

Seasonal variability in the diet of juvenile European catfish, *Silurus glanis*, in the Arno River (Italy) in Florence

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Abstract. While approaches for assessing the impact of invasive species are advancing, fundamental autoecological information such as feeding preferences is often unavailable for many invasive species. The European catfish, *Silurus glanis*, is a well-known, high-impact invasive predator; however, there remain considerable gaps in knowledge about its feeding preferences and biotic interactions that limit ecological impact predictions for this predator. This study is an investigation of seasonal variability in this predator's diet during the juvenile life stage using the Arno River, Italy as a model system. This study identified significant differences across seasons, provided further information on the dietary spectrum of this species, and raised important questions for further study.

Keywords: dietary analysis, stomach contents, invasive species

Introduction

As a growing element of anthropogenically-driven global change, biological invasions significantly disrupt ecosystems (Malcolm and Markham 2000, Bellard et al. 2016, Crystal-Ornelas and Lockwood 2020) and show no signs of subsiding (Seebens et al. 2017). The ecological impacts of biological invasions can potentially challenge conservation efforts and management interventions (Hulme et al. 2009, Cuthbert et al. 2021). Freshwater ecosystems are particularly susceptible to increased introductions and the spread of non-native species (Poulin et al. 2011) and are prone to be modified by them (Gallardo et al. 2016, Haubrock et al. 2021a). Indeed, the already complex ecological interactions among species are further complicated by interactions with novel invasive species in communities (Stellati et al. 2019), but interactions among invasive species themselves also pose problems (Haubrock et al. 2019).

In many European countries, the presence of non-native fishes has increased over the past 25 years (Gherardi et al. 2008, Nocita et al. 2017). Despite some unintentional introductions, most species have been introduced intentionally for angling and other purposes (Nunes et al., 2015), despite indications of noteworthy economic burdens (Haubrock et al. 2021b). In Europe, one particular example is

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Italy, where freshwater ecosystems have suffered from the introduction of various aquatic invasive species in the past (Nocita et al. 2017), resulting in the establishment of more than 150 aquatic species (at least 64 invertebrates and 48 vertebrates) contributing to at least 2% of the inland-water fauna (Gherardi et al. 2008). One such species is the European catfish, *Silurus glanis* L., a native of northeastern Europe and Asia. *S. glanis* is known to be the largest freshwater Osteichthyes in Europe (maximum size 2.7 m and 130 kg; Bouletreau et al. 2016). In the last two decades, mostly facilitated by angling activities, drastic increases in its population size and distribution have been observed (Copp et al. 2009, Alexander et al. 2014, Cunico and Vitule 2014).

S. glanis was introduced in Italy in the 1990s (Balma et al. 1989), and its spread was facilitated intentionally throughout the Italian peninsula with diverse impacts on native and newly-introduced species (Castaldelli et al. 2013). In the Florentine stretch of the Arno River, *S. glanis* has been reported by anglers since 2004 (Gualtieri and Mecatti 2005). Within a few years, this species became a popular target of anglers, which was concomitant with considerable positive economic effects (Rees et al. 2017, Cucherousset et al. 2018) but negative environmental impacts (Copp et al. 2009, Castaldelli et al. 2013). Since 2016, a decrease in the abundance of large individuals and a greater relative frequency of juveniles without prey in their guts has been observed in the Arno River. These observations have been linked to (a) competition with another invader, the North American channel catfish, *Ictalurus punctatus* (Raf.), (b) control efforts, and (c) angling pressure (Haubrock et al. 2019, 2020). However, the data on the diet of *S. glanis* presented in these study were constrained to one season and contributed only preliminary or anecdotal information on this invader's diet (Gualtieri and Mecatti 2005). Accordingly, there remains little information on which taxa are impacted by this species through predation.

To tackle this obvious gap, we assembled dietary information from the *S. glanis* juvenile life stage in the Arno River. We hypothesized that a more detailed analysis of this invasive fish's diet could indicate

potential reasons for its decline while simultaneously revealing missing information on its diet.

Method

Study site, sampling, and sample preparation

A detailed description on the flow regime of the Arno River and its species community was summarized previously (Nocita, 2002, Nocita and Zerunian 2007, Haubrock et al. 2019). Sampling was conducted from late spring (April to the beginning of June) and fall (August to September) 2018 in the section of the Arno River that flows through the city of Florence (43.765606, 11.268234; DATUM WGS84). Information on the *S. glanis* diet sampled during spring was obtained from Haubrock et al. (2019). This data was supplemented with additional *S. glanis* specimens caught in fall. Both datasets were created using information from specimens caught with standard fishing rods and by local fishers using a variety of bait fishing techniques. Overall, 71 individuals (spring – 39, Haubrock et al. 2019; fall – 32) were collected. The total length (TL; cm) of all specimens was measured to the nearest 1 mm. Values reported in the text are averages +/- standard deviation. The specimens were stored in a freezer (-20°C) until the stomach contents were extracted for dietary analyses.

Dietary analyses

The dietary analysis was based only on the cardiac, cecum, and pyloric parts of the digestive tract without discarding the contents of the intestine. Both algae and detritus were ranked according to their occurrence in relation to other prey: 0 = no occurrence, 1 = low occurrence, 2 = equal occurrence, and 3 = dominating occurrence. Bait used for the sampling were excluded and not counted in the dietary analysis. Because of difficulties distinguishing between specimens of partially digested *Alburnus alburnus* (L.) and *Pseudorasbora parva* (Temminck &

Schlegel), these two species were classified as small (<5 cm) and large (>5 cm) cyprinids. For both seasons sampled, feeding intensity was calculated using the vacuity index (V_I) individually as the percentage of empty stomachs in relation to stomachs that contained prey items (Batistic et al. 2005). Diet breadth was estimated with Levin's index formula (Whittaker et al. 1973): $B_i = \frac{1}{p \sum_i^2}$ where B_i is the standardized

index of diet breadth for specimen i and p the sum of the squared proportion of each prey item of specimen i (Levin 1968). For further analyses, the stomach content data were expressed as frequency of occurrence ($F\% =$ number of stomachs containing each food item in relation to the total number of full stomachs) and abundance ($N\% =$ the number of individuals of each food item in relation to the total number of individuals). With these, the prominence value (PV) for each dietary component was estimated according to Hickley et al. (1994): $PV = N\% \times \sqrt{F\%}$ to estimate diet-overlap index α using the formula by Schoener (1986): $\alpha = 1 - 0.5 \left(\sum_{i=1}^n |PV_{xi} - PV_{yi}| \right)$,

where n is the number of food items, PV_{xi} the prominence value of food item i in species x_i , and PV_{yi} is the prominence values of food item i in species y . This index ranges between 0, i.e., no overlap, and 1, when diets are identical (Wallace 1981). Pianka's measure of diet based niche overlap (Pianka 1974) for specimens of *S. glanis* (j , individuals caught in spring; k , individuals caught in fall) was calculated with the

formula $0_{jk} = \frac{\sum_j^n p_j p_k}{\sqrt{\sum_i^n p_j^2 \sum_i^n p_k^2}}$, where p_{ij} and p_{ik} are

the proportions of the i^{th} resource used by the j^{th} and k^{th} individuals from each season. Both estimates for diet overlap were used together, as Pianka's measure of niche overlap alone can be insufficient to identify a relation between niche overlap and competition, i.e., resource partitioning (Krebs 1998).

Statistical analyses

First, we tested if the total length of individuals caught in spring and fall differed significantly using the Student t-test (Zimmerman 1987). Second, using the stomach content records from each specimen sampled and both seasons, a permutational analysis of variance (PERMANOVA; two fixed factors – size and season; sums of squares: type II; 9999 permutations; partial permutation of residuals under a reduced model) was used to test if the diet, and, thus, feeding behavior, differed temporally and/or according to fish size. Additionally, we applied a canonical analysis of principal coordinates (CAP), which is a flexible, meaningful constrained ordination suitable for ecological abundance data. CAP was applied to prey abundances for the factors of size and season. Using the *envfit* function in R, we projected significantly correlated factors and prey items onto the relevant CAP axes. All statistical analyses were performed using the R "Vegan" package (Oksanen et al. 2007). The level of significance for all tests under which the null hypothesis was rejected was $\alpha = 0.05$.

Results

The individuals collected differed in size, and those sampled in spring (27.1 ± 1.1 cm) were significantly larger ($t: 3.41$, $df: 53.73$, $p: <0.05$) than those collected in fall (20.58 ± 9.2). A considerable number of stomachs was found empty from both sets of specimens (spring: 25/43, $V_I: 58\%$; fall: 11/32, $V_I: 34\%$). Overall, 18 items were identified in the stomachs, including biotic components from both aquatic and terrestrial environments and abiotic items (i.e., pebbles) (Table 1). The items that occurred resulted in a medium ($O_{ij}: 0.365$) to high overlap ($\alpha: 0.737$) between the two seasons, and they indicated a wider niche in spring ($B: 7.3$) than in fall ($B: 5.6$).

PERMANOVA identified the occurrence of prey as significantly different according to season ($df: 1$; $R^2: 0.04971$; $F: 3.8987$; $p: 0.0477$) but not according to total size ($df: 1$; $R^2: 0.0266$; $F: 2.0850$; $p: 0.06316$)

Table 1

Results of dietary analysis of *S. glanis* in spring and fall; N% = number of occurrence; F% = frequency of occurrence; PV = prominence value; see Haubrock et al. (2019)

Prey	Spring (n = 39)			Fall (n = 32)		
	N%	F%	PV	N%	F%	PV
Detritus (0,1,2,3)	13.79	9.30	0.04	7.79	15.63	0.03
Algae (0,1,2,3)	13.79	9.30	0.04	1.30	3.13	0.00
Snails (aquatic)	0.00	0.00	0.00	1.30	3.13	0.00
Stones	6.90	4.65	0.01	0.00	0.00	0.00
Plant seeds (terrestrial)	3.45	2.33	0.01	0.00	0.00	0.00
Insect larvae	3.45	2.33	0.01	32.47	18.75	0.14
Insect adult	0.00	0.00	0.00	2.60	6.25	0.01
Terrestrial insects	0.00	0.00	0.00	2.60	6.25	0.01
<i>Gammarus</i> sp.	0.00	0.00	0.00	1.30	3.13	0.00
<i>Dikerogammarus villosus</i>	10.34	4.65	0.02	19.48	28.13	0.10
<i>Palemon</i> sp.	3.45	2.33	0.01	14.29	21.88	0.07
<i>Procambarus clarkii</i>	0.00	0.00	0.00	3.90	6.25	0.01
Fish larvae (unidentified)	3.45	2.33	0.01	3.90	9.38	0.01
<i>Lepomis gibbosus</i>	0.00	0.00	0.00	1.30	3.13	0.00
Cyprinids (small:<5 cm)	24.14	16.28	0.10	2.60	6.25	0.01
Cyprinids (large:>5 cm)	0.00	0.00	0.00	3.90	6.25	0.01
<i>Ictalurus punctatus</i>	13.79	6.98	0.04	0.00	0.00	0.00
Rodents	3.45	2.33	0.01	1.30	3.13	0.00

or interaction (df:1; R^2 : 0.04987; F: 1.4424; p: 0.1774).

The CAP1 and CAP2 axes (squared canonical correlation of $\delta_1^2=0.782$ and $\delta_2^2=0.283$, respectively) separated the groups of specimens identified by the factor of season (Figure 1). The correlation of the variable size scored on the two CAP axes (CAP1 δ_1^2 : 0.139; CAP1 δ_1^2 : -0.990; r^2 : 0.3443; p: 0.001) revealed that size characterized prey occurrence.

Discussion

The majority of studies investigating the diet of the European catfish have focused on its native area in Eastern Europe (see Carol et al. 2009 and references therein). Relatively fewer studies have investigated its diet in its introduced range in Italy (Rossi et al. 1991, Haubrock et al. 2019), which limits

understanding the impact of this species. Despite a scarcity of information, the two ecosystems investigated in these studies are notably distinct, and the Arno River (Haubrock et al. 2019) is particularly noteworthy since its ichthyofauna community composition is entirely comprised of non-native species. While similar studies have been conducted on other piscivorous fish (see, e.g., Lorenzoni et al. 2002), this is the first report of seasonal shifts in the European catfish diet in Italy.

Rossi et al. (1991), for instance, noted that at a size of ~30 cm the European catfish shifts from a crustacean-based diet to piscivory. Although their study was limited to one season, Haubrock et al. (2019) observed that immature individuals of a comparable size expressed no obvious prey preference, but rather consumed a diverse diet consisting of small cyprinids (and other small fishes), small crustaceans, and plants. During the present investigation

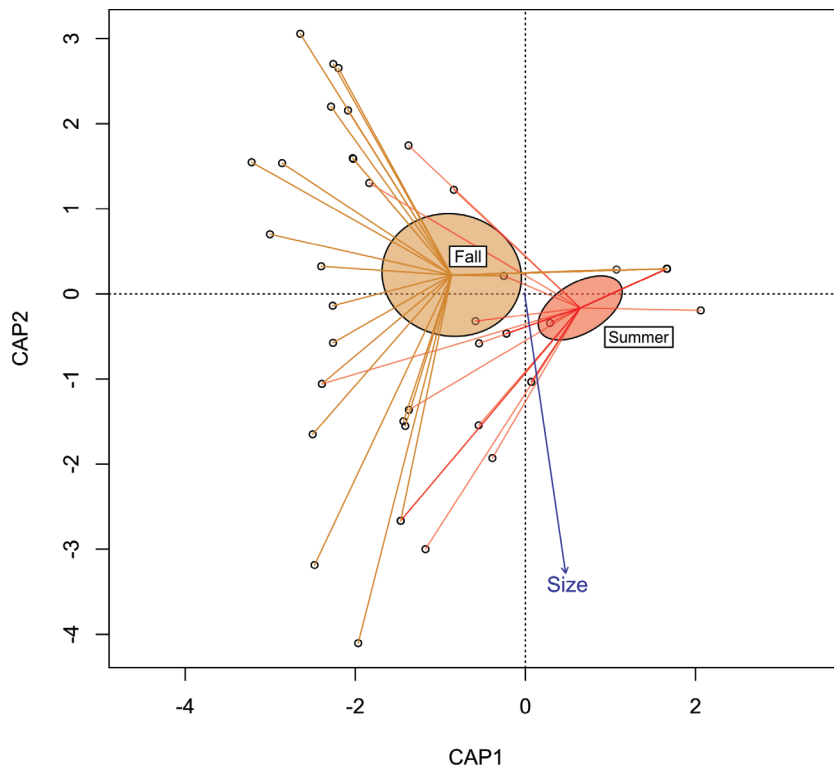


Figure 1. Two-dimensional scatter plot of the first and second principal coordinate axes (after resemblance matrix with Bray-Curtis distance matrix of dietary data, n samples = 73) for the factor of season (summer, fall). Vectors of linear correlations between sizes are superimposed on the graph.

of the diet of *S. glanis* juveniles in fall, a similarly diverse pattern was noted, while a considerable percentage of stomachs contained algae and detritus, individuals predominantly fed on insects, followed by crustaceans, and to a lesser degree on fishes. The diversity of prey occurring in the *S. glanis* diet could have been the result of a heavily disturbed environment. However, while the occurrence of algae and detritus could have originated from accidental ingestion, it is noteworthy as it could be a dietary component, especially of immature individuals (Copp et al. 2009). Haubrock et al. (2019) argued that the strong resource overlap with the co-occurring invasive species *I. punctatus* could be the reason the less competitive *S. glanis* juveniles shifted to other potential food sources. However, invasive, and potentially predatory species like *Dikerogammarus villosus*, *Palaemon* sp., or *P. parva* (the latter combined with *A. alburnus* as cyprinids) are highly abundant in the section of the Arno studied (Haubrock et al. 2021a). Accordingly, we found that cyprinids comprised a considerable

portion of the *S. glanis* diet. Another potential explanation could be that behavioral differences, i.e., the higher density of *I. punctatus* and their greater prey detection sensitivity, and not availability, were limiting factors.

Further, significant seasonal differences in the occurrence of prey species in the diet of *S. glanis* were identified. The reasons for this could be (i) seasonally varying occurrences of prey (Salfina and Burger 1985), (ii) dietary shifts in accordance with physiological requirements (Uys 1989), or (iii) shifts in competition with other co-occurring species. Similarly, interactions and especially niche overlap between the almost analogous cyprinid species *P. parva* and *A. alburnus* in the Arno River (Balzani et al. 2020) were not limiting despite the significant overlap, and this led to competition. While these potential reasons should be the aim of future studies, the variability in this predatory feeding behavior despite being limited to immature individuals is important. Although PERMANOVA ($p = 0.06$) analysis did not


identify this as significantly differentiating prey occurrences, CAP results suggested there was a significant relation. Here, the inclusion of larger sized, mature individuals should clarify whether similar trends observed by Haubrock et al. (2019) or Rossi et al. (1991) are pervasive across a wider fish size range.

Another relevant observation was the high number of empty stomachs. While it is difficult to determine the reason for this, it was previously argued that major drivers could be either competition with *I. punctatus* or increasingly unsuitable environmental conditions (Haubrock et al. 2020). While the Arno River is not the ideal environment to investigate this predator's impact on nearly absent native species (Haubrock et al. 2021a), the present study indicated this species has a generalist diet that could impact multiple resource types. Based on the prey occurrences observed, it can also be argued that the diet of *S. glanis*, and therefore its feeding impact on the community of potential prey, can affect species beyond those previously identified as prey species such as cyprinids and crustaceans. This pertains especially to aquatic insects, but also to various other fishes (e.g., *Lepomis gibbosus* (L.)) and small rodents (*Rattus norvegicus*) that were observed in its diet.

Considering the findings of this study, it can be argued that the reasons for the recent decline in *S. glanis* in the Arno River depends on several factors including (i) increasing competition with other non-native predators, (ii) a decline in native prey species, and (iii) progressive environmental degradation. However, while this study presents novel dietary information in two seasons of a damaging invasive species, it raises further issues that ought to be addressed in the future: (a) information on mature individuals is scarce and it could differ from data on juveniles in the Arno River; (b) the reasons for the ontogenic diet shift remain unclear; (c) the reasons for the decline in *S. glanis* require further elucidation. These issues are all of ecological and economic interest since local anglers have not yet shifted to targeting *I. punctatus* as a valuable game species.

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