


# Invasive round goby *Neogobius melanostomus* has sex-dependent locomotor activity and is under-represented in catches from passive fishing gear compared with seine catches

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The higher proportion of males of the invasive round goby *Neogobius melanostomus* in samples from two activity selective passive fishing gears compared with one activity non-selective fishing gear in three Dutch lakes is related to higher male locomotory activity and is a sex-dependent trait. This difference in activity reflects the different ecology of male and female *N. melanostomus*.

## KEYWORDS

activity, Biesbosch lakes, invasive goby, sex differences

More activity in fish is beneficial for increasing the probability of finding food resources or a mating partner (Smith & Blumstein, 2008; Werner & Anholttt, 1993). However, activity increases the risk of predation and metabolic demands (Brown *et al.*, 2005; Rennie *et al.*, 2005; Werner & Anholttt, 1993). Many differences in the behaviour between sexes are related to sex specific costs of reproduction (Magurran & Maciás Garcia, 2000), reactions to their environment (Slatkin, 1984) and their different reproductive roles (Gross & Sargent, 1985). The intensity of locomotory activity can also differ in one sex

between the reproductive and non-reproductive seasons, especially in nest-guarding species (Gross & Sargent, 1985). No sex-biased differences in activity are expected when local conditions influence both sexes equally (Perrin & Mazalov, 2000). Different activity levels between sexes are widespread in a variety of fish taxa (Croft *et al.*, 2003; Horppila *et al.*, 2011; Hutchings & Gerber, 2002; Leris *et al.*, 2013; Marentette *et al.*, 2011; Srean *et al.*, 2016).

The round goby *Neogobius melanostomus* (Pallas 1814) is a relatively immobile species that moves just a few m/day (Brandner *et al.*,

2015; Cookingham & Ruetz, 2008). It is an invasive fish species in the major part of its current distribution within Europe and North America (Kornis *et al.*, 2012). It first occurred in the western part of the Netherlands in 2004 (van Beek, 2006). In 2012 their presence was confirmed in the Biesbosch lakes using fyke nets (G. Kruitwagen, unpublished data), but they may have been present earlier because no ichthyological survey was carried out between 2009 and 2012. Intensive surveys between 1998 and 2008 did not detect a single individual of *N. melanostomus* (Jůza *et al.*, 2018a).

Males of *N. melanostomus* are larger than females (Kornis *et al.*, 2012) and such difference in body size means that each sex reacts to ecological conditions differently (Slatkin, 1984). Another trait in which sexes can differ is activity. Higher male activity was observed in *N. melanostomus* in North American lakes (Marentette *et al.*, 2011), but there is no information whether activity and concurrently the reaction to ecological conditions differ between sexes in the Biesbosch lakes. The first aim of this study was to determine if there is a difference in locomotory activity between the sexes of *N. melanostomus* in three Dutch lakes. A fish that is more active has a higher probability of capture in a passive sampling method (Prchalová *et al.*, 2010); hence, a higher proportion of males captured during passive sampling would be expected.

*Neogobius melanostomus* has a negative effect on native communities of bottom dwellers due to its aggressive competition (van Kessel *et al.*, 2011). This can result in the exclusion of vulnerable native species (Jůza *et al.*, 2018a). In the Biesbosch lakes, their recent invasion almost led to the local extinction of native ruffe *Gymnocephalus cernua* (L. 1758) (Jůza *et al.*, 2018a). Correct and unbiased sampling is essential to estimate the density of this rapidly spreading invasive species and consequently detect the potential threat to local fish communities. Therefore, the second aim of this study was to compare the proportion of *N. melanostomus* within the total fish community from seining and from gillnets.

The study was conducted in the Biesbosch National Park in the Netherlands, which consists of three interconnected lakes: De Gijster (DG; 51.677° N, 4.804° E; area 320 ha; maximum depth: 27 m); Honderd en Dertig (HD; 51.734° N, 4.774° E; area 219 ha; maximum depth 27 m) and Petrusplaat (PP; 51.757° N, 4.774° E; area 105 ha; maximum depth: 15 m). Fishing is prohibited in the lakes. The banks are asphalt and concrete to a depth of 6 m. This provides a similarity of unstructured shallow habitats within and among lakes. The lakes are described in greater detail by Oskam and van (1992).

Two passive sampling methods (gillnets, minnow traps) and one active (beach seining) were used in the study. Passive gears are selective in that they are dependent on fish activity (Hubert *et al.*, 2012). While beach seining was used as an active, non-selective method (Kubečka *et al.*, 2012) to provide a reference activity-unbiased sex ratio. Two passive methods were used to eliminate different factors that can contribute to a sex-biased capture in a single method. Sampling was done in the second half of August, in 2015 and 2016. Data from minnow traps are presented only from 2016 because sampling in 2015 did not provide a sufficient sample size for statistical analysis (< 5 fish per sampling) because only five traps were used at each locality. DG was not sampled in 2015 because of low water.

Using three sampling methods, *N. melanostomus* were sampled at two localities (PP and DG) and three localities (HD) respectively. Localities were at least 600 m from each other to ensure their independence. Gillnets and minnow traps were set to a depth between 1.5 and 3 m (checked by echo sounder; Piranha Max 10; Hummingbird; www.hummingbird.com) and were installed overnight. Three European standard (CEN, 2015) multi-mesh gillnets were joined by 30 m long ropes and deployed in each locality. Catches from the three gillnets were pooled for analysis. All the fish caught by gillnets were immediately euthanized once removed from the water by an overdose of clove oil anaesthetic. Ten baited minnow traps (25 × 25 × 45 cm, 4 mm mesh, 6 cm openings at both sides) were deployed in 2016. Dog food granules and dead perch *Perca fluviatilis* L. 1758 were used simultaneously as bait. Catches from the 10 traps were pooled for statistical analysis and were not used to compare species composition among methods because only a few individuals of species other than *N. melanostomus* were caught.

One seine haul was performed in each locality between 2200 and 0100 hours. The seine net (30 m long, 3 m deep, mesh size 6 mm) was deployed from a boat 20–25 m from the bank and towed towards the bank for < 2 min. The net was set above 3 m depth (checked by an echo sounder). All fish captured by the seine were kept in a bucket with aerated water and released after all measurements were obtained. The time interval between the use of each sampling method at the same locality was not longer than 3 days and the method used first at each site was randomized.

All captured fish were identified to species and measured (standard length,  $L_S$  to nearest mm). The sex of *N. melanostomus* was determined by shape of the urogenital papilla (Charlebois *et al.*, 1997). Fishing permission was granted by the manager of the lakes, and fish sampling was done as a part of fish-community survey for the manager of the lakes (Evides Waterbedrijf; www.evides.nl). Fish captured using passive methods were not released on the recommendation of the lake manager.

We compared the sex ratios obtained from each passive method to the reference sex ratio from seining using a generalized linear model (GLM) with a binomial error structure and log-link function. Only mature individuals  $L_S > 35$  mm were used in the analysis. To avoid obtaining inaccurate sex ratios, only data with > 5 fish of known sex in a single sample were analysed. The raw binomial data (absolute catches with individual information about sex) were analysed. Gear was a categorical explanatory variable. Covariates ( $L_S$ , locality and year) were added to the model to control their variability. Covariate  $L_S$  was used as a control on different sizes between sexes (Young *et al.*, 2010) to reveal sex-effect only. An interaction term between the sampling method and  $L_S$  was used to reveal potential fish size-related differences in the probability of capture by the sampling methods. Non-significant interaction was omitted from the final model. The significance of variables in the model was tested by the Wald  $\chi^2$ -test.

The second GLM with a binomial error structure compared the relative proportion of all size classes of *N. melanostomus* caught by gillnets and by seining. Data input of the response variable was binary (1 = *N. melanostomus*; 0 = different fish species). The explanatory variable was sampling method in interaction with  $L_S$ . The covariates were locality and year. Residuals of all models were checked by diagnostic

plots. All statistical analysis was carried out in R 3.3.2 (www.r-project.org).

A total of 13,901 individuals (7,712 in gillnets; 6,093 in seine and 96 in minnow traps) of 19 fish species were caught during the survey. The dominant species in the Biesbosch lakes (here presented as raw % uncontrolled on model parameters) were *P. fluviatilis* (84% of relative abundance in gillnets, 41% of relative abundance in seines) and *N. melanostomus* (7% in gillnets, 41% in seines). Other common species were *Rutilus rutilus* (L. 1758) (7% in gillnets, 7% in seines) and *Sander lucioperca* (L. 1758) (1% in gillnets, 3% in seines). Other fish species represented < 2% in relative abundance from each method. In total 3,126 individuals (547 in gillnets, 2,483 in seines, 96 in traps) were *N. melanostomus*. Sex was identified in a sub-sample of 1892 gobies of  $L_S > 35$  mm.

Higher locomotory activity of males was supported by 7.6 and 21.4% higher proportion of *N. melanostomus* males in activity selective gillnets and traps respectively [Figure 1(a) and Table 1] whereas the male proportion of the catch from seining was significantly lower. This trend was consistent in both years for gillnets [Figure 1(a) and Table 1]. There was evident sexual size dimorphism with larger males because the covariate  $L_S$  was significant. There was variability in the sex ratio among localities for gillnets, but not for minnow traps [Table 1]. The probability of *N. melanostomus* being caught by gillnet or trap in comparison with seine was not influenced by  $L_S$  because the interaction between the sampling method used and  $L_S$  was not significant (GLM; gillnet:  $z = -1.28$ ,  $p > 0.05$ ; trap:  $z = 0.52$ ,  $p > 0.05$ ) and so these interactions were omitted from the final models.

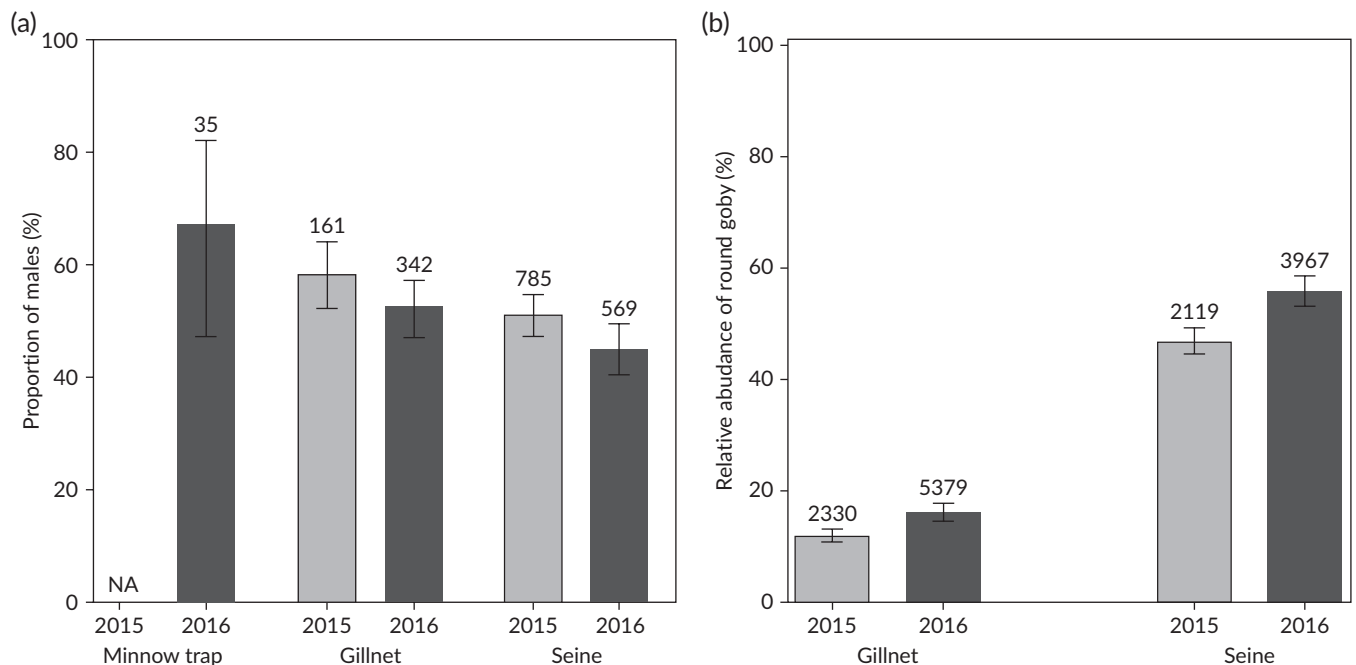
Gillnets and seining offered a significantly different relative abundance of *N. melanostomus* in the catches [Figure 1(b) and Table 1]. The relative abundance of *N. melanostomus* was approximately three times

**TABLE 1** Comparisons of activity-selective gillnets and activity non-selective seining, activity-selective minnow traps and activity non-selective seining and a comparison of relative proportions of *Neogobius melanostomus* in gillnets and seining

Comparison	d.f.	$\chi^2$	P
Gillnets v. seine			
Gear	1	6.51	< 0.05
$L_S$	1	70.30	< 0.001
Locality	6	19.04	< 0.01
Year	1	3.95	> 0.05
Minnow trap v. seine			
Gear	1	4.27	< 0.05
$L_S$	1	10.77	< 0.01
Locality	6	10.56	> 0.05
Gillnet catch v. seine catch			
Gear	1	1075.88	< 0.001
$L_S$	1	4.97	< 0.05
Locality	6	354.30	< 0.001
Year	1	35.46	< 0.001
Interaction gear and standard length	1	32.67	< 0.001

higher in seine nets than in gillnets [Figure 1(b)]. The probability of capturing the larger fish of all taxa together was higher in gillnets. It was supported by the significant interaction between sampling method and  $L_S$  [Table 1].

*Neogobius melanostomus* males were more active than females in the three lakes. This was supported by the higher proportion of males in the two activity selective fishing methods compared with the activity non-selective method. This result was significant despite the control of the model on different sizes of the *N. melanostomus* sexes,



**FIGURE 1** (a) Average proportion (%) of *Neogobius melanostomus* males in catch based on the sampling method and the year of sampling. *Neogobius melanostomus* from minnow traps in 2015 were omitted from analysis due to small sample sizes (NA). (b) Average proportion (%) of *N. melanostomus* in the overall catch based on the sampling method and year. The value above each bar represents total sample size from which the proportion was computed. Presented proportions are from GLM models used for analysis. Error bars represent 95% confidence intervals

meaning that activity is not only a size dependent trait in *N. melanostomus*, but a sex dependent one too.

Higher locomotory activity of *N. melanostomus* males has been confirmed by Marentette *et al.* (2011) under laboratory conditions. On the basis of their findings, Kornis *et al.* (2012) suggested that there would be a higher encounter rate of males to the passive sampling method which would lead to the higher capture of males. This suggestion was confirmed by our study. Lynch and Mensinger (2012) did not observe higher activity of males, but they studied slightly different activities. Our study provides information mainly about locomotory activity inside the *N. melanostomus* home range whereas the Lynch and Mensinger (2012) distance traveled from the place of release. We observed higher male activity despite the fact that August is the *N. melanostomus* spawning season (Gertzen *et al.*, 2016) when males are sedentary and highly territorial during nest guarding (Meunier *et al.*, 2009). Confirmation of activity difference between sexes means that there is surely a sex-specific trade-off between reducing predation risk and finding food or a mating partner (Horppila *et al.*, 2011; Hutchings & Gerber, 2002).

The presence of sexual size dimorphism in *N. melanostomus* means that there are different selective pressures for males and females and consequently each sex reacts differently to the environment (Perrin & Mazalov, 2000; Slatkin, 1984). As a result males are bolder than females and boldness is related to higher activity (Myles-Gonzalez *et al.*, 2015). Larger body size and faster growth of males (Azour *et al.*, 2015; Kornis *et al.*, 2012) means greater foraging demands which can contribute to higher male locomotory activity. Males directly profit from their larger size because they can more easily protect their territory (Stammler & Corkum, 2005). The males' greater willingness to search for food may be why they were caught more often in baited traps than females. This is supported also by the fact that angling often caught more males than females (Brandner *et al.*, 2013b). The risk of predation is very high in the Biesbosch lake systems because it is dominated by *P. fluviatilis* and *S. lucioperca* (Kubečka *et al.*, 2013), which regularly prey on *N. melanostomus* (A. J. Wagenvoort, personal observation). That males are more active suggests that they accept a greater risk of predation to find more food or optimal territory (Werner & Anholttt, 1993; Wilson *et al.*, 1993).

The sex specific locomotory activity in invasive *N. melanostomus* has important implications for its range expansion and dispersal. In accordance with the "individual trait utility hypotheses" (Cerwenka *et al.*, 2017) the individuals with higher performance significantly contribute to invasion success. Attaining high performance is possibly related to high locomotory activity and more active individuals invade new areas sooner (Thorlacius *et al.*, 2015). The higher activity of males can be a reason why there is a higher proportion of males in some newly invaded sites (Azour *et al.*, 2015; Gutowsky & Fox, 2011). On the other hand, some studies observed the opposite sex ratios at invasion fronts (Brandner *et al.*, 2013a, 2018; Gutowsky & Fox, 2012). Such discrepancy could be related to population-specific reactions to various ecological conditions or different sampling methods.

There are differences in activity between *N. melanostomus* at established and newly invaded sites (Thorlacius *et al.*, 2015). This study was done at an established site (Jüza *et al.*, 2018a) where the activity of fish may be driven by intraspecific competition (Thorlacius *et al.*, 2015). The banks of the Biesbosch lakes are unstructured to a

depth of 6 m and do not offer a sufficient amount of shelter or spawning sites for *N. melanostomus*. Territorial males protect these shelters (Stammler & Corkum, 2005) and shelter insufficiency can be a competitive driver which contributes to higher activity of males.

The limitation of this study is that activity levels were not observed directly (for example by scuba divers or by telemetry), but indirectly by comparison of active and passive sampling methods. On the other hand the use of two activity selective methods reduces the risk that the male-biased ratio is influenced by other aspects of *N. melanostomus* behaviour. Moreover, it is generally accepted that passive gears are activity selective in comparison with active gears and higher catches in passive gear can be interpreted as higher fish activity (Diaz Pauli *et al.*, 2015; Prchalová *et al.*, 2008).

Under-representation of *N. melanostomus* was evident in gillnet catches in comparison with beach-seine catches. The lower proportion of *N. melanostomus* in gillnets, was undoubtedly due to their small movements, bodies without hard projections, the shape of their relatively wide heads and their smooth skin, which complicate their capture in gillnets (Finstad *et al.*, 2000; Holmgren, 1999; Pet *et al.*, 1995). The strongly dominant species in all lakes and years in gillnet catches, was *P. fluviatilis*, which is obviously very abundant, but in comparison with *N. melanostomus*, also very active (Prchalová *et al.*, 2008). Moreover, *N. melanostomus* as a species without a swimbladder is caught in gillnets mainly close to the bottom line of the gillnet, whereas *P. fluviatilis* is more easily caught by the whole panel of a gillnet. A detailed description of the *N. melanostomus* population characteristics in the Biesbosch lakes can be found in Jüza *et al.* (2018a).

Our results are in accordance with other studies which identified passive sampling methods as less effective for *N. melanostomus* capture in comparison with active sampling methods (Brandner *et al.*, 2013b; Diana *et al.*, 2006; Nett *et al.*, 2012). A detailed description of method dependent size selectivity of *N. melanostomus* in the Biesbosch lakes can be found in Jüza *et al.* (2018b) but in general, the average size of captured *N. melanostomus* was in ascending order, from traps, then seines and gillnets. On the basis of previously published work and our observations, standard European gillnets cannot be recommended for assessing the population of *N. melanostomus* even in shallow unstructured littoral habitats, due to their relatively low efficiency and skewed sex ratio influenced by the different locomotory activity of the sexes.

The conclusion is that males of invasive *N. melanostomus* have a higher locomotory activity than females in the Biesbosch lakes, supported by the higher proportion of males in activity selective gears. This difference in activity reflects the different ecology of the sexes of *N. melanostomus*. The general activity of this territorial species is however, significantly lower in comparison with other species, which is why *N. melanostomus* is underrepresented in gillnet samples.

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