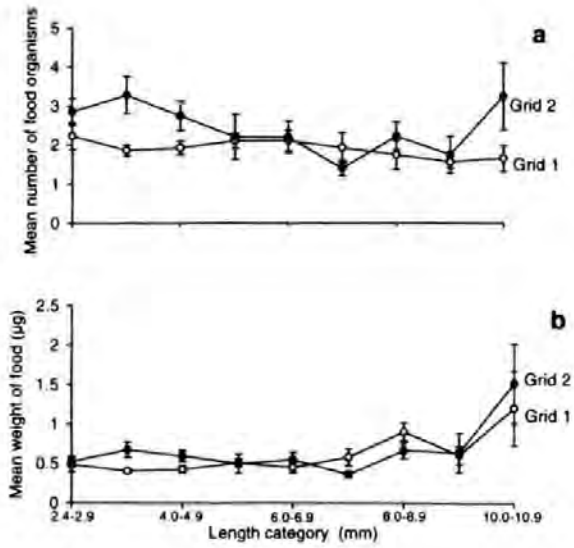

Fig. 4. *Engraulis encrasicolus*. Gut contents per anchovy larva containing food sampled on the 2 Bongo grids, plotted by larval length as (a) mean number and (b) mean dry weight. Error bars show ±1 SE

organisms were converted to dry weight (Table 2, Fig. 4b). In both grids the mean weight of food in the guts remained relatively unchanged at around 0.5 µg per larva containing food, up to a larval length of 7.9 mm, and then increased in the larger larvae. The mean weight of food organisms per larva was significantly different between larval length categories (ANOVA, $F_{8,471} = 5.86$, $p < 0.001$) but did not differ significantly between grids (*t*-test, $t_{478} = 1.38$, $p = 0.167$). The mean weight of gut contents of larvae containing food was 0.52 (± 0.025) µg on Grid 1 and 12% higher at 0.58 (± 0.037) µg on Grid 2.

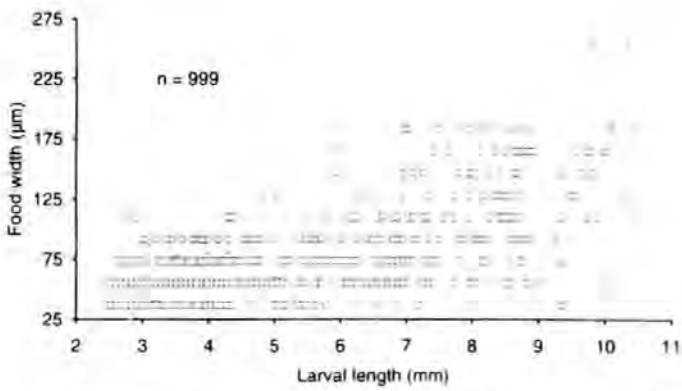

Fig. 5. *Engraulis encrasicolus*. Widths of individual food items taken by anchovy larvae from combined samples of the 2 Bongo grids. n = number of organisms measured

Table 4. *Engraulis encrasicolus*. Diet of anchovy larvae 2.4 to 10.9 mm in length, as the percentage feeding incidence on each food category, by feeding larvae, on each Bongo grid. +: values < 0.5%

Food organisms	Grid 1 (%)	Grid 2 (%)
Copepod eggs and nauplii		
Copepod eggs 73 µm in diameter	11	9
Calanus spp. eggs 183 µm in diameter	3	1
Calanoid nauplii	45	15
Cyclopoid nauplii	43	61
Harpacticoid nauplii	1	9
Copepod copepodites		
Calanus spp.	1	–
Para/Pseudo/Clausocalanus spp.	1	1
Acartia spp.	2	1
Centropages spp.	1	–
Calanoid copepod remains	7	2
Oithona spp.	20	26
Oncaea spp.	–	2
Corycaeus spp.	1	–
Unidentified cyclopoid copepod	+	–
Unidentified harpacticoid copepod	+	1
Other organisms		
Cladoceran remains	+	–
Lamellibranch larvae	–	3
Larvacea	–	1
Tintinnids	–	1
Unidentified remains	2	13

Size of food

There was a progressive increase in the maximum size of food organisms ingested with increase in larval length, but at the same time smaller organisms were still taken (Fig. 5). The smallest sized organism identified in the gut contents of the anchovy larvae was a tintinnid of 27 µm width and the largest a copepod copepodite of 275 µm cephalothorax width.

Dietary composition

The diet of anchovy larvae taken in the Bongo sampling was predominantly the developmental stages of copepods, including eggs, nauplii and copepodite stages (Table 4). Copepod nauplii were the most numerous component of the diet. Some of the more characteristic nauplii, such as the calanoid *Temora* spp. and the harpacticoid *Microsetella* spp., could be identified in the gut contents even when well digested. However, the other nauplii were potentially from a wide range of species and could not be routinely identified with certainty. Most of the harpacticoid copepod

Table 5. Mean concentration (no. l^{-1}) of the preferred food organisms of anchovy larvae 2.4 to 10.9 mm in length taken in the 53 µm samples on the 2 Bongo grids

Organisms	Mean concentration (95% CI) Grid 1	Grid 2	$t_{1,40}$	p
Copepod eggs (73 µm)	8.1 (5.9–11.2)	2.2 (1.7–2.7)	5.49[a]	<0.001
Calanoid nauplii	22.2 (17.1–29.0)	10.3 (8.1–13.3)	6.37[a]	<0.001
Cyclopoid nauplii	33.1 (23.2–43.0)	28.4 (17.7–39.1)	4.69	0.327
Harpacticoid nauplii	1.4 (0.9–1.9)	1.2 (0.7–1.7)	0.70	0.487
Oithona spp.	25.7 (20.0–33.0)	12.3 (9.9–15.4)	5.97[a]	<0.001
Other copepods	19.0 (15.2–23.7)	7.4 (6.3–8.7)	8.11[a]	<0.001
Total organisms	110.1 (82.8–137.4)	64.5 (45.9–83.1)	2.74	0.007

[a]Data were log transformed for statistical analysis

nauplii were probably of non-pelagic species, since, apart from *Microsetella* spp., few harpacticoid copepods were taken in the plankton samples. The copepod eggs 73 µm in diameter were possibly *Acartia* or *Temora* spp. Only in larvae from the LHPR samples were the additional food items, these being the dinoflagellate *Peridinium* spp. and pollen grains, found in low numbers. Most of the copepod eggs, lamellibranch larvae and *Peridinium* spp. showed little signs of digestion, while other organisms were digested to varying degrees.

Diet in relation to food availability

There was a clear difference in the percentage incidence of different food organisms in the diet of feeding anchovy larvae between the 2 Bongo grids (Table 4). In Grid 1, calanoid and cyclopoid nauplii were of similar importance in the diet, while harpacticoid nauplii were rarely taken. In Grid 2, feeding incidence on total copepod eggs, calanoid nauplii and copepods other than *Oithona* spp. was reduced compared with Grid 1, while it increased for cyclopoid nauplii, harpacticoid nauplii and *Oithona* spp.

When species analysis of all 53 µm Bongo samples, taken at the same time as the 280 µm samples for anchovy larvae (Table 1), were compared between the 2 Bongo grids, a change in the abundance of the preferred food organisms in the plankton was noted (Table 5). This change was similar to the dietary changes of the anchovy larvae observed between the grids. Between Grid 1 and Grid 2 there was a reduction of 73% in mean concentration of copepod eggs, a 54% reduction in calanoid nauplii and a 61% reduction in other copepods. However, contrary to the increase shown in the diet of anchovy larvae, there was a 52% reduction in *Oithona* spp. in the plankton. These changes were all significantly different (*t*-test, $p < 0.001$). Numbers of cyclopoid and harpacticoid nauplii were not significantly different between the 2 grids ($p > 0.05$). The mean overall concentration of the preferred food organisms was significantly reduced by 41% in Grid 2 compared to Grid 1 ($p = 0.007$).

Changes in the composition of diet with larval length

Copepod eggs and nauplii were taken by a wide size range of larvae, both of these dietary components diminishing in importance as the dietary range broadened with increasing larval length (Fig. 6). With increase in length, larvae took progressively larger food items, first the small cyclopoid copepod *Oithona* spp. and then other larger copepod copepodites. The most apparent difference between the 2 Bongo grids was the shift to larger larvae feeding on *Oithona* spp. between Grids 1 and 2.

Vertical distribution of anchovy larvae and zooplankton

Six LHPR hauls (Table 3) had sufficient larvae to compare the vertical distributions of larvae and potential food organisms (Fig. 7). The plots are restricted to data in the upper 18 m of the water column since this is the depth range in which most larvae occurred and was a common depth range sampled on all LHPR hauls.

LHPR Haul 1 (21 June) was taken after the first Bongo grid, when stable conditions had just given way to periods of strong wind mixing. Hauls 17, 19, 22 and 23 (7, 9, 10 and 10 July, respectively) were taken after the second Bongo grid during a period of gradually improving weather. Haul 26 (11 July) was taken during very settled conditions. On only 3 hauls (Hauls 1, 17 and 26) was there pronounced temperature structuring (Fig. 7), but with near isothermal conditions in the top 10 m of the water column. Because all LHPR hauls were taken during or closely following periods of variable wind mixing, and because the inertial response of larval feeding is not known, a direct comparison

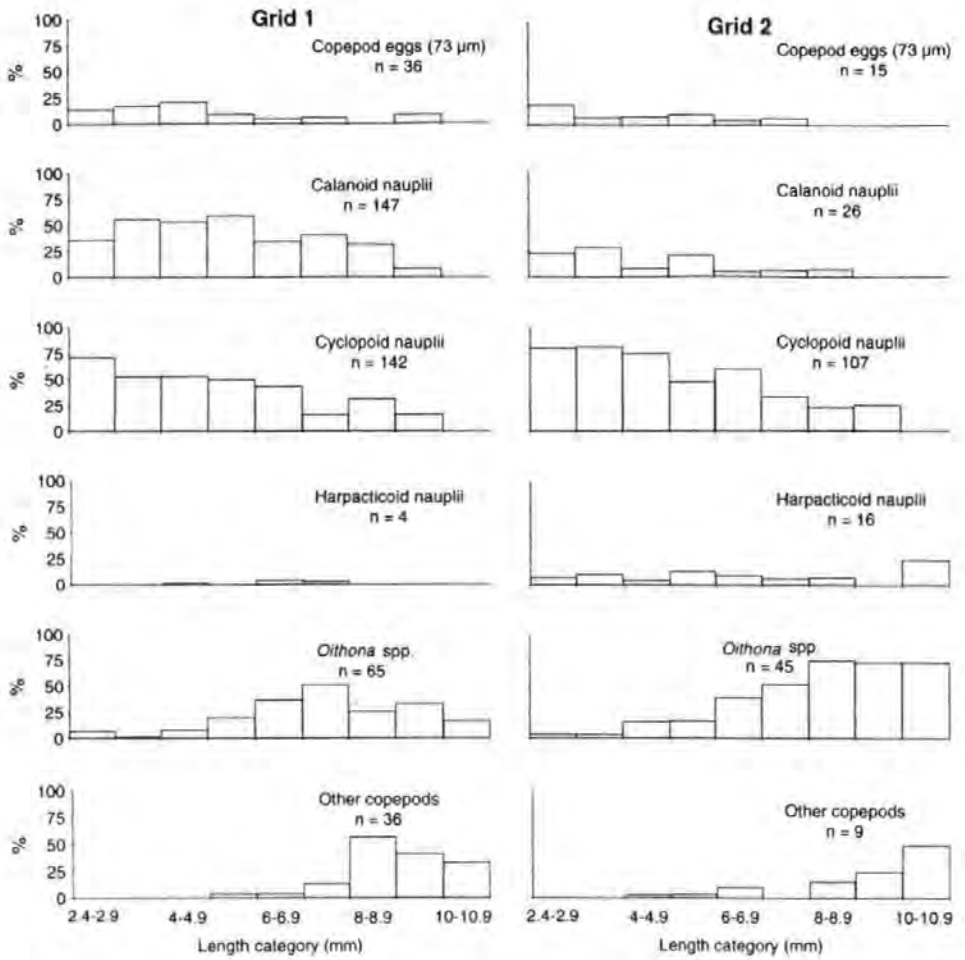

Fig. 6. *Engraulis encrasicolus*. Diet of anchovy larvae plotted as percentage distribution of the principal food items in each larval length category for the 2 Bongo grids. n = number of food items on which the distributions are based

between feeding and hydrographic conditions at the actual time of sampling was not considered valid. Analysis was restricted to larvae within the 2.4 to 6.9 mm length category, this being the size range which represented 86% of all larvae sampled on the LHPR hauls. This length category of larvae had a uniformly restricted diet of copepod eggs, copepod nauplii and copepodites of *Oithona* spp., with these items numerically comprising 96% of all food organisms taken.

Excluding LHPR Haul 17, larval anchovy concentrations tended to be relatively low in the upper 2 m of the water column and either highest just below this, decreasing in numbers with depth, or more evenly distributed throughout the water column. The concentration of preferred food items tended to be lower in the top 2 m of the water column than at greater depths (Fig. 7). Highest total concentrations of food items were mainly located between depths of 3 and 10 m, varying in this depth zone between 34 and 135 organisms l^{-1} on Hauls 1, 17 and 23, and at lower levels between 22 and 44 organisms l^{-1} on Hauls 19 and 22. On Haul 26, stratification of food organisms was most extreme, with concentrations varying between 18 and 188 organisms l^{-1}. Calanoid nauplii predominated over cyclopoid nauplii in Haul 1 but not in the other hauls. It is significant that LHPR Haul 1 was taken early in the cruise (21 June), before the reduction in numbers of calanoid nauplii observed in the 53 µm Bongo samples of the second grid (2 to 4 July).

Of the less important food items for anchovy larvae 2.4 to 6.9 mm in length, copepod copepodites were fairly evenly distributed through the water column, with numbers varying considerably between hauls. Lamellibranch larvae occurred in high numbers in most of the hauls, in concentrations up to 157 l^{-1}. *Peri-*

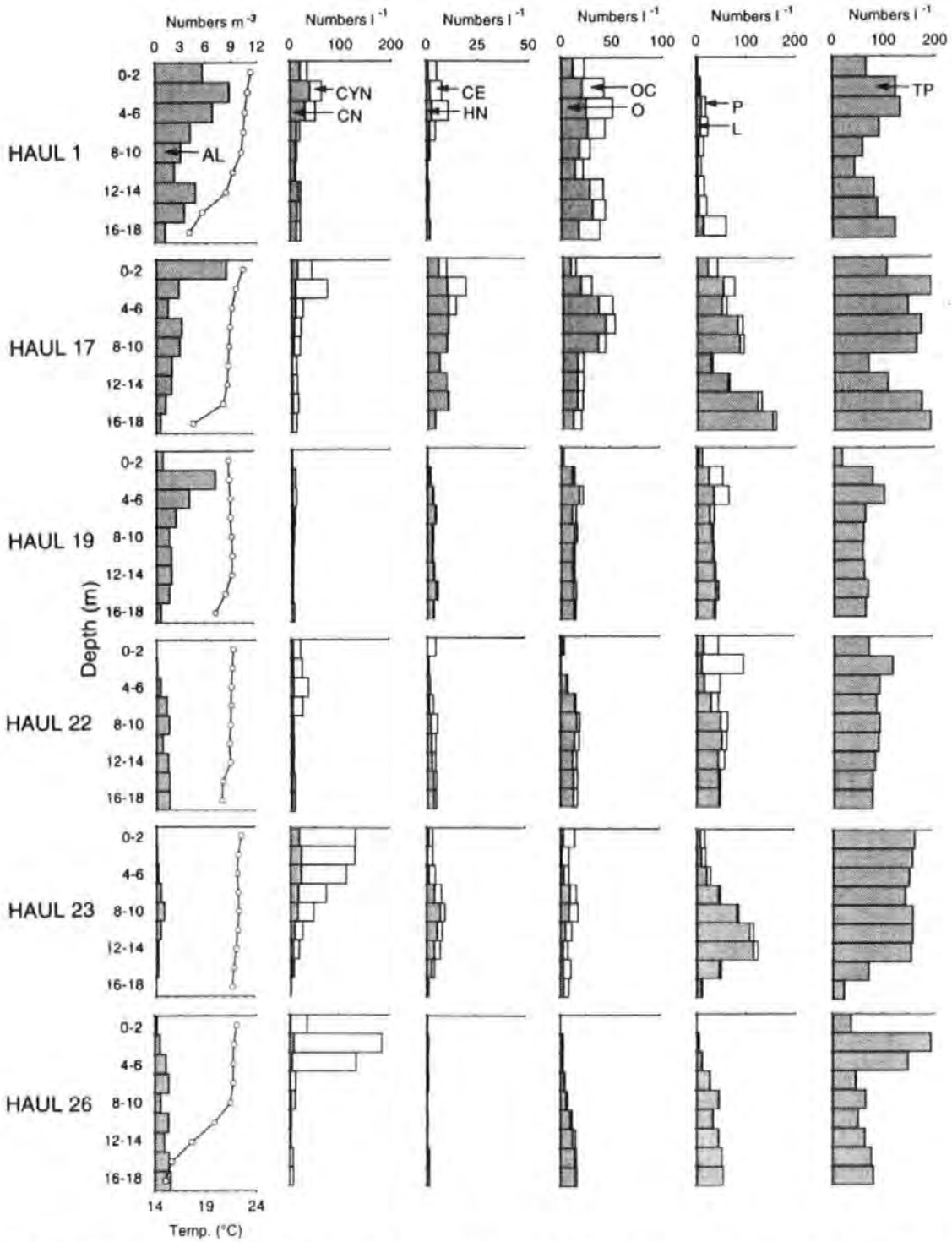

Fig. 7. Vertical distribution of potential food items for anchovy larvae (2.4 to 6.9 mm in length), from 53 µm mesh LHPR samples for the 6 hauls on which there were adequate numbers of anchovy larvae. Vertical distribution of anchovy larvae from the 200 µm mesh LHPR samples on the same hauls and temperature structure of the water column are also shown. AL: anchovy larvae; CYN: cyclopoid nauplii; CN: calanoid nauplii; CE: copepod eggs (73 µm); HN: harpacticoid nauplii; OC: other copepods; O: *Oithona* spp.; P: *Peridinium* spp.; L: lamellibranch larvae; TP: total potential food organisms

dinium spp. were found throughout the water column but tended to be present in highest concentrations, up to 85 l^{-1}, within the upper 10 m.

Comparisons between hauls of the highest concentrations of total potential food organisms in the individual 2 m depth intervals showed a variation of 18 l^{-1} (LHPR Haul 19) and 200 l^{-1} (LHPR Haul 17). The overall vertical distribution of food items was in most cases relatively uniform and showed no clear correspondence with temperature structure.

Changes in feeding success in relation to depth sampled and food concentration

There were insufficient numbers of anchovy larvae on any individual LHPR haul (Table 3) to permit a within-haul comparison of feeding success and concentration of the preferred food organisms (copepod nauplii, copepod eggs and *Oithona* spp.) by depth. For this analysis it was therefore necessary to pool the data from samples with >10 anchovy larvae per sample for the 6 LHPR hauls. This provided 28 samples with 797 larvae for statistical analysis.

For these combined hauls/samples, food concentration did not vary significantly with depth between the surface and 18 m depth (r^2 = 9.4%, $F_{1,26}$ = 2.70, p = 0.112), these 2 variables being subsequently treated as independent. However, there were significant negative relationships between depth and both the percentage of larvae containing food (r^2 = 40.4%, $F_{1,26}$ = 17.62, p < 0.001; Fig. 8a) and the mean number of food items in the gut contents (r^2 = 16.2 %, $F_{1,26}$ = 5.04, p = 0.033; Fig. 8b). There were also significant positive linear relationships between food concentration and the percentage of larvae with food (r^2 = 31.5%, $F_{1,26}$ = 11.94, p = 0.002; Fig. 8c) and the mean number of food items in the gut contents of larvae (r^2 = 29.2%, $F_{1,26}$ = 10.71, p = 0.003; Fig. 8d). A multiple regression of percentage of larvae with food using larval depth and food concentration as independent variables yielded an r^2 of 55.2% ($F_{2,25}$ = 15.39, p < 0.001) and takes the form:

arcsine(proportion of larvae feeding) = 0.12 + 0.42 log food concentration (items l^{-1}) − 0.40 log depth (m)

There was a possible interaction among variables (Lack of Fit Test, p = 0.007). However, the overall significance of the multiple regression remains valid.

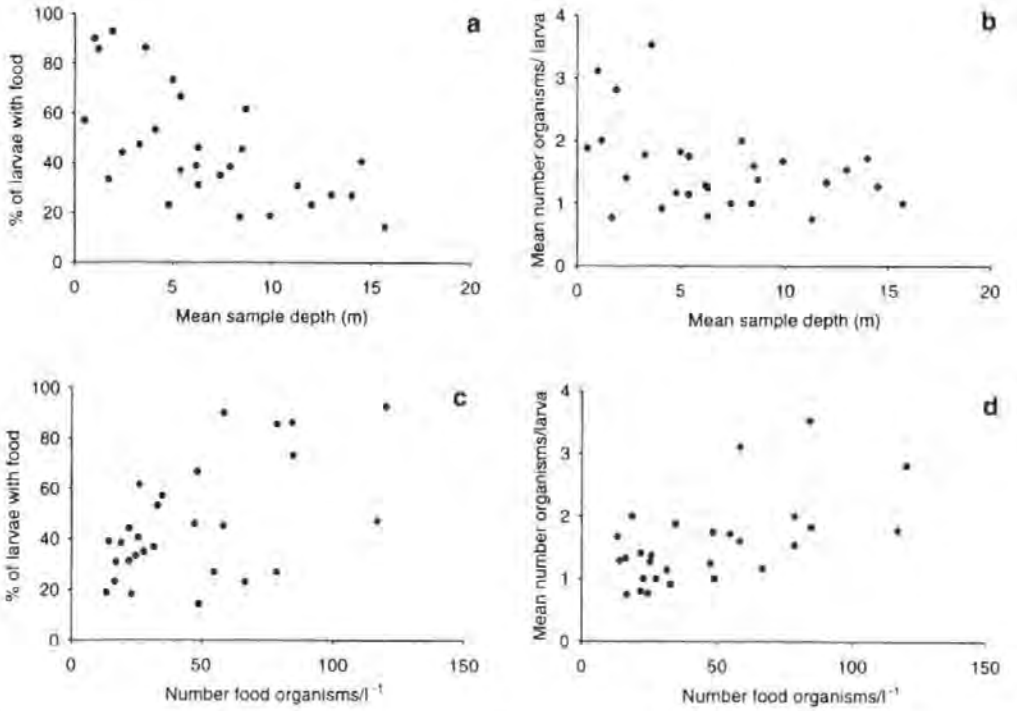

Fig. 8. *Engraulis encrasicolus*. Relationship on the 6 LHPR hauls (Table 3) between (a) sampling depth and percentage of anchovy larvae containing food organisms, (b) sampling depth and mean number of food organisms per feeding larva, (c) ambient food concentration and percentage of larvae containing food items, and (d) ambient food concentration and mean number of food organisms per feeding larva. Data for all plots are for larvae 2.4 to 6.9 mm in length from samples on the LHPR hauls containing >10 larvae

Table 6. *Engraulis encrasicolus*. Percentage occurrence of the preferred food organisms in the gut contents of anchovy larvae 2.4 to 6.9 mm in length and their percentage occurrence over the corresponding depths in the water column in the 53 µm mesh samples from the same LHPR hauls. Results of the Odds Ratio selectivity analysis (± 1 SE) are also shown where there were comparative data: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$

Prey percentages

Haul no.	Calanoid nauplii		Cyclopoid nauplii		Harpacticoid nauplii		Copepod eggs (73 µm)		*Oithona* spp.	
	Diet	Water column	Diet	Water column	Diet	Water column	Diet	Water column	Diet	Water column
1	40	34	52	17	1	2	3	5	3	41
17	12	14	83	40	1	12	3	7	1	27
19	25	11	58	22	–	10	6	2	11	54
22	21	9	64	35	–	4	7	8	7	44
23	56	26	19	42	–	14	6	3	19	15
26	22	8	59	64	3	2	1	2	14	24
All hauls combined	29	17	56	37	1	7	4	5	9	34

ln of Odds Ratio

Haul no.	Calanoid nauplii		Cyclopoid nauplii		Harpacticoid nauplii		Copepod eggs (73 µm)		*Oithona* spp.	
1	0.27	(0.17)	1.65***	(0.18)	−0.78	(0.76)	−0.56	(0.45)	−3.07***	(0.42)
17	−0.14	(0.23)	2.02***	(0.19)	−2.76***	(0.62)	−0.89*	(0.39)	−4.07***	(0.71)
19	0.94**	(0.36)	1.58***	(0.29)	–		0.98	(0.69)	−2.28***	(0.36)
22	1.05	(0.67)	1.20*	(0.51)	–		−0.15	(0.94)	−2.32*	(0.88)
23	1.27*	(0.59)	−1.15	(0.69)	–		0.79	(1.29)	0.29	(0.74)
26	1.23*	(0.35)	−0.21	(0.27)	0.30	(0.78)	−0.49	(1.28)	−0.64	(0.36)
All hauls combined	0.71***	(0.11)	0.78***	(0.09)	−2.25***	(0.42)	−0.03	(0.22)	−1.64***	(0.14)

Thus, most of the variability in the proportion of larvae with food can be explained by variation in larval depth and food concentration.

Food selection

Based on the vertical distribution LHPR data, there were no significant differences between the relative abundance of food organisms in the gut contents of anchovy larvae and food concentration at the same depth (Mann-Whitney *U*-test, $p > 0.05$). However, considering the preferred prey organisms, anchovy larvae (2.4 to 6.9 mm in length) significantly selected for calanoid and cyclopoid nauplii and significantly selected against harpacticoid nauplii and *Oithona* spp. (Table 6). Copepod eggs were present in the larval gut contents in proportion to their concentration in the water column.

Although there were inadequate data for statistical analysis, the few occasions when anchovy larvae fed on lamellibranch larvae and *Peridinium* spp. are of interest. On LHPR Haul 19, 13% of anchovy larvae with food in their guts were noted to have fed on lamellibranch larvae and 4% on *Peridinium* spp., all these anchovy larvae occurring within the top 8 m of the water column; on this haul there was the lowest concentration of copepod nauplii and some of the highest combined concentrations of lamellibranch larvae and *Peridinium* spp. in the top 8 m of all the LHPR hauls (Fig. 7). On LHPR Haul 22, 12% of anchovy larvae with gut contents were noted as having fed on *Peridinium* spp., all the larvae occurring within the top 8 m of the water column; on this haul there was the second lowest concentration of copepod nauplii and the highest concentration of *Peridinium* spp. in the top 8 m of all the LHPR hauls.

DISCUSSION

Brief descriptions of the diet of European anchovy larvae have been given for the northwestern Adriatic Sea by Coombs et al. (1997), for the Croatian coastal region of the mid-Adriatic by Regner (1971), from the Black Sea by Pavlovskaja (1961) and from Spanish coastal waters in the northwestern Mediterranean by Tudela & Palomera (1995). In all of these limited feeding studies the diet was a restricted range of organisms, predominantly the developmental stages of copepods, especially nauplii, similar to the diet of most larval fish (e.g. Last 1980). Ferreira & Ré (1993)

described the diet of anchovy from a Portuguese estuary, but the dietary composition (>80% tintinnids) differed considerably from that found in more open sea studies. In the present study, apart from low numbers of *Peridinium* spp., phytoplankton did not appear to be important in the diet of first feeding anchovy larvae, which has been noted in other studies (Tudela & Palomera 1995), although Regner (1971) recorded green amorphous material in the guts of the earliest stage larvae. Larvae at all lengths fed on copepod nauplii, and with increase in length and mouth gape also took larger organisms. Larval fish, as they develop, typically show alterations in prey size at a considerably lower rate than their physical capacity allows (Pepin & Penney 1997). *Oithona* spp., which are intermediate in size between copepod nauplii and larger copepod species, were an important food item, as they are in the diets of a wide range of larval fish (Last 1980). While only small numbers of copepod eggs, lamellibranch larvae and *Peridinium* spp. were taken, these are all items which may resist digestion (Conway et al. 1994b) and usually appeared to be undigested. These organisms have good nutritional potential and have been shown to be digested by some larval fish species (e.g. Bainbridge & McKay 1968, Jenkins 1987).

Larval fish typically have a higher feeding incidence and intensity during the day than at night (Last 1980); this is associated in the majority of cases with a reliance on visual feeding (e.g. Batty 1987). However, for the larvae of most fish species, food is retained in the guts for at least a substantial part of the night (Last 1980). During this period of inactivity they can digest and assimilate food efficiently (Canino & Bailey 1995). Anchovy larvae are unusual among fish larvae in that their guts were completely emptied within 30 min of sunset, suggesting a different energetic strategy from the larvae of the majority of other fish species. This apparent rapid passage rate of food should allow a more accurate comparison of the gut contents with the ambient food in the plankton than for other species with slower gut passage rates which may ingest their food over a longer time prior to capture.

In samples taken during the day in the present study, the percentage of anchovy larvae containing food decreased rapidly with increase in larval length up to about 5 to 6.9 mm in length and then levelled off before increasing again, at least in Grid 1, at a length of 10 to 10.9 mm. This observation is similar to the results of Arthur (1976), who compared the feeding incidence/length relationship of *Sardinops sagax* and *Engraulis mordax* with that found in 5 other studies on *Engraulis* spp. All showed initial decreases in feeding incidence with length, followed by an increase from 12 mm in length. It is possible that there is either a lack of suitable food for larger larvae, which was not apparently the situation in the present study, or that, over this intermediate stage of gut development, clupeoid larvae are particularly susceptible to evacuation of food as a result of sampling trauma.

The effects of food evacuation may also be reflected in the mean number of organisms per anchovy larvae with food in their gut contents. This varied from 1.4 to 3.3 particles per larva, with no evidence of an increase in numbers taken with increasing larval length. The observed number of food organisms in the gut contents of anchovy larvae is typical of most field studies on clupeoids (Conway et al. 1994a, Viñas & Ramírez 1996). However, Ferreira & Ré (1993) recorded a higher mean number of food organisms in anchovy larvae, this being a maximum of 8 food items, although these food particles were predominantly small tintinnids.

The LHPR vertical sampling demonstrated that the most abundant length group of anchovy larvae (2.4 to 6.9 mm) was distributed mainly in the upper 18 m of the water column, with highest concentrations between 2 and 10 m depth. The preferred food organisms for this size group of larvae (copepod eggs, nauplii, and *Oithona* spp.) were also most concentrated in this depth range, although there was considerable variability in absolute concentration between hauls (18 to 188 l^{-1}). Apart from results for 1 of the LHPR hauls (Haul 26, which was taken following a period of light winds), potential food was not strongly vertically stratified and would generally have required a significant migration by larvae to move between substantially different food concentrations.

There is evidence from field studies that food availability can directly affect larval feeding success (Anderson 1994, Fortier et al. 1995) and from experimental studies that it can affect growth rate (Gotceitas et al. 1996). In the present study, while the subset of LHPR samples tested statistically showed no significant difference in prey concentration with depth, larval feeding success, as measured both by the proportion of larvae feeding and the number of items in the gut contents, was seen to be independently related both to prey concentration and to the depth at which the larvae were sampled. Prey concentration appeared to affect food intake only at levels below ~50 food particles l^{-1}. However, even at low food concentrations, larvae were not necessarily subjected to nutritional deprivation, since there remains the balance between food intake, gut passage rate and assimilation efficiency to be considered (Govoni et al. 1986).

The reduction in feeding success with depth is interpreted as not being due to gut evacuation due to differences in time spent in the sampling net, since on all LHPR hauls the surface samples were collected first. It may be related to a reduction in light levels, which can affect larval feeding activity (Batty 1987),

However, it is surprising that this possible feeding/depth relationship was observed at such shallow depths (0 to 18 m), although light reduction in the highly turbid superficial waters of the northern Adriatic (Justić 1988) might be sufficient to affect feeding performance. Alternatively, it is possible that the larvae without food at depth were larvae in poor condition. Starvation in gadoid larvae results in increased buoyancy due to increasing tissue water content and decreasing protein levels (Frank & McRuer 1989), so that larvae in poor condition can be found distributed towards the surface. However, when they suffer osmoregulatory failure they tend to become less buoyant and occupy a deeper position in the water column (Sclafani et al. 1997).

Fish larvae do not feed indiscriminately, particular organisms being taken selectively, and co-occurring species can have completely different diets (Last 1980). Lamellibranch larvae and *Peridinium* spp. were frequently abundant in the plankton, yet were only taken by small larvae (2.4 to 3.9 mm in length) when availability of other food was low and the concentration of lamellibranch larvae and *Peridinium* spp. was high. Similar selectivity has been noted for cod larvae (Munk 1995), which were less discriminating when food was scarce. Evidence for a high degree of feeding discrimination in fish larvae has been shown by selection of copepod nauplii even at the species level (Hillgruber et al. 1995), possibly due to differential perception by larvae resulting from variations in nauplii swimming behaviour (Paffenhöfer et al. 1996). For anchovy larvae 2.4 to 6.9 mm in length, calanoid and cyclopoid nauplii were selected in preference to harpacticoid nauplii and *Oithona* spp. However, motility is not an essential prerequisite for feeding, since copepod eggs which were only present in the plankton in low numbers were taken in the same proportions as they were available.

Following the period of increased wind mixing between the 2 Bongo grids, there was a marked overall change in the plankton in concentration (41% reduction) and relative composition of the principal food items of larvae 2.4 to 10.9 mm in length. It is not known whether these changes were due to advection or to disruption of zooplankton production. Observed changes were clearly reflected in the diet of anchovy larvae; in Grid 2, larvae compensated for reduction in numbers of calanoid nauplii and 'other copepods' by increasing their intake of cyclopoid nauplii and *Oithona* spp. Dietary flexibility of fish larvae in response to differing species composition of available food has been detected in other situations, for example over several months in an evolving plankton community (Last 1980), in adjacent water masses within and outwith a river plume (Govoni & Chester 1990) and where there are interannual changes in food composition (Anderson 1994).

Strong wind mixing is generally considered to lead to increased mortality in fish larvae (Lasker 1975, Peterman & Bradford 1987), possibly as a result of poor feeding success due to dispersal of food concentrations. In the present study, the vertical distribution of total potential food organisms was reasonably uniform under a range of wind mixing conditions. Despite the lower overall food concentration on the second grid, feeding success was not reduced and the mean number of food organisms taken by anchovy larvae was significantly higher. However, the increase in food intake may not represent enhanced feeding success, because the estimated weight of food was not significantly higher. This was due to replacement of calanoid nauplii in the diet by greater numbers of smaller, lighter cyclopoid nauplii. There may even have been a reduction in nutritional quality due to consumption of greater numbers of these smaller nauplii, since on a consideration of the ratio of surface area to volume they will have a greater proportion of indigestible exoskeleton. Additionally, there may have been a higher energetic expenditure in the capture of greater numbers of smaller prey. Energetically, there was probably little difference in feeding success before and after the period of increased wind mixing and thus no evidence, under the particular environmental conditions studied, that the substantial changes in food concentrations observed would have modified overall larval mortality.

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LITERATURE CITED

Anderson JT (1994) Feeding ecology and condition of larval and pelagic juvenile redfish *Sebastes* spp. Mar Ecol Prog Ser 104:211–226

Arthur DK (1976) Food and feeding of larvae of three fishes occurring in the California Current, *Sardinops sagax*, *Engraulis mordax* and *Trachurus symmetricus*. Fish Bull US 74:517–530

Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. Adv Mar Biol 25:1–83

Bainbridge V, McKay BJ (1968) The feeding of cod and redfish larvae. Spec Publs Int Commn NW Atl Fish 7: 187–218

Batty RS (1987) Effect of light intensity on activity and food-

searching of larval herring, *Clupea harengus*: a laboratory study. Mar Biol 94:323-327

Bradford MJ, Cabana G (1997) Interannual variability in stage-specific survival rates and the causes of recruitment variation. In: Chambers RC, Trippel EA (eds) Early life history and recruitment in fish populations. Chapman & Hall, London, p 469-493

Canino MF, Bailey KM (1995) Gut evacuation of walleye pollock larvae in response to feeding conditions. J Fish Biol 46:389-403

Conway DVP, Coombs SH, Fernández de Puelles ML, Tranter PRG (1994a) Feeding of larval sardine, *Sardina pilchardus* (Walbaum), off the north coast of Spain. Bol Inst Esp Oceanogr 10:165-175

Conway DVP, McFadzen IRB, Tranter PRG (1994b) Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage. Mar Ecol Prog Ser 106:303-309

Coombs S, Giovanardi O, Conway D, Manzueto L, Halliday N, Barrett C (1997) The distribution of eggs and larvae of anchovy (*Engraulis encrasicolus*) in relation to hydrography and food availability in the outflow of the river Po. Acta Adriat 38:33-47

Dower JF, Miller TJ, Leggett WC (1997) The role of microscale turbulence in the feeding ecology of larval fish. Adv Mar Biol 31:169-220

Ferreira PL, Ré P (1993) Feeding of larval anchovy, *Engraulis encrasicolus* (L.), in the Mira estuary (Portugal). Port Zool 2:25-37

Fortier L, Ponton D, Gilbert M (1995) The match/mismatch hypothesis and the feeding success of fish larvae in ice-covered southeastern Hudson Bay. Mar Ecol Prog Ser 120: 11-27

Fox CJ (1996) Length changes in herring (*Clupea harengus*) larvae: effects of capture and storage in formaldehyde and alcohol. J Plankton Res 18:483-493

Frank KT, McRuer JK (1989) Nutritional status of field collected haddock (*Melanogrammus aeglefinus*) larvae from southwestern Nova Scotia: an assessment based on morphometric and vertical distribution data. Can J Fish Aquat Sci 46(Suppl 1):125-133

Gabriel WL (1978) Statistics of selectivity. In: Lipovsky SJ, Simenstad CA (eds) Gutshop '78. Fish food habit studies. Proc 2nd Pacific Northwest Technical Workshop. Washington Sea Grant, Division of Marine Resources, University of Washington H-30, Seattle, p 62-66

Geffen AJ (1996) Effect of experimental manipulation of feeding conditions on the population structure of larval cod (*Gadus morhua*) and herring (*Clupea harengus*). Mar Freshw Res 47:291-300

Gotceitas V, Puvanendran V, Leader LL, Brown JA (1996) An experimental investigation of the 'match/mismatch' hypothesis using larval Atlantic cod. Mar Ecol Prog Ser 130:29-37

Govoni JJ, Boehlert GW, Watanabe Y (1986) The physiology of digestion in fish larvae. Environ Biol Fish 16:59-77

Govoni JJ, Chester AJ (1990) Diet composition of larval *Leiostomus xanthurus* in and about the Mississippi River plume. J Plankton Res 12:819-830

Hay SJ, Evans GT, Gamble JC (1988) Birth, growth and death rates for enclosed populations of calanoid copepods. J Plankton Res 10:431-454

Hillgruber N, Haldorson LJ, Paul AJ (1995) Feeding selectivity of larval walleye pollock *Theragra chalcogramma* in the oceanic domain of the Bering Sea. Mar Ecol Prog Ser 120:1-10

Jenkins GP (1987) Comparative diets, prey selection, and predatory impact of co-occurring larvae of two flounder species. J Exp Mar Biol Ecol 110:147-170

Justić D (1988) Trend in the transparency of the northern Adriatic Sea 1911-1982. Mar Pollut Bull 19:32-35

Lagadeuc Y, Boulé M, Dodson JJ (1997) Effect of vertical mixing on the vertical distribution of copepods in coastal waters. J Plankton Res 19:1183-1204

Lasker R (1975) Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fish Bull US 73:453-462

Last JM (1980) The food of twenty species of fish larvae in the west-central North Sea. Fish Res Tech Rep No. 60, MAFF Directorate of Fish Res, Lowestoft

Leggett WC, Deblois E (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth J Sea Res 32:119-134

MacKenzie BR, Leggett WC, Peters RH (1990) Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar Ecol Prog Ser 67: 209-225

Maillet GL, Checkley DM (1991) Storm-related variation in the growth rate of otoliths of larval Atlantic menhaden *Brevoortia tyrannus*: a time series analysis of biological and physical variables and implications for larval growth and mortality. Mar Ecol Prog Ser 79:1-16

McFadzen IRB, Franceschini G (1997) The nutritional condition of larvae of anchovy (*Engraulis encrasicolus*) in the outflow of the River Po (Northern Adriatic). Acta Adriat 38: 49-64

Munk P (1995) Foraging behaviour of larval cod (*Gadus morhua*) influenced by prey density and hunger. Mar Biol 122:205-212

O'Connell CP (1976) Histological criteria for diagnosing the starving condition in early post yolk sac larvae of the northern anchovy, *Engraulis mordax* Girard. J Exp Mar Biol Ecol 25:285-312

Paffenhöfer GA, Strickler JR, Lewis KD, Richman S (1996) Motion behavior of naupli and early copepodid stages of marine planktonic copepods. J Plankton Res 18: 1699-1715

Pavlovskaja RM (1961) La survie des larves de l'anchois de la mer Noire en fonction de leur alimentation. Rapp Comm Int Mer Médit 16:345-350

Pepin P, Myers RA (1991) Significance of egg and larval size to recruitment variability of temperate marine fish. Can J Fish Aquat Sci 48:1820-1828

Pepin P, Penney RW (1997) Patterns of prey size and taxonomic composition in larval fish: are there general size-dependent models? J Fish Biol 51(Suppl A):84-100

Peterman RM, Bradford MJ (1987) Wind speed and mortality rate of a marine fish, the northern anchovy (*Engraulis mordax*). Science 235:354-356

Regner S (1971) Feeding of anchovy postlarvae, *Engraulis encrasicolus* (Linnaeus, 1758), in the Middle Adriatic. Ekologija 6:157-164 (in Serbo-Croatian)

Regner S (1996) Effects of environmental changes on early stages and reproduction of anchovy in the Adriatic Sea. Sci Mar 60:167-177

Sclafani M, Stirling G, Leggett WC (1997) Osmoregulation, nutritional effects and buoyancy of marine larval fish: a bioassay for assessing density changes during the earliest life-history stages. Mar Biol 129:1-9

Theilacker GH, Bailey KM, Canino MF, Porter SM (1996) Variations in larval walleye pollock feeding and condition: a synthesis. Fish Oceanogr 5(Suppl 1):112-123

Thompson AB, Harrop RT (1991) Feeding dynamics of fish larvae on Copepoda in the western Irish Sea, with partic-

ular reference to cod *Gadus morhua*. Mar Ecol Prog Ser 68:213–223
Tudela S, Palomera I (1995) Microzooplancton and feeding of anchovy larvae in northwestern Mediterranean. Rapp Comm Int Mer Médit 34:259
Viñas MD, Ramírez FC (1996) Gut analysis of first-feeding anchovy larvae from the Patagonian spawning areas in relation to food availability. Arch Fish Mar Res 43: 231–256
Williams R, Collins NR, Conway DVP (1983) The double LHPR system, a high speed micro- and macroplankton sampler. Deep-Sea Res 30:331–342
Zore-Armanda M, Gačić M (1987) Effects of bura on the circulation in the North Adriatic. Ann Geofis 5b:93–102

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Paper VI

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Carbon content and nutritional condition of sardine larvae (*Sardina pilchardus*) off the Atlantic coast of Spain

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Abstract As part of a "European Sardine/Anchovy Recruitment Program" (SARP), sardine larvae (*Sardina pilchardus*) were sampled off the Atlantic coast of Spain through the spawning season from March to June. The larvae were analysed for carbon and nitrogen content as a measure of nutritional condition and survival potential. There was no significant diel variation in larval carbon content, but there was a small significant diel variation in nitrogen; the absence of a strong diel signal in elemental composition was ascribed to the overnight retention of the gut contents. There was an increase in carbon content with increase in body length which reached an asymptote at ~40% carbon content at a larval length of 20 mm. It is argued that larvae with a carbon content of < 25% of body weight were nutritionally stressed, with the smaller larvae (< 10 mm in length) appearing to be more vulnerable to food limitation. Although larvae with the lowest age-specific carbon content (poorest condition) occurred on the cruise with the lowest food availability, there was no consistent relationship between carbon content and food availability. While the successive monthly estimates of carbon content revealed differences in potential recruitment between months, these were not related to the birth-date distribution of the surviving juveniles.

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Introduction

Investigations of variations in fish-stock recruitment often include morphometric, histological or biochemical assessments of the nutritional condition of fish larvae (see Ferron and Leggett 1994). The implicit hypothesis under test has been that food availability is a determining factor in survival of the larval stages (e.g. Buckley and Lough 1987), whether directly by starvation due to low food availability (e.g. Anderson 1994; Fortier et al. 1995), or indirectly through a decrease in the growth rate (e.g. Gotceitas et al. 1996; von Westernhagen et al. 1998) thereby prolonging the period of vulnerability of the early development stages to predation (Bailey and Houde 1989). Analyses of larval condition, taken as measures of incipient starvation, are then used in empirical correlations with indices of plankton abundance and hydrographic events (e.g. Håkanson et al. 1994; Theilacker et al. 1996). The development of techniques for ageing fish larvae within a season using otolith daily growth-rings (Brothers et al. 1976), has allowed an extension of such studies to intra-seasonal events (e.g. Campana 1996).

The practical application of the various methods of condition assessment has been encompassed in a series of parallel "Sardine Anchovy Recruitment Programs" (SARP), designed to relate clupeoid survival at the juvenile stage to hydrobiological conditions experienced during larval development (International Oceanographic Commission 1989). As part of a European SARP, a series of cruises were undertaken in 1991 and 1992 to study the survival of larvae of sardine (*Sardina pilchardus*) off the north and north-west coasts of Spain (López-Jamar et al. 1995). In the present study, the results from carbon and nitrogen analysis of sardine larvae are presented and discussed in relation to environmental conditions and larval survival potential.

The rationale for using elemental analysis as an indicator of nutritional condition is based on a number of experimental studies which have demonstrated the

Table 1 Cruise dates and sampling information (*No. stations* number of stations at which larvae were sampled; *No. larvae* number of larvae analysed for carbon and nitrogen)

Ship	Dates	No. stations	No. larvae
B.O. "Cornide de Saavedra"	17 April–12 May 1991	27	91
B.O. "Cornide de Saavedra"	6–20 March 1992	18	86
B.O. "Cornide de Saavedra"	31 March–15 April 1992	24	113
F.S. "Valdivia"	10–25 May 1992	39	141
R.R.S. "Challenger"	28 May–8 June 1992	30	143

validity of carbon and nitrogen in assessments of the condition of fish larvae: e.g. grunion (*Leuresthes tenuis*: May 1971), herring (*Clupea harengus*: Ehrlich 1974a; Checkley 1984), plaice (*Pleuronectes platessa*: Ehrlich 1974b), walleye pollock (*Theragra chalcogramma*: Harris et al. 1986) and blenny (*Blennius pavo*: von Westernhagen et al. 1998). Practical advantages of elemental analysis are that it is relatively insensitive to post-mortem deterioration (which is a problem with some other methods: Lochmann et al. 1996) and automated analysis methods are readily available.

Carbon and nitrogen show a more or less gradual decrease with duration of starvation, although the metabolic pathways through which they are derived are complex and may differ between species and life stages (Ferron and Leggett 1994). One conclusion has been that, due to parallel variations in carbon and nitrogen, the ratio between these two elements may be of less value as a condition index than percentage composition of each element separately (Ehrlich 1974b). Furthermore, changes in the body composition of carbon are more sensitive to feeding level than nitrogen (May 1971; Ehrlich 1974a, b), reflecting preferential metabolism of lipids before proteins under feeding stress (Harris et al. 1986).

Materials and methods

Sampling was carried out off the north and north-west coasts of Spain during a preliminary cruise in April/May 1991, and during the main series of four cruises between March and June 1992 (Table 1). On each cruise, sampling was based on a series of stations at spacings of 8 nautical miles (14.8 km) along transects ≈20 nautical miles (37 km) apart extending out from the coast (Fig. 1). At each station, hydrographic observations were obtained by a CTD (conductivity–temperature–depth) cast together with plankton sampling with a 50 cm-diam Bongo net (200 µm-mesh aperture) on oblique tows at 2 to 3 knots to 100 m depth or to within 10 m of the bottom. A flowmeter was fitted to one side of the Bongo nets to measure the volume of water filtered, and a depth sensor was attached to the frame to record the maximum depth sampled. In order to minimise damage to the larvae, soft, partially-filtering cod-end bags were used.

On completion of a plankton tow, the nets were rinsed briefly before removal of the cod-ends, and the samples were sorted for *Sardina pilchardus* larvae to be used in a range of condition analyses (McFadzen et al. 1997; Chicharo et al. 1998). This procedure was completed as quickly as possible (within ~10 min from net recovery) to minimise post-mortem deterioration. Specimens for elemental analysis were measured under a binocular microscope

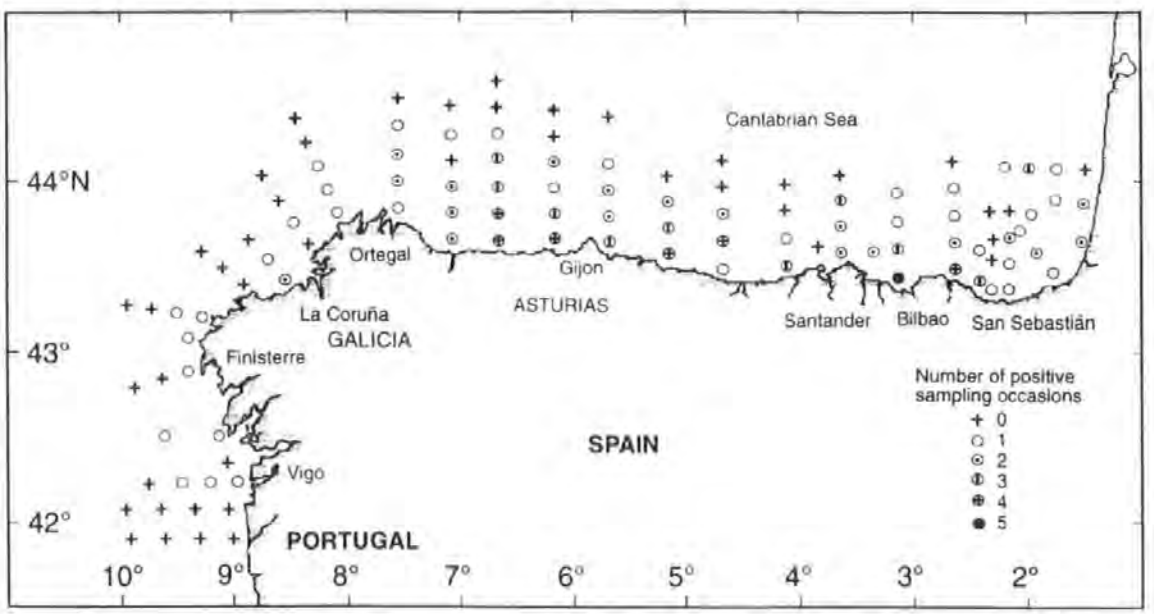

Fig. 1 Sampling grid in 1991 and 1992, showing station positions and number of cruises on which *Sardina pilchardus* larvae were obtained at each station for elemental analysis

(total length to nearest 0.1 mm), and transferred individually with the minimum of adherent water to plastic compartmented trays. They were then dried and stored over silica gel in a desiccator cabinet at room temperature. Larvae were not rinsed in fresh water prior to preservation, since rinsing does not necessarily ensure the most accurate subsequent weight determinations (Conway and Robins 1991).

Over a period of several months subsequent to the cruise, the larvae were transferred to pre-weighed tin cups for dry weight determination (Cahn 25 electrobalance), following a standard rapid procedure to minimise moisture uptake by the larvae. Carbon and nitrogen analysis was then carried out using a Carlo-Erba model NA 1500 Series 2 elemental analyser, with acetanylide as the calibration standard. All results for percentage carbon and nitrogen are expressed as percentage composition of body dry weight. The carbon:length regressions were compared by an analysis of covariance.

Results

Distribution and abundance of larvae

The mean distribution and abundance of all *Sardina pilchardus* larvae sampled (length range 4.0 to 23.4 mm, mean = 10.4 mm) on the four cruises in 1992 is shown in the contour plot of Fig. 2. The majority of larvae were collected along the north coast of Spain, where there were two main centres of abundance, one off Santander and the other west of Gijón. A secondary centre was evident in the Gulf of Biscay in the extreme east of the sampled area. A notable feature is the northwesterly offshore-directed tail of the distribution off Cape Ortegal. For a detailed description of the 1992 larval distributions see López-Jamar et al. (1995). A similar distribution pattern was observed for the more limited sampling in April May 1991 (López-Jamar et al. 1991).

Carbon:nitrogen relationship

The percentage carbon and nitrogen composition for all analysed sardine larvae from 1991 and 1992 showed a parallel variation in these two elements, both increasing with larval length, such that the ratio of carbon:nitrogen varied around a value of 4 (Fig. 3). The spread of carbon:nitrogen values at the smaller lengths may be partly a reflection of the potentially greater proportional error in weighing and tissue loss during sample manipulation of the smaller specimens. Because of the similar variation in carbon and nitrogen, and since carbon is generally considered to be a more sensitive index of larval condition than nitrogen (Ehrlich 1974b), the subsequent results are presented mostly in terms of carbon.

Day/night variation

There was no clear diel pattern in changes in carbon or nitrogen content of sardine larvae (Fig. 4). A comparison of results for larvae sampled during the day (n = 380) with those sampled at night (n = 194) revealed no significant difference in the proportion of body carbon (mean: 31.10 ± 0.33 day, 31.77 ± 0.42 night; Student's t-test: t = 1.21, p = 0.22). However, larvae from night samples had a small but significantly higher nitrogen level than larvae sampled during the day (mean: 8.27 ± 0.09 day, 8.57 ± 0.11 night; t-test: t = 2.08, p = 0.04).

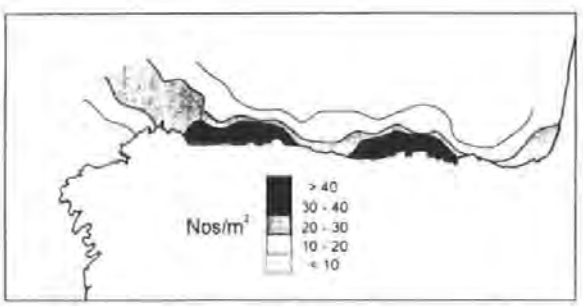

Fig. 2 *Sardina pilchardus*. Contoured mean distribution and abundance of all sardine larvae sampled on the four 1992 cruises combined (see Fig. 1 for station positions)

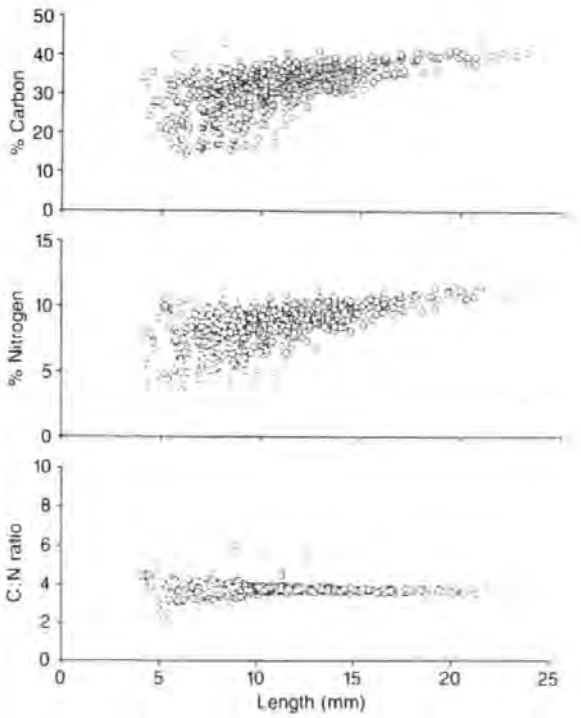

Fig. 3 *Sardina pilchardus*. Relationship between carbon, nitrogen and carbon:nitrogen as a function of length for all sardine larvae analysed (n = 574) in 1991 and 1992

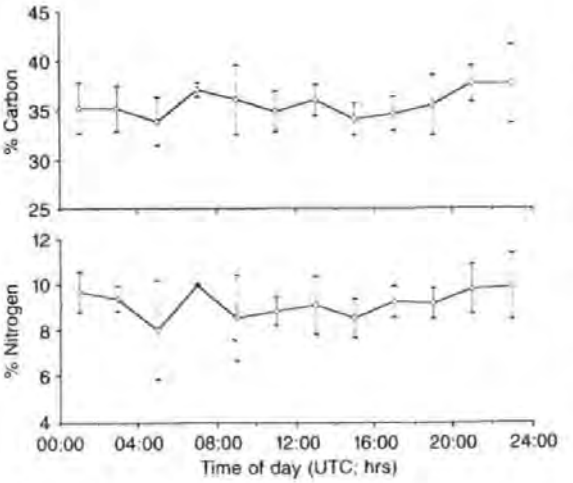

Fig. 4 *Sardina pilchardus*. Mean (±1 SE) percentage carbon and nitrogen (two-hourly sampling periods), plotted at mid-point of each time period, for all sardine larvae analysed (n = 574) in 1991 and 1992. Dawn was between 04:42 and 06:54 hrs and dusk between 18:18 and 20:01 hrs Coordinated Universal Time (*UTC*)

Carbon/length relationships

The relationships between carbon composition and length of larvae on the single cruise in 1991 and on the four cruises in 1992 are shown in Fig. 5, with fitted regression lines (Table 2). Based on the regression values, larvae were in similar condition on the March, March/April and May/June 1992 cruises (slopes and intercepts not significantly different at $p > 0.5$). Larvae on the April/May 1991 cruise had a greater proportion of carbon compared with all the 1992 cruises, the carbon/length relationships for larvae from April/May 1991 being significantly different (at $p < 0.01$) from all other groups of larvae (except that the slope was not significantly different from the May/June 1992 cruise). Results for larvae from the May 1992 sampling period showed both a significantly (at $p < 0.01$) steeper slope and lower intercept compared with all the other cruises; i.e. a lower percentage carbon for the small larvae and higher percentage for the larger larvae. This result was largely a reflection of the low carbon values for a high proportion of larvae < 10 mm in length.

Geographical variation

Insufficient data were available to distinguish regional patterns of carbon content on individual cruises, and it was only possible to show geographical variations based on the pooled data from all the 1992 cruises (Fig. 6). These results are presented as the contoured residuals from a single carbon/length regression for all specimens from 1992 (i.e. showing positive and negative deviations allowing for larval length differences). Two areas of relatively high larval carbon content (i.e. good nutritional condition) are apparent along the north coast, one in a central section off Gijón and the other further to the east off Santander, Bilbao and San Sebastián. The distribution of larval abundance in these two areas (Fig. 2) is similar, but not identical.

Discussion

Various techniques have been used to assess the condition of fish larvae. Some, such as gut-enzyme activity (e.g. Ueberschär 1995), are indicative of patterns of relatively short-term feeding activity, whereas others, such as lipid levels (e.g. Håkanson et al. 1994), represent energy accumulation over a longer period and may therefore be more relevant as integrated indices of survival potential. Carbon content responds to short periods of food deprivation, for example in the blenny *Blennius pavo* and plaice *Pleuronectes platessa* (von Westernhagen et al. 1998), and also shows a progressive decline over extended periods of food deprivation, for example when larvae of grunion *Leuresthes tenuis* (May 1971), herring *Clupea harengus* (Ehrlich 1974a) and plaice (Ehrlich 1974b) are starved for ~20 d. However, since the source of carbon utilised during starvation varies both with larval species and developmental stage (Ferron and Leggett 1994), and the mobilisation process is not well documented, carbon content can be regarded only as a gross index of condition. Despite these reservations and the lack of laboratory validation for *Sardina pilchardus* larvae, there is still a sufficient basis for using carbon content as a measure of their nutritional condition.

Lochmann et al. (1996) estimated that in larval cod (*Gadus morhua*) an average of 56% of total body lipids, and thus a large proportion of the total body carbon content, could be derived from the gut contents. In the present study, although it was not feasible to exclude the gut contents, the potential variability and bias introduced from this source may have been less of a problem, because both the typical number of food organisms in the gut contents of field-caught sardine larvae is low (Conway et al. 1994) and there is a significant retention of food in their guts at night. This low level of diel feeding-variability is reflected in the absence of any clear day/night signal in carbon and nitrogen content. Diel changes in RNA:DNA ratios of sardine larvae were obtained by Chicharo et al. (1998), but this diel variation was ascribed to an endogenous rhythm of RNA production rather than to any alteration in nutritional condition.

The observed increase in percentage carbon composition with increasing body length is common for fish larvae (May 1971; Ehrlich 1974a, b) and other organisms during early development, when rapid morphological changes and energy storage take place (e.g. copepods: Bottrell and Robins 1984). Some element of the observed increase in proportion of body carbon may also be due to preferential mortality of specimens in

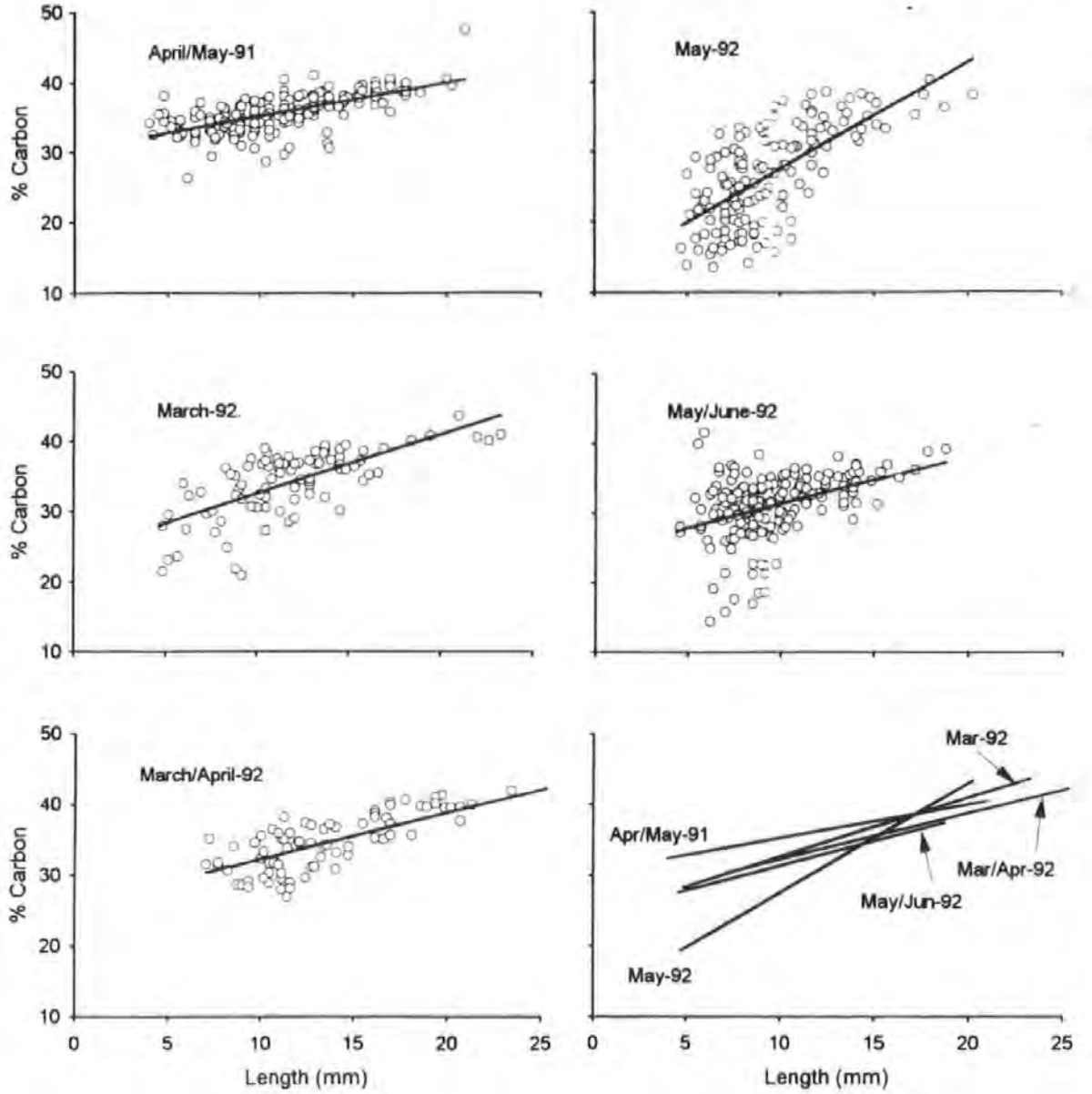

Fig. 5 *Sardina pilchardus*. Relationship between carbon content and larval length for each cruise, with fitted regression lines (see Table 2 for regression values)

poor condition and, hence, with a lower carbon content. In sardine larvae, carbon content stabilises at ~40% by ~20 mm in length, consistent with similar values (35 to 44%) for the carbon content of other healthy fish larvae (May 1971; Ehrlich 1974a, b; Harris et al. 1986). In making comparisons between small larvae, it is important to allow for the variation in carbon content with body length. However, all condition indices which include length parameters are susceptible to problems associated with changes in growth rate due to starvation (von Westernhagen et al. 1998), so that length can be a relatively poor estimator of developmental age.

Based on the observed carbon/length relationships, the smaller larvae sampled on the May 1992 cruise were

Table 2 *Sardina pilchardus*. Regression values for carbon length relationships

Cruise	Slope (SE)	Intercept (SE)	R^2
April May 1991	0.47 (0.040)	30.51 (0.46)	0.65
March 1992	0.84 (0.095)	24.29 (1.18)	0.69
March April 1992	0.65 (0.063)	25.75 (0.92)	0.76
May 1992	1.54 (0.14)	12.11 (1.34)	0.69
May June 1992	0.69 (0.12)	24.46 (1.32)	0.42

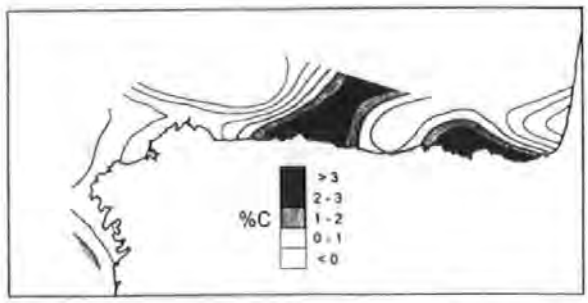

Fig. 6 *Sardina pilchardus*. Contoured residuals of carbon content from carbon length regression, for pooled data the four 1992 cruises (see Fig. 1 for station positions)

in significantly poorer condition than on the other 1992 cruises, and the larvae from the April/May 1991 cruise were in the best condition overall. There is some correspondence between these relative-condition assessments and food availability (Conway et al. 1994); for example, the lowest food availability in 1992 was observed on the May cruise (mean of 7.1 food particles l^{-1}), with carbon analysis showing larvae in the poorest condition. Conversely, the highest food availability (21.6 particles l^{-1}) was recorded on the May/June 1992 cruise, when larvae were in intermediate condition, and the lowest food availability was on the April/May 1991 cruise (7.0 particles l^{-1}), when larval condition was highest. The lack of any clear relationship between food availability and larval condition may not be particularly surprising considering the generalised nature of our data, which do not take into consideration such details as fine-scale distributions and predator/prey contact rates (e.g. Rothschild and Osborn 1988), and an imprecise knowledge of the latency of the response of carbon content to food-deprivation.

Along the north coast, there was some correspondence between the geographical distribution of areas of higher larval carbon content, indicating larvae in good nutritional condition, and the areas of higher larval abundance. An association between larval condition and abundance might be expected, in view of the enhanced survival potential of larvae with a higher carbon content. A similar functional relationship between the geographical distribution of food availability and positive length-specific carbon would also be expected, but this was not apparent from the limited plots of food abundance and potential food (chlorophyll *a*, biomass and particulate volume) presented by López-Jamar et al. (1995).

The relatively poor condition of larvae sampled in May 1992 largely reflects the predominance of specimens < 10 mm in length with a carbon content of < 25% of body weight. Such values, which also occurred sporadically in other months (for example March and May–June 1992), are well below the characteristic carbon composition of > 25% for fish larvae and other zooplankton (e.g. Ehrlich 1974b; Hirota 1981; Ikeda and Skjoldal 1989). Even in the absence of experimental validation specifically for sardine larvae, such low carbon values (< 25%) indicate severely nutritionally stressed larvae, since in other fish species values of ~35% carbon content are typical for larvae deprived of food (May 1971; Ehrlich 1974a, b; Harris et al. 1986). The higher incidence of anomalously low carbon values observed in the present study for the smaller sizes of larvae (< 10 mm in length) agrees with a general expectation that smaller larval sizes are more susceptible to starvation (see Ferron and Leggett 1994).

Various other condition analyses were carried out on larvae sampled on the same set of 1992 cruises, including histological, enzyme and RNA:DNA methods (López-Jamar et al. 1995; McFadzen et al. 1997; Chicharo et al. 1998). A comparison of the carbon analysis with the results from these other techniques (summarised in López-Jamar et al. 1995) showed some agreement in that the carbon, histological and enzyme assessments all identified larvae from the May 1992 cruise as being in the poorest condition; otherwise, there was little clear correspondence between the different condition indices. Such overall inconsistency may be attributable to the different biochemical/physiological processes addressed by each analytical method and the particular response (e.g. latency) to feeding conditions (Ferron and Leggett 1994). This problem is exemplified by the histological results (McFadzen et al. 1997), whereby different assessments of relative monthly condition were obtained depending on which indicators (short-term, long-term or irreversible tissue-damage) were used. Similarly, the poor correspondence between the results of the different condition assessments may be because food was generally not limiting and hence there was little significant response. This explanation is supported by the results of Chicharo et al. (1998) who, of 474 larvae examined, found only four with a DNA:RNA ratio indicating starvation; and by the findings of McFadzen et al. (1977), who reported larvae to be in good long-term histological condition in all months examined. Furthermore, Conway et al. (1994) concluded from an analysis of the gut contents of sardine larvae and comparison with available food in the plankton, that feeding conditions were generally adequate.

The rationale for carrying out nutritional assessments was to use them as indicators of intra-seasonal larval survival and to compare these survival indices with the birth-date distribution of the surviving juveniles, i.e. following the SARP philosophy (International Oceanographic Commission 1989). In terms of carbon content, the May larvae were in poorer condition than those taken in the other months of 1992. However, the subsequent juvenile surveys showed that there was negligible survival of larvae spawned in any of the months from March to June 1992 (López-Jamar et al. 1995; Alvarez and Alemany 1997). Thus, despite being in potentially better condition, the larval populations sampled during the March, March–April and May–June 1992 cruises ultimately fared no better than the May

1992 larvae. This may be attributable to other sources of mortality, such as predation, at any time prior to sampling the juveniles, and be unconnected with their feeding conditions as larvae. One possibility, suggested by López-Jamar et al. (1995), is that offshore transport associated with upwelling in the Finisterre to Cape Ortegal region (and indicated by the offshore directed tail of the larval distribution) resulted in loss of the larvae to deep water and away from their habitual nursery grounds. Thus, in 1992, the ultimate survival of larvae through to the juvenile stage may be ascribable to the overriding influence of advective loss rather than to intra-seasonal variations in food availability (López-Jamar et al. 1995).

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References

Alvarez F, Alemany F (1997) Birthdate analysis and its application to the study of the Atlanto-Iberian sardine *Sardina pilchardus*. Fish Bull US 95: 187–194

Anderson JT (1994) Feeding ecology and condition of larval and pelagic juvenile redfish *Sebastes* spp. Mar Ecol Prog Ser 104: 211–226

Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. Adv mar Biol 25: 1–83

Bottrell HH, Robins DB (1984) Seasonal variations in length, dry weight, carbon and nitrogen of *Calanus helgolandicus* from the Celtic Sea. Mar Ecol Prog Ser 14: 259–268

Brothers EB, Matthews CP, Lasker R (1976) Daily growth increments in otoliths from larval and adult fish. Fish Bull US 74: 1–18

Buckley LJ, Lough RJ (1987) Recent growth, biochemical composition and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. Can J Fish aquat Sciences 44: 14–25

Campana SE (1996) Year-class strength and growth rate in young Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 135: 21–26

Checkley DM (1984) Relation of growth to ingestion for larvae of Atlantic herring *Clupea harengus* and other fish. Mar Ecol Prog Ser 18: 215–224

Chicharo MA, Chicharo L, Valdez L, López-Jamar E, Ré P (1998) Estimation of starvation and diel variation of the RNA DNA ratios in field-caught *Sardina pilchardus* larvae off the north of Spain. Mar Ecol Prog Ser 164: 273–283

Conway DVP, Coombs SH, Fernández de Puelles ML, Tranter PRG (1994) Feeding of larval sardine, *Sardina pilchardus* (Walbaum), off the north coast of Spain. Boln Inst esp Oceanogr 10: 165–175

Conway DVP, Robins DB (1991) Collection and chemical analysis of chaetognaths and changes due to preservation. In: Bone Q, Kapp H, Pierrot-Bults AC (eds) The biology of chaetognaths. Oxford University Press, Oxford, pp 137–146

Ehrlich KF (1974a) Chemical changes during growth and starvation of herring larvae. In: Blaxter JHS (ed) The early life history of fish. Springer-Verlag, Heidelberg, pp 301–323

Ehrlich KF (1974b) Chemical changes during growth and starvation of larval *Pleuronectes platessa*. Mar Biol 24: 39–48

Ferron A, Leggett WC (1994) An appraisal of condition measures for marine fish larvae. Adv mar Biol 30: 217–303

Fortier L, Pontoon D, Gilbert M (1995) The match mismatch hypothesis and the feeding success of fish larvae in ice-covered southeastern Hudson Bay. Mar Ecol Prog Ser 120: 11–27

Gotceitas V, Puvanendran P, Leader LL, Brown JA (1996) An experimental investigation of the "match/mismatch" hypothesis using larval Atlantic cod. Mar Ecol Prog Ser 130: 29–37

Håkanson JL, Coombs SH, Ré P (1994) Lipid and elemental composition of sprat (*Sprattus sprattus*) larvae at mixed and stratified sites in the German Bight of the North Sea. ICES J mar Sci 51: 147–154

Harris RK, Nishiyama T, Paul AJ (1986) Carbon, nitrogen and caloric content of eggs, larvae, and juveniles of walleye pollock, *Theragra chalcogramma*. J Fish Biol 29: 87–98

Hirota R (1981) Dry weight and chemical composition of the important zooplankton in the Setonaikai (Inland Sea of Japan). Bull Plankton Soc Japan 28: 19–24

Ikeda T, Skjoldal HR (1989) Metabolism and elemental composition of zooplankton from the Barents Sea during early Arctic summer. Mar Biol 100: 173–183

International Oceanographic Commission (1989) IOC workshop report of the expert consultation on the Sardine Anchovy Recruitment Program (SARP). International Oceanographic Commission, UNESCO, Paris, 1–57 (IOC Workshop Rep No. 66)

Lochmann SE, Maillet GL, Taggart CT, Frank KT (1996) Effect of gut contents and lipid degradation on condition measures in larval fish. Mar Ecol Prog Ser 134: 27–35

López-Jamar E, Coombs SH, Alemany F, Alonso J, Álvarez F, Barrett CD, Cabanas JM, Casas B, Díaz del Río G, Fernández de Puelles ML, Franco C, Garcia A, Halliday NC, Lago de Lanzós A, Lavín A, Miranda A, Robins DB, Valdés L, Varela M (1991) A SARP pilot study for sardine (*Sardina pilchardus*) off north and northwest Spain in April/May 1991. Int Counc Explor Sea Comm Meet (Biol Oceanogr Comm) L: 69: 1–36

López-Jamar E, Coombs SH, Garcia A, Halliday NC, Knust R, Nellen W (1995) The distribution and survival of larvae of sardine, *Sardina pilchardus* (Walbaum) off the north and northwest Atlantic coast of Spain in relation to environmental conditions. Boln Inst esp Oceanogr 11: 27–46

May RC (1971) Effects of delayed initial feeding on larvae of the grunion, *Leuresthes tenuis* (Ayres). Fish Bull US 69: 411–425

McFadzen IRB, Coombs SH, Halliday NC (1997) Histological indices of the nutritional condition of sardine, *Sardina pilchardus* (Walbaum) larvae off the north coast of Spain. J exp mar Biol Ecol 212: 239–258

Rothschild BJ, Osborn TR (1988) Small-scale turbulence and plankton contact rates. J Plankton Res 10: 465–474

Theilacker GH, Bailey KM, Canino MF, Porter SM (1996) Variations in larval walleye pollock feeding and condition: a synthesis. Fish Oceanogr 5 (Suppl 1): 112–123

Ueberschär B (1995) The use of tryptic enzyme activity measurement as a nutritional condition index: laboratory calibration data and field application. ICES mar Sci Symp (Act Symp) 201: 119–129

Westernhagen H von, Freitas C, Fürstenberg G, Willführ-Nast J (1998) C N data as an indicator of condition in marine fish larvae. Archs Fishery mar Res 46: 165–179

Authors declaration Paper VI

Coombs, S.H., Conway, D.V.P., Morley, S.A., Halliday, N.C. (1999) Carbon content and nutritional condition of sardine (*Sardina pilchardus*) larvae off the Atlantic coast of Spain. Marine Biology, 134: 367-373.

D.V.P. Conway carried out the sample collection and analysis, data interpretation and preparation of the manuscript to 50% of the total effort.

S.H. Coombs S H Coombs 16/3/2000

S.A. Morley S A Morley 8/3/2000

N.C. Halliday 16/3/2000

Appendix

Full list of refereed scientific publications by David V.P. Conway

1980

1. Conway DVP (1980) The food of larval blue whiting, *Micromesistius poutassou* (Risso), in the Rockall Area. J Fish Biol 16: 709-723
2. Williams R, Conway DVP (1980) Vertical distributions of *Calanus finmarchicus* and *C. helgolandicus* (Crustacea : Copepoda). Mar Biol 60: 57-61

1981

3. Williams R, Conway DVP (1981) Vertical distribution and seasonal abundance of *Aglantha digitale* (O.F. Müller)(Coelenterata : Trachymedusae) and other planktonic coelenterates in the northeast Atlantic Ocean. J Plank Res 3: 633-643

1982

4. Williams R, Conway DVP (1982) Population growth and vertical distribution of *Calanus helgolandicus* in the Celtic Sea. Neth J Sea Res 16: 185-194

1983

5. Williams R, Collins NR, Conway DVP (1983) The double LHPR system, a high speed micro- and macroplankton sampler. Deep-Sea Res 30: 331- 342

1984

6. Williams R, Conway DVP (1984) Vertical distribution, and seasonal and diurnal migration of *Calanus helgolandicus* in the Celtic Sea. Mar Biol 79: 63-73

1986

7. Conway DVP, Williams R (1986) Seasonal population structure, vertical distribution and migration of the chaetognath *Sagitta elegans* in the Celtic Sea. Mar Biol 93: 377-387

1987

8. Williams R, Conway DVP, Collins NR (1987) Vertical distributions of eggs, nauplii and copepodites of *Calanus helgolandicus* (Copepoda : Crustacea) in the Celtic Sea. Mar Biol 96: 247-252

1988

9. Williams R, Conway DVP (1988) Vertical distribution and seasonal numerical abundance of the Calanidae in oceanic waters to the south-west of the British Isles. Hydrobiologia 167/168: 259-266

1990

10. Conway DVP, Ellis CJ, Humpheryes IG (1990) Deep distributions of oceanic cirripede larvae in the Sargasso Sea and surrounding North Atlantic Ocean. Mar Biol 105: 419-428

1991

11. Conway DVP, Robins DB (1991) Collection and chemical analysis of chaetognaths and changes due to preservation. *In*: Bone Q, Kapp H, Pierrot-Bults AC, (eds) Biology of Chaetognaths. Oxford University Press, Oxford, p 137-146
12. Poulet SA, Williams R, Conway DVP, Videau C (1991) Co-occurrence of copepods and dissolved free amino acids in shelf sea waters. Mar Biol 108: 373-385

1992

13. Coombs SH, Nichols JH, Conway DVP, Milligan S, Halliday NC (1992) Food availability for sprat larvae in the Irish Sea. J Mar Biol Ass UK 72: 821-834

1993

14. Conway DVP, Tranter PRG, Coombs SH (1993) Digestion of natural food by larval and post larval turbot *Scophthalmus maximus*. Mar Ecol Prog Ser 100: 221-231
15. Conway DVP (1993) Changes in the nutritional value of *Sagitta setosa* during early stages of decomposition. *In*: Moreno I (ed) Proceedings of the II International

Workshop of Chaetognatha: Palma, 1-6 September, 1992, Universitat de les Illes Baleares, Palma, p 129-134

1994

16. Coombs SH, Robins DB, Conway DVP, Halliday NC, Pomroy AJ (1994) Suspended particulates in the Irish Sea and feeding conditions for fish larvae. Mar Biol 118: 7-15
17. Conway DVP, McFadzen IRB, Tranter PRG (1994) Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage. Mar Ecol Prog Ser 106: 303-309
18. Lindley JA, Williams R, Conway DVP (1994) Variability in dry weight and vertical distribution of decapod larvae in the Irish Sea and North Sea during the spring. Mar Biol 120: 385-395
19. Williams R, Conway DVP, Hunt HG (1994) The role of copepods in the planktonic ecosystem of mixed and stratified waters of the European shelf sea. Hydrobiologia 292/293: 521-530
20. Conway DVP, Coombs SH, Fernández de Puelles ML, Tranter PRG.(1994) Feeding of larval sardine *Sardina pilchardus* (Walbaum) off the north coast of Spain. Bol Inst Español Oceanogr 10: 165-175

1997

21. Conway DVP, Coombs SH, Smith C (1997) Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES J Mar Sci 54: 136-147
22. Coombs SH, Giovanardi O, Conway D, Manzueto L, Halliday NC, Barrett C (1997) The distribution of eggs and larvae of anchovy (*Engraulis encrasicolus*) in relation to hydrography and food availability in the outflow of the River Po. Acta Adriatic 38: 33-47
23. Franceschini G, McFadzen IRB, Conway DVP (1997) Preliminary data on indices of nutritional condition of anchovy larvae (*Engraulis encrasicolus* L.) in the outflow of the River Po (Cruise A.L.I.C.E. '95'). Biolog Mar Medit 4: 298-300
24. Giovanardi O, Coombs SH, Manzueto L, Conway DVP, Halliday NC, Barrett CD (1997) Vertical and horizontal distribution of anchovy (*Engraulis encrasicolus* L.) eggs and larvae, of microzooplankton and particulate in the outflow of the River Po (A.L.I.C.E. '95' Cruise). Biolog Mar Medit 4: 301-302

1998

25. Conway DVP, Coombs SH, Smith C (1998) Feeding success of anchovy (*Engraulis encrasicolus*) larvae in the north-western Adriatic Sea in response to changing hydrobiological conditions. Mar Ecol Prog Ser 175: 35-49

1999

26. Coombs SH, Conway DVP, Morley SA, Halliday NC (1999) Carbon content and nutritional condition of sardine (*Sardina pilchardus*) larvae off the Atlantic coast of Spain. Mar Biol, 134: 367-373
27. Conway DVP, Coombs SH, Lindley JA, Llewellyn CA (1999) Diet of mackerel (*Scomber scombrus*) larvae at the shelf edge to the south-west of the British Isles and the incidence of piscivory and coprophagy. Vie et Milieu 49: 213-220

In Press

28. Coombs SH, Giovanardi O, Halliday NC, Franceschini G, Conway DVP, Manzueto L, Barrett CD, McFadzen IRB (In Press) Wind mixing, food availability and mortality of anchovy larvae (*Engraulis encrasicolus*) in the northern Adriatic. Mar Ecol Prog Ser