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Trophic studies of small pelagic fish in the Bay of Biscay: methodological aspects

Eneko Bachiller[1]

Abstract

In trophic studies of small pelagic fish, comparisons between gut contents and the available food in field are commonly limited by the sampling procedure. In this sense, different sized zooplankton samples can differ depending on the mesh size of the sampling net. In the same way, the variability on the distribution of both predators and preys as a function of environmental variables can be a limiting factor when taking samples and hence, that could be reflected in stomach contents. In this study different aspects are discussed in order to optimize the sampling design addressing trophic studies in the Bay of Biscay, presenting also unpublished information about the distribution of both zooplankton and the main small pelagic species. This way, it is observed that 63 µm and 150 µm mesh Pairovet nets are not enough to catch the whole prey size spectrum found in stomachs of fish. In addition, many geographical areas are proposed in order to detect the variability of the distribution of both predators and preys; in the same way, the importance of sampling in different time ranges is discussed, as well as the regurgitation problem that long trawls could suppose for fish due to the stress caused. Finally, since stomach contents can differ from one fish to other, the significance of the individual fish analysis is highlighted, defining the optimum minimum sample size in order to detect a significant percentage of the prey diversity found in different small pelagic species.

Resumen

En estudios tróficos de especies pelágicas pequeñas, las comparaciones entre contenidos estomacales y el alimento disponible en el mar suelen estar limitadas por el método de muestreo. En este sentido, las muestras de distintos rangos de talla de zooplancton pueden variar dependiendo de la luz de malla de la red de muestreo. Del mismo modo, la distribución diferencial tanto del zooplancton como de los consumidores en función de variables ambientales puede ser un factor limitante a la hora de tomar las muestras y por tanto, esto puede verse reflejado en los contenidos estomacales. En este estudio se discuten distintos aspectos con el objetivo de optimizar los muestreos dirigidos a estudios de alimentación de las principales especies pelágicas en el Golfo de Bizkaia, presentando del mismo modo información no publicada en relación a la distribución de predadores y presas. De este modo, este trabajo concluye que las redes Pairovet con luz de malla de 63 µm y 150 µm no son suficientes a la hora de detectar todo el espectro de tallas de presa identificables en los estómagos de los peces. Además, dada la variabilidad en la distribución de predadores y presas, se proponen distintas áreas de muestreo; del mismo modo también se discute la importancia de muestrear en distintos momentos del día, así como el posible problema de regurgitación que los largos arrastres pueden causar por el estrés en determinados casos. Finalmente, dado que los resultados pueden diferir significativamente de un pez a otro, se remarca la relevancia que tiene el análisis individual de los contenidos estomacales, definiendo además un número óptimo de muestra para obtener un porcentaje significativo de la diversidad de presas encontradas en los estómagos de distintas especies pelágicas.

Key Words: Bay of Biscay, sampling design, sample size, small pelagic species, stomach content analysis, zooplankton

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Introduction

Since review of the stomach analysis methods applied from 1950's onwards (Hyslop, 1980), trophic studies have evolved both in terms of methodology and accuracy, but there is still not optimum single standard method. The variability of zooplankton distribution and stomach contents of fish presents a challenge that requires to accept several assumptions: 1) zooplankton description is that of the sampling point whereas fish move around and can be feeding between zooplankton patches not properly described by the zooplankton sample. As an example, in the Bay of Biscay the physical and oceanographic features –e.g. currents, eddies and main river plumes (Gironde, Adour…)– affecting biological variables (Fernández *et al*., 1993; Albaina & Irigoien, 2004; Fernández *et al*., 2004; Albaina, 2007; Zarauz *et al*., 2007; Albaina & Irigoien, 2007; Irigoien *et al*., 2009) can be limiting when designing the sampling area. 2) The same occurs in the vertical scale, where we cannot determine the depth corresponding to the observed stomach contents. 3) The mesh size used determines the comparison between what is observed in the water column and in the stomach. An apparent selectivity towards small or large organisms can be simply due to an underestimation by the net (small organisms passing through the mesh or large organisms escaping the device). Therefore, a comparison is only valid for the size ranges properly sampled by the net. The use of different mesh sizes and sampling devices partially solves the problem, but requires a huge increase of the zooplankton sample analysis effort.

On the other hand, it is generally accepted that prey size spectrum is limited by predator size (Scharf *et al*., 2000; Barnes *et al*., 2010). This way, many studies have treated similar sized fish gut contents of one station together, assuming obtained results from the subsample for the whole size-range of this predator species as well as for the whole area represented by that sampling station. However, it has been observed that in the same shoal and with a similar size range one fish can significantly differ from other both in terms of total stomach weight and diet characterization (this study). Moreover, empty stomachs could be caused not only due to starvation but also to regurgitation (Bowman, 1986) and for some species, this last effect can be correlated with fish length (Staniland *et al*., 2001); during this study, although we firstly considered that the regurgitation was mainly a cause of the stress caused by a long trawling, even in long trawls individuals with full stomachs were acquired. Therefore, recent studies are processing fish gut contents individually, e.g. diet studies with anchovy (Borme *et al*., 2009; Bacha *et al*., 2010; Yasue *et al*., 2010; Raab *et al*., 2011).

Taking into account those points in this study we have avoided point by point comparisons between zooplankton samples and fish stomachs, restricting the comparisons to large areas. Further, rather than addressing the selectivity of each species in relation to what was collected by the net, we have concentrated on comparing the diet of different fish species collected in the same area, and therefore with access to the same zooplankton (including patches and vertical layers).

In addition, day-night variations on feeding behaviour have been observed not only for anchovies (Bulgakova, 1996; Plounevez & Champalbert, 1999, 2000; Borme *et al*., 2009) but also for other small pelagic fish such as sardines (Conway *et al.*, 1991; Garrido, 2007; Nikolioudakis *et al.*, 2011), Atlantic horse mackerels (Olaso *et al.*, 1999) or sprats (Conway *et al.,* 1991). That could suggest the visibility as an additional affecting factor (Benfield & Minello, 1996). Thus, the sampling time should be also considered when making the sampling design. Moreover daily migration pattern (DVM) of zooplankton (Ribera Maycas *et al*., 1999; Irigoien *et al*., 2004; Heidelberg *et al*., 2010; Hylander & Hansson, 2010) should support such a consideration.

Many oceanographic surveys have sampled both zooplankton and small pelagic species around the Bay of Biscay with different aims during last years – BIOMAN, ECOANCHOA, JUVENA (AZTI-Tecnalia), PELACUS (IEO), PELGAS (Ifremer) – but to our knowledge there is no methodological evaluation concerning trophic studies.

Thus, the present study aims to assess the sampling design for trophic studies with small pelagic fish in the Bay of Biscay. In this sense the methodology of fish sampling is discussed in order to optimize it for gut content analyses. In the same way, since trophic studies require information about the available food on field, the zooplankton sampling methodology used with that purpose is also discussed, comparing two net mesh sizes used in different surveys. In addition, unpublished information about both predator (i.e. small pelagic fish) and prey (i.e. zooplankton abundance and biomass, by size class) distribution in the area is provided.

Materials and methods

Area of study

The Bay of Biscay is a part of the sub-temperate eastern North Atlantic and consists of an open oceanic bay located at 43.5-48.5º N and surrounded by the north coast of Spain and French west coast (Figure 1). The ecosystem consists of two shelves with different orientation and width and so that distinct current and tidal patterns are observed forming a highly dynamic region where many mesoscale oceanographic structures occur in a constrained area (Koutsikopoulos & Le Cann, 1996; González *et al*., 2004; Ferrer *et al*., 2009; Ferrer & Caballero, 2011). The Cantabrian coast is characterized by an east-west orientated narrow shelf (15-20 nautical miles) and the absence of important river outflows (Koutsikopoulos & Le Cann, 1996; Prego & Vergara, 1998), whereas the French part has a north-south shelf orientation (Koutsikopoulos & Le Cann, 1996), with an increasing width northwards from 30 to 80 nautical miles (the Landes and Aquitaine Shelf shelves), and two noticeable river plumes, Gironde and Adour, with respectively 900 and 300 m^3 s^{-1} mean freshwater outflows (Puillat *et al*., 2004; Albaina, 2007; Albaina & Irigoien, 2007).

As in other temperate seas, oceanographic processes in the Bay of Biscay are highly influenced by seasonal variability (Koutsikopoulos & Le Cann, 1996; Koutsikopoulos *et al.,* 1998; Puillat *et al*., 2004; Goikoetxea *et al*., 2009). In terms of mesoscale oceanographic structures, Adour and Gironde river

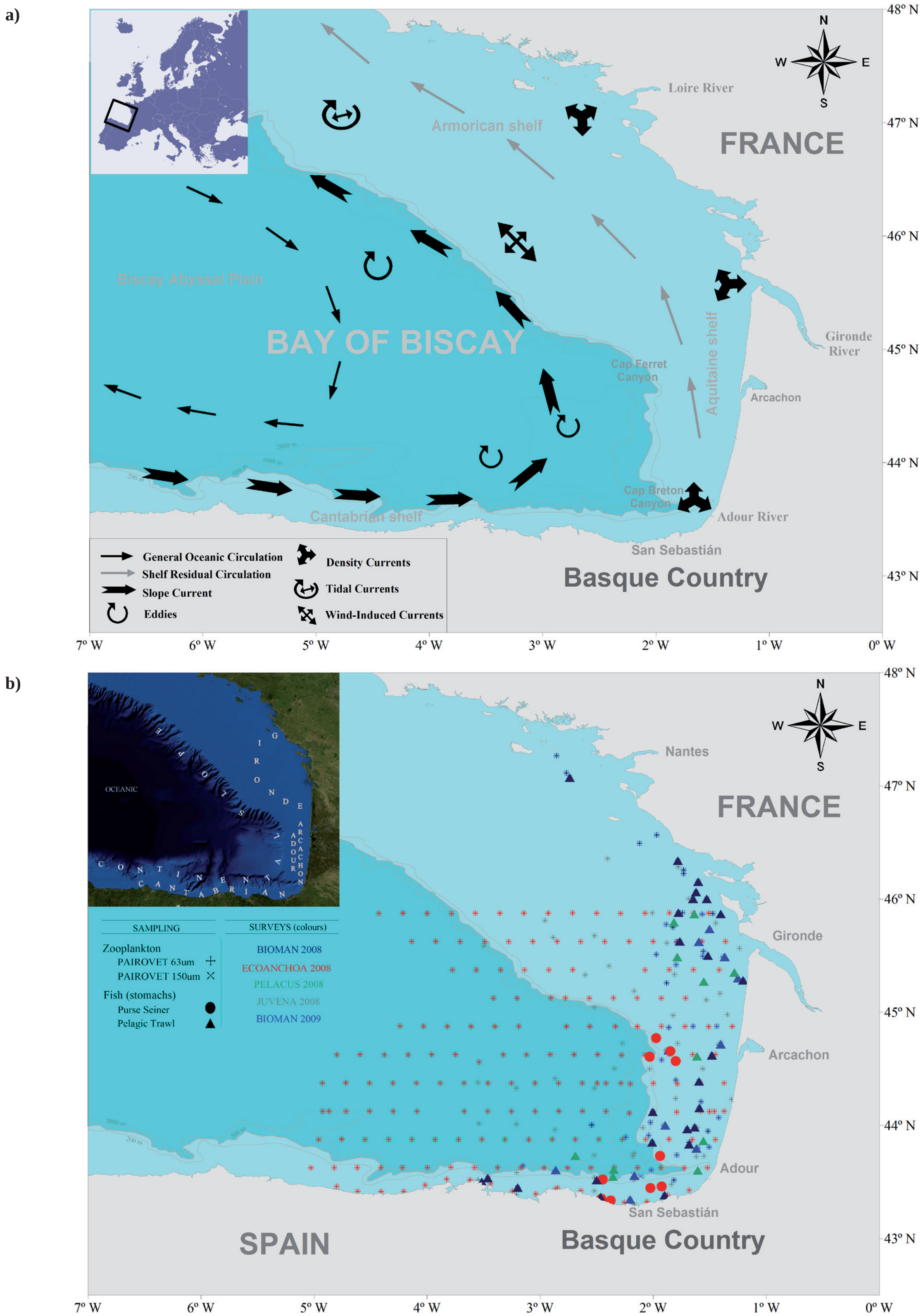


Figure 1. Area of study. **(a)** Main oceanographic features of the Bay of Biscay (modified from Ferrer *et al.*, 2009), and **(b)** sampling stations ($N_{PAIROVET\ 63\mu m}$ = 298 samples, $N_{PAIROVET\ 150\mu m}$ = 298 samples; $N_{PurseSeiner}$ = 10 stations, $N_{PelagicTrawler}$ = 47 stations); note the proposed environmental areas on the small photo in the upper left part of the image.

plumes spreading over the shelf are part of the most characteristic (Albaina & Irigoien, 2004; Albaina, 2007; Albaina & Irigoien, 2007). In fact, the extension of these low salinity waters over the shelf depends on the precipitations and prevalent winds and occasionally cover a great part of the continental shelf (Puillat *et al*., 2004). In addition to these density currents, some occasional upwellings are observed as important mesoscale oceanographic structures depending on wind regime, both in Spanish and Basque (Botas *et al*., 1990) and French shelves (Jégou & Lazure, 1995). The same pattern is observed for residual currents over the shelf since they are mainly affected by the wind, the tides in the northern part and the water density.

Zooplankton

Zooplankton samples were collected in four surveys during 2008 and 2009 (Table 1, Figure 1b). Vertical hauls of 63 and 150 µm PAIROVET nets (Smith *et al*., 1985; Wiebe & Benfield, 2003) were used; the nets were lowered to a maximum depth of 100 m or in case of shallower stations, 5 m above the bottom. Net samples were preserved immediately after collection with ph7 buffered formaldehyde (4%) stored in 250 mL jars, to keep them until the analysis in the laboratory (Harris *et al*., 2000).

After staining samples (24 hours with 1mL Eosin 5 g L^{-1}) samples were digitized and image analysis (*ZooImage* software) was used to obtain mesozooplankton abundance and size (Bachiller & Fernandes, 2011; Bachiller *et al*., 2012). Size was transformed into biomass using minor diameter measurements (i.e. the smallest axis of the ellipsis containing the individual, Fernandes *et al.,* 2009) as basis and applying the equation proposed by Alcaraz *et al*. (2003).

In order to simplify comparisons between species we established 1 and 2 mm as boundaries to separate *small, medium* and *large* sized zooplankton. Labels assigned to each of the identified zooplankton taxonomic group are defined in Table 2.

Small pelagic species

Samples of European anchovies (*Engraulis encrasicolus,* "ANE"), sardines (*Sardina pilchardus*, "PIL"), Atlantic horse mackerels (*Trachurus trachurus*, "HOM"), Mediterranean horse mackerels (*Trachurus mediterraneus,* "HMM"), Atlantic mackerels (*Scomber scombrus*, "MAC"), Atlantic Chub mackerels (*Scomber colias*, "MAS"), bogues (*Boops boops,* "BOG") and sprats (*Sprattus sprattus,* "SPR") were caught around the Bay of Biscay during different surveys in 2008 and 2009 (Table 1, Figure 1b). Caught fish were sized to the nearest 1 mm and weighted to the nearest 0.1 gr and then preserved frozen.

According to the fishing time, samples caught between 04:00 and 20:00 GMT were considered as "day time samples", whereas those caught between 20:00 and 04:00 GMT were defined as "night samples".

Night catches caught by purse seiner were not included in the analysis since fish and prey are attracted by the lights of the fishing vessel and that could distort the behaviour.

Gut content analysis

Once in laboratory stomachs were extracted, weighted to the nearest 0.1 g and then preserved in ph7 buffered formaldehyde (4%) for later stomach content extraction.

A stereo microscope (model NIKON SMZ 645) was used to extract stomach contents manually with claws and scalpel. Quantification and classification of all identifiable prey was done to the lowest possible taxonomic group. No broken parts or appendixes of preys were included in the counting; this way it could be said that at least all counted preys had been ingested by predators, in spite of not taking into account more highly digested unidentifiable or uncountable preys.

Table 1. Description of different oceanographic surveys from which data were obtained for the study.

Year	Survey	Vessel	Sampling period	Area limits	$N_{P\ 150\mu m}$ [1]	$N_{P\ 63\mu m}$ [2]	Fishing Art [3]	N_{hauls}	N_{ANE}	N_{PIL}	N_{HOM}	N_{HMM}	N_{MAC}	N_{MAS}	N_{BOG}	N_{SPR}
2008	BIOMAN	R/V Emma Bardán	May 6th-May 26th	3°33'W / 48°10'N	38	38	PT	25	123	40	39	20	41	2	10	10
2008	ECOANCHOA	R/V Regina Marís	June 27th-July 11th	5°W / 46°30'N	172	172	-	-	-	-	-	-	-	-	-	-
		F/V Ama Antiguakoa	July 8th-July 18th	2°28'W / 44°49'N	-	-	PS	10	20	0	18	0	16	0	0	0
2008	PELACUS	R/V Thalassa	Sept. 22nd-Oct. 10th	3°W / 47°N	-	-	PT	11	30	30	50	15	40	23	0	10
2008	JUVENA	R/V Emma Bardán	Aug. 26th -Sept. 26th	5° W / 47°45'N	53	53	PT	-	-	-	-	-	-	-	-	-
2009	BIOMAN	R/V Emma Bardán	May 5th-May 25th	5° W / 47°N	35	35	PT	11	60	60	64	40	48	4	44	40

[1] PAIROVET 150µm; [2] PAIROVET 63µm; [3] PT: Pelagic Trawl; PS: Purse Seiner

Table 2. Label description of zooplankton taxonomic groups identified in this study.

Phylum	*Subphylum*	*Class*	*Subclass*	*Infraclass*	*Order*	*Suborder*	*Family*	*Label*	*Description*
Platyhelminthes		Trematoda						**TREM**	Trematoda larvae (or Digenea). Parasitic organism
Mollusca		Gastropoda						**GAST**	Veliger larvae of Prosobranchia
		Bivalvia						**BIV**	Veliger larvae of bivalves
		Branchiopoda	Phyllopoda		Diplostraca	Cladocera		**POD**	*Podon sp.*
								EVAD	*Evadne sp.*
							Metridinidae	**METR**	*Metridia lucens, Pleuromamma spp.*
							Acartiidae	**ACAR**	*Acartia spp.*
							Candaciidae	**CAND**	*Candacia armata*
								CENTR.SPP	*Centropages spp.*
							Centropagidae	**CENTR.TYP**	*Centropages typicus*
								CENTR.CH	*Centropages chierchiae*
								ISIAS	*Isias clavipes*
								TEM.L	*Temora longicornis*
					Calanoida		Temoridae	**TEM.S**	*Temora stylifera*
								EURYT	*Eurytemora spp.*
							Euchaetidae	**EUCH**	*Euchaeta sp.*
							Calanidae	**CAL**	*Calanus spp., Calanoides spp.*
							Eucalanidae	**EUCAL**	*Subeucalanus sp.*
			Copepoda				Clausocalanidae	**CLAUSCAL**	*Clausocalanus sp., Ctenocalanus sp., Microcalanus sp., Pseudocalanus sp.*
		Maxillipoda						**S.CAL**	Small Calanoids (unidentifyable small calanoids)
								CAL.UNDF	Undefined Calanoids: *Calocalidae, Paracalanidae, Diaixidae, Eucalanidae, Scolecitrichidae* families
							Oithonidae	**OITH**	*Oithona spp.*
					Cyclopoida		Oncaeidae	**ONC**	*Oncaea spp.*
							Corycaeidae	**CORYC**	*Corycaeus anglicus*
Arthropoda	Crustacea						Euterpinidae	**EUTER**	*Euterpina acutifrons*
							Ectinosomatidae	**MICR**	*Microsetella spp.*
					Harpacticoida		Clytemnestridae	**CLYT**	*Clytemnestra spp.*
							Thalestridae	**THAL**	*Thalestris sp.*
							Caligidae	**CALIG**	*Caligoid sp.* Parasitic copepod
								COP.NAUP	Copepoda nauplii
								COP.JUV	Juvenile copepods
				Cirripedia				**CIRR.NAUP**	Cirripedia nauplii
			Thecostraca					**CIRR**	Cirripedia (cipris larvae)
				Facetotecta				**FACET**	*Facetotecta* larvae
					Mysida			**MYSD**	Mysid
					Stomatopoda			**STOM**	Stomatopoda
					Amphipoda			**AMPH**	Amphipoda
					Isopoda			**ISOP**	Isopoda
								EUPH.NAUP	Euphausiid meta-naupli
								EUPH.CALYP	Euphausiid calyptopis larval phase
		Malacostraca Class			Euphausiacea			**EUPH.LV**	Euphausiid furcilia larval phase
								EUPHS	Euphausiid (adult). *Lengths of broken euphausiids estimated from EYE-BODY size regression-model
								DECAP.ZOEA	Decapoda ZOEA larval phase
					Decapoda			**DECAP.MEGAL**	Decapoda megalopa larval phase
								DECAP.LLV	Decapoda late larval phase
								DECAP	Decapoda (adult)
Nematoda								**ANISK**	*Anisakis sp.* Parasitic organism
Annelida		Polychaeta						**POLYCH**	Polychaete larvae
Cnidaria		Hydrozoa	Siphonophorae		Calycophorae			**SIPH**	*Muggiaea sp.*
			Hydroidomedusae		Anthomedusae			**MED**	Anthomedusae (jellyfish)
Chaetognatha								**CHAET**	*Sagitta setosa* Chaetognath
		Appendicularia						**APP.FRIT**	*Fritillaria sp.* Appendicularia
	Urochordata							**APP.OIKO**	*Oikopleura spp.* Appendicularia
		Thaliacea					Salpida	**SALP**	Salps
Chordata							Engraulidae	**FEGG.ANE**	Anchovy Eggs (*Engraulis encrasicolus*)
								FLV.ANE	Anchovy Larvae (*Engraulis encrasicolus*)
	Vertebrata	Osteichthyes						**FEGG.UNDF**	Undefined Fish Eggs
								FLV.UNDF	Undefined Fish Larvae
								FISH	Undefined fish
								EGG.UNDF	Undefined eggs (crustacean eggs, fish eggs…)
								OTHERS	Others

Results

Zooplankton

No significant differences (t test, $P = 0.45$) were observed in the minor diameter measurements extracted from *ZooImage* between the two mesh sizes (Table 3). The maximum length of prey catchable with plankton sampling nets did not differ significantly depending on the mesh size (t test $P = 0.871$; $N_{PAIROVET\ 63\mu m} = 38$ stations, $N_{PAIROVET\ 150\mu m} = 38$ stations) as also observed in the relation between the length of the largest preys caught with different mesh sizes and their abundance (Figure 2). However, in terms of size distribution the estimated abundance was significantly different for the two mesh sizes (Chi Squared tests: $P < 0.0005$ for small sized, $P < 0.03$ for medium sized and $P < 0.02$ for large sized), being slightly higher in the 150 µm mesh size net (Figure 3a). Nevertheless, in case of the smaller fraction the difference is not enough as to result in significant differences in biomass (Figure 4a) after application of the Alcaraz *et al.* (2003) conversion factor (Chi Squared tests: $P < 0.5$ for *small* sized, $P < 0.0001$ for *medium* and *large* sized). On the other hand, if we consider size fractions as classes, both in case of abundance and biomass estimations were slightly higher for 150 µm mesh size net (Figures 3b & 4b), showing significant difference only for biomass (Chi Squared tests: $P < 0.07$ for abundance and $P < 0.0001$ for biomass).

Concerning to the spatial distribution higher abundance and biomass areas are observed in the coastal areas. However, there are size related differences in zooplankton distribution; small zooplankton abundance and biomass were higher in the coast, whereas large zooplankton was more abundant in the shelf and shelfbreak (Figures 5 & 6).

Small pelagic species (predators)

Figure 7 shows the spatial distribution of small pelagic species caught during the four surveys between 2008 and 2009. Note that two extremely large catches –marked with an asterisk on maps– have not been considered to plot maps in order to avoid figure distortion.

In late spring (BIOMAN 2008 and 2009) the Atlantic horse mackerel was the most abundant pelagic specie, especially in the Cantabrian coast but also in the southern part of the French continental shelf i.e. Cap Breton and the area under the influence of the Adour River plume. According to Cap Breton area, mackerels were also frequently found despite being less in number, the distribution extending towards the Gironde River plume. This was also the area were all sampled sprats were caught. Sardines,

Table 3. Length measurements (minor diameter) obtained with *ZooImage* software from samples collected with different mesh sized PAIROVET nets (i.e. 150 and 63 µm) according to the three predefined prey size-ranges.

		PAIROVET 63 µm							PAIROVET 150 µm						
						Minor Diameter (mm)							Minor Diameter (mm)		
Length Range	Range Description (Zooplankton groups)	$N_{BIO'08}$	$N_{ECO'08}$	$N_{JUV'08}$	$N_{BIO'09}$	**Mean (±St.Dev)**	**Min**	**Max**	$N_{BIO'08}$	$N_{ECO'08}$	$N_{JUV'08}$	$N_{BIO'09}$	**Mean (±St.Dev)**	**Min**	**Max**
<1 mm	GAST, BIV, EVAD, OITH, ONC, CORYC, EUTER, MICR, COP.NAUP, COP.JUV, CIRR.NAUP, CIRR, FACET, ISOP, EUPH.NAUP, FEGG.UNDF, EGG.UNDF	19280	26980	10869	15052	**0.31 (± 0.16)**	**0,15**	**0,99**	8869	30422	9775	9381	**0.38 (± 0.15)**	**0,19**	**0,99**
1-2 mm	POD, ACAR, CENTR.SPP, CENTR.TYP, CENTR.CH, ISIAS, TEM.L, TEM.S, EURYT, S.CAL, CAL.UNF, CLYT, EUPH.CALYP, POLYCH, FEGG.ANE, OTHERS	634	157	65	362	**1.30 (± 0.26)**	**1**	**1,98**	323	759	102	197	**1.31 (± 0.26)**	**1**	**1,99**
>2 mm	TREM, METR, CAND, EUCH, CAL, EUCAL, CLAUSCAL, THAL, CALIG, MYSD, STOM, AMPH, EUPH.LV, EUPHS, DECAP.ZOEA, DECAP.MEGAL, DECAP.LLV, DECAP, ANISK, SIPH, MED, CHAET, APP.FRIT, APP.OIKO, SALP, FLV. ANE, FLV.UNDF, FISH	99	12	23	49	**3.45 (± 2.24)**	**2**	**15,65**	50	189	14	45	**4.38 (± 4.11)**	**2,01**	**42,71**

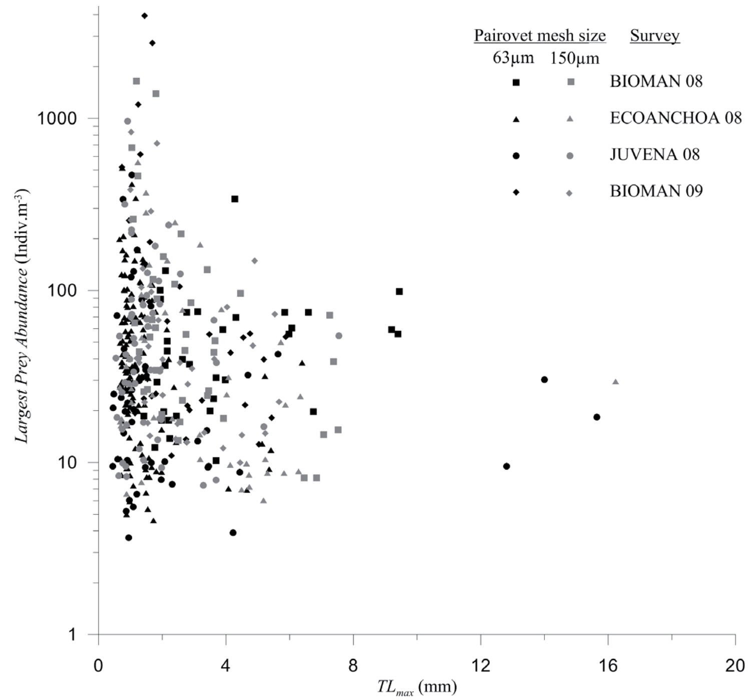


Figure 2. Length of the largest preys caught with different (150 and 63 µm) PAIROVET nets plotted against their abundance on samples ($N_{PAIROVET\ 63\mu m}$ = 244 samples, $N_{PAIROVET\ 150\mu m}$ = 173 samples).

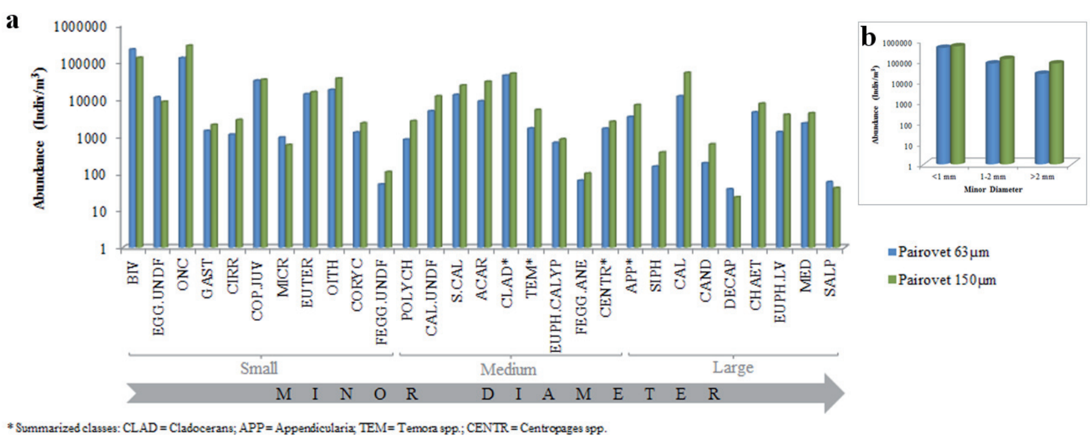


Figure 3. Size distribution of zooplankton total abundance (indiv. m^{-3}) collected with the two mesh size nets (**a**) for all groups and (**b**) for size fractions.

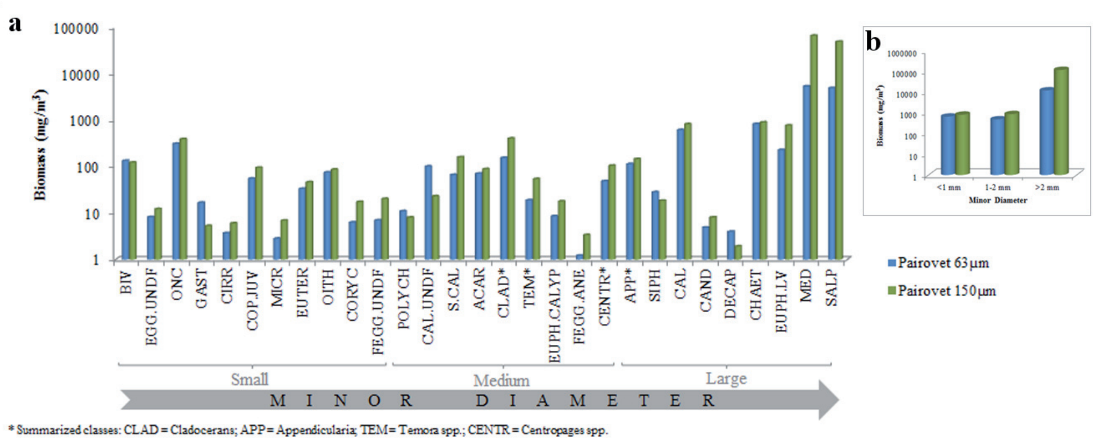


Figure 4. Size distribution of zooplankton total biomass (mg m^{-3}) collected with the two mesh size nets (**a**) for all groups and (**b**) for size fractions.

a)

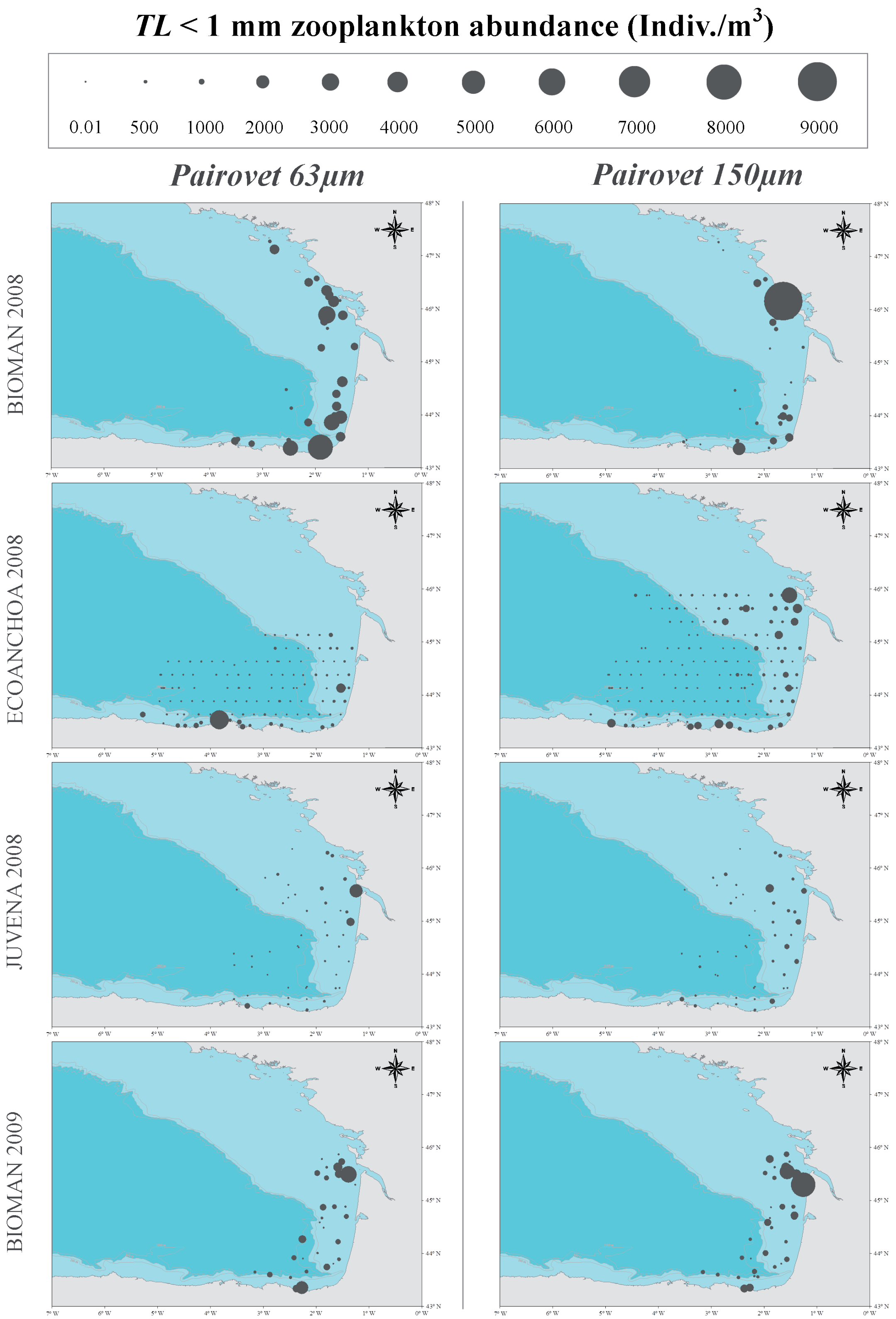


Figure 5. (**a**) Small zooplankton abundance (Indiv. m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

b)

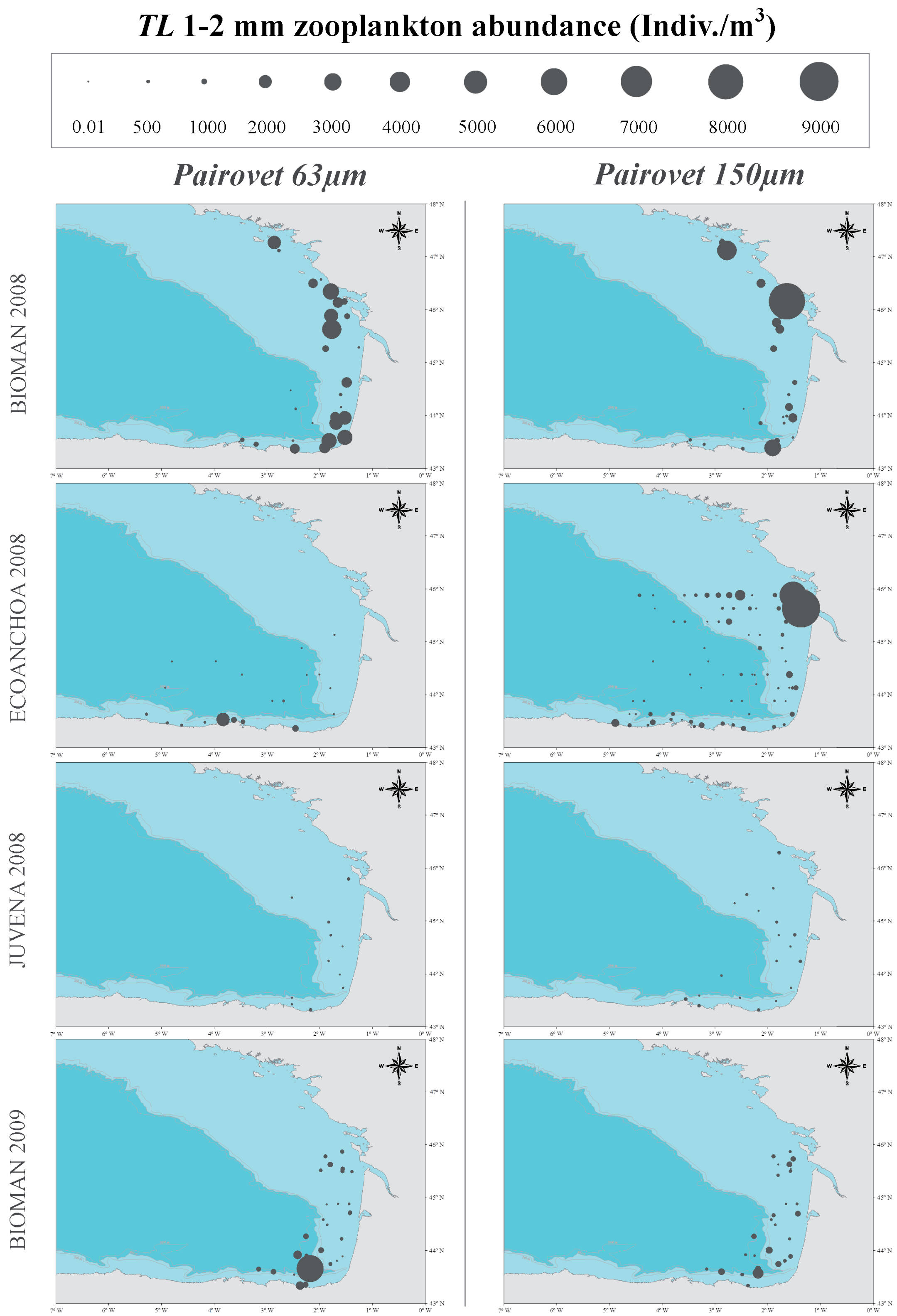


Figure 5. (**b**) Medium zooplankton abundance (Indiv. m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

c)

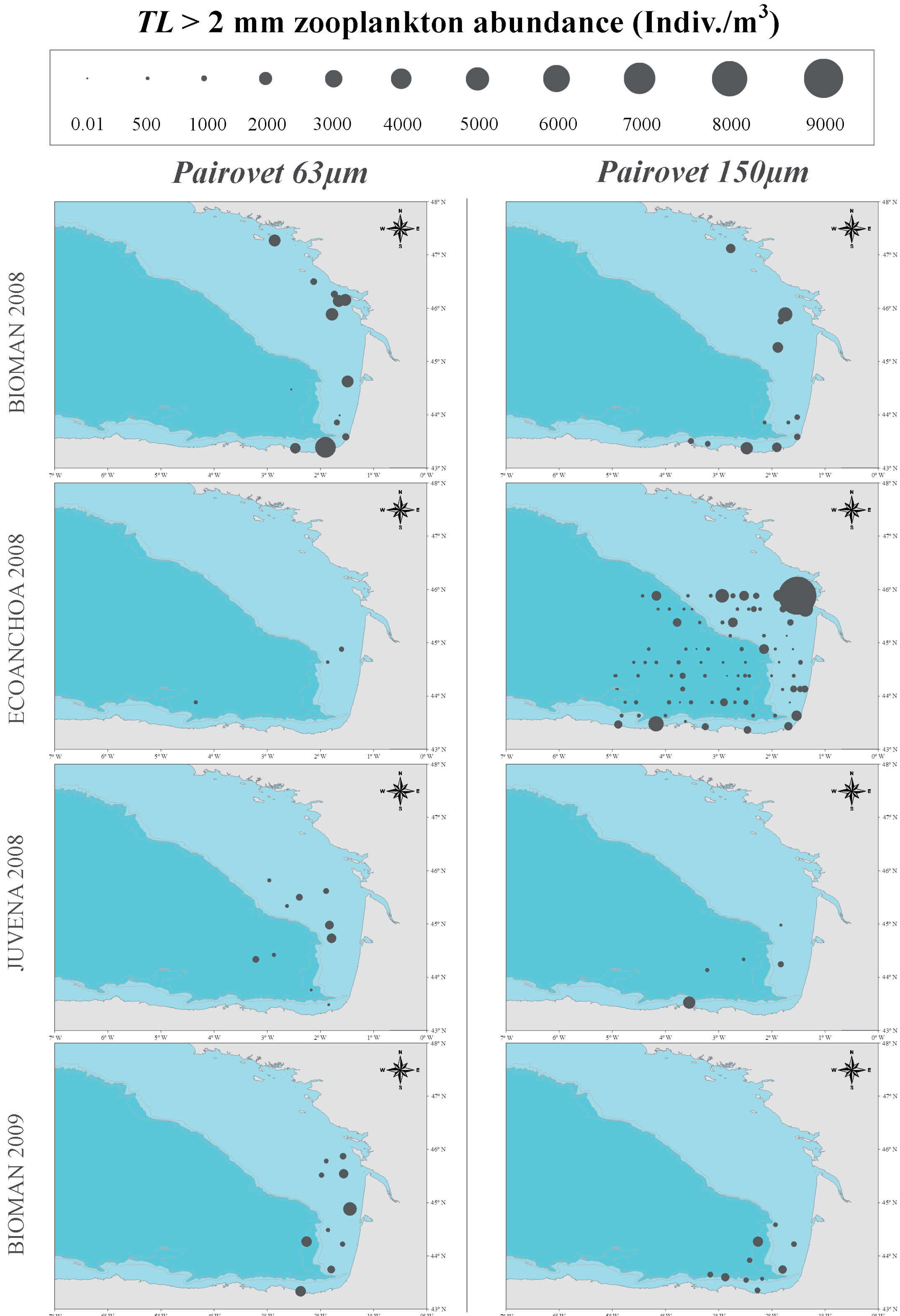


Figure 5. (**c**) Large zooplankton abundance (Indiv. m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

a)

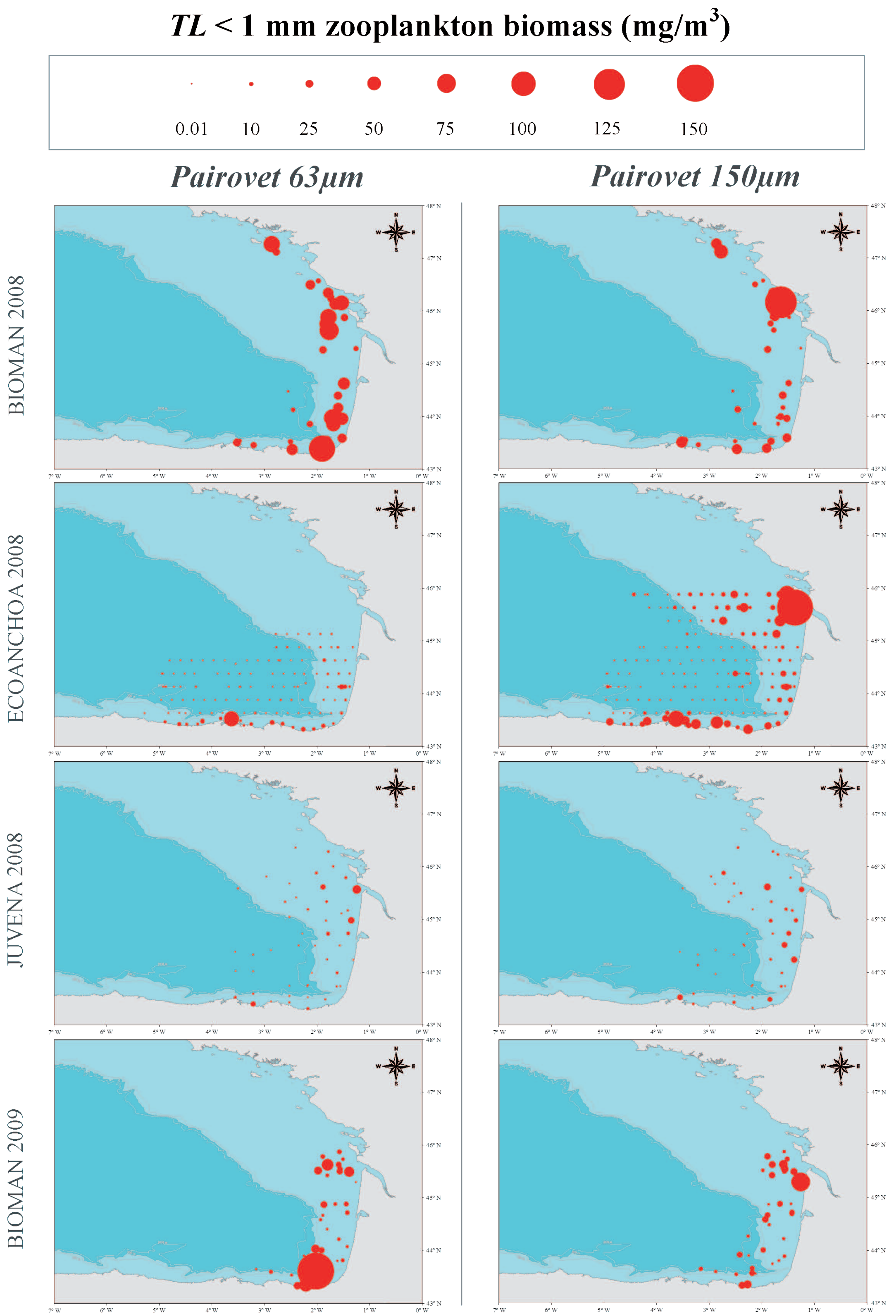


Figure 6. (**a**) Small zooplankton biomass (mg m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

b)

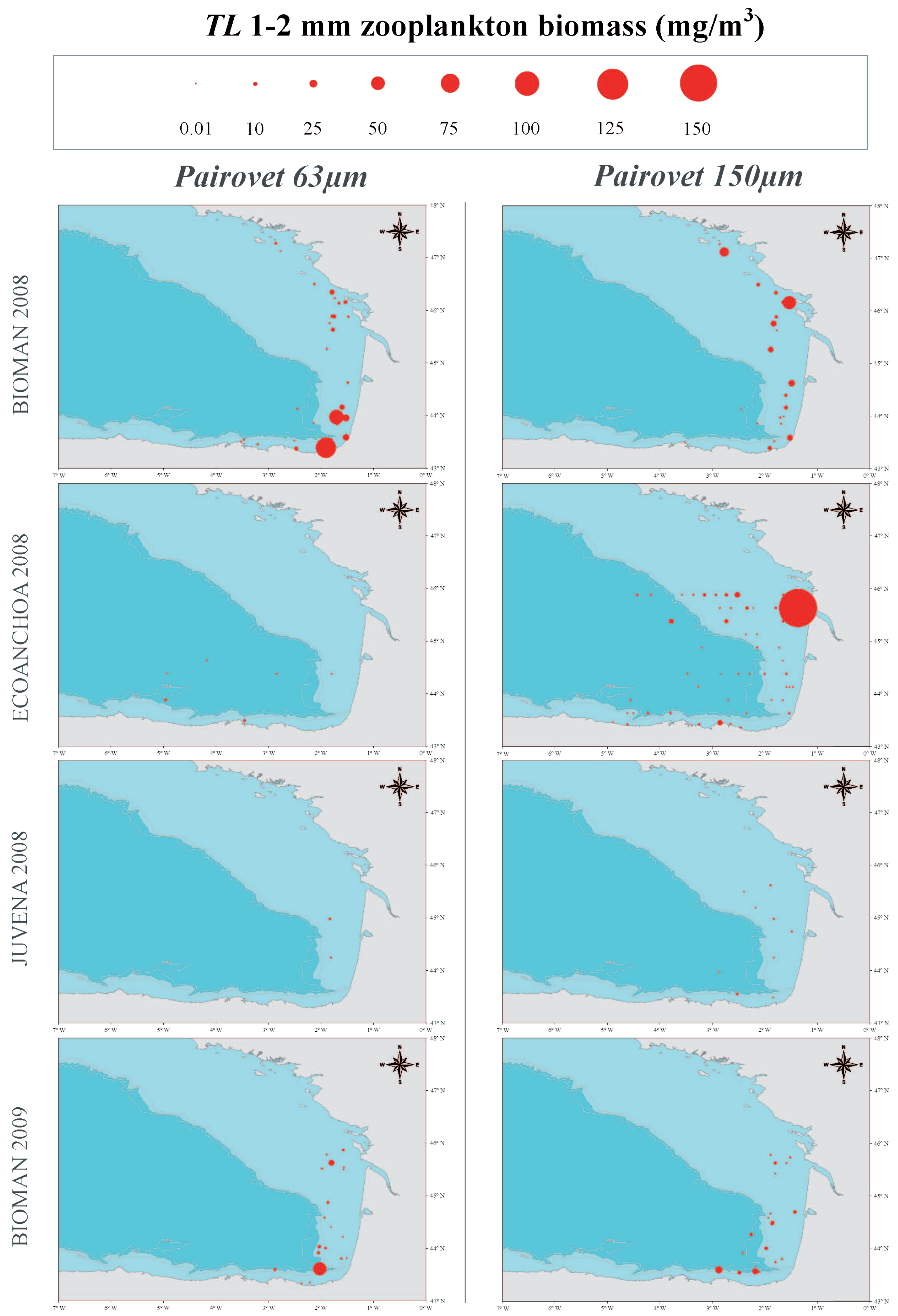


Figure 6. (**b**) Medium zooplankton biomass (mg m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

c)

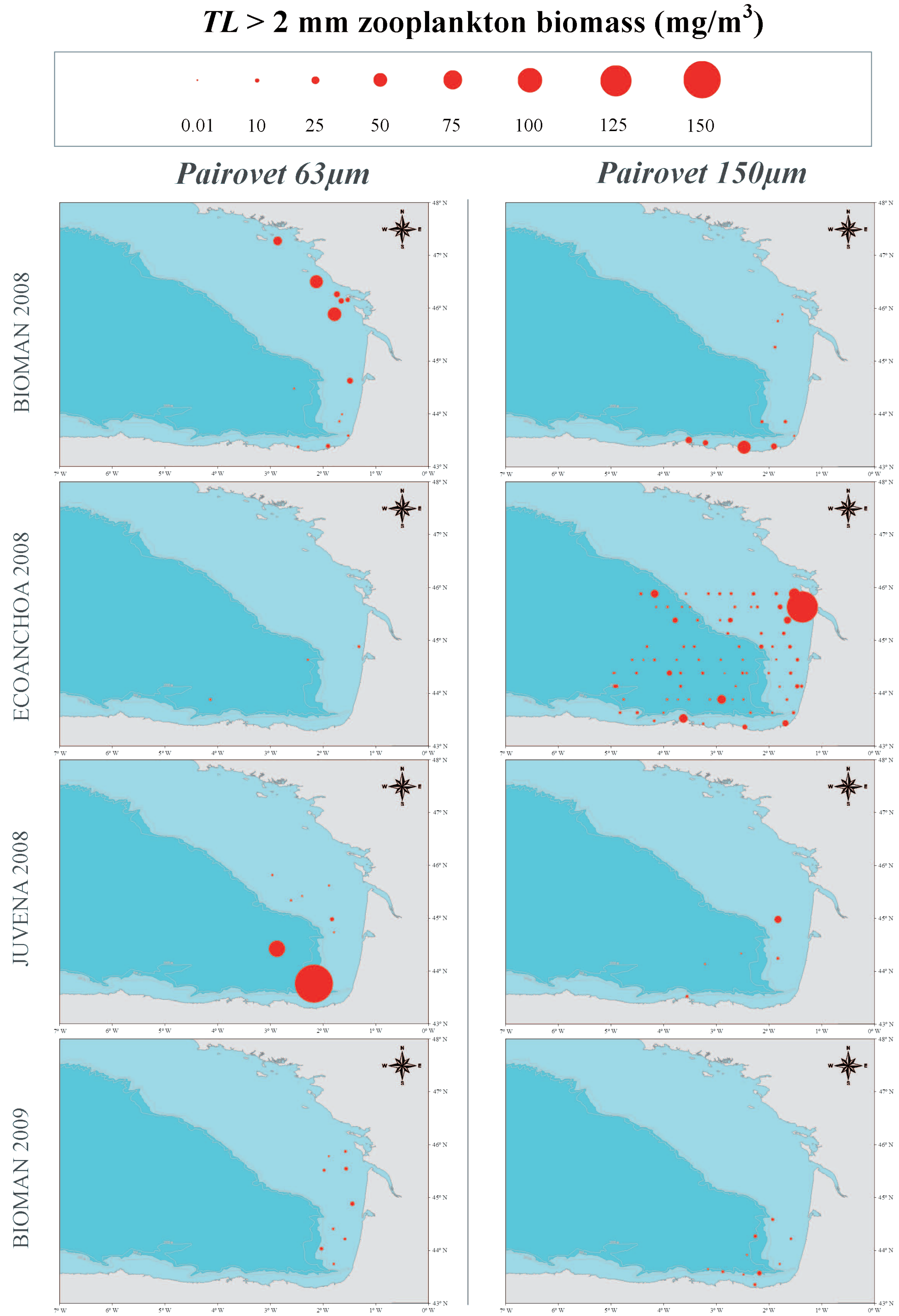


Figure 6. (**c**) Large zooplankton biomass (mg m^{-3}) distribution maps estimated from samples collected with the two mesh size nets for different oceanographic surveys. Differences in number of stations for ECOANCHOA 2008 are due to the breaking of the 63 µm Pairovet net during the survey.

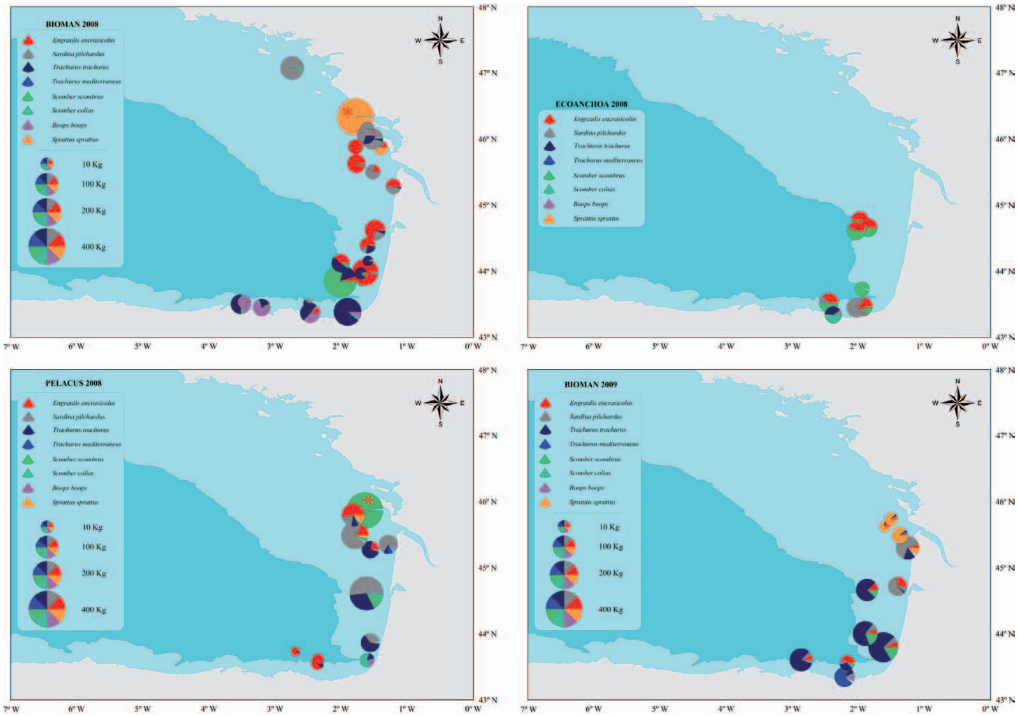


Figure 7. Small pelagic species (predators) distribution around the Bay of Biscay during sampling surveys (2008-2009). *BIOMAN 2008: *46.34°N / 1.78°W*; *S. pilchardus*: 3Kg; *T. trachurus*: 0.6Kg; *S. scombrus*: 3Kg; *S. sprattus*: **>2000Kg**. *PELACUS 2008: 4*5.86°N / 1.64°W*; *E. encrasicolus*: 0.64Kg; *S. pilchardus*: 1.64Kg; *T. trachurus*: 10.82Kg; *T. mediterraneus*: 1.31Kg; *S. scombrus*: 948.5Kg; *S. colias*: 3.61Kg; *B. boops*: 1.18Kg.

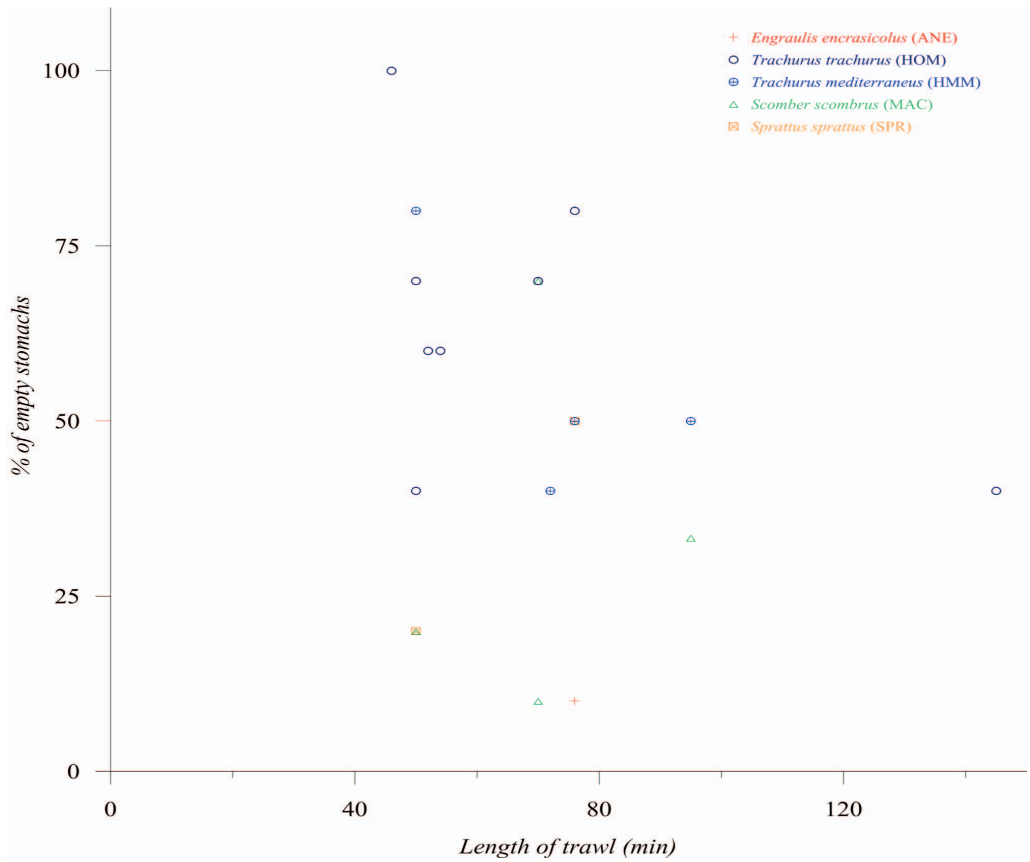


Figure 8 Comparison between the length of pelagic trawl (min) and the percentage of obtained empty stomachs (*N* =19 trawls).

bogues and anchovies were found in all areas, last ones being especially frequent within the French continental shelf. In summer (ECOANCHOA 2008) the sampling was more focused on the shelfbreak area and anchovies, sardines and mackerels were the most abundant fish. In early autumn (PELACUS 2008) anchovies were especially found in the Cantabrian coast and in the Gironde area, although in this second area horse mackerels, sardines and mackerels were more abundant (Figure 7).

However, these results should be treated with caution since the sampling design of different surveys did not cover the whole area of the Bay of Biscay, being focused in areas within the Cantabrian and French continental shelf. In the same way, it should be noted that BIOMAN surveys are aimed to catch anchovies, and especially at night time, optimizing the trawls in order to catch them.

Stomach contents

For the samples collected with trawling nets there was not a significant relation ($R^2 < 0.05$) between the trawling time and the % of empty stomachs (Figure 8).

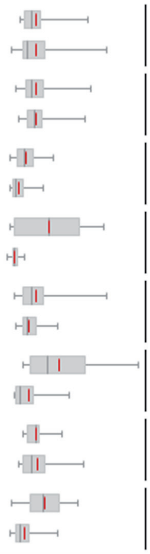
In addition, it was observed that empty stomachs were most commonly found in horse mackerels (Figure 8).

According to the fishing time higher stomach wet weights (*SWW*) were found during daytime for all species, although the difference was only significant in the case of horse mackerels, mackerels and sprats (Table 4).

Regarding the diet characterization, the average cumulative number of new prey types achieved in different surveys during the stomach content identification and counting was plotted against the number (*N*) of stomachs (Figure 9) in order to find an optimum minimum *N* for our target predator species. An average *N* of 20 stomachs per target predator species and station would ensure the detection of 75% (Q_3) of the ingested prey groups (Table 5).

Table 4. Stomach fullness depending on fishing time: day vs. night. Note that purse seiner data have been excluded from the analysis since light attraction method is used both for predators and preys.

Specie	*Time**	*N*	*Mean SWW% (± St.Dev)*	*Box Plot*	*t test P*
Engraulis encrasicolus	D	40	2.454 (± 1.05)		0.767
	N	79	2.534 (± 1.54)		
Sardina pilchardus	D	50	2.513 (± 0.97)		0.941
	N	80	2.5 (± 0.84)		
Trachurus trachurus	D	74	1.816 (± 0.8)		**0***
	N	79	1.273 (± 0.46)		
Trachurus mediterraneus	D	15	3.407 (± 2.52)		**0***
	N	40	1.058 (± 0.22)		
Scomber scombrus	D	48	2.522 (± 1.38)		**0.014***
	N	65	2.042 (± 0.61)		
Scomber colias	D	23	4.129 (± 2.56)		0.066
	N	6	2.023 (± 1.49)		
Boops boops	D	19	2.56 (± 0.76)		0.81
	N	34	2.637 (± 1.27)		
Sprattus sprattus	D	29	3.138 (± 1.26)		**0***
	N	29	1.7 (± 0.87)		



*[D] Day time: 04:00 - 20:00 GMT; [N] Night time: 20:00 - 04:00 GMT

Table 5. Maximum number of prey types in stomach contents, number of sampled stomachs and the number of samples required to achieve the 75% of the prey types in each of our target predator species (N_{Q3}). Note the average N_{Q3} defined as reference for further sampling designs with those eight predator species.

Specie	**Max. num. of prey types**	$N_{stomachs}$	***Q3* (prey types)**	N_{Q3}
Engraulis encrasicolus	36	40	28	18
Sardina pilchardus	36	60	29	28
Trachurus trachurus	28	50	22	32
Trachurus mediterraneus	14	15	11	6
Scomber scombrus	39	41	31	23
Scomber colias	36	23	29	6
Boops boops	44	44	35	26
Sprattus sprattus	23	40	18	25
			Average N_{Q3} =	**20**

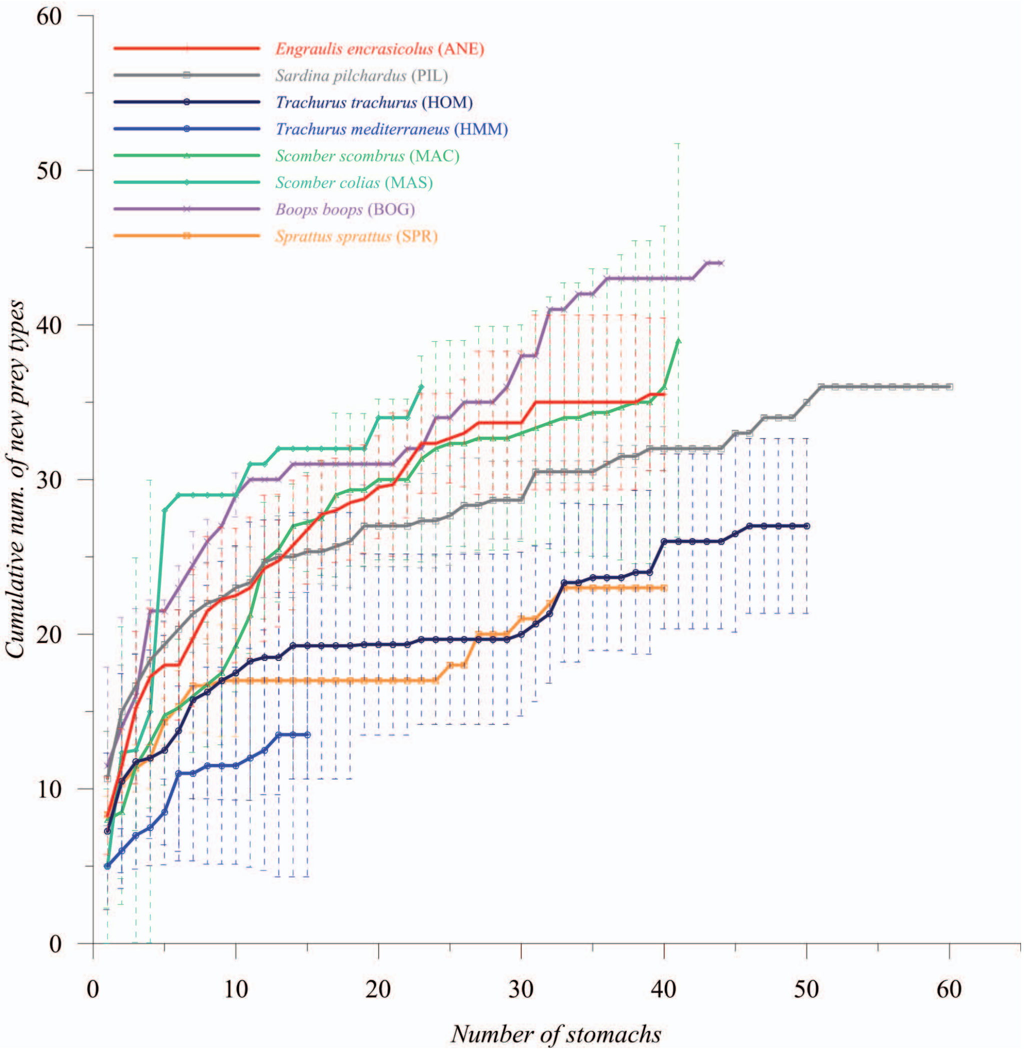


Figure 9. Prey species accumulation plot as an average of BIOMAN'08, ECOANCHOA'08, PELACUS'08 and BIOMAN'09 surveys (N_{ANE} = 40, N_{PIL} =60, N_{HOM} = 50, N_{HMM} = 15, N_{MAC} = 41, N_{MAS} = 23, N_{BOG} = 44, N_{SPR} = 40). Vertical discontinuous bars represent standard deviation between different surveys.

Discussion

We find little differences in abundance and biomass between the 63 and 150 µm mesh nets. However, the small difference in terms of abundance of the smaller sizes (bivalves, eggs and other smaller groups not detectable with image analysis but under the microscope, e.g. naupli, etc.) may be important when comparing field concentrations and stomach contents of the smaller fish or filter feeders (e.g. sardines). On the other hand, largest organisms such as gelatinous (i.e. medusae and salps) seem to be better collected with the 150 µm mesh size. It is also likely that because the slow hauling and small volumes filtered, the Pairovet net (specially the 63 µm mesh size) is not adequate to properly represent the distribution of large motile organisms such as Euphausiids that we commonly find in the stomachs. Therefore, although both nets seem to be similar in terms of characterization, it is likely that they underestimate both the smallest and largest preys that are in the range captured by small pelagic fish in the Bay of Biscay. To be able to compare directly stomach contents with what is in the water the sampling should be complemented with a finer mesh for items captured filter feeding, as well as a high speed or larger sampler for krill.

Zooplankton is observed to be more concentrated within the coastal areas i.e. continental shelf and especially areas under the influence of the main River plumes. That is also supported by many studies showing that oceanographic and geographical variables can affect the zooplankton distribution in the Bay of Biscay (Castel & Courties, 1982; Fernández *et al*., 1993; Albaina & Irigoien, 2004; Fernández *et al*., 2004; Albaina, 2007; Albaina & Irigoien, 2007; Irigoien *et al*., 2011) and consequently the stomach contents of planktivorous species (Murta *et al*., 1993; Plounevez & Champalbert, 1999, 2000; Cabral & Murta, 2002). Therefore, the sampling design should also follow an area based criteria considering the main geographical features. Cotano *et al*. (2008) proposed five environmental areas according to a PCA based on temperature, stratification, chlorophyll and plankton concentration. Taking into account previous analysis together with the spatial distribution patterns of both zooplankton (i.e. preys) and small pelagic species (i.e. potential predators), we propose the following five areas for trophic studies of small pelagic fish in the Bay of Biscay (see on the top left of Figure 1b): *Cantabrian Area* (*C*: Cantabrian Shelf), *Continental Slope Area* (*CS*: Shelfbreak), *Adour-Arcachon Area* (*A*: French shelf covering the area from the Adour River plume to the Arcachon Bay and including the upwelling area), *Gironde Area* (*G*: French shelf area under the influence of the Gironde River plume) and *Oceanic Area* (*O*: areas out of the continental shelf).

The determined areas show differences in the zooplankton abundance and size distribution that we expect to be reflected in the stomach contents. This way, the zooplankton abundance and biomass distribution variability (also by size class) as a function of environmental variables (i.e. main oceanographic features affecting each of the areas), as well as any possible effect that such a distribution could suppose on the diet of predators (i.e. gut contents), should be covered by this sampling methodology. In the same way, the fish sampling design should also take into account small pelagic species distribution patterns and seasonal variations, e.g. anchovy distribution is related with the stage of the life cycle (Uriarte *et al*., 1996; Uriarte *et al*., 2001; Allain et al., 2007; Irigoien et al., 2007; Somarakis & Nikolioudakis, 2007; Cotano *et al*., 2008; Irigoien *et al*., 2008; Aldanondo *et al*., 2010), sprat distribution seemed to be closely related to the Gironde River plume (this study), mackerel distribution is affected by hydrographic conditions (Villamor *et al.*, 2011), etc.

To our knowledge very few studies have treated the regurgitation of food from the stomachs during a trawl and corresponding effect on results (Bowman, 1986; Staniland *et al*., 2001). Although it is clear that *Trachurus* species shows a difference (i.e. the highest regurgitation rate) that requires further attention, in this study no relation is observed between trawling time and percentage of empty stomachs.

According to the fishing time, although no significant differences from day to night are found for some species, a tendency of getting fuller stomachs from daily catches is observed. This confirms previous observations on dial feeding activities (Dahl & Kirkegaard, 1986, 1987; Bulgakova, 1993; Conway *et al.*, 1994; Bulgakova, 1996; Conway *et al*., 1999; Olaso *et al*., 1999; Plounevez & Champalbert, 1999, 2000; Hillgruber & Kloppmann, 2001; Darbyson *et al*., 2003; Garrido, 2007; Borme *et al*., 2009) and that indicates the interest on taking samples in different time ranges in order to detect such differences.

Since gut contents can differ significantly from one fish to other, individual stomach sampling should be considered as a tool to understand individual variability in feeding patterns. A study made with tuna fish by Campo *et al*. (2006) concluded that once we get a number of stomachs sampled, almost all prey types could be found. For the Bay of Biscay small pelagic fish, regarding sampling limitations in terms of space and preservation resources on board vessel as well as the time-effort for later analysis, we could conclude that 20 stomachs would be the minimum for a correct characterisation of the diet. Although not enough fish was collected in case of *T.mediterraneus* and *S.colias* (this could explain the N_{Q3} at 6 individuals for those two species) the 75% of the taxonomic diversity would be detected in most predator species (N_{Q3}). It has to be considered that subsampling from grouped stomachs also presents limitations in terms of finding the rare food items. The objective of the trophic study should determine the method to use. A complete characterisation with understanding of individual variability and small scale differences between areas is better performed in individual analysis. However, routine monitoring or large scale comparisons can probably be carried out with grouped stomachs without losing much information.

Hence, we could conclude that in order to optimize the sampling design addressing trophic studies in the Bay of Biscay, many factors should be considered: firstly, both zooplankton and fish sampling should be carried out in different predefined areas and in different time ranges, in order to detect possible effects the distribution variability of both predators and preys could cause on gut contents. Zooplankton should be sampled with different Pairovet mesh sizes, since different target groups (i.e. size classes) are properly caught in each case; finer than 63 µm mesh net would be appropriate to catch the smallest plankton groups caught by predators by filter feeding activity, whereas larger than 150 µm mesh net samplers should be used in order to catch large preys caught by active predation, such as krill. Regarding predators, a minimum *N* of 20 fish is established as optimum in order to detect at least the 75% of ingested prey diversity. Further research is required in order to solve the regurgitation problem presented in some species such as Atlantic horse mackerels, probably due to the stress caused by long trawls.

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