

Spring zooplankton distribution in the Bay of Biscay from 1998 to 2006 in relation with anchovy recruitment

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The objective of this study was to investigate whether successive recruitment failures in the anchovy fishery in the Bay of Biscay were due to changes in the zooplankton biomass or composition. Image analysis and automatic recognition were used to analyse zooplankton samples collected during diel egg production method spring surveys from 1998 to 2006. We were not able to detect any trend in zooplankton biomass during this period. The zooplankton spatial distribution showed permanent features with large organisms being more abundant over the shelf break and outer areas. Finally, we found a negative correlation between anchovy recruitment and zooplankton biomass which suggests that the 2002–2006 failures in anchovy recruitment in the Bay of Biscay are not due to a decrease in mesozooplankton biomass.

INTRODUCTION

The key role of zooplankton to transfer energy from phytoplankton to the upper trophic levels is widely recognized and has been the subject of major international programmes (www.GLOBEC.org). Several studies have suggested that climate-mediated changes in zooplankton abundance and composition might influence fish recruitment (e.g. Beaugrand *et al.*, 2003) with consequences for fish populations and fish management. On the other hand, fisheries science now recognizes the influence of environment and an ecosystem approach to management is being proposed as one of the solutions to management problems. Because zooplankton are the prey of most fish at one or other stage (larvae to adult), understanding the spatial–temporal variations of zooplankton distribution remains a key element of an ecosystem approach. However, a gap remains between zooplankton and fisheries research. This is mainly due to the differences in the typical

spatial scales and the labour involved in zooplankton samples analyses. This gap results in a lack of appropriate biological information on the prey field for adult fish and their offspring. As a result, relevant questions as to whether larval survival and recruitment are limited by food or predation (e.g. Agostini *et al.*, 2007) often remain unanswered because zooplankton data are not available at the spatial scales of the fish population. This problem is not restricted to the relationship with fisheries, our knowledge of the factors affecting the distribution of zooplankton is very limited because of the difficulties of sampling zooplankton with the relevant spatial (mesoscale) temporal and taxonomic resolution (Kushnir *et al.*, 1997; Moore *et al.*, 2003).

Fish stock estimation cruises can provide the platform to sample zooplankton and some of the stock estimation methods, such as the daily egg production method (DEPM) even include taking zooplankton samples at a high spatial resolution. Furthermore, recent developments in image analysis and automatic recognition using

machine learning techniques allow for a rapid analysis of a large number of samples with some degree of taxonomic resolution (Grosjean *et al.*, 2004; Benfield *et al.*, 2007).

In the Bay of Biscay, the population of anchovy (*Engraulis encrasicolus*) has crashed due to low recruitment during successive years (2003–2007). As consequence of the succession of poor recruitment, the question of a potential regime change has been raised although with the data available at the moment it has not been possible to conclude that there has been a shift in the Bay of Biscay pelagic ecosystem (ICES, 2008). Recruitment of anchovy in the Bay of Biscay appears to be at least partially related to the wind regime and upwelling intensity (Borja *et al.*, 1998), but the mechanisms involved remain unknown and the reasons for the low recruitments unclear (Irigoien *et al.*, 2007). In addition, as for other fisheries, it remains a challenge to understand the respective role of bottom-up and top-down controls on recruitment (Beaugrand *et al.*, 2003; Bakun Broad, 2003; Irigoien *et al.*, 2007). One of the issues that can be addressed in relation to the successive low recruitment is whether there has been a significant decrease in the productivity of the system during recent years.

In this paper, we have used image analysis and automatic recognition with machine learning to analyse the zooplankton samples collected during DEPM spring surveys from 1998 to 2006 so as to explore whether there has been a significant change in the zooplankton abundance or composition in recent years that could explain the consecutive low recruitments.

METHOD

The samples were obtained during Bioman surveys covering the southeast of the Bay of Biscay in spring from 1998 to 2006 (see Table I and Fig. 1 for dates, number of samples and coverage of each cruise). These cruises generally take place in May, at the peak spawning period and covering the spawning area of anchovy in the Bay of Biscay. The objective of the cruises is to evaluate anchovy biomass using the DEPM method. A synthesis of the anchovy spawning areas, larvae and juvenile distribution, and hypotheses about recruitment mechanisms can be found in Irigoien *et al.* (Irigoien *et al.*, 2007). Stations were located every 3 nautical miles (nm) along transects 15 nm apart perpendicular to the coast. A vertical plankton haul was made at each sampling station, using a 150 μm PairoVET net (2-CalVET nets, Smith *et al.*, 1985). The net was lowered to a maximum depth of 100 m or 5 m above the bottom at shallower stations. Samples were preserved in 4% formaldehyde buffered with sodium tetraborate.

Table I: Cruise, dates, average temperature and number of samples collected in each cruise

Cruise	Dates	N samples	Average T (°C)
Bioman 1998	18 May–06 June 1998	657	16.5
Bioman 1999	22 May–04 June 1999	343	17.1
Bioman 2000	02 May–19 May 2000	405	16.5
Bioman 2001	14 May–06 June 2001	614	16.8
Bioman 2002	07 May–20 May 2002	375	14.7
Bioman 2003	22 May–08 June 2003	505	17.3
Bioman 2004	02 May–22 May 2004	410	13.7
Bioman 2005	08 May–27 May 2005	419	14.9
Bioman 2006	04 May–23 May 2006	396	15.6

Samples were stored in 150 mL jars. The sample was thoroughly mixed in a measuring cylinder, the total volume measured (usually around 150 mL) and an aliquot of 6 mL was taken with a pipette from each sample. The average number of organisms counted in each plate with this sub sampling set up is around 400 individuals. The aliquot was stained for 24 h with 4 mL 1% eosin, which stains the cell cytoplasm and the muscle protein. This stain creates sufficient contrast to be recognized by image analysis and reduces counting of detrital material. The sub-samples were scanned on polystyrene plates (12.7 \times 8.5 cm) in 24 bit colour, at a resolution of 600 dpi using an HP Scanjet8200 series scanner (reflective). The samples were not manually separated. Preliminary work (unpublished) has shown that as long as the percentage of the image covered by the sample remains below 3%, there is a linear relation between the number of items and the automatic counting. Over that threshold, the percentage of organisms touching each other increases and results in an underestimation of the abundance. For samples from the Bay of Biscay the aliquot taken and the plastic plate size assure that this limit is not reached. These images were analysed using Zooimage (www.sciviews.org/zooimage). A total of 17 classes were selected combining expert opinion and the class selection method proposed by Fernandes *et al.* (Fernandes *et al.*, 2008). Classification was carried out using a Random Forest algorithm which provided the best results with an estimated accuracy 88.23% using 10-fold cross-validation. Accuracy for each of the classes and the confusion matrix are provided in Tables II and III. The spatial distribution of

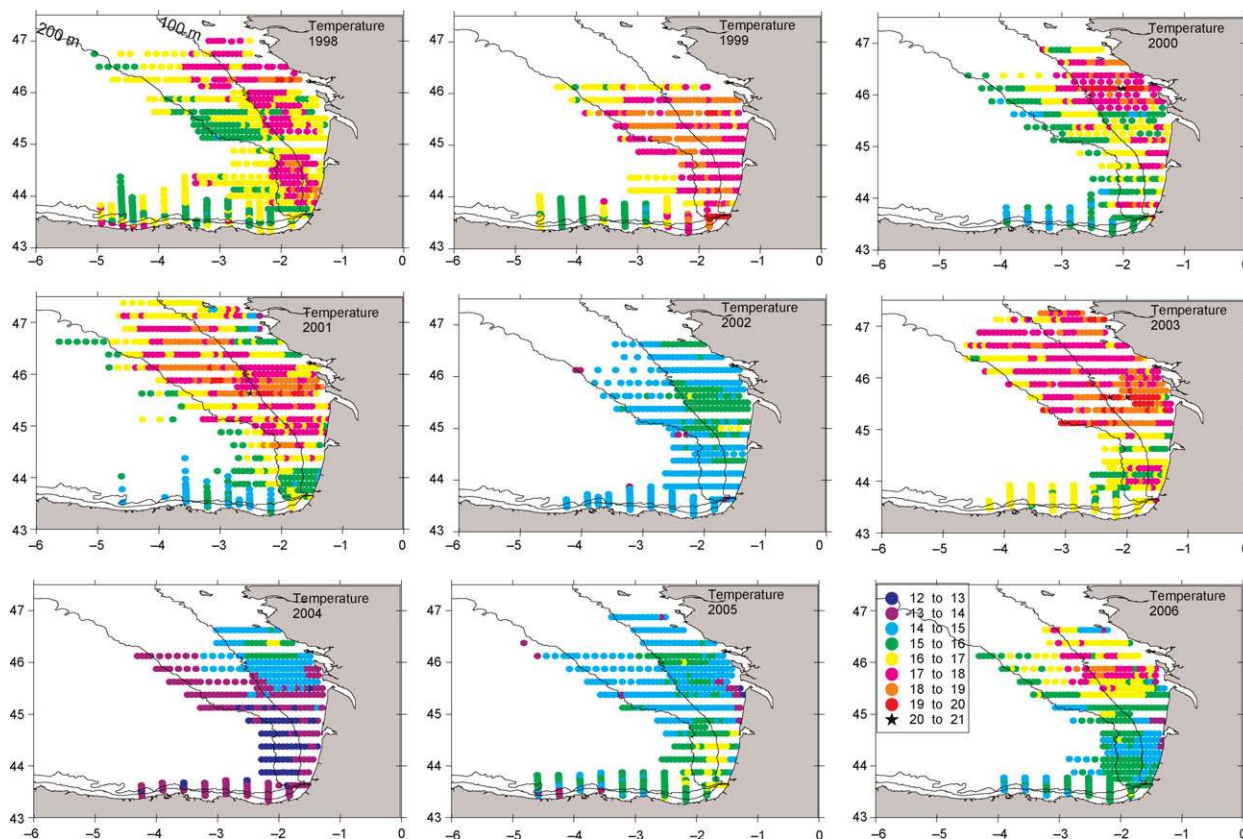


Fig. 1. Sea surface temperature in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

the classes that were rare and resulted in poor classification is not presented. However, because individual biomass were estimated based on the size—biomass relationship provided by Alcaraz *et al.* (Alcaraz *et al.*, 2003) which is not taxon specific, all individuals were used for total biomass estimation. Three classes were excluded from the biomass estimation because these particles are not zooplankton: “scanning artefacts”, “marine snow” and “small marine snow”. Mean abundances and biomass (Table IV) were calculated for a common area corresponding to the area of the year with the minimum coverage (1999).

Biomass was distributed in size classes according to width (minor axis of an ellipsoid of the same area as the particle) instead of other more frequently used size estimates, like the length or the equivalent spherical diameter, because for fish the organism width determines what can be eaten and what cannot (fish mouth gape). In fish dietary research, the width of the prey is a common measurement (e.g. Conway *et al.*, 1998).

The slope of the normalized biomass spectrum was calculated according to Zhou (Zhou, 2006). In this case,

Table II: Percentage of error in the classification of each class (estimated by 10-fold cross-validation and the Random Forest algorithm)

Class	Training-set individuals	Error per class (%)
Marine snow I (small, 0.48–0.8 mm equivalent circular diameter)	482	2.76
Copepods II (medium large, 0.58–3.07 mm ECD)	2063	3.23
Marine snow II (large, 0.8–6.55 mm ECD)	1136	4.78
Artefacts	467	7.59
Copepods I (small, 0.48–0.58 mm ECD)	2228	8.49
Euphausiids and mysids	1838	9.3
Chaetognaths	1123	12.29
Appendicularians	209	39.71
Decapod larvae	330	43.42
Fish larvae	200	45.98
Polychaetes	279	50.21
Doliolids and siphonophorans	309	69.78
Gelatinous (not in other classes)	57	75.93
Cephalopod larvae	17	82.35
Fishes	48	90
Crustaceans (not in other classes)	31	93.33
Polychaete larvae	12	100

Table III: Confusion matrix (10-fold cross-validation with Random Forest algorithm; same training set and class names as in Table II)

	Polychaete larvae	Appendicularians	Scanning Artefacts	Copepods II	Copepods I	Crustaceans	Chaetognaths	Decapod larvae	Doliolids and siphonophorans	Euphausiids and mysids	Fishes larvae	Fishes	Gelatinous	Marine snow II	Marine snow I	Polychaetes	Cephalopod larvae
Polychaete larvae	0	9	0	0	0	0	36	9	0	18	0	0	0	18	0	9	0
Appendicularians	0	58	2	3	0	0	7	1	0	12	0	4	0	3	3	7	0
Scanning artefacts	0	0	92	0	0	0	6	0	0	0	0	0	0	0	1	0	0
Copepods II	0	0	0	97	2	0	0	0	0	1	0	0	0	0	0	0	0
Copepods I	0	0	0	8	92	0	0	0	0	0	0	0	0	0	0	0	0
Crustaceans	0	0	0	40	0	7	0	17	0	30	0	0	0	3	0	3	0
Chaetognaths	0	1	3	0	0	0	87	0	0	2	0	3	0	3	0	1	0
Decapod larvae	0	0	0	9	0	0	0	58	0	30	0	0	0	1	0	1	0
Doliolids and siphonophorans	0	0	1	3	0	0	4	1	30	3	0	0	0	53	1	4	0
Euphausiids and mysids	0	0	0	4	0	0	0	2	0	91	0	0	0	0	0	1	0
Fishes	0	0	0	13	0	0	0	3	0	68	13	5	0	0	0	0	0
Fishery larvae	0	10	0	0	0	0	18	1	1	16	0	52	0	2	0	1	0
Gelatinous	0	0	0	0	0	0	2	11	0	0	0	0	24	63	0	0	0
Marine snow II	0	1	0	0	0	0	1	1	2	0	0	0	0	95	0	1	0
Marine snow I	0	0	1	0	1	0	0	0	0	0	0	0	0	0	97	1	0
Polychaetes	0	2	1	6	0	0	8	1	0	18	0	0	0	11	1	51	0
Cephalopod larvae	0	0	0	59	0	0	0	6	0	6	0	0	0	12	0	0	18

Percentage of items identified in each class. Corresponding identifications are in the diagonal (bold number); errors are outside of the diagonal.

Table IV: Mesozooplankton abundance (ind m^{-3}) and biomass (mg C m^{-3}) in the common area from 1998 to 2006

Year	Abundance (ind m^{-3}) mean (min-max)	Biomass (mg C m^{-3}) mean (min-max)
1998	1880 (16–17 274)	11.4 (0.2–168.3)
1999	1005 (120–4500)	5.3 (0.4–33.8)
2000	2156 (26–12 011)	17.1 (0.5–121.4)
2001	2270 (100–11 810)	25 (0.3–1266.6)
2002	4824 (296–21 280)	21.2 (1.1–90.3)
2003	2510 (628–15 381)	18.4 (4.1–175.3)
2004	2019 (86–16 900)	16.7 (1–342.3)
2005	2440 (346–16 216)	14.6 (0.4–87.4)
2006	3441 (93–96 367)	31.9 (0.3–774.9)

the weight classes for the slope of the biomass spectrum were selected in base 2 logarithmic intervals from 0.8 to 25.6 $\mu\text{g C}$ that appeared to be well sampled by PairoVET nets (no 0 values in the large classes, and fitting a negative slope for the small classes).

Temperature and salinity were measured at the surface with a RBR XR-420 CTD. Anchovy recruitment and biomass data were obtained from ICES (www.ices.dk).

RESULTS

Temperature and salinity

Interannual and spatial variations in temperature have to be carefully considered because the surveys were not carried out exactly on the same dates and also because there was a 10–15 day delay between the first (Southwest corner of the sampled area) and last (Northeast) stations (Fig. 1). The same applies to salinity as it is highly dependent on river run-off (Fig. 2). Nevertheless for cruises carried out during the same dates such as 2000 and 2004, it can be observed that the difference in temperature between years might reach 3°C (Table I). For salinity, the expected pattern of a salinity gradient between coastal and oceanic waters was observed (Fig. 2).

Mesozooplankton abundance and distribution of taxa

The highest abundances of mesozooplankton were observed in 2002 and the lowest in 1999 (Figs 3–6 and Table IV). However, the highest abundances of medium

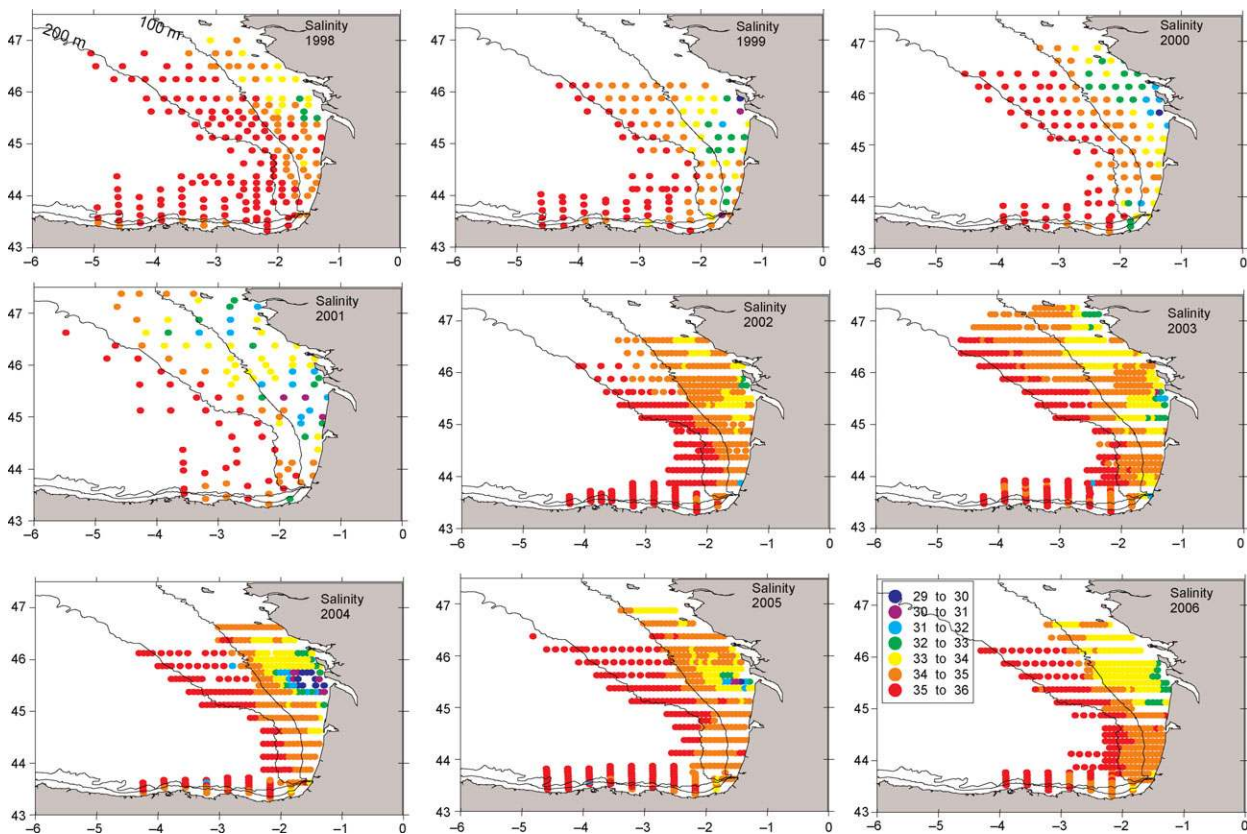


Fig. 2. Surface salinity in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

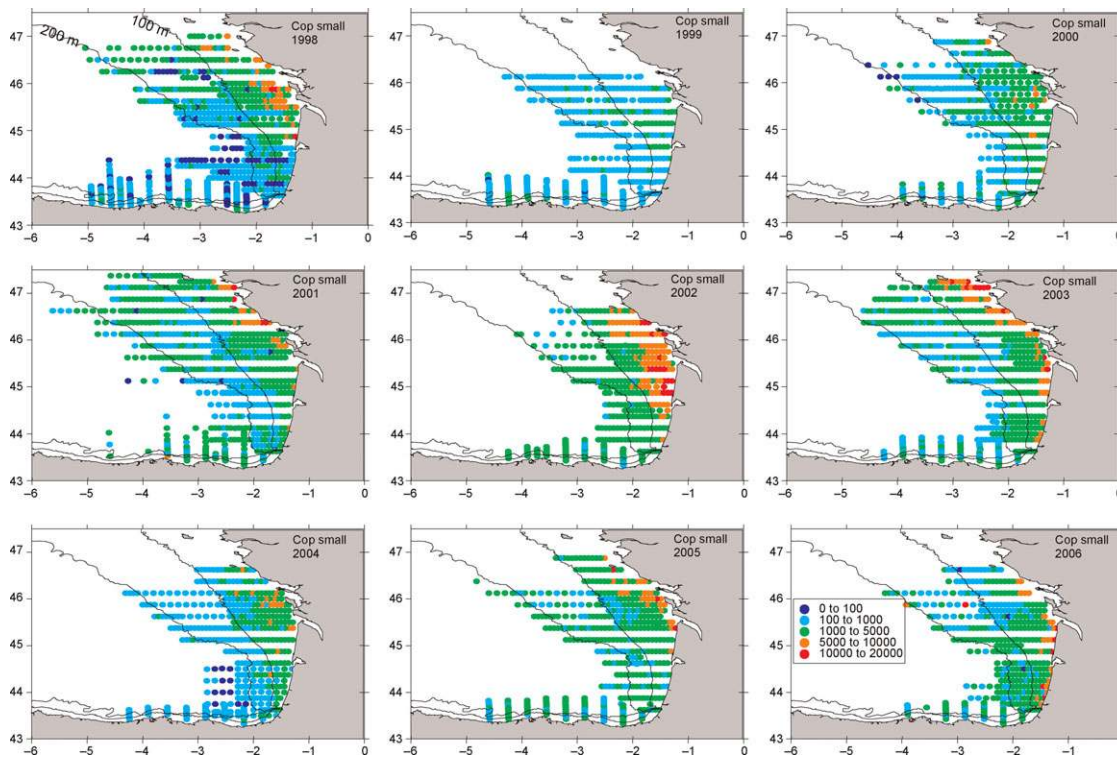


Fig. 3. Distribution of the small copepods abundance (ind m^{-3}) in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

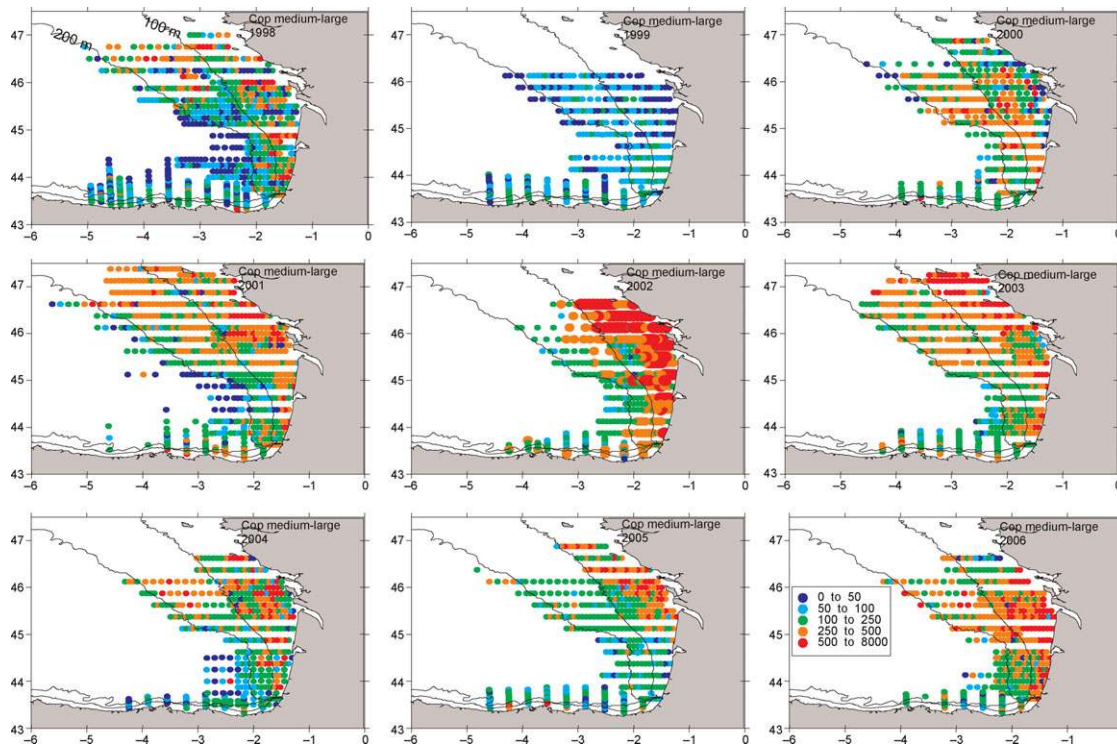


Fig. 4. Distribution of the medium large copepods abundance (ind m^{-3}) in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

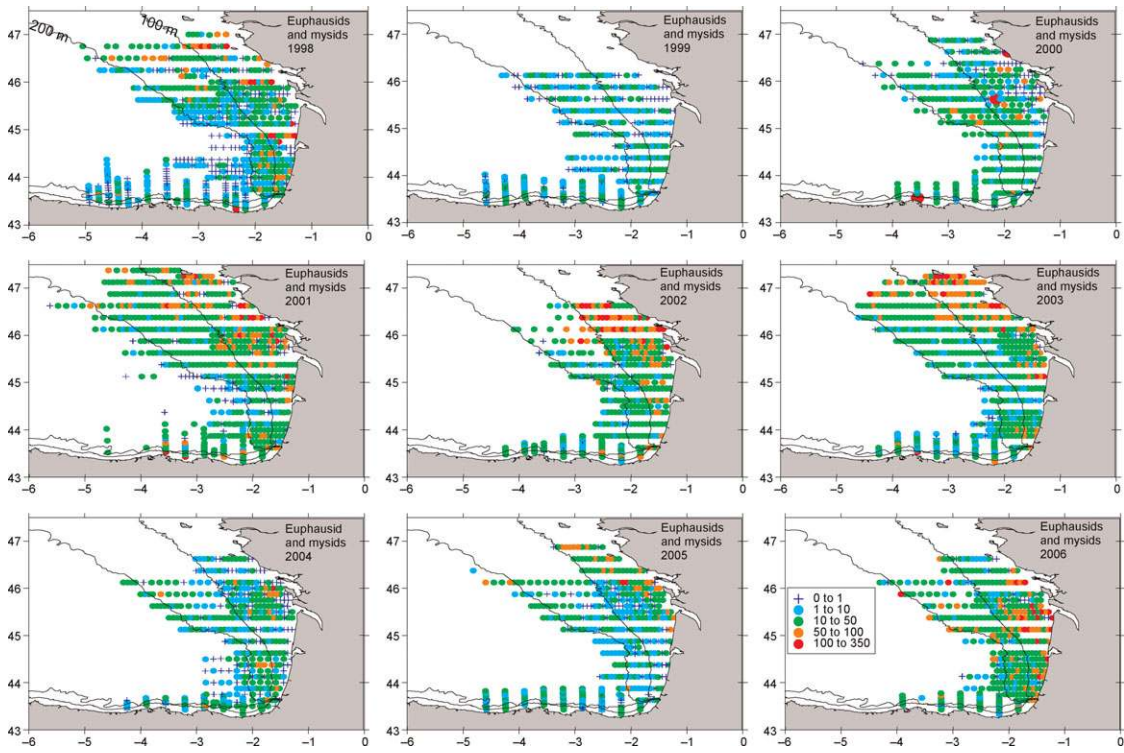


Fig. 5. Distribution of the euphausiid-like organisms (euphausiids and mysids) abundance (ind m^{-3}) in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

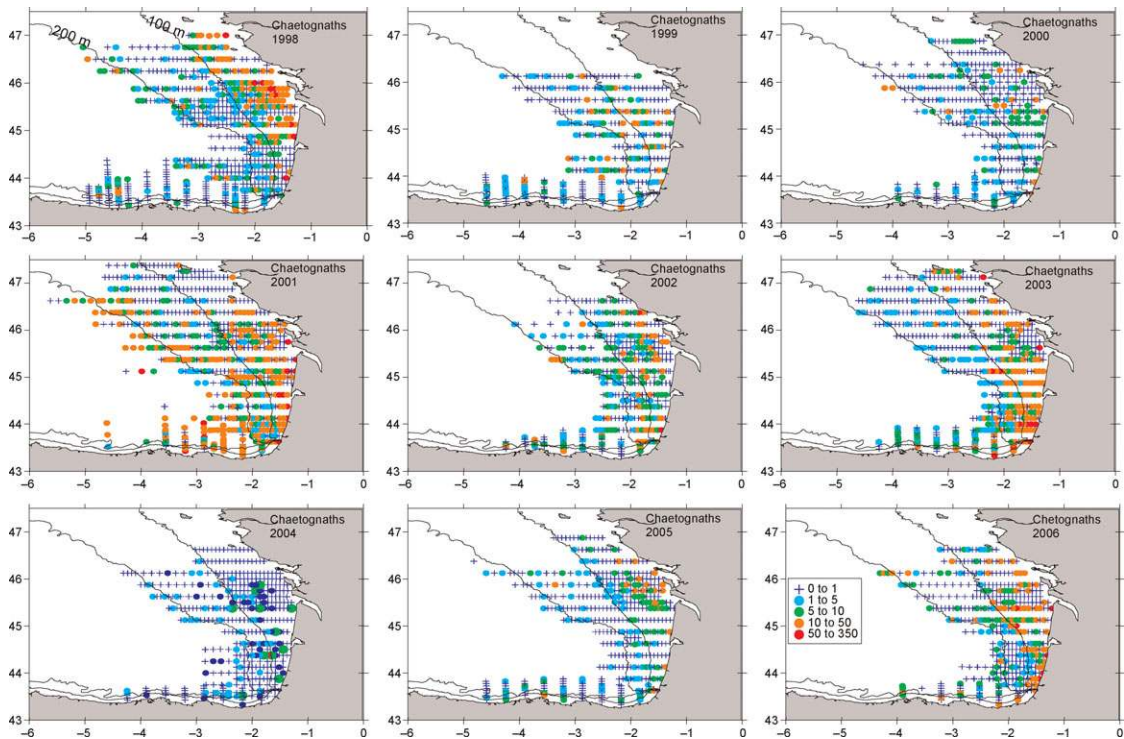


Fig. 6. Distribution of the chaetognaths abundance (ind m^{-3}) in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

and large copepods and those of euphausiid-like organisms (euphausiids and mysids) were observed in 2006 (Figs 4 and 5). On the other hand, the highest concentration of chaetognaths was observed in 2001 and 2003 (Fig. 6). The concentrations of small copepod were higher close to the coast (Fig. 3), whereas medium large copepods showed a more homogeneous distribution on the shelf although concentrations were higher along the coast. Euphausiid-like organisms and chaetognaths also were more abundant inside the 100 m isocline (Figs 5 and 6) although the years of higher abundance (2006 for euphausiid-like organisms and 2001 and 2003 for chaetognaths) showed a wider distribution.

Mesozooplankton biomass and size distribution

The highest mean biomass for the common area (the area that was covered on all the cruises) was observed in 2006 and the minimum in 1999 (Table IV). When divided in terms of width classes, a clear spatial pattern appears with the highest biomass of the smaller width classes being closer to the coast (Figs 7 and 8), whereas

the highest biomass of the largest width classes are higher in the mid-shelf (Figs 9 and 10). This size distribution is reflected in the slopes of the normalized size spectra with steeper slopes near the coast than on the outer shelf area (Fig. 11). When average biomass in the common area increases all size classes contribute to the increase but with different slopes (Fig. 12). This results in a correlation between the total biomass and the percentage of the biomass represented by large organisms, such as euphausiids-like (Table V).

Relations with anchovy biomass and recruitment

A significant negative relationship was observed between mean biomass for the common area and anchovy recruitment in the year ($y = -58\ 128 \ln(x) + 207312$, $r^2 = 0.62$, $n = 9$, $P < 0.05$, Fig. 13). When the biomass was split into the different minor axis size classes the negative relation remained for all the size classes (Fig. 13). There was also a negative relation between the sum of biomass of euphausiid-like and chaetognaths and the anchovy recruitment

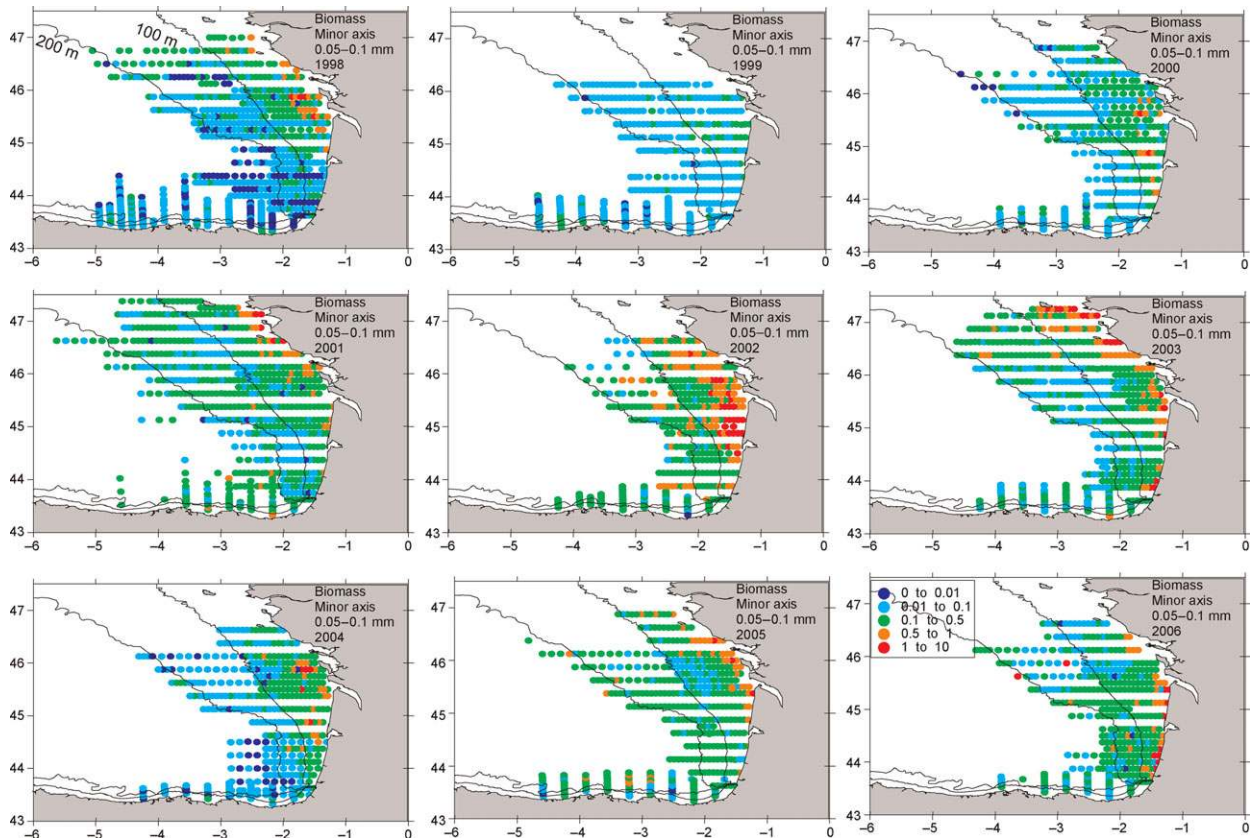


Fig. 7. Distribution of the biomass (mg C m^{-3}) of organisms in the 0.05–0.1 mm width (minor axis) size class in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

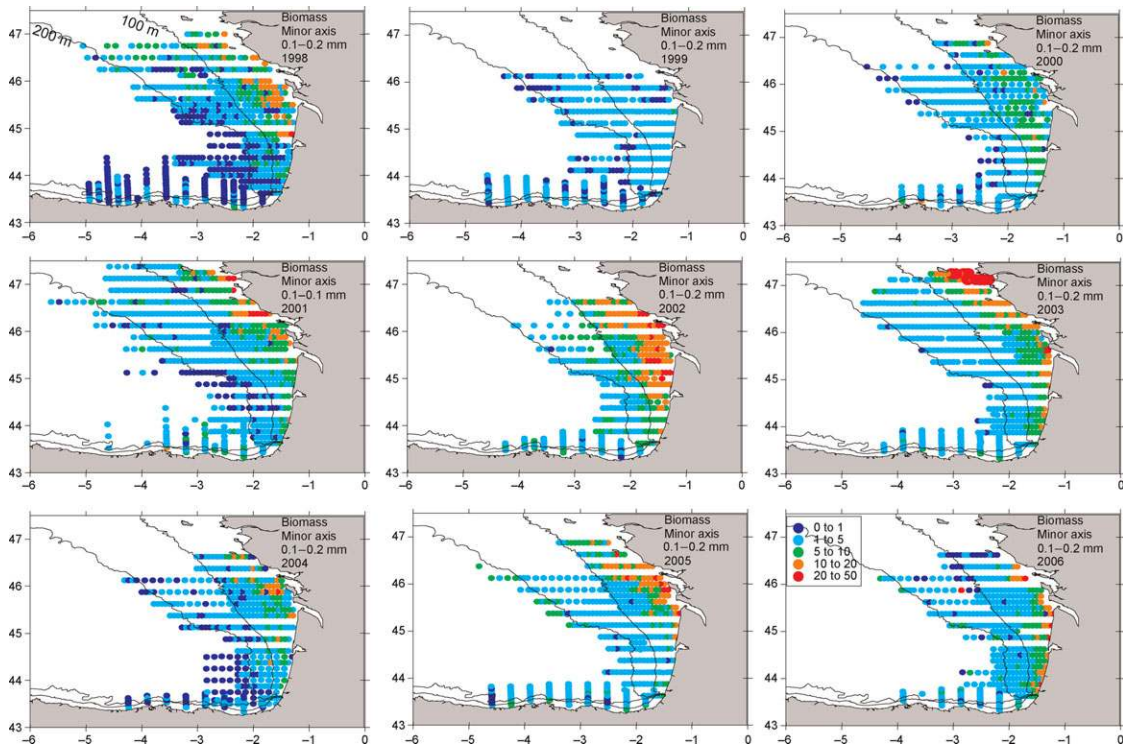


Fig. 8. Distribution of the biomass (mg C m^{-3}) of organisms in the 0.1–0.2 mm width (minor axis) size class in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

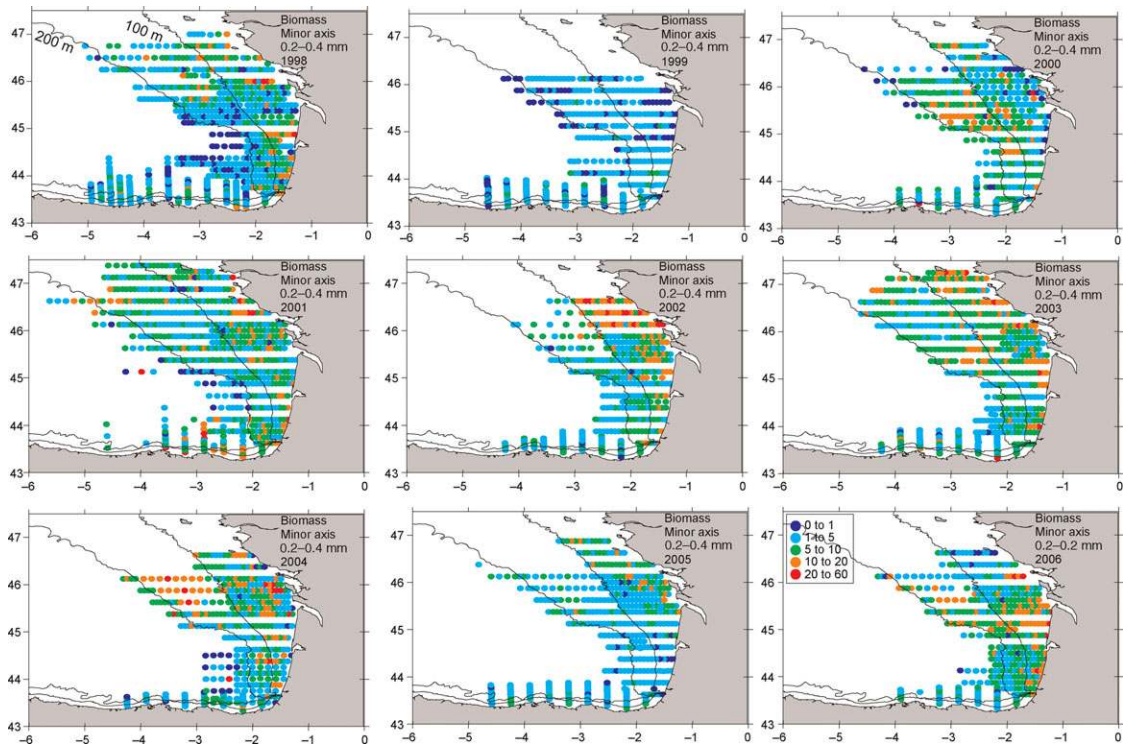


Fig. 9. Distribution of the biomass (mg C m^{-3}) of organisms in the 0.2–0.4 mm width (minor axis) size class in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

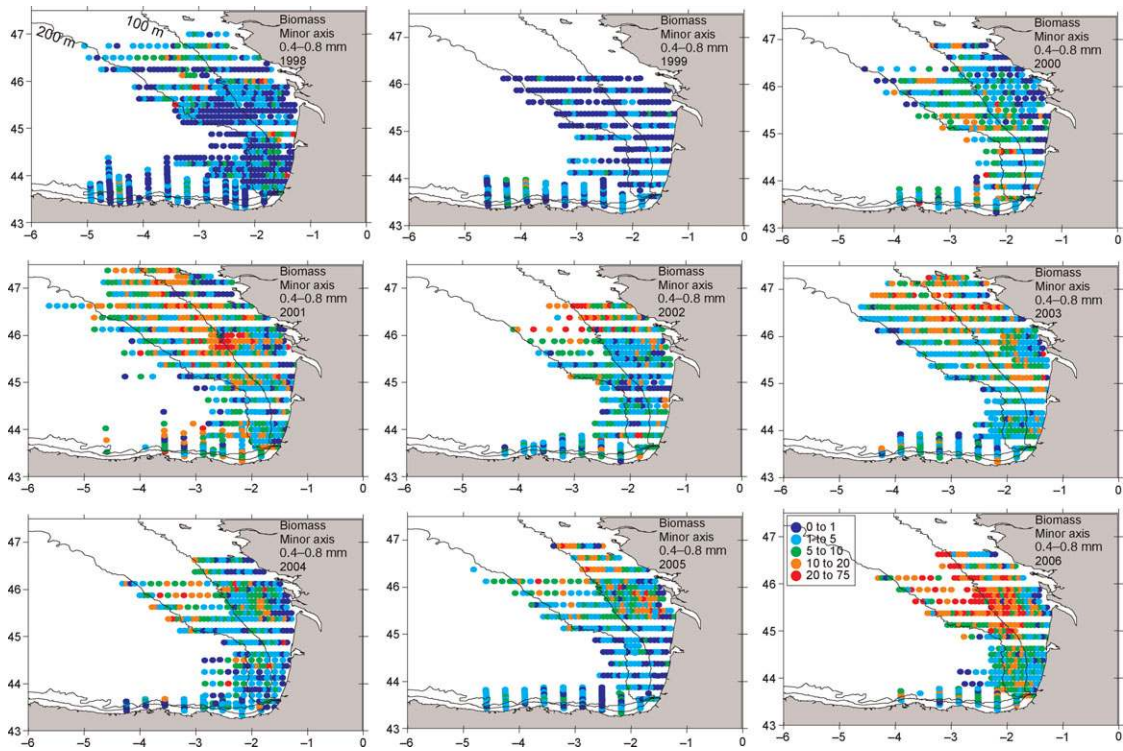


Fig. 10. Distribution of the biomass (mg C m^{-3}) of organisms in the 0.2–0.4 mm width (minor axis) size class in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

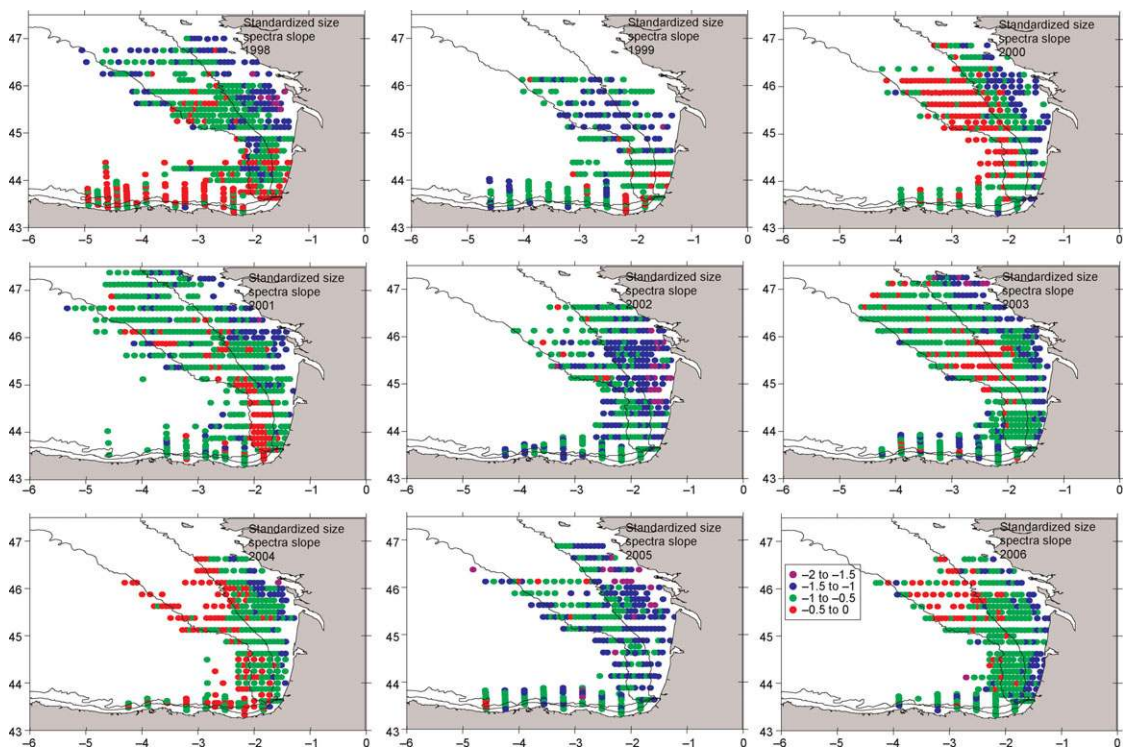


Fig. 11. Distribution of the slope of the standardized size spectra in the surveyed area in spring from 1998 to 2006 (see Table I for survey dates).

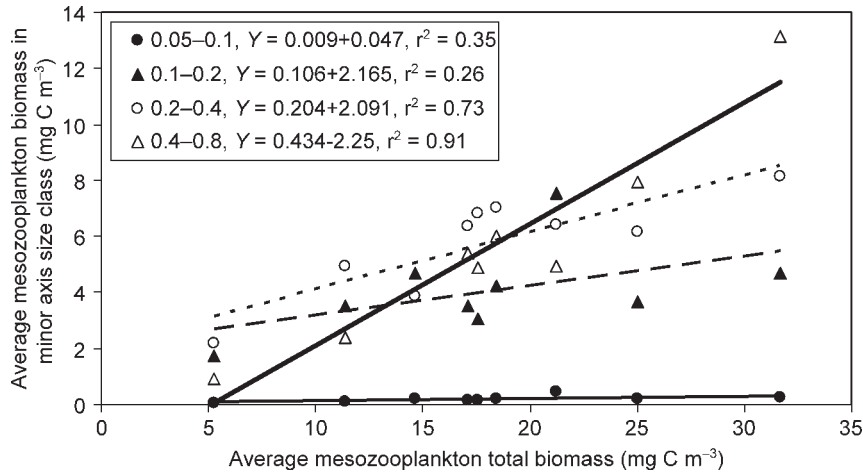


Fig. 12. Relation between mesozooplankton average total biomass (mg C m^{-3}) in the common survey area and the average biomass in the different minor axis size classes.

(Fig. 13). When expressed in terms of percentage contribution to total biomass, the relation of recruitment with size classes 0.1–0.2 and 0.2–0.4 was positive, whereas the relation with size class 0.4–0.8 was negative. There was no apparent relation with size class 0.05–0.1 or with the sum of euphausiid-like+chaetognath category (Fig. 14). On the other hand, there was no significant relationship between anchovy biomass and mesozooplankton mean biomass for the common area (Fig. 15).

DISCUSSION

This work presents, at least, three major results: (i) illustration of the capacity of image analysis to investigate zooplankton distribution at high resolutions (spatial and temporal) without sacrificing taxonomic information (in contrast to other techniques such as biomass, biovolume or the optical plankton counter). (ii) The observation of permanent spatial differences in the size structure of the mesozooplankton in the Bay of Biscay and (iii) a negative correlation between anchovy recruitment and mesozooplankton biomass.

Image analysis combined with automatic recognition using machine learning techniques (here, the Random Forest algorithm was used) appears to be a useful tool to rapidly analyse stored sample collections or large numbers of samples and obtain a minimum of meaningful ecological information. In this study, we were able to analyse 4124 samples in about six months of work by a non-specialist. Even using low-resolution imaging (600 dpi, while other studies use a resolution around 2400 dpi for analysis of similar mesozooplankton samples e.g. Grosjean *et al.*, 2004) for rapid analysis,

enough information is provided in these images of the major zooplankton groups to perform an ecological analysis of this community over a large area.

Our results are within the range of abundances and biomass values previously estimated in the area (Albaina and Irigoien, 2004; Nogueira *et al.*, 2004; Sourisseau and Carlotti, 2006; Albaina and Irigoien, 2007; Zarauz *et al.*, 2007). However, this study is the first one to provide high resolution sampling for several years and with a minimum taxonomic resolution in contrast to those with limited temporal or spatial resolution (Albaina and Irigoien, 2004; Albaina and Irigoien, 2007) or those without taxonomic resolution (Nogueira *et al.*, 2004; Sourisseau and Carlotti, 2006; Zarauz *et al.*, 2007).

During the 9 years which were analysed, we did not observe any significant trend in biomass. This could be due to the period being too short to observe any long-term change. However, it should be mentioned that in nearby areas and in contrast to more northerly locations, the changes have been limited (Pitois and Fox, 2006; Valdes *et al.*, 2007).

A second interesting observation is the permanent spatial difference in the size spectra or biomass distribution between size classes. We observed that in the coastal area, the small zooplankton fraction is more important (steeper slope) than in the mid-shelf and shelf break (flatter slope). This was already observed by Sourisseau and Carlotti (Sourisseau and Carlotti, 2006) for surveys in 2000 and 2001. Our results confirm this spatial pattern of the biomass size distribution to be a permanent feature. The slope of the size spectrum is a compromise between growth and mortality. However, the interpretation of the slope may be slightly different

Table V: Correlation matrix between the different parameters considered

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Temperature	1.00															
2. Anchovy biomass	0.45	1.00														
3. Anchovy recruitment	0.61	0.52	1.00													
4. Average zooplankton biomass	-0.24	-0.22	-0.70	1.00												
5. Average biomass in the range 0.05–0.1 mm width	-0.42	-0.49	-0.58	0.59	1.00											
6. Average biomass in the range 0.1–0.2 mm width	-0.38	-0.44	-0.51	0.51	0.97	1.00										
7. Average biomass in the range 0.2–0.4 mm width	-0.31	-0.18	-0.61	0.86	0.50	0.44	1.00									
8. Average biomass in the range 0.4–0.8 mm width	-0.14	-0.25	-0.58	0.95	0.42	0.33	0.78	1.00								
9. Average biomass large copepods	-0.26	-0.24	-0.59	0.94	0.42	0.35	0.90	0.96	1.00							
10. Average biomass small copepods	-0.41	-0.46	-0.49	0.49	0.97	1.00	0.43	0.31	0.34	1.00						
11. Average biomass chaetognaths	0.45	0.20	-0.38	0.42	0.07	0.05	0.32	0.35	0.27	-0.02	1.00					
12. Average biomass euphausiid-like organisms	-0.14	-0.02	-0.58	0.96	0.42	0.32	0.81	0.92	0.89	0.29	0.47	1.00				
13. Average percentage biomass in the range 0.05–0.1 width	-0.33	-0.47	-0.25	-0.01	0.78	0.79	-0.11	-0.18	-0.22	0.80	-0.18	-0.17	1.00			
14. Average percentage biomass in the range 0.1–0.2 width	0.04	-0.15	0.36	-0.62	0.20	0.29	-0.69	-0.69	-0.75	0.30	-0.40	-0.71	0.73	1.00		
15. Average percentage biomass in the range 0.2–0.4 width	0.17	0.42	0.54	-0.61	-0.66	-0.65	-0.22	-0.60	-0.46	-0.63	-0.22	-0.43	-0.46	-0.05	1.00	
16. Average percentage biomass in the range 0.4–0.8 width	-0.12	-0.13	-0.62	0.87	0.23	0.16	0.69	0.93	0.90	0.13	0.46	0.84	-0.32	-0.78	-0.58	1.00

Temperature and zooplankton biomass parameters are average for the common areas. Anchovy recruitment and biomass data source: ICES (www.ices.dk).

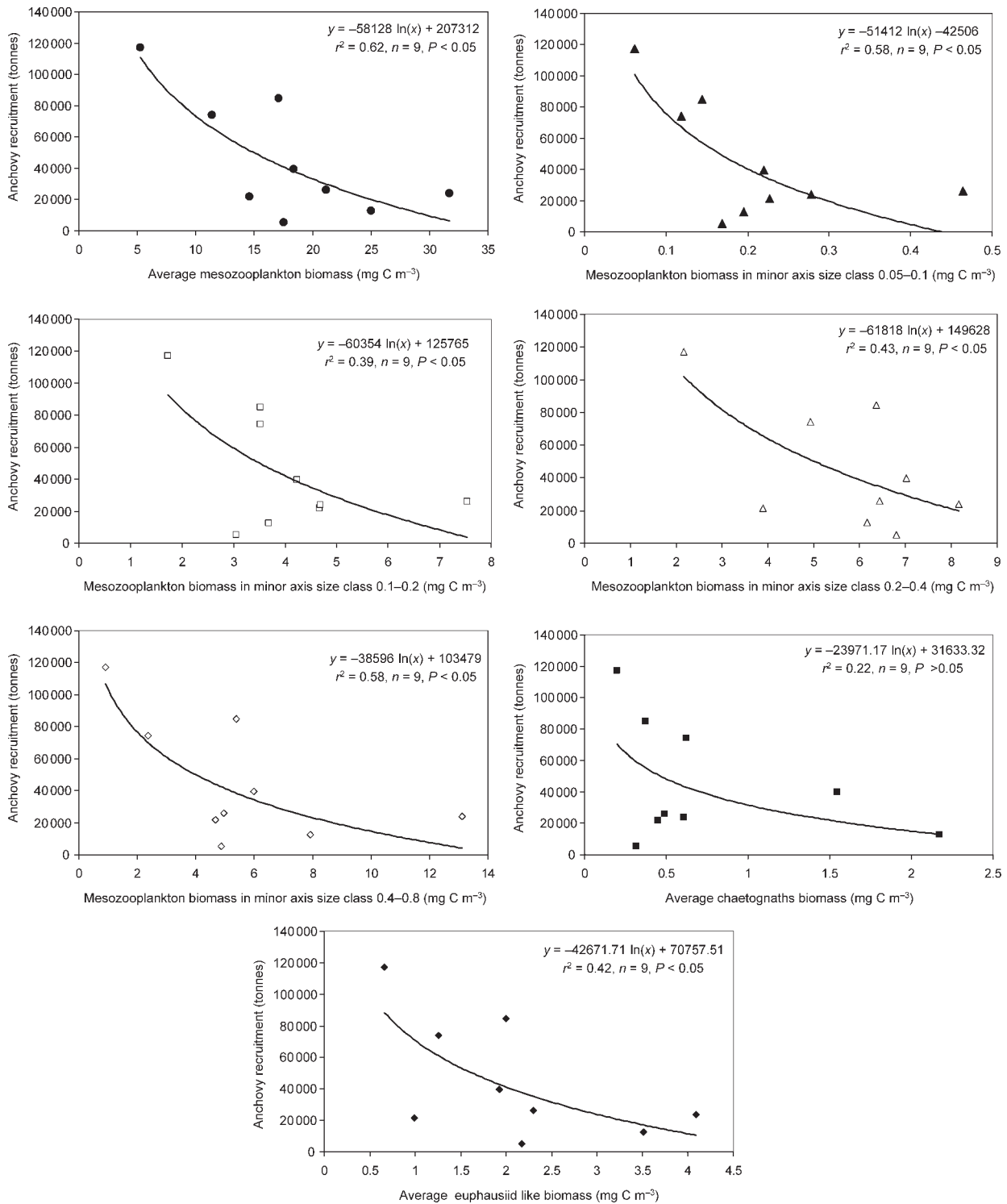


Fig. 13. Relation between mesozooplankton average biomass (mg C m⁻³), average biomass in the different categories (minor axis size classes and the sum of chaetognaths and euphausiid like class) and anchovy recruitment (tonnes) from 1998 to 2006.

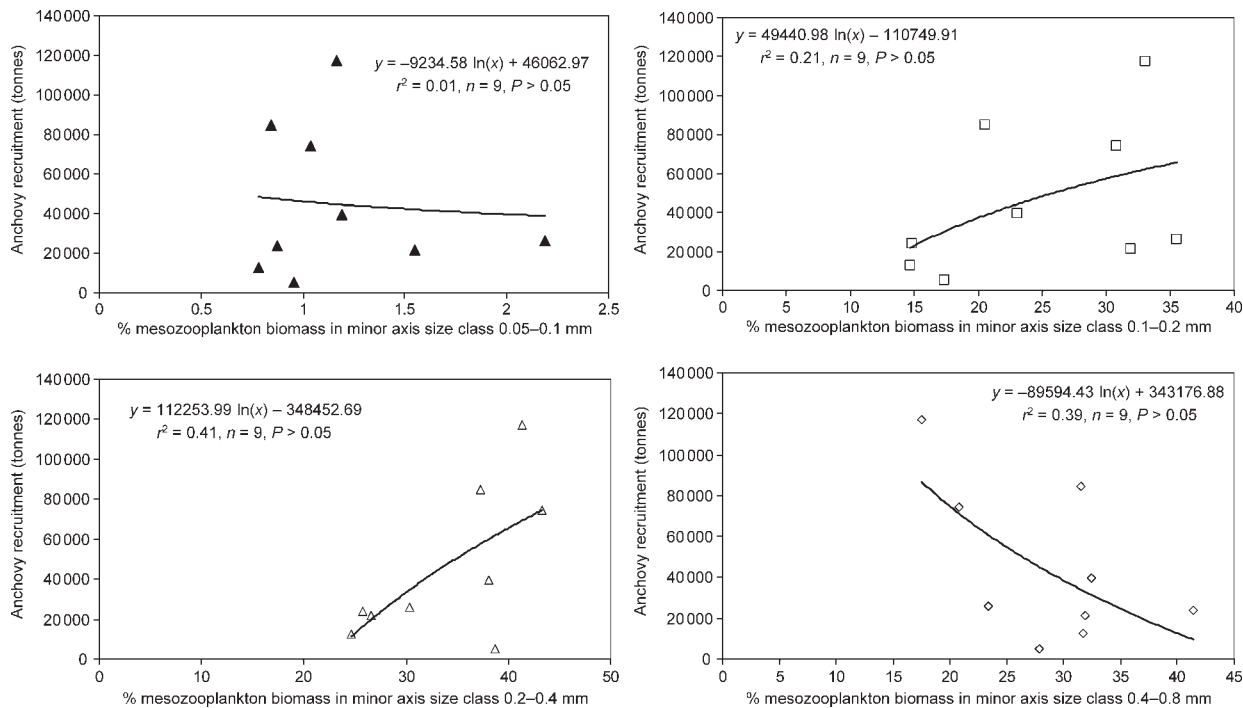


Fig. 14. Relation between the percentage of mesozooplankton average biomass in the different categories (minor axis size classes and the sum of chaetognaths and euphausiid-like class) and anchovy recruitment (tonnes) from 1998 to 2006.

depending on the number of trophic levels included in the analysis. If the size range analysed encompasses several trophic levels, the slope is a measure of the energy transfer efficiency between trophic levels (e.g. San Martin *et al.*, 2006). On the other hand, when applied to a single species, the size spectrum slope is a reflection of the population dynamics (Zhou and Huntley, 1997). In our case, although not restricted to a single species, the size range used should be more or less limited to a single trophic level (mesozooplankton). The results indicate that in the neritic zone, the largest

organisms suffer higher mortality or reduced growth. This is not likely to be related to the total amount of food as the two main peaks of primary production in the surveyed area are in the river plumes and over the shelf-break, due to the input of nutrients from the river (Herbland *et al.*, 1998) and internal waves over the shelf-break (Holligan *et al.*, 1985; Pingree *et al.*, 1986). The observed difference could be related to differences in the nature of the primary production as the bloom in the river plumes seems to occur early in the year, primary production to be then limited by phosphorous, with a microbial loop dominating the river plumes in spring (Herbland *et al.*, 1998). However, we cannot determine why a system dominated by the microbial loop should result in the mesozooplankton biomass dominated by the small size classes as both small and large copepods have been shown to consume microzooplankton (e.g. Castellani *et al.*, 2008). It could also be related to the variability in production instead of the total primary production or the type of food web. Higher variability in the production at the shelfbreak (conditioned by thermocline establishment and internal waves in contrast to permanent river flow) would favour larger zooplankton being able to store energy reserves.

Two other aspects that should significantly differ between both areas are the light regime and the vertical motion. In the coastal area, due to the river

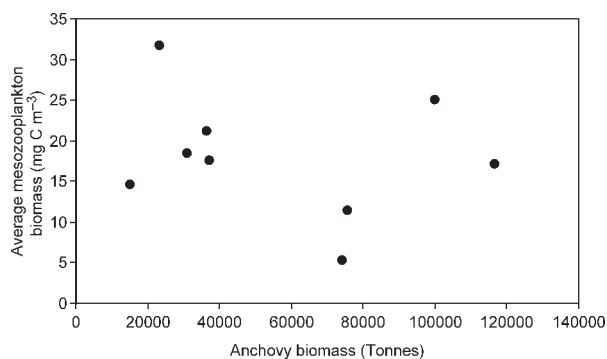


Fig. 15. Relation between anchovy biomass (tonnes) and mesozooplankton average biomass (mg C m⁻³) in the common survey area.

contribution of particles and dissolved humic substances, the penetration of light is much lower than at the shelf-break (a factor 10 of difference can be expected; Guillem Chust, personal communication). This may make a difference in the susceptibility to predation between large and small organisms. Also, due to the internal waves, the vertical motion at the shelfbreak is expected to be higher, which may have an effect on the size structure of the community as has already been shown for phytoplankton (Rodríguez *et al.*, 2001).

Finally, the difference may also be due to the reproductive and overwintering strategies of copepods (90% of the zooplankton). It is known that there is a higher percentage of the small copepod species that carry their eggs in sacs, whereas mid-size copepod species tend to be free spawners (Ohman and Townsend, 1998). Carrying the eggs may be an advantage in shallow waters if contact with sediment results in the death of the egg (e.g. Uye, 2000). Furthermore, small and large copepods differ in their overwintering strategies. Whereas free spawning small copepods often have resting eggs as an overwintering stage (e.g. *Acartia* spp., *Temora* spp.), larger copepods overwinter in deep waters as late copepodites (e.g. *Calanus* spp.). Obviously, a resting egg strategy benefits from shallow waters and is not viable in deep areas, whereas overwintering as copepodites requires access to deep waters. This way, a simple combination of reproductive and overwintering strategy combined with depth could explain a permanent difference in the mesozooplankton size structure in spring in the Bay of Biscay.

Another interesting outcome of this study is the negative relationship between mesozooplankton biomass in spring and anchovy recruitment. This negative relation between zooplankton biomass and recruitment should be considered with caution as it can be argued that anchovy in the Bay of Biscay recruits from May to August (Irigoien *et al.*, 2007) and the survey is only a snapshot of that period. Also, earlier stages of anchovy larvae are likely to eat microzooplankton (not studied here) in addition to mesozooplankton. The biomass of smaller size classes that are most likely to contribute to the diet of larvae (Average prey width for *E. encrasicolus* [larvae (mm) = 0.0131 larvae length (mm) + 0.0074, from Conway *et al.*, 1998] also show a negative relation with recruitment. However, this is normal because the biomass of all size classes increases when total biomass increases (Fig. 12). Furthermore, the time-series is too short as to extract strong conclusions.

However, the surveys cover the peak spawning period of anchovy and the mesozooplankton biomass is likely to be a good index of the productivity of the area. Also, the sampling does cover the diet of the largest larvae

(Conway *et al.*, 1998). A similar negative relation between recruitment and zooplankton abundance has been observed in Pacific sardines (Agostini *et al.*, 2007). Agostini *et al.* (Agostini *et al.*, 2007) attribute the result to predation on eggs and larvae either by the predator being attracted to high mesozooplankton concentrations or by macrozooplankton itself. A similar explanation could apply to the Bay of Biscay: the recruitment correlates positively with the percentage of small zooplankton (potential food, Fig. 14) and negatively with the percentage of large zooplankton (potential predators, Fig. 14). But as the percentage of large zooplankton increases with biomass (Fig. 12), it would imply that potential predators increase proportionally more than food abundance when total biomass increases. Some of those macrozooplankton organisms such as euphausiids are known predators of anchovy eggs (Bailey *et al.*, 1993; Theilacker *et al.*, 1993; Krautz *et al.*, 2007) and we also observed a negative relation between euphausiid abundance and recruitment (Fig. 14). Another potential explanation is the higher abundance of mesozooplankton to attract more planktivorous fish to the area. It has been shown that planktivorous fish might be the main source of mortality for anchovy eggs (Szeinfeld, 1991). Unfortunately, there are no time series in the Bay of Biscay of other planktivorous fish stocks so as to test this hypothesis.

Clearly our results are insufficient to test the different potential explanations for the low recruitment but do shed light on the factors that are not responsible. The succession of low recruitments cannot be attributed to a decrease of the mesozooplankton abundance in the Bay of Biscay.

The lack of a relationship between anchovy biomass and mesozooplankton biomass indicates that there is no top-down control by adult anchovies on mesozooplankton biomass. Actually, this is not surprising, as commercially valuable anchovy is not the only pelagic fish in the area. Different species such as sardine, mackerel, horse mackerel and sprat have biomass equivalent to, or higher than that of anchovies (Masse, 1996; ICES, 2007). Therefore, anchovies only constitute a fraction of the predation pressure on mesozooplankton. Unfortunately, time series of the regional (Bay of Biscay) abundance of the different small pelagic fishes are not readily available to evaluate whether there is, globally, a top-down control on mesozooplankton abundance.

In any case, this study demonstrates that the use of image analysis combined with automatic recognition using machine learning offers the possibility to investigate mesozooplankton at spatial and temporal scales equivalent to those used in the research on their prey and predators (phytoplankton and fish).

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