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Sexual segregation in monomorphic minnows

Siân W. Griffiths [a,*], James E. Orpwood [a,b], Alfredo F. Ojanguren [a,c], John D. Armstrong [b], Anne E. Magurran [c]

[a] Cardiff School of Biosciences, Cardiff University, Cardiff, U.K.
[b] Marine Scotland Science, Freshwater Laboratory, Pitlochry, U.K.
[c] School of Biology, University of St Andrews, St Andrews, U.K.

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Sexual segregation, where males and females use habitat in different ways, is widespread among animals including fish, and has important consequences for key aspects of population ecology including foraging success, predator avoidance and growth. However, currently, evidence for sexual segregation is based on observations of sexually dimorphic species or species with differing reproductive strategies. We used European minnows, *Phoxinus phoxinus*, to test the null hypothesis that sexual segregation does not occur in sexually monomorphic species. A large, seminatural stream channel equipped with passive integrated transponder (PIT) detectors monitored the activity of 70 fish for 98 days on ecologically relevant spatial and temporal scales. Sexual segregation was evident spatially (with males and females using different habitats within the stream), temporally (males switched patches more frequently than females at night, but not during the day) and socially (with males, but not females, demonstrating same-sex association preferences). Our results are the first to demonstrate sexual segregation in monomorphic species outside the breeding season. We discuss potential explanations for our observations and ways in which patterns of variation in activity, space use and social interactions have important implications for population dynamics.

The contrasting behaviour patterns of males and females are well documented in a vast literature that ranges from sex differences in offspring provisioning (Bird, 1999) to sex differences in spatial distribution (Ruckstuhl, 2007; Ruckstuhl & Neuhaus, 2005). The latter is of particular interest for two reasons. First, sexual segregation has important consequences for population processes such as growth and breeding success. For example, gene flow and thus population viability will be reduced in species in which males and females have distant home ranges and only a subset of the sexes are likely to meet during breeding (Komdeur & Deerenberg, 1997). Second, understanding more about the factors underlying separation of males and females has practical implications for management of animals. For example, in marine long-line fisheries a disproportionate number of female fish are captured (Freon & Misund, 1999; Wirtz & Morato, 2001) despite unbiased operational sex ratios. The explanation for this lies in females maximizing growth and fecundity by prioritizing foraging behaviour in the resource-rich habitats of lines with baited hooks. Management of such sex-biased fishing methods can reduce skewed mortality and local species extinctions.

However, explanations of the evolution of sexual segregation and investigations of the factors influencing it have not distinguished between the effects of sex and the effects of sexual body size dimorphism (Wearmouth & Sims, 2008). In ungulates, for example, observations of males occupying different habitats from females are more parsimoniously explained by body size differences rather than sexual segregation per se (Conradt, 1998a, 1998b; Ruckstuhl & Neuhaus, 2002). Similarly, in seabirds such as northern giant petrels, *Macronectes halli*, where females forage mainly at sea whereas males scavenge for seal and penguin carcasses on coastlines (González-Solís, Croxall, & Wood, 2000), these sex-related foraging distributions are explained by the effects of sexual dimorphism on fasting endurance, competition for food items and flight metabolic rates: northern giant petrels are the most sexually dimorphic of all seabirds. In addition to sex differences in morphology, sex differences in reproductive strategies can also cause variation in resource use and segregation: females choose habitats with resources important for their disproportionately large parental investment compared to males (Ruckstuhl, 2007; Ruckstuhl & Neuhaus, 2002). Thus, while it is well established that sexual segregation occurs in taxa exhibiting a high degree of sexual dimorphism or sex differences in reproductive tactics, including ungulates (Bowyer, 2004), reptiles (Shine et al., 2000), birds (González-Solís et al., 2000; Stauss et al., 2012) and fish (Croft,

* Correspondence: S. W. Griffiths, Cardiff School of Biosciences, The Sir Martin Evans Building, Cardiff University, Museum Avenue, Cardiff CF10 3AX, U.K.
E-mail address: GriffithsSW@cardiff.ac.uk (S. W. Griffiths).

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James, & Krause, 2006; Wearmouth & Sims, 2008), the extent to which sexual segregation occurs in sexually monomorphic species, that is, those species in which males and females are not phenotypically distinct, is less clear. Testing monomorphic species, in which sexual segregation may occur in the absence of sexual dimorphism, is important for understanding the proximate and ultimate causes underlying sexual segregation in vertebrates.

European minnows, *Phoxinus phoxinus*, a small species of shoaling cyprinid, are ideal for assessing sexual segregation in a sexually monomorphic species since the sexes are phenotypically distinct only during the breeding season. For the remainder of the year the sexes are phenotypically indistinguishable thus allowing the sex of known individuals to be determined during the breeding season and their behaviour to be monitored remotely thereafter using passive integrated transponder (PIT) technology (Griffiths, Ojanguren, Orpwood, Magurran, & Armstrong, 2007; Orpwood, Magurran, Armstrong, & Griffiths, 2008).

In this study we investigated whether European minnows exhibit sexual segregation outside the breeding season, when individuals are sexually monomorphic. We sought to address whether sex-specific behaviours translate into either spatial and temporal sexual segregation (habitat segregation) with males and females using different habitats or social segregation of the sexes (males and females found in different groups; Conradt, 1999). Our null hypothesis predicted no sexual segregation among monomorphic minnows outside the breeding season. This hypothesis was addressed using wild-caught fish in a large, seminatural indoor stream channel in which the activity and space use of uniquely identifiable individuals were tracked continuously using PIT technology over a period of 14 weeks postspawning under conditions of seasonally ambient temperature and daylength.

METHODS

Study Species

European minnows are a schooling fish which forms single-species or mixed-species schools (Allan & Pitcher, 1986). Because they are found in large numbers and in discrete schools, they are a valuable species for the study of schooling behaviour. Magurran and Pitcher (1987) found that although there was variation in shoal size, the mean elective group size of minnows, in the absence of predation, was four for fish from a population where predatory pike, *Esox lucius*, were absent and five for fish from a habitat where pike were present. Elective group size was defined as shoals of interacting fish within four body lengths of each other. If a predator was present all fish in the vicinity congregated into a single large school.

Male and female minnows are similar in appearance except during the breeding season. At this time (April to June) males typically display a relatively dark and distinctive green and red body coloration, red fins and white tubercles on the head (Frost, 1943). These tubercles increase the tactile effect of butting and mating embraces which occur during spawning (Smith, 1991). Although individual males defend territories over areas of gravel, spawning is best described as communal (Winfield & Nelson, 1991).

Wild European minnows were caught by electrofishing, netting or angling between 17 and 20 May 2004 from the River Almond (56°25′34′N, 3°32′38′W) and two points on the River Braan (56°30′32′N, 03°47′03′W and 56°33′42′N, 03°46′60′W), both tributaries of the River Tay, Perthshire, U.K., where minnows are commonly found. After capture, fish were transported immediately by road to the Marine Scotland Science Almondbank Field Station, Perthshire in a separate container of oxygenated river water for fish originating from each river until the start of the experiment. No mortality was observed in response to capture and transport. In total, 108 fish were caught, representing <1% of the natural population, and consisting of eight groups of fish caught from the same 100 m of river (four groups from each of the Rivers Almond and Braan). Each group of fish comprised either 13 or 14 fish. Between capture and the start of the experiment (21–24 days depending on capture date), each group of fish was kept in a 1 m diameter flow-through circular tank with a continuous supply of water from the River Almond and fed with defrosted chironomid larvae (Aquafresh, Manchester, U.K.) twice a day. A number of plastic tubes were placed on the bottom of each tank to provide shelter. Each fish was assigned a unique alphanumeric identity code by subcutaneous insertion of a PIT tag into the abdominal cavity, 15–17 days before the start of the experiment. This procedure involved anaesthetizing small groups of five or six fish in baths of MS222 at river water temperature for approximately 20 s before measuring fork length (L_F, to the nearest mm) and wet mass (M, to the nearest 0.1 g). A PIT tag was then inserted into the abdominal cavity of each fish via a small (<3 mm) incision made in the ventral side of the fish, and then placing the fish individually into a recovery bath of well-oxygenated river water for approximately 10 min until they resumed normal swimming and respiratory behaviour. Each PIT tag weighed 0.1 g which was typically less than 3% of the wet mass of the fish (mean body mass ± SD = 2.2 ± 0.7 g, $N = 191$). Tags were sterilized before use by soaking them for 24 h in absolute ethanol. Tagging occurred during late May when the sexes were easily distinguishable from colour variations (Frost, 1943).

Neither fork length (L_F) nor wet mass (M) differed significantly between males and females (general linear model, GLM, two-way ANOVA on $\log_{10} x$ transformed data: L_F: $F_{1,\ 66} = 1.90$, $P = 0.173$; M: $F_{1,\ 66} = 3.06$, $P = 0.085$; mean $L_F \pm$ SD: River Almond males = 66.6 ± 4.7 mm, $N = 13$; River Almond females = 67.2 ± 5.0 mm, $N = 25$; Cochill Burn males = 69.7 ± 3.3 mm, $N = 14$; Cochill Burn females = 72.9 ± 6.1 mm, $N = 18$; mean $M \pm$ SD: River Almond males = 3.3 ± 0.7 g, $N = 13$; River Almond females = 3.6 ± 0.8 g, $N = 25$; Cochill Burn males = 3.9 ± 0.4 g, $N = 14$; Cochill Burn females: 4.6 ± 1.3 g, $N = 18$). Fish originating from the River Almond were significantly smaller than fish originating from the Cochill Burn (L_F: $F_{1,\ 66} = 12.48$, $P = 0.001$; M: $F_{1,\ 66} = 12.89$, $P = 0.001$). The interaction between sex and river of origin was not significant for either L_F ($F_{1,\ 66} = 0.90$, $P = 0.346$) or M ($F_{1,\ 66} = 0.19$, $P = 0.667$).

Experimental Set-up

The experiment was conducted in part of a glass-sided indoor stream channel through which water from the River Almond flowed at ambient temperature. Part of the stream channel was divided into four sections with stainless steel wire-mesh screens. Each section (7.5 × 1.5 m) comprised a pool (1.5 × 1.5 m; 0.5 m water depth) positioned between an upstream riffle (3 × 1.5 m; 0.2 m water depth) and a downstream riffle (3 × 1.5 m; 0.2 m water depth). Water temperature was measured continuously using an automated data logger. Daily water temperature (mean ± SD: 13.2 ± 2.2 °C; range 8.4–18.3 °C; $N = 98$) was calculated as the mean value of eight measurements per day taken 3 h apart from 0000 hours. An ambient photoperiod was maintained throughout the experiment by supplementing natural light with full-spectrum lamps between 0700 and 1900 hours. The stream channel was landscaped with a gravel and pebble substratum and several boulders, and supported a natural community of aquatic invertebrates. In addition to this natural food source, defrosted chironomid larvae were provided by an automatic fish feeder (ASU 2000) operated via a computer (MacLean, Miles, & Armstrong, 2003). Two buckets containing a suspension of defrosted