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Research Article

# Predicting the effects of reintroducing a native predator (European eel, *Anguilla anguilla*) into a freshwater community dominated by alien species using a multidisciplinary approach

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## Abstract

Multiple introductions of alien species can lead to the formation of new and diverse communities with diverse interactions, particularly in isolated freshwater ecosystems. In Lake Arréo (currently known as Caicedo-Yuso), located in Basque Country, Northern Spain, the introduction of several North American species (largemouth bass *Micropterus salmoides*, pumpkinseed *Lepomis gibbosus* and the red swamp crayfish *Procambarus clarkii*) and the common carp *Cyprinus carpio* has resulted in a unique community composed mainly of alien species. Previous efforts to eradicate them by intensive fishing/trapping have failed. Reintroduction of native predators could represent a complementary method, with an added biodiversity value. The reintroduction of the European eel *Anguilla anguilla* could lead to an increased predation on crayfish as shown by previous studies, but also affects the abundance of juvenile fish. To investigate the current situation of Lake Arreo, stable isotope analyses were conducted using 15 muscle tissue samples per each fish and crayfish species, while stomach contents of the same species were analysed. Additionally, samples from the common reed *Phragmites australis*, the trophically lowest food source available for fish and crayfish species, were collected and used as baseline for the isotope analysis. To investigate the usefulness of stable isotopes to predict the effects of species reintroductions on present species communities, available stable isotope and diet data from *A. anguilla* in a German freshwater lake with a similar species composition were retrieved and included in the Arreo community analysis. While results from both, dietary and stable isotope analyses, indicate high interactions among alien species with *P. clarkii* having a central position, the modelled reintroduction of *A. anguilla* shows to possibly affect recruits of alien fish species as well as an increased feeding of *M. salmoides* on reintroduced eels.

**Key words:** stable isotope, biocontrol agent, invasive species, eradication, modelling, risk assessment

## Introduction

There is no saturation in the global accumulation of alien species and this has important implications for local biodiversity (Seebens et al. 2017). Introductions of species and their subsequent dispersal can indeed change the structure and composition of entire communities (Huxel et al. 2002; Strayer 2010). Moreover, simultaneously present alien species tend to occupy different trophic levels (Gamradt and Kats 1996; Kiesecker and Blaustein 1998) and interact across trophic levels, leading to increased and often unforeseeable effects due to the prevalence among predators (Peckarsky and MacIntosh 1998; Pringle and Hamazaki 1998; Sih et al. 1998). The complex interactions among alien species within the same ecosystem are so far not well understood (Parker et al. 1999; Huxel et al. 2002; Strayer 2010; Bissattini et al. 2018). As described in the “invasional meltdown hypothesis” (Simberloff and Von Holle 1999), they can favour the chance of a species establishment success and increase the impact on the recipient environment (Simberloff and Von Holle 1999; Simberloff 2006), while in certain cases lowering the impact of predatory invasive alien species on native species (Soluk and Collins 1988a, b; Soluk 1993; Rosenheim 1998; Bissattini et al. 2018).

Lake Arreo in Basque Country (Northern Spain), currently known as Lake Caicedo-Yuso, provides an interesting example of a multiple invaded freshwater ecosystem. The dominant species (*Micropterus salmoides* Lacépède, 1802; *Lepomis gibbosus* Linnaeus, 1758; *Cyprinus carpio* Linnaeus, 1758 and *Procambarus clarkii* Girard, 1852) are all considered invasive. They dominate the communities’ biomass and change its composition and structure, negatively affecting the remaining native flora and fauna and thus the lake’s status as a protected area (see e.g. Costantini et al. 2018).

From 2014 to 2017, Lake Arreo has been monitored, and the four invasive alien species (IAS) have been controlled: Their abundance was reduced, but eradication was not achieved (Haubrock et al. 2018). The reintroduction of a native predator, the European eel *Anguilla anguilla* Linnaeus, 1758, which was once present in the lake, has proven to be an efficient biocontrol for the population of the *Procambarus clarkii* (Aquiloni et al. 2010; Musseau et al. 2015). However, the introduction of predators has major impacts on trophic structures within and across trophic levels (Codron et al. 2018; Costantini et al. 2018). While effects of such reintroduction might be negative for present IAS, positive effects may occur when the activity of the reintroduced predator provides a stable food source for other present species (Codron et al. 2018).

In general, European eels are considered to be night-active predators (Tesch 1999) that hunt close to the bottom rather than in the open water column (Barak and Mason 1992) but are highly opportunistic (Lammens et al.

1985; Schulze et al. 2004). Nonetheless, being a second-order carnivorous species, the species can shift its diet and feeding strategy according to the available potentially seasonally and varying resources presented by the surrounding ecosystem (Bouchereau et al. 2006). Considering this and also its ability to survive in saline environments such as Lake Arreo (Skadhauge 1969), makes *A. anguilla* an ideal candidate for biocontrol in Lake Arreo. But since the interaction between reintroduced predator and omnivorous IAS such as *P. clarkii*, and hence successive effects, are unpredictable, being able to provide such a prediction will be of increasing interest.

Using Stable Isotopes Analysis (SIA) of carbon and nitrogen, can provide long-term information on species, enabling to have a trophic snapshot of a community (Boecklen et al. 2011; Layman et al. 2012; Middelburg 2014), estimate trophic levels (Post 2002), and finely quantify ecological niches (Newsome et al. 2007). Hence, SIA proved to be a useful tool in investigating IAS (Vander Zanden et al. 1999a, b; Balzani et al. 2016). While carbon signatures identify the major energy sources, nitrogen signatures relate to the trophic position within a food web (Layman et al. 2012). This relationship relies on predictable changes in the isotopic values from prey to consumer, being enriched of 1‰ for C and 2.5–5‰ for N between consecutive trophic levels (Post 2002; Vanderklift and Ponsard 2003).

Combined with the analysis of dietary contents (DA), which provide a direct short temporal insight into the feeding habit of a species, relationships among species can be investigated (Polis and Strong 1996; Bissattini et al. 2018; Meeuwig and Peacock 2017). By means of SIA and DA, the trophic web and interactions of Lake Arreo were assessed. The use of overlapping isotopic niches has previously been taken into consideration when predicting consequences at the community level following the introduction of a new species (Vander Zanden et al. 1999a; Gorokhova et al. 2005; Maguire and Grey 2006). Thus, isotope and dietary data of *A. anguilla* retrieved from different ecosystems were used to model and predict the effect of this species reintroduction and the potential consequences for other species, helping us predict the potential effectiveness of biocontrol agents for the management of IAS and the response of native species. Moreover, this approach could be an efficient risk assessment tool and way to evaluate impacts in the future (Kolar and Lodge 2001; Ricciardi 2003; Keller et al. 2007; Ricciardi et al. 2013). Resultant, using this technique could furthermore help increase our understanding of long-term species invasions and bio-manipulation (Wysujack and Mehner 2002; Strayer et al. 2006). Considering the diet preferences of *A. anguilla*, we hypothesize that the applied mixing models will predict the predation of reintroduced *A. anguilla* on the abundant alien species.

## Materials and methods

### Study area

Lake Arreo (42°46′42″N; −2°59′28″E; surface area: 6 ha; max. depth: 24 m) in Basque Country, Spain, is a "Nature 2000 Network – Special Areas of Conservation (ZEC)" (ES2110007) habitat and the largest most unique and natural continental lake system among the scarce lakes of the Iberian Peninsula. Being fed by hypersaline water from the diapiric substratum, there are small areas with crusts of salt allowing the existence of plant communities atypical for the area. Concerning the Basque Catalog of Endangered Species, two present plant species are listed as endangered (*Berula erecta* Hudson Coville, 1893; *Utricularia* australis Brown, 1810), one other as vulnerable (*Puccinellia fasciculate* Parlatore, 1850) and two others as near threatened (*Cistus crispus* Linnaeus, 1753; *Juncus acutus* Linnaeus, 1753). Nutrient-drag and fertilizers used around the ecosystem reduced peripheral vegetation, the introduction of alien species has led to a poor ecological state, as determined by the Monitoring Network of the Ecological State of Wetlands of the autonomous community of the Basque Country (CAPV). The lake state has thus worsened in recent years. Lake Arreo is frequently visited by the common pochard *Aythya farina* Linnaeus, 1758, the common reed bunting *Emberiza schoeniclus* Linnaeus, 1758, the common moorhen *Gallinula chloropus* Linnaeus, 1758, the common snipe *Gallinago gallinago* Linnaeus, 1758 and migrating birds. Additionally, 27 families of invertebrates were identified from 2010 to 2012 but no information on their current status has been assessed. The native crayfish *Austrapotamobius pallipes* Lereboullet, 1858 is considered to have disappeared as a result of the introduction of the North American crayfish *P. clarkii*. Moreover, the conservation of the Lake Arreo endemic aquatic beetle *Gyrinus paykulli* Müller, 1764 is listed in the management plan of the "Lago de Caicedo Yuso y Arreo (ES2110007)" as desirable. The entire fish community consists of the common carp (*C. carpio*), the largemouth bass (*M. salmoides*) and pumpkinseed (*L. gibbosus*), that are the most dominant species in terms of abundance and biomass, while barbel (*Barbus graellsi* Steindacher, 1866), tench (*Tinca tinca* Linnaeus, 1758), European eel (*A. anguilla* Linnaeus, 1758) and the exotic *Carassius auratus* Linnaeus, 1758 are considered extinct (Haubrock et al. 2018).

### Field work

In September 2017, as part of the annual monitoring and control effort, 15 specimens of *M. salmoides* and *L. gibbosus,* 11 specimens of *C. carpio* as well as 10 specimens of *P. clarkii* from all size classes were collected under the consideration of an even spatial distribution across the lake ecosystem (Willson et al. 2010). Additionally, five samples of the dominant introduced aquatic plant, the common reed *Phragmites australis* were collected to complement the primary consumer (*P. clarkii*) as primary producer (Vander Zanden and Rasmussen 1996; Vander Zanden and Vadeboncoeur 2002). Other macroinvertebrates were not sampled as they were not found during sampling. Samples were immediately put on ice before being transported to the laboratory for further processing.

### *Laboratory procedure*

Total length (TL) of fish and carapace length (CL) of crayfish were measured with a calliper (accuracy: 0.01 mm) and body mass (BM) was measured using an electronic balance (accuracy: 0.01 g). From twenty randomly chosen individuals of each species, muscle tissue for SIA was extracted. Muscle tissue was cleaned from fat, skin, scales, bones and shell (for crayfish), and for plants, a 5–10 cm leaf was cut off. Samples were placed into separated glass trays, dried for 48 hrs in an oven at 60 °C and afterwards grinded into fine powder with an agate mortar and pestle. For each sample of fish and crayfish, two replicates of each 0.20–0.30 mg (respectively 1.00–1.10 mg for plants) were weighed on a Mettler Toledo AG245 microscale and enclosed into a tin capsule to be combusted with a FlashEA 1112 elemental analyser. Samples were subsequently analysed by a Thermo Finnigan Delta Plus Advantagean isotope ratio mass spectrometer at the Istituto di Geologia Ambientale e Geoingegneria of the National Research Council (CNR) in Montelibretti, Rome (Italy). Isotope compositions were expressed as ‰ with the δ notation (based on $\delta^{13}C$ or $\delta^{15}N = ((R_{sample}/R_{standard}) - 1) \times 1000$, where R is $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$ ratios). Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric $N_2$ for nitrogen. Arithmetic mean ± 1 SE was calculated for each species. The trophic positions of all species ($TP_c$) were calculated by applying the equation $TP_c= ((\delta^{15}N_c - \delta^{15}N_{base})/\Delta N) +\lambda$, where $\delta^{15}N_c$ is the mean $\delta^{15}N$ of the consumer, $\delta^{15}N_{base}$ is the mean $\delta^{15}N$ of primary consumers, Δ Nis the standard enrichment of 3.4 ‰ between trophic levels and λ is the basal trophic level (= 1 for plants in our case) (Post 2002; Bissattini et al. 2018; Britton et al. 2018).

### *Statistical analyses*

DA of IAS in Lake Arreo were previously performed (Haubrock et al. 2018; Supplementary material Table S1) but were used to compare a potential diet overlap with the results presented by SIA. To estimate and quantify intra-specific and community niche width, Layman metrics (Layman et al. 2007) were calculated with the R-package SIAR (Stable Isotope Analysis in R; Parnell et al. 2010). Additionally, the corrected standard ellipse areas (SEAc) for all species and community were calculated (Jackson et al. 2011). The application of dual plot graphs for $\delta^{15}N$ versus $\delta^{13}C$ of consumer tissues and food sources enabled the determination of probable prey sources and combinations of prey contributing to the diet of predators (Phillips and Gregg 2003). Thus, to investigate how sampled species contribute to the isotopic signatures of each other, Stable Isotope Mixing Models (SIMMs) were applied using the R-package SIMMr (Parnell et al. 2013). SIMMs were conducted for all *M. salmoides* present in Lake Arreo and potential prey items. Results are presented as the average percent values with the possible range percentage for each prey item. A Canonical Analysis of Principal Coordinates (CAP) for both factors, i.e. $\delta^{15}N$ and $\delta^{13}C$, was applied to investigate which contributed more consistently in differentiating species. Spearman correlations for each variable with CAP1 axis, the only one found informative in differentiating the species' niches are reported. Additionally, a PERMANOVA and pairwise post-hoc tests were used to identify significant differences in $\delta^{15}N$ and $\delta^{13}C$ among species and to eventually determine niche overlap between species using the PRIMER software (Clarke et al. 2001).

### *Predicting the effect of reintroducing* Anguilla anguilla

The probable long-term impact of reintroducing *A. anguilla* as a biocontrol agent for the invasive species in Lake Arreo was predicted using the previously described application of mixing models with the inclusion of isotope data of *A. anguilla*. Moreover, these were discussed in respect to information on *A. anguilla* feeding behaviour. Additionally, the corrected standard ellipse areas (SEAc; considering 40% of central data points) as an indicator of niche overlap and the corresponding 95% standard ellipse areas (SEAb) for all species, indicating effects due to common resource usage, were calculated. With these, the degree of isotopic niche overlap, which lays between 0, i.e. no overlapping, and 1, completely overlapping, can be estimated and subsequently used as "a quantitative measure of dietary similarity among populations" (Jackson et al. 2011). Calculations were computed using the R package SIBER (Jackson et al. 2011). Therefore, $\delta^{15}N$ and $\delta^{13}C$ values of IAS in Lake Arreo were standardized with the mean of the primary producer *P. australis*, and $\delta^{15}N$ and $\delta^{13}C$ isotope values of *A. anguilla* (from Dörner et al. 2009) were standardized with the combined means of the two present primary producers in Großer Vätersee (Germany), a species community containing native counterparts with similar ecological roles to the alien species present in Lake Arreo, to make communities comparable (Taylor et al. 2017). In the Großer Vätersee, piscivorous eels ranged from 31.5 to 77 cm and 17.8% stomachs contained only fish (overall diet: 40% perch, 34% roach, 17% crayfish, 8% insects, 2% gastropods). Results were complemented with a short review of dietary analysis of *A. anguilla* (Costa et al. 1992; Dörner and Benndorf 2003, 2009; Bouchereau et al. 2006) and discussed.

These analyses were complemented with an assessment of the predation effect of 1,000 potentially reintroduced eels (average weight: 600 g) into Lake Arreo over time on the basis feeding rates (e.g. fish biomass consumed per unit time; data retrieved from Aquiloni et al. 2010) and offtake-rates of alien fish (i.e. predation impact of eels on aliens; data retrieved from Aquiloni et al. 2010). This information was inferred to the i) abundance and ii) biomass of *L. gibbosus* and *P. clarkii* in Lake Arreo from 2015 to determine how long it would take for reintroduced eels at the previously identified feeding rates and thus per capita effect to remove alien fish (assumption: 0 mortality; 0 recruitment). For this purpose, the mean percentage of consumed prey per day in relation to the bodyweight of European eels (0.5% day$^{-1}$ (large individuals, 59–65 cm), 5.5% day$^{-1}$ (small individuals, 30–36 cm), 1.3% day$^{-1}$ (combined size classes)) as well as the consumption rate of approximately one crayfish every four days as identified by Aquiloni et al. (2010) were used. Data from 2015 was chosen, because population size of *P. clarkii* was not available for 2017 and moreover presents in contrast to 2014 data during ongoing electrical removal.

## Results

### *Lake Arreo community structure*

IAS in Lake Arreo occupied all trophic levels, with *M. salmoides* being the top predator, *L. gibbosus* predator, *C. carpio* occupying an intermediate position between primary consumer and predator and *P. clarkii* being the single primary consumer.

The dietary data from Haubrock et al. (2018) showed a relatively low diet overlap (< 0.4) and diet-based niche overlap between species (0.18 overall, 0.21 between *L. gibbosus* and *M. salmoides*) and was subsequently linked to a rather enclosed trophic web and low competition within the North American species community in Lake Arreo. Additionally, common carp *C. carpio* was mostly excluded from these species' interactions. While *M. salmoides* was shown to have a rather specialized diet, targeting *L. gibbosus* and *P. clarkii*, *L. gibbosus* showed a wider diet feeding mainly on crayfish but also including a variety of insects (Figure 1; insects not included).

Based on the assumption of an enrichment of $\delta^{15}N$ by 3.4 ‰ for each trophic level (Post 2002), Layman's metrics of the entire species community showed a wide range of nitrogen (NR = 8. 62), indicating that the community spans over approximately two to three trophic levels. *Procambarus clarkii* showed the widest isotope ranges for NR and carbon range (CR = 4.81 and 8.31, respectively). *Procambarus clarkii* also expressed the highest convex hull (TA = 16.29; the smallest polygon containing all data points) and SEAc (corrected standard ellipse area) with 10.17, indicating a high trophic plasticity. Its CR values revealed that this is especially due to a plasticity in the carbon source use, making this species a potential omnivore rather than

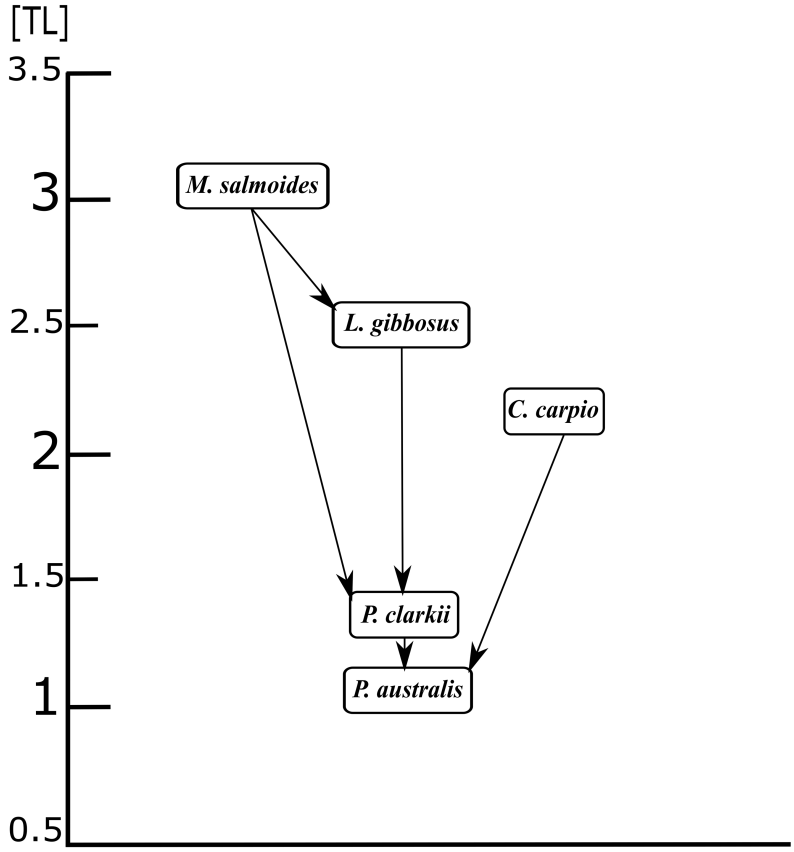


**Figure 1.** Trophic position and feeding interactions displaying the trophic web of alien species in Lake Arreo. The y-axis indicates the trophic level of species.

**Table 1.** Estimated Layman's metrics and Stable Isotope results for $\delta^{15}N$ and $\delta^{13}C$ of alien species in Lake Arreo. NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance, SEAc = Standard Ellipse Area.

| Group | Layman metrics and Stable Isotope Analysis results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean $\delta^{15}N$ | Mean $\delta^{13}C$ | NR | CR | TA | CD | MNND | SDNND | SEAc |
| *Cyprinus carpio* | 12.2 | −33.9 | 2.8 | 3.8 | 5.4 | 1.0 | 0.6 | 0.5 | 2.5 |
| *Lepomis gibbosus* | 13.5 | −32.2 | 3.8 | 3.4 | 4.9 | 1.2 | 0.5 | 0.4 | 2.2 |
| *Micropterus salmoides* | 15.2 | −30.8 | 2.2 | 2.3 | 2.6 | 0.9 | 0.3 | 0.2 | 2.3 |
| *Procambarus clarkii* | 9.4 | −28.2 | 4.8 | 9.6 | 16.3 | 3.2 | 0.8 | 0.7 | 10.2 |
| *Phragmites australis* | 8.4 | −28.6 | 1.0 | 1.4 | 0.1 | 0.6 | 0.3 | 0.2 | 0.1 |
| Community | na | na | 8.6 | 12.3 | 53.4 | 3.0 | 0.4 | 0.4 | 17.1 |

primary consumer and herbivore. *Cyprinus carpio* and *L. gibbosus* had similar Layman's metrics values, with the only difference being in NR, which was considerably higher for *L. gibbosus* (Table 1). Among fish species, *M. salmoides* expressed the lowest values for each metric. Moreover, SIMMs (Figure 2) of *M. salmoides* showed it feeds mainly on *L. gibbosus*, less on crayfish (Figure 2), this high feeding specialization being confirmed by the low values of each Layman metrics.

The applied PERMANOVA (Table 2) and post-hoc test (Table S2) as well as CAP (Figure 3) analysis of isotope data highlighted that all species are clearly distinct and separated from each other for carbon and nitrogen levels ($F_{3,52}$ = 41.78, p = 0.001), confirming the results of trophic position estimations.

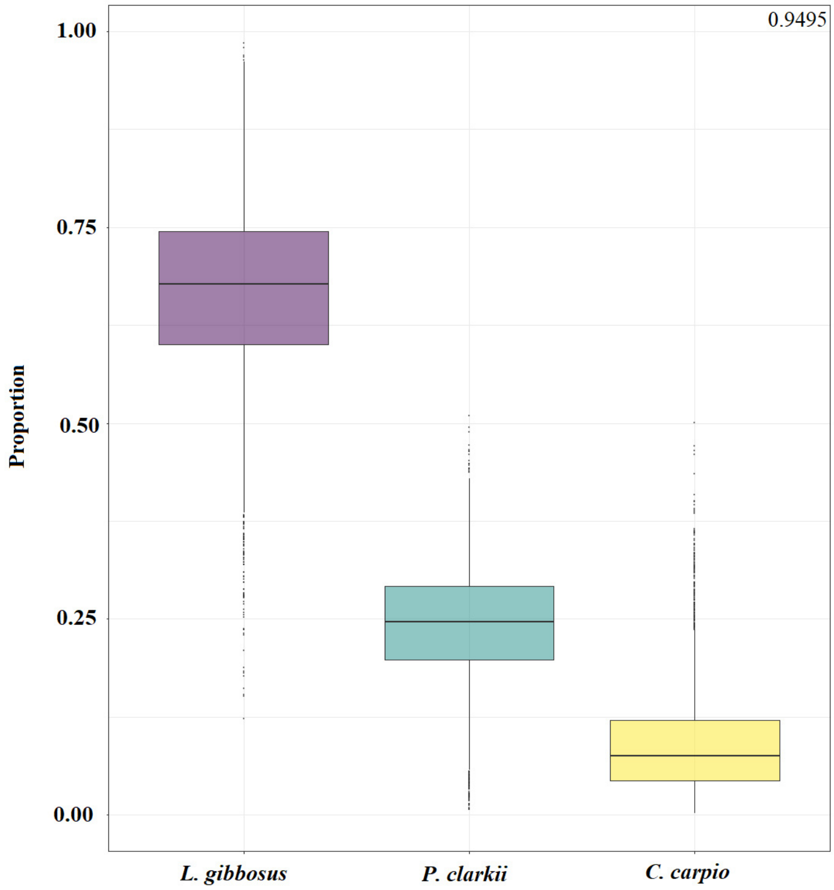
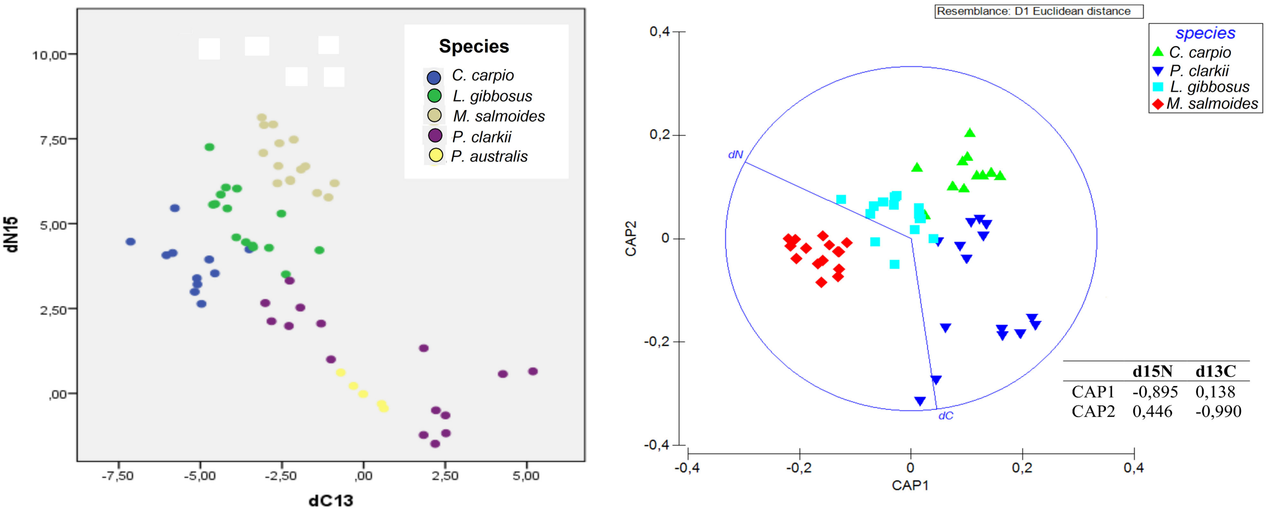


**Figure 2.** Results of Stable Isotope Mixing Models for *Micropterus salmoides*: the statistical probability of the applied model is reported in the top right corner (y-axis; mean plus standard deviation of prey items in x-axis) contribution to trophic level.

**Table 2.** PERMANOVA main test result for factor "species"

| Source | df | SS | MS | Pseudo-F | P(perm) | perms | P(MC) |
|---|---|---|---|---|---|---|---|
| Species | 3 | 502.55 | 167.52 | 41.777 | 0.001 | 999 | 0.001 |
| Residuals | 52 | 208.51 | 4.0098 | | | | |
| Total | 55 | 711.06 | | | | | |



| | d15N | d13C |
|---|---|---|
| CAP1 | -0,895 | 0,138 |
| CAP2 | 0,446 | -0,990 |

**Figure 3.** Stable Isotope distribution of sampled species (left) and visualization of CAP analysis (right) with the Spearman correlation of each isotope and the respective CAP axis (bottom right).

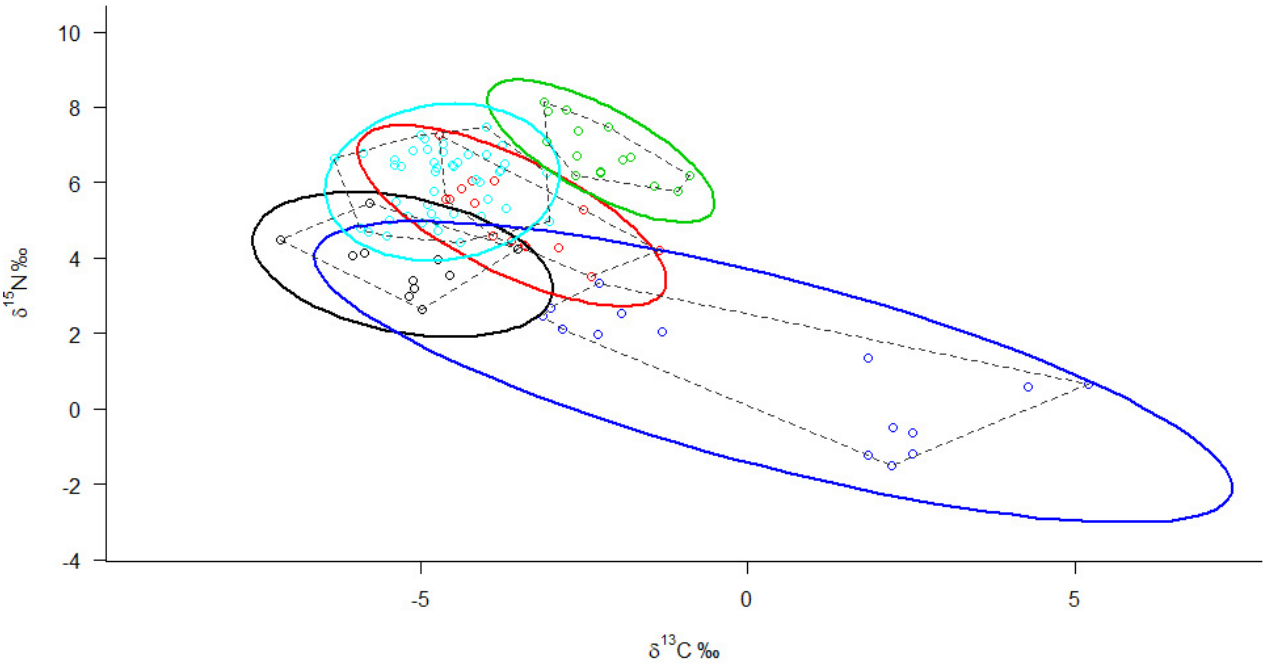


**Figure 4.** Distribution of $\delta^{15}N$ and $\delta^{13}C$ and standard ellipse area (95%) for Lake Arreo IAS *Micropterus salmoides* (green), *Lepomis gibbosus* (light blue), *Cyprinus carpio* (black), *Procambarus clarkii* (dark blue) and the potentially reintroduced biocontrol agent *Anguilla anguilla* (red).

### *Predicting the effect of introducing the European eel* Anguilla anguilla

*Anguilla anguilla* relies on macrozoobenthos and fish (Moriarty 1972, 1973; De Nie 1987) while being able to strongly influence the abundance of age 0 fish through intense predation (Radke and Eckmann 1996; Dörner and Benndorf 2003). In a study by Lammens et al. (1985), the low abundance of chironomid larvae resulted in a diet shift of eels over 40 cm towards smelts (*Osmerus eperlanus* Linnaeus, 1758). In the Mediterranean Ingril lagoon, France, fish constituted to only 1.01–3%, while *Gammarus* gr. *locusta* (24–48%) and Chironomidae (12.01–24%) overweighed. Nonetheless, in February fish (44%) and decapods (44%) dominated, implying an overall mostly benthic predation. However, Costa et al. (1992) determined that fish are always secondary but significant food item, fluctuating due to availability and habitat.

Having a trophic level of 2.8, *A. anguilla* was positioned between *L. gibbosus* and *M. salmoides* but showed a high overlap in the distribution of $\delta^{15}N$ and $\delta^{13}C$ with *L. gibbosus* (Figure 4).

The review of dietary data and feeding habits showed a mixed diet between macrozoobenthic species in all size class of *A. anguilla* and also fish with an increase of predator size (according to abundance of potential prey items) (Figure 4). Layman's metrics for European eels showed a broad isotopic range similar to those of other fishes. TA (7.5) was considerably greater than other species present in the lake, although SEAc value, which is generally less affected by extreme samples and thus outliers, was comparable with other species. The standard deviation of the nearest neighbour distance

**Table 3.** Overlap of the corrected Standard Ellipse Area (SEAc = 40%) and standard ellipse area (SEAb = 95%) for all IAS present in Lake Arreo and the potentially reintroduced biocontrol agent *Anguilla anguilla*.

| | *Cyprinus carpio* (SEAc / SEAb) | *Lepomis gibbosus* (SEAc / SEAb) | *Micropterus salmoides* (SEAc / SEAb) | *Procambarus clarkii* (SEAc / SEAb) | *Anguilla anguilla* (SEAc / SEAb) |
|---|---|---|---|---|---|
| *C. carpio* | x | 1.301043e-18<br>0.583 | 0<br>0.659 | 0<br>0.223 | 1.431147e-17<br>0.644 |
| *L. gibbosus* | | x | 8.673617e-18<br>0.645 | 0<br>0.195 | 0.4067617<br>0.752 |
| *M. salmoides* | | | x | 5.155355e-17<br>0.110 | 0<br>0.404 |
| *P. clarkii* | | | | x | 0<br>0.868 |
| *A. anguilla* | | | | | x |

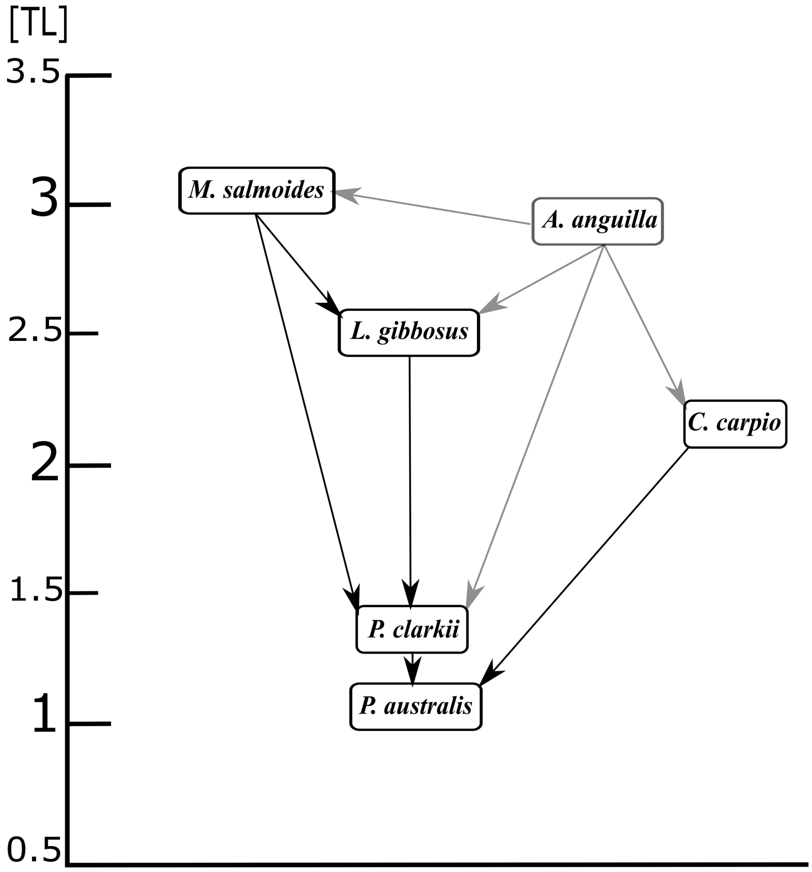


**Figure 5.** Likely feeding habits of *Anguilla anguilla* in Lake Arreo. Grey lines and values indicate predation according to reviewed literature. The y-axis indicates the trophic level of species.

(SDNND) showed little inter-individual variability in this species. Metrics for the community including *A. anguilla* showed very little variation, with a slight increase in TA.

The estimated standard ellipse area revealed no considerable overlap between species except for *L. gibbosus* and *A. anguilla* (0.4). However, under consideration of 95% of data points, overlap among species increased, indicating high values all IAS in Lake Arreo and potentially with *A. anguilla* (Table 3).

Applying SIMMs on combined IAS in Lake Arreo for *M. salmoides* and *A. anguilla* from Germany indicated that *M. salmoides* might mainly feeds on *A. anguilla* and then secondarily on other species (Figures 5, 6). Additionally,

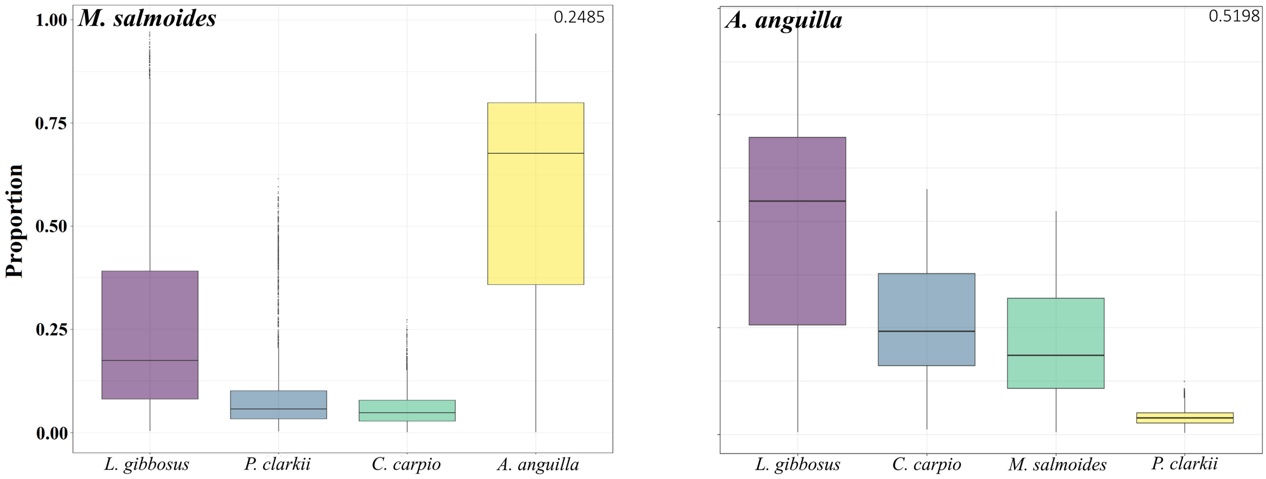


**Figure 6.** SIMMs for *Micropterus salmoides* (a) and *Anguilla anguilla* (b) by combining data from Lake Arreo and Lake Großer Vätersee (Dörner et al. 2009). The number on the top right corner of each diagram indicates the statistical probability of each result presenting the proportion (y-axis; mean plus standard deviation of prey items in x-axis).

**Table 4.** Population estimated for *L. gibbosus* and *P. clarkii* from Lake Arreo from 2015 (Haubrock et al. 2018).

| Species | Mean weight / individual [g] | Number individuals | Total Biomass [g] |
|---|---|---|---|
| *Lepomis gibbosus* | 22.2 | 52062 | 1155776 |
| *Procambarus clarkii* | 52.8 | 70500 | 3722400 |

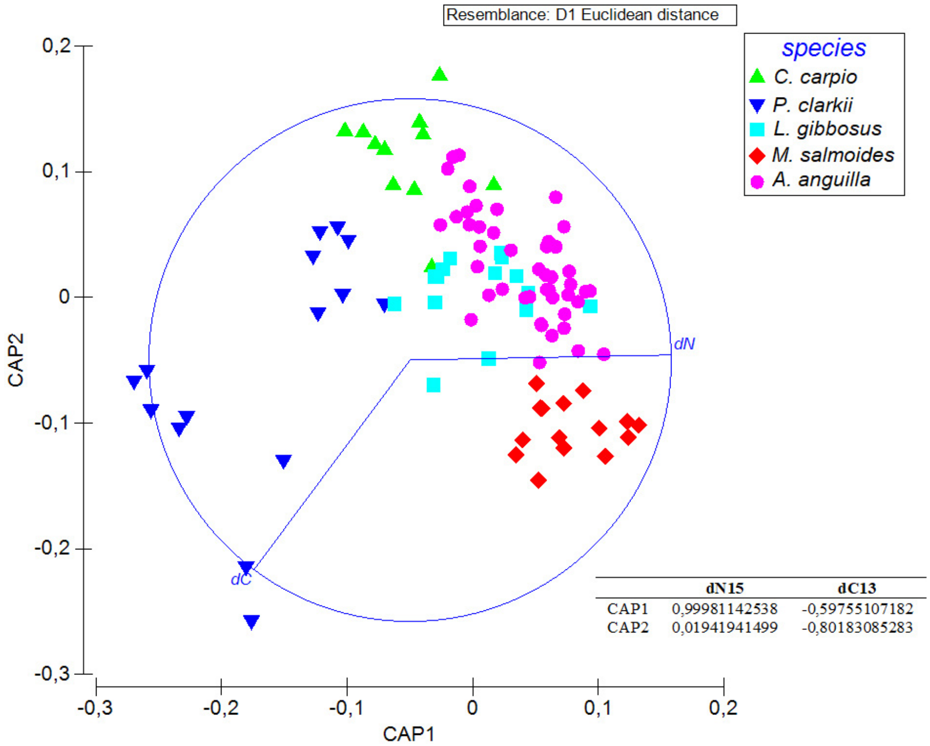
**Table 5.** Calculated days needed for three different estimated consumption rates (Aquiloni et al. 2010).

| Species | Consumption rate | | | |
|---|---|---|---|---|
| | $0.5\%\text{day}^{-1}$ Adults | $5.5\%\text{day}^{-1}$ Juveniles | $1.3\%\text{day}^{-1}$ combined | 1 prey items / 4 $\text{days}^{-1}$ |
| *Lepomis gibbosus* | 1240.8 | 112.8 | 477.2 | 282 |
| *Procambarus clarkii* | 385.3 | 35.0 | 148.2 | 208.2 |

results indicated an exclusively piscivorous diet of *A. anguilla* although crayfish (spinycheek crayfish *Orconectes limosus* Rafinesque, 1817) is present in the German lake, thus indicating a very low probability of predation on crayfish (Figure 6).

Using data from Haubrock et al. (2018), estimated population sizes for *L. gibbosus* and *P. clarkii* from 2015 (Table 4) and the estimated mean weight of each species were used to calculate the amount of days needed to eradicate the populations of both respective species without consideration of recruitment and 0 mortality (Table 5). These surrogates for the per-capita effect resulted in 35 to 385 days for *L. gibbosus* and 113 to 1241 days for *P. clarkii* until either species is reduced to 0. However, using one crayfish every four days resulted in 200–280 days to for both species.

PERMANOVA as well as post-hoc tests (Table S3) and CAP analysis (Figure 7) on the combined and individually standardized datasets confirmed that all species remain distinct and separated for both carbon and nitrogen levels (Pair wise test: p = 0.001). Cross validation showed that *A. anguilla* overlaps with all fish species, which agrees with the PERMANOVA main test and left-one-out allocation explained 66.67% of correct reallocation.



| | dN15 | dC13 |
|---|---|---|
| CAP1 | 0,99981142538 | -0,59755107182 |
| CAP2 | 0,01941941499 | -0,80183085283 |

**Figure 7.** CAP analysis for IAS of Lake Arreo and *Anguilla anguilla*. Table in lower-left corner indicates the correlation of Stable Isotopes with CAP1 and CAP2 axis.

## Discussion

Studying communities of IAS is challenging due to the lack of information on species interactions and potential adaptations to the invaded ecosystem. However, these interactions can lower the impact on native species or facilitate it. To our knowledge, this is the first attempt of using Stable Isotopes to predict potential effects of a predator reintroduction as a biocontrol agent.

Our study in Lake Arreo revealed that IAS were highly interconnected through feeding activities, but their effect on native species could not be directly assessed due to their lack in the sampling. However, the native species abundance has been observed to steadily decrease in the last years, which has been hypothesized to be linked to the presence of IAS (Jose Augusto Monteoliva, *pers. comm.*). Moreover, the novelty of the applied approach showed a potentially high predation on *L. gibbosus* by *A. anguilla*, but also on *A. anguilla* by *M. salmoides*, highlighting how already present predators might respond to the presence of this reintroduced species.

### *Lake Arreo community structure*

Isotope values of sampled fish for $\delta^{15}N$ in Lake Arreo were high, likely due to the already high value of the primary producer *P. australis*, a species that is known to extract nitrates from the surrounding ecosystem and accumulating it in its tissue (Wigand et al. 2007). The food chain is short, including two-three trophic levels. This simple community shows no niche overlap among species, that are distinct, especially for their N isotope values. Moreover, species are highly linked, with both *M. salmoides* and *L. gibbosus* relying on the abundance of *P. clarkii* as food source (aside from *P. australis* as the primary producer) in terms of dietary and SIMMs results. *Cyprinus carpio* stomach content was composed only of detritus, confirming the benthic feeding habit of this species. Nonetheless, its N isotopic signature placed carp in an intermediate position between primary consumers and predator, which could be explained by either high content of consumed detritus due to the eutrophic state of Lake Arreo or a considerable proportion of crayfish in the diet of carp, as *C. carpio* is known to actively prey on highly abundant macrozoobenthos (Britton et al. 2007; Anton-Pardo et al. 2014), i.e. small crayfish in our study site. Hence, SIMMs results supported the observation of Anton-Pardo et al. (2014) and furthermore confirm the highly interconnected community of North American species that simultaneously show low niche overlap, as previously observed by Haubrock et al. (2018) based on pure dietary analyses.

### *Predicting the effect of reintroducing* Anguilla anguilla

The increasing number of isotope studies on a wide variety of species in an even wider range of ecosystems and community compositions allow the prediction of IAS impacts (France 1995; Vander Zanden et al. 1999a, b) as well as the effectiveness of a species reintroduction as a biological control agent (McNabb et al. 2001; Kraiss and Kullen 2008). Although the potential outputs would be inevitably imprecise due to the various differences (i.e. origin of isotope values, different primary producers and soils, species number, abundance and composition of species and geographical and climatic variation), they could provide a valuable tool to forecast how species interactions could affect further introductions (Britton et al. 2010). Indeed, the use of stable isotope data from different areas has a relevant importance and manifold applications, as it was recently pointed out by Pauli et al. (2017), who called for a centralized database for such data in order to explore further applications. Moreover, potential results can refer to a system in equilibrium, given that used data originate from presumably stable communities. However, this is not always true, because at the beginning of an introduction relationships among species will vary depending on various factors (population growth, interactions, presence of other species, behavioural plasticity) from those predicted. This way of approaching IAS introductions could approximate the outcome in terms of ecological niches when using isotope data from the most comparable system available and dietary and behavioural studies. Such an approach could be also integrated in invasive species risk assessment models.

Benndorf (1995) reports that *A. anguilla* were successfully used with other species of piscivorous predators such as the pike *Esox lucius*

Linnaeus, 1758 to decrease the abundance of smaller planktivorous species, emphasising the fact that predators introduced as biomanipulator would need to cover all age classes to show a significant effect on the target community. Nonetheless, such studies (including Dörner and Benndorf 2003) did not sufficiently assess the effectiveness of these introduced species and predation on non-target species. Isotope data on *A. anguilla* are rare, due to the protected status of this species, and concern different ecosystems. Dörner et al. (2009) investigated the trophic position and diet of *A. anguilla* from two lakes in Germany. Due to the obvious climatic differences between Lake Arreo and the Große Vätersee, extracted information have to be supplemented with behavioural data from climatic more comparable ecosystems. Although dietary information on *A. anguilla* is sparce, especially for northern Spain, the available information agrees that, although fish appear to be of secondary importance, piscivorous feeding activity can dominate seasonally due to prey abundances (Lammens et al. 1985; Costa et al. 1992). However, predation of potentially present native macroinvertebrates cannot be excluded from significant side effects. Therefore, results presented by the applied mixing models, predicting an intense feeding of *A. anguilla* on *L. gibbosus* (a small sized Perciformes), are consistent with the observed predation on perch in the Großer Vätersee (Dörner et al. 2009), where, although less frequently consumed than chironomid larvae, fish were major contributors to the biomass of analysed diets. Moreover, crayfish were also predated in a considerable amount, which was not the case for estimated SIMMs in Lake Arreo, but is in accordance with the 2017 observed higher abundance of fish than crayfish (Haubrock et al. 2018). Although the high contribution of Perciformes to the trophic position of eels reintroduced into Lake Arreo might be based on i) the long-time mediated information provided by SIA as well as ii) the diet of specimens from which data was used, the remarkably low proportion of *P. clarkii* indicates high piscivorous feeding activity and fits to the in 2017 (year samples were taken from Lake Arreo) observed higher abundance of *L. gibbosus* (frequency and biomass; Haubrock et al. 2018). In addition to this potential predation on *L. gibbosus*, the estimated SEAc indicated an overlap between *A. anguilla* and *L. gibbosus*, likely indicated a similar use of resources and prey (e.g. insects and small sized *P. clarkii*). However, the SEAb overlap underlined that introduced *A. anguilla* would affect all IAS in Lake Arreo. Additionally, the SEAc values estimated for *A. anguilla* showed a lower value in this reintroduction scenario, indicating that the total community niche space would be more compact. This would relate to less niche space for present species, after the reintroduction of *A. anguilla*, likely due to the occupied niche falling in the already defined community cloud (trophic redundancy, Layman et al. 2007).

Concerning the diet of eels, the difference in carbon signatures should be considered. Dörner et al. (2009) stated that the difference in benthic reliance, i.e. carbon deriving from the benthic pathway, was about 45%. This result would indeed indicate that eels serve as a link between benthic and pelagic food webs when availability of insects is low, meaning that, when the abundance of macrozoobenthos (including the in Lake Arreo abundant *P. clarkii*) is low, eels may shift to a more piscivorous diet (i.e. piscivory is controlled by availability of insects), as in the case of Lake Arreo.

Using the determined feeding-rates by Aquiloni et al. (2010), there is a high variability in the number of days required by small or large *A. anguilla* to eliminate the alien species populations has been revealed. Moreover, the consumed biomass per day is providing only few information, partially due to the high variability, but also due to temperature depending feeding activity (Kuhlmann 1975; Seymour 1989) and seasonal variability of prey abundance and thus, diet shifts in *A. anguilla* (Lammens et al. 1985). Therefore, using the estimated feeding activity of one consumed crayfish every four days is probably more reliable, considering the estimated 35 days at a consumption rate of 5.5% day$^{-1}$. However, without available information on mean daily population growth for both alien species and considering the decreased activity of *P. clarkii* in the presence of *A. anguilla* as well as the increased predation during moult of crayfish, the results of the present study can only be seen as a rough indication. Moreover, considering ongoing electrical removal, these numbers indicate a potentially high contribution of reintroduced eels to the aimed eradication of alien species.

The American eel (*Anguilla rostrate* Le Sueur, 1817) is known to predate on eggs in nests of *M. salmoides* and *L. gibbosus*. Therefore, *A. anguilla* could be a likely competitor for *M. salmoides* (depending on niche overlap; Schiphouwer et al. 2017). On the other hand, a possible predation of IAS and aquatic birds on *A. anguilla* has to be considered possible. The applied SIMMs estimated a potential high predation of *M. salmoides* on eels. Indeed, *Micropterus* sp. are known to occupy wide and variable trophic niches (Costantini et al. 2018) as well as to eat small sized *A. rostrata*, when available (McCord 2005).

Moreover, Lake Arreo provides a productive fishery with high abundances of prey (small sized *L. gibbosus*, *M. salmoides* and *P. clarkii*) and a suitable habitat in the shallow hygrophyte areas for eels. The Lake is also considered as highly saline, a factor that likely contributes to the success of *A. anguilla* as a predator. Nonetheless, problems might arise on the long term if the mature specimens cannot migrate for reproduction: In this case, individuals showed a decrease in weight and fat content but stayed alive for up to 10 years (Westin 2003). However, Lake Arreo's inlet provides the opportunity to migrate during the late fall and winter period (Alberto Criado, *pers. comm.*), but a return of individuals is not certain.

## Conclusion

Reintroducing eels to decrease the abundance of IAS as a complementary technique, to control methods like electrofishing under an integrated pest management approach is an interesting and potentially promising approach (Aquiloni et al. 2010). This approach can provide good indications of potential effects of eels on the IAS of Lake Arreo. It can furthermore be assumed that reintroduced eels would exert a high predation effect on available prey in Lake Arreo, which would be increased by manual removal of *L. gibbosus*. The reintroduction of *A. anguilla* could affect at least the population of *P. clarkii* or *L. gibbosus*, but a more accurate prediction is currently impossible, as outcomes highly depend on the abundance of potential prey, feeding behaviour of eels and lastly the arising interactions after the reintroduction of *A. anguilla*. This approach needs to be validated using data collected during sampling pre- and post to a species reintroduction. Another possibility to assess the potential impact of a predator on a species community, native or alien, would be analysis of comparative functional responses (Dick et al. 2013). Such studies are usually conducted in model systems by investigating the feeding response on differing prey densities. Furthermore, SIA have been considered to be an interesting and potentially valuable addition for these studies (Dick et al. 2013, 2014). For Lake Arreo and the assessment of reintroducing a biocontrol agent, conducting a functional response study, even if only on a theoretical model, could be worthwhile, as the effect of reintroducing a biomanipulator would differ according to its propagule pressure as well as the resident species densities.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Identified dietary components for *L. gibbosus* (N = 38), *M. salmoides* (N = 38) and *C. carpio* (N = 11). Results are displayed as frequency of occurrences [F%] in percentages. Data taken from Haubrock et al. 2018.

**Table S2.** Post hoc comparison (pair-wise tests) after PERMANOVA.

**Table S3.** PERMANOVA main test result for factor SPECIES using all species including data of *Anguilla anguilla* from the Großer Vätersee.

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