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Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*)

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Alternative male reproductive tactics are taxonomically widespread. In such species, parental, or conventional, males express secondary sexual characteristics, court females and guard offspring, while smaller parasitic or sneaker males avoid the costs of courtship and parental care by performing sneak fertilizations. Theory predicts that sneakers will invest more in testes mass and produce more competitive ejaculates than parentals because sneakers always experience sperm competition while parental males experience sperm competition only when a sneaker is present. Here we present convergent lines of evidence supporting the existence of alternative male reproductive tactics in round gobies (*Apollonia melanostoma*, formerly *Neogobius melanostomus*), a recent invader in the Great Lakes. Dark morph males exhibited secondary sexual characteristics, were larger and had higher plasma 11-ketotestosterone concentrations than light morphs, while light morph males invested more in ejaculates (both testes mass and sperm density). Both male morphs had enlarged urogenital papillae, but papillae were relatively longer in light morph males. Sperm tail length did not differ between morphs, and sperm from dark morphs swam faster than sperm from light morphs. Our data strongly argue for the presence of alternative tactics in round gobies, support some predictions from sperm competition theory and align with empirical observations in other taxa. For species of concern like the invasive round goby, it is critical to consider such evidence of alternative male mating tactics when constructing population growth models and assessment of invasion success and impacts.

Introduction

Male alternative reproductive tactics (MARTs) emerge when competition for mating opportunities is fierce and the potential exists for some males to reduce fitness costs by exploiting the reproductive investment of other males (Oliveira et al., 2008). Among fishes, MARTs are particularly common and are taxonomically widespread, because external fertilization is prevalent (allowing simultaneous sperm release from several males), somatic growth is indeterminate (creating large variance in body size and resource sequestering abilities among males), and paternal care is common (providing strong fitness pay-offs to male sneakers that avoid the costs of courtship and parental care; Oliveira, 2006; Taborsky, 1998). The aim of this study was to comprehensively examine the possibility of MARTs in the round goby (*Apollonia melanostoma*, formerly *Neogobius melanostomus*), a recent prolific Ponto-Caspian invader of the Laurentian Great Lakes and aquatic biotas in northeastern Europe (Corkum et al., 2004).

Although the round goby poses a serious threat to native fauna ecology and conservation (e.g., Jude et al., 1995), its reproductive habits are still not fully understood. Breeding fish are difficult to observe in the wild (Wickett and Corkum, 1998) and spawning is rarely achieved under laboratory conditions (L.D. Corkum, University of Windsor, personal communication; J.R. Marentette, personal observation), complicating efforts to study reproduction directly. However, understanding round goby reproduction is critically important in terms of predicting the ecological impacts of this invasive species. Round gobies belong to the speciose teleost family Gobiidae that contains several species with male alternative reproductive tactics, including the common goby, *Pomatoschistus microps* (Magnhagen, 1992), black goby, *Gobius niger* (Mazzoldi and Rasotto, 2002), and the sand goby, *Pomatoschistus minutus* (Svensson, 2004). Sneaking behaviour has been observed in the round goby (C. Murphy, University of Alberta, personal communication) and has been reported to occur in a number of publications (Corkum et al., 1998; MacInnis, 1997; Marentette and Corkum, 2008). However, these

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reports are based on limited laboratory behavioural observations and a few morphological observations.

To investigate the claim of MARTs in round gobies, we examined the external morphology, internal anatomy, endocrinology and sperm characteristics of male round gobies from Lake Ontario in light of sperm competition theory and current knowledge of MARTs in vertebrates. In general, male tactics can be divided into two categories. Parental males, sometimes called conventional, type I or bourgeois males, are large, invest more in growth than in reproduction, defend territories, court females, exhibit secondary sexual characteristics, and have elevated androgen concentrations (Oliveira et al., 2008). In contrast, sneaker, type II or parasitic males are smaller, invest in reproduction at the cost of growth, and lack secondary sexual characteristics. Rather than court females, these sneaker males add their ejaculate surreptitiously to spawnings in progress by stealth, speed, or by imitating females (Oliveira et al., 2008). Males will be subject to asymmetric risks of sperm competition, the competition between sperm from rival males to fertilize a female's ova (Parker, 1970), depending on the reproductive tactic employed. Because parental males are sometimes able to sequester mates and drive off competitors including sneakers, they experience a relatively lower risk of sperm competition. In contrast, sneaker males experience sperm competition during every mating, as by definition they only release sperm in the presence of a parental male. Thus, in order to overcome their disadvantage, sneaker males are expected to invest more in sperm number than parental males (Parker and Ball, 2005). Sperm competition is also thought to lead to sperm with longer flagella that swim faster (Ball and Parker, 1996). Sperm tail length is associated with sperm swimming speed (Fitzpatrick et al., 2009) and, in external fertilizers, sperm swimming speed predicts fertilization success in competitive matings (Gage et al., 2004).

We predicted that if two alternative reproductive tactics exist in round gobies, one morph (presumably the parental male morph) would be larger than the other morph (the sneaker male morph). Since secondary male sexual traits are associated with high levels of androgens, we predicted that plasma concentrations of 11-ketotestosterone (11-KT), the primary fish androgen, would be higher in parental males than in sneaker males (Oliveira et al. 2008). We also predicted that parental males would invest comparatively less in testes mass than sneaker males, and that sneaker males, which encounter higher levels of sperm competition would produce more sperm that have longer flagella and swim faster than the sperm of parental males (Ball and Parker, 1996; Parker and Ball, 2005).

Methods

Round gobies ($n = 1295$) were collected in minnow traps or by electrofishing from Hamilton Harbour between June 26 to August 23, 2006, and May 16 to August 29, 2007, and from nearby Jordan Harbour in Lake Ontario on July 21, 2006. Traps baited with 25 g frozen corn were set in <1 m of water every 2 weeks, and were collected after 24 h. Fish were then transported to McMaster University and maintained in aerated laboratory aquaria before processing.

The sexes were differentiated by the shape of the urogenital papilla, which is broad and square in females, but narrow and pointed in males (Miller, 1984). Fish that did not possess identifiable papillae were classified as juveniles, and all males with small flat papillae were classified as non-reproductive (non-spawning males). Dissections revealed the size and maturity of testes and confirmed this male classification scheme. All males with erect papillae were shown by dissection to have mature testes (see measurements below). Males with erect papillae that had dark to black bodies were classified as dark morph males and we considered these to be putative parental males, since nest-holding round goby males have been reported to have black nuptial coloration (Corkum et al., 1998). Males with erect urogenital papillae that had light, mottled juvenile or female-shaped bodies (see below) were then classified as light morph males and putative sneakers. Using this classification scheme, of the 752 adult males captured, 144 were classified as dark morph and 151 as light morph males, with the remainder classed as non-reproductive males.

Fish were all measured for total length (TL), head width (taken across the posterior orbital edge), and urogenital papilla length (anterior insertion to posterior tip), all to 0.1 mm (Fig. 1). Total body mass and the mass of the gonads were measured to 0.001 g. The gonads of male round gobies have two sets of paired organs: 1) testes and 2) accessory structures also called seminal vesicles or sperm-duct glands (Miller, 1984). The function of these accessory glands has been linked to sperm storage, production of mucus for laying sperm trails (lines of sperm embedded in mucus, laid on nest surfaces), and pheromone production (Jasra et al., 2007; Mazzoldi et al., 2005; Fig. 1). Hence the mass of testes and accessory glands were recorded separately. Based on these measures we calculated a somatic mass as the total body mass − total gonad mass (testes and accessory glands combined). We also calculated a papilla index or PI (papilla length / TL × 100%), testicular somatic index or TSI (testes mass / somatic mass × 100%), and accessory gland somatic index or AGSI (accessory gland mass / somatic mass × 100%).

Blood samples were collected in heparinized 10 µL micro-capillary tubes from a representative sample of 14 dark morph and 14 light morph males by caudal severance 0–2 days after capture, and spun at 14,500 RPM for 10 min. Collection day was random, did not vary between the two morphs and had no correlation with 11-KT concentrations for either morph (Spearman rho, $p > 0.2$ for both dark and light morphs). The plasma was then removed and frozen at −80 °C. Steroids were extracted from the samples by shaking 5 µL of plasma with 5 ml of diethyl ether. After freezing the aqueous phase in

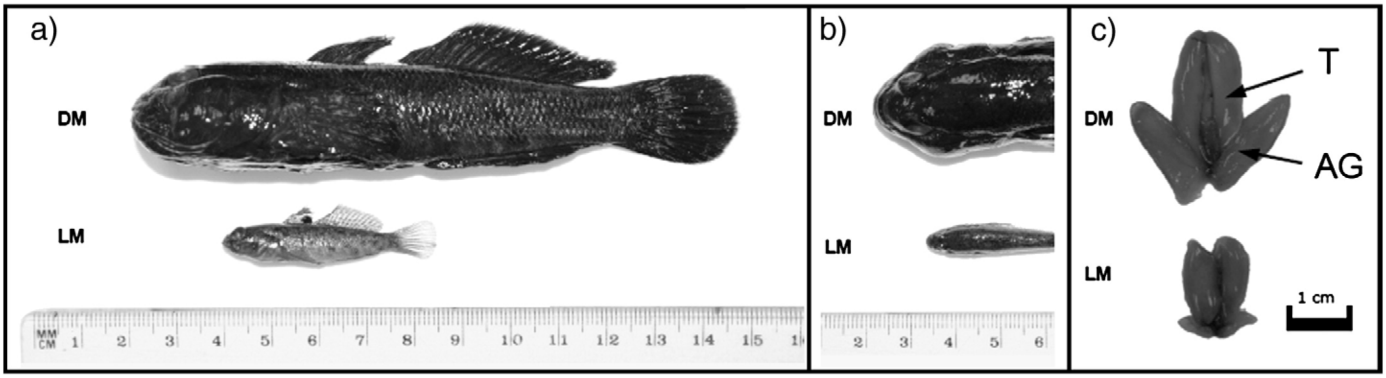

Fig. 1. (a) External morphology (lateral view) of a dark morph male (DM) and a light morph male (LM) showing differences in colour and body size. (b) Anterior dorsal view of a dark morph male (DM) and a light morph male (LM) showing differences in head width. (c) Dissected testes of dark morph males (range 96.9–131.3 mm TL, top row) and light morph males (range 45.0–70.6 mm TL, bottom row). This photo shows the relative investment in lobes of the testes (T) versus accessory glands (AG). A scale appears below (in cm).