Journal of Sea Research 78 (2013) 8–17

Contents lists available at SciVerse ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Feeding habits of European pilchard late larvae in a nursery area in the Adriatic Sea

Diego Borme [a,*], Valentina Tirelli [a], Isabel Palomera [b]

[a] OGS (Istituto Nazionale di Oceanografia e di Geofisica Sperimentale), Via A. Piccard 54, 34151, Trieste, Italy
[b] Institut de Ciències del Mar (CSIC), Passeig Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form 21 November 2012
Accepted 27 December 2012
Available online 17 January 2013

Keywords:
Sardine Late Larvae
Diet Composition
Feeding Selectivity
Ontogenetic Dietary Shift
Mediterranean

ABSTRACT

European pilchard *Sardina pilchardus* late larvae were collected in the Gulf of Manfredonia, an important nursery area, during their seasonal inshore occurrence. Thanks to diel cycle sampling and to the wide range of larval lengths (from a minimum of 27 mm to a maximum of 45 mm), both feeding rhythm and ontogenetic changes were analysed. The feeding peak was observed in the afternoon, before sunset. Sardine larvae were exclusively zooplanktivorous, their diet being based on Calanoid Copepods from the genus *Paracalanus* (*IRI%* = 65.7), on the species *Temora longicornis* (*IRI%* = 15.5) and other small-sized Copepods. Other planktonic organisms appeared in the stomach contents occasionally and never reached *IRI%* values >1. The number of prey per stomach increased suddenly at larval lengths around 40 mm, corresponding to the development of the stomach. Prey composition in the environment was established by contemporaneous sampling of plankton, performed by means of two plankton nets with different meshes. The main prey items were positively selected among those available in the field, but some other prey (*Centropages* spp., Harpacticoids, Corycaeids, *Temora stylifera* and *Acartia* spp.) were also preferred, although rare in the plankton samples. In contrast, copepod nauplii, despite their abundance in the environment (15,848 ± 4441 individuals m^{-3}), were only occasionally recovered in the larval gut contents ($N = 0.26\%$). This shows that sardine late larvae have switched to larger prey items.

1. Introduction

The European pilchard *Sardina pilchardus* (Walbaum, 1792), commonly known as sardine, is a small pelagic marine fish widely distributed in the northeastern Atlantic (from Senegal to Iceland) and the Mediterranean (including the Black Sea) (Muus and Nielsen, 1999). It is a very important commercial species, being targeted by purse seiners and pelagic trawlers all around the Mediterranean. In the Adriatic, the total catch of sardine between 1970 and 2005 varied between a maximum of 88,518 tonnes (recorded in 1981) and a minimum of 20,372 tonnes (reached in 2005), accounting, together with anchovy, for approximately 41% of total marine catches for this basin (Official GFCM catch time series, in Morello and Arneri, 2009). Moreover, in the western part of the Adriatic Sea, there was also a long tradition of the fishery for *bianchetto* (i.e. "whitish"), terms generally used for juveniles of Clupeids but principally referred to sardines late larvae (Marano et al., 1981; Ungaro et al., 1994). Morello and Arneri (2009) reported that in the 1980s the average total catch of sardine *bianchetto* in the Gulf of Manfredonia, along the Apulian coast, was approximately 20 tonnes per day but in 1991 it was decreased to on average of 6 tons per day.

The highly variable recruitment success and hence the large stock fluctuations of European sardine make its management challenging. The most vulnerable life stage in many stocks of teleost fish is considered the larval one, and its varying success influences future stock abundance (e.g. Beaugrand et al., 2003; Cury and Roy, 1989; Fortier and Villeneuve, 1996; Köster et al., 2003). Temporal coupling or decoupling of peak production of fish larvae with their prey has been suggested as one of the most important factors influencing survival of larvae, thus regulating the recruitment success (Cushing, 1974). There is no complete agreement among authors about the period of larval life during which mortality may be concentrated. Hjort (1914) and Karlovac (1967) indicated the critical period as the phase following the absorption of the yolk sac, when the individual has to start feeding exogenously. Several reports, however, suggest that critical periods may also occur at other stages of life history such as metamorphosis (Blaxter, 1988; Chambers et al., 2001; Thorisson, 1994; Vladimirov, 1975), thus no more at larval stage of life. In any case, it seems that fast growing rates enhance survival (e.g. Hovenkamp, 1992; Meekan and Fortier, 1996; Rice et al., 1993). Acceleration of growth may be achieved through size-selective foraging by fish that shift their diets from smaller to larger prey as they grow (Islam and Tanaka, 2006). Therefore stage duration and diet flexibility seem to be key in shaping larval success.

The ecological role of small pelagic fish, like sardine, through channelling energy from lower (i.e. the plankton) to higher trophic

* Corresponding author at: OGS (Istituto Nazionale di Oceanografia e di Geofisica Sperimentale), Via A. Piccard 54, 34151 Trieste, Italy. Tel.: +39 040 2249726; fax: +39 040 2249770.
E-mail addresses: dborme@ogs.trieste.it (D. Borme), vtirelli@ogs.trieste.it (V. Tirelli), isabel@icm.csic.es (I. Palomera).

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http://dx.doi.org/10.1016/j.seares.2012.12.010

levels, is of essential importance for marine ecosystems (Bakun, 2006; Coll et al., 2006). The determination of diet compositions during early life stages (which are still poorly known) is fundamental to improving the current ecosystem models that have been developed for the northwestern Mediterranean and the Adriatic Sea during recent years (Coll et al., 2006, 2007). Detailed trophic aspects of the ontogenetic stages of key species (such as sardine and anchovy) which are included in the models are one of the most important elements to be incorporated. Such information is needed in order to evaluate the environmental effect on survival and to predict potential future recruitment scenarios. Nevertheless, there are just a few studies dealing with the diet of sardine larvae in the Mediterranean Sea (Massutì and Oliver, 1948 in the Balearic Islands; Karlovac, 1967 in the eastern Adriatic Sea; Rasoanarivo et al., 1991 in the Gulf of Lions; Dulčić, 1999 in the eastern Adriatic Sea; Morote et al., 2010 in sardines from the northwestern Mediterranean), and the majority of these refer to larvae smaller than 24 mm. Recently Costalago et al. (2012) analysed the prey categories contributions to the diet by means of stable isotopes in sardine from the Gulf of Lions. On the basis of the differences found between late-larvae, juveniles and adults, they suggested the hypothesis that the diet shift occurred primarily at the time of metamorphosis, whereas juveniles and adults maintained similar diets. Nevertheless the present study is one of the first on the qualitative and quantitative description of sardine late larvae, based on taxonomical analysis, across the metamorphic phase. The aim of this work is to improve our knowledge on European pilchard late larvae diet and in particular in the Adriatic Sea, where target fishery for these life stages occurred since 1 July 2010, when sardine fry fishery was no longer allowed (Morello, 2011).

It is reported that in the Adriatic *S. pilchardus* larvae are rather dispersed and start aggregating in schools at the late post-larval stage, when they move to the nursery grounds and are recruited to the fishery (Kačić et al., 1988; Sinovčić, 2000). One of the main nursery areas on the western coast of the Adriatic Sea is considered the Gulf of Manfredonia, already mentioned for its important fishery for bianchetto (Ungaro et al., 1994). In this area sardine fry are concentrated in shallow areas until April but, as the coastal water temperatures rises, the sardine juveniles are observed to migrate offshore and have completely left the shallow waters by May (Marano et al., 1981).

In view of the high concentrations of sardine larvae reported in winter in the Gulf of Manfredonia, a sampling cruise was carried out in this region during February 2008, with the aim of investigating the biology of their late larval stages. The specific objectives of this work are: i) to describe the feeding rhythm of sardine late larvae and juveniles; ii) to examine feeding patterns in relation to ontogenesis; iii) to determine the major dietary components; and iv) to assess the feeding selectivity by estimating food availability in the environment. These objectives do not provide information of direct use for immediate fishery management, but they aim to build up knowledge necessary to improve the ecological modelling which is currently being developed (Coll and Libralato, 2012; Coll et al., 2007).

2. Materials and methods

2.1. Plankton sampling and analysis

Sampling took place from 13 to 20 February 2008 in the Gulf of Manfredonia (southwestern Adriatic Sea). A grid of 12 stations, located at approximately the same distance from each other (5.5–7 nautical miles), was defined to cover the study area (Fig. 1). Plankton samples were collected by vertical tows, from bottom to surface, performed with two different nets: a standard WP2 net (mesh size 200 μm; mouth opening diameter 58 cm) and a Calvet net (mesh size 53 μm; mouth opening diameter 25 cm). The volume of filtered water was estimated from the net-mouth area and the sampling depth. Immediately on retrieval of the nets, plankton samples were sieved in succession through 200 μm and 50 μm, and 3000 μm and 200 μm, mesh to obtain two different size fractions (a 50–200 μm fraction, named hereafter "microplankton" and a 200–3000 μm fraction named hereafter "mesozooplankton") from the Calvet net and the WP2 net respectively. All samples were fixed and preserved in a seawater-buffered formaldehyde solution (4% final concentration) for later determination of composition and abundance.

The mesozooplankton was analysed using a stereo-microscope Olympus SXZ12 (up to 90× magnification) considering subsamples sufficient to count at least 400 copepods. The small copepods (mainly copepodites) present in the microplankton were also analysed in subsamples of at least 400 individuals, while the presence of nauplii, dinoflagellates, ciliates and diatoms was estimated analysing subsamples of 1–1.5 ml (representing 0.33–0.66% of the sample original volume). All the planktonic organisms encountered in the subsamples were identified at the lowest possible taxonomical level using keys for identification (Jörgensen, 1924; Rose, 1933; Tomas, 1997; Trègouboff and Rose, 1957). Individuals of each identified taxon were counted and their relative abundances were calculated as individuals per cubic meter. Samples obtained from the WP2 and Calvet nets were combined to define food availability in the field.

2.2. Fish sampling and gut content analysis

Fish were sampled with an experimental semipelagic trawl net equipped with a fine-meshed cod-end (stretched mesh length 5.5 mm, ISO 1107), towed at an approximate speed of 3.0 knots for 30 min. Fish sampling was carried out for 2 days (14 and 15 February 2008) of 24 h continuous trawling, with fish samples taken every 2–6 h, in order to analyse the diel feeding cycle. A total of 12 fish tows took place during cruising between the biological stations, in order to get an almost simultaneous sampling of fish and their potential prey. Late larvae of *S. pilchardus* were found in 9 of the 12 performed tows, all located inshore, near station 2 (41°45.03′ N; 16°13, 47′ E; depth 14 m). The sampling period included a Bora (east-northeast stormy wind) event which stopped the sampling for 3 days. Due to the hydrological changes (lower values of temperature and density near to the coast and higher offshore), it was decided to analyse only fish caught before that event, i.e. from the first seven tows. During the sampling period (14–15 February, 2008) the water column had a mean temperature of 10.34 °C and a mean salinity of 37.94; the sunrise was at 06:54 and sunset at 17:31 (GMT).

After the end of each tow, fish larvae were sorted and immediately put in small vials containing sea water. Samples were frozen onboard at −20 °C to stop digestive processes and preserved at the same temperature until laboratory analysis.

At the laboratory fish were defrosted, measured to the nearest 0.1 mm of total length (TL) and weighed on an analytical balance to the nearest 0.0001 g of total wet body weight. About 30 individuals from each tow (Table 1) were dissected under a stereo-microscope and their stomachs were removed and preserved individually in a buffered 4% formaldehyde–seawater solution.

For diet analysis, dissection of the digestive tracts took place under a stereo-microscope and the whole gut content of each fish was washed out onto a Petri dish and examined individually (at 90X magnification). Regurgitation during sampling was not detected since no presence of food was found in any oesophagus. Prey items were identified, when possible, to species level and counted. When items appeared damaged, to avoid prey number overestimation only the most characteristic part of the species was counted as an individual (e.g. head or 5th thoracic somite in copepods).

To describe feeding rhythm in larvae whose stomachs were not yet developed the entire digestive tract was examined, from oesophagus to anus. In larvae presenting a differentiated gut, the stomach content was considered to be the material contained in the pyloric and cardiac stomachs, while the contents of the intestine were discarded to reduce bias caused by different time of ingestion.

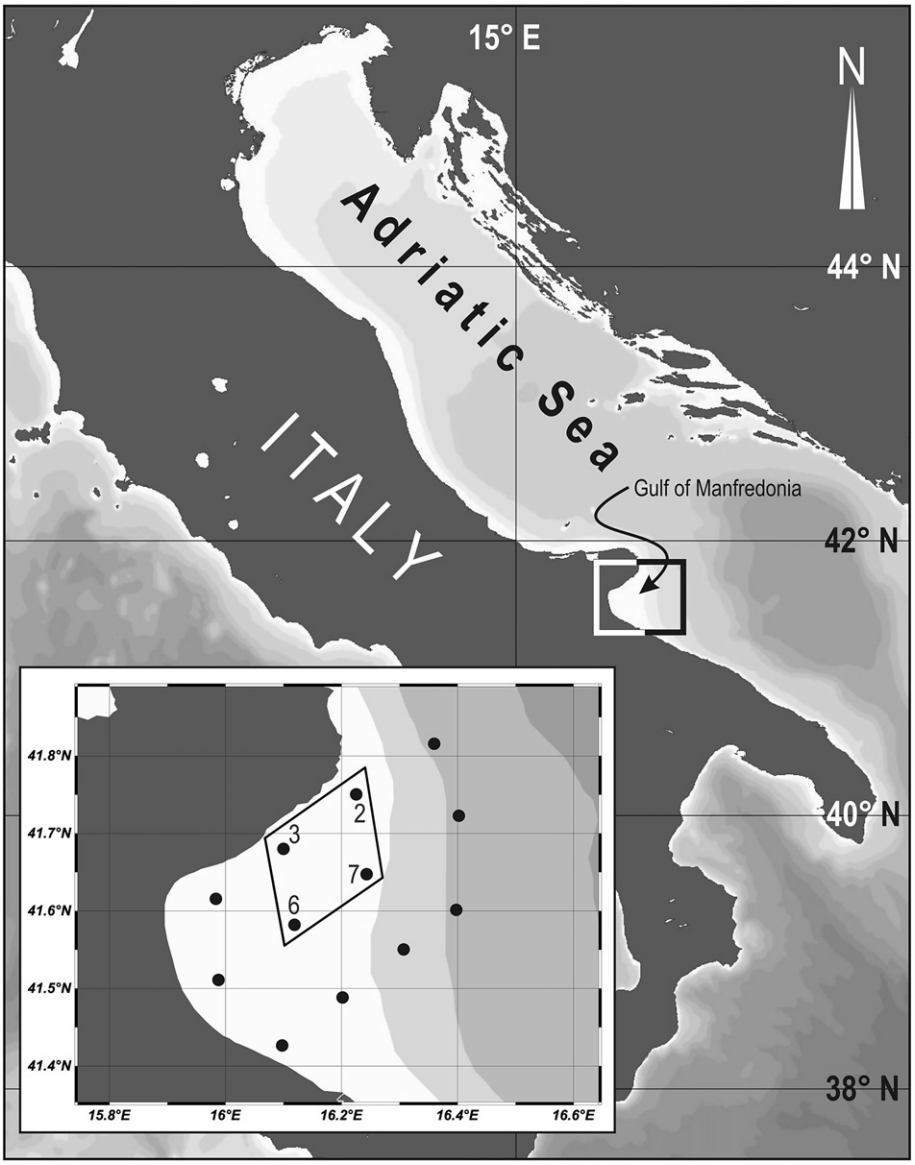

Fig. 1. Map of the study area in the Gulf of Manfredonia (southwestern Adriatic Sea), with the positions of plankton stations (black dots) and main trawling area for fish samples.

Test for significance ($p<0.05$) was performed using Student's t test after checking the normality of gut contents data set. The statistical analysis was performed by STATISTICA software.

2.3. Dietary indexes, feeding strategy and selectivity estimates

The diet was quantified by calculating *N* (%), the relative contribution of a prey item, calculated as the number of that prey expressed as a percentage of the total number of prey items counted in the total gut content from all larvae, and *O* (%), the number of larvae which ate a particular prey type expressed as a percentage of those larvae with some food in their guts (Hyslop, 1980). The product of these two values was taken as an index of relative dietary importance of each food item (*IRI*) and then expressed as percentage (*IRI*%) (Govoni et al., 1983; Laroche, 1982). The vacuity index *V* (%) was calculated as the ratio between the number of empty stomachs and the total number of stomachs analysed.

To assess the feeding strategy a modification of the Costello method (Amundsen et al., 1996; Costello, 1990) was applied to the whole data set of prey categories identified. The prey-specific abundance P_i, defined as the number of a prey taxon expressed as a percentage of all prey from the guts of only those predators in whose gut the prey category was actually found, was plotted against the frequency of occurrence *O*, providing a two-dimensional graph (Amundsen et al., 1996). In mathematical terms, the prey-specific abundance was expressed as follows:

$$P_i = (\sum_i S_i / \sum S_{ti})\ 100$$

where P_i is the prey-specific abundance of prey *i*, S_i the stomach content comprised of prey *i*, and S_{ti} the total stomach content in only those fish with prey *i* in their stomachs.

The resulting plot provides information on prey importance, feeding strategy and niche width contribution inferred through the position of prey categories in the diagram.

Feeding selectivity was studied from the relationships between stomach contents and the abundance of potential prey in the sea. For this analysis only four plankton stations, located around the area where fish assemblages were more consistent (on the basis of fishermen's indications and echosounder prospections), were considered (Fig. 1; stations 2, 3, 6, 7 with bottom depth of 14, 11, 14 and

Table 1
Sampling information: date and times when trawling started, mean bottom depths, mean trawling depths, number (N) and mean length of sardine larvae specimens considered for diet analysis.

Tow	Date	Time of catch (h GMT)	Mean bottom depth (m)	Mean trawling depth (m)	N (individuals)	Total length mean ± sd (mm)
39	14/02/08	14:30	11.0	11.0	35	32.4 ± 3.1
40	14/02/08	16:30	11.0	11.0	30	34.7 ± 2.0
41	14/02/08	20:00	11.0	9.0	30	32.7 ± 2.6
42	14/02/08	23:00	10.9	10.3	30	34.8 ± 4.3
43	15/02/08	05:00	11.0	11.0	30	32.4 ± 3.7
44	15/02/08	10:30	10.8	11.0	27	32.7 ± 2.7
45	15/02/08	15:00	16.9	17.0	35	41.6 ± 1.9

18 m respectively). Selectivity was estimated as the Ivlev electivity index *E* (Ivlev, 1955), calculated as follows:

$$E = (r_i - a_i)/(r_i + a_i)$$

where r_i is the relative abundance of prey category *i* (*%N*) in the stomachs of fish and a_i is the abundance of that prey in the water column. *E* ranges from −1 to +1; negative and positive values indicate avoidance or positive selection for a prey category, respectively, and a zero value indicates neutral selectivity.

3. Results

3.1. Feeding rhythm

The digestive tracts of 217 individuals of *S. pilchardus* late larvae (Table 1) were analysed. The majority of the analysed larvae had empty stomachs (Fig. 2). Only 64 guts contained food and only 24 of them contained more than 10 prey/stomach. Prey were found only in larvae caught during the daytime (Fig. 2). During that period the prey number per stomach varied from 0 to 359, with a mean value of 16.9 ± 52.4 prey/stomach. Nevertheless, the feeding activity was clearly centred in the first hours of the afternoon, around 15:00 h (Fig. 3), when the highest numbers of prey/stomach were observed.

3.2. Ontogenetic changes

All the examined larvae were at post-flexion stage, with lengths (TL) ranging from a minimum of 27.5 mm to a maximum of 45.0 mm, with a mean of 34.6 ± 4.4 mm (Table 1).

Considering only larvae caught during day time, larvae with 28 mm<TL<32 mm (n = 36) had 1.5 ± 3.01 prey/gut, larvae with 32 mm<TL<37 mm (n = 48) had 0.56 ± 1.58 prey/gut and larvae with 37 mm<TL<42 mm (n = 25) had 5.84 ± 5.98 prey/gut, vacuity index being 56%, 81% and 16% respectively (Fig. 2). The number of prey per gut suddenly increased as sardines' length reached 42 mm, attaining a mean value of 106.39 ± 101.67 prey/stomach and a vacuity index of only 6% (n = 18), being significantly higher than in larvae <42 mm TL (t test, $p<0.05$). Generally, at lengths <42 mm the gut did not appear differentiated, presenting a simple straight and tubular shape (Fig. 4A), while in individuals >42 mm the stomach was already developed, presenting a sack-like shape (Fig. 4B).

3.3. Diet composition

To investigate the diet composition both stomach and intestine contents were considered. A total of 2338 prey items, belonging to 35 taxa, were identified. Dietary indexes values referring to each identified food category are presented in Table 2. Copepods were by far the most important food category recovered in the sardine late-larvae guts. Diet was principally based on the copepods belonging to the genus *Paracalanus*

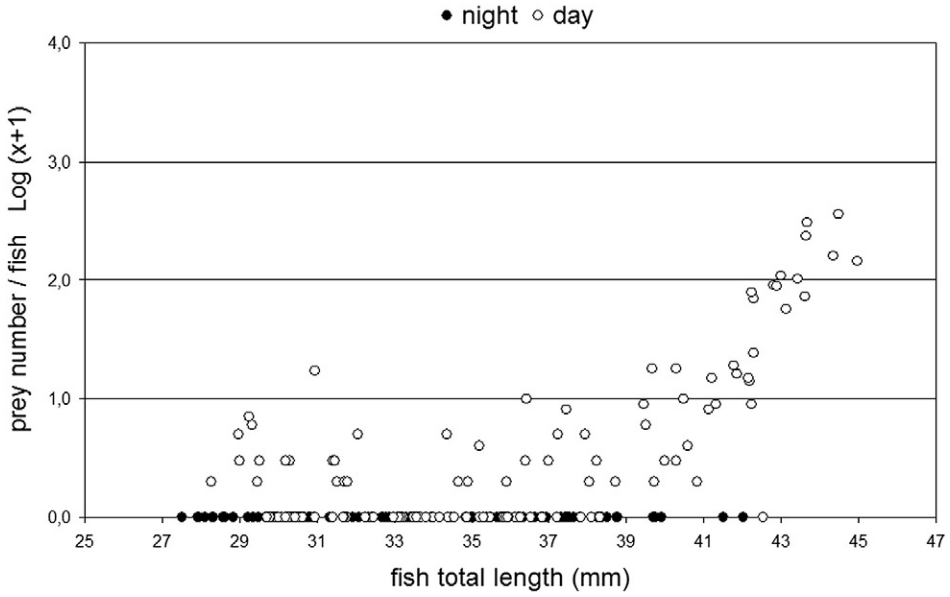

Fig. 2. Number of prey per stomach, expressed as ln (x + 1), relative to total length of each sardine larva. White dots represent sardines caught during the day, black dots represent sardines caught at night.

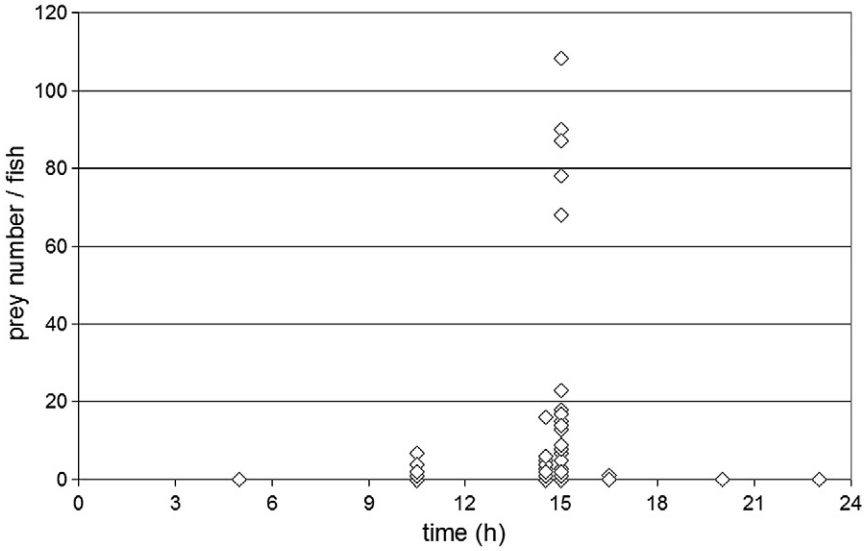

Fig. 3. Sampling time vs prey number found in stomach contents; stomachs containing more than 120 prey are not shown.

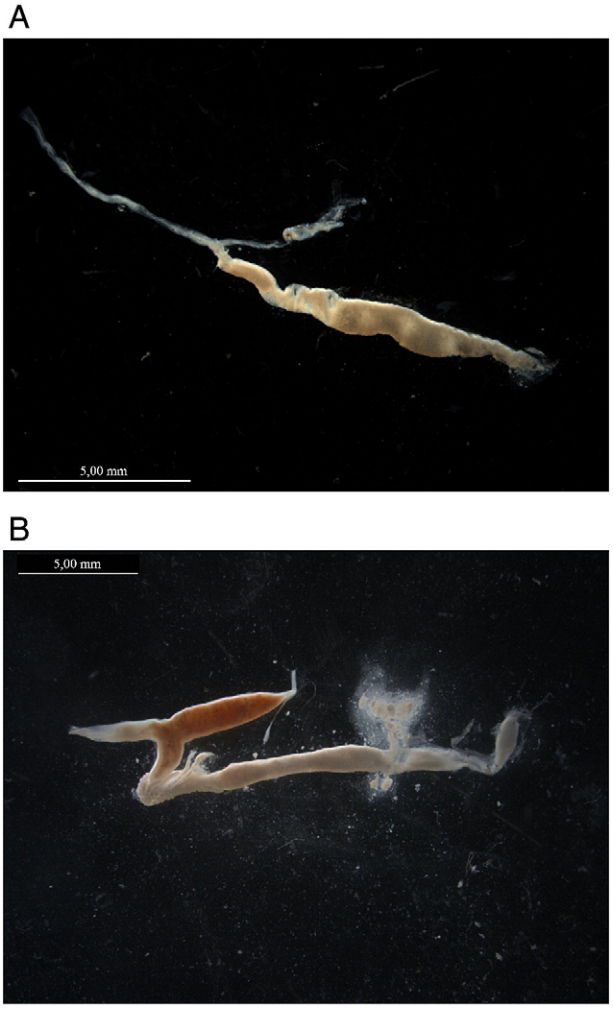

Fig. 4. Digestive tract in sardine late larvae at different developmental stages: A) *straight gut* in a 31.70 mm TL larva, composed of the oesophagus (the thin tubular tract on the left side) and the undifferentiated digestive tract (the larger tube); B) gut in a 43.64 mm TL larva, composed of the oesophagus (the short and thin tube on the left side), followed by the stomach which is already formed with its almost complete shape, and the intestine (the long tube); the pyloric caeca are visible at the junction between stomach and intestine.

(*IRI%* = 65.7) (present at sea mainly as *Paracalanus denudatus*, *Paracalanus parvus* and *Paracalanus* copepodites), other unidentified Calanoid copepods (*IRI%* = 16.48) and the species *Temora longicornis* (*IRI%* = 15.5). Small-sized copepods and other planktonic organisms were present, but never reaching *IRI%* > 1. Prey size ranged from 1210 μm for *Centropages typicus* (prosoma length) to 33 μm for copepod eggs. Nevertheless it was impossible to determine whether these eggs (whose diameters ranged from 33 to 88 μm) were intentionally ingested or if they were accidentally ingested as egg mass carried by the predated copepods.

Table 2
Dietary indexes of *S. pilchardus* late larvae from the Adriatic Sea based on taxonomical composition of the entire gut content, both stomach and intestine. N%, numerical percentage; O%, frequency of occurrence; IRI%, index of relative importance.

Prey group	Prey item	N%	O%	IRI%
Bacyllariophyceae	*Coscinodiscus* spp.	0.09	3.13	0.00
Dinoflagellida	*Ceratium* sp.	0.04	1.56	0.00
Mollusca	Bivalvia veliger	0.34	7.81	0.04
Cladocera	*Podon intermedius*	0.13	3.13	0.01
Ostracoda	Ostracoda juv.	0.04	1.56	0.00
Copepoda Calanoida	*Acartia* spp.	1.75	18.75	0.52
	Calocalanus spp.	0.13	3.13	0.01
	Centropages typicus	1.33	18.75	0.39
	Centropages spp.	0.43	10.94	0.07
	Paracalanus spp.	52.40	79.69	65.72
	Temora longicornis	21.00	46.88	15.50
	Temora stylifera	0.51	12.50	0.10
	Calanidae	0.09	1.56	0.00
	Calanoida unidentified	15.95	65.63	16.48
Copepoda Cyclopoida	*Ditrichocorycaeus brehmi*	0.09	3.13	0.00
	Corycaeus spp.	0.56	17.19	0.15
	Oithona nana	0.81	14.06	0.18
	Oithona similis	0.47	7.81	0.06
	Oithona spp.	0.21	4.69	0.02
	Oncaea spp.	1.24	25.00	0.49
Copepoda Harpacticoida	*Euterpina acutifrons*	0.04	1.56	0.00
	Microsetella spp.	0.04	1.56	0.00
	Harpacticoida spp.	0.17	4.69	0.01
Copepoda	Copepoda eggs	1.45	7.81	0.18
	Copepoda nauplii	0.26	7.81	0.03
	Copepoda unidentified	0.34	6.25	0.03
Cirripedia	Cirripedia nauplii	0.04	1.56	0.00
Urochordata	*Oikopleura* sp.	0.04	1.56	0.00

3.4. Feeding strategy

The feeding pattern observed in the late larvae of *S. pilchardus* is summarised in the Costello plot (Fig. 5). The genus *Paracalanus* dominated the diet as it was eaten by a great number of larvae ($O = 79.7\%$) and in great quantity (high values of specific abundance, $P_i = 52.8\%$). *T. longicornis* was predated by about half of the analysed larvae ($O = 46.9\%$), but its contribution in terms of abundance was moderate ($P_i = 22.2\%$). More frequent ($O = 65.6\%$), but slightly less abundant ($P_i = 16.5\%$), were the other Calanoids. The genera *Oncaea*, *Centropages*, *Oithona* and *Acartia*, and the family Corycaeidae, were found with a certain frequency (in about 20% of larvae), but with very low abundance ($P_i < 3\%$), and were thus a less important food category and indicate a scarce within-phenotype component. The remaining prey (Fig. 5; grouped in the lower left corner) presented very low values of both frequency ($O < 10\%$) and abundance ($P_i < 2\%$); they were occasional or rare food items.

3.5. Food availability and prey selection

The strategy of sampling zooplankton with two different nets enabled proper estimation of the density of potential sardine prey. A large number of small organisms, such as small copepods, copepod nauplii and bivalve larvae, were recovered in the samples collected by the Calvet net. At the same time, the Calvet net underestimated the presence of larger copepods, sampled by the WP2 net. Copepods were always the bulk of the mesozooplankton community while microplankton were numerically dominated by the dinoflagellates. The abundance of the planktonic organisms sampled by the two kind of nets were combined in order to describe the plankton community as food available to fish larvae (see Appendix A). The most numerous planktonic organisms were dinoflagellates ($268{,}388 \pm 134{,}761$ individuals m^{-3}). Copepods were the second most numerous group, with naupliar stages reaching a mean density of $15{,}848 \pm 4441$ individuals m^{-3} and the orders Calanoida and Cyclopoida reaching $10{,}707 \pm 4346$ individuals m^{-3} and $11{,}966 \pm 11{,}508$ individuals m^{-3} respectively. Bivalve larvae occurred with similar abundance (9749 ± 8214 individuals m^{-3}). In contrast, diatoms presented rather lower density (3623 ± 3031 individuals m^{-3}), only the bigger cells being retained by the 53 μm mesh of the Calvet net. Detailed information is available in the Appendix A.

Ivlev's index was calculated for 25 prey items, considering only those prey which were numerically present as >0.1% in the plankton or in the gut contents. Values of Ivlev's index (Table 3) confirmed the preference of sardine larvae for the calanoid copepods *T. longicornis* and those belonging to the families Clauso-Paracalanidae. Unexpectedly, almost at the same level of preference were found *Centropages* spp., Harpacticoids and Corycaeids. Positively selected prey, at high levels, were also *Temora stylifera* and, slightly behind, *Acartia* spp. Even if none of these prey were abundant food items, with the exception of *T. longicornis* and the families Clauso-Paracalanidae, nevertheless sardine late larvae preyed on them in the field. Copepod eggs, *Oithona* spp. and *Oncaea* spp. showed values close to 0, being present almost in the same proportion in both the plankton and the guts. Copepod nauplii were negatively selected, suggesting that the late larvae analysed in this work had already targeted bigger prey. Negative values of Ivlev's index also applied to *Oikopleura* spp., bivalve larvae and *Coscinodiscus* spp. Totally avoided prey were polychaet larvae and dinoflagellates. Unidentified Calanoids and Copepods were not considered for prey selectivity calculations because their abundance in the gut was biased by the difficulties in identifying them at genus or species level.

4. Discussion

4.1. Feeding rhythm

The analysis of stomach contents showed that all the late larvae caught at night had empty gut, suggesting that sardine larvae probably needed light for prey detection and/or capture. Daytime feeding in *S. pilchardus* larvae has already been observed in several regions (Ercegović, 1940 in the Adriatic; Conway et al., 1994 off the northern coast of Spain; Munuera-Fernández and González-Quirós, 2006 in the Cantabrian sea; Morote et al., 2010 in the Catalan Sea) and it has also been described for larvae of herring (Jespersen, 1928), of *Sardinops sagax* and *Engraulis mordax* (Arthur, 1976), and of *Engraulis encrasicolus* (Borme et al., 2009; Morote et al., 2010; Tudela et al., 2002). We observed a feeding peak at 15:00 (few hours before the sunset which was at 17:31), confirming the results of Ercegović (1940) who found exactly the same feeding rhythm, with maximal level of stomach replenishment at the same hour of the afternoon, in coastal areas of the eastern central Adriatic Sea. Munuera-Fernández and González-Quirós (2006) found the highest feeding incidence of *S. pilchardus* larvae early in the morning whereas Rasoanarivo et al. (1991) found that feeding intensity of sardine post-larvae rises considerably at dusk, in correspondence with the drop in light intensity. Conway et al. (1994),

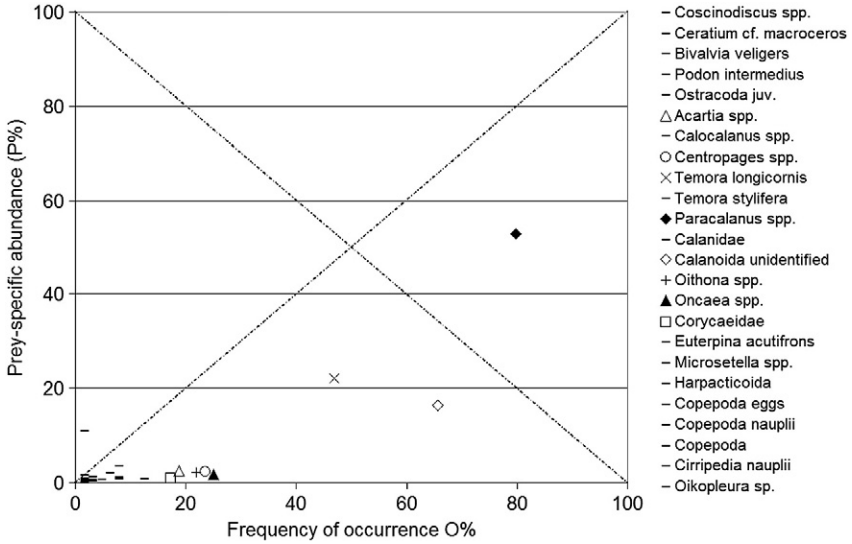

Fig. 5. Graphic representation of diet composition of *S. pilchardus* late larvae according to the modified Costello method. *Paracalanus* spp. (♦); Calanoida unidentified (◊); *Temora longicornis* (x); Oncaea spp. (▲); *Centropages* spp. (○); *Oithona* spp. (+); *Acartia* spp. (Δ); Corycaeidae (□); others (–).

Table 3
Ivlev's electivity index (E), calculated for sardine late larvae.

Prey group	Prey item	Ivlev's index
Bacyllariophyceae	*Coscinodiscus* spp.	−0.75
	Pleurosigma spp.	−1.00
Dinoflagellida	*Ceratium* spp.	−0.99
	Dinophysis spp.	−1.00
	Diplopsalis group	−1.00
	Gonyaulax spp.	−1.00
	Protoperidinium spp.	−1.00
	Noctiluca scintillans	−1.00
	Gymnodinium spp.	−1.00
Mollusca	Bivalvia veliger	−0.79
Plychaeta	Polychaeta larvae	−1.00
Copepoda Calanoida	*Acartia* spp.	0.60
	Centropages spp.	0.97
	Temora longicornis	0.99
	Temora stylifera	0.85
	Clauso−Paracalanidae	0.97
	Calanoida (unidentified)	0.79
Copepoda Cyclopoida	*Oithona* spp.	−0.16
	Oncaea spp.	−0.11
	Corycaeidae	0.90
Copepoda Harpacticoida	Harpacticoida (unidentified)	0.93
Copepoda	Copepoda eggs	0.16
	Copepoda nauplii	−0.90
	Copepoda (unidentified)	1.00
Urochordata	*Oikopleura* spp.	−0.88

on larval sardine off the northern coast of Spain, reported daytime feeding with peaks at dusk and soon after dawn.

Illumination and prey contrast may be of great importance in feeding of fish larvae as many zooplanktonic species are nearly transparent, so that only some particular pigmented portions of their body may be noticed by a visual predator (Zaret, 1972). These prey are difficult to see in light with a natural angular distribution (low image contrast) but Janssen (1981) observed that planktivorous fish can enhance the contrast of their prey by searching for them at angles greater than 48.6° from the vertical, making them appear bright against a dark background. This finding may explain the feeding peak found in late afternoon during winter, when the sun was already low on the horizon and the angle from the vertical was greater.

4.2. Ontogenetic changes

Although for several fish species a considerable amount of literature is available on morphological and physiological changes associated with metamorphosis (i.e. D'Ancona, 1931; Fahay, 1983; Lebour, 1921; Moser, 1981; Olivar and Fortuño, 1991; Ré and Meneses, 2009; Russell, 1976), empirical evidence of ontogenetic changes in foraging behaviour and prey selectivity in the field is relatively sparse. Costalago et al. (2012) analysed the stable isotopes values in sardine from the Gulf of Lions at different developmental stages (larvae, juveniles and adults) and, on the basis of the differences found, they suggested the hypothesis that the diet shift occur primarily at the time of metamorphosis, whereas juveniles and adults maintain similar diets.

In the present work the sardine diet was analysed during the transition from the late larval to the juvenile stage. This life stage occurs when sardines obtain a size which allows them to avoid plankton nets, which are properly used for ichthyoplankton. On the other hand these individuals are not efficiently retained by pelagic trawling nets, which are commonly used to catch adults.

In clupeoid fish, food passage through the larval alimentary canal is generally very rapid, especially in those species whose larvae present straight alimentary canals. Indeed, the low level of food found in larvae smaller than 30 mm may often be explained by the fact that, at those lengths, the tubular shape of the gut makes its emptying very easy. Regurgitation or defecation of gut content due to traumatic capturing or fixing is reported for sardine larvae (Conway et al., 1991, 1994; Morote et al., 2010; Munuera-Fernández and González-Quirós, 2006), for other clupeids (Hay, 1981) and, generally, for fish larvae with a straight gut (Dekhnik, 1974). Even if we cannot assume that prey were not extruded due to stress in sampling procedures, their abundance in the smallest sardine larvae analysed was similar to that found in larvae from 5 to 24 mm standard length by other authors (Dulčić, 1999; Morote et al., 2010; Munuera-Fernández and González-Quirós, 2006). Moreover, in sardine larvae from about 42 mm (TL), the number of prey in the gut had dramatically increased. This change was concomitant with the formation of the stomach, which was observed in fish of total lengths between 35 and 40 mm. Govoni et al. (1986a) reported that the changing complexity of the alimentary tract coincided with marked differences in diet. In this study, the development of the stomach was reflected in the amount of prey recovered in the gut content, due to the increased ability to store greater quantities of food. In fact the posterior region of the foregut (Watanabe and Sawada, 1985) and the midgut (Govoni, 1980) can expand and function as a food store in some larvae. Similar results were found for anchovy late larvae, which show a notable increase in the amount of ingested prey at lengths between 30 and 39 mm, and maximal levels of stomach replenishment at 40–60 mm (Borme et al., 2009). Most marine fishes undergo several larval stages, which end with completion of a final metamorphosis, leading to a juvenile, adult-like, stage. Because metamorphosis is associated with morphological, physiological and behavioural restructuring, during this process additional energy is required above that normally used for maintenance of metabolism and growth (Tanaka et al., 1996; Thorisson, 1994). Thus, a critical period may occur at metamorphosis during late larval–juvenile transition, as reported by several authors (see Blaxter, 1988; Thorisson, 1994; Vladimirov, 1975). The increasing energy demand may be met by capturing a greater number of prey and/or by addressing the research for food toward larger prey (Islam and Tanaka, 2006; Thorisson, 1994). An increase in the number of prey may have been the response adopted by the sardines considered in this work, which were sampled during a particular life stage, immediately prior to metamorphosis, occurring at a length ranging from 40 to 50 mm (Ré and Meneses, 2009).

4.3. Diet composition

The results of the present study demonstrate that the diet of the late larval stage of *Sardina pichardus* is principally composed of copepods, even at adult stages, but belonging to small-sized species whose prosoma length is <1 mm. Diet was principally based on the genus *Paracalanus* and on other copepods such as *T. longicornis*, *Acartia* spp., *Centropages* spp., *Oncaea* spp. and *Oithona* spp. Overall only 3 cells of phytoplankton and, surprisingly, very few copepod nauplii were found in the digestive tracts of all the analysed specimens.

To our knowledge, further information on prey species composition for sardine larvae >25 mm are nearly nonexistent, with the exception of the study by Ercegović (1940). This author, in larvae from 30 to 50 mm, caught in sheltered bays of the eastern central Adriatic Sea, indicated copepod nauplii and copepodites as the main food items, although he also found many species of Dinoflagellates, the most frequent genera being *Prorocentrum*, *Protoperidinium* and *Ceratium*. Among adult copepods, he pointed out as very abundant *Oithona nana* and *P. parvus*, and very frequent, but less numerous, the calanoid copepod *Isias clavipes*. He frequently also found *Corycaeus* spp., *Centropages* spp., *Temora stylifera* and the cladoceran *Evadne* spp.

The same copepod taxa, but at naupliar and copepodite stages, have been reported in the diet analysis of smaller sardine larvae (Conway et al., 1991, 1994; Dulčić, 1999; Karlovac, 1967; Morote et al., 2010; Munuera-Fernández and González-Quirós, 2006; Voss et al., 2009). Voss et al. (2009), for example, in sardine larvae from the North Sea, recognised naupliar stages belonging to *Pseudo-Paracalanus*, *Oithona*, *Acartia* and *T. longicornis*. In the stomach contents of larval sardines from the central Adriatic Sea, Dulčić (1999) found copepod developmental stages (eggs,

nauplii and copepodites) as the most common food organisms, with an increasing percentage of copepodite stages with increasing larval size. Results suggesting an increase in the prey size with the increase in larval size were also obtained in the Northwest Mediterranean by Morote et al. (2010) who reported that *S. pilchardus* larvae < 10 mm (standard length) fed mainly on tintinnids and copepod nauplii whereas the diet of larger larvae (10–15.8 mm) was based on copepod nauplii and *Clausocalanus* postnauplii. Regarding the time-course of the dietary shift, Conway et al. (1994) found that the increase in the consumption of copepodites was gradual; in contrast, Munuera-Fernández and González-Quirós (2006) reported that the dietary shift from nauplii to copepodites was abrupt, occurring at 13 mm in length.

As sardine late larvae inhabit coastal areas during winter and early spring, their preference for copepods can probably be considered a metabolic need for high-energy prey, including to compensate for the cooler water temperatures of that period.

4.4. Food availability and prey selection

The presence, in the gut contents, of prey species which were less abundant in the plankton samples (*Centropages* spp., Harpacticoids, Corycaeids, *Calocalanus* spp., *Podon intermedius*, *Temora stylifera* and *Acartia* spp.), suggests that *S. pilchardus* larvae actively searched for and selected food particles. This interpretation is confirmed by other authors, who describe zooplanktonic prey as visually sighted (Hunter, 1980) and individually captured by nearly all fish larvae (e.g. Checkley, 1982; Govoni et al., 1986b; Hunter, 1980; Last, 1980; Sampey et al., 2007).

Paracalanus, *Pseudocalanus*, *Temora* and *Acartia* are all copepod genera which are very frequently found in gut contents of sardine larvae from several regions (Arthur, 1976; Conway et al., 1991, 1994; Dulčić, 1999; Ercegović, 1940; Karlovac, 1967; Morote et al., 2010; Muck et al., 1989; Voss et al., 2009). The constant presence of these copepods in the gut contents suggests that some prey characteristics are more likely to provoke attacks by larval fish. Although an experimental approach with laboratory observations is probably needed to define what makes these prey more vulnerable, it is plausible that prey movement plays an important role in their detection. In fact, in coastal environments, where planktivorous larvae are generally abundant, the density of suspended matter may be high, making it very difficult to recognize planktonic prey based on size and image contrast alone. Additionally, there is increasing evidence that prey recognition by planktivores may be strongly influenced by prey movement (Wright and O'Brien, 1982; Zaret, 1980).

Avoidant prey, however, can significantly alter prey selection, feeding on less evasive prey being more successful (Drenner and McComas, 1980). Larval fish and their copepod prey have similar swimming speeds (in terms of body length), ranging from less than one to about five body lengths per second [summarised in Mauchline (1998) about copepods; Miller et al. (1988) about fish larvae]. Jiang and Paffenhöfer (2004) suggest that continuous swimmers are less sensitive to hydrodynamic signals and not efficient at remotely detecting predators, thus being more vulnerable to predation, compared to those moving in a 'jump–sink' pattern. Adult *Pseudocalanus* spp. show a small sensitivity to hydrodynamic signals (Viitasalo et al., 2001) and are unable to accelerate rapidly (Checkley, 1982). On the other hand, *Acartia* spp. are able to perform just a weak jump with a small escape distance (Viitasalo et al., 2001). Both the poor alertness and the very weak escape responses of prey are positively related to a predator's success of attack and could have contributed to the observed selection of these prey types.

4.5. Conclusions

This study was carried out in an important sardine nursery area in the Adriatic Sea, where fry of this species were traditionally fished. We demonstrated that sardine late larvae are exclusively zooplanktivorous and able to positively select their prey. Moreover, we showed that during the translation from the late larval to the juvenile stage, the development of the stomach is associated to a considerable increase of the prey number recovered in the gut content. These findings points out the crucial role of late larval stages for *S. pilchardus* recruitment and the importance to improve our knowledge on the trophic ecology of late larval stages of small pelagic species.

Acknowledgements

The present study was financially supported by the European Project SARDONE (Improving assessment and management of small pelagic species in the Mediterranean, FP6-44294). Special thanks to Alessandra de Olazabal for her support in taxonomic identification of prey, to Sara Legovini for her help in laboratory analysis, and to the crew of the RV *G. Dalla Porta* for their assistance in field work. The authors are also grateful for the valuable comments of two anonymous reviewers, which improved the quality of the manuscript.

Appendix A

Mean abundance of zooplanktonic taxa expressed as ind. m^{-3}; SD (standard deviation).

Group	Species	Mean	SD
Foraminiferida	*Bolivina* sp.	223	±446
Bacyllariophyceae	*Coscinodiscus* spp.	1944	±1344
	Navicula spp.	298	±595
	Pleurosigma spp.	1158	±1655
	Undetermined centric diatoms	112	±223
	Undetermined pennata diatoms	112	±223
Dinoflagellida	*Ceratium candelabrum*	800	±318
	Ceratium furca	13,481	±4780
	Ceratium fusus	25,195	±13,532
	Ceratium hexacanthum	153	±211
	Ceratium horridum	1009	±673
	Ceratium minutum	149	±298
	Ceratium tripos	7045	±5036
	Ceratium symmetricum	112	±223
	Dinophysis acuta	112	±223
	Dinophysis caudata	460	±632
	Dinophysis fortii	112	±223
	Dinophysis odiosa	42	±84
	Diplopsalis group	1679	±1338
	Gonyaulax polygramma	201,976	±108,179
	Gonyaulax spp.	637	±806
	Protoperidinium brochi	112	±223
	Protoperidinium claudicans	149	±298
	Protoperidinium conicum	530	±852
	Protoperidinium crassipes	2646	±2304
	Protoperidinium depressum	112	±223
	Protoperidinium divergens	3288	±1770
	Protoperidinium oceanicum	1576	±456
	Protoperidinium pallidum	344	±253
	Protoperidinium pyriforme	42	±84
	Protoperidinium steinii	539	±381
	Protoperidinium spp.	818	±744
	Noctiluca scintillans	3707	±4282
	Gymnodinium spp.	1116	±1946
	Gyrodinium spp.	446	±893
Ciliophora	*Tiarina fusus*	223	±446
Hydrozoa	Anthomedusae (undetermined)	2	±3
	Obelia spp.	2	±3
	Siphonophora (undetermined)	34	±27
Ctenophora	Ctenophora (undetermined)	2	±3
Gastropoda	*Creseis acicula*	19	±37
	Gastropoda pediveliger	262	±338
Bivalvia	Bivalvia veliger	9749	±8214
Polychaeta	Polychaeta larvae	803	±861
Nemertea	Nemertea pilidium	42	±78

(continued on next page)

Appendix A (*continued*)

Group	Species	Mean	SD
Cladocera	*Evadne nordmanni*	1	±3
	Podon intermedius	2	±3
	Podon juv.	29	±36
Copepoda	Copepoda nauplii	15,848	±4441
Calanoida	*Acartia clausi*	231	±149
	Acartia tonsa	11	±15
	Acartia copepodites	1174	±1036
	Calanus helgolandicus	10	±11
	Calanus helgolandicus copepodites	33	±20
	Calocalanus contractus	2	±3
	Calocalanus styliremis	9	±10
	Calocalanus copepodites	12	±10
	Centropages typicus	10	±12
	Centropages copepodites	76	±46
	Clausocalanus arcuicornis	3	±4
	Clausocalanus furcatus	2	±4
	Clausocalanus jobei	4	±7
	Clausocalanus parapergens	1	±3
	Clausocalanus pergens	3	±4
	Clausocalanus copepodites	86	±42
	Ctenocalanus vanus	13	±4
	Ctenocalanus vanus copepodites	34	±18
	Mecynocera clausi	2	±4
	Paracalanus denudatus	994	±1419
	Paracalanus nanus	22	±15
	Paracalanus parvus	414	±320
	Paracalanus copepodites	1067	±471
	Pseudocalanus elongatus	3	±4
	Temora longicornis	44	±45
	Temora longicornis copepodites	246	±238
	Temora stylifera	1	±1
	Temora stylifera copepodites	133	±176
	Calanoida copepodites	6067	±5934
Cyclopoida	*Corycaeus* spp.	34	±15
	Corycaeus copepodites	80	±85
	Oithona nana	83	±104
	Oithona plumifera	8	±10
	Oithona setigera	6	±7
	Oithona similis	245	±196
	Oithona copepodites	6408	±5672
	Oncaea spp.	1483	±2019
	Oncaea copepodites	3618	±3830
Harpacticoida	*Euterpina acutifrons*	7	±10
	Euterpina acutifrons copepodites	60	±119
	Microsetella rosea	298	±595
	Microsetella spp.	4	±7
	Harpacticoida (undetermined)	20	±40
Cirripedia	Cirripedia nauplii	16	±20
Decapoda	Decapoda zoee	2	±3
	Decapoda larvae	1	±3
Isopoda	Epicaridae (undetermined)	2	±4
Echinodermata	Auricolaria larvae	118	±190
Chaetognatha	*Sagitta* spp.	12	±20
Urochordata	*Oikopleura* spp.	2159	±2382
Others	Invertebrata eggs	3478	±1344

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