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PROOF

The Diet Composition and Trophic Position of Introduced Prussian Carp *Carassius gibelio* (Bloch, 1782) and Native Fish Species in a Turkish River

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Abstract

Carbon and nitrogen stable isotope analyses were carried out to determine the diet composition and trophic position of the invasive species *Carassius gibelio* to help understand the potential impact of this species on the native fish fauna in the Karamenderes River, northwest Turkey. Filamentous algae were the most important diet component according to stable isotope mixing models. Filamentous algae and detritus formed also considerable part of the gut contents. The trophic position of *C. gibelio* was 2.43±0.5, 2.04±0.4 and 3.35±0.5 estimated by three different methods. The trophic niche width of this invasive species was larger than those of the native species. Our results indicate that the high dietary plasticity of *C. gibelio* and its lower trophic position than the other fish community members, can contribute to its success as an invasive species.

Keywords: Feeding ecology, stable isotopes, fish ecology, invasive.

Türkiye'nin Bir Akarsuyunda Yabancı *Carassius gibelio* (Bloch, 1782) ve Yerli Balık Türlerinin Besin Kompozisyonları ve Trofik Pozisyonları

Özet

Kuzey Batı Türkiye'de bulunan Karamenderes Nehrinde yaşayan yerli balık türleri üzerine potansiyel etkilerini anlamaya yardımcı olmak üzere istilacı *Carassius gibelio* türünün besin kompozisyonu ve trofik pozisyonu karbon ve azot kararlı izotop analizleri ile belirlenmiştir. Kararlı izotop karışım modeline göre ipliksi algler en önemli besin grubunu oluşturmuştur. İpliksi algler ve detritus sindirim kanalı içeriğinde de önemli bir oranda bulunmuştur. *C. gibelio* türünün trofik pozisyonu üç farklı medoda göre 2,43±0,5, 2,04±0,4 ve 3,35±0,5 olarak tahmin edilmiştir. *C. gibelio* türünün beslenmede yüksek derecede esneklik göstermesi ve diğer balık türlerine göre daha düşük bir trofik düzeye sahip olması istilacı bir tür olarak başarılı olmasına katkı sağlayabilir.

Anahtar Kelimeler: Beslenme ekolojisi, kararlı izotoplar, balık ekolojisi, istilacı.

Introduction

Aquatic ecosystems all over the world are enhanced for fishing by introductions of non-native species. However, invasive species are known to cause a range of adverse environmental and economic effects (Vitousek *et al.*, 1996; Pimentel *et al.*, 2000). Prussian carp, *Carasius gibelio* (Bloch 1782) is one of the most common invasive species in European and also Turkish waters (Özuluğ *et al.*, 2004). It was first introduced to Turkish waters in the early 1990s, and after a rapid population increase now dominates many aquatic ecosystems, both rivers and lakes (Balık *et al.*, 2004), but has a low commercial value.

The rapid expansion and high abundance of Prussian carp in Turkey and also in some parts of Europe makes it problematic (Özcan, 2007; Gaygusuz *et al.*, 2007; Lusková *et al.*, 2010; Liasko *et al.*, 2011; Tarkan *et al.*, 2012). Recent studies have focused on the possible negative impact of this species to distribution (Özcan 2007), abundance (Gaygusuz *et al.*, 2007; Perdikaris *et al.*, 2012), reproduction (Aydın *et al.*, 2011) and some growth parameters (Leonardos *et al.*, 2008; Tarkan *et al.*, 2012). The possible negative effects of Prussian carp on water quality and fish fauna have also been discussed (Crivelli, 1995; Leonardos *et al.*, 2001; Povž and Šumer, 2005; Emiroğlu *et al.*, 2012; Kırankaya and Ekmekçi 2013). However, direct and indirect effects of this species on community structure by competition with native species are still poorly understood (Specziár and Rezsu, 2009). Prussian carp is

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omnivorous and feeds on detritus, zooplankton, zoobenthos and macrophytes (Penaz and Kokes, 1981; Specziár *et al.*,1997). Balık *et al.* (2004) indicated that *C. gibelio* in Eğirdir Lake, Turkey fed mainly on benthic and planktonic invertebrates. Better knowledge of the trophic position and trophic niche of this introduced species when living in sympatry with native species will help in understanding its establishment success.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are widely used to evaluate the food resources (DeNiro and Epstein, 1978; Fry and Sherr, 1984) and trophic positions (França *et al.*, 2011) of consumers. Because consumers often display $\delta^{15}N$ values 3-4 ‰ higher than their food (Minagawa and Wada, 1984) trophic relationships can be studied by stable isotope analysis. Benthic algae are enriched in $\delta^{13}C$ relative to phytoplankton (Hecky and Hesslein, 1995), so stable carbon isotopes ($\delta^{13}C$) can be used for identifying the source of production for consumers in lakes (France, 1995). Hence the isotopic compositions of fish species can give information about their trophic positions and the relative contributions of different potential food sources to the diets of fish species (Peterson and Fry, 1987; Vander Zanden *et al.*, 1997; Vander Zanden and Rasmussen, 1999; Post, 2002). We therefore used stable isotope analysis to study the trophic position and and diet compositon of exotic Prussian carp and native species in the Karamenderes River in northwest Turkey.

Materials and Methods

Study Area and Sampling

The Karamenderes River is located on the Biga Peninsula in northwest Turkey (Figure 1). It originates from the Ağı and Kaz Mountains and flows first from NE to SW, and after passing the Bayramiç basin turns to the north to discharge finally into the Çanakkale strait near the ancient city of Troy. The river is about 110 km in length and has minimum and maximum flows of 60-70 m^3 and 1530 m^3, respectively (Baba *et al.*, 2007; Sarı *et al.*, 2006). There are two reservoirs along the river at Bayramiç and Pınarbaşı. Material for the study was collected below the second reservoir at Pınarbaşı, where the river channel is around 3-60 m wide and 20-200 cm deep, and the substratum is mainly small stones, sand and silt materials. The substratum is covered by filamentous algal mats in summer.

A total of 29 fish individuals belonging to three native species, *Barbus oligolepis* (Battalgil, 1941), *Squalius cii* (Richardson, 1857) and *Alburnus chalcoides (*Güldenstädt, 1772) , and the invasive *Carassius gibelio* were caught with a cast net from the Karamenderes River in June 2011. A five kg net (1.5x1.5cm mesh size) was thrown 30 times in a 200 m reach of the river during one and a half hours of fishing. The mean number of individuals per sample (Catch Per Unit Effort, CPUE) was calculated.

Fork length and weight of individuals were recorded, and dorsal muscle tissue samples and gut contents of individuals were obtained for stable isotope analysis (SIA). The gut contents of the specimens were removed carefully without taking the mucus and/or any part of the digestive tract. From the same area, macrophytes and filamentous algae with molluscs and insects from both bottom and surface, as well as detritus, were collected with a grab and a scoop, and seston was collected with nets for SIA. Macrophytes and filamentous algae samples were washed with tap water and molluscs and insects and/or larvae were removed and stored separately for SIA.

Stable Isotope Analyses

Samples of muscle, gut contents, detritus, macrophytes, algae and macroinvertebrates were

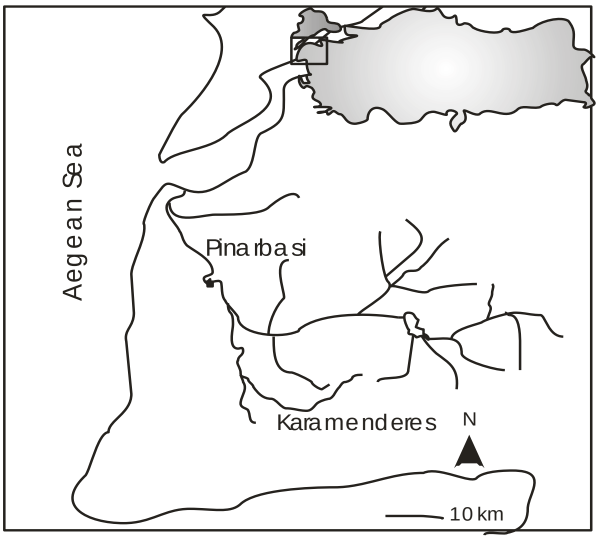

Figure 1. The study area showing the Karamenderes River and the location of the sampling station at Pınarbaşı.

dried at 60°C for 24 hours and and homogenized with a microdismembrator-U (2 min at 1500 rpm) or a mortar and pestle into a fine powder in preparation for isotopic analysis. SIA of samples from fish muscle and from the potential food sources were conducted using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific Corporation, Waltham, MA, U.S.A.) coupled to a Thermo Finnigan DELTA^{plus} Advantage mass spectrometer at the University of Jyväskylä, Finland. From each sample, 0.500–0.600 mg of homogenized powder was weighed into tin capsules prior to analysis. Stable carbon and nitrogen isotope ratios are expressed as delta values (δ^{13}C and δ^{15}N, respectively) relative to the international standards for carbon (Vienna PeeDee Belemnite) and nitrogen (atmospheric nitrogen). Pike (*Esox lucius* L.) white muscle tissue or dried and homogenized potato leaves with known isotopic composition were used as internal working standards for animal tissue and plant material/detritus respectively and were inserted in each run after every five samples. Standard deviation of the internal standards was less than 0.16 ‰ for δ^{13}C and 0.12 ‰ for δ^{15}N in each run. The muscle δ^{13}C values were not corrected for lipids because the C:N ratios (average = 3.4, minimum-maximum = 3.2–3.8) indicated low lipid content (Kiljunen *et al.*, 2006; Post *et al.*, 2006).

Data Analyses and Statistics

The trophic positions of fish species were calculated using the equation:

$$TP_{fish} = [(\delta^{15}N_{fish} - \delta^{15}N_{primary\ consumer})/3.4] + 2$$

in three ways (Rybczynski *et al.*, 2008). (a) TP1: primary consumer δ^{15}N was calculated as the mean δ^{15}N of all primary consumers (mean δ^{15}N value of all macroinvertebrates) collected during sampling which is general baseline for using quantitative estimates of trophic positon (Post, 2002; Rybczynski *et al.*, 2008). In this way, we assumed that fishes assimilate equally all macroinvertebrates collected from the same habitat of fishes. (b) TP2: the total gut contents δ^{15}N was taken to represent the primary consumer δ^{15}N with the assumption that the gut contents are the mixed diet and that fish assimilate equally all food materials in the gut contents. Because these species are known omnivorous and an omnivore might well assimilate substantially equal amounts of nitrogen from each source. (c) TP3: first the proportions of every food category in the gut contents were analysed using the SIAR package(two isotopes five sources), and primary consumer δ^{15}N values were then calculated using these proportions for the different fish species. In this method we assumed that the gut contents are the mixed diet and that fish use each food category according to its proportion.

The SIAR (Stable Isotope Analysis in R) package (Parnell *et al.*, 2013) in R 2.15.3 (R Development Core Team, 2009) was used to calculate the relative contributions of different potential food sources to the diets of each of the three native and one non-native fish species. The potential food sources were grouped into macrophyte (*Potamogeton* sp.), filamentous algae, seston, detritus and macroinvertebrates. The SIAR isotope mixing model uses Bayesian inference to estimate source contributions and allows the input of uncertainties, such as variation around the mean isotope values of sources (prey) and fractionation factors, into the final model. Fractionation factors were assumed to be 0.5 ± 0.2% for δ^{13}C and 3.0 ± 0.5% for δ^{15}N (Peterson and Fry, 1987;Vander Zanden and Rasmussen, 1999; Post, 2002). The paired Student t-test was used to compare the mean of the gut and muscle δ^{13}C and δ^{15}N values for the four fish species. Estimated TPs were compared within each species using analysis of variance (ANOVA) after running normality (shapiro-wilks) and homogeneity of variance (Levene's) tests. ANOVA was also used to compare the mean TP2 of species. However, TP1 and TP3 estimates of species were compared using Kruskal Wallis test due to lack of homogeneity of these groups. All the statistical analyses for comparison of samples were made by SPSS 15.0 (SPSS Inc., 1989-2006).

Results

C. gibelio was the dominant fish species caught, with a CPUE value of 0.78 per net/hour. *A. chalcoides, S. cii* and *B. oligolepis* had respective CPUE values of 0.18, 0.16 and 0.11.

Most of the gut and muscle δ^{13}C and δ^{15}N values of the four fish species were not statistically different (Table 1), except for δ^{15}N values of gut content and muscle pairs of *S. cii* ($t_{(5)}$ = 3.9; p= 0.01) and *A. chalcoides* ($t_{(7)}$=7.6; P<0.001). For muscle and gut δ^{13}C and δ^{15}N values, only muscle nitrogen values differed between fish species ($F_{(3,25)}$=3.06; p=0.03).

The δ^{13}C and δ^{15}N biplot (Figure 2) illustrates the food web relationships for the Pınarbaşı station of the Karamenderes River. The δ^{13}C values of potential plant foods, including detritus, from the study ranged from -31.7‰ to -21.4‰ and the greatest ^{13}C depletion was found in the filamentous algae. The δ^{13}C values of invertebrates ranged from -33.9‰ to -26.0‰.

Individuals of all fish species with δ^{15}N between 11.0 and 16.6‰ were clearly feeding on invertebrates (molluscs, insect larvae, *Asellus* sp., annelids). However, Prussian carp, with lower mean δ^{15}N, was perhaps taking more plant material and also more surface molluscs and insects than other macroinvertebrates such as annelids and *Asellus* sp. *A. chalcoides* had the highest mean δ^{13}C and δ^{15}N values of the fish species.

According to the SIAR model outputs (Figure 3a), macroinvertebrates were the most important food components of the fish community, particularly for *A. chalcoides* (35.1%). Filamentous algae was also an

Table 1. Stable N and C isotope values (mean ± SD) of gut contents and muscle of four fish species

Species	$\delta^{13}C$ ‰		$\delta^{15}N$ ‰	
	Gut contents	Fish Muscle	Gut contents	Fish Muscle
Alburnus chalcoides	-27.3±1.92	-27.2±1.01	13.6±0.62	15.2±0.95
Barbus oligolepis	-28.8±1.68	-28.4±0.96	14.0±0.85	14.5±0.70
Carassius gibelio	-26.4±1.37	-27.4±1.35	13.2±0.70	13.4±1.59
Squalius cii	-26.9±2.72	-27.9±1.70	13.4±0.84	14.5±0.74

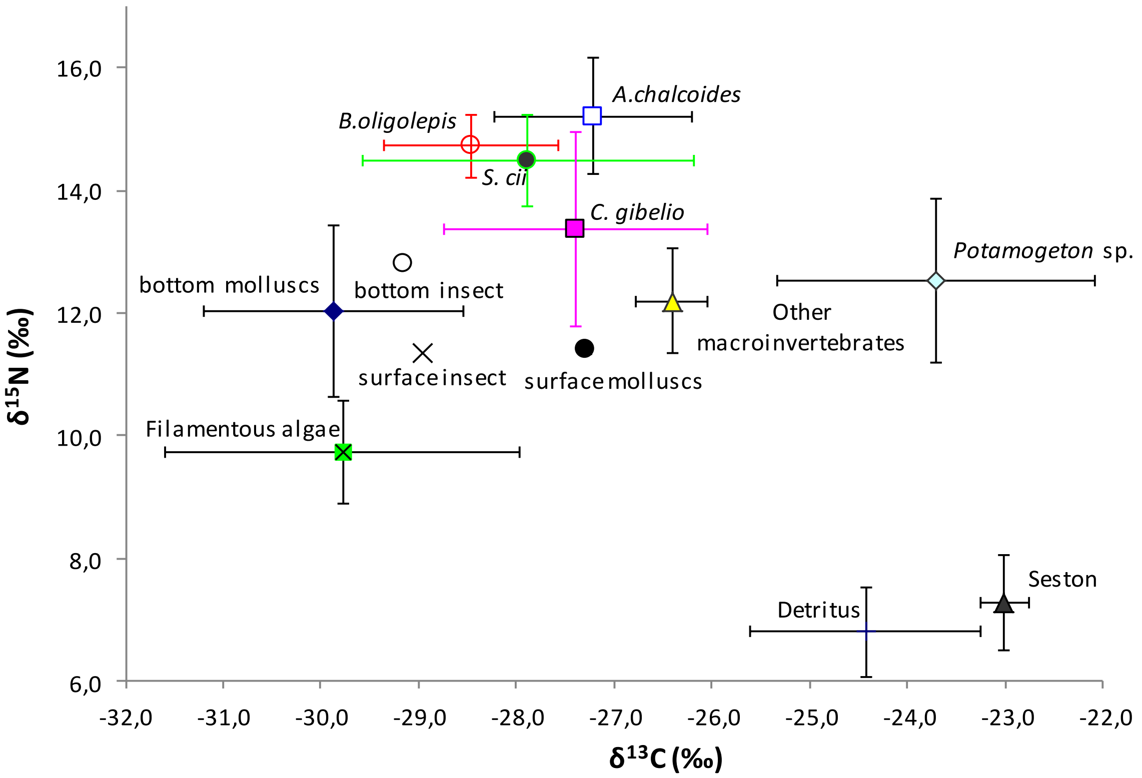

Figure 2. $\delta^{15}N$ and $\delta^{13}C$ biplot representing the food web at the Pınarbaşı station on the Karamenderes River. Mean values with standard deviations are given for four fish species and possible diet components.

important food component for all fish species, and the most important for *C. gibelio* (32.7 %). *Potamogeton* sp. had low importance as food for all the fish species. The percentages of food components in the gut contents (Figure 3b) differed somewhat from those estimated by SIA from muscle samples. In particular, macroinvertebrate percentages in gut contents were lower than those from the muscle samples in all fish species. Similarly, the percentages of filamentous algae in gut contents were slightly lower than those obtained from muscle samples in *B. oligolepis*, *S. cii* and *C. gibelio*. Conversely, the percentages of detritus were higher in gut contents.

The trophic position of fish species was calculated according to three different assumptions (Table 2). When the total macroinvertebrate mean $\delta^{15}N$ value was used as a prey value, the estimated trophic position of *B. oligolepis* was higher than that of *S. cii*. However, when using the total gut contents $\delta^{15}N$ as a prey value or the percentages of each food category calculated from gut content analyses, *S. cii* had a higher estimated trophic position than *B. oligolepis*.

The mean $\delta^{13}C$ ($F_{(5,29)}$ = 2.42; P>0.05) and $\delta^{15}N$ ($F_{(5,29)}$ = 1.33; P>0.05) values of each fish species were not statistically different. However, trophic positions of species varied significantly among species ($F_{(3,25)}$ = 3.71; P<0.05) when trophic position was calculated using gut contents (TP2) (Table 2). The trophic position of *A. chalcoides* was significantly higher than that of *C. gibelio* (P<0.05). TP1 and TP3 of the fish species were compared using Kruskall Wallis test and they were not significantly different (P>0.05). The estimated trophic positions of species were highest when calculated by the third method (Table 2).

Discussion

According to our stable isotope and gut contents results, all four fish species can be considered

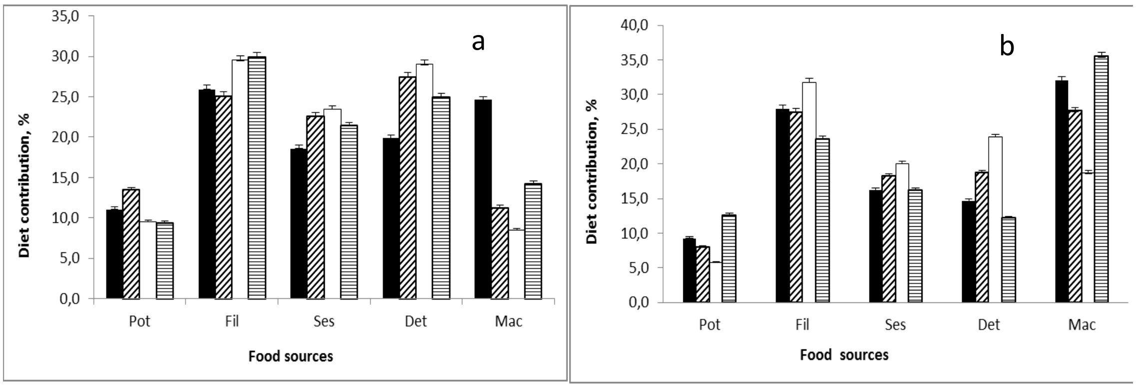

Figure 3. The percentages of diet compenents four four fish species based on SIA of (a) muscle samples and (b) gut contents. Bold bars: *B. oligolepis;* inclined striped bars: *S. cii*; white bars: *C. gibelio;* and horizontal striped bars: *A. chalcoides*. Pot=*Potamogeton* sp., Fil=filamentous algae, Ses=seston, Det=detritus Mac=macroinvertebrates.

Table 2. Trophic positions (TP) of four fish species estimated by using mean total macroinvertabrate $\delta^{15}N$ (TP1), total gut content $\delta^{15}N$ values (TP2), or percentages of each food category taken into consideration (TP3) with the number of specimens (N) examined of both females (F) and males (M) and the fork length (FL) in cm, with standard deviations (SD), range (min-max) and significance level (Sig.)

Species	N (F/M)	mean FL ± SD	(min-max)	TP1 ± SD	TP2 ± SD	TP3± SD	Sig.
Alburnus chalcoides	8/0	13.6 ±1.6	10.6-16.0	2.97 ± 0.3	2.49±0.2	3.82±0.3	F=59.03, P<0.001
Barbus oligolepis	4/1	23.2 ±8.7	13.5-32.6	2.83 ± 0.1	2.21±0.2	3.53±0.1	F=56.02, P<0.001
Carassius gibelio	10/0	18.6 ±3.4	13.5-21.1	2.43 ± 0.5	2.04±0.4	3.35±0.5	F=23.30, P<0.001
Squalius cii	1/5	22.5 ±6.8	14.4-32.4	2.76± 0.4	2.33±0.2	3.59±0.2	F=52.72, P<0.001
Sig.				χ^2= 6.09 P>0.05	*F*= 3.71 P<0.05	χ^2= 5.93 P>0.05	

generalist omnivores. Filamentous algae were an important food, especially for *C. gibelio*, being highly nutritious and readily assimilated by most animals (Waslien, 1979). The other favoured food items were especially macroinvertebrates but also detritus and seston. Specziár and Rezsu (2009) indicated that *C. gibelio* fed on diatoms, which occured as epiphytes on filamentous algae, hence diatoms may be an important food resource also in the Karamenderes River. To evaluate this possibility, the epiphytic diatoms would need to be removed from the filamentous algae and assessed separately as a food source by stable isotope analysis in a future study.

The gut contents indicates the proportions of ingested food resources at the time of sampling, while the stable isotope values of the muscle indicate the proportions of food resources assimilated over a period of several weeks prior to sampling. Macroinvertebrates are particularly important food components for *A. calcoides, B. oligolepis* and *S. cii*, but constitute a somewhat smaller part of the diet of *C. gibelio*. Macroinvertebrates were a lower percentage in gut contents than the SI-based muscle percentages for all the species. In contrast, detritus was a higher proportion of gut contents than in the diet proportions obtained from SIA of muscle. These differences presumably reflect the different assimilation efficiencies from various ingested foods, and illustrate the value of SIA in providing a picture of time-integrated, assimilated diet sources.

According to Rounick *et al.* (1982) molluscs and insects collected from the bottom depend more on autochthonous material than their surface relatives. Lower $\delta^{13}C$ in macroinvertebrates indicates that filamentous algae (or associated epiphytic diatoms) was an important food source particularly for bottom molluscs and insects. The $\delta^{13}C$ of the filamentous algae in our study (-31.7‰) is not so low as values (-35.0‰) reported from New Zealand fresh waters by Rounick *et al.* (1982). But in contrast to the results of Rounick *et al.* (1982), macrophytes in our study showed high mean $\delta^{13}C$ (-23.7 ‰) than algae (-29.8‰). This could reflect the availability of dissolved inorganic carbon to macrophytes. Rounick and Winterbourn (1986) reported that the $\delta^{13}C$ value of *Potamogeton perfliatus* was -5 ‰ in a slow-flowing stream and -30 ‰ in a turbulent, fast-flowing river due to the thickness of the unstirred boundary layer which determine the availability of dissolved inorganic carbon in a stream.

Despite no direct calculation of the trophic positions of these cyprinid species, our results (~2.0

to 3.82) are comparable with the the estimated trophic position of cyprinids (2.5) (Vander Zanden *et al.*, 1997) and the trophic level (~2.2 to 4.5) of an invertebrate-feeding fish in the St. Lawrence river watershed (Anderson and Cabana, 2007).

C. gibelio had the lowest trophic position of the fish species according to all three methods of estimation. Since higher trophic order vertebrates are generally more vulnerable to anthropogenic threats (Duffy, 2003), *C. gibelio* may have a slightly lower risk than the other fish community members, and this can contribute to its success as an invasive.

The trophic position of *A. chalcoides* was the highest of the other species according to all three methods of estimation. However, *S. cii* had a higher trophic level than *B. oligolepis* according to the TP2 and TP3 methods. Variability in the $\delta^{15}N$ of any types of food component used in the baseline $\delta^{15}N$ might be responsible for variation in trophic position, and selection of an appropriate baseline indicator in a community is an important step (Anderson and Cabana, 2007). In our study the differences in the baseline food source were an important factor explaining the variation of the trophic positions of species. The gut contents might be a helpful indicator of the instantaneous food intake and hence of the baseline food $\delta^{15}N$ value for omnivorous fish.

The trophic position of all species including *C. gibelio* was evidently overestimated by the third method, in which we used the percentages of all kinds of food categories, not only macroinvertebrates, in the gut contents. The ingestion of high percentages of food materials with low ^{15}N value but which are only poorly assimilated would then bias trophic position estimates by this approach.

There were high variations in the gut contents $\delta^{13}C$ values of all fish species except *C. gibelio*. This suggests that the other species collect and ingest many kinds of food but can only effectively utilize some parts of these foods, whereas *C. gibelio* can utilize a wider range of foods and can assimilate more of what is ingested under poor feeding conditions. Specziár and Rezsu (2009) reported that *C. gibelio* mostly fed on foods not utilised by other species and observed a significant diet overlap only with 41–120 mm *Rutilus rutilus*. In order to understand the ecological impact of this species, the diet overlap of *C. gibelio* with the native species should be investigated in Karamenderes River.

One of the negative indicators of an invasive species is high dominance in the new environment. Didham *et al.* (2005) discussed how dominance of an exotic could be an indirect consequence of habitat modification driving biodiversity loss of native species. Prussian carp is recorded in high abundance (Gaygusuz *et al.*, 2007) in invaded environments including this study. Our study indicates that Prussian carp, with its high diet plasticity, will increase in the future, facilitated by its high dominance, rapid growth and reproductive characteristics, with potential indirect consequences for native communities. However, further studies are required including spatial, temporal and ontogenitic competition, as well as other community interactions such as predation and parasitism.

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