Feeding and niche differentiation in three invasive gobies in the Lower Rhine, Germany

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ABSTRACT

Since 2006, three invasive Gobiids from the Ponto-Caspian area established in the River Rhine and their abundances nowadays regularly exceed 80% of the fish community. Between 2009 and 2011, densities of gobies in the Rhine increased while their condition decreased, assuming that the populations are approaching or even reached the capacity of the ecosystem. Consequently, we hypothesized a high level of competition on food resources within this group of invasive gobies that all exhibit the same sedentary life style, which might strengthen the differentiation of the ecological niche on a spatial and temporal axis. Invasive gobies were caught with electro fishing and beach seining in different types of habitats over a period of two years in the Lower Rhine, analyzing the food of more than 1500 gobies of the three species *Neogobius fluviatilis* (NF), *Neogobius melanostomus* (NM) and *Ponticola kessleri* (PK). All species showed an opportunistic feeding strategy. In NF and PK, a clear shift in preferred food resources was observed between individuals smaller and larger 50 mm that occurred in parallel with a habitat shift from sandy areas to riprap structures in PK, but not in NF that were only found on gravel and sand. In contrast, there were no distinct changes in food and habitat preference in NM. Small NM were found from spring to autumn on the sandy nearshore areas where they competed on food resources with juvenile PK in spring, and with NF in late summer, respectively. Abundance of juvenile NF and NM increased during the night in sandy nearshore areas in October. This behavior is assumed as predator avoidance against large piscivorous NF as well as native pikeperch, because active feeding occurred mainly during the day. The results on the three invasive Gobiids in the Lower Rhine give important hints how fine-tuned spatial and temporal characteristics in intra- and inter-specific competition shape the ecological niche of these invaders in their new environment.

Introduction

The diet of fishes as an important part of the ecological niche has widely been analyzed, whereby many factors were documented to influence resource use on a spatial and temporal axis (e.g., Ross, 1986 for a review). The diet choice of individuals can be connected to ontogenetic (Mittelbach and Persson, 1998), behavioral or morphological aspects (Skúlason and Smith, 1995 for a review). In addition and most important, various aspects of food intake are connected to size-dependent morphological (e.g., gape size, Mittelbach and Persson, 1998; Nilsson and Brönmark, 2000), physiological (e.g., vision: Hairston and Li, 1982, reactive distance: Breck and Gitter, 1983, digestion: Persson et al., 2004) or behavioral traits (e.g., Magnhagen and Borcherding, 2008; Nakayama and Fuiman, 2010). Thereby, changes of foraging ability or growth rates with size

(Wilson, 1975) can cause discrete ontogenetic diet shifts, executed both toward lower (Benavides et al., 1994; Durtsche, 2000) and higher trophic positions (Mittelbach and Persson, 1998; Cereghino, 2006). As fish assemblages are often strongly size-structured, not only different species but also different size- or age-cohorts of one species (often named ecological species) interact with each other, such that resource partitioning (Ross, 1986) can be related to the avoidance of intra- (Persson and Greenberg, 1990a,b) and inter-cohort competition (Werner and Hall, 1977; Persson, 1986) or predation pressure (Werner et al., 1983).

Neozoans have to successfully meet new environmental conditions, such as climate, predators, diseases or parasites (deRivera et al., 2005). Besides environmental conditions and predators, also competition experienced in non-native areas determines invasive success of neozoans (see Sakai et al., 2001 for a review). In almost all cases reported the invader was shown to be competitively superior to native species in both, interference and exploitative competition, leading to reduced survival, growth (Sakai et al., 2001) and even competitive displacement of native species (Williamson, 1997). For

multiple invasions Simberloff and Von Holle (1999) suggested the term "invasional meltdown" to describe "the process by which a group of nonindigenous species facilitate one another's invasion in various ways, increasing the likelihood of survival and/or of ecological impact". This hypothesis was tentatively supported for the ecosystem of the Great Lakes, where "direct positive (mutualistic and commensal) interactions among introduced species are more common than purely negative (competitive and amensal) interactions" (Ricciardi, 2001).

Strong inter-specific competition might also be found in Ponto-Caspian gobies invading Europe and North America (Grabowska and Grabowski, 2005; Gozlan et al., 2010), when negatively interacting with the native fauna (Jude et al., 1992; Corkum et al., 2004) as competitors for habitats (Bauer et al., 2007) and food (Bergstrom and Mensinger, 2009), and as predators on eggs of other species (Chotkowski and Marsden, 1999) and small fishes (Grabowska and Grabowski, 2005). At this point four species are documented in the River Rhine, Germany, namely tubenose goby (*Proterorhinus seminularis*), bighead goby (*Ponticola kessleri*), round goby (*Neogobius melanostomus*) and monkey goby (*Neogobius fluviatilis*) (in chronological order, cf., Borcherding et al., 2011). Thereby, gobies not only interact with native species but might also be affected by each other, as all invasive gobies have the same sedentary life style. Although interaction between invasive gobies has not yet been studied in detail, data on pairs of other closely related fish species revealed high dietary overlap (Copp and Kovac, 2003; Salgado et al., 2004; Specziar, 2005), hence also suggesting strong inter-specific competition on food between invasive Ponto-Caspian gobies.

Assuming a high level of differentiation for species competing on a similar ecological niche, we studied invasive gobies in the Lower Rhine to disentangle factors influencing diet, and related to this, performance of invasive species. In this sense the present study focussed on the most abundant three invasive goby species in the River Rhine, Germany, analyzing (1) size-dependent (ontogenetic) differences in diet, (2) habitat-specific diet use, (3) and seasonal (4) as well as dial aspects in the usage of food resources. Such results are essential to understand the different spatial and temporal characteristics of the ecological niche of these invaders and to estimate their future potential impact on the ecosystem.

Materials and methods

Fish sampling

All gobies in this study were caught at the Lower Rhine (Rhine km 832–846) using different methods at the different types of habitat (for details on the sampling sites and habitat description cf., Borcherding et al., 2011). At rip-rap structures, fish were caught at daytime by electro fishing with portable backpack units (maximum output 225/300 V, frequency: 75–85 Hz; fitted with a 40/20 cm, elliptical, anode of stainless-steel with netting of 4 mm mesh size, Lena f. Bednář, Czech Republic, cf., Borcherding et al., 2011). In areas of gravel and sand at the groin fields samples were taken with a beach seining net (10 m × 1.5 m, mesh size 1 mm) that was regularly dragged 20 m against the current (all sampling dates are given in Table S1, Supporting information).

Based on preliminary studies that indicated rapid re-colonization of freshly sampled areas, beach seining in October 2010 was repeatedly conducted in the morning (directly after sunrise), the afternoon and at night (about 2 h after sunset) with always 3 replicate samples at fixed locations (Rhine km 842; 20 m length, around 5 m width, thus approx. 100 m²). This sampling procedure was repeated three times within 10 days, only changing the starting time of each consecutive sampling series (first series start in the afternoon, second series start at night, third series start in the morning), resulting in a full factorial design and a total of 27 samples. For all samples, smaller individuals were directly fixed in ethanol (approx. < 50 mm total length (TL)), while larger fish were stored in a cooling bag on ice and subsequently conserved at a temperature of $-18\,^{\circ}$C.

Gastrointestinal analysis

With the gastrointestinal analysis of fish information on the food uptake during the last approximately 8 h can be obtained (cf., Hyslop, 1980). After sex was noticed of at least all invasive gobies ≥ 70 mm TL (roughly 25%), total length was measured to the nearest 1 mm and the fish was weighted to the nearest 0.001 g. In the next step the stomach was removed from the fish following the instructions of Gertzen (2010) and weighted to the nearest 0.0001 g. Afterwards the digestive tract content was removed and finally the empty digestive tract was re-weighted. In 23 out of the more than 1500 dissected individuals the alimentary tracts were empty and not considered in further analysis. The volumetric amount of each prey taxon was visually estimated as percentage of the total sample volume (Polacik et al., 2009).

For each fish the weight of the consumed prey, the index of stomach fullness (ISF) (Hyslop, 1980) and the adjusted condition factor (ACF), based on Fulton's condition factor (Bagenal and Tesch, 1978) were calculated by using the following formulas:

$$W_{\mathrm{prey}} = W_{\mathrm{stomach\ full}} - W_{\mathrm{stomach\ empty}}$$

with W_{prey} = weight of the prey items [g], $W_{\mathrm{stomach\ full}}$ = weight of the full stomach [g] and $W_{\mathrm{stomach\ empty}}$ = weight of the stomach without prey items [g].

$$\mathrm{ISF} = \frac{W_{\mathrm{prey}}}{W} \times 100$$

with W = weight of the fish [g]

$$\mathrm{ACF} = 10^5 \times \frac{W - W_{\mathrm{prey}}}{\mathrm{TL}^{-3}}$$

with TL = total length of the fish [mm]

Although we did not eviscerated body cavity prior to analysis (e.g. potential differences of testis and gonads that may affect results), regularly no sex-dependent differences in condition and stomach fullness were observed (Knutzen & Krüger, University of Cologne, pers. comm.). Further, no consistent differences in the sex ratio occurred in our samples. This allowed to analyze condition and stomach fullness independently of the factor gender.

Analysis of the dietary overlap (O_D) was calculated according to Schoener (1970) using all 55 types of food items observed in this study:

$$O_{\mathrm{D}} = 1 - 0.5 \times \sum \left| (p_{ix} - p_{iy}) \right|$$

where p_{ix} is the percentage of a food item i in species or size class x, and p_{iy} percentage of a food item i in species and or size class y. The dietary overlap varies between 0 and 1 with the value 0 as no overlap and 1 as a total overlap. Wallace (1981) considered the overlap to be biologically significant when the index value exceeds 0.6 (i.e. 60% similarity).

Feeding strategy and prey importance are graphically presented using the modified Costello method (Amundsen et al., 1996) to point out differences between species and size classes. For this analysis as well as the graphical presentations the prey categories were computed for different categories (e.g. zooplankton, crustacean, insects), including the category "Rest" (e.g. fish spawn, annelida, plant material) and indeterminable compounds. The prey specific

abundance (P_i) of every prey category was plotted against the frequency of occurrence of the prey category (%F_i), with:

$$\%F_i = \frac{N_i}{N} \times 100$$

with N_i = the number of fish with prey i in their stomachs and N = the number of all analyzed fish, and

$$P_i = \frac{\sum S_i}{\sum S_{ti}} \times 100$$

where S_i = the summarized amount of prey i and S_{ti} = the stomach content weight of those fish who consumed prey i.

The feeding strategy is constituted along the vertical axis with specialization in the upper part and generalization in the lower part of the diagram. A fictive axis from the lower left to the upper right describes the importance of every prey category. A point in the upper right represents a dominant prey, while in the lower left the prey is rare. Information about the niche width contribution is shown from the upper left (high between-phenotype component) to the lower right (high within-phenotype component) (Amundsen et al., 1996).

Statistical analysis

To achieve homogenous variances when Levene-tests revealed inequality, statistical analysis of the ACF was performed on log transformed data. The log transformed ACF for each species was used as dependent variable in an ANCOVA, with "Year" (2009–2011) as independent factors and "TL" as covariate, followed by a *post hoc* analysis, in which the ACF was described with a linear regression in relationship to the TL. Also based on log transformed data, the ISF as dependent variable was analyzed with an ANOVA using "Species" (*N. fluviatilis*, *N. melanostomus*, *P. kessleri*) and "Season" (spring, summer and autumn) as independent factors. This analysis was performed separately for gobies <50 mm TL caught on gravel and sand, and for fish ≥50 mm TL that were caught in the riprap structures.

Goby densities in the sampling experiment on gravel and sand in October 2010 were compared on a dial basis using Kruskal–Wallis tests. For this sampling, the Levene test revealed equal variances of the ISF between the different samples, allowing an ANOVA on the ISF as dependent variable using "Species" (*N. fluviatilis*, *N. melanostomus*), "Size-class" (gobies smaller and larger 50 mm TL) and "Daytime" (morning, afternoon, night) as independent factors. For the interaction term "Species × Daytime", different groups were compared using a *t*-test. All statistics were computed using SPSS (Ver. 19.0, SPSS Corp.).

Results

Condition

In total more than 1500 gobies of the three species *N. fluviatilis*, *N. melanostomus* and *P. kessleri* were analyzed (Table S1, Supporting information). For all three species the adjusted condition factor (ACF) significantly increased with increasing size of the fish (as covariate within the ANCOVA model: $F_{1,1554} = 2001.4$, $p < 0.0001$; *N. fluviatilis*: ACF = 0.368 + 0.0058 TL, $n = 258$, $r^2 = 0.417$; *N. melanostomus*: ACF = 0.430 + 0.0117 TL, $n = 675$, $r^2 = 0.702$; *P. kessleri*: ACF = 0.423 + 0.0079 TL, $n = 629$, $r^2 = 0.636$). In parallel to increasing densities of gobies in the riprap structures of the Lower Rhine, condition significantly decreased between 2009 and 2011 (ANCOVA: $F_{2,1554} = 16.1$, $p < 0.0001$), at least for *N. melanostomus* and *P. kessleri* (Fig. 1).
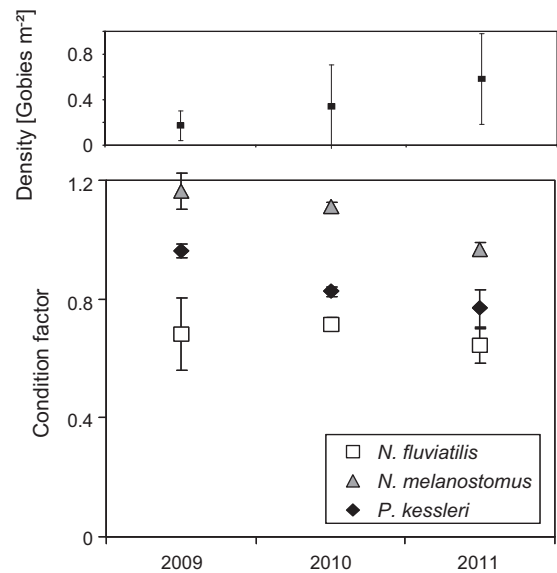

Fig. 1. Density (mean ± standard deviation) of invasive gobies in the Lower Rhine (km 832–846) based on electro fishing data from riprap structures between 2009 and 2011 (top), and adjusted condition factor (mean ± 95% conf. limits) of three Gobiid species, presented as result from the ANCOVA ($r^2 = 74.1\%$) and calculated for a mean goby size of 56.3 mm TL (bottom).
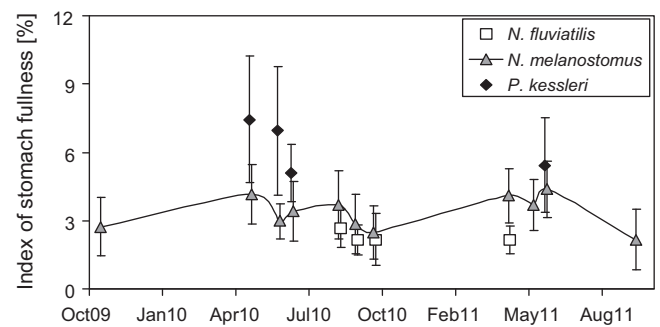
Size dependent feeding

Averaging all analyzed fish, nearly 90% of the ingested and identified food items belonged to only three categories, Crustacea (approximately 56%, mainly invasive *Dikerogammarus villosus*), Chironomid larvae (approximately 21%) and Mollusca (approximately 11%, mainly invasive *Dreissena* sp. and *Corbicula* sp.). The three most important food items for each species showed considerable variation with the size of the gobies. Chironomid larvae and Crustacea were most important for the smallest individuals in all three species. While in *N. melanostomus* the importance of these food items changed more steadily with increasing size, there was a distinct switch between the smallest and the second smallest size class in *N. fluviatilis* and especially in *P. kessleri*, from Chironomid larvae to Crustacea as most important food resource (Fig. 2). With increasing size of the fish the importance of Chironomid larvae decreased for all three species close to zero and Crustacea was then the most important food resource. Although only based on low numbers of the largest individuals (>125 mm TL) of all three species (*N. fluviatilis* and *N. melanostomus* each $n = 3$, *P. kessleri*, $n = 9$), a second switch of the most important food item loomed, revealing Mollusca for *N. melanostomus*, and fish for *N. fluviatilis* and *P. kessleri* as dominant resource in the diet, respectively.

With respect to the first distinct switch in food items between the smallest and the second smallest size class in *N. fluviatilis* and *P. kessleri* and less fundamental changes with further increasing size (also only low numbers of individuals in the largest size class), the following analysis of the feeding and niche differentiation was performed for two size classes, individuals smaller and larger than 50 mm TL (Table S1, Supporting information). There were no clear hints of individual specialization for both size classes (i.e. no data points in the upper left corner of the Costello plots) demonstrating a more mixed and opportunistic feeding strategy of the three Gobiid species in the Lower Rhine, with varying degrees to use different prey types (Fig. 3). With the change of the feeding strategy between both size classes in *P. kessleri*, this species also changed the habitat from gravel and sand when being small, to the riprap structures when increasing in size. Although a comparable change of the feeding strategy between the two size classes occurred in
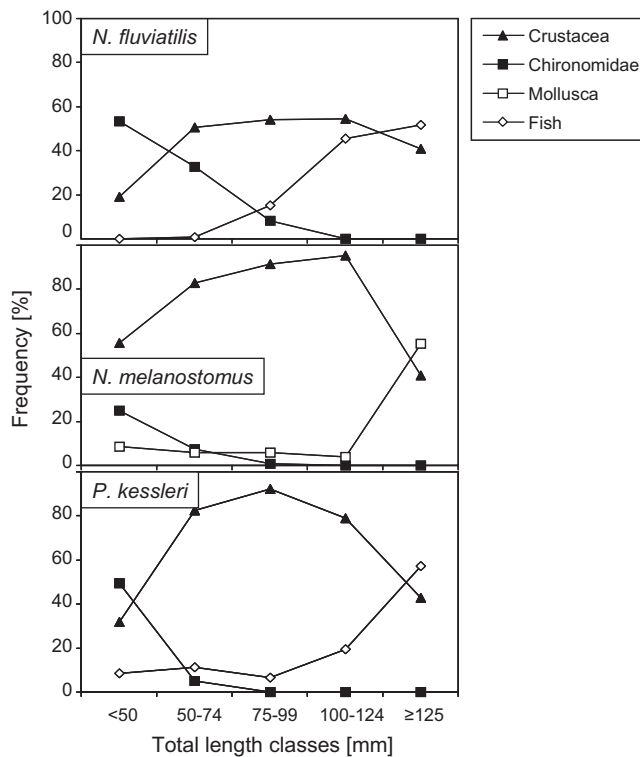
Fig. 2. Frequency of the three most important food categories in dependence to the size of the three Gobiid species (separated in 25 mm size classes) at the Lower Rhine between 2009 and 2011.

N. fluviatilis, this species was found on gravel and sand only, independently of its size. In *N. melanostomus* no clear change of the feeding strategy became obvious with increasing size and larger individuals were found in nearly every catch in both habitats, the riprap structures of the banks and the gravel and sand areas of the groin fields (Fig. 3).
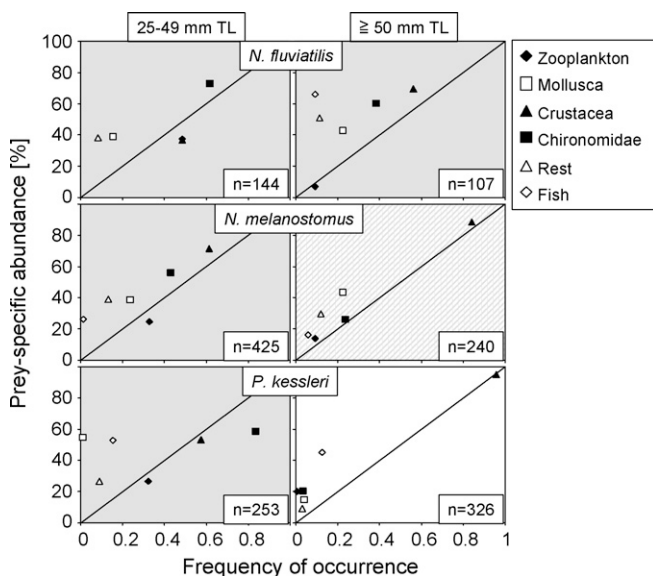

Fig. 3. Feeding strategy (prey-specific abundance plotted against frequency of occurrence of prey in the diet of the predator for the major food items) of two size classes of the three Gobiid species at the Lower Rhine described with the modified Costello diagram (Amundsen et al., 1996). Plots are based on catches of fish <50 mm TL (left) and ≥50 mm TL (right) of each species originating from gravel and sand (highlighted in grey), from riprap structures (highlighted in white) or from both habitats (grey-white striped), respectively.

Fig. 4. Index of stomach fullness (mean ± standard deviation) of fish <50 mm TL of the three Gobiid species caught on gravel and sand in the Lower Rhine between October 2009 and October 2011.

Seasonal feeding of small gobies on gravel and sand

Gobies of all species <50 mm TL (i.e. young-of-the-year, YOY) were only caught in areas of gravel and sand, indicating this habitat as rearing area of all three species in the Lower Rhine. While small *N. melanostomus* were found here at any time, juveniles of both other species occurred in abundance on gravel and sand seasonally, thus, creating inter-specific competitive situations only during distinct periods. The index of stomach fullness (ISF) gives a solid estimation on the quantities of ingested food. For juvenile gobies < 50 mm TL the ISF differed significantly between the species (ANOVA: $F_{2,799}$ = 75.0, $p < 0.0001$, *P. kessleri* ≫ *N. melanostomus* > *N. fluviatilis*) and the season (ANOVA: $F_{2,799}$ = 17.7, $p < 0.0001$, as all three species had significantly more food ingested in spring and summer compared to autumn (Fig. 4). As already indicated by the Costello plots, Chironomid larvae and Crustacea were most important food items for all three species also over the course of the season (Fig. 5). For the small individuals zooplankton occasionally became an important food resource (e.g., at the end of June 2010). In contrast to both *Neogobius* species, juvenile *P. kessleri* < 50 mm TL preyed also on fish to a small amount (on average about 8% of the intestine content). On the other hand *N. melanostomus* used more Mollusca than both other species, especially in autumn (on average about 10% of the intestine content). Despite such small differences in the usage of different food resources, the overall dietary overlap between the species was always above 60% (cf., Fig. 5), indicating an important criterion for significant inter-specific competition when species occur simultaneously on areas of gravel and sand.
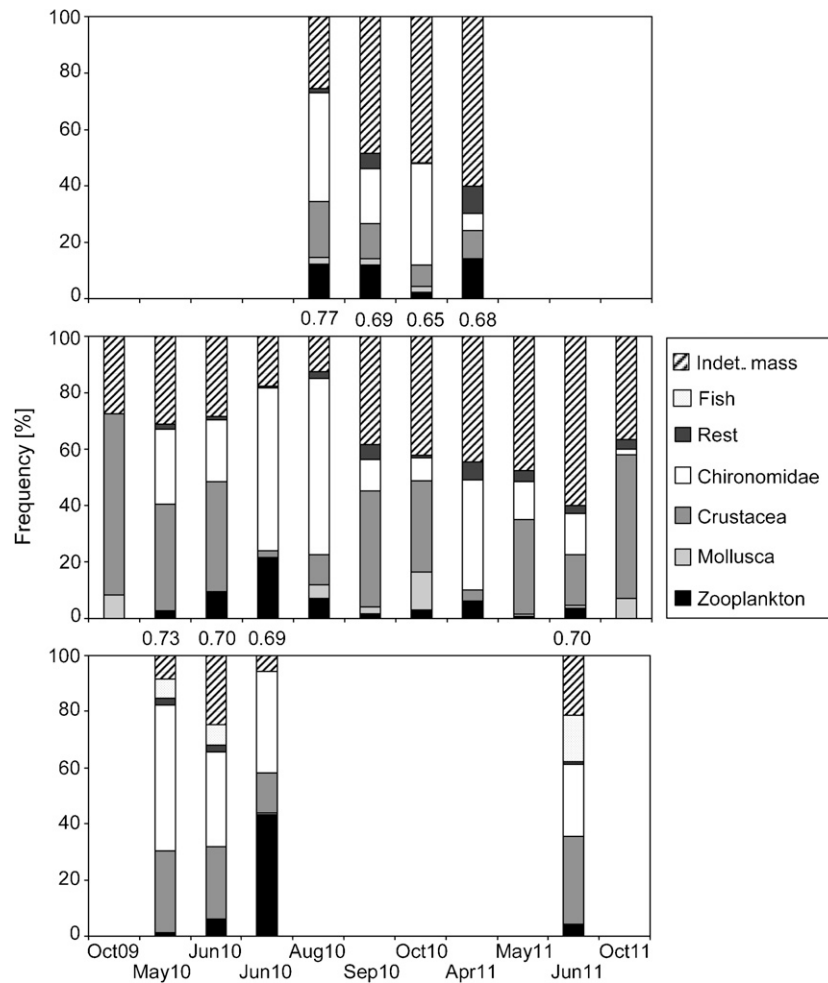
Seasonal feeding of large gobies in riprap structures

Only *P. kessleri* and *N. melanostomus* were caught in the riprap structures of the banks at the Lower Rhine which were never <57 mm TL. Mean size of *P. kessleri* was 89 ± 13.8 mm TL and the majority of *N. melanostomus* was even larger (102 ± 29.3 mm TL). Quantitative analysis of food uptake of these large individuals in the riprap structures revealed comparable results as obtained for small individuals on gravel and sand, with a significantly higher ISF for *P. kessleri* than *N. melanostomus* (ANOVA: $F_{1,384}$ = 13.1, $p < 0.0001$), and significantly lowest ISF values in autumn compared with spring and summer samples (ANOVA: $F_{2,384}$ = 26.1, $p < 0.0001$; Fig. 6). Regularly Crustacea were the main food resource for both species, except for *N. melanostomus* mainly in the solely riprap structures (compared to riprap structures at the groin fields), where Mollusca dominated the diet in autumn (Fig. 7). Similar to small *P. kessleri* on gravel and sand and in contrast to all size classes of *N. melanostomus*, large *P. kessleri* added a certain amount of fish to its diet, especially in the riprap at the groin fields. While in autumn and spring fish prey consisted mainly of gobiids, it changed in early summer to small cyprinids (detailed data not shown). The dietary overlap between

Fig. 5. Frequency of major food categories found in the intestine of fish <50 mm TL of the three Gobiid species (top: *Neogobius fluviatilis*; middle: *Neogobius melanostomus*; bottom: *Ponticola kessleri*) caught on gravel and sand in the Lower Rhine between October 2009 and October 2011. Indet. mass: food items not identifiable; rest: mainly mites, annelids etc. The dietary overlap between species when occurring together is indicated between the single columns.

both species occurring in the same habitat was much higher in early summer than in autumn (Fig. 7).

*Dial feeding of small and large gobies on gravel and sand*

In October 2010, beach seinings during the daytime and early night caught nearly 1200 fish. More than 87% of these fish were
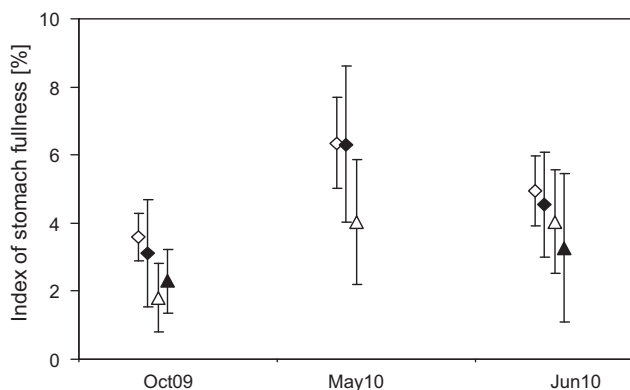

Fig. 6. Index of stomach fullness (mean ± standard deviation) of *Neogobius melanostomus* (triangles) and *Ponticola kessleri* (diamonds) ≥50 mm TL caught in riprap structures at a groin field (white) or at solely riprap structures (black) in the Lower Rhine between October 2009 and June 2010.

gobies, including few *P. kessleri* (6%) not further analyzed here. Some small cyprinids (about 6% of the total catch) were mainly caught during daytime and the percids ruffe (*Gymnocephalus cernuus*, about 2%) and pikeperch (*Sander lucioperca*, about 2%) occurred only during the night. The length-frequency distribution of *N. fluviatilis* and *N. melanostomus* (Fig. S1, information) revealed a distinct increase in small individuals during the night for both species (Kruskal–Wallis-test: $df = 2$, *N. fluviatilis* $p = 0.002$; *N. melanostomus* $df = 2$, $p = 0.011$, Fig. 8). For the individuals ≥50 mm TL a significant increase in abundance during the night was only proven for *N. fluviatilis* (Kruskal–Wallis-test: $df = 2$, $p = 0.011$), but not for *N. melanostomus* (Kruskal–Wallis-test: $df = 2$, $p = 0.823$, Fig. 8).

Dial feeding periods of both species in October 2010 were estimated with the ISF, again revealing the significantly higher gastrointestinal content in *N. melanostomus* than in *N. fluviatilis* (ANOVA $p < 0.001$, Table 1). For both species and independently of size, lowest values of the ISF were observed for individuals that were directly caught after sunrise, evincing lowest food uptake during the preceding night (Fig. 9). Beneath the independent factors "Species" and "Daytime" also the interaction term "Species × Daytime" was significant in the statistical analysis (Table 1). *Post hoc* comparisons of the ISF revealed a significant increase from the morning till the afternoon for both species (*t*-test: *N. fluviatilis* $df = 67$, $p < 0.001$; *N. melanostomus* $df = 112$, $p = 0.012$). While a further significant increase of the ISF until the early night
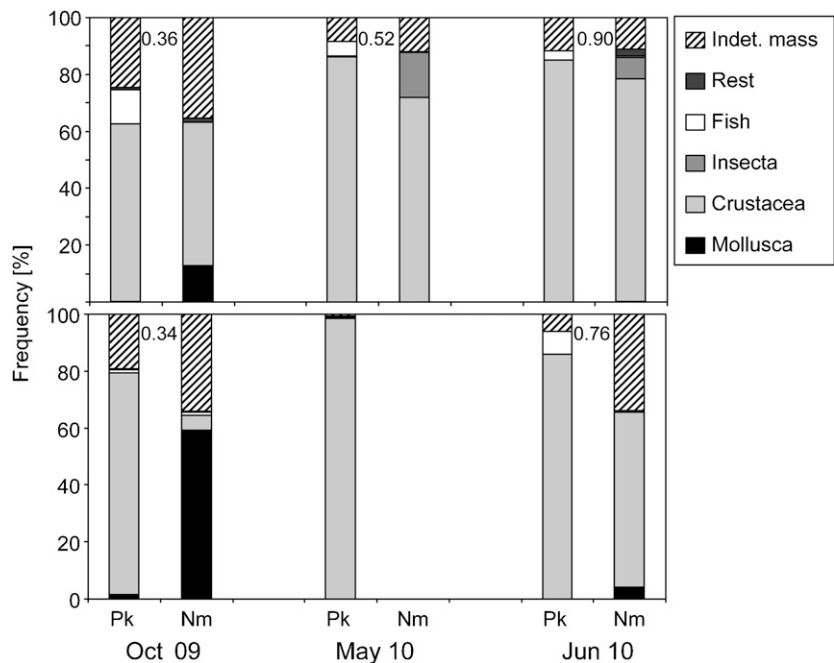
Fig. 7. Frequency of major food categories found in the intestine of *Neogobius melanostomus* (Nm) and *Ponticola kessleri* (Pk) ≥50 mm TL caught in riprap structures at a groin field (top) or at solely riprap structures (bottom) in the Lower Rhine between October 2009 and June 2010. Indet. mass: food items not identifiable; Rest: mainly mites, annelids etc. The dietary overlap between species when occurring together is indicated between the single columns.
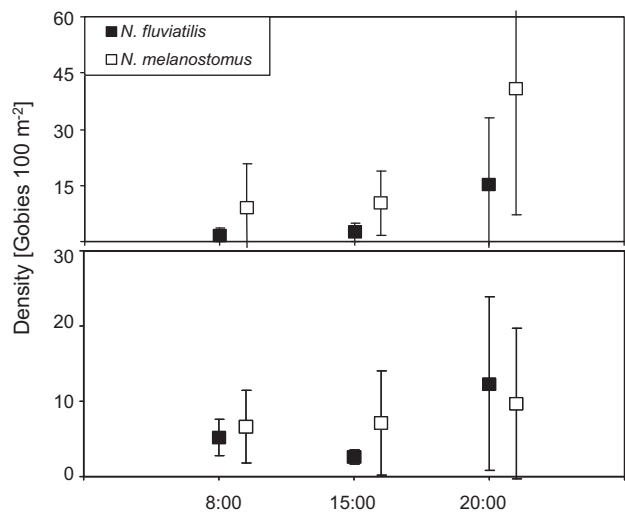
Fig. 8. Density of *Neogobius fluviatilis* (black) and *Neogobius melanostomus* (white) (mean gobies 100 m^{-2} ± standard deviation) caught at three sampling times (8:00 directly after sunrise, 15:00 daytime; 20:00 two hours after sunset) during the sampling experiment on gravel and sand at the Lower Rhine in October 2010, separately for fish <50 mm TL (top) and ≥50 mm TL (bottom).

Table 1
Three-factor ANOVA on the ISF (index of stomach fullness) of gobies sampled on gravel and sand in the River Rhine in October 2010. Independent factors: species (*N. fluviatilis* and *N. melanostomus*), sizeclass (<50 mm TL and ≥50 mm TL) and daytime (morning, afternoon, night). Significant effects are printed in bold.

Source	d.f.	F	p
Species	1	16.07	**<0.001**
Sizeclass	1	0.42	0.519
Daytime	2	18.46	**<0.001**
Species × Sizeclass	2	2.65	0.156
Species × Daytime	2	3.12	**0.045**
Sizeclass × Daytime	2	0.70	0.586
Species × Sizeclass × Daytime	2	0.02	0.989
Error	355		

was found in *N. melanostomus* (*t*-test: *df* = 157, *p* = 0.012), there was a slight tendency in *N. fluviatilis* that the ISF decreased until the night; this change, however, was not significant (*t*-test: *df* = 121, *p* = 0.089). *N. melanostomus* preyed mainly on Crustacea in both size classes, while YOY *N. fluviatilis* fed predominantly on Insecta (mainly Chironomid larvae). Larger individuals of *N. fluviatilis* preferred to feed also on Crustacea, but added a certain amount of fish to its diet, especially during the early night (Fig. 10). Except for the diet of individuals from the morning samples (with significantly less feeding activity in the preceding period, cf., Fig. 9), inter-specific dietary overlap was always higher than the intra-specific dietary overlap between size classes of both species.

Discussion

Based on the analysis of more than 1500 gobies, we found a significant and isometric increase of the condition with size for
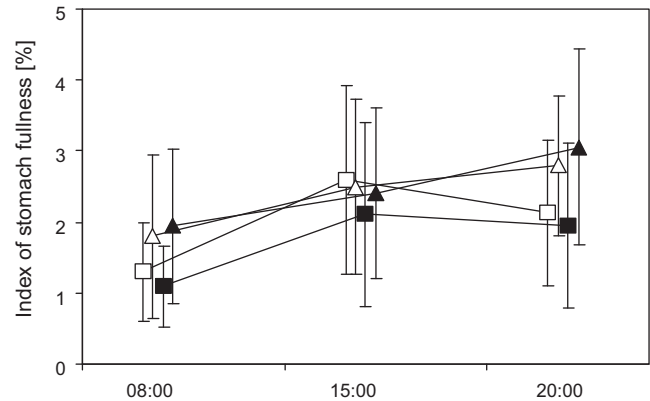
Fig. 9. Index of stomach fullness (mean ± standard deviation) of *Neogobius fluviatilis* (squares) and *Neogobius melanostomus* (triangles) caught at three sampling times (8:00 directly after sunrise, 15:00 daytime; 20:00 two hours after sunset) during the sampling experiment on gravel and sand at the Lower Rhine in October 2010, separately for fish <50 mm TL (white) and ≥50 mm TL (black), respectively.
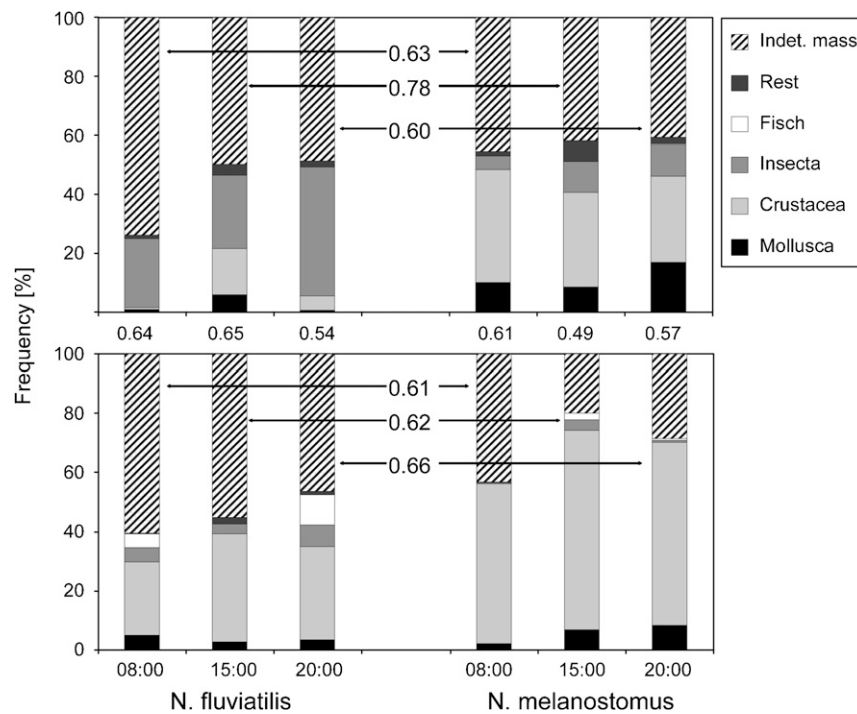
Fig. 10. Frequency of major food categories found in the intestine of *Neogobius fluviatilis* (left) and *Neogobius melanostomus* (right) caught at three sampling times (8:00 directly after sunrise, 15:00 daytime; 20:00 two hours after sunset) during the sampling experiment on gravel and sand at the Lower Rhine in October 2010, separately for fish <50 mm TL (top) and ≥50 mm TL (bottom), respectively. Indet. mass: food items not identifiable; Rest: mainly mites, annelids etc. The intra-specific dietary overlap between both size classes is indicated between the single columns, and the inter-specific dietary overlap between both species is given within the connecting arrows.

all three invasive Gobiid species that is in good agreement to morphological studies on these species (Kovac and Siryova, 2005; L'avrincikova et al., 2005; Capova et al., 2008). Particularly our results revealed, however that condition, at least in *N. melanostomus* and *P. kessleri* significantly decreased between 2009 and 2011 (Fig. 1). As this decrease was in correlation to increasing densities of gobies, we assume our condition data on invasive gobies as a hint that food capacity for fishes in the Lower Rhine is actually at its limits. Especially in a limited environment "ecological differentiation is the necessary condition for coexistence" (competitive exclusion principle, Hardin, 1960), thus, a high level of differentiation is to expect for species competing on a similar ecological niche. In the following, we focussed on spatial and temporal characteristics of inter-specific food consumption that may shape the ecological niche of the three invasive Gobiid species at the Lower Rhine. However, prior to the analysis of inter-specific interactions, knowledge of intra-specific aspects must be obtained, as different size- or age-cohorts of one species may act as ecological species also competing on limited resources.

Size dependent feeding

The most important food items of the gobies in our study (Crustacea, mainly invasive *D. villosus*, Chironomid larvae and Mollusca, mainly invasive *Dreissena* sp. and *Corbicula* sp.) are to a comparable extend also the most abundant macroinvertebrates in the River Rhine (e.g., Borcherding and Sturm, 2002; Bij de Vaate et al., 2002), clearly demonstrating the opportunistic feeding strategy of the three invasive Gobiid species in the Lower Rhine. Within this diet spectrum, Chironomid larvae were obviously preferred by smaller individuals of all species (Fig. 2). In contrast, Mollusca were increasingly preyed on with increasing size, which became obvious, however, mainly in the round goby *N. melanostomus*, while larger individuals of the bighead goby *P. kessleri* and the monkey

goby *N. fluviatilis* increasingly used fish as most important resource, beside Crustacea. The observed diets of the invasive Gobiids in the Lower Rhine are in good agreement to results from different freshwater habitats through Europe (e.g., *N. fluviatilis*: Grabowska et al., 2009; *N. melanostomus*: Polacik et al., 2009; *P. kessleri*: Borza et al., 2009) and North America (here only *N. melanostomus*: Barton et al., 2005) that always exhibited a generalistic and highly flexible feeding strategy. While feeding in competition between the adults of different invasive gobies was analyzed in some occasions (and will be discussed later), size specific differences in resource use of the different species are hardly to obtain in literature.

In *N. fluviatilis* and *P. kessleri* a clear shift in diet was observed between individuals smaller and larger than 50 mm TL (Fig. 3), resembling an ontogenetic diet shift as described for several fish species (e.g., Post and McQueen, 1988; Olson, 1996; Hjelm et al., 2000; Amundsen et al., 2003). In *P. kessleri* this observed dietary shift occurred in parallel with a strict habitat shift (similar to observations e.g. in *Perca fluviatilis*, Persson and Greenberg, 1990c), while all size classes of *N. fluviatilis* were always found on gravel and sand only. In contrast to *N. fluviatilis* and *P. kessleri*, no distinct changes in diet of *N. melanostomus* < 125 mm TL were found in our samples from the Lower Rhine. Additionally, all size classes of this species also occurred simultaneously on gravel and sand, not proving a size-specific habitat shift like in *P. kessleri*. To represent true ontogenetic thresholds or transitions changes in the ontogeny of a population, such shifts should occur to some extent simultaneously, e.g. in ecological interactions, feeding, physiological process, behavioral patterns, and/or shifts in morphology (Werner and Gilliam, 1984; Kovac et al., 1999). Consequently, our results on size-specific feeding and habitat preference give a preliminary systematic order based on ontogenetic transitions, with (1) clear changes in habitat preference and feeding in *P. kessleri*, (2) a clear shift in the use of food resources in *N. fluviatilis* but no changes in habitat preference, and (3) obviously neither a dramatic change in the usage of food resources nor in habitat preference in *N. melanostomus*.

Seasonal aspects of feeding

Species-specific ontogenetic transitions give the overall framework to analyze and evaluate inter-specific interactions, as e.g. distinct habitat shifts may limit the periods of competition. *N. melanostomus* was the only Gobiid species in the Lower Rhine that was found at small sizes in every catch in the areas of gravel and sand. In late spring and early summer the juveniles of *N. melanostomus* (fish that hatched in the previous spawning season) mainly co-occurred with YOY *P. kessleri* that left this area with their habitat shift to the riprap structures in July (Gertzen, 2010). During the period of co-occurrence, high values of dietary overlap were found (mean above 70%, Fig. 5), indicating high levels of inter-specific competition on food resources of juvenile *N. melanostomus* and *P. kessleri* on gravel and sand. This high inter-specific competition was accompanied by significant differences in the index of stomach fullness (ISF) revealing juvenile *P. kessleri* to feed on average more than twice as much as *N. melanostomus* (Fig. 4). These observations raise the question whether one of the species is the better competitor or if there are for instance physiological constraints for either species that are expressed in, or are the reason for the differentiated usage of food resources. Although we have no answer to this question until now, there are, however, some observations during recent behavioral experiments that may give first hints: (1) in single species experiments on behavioral reactions toward food and shelter as well as during periods of acclimatization in the laboratory, *P. kessleri* showed always much higher interest in food than *N. melanostomus* (Borcherding, Hertel, Breiden, unpublished data). (2) Observations revealed that *P. kessleri* clearly lost more weight over longer periods in the laboratory although being fed the same amount of food as were *N. melanostomus* (S. Gertzen, personal communication). These observations may lead to a first hypothesis that metabolic constraints may force *P. kessleri* to feed more than *N. melanostomus* or has to use food with higher energetic value (fish versus Crustacea, cf., Borcherding et al., 2007). However, to prove such a hypothesis on an increased metabolic rate in *P. kessleri* compared to *N. melanostomus*, either daily increments in otoliths have to be analyzed in detail (e.g. Morales-Nin, 2000) or appropriate experiments and measurements have to be conducted.

Whenever juvenile gobies occurred together on gravel and sand there was a high dietary overlap averaging to about 70% (Fig. 5). Competition for food resources between juveniles of invasive Gobiid species were, to the best of our knowledge, never studied in detail until now. Studying a pair of marine gobies (sand goby *Pomatoschistus minutus* and common goby *Pomatoschistus microps*) Salgado et al. (2004) revealed also high dietary overlaps over the season between both species of similar size. Comparable results were shown for stickleback species in English lowland streams (Copp and Kovac, 2003), and the dietary overlap of two species of Sander in Lake Balaton were also high at small sizes, however, rapidly decreased with increasing size (Specziar, 2005).

With increasing size and, thus, decreasing gape size limitations, the potential to use more diverse prey items regularly increases, and consequently, competition on food resources may be reduced (Beeck et al., 2002; De Roos et al., 2003; Borcherding et al., 2010). Similar as for the juveniles, the adults of *P. kessleri* had a significantly higher ISF than *N. melanostomus* while co-occurring in the riprap structures (Fig. 6). The dietary overlap between the larger individuals of both species, however, clearly differed over the season, with high values that indicated biological significant overlaps in summer and only a low dietary overlap in autumn (Fig. 7). High dietary overlaps between the two species were also reported by Copp et al. (2008), without further differentiating between sizes and season. High overlap values may evolve in the absence of competition if shared resources are not limiting, but they can also reflect competition for shared resources (Borza et al., 2009). Studying

three-spined sticklebacks (*Gasterosteus aculeatus*), Svanbäck and Bolnick (2007) demonstrated that increased population density led to reduced prey availability, causing individuals to add alternative prey types to their diet, and confirming that resource competition promotes niche variation within populations. We interpret our diet data in the same sense, assuming increased competition in autumn on lowered food resources that forced differential feeding between *N. melanostomus* and *P. kessleri* in the riprap structures of the Lower Rhine (cf., Borcherding and Sturm, 2002 for seasonal abundance of macroinvertebrates in the Lower Rhine). In contrast to our results, *N. melanostomus* and *P. kessleri* in the riprap structures of the River Danube, Hungary, showed highest dietary overlap in summer and autumn and lowest in spring (Borza et al., 2009). The authors suggested a combination of varying seasonal abundances of macroinvertebrates in combination with morphological constraints between both species as most important to shape the food usage in the River Danube (Borza et al., 2009), however, without addressing changing levels of food abundance.

Dial aspects of feeding

While levels of food abundance and composition of the macroinvertebrate community are known to change seasonally (e.g., Borcherding and Sturm, 2002), occurrence and feeding of fish was shown in many instances to depend also on dial aspects (e.g., Borcherding et al., 2002; Jacobsen et al., 2002). Dial dynamics of YOY chub (*Squalius cephalus*) and dace (*Leuciscus leuciscus*) at the River Ourthe, Belgium, essentially emerge as a size specific trade-off between feeding and hiding (Baras and Nindaba, 1999), and the authors recommended that future studies should focus on spatial and temporal occurrence and feeding of fish. The results of our fishing campaign in the nearshore areas on gravel and sand in October clearly revealed: (1) juveniles of *N. fluviatilis* and *N. melanostomus* migrated into this habitat during the night (Fig. 8); (2) the main feeding time of both species was directly after sunrise but not during the night (Fig. 9). From these results a first hypothesis on the mechanistic relationships can be drawn, with the juveniles of both species that primarily hide during the night in nearshore areas on gravel and sand.

Conducting a similar fishing campaign in late spring in the Vistula River system, Poland, Grabowska et al. (2009) found neither differences in the consumption of food categories among three size-groups of *N. fluviatilis* nor differences in their gut fullness coefficient over the 24 h period. These results were in contrast to similar studies on the invasive racer goby (*Babka gymnotrachelus*) in the same reservoir that revealed this species to be primarily a nocturnal feeder (Grabowska and Grabowski, 2005). Similar to our results, however, Grabowska et al. (2009) also observed that *N. fluviatilis* were more numerous during sunset and night, and the authors suggested that they migrate into shallower waters at this time (without any assumptions on the reasons). A higher abundance of four different invasive Gobiid species during the night was also observed in the littoral zone of the River Danube, Hungary (Erős et al., 2005). Nevertheless, the present results give clear evidence that, beside seasonal characteristics, also dial aspects have to be considered when describing the ecological niche of the different Gobiids at the Lower Rhine.

Conclusions

The extremely high amounts of gobies within the fish community of the Rhine (actual values are always in the range of 80% or even more of all fish) together with decreasing condition of the invasive gobies are a clear hint that populations/communities are approaching or even have reached the capacity of the ecosystem.

Our results further give important hints how fine-tuned spatial and temporal characteristics in intra- and inter-specific competition shape the ecological niche of these invaders, providing some essential and conclusive correlations that allow specifying certain hypotheses to be experimentally tested in the future.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.limno.2012.08.003.

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