Roach in Lake Maggiore: A Peaceful Invasion Detected with C, N Stable Isotope Analysis

By Visconti Anna, Volta Pietro, Fadda Amedeo & Manca Marina

Abstract - Invasions by non-native species are increasingly detected all over the World, as a consequence of globalization. Successful establishment implies the presence of unexploited niches and/or competitive advantage of invader vs. native closely related species. As a consequence, invasion also impacts functional diversity and trophic relationships in ecosystems. Feeding niche is a relevant component of ecological niche; it can be investigated by means of C, N stable isotopes analyses (SIA). Here we present results of a study in which feeding niche of the invader *Rutilus rutilus* is compared with those of *Coregone lavaretus* and Alosa agone in a deep, subalpine lake (Lago Maggiore, Italy). By applying SIA we quantified seasonal shifts in pelagic vs. littoral diet sources and in their percentage contributions for the three species. Feeding plasticity, namely spatial and temporal displacement, allowed for a peaceful coexistence of the invader and the other two potential competitors.

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I. Introduction

Invasions of non-native species usually cause negative perturbations on invaded environments and are often associated with environmental degradation (Wilcove et al. 1998; Byers 2002; Volta et al. 2013). One of the main impacts is the change in native communities with an imbalance of established trophic relationships between native species (Nillson et al., 2012). Such imbalance in turn drives environmental consequences which can result into ecological and economical damages (Welcomme 1998; Rahel 2000; Richardson, 2011), hardly reversible.

Successful establishment of non-native species has been reported to deeply affect diversity of communities, driving changes in taxonomic composition and in functional diversity (Richardson, 2011; Strayer, 2012). Changes in functional diversity of the community in turn have strong impact on food webs and ecosystem functioning (Hooper et al. 2005; Britton et al. 2010; Richardson, 2011; Simberloff 2011; Volta et al. 2013). Species invasion largely depends on the degree of niche overlapping and competition among native and non-native species. Often, success of replacement of native by non-native species is linked to their taxonomical similarity, based on which the two are also ecologically similar. In other cases, however, also taxonomically distant species can be characterized by a similar morphology (e.g. body size), which makes them suitable for the same habitat/niche (Visconti et al. 2009; Ricciardi and Mottiar 2006).

Consequent to global changes and increasing environmental vulnerability, number of invasions are exponentially recorded all over the World (Moss et al. 2009). Such invasions are increasingly detected also in aquatic environments which were traditionally regarded as resilient to environmental impacts and characterized by stable taxonomic structure of their communities, and a high level of predictability in seasonality. Such is the case of deep lakes, increasingly reported as becoming vulnerable to invasions (e.g. Kamburska et al. 2013; Visconti et al. 2009; Volta et al. 2008, Volta et al. 2013). Studying impact of successful establishment of alien taxa in this type of environments therefore allows for predicting new scenarios driven by global changes.

Environments such as Lake Maggiore, object of long term monitoring programs, are reasonably highly informative in detecting changes. Being their biotic communities investigated in detail over the years, they are certainly deemed to prompt detection of the appearance of non-native species. Increasing records of new taxa and of their successful establishment in the lake, are likely to be representative of increasing vulnerability of the lake to changes directly or indirectly driven by globalization, thus changing our perspective on highly stable and resilient environments (de Bernardi et al. 1990).

Invasions in Lake Maggiore and in its catchment basin were detected at different levels of the biota from plankton (e.g. zooplankton copepods, Visconti et al. 2009), benthos (e.g. mussels: Kamburska et al. 2013; Piscia et al. 2011) and fish (Volta and Jepsen 2008; Volta et al. 2013). Alteration in fish community resulting from invasion by aliens, is likely that having also major impacts on social and economical activities, being commercial fishing an important resource in the economy of deep lakes region.

A successful, and relatively fast invasion by the non-native roach, Rutius rutillus (Linnaeus 1758), was recently recorded in Lake Maggiore (Volta and Jepsen 2008). Thanks to the prompt colonization of lake waters, roach is nowadays one of the most abundant fish species of the lake as registered by commercial catches (Volta et al. 2013). The others being the autochthonous shad, Alosa agone (Scopoli 1786) and the allochthonous but naturalized coregonids (C. lavaretus...
Linnaeus 1758. and *C. macrophthalmus* Nusslin 1882 introduced since the end of 1800s). Depending on their life cycles, those species can be more related to pelagic or to littoral zones (Berg and Grimaldi 1966; Visconti et al., 2013). However, due to the steep profile of the shore banks, fish can easily switch between littoral and pelagic zones, as detected by means of diet investigations. Being pelagic and littoral food sources characterized by peculiar carbon and nitrogen stable isotope signatures, tracing their contribution to fish diet, by means of detection of assimilated food, is allowed by Stable Isotope Analysis (SIA) of their tissues. This type of analysis also allows for quantifying relative contribution of the two potential food sources along the season, irrespective of catchment/sampling zone.

While interactions between roach and littoral fish were largely discussed (e.g. Volta and Jepsen 2008), relatively less information is available on interference of roach with pelagic fish species (Persson and Greenberg 1990; Bergman and Greenberg 1994). Comparative studies on the biology of coregonids, shad (Berg and Grimaldi 1965,1966, Volta 2010) and roach (Volta and Jepsen 2008) allow for hypothesizing that the “genuine invasion” (as defined by Volta and Jepsen 2008) of the roach can lead to a peaceful coexistence.

In this respect, the aim of the present study is to define/quantify 1) the degree of trophic niche overlap along the season; 2) seasonal pelagic vs. littoral carbon sources exploitation; 3) seasonal changes in trophic level with respect to isotopic baselines for roach, shad and the European whitefish *C. lavaretus*. All these information will contribute to quantifying the degree of competition among these species.

II. Materials and Methods

a) Study Site

Lake Maggiore (45°58′30″N latitude; 8°39′09″E longitude; 194 m a.s.l. altitude) is the second-deepest (370 m maximum depth) and largest (212.5 km²) subalpine lake in Italy. Long-term (LTER) limnological and paleolimnological studies have traced the impact of multiple stressors (i.e. changes in algal nutrients, climate warming and meteo-climatic inter-annual variability, introduction of non native fish) on the lake (e.g., Guilizzoni et al. 2011; Jeppesen et al. 2012; Manca and DeMott 2009; Visconti et al. 2008; Visconti and Manca 2010; Volta and Jepsen 2008).

b) Sample collection and laboratory analyses

Stable isotopic signatures of primary consumers, i.e. the pelagic cladoceran *Daphnia galeata* and chironomid larvae and amphipod, to trace pelagic and littoral baselines, respectively.

*Daphnia* was collected monthly during 2008 at three Lake Maggiore pelagic stations (Visconti et al. 2011): a station located at the point of maximum depth of the lake (Ghiffa: 45°58′30″N; 8°39′09″E); a second station located near the inflow of the Toce River (Baveno: 45°54′28″N; 8°31′44″E); and a third station located in the southern, shallower part of the lake basin, known for being more influenced by littoral inputs (Lesa: 45°49′70″N; 8°34′70″E). The three stations were sampled on the same day and isotopic data from the three were integrated for the pelagic baseline signature. Live samples for isotopic analysis were collected with large (58-cm opening mouth diameter), 450-μm nylon net, to ensure that large phytoplankton colonies were avoided and small-bodied zooplankton taxa (such as rotifers, and early developmental stages of copepods) were excluded. Organisms were kept overnight in filtered (1.2 μm GF/C filters) lake water for gut clearance, then isolated from other zooplankters in a quantity suitable for isotopic analyses (about 700 individual, to reach 1 mg dw/sample).

Samples of benthic littoral organisms (chironomid larvae and amphipods) were seasonally collected with benthic nets at three littoral stations located at the shore along the major axis of the lake (integrating the samples of three stations: Cannero, Baveno, Meina) and at a station (Toce) near the mouth of the River Toce. Samples were processed as described for *Daphnia*. Approximately 15 organisms/sample were necessary to reach the weight requested for SIA.

European whitefish and shad were sampled monthly in the central zone of the lake (Ghiffa station), with a set of drifting nets of different mesh size (32-34-40-50 mm knot to knot). Roach was sampled in the littoral area of the same region using benthic multimesh survey gillnets (Nordic type). Nets were set at dusk and retrieved the following morning. On each sampling date, the dorsal muscle (located between the head and the dorsal fin and above the lateral line) of three individuals/species (≤ 3 years old, as determined by scales analyses) was dissected for SIA.

All samples (baselines and fish) were oven-dried at 60°C for 48 hours (3 days for fish) and finely powdered. Subsamples of about 1 mg dry weight each were transferred to 5x9 mm tin capsules for Carbon, Hydrogen, Nitrogen (CHN) and Continuous Flow-Isotope-Ratio Mass Spectrometry (CF-IRMS) Stable Isotope Analyses. Three replicates were performed for each sample.

Samples were sent to the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada) where the isotopic composition of organic carbon and nitrogen was determined by a DeltaPlus Advantage CF-IRMS. The standard deviation of the analyses (S.D.), based on the laboratory internal standards, was < 0.2‰ for both δ^{13}C and δ^{15}N. Isotope ratios were expressed as parts per thousand (%) differences from a standard reference (PeeDee Belemnites for carbon and atmospheric N₂ for nitrogen):
where $R$ is the isotopic ratio: $^{13}$C / $^{12}$C and $^{15}$N / $^{14}$N.

c) Integration of baseline fluctuations into DBMM

Sources exploited by fish were assessed by comparing their $\delta^{13}$C signature to the signatures of the two potential baselines, i.e. pelagic and littoral. A consumer maximum fractionation ($F = \delta^{13}$C$_{\text{fish}}$ - $\delta^{13}$C$_{\text{baseline}}$) was assumed at $0.8 \pm 1.1$‰ (De Niro and Epstein 1978). Nitrogen stable isotopes were used to define steps in the trophic web based on the general assumption of a gradual $\delta^{15}$N enrichment of $3.4$‰ $\pm 1.0$ (Post 2002) from food to consumer ($E$ = $\delta^{15}$N$_{\text{fish}}$ - $\delta^{15}$N$_{\text{baseline}}$).

When a single food source was used, fish trophic position ($T$) relative to baseline was calculated applying the equation:

$$T = (E/3.4) + \lambda;$$  $(1)$

Where: $\lambda = 2$ is a constant value usually assigned to primary consumers (e.g. $\text{Daphnia}$ for the pelagic and chironomids/amphipods for the littoral).

When fish $\delta^{13}$C signature appeared linked to both baselines (littoral and pelagic), their contribution to fish diet was quantified using the Dynamic Baseline Mixing Model (DBMM; Woodland et al. 2012). The model assumes that carbon isotopic signature of a consumer tissue is the result of a linear mass balance average of elemental mass from each metabolized food source (Phillips 2001). DBMM takes into account both seasonal shifts in baseline signatures integrated in fish isotopic signature and the specific metabolic and growth rates of investigated fish. It was assumed that monthly carbon signatures of investigated fish integrated diet isotopic signatures over 60 days, i.e. the time delay necessary for fish dorsal muscle to reach equilibrium (Hesslein et al. 1993; Perga and Gerdeaux 2005; Phillips and Eldridge 2006). Percentage contributions of pelagic ($p$) and littoral ($q$) sources to the fish carbon signature result from the equation:

$$p = (\delta^{13}$C$_{\text{fish(t)}}) e^{-k*m*t} + h_{\text{litt(t)}} e^{-k*m*t} - \delta^{13}$C$_{\text{fish(t)}} / (h_{\text{litt(t)}} e^{-k*m*t} - h_{\text{pel(t)}} e^{-k*m*t});$$  $(2)$

$$q = 1-p;$$

Where: $\delta^{13}$C$_{\text{fish(t)}}$ is the initial isotopic value of fish in equilibrium with its diet at time $t=0$; $\delta^{13}$C$_{\text{fish(t)}}$ is the isotopic signature of fish in equilibrium with its diet at time $t$ (where $t=60$ days); and $k$ and $m$ are fish growth rate ($d^{-1}$) and tissue turnover metabolic constant ($d^{-1}$), respectively.

The model is based on a system of linear equations ($h$) best fitting baseline data through time ($h_{p}$ and $h_{l}$ = functions associated with pelagic and littoral signatures, respectively). For fish dorsal muscle tissue, $m$ was assumed to be 0.0018 $d^{-1}$ for both $\delta^{13}$C and $\delta^{15}$N (Hesslein et al. 1993). Growth rates $k$ assigned to each fish species were calculated according to Hesslein et al. (1993): $k_{\text{shad}} = 0.01 d^{-1}$; $k_{\text{whitefish}} = 0.03 d^{-1}$; $k_{\text{roach}} = 0.03 d^{-1}$.

Time-specific percentage contributions of pelagic ($p$) vs. littoral ($q$) carbon signature were used to calculate mean year contribution of each source, i.e. the number of months/year during which each source was exploited.

When both food sources (littoral and pelagic) were exploited, the fractional carbon contribution from each source ($p$ and $q$) was entered into the trophic level equation:

$$T = (\delta^{15}$N$_{\text{fish}} - (p * \delta^{15}$N$_{\text{pelagic}} + (1-p) * \delta^{15}$N$_{\text{littoral}})/\lambda) + 2;$$  $(3)$

III. Results

a) Seasonal fluctuations in baselines and fish carbon signatures

Seasonal changes in carbon isotopic values ($\delta^{13}$C) of the pelagic baseline were substantial, following a common trend of deep, subalpine lakes, with more $^{13}$C-depleted values in winter (-35.78‰ $\pm$ 0.06 SE) and less depleted values in summer (-25.72‰ $\pm$ 0.72 SE). Overall, pattern of change in $\delta^{13}$C signature of pelagic baseline was related to water temperature and thermal stratification (Caroni et al. 2012), with a gradual thermal de-stratification and increasing contribution of dissolved carbon from the hypolimnion, leading to a seasonal minimum during winter mixing (Zohary et al. 1994).

Seasonal changes in littoral baselines were very rarely investigated (e.g. in Lake Maggiore, 2008: Visconti and Manca, 2010). Indeed, littoral baseline was found to change seasonally, in a step-by-step temporal pattern similarly to what observed for the pelagic baseline, although shifted towards less $^{13}$C-depleted values in summer (-18.3‰ $\pm$ 0.14 SE) and more $^{13}$C-depleted values in winter (-25.28‰ $\pm$ 0.6 SE) (Visconti et al., 2013).

Owing to results showed in trophochemical plots of the three fish species analyzed (Fig. 1), is clear that both truly pelagic species, whitefish and shad, closely related to the pelagic food sources from June ($\delta^{13}$C signatures: -28.6‰ $\pm$ 0.5 and -29.1‰ $\pm$ 0.6, respectively) to December ($\delta^{13}$C: -29.2‰ $\pm$ 0.4 for shad and -28.5‰ $\pm$ 0.2 for whitefish), are able to exploit also littoral food sources in winter and early spring ($\delta^{13}$C: -27.8‰ $\pm$ 0.7 and -28‰ $\pm$ 0.4, in February and -28.6‰ $\pm$ 0.2 and -26.9‰ $\pm$ 0.4, in April, for shad and whitefish, respectively), i.e. when both pelagic and littoral carbon baselines reached their most $^{13}$C-depleted values. Similarly, the truly littoral invader, related to littoral sources from December (-25.7‰ $\pm$ 0.4) to June (-25.3‰ $\pm$ 0.2), was found to incorporate pelagic isotopic signatures from August (-26.3±0.2‰) to October (-25.6±0.2‰). Seasonal pattern of change in isotopic signatures of the two mainly pelagic fish species allowed for tracing their moving into the littoral for feeding in winter. In Summer, however, when all three fish species fed in the pelagia, their C and N isotopic
The trophochemical graph also suggests that whitefish were, overall, on a higher trophic level (δ¹³C signatures ranging from 9.65‰ ± 0.7 in June to 11.79‰ ± 0.3 in September) than the other two species (δ¹³C: between 8.58‰ ± 0.3 in August and 11.74‰ ± 0.2 in December for shad; between 8.59‰ ± 0.3 in November and 10.41‰ ± 0.8 in September, for roach). Thus suggesting that whitefish preferentially exploit, when available, zooplankton secondary consumers.

When relying on littoral sources, the trophic position of the three fish were of 4 or 5, indicating the presence of one or two intermediate preys between fish and organisms used to trace littoral baseline (T=2). In August-October, when roach fed entirely in the pelagic, T was always 3, i.e. one step above primary consumers (T=2) used as pelagic baseline (Daphnia). When relying on pelagic food sources (May-December), shad occupied level 3 from June to November, feeding directly on cladoceran (Daphnia) or copepod (diaptomids and the small-sized Mesocyclops leuckarti (Visconti et al. 2013)) primary consumers. In November, whitefish trophic position T= 4 was due to the presence of intermediate secondary consumers (namely, Bythotrephes and Leptodora among cladocera; the large Cyclops abyssorum among copepods (Visconti et al. 2013)) among zooplankton potential preys.

When a mix of pelagic and littoral δ¹³C isotopic signatures were integrated into fish tissues, DBMM allowed to calculate percentages of pelagic (p) vs. littoral (q) contributions (equation 2; Tab. 1). For roach, intermediate carbon isotopic values between pelagic and littoral were measured in July and November, suggesting simultaneous exploitation of the two carbon sources. A p contribution of 64% in July and of 46% in November were estimated (equation 2; Tab. 1). For both whitefish and shad, isotopic carbon signatures were intermediate between pelagic and littoral carbon sources in January and May. We estimated 78% of littoral sources (q) in January and 79% of pelagic (p) sources in May for shad. A p-contribution of 51% and of 53% were estimated for whitefish in January and May, respectively (equation 2; Tab. 1).

On an annual basis, mean contributions of pelagic sources exploited by the three fish species were of 33%, 75% and 67 %, for roach, whitefish and shad, respectively.

IV. DISCUSSION AND CONCLUSIONS

Being omnivorous and opportunist, roach is characterized by a wide trophic spectrum, directly influenced by seasonality. SIA results confirm previous studies (Volta and Jepsen 2008) indicating that roach switches towards a zooplanktonic diet in summer (Volta and Jepsen, 2008), while relying upon littoral food sources (such as algae, benthos, detritus and zoobenthos) in winter and spring, i.e. until the spawning period.

On the other hand, we found that whitefish and shad relied upon littoral food sources although for a short time, three and four months, respectively. Littoral feeding was restricted to the spawning periods (winter for whitefish and late spring for shad). During this period, whitefish preferentially eat on chironomid larvae and amphipods (Berg and Grimaldi 1965; Perga and Gerdeaux 2005), while shad mainly feed on ostracods, Tendipedidae, Turbellarii and detritus (Berg and Grimaldi 1966). Owing to results of the present study, when whitefish and shad exploited pelagic food sources, however, competition was not strong: based on isotopic signatures of zooplankton available preys (Visconti et al. 2013) and on their own isotopic signatures, in fact, they fed on different preys. Whitefish mainly fed on cladocera. When shad shifted towards cladoceran primary consumers (i.e. Daphnia), whitefish shifted towards cladoceran secondary consumers (i.e. Bythotrephes and Leptodora), by shifting to a higher trophic level thus releasing competition with shad.

During pelagic co-occurrence with the other two species (i.e. from July to October), roach tended to overlap with shad for 15N-enrichment, by sharing the same primary consumers (Daphnia and Bosmina). During pelagic feeding, however, less- and more- 13C-depleted isotopic signatures of roach and whitefish, respectively, indicate horizontal segregation in feeding zones of the two fish species: likely, roach was related to nearshore and shad the open water pelagic zones of the lake, thus not exploiting the same preys. Absence of competition was also suggested by a similar increase in abundance of these two species during the last ca. five years (Volta et al. 2013).

Despite a similar ability to shift between pelagic and littoral food sources, roach invasion did not result apparently into an heavy competition with to the two naturalized fish species. Their trophic niches were not only displaced in time, with only a 25 (with whitefish) and 33 (white shad) % overlapping in foraging zones, but they were also displaced in space in periods of apparent overlap in diet (i.e. with shad, in summer).

REFERENCES Références Referencias


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**Legends to Figures**

- ◇ Whitefish
- ● Shad
- △ Roach

*Figure 1*: Trophochemical graph of monthly δ¹³C and δ¹⁵N isotopic signatures of roach, whitefish and shad from Lake Maggiore, 2008. White and grey symbols identify pelagic and littoral sources, respectively. For further explanation refer to text.
Table 1: Monthly values of \( p \) (pelagic, white cells) and \( q \) (littoral, grey) contributions (%) to carbon isotopic signature of the three Lake Maggiore’s fish species. Numbers in bold refer to values calculated by applying a DBMM (Woodland et al. 2012). For further explanation see text.

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