Resource partitioning among early colonizing *Siganus luridus* and native herbivorous fish in the Mediterranean: an integrated study based on gut-content analysis and stable isotope signatures

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The present study attempts to give timely information on the resource partitioning between the lessepsian migrant *Siganus luridus* and two ecological native analogues, *Sarpa salpa* and *Sparisoma cretense*. Sampling was carried out in concomitance with the first record of *Siganus luridus* in Linosa and fish were caught simultaneously, allowing direct comparison of diets. Gut-contents analyses provided a snapshot of the feeding habits of the three species: *Siganus luridus* fed on 34 taxa of benthic algae (mostly represented by *Dictyota dichotoma*) and a total of 27 taxa was identified in the stomach of *Sarpa salpa*, with the predominance of *Sargassum vulgare*. For the first time, a detailed picture of *Sparisoma cretense* diet was given (N=22 taxa of identified algae) albeit, due to the high percentage of digested food, this species was excluded from gut-content comparisons. A certain resource partitioning between *Siganus luridus* and *Sarpa salpa* was resolved on the basis of trophic indices and multivariate analyses, these latter also highlighting a more dispersed diet for *Siganus luridus* with respect to *Sarpa salpa*. Isotopic signatures were important towards defining the trophic level of the three species and particularly of *Sparisoma cretense*, whose gut-contents analysis was only partially informative. The values of $\delta^{15}\text{N}$ confirmed a strictly vegetal diet for *Siganus luridus* and *Sparisoma cretense* while *Sarpa salpa* was significantly more enriched. According to $\delta^{13}\text{C}$, observed values matched the predicted ones for *Siganus luridus* and *Sarpa salpa* while both species presented less enriched $\delta^{15}\text{N}$ values than expected.

INTRODUCTION

The ongoing spread of lessepsian species in the Mediterranean, i.e. migrants from the Red Sea across the Suez Canal, is a matter of growing concern, with new evidence indicating their western expansion across the central sectors of the basin (Azzurro, 2006). Entering into the Mediterranean through the Suez Canal, these invaders have generated remarkable changes in the native communities, especially in the eastern sectors of the basin (Golani, 1998), so that specific studies are highly required. Actually, one of the major constraints in addressing ecological questions is the lack of information from the period prior to invasion (Golani, 1998) or even during the early stages of invasion, when the interaction between exotic and native species are supposed to be negligible. Such information would provide critical power towards assessing the importance of invading events and timely insight as to how to manage the same, but often scientific actions are not undertaken until impact becomes detectable.

Recently, the finding of a newly settled population of the dusky spinefoot *Siganus luridus* (Rüppel, 1829) (rabbitfish, Siganidae) at the Italian island of Linosa (Azzurro & Andaloro, 2004) provided a valuable opportunity to study the lessepsian phenomenon (Azzurro, 2006). *Siganus luridus* belongs to the family of rabbitfish (Siganidae), a group of

herbivorous species inhabiting the Indo-West Pacific). Together with its congeneric *Siganus rivulatus* Forsskal, 1775, *S. luridus* has a story of successful invasion in the Mediterranean and represents one of the prevailing invaders in the eastern basin (Golani, 1998). In the last two decades, the feeding habits of the dusky spinefoot have been widely studied in the Levantine Sea (e.g. Bariche, 2006 and references therein included) but no information is available from the central Mediterranean.

According to the available literature, only two native fish species are assumed to have feeding analogies with siganids, e.g. *Sarpa salpa* (Linnaeus, 1758) and *Sparisoma cretense* (Linnaeus, 1758). Some authors (Bariche et al., 2004) suspected a competitive superiority of Siganidae (particularly *Siganus rivulatus*) vs *Sarpa salpa* and others (e.g. Lundberg et al., 2004) claimed that rabbitfish out-competed it but, surprisingly, comparative studies are missing. Moreover, Bariche et al. (2004) hypothesized that the maintenance of the *Sparisoma cretense* population in the Lebanese coasts may be due to unexamined ecological differences with rabbitfishes, such as in feeding behaviour. In fact, very little information is available on the diet of *S. cretense* (Petrakis & Papaconstantinou, 1990).

The recent incoming of *Siganus luridus* in the island of Linosa adds a new potential link to the use of local trophic resources and stimulates research on the nature of

Figure 1. Map showing the study area (Linosa Island) and the sites of fish collection (black rectangles).

competitive interactions between this lessepsian migrant and its native ecological analogues (*sensu* Herbold & Moyle, 1986). Gut-contents analysis is a traditional tool with which to address this objective but often temporal and spatial variation is ignored and caution is required when drawing conclusions (Pinnegar & Polunin, 2000). On the other hand, stable isotope analyses (mainly the ^{13}C and ^{15}N) yield information on what is actually assimilated and provide relatively long-term and time-integrated measurements of feeding preferences, because they are less subject to temporal bias (Pinnegar & Polunin, 2000; Vizzini et al., 2002). Animals typically possess more of the heavier isotopes ^{13}C or ^{15}N than their food and an enrichment occurs with each trophic level in food webs/chains, providing the basis for analysis of complex food chain interrelationships, trophic pathways and food webs.

The goal of the present study was to provide timely information about the diet of *Siganus luridus* in its newly colonized habitat and the native herbivorous fish *Sarpa salpa* and *Sparisoma cretense*. To address this aim we attempted to: (1) characterize the feeding habits of the three sympatric species in the study area; (2) compare their diets; and (3) determine the ^{13}C and ^{15}N composition of these fish and their main food items.

MATERIALS AND METHODS

Study site

Linosa ($35^{\circ}51'\text{N}$ $12^{\circ}51'\text{E}$) (Figure 1) is a small volcanic island (5.4 km^2 wide) originating in the Quaternary period. It belongs to the Pelagean archipelago, located in the middle of the Sicily Strait at the western limit of the so called 'lessepsian province'. The coastline has a limited extension (11 km) and the surrounding bottoms reach rapidly depths of 300 m. Due to a reduced anthropogenic pressure, the island

is largely undisturbed and recently (in October 2002) it was declared a Marine Protected Area, even though effective protection measures were still not in force during the study period. Along the Linosa infra-littoral rocky bottoms, *Sarpa salpa* and *Sparisoma cretense* occur with high abundances, representing two of the prevailing fish species, whilst the recently settled *Siganus luridus*, scattered in small groups of individuals, is rather uncommon in the island (Azzurro et al., 2007).

Sample collection and processing

Sarpa salpa ($N=18$), *Sparisoma cretense* ($N=36$) and early settled *Siganus luridus* ($N=40$) were caught through July–August 2003 during daylight hours (0800–1800 h). Total length (TL) was 13.5–27.5 cm, 12.8–25.3 cm and 13.6–24.5 cm, respectively. All fish were caught at two different locations along the western and eastern infra-littoral rocky bottoms (depth range 1–8 m) of Linosa (Figure 1). The *S. luridus* specimens represented the first record of rabbitfish in the Pelagean archipelago (Azzurro & Andaloro, 2004). The sampling technique, by spear-fishing, allowed for the capture of fish simultaneously from the same sites and prevented the rapid digestion of food. Immediately after capture, length and weight were determined and digestive tracts were extracted and preserved in 6% buffered formalin.

For the stable isotope analyses, six specimens of each fish species *Siganus luridus*, *Sparisoma cretense* and *Sarpa salpa* were immediately frozen at -20°C . Mean size of samples was 15.8 ± 0.9 cm TL, 18.3 ± 2.3 cm TL, 17.3 ± 2.6 cm TL for *Siganus luridus*, *Sparisoma cretense* and *Sarpa salpa*, respectively. At the same time and in the same sites of study, dominant macrophytae were collected and immediately frozen at -20°C for isotope analyses.

Gut-content analysis

Digestive tracts were opened in the laboratory, and contents were blotted dry with absorbent paper. Total wet weight was recorded to the nearest 0.001g and an index of stomach fullness was calculated as the ratio between the total weight of stomach content and total fish weight. Subsequently all food items were identified to the lowest possible taxon under a dissecting microscope. Each taxon was then separated, blotted dry and weighted. In order to measure sample size sufficiency, the cumulative number of food items against the cumulative number of gut analysed was plotted (Ferry & Cailliet, 1996). The logistic and linear regressions were calculated and the respective goodness of fit coefficients R^2 were compared. The sample size was considered sufficient when R^2 for the logistic curve was higher than the R^2 for the linear relation (Castricola et al., 2005). The contribution of each food item to the diet was expressed as percentage frequency of occurrence (%F) and percentage by weight (%W). The most important food items were determined using the index of relative importance (IRI) modified according to the requirements of the present study as $\text{IRI} = \%W * \%F$. This index was then expressed on a per cent basis as: $\% \text{IRI} = 100 * \text{IRI} / \sum \text{IRI}_i$.

The Schoener index of overlap was used to assess the diet similarity of the two species. The mean per cent wet weight was calculated for each food item. This index was then

Table 1. Results of the sample size sufficiency analysis for the total number of *Siganus luridus*, *Sarpa salpa* and *Sparisoma cretense* examined guts.

	Logistic regression			Linear regression		
	R ²	F	P	R ²	F	P
<i>Siganus luridus</i>	0.892	F _{1,36} =296.12	<0.001	0.701	F _{1,36} =84.51	<0.001
<i>Sarpa salpa</i>	0.942	F _{1,16} =259.06	<0.001	0.874	F _{1,16} =111.25	<0.001
<i>Sparisoma cretense</i>	0.690	F _{1,34} =76.15	<0.001	0.548	F _{1,34} =41.21	<0.001

calculated as follows:

$$\alpha = 1 - 0.5 \left(\sum |p_{ij} - p_{ik}| \right) \quad (1)$$

where p_{ij} is the proportion of food item used by species j , and p_{ik} is the proportion of food item i used by species k . α varies between 0 and 1.0, representing no to complete food overlap, respectively. The degree of overlap between two species diets is considered significant when the index is greater than 0.60.

The diet breadth was calculated using Levin's standardized index:

$$B_i = \frac{1}{n-1} \left(\frac{1}{\sum p_{xi}^2} - 1 \right) \quad (2)$$

where B_i = Levin's standardized index for predator i , p_{xi} = proportion of diet of predator i that is made up of prey x , n = number of prey categories. This index ranges from 0 to 1; low values indicate diets dominated by few prey items (specialist predators); higher values indicate generalist diets.

Isotope analyses

Isotopic analyses were performed on muscle tissue samples (portions of white muscle close to dorsal fin) of the three fish species and nine macrophytae (chosen on the basis of their relevance in gut-content analysis). According to each fish species, we analysed six individuals of the same size (ANOVA $P > 0.05$). For each species of macrophytae, we analysed two samples taken from a composite sample of specimens.

Samples of both fish and macrophytae were dried to constant weight at 60 °C in a fan-assisted oven before being ground to a fine powder with a mortar and pestle.

Using continuous flow, the samples were introduced into a Finnigan Delta plus XP isotope ratio mass spectrometer for isotopic analysis. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 10^3 \quad (3)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

For each species, the nitrogen and carbon isotope signatures predicted from their diets were calculated using a weighted-average equation of the following form (shown for $\delta^{15}\text{N}$):

$$\sum_{i=1}^k \delta^{15}\text{N} \frac{b_i}{b_t} + f \quad \text{and} \quad b_t = \sum_{i=1}^k b_i \quad (4)$$

where $\delta^{15}\text{N}$ is the nitrogen isotope signature of the i th prey item, b_i/b_t is the proportion of diet biomass observed for that prey item, and f is the trophic fractionation coefficient which was chosen of 3.4‰ for $\delta^{15}\text{N}$ and 1‰ for $\delta^{13}\text{C}$.

Statistical analyses

Multivariate analyses of gut-contents data were performed only for *Siganus luridus* and *Sarpa salpa* while *Sparisoma cretense* was excluded, due to the high amount (92–95%) of digested unidentifiable material in the gut contents. Firstly, analysis of similarities (ANOSIM) was done in order to test for possible differences between the diet of each species sample belonging to the two sampling locations. Principal component analysis (PCO) was performed on Bray–Curtis distance based on 4th root transformed and standardized biomass data. Based on the null-hypothesis that no differences exist among species diet, a permutational multivariate analysis of variance (PERMANOVA) design was created on one factor to detect significant differences between *Siganus luridus* and *Sarpa salpa* diets. A PERMDISP test (Anderson, 2006), a distance-based test for homogeneity of multivariate dispersions, was then performed in order to compare the multivariate dispersions among species. The similarity percentage analysis (SIMPER) procedure was used to identify those species that characterized each diet and those responsible for discriminating between diets. Uncommon species that were seldom present or exhibited low abundance levels were not included in the data matrix. Multivariate analyses were performed by means of the software PRIMER 6 plus PERMANOVA (Clarke & Warwick, 1994; Anderson, 2006).

One-way ANOVA was used to compare isotopic signatures within all fish species. The Tukey HSD test was applied a posteriori to locate the source of any difference.

RESULTS

Gut-contents analysis

All of *Siganus luridus*, *Sarpa salpa* and *Sparisoma cretense* digestive tracts examined were full and the index of fullness exhibited high values (8.26 ± 2.18 , 7.93 ± 1.87 , 6.83 ± 3.77 , respectively). The cumulative prey types curve resulted as fitting better with a logistic curve than with a linear relation for *Siganus luridus*, *Sarpa salpa* and *Sparisoma cretense* when 100% of the guts examined was considered; then sample size was considered sufficient to describe the diet of each species (Table 1).

The analysis of *Siganus luridus* gut contents led to the identification of 34 taxa of benthic algae and one sea grass

Table 2. Overall food items of *Siganus luridus*, *Sarpa salpa* and *Sparisoma cretense* in Linosa Island.

Food items	<i>Siganus luridus</i>			<i>Sarpa salpa</i>			<i>Sparisoma cretense</i>		
	%F	%W	%IRI	%F	%W	%IRI	%F	%W	%IRI
Chlorophyta	63.16	3.96	2.58	38.89	3.23	1.63	19.44	0.13	0.38
<i>Anadyomene stellata</i>	28.95	0.84	0.32	5.56	0.01	<0.01	2.78	0.02	<0.01
<i>Bryopsis</i> sp.	7.89	0.01	<0.01	5.56	<0.01	<0.01	—	—	—
<i>Caulerpa racemosa</i>	28.95	1.55	0.58	—	—	—	—	—	—
<i>Cladophora</i> sp.	34.21	1.50	0.67	27.78	0.07	0.03	8.33	0.05	<0.01
<i>Dasycladus vermicularis</i>	7.89	0.02	<0.01	—	—	—	—	—	—
<i>Microdictyon tenuius</i>	2.63	0.05	<0.01	—	—	—	—	—	—
<i>Ulva</i> sp.	—	—	—	5.56	3.15	0.28	—	—	—
<i>Flabellia petiolata</i>	—	—	—	—	—	—	2.78	0.02	<0.01
<i>Valonia</i> sp.	—	—	—	—	—	—	5.56	0.05	<0.01
Heterokontophyta	100.00	89.20	92.26	100.00	66.59	86.53	100	3.69	53.96
<i>Asperococcus</i> sp.	—	—	—	5.56	0.26	0.02	—	—	—
<i>Cystoseira humilis</i>	13.16	0.19	0.03	55.56	5.42	4.85	5.56	0.12	0.01
<i>Cystoseira</i> sp.	26.32	12.43	4.25	50.00	0.66	0.53	19.44	0.28	0.06
<i>Dictyopteris polypodioides</i>	28.95	0.07	0.03	66.67	3.14	3.37	—	—	—
<i>Dictyota dichotoma</i>	34.21	2.41	1.07	16.67	0.43	0.11	—	—	—
<i>Dictyota dichotoma</i> var. <i>intricata</i>	47.37	1.86	1.15	5.56	1.05	0.09	—	—	—
<i>Dictyota</i> sp.	97.37	64.31	81.47	77.78	16.96	21.23	16.67	0.14	0.02
<i>Halopteris filicina</i>	97.36	4.16	5.27	100	3.76	6.05	75	1.34	0.55
<i>Lobophora variegata</i>	15.79	0.29	0.06	5.56	<0.01	<0.01	—	—	—
<i>Padina pavonica</i>	42.11	0.78	0.42	83.33	3.37	4.52	—	—	—
<i>Sargassum</i> sp.	13.16	0.07	0.01	5.56	5.30	0.47	—	—	—
<i>Sargassum vulgare</i>	36.84	1.31	0.63	83.33	25.02	33.57	2.78	0.05	<0.01
<i>Sphacelaria cirrosa</i>	10.53	0.05	0.01	—	—	—	11.11	0.16	0.02
<i>Sphacelaria</i> sp.	55.26	0.71	0.51	61.11	0.85	0.84	66.67	0.95	0.66
<i>Sphacelaria tribuloides</i>	7.89	0.02	<0.01	11.11	0.09	0.02	—	—	—
<i>Stypocaulon scoparium</i>	44.74	0.54	0.31	33.33	0.28	0.15	55.56	0.65	0.38
Rhodophyta	94.74	5.15	5.05	77.78	5.93	5.99	91.67	2.06	27.58
<i>Acrosorium ciliolatum</i>	26.31	1.88	0.64	11.11	0.11	0.02	—	—	—
<i>Alsidium</i> sp.	21.05	0.07	0.02	5.56	0.03	<0.01	—	—	—
<i>Botryocladia</i> sp.	39.47	0.27	0.14	16.67	0.03	0.01	8.33	0.06	<0.01
<i>Corallophila cinnabarina</i>	21.05	0.18	0.05	—	—	—	—	—	—
<i>Dasya</i> sp.	2.63	0.05	<0.01	—	—	—	—	—	—
<i>Eupogodon planus</i>	13.16	0.07	0.01	5.56	<0.01	<0.01	—	—	—
<i>Ceramium</i> sp.	—	—	—	—	—	—	5.56	0.02	<0.01
<i>Gelidium</i> sp.	13.16	0.07	0.01	—	—	—	25.00	0.12	0.03
<i>Hypnea</i> sp.	—	—	—	16.67	3.11	0.84	—	—	—
<i>Hypoglossum hypoglossoides</i>	10.53	0.24	0.03	—	—	—	2.78	0.01	<0.01
<i>Halitilon virgatum</i>	—	—	—	—	—	—	47.22	0.81	0.40
<i>Jania rubens</i>	—	—	—	—	—	—	27.78	0.58	0.17
<i>Laurencia</i> sp.	10.53	0.01	<0.01	33.33	0.78	0.42	13.89	0.04	0.01
<i>Monosporus pedicellatus</i>	2.63	0.04	<0.01	—	—	—	—	—	—
<i>Nitophyllum</i> sp.	—	—	—	5.56	1.03	0.09	—	—	—
<i>Peyssonnelia</i> sp.	60.53	2.23	1.76	55.56	0.84	0.75	13.89	0.16	0.02
<i>Polysiphonia</i> sp.	5.26	0.04	<0.01	—	—	—	38.89	0.19	0.08
<i>Polysiphonia opaca</i>	2.63	<0.01	<0.01	—	—	—	2.78	0.08	<0.01
Spermatophyta	57.89	0.18	0.11	38.89	11.57	5.85	80.56	1.54	18.08
<i>Posidonia oceanica</i>	57.89	0.18	0.14	38.89	11.57	7.25	80.56	1.54	1.29
Other items									
Unidentified algae	34.21	0.19	0.08	16.67	1.07	0.29	2.78	0.02	<0.01
Digested materials	23.68	0.81	0.15	88.89	11.47	14.14	100.00	92.51	96.30
Inorganic materials	21.05	0.39	0.11	11.11	0.09	0.02	2.78	0.05	<0.01
Amphipods	21.05	0.06	0.02	22.22	0.02	0.01	—	—	—
Foraminiferans	50.00	0.07	0.05	66.67	0.03	0.03	2.78	<0.01	<0.01

%F, occurrence frequency of prey; %W, percentage by weight; %IRI, index of relative importance.

Figure 2. Principal component analysis ordination plot of 4th root transformed gut-contents data for *Siganus luridus* (black triangles) and *Sarpa salpa* (grey triangles) specimens. Overlay variables were the most typifying species as evidenced by SIMPER analysis.

(*Posidonia oceanica*) (Table 2). Brown algae were, according to all indices, the most important food item (%F=100, %W=89.20, %IRI=92.26). Among them, *Dictyota* sp. was the dominant taxon according to all indices. *Cystoseira* sp. was the second most represented food item with reference to %W and %IRI. According to %W, other important species

were *Halopteris filicina*, *Dictyota dichotoma* and *Peyssonellia* sp. Several species were recorded frequently but were of minor importance according to %W and %IRI. The introduced green alga *Caulerpa racemosa* was exclusively present. Foraminiferans were found frequently even if their contribution in terms of biomass was low. Amphipods were recorded occasionally. *Siganus luridus* showed very low values of niche width ($B=0.027$).

In the gut-contents of *Sarpa salpa*, 27 taxa of benthic algae plus *P. oceanica* were identified (Table 2). Overall, brown algae constituted the dominant group according to %F (100%), %W (66.59%) and %IRI (86.53%). Among them, the most represented species was *Sargassum vulgare* followed by *Dictyota* sp. *P. oceanica*, even if not very frequent, showed a good contribution with reference to %W and %IRI. Other relevant species were *Cystoseira* sp., *Padina pavonica*, *Dictyopteris polypodioides* and *H. filicina*. *Sarpa salpa* showed low values of niche width ($B=0.212$).

The diet of *Sparisoma cretense* was characterized by the substantial incidence of highly digested materials (%W=92.51, %IRI=96.30) while, among the remaining percentage of identifiable food items, 22 taxa of algae and *Posidonia oceanica*, were identified (Table 2). According to %IRI, brown algae were the predominant food item, followed by red algae and *P. oceanica*. The latter was the most frequent and important species. Other food items such as *Sphacelaria* sp., *Stypocaulon scoparium*, *Halitilon virgatum* and *Halopteris filicina* were frequent but showed low values in terms of %W and %IRI.

Since no differences among samples from different locations were found for both *Siganus luridus* and *Sarpa salpa* (ANOSIM $R=0.003$, $R=0.004$ respectively and $P>0.05$), the analyses were performed on the whole sample, excluding the factor 'location'. Schoener's index was 0.262, indicating a very low overlap between *Siganus luridus* and *Sarpa salpa* diets.

Figure 3. Plot of the $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ values (mean $\pm$ SD) for food items and fish. The empty triangle and square represent predicted values by means of eqn 4. Svul, *Sargassum vulgare*; Aste, *Anadyomene stellata*; Chum, *Cystoseira humilis*; Psqu, *Peyssonellia squamaria*; Halo, *Halopteris* sp.; Ddic, *Dictyota dichotoma*; Ppav, *Padina pavonica*; Crac, *Caulerpa racemosa*; Poce, *Posidonia oceanica*.

The PCO analysis (Figure 2) highlighted a clear separation among species diets with the first two axes explaining 45.1% of total variance. The PERMANOVA provided evidence for statistically significant differences in species diets ($F_{1,55}=10.58$; $P=0.001$). The PERMDISP procedure also showed a significantly higher dispersion ($F=6.1$; $P=0.03$) for *Siganus luridus* with respect to *Sarpa salpa* (34.7 and 27.4 respectively).

The SIMPER analysis showed a higher value of intra-specific similarity for *Sarpa salpa* (59.2%) than for *Siganus luridus* (50.6%). *Dictyota* spp. and *Halopteris* spp. accounted for 47.7% and 18.9% of the average similarity in *S. luridus*, while *Dictyota* spp. (22.4%), *Sargassum* spp. (20.9%), *Cystoseira* spp. (14.6%) and *Halopteris* spp. (14.5%) were the most typifying species in *Sarpa salpa*. Finally, *Dictyota* spp., *Sargassum* spp. and *Cystoseira* spp. were the food items that contributed the most to the average dissimilarity between these two species.

Isotope analysis

The $\delta^{15}\text{N}$ isotopic value of *Siganus luridus* was $5.9\text{‰} \pm 1.1$, of *Sarpa salpa* $7.2\text{‰} \pm 1.5$ and of *Sparisoma cretense* $5.5\text{‰} \pm 0.2$. ANOVA tests showed significant differences both in $\delta^{15}\text{N}$ ($F_{1,15}=3.8$; $P<0.05$) and $\delta^{13}\text{C}$ ($F_{1,15}=4.5$; $P<0.05$) values among fish. According to the *post-hoc* Tukey HSD test, the $\delta^{15}\text{N}$ values were significantly higher in *Sarpa salpa* than *Sparisoma cretense* and the $\delta^{13}\text{C}$ values more depleted in *S. cretense* ($-19.0\text{‰} \pm 0.5$) than in *Sarpa salpa* ($-17.2\text{‰} \pm 1.2$) ($P<0.05$).

There was a very wide variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the studied food items, with *Padina pavonica* being particularly enriched in $\delta^{15}\text{N}$ (-9.1‰) and *Pyssonnellia squamaria* particularly depleted (-24.7‰). According to $\delta^{15}\text{N}$, the highest values were recorded for *Posidonia oceanica*, *Cystoseira humilis* and *Sargassum vulgare*, while the lowest were recorded for *D. dichotoma* and *Anadyomene stellata* (Figure 3).

The $\delta^{15}\text{N}$ values of *Siganus luridus* and *Sarpa salpa* were less enriched than expected on the basis of consumed food items (Figure 3). In fact, predicted values (through eqn 4) for $\delta^{15}\text{N}$ signatures were respectively 1.4‰ and 1.3‰ more enriched than the mean observed values. Conversely, the predicted $\delta^{13}\text{C}$ signatures matched with the observed ones.

DISCUSSION

Our study provides timely information on the feeding habits of Linosa herbivorous fish species, at a stage where slightly invasive versus native species interaction is supposed to act. Results verify the capacity of the lessepsian rabbitfish *Siganus luridus* to adapt to local trophic resources and confirmed the success of these first colonizers. Gut-content analysis showed clear differences between the feeding preferences of *S. luridus* and *Sarpa salpa* and a good resource partitioning among them. Clearly, these results are limited to the period of study (summer) and seasonal variations have been not taken into account. Moreover, we do not know whether changes in the herbivorous abundance (a possible expansion of rabbitfish) and the subsequent increase of feeding pressure may lead to a lowering of the algal resource. In any case, a weakening of dietary niche-partitioning is expected only if the preferred algae should turn into a limited resource. Given the recent spreading of *Siganus luridus* across the central Mediterranean, studies on early settled populations assume a certain

importance. However, up to date, their limited abundances in the newly colonized areas do not support extensive temporal and spatial samplings which would allow to better define their trophic niche.

Diet of single species

Plotting the number of food items versus the number of analysed gut-contents, data resulted which were representative of the full array of preys. Notwithstanding the small number of samples, this is not surprising. In fact, the study of herbivore species diet has some advantage presenting much less intra-specific variability, with respect to carnivores or omnivores (Stergiou & Karpouzi, 2002).

The stomach fullness was high in *S. luridus*, even if these specimens were captured during the reproductive period (Azzurro, 2006), when the feeding should be at its minimum (Stergiou, 1988). Actually, Stergiou (1988) provided very low values of the fullness index in comparison with our study (values around 1 against 8.26 ± 2.18) which were possibly due to the different sampling methods. With 34 identified taxa of benthic algae and one sea grass, the qualitative screening of *S. luridus* diet resulted as being considerably broad compared with previous studies (see Bariche, 2006) testifying to the wideness of the *S. luridus* trophic spectrum in its newly colonized area, and a wide availability of algal resources. According to Serio et al. (2006), 233 species characterize the phytobenthos of Linosa Island, accounting for numerous tropical affinity taxa consumed by *S. luridus*. Particularly abundant and rich species are brown algae, which are the favourite food items for *S. luridus*, and this abundance could have facilitated the establishment of the dusky rabbitfish across the central Mediterranean (Lundberg & Golani, 1995).

Gut-content analysis showed some similarities between the early settled *S. luridus* and the eastern Mediterranean populations (Bariche, 2006). In Linosa, *Dictyota* spp. were the main and favourite food items followed by *Cystoseira* sp. Instead *Sargassum* sp. and calcareous algae, which are widely distributed in this area (Serio et al., 2006) were of minor importance in *Siganus luridus* diet. Particularly noteworthy was *Caulerpa racemosa*, an introduced Red Sea macroalga which occurs along Linosa's shallower waters in the form of occasional patchy mats (E. Azzurro, personal observation). The spread of *C. racemosa* across the Mediterranean coastal habitats is a matter of growing concern and its significance for *S. luridus* diet represents an interesting example of the way in which some invading species interact with other not-native ones, possibly facilitating their establishment. Previously, *C. racemosa* was found for *S. luridus* caught in Cyprus (Lundberg et al., 1999) and some caution has been recommended regarding the consumption of siganids which ingested this toxic alga (Bariche, 2006).

For *Sarpa salpa*, the most represented food item was *Sargassum vulgare* followed by *Dictyota* sp. The sea grass *Posidonia oceanica* showed a good contribution in terms of %W and %IRI, in accordance with a variety of other studies (Havelange et al., 1997 and references therein included). The green alga *Ulva* sp. resulted as being exclusive for *Sarpa salpa* together with *Nitophyllum* sp. and *Hypnea* sp.

The feeding habits of *Sparisoma cretense* are mostly unknown (Petrakis & Papaconstantinou, 1990) and our results, with 23

identified taxa of macrophytae, represented the first detailed information about its diet. As well as in other scarids, the identification of food items in *S. cretense* was particularly difficult, the food being highly fragmented by the grinding of pharyngeal teeth and highly digested. Reasonably, the less leathery items (such as tender or filamentous algae) are digested with more facility and could be underestimated by the analysis. The opposite happens to tough macrophytae, such as calcareous algae or sea grass, which are more resistant to digestion.

Siganus luridus vs *Sarpa salpa*

The simultaneous capture of fish and their similar size-range enabled us to perform a direct comparison of diets (Lundberg & Golani, 1995), at least for the temporal and spatial range considered here. According to the statistical analyses, the two diets were characterized by a low overlap and a high degree of dissimilarity, with *Sargassum* spp., *Cystoseira* spp. and *Dictyota* spp. as the food items that mostly contributed to such differentiation. Notwithstanding the predominance of *Dictyota* spp. in the gut contents of *Siganus luridus*, the diet of this species seems to be more dispersed (many other rare food items are grazed) in comparison with *Sarpa salpa*. It has to be noticed that these habits are expected to vary on a seasonal basis as observed in the eastern Mediterranean, where the dusky spinefoot presented low values of trophic breadth in autumn (October), intermediate values in summer (July) and high values in spring (March–April) (Stergiou, 1988).

Assuming that the same algal resources were available for both species (that is reasonable considering that all the individuals were sampled in the same sites), results indicated that the early settled *Siganus luridus* and the native *Sarpa salpa* tend to choose their own diet with a good trophic partitioning. Considering the short period of time since colonization and the rarity of *Siganus luridus* in Linosa (Azzurro et al, 2007), the differences between the diets of these two species cannot be related to some ecological switch. This is instead a common and predictable consequence of competitive interactions within fish communities (Hanson & Leggett, 1986).

Elton (1958) suggested that 'empty niches', i.e. the existence of ecological space and the lack of competition within some communities, facilitated the establishment of invading species. In recent times, this hypothesis has received much attention, even if some controversy has grown (Herbold & Moyle, 1986). In many cases, the success of lessepsian species in the Mediterranean has been explained with this assumption (e.g. Golani, 1994) and siganids are thought to be one among the clearest examples of that (Lundberg & Golani, 1995). Actually other potentially important biological and ecological characters could lie behind the success of the dusky spinefoot and future studies should be aimed at better defining its ecological niche and making specific comparison with native species.

The isotopic signatures

Isotopic signatures were important towards defining the trophic level of the three species and particularly of *Sparisoma cretense*, whose gut-contents analysis was only partially

informative. Both isotopic signatures assigned *Siganus luridus*, *Sarpa salpa* and *Sparisoma cretense* to the same functional group of herbivores. This information, which is new for *Sparisoma cretense*, agrees with the review of Stergiou & Karpouzi (2002), which indicated the rabbitfish (*Siganus luridus* and *Siganus rivulatus*) and *Sarpa salpa* as the fish species with the lowest trophic level in the Mediterranean (TROPH=2.00, mean=2.00, TROPH=2.00–2.50, mean=2.13 respectively). However, the values of $\delta^{15}\text{N}$ suggested that the diet of *Siganus luridus* and *Sparisoma cretense* is based exclusively on vegetal items, while *Sarpa salpa* was significantly more enriched. The $\delta^{15}\text{N}$ values of *S. salpa* were similar to those observed by Pinnegar & Polunin (2000) while both Jennings et al. (1997) and Vizzini et al. (2002) observed $\delta^{15}\text{N}$ higher values. The highest trophic level of *Sarpa salpa* could be explained by the presence of food items with a higher $\delta^{15}\text{N}$ such as some invertebrates, as shown by Jennings et al. (1997) and Pinnegar & Polunin (2000). According to Verlaque (1985) another possible source of nitrogen may be the faeces of planktivorous fish such as *Chromis chromis* or *Spicara maena* consumed by young *Sarpa salpa*.

The values of $\delta^{15}\text{N}$ for several macrophytae, were higher in comparison with other Mediterranean areas (Pinnegar & Polunin, 2000) and comparable to eutrophic habitats (Vizzini et al., 2002). These differences may be due to physiological factors (enzymatic discrimination during photosynthesis) and to a different ability to use different nitrogen sources.

According to the food items, the substantial variation in the isotopic signature for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values combined with their high number gave little information on resource partitioning between the species. However, according to $\delta^{13}\text{C}$, observed values matched the predicted ones for *Siganus luridus* and *Sarpa salpa* while both species presented less enriched $\delta^{15}\text{N}$ values than expected (Figure 3). Assuming that food items were assimilated into tissue at the same proportions which were ingested, three explanations could be inferred to explain such bias: (1) the actual $\delta^{15}\text{N}$ fractionation value could be lower than 3.4‰; (2) *Siganus luridus* and *Sarpa salpa* feed on less enriched food items during the other seasons; and (3) the $\delta^{15}\text{N}$ values of macroalgae in summer are more enriched in comparison with the rest of the year. Future studies which consider the seasonal variability in the diet of these species and in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ food sources are required in order to clarify this aspect.

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