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# Assessment of possible diel and sex-related differences in round goby (*Neogobius melanostomus*) diet

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**Abstract.** Several aspects of invasive round goby (*Neogobius melanostomus*) biology are based on sporadic observations or anecdotal reports only, e.g. they are night feeders, they prey on eggs and larvae of native fish and male feeding ceases or is highly restricted during breeding due to nest-guarding. To test the general validity of such hypotheses, we assessed diel and inter-sex differences in diet and feeding intensity of 232 gobies (144 female [54 day:90 night] and 88 male [39 day:49 night]) caught during the breeding season. Gobies took primarily aquatic insect larvae and did not predate on eggs, larvae or juveniles of native fish. Unlike previous studies, we observed no diel difference in feeding intensity or diet composition; hence no universal diel pattern can be implied for round goby feeding. On the other hand, we observed significant inter-sex differences in both feeding intensity and diet composition, with males consuming less food than females, presumably due to restricted feeding activity during nest-guarding. Inter-sex shifts in both diet composition and amount of food were less than expected, however, suggesting that, under most conditions, there is no reason to expect a fatal decrease in male energy intake, as suggested by some earlier studies.

**Key words:** invasive species, diel feeding, inter-sex differences, nest-guarding, breeding season

## Introduction

Introduction of non-native species can have severe effects on recipient ecosystems. In order to evaluate the potential impact of such species, however, it is necessary to collect detailed information on their ecology, with information on feeding habits being of particular importance. Such data can reveal potential impacts on native organisms through competition, predation or inclusion into the food chain as prey.

The round goby (*Neogobius melanostomus* Pallas, 1811), an invasive Ponto-Caspian species of particular interest at the present time, first expanded its range up main rivers, such as the Danube, and has more recently entered completely new watersheds such as those of the Rhine and the Baltic (see review in Sapota & Skóra 2005, Roche et al. 2013). It has also established itself in the Great Lakes Basin in the United States, having apparently been transported there in ballast water (Brown & Stepien 2009). Indeed, it was in the Great Lakes that the species was first considered a potential threat to native ecosystems (Charlebois et al. 1997, Kornis et al. 2013).

While the large number of dietary studies instigated since this multi-locus invasion have revealed a high degree of dietary plasticity (e.g. Borcherding et al. 2013, Brandner et al. 2013), round goby diet is usually reported as consisting of aquatic insect larvae, amphipods, chironomids, molluscs and small fish (e.g. Simonovič et al. 1998, Djuricich & Janssen 2001, Adámek et al. 2007, Kornis et al. 2012, Brandner et al. 2013). Several studies have shown ontogenic dietary shifts in round goby, with smaller fish (< 6 cm total length) consuming mostly chironomids and larger fish shifting to larger items, such as molluscs (Jude et al. 1995, Brandner et al. 2013). The round goby has long been regarded as showing a strong preference for zebra mussels (*Dreissena* sp.) based on their prevalence in gut samples in earlier studies (e.g. Ray & Corkum 1997). More recent laboratory experiments, however, have clearly shown that, given a choice, gobies prefer other dietary items (e.g. crustaceans, aquatic insect larvae) over molluscs (Diggins et al. 2002, Polačik et al. 2009). Round gobies are also considered a potential threat to native ichthyofauna due to consumption of eggs and larvae of native fish (Chotkowski & Marsden 1999, Thompson & Simon 2014).

Despite our increasing knowledge of round goby dietary preferences, some aspects of their biology

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are still based on either sporadic observations or anecdotal reports. As an example, during the breeding season, males guard the nest and eggs (see Wickett & Corkum 1998, Meunier et al. 2009). Early studies considered male feeding to be so restricted at this time that it was thought most males ceased feeding altogether, eventually dying after the breeding season (Kostyuchenko 1961, 1970). This was later questioned by Charlebois et al. (1997). Today, it is generally accepted that, rather than ceasing to feed, the male's feeding range, and hence dietary choice, becomes severely restricted while nest-guarding. As a consequence, males may consume food less frequently than females and/or consume a wider range of prey that includes a greater proportion of "less preferred" items. To our knowledge, however, the only study that has touched on the topic of sex-related differences in round goby diet has shown the opposite, i.e. that males show increased feeding at this time (Thompson & Simon 2014).

Similarly, round gobies are generally considered as feeding primarily during the night. This prediction, however, is only supported by a single field study by Johnson et al. (2008), who documented a night-time increase in feeding intensity. Indirect support also comes from a laboratory study of Dubs & Corkum (1996), who observed that round gobies spent more time outside their refuge during the night. To our knowledge, no other study supports this belief. Only two studies have demonstrated diel differences in diet composition, with Carman et al. (2006) observing that round goby diet in a warm North American stream shifted from caddisfly (Hydropsychidae) and chironomid larvae during the day to mayfly (Heptageniidae) larvae at night, mainly due to an increase in mayfly larvae activity during the night and their subsequent presence in drift. Johnson et al. (2008) reported that, along with a night-time increase in feeding intensity, round gobies in Lake Ontario also ceased eating quagga mussels (*Dreissena bugensis*), which represented 25 % of their daytime diet, and switched to chironomids.

In this study, we assess whether the dietary paradigms mentioned above can be generalised by comparing day and night feeding intensity and diet composition of male and female round gobies during the breeding season. We hypothesise that (1) males will eat less food than females and that the dietary items taken will differ from those of females; and (2) that fish captured during the night will have consumed more prey than fish captured during the day, with different prey types consumed during the two periods.

## Material and Methods

*Study area*

This study took place on the River Dyje (Danube Basin, Czech Republic), near the town of Břeclav (48°44′30.079″ N, 16°53′31.366″ E), 22 km from its confluence with the River Morava, a main tributary of the River Danube. The river is regulated along the whole length of the study stretch, with riverbanks consisting of 10-50 cm rocky rip-rap. The river at this point is 46 m wide and has a maximum depth of 1.5 m and a mean annual discharge of 35 $m^3.s^{-1}$. With its linear currents and long homogenous nearshore rip-rap habitat, the River Dyje is typical of the channelised European rivers into which round gobies have recently dispersed.

The macroinvertebrate assemblage at the site during the breeding season, based on three standardised kick-samples with a 500 µm mesh sweep net (Kokeš & Němejcová 2006), consisted mostly of chironomid larvae, mayfly larvae (mainly *Potamanthus* sp. and *Caenis* sp.), caddisfly larvae (*Hydropsyche* sp. and *Neureclipsis* sp.), *Bithynia tentaculata* (Gastropoda), zebra mussels *Dreissena polymorpha* (Bivalvia) and *Erpobdella* sp. (Hirudinea).

Round gobies first appeared in the River Dyje in 2008, having migrated up the non-navigable the River Morava and on into the Dyje from the River Danube (Lusk et al. 2008). The species is now well established and forms a viable population all along the river's length (Janáč et al. 2013).

*Fish sampling*

Sampling took place on seven occasions between June and August 2011, covering the expected breeding season for round goby (Skóra & Stolarski 1993, Macinnis & Corkum 2000). Fish were captured along a 100 m stretch of bankside rip-rap (preferred habitat for round goby, see Jude & DeBoe 1996) during the day (14:00) and night (01:00) using electrofishing gear (SEN, Fa. Bednář, Olomouc). All round gobies were euthanised with clove oil and preserved in 4 % formaldehyde for further analysis in the laboratory. Fish were measured to the nearest 0.01 mm (standard length, SL) and weighed to the nearest 0.01 g before and after evisceration. The removed digestive tract was also weighed, with and without its contents. Only fish > 50 mm (SL) were subjected to further analysis as sex determination was uncertain below this size. Note that in previous studies, this is the approximate size at which round gobies are believed to switch their diet and start eating molluscs (e.g. Carman et al. 2006). Digestive



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tract contents were examined under a 10× binocular microscope, the constituents being determined to the lowest possible taxonomic group and weighed. In order to simplify analysis, the following dietary categories were used: molluscs (Mollusca), leeches (Hirudinea), zooplankton, crustaceans (Crustacea, represented solely by waterlouse *Asellus aquaticus*), mayflies (Ephemeroptera), caddisflies (Trichoptera), chironomid larvae and pupae (Chironomidae), dipterans (Diptera, except Chironomidae), terrestrial insects, other (Odonata, Heteroptera, Megaloptera, Coleoptera), fish eggs, fish fry and detritus (detritus, sand and macrovegetation residue; not included in analysis).

*Data analysis*

Index of gut fullness (IF, in $^{o}/_{ooo}$) was used to describe feeding intensity (following the methods of Johnston et al. 2008) and was calculated as $IF = 10^4*(W_F/W_{evi})$; where $W_F$ = food weight (food bulk in each digestive tract was weighed to the nearest 0.001 g) and $W_{evi}$ = eviscerated fish weight.

The representation of each dietary item in each digestive tract was expressed as relative weight $W_R$ using the calculation $W_{Ri} = 100*(Vi/\Sigma Vi)*W_F$, where Vi = estimated original volume of dietary item i in a digestive tract and $W_F$ = weight of food in a digestive tract (see Hyslop 1980). Analysis of diel and inter-sexual differences in diet composition were based on these $W_{Ri}$ values.

The index of preponderance (IP, Natarajan & Jhingran 1961) was used to evaluate the "importance" of a dietary item in round goby diet and was calculated as $IP_i = (\%W_i*\%F_i)/(\Sigma\%W_i*\%F_i)*100$; where $\%W_i$ is the percentage bulk weight of dietary item i (calculated as $\%W_i = 100*(W_i/\Sigma W_i)$, where $W_i$ = the weight of a particular dietary item) and $\%F_i$ = the percentage frequency of occurrence of the same item (calculated as $\%F_i = 100*(n_i/n)$; where $n_i$ = the number of fish with dietary item i in the digestive tract and n = the total number of fish with food recorded in the digestive tract).

*Statistical analysis*

The effect of diel period and sex on IF was assessed using a linear mixed model, with sampling date as a random predictor. IF data were log-transformed prior to analysis in order to attain normality and homoscedasticity (models based on log-transformed data displayed "normality of residuals" and "no shape in residuals *vs.* fitted values", thereby confirming suitability). Effect of diel period and sex on diet composition was assessed using non-parametric permutational multiple analysis of variance (PERMANOVA, Anderson 2001) with sampling data as a "strata" parameter (i.e. permutations were conducted only within each "date" level). The response variable (distance matrix of samples, i.e. digestive tracts) for PERMANOVA was calculated from an original "sample × diet-item" matrix of $W_{Ri}$,

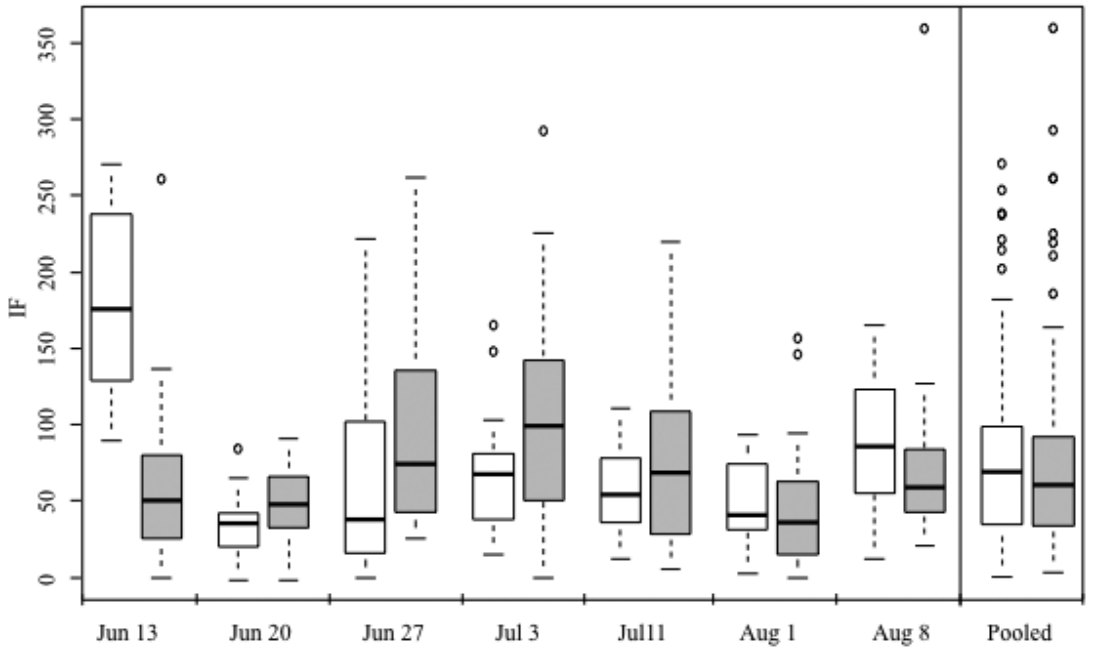



**Fig. 1.** Index of fullness (IF) for round gobies captured during the day (white boxes) and night (grey boxes) on each sampling date and pooled for all dates. Horizontal bar = median, boxes = interquartile range, whiskers = non-outlier range, points = outliers.



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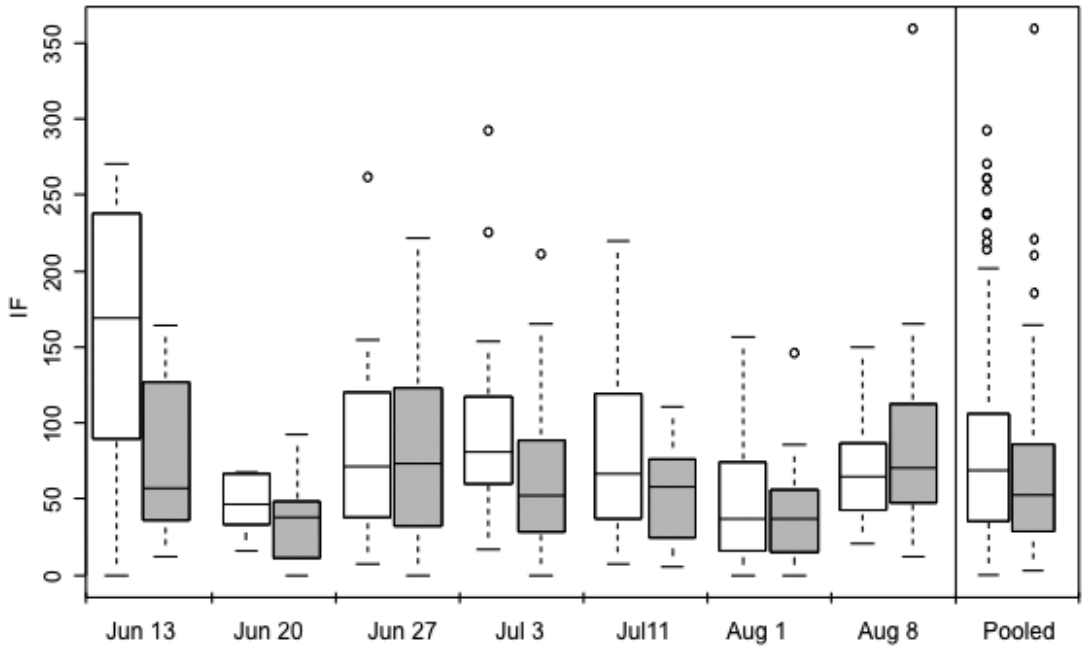



**Fig. 2.** Index of fullness (IF) for round goby females (white boxes) and males (grey boxes) on each sampling date and pooled for all dates. Horizontal bar = median, boxes = interquartile range, whiskers = non-outlier range, points = outliers.

**Table 1.** Round goby diet composition (index of preponderance, IP), with index of fullness (IF), mean size (SL), mean weight ($W_{evi}$) and number of full/empty guts also indicated. Note that fish with empty guts were excluded from the analysis. L. = larva, P. = pupae, * = (IP < 0.1).

| | Female | | Male | | Sex | | Diel period | |
|---|---|---|---|---|---|---|---|---|
| | day | night | day | night | female | male | day | night |
| Trichoptera | 31.6 | 26.8 | 35.5 | 38.7 | 28.7 | 37.3 | 33.5 | 30.7 |
| Ephemeroptera | 25.9 | 32.8 | 27.4 | 31.8 | 29.9 | 29.7 | 26.7 | 32.6 |
| Chironomidae L. | 36.7 | 31.2 | 18.3 | 19.5 | 33.0 | 18.9 | 29.0 | 27.3 |
| Mollusca | 4.5 | 7.6 | 18.3 | 9.2 | 7.1 | 13.1 | 9.9 | 8.2 |
| Hirudinea | * | 0.1 | 0.2 | 0.7 | 0.1 | 0.6 | 0.1 | 0.3 |
| Crustacea | 0.7 | 0.4 | * | * | 0.5 | * | 0.3 | 0.2 |
| Chironomidae P. | 0.6 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.4 | 0.1 |
| Terrestrial insect | | 0.1 | | | * | | | 0.1 |
| Diptera L. | * | * | | | * | | * | * |
| Zooplankton | | | | | * | | * | |
| Fish eggs | 0.1 | * | | | * | | * | * |
| Fish tissue | | | | | * | | * | |
| Other | * | 0.9 | * | * | 0.4 | * | * | 0.6 |
| No. guts examined | 54 | 90 | 39 | 49 | 144 | 88 | 93 | 139 |
| No. empty guts | 1 | 4 | 4 | 5 | 7 | 9 | 5 | 9 |
| No. non-empty guts | 53 | 86 | 35 | 44 | 139 | 79 | 88 | 130 |
| IF (Φ ± SD) | 85.4 (± 67.2) | 80.7 (± 60.6) | 69.2 (± 52.9) | 57.7 (± 60.9) | 82.5 (± 63.2) | 62.7 (± 57.8) | 78.3 (± 61.9) | 72.3 (± 61.7) |
| SL (Φ ± SD) mm | 76.6 (± 13.8) | 82.2 (± 16.7) | 80.4 (± 13.7) | 83.2 (± 17.1) | 80.2 (± 16) | 82 (± 15.8) | 78.3 (± 13.9) | 82.6 (± 16.9) |
| $W_{evi}$ (Φ ± SD) g | 14.0 (± 8.8) | 17.7 (± 12.3) | 16.1 (± 8.6) | 18.8 (± 11.9) | 16.4 (± 11.3) | 17.6 (± 10.7) | 14.9 (± 8.8) | 18.1 (± 12.2) |



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using Bray-Curtis dissimilarity as a distance measure. PERMANOVA uses a multivariate analogue of Fisher's F ratio to compare variability within groups versus variability between different groups, P-values being obtained using permutations (Anderson 2001). In this study, 9999 permutations were conducted in PERMANOVA. All statistical analyses were conducted using R 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) using the *vegan* and *nlme* packages.

## Results

In total, 232 round gobies were examined, of which 144 were female (54 day:90 night) and 88 male (39 day:49 night).

*Feeding intensity*

Diel period had no significant effect on IF (mixed-model, $F_{1, 223} = 0.46$, $P > 0.05$; Fig. 1). There was, however, a significant difference between the sexes, with IF significantly higher in females (mixed-model, $F_{1, 223} = 8.72$, $P < 0.01$), though the difference was marginal (mean IF ± SD = 68.4 ± 57.1 in males and 84.2 ± 62.7 in females; Fig. 2).

*Dietary composition*

Diel period had no significant effect on diet composition (PERMANOVA, $F_{1, 215} = 1.39$, $P > 0.05$). There was, however, a significant sex-related difference, with diet composition non-randomly distributed between the sexes (PERMANOVA, $F_{1, 215} = 3.22$, $P < 0.01$). The shift in diet preference was minimal, however, as both males and females consumed similar dietary items; with mayflies, caddisflies, molluscs and chironomids the most important items taken (Table 1). Inter-sex differences were only slight, with molluscs and caddisflies slightly more important in the diet of males and chironomids taken slightly more by females. Fish eggs occurred only in female diet, and then in the digestive tracts of just three females (Table 1). All eggs and all fry taken were of round goby, with very few examples of each found.

## Discussion

In our study, the diet was dominated by readily digestible (i.e. little or no indigestible matter, such as shell) and easily accessible aquatic insect larvae such as caddisfly (mainly those lacking protective cases such as *Hydropsyche angustipennis* [Hyropsychidae] and *Neureclipsis bimaculata* [Polycentropodidae]), chironomids and mayflies. The low consumption of both crustaceans and molluscs (which dominate in round goby diet elsewhere, e.g. Kornis et al. 2012) in our study reflects their low representation in the macrozoobenthos assemblage, suggesting that round goby is a generalist feeder with prey availability determining diet composition. Low mollusc consumption may also reflect higher availability of other, more profitable prey items. Note also that most of the molluscs consumed in this study were represented by species such as *Pisidium* sp., *Sphaerium* sp., *Lymnea* sp. and *B. tentaculata*, which all have thin, easily digested shells. Of the molluscs taken, *D. polymorpha* represented just 27 %. Despite the presence of easily accessible eggs, larvae and juveniles of native fish (and gobies), our results demonstrated no predation pressure on the native fish fauna (see also Vašek et al. 2014), indicating rather a preference for bottom living, "sedentary" prey types. Indeed, there is little or no evidence of eggs or larvae as a common dietary item of round gobies throughout Europe, despite studies from the U.S. (e.g. Thompson & Simon 2014) recording up to 24 % of guts containing eggs, larvae and juveniles of a range of native species. While it is not known why this should be so, we can speculate that round gobies in the U.S. may be making new dietary choices as they adapt to a novel environment.

*Diel pattern*

Our results did not confirm the hypothesis that round goby feeding intensity increases at night, suggesting that there is no general diel pattern in round goby feeding behaviour. The original hypothesis was based on relatively few studies, all of which originate from the Laurentian Great Lakes Basin. Dubs & Corkum (1996) and Dopazo et al. (2008), for example, both describe a night-time increase in round goby activity, connecting this with an increase in feeding intensity (Johnson et al. 2008) and a diel shift in diet composition (Carman et al. 2006). As our study took place on a typical channelised European river, this suggests that environmental conditions may play a role in determining round goby activity patterns, and on diel feeding behaviour in particular.

Intuitively, diel feeding patterns are defined by either morphological adaptations (apparently not in round goby) and/or diel changes in a range of interacting factors, such as competition, predator pressure, habitat and prey availability and/or activity. At sites with homogeneous and stable habitat features, such as those found along our channelised study site (e.g. constant flow regime, constant presence of shelter), there is little or no potential for diel feeding changes.



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Similarly, there is little potential for diel change due to competition or predatory pressure as few other species inhabit the rip-rap habitat along the River Dyje (see Valová et al. 2006) and a diverse range of both day-active (e.g. pike *Esox lucius*, larger chub *Squalius cephalus*) and night-active (e.g. burbot *Lota lota*, pikeperch *Sander lucioperca*, European catfish *Silurus glanis*) predators constitute diurnally constant predatory pressure (see also Janáč & Jurajda 2013).

The only obvious way in which prey availability could change diurnally at this type of site is through night-time drift. While drift was not specifically sampled for this study, samples assessing larval drift were taken on the same nights as our samples for a different study. These showed that the drifting assemblage consisted mostly of gobiid larvae and *Leptodora kindti* (Crustacea, Cladocera) (Janáč et al. 2013, K. Roche, pers. observ.). None of these items, however, was important in round goby diet (*L. kindti* was never found) during the day or night, strongly suggesting that gobies do not feed on drift, at least at our study site (but compare with the suggestions of Carman et al. 2006 and Johnson et al. 2008).

Diel changes in prey activity (aside from drift) were also unlikely. Most taxa present in round goby diet were sedentary and exposed to both day and night predation (e.g. molluscs, chironomids), those that could theoretically hide during the day (e.g. mayfly larvae, see Carman et al. 2006) would have to hide in the very habitats where gobies spend much of their time sheltering and feeding (i.e. interstitial spaces and sediment).

*Inter-sex differences*

Unlike diel differences, there were significant (albeit relatively small) inter-sex differences observed in diet, thus confirming the paradigm-based hypothesis. We assume that the inter-sex differences observed in feeding intensity in our study resulted from the nest-guarding behaviour of males, i.e. even if males do leave the nest to feed, they will be restricted to the food available within a limited area close to the nest (herein termed the "nest range"). In our study, the significant inter-sex differences observed were marginal, with male IF 80 % of female and displaying only slight differences in diet composition. The inter-sex similarity in diet strongly suggests that a) the male nest range in our study area was large enough to contain prey in numbers/composition similar to that available over the whole river (i.e. reflecting that available to non-nest guarding females), b) that guarding of the nest only marginally restricts male feeding, and/or c) only a low proportion of guarding males were present in our sample. Although the majority of males caught consisted of larger (> 8 cm SL), mature individuals, many of which had the distinctive dark colouration of dominant males, we could not directly quantify the proportion of guarding males in the population sampled in our study.

Our results can thus be interpreted in two ways. First, that nest-guarding only has a limited effect on feeding; and hence there would be no reason to expect males to stop feeding over the nesting period (resulting in poor condition or mortality in the majority of guarding males) as previously suggested by Kostyuchenko (1961, 1970). Males may indeed display poor condition following the breeding season; however, this may be the result of a range of factors, including variable prey availability within the nest-range, prey types available and competition and energetic costs associated with nest defence. These factors will differ both over time and between sites. As an example, male post-breeding condition is likely to be negatively affected at sites where the available macrozoobenthos community is relatively inaccessible or dominated by high cost/low energy species, such as the zebra and quagga mussel-dominated communities of the Great Lakes.

Alternatively, the proportion of guarding males present in the sample may have been too low to produce large inter-sex differences. Hence, our results would suggest that inter-sex differences in diet are hard to generalise and will depend on the proportion of guarding males in the population and other factors not considered in our study. Indeed, the only current study that has analysed inter-sex differences (Thompson & Simon 2014) obtained results opposite to our study, i.e. higher IF in males than females, which was put down to female fasting.

This study was undertaken in order to assess certain commonly held paradigms regarding round goby diet, i.e. consumption of eggs/larvae of native fish, night-time feeding and male-biased inter-sex differences in diet. Our results suggest that a more critical approach is needed in round goby dietary studies due to a lack of generalisation and the range of factors that can affect dietary choices.

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