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Trophic adaptability shapes isotopic niche of the resident fish *Aphanius fasciatus* across lagoon habitats

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ABSTRACT

Coastal lagoons are characterized by high habitat heterogeneity where natural habitats coexist with artificial ones, historically set up to support human activities. Increasing anthropogenic pressure may lead to progressive degradation of the most vulnerable lagoonal habitats and the associated biological communities. One of the strictly estuarine-dependent fish species that may be affected by the degradation of lagoon habitats is the South European toothcarp *Aphanius fasciatus*, archetype of Mediterranean lagoon residents.

Carbon and nitrogen stable isotopes were used to disentangle the influence of habitat types (natural *vs* artificial) and fish community (multi-trophic context) on the trophic niche features of *A. fasciatus*. Fish communities and organic matter sources were sampled in two Mediterranean lagoons located at the northernmost (Lagoon of Venice) and at the mid-latitude (Stagnone di Marsala, Trapani) of *A. fasciatus* distribution.

Results showed evidence of high trophic adaptability of *A. fasciatus*, whose omnivorous feeding habit resulted in seasonal changes in isotopic niche width and niche partitioning with co-occurring species, constituting an advantage in habitats characterized by high seasonal variation of resources availability. Furthermore, while macrophytes drove the main trophic pathways leading to *A. fasciatus* in natural habitats, artificial habitats were mainly based on sedimentary organic matter routes. These outcomes broaden our knowledge on how natural and artificial habitats trophically support *A. fasciatus* populations and results are discussed in light of the ecological implications for environmental management of coastal lagoons.

1. Introduction

Transitional water ecosystems, such as coastal lagoons, are typically characterized by high habitat heterogeneity and high productivity (Cognetti et al., 2008; Pérez-Ruzafa et al., 2010). The combination of these features contributes to support a large diversity of biological communities that thrive and maintain high levels of biodiversity, not only within the same system, but also in the adjacent marine ones (Elliott et al., 2007).

Fish communities using estuarine habitats have been thoroughly described using the guild approach and can be composed by four categories including marine, estuarine, diadromous and freshwater fish (Potter et al., 2015), which take advantage of the highly productive and secure shallow waters of lagoon habitats and use them as shelter, nursery areas, reproductive and feeding grounds (Franco et al., 2009). Not only lagoon habitat heterogeneity supports high fish production, but has also a crucial role in shaping their trophic dynamics across habitats, thus influencing the life history traits (Brigolin et al., 2016; Elliott and Hemingway, 2002). The diversity of lagoon natural habitats includes saltmarshes, seagrass meadows, mudflats (either vegetated or not) and intertidal creeks (Elliott and Hemingway, 2002). More recently, increasing attention has been paid also to the variety of artificial habitats commonly present in coastal lagoons, typically artificial ditches or ponds which have been historically set up to support human activities related for example to salt production, aquaculture and artisanal fishery. The importance of such marginal habitats for fish species has been highlighted by different studies (Brigolin et al., 2016; Cavraro et al., 2014, 2017, 2021), which found that abundances of some resident fish

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populations were higher in small and sheltered artificial habitats rather than in natural habitats of the same lagoon. This evidence was related to the confined nature of artificial habitats, to the low exposure to predation and to the ability of the resident species to tolerate extreme environmental fluctuations (Cavraro et al., 2017).

Increasing anthropogenic pressure on coastal areas is inducing progressive degradation of the most vulnerable lagoonal habitats (either artificial and natural) and inadequate management practices may lead to the risk of ecological and environmental loss, with consequent impact on the associated biological communities (Gaertner-Mazouni and De Wit, 2012; Pérez-Ruzafa et al., 2011). In this context, particular attention should be paid to (i) the possible role that artificial habitats (in addition to the natural ones) play for species of conservation interest, and consequently (ii) their possible role as key refuge habitats where reduced predation pressure is combined with high resource availability due to the confined nature of the habitat (Cavraro et al., 2017; Ruiz et al., 1993).

One of the few strictly estuarine dependent fish species (*sensu* Potter et al., 2015; Facca et al., 2020) that deserves special consideration is the South European toothcarp *Aphanius fasciatus* Valenciennes 1821 (family Cyprinodontidae), archetype of the Mediterranean lagoon residents, which may be affected by the degradation and loss of lagoon habitats. It is considered one of the few "lagoon endemisms" according to Kara and Quignard (2018) and its distribution includes a wide variety of coastal habitats in the central and eastern Mediterranean, within which it spends the entire life cycle and reaches high population densities (Cavraro et al., 2014; Leonardos, 2008). *A. fasciatus* belongs to the group of *killifish* as it is known to populate shallow brackish-waters, where it is able to tolerate a wide range of temperature, salinity and oxygen concentration, reaching extreme conditions (Kara and Quignard, 2019; Triantafyllidis et al., 2007). It is listed in the Annex II of Habitats Directive (92/43/EEC) as "animal species of Community interest whose conservation requires the designation of special areas of conservation" and due to its high ecological value, it has been extensively used as a model species for different fields of study (Facca et al., 2020). For instance, in the field of genetics it was used to investigate evolutionary processes (e.g. Hrbek and Meyer, 2003; Maltagliati et al., 2003) and genetic diversity (e.g. Triantafyllidis et al., 2007; Pappalardo et al., 2008; Angeletti et al., 2010). Further studies used it as sentinel species to assess the genotoxic effect of pollutants released in Mediterranean lagoon ecosystems (Mosesso et al., 2012) and others have focused on aspects of reproductive behavior (Cavraro et al., 2013, 2021; Malavasi et al., 2010) and life history traits linked to the habitats used (Cavraro et al., 2014, 2021). However, relatively little work has been done examining the trophic niche ecology of the species (e.g. Leonardos and Sinis, 1999; Leonardos, 2008) and its variability across the different types of lagoon habitats along its geographic distribution to infer the potential consequences of habitat loss.

In the last decades, the recent development of quantitative tools to describe the trophic niche by means of stable isotope data, typically carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$), allowed to explore several aspects in the field of trophic niche ecology. Indeed, previous studies demonstrated how stable isotopes can be transposed into the concept of the trophic niche (Bearhop et al., 2004; Newsome et al., 2007). A series of isotopic niche metrics were developed to describe, for instance, the width of the niche and its trophic diversity (see Layman et al., 2007; Jackson et al., 2011). More interestingly, these metrics can respond to changes induced by intraspecific and interspecific competition, prey abundance (Bearhop et al., 2004), trophic habits (Vermeiren et al., 2015), ontogenetic diet shifts (Andolina et al., 2020), ecological invasion (Jackson et al., 2012) and seasonality (Abrantes et al., 2014).

Here, isotopic niche descriptors were used to disentangle the influence of habitat heterogeneity and fish community on the trophic niche features of *A. fasciatus*, by obtaining quantitative information that can be useful to broaden our knowledge of this species as well as the habitats where it is distributed. Particularly, hypotheses were that: (i) the isotopic niche of *A. fasciatus* populations would differ in the two habitat types considered (natural *vs* artificial), being driven by different pathways of organic matter; (ii) pathways of organic matter would be presumably based on macrophytes in natural habitats, due to abundance of this basal source and on sedimentary organic matter in artificial habitats, due to the confined nature of the habitat and the high frequency of resuspension events; (iii) the isotopic niche of the species changes also in dependence of the multi-trophic community context, determining variations in niche width and resource partitioning according to the feeding ecology of the species co-occurring in the same habitat.

2. Materials & methods

2.1. Study areas

This study was carried out in two of the main Mediterranean transitional ecosystems: coastal ponds and Northern Adriatic lagoons (Brambati et al., 1988). The first ones are represented by the Stagnone di Marsala (Italy), and the second ones are represented by the Venice Lagoon (Italy, specifically the Northern sub-basin), which are located respectively at the mid- and the northernmost latitude of the target species' distribution area within the Mediterranean Sea (Valdesalici et al., 2015) (Fig. 1). The Stagnone di Marsala and the Venice Lagoon differ in terms of habitat heterogeneity, types of dominant natural habitats (respectively seagrasses *vs* saltmarshes), artificial habitats (saltworks *vs* artificial creeks), tidal regime (nanotidal *vs* microtidal), hence offering interesting study cases to look at to pursue our aims.

The Stagnone di Marsala (37° 52′ N, 12° 28′ E) is a shallow semi-enclosed coastal environment, 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 than 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 representing 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 and stronger marine influence (La Loggia et al., 2004). The Stagnone di Marsala is also classified as oligotrophic, as the mean chlorophyll-*a* concentration is around 1.0 $\mu g\ l^{-1}$ in the water column (Sarà et al., 1999) and 3.0 $\mu g\ l^{-1}$ in the sediment (Pusceddu et al., 1999). According to the European Directive 92/43/CEE, the area is included in the lists of the Sites of Community Importance (SCI, ITA010026) and Special Protection Areas (SPA, ITA010028).

The Venice Lagoon (45° 26′ N, 12° 20' E), with a surface area of about 550 km^2, is among the largest lagoonal systems in the Mediterranean (Fig. 1). According to its hydrology, it can be divided into three main sub-basins: Northern, Central and Southern (Solidoro et al., 2004). It has mainly shallow waters (mean depth about 1.0 ± 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, which hence has a lower salinity than the other sub-basins. The area is subjected to the widest tidal range of the Mediterranean Sea, which varies from ±30 cm during neap tide to ±110 cm during spring tide (Rapaglia et al., 2011). The high variability of morphological and physico-chemical parameters induces high levels of spatial and habitat heterogeneity that characterizes the whole lagoon (Brigolin et al., 2014). Among the different levels of protection of the area, the Habitats Directive (92/43/CEE) includes the northern and the central-southern areas of the Venice Lagoon within the Sites of Community Importance (SCI, respectively IT3250030 and IT3250031), and the whole lagoon is designated as Special Protection

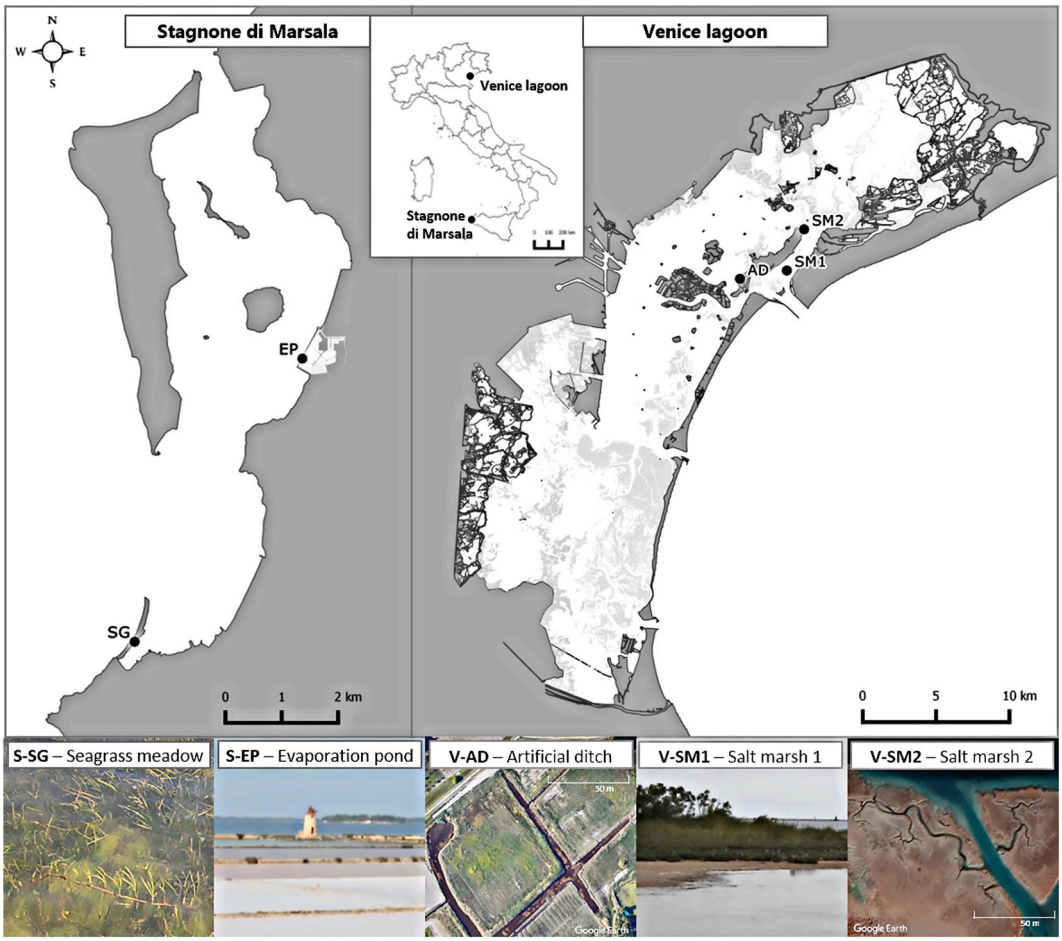

Fig. 1. Location of the study areas and relative position of the habitats examined. S-SG: seagrass meadow, S-EP: evaporation pond, V-AD: artificial ditch, V-SM1: salt marsh 1, V-SM2: salt marsh 2.

Areas (SPA, IT3250046).

2.2. Habitat selection

According to each lagoon habitat heterogeneity, natural and artificial habitats most widely represented were selected, after conducting a preliminary survey intended to verify the actual presence of *A. fasciatus* as suggested by the available literature (Stagnone di Marsala: Vizzini and Mazzola, 2006, 2008; Mazzola et al., 2010; Venice Lagoon: Cavraro et al., 2017; Franco et al., 2006; Franzoi et al., 2010). In each habitat, the presence of macrophytes (seagrasses and macroalgae) was estimated by visual census and the percent coverage within the habitat was recorded according to the Braun-Blanquet method (Braun-Blanquet, 1932).

In the Stagnone di Marsala (S), two habitats were selected: a seagrass meadow as natural habitat and an evaporation pond of a saltwork system as artificial habitat (Fig. 1). The seagrass meadow (SG) was located in the southern sub-basin of the Stagnone di Marsala (37.8143° N, 12.44241°E). Depth was about 40 cm and the sediment was sandy-pelitic, fully covered by *Cymodocea nodosa* Ucria Ascherson, 1870 (90–95%), while macroalgae represented a minor component (5–10%) and the halophyte species *Sarcocornia fruticosa* (L., A.J. Scott) covered the entire coastline. The evaporation pond (EP), also called "cooling vat", was one of the ponds of the saltwork system located in the central area of the Stagnone di Marsala (37.85940°N, 12.47611°E). The selected pond was approximately 0.06 km^2 in surface area and about 50 cm deep, it was enclosed from the adjacent sea and, only occasionally, emptied and refilled through a small channel to allow new salt production. Sandy-pelitic bottom was covered by mixed patches of *C. nodosa* (~30%) and macroalgae species (~25%). Among the halophytes, *S. fruticosa* was present on the edge of the pond.

In the Venice Lagoon (V), *A. fasciatus* can be found mainly in saltmarsh habitats and artificial creeks within lagoon islands (Franzoi et al., 2010). Given the wider extension of the Venice Lagoon and to cover its higher heterogeneity compared to the Stagnone di Marsala, two examples of saltmarshes were selected as natural habitat, and one artificial ditch as artificial habitat (Fig. 1). The first natural site was a small saltmarsh (SM1) situated close to the northern mouth of the lagoon (45.44741°N, 12.41583°E). Water depth was about 30 cm, the sediment was muddy with low coverage of seagrasses (5–25% of *Zostera noltei* Hornemann 1832) and sparse drift macroalgae. Multiple halophyte species (mainly *Limonium narbonense* Mill. and *S. fruticosa*) covered the border of the saltmarsh. The second natural site was a saltmarsh (SM2) placed in an inner area of the lagoon (45.47190°N, 12.43080°E). The sampling site was about 40 cm deep on average and the substrate was mainly muddy and bare, with a very low (5–10%) presence of seagrasses (*Z. noltei* and *Zostera marina* Linnaeus 1753) and macroalgae. It was also characterized by the presence of small adjacent intertidal creeks, about 10 cm deep. Different halophyte species (mainly *Atriplex portulacoides* L. Aellen and *S. fruticosa*) covered the border of the saltmarsh. The artificial site was a system of small connected ditches (AD) situated in Vignole Island (45.44259° N 12.37538°E), historically used to stock temporarily live juvenile fish. Ditches were isolated from the rest of the lagoon, had about 60 cm depth and were periodically dug to avoid landfilling. The bottom of the ditches was mainly muddy and bare, with a very low (5–10%) presence of seagrasses (*Ruppia maritima* Linnaeus 1753) and drift macroalgae, while the borders were mainly covered by the

halophyte *A. portulacoides*.

2.3. Sample collection

Fish specimens and all the dominant basal sources of organic matter were collected in spring and autumn 2014, following a standardized protocol used in all the habitats of both study areas (Stagnone di Marsala and Venice Lagoon). Fish communities were sampled by semi-quantitative catch, using a small beach seine net (mesh size 2 mm) that was towed two times in each habitat to obtain two sampling replicates in each sampling occasion. Length and width of each tow were measured and, depending on the habitat extension, surface area sampled varied between 20 and 600 m^2.

Primary producers such as macroalgae, seagrasses and terrestrial halophytes were randomly collected by hand in triplicate in each habitat and season. Among the halophytes species present in the study habitats, only the most abundant were collected and considered for trophic assessments. In each site seawater samples (10 l in Stagnone di Marsala and 2 l in Venice Lagoon according to the different level of turbidity of the two lagoons) 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). All the samples collected were kept cool until arriving at the laboratory and stored at −20 °C prior to the processing.

2.4. Sample processing and laboratory analysis

Fish sampled were identified at species level and the standard length (SL) was measured. At least 15 individuals for each species were selected across the whole size range found per sampling occasion, dissected by means of scalpel and tweezers to extract the dorsal muscles. Macroalgae, seagrasses and terrestrial halophytes were identified at species level; when present, epiphytes were scraped and separately considered for subsequent analysis. Water samples were pre-filtered (200 μm) and then filtered on precombusted (450 °C, 4 h) filters (GF/F Whatman, pore size 0.7 μm) to concentrate the POM. The top 1.5 cm of sediment cores was sliced and homogenized as proxy for SOM.

Once processed, all samples intended for the analysis of stable isotopes were oven-dried at 60 °C to constant weight for about 48 h and then ground to fine powder using a micro mill or a mortar and pestle. Lastly, an aliquot of each sample was packed into tin capsules and analysed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo Delta Plus XP) coupled to an elemental analyser (Thermo Flash EA1112) at the Stable Isotope Ecology Laboratory of the Department of Earth and Marine Sciences, University of Palermo. Part of the samples containing any carbonate component (i.e. sediment, POM, macrophytes, seagrasses and epiphytes) that might interfere with the determination of carbon isotopic composition was acidified (HCl 1M) prior to $\delta^{13}C$ analysis, which was run separately.

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. Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA- CH-6 for $\delta^{13}C$ and IAEA-NO-3 for $\delta^{15}N$) was 0.1 for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.5. Data analysis

A preliminary screening of the fish fauna data was performed to assess the composition of the community where the target species occurred. The number of individuals of each species collected in each habitat was standardized to 100 m^2. In addition, each species was assigned to an ecological and feeding group, as defined in Franco et al. (2008), to outline the main ecological features of the populations found within the fish assemblage.

To test for differences in composition and densities of fish communities and *A. fasciatus* populations among habitat types, seasons and lagoons, multivariate permutational analysis of variance (PERMANOVA) was performed on fish community density, while univariate PERMANOVA was run on fish total density and *A. fasciatus* density. PERMANOVA was based on the Bray-Curtis similarity index applied on square root-transformed density data. Habitat type (artificial and natural), season (spring and autumn) and lagoon (Stagnone di Marsala and Venice Lagoon) were considered as fixed factors. Similarity matrix of *A. fasciatus* was built including a dummy variable in order to avoid undefined data in the output. Differences in fish isotopic values between habitats, seasons and lagoons were conducted separately for $\delta^{13}C$ and $\delta^{15}N$, using PERMANOVA test based on Euclidean distance similarity index, following the same experimental design mentioned above.

Two quantitative metrics of the isotopic niche width were estimated for all fish populations sampled: standard ellipse areas (SEAc, corrected for small sample size) and Bayesian standard ellipse areas (SEAb). The first metric provided a unique value and was discussed in terms of position and overlap, the second one was generated by 100,000 posterior iterations and allowed for statistical comparison between *A. fasciatus* and the co-occurring species and between seasons. Additional isotopic metrics (Layman metrics, Layman et al., 2007) were estimated only for *A. fasciatus* populations, in order to provide further quantitative details of the isotopic niche features and were:

i) $\delta^{13}C$ 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;
ii) $\delta^{15}N$ Range (NR), difference between the most enriched and most depleted $\delta^{15}N$ values, estimates the trophic length of each population;
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.

Layman metrics were bootstrapped (n = 10000) and then presented as mode along with 95% credible intervals (Jackson et al., 2011, 2012). Bootstrapped metrics are indicated with a subscript 'bt'. Differences in SEAb between *A. fasciatus* and the co-occurring species within season, and between seasons for *A. fasciatus* only were tested through pair-wise comparisons, by calculating the probability that the metric of one group was larger than that of the other, hence a significant difference was regarded as a probability of at least 95% (Jackson et al., 2011). The same test was used to look for differences in *A. fasciatus* bootstrapped metrics between season within habitat and between habitats within season.

A further test was used to check for differences only for *A. fasciatus* niche width between habitat types, seasons and lagoons. For this purpose, 50 SEAb values were randomly sampled from the SIBER data output of each habitat and season and used to perform a PERMANOVA analysis based on Euclidean distance similarity index, following the same experimental design mentioned above: a three-factor design with habitat type (artificial and natural), season (spring and autumn) and lagoon (Stagnone di Marsala and Venice Lagoon) considered as fixed factors.

Lastly, to investigate the trophic pathway of the basal sources that sustain *A. fasciatus* in the different habitats and areas, Bayesian mixing models were performed, using multiplicative error structure, Process * Residual error (Stock and Semmens, 2016). All the sources of organic matter available in the specific habitat for each area were included in the models: sedimentary and particulate organic matter (SOM and POM), macroalgae, seagrasses, pooled halophytes and epiphytes when present. Before running the models, bivariate isotope data $\delta^{13}C$-$\delta^{15}N$ of all sources were tested for their significant difference through PERMANOVA analysis, using a three-factor design (factors Source, Habitat and Season) on Euclidean distance matrix of data for each study area

separately. When sources did not show significant differences, they were grouped before running the mixing model. 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: 0.4 ± 1.3‰ for $\delta^{13}C$ (Post, 2002) and 2.5 ± 1.0‰ for $\delta^{15}N$ (Vander Zanden and Rasmussen, 2001). In addition, to account for the second trophic level of consumers, the standard deviations of the TEFs were amplified by doubling its corresponding variance. No results were available for SM1 spring data due to the lack of the prerequisites needed to run the model correctly (i.e. consumer data did not fall within the source polygon) (Phillips et al., 2014).

All PERMANOVA analyses were performed using PRIMER 6 +PERMANOVA (Plymouth Marine Laboratory, UK). SEAc, SEAb, Layman metrics and mixing models were run in R v. 4.0.2 (R Development Core Team, 2015). SEAc, SEAb and Layman metrics were estimated using the package SIBER v2.1.5 (Stable Isotope Bayesian Ellipses in R) (Jackson et al., 2011), mixing models were performed using MixSIAR package v3.1 (Stock and Semmens, 2016; Stock et al., 2018).

3. Results

Overall, a total of 2641 fishes, belonging to 13 species and 7 families were collected across all the lagoon's habitats and seasons (Supplementary material, Table 1S). Fish communities comprising *Aphanius fasciatus* were characterized by different assemblages across the investigated habitats.

Natural and artificial habitats of the two lagoons supported different fish assemblages in terms of species richness and density. The saltmarsh habitat in the Venice lagoon (S-SM2) showed the highest species richness with 11 species found in spring, whereas the seagrass meadow in the Stagnone di Marsala (S-SG) and the artificial ditch in the Venice lagoon (V-AD) showed the lowest richness, with only two species found in both seasons.

Overall, significant differences emerged in fish community density either for the interaction between Habitat type (artificial *vs* natural) and Lagoon (Stagnone di Marsala *vs* Venice Lagoon) and overall between seasons (spring *vs* autumn) (see PERMANOVA results in Table 2Sa, *p*-value <0.05). Significant differences in fish assemblages between habitat types emerged only within the Venice Lagoon and between lagoons only for the artificial habitat (Table 2Sbc). Total fish density did not show significant differences among habitat types, seasons or lagoons (Table 3S), while density of *A. fasciatus* alone showed significant differences between seasons (Table 4S), with mean density being 145.90 ± 109.28 ind. 100 m^{-2} in spring and 40.74 ± 46.56 ind. 100 m^{-2} in autumn.

3.1. Fish community isotopic values

$\delta^{13}C$ values of fish communities showed significant differences in the interaction of Habitat type with Lagoon and Season with Lagoon (see PERMANOVA results in Table 5Sa, Fig. 2a). Fish communities in artificial habitats were more ^{13}C-depleted than in natural habitats in both lagoons and in Venice Lagoon were more ^{13}C-depleted than in Stagnone di Marsala (Table 5Sbc, $\delta^{13}C$ ranges: V-AD: 26.2 to −17.5‰, V-SM1-2: 21.5 to −11.5‰, S-EP: 15.6 to −10.2‰, S-SG: 13.4 to −11.72). Seasonal differences showed opposite trends in the two lagoons, with fish communities showing lower $\delta^{13}C$ values in autumn than in spring in Venice Lagoon and vice-versa in Stagnone di Marsala (Table 5Sd).

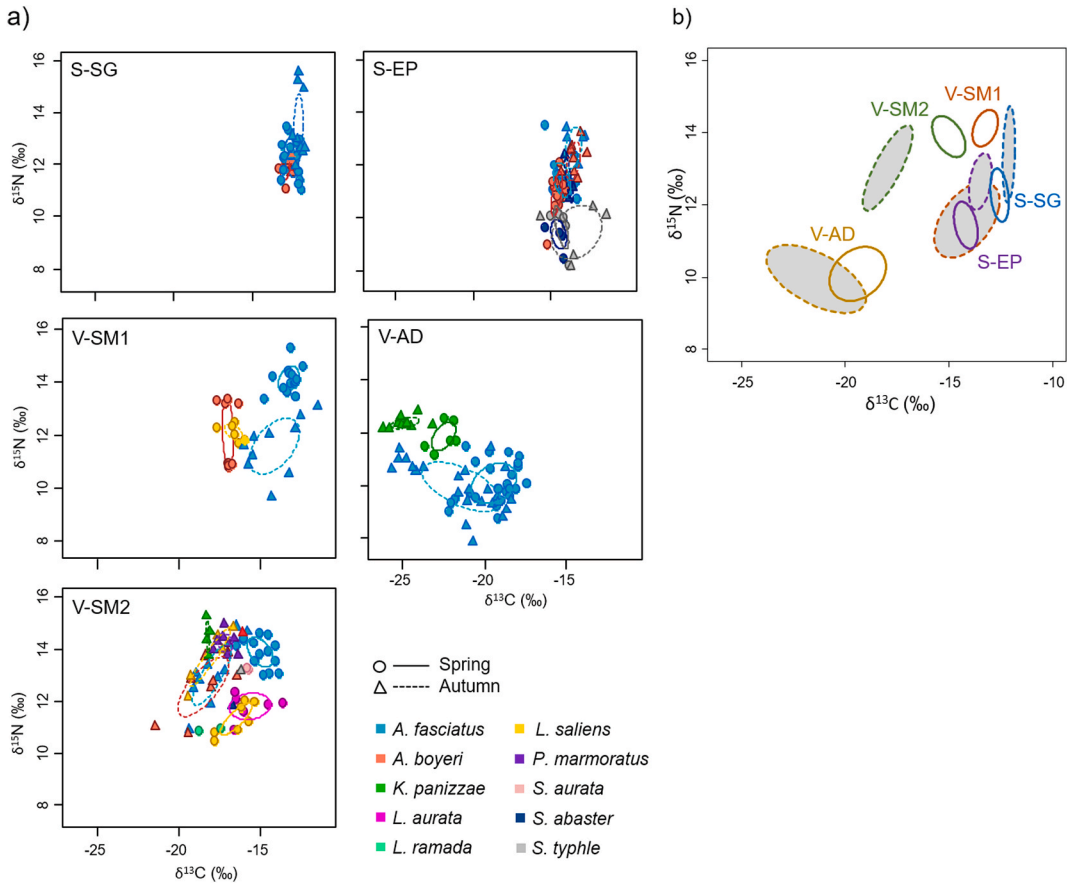

Fig. 2. Distribution of fish species and relative Standard Ellipse Areas (SEAc) within the isotopic space of each habitat examined in spring (circles) and autumn (triangles) (a), and focus comparing *A. fasciatus* SEAc among habitats and seasons (full line ellipses for spring, dashed line ellipses for autumn) within the same isotopic space (b).

As regards $\delta^{15}N$, significant differences emerged for the interaction of the factors Habitat type with Lagoon and Season with Lagoon (see PERMANOVA results in Table 6Sa). $\delta^{15}N$ of fish in artificial habitats distinguished the two lagoons and, in each lagoon, showed lower values in artificial than natural habitats (Table 6Sbc, Fig. 2a, ranges: V-AD: 7.8–12.8‰, V-SM1-2: 9.6–15.3‰, S-EP: 8.1–13.5‰, S-SG: 11.0–15.6‰). Seasonal differences were highlighted only for the Stagnone di Marsala, with fish $\delta^{15}N$ values significantly higher in autumn than in spring (Table 6Sde) and being different from the Venice Lagoon only in autumn.

3.2. Isotopic niche

A. fasciatus' isotopic niche varied in position within the isospace, width and shape across habitats and seasons (Fig. 2). In most of the habitats, the isotopic niche of the target species was located into a more ^{13}C- and ^{15}N-enriched area of the isotopic space compared to the other species, with exception for the population inhabiting V-AD where it showed lower $\delta^{15}N$ values than the co-occurring species (Fig. 2a). A clear segregation of *A. fasciatus* niche occurred in V-AD and V-SM1 in both seasons, in V-SM2 in spring and S-SG in autumn (Fig. 2a).

Niche overlap between the target species *A. fasciatus* and the other species of the community occurred mainly with *Atherina boyeri* when present (Table 1). In spring, in both natural and artificial habitats of the Stagnone di Marsala (S-SG and S-EP), *A. boyeri* occupied respectively 21 and 28% of *A. fasciatus* isotopic niche. In autumn, 48% of *A. fasciatus* niche was overlapped by *A. boyeri* niche in S-EP and 84% in saltmarsh habitat of the Venice lagoon (V-SM2). Overlap with other species were negligible, accounting for less than 20%, in V-SM2 and S-EP in autumn.

Whereas the position of isotopic niches is strictly dependent on the values of the basal sources that characterize the specific habitat at a season, niche shape, size and spacing of individuals within them are related to the trophic diversity of the populations taken into account. In terms of width, the isotopic niche of *A. fasciatus* was wider than that of the co-occurring species in most of the habitats only in autumn, with a few exceptions. In spring, isotopic niche of *A. fasciatus* was significantly wider than that of the co-occurring species, *K. panizzae*, only in V-AD (Table 1).

Seasonal differences emerged in the isotopic niche position of the target species (Fig. 2b). In both habitats of the Stagnone di Marsala, S-SG and S-EP, *A. fasciatus* shifted the isotopic niche towards a more ^{13}C- and ^{15}N-enriched area of the isotopic space, from spring to autumn, with a minor between-season overlap only in S-SG (15%, Table 2). In contrast, in the Venice Lagoon, the isotopic niche of *A. fasciatus* shifted towards a more ^{13}C- and ^{15}N-depleted area of the isotopic space, from spring to autumn. While the shift was complete for populations in both saltmarsh habitats, V-SM1 and V-SM2, 40% of the spring niche was overlapped with the autumn niche in the artificial ditch (V-AD, Fig. 2b, Table 2). Also, the population in the artificial ditch (V-AD) remarkably stood out of all the other habitats.

Looking at the niche width of *A. fasciatus*, significant differences emerged for the interaction of the factor Habitat type with Lagoon and Season with Lagoon (Fig. 2b, PERMANOVA results in Table 7Sabc). Only in Venice Lagoon *A. fasciatus* niche in the artificial habitat was wider than in the natural habitats (Table 2, 7Sb). Seasonal differences between lagoons were due to the enlargement of isotopic niches in autumn

Table 1
Isotopic niche width and area of niche overlap between *Aphanius fasciatus* (AFA) and co-occurring species: standard ellipse area corrected for small sample size (SEAc, ‰^{2}) of *A. fasciatus* (AFA) and co-occurring species; between-species SEAc overlap (‰^{2}); % of *A. fasciatus'* SEAc occupied by the SEAc of co-occurring species; probability that Bayesian standard ellipse area (SEAb) of *A. fasciatus* is different from that of co-occurring species ($^+$= significantly bigger, $^-$= significantly smaller).

Lagoon-Habitat	Season	AFA SEAc (‰^{2})	Co-occurring species	Co-occurring species SEAc (‰^{2})	SEAc overlap AFA co-occurring species (‰^{2})	% AFA SEAc overlapped by co-occurring species	Probability SEAb AFA ≠ SEAb co-occurring species
S-SG	spring	1.04	*A. boyeri*	0.6	0.2	21.0	0.92
	autumn	1.35	*A. boyeri*	0.1	–	–	1.00$^+$
S-EP	spring	1.15	*A. boyeri*	0.8	0.3	27.8	0.80
			S. abaster	1.1	–	–	0.67
			S. typhle	1.1	–	–	0.56
	autumn	1.54	*A. boyeri*	1.1	0.7	48.1	0.72
			S. abaster	1.0	0.2	16.1	0.79
			S. typhle	6.7	–	–	0.01$^-$
V-SM1	spring	1.00	*A. boyeri*	1.5	–	–	0.18
	autumn	5.03	*C. saliens*	0.6	–	–	1.00$^+$
V-SM2	spring	1.37	*C. aurata*	2.5	–	–	0.16
			C. saliens	1.3	–	–	0.43
	autumn	2.91	*A. boyeri*	4.6	2.4	84.2	0.21
			C. saliens	2.0	0.5	18.9	0.50
			P. marmoratus	0.6	0.2	8.1	1.00$^+$
			K. panizzae	0.2	–	–	1.00$^+$
V-AD	spring	3.28	*K. panizzae*	1.4	–	–	0.96$^+$
	autumn	6.00	*K. panizzae*	0.6	–	–	1.00$^+$

Table 2
Comparison of *Aphanius fasciatus* isotopic niche width between seasons in each habitat: standard ellipse area corrected for small sample size (SEAc) overlap (‰^{2}), % of spring SEAc occupied by autumn SEAc and probability that Bayesian standard ellipse area (SEAb) of *A. fasciatus* in spring is different from that in autumn ($^+$= significantly bigger, $^-$= significantly smaller).

Lagoon-Habitat	Spring SEAc (‰^{2})	Autumn SEAc (‰^{2})	SEAc overlap spring – autumn (‰^{2})	% spring SEAc overlapped by autumn SEAc	Probability SEAb spring ≠ autumn
S-SG	1.0	1.3	0.2	15.3	0.29
S-EP	1.1	1.5	–	–	0.32
V-SM1	1.0	5.0	–	–	0.00$^-$
V-SM2	1.4	2.9	–	–	0.02$^-$
V-AD	3.3	6.0	1.3	40.1	0.02$^-$

compared to spring. Differences between lagoons in spring were driven mainly by the higher niche width in V-AD compared to the other habitats, which were comparable to each other regardless of habitat type and lagoon. In autumn, the significant enlargement of the niche in the Venice Lagoon was not observed in the Stagnone di Marsala, where it increased to a lesser extent (see SEAb comparisons in Table 2).

Overall, *A. fasciatus*' isotopic niches showed similar extension in terms of nitrogen (NR_{bt}) and carbon (CR_{bt}) ranges between habitats in spring, with the exception of niche in V-AD that showed a significantly wider CR_{bt} compared to all other habitats (Table 3). Relevant seasonal changes also occurred, as CR_{bt} significantly decreased from spring to autumn in both populations of Marsala habitats (S-SG and S-EP). Also, CR_{bt} significantly increased in two populations of Venice habitats (V-SM1 and V-AD) but not in the inner saltmarsh (V-SM2), in which *A. fasciatus* showed the only increase in NR in autumn (Table 3). Lastly, the trophic diversity of the populations (CD_{bt}), significantly increased in V-SM1 and V-AD from spring to autumn, but remained comparable between seasons for all the other habitats (Table 3).

3.3. Sources of organic matter and mixing models results

Overall, $\delta^{13}C$ values of the sources across seasons were comprised between −28.4 and −6.9‰ and between −26.6 and −8.8‰ in the natural and artificial habitats of the Stagnone di Marsala respectively (Fig. 3, see Table 8S for complete list of the species used as sources of organic matter). In the Venice Lagoon, $\delta^{13}C$ values ranged between −27.8 and −10.3‰ in the natural habitats and shifted towards more ^{13}C-depleted values, comprised between −33.8 and −16.3‰ in the artificial habitat. Regarding $\delta^{15}N$ of the sources collected, values across seasons ranged similarly between 4.8 and 11.2‰ in natural habitat and between 5.8 and 12.8‰ in artificial habitat at the Stagnone di Marsala. In the Venice Lagoon, $\delta^{15}N$ ranges were wider in the natural habitats (2.0–14.0‰) and narrower in the artificial habitat (2.2–10.2‰) (Fig. 3). PERMANOVA performed on bivariate isotope data of the sources showed a significant interaction of all the factors considered: Source, Habitat and Season (Table 9S). A few sources which were not significantly different and were grouped before running mixing models (see Table 8Se).

Results of Bayesian mixing models showed that the most likely contribution of organic matter sources to the trophic pathway sustaining *Aphanius fasciatus* populations was given by macrophytes (namely seagrasses and macroalgae) in all the natural habitats of both lagoons in both seasons, ranging from 12 to 51% in mode (Fig. 4). In the artificial evaporation pond of the Stagnone di Marsala, the role of the SOM (and a mixture of SOM and epiphytes in autumn) was predominant as it provided between 58 and 86% of the total contribution (respectively in autumn and spring). In the artificial ditch in Venice Lagoon, together with macrophytes, a notable contribution was provided by halophytes in spring (33%) and SOM in autumn (32%). The contribution of the halophytes was marginal in all the other habitats (≤3%), even in the ones dominated by terrestrial plants, namely the saltmarshes.

4. Discussion

This study showed evidence of high trophic adaptability of *A. fasciatus*, whose omnivorous feeding habit resulted in seasonal changes in isotopic niche width and niche partitioning with co-occurring species, constituting an advantage in habitats characterized by high seasonal variation of resources availability. Furthermore, while macrophytes drove the main trophic pathways leading to *A. fasciatus* in natural habitats, artificial habitats were mainly based on sedimentary organic matter routes.

4.1. Variability of A. fasciatus trophic niche and supporting pathways of organic matter

The observed variability of *Aphanius fasciatus* isotopic niche, in terms of position within the isotopic space mirrors that of basal sources. This supports the hypothesis that the trophic features of habitat types and lagoons shape its trophic niche. Indeed, it is widely known that the isotopic values of a consumer species, hence the overall population niche, is strictly dependent on the values of the trophic sources at the base of the pathway of organic matter (Vizzini and Mazzola, 2006). The overall depletion of carbon and nitrogen isotopic ratios that has emerged in both fish and organic matter sources of the artificial habitats compared to the natural ones and overall in the Venice Lagoon compared to the Stagnone di Marsala, can be addressed to differences in the main abiotic factors characterising the two lagoons located at two different latitudes, from the level of salinity to the level of confinement. Then local features contribute to exacerbate some patterns, as in the case of the artificial ditch in Venice Lagoon, where the particularly low values of $\delta^{13}C$ 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 and Koprivnjak, 2007), probably related to digging activities occasionally carried out in the channels investigated, and also to the indirect influence of the detritus of the terrestrial halophytes surrounding the ditch border.

Assessing the spatio-temporal changes in resource use is crucial to understand the trophic structure of a given habitat (Nordström et al., 2015). Trophic pathways estimated by isotope mixing models aided to highlight the different role of organic matter sources supporting the target species in natural and artificial habitats of the two lagoons. While the main trophic pathway of *A. fasciatus* seems definitely driven by the macrophytes in the natural habitats (the seagrass meadow in the Stagnone di Marsala, and the two saltmarshes in the Venice Lagoon), in the artificial habitats of both lagoons the role of SOM seems to give an important contribution.

Our findings suggested that the central role of macrophytes (seagrasses followed by macroalgae) is not density dependent, since the contribution to the trophic web was significant not only in the seagrass habitat, where it was fully expected, but also in the saltmarshes, where macrophytes showed a patchy distribution. Despite the direct utilization of these sources is generally low because of the high content of

Table 3
Bootstrapped isotopic metrics (‰) calculated for *A. fasciatus* by habitat and season. Relative 95% credible intervals for each metric are reported in brackets. Same letters in apex indicate not significant differences ($p > 0.05$) based on the probability associated with paired comparisons between habitats (within the same season), whereas asterisks in apex indicate significant differences ($p < 0.05$) between seasons based on the probability associated with paired comparisons (within the same habitat).

Metrics	Spring					Autumn				
	S-SG	S-EP	V-SM1	V-SM2	V-AD	S-SG	S-EP	V-SM1	V-SM2	V-AD
NR_{bt}	2.4 (1.6–2.4)^{ab}	2.8 (0.9–2.8)^{abc}	1.9 (0.7–2.0)^{ac}	1.6* (0.9–1.6)^{c}	2.7 (1.9–2.8)^{b}	3.6 (1.0–3.6)^{A}	2.3 (0.4–2.3)^{B}	3.4 (1.4–3.4)^{AB}	4.0* (1.3–4.0)^{AB}	3.6 (2.1–3.6)^{A}
CR_{bt}	1.2* (1.0–1.2)^{a}	2.0* (1.3–2.0)^{b}	2.4* (0.6–2.4)^{ab}	2.6 (1.4–2.6)^{b}	4.7* (3.2–4.7)^{c}	0.9* (0.4–0.9)^{A}	1.4* (0.3–1.4)^{A}	4.5* (2.5–4.5)^{B}	3.6 (1.5–3.6)^{B}	7.3* (6.0–7.3)^{C}
CD_{bt}	0.7 (0.5–0.9)^{a}	0.6 (0.5–1.0)^{a}	0.6* (0.3–0.9)^{a}	0.8 (0.6–1.1)^{a}	1.4* (1–1.7)^{b}	1.3 (0.4–1.5)^{AB}	0.5 (0.3–1.1)^{B}	1.6* (0.9–2.0)^{AC}	1.3 (0.7–1.9)^{AB}	2.2* (1.6–2.7)^{C}

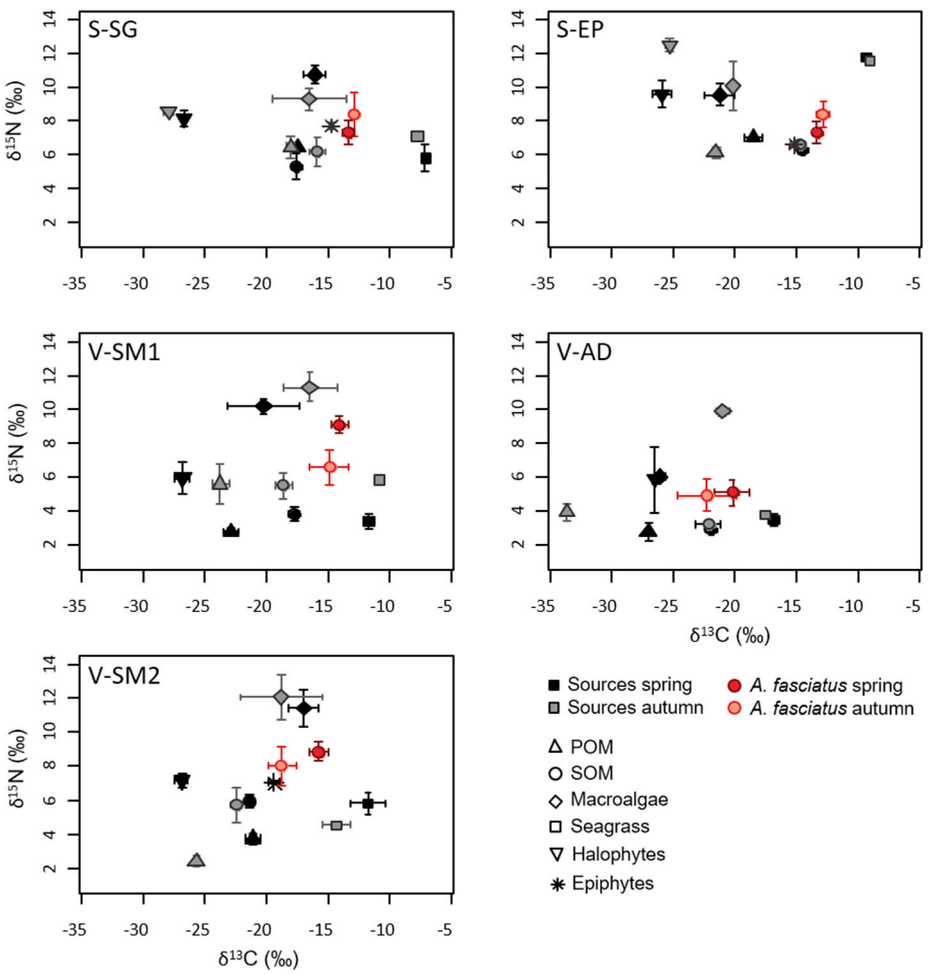

Fig. 3. Mean (±s.d.) $\delta^{13}C$ (‰) *vs.* $\delta^{15}N$ (‰) of organic matter sources sampled in each habitat and *Aphanius fasciatus* corrected by TEF. S-SG is seagrass meadow and S-EP evaporation pond in the Stagnone di Marsala; V-SM1 and V-SM2 are saltmarshes and V-AD is artificial ditch in Venice Lagoon.

deterrents (such as phenols) and high C/N (Poore et al., 2012), macrophytes play a central role in sustaining the energy flow 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 and Mazzola, 2008). In both artificial habitats, which are structurally enclosed and characterized by patchy primary producers, a relevant contribution was given by SOM. This contribution was dominant in the evaporation pond in both seasons, while in the artificial ditch it was as important as the seagrass contribution in autumn (despite the low coverage). This is in accordance with studies that found that the role of SOM increases when local conditions enhance frequent event of resuspension and sedimentation (Vizzini and Mazzola, 2006). Also, in sites with patchy distribution of macrophytes, the role of primary producers decreases in favour of SOM and POM (Rossi et al., 2015). As a consequence, the use of basal sources supporting *A. fasciatus* trophic pathway is habitat-specific, depending not only on their specific composition and availability, but also on the abiotic factors that characterize the structure of such habitats. Conversely, halophytes that in general are considered important sources in lagoon food webs, given their extensive distribution along the border of saltmarshes (Short and Neckles, 1999), in our findings only played a very marginal role in the energy flow towards the target species, accounting for very low percentage contribution in most of the habitats. Again, the artificial ditch in Venice Lagoon is an exception, due to the isolated nature of the habitat that might contribute to the detention of this source inside the ditch.

If the position of the isotopic niche is dependent on the isotopic values of the basal sources, other aspects of the isotopic niche, describing the width (SEAc, NR, CR) and trophic diversity (CD) of the population, are entirely dependent on the feeding ecology of the species and the ability to perform individual specialization to reduce intraspecific competition (Matthews and Mazumder, 2004; Leonardos, 2008). This is consistent with the seasonal pattern observed for the isotopic niche width of *A. fasciatus*, suggesting that the omnivore feeding habit (Franco et al., 2008) allows the species to enlarge the niche in autumn, exploiting a wider spectrum of sources not normally exploited in spring. Previous studies, in fact, assert that niche width can increase 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). Hence, the high trophic adaptability of *A. fasciatus* allows to maintain and even enlarge the trophic niche, hence constituting an advantage in those environments characterized by high seasonal variation of quali-quantitative availability of resources. This can also be linked to the necessity to store resource energy before the winter season approaches, as demonstrated in various fish species that increase their condition index in autumn (Blackwell et al., 2000; Froese, 2006).

In addition, the isotopic niche width and trophic diversity of *A. fasciatus* populations seemed to be overall diminishing at increasing density of the local community in autumn, when sources availability is limited in the lagoon systems (Ceccherelli et al., 1994). High densities of the various fish populations within the community might contribute to limit the niche width expansion and the individual diversification of *A. fasciatus* both in natural habitats and artificial habitats.

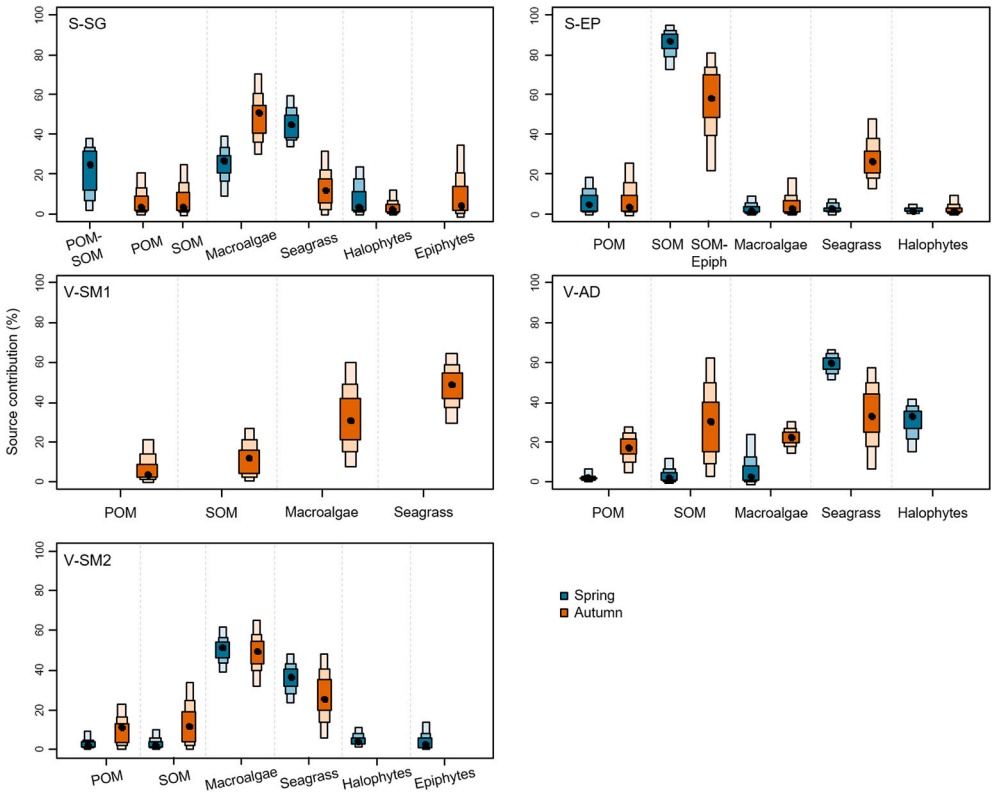

Fig. 4. Percentage contribution of organic matter sources for *A. fasciatus* in the habitats investigated in spring and autumn, with relative credibility intervals of 95% (light blue and orange respectively in spring and autumn), 75% (intermediate blue and orange) and 50% (darker blue and orange), black points indicate the mode. S-SG is seagrass meadow and S-EP the evaporation pond in the Stagnone di Marsala; V-SM1 and V-SM2 are the saltmarshes and V-AD is the artificial ditch in Venice Lagoon. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.2. Dynamics of trophic interaction within the community context/niche overlap

The interpretation of trophic 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 coexisting in the same community. Patterns observed in isotopic niche segregation and overlap are consistent with the feeding ecology of the co-occurring species and vary between habitats.

A. fasciatus is an omnivorous species, a generalist benthic micro-carnivore feeding mainly on small benthic invertebrates such as small crustaceans, gammarids and copepods, chironomid larvae, unicellular and multicellular algae such as diatoms (Leonardos, 2008) and showed a considerable variability of the niche at both inter- and intra-lagoon level.

In the artificial ditch, where *Knipowitschia panizzae* is the only species sharing the habitat with the target species, and it is realistically the only one throughout the year since the site has no communication with the external waters of the lagoon (Cavraro et al., 2017), a clear separation of the isotopic niche was observed in both seasons. Differently form *A. fasciatus*, in fact, *K. panizzae* is invertivore/microbenthivore (Franco et al., 2008) and showed markedly lower values of $\delta^{13}C$ and higher values of $\delta^{15}N$ than *A. fasciatus* in both seasons, indicating a diet based on higher trophic levels (Vizzini and Mazzola, 2008).

Isotopic niche overlap of *A. fasciatus* with other species seemed to occur more frequently in the Stagnone di Marsala with the sandsmelt *Atherina boyeri*, in both the seagrass meadow habitat and the evaporation pond, probably due to a higher source homogeneity being carbon isotopic values of the sources less variable than in the Venice Lagoon. In contrast, no or very low overlap occurs in both lagoons with other microbenthivorous (e.g. *Syngnathus abaster* in the evaporation pond) or detritivorous species (e.g. *Chelon saliens* in the saltmarsh), with major differences occurring along the carbon isotope axis, as a result of different use of alternative basal sources (Vizzini and Mazzola, 2008).

In the inner saltmarsh, the habitat displaying the highest seasonal variation in structure and composition of the fish community, *A. fasciatus* seems to concentrate its niche in a portion of the isotopic space not used by other species in spring, but in autumn enlarges its niche reaching the highest degree of overlap with *A. boyeri* and very limited overlap with other benthivorous or detritivorous species. Both species show a markedly opportunistic feeding behavior, preying on the most abundant invertebrate populations in lagoon environments (e.g, peracarid crustaceans, copepods, decapoda, molluscs) (Shaiek et al., 2015), but there are detectable differences in feeding behavior of the two species. *A. boyeri* is a hyperbenthivorous/zooplanktivorous species (Franco et al., 2008), mostly dependent on preys moving or swimming in the free water column above the bottom (Kara and Quignard, 2019), and this explains the mostly lower $\delta^{15}N$ value than *A. fasciatus*. In contrast, *A. fasciatus* feeds on a wider variety of sources, from the surface of the water to the bottom, due to the independent mobility of both upper and lower jaws (Leonardos, 2008). Hence, the two species, despite displaying a similar position of the niche in autumn, might segregate the spatial niche within the habitat, limiting competing interactions.

Overall, *A. fasciatus* seems to shift its autumn feeding habit (or just to enlarge is trophic niche) towards the benthic compartment in the Venice Lagoon; towards the pelagic compartment in the Stagnone di Marsala, accessing preys placed over higher trophic positions.

4.3. Importance of A. fasciatus and habitat conservation

In spite of its high tolerance to extreme environmental conditions (Alcaraz et al., 2008), *A. fasciatus* is strictly associated with some coastal habitats that have undergone to progressive destruction or fragmentation (Cavraro et al., 2014, 2017). It is also considered a vulnerable species as its intra-population genetic variation is lower than the among-population variation (Maltagliati, 1999). This situation may severely affect the evolutionary potential of natural populations (Maltagliati, 2002; Toro and Caballero, 2005), calling major attention to

their conservation status.

On the other hand, the trophic adaptability of *A. fasciatus* described here, 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, represents a key feature for successful persistence, even in the marginal and, often isolated, habitats studied. Indeed, 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, also by partitioning the sources available in case of competition with other species (Barros et al., 2013). In this context, lagoonal artificial habitats such as the ones considered in this study deserve major consideration when discussing about the management of coastal lagoon ecosystems.

5. Conclusions

Isotopic niche descriptors were good tools to explore the variability of trophic niche in a quantitative way and to compare the variability of target fish populations across different communities and systems. The results highlight that the trophic niche of *A. fasciatus* varied both at *inter*- and *intra*-lagoon level and broaden the knowledge on how a species adapts to local conditions (i.e. habitat and community context).

The target species appears to shift its trophic niche along different trophic levels (δ^{15}N-axis of the isotopic space), but also different basal sources (δ^{13}C-axis of the isotopic space), thus adapting to different habitats within the same lagoon system. Such habitats, both natural and artificial ones, deserve special attention to be in line with the aims of the Habitat Directive, according to which the conservation of threatened species necessarily implies the preservation and fair management of the habitats where the species live and reproduce.

Author contributions

Cristina Andolina: Conceptualization; Formal analysis; Investigation; Visualization; Writing - original draft; Writing - review & editing.

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Francesco Cavraro: Investigation; Methodology; Writing - review & editing.

Andrew L. Jackson: Formal analysis; Writing - review & editing.

Antonio Mazzola: Funding acquisition; Resources; Writing - review & editing.

Salvatrice Vizzini: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2021.107685.

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