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# First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus*)

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ABSTRACT

Although the round goby, *Neogobius melanostomus*, is widespread in the Great Lakes and has an extended breeding season with a high reproductive rate; its spawning behaviour remains elusive. We present the first reported accounts of spawning by the round goby in the laboratory. By simulating winter conditions and restoring spring conditions, we induced round gobies to spawn in October 2007, March 2008, May 2008, and January 2009. In one case, fanning by the nest-holding male began 10 days before egg deposition and, during this period, the male rubbed secretions along the ceiling of the nest. Males were choosy about which gravid females entered the nest and prevented entry by some females. Spawning involved repeated inversions by females and males releasing gametes on the ceiling of the nest. Males guarded the nest by blocking the entrance, producing agonistic vocalizations and chasing intruders. Inside the nest, eggs were regularly inspected by the males and constantly ventilated using pectoral and caudal fins. Up to three gravid females spawned sequentially in a nest. Peak ventilation occurred after egg deposition and declined with time until the parental male ate the eggs. The decline of parental care and egg cannibalism was likely an artifact of laboratory conditions and small brood size. Our findings offer new information on the reproductive habits of the invasive round goby. Because the reproductive sequence in the laboratory seems easy to disrupt, the procedures may lead to a management tool to control the spread of the species into new areas.



## Introduction

Since it was first discovered in 1990 (Jude et al., 1992), the round goby (*Neogobius melanostomus*) has been a prolific invader in the Laurentian Great Lakes (Charlebois et al., 2001). Potential reasons for the proliferation of the round goby include its broad diet and availability of molluscan prey (adults eat mainly dreissenids), aggressiveness, high fecundity, high frequency of spawning (up to six times per year), and male parental care (Corkum et al., 2004). In the central region of the Laurentian Great Lakes, round gobies spawn from early spring throughout the summer (Wickett and Corkum, 1998; MacInnis and Corkum, 2000). Previous field studies have provided evidence for nest defense and egg fanning (Wickett and Corkum, 1998), but there has been no account of spawning behaviour of the round goby in the field or laboratory.

There is a distinction between factors that attract or guide conspecifics to a nest and factors associated with courtship that lead to spawning. Washings from reproductive male round gobies (Gammon et al., 2005) help guide gravid round goby females to the nest. Also, in the laboratory, playback of vocalizations of a reproductive male round goby have been shown to attract male and female round gobies to within 107 cm of a speaker (Rollo et al., 2007).

In this study, we describe nest preparation by the male, courtship at the nest entrance, spawning within the nest and parental care. Despite our success in documenting the first induced round goby spawnings in a laboratory by simulating winter and spring conditions, none of the four parental males that spawned in the laboratory completed their brood cycle (i.e., we did not observe egg hatching). However, larval hatching in the lab is successful when embryos are isolated from males. Understanding the reproductive habits of the round goby may enable researchers to control the spread of this invasive species by manipulating factors associated with its reproductive success.

## Methods

Round gobies were collected in August 2007 and 2008 by angling along the Canadian shore of the Detroit River at Windsor, ON, and brought to the Animal Care Facility at the University of Windsor. We observed four separate reproductive males and associated females spawn in the laboratory (Table 1). From fish collected in August 2007, we recorded three spawning events in October 2007, March 2008 and May 2008. From fish collected in August 2008, we recorded one male and associated females spawn in January 2009. Because we were

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**Table 1**
Summary of spawning events after simulated winter and spring conditions.

| Trial | Male total length (cm) | Winter conditions initiated | Spring conditions initiated | Spawning date | Time spawning initiated (h) | Video documentation |
|---|---|---|---|---|---|---|
| 1 | 15.8 | Sep. 3, 2007 | Sep. 24, 2007 | Oct. 14, 2007 | 22:00 to 23:00 | After egg deposition |
| 2 | 15.5 | Jan. 21, 2008 | Feb. 11, 2008 | Mar. 3, 2008 | 15:00 to 15:30 | Eggs deposited by females |
| 3 | 13.5 | Apr. 1, 2008 | Apr. 22, 2008 | May 21, 2008 | 22:00 to 23:00 | After egg deposition |
| 4 | 14.3 | Nov. 3, 2008 | Nov. 25, 2008 | Jan. 4, 2009 | 01:00 to 0:200 | Continuous documentation, beginning 10 days prior to spawning |

Male size was determined after the spawning event.

unable to record video constantly, our recordings captured different aspects of spawning behaviour among trials (Table 1).

In August 2007, ten mature fish (three males and seven females) and juveniles were placed into a 90-L flow-through tank lined with aquarium gravel and filled with dechlorinated, aerated water. None of the fish exhibited reproductive characters as described in Miller (1984). We provided each male with a nest, which was a rectangular black PVC (16 cm × 11.5 cm × 5 cm) box with sheets that were glued together with a single opening (5 cm × 5 cm). Males ate several pieces of an earthworm each day in addition to Nutrafin® flakes.

Spawning was induced by changing environmental factors. Photoperiod was changed from 16L:8D to 8L:16D, water temperature was decreased from 20 to 10 °C and food supply was restricted to simulate "winter conditions." After 3 weeks, artificial "spring conditions" were gradually restored (water temperature (20 °C), light exposure (16L:8D), daily food supply). Within a few days, a male displayed reproductive traits; i.e., the male became black and exhibited territorial behaviour (Miller, 1984). During the following weeks, two to three females developed swollen abdomens, indicative of gravid conditions. The same protocol was repeated to initiate spawning in subsequent trials with different fish (Table 1).

Once males developed secondary sexual characters, male–female interactions were monitored every 2 h (from 9:00 a.m. to midnight). We began video recording once spawning was confirmed. We used a colour video camera (Hitachi VKC-370) and a DVD recorder (SONY RDR-GX330) in each of the first three spawning events. In the final spawning event (January 2009), we used an HDD SONY recorder, enabling us to record courtship, spawning and parental care without interruption. After analysing the digital images from four spawning events, we were able to describe the main phases of round goby reproduction (nest preparation, courtship, spawning and parental care) and associated activities. Recordings of sample spawning behaviours may be viewed on the website: www.uwindsor.ca/goby. Because round gobies produce sound (Rollo et al., 2007), we placed a hydrophone (Interocean Systems Inc., model 902) in the tank 30 cm from the nest entrance during the first spawning event.

## Results and discussion

### *Nest preparation*

In preparation for spawning, the resident male modified the interior of the nest by picking up gravel from the bottom of the nest and spitting it out at the nest entrance (Fig. 1a). Once the activity stopped, the nest floor still contained some gravel and a gravel mound appeared at the exterior of the nest opening. This nest excavation occurred whenever a reproductive male occupied a nest in which there was gravel on the floor of the nest. In the field, nesting round goby males occupy hard smooth crevices, either artificial or natural (MacInnis and Corkum, 2000). In sand gobies, *Pomatoschistus minutus*, Svensson and Kvarnemo (2007) showed that males in the presence of sneaker males built the smallest nest openings, apparently because the nest-holding males perceived sneakers to be a threat to competition for females and for fertilizing eggs, but not to reduce egg predation. A small nest opening may also aid in nest concealment, whereas larger entrances enhance ventilation (Jones and Reynolds, 1999a).

In January 2009, a reproductive male began fanning within the nest (Fig. 1b) and rubbing the ceiling surface with his genital papilla 10 days before females deposited eggs. Rubbing surfaces within nests has been reported for three species of Mediterranean gobies (*Gobius niger*, *Knipowitschia panizzae*, *Zosterisessor ophiocephalus*) where sperm trails are laid on the nest surface before females enter the nest; i.e., before eggs are deposited (Marconato et al., 1996; Scaggiante et al., 1999). These sperm trails contained viscous material in which sperm were contained, explaining the prolonged release (up to 80 min) of sperm into the surrounding water (Scaggiante et al., 1999). This early sperm trail production enabled the male to defend the nest and female from intruders when females deposited eggs (Marconato et al., 1996). The round goby males that we observed repeatedly fertilized eggs deposited by the female despite the deposition of secretions on the nest surface before the female entered the nest. The slime was analysed post-spawning and was found to contain sperm, however the sperm could have been tangled in the mucus from sperm released after egg deposition.

### *Courtship behaviour at the nest entrance*

Round goby courtship is initiated at the nest entrance. Females spend more time near a nest occupied by darkly pigmented males than mottled males (Yavno and Corkum, in press). Not all black round gobies are reproductive, but black males that defend nests and exhibit other secondary sexual characteristics (e.g., swollen cheeks) are reproductive (Marentette and Corkum, 2008).

Males appear to be choosy about which females enter the nest and about the timing of spawning events. In January 2009, we observed pectoral and caudal fanning activity before egg deposition, suggesting that this behaviour may play a role in mate attraction perhaps by dispersing odours. The association of fanning with courtship has been reported in the sand goby, whose males increase fanning in the presence of potential mates (Pampoulie et al., 2004). Like the round goby, three-spined stickleback *Gasterosteus aculeatus* males performed courtship fanning in the absence of eggs in their nests (Sevenster, 1961).

Our January 2009 video recordings showed that males prevented females from entering the nest by blocking the entrance. Females either faced the entrance or aligned themselves perpendicular to the male within a body length of the nest entrance, perhaps advertising their swollen belly, and erect dorsal fins to the male. Information exchange between males and females also may occur through ventilation. Reproductive males respond by increased ventilation to gonadal extracts and the putative pheromone estrone, from gravid females, indicating that reproductive males can recognize potential mates based on olfactory cues produced by females (Belanger et al., 2006). Earlier, Murphy et al. (2001) showed that ventilation responses of round gobies to steroids were sexually dimorphic.

Vocalization in fishes has been well studied (Tavolga et al., 1981). Fish are known to emit calls during mate attraction and courtship (Lugli et al., 1995) and during agonistic encounters (Thorson and Fine,