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Non-native fish assemblages display potential competitive advantages in two protected small and shallow lakes of northern Italy

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ABSTRACT

The introduction and establishment of non-native fish species is a widespread phenomenon in freshwater ecosystems, including small and shallow lakes. However, these ecosystems are often not considered in conservation and ecological studies and a few information is available on their fish communities and the impacts of biological invasions. Here, standardized fish surveys (gill-netting and electrofishing) and stable isotopes analysis (SIA) of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) were combined to assess fish community composition, trophic structure and trophic diversity, and to characterize the interspecific trophic interactions (as isotopic niches and their relative asymmetric overlap) of the native and non-native species (NS and NNS, respectively) found in two protected small and shallow lakes, San Michele and Campagna (northern Italy). In San Michele, 92% of the fish caught were NNS and both the individual and biomass per unit effort were dominated by NNS, particularly by the invasive *Lepomis gibbosus* L. and *Ictalurus punctatus* (Rafinesque, 1818). In Campagna, 5.6% of the fish in the total catches belonged to NNS and the numeric and biomass abundances of the assemblage were dominated by the NS *Alburnus alborella* (Bonaparte, 1841). SIA revealed that NNS had greater trophic structure and were exploiting a wider range of resources (as per Layman metrics) and had a higher asymmetric overlap than NS assemblages in both lakes. This was also evident when species-specific isotopic ecology was considered, underlining that, in both lakes, NNS (particularly the NNS *Ameiurus melas* (Rafinesque, 1820)) may have a competitive advantage over NS in case of limiting resources. The results thus pointed to a worrying conservation status of these lakes that may mirror the condition of other Italian shallow and small lakes highlighting the need to implement management actions to preserve these valuable ecosystems.

1. Introduction

Small ($\leq$ 10 ha) and shallow lakes (depth $<$ 20 m) are key aquatic ecosystems sustaining a wide range of ecological services and functions (Kristensen and Globevnik, 2014). These aquatic ecosystems, together with small rivers and ponds, greatly contribute to

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water supply, hydrological and nutrient regulations, fish production and provide recreational activities (Céréghino et al., 2007; Bolpagni et al., 2019). Nevertheless, small lakes are often not considered in management and conservation plans – e.g., they are not included in the European Water Framework Directive (2000/60/EC) monitoring programs – and, consequently, little information is available on the spatio-temporal evolution of their biological communities. This is despite small ecosystems being relatively more susceptible than they larger counterpart to anthropogenic pressures such as pollution, invasive species, and habitat destruction (Kristensen and Globevnik, 2014; Reynolds and Aldridge, 2021). Although in the last years there has been a growing interest towards small water bodies (Céréghino et al., 2007), this was mainly addressed to ponds and wetlands, whilst small lakes remained marginally considered (Bolpagni et al., 2019). This is particularly true for fish communities and biological invasions, which are inadequately considered in conservation and ecological studies addressing small lakes (Bolpagni et al., 2019).

In the last centuries, thousands of terrestrial and aquatic organisms have dispersed outward from their native regions due to human activities (Elton, 1958; Pimm et al., 1995; Vitousek et al., 1997; Ricciardi, 2006), and nowadays biological invasions are a major global environmental and economic problem (Cohen and Carlton, 1998; Leprieur et al., 2008; Gozlan et al., 2010; Simberloff et al., 2013). The introduction of invasive species in a recipient community has important implications for the structure and functioning of terrestrial and aquatic ecosystems (Moyle and Light, 1996a; Mack et al., 2000; Simberloff et al., 2013; Gallardo et al., 2016). Biological invasions involve multiple adverse ecological processes including competition for resources (Race, 1982; Hooper et al., 2005; Copp et al., 2017; Jackson et al., 2017) and novel predation pressure (Gozlan, 2008; Strayer, 2010; Jackson et al., 2017). When invading a community, non-native species (hereafter referred as NNS) can occupy vacant niches in the invaded ecosystem (Herbold and Moyle, 1986) thus avoiding competition. Alternatively, trophic niches of NNSmay overlap with those of native species (hereafter referred as NS) and, if resources are limited, NNS may outcompete the NS (Elton, 1958; Shea and Chesson, 2002; Haubrock et al., 2019). Understanding the trophic strategy that guides the success of new invaders into receiving communities is fundamental to detect changes in trophic structure and trophic re-organization following the invasion, which in turn is necessary for an effective NNS management.

Stable Isotopes Analysis (SIA) is widely used across broad areas of ecology for the study of isotopic niche (Zambrano et al., 2010; Layman and Allgeier, 2012; Newsome et al., 2012), fish trophic ecology (Gu et al., 1996; Abrantes et al., 2014; Córdova-Tapia et al., 2015; Costantini et al., 2018; Cicala et al., 2020), to quantify food web structure and functioning (Layman et al., 2007a; Perkins et al., 2014), and to evaluate the impacts of biological invasions on the trophic structures of the recipient communities (Vander Zanden et al., 1999; Sagouis et al., 2015). The isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) give information about the potential resources exploited by a consumer and its trophic position in the food web, respectively (Post, 2002; Inger and Bearhop, 2008; Gallagher et al., 2017). Moreover, $\delta^{13}C$ and $\delta^{15}N$ ratios of consumers within a given environment can be plotted on an isotopic biplot, allowing the quantification of isotopic niche metrics (Layman et al., 2007a; Jackson et al., 2011), which can provide useful insight on the trophic structure of a population and on potential interspecific interactions (Jackson et al., 2012).

Fresh waters are among the most vulnerable ecosystems to biological invasions (Gallardo et al., 2016) and freshwater fishes are the most introduced vertebrate worldwide (Gozlan et al., 2010). This is particularly true for Italian inland waters, where the most updated fish species list identified 57 established NNS against 55 NS (Lorenzoni et al., 2019). In some Italian systems, entire fish communities have been completely substituted by NNS (e.g., Lanzoni et al., 2018; Haubrock et al., 2021). Information on the spread of NNS in main rivers and large lakes is available (Carosi et al., 2015; Volta et al., 2018; Milardi et al., 2020; Haubrock et al., 2021), but restricted knowledge is present for small water bodies. Similarly, no SIA studies have been carried out on fish assemblages of small and shallow lakes in Italy, leaving unexplored the quantification of the spread of NNS in these ecosystems and the quantification of the interspecific

Fig. 1. a) Location of the study area in northwestern Italy and b) inset of the hydrography of Lake San Michele (top right – 45.28°N 7.53° W) and Lake Campagna (bottom right – 45.29°N 7.53° W).

trophic interactions between NNS and NS. The absence of this information limits the planning and execution of adequate management actions to protect these valuable environments.

In this study, the fish assemblages of two small and shallow Italian lakes were characterized using electric fishing and standardized gillnetting while SIA was applied to investigate the interspecific trophic interactions (as isotopic niches and their relative asymmetric overlap), the trophic diversity and the trophic structure of the NS and NNS found in both lakes. Although the two lakes are included within the European network of protected areas (i.e., Natura 2000 EU (1992)), there was a lack of information on their fish community composition, and it was then likely that a high number of NNS were present. We hypothesized that a) both the lakes had similar fish communities dominated by NNS b) with NNS communities exploiting a wider range of resources and displaying a higher asymmetric trophic overlap than NS. To verify these two hypotheses, we: (i) characterized the composition of the fish assemblage as resulted from standardized gillnet and electrofishing samplings, (ii) analyzed the species-specific isotopic composition of the fish assemblage within each lake and iii) we quantified the trophic structure, the trophic diversity, and resource sharing (i.e., isotopic overlap) of NNS and NS assemblages in each lake.

2. Material and methods

2.1. Study area

The studied systems included two lakes, San Michele and Campagna, belonging to the protected area called “Five lakes of Ivrea” (SCI IT1110021) comprised within the Natura 2000 European network (EU, 1992). The two lakes are situated within the Morainic Amphitheatre of Ivrea, in the northeastern part of the Canavese region (Piedmont region – northwestern Italy; Fig. 1a), an area straddling in a tectonic lineament (Linea Insubrica) (Duregon et al., 1999; Minuzzo et al., 2005). The lakes are adjacent (i.e., are located at about 1 km apart; Fig. 1b) and are situated at the same latitude and altitude (240 m a.s.l.). The lakes have a surface comprised between 0.07 km^2 (San Michele) and 0.11 km^2 (Campagna) and a maximum depth ranging from 7 m (San Michele) to 16 m (Campagna). Average depths are 3 m and 5 m, respectively. Campagna is relatively more productive than San Michele being the phosphorus concentration of the first equal to 130 μg/l and that of the second equal to 70 μg/l (Minuzzo, 2005). Even though these lakes are comprised in a protected area, any qualitative and quantitative information on the fish community composition was not available before the study and consequently no management or conservation actions were undertaken by the competent authority, yet.

2.2. Fish sampling and sample selection for SIA

Fish surveys were carried out during 2-days campaigns in November and December 2019 in San Michele and Campagna, respectively. Sampling was performed following the national guidelines for fish sampling in lakes (ISPRA, 2014), using a standardized multi mesh benthic gillnet approach (see details in Volta et al., 2018). Considering surface and maximum depth for each lake, eight gillnets were used in Campagna (for a total of 480 m^2 of net surface) and nine were used in San Michele (for a total of 540 m^2 of net surface). Only in the deepest San Michele, sampling was implemented with one mesopelagic gillnet, set at the deepest point of the lake. Each benthic gillnet was 40 m long and 1.5 m high and was made of 16 panels of equal length, with mesh size 5.5–135 mm. The mesopelagic gillnet used was composed by a set of two nets 27.5 m long and 6 m high, each composed of 11 panels with mesh size 8–55 mm knot to knot. Benthic gillnets were distributed following a depth-stratified sampling design with depth measured with an Echosounder (Lowrance HDS7, transducer 50/200 kHz, 300 W). Gillnets were set at dusk (around 6 pm) and retrieved the following morning at around 8 am, leaving the nets set for 12 h. In addition, the littoral habitats of the two lakes were sampled through electrofishing. Fishes were sampled alongshore on a line transect of 100 m length, from a boat and during daytime with a built-in-frame EL64GII electrofishing device (Scubla Aquaculture, 7000 W, 600 V, DC current) constituted by a copper cathode (width 2.5 cm, length 300 cm) and a steel ring anode (thickness 0.8 cm, diameter 50 cm).

All the fish taken were measured (total length up to 0.1 cm) and weighed (body mass up to 1 g) after being taxonomically identified up to the species level following Kottelat and Freyhof (2007). A subset of individual fish per species (2−25) was selected for stable isotope analysis (SIA). These individuals were euthanized with an overdose of an ethanol suspension of eugenol, placed into refrigerated plastic bags and subsequently transferred to the laboratory where they have been kept frozen until processing. A minimum of five individuals per species was set for species-specific isotopic ecology inferences.

2.3. Laboratory procedures for SIA

In the laboratory, fish were processed for SIA with this processing involving defrosting of each fish and the excision of a sample of dorsal muscle (Darnaude et al., 2004; Cicala et al., 2019). Muscles were subsequently freeze dried and finely powdered and homogenized using a ball mill (Fritsch Mini-Mill Pulverisette 23). Approximately 200–400 μg of powdered fish muscle was then placed in a tin capsule (5 mm × 9 mm, Säntis Analitical TM), oven-dried for at least 24 h at 60∘C and then analyzed for the stable isotope ratios of carbon and nitrogen ($^{13}C/^{12}C$, $^{15}N/^{14}N$) on a continuous flow (MAT ConfloIII) elemental stable isotope ratio mass spectrometer (CF-EA-IRMS) constituted by a FLASHEA 1112 CHN elemental analyser (ThermoFisher, Milan, Italy) coupled with a DeltaPlusXP mass spectrometer (ThermoFisher, Milan, Italy) available at the IGG-CNR laboratory in Pisa. The following operational conditions were used: oxidation column temperature: 1050 °C; reduction column temperature: 650 °C; and gas chromatography column: 55 °C. The results of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were expressed as parts per thousand (‰) on the relative δ-scale referred to the V-PDB (Vienna Pee Dee Belemnite) and atmospheric air, international standards selected for carbon and nitrogen isotopic ratios,

respectively. Ratios were then calculated as follows:

$$\delta\ (‰) = [(R_{Sample}/R_{Reference}) - 1] \times 1000$$

where R is the ratio of the heavy to light stable isotope (e.g., $^{15}N/1^{4}$ N) in the sample (R_{Sample}) and in the standard ($R_{Reference}$). Average analytical reproducibility was ± 0.15‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. Samples were analysed in duplicate or triplicate with a standard deviation on average lower than 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$ measurements. International standards USGS-40 ($\delta^{13}C = -26.4‰$ and $\delta^{15}N = -4.5‰$), IAEA-N1 ($\delta^{15}N = +0.4‰$), IAEA-CH6 ($\delta^{13}C = -10.4‰$) and IAEA-CH7 ($\delta^{13}C = -32.1‰$) were analysed during each run to calibrate the results and within-run standards (graphite and ammonium chloride) were measured to check the instrument functioning and possible drift deviations. Since dorsal muscles of different fish species may contain different level of lipids that can bias the $\delta^{13}C$ values leading to erroneous diet interpretation, the C:N of each species in each lake was examined. The ratio was below 3.5 for every species in each lake, hence no lipid correction was necessary (Post et al., 2007; Skinner et al., 2016).

2.4. Data analyses

Diversity of the two fish assemblages was characterized using the Shannon's diversity index (H; Shannon, 1948) and fish community composition was described and compared using relative numeric and biomass estimates. For each species within each lake, the percent number over the total catch of each fish species was calculated for the whole sampling (gillnetting + electrofishing) together with separated calculation per sampling method: gillnetting (GN %) and electrofishing (EF %). In addition, for the gillnet samplings, the relative number (NPUE) and biomass (BPUE) of individuals per unit effort (m^{-2}; measured as total net surface) were calculated.

Before calculation of species-specific isotopic ecology, differences in length between species collected in both lakes were tested using the Student *t* test and possible effects of ontogenetic variations on stable isotope ratios were assessed through linear regression using fish total lengths as a proxy of ontogenetic stage for each species in each lake. For those species in which a significant relationship between length and isotope ratios was found, if at least five individuals per size class were available, calculations of isotopic niche and asymmetric overlap were performed splitting the fish in different size class.

Isotopic niche width, niche orientation into the isotopic space and niche asymmetric overlap were calculate at the species-specific level and splitting NS and NNS assemblages in each lake. To graphically display the isotopic niches, the maximum likelihood derived standard ellipse areas (SEA) encompassing the 95% of the data were used (Jackson et al., 2011). To statistically compare the width of the isotopic niches, the Bayesian-derived standard ellipse area (SEA_B) was instead used, which is less sensitive to small sample sizes than SEA (Jackson et al., 2011). The posterior values of the covariance matrix and means of the sampled isotopic values were estimated from a set of iterative draws (20,000 iterations) of a Markov chain Monte Carlo (MCMC) simulation, assigning vague normal priors to the means of the isotopic ratios, and a vague Inverse–Wishart prior to the covariance matrix (Jackson et al., 2011). To assess the proportion of potential resource sharing between species and between NS and NNS assemblages, percent pairwise asymmetrical overlaps were estimated. Pairwise asymmetrical overlap is a directional metric estimating the probability that the isotopic group A is found in the niche area (a) of isotopic group B (pAB), which is different from the probability that an individual of isotopic group B is found in the niche area of group A (pBA), since A and B typically have different niche areas (aA, aB).

Bayesian estimates of Layman's metrics (Layman et al., 2007a) where used to assess isotopic structure and isotopic diversity for NNS and NS assemblages within each lake. These metrics were: the carbon range (CR) and nitrogen range (NR), which measure the distance between the lowest and the highest $\delta^{13}C$ or $\delta^{15}N$ values indicating respectively the range of primary resources consumed by a community and the range of trophic levels involved; the mean distance to centroid (CD), which measures the trophic diversity of a given community as the mean Euclidean distance of each individual from the centroid into the isotopic bivariate space; the mean nearest neighbor distance (MNND) and associated standard deviation (SDNND), which offer a measure of trophic redundancy as they estimate the density of individual's isotope ratios and the spatial variation of this packaging, respectively.

Finally, to assess the health status of the most abundant fish species in each lake, two complementary indices of body condition were calculated based on standard length-weight relationships (LWR). First, for each species, LWRs parameters were retrieved from FishBase database (Froese and Pauly, 2000; Froese et al., 2014; Table S1). Then, the relative body condition (Rn; Blackwell et al., 2000) and the Residual Index (RI; Jakob et al., 1996) were calculated. RI is calculated as the difference between the observed total weight and that expected based on the standard LWR equation. Rn is calculated as the ratio between observed weight and expected weight based on the standard LWR equation multiplied by 100. Both indices offer a straightforward interpretation: fishes with Rn or RI less than 100 or 0, respectively, are in a worse condition than expected. ANOVA was used to test for differences between species within each lake and post hoc Tuckey's test was used for pairwise comparisons.

All the isotopic metrics and associated testing were calculated and performed in R, using SIBER package (Jackson et al., 2011; R Core Team, 2018). The Bayesian credible intervals (CI) were used to test for significant differences for Bayesian derived metrics (SEA_B and Bayesian Layman's metrics). Student *t* test, Mann Whitney nonparametric test, linear regressions, H index, ANOVA and Tuckey's tests were performed in PAST v. 4.0. (Hammer et al., 2001).

3. Results

3.1. Fish assemblage characterization

Fish assemblages of the two lakes differed considerably in diversity, species composition and species relative abundances (number

Table 1
Results of fish sampling in San Michele and Campagna. NNS= non-native species, NS= native species; n = number of individuals; Length range (Min–Max) cm; Weight (Min–Max) g; n% = percentage number in the total catches; EF% = percentage number in the Electric Fishing catches; GN % = percentage number in the gillnets catches; NPUE = number of individuals per square meter of gillnet; BPUE = fish biomass (g) per square meter of gillnet.

Lake	Species	Status	n	Length range (cm)	Weight range (g)	n (%)	EF (%)	GN (%)	NPUE (individuals/m^2) (± st. dev.)	BPUE (grams/m^2) (± st. dev.)
San Michele	*A. melas*	NNS	8	18–25.6	31–130	4	–	6.2	0.01 ± 0.01	0.97 ± 1.22
	C. carassius	NNS	3	32–36.5	487–648	1.5	–	2.3	0.01 ± 0.01	5.04 ± 10.17
	I. punctatus	NNS	4	47–65	788–3239	2	2	2.3	0.01 ± 0.01	12.64 ± 21.87
	L. gibbosus	NNS	133	2.8–15.6	2–72	67	65	68.2	0.16 ± 0.12	4.74 ± 2.77
	M. salmoides	NNS	35	12.9–29.5	19–260	17	33	8.5	0.02 ± 0.02	1.29 ± 1.50
	P. fluviatilis	NS	9	10.5–21	13–95	5	–	6.9	0.02 ± 0.03	0.66 ± 1.06
	S. hesperidicus	NS	6	7–22.5	9.2–161	3	–	4.6	0.01 ± 0.02	0.66 ± 1.21
	S. squalus	NS	1	43.5	1243	0.1	–	0.8	0.00 ± 0.01	2.30 ± 6.91
	TOTAL NNS		183			92	100	87.5	0.19 ± 0.14	22.31 ± 22.16
	TOTAL NS		16			5.4	–	12.3	0.03 ± 0.03	3.26 ± 6.39
	TOTAL catch		199						0.22 ± 0.15	25.6 ± 22.05
Campagna	*A. melas*	NNS	32	6–16.8	5–46.8	2	–	2.2	0.07 ± 0.07	1.47 ± 1.68
	C. carassius	NNS	3	19–21.5	114.3–208.6	0.1	–	0.3	0.01 ± 0.02	1.39 ± 2.67
	L. gibbosus	NNS	12	4–10.8	2–20.4	1	–	0.8	0.03 ± 0.04	0.10 ± 0.18
	R. amarus	NNS	30	4.8–8.2	1.8–6.6	2	18	1.4	0.04 ± 0.06	0.13 ± 0.19
	S. glanis	NNS	1	60	1322	0.1	2	–	–	–
	A. arborella	NS	1269	0.5–13	0.1–10.8	85	20	87.5	2.69 ± 1.41	4.99 ± 3.72
	P. fluviatilis	NS	15	14–22.4	28–103	1	12	0.6	0.02 ± 0.02	1.27 ± 1.58
	R. aula	NS	125	4.8–17.7	0.93–34.4	6	22	5.7	0.18 ± 0.30	2.38 ± 2.89
	S. hesperidicus	NS	35	5–23.2	2–97	3	26	1.5	0.05 ± 0.07	1.60 ± 2.60
	TOTAL NNS		78			5.2	20	4.7	0.14 ± 0.13	3.10 ± 2.96
	TOTAL NS		1444			95	80	95.3	2.39 ± 1.56	10.24 ± 7.28
	TOTAL catch		1522						3.07 ± 1.54	13.34 ± 9.48

and biomass). In San Michele, the fish assemblage was the most diverse (H = 1.18), with this reflected by the lower number of individuals (n = 199; Table 1) found distributed between 8 species (Table 1). Campagna presented a higher number of species (9 species; Table 1) but the fish community was less diverse (H = 0.7) than in San Michele since in Campagna the species were represented by a higher number of individuals (n = 1523; Table 1). Correspondingly, Campagna had a higher relative number of individual fish caught (as NPUE; Table 1) than San Michele but the latter presented a higher relative biomass (as BPUE; Table 1).

Considering the species composition, the two lakes had the same number of NNS present but different number of NS, with San Michele registering only three NS (Table 1). Five species were captured in both lakes, three were NNS and included the pumpkinseed sunfish *Lepomis gibbosus* L., the black bullhead *Ameiurus melas* (Rafinesque, 1820) and crucian carp *Carassius carassius* L. The other two species were NS and comprised the perch *Perca fluviatilis* L. and rudd *Scardinius hesperidicus* Bonaparte, 1845 (Table 1).

However, the major differences between the two fish assemblages were observed when considering the species-specific relative abundances in the catches, both by number and biomasses. Catches in San Michele were dominated both as relative number and biomass abundances (NPUE and BPUE) and as percentages (total (n %) and per sampling method (EF % and GN %)) by NNS. In particular, the NNS *L. gibbosus* dominated both the gillnet and electrofishing catches by percentages and relative numbers, whilst the channel catfish *Ictalurus punctatus* (Rafinesque, 1818) had the highest biomass contribution (Table 2), followed by *C. carassius* and *L. gibbosus*. The mesopelagic gillnet caught only one individual *A. melas*, which was thus analyzed together with fishes caught with benthic gillnets. On the contrary, catches in Campagna were dominated by NS, and specifically by the Italian bleak *Alburnus alborella* (Bonaparte, 1841), which was the most abundant species both by biomass and number, followed by the triotto *Rutilus aula* Bonaparte, 1841, an Italian endemic (Table 1).

3.2. Isotopic composition and species- specific isotopic ecology in each lake

A total of 166 fish were analyzed for stable isotopes, 62 in San Michele and 104 in Campagna (Table 2). $\delta^{13}C$ ranged from − 23.8‰ (*I. punctatus*) to − 29.2‰ (*A. melas*) in San Michele and from − 21.9‰ (European bitterling *Rhodeus amarus* (Bloch, 1782)) to − 29.3‰ (*A. melas*) in Campagna. Nitrogen values ranged from 7.9‰ (*S. hesperidicus*) to 13.7‰ (largemouth black bass *Micropterus salmoides* (Lacepède, 1802)) in San Michele and from 6.6‰ (*R. amarus*) to 14.4‰ (*A. arborella*) in Campagna.

Among the four species captured in both lakes (excluding *C. carassius* which was not analyzed further due to the low sample size), the NNS *A. melas* and *L. gibbosus* and the NS *P. fluviatilis* specimens analyzed for SIA were significantly different in length between San Michele and Campagna. Specifically, *A. melas* and *L. gibbosus* individuals were significantly longer in San Michele than in Campagna (Student *t* test, $t_{A.melas} = 12.5$, $p = 0.0001$; $t_{L.gibbosus} = 2.36$, $p = 0.03$) (Table 2) while *P. fluviatilis* specimens were longer in Campagna ($t = 2.58$, $p = 0.02$). *Ameiurus melas* in San Michele showed a significant relationship between length and nitrogen ratios ($R^2 = 0.66$; $p = 0.01$) suggesting a ^{15}N-enrichment in larger individuals (length > 23 cm; Figure S1), whilst there was not a significant variation in carbon ratios with length ($R^2 = 0.15$; $p = 0.33$) and in Campagna, no significant variation of diet with body size was detected ($\delta^{13}C$: $R^2 = 0.03$, $p = 0.68$; $\delta^{15}N$: $R^2 = 0.44$, $p = 0.07$), since all the individuals analyzed were of small/medium size (6 – 10.5 cm; Table 2). Similarly, *L. gibbosus* showed a significant relationship between total length and $\delta^{15}N$ but unlike *A. melas*, the relationship was significant both in San Michele ($R^2 = 0.27$, $p = 0.02$) and in Campagna ($R^2 = 0.67$, $p = 0.04$), regardless of the limited size range of *L. gibbosus* individuals in Campagna (Table 2). Also in this species, no relationship between length and carbon ratios were observed in each lake (Campagna: $R^2 = 0.14$, $p = 0.46$; San Michele: $R^2 = 0.16$ $p = 0.08$). On the contrary, *P. fluviatilis* individuals displayed an ontogenetic shift in diet, but this was only apparent for carbon ($R^2 = 0.40$, $p = 0.02$) in Campagna indicating that smaller individuals

Table 2
Bulk stable isotope mean ratios (± standard deviations) of carbon and nitrogen stable isotope ratios and isotopic niche width (as maximum likelihood standard ellipse area corrected for small sample sizes (SEA_c) and the mode and 95% credible interval of the corresponding Bayesian estimate (SEA_B) of each species found in each lake. Mean fish length and range (min–max), status of the species (NS = native species; NNS = non-native species) and sample size (s) for each species are also reported.

Lake	Species	Status	s	Length	$\delta^{13}C$	$\delta^{15}N$	SEA_c	SEA_B
San Michele	*A. melas*	NNS	8	21.8 (18–25.6)	-27.5 ± 0.6	10.3 ± 1.2	6.8	5.5 (2.5–12.9)
	C. carassius	NNS	2	34.2 (32–36.5)	-27.6 ± 0.3	10.5 ± 2.9	–	–
	I. punctatus	NNS	4	58.5 (47–65)	-24.5 ± 0.6	10.1 ± 0.2	–	–
	L. gibbosus small	NNS	15	7.5 (4.9–10.5)	-26.6 ± 1.1	10.5 ± 0.9	3.4	2.9 (1.8–5.1)
	L. gibbosus large	NNS	5	14.8 (14.1–15.6)	-27.8 ± 0.7	12.0 ± 0.5	1.5	1.0 (0.4–2.9)
	M. salmoides	NNS	13	19.2 (12.9–29.5)	-25.9 ± 0.7	12.6 ± 0.9	1.5	1.4 (0.7–2.5)
	P. fluviatilis	NS	9	15.7 (13–21)	-27.4 ± 0.3	11.5 ± 0.3	0.3	0.3 (0.1–0.6)
	S. hesperidicus	NS	6	14.7 (9.2–24)	-26.7 ± 0.6	10.6 ± 1.3	2.9	2.1 (0.9–5.5)
Campagna	*A. melas*	NNS	8	9.0 (6–10.5)	-27.1 ± 1.3	11.3 ± 1.5	6.6	5.7 (2.3–12.2)
	C. carassius	NNS	3	19.6 (18.4–21.5)	-25.3 ± 0.4	7.9 ± 0.3	–	–
	L. gibbosus	NNS	6	5.6 (4.0–7.4)	-26.9 ± 0.6	10.9 ± 0.9	1.7	1.6 (0.6–4.0)
	R. amarus	NNS	9	5.2 (4.6–6.5)	-23.2 ± 1.4	7.7 ± 1.1	4.8	4.0 (1.8–8.2)
	A. arborella small	NS	10	4.9 (4.0–5.6)	-26.4 ± 0.3	9.1 ± 0.5	0.4	0.4 (0.2–0.8)
	A. arborella large	NS	9	9.0 (7–12)	-28.2 ± 0.8	12.1 ± 1.7	3.3	3.1 (2.5–3.9)
	P. fluviatilis	NS	13	18.3 (14.0–22.4)	-25.5 ± 1.3	11.0 ± 0.6	2.6	2.5 (1.4–4.5)
	R. aula small	NS	5	4.8 (4.3–5.7)	-25.5 ± 0.4	8.7 ± 0.7	1.1	0.8 (0.2–2.2)
	R. aula large	NS	20	13.1 (9.0–16.8)	-27.0 ± 0.9	9.0 ± 0.4	1.3	1.2 (0.7–1.9)
	S. hesperidicus	NS	21	14.8 (5.9–23.2)	-25.6 ± 1.2	10.1 ± 0.7	2.6	2.6 (1.7–4.0)

(total length < 17 cm) tended to have less negative carbon values than larger ones (Figure S2). In San Michele, length-$\delta^{13}C$ relationship was not significant ($R^2 = 0.20$, $p = 0.22$) and all the individuals (except one) were smaller than 17 cm (Figure S1). In both lakes, no variations in nitrogen values with body size were observed (Campagna: $R^2 = 0.04$, $p = 0.50$; San Michele: $R^2 = 0.30$, $p = 0.21$). *Scardinius hesperidicus* specimens did not show any significant difference in mean length ($t = 9.67$, $p = 0.97$) between the two lakes and in both lakes, any significant relationship between body size and carbon (San Michele: $R^2 = 0.30$, $p = 0.21$; Campagna: $R^2 = 0.30$, $p = 0.21$) or nitrogen (San Michele: $R^2 = 0.30$, $p = 0.21$; Campagna: $R^2 = 0.30$, $p = 0.21$) ratios was not recorded.

Among the species found in only one of the two lakes, significant possible ontogenetic variations in diet were recorded for the NS *A. alborella* and the NS *R. aula* in Campagna (Figure S2). Specimens of *A. alborella* showed a significant variation both of carbon ($R^2 = 0.84$, $p = 0.001$) and nitrogen ($R^2 = 0.88$, $p = 0.001$) with total length, with individuals larger than 8 cm having higher nitrogen values and more negative carbon values (Figure S2). *Rutilus aula* individuals instead showed a significant relationship between length and carbon ($R^2 = 0.25$, $p = 0.009$) but not with nitrogen ($R^2 = 0.05$, $p = 0.26$) with smallest individuals (< 5 cm) displaying less negative carbon values than the largest ones (Figure S2).

Generally, *A. melas* had the largest isotopic niche area (SEA_c and SEA_B) in both lakes (Fig. 2) whilst the smallest niche was recorded

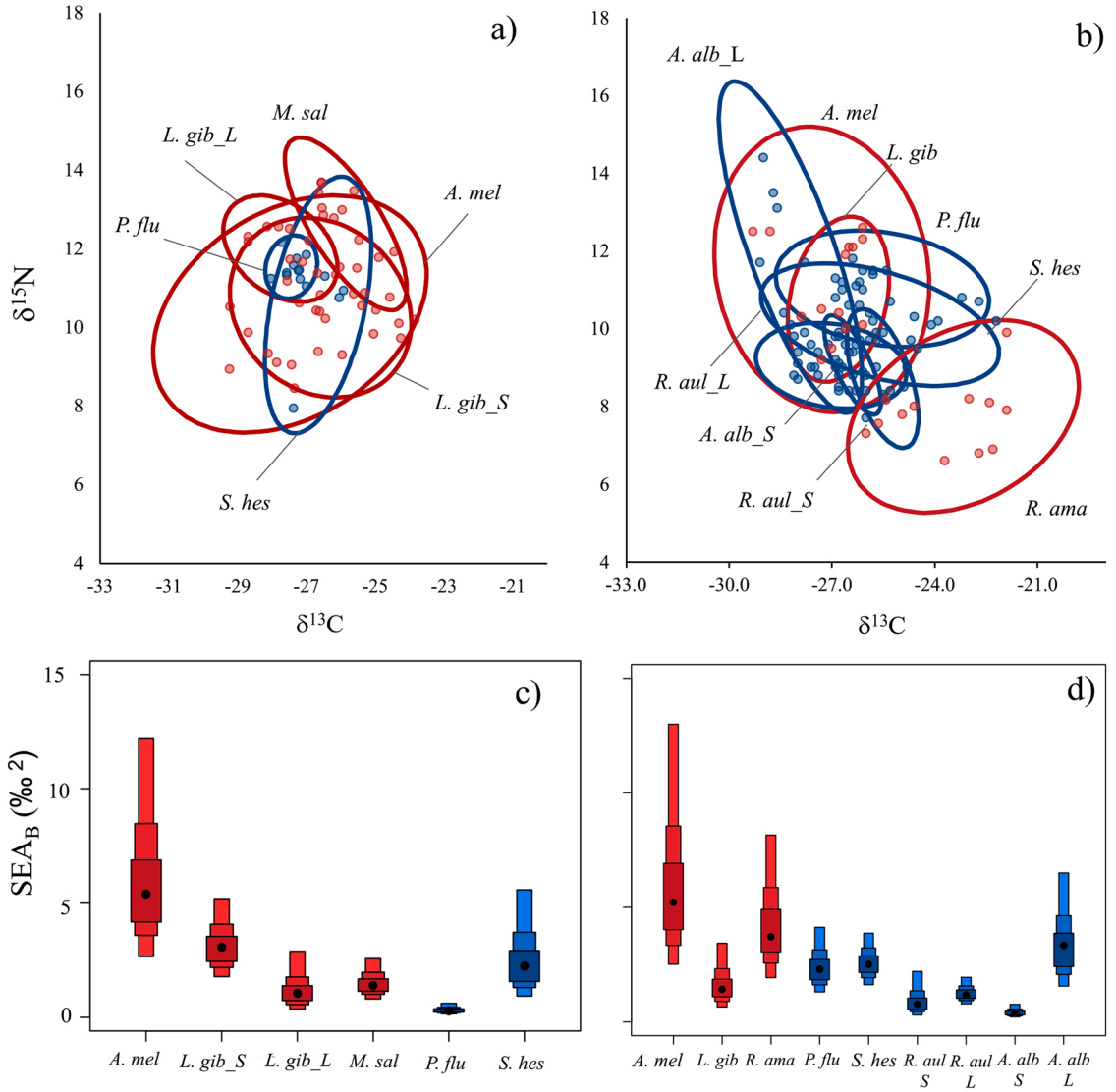

Fig. 2. Isotopic biplot of $\delta^{13}C$ and $\delta^{15}N$ ratios with isotopic niches indicated as standard ellipse areas (SEA) of NNS (red ellipses) and NS (blue ellipses) of (a) San Michele and (b) Campagna and density plots of the Bayesian standard ellipse area (SEA_B) estimates and relative credible intervals (CI:0.50, 0.70 and 0.95) of the of fish sampled in (c) San Michele (d) and Campagna. *A. mel: Ameiurus melas; A. arb: Alburnus arborella; L. gib: Lepomis gibbosus; P. flu: Perca fluviatilis; R. ama: Rhodeus amarus: R. aul: Rutilus aula; S. hes: Scardinius hesperidicus.* Fish species with < 5 analyzed specimens were not included in the isotopic niche analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for the NS *P. fluviatilis* in San Michele and *A. alborella* of small size class in Campagna. All the other species showed similar niche sizes and most of the isotopic niches were largely overlapped in each lake (Fig. 2; Table S2 a and b). In San Michele, most species shared a great part of their isotopic niches with asymmetric overlap often exceeding 70% (Table S2a). This indicates that there is a reduced resource partitioning between these species which are occupying similar trophic positions (as shown by their nitrogen ratios) and are exploiting similar carbon sources. In this lake, NNS *A. melas* and *M. salmoides* had the most distinct isotopic niches (sharing always < 70% of their niches with other species) but with *A. melas* occupying a great portion of other species' niches (Table S2) contrary to *M. salmoides* interesting only marginally (< 30%) the isotopic niches of the other species. Remarkably, the niche of the NS *P. fluviatilis* was extensively asymmetrically overlaid by the isotopic niches of the NNS *A. melas*, *L. gibbosus* and the NS *S. hesperidicus* (Fig. 4; Table S2a) while occupying the smallest portion of other's isotopic space (i.e., asymmetric overlap < 10%). In Campagna, the NNS *R. amarus* had the lowest level of overlap, sharing less than the 20% of its isotopic niche but also occupying less than the 30% of other's species niche with an exception for the niche of the small size class of the NS *R. aula* overlaid by 60% with that of *R. amarus* (Table S2 b). Contrastingly, the NNS *A. melas* had strong asymmetric (> 70%) overlap with most of the other species (both NNS and NS), while sharing less than 40% of its niche.

3.3. Isotopic comparisons between NNS vs. NS assemblages in each lake

Among NNS and NS assemblages in San Michele there was a significant variation in median $\delta^{13}C$ ratios (Mann–Whitney U tests, U = 216 $p < 0.05$) with the NNS assemblage having less negative values compared to the NS assemblage (Table 3, Fig. 3). This was also reflected by a substantially greater CR in NNS assemblage compared to the NS one (> 80% of posterior drawings; Figure S3). In contrast, nitrogen median ratios did not differ considerably between the two assemblages (Mann–Whitney U tests, U = 329 $p > 0.05$) despite the NNS assemblage displayed a generally wider NR than NS (> 80% of posterior drawings; Figure S3). NNS assemblage also showed a wider CD that, together with the greater CR and NR, indicate a wider trophic structure for NNS than NS. On the contrary, trophic redundancy (as MDNND) was comparable among the two assemblages whilst it was not possible to calculate posterior drawings of TA and SDNND of the NS assemblage due to the small sample size. However, SEA_B supported a considerable difference in the isotopic niche breath (Figure S3; Table 3), with NS assemblage showing a more restricted niche, which also appeared completely overlapped with that of the NNS assemblage (Table 3, Fig. 3).

In Campagna, no significant differences were observed for both median values of nitrogen (Mann–Whitney U tests, U = 897 $p > 0.05$) and carbon (Mann–Whitney U tests, U = 256 $p > 0.05$). Nevertheless, like in San Michele, NNS assemblage displayed a significantly greater trophic structure as indicated by wider NR, CR and CD (95% posterior probability, Figure S4). Trophic redundancy resulted also in this case comparable between the two assemblages while TA appeared generally more restricted in NS assemblage although it did not have a strong statistic support (posterior probability < 75%) (Figure S4). Like in San Michele, SEA_B indicate that the difference in isotopic niche breath was consistent, with NS having a more restricted niche and with a high asymmetric

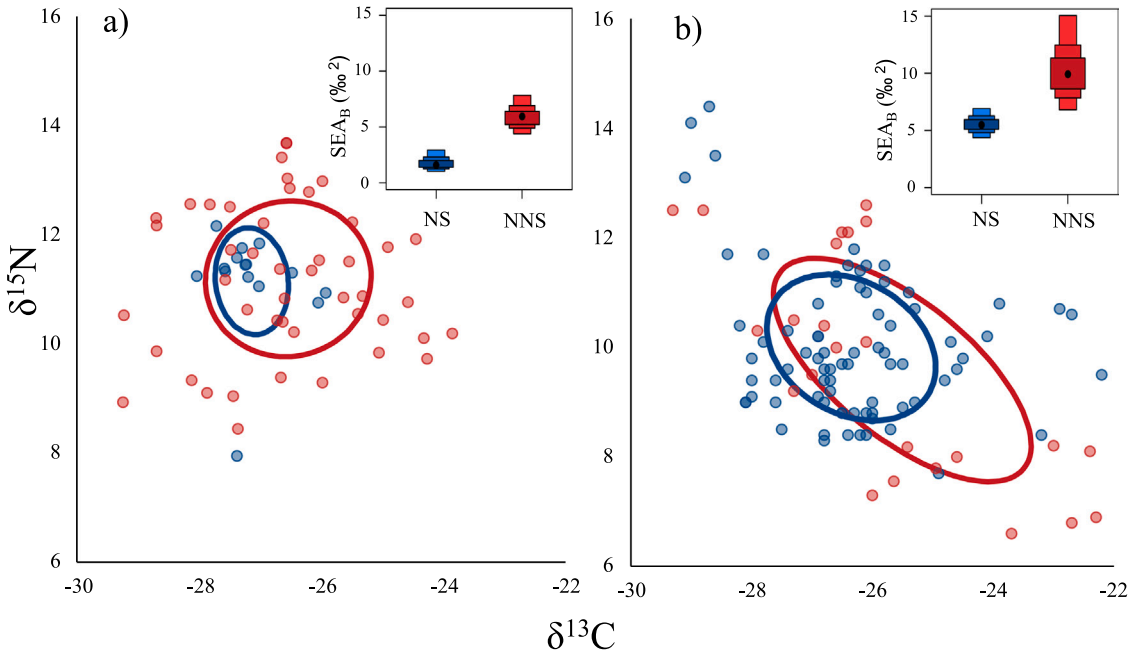

Fig. 3. Isotopic biplot of $\delta^{13}C$ and $\delta^{15}N$ ratios with isotopic niches indicated as standard ellipse areas (SEA_c) and density plots of the Bayesian standard ellipse area (SEA_B) with relative credible intervals (CI:0.50, 0.70 and 0.95) for the NNS (red ellipses) and NS (blue ellipses) in (a) San Michele and (b) Campagna. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

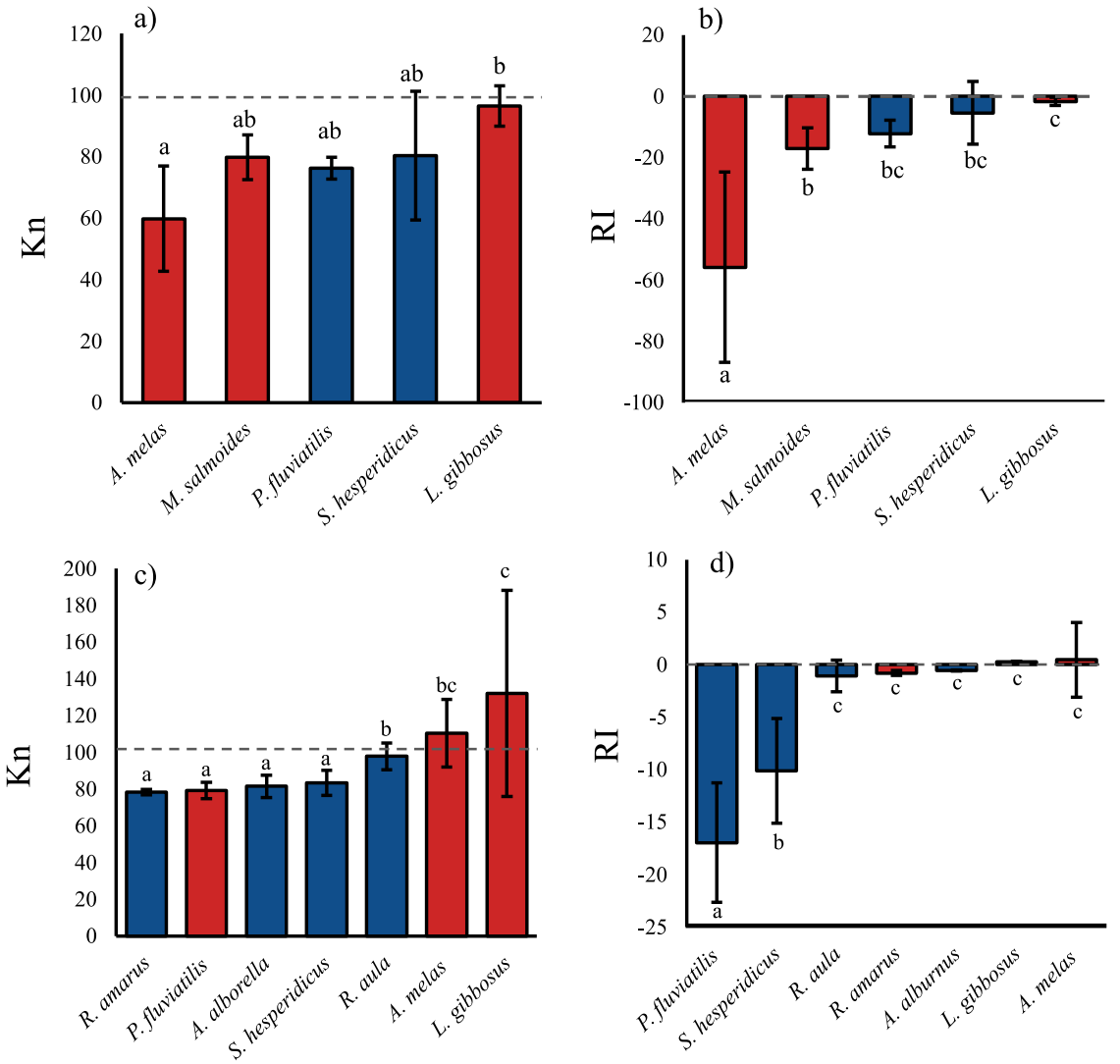

Fig. 4. Barplots of the relative condition factor (Kn) and the residual index (RI) body condition indices calculated for the most abundant fish species in San Michele (a, b) and Campagna (b, c). Error bars indicate 95% confidence interval and grey dashed lines indicate the value of each body condition index above or below which the fish is considered in a good or bad condition. Letters above error bars represent differences in mean values as per Tukey's post hoc test: species with no letter in common have significantly different mean body condition ($p < 0.05$).

overlap with NNS' isotopic niche (Table 3).

3.4. Body condition of the most abundant species

Across the most abundant species found in each lake, significant differences in both relative condition factor (Kn) and residuals index (RI) were found in both lakes (Table 4).

In Campagna, pairwise post hoc analysis revealed that Kn of the NNS *A. melas* and *L. gibbosus* were significantly higher than in all the other species present except for the NS *R. aula*, which among the NS, was the species with the best body condition (Fig. 4). Similarly, *A. melas* and *L. gibbosus* had also significantly higher residual indices than the other species, whilst the NS *P. fluviatilis* and *S. hesperidicus* displayed significantly lower residual indices, indicating that these species were substantially thinner than expected.

In San Michele, the post hoc test revealed that there was a significant difference in Kn only between *A. melas* and *L. gibbosus* (Fig. 4), whilst all the remaining pairwise comparisons were non-significant. *Ameiurus melas* presented also significantly lower values of RI than the other species, but all the species had negative values for this index (Fig. 4).

Table 3
Bayesian Layman's metrics and isotopic niches as Bayesian standard ellipse areas (SEA_B) (modes and 95% credible intervals) of the native (NS) and non-native (NNS) fish assemblages in each shallow lake together with mean carbon and nitrogen ratios (± standard deviation). CR = carbon range; NR = nitrogen range; CD = mean distance to centroid; SEA_c= standard ellipse area corrected; SEA_B = standard ellipse Bayesian area; MNND = mean nearest neighbor distance; SDNND = standard deviation of nearest neighbor distance; Asym. Overl. = asymmetrical overlap of isotopic niche.

Lake	San Michele		Campagna	
Assemblage	NS (n = 15)	NNS (n = 43)	NS (n = 78)	NNS (n = 26)
$\delta^{13}C$	-27.1 ± 0.6	-26.5 ± 1.3	-26.3 ± 1.4	-25.5 ± 2.1
$\delta^{15}N$	11.1 ± 1.0	11.2 ± 1.4	10.0 ± 1.3	9.6 ± 2.0
NR	1 (0.0–2.0)	2.3 (1.4–3.3)	2 (1.4–2.7)	3.8 (2.7–5.4)
CR	0.7 (0.0–1.8)	1.6(0.7–2.6)	1.9 (1.2–2.5)	4.1 (3.1–5.3)
TA	–	0.2 (0.0–1.4)	1.9 (0.9–3.0)	4.4 (0.4–9.4)
CD	0.7 (0.2–1.2)	1.1 (0.8–1.4)	1 (0.8–1.3)	2.3 (1.8–2.8)
MNND	1.4 (0.4–2.5)	1.3 (0.8–1.9)	1.2 (0.8–1.6)	1.6 (0.7–2.5)
SDNND	–	0.6 (0.0–1.0)	0.3 (0.0–0.7)	0.8 (0.0–1.7)
SEA_c	1.9	6	5.6	10.3
SEA_B	1.7 (1.0–3.0)	5.9 (4.3–7.8)	5.5 (4.3–6.8)	10 (6.4–14.6)
Asym. Overl.	1	0.3	0.9	0.5

Table 4
Results of the ANOVA test conducted on the Relative condition factor (Kn) and the residual index (RI) calculated for each most abundant species found in the assemblages of San Michele and Campagna.

Lake	Parameter	F	*df*	p-value
San Michele	Kn	17.04	6, 1085	0.001
	RI	73.27	6, 1085	0.001
Campagna	Kn	4.50	4, 175	0.002
	RI	38.67	4, 175	0.001

4. Discussion

The fish assemblages of the two small and shallow lakes investigated in this study has resulted to be populated by a mixture of NNS and NS, with NNS dominant by species number in both lakes, confirming our initial hypothesis (62% and 55% were NNS in San Michele and Campagna, respectively). Although the lakes are located in protected areas, this is not surprising given that the fish assemblages of north Italian lakes have suffered drastic ecological changes, on top of which there was the increasing introduction of non-native fishes, which started during Roman and Middle Ages for some species (e.g., common carp *Cyprinus carpio* L.) and peaked in late 1900 s (Bianco, 1998; Volta and Jepsen, 2008; Volta et al., 2013a; 2013b; Bianco, 2014; Nocita et al., 2017; Volta et al., 2018). Since there is a scarcity of information on the historical fish community composition of these two lakes, their evolution can only be surmised. According to the historical reconstruction of fish assemblages in Italian lakes (Volta et al., 2011), it is possible to broadly reconstruct the near-natural communities of the two lakes. Specifically, both the lakes may be grouped into "type I lakes" (*sensu* Volta et al., 2011), a group of relative shallow and small lakes, located in the lowland part of the Alpine ecoregion and characterized by a relatively restricted number of species (mean number of species = 7.9 ± 1.6; Volta et al., 2011). These lakes are typically dominated by phytophilic species such as Italian pike *Esox cisalpinus* Bianco and Delmastro 2011, tench *Tinca tinca* L. and *S. hesperidicus* and accompanied by *A. alborella*, *P. fluviatilis*, *C. carpio* and Padanian goby *Padogobius bonelli* (Bonaparte 1846). Comparing thus the assemblages found in the two analyzed lakes with the potential reference community, it is evident that both lakes have undergone a substantial alteration of their fish populations. Between the seven indicator species, only two to three were recorded in San Michele and Campagna respectively, and these included the only two NS the two lakes had in common, *S. hesperidicus* and *P. fluviatilis*. However, the lack of some species may not indicate the total absence of the species in the lake since this study is based on a single sampling event. Thus, attention should be applied in the interpretation of the provided results, which however offered a first picture of the most abundant fish species present in Campagna and San Michele.

Considering the NNS assemblages, in San Michele only NNS introduced to Italy before the 1960 (Volta et al., 2018) were recorded whilst in Campagna, there were also invaders of relatively more recent introduction (i.e., introduced after 1960; Volta et al., 2018), namely *R. amarus* and the European catfish *Silurus glanis* L. This suggests that, although the two lakes are adjacent, they may have experienced two different invasion histories and mechanisms, potentially explaining the contrasting relative abundances (both numeric and biomass) detected for NNS and NS between the two lakes. In fact, in San Michele the abundance of NNS was greater than that of NS - mainly affected by the presence of *L. gibbosus* and the biomass of *I. punctauts,* respectively -, whilst in Campagna fish assemblage was dominated by NS, particularly by the epipelagic NS *A. arborella*. Generally, NNS have the ability to increase densities and biomass of their individuals that act in different trophic roles into the recipient community, leading them to dominate the fish assemblage (Moyle and Light, a, 1996 and b; Simon and Townsend, 2003). It is thus possible that in San Michele this well-established eco-evolutionary mechanism of limitation of NS populations occurred via the combination of inter-specific competitive interactions and predation pressure phenomena (Moyle and Light, b, 1996; Simon and Townsend, 2003; Cucherousset and Olden, 2011; Huges and

Herlihy, 2012). Fish assemblage of Campagna could instead reflect a more recent invasion history in which the dominance shift from NS to NNS population had yet to occur or, alternatively, may be a result of the mechanism of biotic resistance (Elton, 1958; Tilman, 1997; Levine and D'Antonio, 1999; Maron and Vilà, 2003) by which NS exert resistance on NNS. Indeed, the recipient community in which NNS are introduced can repel the invaders suppressing population growth of NNS (Levine et al., 2004) mainly through dynamics of competitive interaction or performing a numerical increase as a response to NNS expanding populations (Maron and Vilà, 2003; Charles and Dukes, 2008). The NNS introduction into a new recipient community can create a selection pressure to which the NS can show rapid adaptive changes (Moyle and Light, 1996b; Mooney and Cleland, 2001; Strauss et al., 2006). As an adaptive response, an increase in the number of individuals (abundance) and/or in the body size structure (biomass), through phenotypic and behavioral plasticity can occur (Graves and Shapiro, 2003; Strauss et al., 2006; Fisk et al., 2007; Nunes et al., 2014), reducing predation risk and inter-specific competitive interactions thus minimizing the potential for extirpation.

SIA assisted in the interpretation of the ecological status of the fish assemblages in the two lakes. In fact, although it was not possible to directly compared SIA between the two lakes due to the lack of baselines, it was possible to compare isotopic metrics between NS and NNS within each lake. This approach revealed a similar pattern within the two assemblages despite the two lakes presented different dominant species by number and biomass. In both lakes, NNS assemblages displayed a greater trophic structure suggesting NNS were likely exploiting a wider range of resources as evidenced by wider CR, NR, TA, and SEAs but also by the higher asymmetric overlap than NS assemblages. The similar pattern observed in the two lakes may suggest that the isotopic metrics used in this study are not sensitive to the observed differences between the two fish assemblages. Most of the isotopic metrics used (i.e., Layman's metrics and standard ellipse areas), are influenced by several factors, including the number of species (Brind'Amour and Dubois, 2013) whilst do not consider the abundance of organisms (Cucherousset and Villéger, 2015). Moreover, these metrics do not incorporate basal resources variations, and this aspect was also not considered in this study, potentially leading to the detection of erroneous differences or similarities between ecosystems (Hoeinghaus and Zeug, 2008). Nevertheless, numerous studies have demonstrated the utility of these isotopic derived metrics in evaluating the ecological status of aquatic ecosystems, linking their trophic structures to the impacts deriving from eutrophication (Rawcliffe et al., 2010), habitat fragmentation (Layman et al., 2007b) and invasive species (Sagouis et al., 2015). SIA has been proved efficient to track the effects of multiple stressors in shallow lakes (Kelly and Schallenberg, 2019), showing a high sensitivity towards subtle different combinations of anthropogenic stressors of different nature and their relative interactions with natural features (Pingram et al., 2020). Thus, another potential explanation of the similarity detected in the two investigated shallow lakes points to similar invasion consequences in the two ecosystems, sustaining the hypothesis that trophic plasticity and generalism allows NNS to easily colonize new environments (Kolar and Lodge, 2002; Moyle and Marchetti, 2006; Ribeiro et al., 2007; Gido and Franssen, 2007; Jackson et al., 2016). Generalist species usually display large trophic niche areas (SEA_C, SEA_B, CR), high trophic diversity (CD), and broad use of resources spectrum (Shea and Chesson, 2002; Feiner et al., 2013; Jackson et al., 2016), which can reduce both intra- and inter-specific competitive interactions (Svanbäck and Persson, 2004; Svanbäck and Bolnick, 2005; Araújo et al., 2011) facilitating the colonization of new ecosystems (Elton, 1958; Shea and Chesson, 2002; Jackson et al., 2016). This is also in agreement with previous studies (Shea and Chesson, 2002; Jeschke and Strayer, 2006; Romanuk et al., 2009; Layman and Allgeier, 2012; Arbaciauskas et al., 2013; Hayden et al., 2014; Jackson et al., 2016) that suggested a positive association between trophic generalism and invasive success, recognizing it as one of the determinant mechanisms of NNS invasion success outside their natural range (Feiner et al., 2013; Jackson et al., 2016; Penk et al., 2017; Cathcart et al., 2019). Moreover, the large asymmetric overlap found between NNS and NS assemblages, suggests a potential advantage in inter-specific competition for the NNS assemblages, which in case of limiting resources, may out-compete the few remaining NS (Balzani et al., 2016; Wang et al., 2019; Pennock et al., 2021).

When SIA was applied to explore the feeding ecology at the species-specific level in each lake, a high degree of niche overlap between species was also apparent and some NNS appeared to be advantaged by their wider isotopic niches and opportunism. Although trophic position and resource use were not estimated since baseline organisms were not sampled, both the raw ratios of carbon and nitrogen assisted in the interpretation of the feeding ecology of the species in each lake. $\delta^{15}N$ in animals is strictly related to protein intake through diet and typically, the higher the protein intake and the more enriched in ^{15}N the tissue of the animal becomes (e.g., Bloomfield et al., 2011). However, the diet (and thus $\delta^{15}N$) of fish can vary across life stages (ontogenetic variations), and according to the level of specialism of individuals within a species, there may be more pronounced intraspecific variations in the diet that can ultimately determine variation in niche sizes (e.g., Stewart et al., 2021). Most of the species recorded in the two lakes were flexible predators or meso-predators such as *M. salmoides* (Lorenzoni et al., 2002; Marinelli et al., 2007; Feiner et al., 2013; Costantini et al., 2018), *P. fluviatilis* (Lorenzoni et al., 2007; Svanbäck et al., 2015; Linzmaier et al., 2018), *L. gibbosus* (Osenberg et al., 1988; Godinho et al., 1997) and *A. melas* (Kline and Wood, 1996; Leunda et al., 2008), able to shift their diet from macro-invertebrates to prey fish of different size class (including larvae), according to prey availability or shifting with ontogeny as demonstrated by the significant relationships found in this study between nitrogen ratios and body size for most of these species. Other fishes included omnivorous species such as the littoral *S. hesperidicus* that feeds on macrophytes, zooplankton and zoobenthos (Ravera and Jamet, 1991; Garcìa-Berthou and Moreno Amich, 2000), and *A. arborella*, an epipelagic zooplanktivore feeding also on algae (Giussani and Ruffoni, 1985; Soric, 2006), which had similar $\delta^{15}N$ values to meso-predators. Fishes with lower nitrogen ratios were also omnivorous and included the NNS *R. amarus* that forages on filamentous algae, organic material, and zooplankton (Koutrakis et al., 2003; Bo et al., 2012; Moreva et al., 2017) similarly to the NS *R. aula* (La Porta et al., 2010). The differences between the mean highest and lowest nitrogen ratios were of 2.5‰ in San Michele and 3.6‰ in Campagna, which, considering a general 3.4‰ enrichment across trophic levels (Post et al., 2002), suggests feeding activity of the species were limited to one and maximum two trophic levels. This is consistent with the fact that any top-predator fish was not recorded (except for a single *S. glanis* individual collected in Campagna) and most of the analyzed meso-predators were of small to medium size and had yet to reach their piscivorous phase thus probably foraging on

zooplankton and macroinvertebrates like the other omnivorous fishes. Similarly for carbon, mean differences between ^{13}C-most enriched and ^{13}C-most depleted fishes were limited to 1.5‰ and 1.6‰ in San Michele and Campagna respectively, this latter excluding the NNS *R. amarus* which clearly presented less negative δ^{13}C ratios than the rest of the community. This is despite lakes being complex ecosystems composed of different interconnected habitats (e.g., littoral, benthic and pelagic) that may be reflective of different carbon fractionation processes and thus different δ^{13}C ratios (France, 1995). Overall, species-specific SIA suggests that the most abundant fishes were exploiting similar resources, highlighting the potential for strong competitive interactions in the two studied systems.

Given that, in this situation, fish with broader trophic niches may then be advantaged, the NNS *A. melas* emerges potentially as one of the most favored species. In fact, in both lakes, this species displayed the widest isotopic niches and had the highest asymmetric overlap, with this indicating the ability to invade other species' niches while also consuming unexploited resources, thus lessening the strength of competition. The black bullhead *A. melas* has been recently included in the Italian blacklist of invasive species (Monaco et al., 2020) and thus, also considering the results of the present study, management actions should be considered to contain this species and limit the potential competition with the few native species present. In Campagna, the NNS *R. amarus* also appeared to be favored, but as it occupies an apparently empty niche it does not compete directly for resources with the rest of the fish community although potential bottom-up impacts should be monitored. Moreover, although bitterling may not constitute an issue for the native fish fauna, it is a freshwater mussel parasite and could therefore constitute a threat for this group (Van Damme et al., 2007).

The potential for competition and relative advantage of NNS species was partly supported also by the fish body condition analysis. In Campagna, a substantial better condition than expected was detected for the NNS *A. melas* and the NNS *L. gibbosus*. However, results in San Michele contrasted that of Campagna with *A. melas* displaying the worst condition of all the species considered. Nevertheless, all fishes in this lake had their residual indices shifted towards negative values and relative condition always < 100, indicating that all the species were in worse condition than expected. This might be a result of less available resources in San Michele, also considering that this lake is relatively less productive than Campagna but hosted a higher fish biomass. Although morphometric condition indices such those used in this study have been widely applied to test fish health responses to a series of problematics including habitat alteration (e.g., Cavraro et al., 2019) and fishing impacts (e.g., Mello and Rose, 2005), they do not always correlate with the energetic metabolisms of the fish (McPherson et al., 2011) and thus their use to infer competition outcomes may be questionable. Thus, the use of these indices in the present study was explorative and had the aim to provide information on the overall status of the fish assemblage in the two lakes rather than strictly test for effect of competition.

The application of SIA allowed to point out the attention on the worrying conservation and ecological status of the fish assemblages inhabiting two Italian small and shallow lakes. Also, it provided consistent and previously not available information on the trophic ecology and trophic structure of the analyzed fish assemblages. However, the use of SIA alone did not allow to obtain detailed information on the feeding habits of the fishes (taxonomic composition of the diet and relative proportions of diet items) and on the contribution of different resources such as allochthonous (e.g., terrestrial) or autochthonous carbon sources. Moreover, SIA of consumers are also influenced by the metabolism and physiology of the organisms thus limiting the reliability of the trophic niche reconstructions and the estimate of competition. More robust information could have been obtained if bulk SIA would have been coupled with other methods such as gut content analysis (De Santis et al., 2021), DNA metabarcoding (Stewart et al., 2021) or compound specific SIA (i.e., stable isotopes of amino acids (Chikaraishi et al., 2014; Alp and Cucherousset, 2022)). This detailed information is useful to fully understand the extent and strength of inter-specific trophic interactions and to better understand and mitigate the impacts of NNS in receiving ecosystems. As such, the combination of SIA with other methods should be considered for future studies.

This is particularly true considering the risks associated to the spread of some NNS that is foreseen to increase in the future (Seebens et al., 2020), both due to natural dispersion related to the ongoing climate change (e.g., Vilizzi et al., 2021; Souza et al., 2022) but also because of socio-economic changes (e.g., human land use) and changes in trade and transport (i.e., new trade routes and modes, e.g., Essl et al., 2020). Given the high vulnerability of small water bodies to present and future introductions (Reynolds and Aldridge, 2021), this study provides a relatively cost-effective and accessible methodology to assess present and future changes in interspecific trophic interactions that can then be used to inform the local-scale management of ecosystems by detecting of the most dangerous invasive NNS that requires prioritization.

5. Conclusions

Overall, this work presents the first assessment of the ecological status of the fish assemblages in two shallow and small lakes included in protected areas in northern Italy. Traditional community survey was coupled with stable isotopes analysis used to infer the trophic ecology and structure of the NS and NNS fish assemblages. Although the two lakes are included in protected areas, their fish community resulted to be deeply altered compared to the most probable near-natural conditions, lacking most of the typical fish species. The two lakes had fish assemblages contrastingly dominated by NNS and NS in biomass and number, suggesting a slightly better ecological status for the NS dominated lake (i.e., Campagna). Despite this difference, stable isotopes revealed a worrying situation in which NNS may have competitive advantages over the already reduced NS in both lakes. Given its broadest niche and its ability to invade other species' niches while consuming other resources, the NNS *A. melas* emerged as the most problematic species. Thus, the results pointed to the need of implementing conservation and management actions to mitigate the ecological damaging of this valuable ecosystems and restore, if possible, their ecological status. Despite the crucial role that shallow and small lakes have in preserving aquatic ecosystems diversity and services, their ecological status is seldom evaluated, particularly regarding fish communities and their trophic interactions. It is likely that the situation of the two lakes investigated in this study mirrors that of many other Italian small and shallow lakes. The use of SIA, possibly combined with other methodologies, may be thus extended to a higher number of understudied shallow Italian lakes. This will allow to obtain a more comprehensive picture of the inter-specific interactions

and possible trophic re-organizations of the receiving food webs induced by NNS, also in the view of the future increase of NNS.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2022.e02082.

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