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

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A B S T R A C T

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.

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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
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, daily food supply was restricted to simulated “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; Scagillaume 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 (Scagillaume 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 (Pompoule 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, 2009).
We noted “barking calls” before, during and after egg deposition. As shown in Rollo et al. (2007), the vocalizations were low frequency (1–400 Hz) calls. The acoustic properties of the calls also were similar to what Rollo et al. (2007) described as “pulse series.” In our study, there were variations within and among individuals in the amplitude of these calls and the number of times that they were repeated. However, episodes of intense vocalization had a constant period of 0.4–0.6 s.

**Spawning behaviour**

In January 2009, reproductive male 4 (Table 1) occupied a nest in the presence of three gravid females, but it was 10 days before the male permitted a female to enter the nest to deposit eggs. Perhaps, the male blocked the nest entrance because females may not have been ready to release gametes. The three reproductive females entered the nest head first, sideways and tail first at different times. Once a reproductive female entered the nest, the male and the female alternately flipped over upside down (inverted) to deposit their gametes onto the ceiling of the nest. Females in other species within the genus *Neogobius* also deposited eggs on the ceiling of the nest (Biro, 1971; Grabowska, 2005).

Whenever the female deposited eggs, she performed small, but rapid, undulating upside down movements on the ceiling, pressing her papilla against the roof of the nest. The male made similar undulating movements when fertilizing the eggs. Eggs and sperm were released through the urogenital papilla, which was erected at about 45 degrees from the body to make contact with the nest ceiling (Fig. 1c and d). Observations of round goby spawning events in the Detroit River (MacInnis and Corkum, 2000) and western Lake Erie (Wickett and Corkum, 1998) revealed that fertilized eggs were deposited in a monolayer on all surfaces of the interior of a nest (MacInnis and Corkum, 2000). Perhaps initial egg deposition begins on the ceiling of the nest and subsequent egg deposition by other females fills the remaining available surfaces.

Female inversions were almost twice as frequent as male inversions, and each female inversion lasted twice as long as male inversions. For the March 2008 spawning event, we quantified spawning behaviour over a 30-min observation period. The female inverted 39 times, while the male inverted 21 times. In January 2009, females inverted 272 times and males inverted 151 times over 118 min of spawning. Three females spawned sequentially with the same parental male. Although two females occurred together in a nest once, spawning occurred with only one female and one male in the nest. There was a difference in mean (standard error) time for inversions between females (10.44±2.16 s) and males (5.44±1.13 s). Despite the presence of other round gobies in the tank, no intruders entered the nest during spawning between the resident male and a single female. Moreover, we did not observe any intruding males, sneaking into the nest to fertilize eggs. Although there is evidence for the presence of sneakers in the round goby (Marentette et al., 2009), behavioural confirmation is lacking.

During periods in which fish were not inverted, both the male and female were located in the center of the nest. Occasionally, the male would dart out of the nest to chase an intruder. After spawning, the female appeared to depart without being chased and the male continued to guard the nest. In January 2009, there were 683 eggs deposited in a single layer on the ceiling of the nest as a result of egg deposition by three reproductive females and a single reproductive male.

**Parental care**

Once eggs were deposited in the nest and fertilized, the reproductive male exhibited parental care. Nest-guarding males raised their pectoral and dorsal fins upon the approach of an intruder, possibly to...
increase their body size and better block the access to their nest. At the next level of aggression, the male initiated vocalizations and erected its pectoral and dorsal fins in response to the intruder. At the highest aggression level, the male darted out of the nest to chase the intruder. Once the intruder left, the male returned immediately to the nest.

In October 2007, we observed behavioural displays associated with vocalizations by the reproductive male at the nest entrance for 30 continuous minutes (selected at random) within each of 4 h after egg deposition. The calls (barks) did not create any currents out of the mouth of the male, eliminating speculations that the sound had hydrodynamic origins. Barks were typically initiated when another fish approached the nest. For example, barks were recorded in the presence of reproductive females (n = 12), reproductive males (n = 10), juveniles (n = 38), and in the absence of fish at the nest entrance (n = 3). Twice intruders were repelled from the nest entrance in response to the sound, but at other times intruders did not move. In addition to the calls, the resident male appeared agitated often darting about in the presence of intruders. We speculate that this agonistic behaviour helps to deter egg cannibalism by intruders.

The male calls of the round goby were directly related to the opening and closing of the jaws. When stationed at the nest entrance, the male kept its mouth open, revealing white teeth and gums against a black mouth cavity. This type of display was also described in the river bullhead Cottus gobio (Morris, 1954) and the cichlid fish Tilapia natalensis (Baerends and Baerends van Roon, 1950). Agonistic vocalizations during territorial defense have been identified in eight other species of the Gobiidae (Amorim and Neves, 2007). In contrast, Rollo et al. (2007) reported that recorded vocalizations from speakers were associated with the approach of an intruder. The presence of an active nest-holding reproductive male and/or the tank environment accounts for the different observations between our study and that of Rollo et al. (2007). We speculate that the vocalizations we observed are agonistic behaviours intended to deter egg cannibalism by intruders. However, the findings by Rollo et al. (2007) that males and females are attracted to playbacks of vocalizations suggest that vocalizations may not be used exclusively in agonistic contexts.

In some cases, eggs were consumed by round gobies that successfully entered the nest after spawning despite the male’s guarding activities. In other cases, the parental male terminated his brood cycle by eating the eggs within its nest. Small clutches have a high egg cannibalism rate. In the present study, we observed that the male sometimes nibble them (Figs. 1e and f). This snifing behaviour by males may be a technique to spot unhealthy or dead eggs (Jones and Reynolds, 1999b), preventing the spread of diseases in the clutch.

Once spawning occurs, one can hatch round goby larvae from eggs by removing the male from the nest (to avoid cannibalism of eggs) and by aerating the embryos attached to the interior of the nest. Using this technique, eggs have been recorded to hatch at 21 °C in 13 days (J. Marentette, personal communication). In our laboratory, one of us (S. Yavno) observed round goby spawning (July 6, 2009) under summer conditions (24 °C at a 16L:8D light regime). Once embryos were isolated from the parental male, they were aerated and maintained at the same spawning condition until hatching, 15 days after spawning. The larvae were planktonic, entering the water column repeatedly even during day light hours. These recent laboratory observations support earlier field observations of vertical (albeit, night time) migration of round goby larvae in Lake Erie, Lake Michigan and the Muskegon River mouth (Hensler and Jude, 2007). The vertical migrations enhance the dispersal of this invasive species.

**Conclusions**

The reproductive behaviours exhibited by the round goby, such as uniparental care, inversions during spawning, fanning, vocalizations, and filial cannibalism, are shared by many other species of Gobiidae.

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**Table 2**

Summary of common spawning behaviours observed within the Gobiidae.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Uniparental male care</th>
<th>Inversion</th>
<th>Vocalization</th>
<th>Filial cannibalism</th>
<th>Fanning</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblygobius phalaena</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Takegaki, 2000</td>
</tr>
<tr>
<td>Gobius cruentatus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sebastianutto et al, 2008</td>
</tr>
<tr>
<td>Gobiusculus flavescens</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Skolbekken and Utne-Palm, 2001</td>
</tr>
<tr>
<td>Knipowitschia punctatissima</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lugli et al., 1995</td>
</tr>
<tr>
<td>Neogobius fluviatilis</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biro, 1971</td>
</tr>
<tr>
<td>Neogobius gymnotrachelus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grabowska, 2005</td>
</tr>
<tr>
<td>Neogobius melanostomus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miller, 1984; Rollo et al., 2007; Wickert and Corkum, 1998</td>
</tr>
<tr>
<td>Podagogobius martensi</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lugli et al., 1995; Torricelli and Romani, 1986</td>
</tr>
<tr>
<td>Pomatoschistus minutus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Järvi-Laturi et al., 2008; Lindström and Hellsström, 1993; Lindström and Wennström, 1994; Lugli et al., 2003</td>
</tr>
<tr>
<td>Pomatoschistus pictus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amorim and Neves, 2007; Amorim and Neves, 2008</td>
</tr>
<tr>
<td>Priolepis nocturna</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wittenrich et al., 2007</td>
</tr>
<tr>
<td>Rhinogobius brunneus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suk and Cho, 2002; Takahashi and Kohda, 2004</td>
</tr>
<tr>
<td>Valenciennea longipinnis</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Takegaki and Nakazono, 1999; Takegaki and Nakazono, 2000</td>
</tr>
<tr>
<td>Zosteriaspis ophiocephalus</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ota et al., 1996; Mazzoldi et al., 2000; Malavasi et al., 2008; Seagjante et al., 1999</td>
</tr>
</tbody>
</table>

Species names are listed alphabetically. Confirmed behaviours exhibited by males of each species are indicated by a check mark.
(Table 2). Although, fertilized eggs have been observed in round goby nests in the field during spring and summer (Wickett and Corkum, 1998; Macninis and Corkum, 2000), our study showed that it is possible to induce round goby reproduction in the laboratory outside of the reproductive season by manipulating environmental conditions. This finding will have important implications in future studies that investigate mate attraction within round goby. On the basis of these laboratory observations, it appears that it is feasible to interrupt these laboratory observations, it appears that it is feasible to interrupt

Acknowledgments

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