Competition for shelter between four invasive gobiids and two native benthic fish species

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Abstract Recent invasions by non-native gobiid fish species that are ongoing in the Western European rivers Rhine and Meuse, will lead to interactions with native benthic fish species. Since both non-native gobiids and native benthic species are bottom dwelling species with a preference for shelter during at least part of their life cycle, it is likely that competition for shelter will occur between these non-native and native species when shelter is a limiting factor. To investigate the importance of this mechanism for species replacements, various habitat choice experiments were conducted between two common native benthic fish species (Cottus perifretum and Barbatula barbatula) and four invasive non-native gobiid species (Proterorhinus semilunaris, Neogobius melanostomus, N. kessleri and N. fluviatilis). The first series of single specimen experiments determined the habitat choice of each individual fish species. In a second series of competition experiments, shifts in habitat choice in comparison with the previously observed habitat choice, were determined when a native benthic fish species co-occurred with non-native gobiid species. Native C. perifretum displayed a significant shift in habitat choice in co-occurrence with the gobiids N. kessleri or P. semilunaris. C. perifretum was outcompeted and moved from the available shelter place to less preferred habitat types. During the competition experiments no change in habitat choice of B. barbatula was shown. Our study therefore suggests that competition for shelter is likely to occur in rivers invaded by N. kessleri and P. semilunaris at sites where shelter is limiting [Current Zoology 57 (6): –, 2011].

Key words Bullhead, Exotic species, Habitat choice, Invasive gobies, Rivers Rhine and Meuse, Stone loach

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The settlement of non-native species that become invasive often results in negative ecological effects that may disturb ecosystems and subsequently result in local extinctions of native species (Vanderplaats et al., 2002; Clavero and Garcia-Berthou, 2005), particularly in aquatic systems (Marchetti et al., 2004). These invasive species often enter new aquatic habitats as a result of ballast water transport by ships, deliberate or unintentional release of species (e.g. aquaculture or escapes of ornamental species) or immigration through artificial canals constructed to connect river basins for shipping, such as the Rhine-Main-Danube Canal (Leuven et al., 2009). Invasive fish species can affect native fish assemblages by means of direct predation, competition for resources, hybridization in the case of closely related species or subspecies, habitat modification or transmission of diseases (reviewed in Gozlan et al., 2010).

During the last decades many non-native fish species invaded Dutch inland waters (Van der Velde et al., 2002). Since 2002, four non-native freshwater gobiid species have established viable populations in the rivers Rhine and Meuse in the Netherlands, i.e. Western tubenose goby *Proterorhinus semilunaris* since 2002, round goby *Neogobius melanostomus* since 2004, bighead goby *Neogobius kessleri* since 2007, and Pontian monkey goby *Neogobius fluviatilis* since 2009 (Van Beek, 2006; Van Kessel et al., 2009). In contrast to earlier settlement patterns of non-native fish species in Dutch rivers, these species showed an invasive pattern characterized by a rapid colonization of a large part of the Dutch Rhine and Meuse system (Van Kessel et al., 2009; Cammaerts et al., 2011).

The four gobiid species are all native to the Ponto-Caspian area, i.e. the rivers discharging into the Black, Caspian and Azov seas and their tributaries (Kottelat and Freyhof, 2007). From there, they invaded other water systems through the network of waterways existing in Europe, in particular from the river Danube through the Main-Danube canal to the river Rhine (i.e. Southern invasion corridor: see Bij de Vaate et al., 2002; Leuven et al., 2009). The egg capsules of these species might be transported fixed on the hulls of ships sailing on inland waterways, while transport in the ballast water of seagoing ships is also possible (Jude et al., 1995; Wonham et al., 2000; Corkum et al., 2004). Transport in ballast water and consequent discharge was the likely way that *N. melanostomus* and *P. semilunaris* invaded the Great Lakes of North America (Carlton and Geller, 1993; Corkum et al., 2004).

For *N. melanostomus*, various authors indicated negative effects on populations of native benthic freshwater fish species in the Great Lakes district of North America (Dubs and Corkum, 1996; French and Jude, 2001; Janssen and Jude, 2001; Balshine et al., 2005; Vélez-Espinò et al., 2010). Vaas et al. (1975) suggested that the introduction of the black goby *Gobius niger* into Lake Veerse Meer (South-West Netherlands) led to a decline of the native sand goby *Pomatoschistus minutus* and the extinction of shorthorn sculpin *Myoxocephalus scorpius*. However, this observation coincided with the isolation of this former estuary from the sea by dams, resulting in a decline in salinity.

The freshwater sections of the rivers Rhine and Meuse in the Netherlands are characterized by the presence of four non-native invasive gobiid species that have shown strongly increasing populations in previous years. Therefore, negative effects on small native benthic fish species with overlapping ecological niches, such as the bullhead *Cottus perifretum* (= *C. gobio* pro parte; see Freyhof et al., 2005) and stone loach *Barbatula barbatula*, are likely.

One of the underlying mechanisms driving the possible negative impact of these invasive gobiid species on small native benthic fish, may be competition for shelter (Balshine et al., 2005). The body morphology of the four non-native gobiid species shows adaptation to either a life on substrate rich habitats such as stones and rip rap (*N. kessleri*, *N. melanostomus* and *P. semilunaris*) or on bare sand *N. fluviatilis*, habitats that frequently occur in the rivers Rhine and Meuse. Competition for resources and/or aggressive territorial behaviour may give these species an advantage with respect to the small native benthic fish that show similar preferences and resource use.

We investigated whether the presence of these four non-native gobiids affects the natural habitat choice of these two small native benthic fish species. It was hypothesized that when shelter is limiting, the non-native species mainly use the available shelter and cause a shift in habitat choice of the native species to suboptimal adjacent habitats, when there is a choice.

1 Materials and Methods

1.1 Fish sampling and stock

Gobiid species were collected in various parts of the rivers Rhine and Meuse in the central part of the Netherlands: the rivers Waal, Hollandsch Diep, Nieuwe Merwede and Neder-Rijn, and the Meuse. Fish were caught using electrofishing (generator pulsating D.C. 200–300V) and seine netting (smallest mesh size 5 mm). Seine netting was used to collect *N. fluviatilis* only. Individuals of *C. perifretum* and *B. barbatula* were collected in tributaries of the rivers Rhine and Meuse using a hand net. All fish were collected between December 2009 and February 2010.
Species were housed separately in stock tanks of 150 L. The water of each stock tank was continuously filtered using an external canister filter. Stock tanks did not contain any substrate. Water temperature of the tanks was maintained between 13.1–14.8 °C (mean ± SE: 14.1 ± 0.11 °C). Fish were fed every evening with live food (i.e. *Daphnia* sp. and chaoborid larvae).

Fish species were caught at random and the most abundant size class was collected in the field. Differences in standard length (SL) of the species used in the present study reflect the differences in SL within their natural habitat. Collected *N. kessleri* ranged in SL from 7.4 to 10.5 cm SL (mean ± SE: 8.9 ± 1.6 cm SL), *N. melanostomus* from 5.3 to 9.4 cm SL (mean ± SE: 7.2 ± 1.4 cm SL), *N. fluviatilis* from 5.9 to 8.5 cm SL (mean ± SE: 7.5 ± 1.0 cm SL), *P. semilunaris* from 5.1 to 9.2 cm SL (mean ± SE: 6.5 ± 1.4 cm SL), *C. perifretum* from 6.0 to 8.5 cm SL (mean ± SE: 6.8 ± 0.8 cm SL) and *B. barbatula* from 6.1 to 9.5 cm SL (mean ± SE: 7.6 ± 1.4 cm SL). Table 1 summarizes the significance of differences in mean SL of specimens that were used in the experiments (One-Way ANOVA).

1.2 Study design

All tanks were housed at the same locality to maintain a similar water temperature between housing and experiment tanks. During the experiments, 10 L non-transparent water tanks were used to avoid visual interference. Experiments were replicated using five separate water tanks. A light regime of 14/10 hours light/dark was used to simulate a natural day-night rhythm.

Each of the five experimental tanks was equipped with eight different habitat types arranged in a similar sequence in each tank (Fig. 1). Each habitat type measured 10 × 13 cm. The habitat types reflected the most abundant natural habitats within Dutch rivers. We used an upside-down terracotta (clay) pot to create the habitat type ‘shelter’. On the edge of the pot an artificial entrance was made with a radius of 15 mm. Only one shelter place was available in each experimental tank to ensure shelter (i.e. an artificial rock cavity) was limiting. No other substrate was used in the habitat shelter. In addition to this shelter, we used seven other habitat types that differed in complexity: (1) vegetation (plastic Canadian pondweed *Elodea canadensis*) fixed to the bottom with sand (Ø <1 mm), (2) large (Ø 15–30 mm) and (3) small gravel (Ø 3–6 mm), equal mixtures of sand with (4) large (Ø 15–30 mm) and (5) small gravel (Ø 3–6 mm), (6) homogeneous sand (Ø <1 mm), and (7) a habitat type with no substrate. In the centre of each experimental tank we created a release location (Fig. 1). In comparison with the eight habitat types, this location was elevated (3 cm) and contained no structure. During experiments, no fish were recorded at the release location and the release location was not considered to a habitat type.

Two types of experiments were conducted. Firstly, single individual tests (referred to as solo experiments hereafter) were conducted to determine the habitat choice of each species without interactions with other species or individuals of the same species. Therefore, in separate experiments one individual per species was placed in each of the five experimental tanks at the release location at 07:00 a.m. After an initial acclimatization period of four hours, each experiment was initiated between 11:00 and 12:00 a.m. Habitat choice (i.e. the presence of a fish in one of the eight habitat types) was recorded at 14:00, 17:00, 20:00 p.m. and the next day at 08:00 and 11:00 a.m. The five replicate experiments were conducted simultaneously per tested species. Observations were only made during daytime since the species are mainly nocturnal feeders and change their habitat choice for feeding purposes, whereas the present experiment only focuses on shelter habitats. Secondly, a series of competition experiments were conducted to determine the effects of the presence of a non-native gobiid species on the habitat choice of the two small native benthic fish species. During these competition experiments all combinations of the native species and the four non-native species were examined (see Table 2 for an overview). In each competition experiment, two fish of different species were
simultaneously released in each of the five experimental tanks. Habitat choice for each individual was recorded in a similar way to the solo experiments. Additionally, for each native species a competition experiment with two individuals of the same species was conducted to investigate intra-specific competition. To prevent any influence of hunger stress, fish were fed daily. However, during the experiments fishes were not fed at all. To prevent any learning effect during the experiments, individual fish were only used once. To avoid any effect of habitat choice as a result of spawning behaviour, all experiments (12 competition experiments and six solo experiments, Table 2) were conducted in January–February 2010.

1.3 Data analyses

For each experiment (replicates of either solo or competition experiments), the number of observations (frequency of choice, i.e. the presence of a fish in a specific habitat type) of the five measurements were pooled per habitat type. These pooled observations were then used to calculate the degree of habitat choice for each replicate experiment. Total frequency of occurrence for each of the eight habitat types within each solo or competition experiment was calculated by pooling observations of the five replicate experiments.

Habitat choice and/or shifts within each solo or competition experiment was tested for significant differences by means of replicated G-tests of goodness of fit (McDonald, 2009), a test also known as the likelihood ratio test for goodness of fit. The statistical null hypothesis of a G-test is that the number of observations in each category (i.e. habitat type) is equal to that predicted by theory, whereas the alternative hypothesis is that the number of observations is different from the expected ratio.

For each solo experiment (\(n=6\); two native species and four non-native species), the expected observation ratio for each habitat type (eight possible habitat types) at random distribution was calculated by \(1/8 = 0.125\). For the competition experiments (i.e. the two native species tested in combination with one native species and four non-native species), the mean choice (i.e. the mean number of observations per habitat type based on the five replicate experiments) derived from the solo experiments of the native species was used as the expected observation ratio.

For each solo or combination experiment, total G, pooled G and heterogeneity G values were calculated. Within a solo or competition experiment, total G indicates whether all the observations from the different replicates differ from the expected ratio. Pooled G indicates when observations from the different replicates are pooled per habitat type and differ from the expected ratio, whereas heterogeneity G indicates whether the observations of the different replicates differ from each other. However, when heterogeneity G is significant, the pooled G cannot be interpreted since the replicate observations significantly differ from each other. Habitat choice or competition of each solo or combination experiment was considered significantly different from the expected observation ratio when both total and pooled G exhibited a significance of \(P < 0.05\).

For competition experiments, a significant total G and/or pooled G was considered to be a significantly different habitat choice of a native species as a result of competition with the other fish species (either native or non-native). G-tests were conducted based on an Excel-model available at http://udel.edu/~mcdonald/statrepgtestgof.html.

2 Results

2.1 Solo experiments

Both native fish species showed a habitat choice that significantly differed from the expected observation ratio (Fig. 2a; Table 2). For both species, the habitat type shelter was chosen most frequently in comparison with the other habitat types. Of the non-native species, only \(P. \text{semilunaris}\) showed a similar preference for shelter as native species (Fig. 2b;
Table 2). On the contrary, *N. kessleri*, *N. melanostomus* and *N. fluviatilis* chose different habitat types during the experiments. These species did not show a habitat choice that significantly differed from the expected observation ratio and were recorded in various habitats. *N. kessleri*, *N. melanostomus* and *N. fluviatilis* showed a relatively generalistic habitat choice and were frequently observed in the habitat types shelter, vegetation, large gravel, sand and mixtures with sand, small and large gravel. No fish at all (both native and non-native species) were observed in the habitat types small gravel and no substrate.

### 2.2 Competition experiments

In the competition experiments where the native species interacted with the non-native gobiids, significant effects were observed for *C. perifretum*. In comparison with the expected observation ratio as derived from the solo experiment with *C. perifretum*, the species showed a significantly different habitat choice in the competition experiments with *P. semilunaris* and *N. kessleri* (Fig. 3; Table 2). In competition with these species, *C. perifretum* did not occupy its preferred habitat (*i.e.* shelter), as derived from the solo experiment and was observed frequently in the habitat types vegetation, sand, mixtures of sand, small and large gravel, and no substrate. *N. kessleri* did not increase its preference for shelter in combination with *C. perifretum*, but did increase its preference for vegetation. *C. perifretum* also increased its preference for vegetation in combination with *N. kessleri*, but both species were only recorded simultaneously in this habitat once. The same observation was made with *C. perifretum* in combination with *P. semilunaris* and the species were not observed simultaneously in the habitat vegetation. *C. perifretum* also increased its preference for the habitat ‘no substrate’, were it was observed together with *P. semilunaris* once.

In the competition experiments with *B. barbatula*, *N. fluviatilis*, *N. melanostomus*, or a second individual of *C. perifretum*, this shift was not observed (results not shown), and habitat choice did not significantly differ from the expected observation ratio (Table 2).

As in the latter competition experiments, *B. barbatula* did not show a shift in habitat choice in the competition experiments that significantly differed from the expected observation ratio as derived from the solo experiment (Table 2). In each competition experiment, *B. barbatula* occupied primarily the shelter during the entire experiment and almost no other habitat use was recorded. This species occupied the habitat type shelter often simultaneous with other species present (either a second *B. barbatula*, *C. perifretum* or one of the four non-native gobiid species), without clear interaction between them.

### 3 Discussion

The solo experiments revealed a strong habitat choice for shelter in the case of the native *C. perifretum*, *B. barbatula* and the non-native *P. semilunaris* in comparison to the expected observation ratio. In contrast, the other non-native species *N. kessleri*, *N. melanostomus* and *N. fluviatilis* showed a more generalistic pattern in which various habitat types were occupied. The strong habitat choice of *C. perifretum* for shelter was markedly changed in competition with the non-native *P. semilunaris* and *N. kessleri*. *P. semilunaris*. *N. kessleri* outcompeted *C. perifretum* from the habitat with shelter to habitat types that contained less shelter. *N. kessleri* and *P. semilunaris* in the presence of *C. perifretum* did not increase their preference for shelter in comparison to the results as showed in the solo experiments. *C. perifretum* was not outcompeted by *B. barbatula* in the presence of *C. perifretum* and *N. kessleri* did not increase their preference for shelter. In contrast, intraspecific competition was not recorded for *C. perifretum* as two specimens of *C. perifretum* often shared the shelter together and no change in habitat choice was recorded. This indicates that when native and non-native species meet each other and interact with limited shelter, some non-native gobiid species outcompete native benthic species that live in the same ecological niche.
Although the present study is not an in situ field experiment, it is one of the first experimental studies to show that competition for shelter is a feasible mechanism by which some non-native gobiids such as \textit{P. semilunaris} and \textit{N. kessleri} evict or prevent small benthic native fish species from accessing preferred habitat. This mechanism was demonstrated in a comparable experiment on interactions between \textit{N. melanostomus} and logperch \textit{Percina caprodes} and between \textit{N. melanostomus} and mottled sculpin \textit{C. Bairdi} in North America (Dubs and Corkum, 1996; Balshine et al., 2005).

In contrast to the negative effect of \textit{P. semilunaris} and \textit{N. kessleri} on \textit{C. perifretum}, no negative impact on the behaviour of \textit{B. barbatula} was recorded during our experiments. This species was not evicted from its preferred habitat, \textit{i.e.} shelter, and did not display any shifts in habitat choice as a result of the introduction of one of the non-native species, or as a result of inter- or intraspecific competition between the two native species. \textit{B. barbatula} was often found in the shelter together with the second individual of its own species or with the other present species. The lack of competitive behaviour of \textit{B. barbatula} (Smyly, 1955) might explain the absence of competition observed in the present study.

In contrast to the previously suggested negative effect of \textit{N. melanostomus} on small native benthic fish species by (Dubs and Corkum, 1996; Janssen and Jude, 2001; French and Jude, 2001; Balshine et al., 2005), no effect of \textit{N. melanostomus} on the habitat choice of the native benthic fish species were recorded here. However, the negative impact of \textit{N. melanostomus} on native benthic fish species could be underestimated by the absence of aggressive behavioural interactions that only occur during its spawning season and subsequent brood care. Since the present study was conducted outside the spawning season of the investigated species, none of the fish displayed any spawning behaviour. Reproductive behaviour could increase territorial behaviour and consequent competition for shelter. Janssen and Jude (2001) suggested that \textit{N. melanostomus} evicted individuals of \textit{Cottus Bairdi} from their shelter to seize it as spawning territory. As a result, when spawning events are considered, better reflecting natural conditions, it could be possible that competition between \textit{N. melanostomus} and small native benthic fish does exist. Immigration of \textit{N. melanostomus} coincided with a decline of \textit{Cottus Bairdi} and logperch \textit{Percina Caprodes} in the Great Lakes. In the Gulf of Gdansk round goby increased and three-spined sticklebacks \textit{Gasterosteus aculeatus} decreased (Corkum et al., 2004).

The presence of \textit{N. fluviatilis} did not significantly shift habitat use of the small native benthic fish species. \textit{N. fluviatilis} did not display a clear preference for shelter and this species did not interact with \textit{C. perifretum} or \textit{B. barbatula}. In a similar way to \textit{N. melanostomus}, competitive behaviour of \textit{N. fluviatilis} could also be underestimated since the experiments were conducted outside the spawning season of the latter species. During spawning season, \textit{N. fluviatilis} is known to excavate nests beneath objects such as stones and displays brood care (Pinchuk et al., 2003a).

Another mechanism that may influence competition is a difference in fish size. However, in our study the smallest species (\textit{P. semilunaris}), as well as the largest species (\textit{N. kessleri}) appeared to be the most competitive species. This indicates that fish size is not the most important mechanism to explain the observed competition. Nevertheless, in their native range the body size of adult \textit{N. melanostomus} and \textit{N. fluviatilis} is considerably larger (Jude et al., 1995; Pinchuk et al., 2003a, 2003b; Vasil’eva and Vasil’ev, 2003) than that of \textit{C. perifretum} and \textit{B. barbatula} (Kottelat and Freyhof, 2007). Since it is likely that larger gobiids such as adult \textit{N. kessleri} and \textit{N. fluviatilis} are stronger competitors than smaller individuals, competition effects between native species and larger individuals of non-native species could be much stronger than that reflected by our experiments.

In conclusion, our results show that competition for shelter induced by invasive non-native gobiids may be a realistic threat to small native benthic fish species in the Rhine and Meuse systems. This could at least be demonstrated for \textit{C. perifretum} in competition with \textit{P. semilunaris} and \textit{N. kessleri}. Furthermore, the results suggest that a species with
territorial behaviour such as *P. semilunaris* shows stronger competitive mechanisms in the presence of native species with similar territorial behaviour, such as *C. perifretum*. Eviction from shelter places in the wild may result in the use of less preferred habitat, where food is less abundant, predation is more frequent, higher stress occurs due to stronger current and spawning habitat is not available (Dubs and Corkum, 1996). As densities of non-native gobies in the rivers Rhine and Meuse are locally high, interactions between these invasive species and small native benthic fish species are likely. The presence of these gobies can therefore lead to a decline in native fish populations, although a negative impact has not yet been demonstrated in the Netherlands.

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**References**


Figure legends

Fig. 1 Experimental setting of the eight tested habitat types during experiments

Fig. 2 Pooled habitat choice (n=5) in the solo experiments using the two native species

a. Barbatula barbatula, Cottus perifretum and the four invasive species.  
b. Neogobius fluviatilis, Neogobius kessleri, Neogobius melanostomus,  
Proterorhinus semilunaris. Asterisks indicate significant differences (P < 0.001) from the expected observation ratio (replicated G-tests, see text and table 2).

Fig. 3 Pooled habitat choice (n=5) of Cottus perifretum in the competition experiment with two native species (C. perifretum and Barbatula barbatula) and four invasive species (Neogobius fluviatilis, Neogobius kessleri, Neogobius melanostomus and Proterorhinus semilunaris)

a) shows the competition experiment with N. kessleri, panel b) with P. semilunaris. Only the competition experiments are shown that significantly differed from the habitat choice as determined by the solo experiment of C. perifretum (Fig. 2a; see table 2 for statistical details).
<table>
<thead>
<tr>
<th>Large gravel</th>
<th>Sand</th>
<th>No substrate</th>
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<tr>
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<td>Release location</td>
<td>Sand and large gravel</td>
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<tr>
<td>Vegetation</td>
<td>Shelter</td>
<td>Small gravel</td>
</tr>
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Fig. 1.
Fig. 3. Pooled habitat choice (%)

a) Neogobius kessleri vs Cottus perifretum
   Cottus perifretum vs Cottus perifretum
   Cottus perifretum solo

b) Proterorhinus semilunaris vs Cottus perifretum
   Cottus perifretum vs Cottus perifretum
   Cottus perifretum solo
Table 1 Mean SL of each fish species used in the 12 competition experiments (six for Barbatula barbatula, six for Cottus perifretum)

<table>
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<th>Native species</th>
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<td>vs. Neogobius fluviatilis</td>
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In each competition experiment mean SL between the two species (or within the same species) was compared by means of an ANOVA. df: degrees of freedom; NS: not significant (P > 0.05); vs.: versus.
Table 2  Statistical values of the replicated G-tests of goodness of fit for both the solo and competition experiments between the two native (Barbatula barbatula, Cottus perifretum) and the four invasive species (Neogobius fluviatilis, N. kessleri, N. melanostomus, Proterorhinus semilunaris)

<table>
<thead>
<tr>
<th>Solo experiments:</th>
<th>Species:</th>
<th>Total G</th>
<th>df</th>
<th>$P$</th>
<th>Pooled G</th>
<th>df</th>
<th>$P$</th>
<th>Het G</th>
<th>df</th>
<th>$P$</th>
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<tr>
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<td>93.96</td>
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<tr>
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<td>35</td>
<td>&lt;0.001</td>
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<td>35</td>
<td>NS</td>
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<td>N. melanostomus</td>
<td>invasive</td>
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<td>35</td>
<td>NS</td>
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Het: Heterogeneity; vs.: versus; NS: not significant ($P > 0.05$).