

Diet and trophic level of scaldfish *Arnoglossus laterna* in the southern Tyrrhenian Sea (western Mediterranean): contrasting trawled versus untrawled areas

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*This study assesses the effects of bottom trawling on the feeding ecology of the scaldfish *Arnoglossus laterna*, by contrasting diets in an untrawled area and in two trawled areas off northern Sicily (western Mediterranean): the Gulf of Castellammare (which hosts a 200 km² no-trawl area) and the gulfs of Termini Imerese and Sant'Agata. Scaldfish were collected in May–June 2005 using a commercial otter trawler between 40 and 80 m on muddy bottoms. Our data show that scaldfish is a selective feeder and consumes similar prey items in the three gulfs, although between-site differences in the relative prey abundance in the stomachs were detected. In the three gulfs scaldfish fed mainly on the crab *Goneplax rhomboides* and on the fish *Lesueurigobius suerii*, but there was an increase in the consumption of *L. suerii* and the shrimp *Alpheus glaber* in the trawled areas. Ontogenetic changes were evident in the diet of scaldfish, with juveniles preying mainly upon suprabenthic species, e.g. mysids and small decapods. While significant differences occurred in the diet of juveniles among all areas due to natural spatial variability, variations in the diet of adults, which feed on benthic prey, occurred between untrawled and trawled areas. These variations were clearly due to trawling disturbance, and no significant difference was detected in the diet of adults between the two trawled areas. On the contrary, the trophic level ($\delta^{15}\text{N}$ values) of scaldfish did not vary between trawled and untrawled areas and between juveniles and adults. Changes in the source of carbon, as detected by $\delta^{13}\text{C}$ values, were evident between juveniles and adults, evidencing a shift from a suprabenthic (juveniles) to a benthic (adults) diet.*

Keywords: feeding ecology, stable isotopes, trawl ban, *Arnoglossus laterna*, Mediterranean Sea

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INTRODUCTION

Two of the most important effects of trawl fishing are the reduction in habitat complexity and the alteration of benthic community structure (Turner *et al.*, 1999; Kaiser *et al.*, 2000a; Callaway *et al.*, 2002; Kaiser *et al.*, 2002). Trawling directly disturbs benthic organisms, by physical removal and/or resuspension of superficial sediment (Palanques *et al.*, 2001; Smith *et al.*, 2003). Indirectly trawling increases the vulnerability to predation of many benthic species by physically removing shelters provided by holes, ripples and emergent seabed organisms (Kaiser *et al.*, 2002b; Colloca *et al.*, 2004). There is a consensus that vulnerable species, such as large-size bottom-dwelling species directly impacted by the gear, are replaced by less vulnerable organisms, such as small-size burrowers (Collie *et al.*, 1997; Thrush *et al.*, 1998; Bergman & Van Santbrink, 2000) and/or scavengers (Kaiser *et al.*, 1998, 2000a). Thus the most intensively fished areas are likely to be in a permanently altered state, dominated

by a resilient fauna adapted to frequent trawling disturbance (Kaiser *et al.*, 2000b; Thrush & Dayton, 2002).

As benthos is an important food source for demersal fish, a high level of trawling disturbance could affect the diet of the latter, with implications for whole food web dynamics (Pinnegar *et al.*, 2000, 2002; Jennings *et al.*, 2002; Sanchez & Olaso, 2004). However, detecting the effects of trawling on benthic habitats and communities is not easy due to natural variability, thus an effort should be made to select homogeneous areas with different fishing pressures. Also, while direct effects of trawling on target stocks are generally evident (Hiddink *et al.*, 2006), indirect changes on the feeding habits of demersal fish are more difficult to detect. Contrasting the diet of fish from trawled and untrawled areas may lead to a better understanding of the trophodynamic response of demersal fish to trawling. The trawl fishing ban imposed in the Gulf of Castellammare, north-western Sicily in 1990 (Pipitone *et al.*, 2000a) gave us the opportunity to explore changes in the diet and trophic level of fish, by comparing them with those from intensively trawled control areas.

The scaldfish *Arnoglossus laterna* is a highly motile predatory fish with medium vulnerability to trawling (de Juan *et al.*, 2007), ten-times more abundant in the Gulf of Castellammare

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than in the two control areas (personal unpublished data). Adult scaldfish prey on benthic prey such as polychaetes, bivalves, peracarids and decapods (Avsar, 1994; Darnaude *et al.*, 2001, 2004; Cabral *et al.*, 2002). Conversely, juveniles prey mainly on copepods, amphipods and mysids (Avsar, 1994) which are part of the suprabenthos that is a group of small-sized animals with low swimming capacity occupying the benthic boundary layer (Brunel *et al.*, 1978; Mees & Jones, 1997). The suprabenthos in general does not seem to be affected by trawling (Ligas, 2005).

The main objective of this paper was to assess if and how trawling affects the feeding ecology of a benthic feeder such as scaldfish, and to evaluate whether its diet can be used as an indirect indicator of trawling disturbance. Three hypotheses were tested: (1) the diet of scaldfish is more specialized in the untrawled area because of the expected higher availability of benthic food; (2) there is a difference between the diet of larger scaldfish (feeding on benthos) and the diet of juveniles (feeding on suprabenthos, which is not or less affected by trawling); and (3) the mean trophic level of scaldfish in the untrawled area is higher than in trawled areas, as a consequence of a diet switch to small fish prey which are particularly abundant in the untrawled area (Sweeting *et al.*, in press). This paper uses stomach content analysis along with

nitrogen and carbon stable isotope analysis ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) contrasting trawled versus untrawled areas to assess the impact of trawling on the trophic ecology of juveniles and adults of scaldfish.

MATERIALS AND METHODS

Study area

The study was carried out in three areas along the coast of northern Sicily (Figure 1): the Gulf of Castellammare (GCAST, $38^{\circ}04'N$ $12^{\circ}56'E$) where trawling has been banned since 1990 (Pipitone *et al.*, 2000a) and the Gulfs of Termini Imerese (GTERM, $38^{\circ}02'N$ $13^{\circ}46'E$) and Sant'Agata (GSANT, $38^{\circ}04'N$ $14^{\circ}20'E$), where trawling is permitted at >50 m depth according to the Italian legislation.

Sampling

A total of 239 scaldfish were collected by bottom otter trawl in the three areas in May–June 2005: 102 in GCAST, 91 in GTERM and 46 in GSANT. Samples were taken between 40 and 80 m depth over muddy bottoms (VTC *sensu* Pérès &

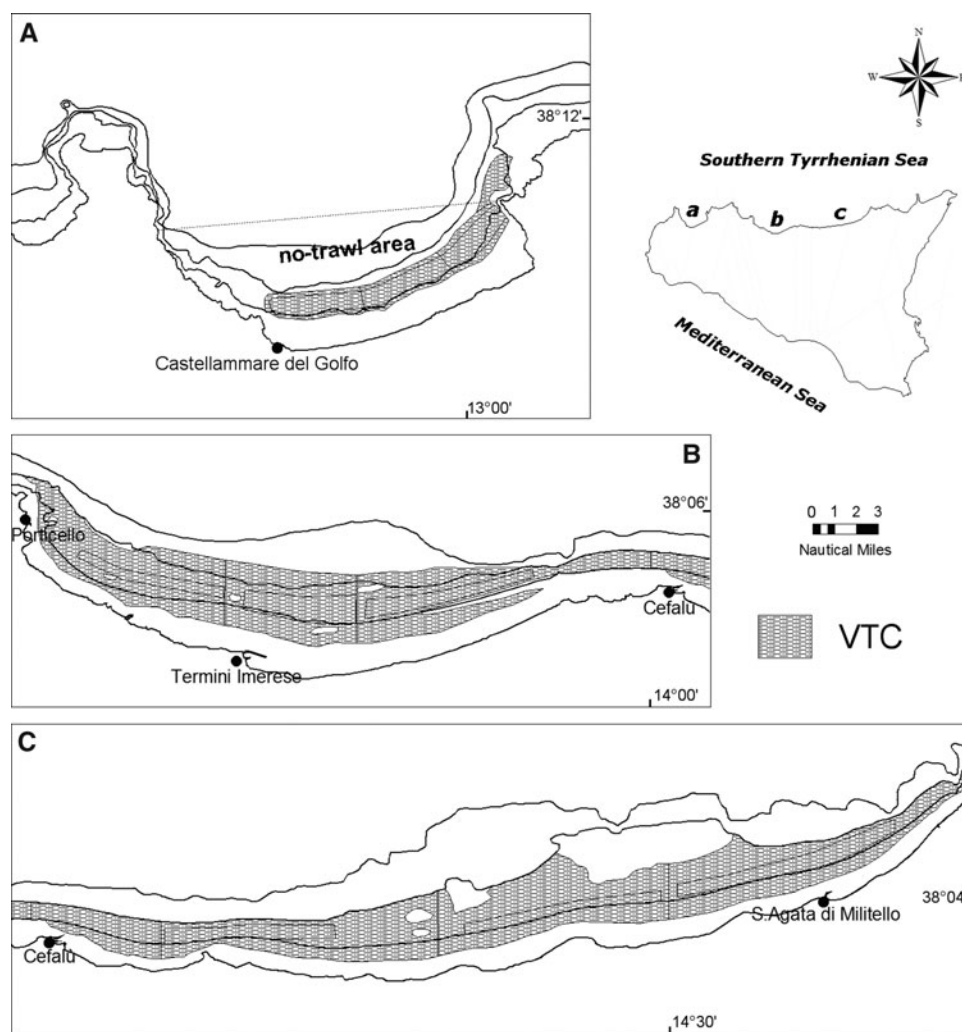


Fig. 1. Map of (A) the Gulf of Castellammare; (B) the Gulf of Termini Imerese; and (C) the Gulf of Sant'Agata in northern Sicily. The dashed line in the Gulf of Castellammare represents the northern boundary of the no-trawl area. Shaded areas (VTC) indicate the terrigenous mud-shelf assemblage.

Picard, 1964; terrigenous mud-shelf assemblage *sensu* Pérès, 1982) with the aid of maps drawn in previous studies (Arena & Bombace, 1970; Andaloro, 1994; Pipitone *et al.*, 2000b) (Figure 1). Benthic samples were collected and analysed with a Van Veen grab during the present study (D'Anna & Pipitone, 2007); although a sedimentary study was not carried out, the presence of species such as the polychaete *Sternaspis scutata*, the crab *Goneplax rhomboides* and the sea cucumber *Labidoplax digitata* allowed us to confirm the type of benthic assemblage.

Scaldfish were frozen on board, then dissected for stomach content analysis in the laboratory after taking total length (TL) to the nearest mm and total weight to the nearest 0.1 g. All the specimens examined were subdivided in to two size-classes: juveniles with TL < 100 mm and adults with TL > 105 mm (Avsar, 1994).

A portion of white muscle close to the dorsal fin was taken from two juvenile and three adult fish of similar total length from each area (juveniles TL = 87.5 ± 6.9 mm, $F_{2,3} = 2.48$, $P > 0.05$ and adults TL = 112.8 ± 8.7 mm, $F_{2,6} = 5.18$, $P > 0.05$) and prepared for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis. Unfortunately, due to problems with the instruments it was possible to analyse only nine samples (one juvenile and two adults from each area) for $\delta^{13}\text{C}$ instead of fifteen. Thus, differences for carbon were tested separately for juveniles and adults and for areas on pooled adult and juvenile data.

Using continuous flow, the muscle samples were introduced into a Finnigan Delta XP Plus isotope ratio mass spectrometer for isotopic analysis. Three capsules of an internal reference (international certified leucine) were analysed at the beginning of each sequence and one every six samples to compensate for machine drift and as a quality control measure. Experimental precision (based on the standard deviation of replicates of the internal standard) was <0.2‰ for $\delta^{15}\text{N}$ and <0.1‰ for $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were obtained in parts per thousand (‰) relative to Vienna Pee Dee Belemnite (vPDB) and atmospheric N_2 standards, respectively, according to the following formula: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

Stomach content analysis

All prey items found in the stomach contents were identified under a stereomicroscope to the lowest possible taxonomic level, then counted and weighed (precision: 0.0001 g) after blotting on tissue paper to remove excess moisture. All undamaged prey items were measured using a micrometer. Vacuity coefficient (%V = per cent number of empty stomachs), stomach fullness (=weight of stomach contents/weight of fish*100, used as a proxy of feeding intensity) and the following indices of prey importance were calculated (see Hyslop, 1980 for a review): per cent frequency of occurrence (%F), per cent numerical composition (%N), per cent gravimetric composition (%W) and index of relative importance (IRI) = (%N + %W)*%F (Pinkas *et al.*, 1971), expressed as %IRI = (IRI/ΣIRI)*100. Prey items were sorted in decreasing order according to their %IRI contribution, and then cumulative %IRI was calculated. The Shannon diversity index (H') calculated using natural logarithm was used as a measure of diet diversity. In order to measure sample size sufficiency (i.e. the number of stomachs to be examined), the cumulative trophic diversity (H') was plotted against the cumulative number of analysed stomachs (Ferry & Cailliet, 1996) for

each area and size-class. The logistic curve and the linear regression were calculated and the respective goodness of fit coefficients (R^2) estimated. The sample size was considered sufficient when R^2 from the logistic curve was higher than R^2 from the linear regression (Castrioni *et al.*, 2005).

Diet breadth was calculated separately for each area and size-class, using Levin's standardized index (Hulbert, 1978; Krebs, 1989), $B = (1/n - 1) \times (1/\sum_j p_{ij}^2 - 1)$, where p_{ij} = proportion of prey j in the diet of predator i , and n = number of prey categories. This index ranges between 0 and 1, with lower values indicating a specialist diet dominated by few prey items and higher values indicating a generalist diet with a broad food spectrum (Gibson & Ezzi, 1987; Krebs, 1989).

Trophic level estimates

The trophic level (TrL) was calculated from $\delta^{15}\text{N}$ data of scaldfish using the shrimp *Alpheus glaber* as reference (mean $\delta^{15}\text{N} = 5.66\text{‰} \pm 0.27$; one-way ANOVA of $\delta^{15}\text{N}$ values between areas: $F_{2,6} = 0.99$, $P > 0.05$). *Alpheus glaber* is one of the dominant deposit feeders in the benthic assemblages of the study areas (D'Anna & Pipitone, 2007; Fanelli, 2007). The rationale behind this choice was that benthic food webs of shelf muddy bottoms are generally detritus-based, hence a deposit feeder is better suited as reference material than copepods, which are the standard reference material for plankton-based food webs. $\delta^{15}\text{N}$ values of scaldfish were converted to trophic level based on the assumption that there is a fractionation of 3.4‰ at each trophic level (Minagawa & Wada, 1984; Post, 2002) and that the reference material (*A. glaber*) has a trophic level of 2: $\text{TrL} = (\delta^{15}\text{N} - \delta^{15}\text{N}_{\text{ref}}/3.4) + 2$, where $\delta^{15}\text{N}$ is the mean $\delta^{15}\text{N}$ value of predator, and $\delta^{15}\text{N}_{\text{ref}}$ is the mean $\delta^{15}\text{N}$ of the reference material.

Data analysis

Differences between the %IRI of the main prey items in the three areas were tested by a Chi-square test (Yates' correction). A two-way permutational univariate ANOVA (Anderson, 2001) based on Euclidean distances, with area (three levels: GCAST, GSANT and GTERM) and size (two levels: adults and juveniles) as fixed factors, was adopted to analyse stable isotope data, fullness, diversity and Levin's niche breadth. While permutational ANOVA was designed for multivariate analysis on distance matrices, it can be used for univariate ANOVA. When we have only one variable in the analysis and we choose to use Euclidean distances, the resulting sums of squares and F-ratios are exactly the same as Fisher's univariate F-statistic in traditional ANOVA. Variance was partitioned for factor area by contrasting untrawled versus trawled (UtrvsTr) areas and the two trawled areas (GSANT versus GTERM). Significance was set at $P = 0.05$; P values were obtained using 9999 permutations of residuals under a reduced model (Anderson, 2001). The same design was used to analyse stomach content weight data. In this case, the PERMANOVA was based on modified Gower distance after a fourth root transformation of the multivariate set of data. This measure of resemblance was used because it takes into account the joint absence of food items in the stomachs of fish in the three areas. It was deemed the most suitable for stomach content data, which are often

highly skewed and whose matrices contain many zeros (Anderson, 2001; Cannicci *et al.*, 2007; Fanelli & Cartes, 2008).

The fullness index data were arc-sin transformed before performing the analysis, because arc-sin transformation is appropriate for data expressed as decimal fractions or percentages (Underwood, 1997). The distribution of percentages is binomial and this transformation makes the distribution normal. Since we could not analyse $\delta^{13}\text{C}$ data from an equal number of specimens of different sizes from each area, a one-way ANOVA was used to test first for differences in $\delta^{13}\text{C}$ between areas and then between sizes.

A distance-based test for homogeneity of multivariate dispersions (PERMDISP) was then performed on factors that resulted significant in the PERMANOVA (Anderson, 2006). A SIMPER analysis (Clarke, 1993) was also applied to stomach content data in order to determine the most typical prey species in each area and size-class.

All the statistical analyses were performed with PRIMER 6 and PERMANOVA plus (Clarke & Warwick, 1995; Anderson, 2006) and with STATISTICA 6.0.

RESULTS

Stomach content analysis

The cumulative prey types curve fitted better a logistic curve than a linear relation for *A. laterna* when 100% of the guts examined were taken into consideration; then sample size

Table 1. Results of the sample size sufficiency analysis for the total number of *Arnoglossus laterna* examined stomachs in each area. All values are significant at $P < 0.001$.

	Logistic regression		Linear regression	
	R ²	F	R ²	F
GCAST juveniles	0.922	F _{1,32} = 68.66	0.682	F _{1,32} = 49.99
GCAST adults	0.934	F _{1,38} = 182.78	0.827	F _{1,38} = 102.46
GTERM juveniles	0.763	F _{1,24} = 59.33	0.712	F _{1,24} = 42.43
GTERM adults	0.749	F _{1,46} = 28.31	0.381	F _{1,46} = 25.37
GSANT juveniles	0.873	F _{1,21} = 42.06	0.750	F _{1,21} = 34.97
GSANT adults	0.733	F _{1,19} = 24.24	0.660	F _{1,19} = 23.23

was considered sufficient to describe the diet of this species for each area and size (Table 1).

The fullness index of the analysed stomachs varied significantly between the three areas, with a lower value in the untrawled area (GCAST = 0.55 ± 0.4 , GTERM = 1.07 ± 1.4 , GSANT = 0.87 ± 1.2) (Table 2A). Adults had significantly fuller stomachs than juveniles (adults: GCAST = 0.60 ± 0.5 , GTERM = 1.74 ± 1.3 , GSANT = 1.47 ± 1.2 ; juveniles: GCAST = 0.33 ± 0.2 , GTERM = 0.81 ± 1.2 , GSANT = 0.31 ± 0.2) and, although the interaction area \times size was not significant, there were significant differences in fullness between adults from GCAST and GSANT-GTERM (pairwise test within level adult of factor size: GCAST versus GTERM $t = 2.43$, $P < 0.05$; GCAST versus GSANT $t = 3.31$, $P < 0.05$; GTERM versus GSANT $t = 0.46$; $P > 0.05$).

Table 2. Two-way permutational univariate ANOVA on: (A) fullness; (B) Shannon trophic diversity index; (C) Levin's niche breadth index; and (D) $\delta^{15}\text{N}$ values of scaldfish based on the experimental design described in the text (9999 permutations). Utr, untrawled area (GCAST); Tr, trawled areas (GTERM, GSANT). Pairwise tests on the significant interaction term area \times size are reported. GCAST, Gulf of Castellammare; GSANT, Gulf of Sant'Agata; GTERM, Gulf of Termini Imerese; Juv, juvenile scaldfish; Ad, adult scaldfish; * $P < 0.05$; *** $P < 0.001$; n.s., not significant.

(A) Fullness				(B) Shannon (H')		
Source of variation	df	MS	Pseudo-F	df	MS	Pseudo-F
Area	2	22.98	3.30*	2	0.01	0.05 ^{n.s.}
Utr versus Tr	1	38.72	5.61*	1	0.01	0.05 ^{n.s.}
GSANT versus GTERM	1	7.31	0.88 ^{n.s.}	1	0.01	0.10 ^{n.s.}
Size	1	149.53	21.49***	1	9.60	43.93***
Area \times size	2	8.26	1.19 ^{n.s.}	2	0.75	3.43*
Utr versus Tr \times size	1	16.16	2.34 ^{n.s.}	1	1.25	5.76*
GSANT versus GTERM \times size	1	0.06	0.01 ^{n.s.}	1	0.24	1.66 ^{n.s.}
Res	107	6.96		129	0.22	
Total	112			134		
				(B) area \times size interaction		
				Juv GCAST = GTERM = GSANT		
				Ad GCAST = GSANT > GTERM		
(C) Levin's				(D) $\delta^{15}\text{N}$		
Area	2	0.08	96.05***	2	0.46	1.08 ^{n.s.}
Utr versus Tr	1	0.08	17.08***	1	0.15	0.31 ^{n.s.}
GSANT versus GTERM	1	0.07	73.51***	1	0.77	1.84 ^{n.s.}
Size	1	0.29	349.64***	1	0.09	0.21 ^{n.s.}
Area \times size	2	0.05	62.84***	2	0.52	1.23 ^{n.s.}
Utr versus Tr \times size	1	0.09	18.58***	1	0.24	0.51 ^{n.s.}
GSANT versus GTERM \times size	1	0.01	11.21***	1	0.81	1.92 ^{n.s.}
Res	129	0.00		9	0.42	
Total	134			14		
(C) area \times size interaction						
Juv GSANT > GCAST > GTERM						
Ad GSANT > GTERM > GCAST						

Thirty-six different prey items were identified in the 192 stomachs (GCAST juveniles: 34, GCAST adults: 40; GTERM juveniles: 26; GTERM adults: 48; GSANT juveniles: 23; GSANT adults: 21) of scudfish analysed for diet composition (Annex 1). The diet consisted mainly of fish (mean %IRI in the three Gulfs = 65) and decapods (mean %IRI = 28). At the species level, the decapods *Goneplax rhomboides* and *Alpheus glaber* and the fish *Lesueurigobius suerii* dominated the diet.

The proportion (%IRI) of decapods and fish ingested was higher in GCAST and GSANT (χ^2 test, $P < 0.05$), while mysids were an important prey in GTERM (Annex 1; Figure 2).

On the basis of the SIMPER analysis the preferred prey in the trawled areas was *L. suerii*, which contributed with 45.6% and 62% to the diet in GSANT and GTERM, respectively. In GCAST *G. rhomboides* was the most typical species, adding 78.9% to similarity among samples (Table 3).

Asymmetrical PERMANOVA provided evidence for separation of stomach contents between untrawled and trawled areas (Utr versus Tr), between the two trawled areas, between size-classes (juveniles versus adults) and for the interactions (Table 4). The pairwise comparison based on the area \times size interaction showed that the diet of juvenile scudfish differed significantly among all areas; however the diet of adults varied significantly between untrawled and trawled areas, but not between trawled areas. Moreover, the diet of juveniles and adults was different among the three areas. Although the PERMDISP test based on factor area was not significant ($F_{3,135} = 3.43$, $P = 0.068$), the pairwise test (Table 5) evidenced a significant difference in the homogeneity of multivariate dispersions between GCAST and GTERM–GSANT, while GTERM and GSANT did not differ from each other. Data from GCAST were less dispersed than those from GTERM and GSANT.

The mean Shannon diversity index (GCAST = 1.03 ± 0.31 , GTERM = 0.73 ± 0.17 , GSANT = 0.97 ± 0.38) was significant only for the area \times size interaction, but not for the differences arising from the pairwise comparison (Table 2B). As far as the Levin's index is concerned, juveniles had a significantly wider niche breadth than adults (Table 2C). The lowest value, indicating the narrowest niche breadth, was found for adults in GCAST (Table 6).

The diets of juvenile and adult scudfish were different, as shown by the SIMPER analysis (Table 7). Juveniles fed mostly on suprabenthic species such as amphipods (mainly *Ampelisca* sp.), small decapods (mainly *Philocheira bispinosus* and small *G. rhomboides* with 4–6 mm carapace width, CW) and mysids (*Leptomysis gracilis* and *Erythropeleus elegans*), which contributed largely to the diet in the three Gulfs (Table 7a). Adults preyed mainly on large *G. rhomboides* (CW: 6.1–8.4 mm) and *L. suerii* in GCAST and on *L. suerii* and *A. glaber* in GTERM and GSANT (Table 7a). SIMPER performed on adults evidenced higher dissimilarity between untrawled and trawled areas (Table 7b) than between the two trawled areas, where the average abundance of the main prey items (*L. suerii* and *A. glaber*) was very similar.

Stable isotope data

$\delta^{15}\text{N}$ values of scudfish did not differ between areas ($\delta^{15}\text{N}$ GCAST: $9.61\text{‰} \pm 0.87$; $\delta^{15}\text{N}$ GTERM: $9.26\text{‰} \pm 0.32$; $\delta^{15}\text{N}$ GSANT: $9.80\text{‰} \pm 0.39$) and size-classes ($\delta^{15}\text{N}$ adults:

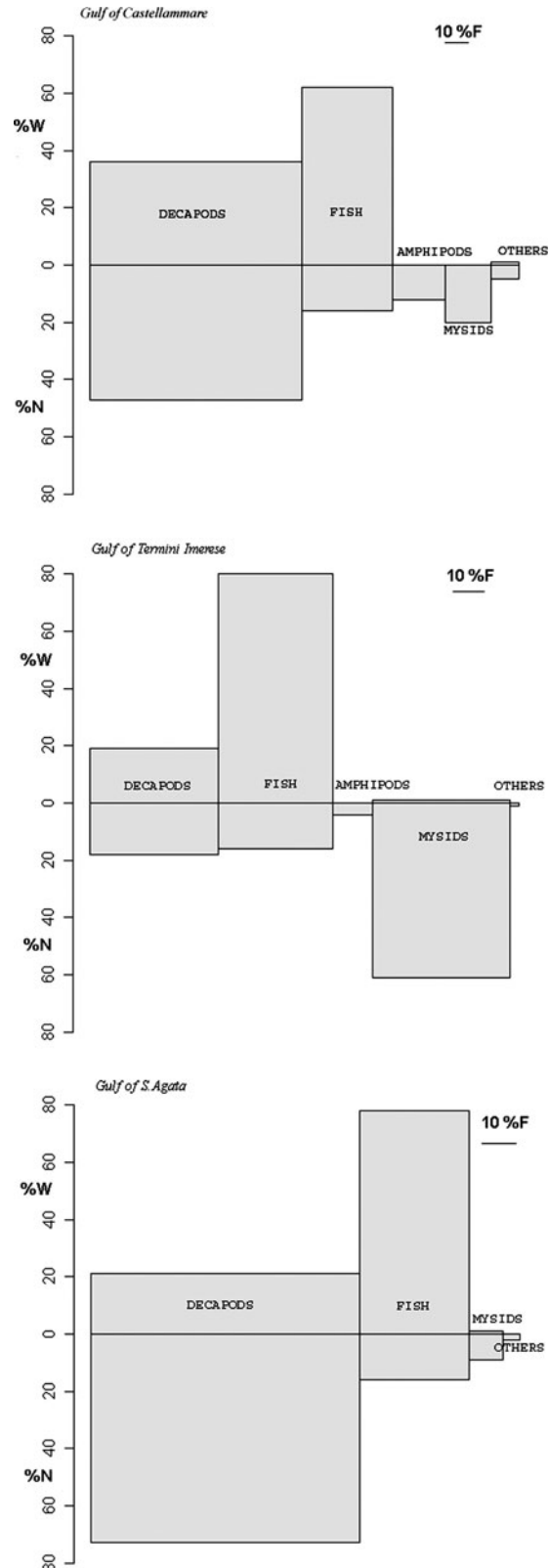


Fig. 2. Indices of prey importance in the diet of scudfish. Only prey items with %IRI > 1 are shown. The area of each rectangle is equivalent to the value of %IRI. For the meaning of %IRI, %N, %W and %F see text.

$9.3\text{‰} \pm 0.4$; $\delta^{15}\text{N}$ juveniles: $9.2\text{‰} \pm 0.9$) (Table 2D). Individuals from GSANT had $\delta^{13}\text{C}$ values significantly more enriched ($\delta^{13}\text{C}$ GSANT: $-17.43\text{‰} \pm 0.01$) than those from GCAST and GTERM ($\delta^{13}\text{C}$ GCAST: $-18.06\text{‰} \pm 0.80$;

Table 3. SIMPER analysis of scaldfish stomach contents from the three areas off northern Sicily (cut-off for low contribution: 90%). GCAST, Gulf of Castellammare; GSANT, Gulf of Sant'Agata; GTERM, Gulf of Termini Imerese.

GCAST					
Average similarity: 27.46					
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Goneplax rhomboides</i>	1.66	21.67	0.64	78.92	78.92
<i>Lesueurigobius suerii</i>	0.72	3.57	0.24	12.99	91.92
GTERM					
Average similarity: 20.32					
<i>L. suerii</i>	1.19	12.6	0.4	61.97	61.97
<i>Erythroptis elegans</i>	0.83	3.78	0.33	18.58	80.55
<i>Alpheus glaber</i>	0.5	1.99	0.15	9.79	90.34
GSANT					
Average similarity: 15.02					
<i>L. suerii</i>	0.93	6.86	0.29	45.65	45.65
<i>A. glaber</i>	0.68	3.07	0.23	20.47	66.12
Crangonidae	0.58	1.81	0.2	12.03	78.15
<i>Philocheras bispinosus</i>	0.44	1.14	0.16	7.6	85.75
<i>G. rhomboides</i>	0.44	0.93	0.13	6.18	91.93

$\delta^{13}\text{C}$ GTERM: $-19.27\text{‰} \pm 0.54$) (one-way ANOVA $F_{2,6} = 8.4$; $P < 0.05$). $\delta^{15}\text{N}$ values did not differ between size-classes (Table 2d), while the $\delta^{13}\text{C}$ value of juveniles was significantly more depleted than in adults ($\delta^{13}\text{C}$ juveniles: $-18.92\text{‰} \pm 0.15$; $\delta^{13}\text{C}$ adults: $-17.66\text{‰} \pm 0.37$) (one-way ANOVA $F_{1,7} = 38.4$; $P < 0.05$). Since no significant differences existed in the scaldfish $\delta^{15}\text{N}$ values by area and by size (Table 2D) and the same reference value was used for the trophic level calculations, we assumed the same mean trophic level value of 3.06 (± 0.19) for scaldfish in the overall study area.

Table 4. PERMANOVA based on the modified Gower dissimilarity of standardized and fourth root transformed stomach contents weight data (36 taxa; 9999 permutations). Utr, untrawled area (GCAST); Tr, trawled areas (GTERM, GSANT). Pairwise tests on the significant interaction term 'area x size' are reported. Juv, juvenile scaldfish; Ad, adult scaldfish; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

Source of variation	df	MS	Pseudo-F
Area	2	4.46	6.92***
Utr versus Tr	1	7.12	10.79***
GSANT versus GTERM	1	1.70	2.60*
Size	1	9.30	14.42***
Area x size	2	2.46	3.81***
Utr versus Tr x size	1	3.41	5.17***
GSANT versus GTERM x size	1	1.54	2.35*
Res	129	0.64	
Total	134		
Pairwise within level 'Juv' of factor size			
GCAST \neq GSANT \neq GTERM			
Pairwise within level 'Ad' of factor size			
GCAST \neq GSANT = GTERM			
Within level GCAST, GTERM, GSANT of factor area			
Juv \neq Ad			

Table 5. Pairwise comparison of PERMDISP test of factor area based on the modified Gower dissimilarities of standardized and fourth root transformed stomach content weight data (36 taxa). ** $P < 0.01$; n.s., not significant.

Pairwise comparisons	
Groups	t
GCAST, GSANT	2.07**
GCAST, GTERM	1.89**
GSANT, GTERM	0.76 ^{n.s.}
Means and standard errors	
Groups	Mean \pm SE
GCAST	0.79 (0.03)
GSANT	0.88 (0.02)
GTERM	0.86 (0.02)

DISCUSSION

Differences in diet composition: fishing impact and predator size

Scaldfish living on the muddy bottom off the northern coast of Sicily preyed mainly upon fish and decapod crustaceans. This species, like other bothid fish, is an active predator, responding primarily to moving prey and thus feeding mainly on active rather than on sedentary prey (De Groot, 1971; Braber & De Groot, 1973; Darnaude *et al.*, 2001). Small gobiid fish, like *Lesueurigobius suerii* in this study, which are the most important prey in the trawled areas and the second most important in the untrawled area in terms of %IRI, constitute a significant component in the diet of scaldfish (Gibson & Ezzi, 1980). Avsar (1994) found that scaldfish from Turkey fed mainly on crustaceans (amphipods, euphausiids, decapods, mysids and copepods), with fish and polychaetes as secondary prey items; similar feeding habits were described by Gibson & Ezzi (1980) off the Scottish coast and by Cabral *et al.* (2002) off Portugal, although in these two areas scaldfish preyed almost exclusively upon crustaceans. Shrimps were the main food item of scaldfish in the Irish Sea (Amezcuea *et al.*, 2003), while amphipods, mysids, hermit crabs, shrimps and occasionally polychaetes were the preferential prey of juvenile scaldfish in the Gulf of Fos (north-western Mediterranean) (Darnaude *et al.*, 2001). Our results partially agree with the above findings, with differences attributable to geographical and bathymetric variability in resource availability. According to existing data on the abundance and biomass of benthos (D'Anna & Pipitone, 2007; Fanelli, 2007), the dominant preys in the diet of adult scaldfish are not the most abundant in the benthos of the study areas. This suggests selective feeding behaviour in adult scaldfish, as supported by the values of the Shannon diversity index calculated on prey items.

Stomach fullness, used as an expression of feeding intensity, was higher in adult than in juvenile scaldfish. The fullness index of juveniles did not vary between trawled and untrawled areas, while adults had fuller stomachs in the trawled areas. This result suggests that trawling does not affect the feeding intensity of juveniles feeding on suprabenthos—which is not affected by trawl gear (Ligas, 2005; Fanelli, 2007)—while it does affect that of the benthic feeding adults. It seems that adult scaldfish benefit indirectly from trawling, since at least two of their main prey (*L. suerii* and *Alpheus glaber*) are more abundant in the trawled areas. It has been shown that, depending on the degree of disturbance, resource availability

Table 6. Levin's index for each size-class and area. Standard deviations are also given.

Area	GCAST		GSANT		GTERM	
	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults
Levin's index	0.55 ± 0.02	0.14 ± 0.01	0.61 ± 0.03	0.45 ± 0.03	0.45 ± 0.04	0.38 ± 0.03

may be higher in disturbed areas, due to the occurrence of opportunistic species (Connell, 1978). The postulated increase in scavengers in areas with higher trawling impact (Kaiser *et al.*, 2002a) was not found for the suprabenthos, as shown by Fanelli (2007).

The diet of juveniles varied among the three areas, thus the detected differences cannot be attributed to fishing pressure. In fact, as also found by Darnaude *et al.* (2001, 2004), juveniles fed on suprabenthic resources such as mysids, amphipods and small decapods like *Philocheiras bispinosus*. These last differ among the three areas in terms of biomass, abundance and assemblage structure, as a response to different environmental characteristics such as primary production and advective fluxes (Fanelli, 2007). That the suprabenthic assemblage is not affected by trawling has been suggested in other studies comparing trawled and untrawled areas (Ligas, 2005; Cartes *et al.*, 2007). In contrast the effect of the trawl ban in the Gulf of Castellammare, and hence of the presence of an undisturbed benthic assemblage, was evident in the diet of adult scaldfish, which differed significantly in trawled versus untrawled areas. Several studies have highlighted that trawling activities heavily affect the structure and diversity of benthic communities (see Jennings & Kaiser, 1998 and references cited therein). In addition, the increase in the abundance of motile burrowing predators and scavengers caused by trawling was correlated to the reduction of highly motile surface predators and of less motile burrowing filter feeders (de Juan *et al.*, 2007). In an undisturbed bottom community, such as in the untrawled Gulf of Castellammare, benthic resources are expected to be more abundant and diversified than in intensively fished areas (Auster & Langton, 1999; Norse & Watling, 1999; Collie *et al.*, 2000; Kaiser *et al.*, 2002a, 2006 and references cited therein). A recent study that included an analysis of the benthic communities in the three Gulfs surveyed in this study showed a general trend of increased benthic abundance in the untrawled area compared to trawled areas, but no difference in the benthic biomass (D'Anna & Pipitone, 2007). Moreover, a higher abundance of highly vulnerable organisms, represented by sedentary surface filter feeders (e.g. bivalves and burrowing species with low motility such as ophiuroids and amphipods like *Ampelisca* spp.) was also found in the Gulf of Castellammare, indicating a well structured and less disturbed community.

In the untrawled Gulf the scaldfish exhibited more selective feeding behaviour, as evidenced by lower data dispersion (PERMDISP) and Levin's index for adults. *Goneplax rhomboides*, a species with medium vulnerability to trawling disturbance (de Juan *et al.*, 2007), was twice as abundant in the Gulf of Castellammare as in the trawled areas (D'Anna & Pipitone, 2007; Fanelli, 2007). This is in agreement with de Juan *et al.* (2007), who observed a higher abundance of this crab in an unfished site than in an intensively trawled area off the Catalan coast. Indeed, *G. rhomboides* seemed to be the preferred prey of adult

scaldfish in GCAST. In the trawled areas, the proportion of other prey increased and the most typical species in the diet of scaldfish were *L. suerii* and *A. glaber*. *Lesueurigobius suerii* is a motile burrowing predator, more abundant in trawled than in untrawled sites off the Catalan coast (de Juan *et al.*, 2007) and off northern Sicily (Fanelli, 2007). The shrimp *A. glaber* was the species that contributed most to the dissimilarity between GCAST and GSANT–GTERM. This species is a sedentary burrowing deposit feeder with low vulnerability to trawling (de Juan *et al.*, 2007) and while equally abundant in trawled and untrawled areas (D'Anna & Pipitone, 2007), it was more frequently ingested in the trawled areas. De Juan *et al.* (2007) noted that sedentary burrowing deposit feeders dominated the infaunal community in the Catalan trawling grounds, but also that it is difficult to ascertain whether this assemblage configuration is solely a result of trawling disturbance history or whether it represents a characteristic community of muddy bottoms.

Stable isotopes

The present study showed a difference in the information provided by the stomach content analysis and the $\delta^{15}\text{N}$ analysis of scaldfish, as far as trawl impact and ontogenetic changes in the diet are concerned. While the diet of adult scaldfish varied significantly between trawled and untrawled areas, the $\delta^{15}\text{N}$ value and the mean trophic level did not. Although trawling disturbance normally leads to a reduction in infauna and epifauna biomass, and to dramatic changes in the composition of the infauna (see Jennings *et al.*, 2001 among others), these changes are not considered to affect the mean trophic level of the community (Jennings *et al.*, 2001). Jennings *et al.* (2001) after studying the impacts of demersal fishing at large spatial scales suggested that 'the trophic structure of intensively fished communities has not change, despite changes in species composition, to ensure the continued processing of production by those remaining invertebrates that can withstand the levels of mortality imposed by trawling'. However, Badalamenti *et al.* (2008) reported a small increase of the trophic level of benthic fish in the GCAST untrawled area. In the case of scaldfish we cannot exclude that, given the small sample size used for the stable isotopes analysis in this study, the absence of a difference is due to the low power of the analysis. Indeed, recent data on the benthic food web revealed greater $\delta^{15}\text{N}$ values for omnivorous and carnivorous invertebrates at GCAST when compared to GTERM and GSANT and no differences for selective benthic deposit feeders (D'Anna & Pipitone, 2007) and for suprabenthic deposit feeders (Fanelli, 2007).

Finally, the ontogenetic shift observed in the diet of scaldfish larger than 105 mm was not paralleled by an increase in the $\delta^{15}\text{N}$ value with size, once again as a possible consequence of the low power of our analysis. Peracarids such as amphipods, cumaceans and mysids, which were preyed by juvenile

Table 7. SIMPER analysis of stomach contents in two size-classes of scaldfish in each area (cut-off for low contribution: 90%). Juveniles, TL < 100 mm; adults, TL > 105 mm. (A) Similarity within each size-class per area; (B) dissimilarity within adults between areas. *Goneplax rhomboides* small ranged between 4 and 6 mm CW. *G. rhomboides* large ranged between 6.1 and 8.4 mm CW. GCAST, Gulf of Castellammare; GSANT, Gulf of Sant'Agata; GTERM, Gulf of Termini Imerese.

(A)						
GCAST juveniles						
Average similarity: 16.33						
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
<i>G. rhomboides</i> small	1.14	8.8	0.38	53.91	53.91	
<i>Ampelisca</i> sp.	0.69	2.45	0.28	15	68.91	
<i>Erythrope elegans</i>	0.66	2.21	0.3	13.53	82.44	
<i>Philocheras bispinosus</i>	0.51	0.99	0.2	6.05	88.49	
<i>Leptomysis gracilis</i>	0.41	0.68	0.16	4.18	92.67	
GCAST adults						
Average similarity: 46.26						
<i>G. rhomboides</i> large	2.14	37.35	0.96	80.74	80.74	
<i>Lesueurigobius sueri</i>	1.07	7.69	0.38	16.62	97.36	
GSANT juveniles						
Average similarity: 16.72						
Crangonidae	1.12	6.97	0.41	41.67	41.67	
<i>P. bispinosus</i>	0.85	4.4	0.33	26.33	68	
<i>G. rhomboides</i> small	0.84	3.58	0.26	21.42	89.41	
<i>Alpheus glaber</i>	0.51	1	0.18	5.99	95.41	
GSANT adults						
Average similarity: 37.51						
<i>L. suerii</i>	1.94	30.86	0.73	82.25	82.25	
<i>A. glaber</i>	0.87	5.55	0.27	14.8	97.05	
GTERM juveniles						
Average similarity: 26.63						
<i>Erythrope elegans</i>	1.84	19.23	1.01	72.19	72.19	
<i>P. bispinosus</i>	0.71	2.65	0.3	9.95	82.15	
<i>A. agilis</i>	0.64	2.26	0.3	8.5	90.65	
GTERM adults						
Average similarity: 40.11						
<i>L. suerii</i>	1.92	32.98	0.76	82.22	82.22	
<i>A. glaber</i>	0.91	6.67	0.29	16.62	98.84	
(B)						
GCAST and GTERM						
Average dissimilarity = 75.83						
Taxon	GCAST Av.Abund	GTERM Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>G. rhomboides</i>	2.14	0.23	28.39	1.4	37.4	37.4
<i>L. suerii</i>	1.07	1.92	23.05	1.1	30.4	67.8
<i>A. glaber</i>	0.45	0.91	14.46	0.73	19.1	86.9
<i>Processa</i> sp.	0	0.22	2.71	0.27	3.6	90.5
GCAST and GSANT						
Average dissimilarity = 80.42						
Taxon	GCAST	GSANT				
<i>G. rhomboides</i>	2.14	0	29.05	1.49	36.1	36.1
<i>L. suerii</i>	1.07	1.94	22.72	1.09	28.3	64.4
<i>A. glaber</i>	0.45	0.87	14.14	0.73	17.6	82.0
Unidentified fish	0.11	0.49	7.37	0.48	9.2	91.1
GSANT and GTERM						
Average dissimilarity = 59.60						
Taxon	GSANT	GTERM				
<i>L. suerii</i>	1.94	1.92	21.95	0.96	36.8	36.8
<i>A. glaber</i>	0.87	0.91	18.46	0.84	31.0	67.8
Unidentified fish	0.49	0	6.94	0.42	11.6	79.5
<i>G. rhomboides</i>	0	0.23	3.51	0.28	5.9	85.3
<i>Processa</i> sp.	0	0.22	2.87	0.27	4.8	90.1

scaldfish, have approximately the same $\delta^{15}\text{N}$ value of *A. glaber* and *G. rhomboides* consumed by adults (mean $\delta^{15}\text{N}$ of peracarids: 5.4‰, mean $\delta^{15}\text{N}$ of *A. glaber* and *G. rhomboides*: 5.2‰; Fanelli, 2007). Similarly, the carnivores *P. bispinosus*

and *L. suerii* (Costa, 1988; Cartes *et al.*, 2002), consumed by juveniles and adults respectively, exhibited mean $\delta^{15}\text{N}$ values of 7.13‰ and 8.21‰ (Fanelli, 2007), hence $\delta^{15}\text{N}$ values of scaldfish did not rise with size.

The value of $\delta^{13}\text{C}$ in scaldfish increased with size and appeared more enriched in adults. $\delta^{13}\text{C}$ is a good indicator of the source of organic matter consumed in the food chain because plants and invertebrates associated with benthic food chains are relatively enriched with $\delta^{13}\text{C}$ and easily distinguished from plankton, which is depleted (Pinnegar & Polunin, 1999). Suprabenthic resources exploited by juvenile scaldfish were mainly composed of mysids like *Leptomysis gracilis*, *Erythrops elegans* and *Acanthomysis longicorne* which consume primarily phytoplankton and zooplankton (Mauchline, 1980; Cartes & Sorbe, 1998), thus juveniles exhibited more depleted $\delta^{13}\text{C}$ values. On the contrary the preferred prey of adult scaldfish were the deposit feeder *A. glaber* (Cartes *et al.*, 2002), the infaunal feeder *G. rhomboides* (Cartes *et al.*, 2002) and the benthic carnivore *L. suerii* (Costa, 1988), linked to a detritus-based food web enriched in $\delta^{13}\text{C}$. The above recognized low power of our analysis is particularly important for carbon, where overall only nine fish were analysed. In this case a type II error is likely to occur in the comparison among areas.

Our results have shown that the diet of a benthic fish such as the scaldfish, at least the adults, can be used as an indicator of trawling disturbance, as observed for other species with similar benthic feeding behaviour (e.g. *Pagellus erythrinus*; Fanelli 2007). In contrast, it remains to be established whether trophic levels are good indicators of fishing disturbance, since no change was observed in the trophic structure of benthic communities that have undergone a re-arrangement process as a response to the impact of trawling gear.

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Annex 1. List of prey items identified in the stomach contents of scaldfish. GCAST, Gulf of Castellammare; GTERM, Gulf of Termini Imerese; GSANT, Gulf of Sant'Agata. For the meaning of %W, %N, %F and %IRI see text. Values less than 0.1 are indicated with asterisks.

Taxon	GCAST				GTERM				GSANT			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
CNIDARIA												
<i>C. appendiculata</i>	–	–	–	–	–	–	–	–	0.1	1.1	4.0	0.1
POLYCHAETA												
Ampharetidae	0.1	1.1	2.7	0.1	–	–	–	–	–	–	–	–
CRUSTACEA												
Ostracoda	*	0.5	1.4	*	–	–	–	–	–	–	–	–
Copepoda	*	1.1	2.7	*	*	1.0	3.9	0.1	*	1.1	4.0	0.1
Decapoda												
Natantia												
<i>A. glaber</i>	3.8	3.2	8.1	0.9	15.5	4.4	15.7	5.8	16.7	8.9	28.0	14.4
<i>P. bispinosus</i>	0.7	4.3	10.8	0.9	0.1	4.4	13.7	1.1	0.5	13.3	20.0	5.6
Unidentified Crangonidae	*	0.5	1.4	*	0.1	4.4	9.8	0.8	1.0	34.4	24.0	17.1
<i>Processa</i> sp.	0.4	1.1	2.7	0.1	2.4	1.5	5.9	0.4	0.2	3.3	12.0	0.9
Natantia post-larvae	*	1.6	2.7	0.1	*	*	*	*	0.5	1.1	4.0	0.1
Reptantia												
<i>U. typica</i>	3.2	0.5	1.4	0.1	–	–	–	–	–	–	–	–
Brachyura												
<i>G. rhomboides</i>	28.0	33.0	58.1	57.3	1.0	2.4	7.8	0.5	1.8	5.6	20.0	3.0
Unidentified Brachyura	–	–	–	–	*	0.5	2.0	*	0.1	4.4	12.0	1.1
Anomura												
<i>Anapagurus</i> sp. juv.	0.1	1.1	1.4	*	–	–	–	–	0.5	1.1	4.0	0.1
<i>Galathea</i> sp. juv.	*	1.6	4.1	0.1	–	–	–	–	*	1.1	4.0	0.1
Unidentified Decapoda	0.1	0.5	1.4	*	*	0.5	2.0	*	–	–	–	–
Mysidacea												
<i>A. agilis</i>	–	–	–	–	0.1	7.3	13.7	1.9	–	–	–	–
<i>A. longicorne</i>	0.1	1.1	1.4	*	*	*	*	*	–	–	–	–
<i>E. elegans</i>	0.1	14.4	12.2	2.8	0.4	44.7	35.3	29.5	0.1	5.6	4.0	0.5
<i>L. mediterranea</i>	0.1	4.3	6.8	0.5	0.1	6.8	7.8	1.0	0.4	1.1	4.0	0.1
<i>L. typicus</i>	–	–	–	–	–	–	–	–	0.4	1.1	4.0	0.1
Unidentified Mysidacea	–	–	–	–	*	2.4	3.9	0.2	0.1	1.1	4.0	0.1
Cumacea												
<i>D. serrata</i>	*	0.5	1.4	*	–	–	–	–	–	–	–	–
Isopoda												
Unidentified Isopoda	0.4	1.1	2.7	0.1	–	–	–	–	–	–	–	–

Continued

Annex 1. Continued

Taxon	GCAST				GTERM				GSANT			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
Amphipoda												
<i>A. typica</i>	*	0.5	1.4	*	–	–	–	–	–	–	–	–
<i>Ampelisca</i> sp.	0.2	5.3	12.2	1.1	0.0	1.5	5.9	0.2	–	–	–	–
<i>A. vexatrix</i>	*	2.1	2.7	0.1	–	–	–	–	–	–	–	–
<i>Corophium</i> sp.	*	0.5	1.4	*	–	–	–	–	–	–	–	–
<i>L. mariae</i>	*	1.1	2.7	*								
<i>M. griseus</i>	–	–	–	–	*	1.0	3.9	0.1	–	–	–	–
<i>W. rectirostris</i>	*	2.1	2.7	0.1	*	–	–	–	–	–	–	–
Unidentified Gammaridea	0.1	0.5	1.4	*	*	1.9	7.8	0.3	–	–	–	–
OSTHEYCHTHYES												
<i>L. suerii</i>	61.4	12.2	29.7	35.4	77.5	10.2	35.3	57.3	73.6	10.0	32.0	53.8
Unidentified Gobidae	0.7	0.5	1.4	*	2.5	1.9	7.8	0.6	–	–	–	–
Fish larvae	0.1	1.1	2.7	0.1	–	–	–	–	–	–	–	–
Unidentified fish	0.1	2.7	5.5	0.1	0.2	3.4	7.8	0.3	4.1	5.5	20.0	2.8
Others												
Vegetal remains	*	0.5	1.4	*	–	–	–	–	–	–	–	–