

Study of the diets of two coexisting species – invasive Chinese sleeper (*Perccottus glenii* Dybowski, 1877) and native European perch (*Perca fluviatilis* Linnaeus, 1758)

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In Poland, the Chinese sleeper *Perccottus glenii* is one of the alien fish species which, by effectively competing with native species, may contribute to the weakening of their population and a reduction of the biodiversity. The aim of this paper is to study the diets of two coexisting species: the native European perch *Perca fluviatilis* and the invasive Chinese sleeper. The fish were collected from a watercourse used for draining water from a fishing farm, located in the Nida River water system (Poland), using the electro-fishing method. The captured fish were killed, preserved in a 4% formaldehyde solution and the stomachs of the fish were dissected. Then, based on the materials collected from these stomachs, the abundance and taxonomic rankings of the individual diet components (quantity of individuals in the individual taxa) were determined. The overlap of the *P. fluviatilis* and *P. glenii* feeding spectra was studied using a principal component analysis (PCA). The analysis of the data collected demonstrated that the diets of these two species overlaps considerably. At the same time, the analysis of the major diet components showed that the niche used by *P. fluviatilis* is broader than that used by *P. glenii*. It seems that, due to its high level of plasticity, *P. fluviatilis* is able to withstand the pressures of *P. glenii* and is also able to limit its abundance. Both species frequently choose fish as their prey, including juvenile common carp *Cyprinus carpio* Linnaeus, 1758, which indicates that together with native predators, *P. glenii* may pose a considerable threat to farmed juvenile Cyprinidae.

Key words: Poland, alien fish species, food competition, predation.

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In nearly all publications on alien species there is a claim that, next to the loss of habitat, biological invasions are a major threat to biodiversity (Wittenberg & Cock 2001; Solarz 2007; Seebens *et al.* 2017). The same applies to aquatic organisms, including fish. Invasive species are also the cause of considerable economic losses (Rosaen *et al.* 2012; Rothlisberger *et al.* 2012).

One of the recently discovered arrivals in Polish waters is the Chinese sleeper *Perccottus glenii*, a fish that originates from Asia and is spreading through, among other means, the transport of stocking materials between fish farms (Nowak *et al.* 2008a; Nehring & Steinhof 2015). *Perccottus glenii* is a small predatory fish that reaches a total length of 25 cm and has a body weight of up to 250 g (Koščo

et al. 2003). In Poland, *P. glenii* was first recorded in 1993 in a Vistula River oxbow lake located near Dęblin (Antychowicz 1994). It is not clear how the first fish found their way into Polish waters. However, the fact that the species is now found especially in the waters of numerous fish farms in the Vistula basin, and unfortunately also in the watercourses that feed them, is a good hint as to how it was introduced to Poland (Nowak *et al.* 2008a). In 2009, *P. glenii* was also found for the first time in the Oder River basin (Andrzejewski *et al.* 2011), which is probably due to the fact that the Vistula and Oder are connected by the Bydgoszcz Canal (via the Noteć River, a tributary of the Oder). It is also believed that anglers using *P. glenii* as live bait for larger predators have participated in the spread of this species (Terlecki & Pałka 2012; Pupina *et al.* 2015).

During studies of the ichthyofauna in the Nida water system in Poland, conducted by the authors in 2016, both *P. glenii* and *P. fluviatilis* were found. The presence of other species in a watercourse draining water from one of the carp farms, including the juvenile common carp *Cyprinus carpio*, were also found. The presence of the Chinese sleeper in the Nida water system was nothing new, as the species had been recorded there earlier (Klaczak *et al.* 2011). Both *P. glenii* and *P. fluviatilis* are predators, but the latter is a species that is native to Polish waters. In a fish farm, both of these species may be a threat to the juvenile *C. carpio*. However, in open waters *P. fluviatilis* may possibly be threatened by the *P. glenii* invasion, because the latter competes for the same food resources and has a predatory nature (Grabowska *et al.* 2009; Sikorska *et al.* 2013). This applies in particular to younger (smaller) *P. fluviatilis* individuals, as the larger individuals (as well as the northern pike *Esox lucius* Linnaeus, 1758) are predators that pursue *P. glenii* as prey (Litvinov & O’Gorman 1996). Experiments conducted in small eutrophic lakes in Lithuania confirmed the hypothesis that systematic stocking of the water with *E. lucius* and *P. fluviatilis* may not only considerably limit the abundance of *P. glenii*, but can also completely eliminate it from such water bodies (Rakauskas *et al.* 2019). Both *P. glenii* and *P. fluviatilis* are generalist species (Kottelat & Freyhof 2007; Grabowska *et al.* 2009). *P. fluviatilis* is a slow-growing species – in the lakes and dam reservoirs of Poland, it reaches a total length (TL) of around 12 cm in the third or even the fourth year of its life (Rechulicz 2008). In numerous water bodies, *P. fluviatilis* individuals reach a total length of up to 20–25 cm. Thus, in such habitats their size is similar to that of the larger *P. glenii* individuals (Koščo *et al.* 2003).

Since the *P. glenii* and *P. fluviatilis* individuals captured in 2016 were of a similar size, the authors deemed this a good opportunity to compare the diets of these two predatory species. The aim of the study was, among other things, to answer the following questions:

- Is (in the conditions of the investigated watercourse) *P. glenii* a significant competitor for food resources with *P. fluviatilis* that is capable of limiting the food resources available to the latter?
- Which of these predators has a stronger preference for vertebrates (especially fish) as its prey?

Material and Methods

Study area and data collection

The study material was comprised of 20 *P. fluviatilis* individuals and 12 *P. glenii* individuals captured in July 2016, in a watercourse draining water from a fishing farm located in the Nida River valley (Poland coordinates: 50.341872, 20.726290), whose main operations were focused on the production of *C. carpio*. The fish were captured in a single pass of the watercourse in the upstream direction using the back-pack electrofishing device IG-600T (Hans-Grassl, Germany). The captured fish were killed using a lethal dose of MS-222 (Sigma-Aldrich, St. Louis, MO, USA), and were then preserved in a 4% formaldehyde solution and stored in a laboratory at the Department of Animal Nutrition and Biotechnology and Fisheries of the University of Agriculture in Kraków. As the fish were not subjected to any testing procedures, the research did not require approval from the Ethics Committee. All procedures involving the fish used in the study complied with the current laws of Poland. After measuring the total length (TL), standard length (SL) (to the nearest 1 mm) and weight (to the nearest 0.01 g), the stomachs of the fish were dissected. Then, based on the materials collected from these stomachs, the abundance and taxonomic rankings of the individual diet components (quantity of individuals included in individual taxa) were determined.

Data analysis

The analysis of the total length, standard length and weight of the captured fish was performed using the two-tailed non-parametric Mann-Whitney U test. Values of $p < 0.05$ were considered to be statistically significant.

The overlap of the *P. glenii* and *P. fluviatilis* diets

was assessed with Schoener's diet overlap index (Z) (1970):

$$Z = 100 - \frac{1}{2} \sum |r_1 - r_2|$$

where:

r_1 and r_2 – the share of a given component in the diet of the species being compared [%].

This index [%] expresses the overlapping part of the diet of the species that we compared. A Z index of 100% denotes a complete overlap of the diets, while 0% denotes a complete lack of such an overlap.

The preference of the captured fish for individual diet components was analysed using the graphical method proposed by Costello (1990). The Costello method is based on a two-dimensional representation, in which every point represents the frequency of the occurrence and abundance of prey in the diet of a specific predator. The diagonals of the plot represent the importance of prey for the predator (dominant or rare) and the predator's feeding strategy (specialist or generalist). Points (prey-specific) near 100% for frequency and 100% for abundance reflect the dominant taxa in the diet; points near 100% for frequency and 1% for abundance represent a generalist diet; while points near 1% for frequency and 100% for abundance represent a high specialisation in feeding on selected taxa (Fig. 1).

The overlap of the *P. fluviatilis* and *P. glenii* feeding spectra was studied using a principal component analysis (PCA). As the PCA was developed for the analysis of continuous random variables, the raw data (abundance of individual taxa, n) was subject to a $\log_{10}(n_i+1)$ logarithmic transformation. The PCA was performed in a covariance matrix.

In order to verify the null hypothesis on the lack of considerable differences in the composition of the *P. fluviatilis* and *P. glenii* diets, we conducted a non-parametric multifaceted variance analysis (PERMANOVA) (Anderson 2001). Both the PCA and PERMANOVA were conducted in the R environment ver. 2.3.2 using the vegan package (Oksanen et al. 2013).

Results

Length and weight of the fish

The mean total length (TL) of the captured *P. fluviatilis* was 100.9 mm, the mean standard length (SL) was 85.50 mm and the mean weight was 14.63 g. In the case of *P. glenii*, these values were: 105.2 mm, 89.33 mm and 18.40 g, respectively (Table 1). The two-tailed non-parametric Mann-Whitney U test ($p < 0.05$) did not demonstrate significant differences between the medians of the distributions of these parameters for *P. fluviatilis* and *P. glenii*.

Diet components and feeding strategy analysis

For both species, the most numerous prey (largest abundance of individuals) were representatives of the invertebrate fauna (Tables 2 and 3, Figs 2 and 3). Also, both *P. fluviatilis* and *P. glenii* often fed on fish, which were found in the stomachs of 9 out of the 12 captured *P. glenii* individuals and in 16 out of the 20 captured *P. fluviatilis* individuals. Fish were the most frequently found (highest frequency)

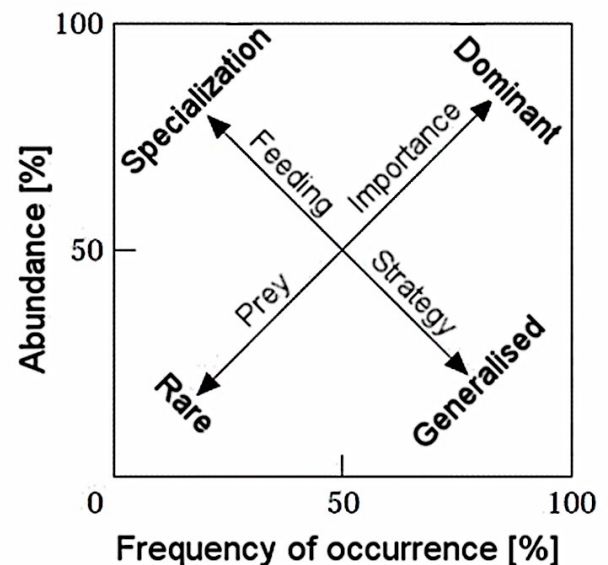


Fig. 1. Explanatory diagram of the graphical method according to Costello (1990)

Table 1

Parameters of the captured fish: TL – total length, SL – standard length

Species	n	TL [mm]			SL [mm]			Weight [g]		
		Mean±SE	Max	Min	Mean±SE	Max	Min	Mean±SE	Max	Min
<i>Perca fluviatilis</i>	20	101±2.55	118	88	85±2.37	100	72	14.6±1.14	25.8	9.24
<i>Perccottus glenii</i>	12	105±4.22	135	83	89.33±3.91	118	71	18.4±2.26	33.5	9.76

Table 2

Abundance and frequency (frequency of occurrence of the diet components) of individual taxa in the diet of *P. fluviatilis*. n – number of components or number of stomachs containing a given component

Main taxon	Component abundance		Frequency	
	n	%	n	%
Heteroptera	77	33.19	18	90.00
Teleostei	35	15.09	16	80.00
Diptera	34	14.66	4	20.00
Ephemeroptera	31	13.36	3	15.00
Odonata	28	12.07	13	65.00
Trichoptera	26	11.21	13	65.00
Haplotaxida	1	0.43	1	5.00
Total	232	100.00		

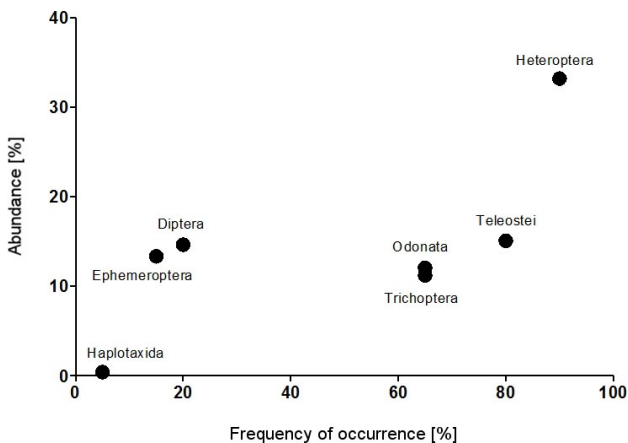


Fig. 2. *P. fluviatilis* feeding strategy plot created using the method (Costello 1990)

component in the diet of *P. glenii* (Table 3, Fig. 3); while in the case of *P. fluviatilis*, fish were surpassed only by members of the Heteroptera order, which were found in great numbers in the stomachs of the European perch (Table 2, Fig. 2). *Percottus glenii* were less prone to choose this as a component of their diet (Fig. 1). Other prey often selected by both species were members of the Odonata order, while only few of the fish studied were found to be feeding on Diptera larvae (Figs 2 and 4).

Cyprinus carpio comprised over 80% of the fish consumed by *P. glenii* (Fig. 5), while its share was considerably smaller in the case of *P. fluviatilis*.

Table 3

Abundance and frequency (frequency of occurrence of the diet components) of individual taxa in the diet of *P. glenii*. n – number of components or number of stomachs containing the component

Main taxon	Component abundance		Frequency	
	n	%	n	%
Trichoptera	20	31.25	8	66.67
Teleostei	12	18.75	9	75.00
Odonata	10	15.63	6	50.00
Diptera	10	15.63	2	16.67
Heteroptera	7	10.94	5	41.67
Ephemeroptera	3	4.69	1	8.33
Megaloptera	1	1.56	1	8.33
Coleoptera	1	1.56	1	8.33
Total	64	100.00		

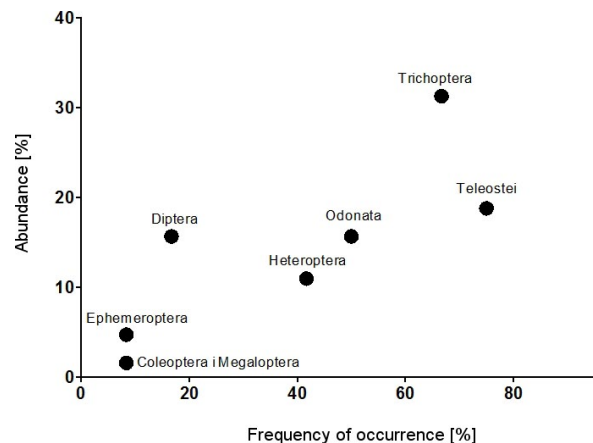


Fig. 3. *P. glenii* feeding strategy plot created using the Costello method (Costello 1990)

Among the other fish likely to be present in the stomachs of the studied fish species were *Pungitus pungitus* (Linnaeus, 1758), *Pseudorabara parva* Temminck & Schlegel, 1846, *Barbatula barbatula* (Linnaeus, 1758), *P. glenii* and *P. fluviatilis*.

The overlap in the diets of the two species studied, expressed using the diet overlap index (Z), was 68.642. The non-parametric multifaceted variance analysis (PERMANOVA) did not demonstrate statistically significant differences in the taxonomic structure of the diets of the European perch and the Chinese sleeper ($p = 0.074$).

The PCA results are presented in Fig. 4 and Table 4.

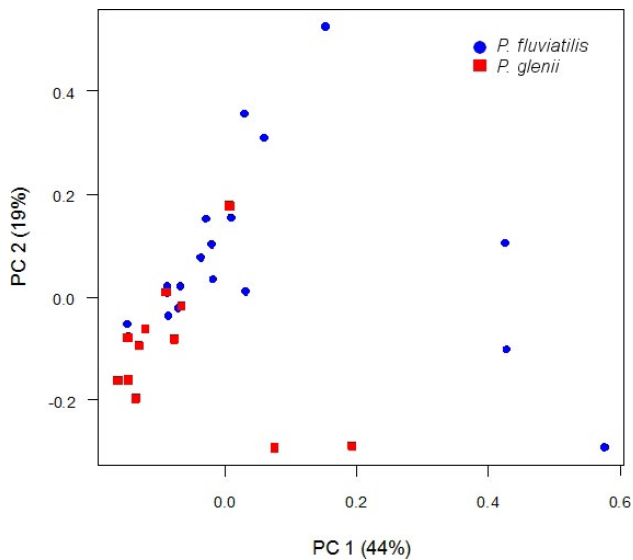


Fig. 4. PCA analysis of the major diet components of *P. fluviatilis* and *P. glenii*.

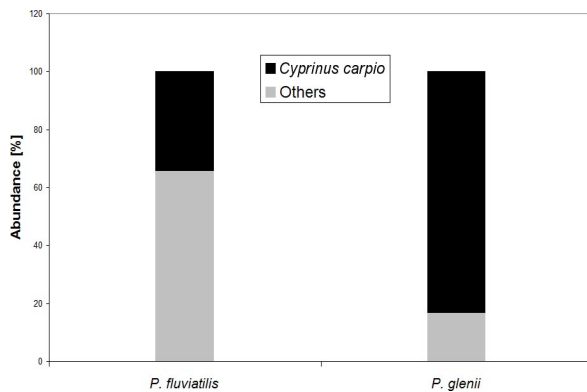


Fig. 5. Share [%] of juvenile carp in the diets of *P. fluviatilis* and *P. glenii*.

Even though in most cases both species used the same resources, it was observed that the niche used by *P. fluviatilis* was slightly broader than that used by *P. glenii*. This stemmed mostly from the differences in abundance of Diptera, Ephemeroptera and Heteroptera, which were noticeably more often consumed by *P. fluviatilis*.

Discussion

There is no doubt that competition from, and the predation of, alien species may result in serious changes in ecosystems, which might eventually lead to the extinction of native species (Khan & Panikkar 2009). Currently, one quarter of all Polish inland water ichthyofauna are of an alien origin (Nowak *et al.* 2008a, 2008b; Witkowski *et al.* 2009; Głowaciński & Pawłowski 2011).

P. glenii, an alien species in Polish waters, is commonly considered to be dangerous and invasive (Nehring & Steinhof 2015). It has been found in the Nida River basin, especially near fish farms, for at least ten years, sometimes together with other invasive alien species that include the Prussian carp *Carassius gibelio* Bloch, 1782, the brown bullhead *Ameiurus nebulosus*, the black bullhead *Ameiurus melas* and the stone moroko *Pseudorasbora parva* (Nowak *et al.* 2010; Klaczak *et al.* 2011). The majority of the species listed above, with the exception of *P. parva*, have been found in this water system for several decades.

Data on the diet of *P. glenii* provided by different authors seems to confirm that it is a typical generalist species that easily adapts to the local conditions. For example, in the lowland canals and oxbow lakes of the Cisa (a tributary of the Danube in Hungary),

Table 4

Major diet components of *P. fluviatilis* and *P. glenii*: results of the PCA analysis

Main taxon	PC1	PC2	PC3	PC4	PC5	PC6
Coleoptera	0.011193	-0.043304	0.029305	-0.037756	-0.02113	0.08400
Diptera	0.773182	-0.437964	0.071403	-0.078710	-0.04805	0.23114
Ephemeroptera	0.781686	-0.208971	0.076755	0.123973	0.20673	-0.24957
Haplotaxida	0.022726	0.077774	-0.007083	0.006394	-0.02151	0.02726
Heteroptera	0.724981	0.599515	-0.010620	-0.228974	-0.20871	-0.02086
Megaloptera	-0.013110	0.001349	0.026989	0.003233	-0.01329	-0.01038
Odonata	0.191520	0.288869	0.147227	0.585113	-0.01131	0.10398
Teleostei	-0.009034	0.280312	0.205793	-0.171341	0.49911	0.10507
Trichoptera	0.199161	0.066687	-0.671105	0.083662	0.17117	0.05437

Data indicating differences in the diet of studied fish are marked in bold

the most abundant components of the diet were Diptera (mostly Chironomidae larvae), mayfly larvae (Ephemeroptera), damselfly larvae (Zygoptera) and crustaceans (Crustacea), while the component with the biggest share in the biomass were molluscs (Kati *et al.* 2015), which were completely absent in our research. In the case of *P. glenii* inhabiting the Włocławek Reservoir (Vistula River, Baltic Sea catchment area, Poland), the main part of the diet consisted of Amphipods and Chironomidae larvae, supplemented by zygopteran larvae and molluscs (Grabowska *et al.* 2009). However, the abundance of Diptera larvae in the stomachs of the *P. glenii* collected in the course of this study was low. This seems to have stemmed from the large size of the Chinese sleeper individuals (above 90 mm) and their tendency to choose larger prey, especially fish, while avoiding small organisms (Diptera and Ephemeroptera larvae) and those which move too quickly, e.g. water bugs (Heteroptera). Koščo *et al.* (2008) also observed that *P. glenii* was less willing to hunt highly-mobile invertebrates.

P. glenii is considered to be a non-discriminating predatory species which may be a direct threat to native macroinvertebrates, but also to fish and amphibians (Koščo *et al.* 2008; Grabowska *et al.* 2009; Reshetnikov & Schliewen 2013). Fish are mostly consumed by the individuals of this species whose length exceeds 50-60 mm (Koščo *et al.* 2008; Grabowska *et al.* 2009; Kati *et al.* 2015), i.e. those that were used in this study. Sinelnikov (1976) stated that *P. glenii* become typical piscivorous predators (that also hunt amphibians) after exceeding a length of 100 mm.

In fact, a large number of Teleostei was found in the stomachs of the fish studied. Among them, juvenile *C. carpio* comprised a large portion. In the case of *P. glenii*, 80% of the consumed fish were recognised as juvenile *C. carpio* that had escaped from ponds; while in the case of the European perch this share was 35%. The presence of *P. glenii* in a watercourse used for draining water from carp ponds suggests that these fish may be present in the ponds themselves, among the juvenile *C. carpio*. Despite its small size, *P. glenii* is a voracious predator, which is capable of feeding on fish whose length exceeds 2/3 of its own body length and even larger prey (Koščo *et al.* 2003; Grabowska *et al.* 2009). Undoubtedly, its presence in ponds cannot be trivialised, as its impact on the production output of fish farms may be considerable. A case in point is a small water reservoir near Sobibór (Poland), which demonstrates the predatory effectiveness of this species: after it had been introduced into the lake due to unknown

circumstances, it almost completely eliminated the lake minnow *Rhynchocypris percunura* Pallas, 1814, a species subject to legal protection, within just one year (Wałowski & Wolnicki 2010). Additionally, in European waters *P. glenii* is known to limit the abundance of native species including the mud minnow *Umbra krameri* Walbaum, 1792, the Crucian carp *Carassius carassius* Linnaeus, 1758, the European bitterling *Rhodeus amarus* Bloch, 1782 and the belica *Leucaspius delineatus* Hackel, 1843 (Koščo *et al.* 2003; Lusk *et al.* 2004; Andrzejewski *et al.* 2011).

The structure of the *P. glenii* diet in various ecosystems confirms the enormous adaptive capabilities of the species. It was demonstrated that, in adapting to the changes resulting from its own environmental pressure, *P. glenii* changes its resources: after eliminating large invertebrates, larvae and the juvenile fish of other species, *P. glenii* started to demonstrate cannibalistic behaviour (Sikorska *et al.* 2013). Furthermore, a group of similar-sized *P. glenii* released into an aquarium quickly reduced itself to one or two of the fastest-growing individuals by consuming all the others (Szczerbik, personal observation).

In new habitats, especially in small water reservoirs and canals without other predatory fish, *P. glenii* often becomes the dominant species (Kottelat & Freyhof 2007; Koščo *et al.* 2003). However, it is also believed that in waters inhabited by native predators (including *P. fluviatilis*), *P. glenii* remains subject to their pressure (Rakauskas *et al.* 2019). As the results of the present study demonstrate, the competition between *P. glenii* and *P. fluviatilis* may be a significant problem, because the diets of the similar-sized individuals of these species overlap to a large extent. The diet overlap among the captured *P. glenii* and *P. fluviatilis*, expressed as the diet overlap index (Z), was 68.642; while the (PERMANOVA) did not demonstrate statistically significant differences in the taxonomic structure of the diets of the European perch and the Chinese sleeper ($p = 0.074$). The analysis of the major diet components in both species indicated that the niche used by *P. fluviatilis* is broader than that used by *P. glenii* (Table 4, Fig. 4). This suggests a great versatility of *P. fluviatilis* in using the available resources, and may also indicate that it reacts to the pressure introduced by *P. glenii*.

Percottus glenii prefers standing or very slow-moving waters, with a large number of macrophytes, as well as ponds and oxbow lakes (Kottelat & Freyhof 2007; Andrzejewski *et al.* 2011). Thus, in terms of its habitat preferences, the species is much less versatile than *P. fluviatilis*, which is found in very diverse habitats ranging from estuaries through various types of lakes to large and small rivers (Kottelat & Freyhof 2007).

As was mentioned before, *P. fluviatilis* is considered to be one of the predators that are able to limit the abundance of *P. glenii* in European waters through predation. However, this can happen only if perch individuals of an appropriate size are present in the ecosystem being threatened by *P. glenii* (larger than the consumed Chinese sleeper individuals). In the present study, it has been demonstrated that by competing for food resources, *P. glenii* may affect the population of *P. fluviatilis*. However, this impact may be minimised by the high plasticity of the latter, including its ability to use a broader dietary niche. Nonetheless, before reaching a total length of 10-14 centimetres, *P. fluviatilis* seems to be potentially at risk (in the case of limited resources) of competing with *P. glenii*, and thus may be forced to search for diet components in a broader dietary niche than before. In Polish waters, *P. fluviatilis* reaches this size only in the third or fourth year of life (RECHULICZ 2008), and it is believed that it switches to feeding on fish when it reaches a total length (TL) of about 15 cm (Antosiak 1963; Bączkowska 1965). However, this is not always the case. It was demonstrated that among *P. fluviatilis* aged 0+, a division may already occur into a slow-growing planktivorous cohort and a fast-growing piscivorous cohort (during the first months of their life), but both these cohorts remain at risk of predation by other fish (Beeck 2003; Borcharding 2006). An early switch to piscivory is typically observed in eutrophic water bodies with a limited abundance of zooplankton, but with a large quantity of larvae and juvenile Cyprinidae (Mehner *et al.* 1996; Beeck *et al.* 2002). One such example is Lake Speldrop on the Lower Rhine in Germany (Borcharding 2006). In controlled conditions, the growth rate of *P. fluviatilis* larvae is greater than that of *P. glenii* larvae (Szczerbowski *et al.* 2003; Sikorska *et al.* 2013), which makes the species more resistant to the *P. glenii* pressure in the initial period of its life. This is different to other fish such as *C. carassius* or *R. percnura*, whose larvae grow slower (compared to *P. glenii*) and remain less active for a longer period of time (Wolnicki *et al.* 2004; Sikorska *et al.* 2013). Even in the early months of its life, *P. glenii* may be a hazard to both the spawn and larvae of these species, especially given the fact that feeding on the spawn and larvae of fish has already been observed in individuals that have reached a total length of 20 mm (Voskoboinikova & Pavlov 2006).

In the present study, it was also demonstrated that *P. glenii* feeds on juvenile *C. carpio* individuals. This is not surprising, given the voracity and versatility of the Chinese sleeper. However, it is important from the perspective of pond aquaculture, as *Perccottus glenii* is very hard to eradicate in the earthen ponds used in the production of *C. carpio* and other Cyprinidae

(Wałowski & Wolnicki 2010).

The observations conducted to date (in waters around the world) have clearly shown that the primary weapon against invasions of alien species should be prophylactic actions. In the case of species that have already settled in open waters, it is necessary to further monitor their habitats and to study their impact on the local ecosystems, taking into consideration the local characteristics.

The authors believe that this paper may serve as an introduction to further studies on the *P. glenii* biology and its possible interactions with species native to European waters.

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Author Contributions

Research concept and design: P.S.; Collection and/or assembly of data: P.S., J.N.; Data analysis and interpretation: P.S., M.N., J.N., E.L.-T., E.D.-K., J.C., W.P.; Writing the article: P.S., J.N., E.L.-T.; Critical revision of the article: M.N., E.L.-T., E.D.-K., J.C., W.P.; Final approval of article: P.S., M.N., J.N., E.L.-T., E.D.-K., J.C., W.P.

Conflict of Interest

The authors declare no conflict of interest.

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