The Diet Composition and Trophic Position of Introduced Prussian Carp

Carassius gibelio (Bloch, 1782) and Native Fish Species in a Turkish River

Şükran Yalçın Özdílek1,*, Roger L. Jones2

1 Çanakkale Onsekiz Mart University, Science and Letters Faculty, Department of Biology, 17100, Canakkale, Turkey.
2 University of Jyväskyla, Department of Biology and Environmental Science, PO Box: 35, FIN-40014, Finland.

* Corresponding Author: Tel.: +90.286 2180018/1796; Fax: +90.286 2180018;
E-mail: syalkincodilek@gmail.com

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.

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, Carassius 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 (Balik 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., 2011; 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 (Aydin 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; Kirankaya and Ekmeği 2013). However, direct and indirect effects of this species on community structure by competition with native species are still poorly understood (Speziär and Rezsu, 2009). Prussian carp is
omnivorous and feeds on detritus, zooplankton, zoobenthos and macrophytes (Penzaz 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 ($^{13}$C) and nitrogen ($^{15}$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 $^{15}$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 $^{13}$C relative to phytoplankton (Hecky and Hesslein, 1995), so stable carbon isotopes ($^{13}$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 diet composition 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 (1.5x1.5 cm 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
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 DELTAplus 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 (δ13C and δ15N, 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 δ13C and 0.12 ‰ for δ15N in each run. The muscle δ13C 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:

\[
TP_{\text{fish}} = \frac{[\delta^{15}N_{\text{fish}} - \delta^{15}N_{\text{primary consumer}}]}{3.4} + 2
\]

in three ways (Rybczynski et al., 2008). (a) TP1: primary consumer δ15N was calculated as the mean δ15N of all primary consumers (mean δ15N value of all macroinvertebrates) collected during sampling which is general baseline for using quantitative estimates of trophic position (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 δ15N was taken to represent the primary consumer δ15N 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 δ15N 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 δ13C and 3.0±0.5% for δ15N (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 δ13C and δ15N 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 δ13C and δ15N values of the four fish species were not statistically different (Table 1), except for δ15N values of gut content and muscle pairs of S. cii (t10 = 3.9; P= 0.01) and A. chalcoides (t17=7.6; P<0.001). For muscle and gut δ13C and δ15N values, only muscle nitrogen values differed between fish species (F1,3,25=3.06; P=0.03).

The δ13C and δ15N biplot (Figure 2) illustrates the food web relationships for the Pınarbaşı station of the Karamenderes River. The δ13C values of potential plant foods, including detritus, from the study ranged from -31.7‰ to -21.4‰ and the greatest 13C depletion was found in the filamentous algae. The δ15C values of invertebrates ranged from -33.9‰ to -26.0‰.

Individuals of all fish species with δ15N between 11.0 and 16.6‰ were clearly feeding on invertebrates (molluscs, insect larvae, Asellus sp., annelids). However, Prussian carp, with lower mean δ15N, 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 δ13C and δ15N 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
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 δ¹⁵N 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 δ¹³N 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 δ¹³C (*F*₅,₂₉ = 2.42; *P*>0.05) and δ¹⁵N (*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*₁₃,₃₂₅ = 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 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Gut contents δ¹³C ‰</th>
<th>Fish Muscle δ¹³C ‰</th>
<th>Gut contents δ¹⁵N ‰</th>
<th>Fish Muscle δ¹⁵N ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alburnus chalcoides</em></td>
<td>-27.3±1.92</td>
<td>-27.2±1.01</td>
<td>13.6±0.62</td>
<td>15.2±0.95</td>
</tr>
<tr>
<td><em>Barbus oligolepis</em></td>
<td>-28.8±1.68</td>
<td>-28.4±0.96</td>
<td>14.0±0.85</td>
<td>14.5±0.70</td>
</tr>
<tr>
<td><em>Carassius gibelio</em></td>
<td>-26.4±1.37</td>
<td>-27.4±1.35</td>
<td>13.2±0.70</td>
<td>13.4±1.59</td>
</tr>
<tr>
<td><em>Squalius cii</em></td>
<td>-26.9±2.72</td>
<td>-27.9±1.70</td>
<td>13.4±0.84</td>
<td>14.5±0.74</td>
</tr>
</tbody>
</table>

Figure 2. δ¹⁵N and δ¹³C 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.
seston. Specziár and Rezsú (2009) indicated that *C. gibelio* fed on diatoms, which occurred 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 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 δ¹³C in macroinvertebrates indicates that filamentous algae (or associated epiphytic diatoms) was an important food source particularly for bottom molluscs and insects. The δ¹³C 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 δ¹³C (-23.7‰) than algae (-29.8‰). This could reflect the availability of dissolved inorganic carbon to macrophytes. Rounick and Winterbourn (1986) reported that the δ¹³C value of *Potamogeton perfoliatus* 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).

Table 2. Trophic positions (TP) of four fish species estimated by using mean total macroinvertebrate δ¹⁵N (TP1), total gut content δ¹⁵N 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.)

<table>
<thead>
<tr>
<th>Species</th>
<th>N (F/M)</th>
<th>mean FL ± SD (min-max)</th>
<th>TP1 ± SD</th>
<th>TP2 ± SD</th>
<th>TP3± SD</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alburnus calcoides</em></td>
<td>8/0</td>
<td>13.6 ±1.6 10.6-16.0</td>
<td>2.97±0.3</td>
<td>2.49±0.2</td>
<td>3.82±0.3</td>
<td>F=59.03, P&lt;0.001</td>
</tr>
<tr>
<td><em>Barbus oligolepis</em></td>
<td>4/1</td>
<td>23.2 ±8.7 13.5-32.6</td>
<td>2.83±0.1</td>
<td>2.21±0.2</td>
<td>3.53±0.1</td>
<td>F=56.02, P&lt;0.001</td>
</tr>
<tr>
<td><em>Carassius gibelio</em></td>
<td>10/0</td>
<td>18.6 ±3.4 13.5-21.1</td>
<td>2.43±0.5</td>
<td>2.04±0.4</td>
<td>3.35±0.5</td>
<td>F=23.30, P&lt;0.001</td>
</tr>
<tr>
<td><em>Squalius cii</em></td>
<td>1/5</td>
<td>22.5 ±6.8 14.4-32.4</td>
<td>2.76±0.4</td>
<td>2.33±0.2</td>
<td>3.59±0.2</td>
<td>F=52.72, P&lt;0.001</td>
</tr>
</tbody>
</table>

| Sig. | χ² = 6.09 | F= 3.71 | χ² = 5.93 |
|      | P=0.05    | P<0.05  | P>0.05    |

Figure 3. The percentages of diet components for 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. calcoides*. Pot=Potamogeton sp., Fil=filamentous algae, Ses=seston, Det=detritus Mac=macroinvertebrates.
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 δ15N of any types of food component used in the baseline δ15N 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 δ15N 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 15N value but which are only poorly assimilated would then bias trophic position estimates by this approach.

There were high variations in the gut contents δ13C values of all fish species except C. gibelio. This suggests that the other species collect and ingest many parts of these foods, whereas 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 ontogenetic competition, as well as other community interactions such as predation and parasitism.

Acknowledgements

We thank fisherman Selahattin Erol from Pınarbaşı district for fishing, and also Dr. Hasan Goksel Ozdilek and Dr. Emel Okur Berberoğlu for field assistance. We thank Dr. Tuula Sinisalo for SIA analysis and Dr. Mikko Kilyunen, Dr. Antti Elanorata and Jyrki Torniainen for help with using the SIBER program.

References


