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Inter-Population Variability of Diet of the Alien Species Brown Bullhead *(Ameiurus nebulosus)* from Lakes with Different Trophic Status

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Abstract

The alien fish species brown bullhead (*Ameiurus nebulosus*) has had a direct impact on native fish species through competition for food and predation in European water bodies. The aim of the study was to determine the diversity, frequency of occurrence and abundance of types of food in the stomachs of 278 individuals of *A. nebulosus* from seven lakes in Central-Eastern Europe with different trophic status.

Altogether, 20 types of food (including taxa) in the diet of *A. nebulosus* were found and the most frequently eaten were detritus, Chironomidae, plants and selected taxa of macrofauna. In the diet of fish from lakes with higher trophic status the higher share of detritus and the lower number of type of food were noted. Fish in the diet were present only in brown bullhead from two lakes, but in the other lakes in the diet, fish scales were reported.

The present study shows that *A. nebulosus* in the lakes of Central - Eastern Europe is an opportunist and the composition of its diet is strictly dependent on habitat. In addition, due to its flexibility, this alien species can easily adapt its food preferences to potential food resources in occupied water bodies.

Introduction

The problems associated with the occurrence of invasive species of fish in water bodies are pertinent in many European countries. The causes and routes of the spread of non-indigenous species, including invasive ones, to new areas are well known (Wheeler, 1978; Holčík, 1991). However, there is still little information on their range of occurrence and particularly on their impact on aquatic ecosystems and on the communities of organisms inhabiting the aquatic ecosystems they have dominated. Invasive species can unquestionably affect specific species and/or entire ecosystems, but their impact has economic importance as well (Copp *et al*., 2005). In recent years, the effects of alien and invasive species on colonised eco-systems and individual species have been widely investigated. These effects include predation, competition for food and habitat, hybridisation, habitat degradation and alteration, and the transmission of pathogens and parasites (Lockwood, Hoopes, & Marchetti, 2007; Grabowska, Kotusz, & Witkowski, 2010; Rabitsch *et al*., 2013). However, the most damaging and undesirable effect seems to be their direct impact on native species through predation and competition for food and habitat.

The brown bullhead, *Ameiurus nebulosus* (Lesueur, 1819) comes from North America, where it occurs naturally in the Mississippi and Missouri River catchment areas. The brown bullhead was introduced to several European countries (e.g. Spain, Germany, England, France, the Netherlands, Belgium and Austria) in the second half of the 19th century (Welcomme, 1988; Elvira & Almodóvar, 2001; Copp *et al*., 2005). Currently, according to many sources listed by Rutkayová, Biskup, Harant, Šlechta, and Koščo (2013), *A. nebulosus* has been reported in 37 European countries. Information about its occurrence is still limited, although several authors report that in some places it can create stable populations and continue to be a significant part of the fish fauna (Holčík, 1991; Keith & Allardi, 1998; Rechulicz, Płaska, Tarkowska-Kukuryk, Mieczan, & Pęczuła, 2015).

Due to its range of occurrence, and sometimes its very large proportion of the fish fauna, the brown bullhead's impact through predation and competition for food seems to be crucial. However, to better understand it, the specific types of food eaten by this invasive species must be identified. In the area of its natural occurrence, several studies have been conducted on the diet of the brown bullhead, providing a basic characterisation of the types of food and also investigating the impact of various factors on the composition of its diet (Massengill, 1973; Klarberg & Benson, 1975; Gunn, Qadri, & Mortimer, 1977; Kline & Wood, 1996). In areas where *A. nebulosus* has successfully invaded (New Zealand), Barnes and Hicks (2003) also attempted to determine the composition of the diet of this species. In addition, the ability of *A. nebulosus* to prey on juvenile fish was studied by Bigun and Afanasyev (2011).

The brown bullhead is known to have a well-developed sense of smell and poor eyesight (Keast, 1985b). The composition of its food may be dependent on the availability of prey in the environment and on the size of fish prey (Keast, 1985a). In the species' natural area of occurrence, the young can feed on zooplankton; later they consume macro-invertebrates, and mature individuals feed on fish and amphibians (Keast, 1985a). As reported by Gunn *et al.* (1977) and Hill, Duffy, and Thomson (1995), macrophytes and filamentous algae may also be a food resource for the brown bullhead. This food can be efficiently digested and absorbed by this species. On the other hand, some authors suggest that the brown bullhead is able to meet its nutritional requirements with detritus, sewage and acid-tolerant invertebrates (Massengill, 1973).

There is a lack of information about the diet of the brown bullhead in invaded areas in Europe, although there are reports about the food of a congeneric, *A. melas*, in the Iberian Peninsula (Leunda et *al*., 2008) and the UK (Ruiz-Navarro, Britton, Jackson, Davies, & Sheath, 2015). As this species is a regular component of the fish fauna of some regions of central-eastern Europe (Kornijów, Rechulicz, & Halkiewicz, 2003; Rechulicz *et al.,* 2015) it is interesting whether it has a variable diet or a clear preference for certain types of food. Another interesting question is whether the choice of food depends on the habitats in which the brown bullhead lives. This study is one of the first attempts to describe the diet and food preferences of the brown bullhead in the lakes of central-eastern Europe. The aims of the present study are i) to determine the diversity of the food of the brown bullhead in lakes with different trophic status; ii) to identify what kind of food is most often eaten by this invasive species in relatively new areas of occurrence, and iii) to determine what kind of food dominates the diet of the brown bullhead.

Materials and Methods

Specimens of *A. nebulosus* were collected from seven lakes located in Łęczna-Włodawa Lakeland (south-eastern Poland) in the summer months (June-September) of 2011-2015 (Table 1). The material was taken from the littoral zone of all the lakes, at depths from 1.0 to 1.5 metres, using four separate sets of multi-mesh gillnets (1.5 m deep, 30 m long, 12 mesh sizes from 5 mm to 55 mm knot-to-knot) (Appelberg, 2000; CEN document, 2005). In each lake, controlled sampling was conducted within a two-year period, always in May, August and October. Lakes Skomielno, Piaseczno and Głębokie were sampled in 2011-2012, Lakes Domaszne, Czarne, and Białe in 2012-2013 and Lake Glinki in 2014-2015. For each fish sampling, nets were exposed for 12 hours, from 6 PM to 6 AM.

The assessment of water quality in all of the lakes studied was based on the physical and chemical parameters of the water, such as total phosphorus (TP), chlorophyll *a* (Chl *a*) and water transparency (Secchi disc - SD). The limnological analyses were carried out according to the relevant methodology, and laboratory analyses were performed. The measurements of the variables listed above were carried out three times each summer in all years of observation (Hermanowicz, Dożańska, Dojlido, & Koziorowski, 1976). To determine the trophic status of the lakes, based on the main water quality parameters (TP, Chl *a* and SD), the average Carlson index (TSI) was calculated by the formula TSI =

Table 1. Morphometric, trophic, and fishery characterisation, as well as some physical and chemical variables (mean ± sd) of water of the studied lakes; * according to Smal, Kornijów, & Ligęza, 2005 - modified); sd - standard deviation, $^{A, B, C, D}$ - values of selected variables in row marked the same letter are not different at P≤0.05

	Skomielno	Piaseczno	Glinki	Głębokie	Domaszne	Czarne	Białe
Years of study	2011 - 2012	2013 - 2014	2013 - 2014	2011 - 2012	2014 - 2015	2014 - 2015	2014 - 2015
GPS coordinates	N 51º29'17'' E 23º0'40''	N 51º23'47'' E 23º1'45''	N 51º30'24'' E 23º33'32''	N 51º28'33'' E 22º55'22''	N 51º28'16'' E 23º0'10''	N 51º30'57'' E 23º1'40''	N 51º31'7'' E 23º2'36''
Maximum depth (m)*	5.5	38.8	8.8	7.1	3.1	15.6	2.7
Average depth (m)*	2.0	12.6	2.8	3.4	2.4	5.1	1.3
Surface area (ha)*	75.3	84.7	40.9	20.3	81.7	39.0	136.9
Water mixing type*	polymictic	dimictic	polymictic	polymictic	polymictic	polymictic	polymictic
Fishery lake type *	tench-pike	bream-vendace	bream-perch	tench-pike	tench-pike	tench-pike	tench-pike
Secchi disc (m)	1.4 ± 0.1^{A}	4.6 ± 0.5^{A}	0.3 ± 0.1^{D}	0.9 ± 0.2^{BC}	0.9 ± 0.2^{B}	$0.5 \pm 0.1B^{CD}$	0.4 ± 0.1^{D}
TP (µg dm^{-3})	104.0 ± 69.8^{C}	28.3 ± 32.6^{A}	163.7 ± 86.9^{D}	73.7 ± 13.6^{BC}	319.3 ± 74.7^{D}	263.7 ± 124.6^{D}	240.3 ± 92.2^{D}
Chl *a* (µg dm^{-3})	6.5 ± 2.5^{A}	5.3 ± 0.9^{A}	218.6 ± 26.8^{C}	44.4 ± 2.3^{C}	22.4 ± 0.6^{B}	13.8 ± 1.7^{AB}	60.6 ± 8.6^{C}
TSI	57.5	43.6	79.2	65.0	69.9	69.6	75.6

TSI(SD) + TSI(CHL) + TSI(TP)/3, where: TSI(SD) = 60 – 14.41 ln(SD), TSI(CHL) = 9.81 ln(CHL) + 30.6, TSI(TP) = 14.42 ln(TP) + 4.15 (Carlson, 1977) (Table 1).

All *A. nebulosus* specimens caught were weighed (W) on a laboratory scale (to the nearest 0.1 g) and their total length (TL) was measured (to the nearest 0.1 mm). After weighing and measuring, the fish were killed with an overdose of 2-phenoxyethanol. Then the fish were dissected and their stomachs were immediately preserved in 4% formaldehyde. Altogether, from all lakes, 299 specimens of the brown bullhead were caught. Analysis of their stomach contents revealed that 21 fish had empty stomachs. The remaining 278 individuals were used to determine their diet (Table 2).

In the laboratory, each individual stomach was weighed (WFS) and dissected and the contents were removed. Then the weight of the empty stomach (WES) and the total weight of the contents (WP) were measured.

During the analysis of the stomach contents of *A. nebulosus*, the prey was sorted and, when possible, identified at the lowest taxonomic level using a Nikon SMZ800 stereomicroscope at maximum magnification of 120 x (Hyslop, 1980; Kołodziejczyk & Koperski, 2000; Rybak, 2000). In each sample, the biomass and/or the number of all types of food and the identified taxa were determined. The unidentified contents, consisting of organic remains, were classified as detritus. All the weight measurements, i.e. full and empty stomachs and all types of food, were made with accuracy within 0.1 mg.

For *A. nebulosus* from all the lakes studied, the relative index of digestive tract fullness (%SF) was calculated according to the following formula: $\%SF = W_P \cdot 100\% / W_{ES}$, where: W_P - biomass of whole food, W_{ES} - biomass of the empty digestive tract (Zacharia & Abdurahiman, 2004). The calculated values of the index of digestive tract fullness of *A. nebulosus* were used to divide the fish into six groups (0 - 5) of different degrees of fullness: 0 - %SF = 0% (digestive tract empty); 1 – 0% < %SF ≤ 25%; 2 - 25% < %SF ≤ 50%; 3 - 50% < %SF ≤ 75%; 4 - 75% < %SF ≤ 100%; 5 - %SF > 100%. For each lake, the percentage share of individual groups of fish with varying degrees of digestive tract fullness was determined.

The frequency of occurrence ($\%F_i$) of individual groups of food and taxa in the stomachs of *A. nebulosus* from each lake were calculated using the formula $\%F_i = N_a/N \cdot 100\%$, where: N_a - number of fish in which taxon/type of food "*a*" was present, N – total number of examined fish. Moreover, the abundance of biomass of a specific food ($\%P_i$) found in the stomachs was determined by the formula $\%P_i = n_a/n \cdot 100\%$, where: n_a – biomass of specific food belonging to taxon/type of food "*a*", n – total biomass of stomach contents. Furthermore, the biomass of a specific food ($\%P_i$) found in the stomachs was determined by the formula $\%P_i = n_a/n \cdot 100\%$, where: n_a – biomass of prey-specific food belonging to taxon/type of food "*a*", n – total biomass of stomach contents (Zacharia & Abdurahiman, 2004). The Shannon-Wiener (H') diversity index was calculated for the macrofauna individuals found in the stomachs of *A. nebulosus*.

To determine the feeding strategy of *A. nebulosus*, Costello's (1990) graphic method was used with modifications suggested by Amundsen, Gabler, and Staldvik (1996). This method is based on a two-dimensional scale, where each point represents occurrence ($\%F_i$) and prey-specific abundance ($\%P_i$) of components in the diet of fish.

The distribution of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) were tested for all data obtained. This made it possible to choose appropriate statistical tests (parametric or non-parametric). The non-parametric Kruskal-Wallis ANOVA test H was used to detect any variability in the trophic status of the lakes, based on physical and chemical variables such as Secchi disc (SD), total phosphorus (TP) and chlorophyll *a* (Chl *a*). To compare the average weight of food, the number of types of food, the number of macroinvertebrates individuals found, and the diversity index (H') from each lake, non-parametric ANOVA (Kruskal-Wallis, test H) was used as well.

Spearman correlation analysis was performed to

Table 2. The total length (TL, in mm), body mass (W, in g) and the groups of degree of fullness of brown bullhead digestive tracts from studied lakes; sd – standard deviation

Lake	N	Total length (TL, in mm)		Body mass (W, in g)	Groups of relative degree of fullness of digestive tracts (%)					
		Mean ± sd	Min - Max	Mean ± sd	0	1	2	3	4	5
Skomielno	52	145.2 ± 18.7	100.0 - 180.0	36.3 ± 14.9	7.7	46.1	15.4	3.9	7.7	19.2
Piaseczno	34	155.9 ± 10.3	140.0 - 181.0	47.6 ± 13.4	0.0	32.0	20.0	24.0	16.0	8.0
Glinki	32	168.0 ± 21.9	134.0 - 210.0	77.3 ± 33.5	0.0	56.2	31.2	12.6	0.0	0.0
Głębokie	78	152.5 ± 27.7	100.0 - 230.0	48.1 ± 30.6	20.8	40.3	9.1	5.1	1.3	23.4
Domaszne	31	172.7 ± 18.4	120.0 - 214.0	60.2 ± 34.2	0.0	67.7	22.6	6.5	0.0	3.2
Czarne	36	166.1 ± 10.7	148.0 - 183.0	51.7 ± 14.2	0.0	50.0	25.0	16.7	8.3	0.0
Białe	36	160.6 ± 11.1	137.0 - 180.0	38.5 ± 23.3	0.0	66.7	8.3	16.7	0.0	8.3

determine the relationship between the abundance of different types of food in the stomach contents and the parameters characterising the trophic status of each lake.

All the analyses and statistical tests were carried out using the Statsoft Statistica package v. 10 for Windows at a significance level of $P \leq 0.05$.

To verify the similarity of food abundance in different populations of *A. nebulosus*, a cluster analysis (with Bray-Curtis as similarity index) was conducted using Biodiversity Pro software (McAleece, Gage, Lambshead, & Paterson, 1997).

Results

The lakes in which the *A. nebulosus* populations were sampled for stomach content analysis had varying trophic status (Table 1). The water from Glinki, Domaszne, Czarne and Białe Lakes had significantly higher values for chlorophyll *a* (Chl *a*) and total phosphorus (TP) than the other lakes. The lowest values of these key physical and chemical variables were recorded in Lake Piaseczno (Kruskal-Wallis, test H): for TP (6, N = 205) = 150.75; P< 0.0001; for Chl *a* (6, N = 205) = 190.18, P< 0.0001). The average calculated for the Carlson index (TSI) ranged from 43.60 for Lake Piaseczno to 79.22 for Lake Glinki, and clearly distinguished these lakes (Table 1).

The total length (TL) of the brown bullhead individuals ranged from 100 mm to 230 mm. The brown bullheads with the highest average TL (172.7 ± 18.4 mm) were found in Lake Domaszne. The analysis of digestive tract fullness showed that *A. nebulosus* individuals with empty stomachs were observed only in Lake Skomielno and Lake Głębokie, where more than 20% of individuals had empty stomachs (Table 2). In addition, in Lakes Domaszne, Piaseczno, Biale, Skomielno and Głębokie, a relatively large portion of the samples (3.2%, 8.0%, 8.3%, 19.2%, and 23.4%) had overfilled digestive tracts (%SF > 100%) (Table 2).

In total, 20 types of food were found in the analysed digestive tracts. Of these, 19 types of food were found in individuals from most lakes, while two taxa (specified as "Other" i.e. the remains of amphibians and other Diptera) were recorded only in Lakes Głębokie, Czarne and Białe. The highest numbers of food types were recorded in the diet of fish from Lake Piaseczno (14) and the least in fish from Lake Białe (10). Lake Białe also differed significantly from the others in the number of food types and in H´ (Kruskal - Wallis, test H for number of food types (6, N = 278) = 27.77; *P* = 0.0001 and for H´ (6, N = 278) = 22.15; *P* = 0.0011). In the digestive tracts of the fish from the other lakes, the number of food types ranged from 11 to 13 (Table 3). The types of food present in the stomachs of all brown bullhead populations were detritus, parts of plants, sand, Chironomidae (larvae) and Coleoptera (adults). The presence of Tubificidae in the food was noted only in the stomachs of fish from Lake Piaseczno. Maize used by anglers as fishing bait was found in the diet only in Lake Domaszne (Table 3). The analysis shows that detritus was the most frequent food type of the brown bullhead in almost every lake. Its presence varied from 34% (Lake Głębokie) to 100% (Lake Czarne). In addition, Chironomidae were found in the diet of a large portion of individuals (from 46% to 84%) from all populations (Table 3).

The greatest biomass of food was recorded in the stomachs of brown bullheads from Lakes Skomielno and Głębokie, with fish making up the largest share. Analysis of the taxonomic features of fish (number of fin rays, shape of fins, etc.) showed that the fish prey were small individuals (TL max. 70 mm) of species such as roach, bleak, rudd and ruffe. In addition, fish prey was present only in the stomachs of specimens with a total length above 135 mm. Plant food (parts of plants and filamentous algae) and detritus also had significant biomass in the stomachs of fish from all lakes (Table 4). Overall, the median biomass of food found in the stomachs of brown bullheads from all the lakes was similar, with statistically significant differences found only between the biomass of the stomach contents in fish from Lakes Skomielno and Piaseczno (Kruskal-Wallis test H (6, N = 278) = 13.99; *P* = 0.03).

Analysis of the percentage share of biomass of specific prey (%P_i) in the food of brown bullheads showed that in Lakes Skomielno and Głębokie their diets were dominated by fish and that this food accounted for 65% and 79.8%, respectively, of all biomass of food (Figure. 1). Moreover, in the stomachs of fish from other lakes (excluding Lake Glinki) the scales of large fish were found. This demonstrates that brown bullheads readily eat carrion. Detritus dominated in the diets of the fish in four of the surveyed lakes, i.e. Białe, Czarne, Domaszne and Glinki. It accounted for 51% to 83.5% of the biomass of their food in these lakes. In addition, in Lakes Skomielno, Piaseczno and Glinki, plants made up a significant share of the biomass of the diet of *A. nebulosus*. In the first two lakes, however, plant parts were found in this category of food, while in Lake Glinki mainly filamentous algae of phytobenthic origin were noted (Figure. 1).

There were differences in the average number of individuals of macrofauna prey in the food of the brown bullheads from each lake. The greatest average numbers of macrofauna prey were noted in the stomachs of brown bullheads from Lake Glinki (29.12 ± 28.23) and Lake Piaseczno (26.60 ± 68.41), while the fewest macrofauna individuals were eaten by fish from Lake Głębokie (6.51 ± 9.43) and Lake Skomielno (10.63 ± 12.26) (Figure. 2). The analysis of the medians showed differences in the number of macrofauna prey eaten only in the case of Lake Głębokie, where the number of macro-fauna prey was significantly lower than in Lakes Glinki and Domaszne (Kruskal-Wallis test H (6, N = 278) = 24.484; *P* = 0.0004) (Figure. 2). Significant differences in the diversity index (H') of

Table 3. Number of types of food and occurrences (%F_i) of prey and others in the stomach contents of brown bullhead from the studied lakes; H' – Shannon–Wiener index for macrofauna; * – significant differences at P≤0.05

Lake Type of food	Skomielno	Piaseczno	Glinki	Głębokie	Domaszne	Czarne	Białe
Detritus	52.08	64.00	75.00	34.43	87.10	100.00	91.67
Plants	29.17	56.00	43.75	21.31	22.58	16.67	8.33
Sand	2.08	48.00	18.75	14.75	19.35	50.00	8.33
Zooplankton	6.25	56.00	12.50	14.75	0.00	0.00	0.00
Chironomidae	60.42	68.00	75.00	45.90	83.87	75.00	66.67
Trichoptera	43.75	16.00	0.00	19.67	22.58	8.33	0.00
Mollusca	22.92	0.00	0.00	0.00	3.23	41.67	8.33
Odonata	0.00	0.00	6.25	13.11	3.23	0.00	0.00
other Diptera	10.42	0.00	6.25	27.87	6.45	50.00	0.00
Ephemeroptera	25.00	8.00	12.50	1.64	0.00	33.33	8.33
Coleoptera	10.42	12.00	6.25	4.92	9.68	41.67	16.67
Assellus aquaticus	14.58	8.00	6.25	6.56	0.00	0.00	8.33
Corixidae	6.25	4.00	0.00	0.00	22.58	0.00	0.00
Ostracoda	0.00	4.00	18.75	0.00	0.00	0.00	0.00
Tubifex	0.00	8.00	0.00	0.00	0.00	0.00	0.00
Hydracarina	0.00	4.00	6.25	0.00	3.23	0.00	0.00
Corn	0.00	0.00	0.00	0.00	19.35	0.00	0.00
Fish	33.33	0.00	0.00	40.98	0.00	0.00	0.00
Scales	0.00	4.00	0.00	0.00	3.23	16.67	33.33
Other	0.00	0.00	0.00	4.92	0.00	8.33	16.67
No. of types of food	13	14	12	13	13	11*	10*
H' for macrofauna	0.21	0.10	0.07	0.14	0.05*	0.18	0.05*

Table 4. Biomass of food types (in mg) of the brown bullhead from studied lakes; life stage: lvr – larve, im – imago; sd –standard deviation, * – significant differences at P≤0.05

Lake	Skomielno	Piaseczno	Glinki	Głębokie	Domaszne	Czarne	Białe
Type of food	mean ± sd	mean ± sd	mean ± sd	mean ± sd	mean ± sd	mean ± sd	mean ± sd
Detritus	35.07 ± 60.57	87.17 ± 11.75	120.75 ± 134.08	19.93 ± 69.52	164.92 ± 176.68	223.65 ± 211.97	189.35 ± 133.19
Plants	59.08 ± 217.71	200.45 ± 334.84	86.94 ± 184.31	45.28 ± 176.26	2.21 ± 6.55	1.64 ± 4.14	10.63 ± 36.83
Sand	0.01 ± 0.06	28.39 ± 56.61	5.91 ± 14.32	3.07 ± 14.00	52.69 ± 290.88	12.88 ± 43.50	-
Zooplankton	0.04 ± 0.21	73.86 ± 155.04	0.35 ± 1.20	4.29 ± 15.04	-	-	-
Chironomidae (lvr)	2.55 ± 6.13	13.91 ± 38.62	20.54 ± 32.44	2.08 ± 3.86	27.63 ± 68.67	2.43 ± 2.41	18.29 ± 41.10
Trichoptera (lvr)	1.80 ± 3.75	0.27 ± 0.70	-	1.00 ± 3.31	4.54 ± 19.36	4.58 ± 15.88	-
Mollusca	4.22 ± 17.39	-	-	-	0.02 ± 0.13	1.38 ± 2.87	0.65 ± 2.25
Odonata (lvr)	-	-	0.56 ± 2.25	2.29 ± 12.31	0.04 ± 0.20	-	-
Other Diptera (lvr)	0.04 ± 0.15	-	0.33 ± 1.30	0.35 ± 0.98	1.44 ± 6.00	9.74 ± 20,58	-
Ephemeroptera (lvr)	0.47 ± 1.37	0.12 ± 0.44	0.19 ± 0.54	0.04 ± 0.37	-	0.23 ± 0.34	0.01 ± 0.03
Coleoptera (im)	0.44 ± 2.20	1.06 ± 2.96	0.36 ± 1.43	6.12 ± 37.94	0.14 ± 0.52	8.49 ± 15.39	0.99 ± 2.32
Assellus aquaticus	1.42 ± 5.48	1.14 ± 5.68	0.76 ± 3.05	0.55 ± 2.85	-	-	0.37 ± 1.27
Corixidae (im)	0.01 ± 0.05	0.20 ± 1.02	-	-	28.07 ± 77.39	-	-
Ostracoda	-	0.01 ± 0.04	0.30 ± 0.80	-	-	-	-
Tubifex	-	80.01 ± 400.00	-	-	-	-	-
Hydracarina	-	0.02 ± 0.10	0.01 ± 0.05	-	0.01 ± 0.05	-	-
Corn (baits)	-	-	-	-	20.29 ± 66.62	-	-
Fish	194.25 ± 583.81	-	-	572.45 ± 1417.86	-	-	-
Scales	-	14.24 ± 71.20	-	-	0.03 ± 0.14	1.21 ± 2.94	14.35 ± 30.82
Other	-	-	-	60.10 ± 529.09	-	1.48 ± 5.14	1.53 ± 4.72
Medians	74.70*	339.30*	177.29	88.30	146.80	182.35	218.49

macrofauna were found only in the case of fish from Lakes Domaszne and Białe (Kruskal-Wallis test: H (6, N = 278) = 22.15; *P* = 0.0011) (Table 3).

The analysis of the brown bullheads' feeding strategy (compilation of frequency of occurrence of food (%F_i) with the share of specific food in the total biomass (%P_i)), showed that the brown bullheads in all lakes ate mainly Chironomidae, but the biomass of this food category was relatively small (Figure. 3). The feeding strategy of the brown bullheads from five of the lakes concentrated on detritus, while fish were the most preferred food only in Lakes Skomielno and Głębokie. In Lake Piaseczno the fish ate mostly plants. In Lakes Glinki and Domaszne, this category of food was also chosen more frequently than other types of food. In Lake Piaseczno most of the brown bullhead individuals used many types of food resources (Figure. 3b).

The cluster analysis of the diet data showed high similarity of the stomach contents of *A. nebulosus* in Lakes Domaszne, Czarne and Białe (Figure. 4). The analysis of the similarities in the biomass of the food of brown bullheads from the surveyed lakes indicated that fish from Lakes Białe and Czarne had very similar

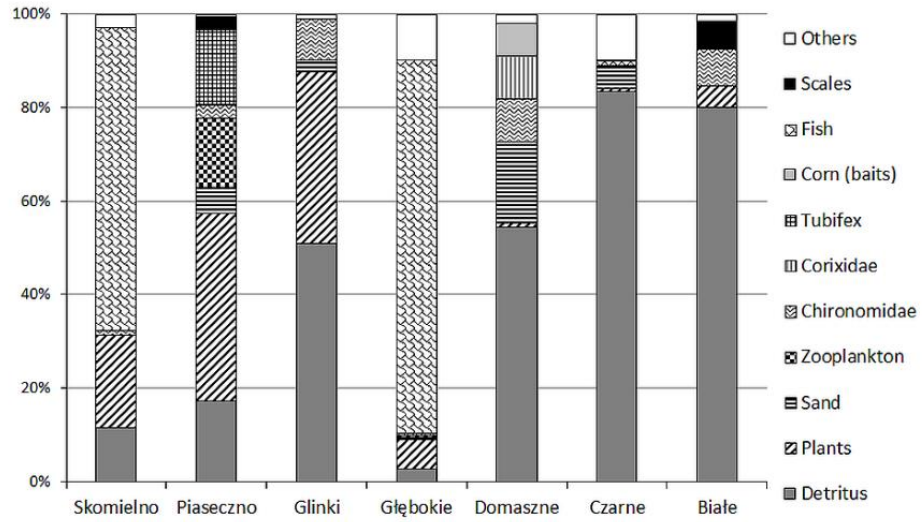

Figure 1. Abundance of biomass (%P) of types of food in the contents of brown bullhead digestive tracts from the studied lakes; taxa with share in biomass %P < 5% marked as Others.

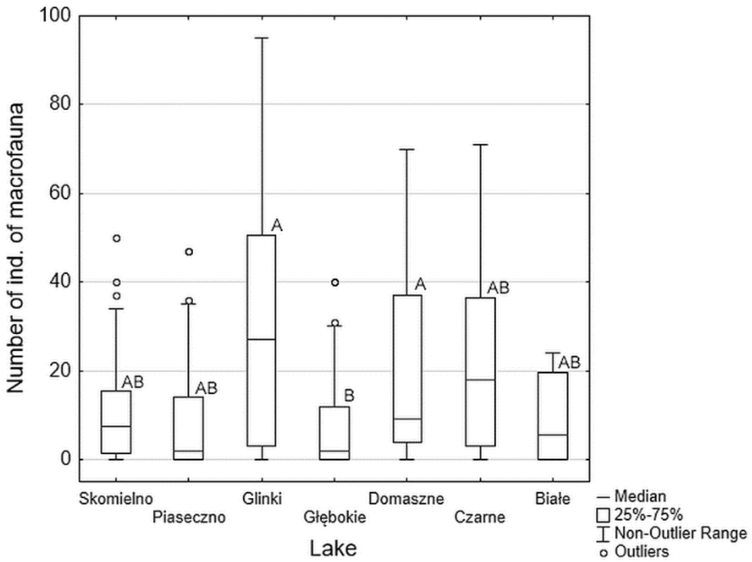

Figure 2. Numbers of individuals of macrofauna prey identified in the brown bullhead digestive tracts in the studied lakes; differences in medians with the same letter are not statistically significant at P≤0.05.

food (similarity more than 70%) (Figure. 4). The brown bullheads from Lakes Glinki and Domaszne also had similar stomach contents to the fish from Lakes Białe and Czarne. The food of the brown bullheads from Lakes Skomielno and Głębokie was different from that of the fish from the other five lakes, but very similar to each other (>70%) (Figure. 4).

Spearman correlation analysis revealed the highest correlations between total phosphorus (TP) and biomass of food type. The large share of detritus in the biomass of the stomach contents of the brown bullheads was significantly correlated with the content of total phosphorus in the lakes ($r = 0.353$, $P<0.05$). Moreover, this water parameter was negatively correlated with the share of biomass of zooplankton and biomass of fish in the diet of *A. nebulosus* (Table 5).

Discussion

This is one of the first studies on the composition of the diet of the alien fish species *A. nebulosus*, which is very common in water bodies in Europe. The results demonstrate the diversity in the diet of populations of this species from lakes of varying trophic status located in central-eastern Europe.

Previous studies of the diet composition of A. *nebulosus* in its native areas (North America) have shown a wide range of contents in their digestive tracts. Food types ranging from detritus to fish eggs, macrofauna and small fish were found in the stomachs

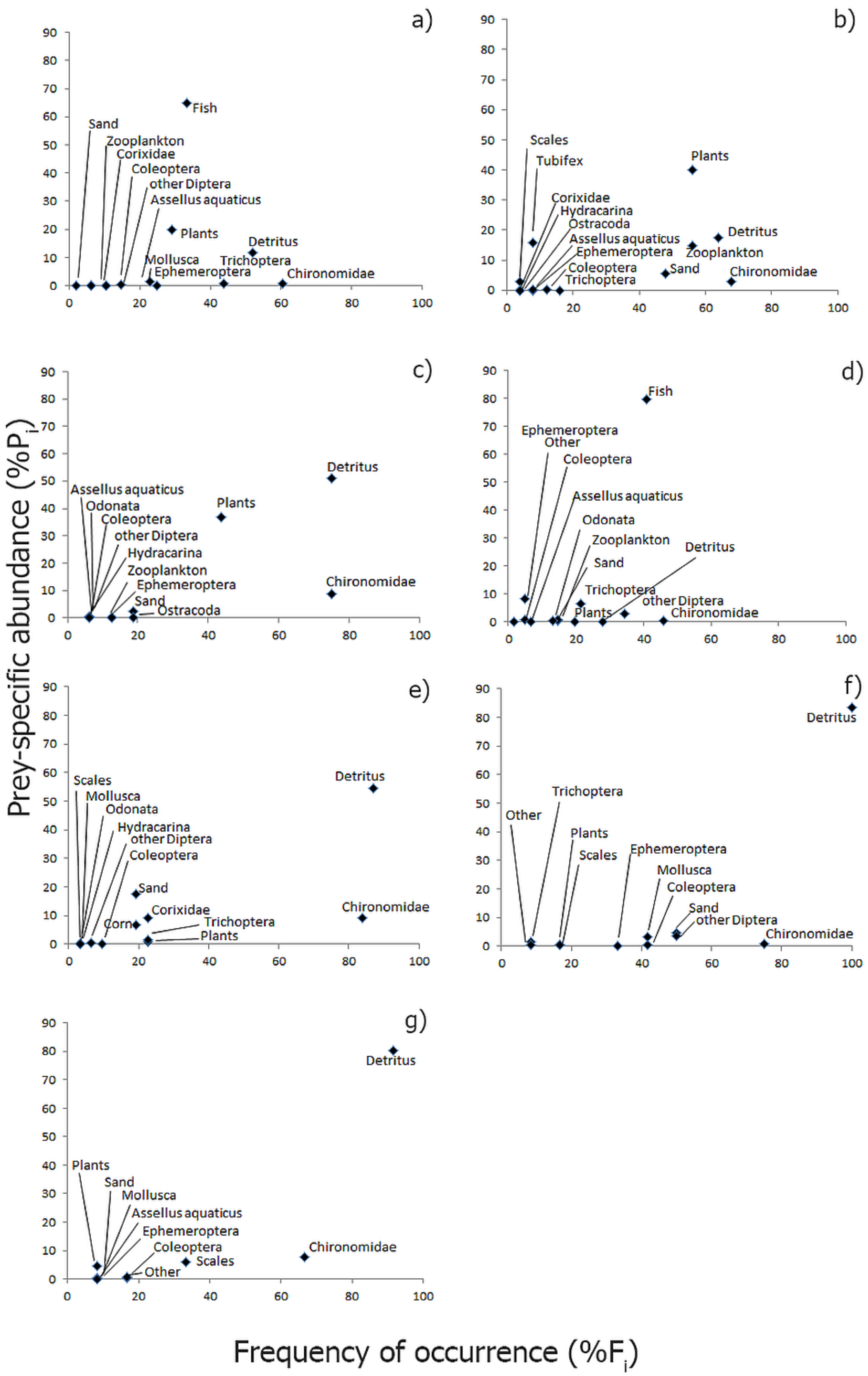

Figure 3. Feeding strategy plots (percentage occurrence of prey (%F_i) and prey-specific abundance (%P_i) for brown bullheads in the studied lakes; a) Skomielno, b) Piaseczno, c) Glinki, d) Głębokie, e) Domaszne, f) Czarne, g) Białe.

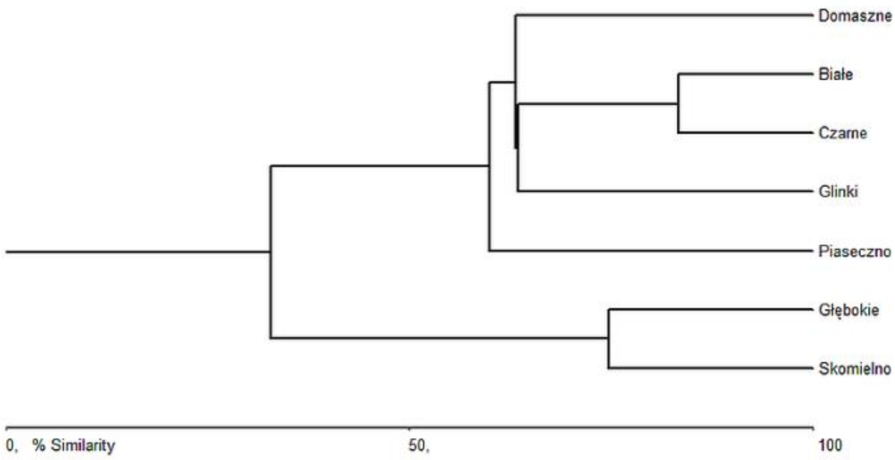

Figure 4. Dendrogram resulting from the cluster analysis of prey abundance in brown bullhead stomachs from different lakes (Bray-Curtis index used).

Table 5. Spearman correlations between the food biomass of *A. nebulosus* and variables characterising the trophic status of the studied lakes (SD - Secchi disc, TP - total phosphorus, Chl *a* - chlorophyll *a*); * - statistically significant difference at P≤0.05

	SD	TP	Chl *a*
Detritus	-0.206*	0.353*	0.033
Plant	0.202*	-0.128	-0.137*
Zooplankton	0.256*	-0.340*	-0.158*
Sand	0.019	-0.055	-0.084
Macroinvertebrates	-0.068	0.167*	-0.035
Fish	0.079	-0.225*	0.038
Scales	-0.176*	0.165*	0.073
Other	-0.108	0.031	0.139*

of these fish (Keast, 1985a). In areas where *A. nebulosus* has been found in recent years (1980s) (New Zealand, Lake Taupo), it has also been shown to eat very diverse food (Barnes & Hicks, 2003). Young fish preferred Chironomids, Cladocera, gastropods, caddis fly larvae, plant material and detritus, whereas crayfish, invertebrates and fish were found in the stomachs of larger specimens. In the present study, the range of food found in *A. nebulosus* digestive tracts was very wide and included 20 food types (Table 3).

Food composition has also been shown many times to be dependent on the type of lake and/or reservoir. Our present observations provide information on both the diet composition of the brown bullhead in newly invaded areas and its variability in lakes with different trophic states. Many authors in Europe (Wheeler, 1978; Holčík, 1991; Grabowska *et al.,* 2010), have previously reported that the brown bullhead, as a non-indigenous, invasive species, competes successfully for food with native fish species. It may also affect the ecosystems at different levels of the trophic pyramid, as an herbivore and as a predator. A possible influence was confirmed by the analysis of food in the lakes, particularly in Lakes Skomielno, Glinki, Głębokie and Piaseczno, where parts of plants and fish constituted the largest portion (Figure. 1). This is very important when this species makes up a significant portion of the fish fauna in reservoirs and lakes in relatively newly inhabited areas (about 100 years) (Holčík, 1991; Kornijów *et al.,* 2003; Rechulicz *et al.,* 2015).

In native areas, invertebrate macrofauna has been shown to be the basic food for *A. nebulosus* (Scott & Crossman, 1973; Keast, 1985b). According Kline and Wood (1996), irrespective of the body size of *A. nebulosus*, Chironomid larvae and pupae and also the amphipod *Hyalella* sp. were dominant components of its diet.

The present observations have shown that the trophic variability of lakes may in some way modify the feeding strategy of brown bullheads. Thus plants or fish were important food items in some lakes (i.e. Skomielno, Piaseczno and Głębokie), and detritus in others (i.e. Domaszne, Czarne and Białe) (Figure. 1 and 3).

Moreover, research conducted by Leunda *et al*

(2008) on the Iberian Peninsula and by Ruiz-Navarro *et al* (2015) in the UK showed that *A. melas* ate similar food. In addition, the digestive tracts of brown bullheads have been shown to be adapted for efficient digestion of flora tissues (i.e. filamentous algae) (Gunn *et al.*, 1977). All of this demonstrates that despite its diverse geographic locations, the brown bullhead has a similar, wide range of food types.

In our observations, there was noticeable variation in food type depending on the trophic levels of the lakes, expressed by Chl *a*, Secchi disc and total phosphorus (TP) values. TP was the indicator that seemed to most influence the occurrence of individual taxa and types of food in the stomachs of *A. nebulosus* (Table 5). In lakes where the TP value increased, the abundance of detritus in the fishes' stomachs was the highest (Figure. 1). While this dietary component was present in most of the brown bullhead stomachs examined, irrespective of the lake, its contribution varied (Table 3). Similarly, a significant share of detritus in the diet of *A. nebulosus* and in that of the congener *A. melas* in their native area was reported by Klarberg and Benson (1975) and Pouilly, Barrera, and Rosales (2006), respectively. The significant contribution of this type of food in the stomachs is probably a result of its passive intake during penetration of the bottom of the reservoirs and lakes. This method of actively searching for potential prey by the brown bullhead was confirmed in an experiment conducted by Bigun and Afanasyev (2011). This was particularly evident in eutrophic lakes, with little vegetation and large areas of exposed deposits in the littoral zone (i.e. Lakes Glinki, Domaszne, Czarne and Białe, Figure. 1). Another food source was plants, which were favoured by fish in Lakes Piaseczno, Glinki and Domaszne. Chironomidae were very frequently found in the stomachs, but they constituted a small part of the food biomass. Nevertheless, the vast majority of individual brown bullheads in all the lakes ate this food (Figure. 3). According to many authors, this is the basic food of fish from the Ictaluridae family in their native areas of occurrence (Scott & Crossman, 1973; Klarberg & Benson, 1975; Hill *et al.,* 1995; Kline & Wood, 1996; Leunda *et al.*, 2008).

Our research has shown that the brown bullhead, due to its high plasticity and opportunism, has a varied diet composition in lakes located in non-native areas of occurrence. This diversity was also observed in the number of taxa and types of food, as well as in the number of macrofauna prey found in the fishes' stomachs. In the lakes with higher values of trophic indicators, fewer taxa and food categories were recorded in the stomachs of the fish (Table 3). In addition, more macrofauna prey individuals were found in the stomachs of fish from lakes with a higher Carlson index (eutrophic lakes) (Figure. 2, Table 3). The larger number of macrofauna prey is probably linked to the greater productivity of the lakes and to foraging methods. It may also be related to the increasing amount and share of detritus in their diet, as explained above. In Lake Skomielno, with a slightly lower Carlson index, more taxa and food categories were recorded in the diet of *A. Nebulosus* diet (Figure. 3b). At the same time, in this lake the brown bullheads specialised in eating fish, which were dominant in their diet. This was also evident in the smaller numbers of macrofauna in the diet of the fish in this lake (Figure. 2).

In general, the statistical analysis (cluster analysis) showed that the diet of *A. nebulosus* was similar in lakes with similar trophic conditions. Therefore, the lakes were clearly divided into two distinct types, in terms of trophic group (Figure. 4).

The brown bullhead can have a very direct impact on native fish in colonised areas where it is invasive. Such a direct impact was confirmed in an experiment conducted by Bigun and Afanasyev (2011), who reported that *A. nebulosus* prefers the spawn of pike than perch. Declerck, Louette, De Bie, and De Meester (2002), in a study conducted in interconnected ponds, found that fish was one of the basic food types for *A. nebulosus*. The prey mainly consisted of fish species occurring in ponds in large numbers, i.e. topmouth gudgeon, rudd and roach. On the Iberian Peninsula, the presence of fish in the diet was also recorded in the stomachs of congener *A. melas* (Leunda *et al*., 2008).

Similarly, an impact of the brown bullhead on fish was found in our research. This type of food was present in the stomach contents of fish from two lakes (Skomielno and Głębokie) and was recorded in 30-40% of individuals (Table 3). In addition, this type of food accounted for more than 60% of the biomass of the food of *A. nebulosus* from these lakes. In addition, the remains of fish were found in the form of scales in the stomachs of the fish from almost all lakes (except Lake Glinki) (Table 3, Figure. 1). These scales came from the remains of larger fish, which brown bullheads would not be able to capture. This is confirmed by the presence of soft tissue parts and the absence of bones in the digestive tracts. This shows that the brown bullheads readily eat flesh of dead fish, and that its source may be carrion. It is possible that at times it may be the most easily available food in the water body. It also confirms the opportunism of *A. nebulosus* in its choice of food type.

The data analysis showed that fish as prey were only recorded in *A. nebulosus* individuals with a TL above 135 mm. This is confirmed by previous studies reporting that individuals bigger than 140 or 150 mm preyed on fish (Declerck *et al.*, 2002; Leunda *et al.*, 2008). Possibly, due to other habitat conditions, nutrients and growth conditions, brown bullheads in Europe can prey upon fish earlier. As shown in a related species, in a study by Copp *et al.* (2016), some features of the species may change when it invades new territories.

In conclusion, *A. nebulosus*, as an alien fish species with a capacity for highly variable and flexible feeding, should be regarded as opportunistic, as evidenced by the highly varied diet composition found for the fish from the lakes studied. Our findings demonstrate that this species can easily use the potential resources available in the water bodies it inhabits as its main food source and can affect other groups of organisms, especially in reservoirs and lakes where it becomes abundant.

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