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# *Trophic niche variability of fish populations and communities as a response to ontogeny, habitat heterogeneity and restoration*

SETTORE SCIENTIFICO DISCIPLINARE DI AFFERENZA: BIO/07
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*"When we try to pick out anything by itself,*
*we find it hitched to everything else in the universe"*
John Muir

# Contents

# Introduction

Understanding natural variability of ecological processes is crucial for interpreting ecosystem functioning and achieving fair management practices (Landres, Morgan & Swanson 1999). Moreover, it allows to interpret response to disturbance, discerning the baseline of natural variability from altered processes (Fraterrigo & Rusak 2008; Young *et al.* 2015).

Natural variability in environmental systems arises from intrinsic dynamics of both abiotic and biotic processes (Fraterrigo & Rusak 2008). Primarily the variability of abiotic factors, considered either at the spatial scale (*e.g.* gradients, heterogeneity) or temporal one (*e.g.* seasonality) induces organisms to adapt to new conditions, which influence all the aspects of the life cycle of an organism and its behaviour, from growth (Russo, Costa & Cataudella 2007) to reproduction (Cavraro, Varin & Malavasi 2014), including feeding habits and resource use (*e.g.* Francis *et al.*, 2011), habitat choice and interactions with other species (*e.g.* Dantas *et al.* 2012; Munk *et al.* 2014). Secondly, biotic processes such as inter- and intra-specific competition, due to food and space limitation, mainly regulate the abundance of populations (*e.g.* Creese & Underwood 1982). The interplay of such dynamics, together with the intrinsic life history traits, contributes to structure the variability at different levels of organization, from individuals to populations to communities (Pantel *et al.* 2014).

Any order of perturbation, either negative (impact) or positive (*e.g.*, management or restoration practices), induces some change in the dynamics mentioned, resulting in structural variation of populations and communities involved, which can remain relatively stable within certain tolerance ranges, or can even advance or regress to one of the known states of an ecological succession (Fraterrigo & Rusak 2008).

In the last decades, increasing attention has been posed to trophic interactions as useful tools to investigate the degree of complexity and stability of a system (Belgrano *et al.* 2005; Dunne 2006), as they represent one of the key interaction typologies that can occur between species (Kéfi *et al.* 2012).

Since ecologists have always tried to identify the role of species in the context of their community, by summarizing all the aspects of their life history traits and habits (Cohen 1977), the concept of trophic niche has become a milestone in trophic structure studies (Elton 1927). The trophic niche represents one of the different aspects of the more comprehensive concept of ecological niche, which includes the overall requirements needed for a species to persist and reproduce in a given environment. This can be interpreted in terms of space/habitat occupied (Grinell's niche), food habits (Elton's niche) and can be imagined as an n-dimensional hypervolume where axes are given by environmental variables (Hutchinson's niche).

Particularly, the trophic niche was originally defined by Elton (1927) as the sum of all the trophic interactions occurring among species in a system. Niche parameters are influenced by any intra- and interspecific change of trophic interactions (Bearhop *et al.* 2004), hence the analysis of trophic structure can provide quantitatively information on the trophic variability of species, which can be assessed either at population or community levels.

Looking at the population level, much of the variability in feeding habits, resource use and habitat choice are linked to the trophic adaptability of the species, which, according to Gerking (1994), defines the ability of an organism to benefit from the most favourable food source available in a particular environment, at a particular time. The greater the ability for a species to shift to the use of different sources, the greater the possibility to persist in a given environment under changing conditions, by partitioning the sources available in case of competition with other species (Barros *et al.* 2013).

Trophic adaptability is typical for example of opportunistic fish species inhabiting ecosystems characterized by high natural fluctuations of environmental variables, such as transitional ecosystems (Vizzini & Mazzola 2002; Vinagre *et al.* 2012; Kopp *et al.* 2013). For this reason, such fish species can be considered as models to study the degree of variability of the trophic niche within the same system, between different systems or across time.

Estuaries, coastal lagoons and ponds are transitional ecosystems, ecotones located at the interface between the marine and the terrestrial domains. Due to their position, the mixing of continental waters with marine waters determines the instability of physico–chemical variables such as salinity, temperature, oxygen, whose fluctuation extent depends also on tide, evaporation, rainfalls and floodings, and results in a high environmental heterogeneity (Cognetti, Sarà & Magazzù 2008; Pérez-Ruzafa *et al.* 2010). On the other side, however, such environmental heterogeneity and variability results in a high habitat heterogeneity, that together with the high productivity characterizing these systems support a large diversity of biological communities that thrive and contribute to maintain high levels of biodiversity, not only within the same system, but also in the adjacent ones, marine and estuarine, playing a crucial role in the connectivity among systems ((Tagliapietra *et al.* 2014). Furthermore, the different typologies of habitats are used by fish populations for several reasons, including spawning, nursery and feeding grounds (Franco, Torricelli & Franzoi 2009). Although describing the life strategies of fish populations that inhabit such coastal-marine and estuarine environments (*sensu* Franco *et al.* 2008a) helps to understand the use at spatial and temporal scales of available resources, reflecting the ecosystem function of these environments, more quantitative studies of such use are necessary.

The changing conditions given by high habitat heterogeneity shape the life history traits (Brigolin *et al.* 2016), and as a consequence, they are predicted to shape also the trophic structure, hence the trophic niche of fish populations.

Among the different techniques used in trophic studies (*e.g.* stomach content, fatty acids), the analysis of stable isotopes, especially of carbon ($^{13}C/^{12}C = \delta^{13}C$) and nitrogen ($^{15}N/^{14}N = \delta^{15}N$), emerged as one of the primary tools to describe the trophic niche with a quantitative approach.

Previous works, in fact, have shown how the concept of trophic niche can be transposed into isotopic key, by considering the δ–space given by the bi-plot $\delta^{13}C$-$\delta^{15}N$ as a two dimensional space where to represent the isotopic niche space (Bearhop *et al.* 2004; Newsome *et al.* 2007). This allows us to use the isotopic niche as descriptor of the trophic niche, although it needs to be kept in mind that they are not exactly equivalent (Jackson *et al.* 2011). In fact, depending on the system involved, information given from the same isotopic data could provide insights on other aspects of the ecological niche, such as the habitat use (*e.g.* latitude, altitude, see Newsome *et al.* 2007).

Further extension of the concept have been applied by Layman *et al.* (2007a), who suggested a series of "community-wide metrics" that provide a quantitative estimate of the width of the isotopic niche, such as the convex hull encompassing all the individuals of a populations, but also the trophic diversity and the trophic redundancy, observable either at community or population level. Moreover, Jackson *et al.* (2011) later refined the aspect of the width of the isotopic niche by applying Bayesian statistics to calculate standard ellipse area that result more precise than the convex hulls previous mentioned. Both applications allow to compare different communities or populations across space (habitats), and the same ones across time (Layman *et al.* 2007a).

The aim of this project is to characterize the trophic niche variability of resident fish populations and communities under changing conditions typical of coastal-marine ecosystems. Three main factors inducing variability to trophic niche were taken into account: (i) habitat heterogenity, (ii) ontogenetic development and (iii) habitat restoration processes, and they were discussed in three chapters:

- Chapter 1 is focused on the trophic niche variability of resident fish populations across habitats at inter- and intra-lagoon level, with a particular focus to a target species of high ecological value, the Mediterranean toothcarp *Aphanius fasciatus;*
- Chapter 2 assesses the variability of trophic niche and trophic position occurring during ontogeny of *S. aurata* and the role of different habitats in supporting the trophic pathway leading to the species over early stages of development within a coastal lagoon*;*
- Chapter 3 examines temporal variation of the trophic niche of a fish community associated to a site under seagrass restoration, in order to evaluate functional recovery of fish communities during habitat restoration processes.

# Chapter 1

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## Trophic niche variability of resident fish across habitats at inter- and intra-lagoon level: focus on a Mediterranean killifish

### Abstract

Coastal lagoons are characterized by high habitat heterogeneity where, often, natural habitats coexist with artificial ones, historically set up to support human activities, for example related to salt production, aquaculture and traditional fishery. Aim of this work is to assess the trophic niche variability of resident fish populations across natural and artificial habitats at inter- and intra-lagoon level, with a particular focus to a target species of high ecological value, the Mediterranean toothcarp *Aphanius fasciatus*.

Fish communities and all the possible sources of organic matter were sampled in spring and autumn 2014 in two habitat typologies, one artificial and one natural, within the Stagnone di Marsala (Trapani) and the Lagoon of Venice. Habitats sampled were respectively a seagrass meadow dominated by *Cymodocea nodosa* and an evaporation pond in a salt work system in the first study area, and two salt marshes and an artificial ditch in the second one. Trophic niche variability was described using carbon and nitrogen stable isotope population and community-wide metrics, while Bayesian mixing models allowed to track the main organic matter pathways supporting the target species.

Main differences, both at community and target population level, occurred between the two study areas and within each, between artificial and natural habitats. Habitat and community structure resulted to influence the trophic niche of *A. fasciatus* that hence varied in dependence of the specific habitat, but they did not influence the overall ecological role of the species. Furthermore, two main trophic pathways seemed to drive the fluxes of organic matter leading to *A. fasciatus* within the two typologies of habitat examined: while the macrophytes definitely support the species in the natural habitats, artificial habitats are mainly based on sedimentary organic matter routes.

Our outcomes allowed to broaden knowledge on how natural and artificial systems support fish populations living within coastal lagoons and results are discussed also in the light of ecological implications for coastal management of such artificial habitats that could represent hotspot for conservation priorities of *A. fasciatus*.

## 1.1. Introduction

Transitional ecosystems, such as coastal lagoons, are typically characterized by high spatio-temporal variability of the environmental features. This, in turn, enhances a great habitat heterogeneity, that is also dependent on intrinsic geomorphology of the specific system, such as the degree of connection to the open sea and the input of freshwaters (Franco, Franzoi & Torricelli 2008b; Vizzini 2009). Depending on the lagoon, the variety of habitats can include salt marshes, seagrass meadows, mudflats (either vegetated or not), intertidal creeks. Moreover, in these ecosystems, natural habitats often coexist with artificial ones, historically set up to support human activities related for example to salt production, aquaculture and artisanal fishery, which makes them important contributors of ecosystem services.

An extensive body of research has assessed the structure, functioning and temporal dynamics of fish assemblages inhabiting coastal lagoons (Elliott & DeWailly 1995; Vizzini & Mazzola 2003; Franco *et al.* 2006a; Manzo 2010; Franzoi, Franco & Torricelli 2010) and depicted their life history traits (Franco *et al.* 2012; Cavraro *et al.* 2014a; Brigolin *et al.* 2016).

Some authors proposed a functional guild approach as a tool to describe the life history traits of fish populations of such transitional environments (Franco *et al.* 2008a). In particular, (Franco *et al.* 2008a) applied the guild approach to depict the link between fish populations and the utilization of coastal systems, where the guild is defined as a group of species that share their niche requirements, exploiting the same range of environmental resources with similar habits (Root 1967).

Among the interactions that relate a fish population to a certain environment, trophic relationships are of crucial importance and are useful tools to assess the functioning and the level of complexity of a system.

Since habitat heterogeneity has been demonstrated to shape the life history traits of fish species populations (Brigolin *et al.* 2016), we predict that this can create some variation in trophic structure of populations and consequently communities, that must be evaluable by descriptors of trophic niche.

The analysis of carbon and nitrogen stable isotopes has been widely used in studies of trophic ecology (Layman *et al.* 2012) and the recent development of descriptors (population/community-wide metrics) and statistical models (mixing model) based on the concept of isotopic niche have emerged as main approaches to describe quantitatively the trophic niche of populations and communities and to infer the pathways of organic matter in a wide variety of systems (Layman *et al.* 2007a; Jackson *et al.* 2011).

Resident fish populations that inhabit a wide variety of environments can be used as suitable models to assess the influence of different habitat types on the variability of the trophic niche, at intra and inter-lagoon level.

Aim of this work is to assess the trophic niche variability of resident fish populations across habitats at inter- and intra-lagoon level, with a particular focus to a target species of high ecological value. The purpose is to investigate how the different habitats of a transitional ecosystem shape the composition and trophic structure of resident fish populations.

Special attention was given to one of the resident fish species that exhibits a very high ecological value, as it is able to populate a wide variety of habitats within many Mediterranean coastal lagoons: the Mediterranean toothcarp *Aphanius fasciatus* (Valenciennes, 1821) (Leonardos & Petridis 1996; Cimmaruta *et al.* 2003; Tigano *et al.* 2006; Cavraro *et al.* 2011), also called killifish for its small size, short life cycle and persistency in small brackish water bodies. It is the only species of Cyprinodontidae family inhabiting Italian coastal waters and as level of protection it is included in the Annex II of Habitats Directive (92/43/EEC), regarding the conservation of natural habitats and wild fauna and flora in the European Union, and additionally it is also included in the Red list of threatened species of IUCN (International Union for Conservation of Nature), whose threat is classified of “Least Concern”.

Two coastal lagoons were taken into account, within which a number of habitats were selected, either natural or artificial, characterized by a different level of confinement and availability of basal sources. Furthermore, the computing of Bayesian mixing models allowed to estimate the variability of basal sources contribution to the trophic pathways that sustain the target fish across habitats.

## 1.2. Materials and methods

*Study areas*

This study was carried out in two lagoon ecosystems: the Stagnone di Marsala and the Venice Lagoon in southern and northern Italy respectively.

The Stagnone di Marsala (37° 52' N, 12° 28' E) is a shallow semi-enclosed basin with a surface area of approximately 21 $km^2$ and an average depth of 1 m (Fig. 1). No substantial inputs of freshwater are present, therefore values of temperature and salinity are generally higher in comparison to the adjacent sea (Mazzola *et al.* 2010), to which the two main sub-basins have a different degree of connection. The northern sub-basin has a narrow and shallow channel (450 m width and 10-30 cm depth) that allows only scarce water exchange, thus represents the actual lagoon basin, with a higher annual variability of the main physico-chemical parameters and the bottom is often dominated by events of sediment resuspension. By contrast, the southern sub-basin is connected to the sea through a wider mouth (1350 m width and about 2 m depth) that induces a higher rate of water turnover (La Loggia *et al.* 2004) and stronger marine influence. The Stagnone is also classified as oligotrophic, as the mean chlorophyll-*a* concentration is around 1.0 μg $l^{-1}$ in the water column (Sarà, Leonardi & Mazzola 1999) and 3.0 μg $l^{-1}$ in the sediment (Pusceddu *et al.* 1999). According to the European Directive 92/43/CEE, the area is included in the lists of the SCI (ITA010026) and SPA (ITA010028).

The lagoon of Venice (45° 26′ N, 12° 20′ E) is the largest lagoonal system in Europe (Fig. 1), has a surface area of about 550 $km^2$ and according to its hydrology can be divided in three main sub-basins: Northern, Central and Southern (Avanzi *et al.* 1979). It has mainly shallow waters (mean depth about 1±0.3 m, Bonfà *et al.* 2004) crossed by deeper shipping channels extending towards the three mouths (Lido, Malamocco and Chioggia) that allow connection and water exchange with the

Adriatic Sea. The major freshwater tributaries are located in the northern sub-basin, hence has a lower salinity than the other sub-basins. The area is subjected to the widest tidal range of the Mediterranean Sea, which is up to approximately 1 m. The high variability of morphological and physico-chemical parameters induces high levels of spatial and habitat heterogeneity that characterizes the whole lagoon. Among the different levels of protection of the area, the Habitats Directive (92/43/CEE) includes the Northern Lagoon of Venice within the Sites of Community Importance (SCI) as well as the Central-Southern areas (respectively IT3250030 and IT3250031), and the lagoon as a whole is designated as a Special Protection Area (SPA, IT3250046).

### *Sampling sites*

Five sites corresponding to different natural and artificial habitats, where the target species is widely distributed, were chosen across the two study areas (Fig. 1).

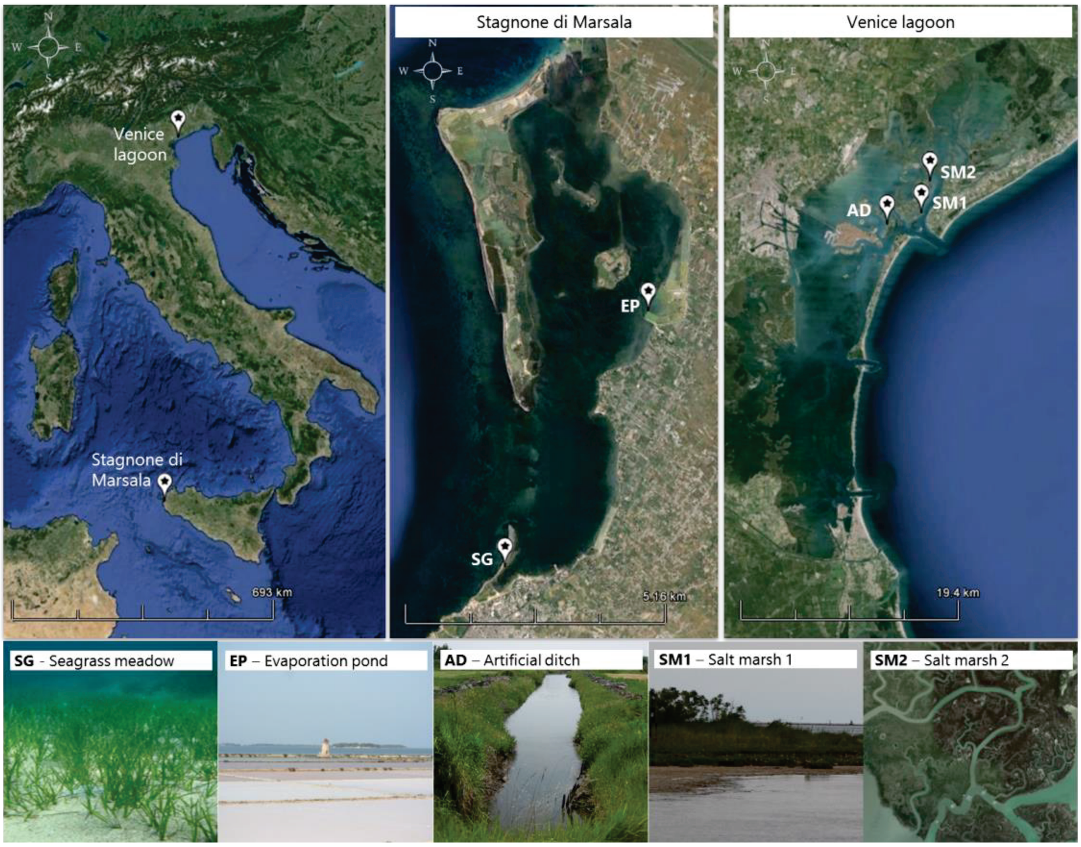

**Figure 1.** Location of the study areas and relative position of the habitats examined.

In the Stagnone di Marsala the following two sites were investigated:

SG: Seagrass meadow – is a dense *Cymodocea nodosa* (Ucria Ascherson, 1870) meadow located near Punta d'Alga, in the southern sub-basin of the Stagnone. Site depth is about 40 cm and the sediment is sandy-pelitic, fully covered in *C. nodosa* and minor macroalgae.

EP: Evaporation pond – also called "cooling vat", is one of the tanks of the saltwork system located in the central area of the Stagnone. The tank is approximately 60.000 $m^2$ in surface area and 50 cm deep, it is enclosed from the adjacent sound and, only occasionally, a small channel is opened to allow the income of water for new salt production. Sandy-pelitic bottom in covered by mixed patches of *C. nodosa* and macroalgae.

In Venice lagoon the following three sites were investigated:

SM1: Salt marsh 1 – is a small salt marsh situated in Baccan (45.44741°N, 12.41583°E), a site close to the northern mouth of the lagoon. Depth is about 30 cm, the sediment is muddy and has a low coverage (5-25%) of seagrasses with sparse drift macroalgae.

SM2: Salt marsh 2 – is a salt marsh placed in an inner area of the lagoon, in the proximity of Crevan island (45.47190°N, 12.43080°E). The site is 40 cm deep and the substrate is mainly muddy and bare, with a very low (5-10%) presence of seagrasses and macroalgae. It is also characterized by the presence of small intertidal creeks, about 10 cm deep.

AD: Artificial ditch – is an artificial system of small ditches situated in Vignole island (45.44259° N 12.37538°E), used in the past as inland fish pond. Now ditches are about 60 cm deep and are isolated from the rest of the lagoon.

### *Sample collection*

Fish communities and all the possible sources of organic matter representative of the habitats examined were collected in spring and autumn 2014, following a standardized protocol used in all the study sites.

Fish were collected by semi-quantitative catch, using small (4-8-10 m) beach seine nets with a mesh size of 2 mm, covering two times a known surface area. Size of the seine net used and surface area sampled varied by study area or site, depending on the specific habitat involved (*e.g.* narrower net covering longer distance when sampling in canals or creeks, wider net over wider surface area when sampling in open salt marshes).

Primary producers such as seagrasses, macroalgae and terrestrial halophytes were collected manually from three random points chosen within the sampled areas.

Seawater samples (10 l needed in the oligotrophic Stagnone di Marsala and 2 l in Venice Lagoon) and superficial sediment cores (3 cm Ø) were collected in triplicate to obtain the sources related respectively to the suspended particulate (POM) and sedimentary organic matter (SOM). In addition, only in Venice Lagoon sites, zooplankton was sampled by horizontal haul with a small plankton net with a 160 µm mesh size. All the samples collected were kept cool until arriving at the laboratory and stored at -20°C prior to the processing.

### *Sample processing and laboratory analysis*

Fish sampled were identified at species level and sex (when possible) and the main biometrics such as standard length (SL), total weight (TW), were measured in order to determine the main demographic traits of the populations examined.

Afterwards, at least 15 individuals for each species and sex, were dissected by means of scalpel and tweezers to extract the dorsal muscles. Seagrasses, macroalgae and terrestrial halophytes were identified at species level; eventual epiphytes were scraped manually.

Water samples were pre-filtered (200 μm) and then filtered on precombusted (450°C, 4h) filters (GF/F Whatman, pore size 0.7 μm) to concentrate the POM. The top 1.5 cm of sediment cores were sliced and homogenized as proxy for SOM. Zooplankton samples were carefully cleaned under a binocular microscope to remove any detrital material and concentrated in eppendorf tubes.

Once processed, all samples intended for the analysis of stable isotopes were oven-dried at 60 °C to constant weight for about 48h and then ground to a fine powder using a micro mill or a mortar and pestle. Part of the samples containing any carbonate component that might interfere with the determination of carbon isotopic signature was acidified (HCl 1M). Lastly, an aliquot of each sample was packed in tin capsules and analysed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo-Electron Delta Plus XP) coupled to an elemental analyser (Thermo-Electron Flash EA1112).

Carbon and nitrogen stable isotope ratios were expressed in δ unit notation, as parts per mil deviation from the international standards (respectively Pee Dee Belemnite and atmospheric $N_2$ for carbon and nitrogen) and determined as follows: $\delta X=[(R_{sample}/R_{standard})-1]\times 10^3$, where X is $^{13}C$ or $^{15}N$ and R is the relative $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

*Data analysis*

*Demographic traits* - Preliminary analysis of the fish fauna data allowed to assess the context of the community where the target species was placed.

The number of individuals of each species collected was related to the specific sampling area and then standardized to 100 $m^2$, in order to compare the density among sites as n° individuals $100^{-2}$ and relative percentage abundance. In addition, each species was assigned to an ecological and feeding group, as defined in Malavasi *et al.* (2004) and Franco *et al.* (2006a), to outline the main ecological features of the populations found within the fish assemblages.

In order to focus on the population level of the target species *A. fasciatus*, the main traits of the demographic structure were described by looking at the frequency distributions of standard length (SL) and the sex ratio female:male (F:M).In addition, trophic positions (TP) were estimated for *A. fasciatus* using the equation reported in (Post 2002): TP = $[(\delta^{15}N_f - \delta^{15}N_b) / \Delta n] + \lambda$, where $\delta^{15}N_f$ and $\delta^{15}N_b$ are respectively the nitrogen isotopic signature of the fish species and that of the source baseline. As source baseline we used the mean value of all the samples of zooplankton collected within the Venice lagoon (7.37‰) and the Stagnone di Marsala (6.5‰). $\Delta n$=3.4‰ is the trophic enrichment expected for each trophic level and $\lambda$ is the trophic position of the species used as baseline, that is 2.

*Trophic structure* - Differences for isotopes between lagoons and among habitats were conducted separately for $\delta^{13}C$ and $\delta^{15}N$, respectively through t-test or non-parametric Kruskal-Wallis after testing for normal distribution of the data.

In order to assess the isotopic niche width and relative metrics, standard ellipse areas (SEAc, corrected for small sample size) were estimated by Bayesian statistics using the R package SIBER v2.0.2 (Stable Isotope Bayesian Ellipses in R) (Jackson *et al.* 2011) and community-wide metrics were calculated using the R package SIAR 4.2.2 (Parnell *et al.* 2010).

Metrics were estimated independently, at first, for all of the species populations found in all of the habitats and for both seasons, in order to have an overall view of the fish community and the variability in time. Secondly, to focus on the target species and assess its trophic niche variability across habitats, the same metrics were estimated only for *A. fasciatus* populations, pooling seasons together.

Among the community-wide metric, in particular the followings were calculated for the purpose:

i) $\delta^{15}N$ Range (NR), difference between the most enriched and most depleted $\delta^{15}N$ values, estimates the trophic length;

ii) $\delta^{13}C$ Range (CR), difference between the most enriched and the most depleted $\delta^{13}C$ values, is a measure of the diversity of basal resources used;

iii) mean Distance to Centroid (CD), average Euclidean distance of each species to the centroid $\delta^{13}C$-$\delta^{15}N$, quantifies the trophic diversity and species spacing within the isotopic space;

iv) mean Nearest Neighbour Distance (NND), Euclidean distance of each individual to the nearest neighbour, provides information on individuals density and packing within the population (trophic redundancy);

v) Standard Deviation of the Nearest Neighbour Distance (SDNND) provides information on the evenness of individuals packing.

Additionally, to investigate the trophic pathway of the basal sources that sustain *A. fasciatus* in the different habitats, Bayesian mixing models were applied to estimate the contribution of each source, using the R SIAR package previously mentioned. As basal sources we considered all of the of organic matter sources available in the specific habitat, that included sedimentary and particulate organic matter (SOM and POM), seagrasses, algae, pooled halophytes and also epiphytes or plankton when present. As fish are considered second level consumers, the trophic enrichment factors (TEFs) used for the model were multiplied by two and, according to literature were: 2.5‰±1 for $\delta^{15}N$ (Vander Zanden & Rasmussen 2001) and 0.4‰±1.3 for $\delta^{13}C$ (Post 2002).

## 1.3. Results

*Habitats macrophytes composition*

Different macrophytes compositions were recorded in the five habitats examined and a total of 14 species were identified (Tab. 1). Main differences occurred between study areas, within which both the artificial habitats showed the lowest diversity in terms of number of macrophytes recorded (respectively 2 in the evaporation pond, EP in Marsala and 3 in the artificial ditch; AD in Venice). Apart from the dense seagrass meadow (SG), all the other habitats investigated were characterised by a patchy distribution of the macrophytes recorded, with macroalgae occurring mainly as drift. Both the two habitats of the Stagnone di Marsala were characterised by the presence of the seagrass *Cymodocea nodosa* associated with the green alga *Chaetomorpha linum* (O.F.Müller) Kützing 1845.

**Table 1.** List of the species recorded in the habitats examined with indication of their presence (+) or absence (-).

| | | Stagnone di Marsala | | Venice Lagoon | | |
|---|---|---|---|---|---|---|
| **Species** | **Code** | **SG seagrass meadow** | **EP evaporation pond** | **AD artificial ditch** | **SM1 salt marsh** | **SM2 salt marsh** |
| **Seagrasses** | | | | | | |
| *Cymodocea nodosa* | Cno | + | + | - | - | - |
| *Ruppia maritima* | Rma | - | - | + | - | - |
| *Zostera marina* | Zma | - | - | - | - | + |
| *Zostera noltei* | Zno | - | - | - | + | + |
| **Macroalgae** | | | | | | |
| *Agardhiella subulata* | Asu | - | - | - | - | + |
| *Caulerpa prolifera* | Cpr | + | - | - | - | - |
| *Chaetomorpha linum* | Cli | + | + | + | - | - |
| *Cladophora* sp. | Cla | - | - | + | - | - |
| *Cystoseira barbata* | Cba | - | - | - | - | + |
| *Glacilaria bursa pastoris* | Gbp | - | - | - | + | - |
| *Lomentaria clavellosa* | Lcl | - | - | - | + | - |
| *Ulva rigida* | Uri | - | - | - | + | + |
| **Halophytes** | | | | | | |
| *Atriplex portulacoides* | | - | - | + | - | + |
| *Limonium narbonense* | | - | - | - | + | - |
| *Sarcocornia fruticosa* | | + | + | - | + | + |

Among the seagrasses in the lagoon of Venice, *Ruppia maritma* Linnaeus 1753 only occurred in the artificial ditch of Vignole (AD), while *Zostera noltei* Hornemann 1832 occurred in both the salt marsh habitats (SM1 and SM2) as well as the green alga *Ulva rigida* Agardh 1823. Among the halophytes species present in the study sites, only the most abundant were collected and considered for trophic assessments. Particularly, *Sarcocornia fruticosa* (L.) A. J. Scott. was found widespread in almost all of the habitats investigated, covering most of the borders of the salt marshes.

*Fish demographic traits*

*Fish assemblages* - Different fish assemblages occurred among all of the habitats investigated, with some variability at seasonal level (Tab. 2). Overall, a total of 12 species belonging to 6 families were sampled, most of which fell in the ecological group of estuarine resident and invertivorous or omnivorous as feeding group. The highest number of species was found in the inner salt marsh (SM2).

The target species *A. fasciatus* showed a common trend in most of the habitats: it was dominant in spring, both in terms of density (number of individuals $m^{-2}$) and percentage abundance (%) and decreased in autumn.

Exceptions were given by the artificial channel (AD) and the inner salt marsh (SM2), where *A. fasciatus* densities were higher even in autumn and by the first salt marsh site (SM1), whose community was dominated by *Atherina boyeri* (Risso, 1810) in spring and by *Liza saliens* (Risso, 1810) in autumn. *A. boyeri* was also dominant in autumn within the sites in the Stagnone di Marsala (SG and EP) and SM2 in the Venice lagoon.

*A. fasciatus demographic structure* - The demographic structure of the target populations was investigated through frequency distributions of the standard length (Fig. 2). All the populations showed distributions skewed to the lower size classes (mainly SL< 40 mm). The only exception was given in SM1 habitat, where few individuals were uniformly distributed. The seasonal trend seems to follow opposite patterns within the two study areas: despite the neat reduction of individuals from spring to autumn, it is clear how the mode shifts from lower to slightly higher classes in Marsala (SG and EP), and *vice versa* in Venice (SM1, SM2 and AD).

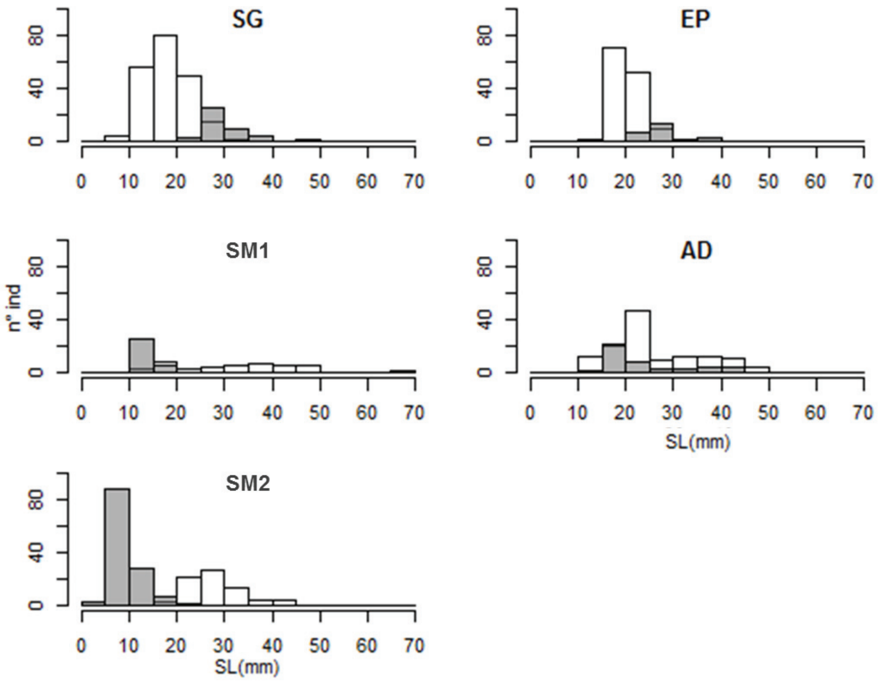

**Figure 2.** Frequency distributions of the standard length of *A. fasciatus* populations collected in the sampling sites in spring (white bars) and autumn (overlapped grey bars).

The sex ratio between females and males recorded was highly variable (Tab. 3) across habitats and seasons, with a general tendency to female prevalence, with the exception of the artificial ditch in Vignole, where males were more abundant in both sampling seasons.

**Table 3.** Sex ratio Female:Male of *A. fasciatus* collected in both sampling seasons across habitats.

| F:M | Stagnone di Marsala | | Venice lagoon | | |
|---|---|---|---|---|---|
| | SG | EP | AD | SM1 | SM2 |
| Spring | 16.08 | 0.90 | 0.53 | 9.50 | 1.25 |
| Autumn | 3.33 | 211.00 | 0.52 | 3.78 | 24.20 |

**Table 2.** Density (number of individuals 100 m$^{-2}$) and percentage abundance (%) of the species sampled in the study sites in spring and autumn. Ecological and feeding groups are also indicated according to Franco *et al.* (2006a) and Leonardos (2008) specifically for *A. fasciatus*. Ecological groups are: ER, estuarine resident, MJ, marine juvenile migrant, MS, marine seasonal migrant. Trophic groups are: CS, carnivores, IS, invertivores, IF, invertebrate-fish feeders, HC, herbivores-carnivores, O, omnivores.

| | | | | | Stagnone di Marsala | | | | Venice lagoon | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **SG**<br>**Seagrass meadow** | | **EP**<br>**Evaporation pond** | | **AD**<br>**Artificial ditch** | | **SM1**<br>**Salt marsh** | | **SM2**<br>**Salt marsh** | |
| **Family** | **Species** | **Ecological group** | **Feeding group** | **Season** | **Density** | **%** | **Density** | **%** | **Density** | **%** | **Density** | **%** | **Density** | **%** |
| **Atherinidae** | ***Atherina boyeri*** | **ER** | **CS** | Spring | 10.0 | 3.8 | 58.7 | 26.5 | - | - | 134.4 | 83.1 | - | - |
| | | | | Autumn | 136.3 | 73.6 | 190.0 | 82.6 | - | - | - | - | 2.0 | 52.2 |
| **Cyprinodontidae** | ***Aphanius fasciatus*** | **ER** | **O** | Spring | 256.3 | 96.2 | 144.6 | 65.2 | 231.9 | 65.3 | 27.3 | 16.9 | 23.4 | 27.3 |
| | | | | Autumn | 48.8 | 26.4 | 27.5 | 12.0 | 58.8 | 50.5 | 15.7 | 31.0 | 157.5 | 90.0 |
| **Gobiidae** | ***Pomatoschistus marmoratus*** | **ER** | **IS** | Spring | - | - | - | - | - | - | - | - | - | - |
| | | | | Autumn | - | - | - | - | - | - | - | - | 0.7 | 17.4 |
| **Gobiidae** | ***Pomatoschistus minutus*** | **MJ** | **IS** | Spring | - | - | - | - | - | - | - | - | - | - |
| | | | | Autumn | - | - | - | - | - | - | - | - | 0.2 | 4.3 |
| **Gobiidae** | ***Knipowitschia panizzae*** | **ER** | **IS** | Spring | - | - | - | - | 123.1 | 34.7 | - | - | - | - |
| | | | | Autumn | - | - | - | - | 57.5 | 49.5 | - | - | 5.0 | 2.9 |
| **Mugilidae** | ***Liza aurata*** | **MS** | **HC** | Spring | - | - | - | - | - | - | - | - | 8.8 | 10.2 |
| | | | | Autumn | - | - | - | - | - | - | - | - | 0.3 | 8.7 |
| **Mugilidae** | ***Liza ramada*** | **MJ** | **O** | Spring | - | - | - | - | - | - | - | - | 14.0 | 16.3 |
| | | | | Autumn | - | - | - | - | - | - | - | - | - | - |
| **Mugilidae** | ***Liza saliens*** | **ER** | **HC** | Spring | - | - | - | - | - | - | - | - | 3.2 | 3.8 |
| | | | | Autumn | - | - | - | - | - | - | 34.7 | 68.5 | 0.5 | 13.0 |
| **Mugilidae** | ***Mugil cephalus*** | **MJ** | **O** | Spring | - | - | - | - | - | - | - | - | - | - |
| | | | | Autumn | - | - | - | - | - | - | 0.3 | 0.6 | - | - |
| **Sparidae** | ***Sparus aurata*** | **MJ** | **O** | Spring | - | - | - | - | - | - | - | - | 0.6 | 0.8 |
| | | | | Autumn | - | - | - | - | - | - | - | - | - | - |
| **Syngnathidae** | ***Syngnathus abaster*** | **ER** | **IS** | Spring | - | - | 9.8 | 4.4 | - | - | - | - | - | - |
| | | | | Autumn | - | - | 3.8 | 1.6 | - | - | - | - | - | - |
| **Syngnathidae** | ***Syngnathus typhle*** | **ER** | **IF** | Spring | - | - | 7.6 | 3.4 | - | - | - | - | - | - |
| | | | | Autumn | - | - | 8.8 | 3.8 | - | - | - | - | 0.2 | 4.3 |

### *Isotopic results*

#### *Organic matter sources and fish communities*

The overall ranges of both $\delta^{13}C$ and $\delta^{15}N$ that include all the aquatic sources of organic matter available in the habitats sampled (namely excluding the terrestrial halophytes) showed values more depleted in the Venice Lagoon than in the Stagnone di Marsala (Fig. 3). A significant shift was recorded for $\delta^{13}C$ between the two study areas (*t-test*, $p\text{-value}<2.2e^{-16}$) and within each lagoon, between natural and artificial habitats (*Kruskal-Wallis post-hoc test*, $p\text{-value}<0.05$). In the Stagnone, the mean of carbon signatures ranged from -17.81±0.41 to -7.45±0.47‰ in the seagrass meadow (SG) and from -20.77±1.01 to -9.18±0.3‰ in the salt evaporation pond (EP). In the Venice Lagoon, instead, the ranges varied from around -23 to about -10.5‰ in both salt marsh habitats (SM1 and cSM2) and were even more depleted in the artificial ditch of Vignole (AD), ranging from -30.31±1.49 to -17.16±0.48‰.

About the $\delta^{15}N$, no evident differences emerged between lagoons, but AD in the Venice Lagoon was significantly shifted (*Kruskal-Wallis post-hoc test*, $p\text{-value}<0.05$) towards a lower range (from 3.1±0.18 to 9.9±0.25‰) than in all the other habitats, that ranged approximately from 4 to 12‰.

Halophytes signatures always fell within the N range, but were more depleted in C, with the exception of AD, where it was placed between the signatures of POM and SOM. The shift observed for the aquatic sources of organic matter was also maintained at fish community level, whose overall ranges, especially of carbon isotopic signatures fell within more depleted values in Venice Lagoon and within each lagoon, they were still more depleted in the artificial habitats (Fig. 3).

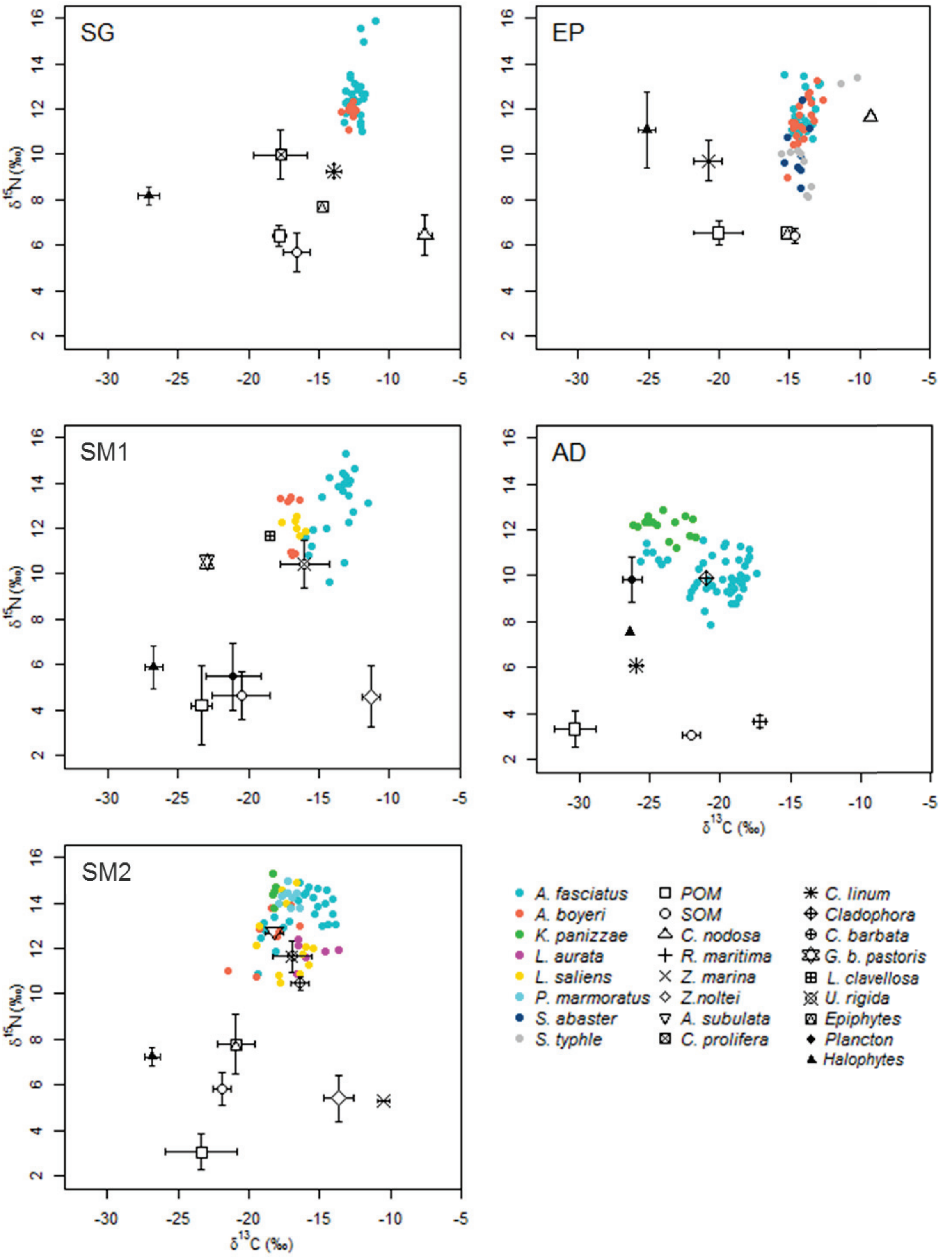

**Figure 3.** $\delta^{13}$C (‰) *vs.* $\delta^{15}$N (‰) of fish species and annual mean (±s. d.) of organic matter sources sampled in each habitat site: SG, seagrass meadow and EP, evaporation pond in Stagnone di Marsala; SM1 and SM2, salt marshes, AD, artificial ditch in Venice Lagoon. Full coloured points are single individuals of fish species, black symbols are organic matter sources, according to the legend specified in the graph.

### *Comparison of* A. fasciatus *isotopic niche* within *habitats*

The isotopic niche of *A. fasciatus* varied in width, shape and position across habitats and seasons (Fig. 4) as well as its descriptive metrics (Tab. 4).

As a general trend, in all the habitats investigated, the metrics describing the isotopic niche of *A. fasciatus* were higher in autumn than in spring (Tab. 4), indicating a higher extension of the isotopic niche (SEAc, NR and CR), and also a higher trophic diversity of the individuals (CD), redundancy (NND) and evenness of the individual spacing within the niche (SDNND). The only exception was given in EP, where NR and CR were lower in autumn than in spring.

**Table 4.** Population-wide metrics calculated for each species and habitat in spring and autumn.

| | Spring | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SEAc** | **NR** | **CR** | **CD** | **NND** | **SDNND** | **SEAc** | **NR** | **CR** | **CD** | **NND** | **SDNND** |
| **SG** | | | | | | | | | | | | |
| *A. fasciatus* | 1.04 | 2.44 | 1.24 | 0.74 | 0.24 | 0.11 | 1.91 | 3.89 | 1.64 | 1.28 | 0.47 | 0.31 |
| *A. boyeri* | 0.55 | 1.22 | 0.86 | 0.42 | 0.39 | 0.34 | 0.10 | 0.43 | 0.42 | 0.20 | 0.15 | 0.09 |
| **EP** | | | | | | | | | | | | |
| *A. fasciatus* | 1.15 | 2.82 | 2.01 | 0.70 | 0.32 | 0.37 | 1.54 | 2.26 | 1.43 | 0.79 | 0.51 | 0.43 |
| *A. boyeri* | 0.83 | 3.11 | 1.20 | 0.67 | 0.43 | 0.42 | 1.10 | 2.55 | 1.68 | 0.73 | 0.49 | 0.38 |
| *S. abaster* | 1.10 | 1.48 | 1.17 | 0.56 | 0.56 | 0.35 | 4.25 | 1.66 | 1.68 | 0.98 | 1.49 | 0.20 |
| *S.typhle* | 1.10 | 1.92 | 1.18 | 0.71 | 0.59 | 0.54 | 2.21 | 1.90 | 2.14 | 1.20 | 1.18 | 1.21 |
| **AD** | | | | | | | | | | | | |
| *A. fasciatus* | 3.28 | 2.75 | 4.72 | 1.38 | 0.39 | 0.23 | 6.00 | 3.59 | 7.28 | 2.24 | 0.51 | 0.25 |
| *K. Panizzae* | 1.43 | 1.39 | 1.97 | 0.81 | 0.56 | 0.12 | 0.57 | 0.67 | 3.02 | 0.65 | 0.36 | 0.29 |
| **SM1** | | | | | | | | | | | | |
| *A. fasciatus* | 1.00 | 1.95 | 2.40 | 0.64 | 0.39 | 0.32 | 4.58 | 3.43 | 5.52 | 1.78 | 0.89 | 0.50 |
| *A. boyeri* | 3.94 | 2.57 | 3.40 | 1.41 | 0.42 | 0.60 | - | - | - | - | - | - |
| *L. saliens* | - | - | - | - | - | - | 0.59 | 0.81 | 1.68 | 0.50 | 0.43 | 0.27 |
| **SM2** | | | | | | | | | | | | |
| *A. fasciatus* | 1.37 | 1.64 | 2.62 | 0.87 | 0.42 | 0.17 | 2.91 | 3.96 | 3.56 | 1.35 | 0.70 | 0.40 |
| *A. boyeri* | - | - | - | - | - | - | 4.56 | 3.14 | 5.03 | 1.58 | 1.14 | 0.68 |
| *K. Panizzae* | - | - | - | - | - | - | 0.16 | 1.48 | 0.20 | 0.37 | 0.34 | 0.24 |
| *L. aurata* | 2.49 | 1.48 | 2.97 | 1.14 | 0.68 | 0.33 | - | - | - | - | - | - |
| *L. saliens* | 1.34 | 1.57 | 2.43 | 0.98 | 0.47 | 0.19 | 1.97 | 2.71 | 2.78 | 1.41 | 0.79 | 0.16 |
| *P. marmoratus* | - | - | - | - | - | - | 0.61 | 1.17 | 1.48 | 0.51 | 0.35 | 0.20 |

A comprehensive look at the metrics of the whole communities where *A. fasciatus* was placed (Tab. 5) showed a similar trend within both lagoons, with the artificial habitats, EP and AD presenting the widest isotopic niche (higher SEAc values) and the highest trophic diversity (higher CD).

**Table 5.** Community-wide metrics of whole communities sampled at each habitat site.

| | SEAc | NR | CR | CD | NND | SDNND |
|---|---|---|---|---|---|---|
| **SG** | 1.54 | 4.81 | 2.43 | 0.89 | 0.24 | 0.22 |
| **EP** | 3.31 | 5.39 | 5.42 | 1.28 | 0.27 | 0.27 |
| **AD** | 7.89 | 4.98 | 8.76 | 2.56 | 0.37 | 0.19 |
| **SM1** | 7.13 | 5.66 | 6.24 | 2.19 | 0.44 | 0.37 |
| **SM2** | 6.58 | 4.76 | 7.82 | 1.88 | 0.36 | 0.28 |

In most of the habitats, the isotopic niche of the target species was clearly separated from the niches of the co-occurring species and located into a more $^{13}$C and $^{15}$N-enriched area of the isotopic space (Fig. 4). A considerable overlap with other species' niche was observed only when more than 3 fish species occurred in the same community. In fact, in SM1 and AD there was no overlap respectively with *A. boyeri* or *L. saliens* and *K. panizzae*. In SG and EP a small overlap with *A. boyeri* occurred in spring, when the isotopic niche of both species had similar width. Differently, in EP and SM2 the overlap between *A. fasciatus* and *A. boyeri* was consistent and persisted in both seasons (only exception in SM2, that had only 3 species in spring).

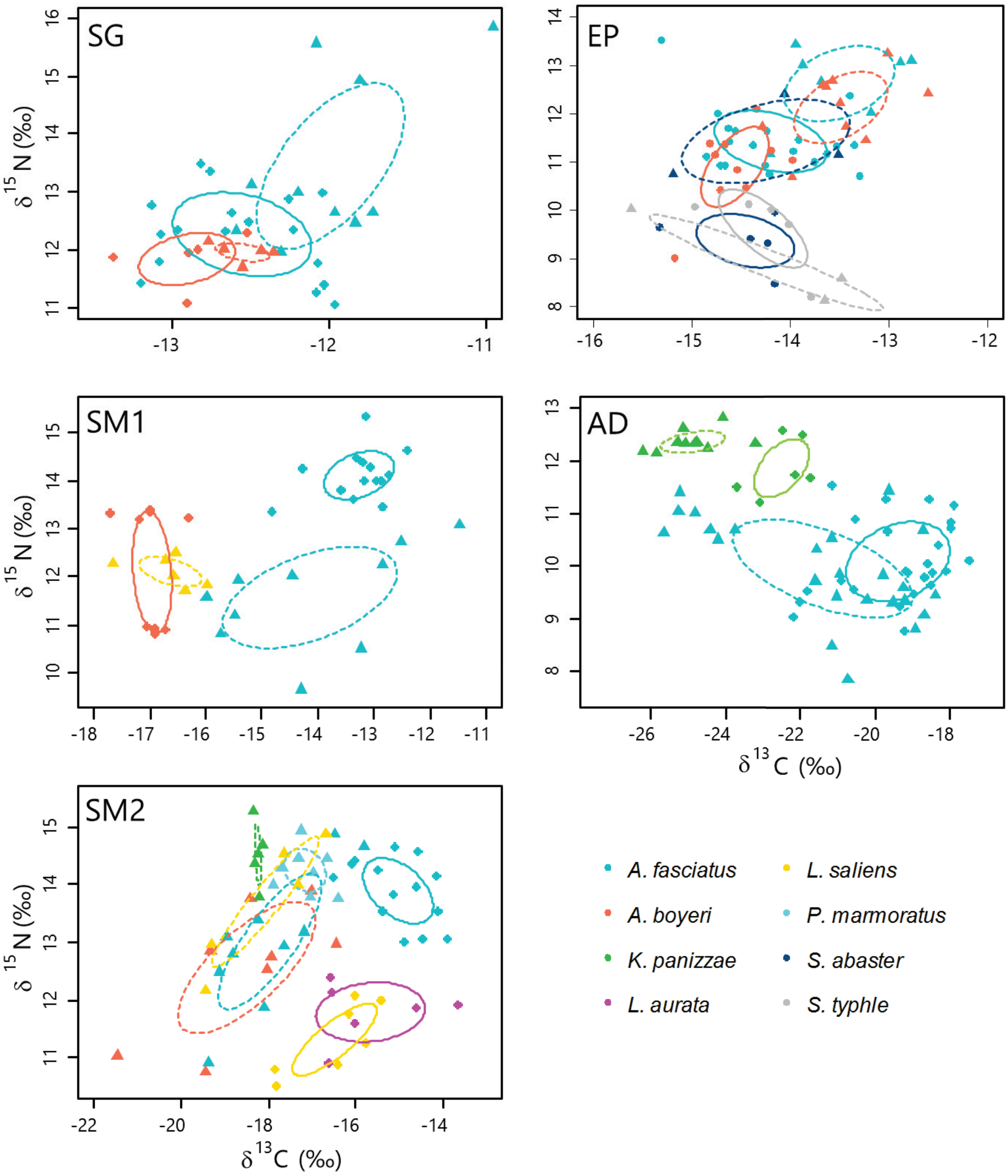

**Figure 4.** Distribution of fish species within the isotopic space of each habitat examined in spring (circles) and autumn (triangles). The isotopic niche of each species is indicated by the bayesian Standard Ellipse Areas (SEAc), calculated for spring (full line) and autumn (dashed line).

### *Comparison of* A. fasciatus *isotopic niche between habitats*

A focus on the metrics calculated for the whole populations of *A. fasciatus* (therefore including both seasons), allowed to assess the variability of the isotopic niche across habitats (Fig. 5). Different habitats showed different and separated isotopic niches. Whereas the position of the niches is strictly dependent on the values of the basal sources that characterize the specific site, shape, size and spacing of individuals within them are related to the trophic diversity and redundancy of the populations taken into account. The most evident difference in terms of extension of the niche occurred between study areas. Overall, the populations in the Stagnone di Marsala presented narrower niches than those of the Venetian Lagoon. Accordingly, this difference was also maintained for all the other metrics (Tab. 6). Similar habitats in the Venice Lagoon showed similar metrics, as it is the case of the two salt marshes (SM1 and SM2). The population in the artificial ditch of Vignole (AD) remarkably stood out of all the other habitats, with a niche shifted towards depleted area of the isotopic space and at the same time the highest trophic diversity (CD), after SM1, due to the high seasonal variability.

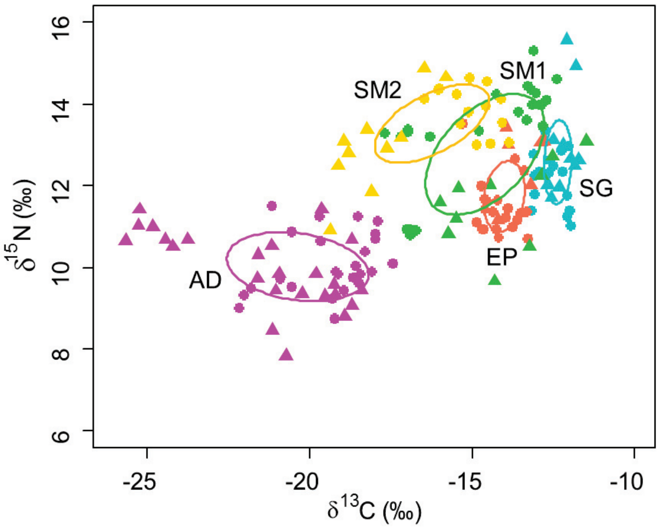

**Figure 5.** Comparison of the overall isotopic niche (expressed as SEAc) of *A. fasciatus* populations sampled in both seasons across all the habitats investigated. Circles are populations sampled in spring, triangles in autumn.

Lastly, looking at the C range, there is a remarkable difference between lagoons, as the populations in Venice seems to depend on a wider diversity of basal sources (higher CR) in comparison to the Stagnone di Marsala that is concentrated around narrower C ranges.

**Table 6.** Community-wide metrics of *A. fasciatus* calculated on the whole populations sampled at each habitat site.

| | SEAc | NR | CR | CD | NND | SDNND |
|---|---|---|---|---|---|---|
| **SG** | 1.43 | 4.53 | 1.41 | 0.88 | 0.27 | 0.16 |
| **EP** | 1.76 | 2.82 | 2.54 | 0.94 | 0.28 | 0.25 |
| **AD** | 5.73 | 3.67 | 8.20 | 1.99 | 0.36 | 0.19 |
| **SM1** | 7.65 | 5.66 | 6.24 | 2.22 | 0.46 | 0.39 |
| **SM2** | 4.53 | 3.96 | 5.50 | 1.79 | 0.52 | 0.31 |

The shift observed between the the artificial habitat in Venice Lagoon (AD) and the other habitats examined was also mirrored in the mean trophic positions (TP) calculated for the different populations of *A. fasciatus* (Fig. 6). While in all the habitats the target species occupied a trophic position approximately between the third and the fourth trophic level, in AD the population laid beneath the third level.

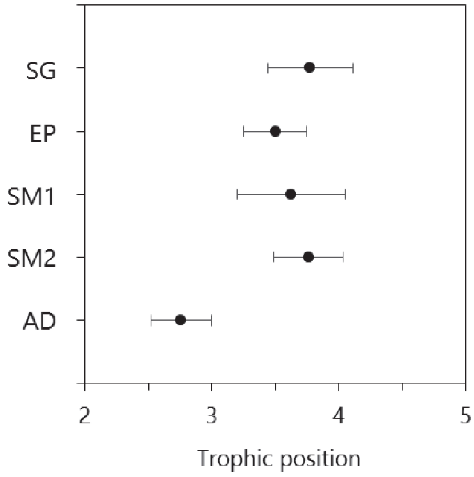

**Figure 6.** Trophic positions (TP) of *A. fasciatus* (mean ±s.d.) at each habitat site.

*Mixing models*

Results of the Bayesian mixing model, applied to estimate the contribution provided by the potential source of organic matter to the trophic pathway of *A. fasciatus* in each habitat investigated, are presented in Fig. 7.

At a general sight, the most likely contribution is given by the macrophytes in the natural habitats (SG, SM1 and SM2), whereas the role of SOM is remarkably higher in the artificial habitats of both lagoons (EP and AD).

Among the natural habitats, in SG and SM1 the main source supporting the trophic pathway were the only seagrass present in the site, respectively *C. nodosa* (mode contribution 32%) and *Z. noltei* (44%), followed by the algae species, respectively *C. prolifera* in SG (22%) and *U. rigida* in SM1 (39%). In SM2 seagrass and algae provide similar contribution (ranging from 12 to 17%).

Among the artificial habitats, as stated above, the role of the SOM is notable, as it provides likely the 46% of contribution in EP and the 20% in AD. However, two other sources seems of relevant importance in these different habitats: the epiphytic component reaches 37% in EP, while in AD the seagrass *R. maritima* represents the most relevant source, with 46% of contribution. Certainly, the contribution of the halophytes is marginal in all the habitats (mode <2%), even in the ones dominated by the terrestrial plants, namely the salt marshes SM1 and SM2.

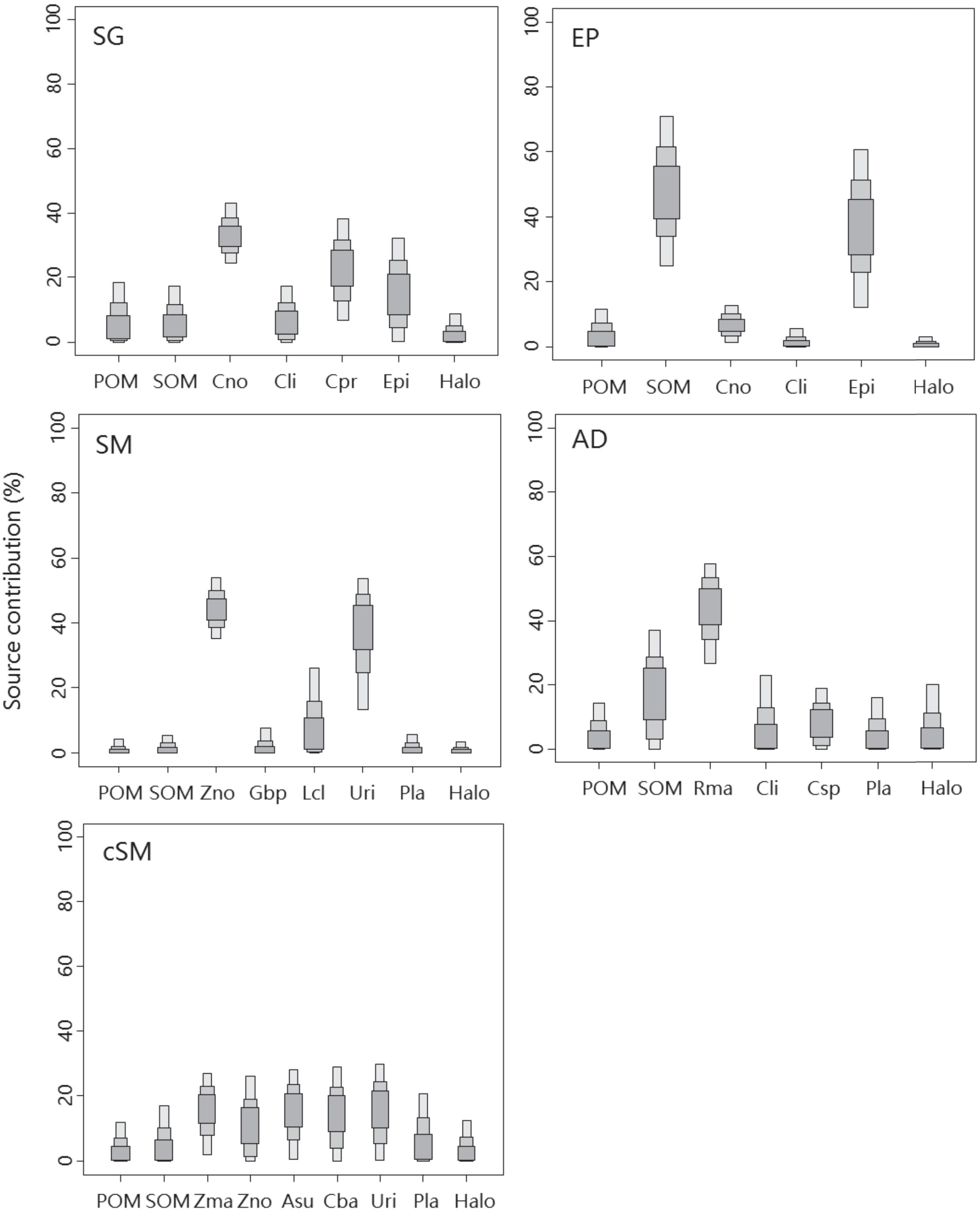

**Figure. 7.** Percentage contribution of organic matter sources for *A. fasciatus* in the habitat investigated and relative credibility intervals of 95% (light grey), 75% (intermediate grey) and 50% (dark grey), where SG, seagrass meadow and EP, evaporation pond in Stagnone di Marsala; SM1 and SM2, salt mash, AD, artificial ditch in Venice Lagoon. Sources are ordered as follows: POM and SOM, respectively particulate and sedimentary organic matter; seagrasses (Cno *C.* nodosa, Zma *Z. marina*, Zno *Z. noltei*, Rma *R. maritima*), macroalgae (Asu *A. subulata*, Cpr *C. prolifera*, Cli *C. linum*, Cla *Cladophora* sp., Cba *C. barbata*, Gbp *G. b. pastoris*, Lcl *L. clavellosa*, Uri *U. rigida*), Epi: epiphytes or Pla, plankton and Halo: halophytes.

## 1.4. Discussion

This study investigates the variability of the trophic structure of resident fish populations occurring in a variety of habitats of two Mediterranean coastal lagoons, with a particular focus on a target species of high ecological value, the Mediterranean toothcarp *Aphanius fasciatus*. Four habitat types were considered to represent the heterogeneity of coastal lagoons, bringing attention also to some artificial habitats commonly found in such environments, to infer their important role in maintaining the preservation of these populations (Cavraro *et al.* 2014a).

A first assessment of the structure of the whole fish communities occurring in these habitats allowed to characterize the community context within which the target species was inserted. In parallel, the stable isotope approach and the interpretation through the most recent descriptors (community-wide metrics) and models (mixing model) allowed to describe its trophic niche and the pathways of the organic matter that support the species in each specific habitat.

Overall, the descriptors used showed clear differences at inter- and intra-lagoon level, highlighting the variability of the trophic niche of *A. fasciatus* as well as the variability of the resource use among the habitats examined. These results provide important information on the trophic processes occurring in coastal lagoons, which should be consulted in a management perspective, not only of species of conservation interest, but also of such lagoon habitats, either natural or artificial.

### *Structural organization of fish assemblages at inter-and intra-lagoon level*

Habitats within the two lagoons supported different fish assemblages in terms of species diversity and density. The highest species diversity was recorded respectively in the inner salt marsh (SM2, with 6 species found) in Venice Lagoon and in the evaporation pond (EP, with 4 species found) in Stagnone di Marsala. The habitats hosting the highest fish densities were the seagrass meadow (SG) and the artificial ditch (AD) in spring and the evaporation pond (EP) and the enclosed intertidal creek of the inner salt marsh (SM2) in autumn, both respectively in Stagnone di Marsala and the Venice Lagoon, suggesting a high seasonal variability at intra-lagoon level.

In all of the habitats investigated, the target populations of *A. fasciatus* were dominant in spring (only exception in the first salt marsh, SM1) and decreased in autumn and in terms of size distribution, were mainly shifted towards the smallest size classes (SL mostly <25 mm). In Venice Lagoon, the most enclosed habitats, both the artificial and the natural channels in Vignole and Baccan salt marsh (AD and SM2) represented the most suitable habitats to support high densities of the species and the same role was played by the seagrass meadow (SG) in the Stagnone di Marsala in spring. .

The second most abundant population was *Atherina boyeri*, the most ubiquitous species of coastal lagoons (Franzoi *et al.* 2010) that dominated all of the habitats in autumn, and the SM1 in spring as well.

The seasonal fluctuation in densities and size class structure has been extensively documented for the target species in particular (Leonardos & Petridis 1996; Cavraro 2013) and more in general for fish assemblages of resident species (*e.g.* Franco *et al.* 2006b; Manzo 2010) and is mainly related to the spawning and the recruiting period of the species. Furthermore, the structure of fish populations in coastal lagoons is highly influenced not only by the environmental complexity, but also by the degree of marine influence (confinement), and the input of freshwaters (Poizat *et al.* 2004). This can also induce a high variability in species diversity in these systems, where few taxa persist in high abundance and are able to tolerate wide ranges of environmental fluctuation (Franco *et al.* 2006b).

### *Trophic organization of fish assemblages at inter- and intra-lagoon level*

The distribution of a species individuals within the isotopic space, namely the biplot $\delta^{13}C$-$\delta^{15}N$, is used to quantify the width of the isotopic niche and therefore represents a valid descriptor of the trophic niche, although by definition isotopic niche and trophic niche can not be considered identical (Jackson *et al.* 2011).

The interpretation of stable isotope patterns and their spatial variation within the isotopic space cannot disregard from the assessment of the community context (Cummings *et al.* 2012). As such, when describing the trophic niche of a fish population by interpreting the pattern of its isotopic niche space, it is appropriate to relate it to the context of the other species in the same community. Here, we assessed the variability of the isotopic niche width and relative metrics of the target species, at first relating it at community level within each different habitat, and then comparing the different populations among habitats.

Main differences at overall community level occurred between the two study areas and within each, between artificial and natural habitats. The higher niche widths (higher SEAc) of the communities recorded in the Lagoon of Venice is mainly attributable to the coexistence of a higher number of species, supported not only by more heterogeneus habitats, but also by a higher variety in basal food sources.

Comparing the isotopic niche metrics among species, the major differences in the metrics occurred when few (two) species were present in the habitat selected. In habitats such as EP and SM2 characterized by higher species diversity, instead, negligible differences were reported in the trophic structure (comparable values of most of the metrics) in spring, but evident differences emerged for some species during the autumn season. In fact, for example *A. fasciatus*, *A. boyeri* and *L. saliens* in SM2, in autumn evidently found suitable conditions to expand their isotopic niches (higher SEAc), to increase the trophic diversity (higher CD) and reduce the redundancy (NND), in comparison to the other species occurring in the same habitat. This suggests that competitive interactions mainly emerge in the autumn season, when food availability is more scarce and species with higher trophic plasticity are able to differentiate more from the others, probably accessing to a wider diversity of sources (Vizzini & Mazzola 2003).

*A. fasciatus* is an omnivorous species, it mainly feeds on small benthic invertebrates, such as small crustaceans, chironomid larvae, unicellular and multicellular algae such as diatoms (Leonardos 2008). In agreement with the omnivorous feeding habit, the killifish showed considerable variability of isotopic niche at both inter- and intra-lagoon level. Indeed, the isotopic signature of a consumer species, hence the overall population niche, is strictly dependent on the signatures of its basal sources (Vizzini & Mazzola 2006), that in this study were significantly more $^{13}$C-depleted in Venice lagoon than in the Stagnone di Marsala, and within each lagoon, in the artificial more than in the natural habitats. On the other hand, the distribution of the individuals within the isotopic space, described as trophic diversity and redundancy of the population, is entirely dependent on the individual specialization (Matthews & Mazumder 2004; Cummings *et al.* 2012). Two interesting observations emerged from the isotopic niche analysis at community level: (i) the isotopic niche of *A. fasciatus* was clearly separated from the niche of the other species, when the community was composed by few species; (ii) the width of the isotopic niche (SEAc) was higher in autumn, when the species exhibited the lowest density.

(i) The first case occurred in three habitats, the artificial ditch AD and salt marsh SM1 in Venice lagoon and the seagrass meadow SG in Stagnone di Marsala (despite a small overlap in spring). In AD, *K. panizzae* is the only one other species with which *A. fasciatus* shares its habitat and is realistically the only one throughout the year since the site has no communication with the external waters of the lagoon. Whereas the other species found in the two natural habitats were, respectively, *A. boyeri* in SG and *L. saliens* (juv) in the SM1. However, the intrinsic nature of these habitats as open structures does not allow us to argue that these assemblages remain unvaried throughout the year. Anyways, these results suggest a good allocation of the resources among co-occurring species. This is particularly likely in AD, where *K. panizzae*, which is invertivorous (Franco *et al.* 2006b), shows markedly higher values of $\delta^{15}N$, indicating a diet based on higher trophic levels (Vizzini & Mazzola 2008), while *A. fasciatus*, which is omnivorous (Leonardos 2008), is able to shift its diet on lower trophic levels. This is not true for the other two habitats, where the main difference occurs along the carbon axis, indicating instead a different use of alternative basal sources (Vizzini & Mazzola 2008). Conversely, in the habitats that exhibited the highest number of species, evaporation pond EP and the inner salt marsh SM2, the isotopic niche of *A. fasciatus* was greatly overlapped with the other species of the community, especially with *A. boyeri*. This can be addressed to high competition processes, enhanced in an enclosed habitat like the EP, where sources can be limiting or restricted to a lower prey diversity.

(ii) In the second case, the seasonal trend occurred in all the habitats investigated. Previous studies assert that niche width can increase or because the whole population uses a wider range of all the available resources, or either because increases the individual specialization of the population members (Bolnick *et al.* 2003; Cummings *et al.* 2012). We also speculate that enlarging the isotopic niche when the population density is very low could be a mechanism of preservation of the trophic niche space, exerted by a species with a high trophic plasticity to face a period of decline of the population. However, further study would be necessary to confirm this hypothesis.

Evaluating the isotopic niche only looking at the width would lead to erroneous conclusions, as it has been demonstrated that, a wider niche does not necessarily correspond to a more generalist behaviour while a smaller niche to a specialist as previously thought (Bolnick *et al.* 2003; Matthews & Mazumder 2004).

When comparing the isotopic niche of annual populations of *A. fasciatus* among habitats, in fact, the single metrics accounting for individual spacing within the niche help to interpret the main results. Overall, the main difference occurred in the isotopic niche width between the two study areas, with the populations in the

Stagnone exhibiting the narrowest ones. Niches of the habitats within the same lagoon were shifted in position and different in shapes; however, looking at the metrics, especially the CD and MNND accounting for trophic diversity and redundancy, did not vary greatly among habitats within the same lagoon.

At a comprehensive look, hence, results indicate that the habitat structure influences the specific population, that will also occupy different trophic position in dependence of the specific habitat (lower in artificial habitats than in natural ones according to our results), but it does not influence the overall ecological role of the species within the same lagoon.

*Basal sources supporting fish species*

An overall depletion of both isotopic ratios has emerged in the Venice Lagoon than in Marsala and can be addressed to a main difference in the main abiotic factors characterising the two lagoons, from the level of salinity to the level of confinement.

In particular, the extremely lower values of $\delta^{13}C$ in the AD of Vignole are noteworthy and differentiate this habitat from all the others investigated. The reason for this strong depletion may be addressed to some terrigenous input of organic matter (Perdue & Koprivnjak 2007), probably related to the periodic digging of the channels investigated. The same pattern results in the trophic structure and trophic pathways involving the fish communities investigated, that vary accordingly to the main differences found at the basal sources level.

Estimating the spatio-temporal changes in resource use is crucial to understand the trophic structure of a given habitat (Nordström *et al.* 2015).

Results from Bayesian mixing models revealed the different role of organic matter sources in the different habitats of the Venice Lagoon and the Stagnone di Marsala.

While the main trophic pathway of *A. fasciatus* seems definitely driven by the macrophytes in the natural habitats (SG, SM1 and SM2), in the artificial habitats of both lagoons the role of SOM gives an important contribution.

With specific differences among habitats, given by the different set of basal sources available, overall, seagrasses, followed by few macroalgae species represent the main end-member of the trophic pathway. Despite the direct utilization of these sources is generally low because of the high content of deterrents (such as phenols) and high C/N (Poore *et al.* 2012), macrophytes play a central role in sustaining the flow energy of the trophic pathways in coastal lagoons (Vizzini 2009) and often a high proportion of them enters back in the food web across the detritus pathway (Vizzini & Mazzola 2008).

This was fully expected in the SG habitat, while in the salt marshes, SM1 and SM2, where there was a patchy distribution of the macrophytes, this demonstrated that their role is not density dependent. Conversely, halophytes that in general are considered important sources in lagoon food webs, given their extensive distribution along the border of salt marshes for example (Short & Neckles 1999), in this study they only played a very marginal role in the flow energy to the target species, accounting only for very low percentage of contribution in all the habitats. As well as for the planktonic-pathway, which can be excluded in these habitats, as the contribution estimated was very low.

In the EP, habitat structurally enclosed and characterized by patchy primary producers, the greatest contribution is given by SOM and epiphytes. In AD the only seagrass specie present drives the whole trophic pathway, followed by the SOM. Hence, the role of SOM increases when the local conditions enhance frequent event of resuspension and sedimentation (Vizzini *et al.* 2005). This is also in accordance with Rossi *et al.* (2015) who found that sites with macrophytes present in patches, the trophic role of primary producers decreases, while increases that of SOM and POM. As a consequence the use of sources at the base of the trophic webs that support the fish species is habitat-specific, depending not only on the specific composition of the basal sources available, but also on the abiotic factor that characterize the structure of such habitats.

## 1.5. Conclusion

In conclusion, this study allowed to broaden knowledge on how natural and artificial systems support fish populations living within coastal lagoons.

All the habitats examined supported fish communities typical of lagoonal ecosystems and in relation to the target species *A. fasciatus*, different habitats shaped differently the trophic structure of specific populations, without influencing the overall ecological role of the species.

Habitats characterized by higher diversity of basal sources promote the coexistence of more species, while in habitats with lower source diversity but high confinement level, *A. fasciatus* population reach notable abundances. This resulted particularly true for the population examined in the artificial ditch in Venice Lagoon, highlighting the importance of such artificial habitats that could represent hotspots for conservation purposes of the target species (Cavraro *et al.* 2014a). In line with the aims of the Habitat Directive, the conservation of threatened species necessarily implies the preservation and fair management of the habitats where the species live and reproduce. Hence, our outcome induce us to pose major attention to this kind of artificial habitats, frequently present in coastal lagoons where are managed for multiple coexisting activities (*e.g.* salt production, aquaculture) or even more marginal, that still need to be surveyed (Pérez-Ruzafa, Marcos & Pérez-Ruzafa 2011).

# Chapter 2

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## Habitat use and isotopic niche variability during ontogeny of the gilthead seabream *Sparus aurata* within a coastal lagoon

### Abstract

The gilthead seabream *Sparus aurata* is known to enter coastal lagoons and estuaries during the first stages of post-larval and juvenile development. The high habitat heterogeneity and shallowness that characterize such ecosystems offer plentiful food and shelter for this species that hence undergoes rapid growth. The juvenile stage development occurs through stepwise changes in anatomy, physiology, and behaviour that is linked strongly to ontogenetic changes in habitat and resource use. Previous works based on morphology studies of dental apparatus assigned post-larvae to the feeding group of zooplanktivorous, juveniles to the micro/meiobenthivorous group and sub-adults to the macrobenthivorous group.

We used carbon and nitrogen stable isotopes to assess: (1) the ontogenetic changes of carbon and nitrogen stable isotope signatures related to the diet shift occurring during ontogeny of *S. aurata* and the reported morphological changes; (2) how they reflect in trophic niche variability and trophic position of the species; (3) the role of different habitats in supporting the trophic pathway leading to *S. aurata* over early stages of development within a coastal lagoon.

Fish and organic matter sources were sampled in spring and summer 2014 and 2015, in different sites of the Venice Lagoon representing salt marsh habitats located along a confinement gradient. Bayesian mixing models and community-wide metrics were applied to the four size classes detected, according to standard length (SL): post-larvae, SL<20 mm, juveniles I 20<SL<40 mm, juveniles II, 40<SL<60 mm and sub-adults SL>60 mm. Organic matter sources considered were zooplankton, particulate and sedimentary organic matter (POM and SOM), seagrasses, macroalgae and halophytes. We observed a clear shift in source contribution through size classes, with slight differences among habitats. The greatest contribution to the trophic pathway of *S. aurata* shifted from a mix of POM, SOM and zooplankton for the post-larval stage, to mainly seagrasses for the middle stages, to a mix of macroalgae and seagrasses for the sub-adults, characterised also by a high trophic plasticity. Additionally, the shift manifest in the isotopic niche, which varied in shape, width and position across size classes, with the main differences occurring between post-larvae and juveniles/sub-adults, in accordance to their diet shift that goes respectively from zooplanktivorous to zoobenthivorous habits. This study emphasises the important trophic role that coastal lagoon habitats provide to the early stages of *S. aurata* life cycle and links it to the implications that may result in terms of coastal management.

## 2.1. Introduction

Coastal lagoons are important nursery areas for many aquatic species, either vertebrates or invertebrates, to which they provide suitable conditions for safe development and rapid growth (Beck *et al.* 2001). A number of different lagoonal habitats, such as salt marshes, intertidal creeks or seagrass meadows, constitute highly productive systems where plentiful availability of food sources grants an optimal foraging and, at the same time, their shallow waters assure an ideal shelter from predators (Able 2005; Franco *et al.* 2006a). As such, they play a central role in maintaining high densities of juveniles, contributing to the biodiversity also of adjacent environments, either marine or freshwater (Tagliapietra *et al.* 2014), as well as the connectivity among these systems.

Fish species using lagoonal habitats as nursery areas, usually are included into the ecological group of marine migrants (Franco *et al.* 2008a), which spend only the early stages of their life cycle within the lagoon, entering as post-larvae and leaving once reached the sub-adult phase.

The gilthead seabream *Sparus aurata* Linnaeus, 1758 is one of the species representing the category of marine migrant widely distributed in the Mediterranean Sea, in the Eastern coasts of the Atlantic Ocean and rarely also in the Black Sea (Bauchot & Hureau 1986). *S. aurata* is a euryhaline and eurythermal species whose juvenile stage development is characterized by stepwise changes in anatomy, physiology, and behaviour, that is strictly linked to ontogenetic changes in habitat and resource use (Cataldi *et al.* 1987; Tancioni *et al.* 2003). Research interest on this species is due not only to its high ecological value, but also to its considerable commercial importance and use in aquaculture (FAO 2016).

Many studies in the past decades have assessed the morphological changes occurring during ontogeny of *S. aurata*, providing detailed knowledge about the development of body structures such as the digestive tract (Elbal *et al.* 2004), the oral cavity with the consequent teeth-age adaptation (Cataldi *et al.* 1987; Elgendy, Alsafy & Tanekhy 2016), the gonadal cycle (Zohar, Abraham & Gordin 1978) or more in general the progressive changes of the body shape (Russo *et al.* 2007). In all of these studies, authors agree that, as well as for other species, slight variations during ontogeny promote progressive capability to perform new behaviours related, above all, to feeding habits and swimming ability (Russo *et al.* 2007; Elgendy *et al.* 2016).

Diet of *S. aurata* has been demonstrated to shift from zooplankton during larval stage, to micro-, meio- and macrozoobenthos once reached the juvenile and adult stages (Ferrari & Chieregato 1981; Cataldi *et al.* 1987; Russo *et al.* 2007; Elgendy *et al.* 2016). In particular, according to Elbal *et al.* (2004) and Cataldi *et al.* (1987), post-larvae up to 20 mm in standard length show only canine teeth and a gastric channel barely developed, that only allow ingestion and digestion of small planktonic preys. In juveniles between 25-35 mm, three concentric rows of canine teeth and the presence of "transitional teeth" that will develop in molars promote a microbenthivorous diet. In adults, strong molars in addition to canines allow to prey on hard preys such as decapods, gastropods and bivalves (Elgendy *et al.* 2016). As a consequence, these changes result in ontogenetic shifts not only in resource but also in habitat use (Pita, Gamito & Erzini 2002; Tancioni *et al.* 2003; Gratwicke, Petrovic & Speight 2006). Few studies, however, have deepened the relationship between resource use and habitat use within the coastal lagoonal environment and very little research have faced this topic in terms of trophic support provided by such systems, in order to trace the trophic pathway of this species that shows high trophic plasticity during ontogeny.

Stable isotope analysis has been extensively used as a useful tool to trace the pathways of organic matter in a wide variety of systems and more recently new applications have also allowed to describe the variability of the trophic niche of a species across time and space (Layman *et al.* 2007a; Jackson *et al.* 2011).

Hence, the aim of this study was to assess: (1) the ontogenetic changes of carbon and nitrogen stable isotope signatures related to the diet shift occurring during ontogeny of *S. aurata*; (2) how they reflect in trophic niche variability and trophic position of the species; (3) the role of different habitats in supporting the trophic pathway leading to *S. aurata* over early stages of development within a coastal lagoon.

We hypothesize that shifts in feeding habits induced by changes in morphology during ontogenetic development, should correspond to a visible differentiation of the isotopic niches of the main size classes previously detected. Particularly, we expect to observe isotopic shifts between the following size classes defined according to Cataldi *et al.* (1987) and Elbal *et al.* (2004): post-larvae (SL<20 mm), juveniles I (20<SL<40 mm), juveniles II (40<SL<60 mm), sub-adults (SL>60 mm).

For this purpose, trophic niche descriptors and Bayesian mixing models allowed to assess the dynamics of ontogenetic shifts occurring at two different levels: the first merely related to the morphological changes (looking at different size classes of the species), the second related to the use of different habitats, to understand their role in supporting the different stages of development of the species.

## 2.2. Materials and methods

*Study area and sampling sites*

The lagoon of Venice was chosen as study area (see chapter 1 for full description of the Venice Lagoon) as it represents an important nursery area of *S. aurata* in the Northern Adriatic Sea (Zucchetta 2009), and field samplings were conducted in 2014 and 2015.

Fish were collected through a small beach seine (8-20 m long, 2 mm mesh size), following the migration path of *Sparus aurata* into the lagoon (Fig. 1). In spring 2014, post-larvae were collected first in a marine site located outside the lagoon, in proximity of the Northern mouth (M1). Afterwards, further post-larvae and bigger sizes were collected in three different salt marsh areas (SM1-3) located within the Northern sub-basin of the lagoon, at different distance from the mouth.

**Figure 1** Study area and sampling sites. M1-3 marine sites located in proximity of the northern (M1-2) and the central (M3) mouth of the lagoon. SM1-3 salt marsh sites located within the lagoon, at three different distances from the northern mouth. SM1c and SM2c are the intertidal creeks flowing within the corresponding salt marsh sites.

In spring 2014 *S. aurata* was sampled in a small intertidal creek flowing in the salt marsh nearest to the mouth (SM1c), in the salt marsh located at intermediate distance from the mouth (SM2) and also in the relative intertidal creek (SM2c). In spring 2015 fish were collected in the salt marsh located in the farthest area (SM3).

Finally, in summer 2015, sub-adults of

*S. aurata* were collected by angling in two additional sites placed in the northern and central mouths of the lagoon, (respectively named M2 and M3).

### *Sample collection*

Sources of organic matter were collected in the three salt marsh sites (SM1-3) located within the Northern sub-basin.

Seagrasses, macroalgae and halophytes were collected randomly by hand in triplicate. Regarding the halophytes, only the most abundant species were collected and considered for trophic assessments. Sedimentary and particulate organic matter (respectively SOM and POM) were obtained by sampling three replicates of superficial sediment cores (3 cm Ø) and 2 L of superficial water respectively. Zooplankton was sampled by horizontal haul with a small plankton net with a mesh size 160 µm. All the samples collected were maintained refrigerated in thermic boxes until arriving at the laboratory and stored at -20°C prior to the processing.

### *Sample processing and laboratory analysis*

Fish sampled were identified at species level and measured for standard length (SL) and total weight (TW). According to the trophic ontogenetic shift previously observed on the basis of the morphological changes (*e.g.* Cataldi *et al.* 1987; Elbal *et al.* 2004), four size classes were *a priori* defined as follows:

I. post-larvae, SL<20 mm;

II. juveniles I 20<SL<40 mm;

III. juveniles II, 40<SL<60 mm;

IV. sub-adults SL>60 mm.

Whenever available, at least 15 individuals for each size class were dissected using scalpel and tweezers to extract the dorsal muscle.

Primary producers (seagrasses, macroalgae and halophytes) were identified at species level and gently scraped to remove epiphytes. Water samples collected for POM were pre-filtered (200 μm) and then filtered on GF/F Whatman filters (pore size 0.7 μm) previously combusted at 450°C (4h). Sediment cores sampled for SOM were sliced to the top 1.5 cm and homogenized. Zooplankton samples were concentrated in eppendorf tubes after accurate cleaning to remove any detrital material under binocular microscopy.

After processing, all fish and source samples designed for isotopic analysis were oven dried (60°C, 48h) to constant weight and ground with a micro mill or a mortar and pestle to obtain a fine powder. An aliquot of each replicate of primary producers, SOM and POM was acidified with HCl 1M in order to dissolve carbonates potentially affecting the carbon isotopic signature. Each sample, encapsulated in tin cups, was analysed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo-Electron Delta Plus XP) coupled to an elemental analyser (Thermo-Electron Flash EA1112).

Carbon and nitrogen stable isotope ratios were reported based on the following equation: $\delta X=[(R_{sample}/R_{standard})-1]\times10^3$, where the $\delta$ notation expresses the parts per mil deviation from the reference international standards (Pee Dee Belemnite and atmospheric $N_2$, respectively for carbon and nitrogen), X is $^{13}C$ or $^{15}N$ and R is the relative $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The analytical precision of the measurement was 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively. Analysis was conducted on single individuals of fish, while only for fish collected in SN (size class I) it was necessary to pool at least 5 individuals to reach the minimum analytical weight.

### *Data analysis*

Carbon and nitrogen isotopic data of *S. aurata* were analysed separately. Normality and homogeneity of variances were tested using respectively Shapiro-Wilk and Bartlett tests. Since none of the data showed suitable conditions for using parametric tests, non parametric Kruskal-Wallis test and relative post-hoc tests were used to assess differences in multiple comparisons among fish size classes or sites. All tests were performed in R (R Core Team, 2015), using a significance level of $p < 0{\cdot}05$.

Trophic positions (TP) were estimated using the following equation according to Post (2002): $TP_f = [(\delta^{15}N_f - \delta^{15}N_b) / \Delta N] + \lambda$. $\delta^{15}N_f$ and $\delta^{15}N_b$ are respectively the nitrogen isotopic signature of the fish species of interest and that of a baseline, for which we used the mean value of zooplankton samples (7.2‰). $\Delta N$ is the trophic enrichment expected for each trophic level (3.4‰) and $\lambda$ is the trophic position of the baseline. According to the ontogenetic shift of the species, *S. aurata* is a direct consumer of zooplankton until approximately the size of 20-30 mm in SL (Ferrari & Chieregato 1981), therefore we used $\lambda=1$ for individuals of the first size class and $\lambda=2$ for bigger individuals.

To assess trophic niche variability across size classes and sites, carbon and nitrogen stable isotope data of each group (size class or site) were used to estimate corrected standard ellipse areas (SEAc, corrected for small sample size) through Bayesian statistics. SEAc were set to contain 60% of the data and were elaborated with R package SIBER v2.0.2 (Stable Isotope Bayesian Ellipses in R) (Jackson *et al.* 2011). Moreover, community-wide metrics were calculated using the R package SIAR 4.2.2 (Parnell et al., 2010), in order to quantify the following descriptors of the isotopic niche: $\delta^{15}N$ Range (NR), $\delta^{13}C$ Range (CR), mean Distance to Centroid (CD), mean Nearest Neighbour Distance (NND), Standard Deviation of the Nearest Neighbour Distance (SDNND) (see chapter 1 for further details on each metrics).

Lastly, Bayesian mixing models were applied for each habitat selected and the four size classes detected, with the purpose to trace the trophic pathway supporting the species at different development stages and investigate the role of different habitats. Models were run with the R SIAR package previously mentioned, using as basal sources SOM, POM, zooplankton, halophytes when present and the seagrasses and algae species found in each site. Trophic enrichment factors (TEFs) used for the model were 2.5‰±1 for $\delta^{15}N$ (Vander Zanden & Rasmussen 2001) and 0.4‰±1.3, for $\delta^{13}C$ (Post 2002) and were multiplied by two to consider fish as second level consumer.

## 2.3. Results

*Demographic characteristics*

Throughout all the sampling sites a total of 174 individuals of *Sparus aurata* were collected, with main biometrics specific for each site reported in Table 1. Following the migration of the species towards the Venice Lagoon, in spring 2014 the smallest post-larvae (size class I), with mean SL 15.00 ± 0.98 mm, were found in the marine site located next to the northern mouth of the lagoon (M1), just before entering the lagoon. A further group of post-larvae were then found within the lagoon in the salt marsh site SM2, at intermediate distance from the mouth and had mean SL 17.60±2.47 mm. Later in the same season, the first class juveniles (size class II) were collected mainly within the two intertidal creeks of both salt mashes SM1c and SM2c (mean SL respectively 24.32±3.43 mm and 27.30±3.48 mm). The second class juveniles (size class III) were collected the following year, in spring 2015 in the most inner salt marsh site, SM3 and were 49.82±4.15 mm long on average. Lastly, sub-adults (size class IV) were caught in summer 2015 at both northern (M2) and central (M3) mouths of the lagoon and had a SL of 191.25±8.63 mm and 182.8±7.80 mm respectively. From the read of the dorsal scales of the sub-adults, we also estimated that they belonged to the same population of the individuals collected in spring 2014, as the number of the circles areas corresponded to 1+ age.

**Table 1.** Size class, standard length (SL, mm ± s.d.) and total weight (TW, g ± s.d.) of *S. aurata* collected in each sampling site. M1-3 are the sites located outside the lagoon, SM1-3 and SM2c are the salt marsh sites located within the lagoon.

| Site | Sampling season | n | Size class | SL | TW |
|---|---|---|---|---|---|
| **M1** | Spring 2014 | 63 | I | 15.00 ± 0.98 | 0.06 ± 0.01 |
| **SM1c** | Spring 2014 | 15 | II | 27.30 ± 3.48 | 0.54 ± 0.20 |
| **SM2** | Spring 2014 | 5 | I | 17.60 ± 2.47 | 0.13 ± 0.05 |
| **SM2c** | Spring 2014 | 57 | II | 24.32 ± 3.43 | 0.42 ± 0.17 |
| **SM3** | Spring 2015 | 16 | III | 49.82 ± 4.15 | 3.55 ± 0.88 |
| **M2** | Summer 2015 | 8 | IV | 191.25 ± 8.63 | 213.67 ± 37.82 |
| **M3** | Summer 2015 | 10 | IV | 182.80 ± 7.80 | 186.53 ± 15.38 |

### *Ontogenetic shift of isotopic signatures, isotopic niche and trophic position*

All the individuals of *S. aurata* analysed for stable isotopes were included within the range between -21.35 and -12.79‰ for $\delta^{13}$C and between 8.12 and 14.48‰ for $\delta^{15}$N (Fig. 3). Looking closely to the relationship between body length and isotopic signatures, there was evidence of a significant trend for the first stage of development of *S. aurata*. Indeed post-larvae (size class I) showed a steep enrichment, shifting towards higher values of both $\delta^{13}$C and $\delta^{15}$N as they grew up to approximately 20 mm in SL (Fig. 3). After this point, the trend becomes less marked and non-significant for the juvenile and adult stages. $\delta^{15}$N decreased slightly, while $\delta^{13}$C kept increasing slightly as the species reached the second phase of juvenile development (up to 60 mm SL, size class III). Lastly, sub-adults showed quite stable signatures for carbon and large variability for nitrogen without any specific trend.

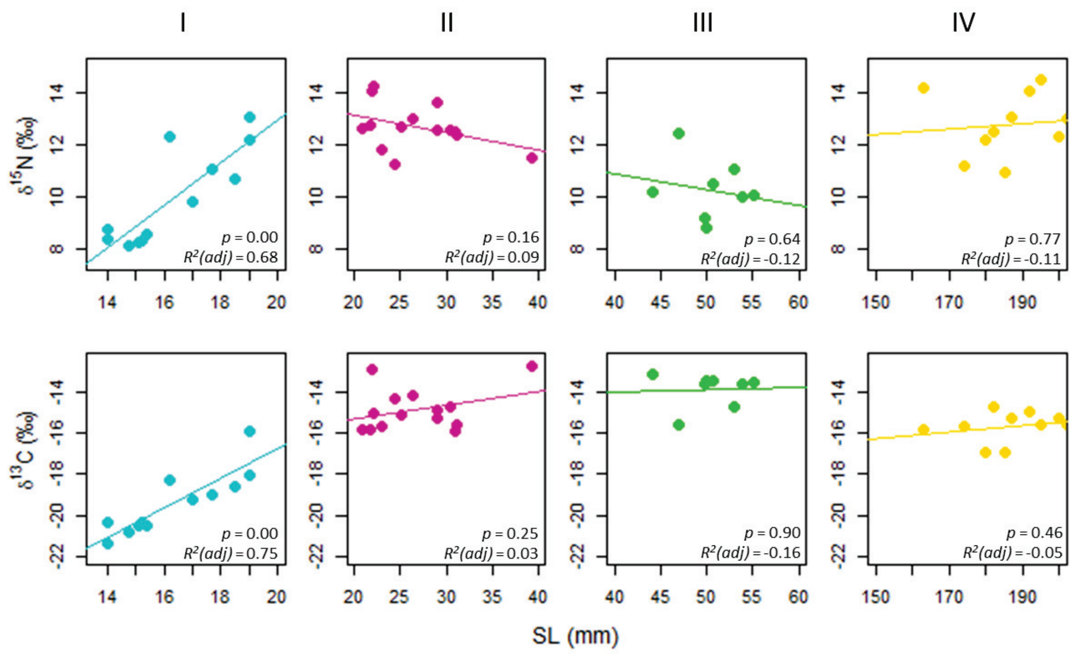


**Figure 3.** Relationship between standard length (SL) and isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) relative to the overall fish sampled and the different size classes. In each graph *p*-value and the adjusted R-squared are also reported.

However, apart form size class I, very little is explained by the linear regression for the older individuals, when considering all the classes separated (very low $R^2_{adj}$ and p-value>0.05). The best fit describing the early stage of development (size classes I-III) is given by a logarithmic curve for $\delta^{13}C$ and a second-order polynomial for $\delta^{15}N$, which explained 68% and 52% of the variability, respectively (Fig. 4).

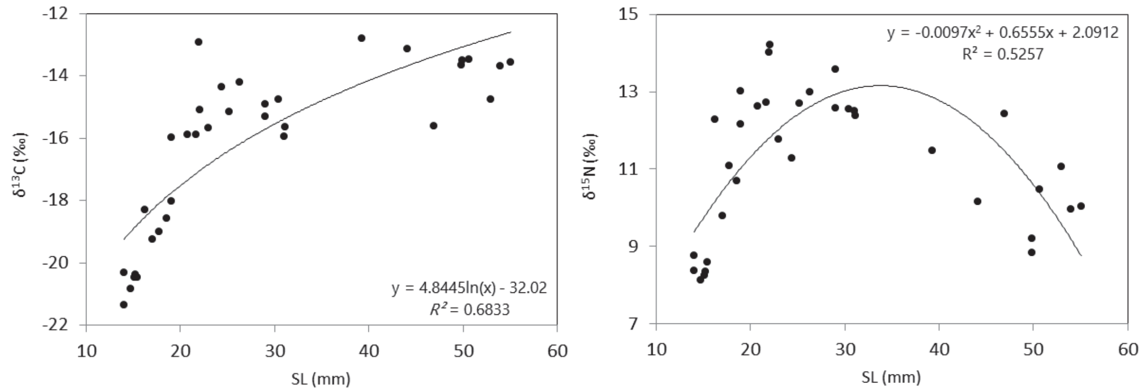


**Figure 4.** Relationship between standard length (SL) and isotopic signatures ($\delta^{13}C$ to the left and $\delta^{15}N$ to the right) of *S. aurata* for size classes I to III and related curves describing respectively a logarithmic and a second order polynomial trend.

The ontogenetic shift was observed looking also at the isotopic niche of each size class within the isotopic space (the biplot $\delta^{13}C$-$\delta^{15}N$), which varied in position, shape and size (Fig. 5). In fact, as the size class increased, the isotopic niche of *S. aurata* represented by the SEAc, moved along both axes, with a remarkable and significant shift from size I to II, towards more enriched isotopic signatures of both carbon and nitrogen (*Kruskal-Wallis post-hoc test*, p-value<0.05). Then, the niche still shifted to higher $\delta^{13}C$, but more depleted values of $\delta^{15}N$ in size class III, before significantly increasing in $\delta^{15}N$ once more with a slight depletion in $\delta^{13}C$ in size class IV.

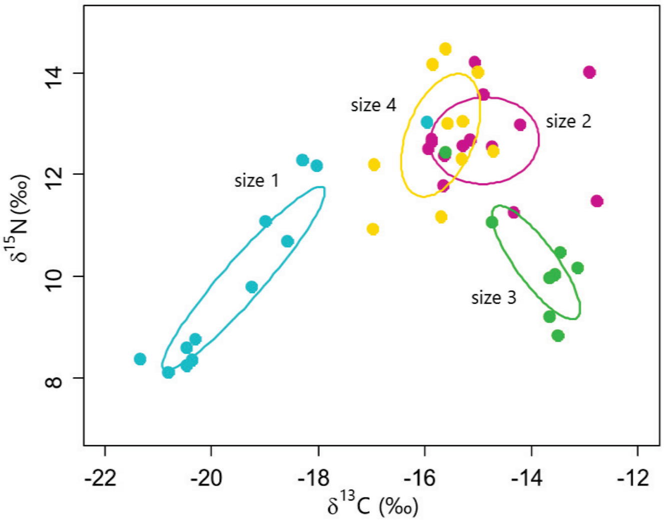


**Figure 5.** $\delta^{13}C$ (‰) *vs.* $\delta^{15}N$ (‰) of *Sparus aurata* samples. Different colours correspond to the different size classes: turquoise I size, magenta II size, green III size, yellow IV size. The isotopic niche of each size class is represented by the bayesian corrected Standard Ellipse Areas (SEAc).

As regards the width of the isotopic niche, values reported for SEAc indicated a progressive reduction from class I to III (respectively 3.13, 2.97 and 1.90) and then an increase in class IV (2.87, Table 2). *Post*-larvae (size class I) showed the highest ranges of both nitrogen and carbon isotope signatures (respectively NR and CR), with both values around 5‰, and also the highest trophic diversity of the individuals (distance from centroid CD= 2.02) in comparison to juveniles and *sub-*

adults (CD~ 1). Trophic redundancy (NND) and evenness of the individuals spacing within the niche (SDNND) were similar for the first three size classes (respectively values ~ 0.60 and 0.56) and only for class IV they were slightly higher (0.71) and lower (0.4) respectively (Table 2).

**Table 2.** Community-wide metrics calculated for each size class of *S. aurata*.

| | I | II | III | IV |
|---|---|---|---|---|
| **SEAc** | 3.13 | 2.97 | 1.90 | 2.87 |
| **NR** | 4.90 | 2.95 | 3.60 | 3.55 |
| **CR** | 5.38 | 3.15 | 2.48 | 2.25 |
| **CD** | 2.02 | 1.10 | 1.07 | 1.21 |
| **NND** | 0.57 | 0.61 | 0.62 | 0.71 |
| **SDNND** | 0.59 | 0.54 | 0.57 | 0.40 |

Trophic positions (TP) calculated at the individual level maintained the ontogenetic trend previously reported with a sharp increase during the early stages and then a sort of stabilization for the last stage cosidered. Values grouped by size class are reported in figure 6 and mean values varied from 1.81±0.53 for *post*-larvae, to 3.32±0.39 and 2.90±0.33 respectively for juveniles of classes I and II, while *sub*-adults occupied the highes position reaching 3.64±0.36.

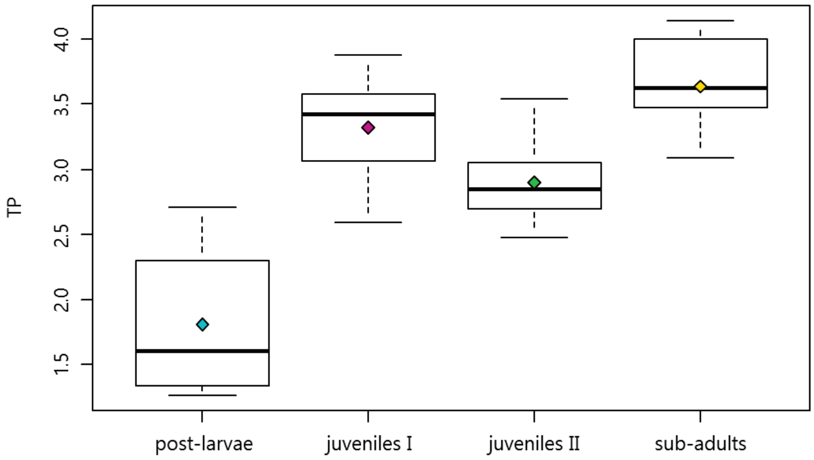


**Figure 6.** Boxplots showing trophic positions (TP) of *S. aurata* grouped by size class. In each box, horizontal line is the median, lower and upper sides of the box are 25th and 75th percentiles, lower and upper whiskers are min and max values, diamond is the mean.

*Inter-site variability of the isotopic niche*

The isotopic niche of *S. aurata* showed a remarkable variability across all the sites sampled and also within different portions of the same site (*e.g.* salt marsh *vs* intertidal creek of the same salt marsh) (Fig. 7).

Following the migration of the species, *post*-larvae catched in spring just before entering the lagoon, in M1, showed the smallest isotopic niche recorded (0.15), with the lowest values of all the relative descriptive metrics (from N and C ranges, to CD, NND and SDNND) (Table 3). Conversely, *post*-larvae that had just entered the lagoon and settled in the intermediate salt marsh (SM2) showed a wider isotopic niche (3.40), over a much extended range of both N and C (respectively 4.25‰ and 5.46‰), the highest level of trophic diversity (CD) and the lowest redundancy (highest NND and SDNND) in comparison to the other sites. Noticeable is the isotopic niche shift occurring within a different portion of the same salt marsh, the inner intertidal creek (SM2c), that hosts slightly bigger individuals (belonging to both *post*-larvae and I class juveniles). Although SEAc and CR are comparable to that of the external portion of the salt marsh, they result shifted towards a more isotopic-enriched area within the bidimensional space, and NR is compressed to approximately half of the range of the previous class. The other intertidal creek located within the first salt marsh (SM1c), instead, presented a more compressed isotopic niche of *S. aurata*, where individuals, exclusively belonging to I class juveniles, showed a close vicinity within the isotopic space, indicating not only a lower trophic diversity (CD), but also a higher redundancy and evenness of the spacing (NND and SDNND).

II class juveniles found in the most inner salt marsh considered, also characterised by a patchy distribution of seagrass, showed a niche (SEAc=2.92) mainly extended along the NR than the CR, shifted to more enriched $\delta^{13}C$ but more depleted $\delta^{15}N$.

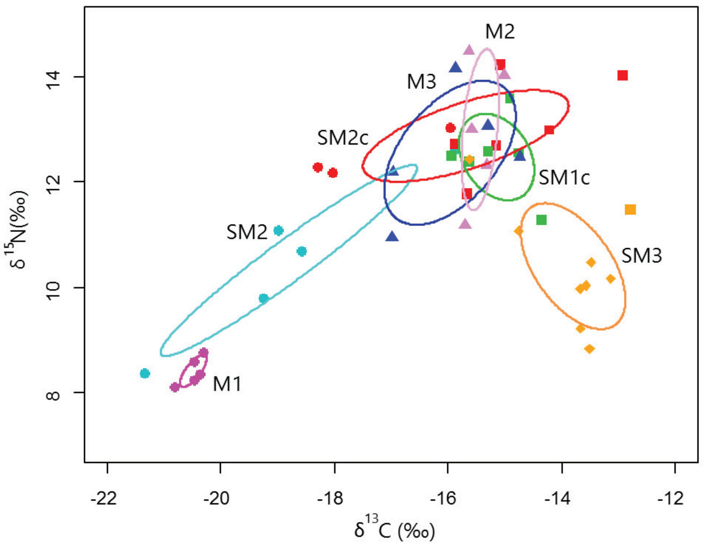


**Figure 7.** SEAc of *S. aurata* grouped by site. Following the chronological order of data collection, sites acronyms are: M1, marine site 1 next to the northern mouth; SM2, intermediate salt marsh; SM2c, intertidal creek of the intermediate salt marsh; SM1c, intertidal creek of the salt marsh nearest to the mouth; SM3, farthest salt marsh; M2, northern mouth and M3, central mouth of the Venice Lagoon. Size classes are highlighted by different symbols: class I circles, II squares, III diamonds, IV triangles.

Lastly, the two sites located at the mouths of the lagoon, the northern (M2) and the central one (M3), were the only points where the sub-adults could be intercepted and showed overlapped isotopic niches with a marked difference in the width extent (respectively 1.47 and 4.28), mainly due to the difference on the CR occupied, that also overlapped with the niches found in SM1c and SM2c. However, they show comparable values of the other metrics, indicating similar trophic structure in terms of diversity, redundancy and evenness of species spacing.

**Table 3.** Community-wide metrics calculated for *S. aurata* in each site sampled.

| | **M1** | **SM2** | **SM2c** | **SM1c** | **SM3** | **M2** | **M3** |
|---|---|---|---|---|---|---|---|
| **SEAc** | 0.15 | 3.40 | 3.71 | 1.62 | 2.92 | 1.47 | 4.28 |
| **NR** | 0.65 | 4.25 | 2.44 | 2.31 | 3.60 | 3.30 | 3.23 |
| **CR** | 0.52 | 5.46 | 5.38 | 1.59 | 2.83 | 0.69 | 2.25 |
| **CD** | 0.27 | 1.73 | 1.48 | 0.74 | 1.12 | 1.05 | 1.33 |
| **NND** | 0.23 | 1.62 | 0.78 | 0.66 | 0.69 | 0.84 | 1.08 |
| **SDNND** | 0.10 | 1.24 | 0.53 | 0.43 | 0.57 | 0.20 | 0.25 |

The inter-site shift in isotopic niche was corroborated by trophic position estimations (Fig. 8). TP values exhibited an increasing trend, following both the migration and the growth of the species. In fact, *S. aurata* had the lowest TP (mean 1.36) in M1, then it progressively raised during the growth in the inner sites of the lagoon (mean values 1.97 in SM2, 3.0 in SM2c, 3.55 in SM1c and 2.94 in SM3), until it reached the highest position, before leaving the lagoon (M2 and M3, mean respectively 3.70 and 3.57).

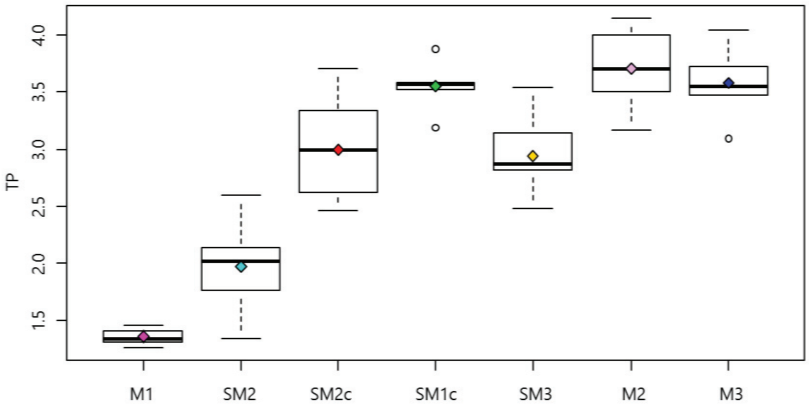


**Figure 8.** Boxplots showing trophic positions (TP) of *S. aurata* grouped by site. In each box, horizontal line is the median, lower and upper sides of the box are 25th and 75th percentiles, lower and upper whiskers are min and max values, diamond is the mean and circles are outliers.

### *Sources of organic matter and mixing models results*

Sites sampled for organic matter sources showed different macrophytes composition (Table 4.). In terms of species recorded, the most confined site (SM3) showed the highest diversity of both seagrasses and macroalgae, followed by the intermediate salt marsh (SM2), and the less confined salt marsh (SM1). The only species found in common to all the sites were the seagrass *Zostera noltei* Hornemann 1832 and the green alga *Ulva rigida* Agardh 1823. Regarding halophytes, 4 species were the most abundant in the sites investigated, with *Sarcocornia fruticosa* (L.) A. J. Scott present in all of them.

13.68±1.03‰ in SM2, and *U. rigida* varied in $\delta^{15}$N, with values ranging from 9.72±0.49‰ in SM3 to 11.66±0.69‰ in SM2.

**Table 4.** List of the species recorded in the three salt marsh examined with indication of their presence (+) or absence (-). For halophytes only, + indicates species pooled together for analysis and – does not necessarily means that the species was not present at the site.

| Species | Code | SM1 | SM2 | SM3 |
|---|---|---|---|---|
| **Seagrasses** | | | | |
| *Cymodocea nodosa* | Cno | - | - | + |
| *Ruppia maritima* | Rma | - | - | + |
| *Zostera marina* | Zma | - | + | - |
| *Zostera noltei* | Zno | + | + | + |
| **Macroalgae** | | | | |
| *Agardhiella subulata* | Asu | - | + | + |
| *Chondria coerulescens* | Cco | - | - | + |
| *Cystoseira barbata* | Cba | - | + | - |
| *Glacilaria bursa pastoris* | Gbp | + | - | + |
| *Lomentaria clavellosa* | Lcl | + | - | - |
| *Spiridia filamentosa* | Sfi | - | - | + |
| *Ulva rigida* | Uri | + | + | + |
| **Halophytes** | | | | |
| *Atriplex portulacoides* | | - | + | - |
| *Limonium narbonense* | | + | - | - |
| *Puccinellia palustris* | | - | - | + |
| *Sarcocornia fruticosa* | | + | + | + |

Overall, all the organic matter sources collected showed similar isotopic structure among sites, with both carbon and nitrogen isotopic ratios falling within similar ranges (Fig. 9). In particular, mean δ13C was approximately between -27 and -9‰ and mean δ15N was approximately between 9 and 13‰. Most of the sources common to all the habitats sampled presented approximately similar carbon and nitrogen isotopic signatures , with the exception of SOM and Z. noltei that showed a certain variability. In fact, δ13C for SOM was more depleted in the most inner sites, SM2 (-21.92±0.67‰), and SM3 (-20.68±0.18‰), than in the less confined one SM1 (-18.19±0.73‰). Z. noltei δ13C ranged from -9.9±0.32‰ in SM3 to -

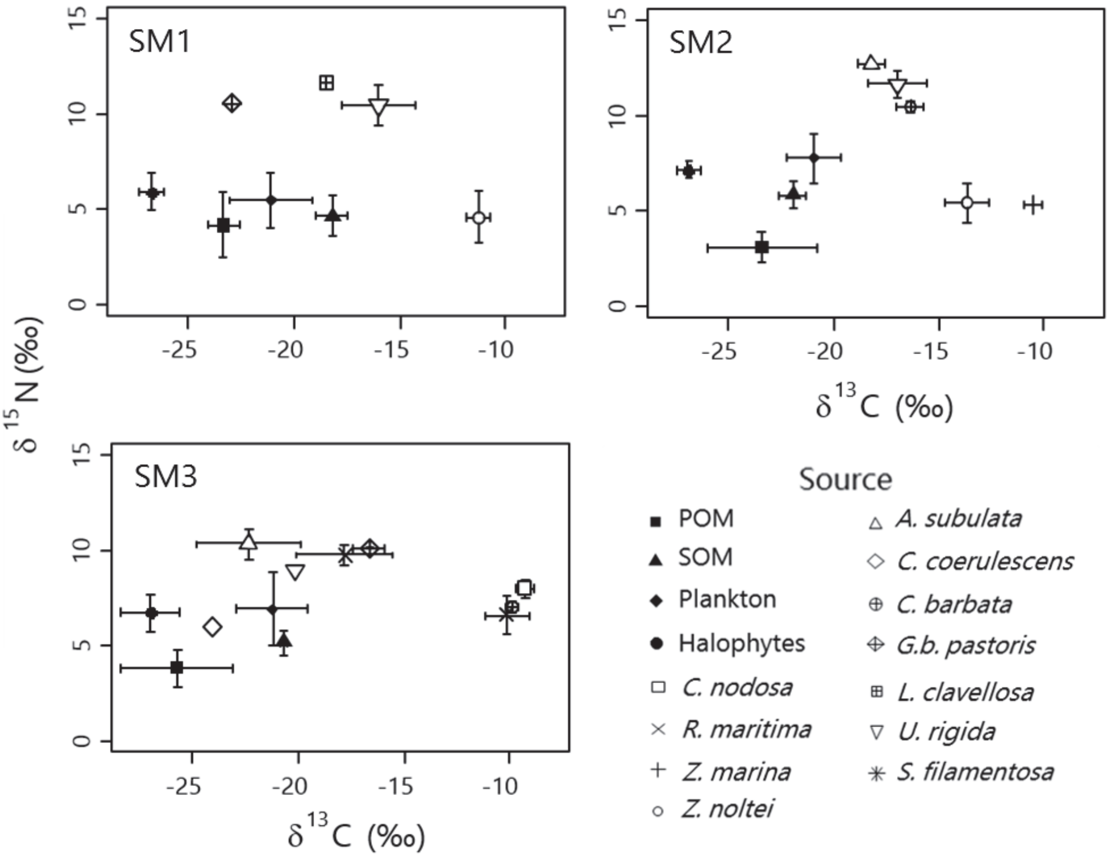


**Figure 9.** Mean (± s.d.) $\delta^{13}C$ (‰) *vs.* $\delta^{15}N$ (‰) of sources of organic matter sampled in each salt marsh habitat located at different distance from the mouth: SM1, closer; SM2, intermediate; SM3, farther.

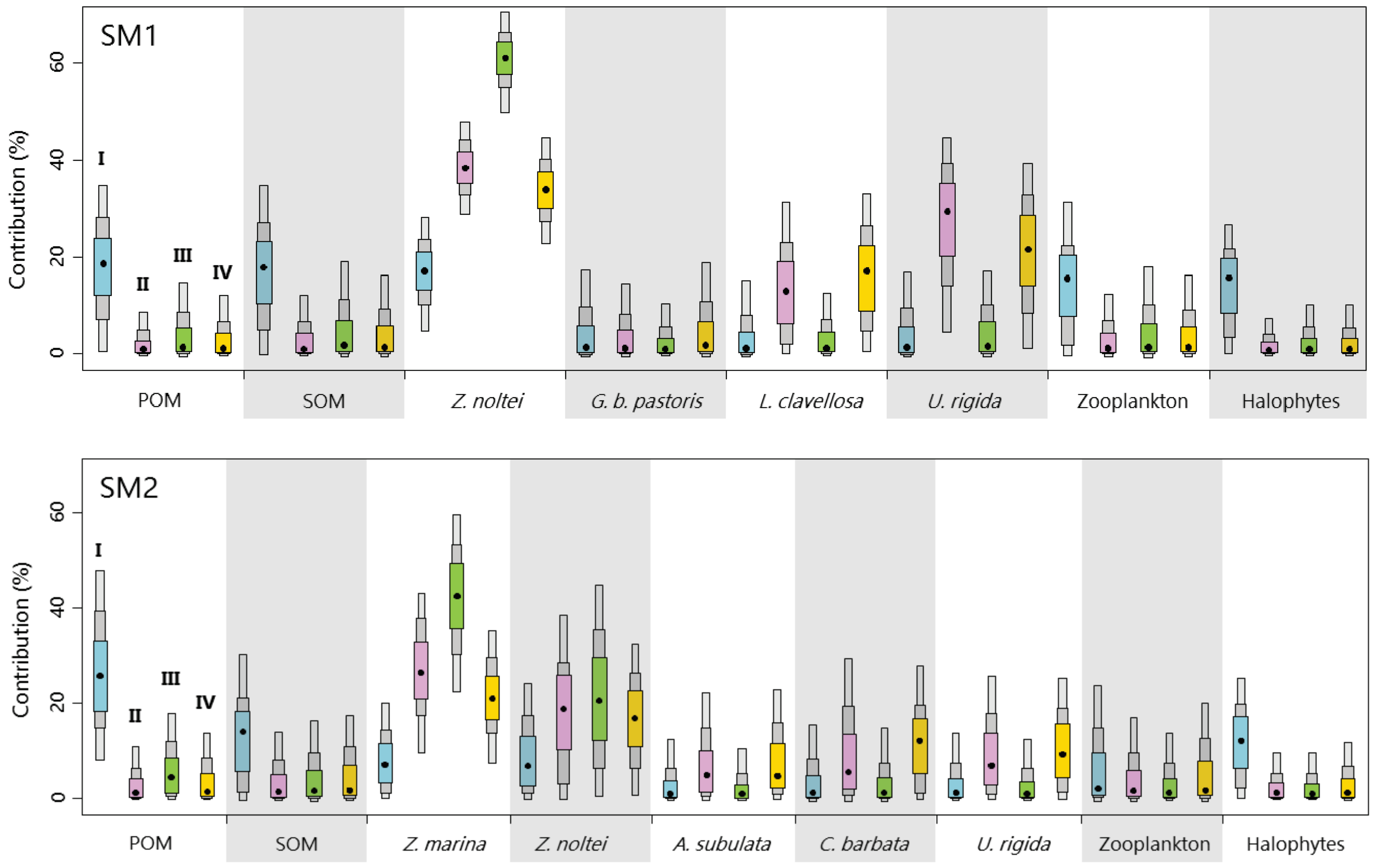
Mixing models elaborated for each class size and habitat investigated allowed to estimate:

- Firstly, the contribution of different habitats in supporting the trophic pathway of organic matter leading to *S. aurata* during the four development stages (size classes I-IV) (Fig. 10). The *a priori* assumption was that the species could potentially inhabit all the three habitats taken into account in this study, during its permanence within the lagoon.
- Secondly, the results were summarized to obtain a model representing the comprehensive contribution of groups of OM sources available within the lagoon (that hence is considered as a whole), to the different ontogenetic phases of *S. aurata* (Fig. 11). For this purpose, the OM sources of each habitat

were sorted into 6 groups: POM, SOM, zooplankton, halophytes, seagrasses and algae, by grouping more species belonging to the same group (seagrasses and algae) when necessary.

Results of mixing models provided evidence that the contribution of the potential sources of organic matter to the trophic pathway of *S. aurata* clearly varied across size classes, particularly between *post*-larvae and older classes, with slight differences across sites and habitats (Fig. 10).

In the most confined habitat, SM3, where seagrasses are heterogeneously distributed, a mixture of POM and SOM provided the most likely contribution to the first size class (modal values respectively 14 and 12%), together with a non negligible role given by the red alga *C. coerulescens* and a mixture of halophytes (mode respectively 11 and 10%). As the species grows, all seagrass species (*C. nodosa*, *R. maritima* and *Z. noltei*) provided the highest contribution that increased progressively until class III when it reached 19% and then slightly decreased to 13% for class IV. A similar trend was maintained also in the intermediate salt marsh (SM2), where the contribution of POM reached the peak of 26% for *post*-larvae that were also highly supported by SOM and halophytes (respectively 14 and 12%). Juveniles I and II and sub-adults instead were mainly supported by the energy pathways based on seagrasses, *Z. noltei* and especially *Z. marina* that provided up to 42% of proportion. When moving to the less confined habitat, the salt marsh closer to the sea (SM1), a not negligible contribution was supplied by zooplankton (15%) to the size class I, in addition to the major mixture of POM and SOM (both ~19%).


SM1
Contribution (%)
0
20
40
60
I
II
III
IV
POM
SOM
*Z. noltei*
*G. b. pastoris*
*L. clavellosa*
*U. rigida*
Zooplankton
Halophytes
SM2
Contribution (%)
0
20
40
60
I
II
III
IV
POM
SOM
*Z. marina*
*Z. noltei*
*A. subulata*
*C. barbata*
*U. rigida*
Zooplankton
Halophytes

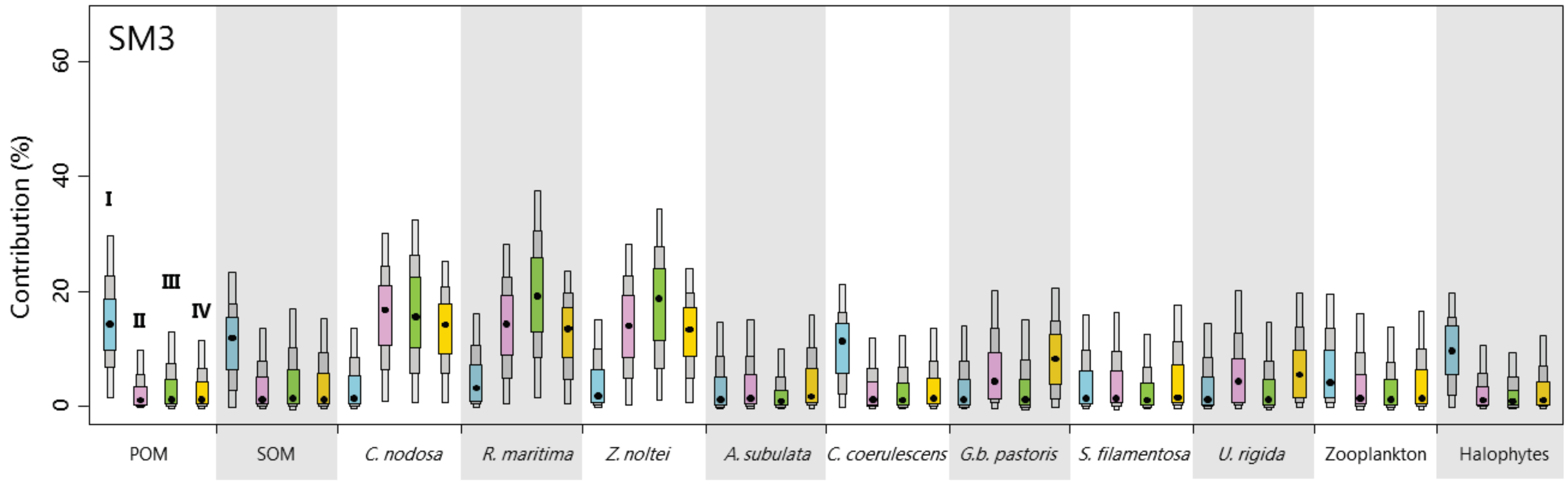


**Figure. 10.** Percentage contribution of organic matter sources of the three salt marsh sites investigated (SM1-3) to the trophic pathway leading to *S. aurata* of the four size classes taken into account (I-IV), with relative credibility intervals of 95% (light grey), 75% (intermediate grey) and 50% (coloured box) and mode (black circle).

In SM1, a remarkable proportion was also given by the only seagrass species present in the site, *Z. noltei*, which contributed 17% to the first size class and then became the main source of organic matter for the following classes, with values 38%, 61% and 34% respectively for class II, III and IV. Furthermore, differently form the most confined sites, in SM1 a more important role of algae emerged. In particular, *L. clavellosa* and *U. rigida* reported percentage contributions around 18% and 25% respectively for size classes II and IV.

Overall, the general results summarized by size revealed a neat distinction between the trophic pathways of post larval stage and successive ones (Fig. 11). Whereas a mixture of POM, SOM seagrasses, algae, and also halophytes, accounting for about 15% each, drove the trophic pathway of post-larvae within the lagoon, the main sources of organic matter supporting juveniles I and II and sub-adults were seagrasses and algae, respectively with modal contributions of 45 and 35% for juveniles I, 45 and 15% for juveniles II and 38 and 42% for sub-adults.

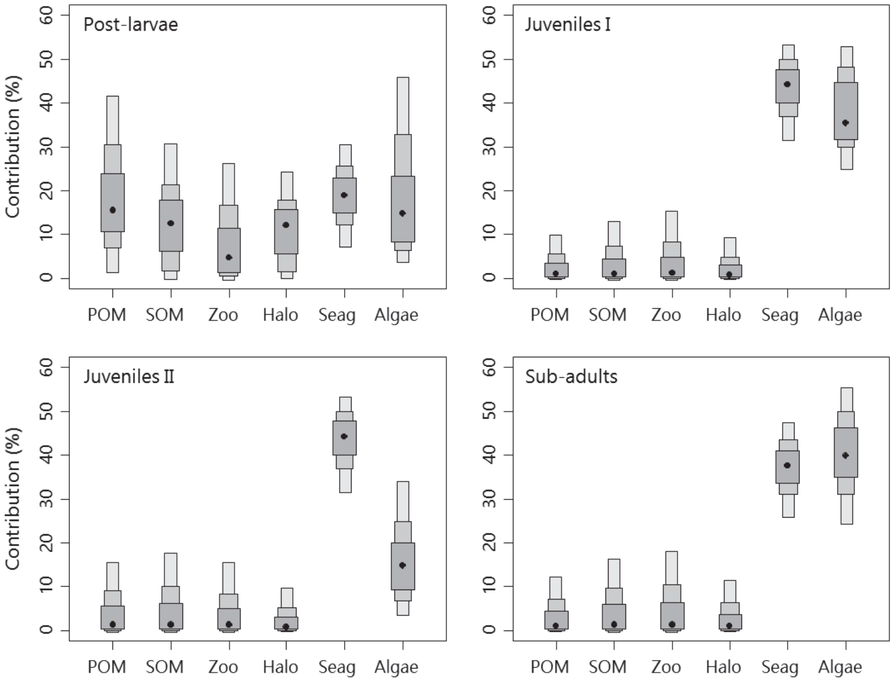


**Figure. 11.** Percentage contribution of organic matter sources to the trophic pathway leading to the different size classes of *S. aurata*, with relative credibility intervals of 95% (light grey), 75% (intermediate grey) and 50% (dark grey) and mode (black circle). Among sources, Zoo is zooplankton, Halo is halophytes and Seag is seagrass.

## 2.4. Discussion

Trophic niche variability and habitat use during ontogeny of the gilthead seabream *Sparus aurata* within the Lagoon of Venice were investigated by means of carbon and nitrogen stable isotope ratios. Previous works assessing the ontogenetic development stages of the fish have reared the species in captivity (*e.g.* Parra & Yúfera 2000; Russo *et al.* 2007), thus this represents the first attempt to study the isotopic ecology of wild populations of *S. aurata* following its migration movement within a lagoon. Size range covered went from post-larvae (min 14 mm SL) to sub-adults (max 202 mm SL), and despite the size interval between 60 and 160 mm was missing due to objective sampling limits, we can state to have covered all of the phases occurring within the lagoon that show the most salient ontogenetic changes.

Results obtained showed evidence of a clear shift in the trophic structure of the population during growth, either in terms of trophic niche and relative population metrics, or in terms of trophic position, either looking at the size class level, or looking at the spatial (site) level. Furthermore, when relating the species to the lagoonal environment used and the specific basal sources available, mixing model results allowed to infer the role of the salt marsh habitats habitat taken into account in supporting the different stages of the species development. Lastly, we propose a summary of the previous outcome in a single model, in order to generalize the overall role of the Venice Lagoon in driving the trophic pathways that lead to the species.

### *Trophic niche variability across size classes*

The relationship among nitrogen or carbon isotopic signatures and body size of *S. aurata* followed an overall increasing trend, with some differences between the two signatures when looking at the size class level.

The $\delta^{15}$N is commonly recognized to increase with body size in fishes due to multiple reasons: changes in preys' size (Fry *et al.* 1999; Vander Zanden *et al.* 2000; Badalamenti *et al.* 2002), alteration of catabolism due to starvation (Sweeting *et al.* 2007; Varela *et al.* 2015) or it may even vary with growth rate (Trueman, McGill & Guyard 2005). The $\delta^{15}$N of *S. aurata* showed a sharp increase across the I size class, after which juveniles I followed a high variability, tendentially decreasing until the juveniles II class and then in sub-adults it raised again. Similarly, the $\delta^{13}$C increased sharply during the post-larval stage, but then stabilized in juveniles I and II, while in sub-adults it was stable around slightly lower values.

This shift towards more enriched values of both $\delta^{15}$N and $\delta^{13}$C during the earliest stage (size class I) of growth and development of *S. aurata* subtends a shift in resource use that is strictly linked to the trophodynamic of the gilthead seabream.

In fact, such development stage realistically corresponds to the transition phase from a pelagic, planktivorous feeding habit, characterizing the post-larvae living in (or entering from) the open sea, to a benthic diet, characterizing the species entered into the shallow environments of the lagoon (Ferrari & Chieregato 1981; Russo *et al.* 2007).

Furthermore, the evident isotopic shift reported for the first size class can also be addressed to the higher turnover rate occurring in the muscles of the younger individuals (Bosley *et al.* 2002; Heady & Moore 2013). Hence, diet shift in early juveniles could be more visible than diet variability of older individuals, where turnover rates decrease with age and longer time is needed to integrate the information into the muscle tissues (Martínez Del Rio *et al.* 2009; Layman *et al.* 2012).

Ontogenetic changes reflected on trophic niche as described by the estimates of the isotopic niche: during different development stages *S. aurata* occupied different isotopic niche spaces within the bi-plot $\delta^{13}$C-$\delta^{15}$N. The main difference emerged between the post-larvae and the older individuals, both in terms of occupied space and width, highlighting two completely different feeding habits.

The absence of overlap between the two main groups (size class I vs size classes II-IV) indicates they evidently rely on different pathways and resources, while the width of the isotopic niche, higher for post-larvae and narrower for older size classes, needs to be described together with its metrics (Layman *et al.* 2007a).

According to previous studies about trophic niche, generalist species show a wider niche width in comparison to specialist species, as the first ones rely on a wider variety of sources, whilst the second ones feed on a restricted type of similar preys (Bearhop *et al.* 2004; Layman *et al.* 2007a). Thus, this reasoning would led us to conclude that post-larvae are somewhat more generalist than juveniles and adults given the differences found in the niche widths. However, in this case the slightly higher isotopic niche width shown by post-larvae is attributable mainly to the trophic diversity, increased by the high individual specialization (Post 2003; Matthews & Mazumder 2004) that characterizes *S. aurata* at this stage. In fact, an "intra-class" shift is clearly visible within the size class of post-larvae, that not only is due to the ability of slightly bigger individuals to feed on slightly bigger preys (Russo *et al.* 2007; Cummings *et al.* 2012), but more importantly is related to the migration of the species within the lagoon, that induces a transition from a planktonic diet to a benthic diet.

This results in a notable difference in the value of the distance of individuals from the centroid of the isotopic space, representing the trophic diversity, that hence for post-larvae was double than for the older size classes.

On the other hand, the distribution of juveniles and adults within their isotopic niches followed a progressive lower trophic redundancy and increasing spacing, that agree with the shift towards a more generalist feeding behaviour of the older individuals of the species. Juveniles II evidently feeding on low trophic levels lied in a lower area of the isotopic space, whilst the sub-adults partially overlapped their niche with juveniles I, meaning that they rely on similar basal sources. Consequently, caution must be taken when analysing isotopic niches just based on the width, especially when dealing with species undergoing ontogeny (Hammerschlag-Peyer *et al.* 2011).

*Trophic niche variability across sites*

Due to the migratory movements of *S. aurata* between the open-sea and costal lagoons and its commercial value, it is worth to undestand which habitats support, from the trophic point of view, the different stages of the species development. Stable isotope ratios, especially $\delta^{13}C$, have been widely used to follow organism movements between different feeding sites (Rubenstein & Hobson 2004; Hobson & Wassenaar 2008; Graham *et al.* 2010; Carlisle *et al.* 2012), as different basal sources vary greatly in carbon isotopic signature and the trophic fractionation between trophic levels is small (about 0.4‰; Post 2002). In addition, quantitative isotopic niche and relative metrics allowed a good description of the trophic niche ontogeny during migratory pattern.

*S. aurata* followed a progressive shift of the trophic niche towards more $\delta^{13}C$-enriched areas of the isotopic space, as the species moved from the sea towards inner sites of the Venice Lagoon, and then a return to $\delta^{13}C$-depleted but more $\delta^{15}N$-enriched area, once *S. aurata* left the lagoon. This pattern indicated a clear correspondence between the migration movements and the diet shift during ontogeny, revealing a progressive shift from pelagic to benthic resources use (Ferrari & Chieregato 1981; Russo *et al.* 2007). Likewise, the width of the isotopic niche increased and trophic positions raised progressively following the same direction, with some exceptions given at particular sites.

While for early juveniles the shift is given to a change in diet, for older groups the shift is due to a different use of the lagoon

Post-larvae collected in spring before entering the lagoon, hence with a marine diet based mainly on zooplankton (Ferrari & Chieregato 1981; Elbal *et al.* 2004), showed the narrowest isotopic niche width typical of specialist feeders. Once entered the lagoon and settled in different habitats, the species evidently started a rapid growth, which resulted in the extension of the trophic niche width, the increase of the trophic level and diversity, due both to the increased feeding ability and to the availability of a wider variety of resources that characterizes such highly productive habitats.

Salt marshes are particularly recognized as the most suitable habitats, being nursery areas for different species (Zucchetta *et al.* 2009). Accordingly, they resulted to play a specific role also for the trophic structure of *S. aurata* in the Venice Lagoon.

Interestingly, post-larvae and juveniles I collected in different parts of the same salt marsh, the outer edge (SM2) and the inner creek (SM2c), displayed similar isotopic niches in terms of width (SEAc). However, the neat shift along both $\delta^{13}$C and $\delta^{15}$N axes of juveniles in SM2c indicated a different use of the resources, compatible with a benthivorous diet, based on a narrower set of sources, higher specialisation, reduced trophic diversity, increased redundancy and reduced evenness within the niche.

In the most confined site of the lagoon (SM3), only the third size class (juveniles II) was found in spring 2015, and showed a trophic niche completely separated from the others, shifted towards the most enriched $\delta^{13}$C but unexpectedly low $\delta^{15}$N portion of the biplot, that can be probably addressed to a different availability of food sources characterizing a different sampling year.

Lastly, the trophic varability of sub-adults catched at the two northern mouths of the lagoon before going back to the sea, can be related to the high trophic plasticity of the species, that during its permanence within the lagoon may have taken advantage of different feeding opportunities, as typical for generalist and opportunistic species.

Indeed, although the isotopic niche of *S. aurata* catched in proximity of the central mouth (M3) was wider than that of the individuals catched in the northern mouth (M2), the metrics confirmed a similar trophic organization of individuals, with similar level of trophic diversity, redundancy and evenness of the species within the isotopic space.

*Trophic role of the Venice Lagoon as a nursery area for* S. aurata

Mixing model estimates provided a quantitative description of the shift in basal sources supporting the early development stages of *S. aurata*. Despite slight differences due to the variability of the basal sources occurring in the salt marsh sites taken into account (SM1, SM2 and SM3), the overall picture of the results highlights a main evident difference between the trophic pathways leading to the post larval stage and the one leading to juveniles and sub-adults.

While the trophic pathway of the early stages of *S. aurata* is driven mainly by a mixture of POM, SOM and plankton, the pathways for older classes shifts mainly to seagrasses for the middle stages, to a mix of macroalgae and seagrasses for the sub-adults.

Habitats chosen to estimate the source contribution were located along a confinement gradient, and were three salt marshes (SM1-3) characterized by the presence of seagrass patches.

In all of the habitats, the mixture of POM and SOM and zooplankton plays a major role exclusively for post-larvae, given their ready availability for such small individuals (Cataldi *et al.* 1987; Elbal *et al.* 2004), while the high contribution of SOM could be attributable to the frequent resuspension and sedimentation that usually characterize such shallow habitats (Vizzini *et al.* 2005; Vizzini & Mazzola 2008). In accordance with different studies about the trophic role of coastal lagoons (*e.g.* Vizzini 2009; Vaslet *et al.* 2011), particularly important was revealed the role of the macrophytes (seagrasses and macroalgae), whose contribution increased overall with fish body size. In all habitats, seagrasses provided the highest individual contribution, either when predominant (in SG and SM) or not (in cSM and cSG), while the role of few algae species, for example the green alga *U. rigida* was particularly noteworthy in the less confined habitat SM and SG.

Given the slight differences occurred between the habitats investigated we were able to summarize the results in a unique model accounting for the role of the Lagoon of Venice itself as a nursery area for *S. aurata*. Once again, the crucial difference emerged among the two trophic pathways supporting the post larvae from the following size classes, in accordance to their diet shift that goes respectively from zooplanctivorous to zoobenthivorous habits (Ferrari & Chieregato 1981; Cataldi *et al.* 1987; Russo *et al.* 2007; Elgendy *et al.* 2016).

## 2.5. Conclusion

The most evident and significant differences among the development stages of *Sparus aurata* occur between post-larvae and the older stages. The size limit of 20 mm in standard length seems to represent a crucial threshold for this species, after which it incurs in significant changes of morphology that perfectly match the shift in trophic habits and habitat exploitation, differently from what claimed by Ferrari & Chieregato (1981), that indicate the same threshold at 30 mm. Stable isotope analysis resulted to be a valid tool to track these changes providing interesting details on the trophic organization so far missing. In general, this study emphasises the importance of coastal lagoon environments as nursery areas for the early stages of the gilthead seabream *S. aurata* life cycle, whose role should be taken into account when discussing the implications that may result in terms of coastal management. Particularly, special attention should be paid to the conservation strategies of those lagoonal habitats, such as the salt marshes, that represent nursery areas offering substratum and refugia, as well as the submerged macrophytes (seagrasses and macroalgae) that are at the base of the food web supporting migrant species. In fact, these terrestrial and marine vegetated habitats are increasingly threatened by the effects of anthropic pressures and climate change, and their irreversible regression would result in loss of crucial ecological function of lagoon systems.

# Chapter 3

## Structural and functional recovery of fish communities during seagrass habitat restoration

**Abstract**

Full success of habitat restoration programs is reached when the recovery of the habitat structure is coupled also to a functional recovery of the system. This includes the re-establishment of the faunal community with a stable trophic structure. Recently, the analysis of trophic structure and trophic niche by means of carbon and nitrogen stable isotopes descriptors has been widely used to quantitatively describe communities, integrating basic information given for example by species composition and abundance.

In the present study, we used community-wide isotopic metrics as descriptors to assess temporal variations of the trophic niche of a fish community associated to a habitat subjected to seagrass restoration in the Venice Lagoon (SeResto, Life12 NAT/IT/00033,). In particular, the fish community was monitored for the first two years since the beginning of the restoration project, comparing it to a reference community associated to a stable seagrass meadow present in the same study area.

Results showed that lower species diversity but similar density characterized the fish community of the restoration site. Descriptors of the trophic niche revealed a slight increase of the isotopic niche width and trophic diversity and a reduced trophic redundancy across time. Outcomes reported suggest similar trophic dynamics to that of the reference community, mainly driven by seasonal fluctuation.

Despite the ecological status and structure of the seagrasses increased during the first two years, longer time is needed for the fish community to restore structurally and functionally. Overall, this study emphasizes the importance of considering the recovery of habitat function, other than structure, among the criteria to assess the restoration success.

### 3.1. Introduction

Seagrass meadows constitute important habitats playing multiple crucial roles within coastal ecosystems, both ecological and economic (Costanza *et al.* 1997). Due to their three-dimensional structure, with the leaves extending into the water column, they provide habitats for many vertebrate (*e.g.* fish) and invertebrate species (*e.g.* epifauna, zoobenthos), refugia for prey species and nursery sites for juveniles (Nagelkerken *et al.* 2000; Strazisar, Koch & Madden 2014), enhancing biodiversity. They are essential primary producers that contribute to water oxygenation, nutrient cycling and absorption, sediment stabilization (Orth *et al.* 2006a; Kun Seop, Sang Rul & Young Kyun 2007). Moreover, they represent one of the main organic matter sources, producing carbon and driving energy fluxes through trophic transfers (Vizzini 2009), that support high secondary production (McArthur & Boland 2006; Vaslet *et al.* 2011).

However, a multitude of anthropogenic pressures exerted on coastal systems, that lead to rapid environmental alterations, is inducing a progressive fragmentation of such habitats, with consequent regression of seagrass meadows (Orth *et al.* 2006a) and decline of associated species. Seagrass loss has recently prompt the scientific community to the implementation of restoration projects worldwide, intended to re-establish the natural habitats in those coastal systems where they underwent severe reduction, such as bays (*e.g.* Fonseca, Kenworthy & Courtney 1996) and coastal lagoons (*e.g.* Orth *et al.* 2006b).

The success of these programs is reached not only when the habitat has regained the seagrass structure and biomass previously lost, but also if the general ecological status of the site is improved and the typical communities, among others the fish communities, and relative trophic structure are re-established (Wozniac *et al.* 2006; Able *et al.* 2008; Chang-Keun *et al.* 2008; McSkimming *et al.* 2016). This means that other than the structural recovery of the habitat, a restoration process must aim also at the functional recovery of the system (Nordström *et al.* 2015; McSkimming *et al.* 2016).

The assessment of trophic structure and trophic niche, using the approach of carbon and nitrogen stable isotopes, can be used to quantitatively describe the trophic function of communities (Abrantes, Barnett & Bouillon 2014), integrating basic information on community structure given for example by species composition and abundance.

The application of community-wide metrics originally proposed by Layman *et al.* (2007a) and based on the concept of the isotopic niche as descriptor of the trophic niche (Bearhop *et al.* 2004; Newsome *et al.* 2007), enabled to quantify some parameters such as niche width, trophic diversity and redundancy of the species within a community. Furthermore, additional developments of such metrics have allowed statistical comparisons among different communities and across time (Jackson *et al.* 2011).

In the present study, we assessed the trophic re-organization of fish communities associated to a habitat subjected to seagrass restoration. We expected that, in parallel to the recovery of the seagrass habitat, the trophic structure, hence the trophic niche of the associated fish community varied progressively across time, displaying trophic characteristics similar to those of a community associated to a corresponding seagrass natural habitat chosen as a reference.

The restoration project taken into account as study case is the SeResto project (SEagrass RESTOration, Life12 NAT/IT/000331, www.lifeseresto.eu), that was carried out in the Northern basin of the Lagoon of Venice (Adriatic Sea), where the extension of seagrass meadows faced critical reduction in the last decades (Sfriso & Facca 2007).

The project SeResto involved the explant of sods and rhizomes of four species of seagrasses from a donor site and the following transplant in 17 sites of the northern basin of the lagoon, previously detected as suitable locations for the rooting, growth and spread of the plants. The transplant process of sods and rhizomes started in spring 2014 and will be completed in 2018; species involved were *Cymodocea nodosa* (Ucria Ascherson, 1870), *Zostera marina* (Linnaeus, 1753), *Zostera noltei* (Hornemann, 1832), and *Ruppia cirrhosa* (Petagna Grande, 1918) (Facca *et al.* 2014).

## 3.2. Materials and methods

### *Sampling sites and sample collection*

The study was conducted in the Northern sub-basin of the Venice Lagoon (see chapter 1 for full description of the study area), where the project of seagrass restoration has been carried out.

Sampling sites were one of the 17 sites of the restoration project (SeResto), hereafter called Resto and a reference site, hereafter called Control or Ctrl.

The Resto site was interested by the transplant of sods and rhizomes of three species of seagrasses: *Zostera marina*, *Zostera noltei* and *Cymodocea nodosa*.

The Control site was chosen in order to represent a stable seagrass meadow constituted by two of the species programmed for transplanting in the Resto site (*Cymodocea nodosa* and *Zostera noltei*) (Fig. 1).

Sample collection was conducted in spring and autumn 2014 and 2015, in a total of four sampling times, from T0 (preceding the first transplant) to T3 in the Resto site, while the Control site was sampled starting from T1 to T3, for a total of three sampling times.

**Figure 1.** Study area and sampling sites. Restoration, restoration site, Control, reference site.

At each sampling time, fish communities were collected in both sites by means of a small beach seine net (10 m long, 2 mm mesh size), by dragging it twice over two known surface area. Aquatic macrophytes (seagrasses and macroalgae) and terrestrial halophytes (only present at Resto site) were collected by hand.

Three replicates of surficial sediment and water were collected through cores (3 cm Ø) and 2 L bottles respectively and used as proxies of sedimentary organic matter (SOM) and particulate organic matter (POM). Once collected, all samples were kept refrigerated during transport to the laboratory, were they were stored at -20°C before to the processing.

*Sample processing and laboratory analysis*

In the laboratory, fish collected were identified at species level and measured for standard length (SL) and total weight (TW). Fish dorsal muscle was extracted from at least 15 individuals for each species found (when available), by means of scalpel and tweezer. Seagrasses and macroalgae were identified at species level and eventual epiphytes were manually scraped out. Pre-filtered (200 μm) water samples were filtered on precombusted (450°C, 4h) filters (GF/F Whatman, pore size 0.7 μm) to concentrate the POM. Sediment cores were sliced to the top 1.5 cm and homogenized as proxy for SOM.

Afterwards, all the samples destined to the stable isotope analysis were oven-dried at 60 °C for about 48h to constant weight and ground to a fine powder using a micro mill or a mortar and pestle. Part of the samples containing carbonate components were acidified (HCl 1M) in order to dissolve inorganic carbon that might interfere with the determination of carbon isotopic signatures. Analysis of $\delta^{13}C$ and $\delta^{15}N$ was then performed on small aliquots of each sample packed in tin capsules, using an isotope ratio mass spectrometer (Thermo-Electron Delta Plus XP) coupled to an elemental analyser (Thermo-Electron Flash EA1112). Carbon and nitrogen stable isotope ratios were expressed in δ unit notation, as parts per mil deviations from the international standards (Pee Dee Belemnite and atmospheric $N_2$ for carbon and nitrogen respectively) and determined as follows: $\delta X=[(R_{sample}/R_{standard})-1]\times 10^3$, where X is $^{13}C$ or $^{15}N$ and R is the relative $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The analytical precision of the measurement was 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

### *Data analysis*

Density of each fish species was calculated by standardizing the number of individuals collected in the specific sampling area to 100 $m^2$ (n° individuals 100 $m^{-2}$), and also the relative percentage abundances were calculated. In order to assess differences occurring in fish assemblages among sites and sampling times, density data were analysed, after square root transformation, firstly applying a non-metric multidimensional scaling (nMDS) based on the Bray-Curtis similarity index and then performing permutational analysis of variance (PERMANOVA) and relative pairwise test to detect eventual differences between sampling times within each site. Analyses were performed using PRIMER 6 +PERMANOVA (Plymouth Marine Laboratory, UK).

Each species was assigned to an ecological and feeding group, in accordance to Franco *et al.* (2008a) and Franzoi *et al.* (2010), in order to outline the main ecological features of the populations found within the fish assemblages.

Trophic positions (TP) of each fish species at each site and sampling time were estimated according to the equation suggested by Post (2002): $TP = [(\delta^{15}N_f - \delta^{15}N_b) / \Delta n] + \lambda$, where $\delta^{15}N_f$ and $\delta^{15}N_b$ are the nitrogen isotopic signatures of the fish species selected and the source baseline, respectively. The mean value of zooplankton samples previously collected in the same sites was used as source baseline (8.3‰). $\Delta n$ is the trophic enrichment expected for each trophic level (3.4‰, Post 2002) and $\lambda$ is the trophic position of the species used as baseline (2 for zooplankton).

Carbon and nitrogen isotopic data were analysed separately. After testing for normality and homogeneity of variances using respectively Shapiro-Wilk test and Bartlett test, if normality conditions were suited, two-way ANOVA and corresponding post-hoc tests were used, otherwise non parametric Kruskal-Wallis test and relative post-hoc tests were used to assess differences in multiple comparisons among sites and sampling times. All tests were ran in R (R Core Team, 2015), using a significance level of $p < 0{\cdot}05$.

Changes occurring in the trophic niche of the community examined during the restoration process were investigated by means of isotopic niche descriptors.

Standard ellipse areas (SEAc, corrected for small sample size) allowed to assess the width of the isotopic niche and were estimated through Bayesian statistics, using the R package SIBER v2.0.2 (Stable Isotope Bayesian Ellipses in R) (Jackson *et al.* 2011), where SEAcs were set to contain 60% of the data.

Community-wide metrics proposed by Layman *et al.* (2007) allowed to describe quantitatively other aspects of the trophic niche such as:

- trophic diversity and species spacing within the isotopic space (expressed as $\delta^{15}$N Range, NR; $\delta^{13}$C Range, CR and mean Distance to Centroid, CD),
- trophic redundancy (expressed as mean Nearest Neighbour Distance, NND)
- trophic evenness of species packing within the isotopic space (expressed as Standard Deviation of the Nearest Neighbour Distance, SDNND).

However, to avoid any bias given by different sample sizes (*e.g.* different number of fish populations) constituting the two communities of Resto and Control sites, community wide metrics were estimated through Bayesian statistics, hence accounting for sampling error and providing a measure of uncertainty that allows comparisons between different communities (Jackson *et al.* 2011). Accordingly, community wide metrics were estimated by using the R package SIBER previously mentioned.

## 3.3. Results

*Macrophytes composition*

Macrophytes composition of the Resto site varied across sampling times (Tab. 1). Small patches of *Z. noltei* were found at all times, with the exception of T2 (spring 2015). *R. maritima* occurred exclusively in both spring seasons (T0 and T2), while *C. nodosa* was only found in T2. Macroalgae were mainly present as drift of varied species, among which the red alga *Agardhiella subulata* was the most constantly present (but it was not found in T3). In the Control site *C. nodosa* and *Z. noltei* constituted a dense meadow, with sparse algal drift mainly dominated by the green alga *U. rigida*.

**Table 1.** List of the species recorded in the habitats examined with indication of their presence (+) or absence (-).

| | **Restoration site** | | | | **Control site** | | |
|---|---|---|---|---|---|---|---|
| | **T0** | **T1** | **T2** | **T3** | **T1** | **T2** | **T3** |
| **Seagrasses** | | | | | | | |
| *Cymodocea nodosa* | - | - | + | - | + | + | + |
| *Ruppia maritima* | + | - | + | - | - | - | - |
| *Zostera noltei* | + | + | - | + | + | + | + |
| **Macroalgae** | | | | | | | |
| *Agardhiella subulata* | + | + | + | - | - | + | - |
| *Chondria coerulescens* | + | - | - | - | + | - | - |
| *Glacilaria* sp. | + | - | - | - | - | - | - |
| *Spiridia filamentosa* | - | + | - | + | - | - | - |
| *Ulva rigida* | - | + | + | - | + | + | + |

*Fish communities*

A total of 12 fish species belonging to 6 families were identified, with differences among sites and sampling times (seasons) (Tab. 2).

In the restoration site fish assemblage was dominated by few species of estuarine residents such as *Atherina boyeri* (Risso, 1810) and *Knipowitschia panizzae* (Verga, 1841), constantly present with fluctuating densities across time. Other resident species, such as the Syngnathidae *Syngnathus abaster* (Risso, 1827), usually associated to seagrass meadows, was found at most of the sampling times (T0, T1 and T3), but with very low densities (<3 n° ind. 100 $m^{-2}$).

**Table 2.** Density (number of individuals 100 $m^{-2}$) and percentage abundance (%) of the species sampled in the study sites from T0 to T3. Ecological and feeding groups are also indicated according to Franco *et al.* (2008). Ecological groups are: ES, estuarine species, MS, marine stragglers, MM, marine migrants. Trophic groups are: Bmi, microbenthivores, Bma, macrobenthivores, PL, planktivores, HZ, hyperbenthivores/zooplanktivores, HP, hyperbenthivores/piscivores, OV, omnivores.

| | | | | | | Restoration site | | Control site | |
|---|---|---|---|---|---|---|---|---|---|
| **Family** | **Species** | **Ecological group** | **Feeding group** | **Time** | **Season** | **Density** | **%** | **Density** | **%** |
| Atherinidae | *Atherina boyeri* | ES | HZ | T0 | Spri | 49.76 | 87.82 | - | - |
| | | | | T1 | Aut | 5.43 | 27.54 | 0.95 | 1.89 |
| | | | | T2 | Spri | 2.27 | 26.92 | 0.97 | 2.14 |
| | | | | T3 | Aut | 35.71 | 84.03 | 11.07 | 29.81 |
| Blenniidae | *Salaria pavo* | ES, MS | OV | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 0.95 | 1.89 |
| | | | | T2 | Spri | 0 | 0 | 0.32 | 0.71 |
| | | | | T3 | Aut | 0 | 0 | 1.43 | 3.85 |
| Engraulidae | *Engraulis encrasicolous* | MM, MS | PL | T0 | Spri | 0.71 | 1.26 | - | - |
| | | | | T1 | Aut | 0 | 0 | 0 | 0 |
| | | | | T2 | Spri | 0 | 0 | 0 | 0 |
| | | | | T3 | Aut | 0 | 0 | 0 | 0 |
| Gobiidae | *Gobius niger* | ES | Bmi, HP | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 0.48 | 0.94 |
| | | | | T2 | Spri | 0 | 0 | 0 | 0 |
| | | | | T3 | Aut | 0 | 0 | 0 | 0 |
| Gobiidae | *Pomatoschistus marmoratus* | ES, MS | Bmi | T0 | Spri | 3.57 | 6.30 | - | - |
| | | | | T1 | Aut | 0.29 | 1.45 | 0 | 0 |
| | | | | T2 | Spri | 0 | 0 | 0 | 0 |
| | | | | T3 | Aut | 0 | 0 | 0 | 0 |
| Gobiidae | *Knipowitschia panizzae* | ES | Bmi | T0 | Spri | 0.95 | 1.68 | - | - |
| | | | | T1 | Aut | 11.14 | 56.52 | 0.95 | 1.89 |
| | | | | T2 | Spri | 5.19 | 61.54 | 0 | 0 |
| | | | | T3 | Aut | 5.71 | 13.45 | 0 | 0 |
| Gobiidae | *Zosterisessor ophiocephalus* | ES | Bmi, Bma | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 0.95 | 1.89 |
| | | | | T2 | Spri | 0 | 0 | 0.65 | 1.43 |
| | | | | T3 | Aut | 0 | 0 | 6.79 | 18.27 |
| Labridae | *Symphodus cireneus* | MS | | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 0 | 0 |
| | | | | T2 | Spri | 0 | 0 | 0.32 | 0.71 |
| | | | | T3 | Aut | 0 | 0 | 0.71 | 1.92 |
| Syngnathidae | *Hippocampus guttulatus* | ES, MS | Bmi | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0.48 | 1.45 | 0 | 0 |
| | | | | T2 | Spri | 0 | 0 | 0 | 0 |
| | | | | T3 | Aut | 0 | 0 | 0 | 0 |
| Syngnathidae | *Nerophis ophidion* | ES, MS | Bmi, HZ | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 1.90 | 3.77 |
| | | | | T2 | Spri | 0 | 0 | 11.04 | 24.29 |
| | | | | T3 | Aut | 0 | 0 | 2.50 | 6.73 |
| Syngnathidae | *Syngnathus abaster* | ES, MM | Bmi | T0 | Spri | 1.67 | 2.94 | - | - |
| | | | | T1 | Aut | 2.57 | 13.04 | 23.81 | 47.17 |
| | | | | T2 | Spri | 0.00 | 0.00 | 5.19 | 11.43 |
| | | | | T3 | Aut | 1.07 | 2.52 | 6.07 | 16.35 |
| Syngnathidae | *Syngnathus typhle* | ES, MS | HZ | T0 | Spri | 0 | 0 | - | - |
| | | | | T1 | Aut | 0 | 0 | 20.48 | 40.57 |
| | | | | T2 | Spri | 0.97 | 11.54 | 26.95 | 59.29 |
| | | | | T3 | Aut | 0 | 0 | 8.57 | 23.08 |

Further species occasionally recorded were manly marine migrants (MA) or visitors (MS), therefore they did not constitute persistent components of the fish assemblage.

Control site confirmed to host a stable fish assemblage (Tab. 2), mainly composed by six species of estuarine residents, among which *Syngnathus typhle* and *S. abaster* (Linnaeus, 1758) represent the dominant species, with some seasonal fluctuations. Spatial analysis given by nMDS clearly highlighted differences between the fish communities of the two sites (Fig. 2), but pairwise test of PERMANOVA did not reveal any significant difference between sampling times within each site (p>0.05 for all pairwise test).

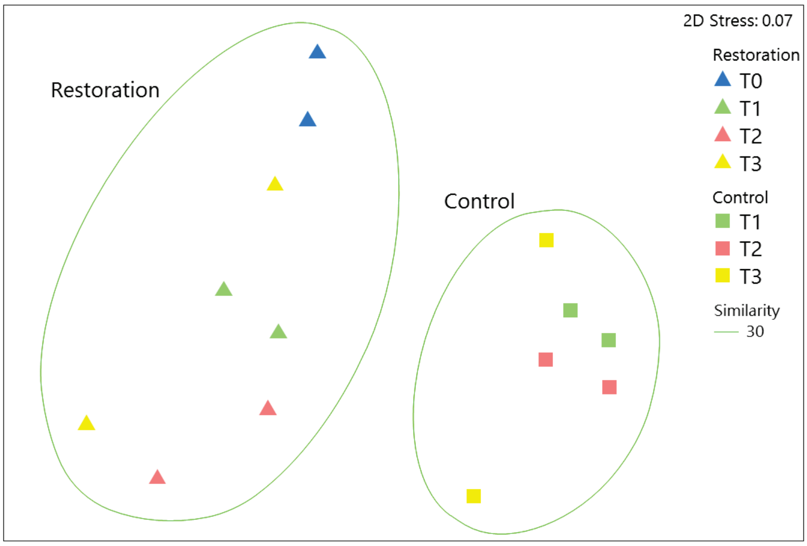


**Figure 2.** nMDS ordination based on fish density values of each site and sampling time. Sample grouping was based on Bray-Curtis clustering from square root transformed data.

### *Isotopic features of organic matter sources and fish communities*

All the sources of organic matter collected in both Resto and Control sites fell within similar ranges of carbon and nitrogen isotopic signatures, with no statistically significant variation among sites or sampling times (*Kruskal-Wallis post-hoc test*, p-value>0.05) (Fig. 3).

The distribution of the basal sources over the $\delta^{13}$C axis of the isotope space (the biplot $\delta^{13}$C-$\delta^{15}$N) followed the same order in all cases, with the SOM showing the most $^{13}$C-depleted values (but not in comparison to *A. subulata* in Resto T2 and Ctrl T2), the POM the intermediate ones and the seagrasses the most enriched, while the macroalgae were dispersed over the whole range.

A slight shift of $\delta^{13}$C towards more depleted values was recorded at the autumn sampling times for both sites. Particularly, $\delta^{13}$C values in Resto ranged from about -25.0‰ to -9.0‰ in spring (T0 and T2), while decreased in autumn ranging from about -29.0‰ to -10.0‰ (T1 and T3). In Control, basal sources ranged over slightly more $^{13}$C-depleted signatures only at T1 ($\delta^{13}$C between -27.6‰ to -10.5‰), in comparison to the two following sampling times (T2 and T3), when they ranged approximately between -25.0‰ and -10.0‰.

The $\delta^{15}$N of all sources ranged similarly between Resto and Control site, with values approximately between 3.0‰ and 10.0‰ from T0 to T2 and then a slight compression occurs in both sites at T3, with values ranging from 4.4‰ to 7.4‰ in Resto and from 3.8‰ to 8.2‰ in Control.

Looking at the general isotopic structure of the fish communities, few significant differences in the overall ranges of carbon and nitrogen isotopic signatures occurred across time (Fig. 3).

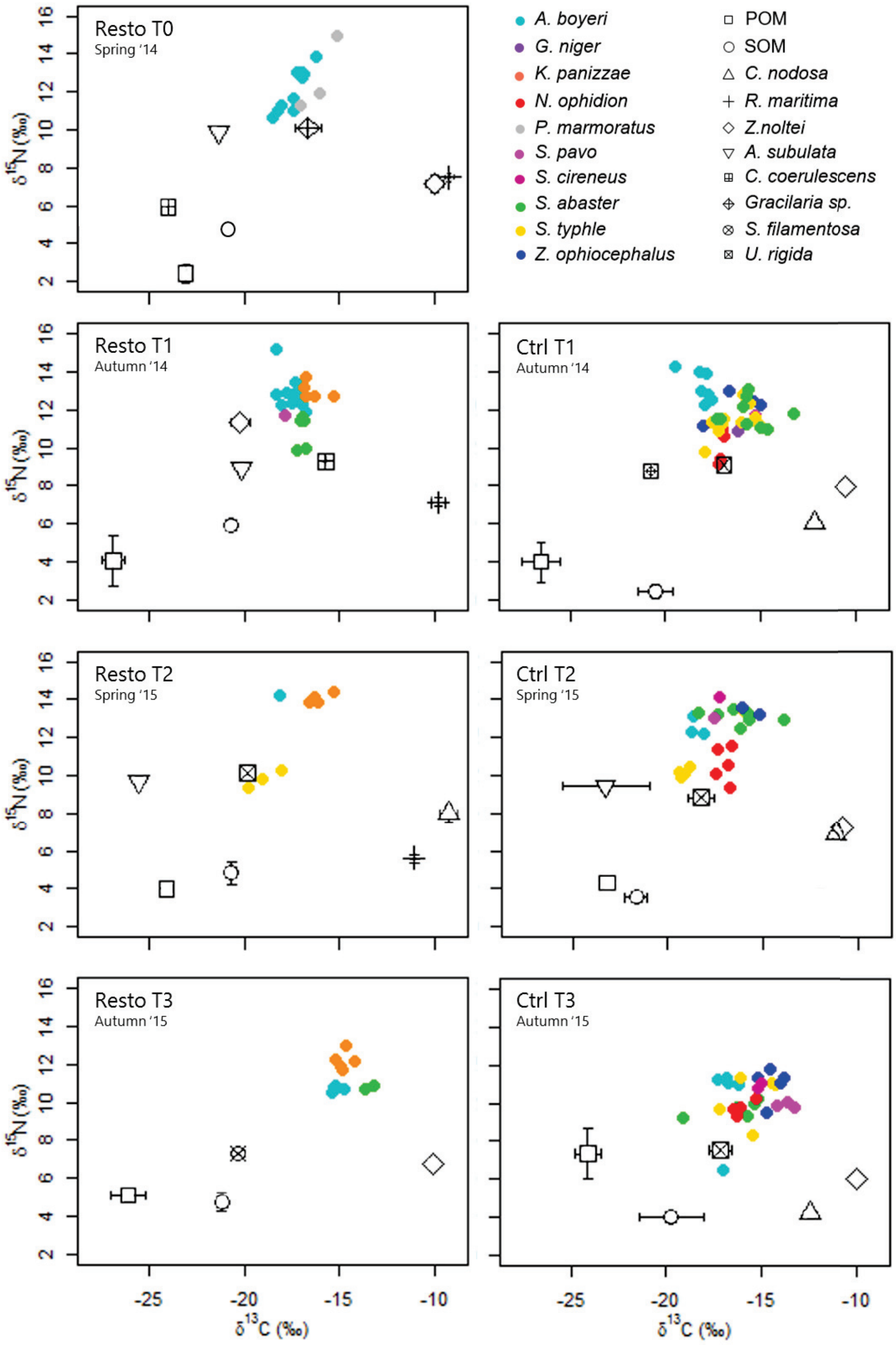


**Figure 3.** $\delta^{13}$C (‰) *vs.* $\delta^{15}$N (‰) of fish species and mean (±s. d.) of organic matter sources sampled in each site (Resto and Control) and sampling time (T0-T3). Full coloured points are single individuals of fish species, black symbols are organic matter sources, according to the legend.

The range of $\delta^{13}C$ significantly shifted in the restoration site from the sampling times T0-T2 to T3 (*ANOVA post-hoc test*, p-value>0.05). In fact, at T0-2 the range was constant, with values between -19.0‰ and -15.0‰ *ca.*, at T3, the community composed by similar species found at previous times (*A. boyeri*, *K. panizzae* and *S. abaster*), shifted to $^{13}C$-enriched values, ranging from -15.4‰ to -13.2‰. On the contrary, in the Control site the $\delta^{13}C$ range was constant, with values included between -19.0‰ and -13.0‰ at all sampling times.

No evident differences occurred for the $\delta^{15}N$ in Resto, where values ranged approximately from 10.0‰ to 15.0‰, while in the Control a significant difference was recorded from T2 to T3 (*ANOVA post-hoc test*, p-value>0.05), with ranges shifting towards depleted signatures, from 9.0‰-14.0‰ in T2 (and similar in T1) to 6.5‰-11.8‰ in T3.

Trophic positions (TP), estimated for each species of the fish communities, varied mainly across time, without any comparable trend between the two study sites (Fig. 4).

Overall, TPs had similar ranges in both Resto and Control sites, with the lowest position equals to 2.4 and the highest to 3.7, but the different species varied differently between sites. *A. boyeri* showed opposite trends in TP, with values increasing in Resto from T0 (3.1±0.3) to T2 (3.7±0) and then decreasing of about one trophic level at T3 (2.7±0), while in Control TP progressively decreased of one level, from T1 (3.2±0.1) to T3 (2.6±0.4).

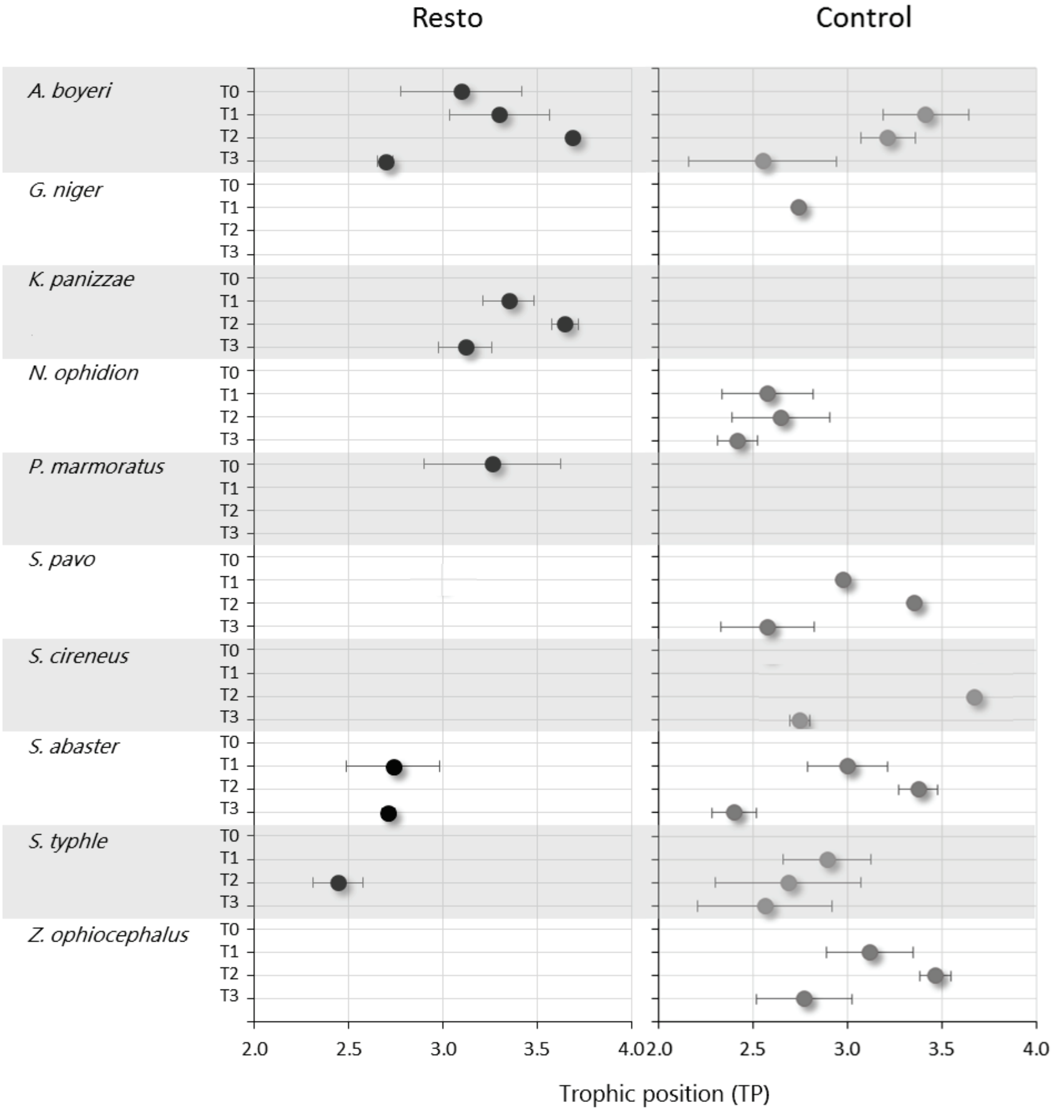


**Figure 4.** Trophic positions (TP, mean ±s.d.) estimated for each fish species collected in each site (Resto and Control) and sampling time (T0-T3).

### *Trophic niche variability of fish communities across time*

Results obtained from the analysis of the isotopic niche revealed that overall, trophic niches of the communities of the restoration site were mostly smaller, across time, than the niche of the reference community (Fig. 5 and 6). The width of the isotopic niche progressively increased in the restoration site until T2 (SEAc from 2.33‰$^2$ at T0 to 6.10‰$^2$ at T2) and then it sharply decreased at T3 (1.95‰$^2$) in parallel to a significant shift towards more $^{13}$C-enriched portion of the isotopic space (Fig. 5). The same trend, but wider niches characterized the communities sampled in the control site, with values of SEAc lower in autumn (respectively 4.69 and 4.98‰$^2$ at T1 and T3) and higher in spring (6.12‰$^2$ at T2).

The overlap between the isotopic niches of the two communities at the same sampling time in Resto and Control was higher in Spring (at T2, with 58% of overlap), indicating a similar positioning of the niches within the isotopic space, rather than in Autumn (respectively 41 and 33% overlap at T1 and T3).

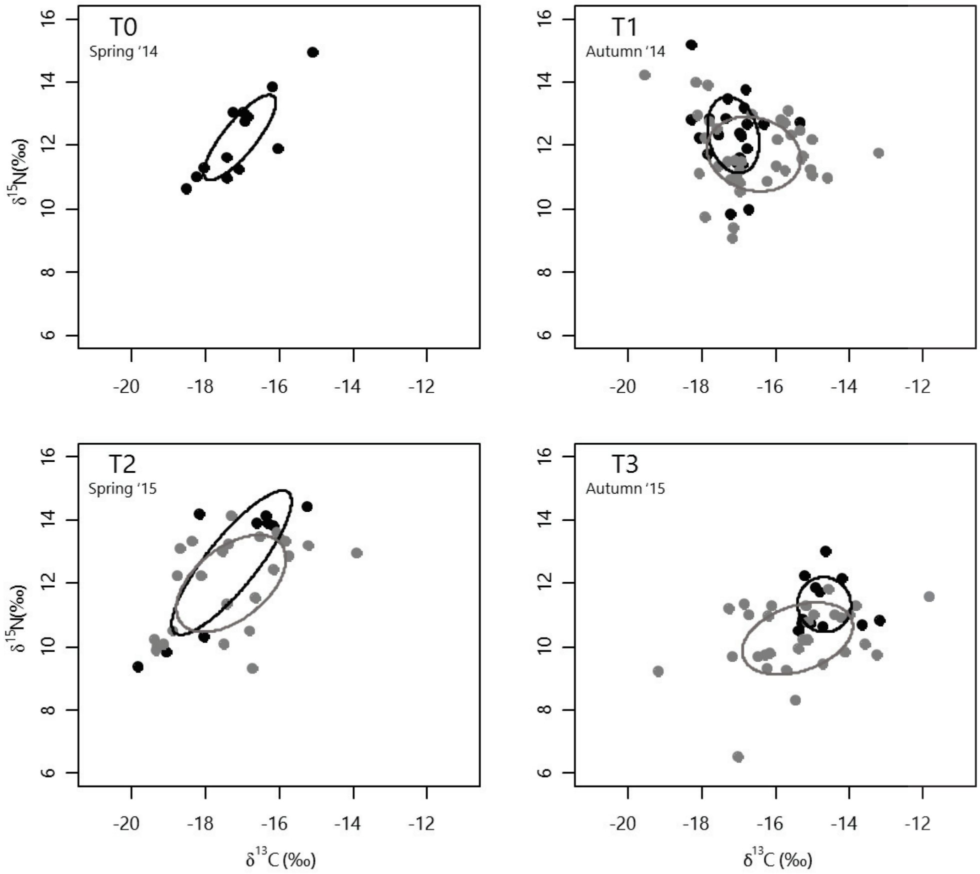


**Figure 5.** Comparison of the isotopic niche (expressed as SEAc) of the whole fish communities sampled in the restoration site (black points and ellipse) and in the control site (grey points and ellipse) across sampling times (T0-T3).

Community-wide metrics, presented as Bayesian estimates in order to allow comparisons between communities with different number of populations (Jackson *et al.* 2011), showed overall comparable variation trends across time, with values for the communities of the restoration site mostly lower than the Control site (Fig. 6).

The three metrics that provide information on trophic diversity within the communities, NR, CR and CD constantly increased from T0 to T2 and then decreased at T3 in the restoration site. In the Control, the same metrics followed mainly a seasonal trend, with higher values in Spring (T2) and lower in Autumn (T1 and T3) and in general showed slightly higher modes than in Resto (the only exceptions were at T2 for NR and CD). Conversely, the trophic redundancy was lower in Resto (higher modal values of NND) than in the control community, while the evenness of the species was slightly higher (lower values of SDNND) than in the control across time.

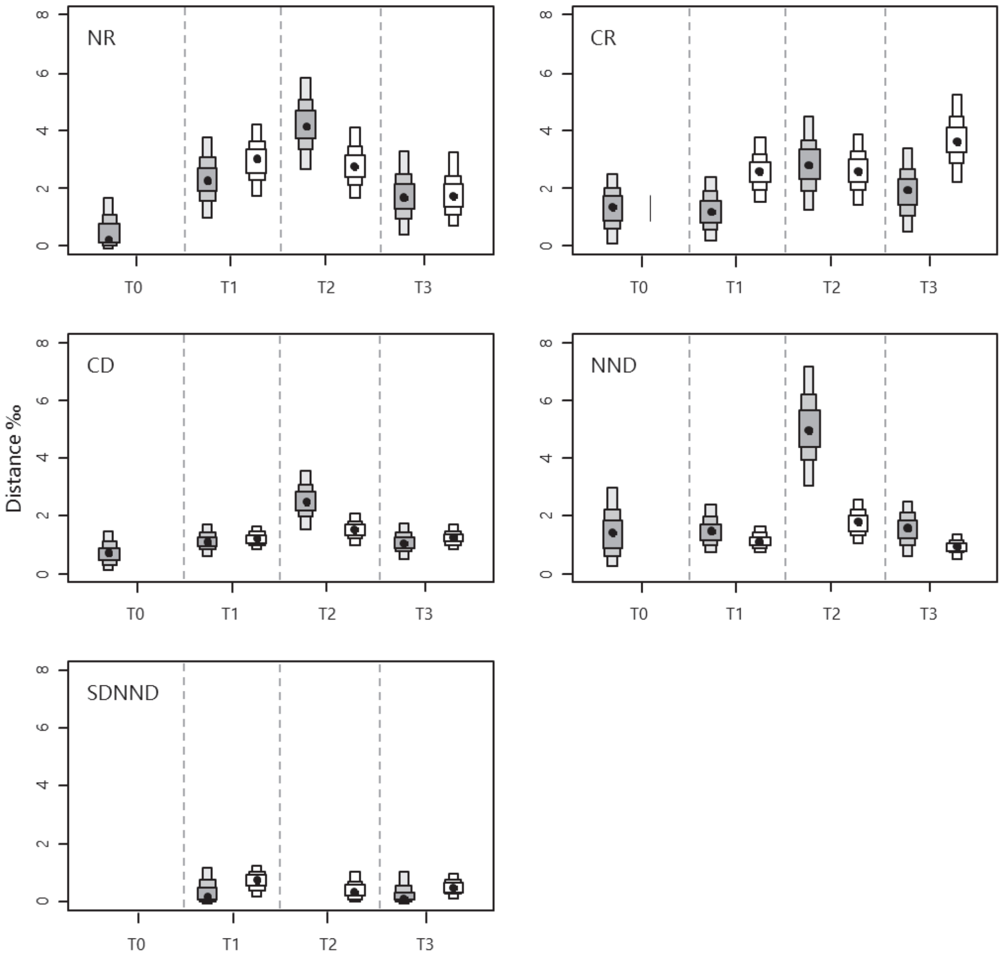


**Figure 6.** Comparison of the Bayesian estimates for the community-wide metrics of the entire fish communities sampled in the restoration site (grey boxplots) and in the Control site (white boxplots) across sampling times (T0-T3). Boxes in each boxplot represent the 50, 75 and 95% of credibility intervals and black points are the mode. NR and CR are respectively the $\delta^{15}$N and $\delta^{13}$C ranges, CD is the mean distance to centroid of the community, NND and SDNND are respectively the mean and the standard deviation of the nearest neighbour distance of the individuals within the community.

## 3.4. Discussion

The trophic structure of a fish community associated to a site subjected to seagrass restoration in the Venice Lagoon was monitored for the first two years since the beginning of the restoration project. In particular, changes in fish community and its trophic niche were examined comparing the community of interest to a reference community associated to a stable seagrass meadow present in the same study area.

Although longer time scale monitoring is needed in order to infer any evaluation of the restoration success (Bell, Middlebrooks & Hall 2014), few early signs of structural and functional recovery of the fish community were detected during the investigation period.

*Fish community features during restoration process*

After two years of restoration, seagrasses transplanted in the site investigated provided a good recovery response, according to the results reported by Scapin *et al.* (2016). Data reported for seagrass coverage and seagrass patch average diameter indicated a consistent increase in time since the beginning of the project, with higher increase recorded during the first year (Scapin *et al.* 2016).

Similarly, two idexes calculated by Scapin *et al.* (2016) to assess the state of the macrophytes (MaQI, Macrophyte Quality Index; Sfriso *et al.* 2014), and the macroinvertebrate community (MAMBI, Multivariate-Azti Marine Biotic Index; Muxika, Borja & Bald 2007) indicated both a good condition at the end of the second year of the project.

Despite this good response of seagrasses, after two years of restoration the fish assemblage was still composed by a lower number of species than in the control site, with any evident increase in species diversity.

Density of individual fish species did not show any relevant increase across time, hence did not let us deduce any improvement given by the restoration process, but overall, the mean density was comparable to that of the control site. This could indicate that the seagrass habitat partially recovered was already able to support similar fish communities structure (in terms of quantity, hence densities) as the corresponding natural habitat, but did not present yet suitable conditions to support similar function (in terms of quality, hence species diversity). Likewise, high densities of few species at the restoration site could be due to high opportunistic behaviour of the species involved, which can be able to dominate bare substrate habitats (Franco *et al.* 2006a). In fact, only the two estuarine residents *Atherina boyeri* and *Knipowitschia panizzae* could be considered dominant species at the restoration site, but usually they are, especially the latter, abundant also in other shallow habitats such as bare mudflats or intertidal creeks (Franco *et al.* 2006b; Franzoi *et al.* 2010). Only a principle of colonization was showed by some of the species typically present in seagrass meadows, such as the Syngnathidae *Syngnathus abaster* and *S. typhle* (Malavasi *et al.* 2004). However, they were only occasionally present in the restoration site and their densities were very low, hence did not allow to consider them as stable components of the fish assemblage.

Further, no presence was recorded of other key species such as the pipefish *Nerophis ophidion*, or the grass goby *Zosterisessor ophiocephalus*, that are expected to represent integral part of the fish community associated to seagrass beds (Franco *et al.* 2006a; Franzoi *et al.* 2010). Accordingly, such species were recorded in the reference site, a stable and dense seagrass habitat, where their morphology and behavior habits allow them to camouflage and hide from predators, or reproduce in suitable conditions (Franco *et al.* 2006b, 2012).

### *Fish community trophic niche changes during restoration process*

The analysis of the trophic niche of the entire community examined at the restoration site revealed some early signs of trophic changes that are indicative of trophic dynamics occurring similarly at the control site, where they are mainly driven by seasonal fluctuations.

As well as for a fragmented habitat described in Layman *et al.* (2007b), we could assume that the restoration site before the beginning of seagrass transplanting was mainly characterized only by null or patchy distribution of macroalgae, hence scarce availability of fish preys usually associated to macrophytes (*e.g.* small benthic invertebrates) in comparison to a dense meadow rich of food sources. As a consequence, a lower availability of food items should result in a lower trophic diversity of the community, where species are constrained to exploit similar food items, hence displaying a narrower isotopic niche (Layman *et al.* 2007b).

Here, the isotopic niche width (SEAc) of the community investigated showed the narrowest width at T0, before the restoration practices and then a slight progressive expansion occurred until T2. After such sampling time, the community seemed to be aligned with the natural seasonal fluctuation, carachterized by wider niches in spring and narrower in autumn, that has induced the sharp restriction in autumn at T3 also showed in the control site.

The high overlap percentage with the niche of the control site, reported mainly for T1 and T2, could be indicative of a similar basal trophic support, according also to the similar isotopic signatures reported for the basal sources of organic matter collected in both sites.

Furthermore, the significant shift of the isotopic niche towards more $^{13}$C-enriched portion of the isotopic space, reported at T3, suggests an actual shift in the trophic pathways supporting the fish community, which is more influenced by the seagrasses (the most $^{13}$C-enriched basal sources).

Community-wide metrics provided further detailed information on the changes occurring in the trophic structure across time and in comparison to the reference site.

The trophic extent of the isotopic niche on the nitrogen and the carbon axis (respectively NR and CR) was lower in comparison to the control (with exception at T2 for NR), indicating a resource use limited to a smaller range respectively of trophic levels and of basal sources (Layman *et al.* 2007a).

Trophic diversity, expressed as CD, slightly increased throughout the monitoring time (except that at T2), following slightly lower trends but comparable across time with the reference community. From one hand, this could be interpreted as a small, early sign of trophic recovery in a community that, although constituted by a little number of species, does not differ greatly from a stable community in terms of trophic diversity. On the other hand, this could be soley due to the fact that the two main species co-occurring in the investigated community, that are *A. boyeri* and *K. panizzae*, show different feeding habits, being respectively hyperbenthivores/zooplanktivores and microbenthivores (Franco *et al.* 2008a), hence are able to partition the few resources available.

The lower species diversity reflects in a reduced trophic redundancy (higher NND) of the community, that is kept constant throughout the monitoring time and tendentially lower than the control community (with lower NND).

The notable exception given at T2 for most of the metrics (NR, CD and NND) is attributable to the presence of *S. typhle* that occupies a distant position from the other species in the isotopic space, at a lower trophic position. This induces a widening of the isotopic niche of the entire community and especially its trophic redundance, that by definition is sensitive to the distance between populations.

Overall, results here reported suggest similar trophic dynamics to that of the reference community, mainly driven by seasonal fluctuation, with early signs of resource use shift towards seagrass basal sources.

Many studies in the past highlighted that the time needed for a seagrass meadow to return to natural, stable conditions is often much longer than the scheme of the present monitoring (Bell *et al.* 2008, 2014; Irving *et al.* 2010) and the success has only been limited to 30% of the cases (Fonseca *et al.* 1996; McSkimming *et al.* 2016). Nowadays, major attention is paid to site selection, which was believed to be the main cause of failure (Fonseca 2011), but there is still need to monitor the restoration process for longer time in order to detect, other than the structural recovery, also a functional recovery of the system (McSkimming *et al.* 2016).

## 3.5. Conclusion

Patterns showed in this study have outlined trophic features of a community associated to a site under seagrass restoration. Lower species diversity but similar density characterized the fish community of the restoration site, while the trophic niche showed early sign of extension and shift towards seagrass basal sources.

Results suggested that, despite the ecological status of macrophytes improved during the first two years, longer time is needed for the fish community to restore structurally and functionally. This represents the first step of a long and complex process that will need to be monitored for years and decades to observe results clearly attributable to the seagrass restoration progress. However, this study emphasizes the importance of considering the recovery of ecosystem function, other than ecosystem structure, among the criteria to assess the restoration success.

# Conclusion

From an ecological and management perspective, it is essential to understand and predict how populations and communities respond to changes induced by environmental variability and perturbations.

The complex web of interactions between species and their environment represents the basic structure on which the ecology of populations and communities is based. Among these, trophic interactions are useful tools to investigate the degree of complexity and natural variability of a system, that summarizes the wide spectrum of abiotic and biotic processes (Fraterrigo & Rusak 2008).

This study examined the natural variability of trophic organization and trophic niche as a response to different drivers of change at level of population and community. Investigation was focused on fish species in the context of lagoon ecosystems, looking at single population, multiple populations or whole communities, as response to three main drivers of change given by habitat heterogeneity as intrinsc feature of transitional environments, morphology changes occurring during species development (ontogeny) and habitat changes induced by restoration management practice (positive perturbation).

Looking at the habitat hetereogenity, outcomes emerged from the focus on the target species *Aphanius fasciatus*, examined in the context of trophic niche variability across natural and artificial habitats at inter- and intra-lagoon level, revealed the high trophic adaptability of the species as a key factor of success to ensure a broad distribution in such wide variety of heterogeneous environments. While habitats characterized by higher diversity of basal sources (*e.g.* seagrass beds and salt marshes) promote the coexistence of more species, some marginal habitats with lower source diversity and high confinement level (*e.g.* artificial ditches or intertidal creeks), could represent hotspots for conservation purposes where the target species is able to reach the highest abundances.

Focusing closely to the trophic ecology of a species of important economic value, *Sparus aurata*, allowed to provide accurate description of the diet shift occurring during the ontogeny of the species, which is strictly influenced by its migration from the sea into coastal lagoons, which are used as nursery areas. The size limit of 20 mm in standard length was detected as a crucial threshold that marks the transition from post-larvae to the juvenile phase, resulting in significant changes of morphology that perfectly match the shift in trophic habits and habitat exploitation. Salt marshes represented particularly important habitats for the species during its migration and movement towards the different areas of the lagoon, from the less to the most confined ones. For this reason, conservation strategies of lagoon systems should pay special attention to such lagoonal habitats, that are increasingly threatened by anthropic impact, and whose irreversible regression would result in severe loss of ecological functioning.

Lastly, assessing the temporal variation in trophic organization of the fish community associated to a site under seagrass restoration provided an evaluation of the functional recovery that should be parallel to the structural recovery expected by successful habitat restoration actions. However, despite results suggested similar trophic dynamics between the restored site and the reference one, mainly driven by seasonal fluctuations, longer time of observation is needed to detect a clear functional recovery of the fish community.

Patterns emerged from the three main topics discussed highlighted the importance of the trophic adaptability of fish species that spend the entire life cycle (residents, *A. fasciatus*) or only one stage (migrants, *S. aurata*) within systems typically dominated by highly changing conditions.

Overall, outcomes obtained from this research contributed to deepen the knowledge of the variability of trophic processes, that is crucial to understand the functioning of transitional ecosystems such as coastal lagoons.

Moreover, these results could have applicative value in terms of conservation and environmental management. In line with the aims of the Habitat Directive, the preservation of a species of conservation interest (*A. fasciatus*) cannot disregard from the contextual preservation of the habitats where the species live and reproduce, including the minor and marginal ones. In addition, fair management practices of lagoonal habitats provide important advantages for those organisms of commercial value (*e.g. S. aurata*), enhancing not only the ecological role of such ecosystems (*e.g.* nursery), but also contributing to the ecosystem services provided.

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