Behavioural responses of female *Neogobius melanostomus* to odours of conspecifics

D. B. Gammon*, W. Li†, A. P. Scott‡, B. S. Zielinski*
and L. D. Corkum*§

*Department of Biological Sciences, University of Windsor, Windsor, ON N9B 3P4,
Canada, †Department of Fisheries and Wildlife, Michigan State University,
East Lansing, MI 48824, U.S.A. and ‡Weymouth Laboratory,
The Centre for Environment, Fisheries, and Aquaculture Science,
Weymouth, Dorset DT4 8UB, U.K.*

The behavioural responses of reproductive and non-reproductive female round gobies *Neogobius melanostomus* to water conditioned by reproductive and non-reproductive males and females were tested. The behavioural responses of reproductive female round gobies exposed to odour of reproductive males included increased time spent near the source of the odour, elevated swimming velocities and directed movement to and around the odour source when compared with their responses to control water. These results suggested that pheromones released from reproductive males may induce spawning behaviour in reproductive females. Non-reproductive females exposed to reproductive female odour spent significantly more time near the odour source of reproductive females compared with control water. Non-reproductive females also showed directed movement towards and around the odour source when exposed to reproductive female odour. These results suggested that round gobies use inter-sexual and intra-sexual pheromones and that both sex and reproductive status are important in the detection and release of these pheromones. © 2005 The Fisheries Society of the British Isles

Key words: inter-sexual signalling; intra-sexual signalling; pheromone; round goby; swimming pathway; swimming velocity.

INTRODUCTION

Olfaction in fishes is among the most highly developed olfactory systems in vertebrates (Kleerekoper, 1969). This powerful sensory system has been linked to various behavioural and physiological functions including: courtship behaviour (Stadler, 1999), mate recognition and attraction (Haberli & Aeschlimann, 2004), spawning (Carolsfeld *et al.*, 1997), discrimination between conspecifics and heterospecifics (McLennan & Ryan, 1997), intra-sexual communication (Losey, 1969; Shohet & Watt, 2004), predator avoidance (Kristensen & Closs, 2004) and regulation of sexual maturation (Aday *et al.*, 2003). Pheromonal cues

§Author to whom correspondence should be addressed. Tel.: +1 519 253 3000 ext. 2717; fax: +1 519 971 3609; email: corkum@uwindsor.ca

released between conspecifics can have several valuable functions, many of which are involved in vital reproductive processes (Stacey *et al*., 2003). Detection of these cues becomes even more important for those species that live in dark, turbid waters where visual communication may be limited.

The round goby *Neogobius melanostomus* (Pallas) is a small benthic fish that is believed to have been introduced to the Laurentian Great Lakes through ballast water in ships originating from its native range in the Ponto-Caspian region (Vanderploeg *et al*., 2002). Since its introduction in 1990, the round goby has spread to all five of the Great Lakes and has started to invade the Mississippi River basin (Charlebois *et al*., 2001). Corkum *et al*. (2004) suggested that the success of the round goby may be due to its broad diet, aggressiveness, high fecundity, repetitive annual spawns and male parental care. Parental male round gobies guard and defend nests into which up to 15 females may deposit eggs (MacInnis & Corkum, 2000). Nests can be located at depths of 11 m or more (Wickett & Corkum, 1998) at which visibility may be limited. While male round gobies may use visual cues (colouration changes) to attract females, these cues may not be as successful in attracting females in dim light as are water-transported chemical cues. Indeed, species recognition and sex discrimination by male notchtongue gobies *Bathygobius curacao* (Metzelaar), a fish commonly found in highly turbid waters, is primarily facilitated through chemical cues (Stadler, 1999). Similarly, sex discrimination in adult blind gobies *Typhlogobius californiensis* Steindachner is done solely through chemoreception (MacGintie, 1939). Pheromone-based signalling, therefore, may play a vital role in the reproductive success of the round goby.

Evidence of pheromonal signalling has been found in other gobiids. For example, Tavolga (1956) demonstrated the presence of sex-specific courtship pheromones in the frillfin goby *Bathygobius soporator* (Valenciennes) by showing that reproductive males were induced to court when exposed to water that had previously contained ovulating females, however, males did not respond to water that had contained other males. This is in contrast to the black goby *Gobius jozo* (= *G. niger*) L., in which parental males produce a pheromone that not only attracts ovulated females (Colombo *et al*., 1980), but also stimulates aggressive behaviour in other males (Locatello *et al*., 2002). Interestingly, males of both *B. soporator* (Tavolga, 1956) and *G. jozo* (Colombo *et al*., 1982) demonstrated a decreased courtship response when exposed to conspecific females that were preovulatory as opposed to ovulated, suggesting the importance of reproductive status in these pheromonal-signalling systems. Further, ovulated *G. jozo* females, but not pre-ovulatory or post-ovulated females, displayed attraction to a sex steroid produced by the testes of males (Colombo *et al*., 1980). Clearly, pheromonal-signalling systems in gobiids often depends on the reproductive status of the sender and the receiver, an evolutionary mechanism that may help only those fishes that are ready to spawn find each other.

The goal of this study was to investigate the behavioural response of female round gobies to a variety of conspecific chemical cues. The response of reproductive (RFs) and non-reproductive (NRFs) females to water containing the odour of either reproductive males (RMs), non-reproductive males (NRMs), reproductive females (RFs) or non-reproductive females (NRFs) was tested. Water conditioned from conspecifics (*i.e*. washings) was used rather than individual (or groups of) steroidal compounds to avoid the possibility of missing an

essential component of the potential sex pheromones. It was hypothesized that RFs would be attracted to sex pheromones released by RMs.

MATERIALS AND METHODS

COLLECTION OF EXPERIMENTAL ANIMALS AND CONDITIONED WATER

Round gobies were collected by angling in the Canadian waters of the upper Detroit River and trawling (Ontario Ministry of Natural Resources) in the western basin of Lake Erie. Fish were collected from May to September 2002 and from May to July 2003. Round gobies were stored in holding tanks (205 l) under a constant photoperiod (16L : 8D) in the animal quarters facilities of the University of Windsor (Department of Biological Sciences). Holding tanks were equipped with an 18° C dechlorinated tap water flow-through system, two air stones, gravel and shelters made from PVC tubing. All round gobies were fed daily with Nutrafin® fish flakes. After 24 h, fish were separated into one of four different holding tanks based on sex and reproductive status (*i.e.* RM, NRM, RF or NRF).

The sex of round gobies was determined through examination of their urogenital papilla: pointed in males and more broad and oval in females (Miller, 1984). Reproductive males were identified by their black nuptial colouration, enlarged cheeks, thickness of their papilla (Miller, 1984), active sperm (Belanger *et al.*, 2004) and histological examination of the testes (Arbuckle *et al.*, 2005). Males lacking secondary sexual characteristics were classified as NRMs. Reproductive females were identified by their swollen abdomens and their reproductive status was verified after use by their gonadosomatic index (I_G; $I_G = 100 M_G M^{-1}$, where M_G is the gonad mass and M is the total mass of the fish), with values of ≥ 8 considered to be reproductive (MacInnis, 1997). Females lacking these traits were classified as NRFs. The mean ± s.e. I_G was $11 \cdot 8 \pm 0 \cdot 7$ for RFs and $1 \cdot 1 \pm 0 \cdot 5$ for NRFs. Although there was a significant difference in the I_G for these two groups (unpaired *t*-test, d.f. = 47, $P < 0 \cdot 001$), there was no significant difference in the mean total length (L_T) between RFs and NRFs (unpaired *t*-test, d.f. = 47, $P > 0 \cdot 05$). Subsets of these females were randomly assigned into two groups, those that would be used as an odour source, and those that would be tested for behavioural responses. All animals were treated under the guidelines for care set out by the University of Windsor Animal Care Committee.

Dechlorinated, aerated tap water (control) and water from RMs ($n = 9$), NRMs ($n = 9$), RFs ($n = 9$) and NRFs ($n = 9$) (termed conditioned water) were used as an odour source for each behavioural experiment (trial). Odours used for each trial were randomly selected. Each donor fish was placed alone in 1 l of dechlorinated aerated tap water for 4 h and each donor fish was used only once. After 4 h, 500 ml of this 'conditioned' water or simply control water was used for trials. The total number of fish used for conditioned water was 36.

EXPERIMENTAL DESIGN

Laboratory experiments were conducted to determine if RFs respond to control water ($n = 4$) or odours from water conditioned by RMs ($n = 4$), NRMs ($n = 4$), RFs ($n = 4$) or NRFs ($n = 4$). Similarly, NRFs were tested for their response to control water ($n = 5$) or odours from water conditioned by RMs ($n = 5$), NRMs ($n = 5$), RFs ($n = 5$) or NRMs ($n = 5$). Each female round goby was used only once. The total number of fish used for behavioural analysis was 45. Sample sizes were low. Despite the large number of round gobies in the study area, it was difficult to obtain live reproductive females during the spawning period, owing in part to the male-biased (6 : 1) operational sex ratio (Corkum *et al.*, 2004). Mean ± S.E. L_T of fish in each group varied: RMs (147 ± 11 mm), NRMs (94 ± 2 mm), RFs (69 ± 2 mm) and NRFs (72 ± 2 mm).

In each trial, either a RF or NRF was placed in a shelter (0·16 m long × 0·11 m wide × 0·05 m high) at one end of a tank (0·88 m long × 0·28 m wide × 0·28 m high) with 20 l of 18° C aerated dechlorinated water. The shelter had a single opening towards the opposite end of the tank. The back of the shelter contained a series of small holes that allowed water to flow-through the shelter and prevented the build up of stagnant water in the shelter. Water [either dechlorinated (control) or conditioned] was introduced at the opposite end of the tank. An air stone was placed at the end of the tank where water was introduced to help circulate the introduced water. A line indicating the midpoint of the length of the tank was drawn across the bottom so that the position of the fish could be recorded as being in one half of the tank or the other. Each trial was 1 h 15 min and consisted of three sequential periods: a 30 min acclimation period in which no water (conditioned or control) was added, a 15 min control period in which dechlorinated water was added and a 30 min stimulus period in which either conditioned water or control water was added. The 30 min acclimation and stimulus periods allowed the trials to begin and end without disturbing the animals as only the last 15 min of the acclimation period and the first 15 min of each stimulus period were used for analysis. Because round gobies can significantly change their ventilation rates solely due to the presence of an observer (R.M. Belanger, pers. comm.), these longer acclimation and stimulus periods were necessary. The control period was used to verify that the process of introducing fluid into the tank did not affect fish behaviour. Introduction of control water both in the control and stimulus periods was examined to detect possible changes in behaviour over time.

For the stimulus period of each behavioural trial, conditioned water or control water was introduced into the tank from an intravenous bag (600 ml) which was secured above the experimental tank and attached to Tygon® delivery tubing. The flow (6 ml min⁻¹) from the bag into the tank was regulated by a valve on the Tygon® delivery tube; outflow was removed from the opposite end of the tank at the same rate and thus total volume remained constant throughout each trial. Dechlorinated water was added and removed in the same manner within the control period of each trial. Preliminary studies showed that this flow rate and the circulation of water by the air stone distributed dye throughout the tank within 4 min of injection. The tubing and tank was flushed with clean dechlorinated water between experiments.

BEHAVIOURAL AND STATISTICAL ANALYSIS

The activity of the fish in the tank was videotaped from overhead using a colour camera (Hitachi Denshi VKC-370). Videotapes were analysed using a 2-D image analysis system (Peak Motus® Version 7.2) to determine: 1) the time spent in the far half of the tank near the odour source by the female, 2) the mean swimming velocity of the female and 3) the pathway of movement by the female. Time spent in the half of the tank near the odour source was recorded for the 15 min of the control period and the first 15 min of the stimulus period. Swimming velocity was calculated for 1 min periods every 3 min for the first 12 min of the control period and first 15 min of the stimulus period. Thus mean velocities were calculated for min 0, 3, 6, 9 and 12 for control periods and for min 0, 3, 6, 9, 12 and 15 for stimulus periods. Two pairs of pathways, illustrating differences in patterns of movement between control and stimulus periods, were calculated for min 3 to 12 in both control and stimulus periods using positional co-ordinates obtained from actual trial data. One pair of pathways corresponded to a trial in which a RF was exposed to RM conditioned water and the second pair of pathways corresponded to a trial in which a NRF was exposed to RF conditioned water.

An ANOVA test was used to examine mean differences in responses between stimulus and control periods for each of the five treatments (RM, NRM, RF, NRF and control). The difference between stimulus and control periods was calculated for a single fish and then the analysis was conducted on the mean differences obtained for all replicates for each of the five treatments. Four separate analyses were conducted for: 1) time spent by either RFs or NRFs in the far half of the tank near the odour source and 2) swimming

velocity by either RFs or NRFs in response to the five odours. If significant results among treatments were obtained, *post-hoc* tests (Student–Newman–Keuls, S-N-K) were performed.

RESULTS

TIME SPENT NEAR ODOUR SOURCE

Results of the ANOVA tests showed that there were significant differences between stimulus and control periods among the five treatments for the time that females spent in the far half of the tank near the odour source for both RFs ($F_{4,19}$, $P = 0.002$) and NRFs ($F_{,4,24}$, $P = 0.013$). Results of the *post-hoc* tests showed that the response of RFs to RM odour was significantly different from all other cases (S-N-K test, $P < 0.05$). Reproductive females spent more time (mean ± s.e.) in the far half of the tank when exposed to RM conditioned water (701 ± 72 s) than when exposed to control water (113 ± 62 s) [Fig. 1(a)]. There was no significant difference in the time that RFs spent near odour sources for the remaining four treatments (NRM, RF, NRF, control).

In contrast to the response by RFs, NRFs spent significantly more time in the far half of the tank when exposed to the RF stimulus (521 ± 61 s) compared with control water (97 ± 47 s) (S-N-K test, $P < 0.05$) [Fig. 1(b)]. Results of the *post-hoc* S-N-K test indicated that there was no significant difference in time spent near the odour source between stimulus and control periods among RM, NRM, NRF and control treatments.

VELOCITY OF FISH MOVEMENT

There were significant differences in the swimming velocity of RFs between stimulus and control periods among the five treatments ($F_{4,19}$, $P = 0.041$). Mean swimming velocity of RFs differed between RM and RF treatments (S-N-K, $P < 0.05$). The RFs swam faster (mean ± s.e.) during the stimulus period (3.68 ± 0.19 cm s^{-1}) than the control period (1.99 ± 0.52 cm s^{-1}) in the RM treatment, whereas RFs swam faster during the control period (1.71 ± 0.545 cm s^{-1}) than the stimulus period (1.054 ± 0.304 cm s^{-1}) in the RF treatment [Fig. 2(a)]. Overlap existed in mean differences between stimulus and control periods in the response of RFs among other treatments (RM, NRM, NRF, control and NRM, RF, NRF, control) (S-N-K, $P > 0.05$).

In contrast to differences in swimming velocities exhibited by RF among treatments, no significant differences in mean swimming velocities of NRF were detected between stimulus and control periods among treatments ($F_{4,24}$, $P = 0.330$) [Fig. 2(b)].

DIRECTION OF MOVEMENT

Overall, RFs and NRFs that responded to conspecific odours approached the odour source within 3–6 min of the odours being added. The response time corresponds to the tank-wide distribution of the dye within 4 min of being added to the tank. Although no quantitative comparisons were performed, the
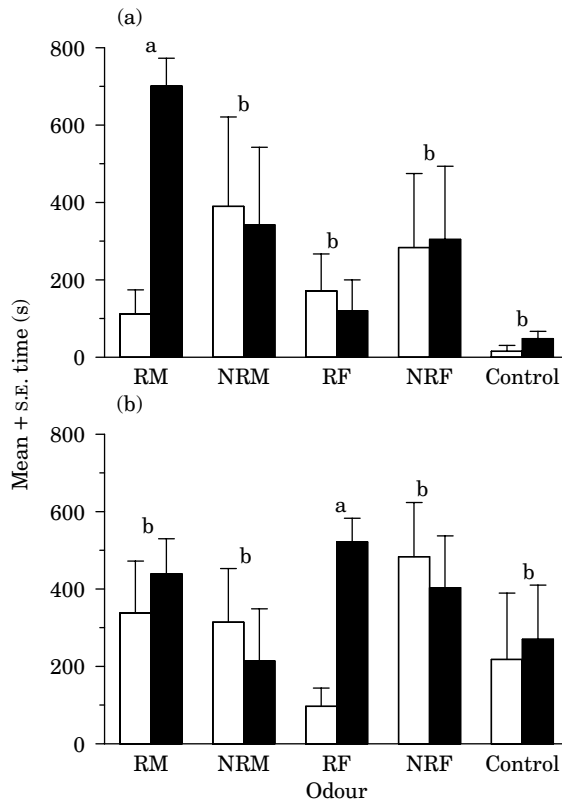
Fig. 1. Mean + s.e. time spent in the far half of the tank near the odour source by (a) reproductive females (RF) and (b) non-reproductive females (NRF) in control (□) and stimulus (■) periods, representing five treatments: water conditioned by reproductive males (RM), non-reproductive males (NRM), reproductive females (RF), non-reproductive females (NRF) and control (*i.e.* flowing water in the stimulus period). Four replicates for each treatment were tested using RF and five replicates for each treatment were tested using NRF. There were significant differences between stimulus and control periods among treatments for RF (ANOVA, $F_{4,19}$, $P = 0.002$) and NRF (ANOVA, $F_{4,24}$, $P = 0.013$). Lower case letters, a and b, represent results of the Student–Newman–Keuls multiple comparison tests. Treatments with the same letter did not differ significantly between stimulus and control periods.

swimming pathways of RFs were clearly different between control and stimulus periods when the odour source was RMs. Swimming patterns of a RF before [Fig. 3(a)] and after [Fig. 3(b)] the addition of RM conditioned water is representative of movement patterns of all RFs tested. During the control period, RFs swam near the shelter with occasional movement to the other end of the tank [Fig. 3(a)]. When exposed to RM conditioned water, RFs exhibited a dramatic change in their swimming pattern. The RFs swam directly from the shelter towards the opposite end of the tank, followed by rapid back and forth movements parallel to and along the width of the tank where the RM-conditioned water was being added.

Similarly, the pathways of NRFs were different between control and stimulus periods when the odour source was RFs. The swimming pattern of a NRF,
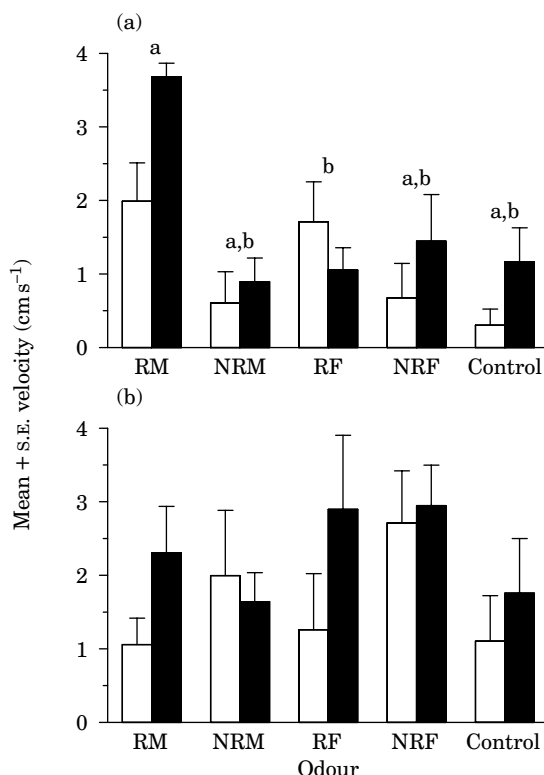
FIG. 2. Mean + S.E. velocity of (a) reproductive females (RF) and (b) non-reproductive females (NRF) in control (□) and stimulus (■) periods, representing five treatments: water conditioned by reproductive males (RM), non-reproductive males (NRM), reproductive females (RF), non-reproductive females (NRF) and control (*i.e.* flowing water in the stimulus period). Four replicates for each odour were tested using RF and five replicates for each odour were tested using NRF. There were significant differences between stimulus and control periods among treatments in swimming velocity of RF (ANOVA, $F_{4,19}$, $P = 0.041$), but not NRF (ANOVA, $F_{4,24}$, $P = 0.330$). Lower case letters, a and b, represent results of the Student–Newman–Keuls multiple comparison tests. Treatments with the same letter did not differ between stimulus and control periods.

before [Fig. 4(a)] and after [Fig. 4(b)] the addition of RF conditioned water, is representative of movement pathways of all NRFs tested. Although NRFs typically remained in or near the shelter during control periods, they exhibited directed movement from or near the shelter to the opposite end of the tank within minutes of the addition of the stimulus. NRFs responding to RF odour also exhibited back and forth movements along the far wall of the tank where odour was added as described for the case of RFs responding to RM odour.

DISCUSSION

The present study demonstrates that reproductively mature male round gobies release a chemical cue which initiates a strong behavioural response in reproductively mature females with observable changes in time spent near the RM odour,
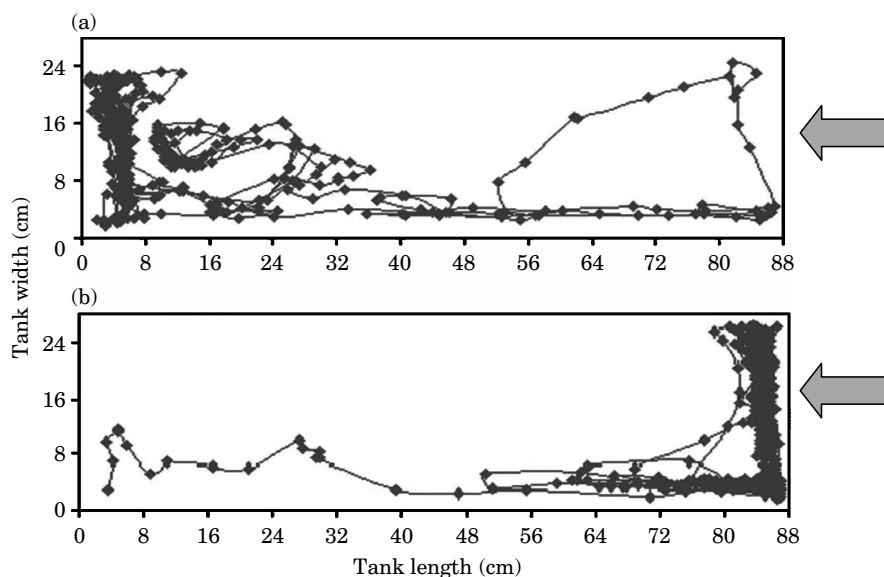
Fig. 3. Pathway of movement of a reproductive female (RF) (*n* = 1) during min 3–12 in the (a) control period when dechlorinated water was added and (b) stimulus period when reproductive male (RM) conditioned water was added. The end of the tank where either control water or conditioned water was added is indicated (⇐).

swimming velocities and direction of movement. Interestingly, NRFs spent more time near the RF odour source compared with control water. These findings suggest that the round goby may use inter- and intra-sexual pheromonal communication. The results, although significant, should be treated with caution owing to the small sample size. Physiological assays on RF and NRF round gobies, however, support the behavioural findings. Electro-olfactogram (EOG) studies showed that reproductive male water was a 'potent olfactory stimulus to reproductive females, but not to non-reproductive females' (Belanger *et al.*, 2004). In addition, NRFs exhibited elevated EOG values when exposed to RF conditioned water, but not to water from NRF, RM or NRM (S. Jasra, unpubl. data). Moreover, sex and reproductive status are important in other fishes exhibiting chemical communication, *e.g. B. soporator* (Tavolga, 1956), African catfish *Clarias gariepinus* (Burchell) (Resink *et al.*, 1989) and fathead minnows *Pimephales promelas* Rafinesque (Cole & Smith, 1992).

Attraction of RFs to RM odour suggests that RFs may respond to male sex pheromones rather than visual cues to find nesting sites, which are often located in dark areas (Wickett & Corkum, 1998). Pheromone-based mate attraction is important in ovulating female sea lamprey *Petromyzon marinus* L., in which females can improve their chances of reaching nesting sites upstream by responding to male-based chemical stimuli through active swimming against currents and searching behaviour (Li *et al.*, 2002). Round goby males that attract RFs increase their mating opportunities and females benefit from depositing their eggs in a shelter where a parental male resides.
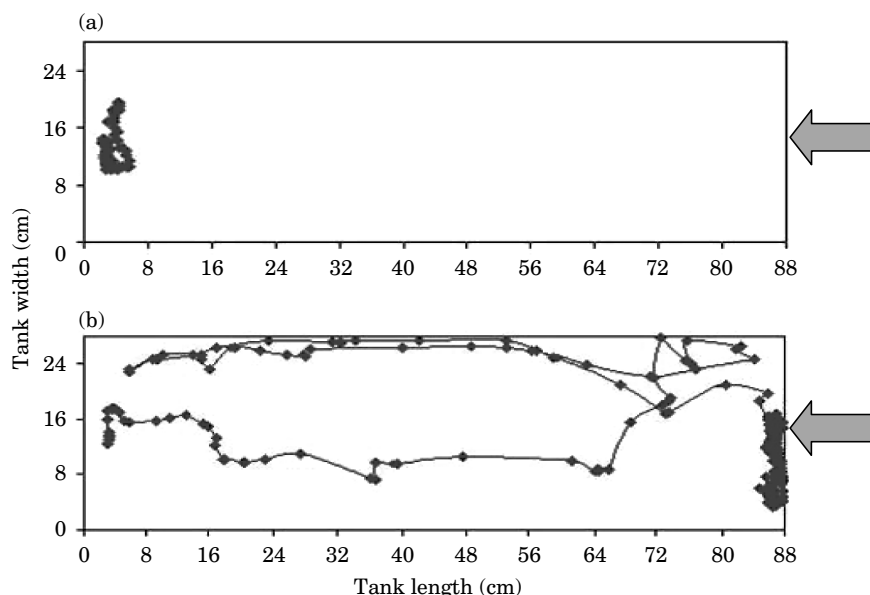
FIG. 4. Pathway of movement of a non-reproductive female (NRF) ($n = 1$) during min 3–12 in the (a) control period when dechlorinated water was added and (b) stimulus period when reproductive female (RF) conditioned water was added. The end of the tank where either control water or conditioned water was added is indicated (⇐).

Intra-sexual pheromones, while not as well studied as inter-sexual pheromones, have been investigated in several groups, *e.g.* Blennidae (Losey, 1969), Cyprinidae (Bloom & Perlmutter, 1977; Cole & Smith, 1992), Gasterosteidae (Haberli & Aeschlimann, 2004), Gobiidae (Tavolga, 1956; Stadler, 1999), Poeciliidae (Shohet & Watt, 2004) and Salmonidae (Newcombe & Hartman, 1973)). The present study is the first to demonstrate pheromone-based attraction of females to other females in the Gobiidae.

Female intra-sexual pheromones may be linked to the development of aggregations of conspecifics as proposed in other species. For example, pheromones released by female zebrafish *Danio rerio* (Hamilton) have been shown to attract other females and it has been suggested this attraction may be involved in shoaling behaviour (Bloom & Perlmutter, 1977). Similarly, Shohet & Watt (2004) demonstrated that female guppies *Poecilia reticulata* (Peters) prefer water containing the odour of sexually mature females over that of odourless water and this attraction also is thought to be involved in shoaling. While round gobies, unlike the cyprinids and poeciliids, are non-shoaling, they are often found aggregated together in large numbers in their benthic environments (Ray & Corkum, 2001). Thus, this attraction between females may function in facilitating aggregations of round gobies.

Although chemical stimuli are not directly linked to the attraction, it has been suggested that female bluehead wrasse *Thalassoma bifasciatum* (Bloch) follow

other females to learn the routes to mating sites (Warner, 1990). During the reproductive season, male round gobies are the first to leave deeper waters and move inshore and are followed later by females (Kovtun, 1979; Moskal'kova, 1996). Odour plumes from colonial nesting males may guide RFs to nests and the NRFs play 'follow-the-leader', tracking RFs.

Many researchers have measured time spent near an odour source (Colombo et al., 1980; Haberli & Aeschlimann, 2004; Kristensen & Closs, 2004), turning movements (Laberge & Hara, 2003) or ventilation changes (Murphy et al., 2001; Belanger, 2002) to examine responses of individuals to conspecific chemical cues, but swimming velocity is seldom determined. The heightened swimming velocity of RFs exposed to RM odour is important because fishes that are negatively buoyant and slow swimmers, such as the round goby, may incur high energy losses trying to stabilize their swimming trajectories (Webb, 2002).

Analysis of swimming pathways is not only important for understanding the type of response elicited by an odour, but may also give indications of how the animal detects it. While detailed quantitative analysis of the locomotor behaviour of round gobies is beyond the scope of this paper, several clues from the behaviour observed indicate that gobies may use chemotropotaxis, directed turns based on the simultaneous comparison of the intensity of a chemical stimulus on either side of the body (Wyatt, 2003). Females responding to RM or RF odour moved back and forth across the wall of the tank near the odour source, keeping their bodies perpendicular to the wall. A study on the locomotor responses of male channel catfish Ictalurus punctatus (Rafinesque) to female sex pheromones indicated that males use chemotropotaxis to locate the source of the odour (Timms & Kleerekoper, 1972). Round gobies may have the ability to actively 'sniff' their surroundings by maintaining constant flow of water over their olfactory epithelium through the pumping mechanism of accessory nasal sacs (Murphy et al., 2001; Belanger et al., 2003).

References

Aday, D. D., Wahl, D. H. & Philipp, D. P. (2003). A mechanism for social inhibition of sexual maturation in bluegill. *Journal of Fish Biology* **62,** 486–490. doi: 10.1046/j.0022-1112.2003.00033.x

Arbuckle, W. J., Belanger, A. J., Corkum, L. D., Zielinski, B., Li, W., Yun, S-S., Bachynski, S. & Scott, A. P. (2005). *In vitro* biosynthesis of novel 5β reduced steroids by the testis of the round goby, *Neogobius melanostomus. General and Comparative Endocrinology* **140,** 1–13.

Belanger, A. J., Arbuckle, W. J., Corkum, L. D., Gammon, D. G., Li, W., Scott, A. P. & Zeilinski, B. S. (2004). Behavioural and electrophysiological responses by reproductive female *Neogobius melanostomus* to odour released by conspecific males. *Journal of Fish Biology* **65,** 933–946. doi: 10/1111/j.1095-8649.2004.00494.x

Belanger, R. M. (2002). Morphology and histochemistry of the peripheral olfactory organ and behavioural responses by the round goby, *Neogobius melanostomus*,

to putative pheromones and conspecific extracts. MSc Thesis, University of Windsor, Windsor, Ontario.

Belanger, R. M., Smith, C. M., Corkum, L. D. & Zielinski, B. S. (2003). Morphology and histochemistry of the peripheral olfactory organ in the round goby, *Neogobius melanostomus* (Teleostei: Gobiidae). *Journal of Morphology* **257,** 62–71.

Bloom, H. & Perlmutter, A. (1977). A sexual aggregating pheromone system in the zebrafish, *Brachydanio rerio* (Hamilton-Buchanan). *Journal of Experimental Zoology* **199,** 215–226.

Carolsfeld, J., Scott, A. P. & Sherwood, N. M. (1997). Pheromone-induced spawning of pacific herring. *Hormones and Behaviour* **31,** 269–276.

Charlebois, P. M., Corkum, L. D., Jude, D. J. & Knight, C. (2001). The round goby (*Neogobius melanostomus*) invasion: current research and future needs. *Journal of Great Lakes Research* **27,** 263–266.

Cole, K. S. & Smith, J. F. (1992). Attraction of female fathead minnows, *Pimephales promelas*, to chemical stimuli from breeding males. *Journal of Chemical Ecology* **18,** 1269–1284.

Colombo, L., Marconato, A., Belvedere, P. C. & Frisco, C. (1980). Endocrinology of teleost reproduction: a testicular steroid pheromone in the black goby, *Gobius jozo* L. *Bollettino di Zoologia* **47,** 355–364.

Colombo, L., Belvedere, P. C., Marconato, A. & Bentivegna, F. (1982). Pheromones in teleost fish. In *Proceedings of the Second International Symposium on Reproductive Physiology of Fish* (Richter, C. J. J. & Goos, T. J. T., eds), pp. 84–94. Wageningen: Pudoc.

Corkum, L. D., Sapota, M. R. & Skora, K. E. (2004). The round goby, *Neogobius melanostomus*, an invader on both sides of the Atlantic. *Biological Invasions* **6,** 173–181.

Haberli, M. A. & Aeschlimann, P. B. (2004). Male traits influence odour-based mate choice in three-spined stickleback. *Journal of Fish Biology* **64,** 702–710. doi: 10.1046/j.1095-8649.2003.00338.x

Kleerekoper, H. (1969). *Olfaction in Fishes*. Bloomington, IN and London: Indiana University Press.

Kovtun, I. F. (1979). Significance of the sex ratio in the spawning population of the round goby, *Neogobius melanostomus*, in relation to year-class strength in the Sea of Azov. *Journal of Ichthyology* **19,** 161–163.

Kristensen, E. A. & Closs, G. P. (2004). Anti-predator response of naïve and experienced common bully to chemical alarm cues. *Journal of Fish Biology* **64,** 643–652. doi: 10.1046/j.1095-8649.2003.00328.x

Laberge, F. & Hara, T. J. (2003). Behavioural and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonid fishes. *Journal of Fish Biology* **62,** 206–221. doi: 10.1046/j.0022-1112.2003.00020.x

Li, W., Scott, A. P., Siefkes, M. J., Yan, H. G., Liu, Q., Yun, S-S. & Gage, D. A. (2002). Bile acids secreted by male sea lamprey that act as a sex pheromone. *Science* **296,** 138–141.

Locatello, L., Mazzoldi, C. & Rasotto, M. B. (2002). Ejaculate of sneaker males is pheromonally inconspicuous in the Black goby, *Gobius niger* (Teleostei, Gobiidae). *Journal of Experimental Zoology* **293,** 601–605.

Losey, G. S., Jr. (1969). Sexual pheromone in some fishes of the genus *Hypsoblennius* Gill. *Science* **163,** 181–183.

MacGintie, G. E. (1939). The natural history of the blind goby, *Typhlogobius californiensis* Steindachner. *American Midland Naturalist* **21,** 489–505.

MacInnis, A. J. (1997). Aspects of the life history of the round goby, *Neogobius melanostomus* (Perciformes: Gobiidae), in the Detroit River. MSc Thesis, University of Windsor, Windsor, Ontario.

MacInnis, A. J. & Corkum, L. D. (2000). Fecundity and reproductive season of the round goby *Neogobius melanostomus* in the upper Detroit River. *Transactions of the American Fisheries Society* **129,** 136–144.

McLennan, D. A. & Ryan, M. J. (1997). Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. *Animal Behaviour* **54,** 1077–1088.

Miller, P. J. (1984). The tokology of gobioid fishes. In *Fish Reproduction: Strategies and Tactics* (Potts, G. W. & Wooton, R. J., eds), pp. 119–153. London: Academic Press.

Moskal'kova, K. I. (1996). Ecological and morphophysiological prerequisites to range extension in the round goby *Neogobius melanostomus* under conditions of anthropogenic pollution. *Journal of Ichthyology* **36,** 584–590.

Murphy, C. A., Stacey, N. E. & Corkum, L. D. (2001). Putative steroidal pheromones in the round goby, *Neogobius melanostomus*: olfactory and behavioural responses. *Journal of Chemical Ecology* **27,** 443–469.

Newcombe, C. & Hartman, G. (1973). Some chemical signals in the spawning behaviour of rainbow trout (*Salmo gairdneri*). *Journal of Fisheries Research Board of Canada* **30,** 995–997.

Ray, W. J. & Corkum, L. D. (2001). Habitat and site affinity of the round goby. *Journal of Great Lakes Research* **27,** 329–334.

Resink, J. W., Schoonen, W. G. E. J., Albers, P. C. H., File, D. M., Notenboom, C. D., van den Hurk, R. & van Oordt, P. G. W. J. (1989). The chemical nature of sex attracting pheromones from the seminal vesicle of the African catfish, *Clarias gariepinus*. *Aquaculture* **83,** 153–166.

Shohet, A. J. & Watt, P. J. (2004). Female association preferences based on olfactory cues in the guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* **55,** 363–369.

Stacey, N., Chojnacki, A., Narayanan, A., Cole, T. & Murphy, C. (2003). Hormonally derived sex pheromones in fish: exogenous cues and signals from gonad to brain. *Canadian Journal of Physiology and Pharmacology* **81,** 329–341.

Stadler, J. H. (1999). Species recognition and sex discrimination by males of the notchtongue goby, *Bathygobius curacao*. PhD Dissertation, University of Miami, Miami, FL.

Tavolga, W. N. (1956). Visual, chemical and sound stimuli as cues in sex discriminatory behaviour of the Gobiid fish *Bathygobius soporator*. *Zoologica* **41,** 49–65.

Timms, A. M. & Kleerekoper, H. (1972). The locomotor responses of male *Ictalurus punctatus*, the channel catfish, to a pheromone released by the ripe female of the species. *Transactions of the American Fisheries Society* **101,** 302–310.

Vanderploeg, H. A., Nalepa, T. F., Jude, D. J., Mills, E. L., Holeck, K. T., Liebig, J. R., Grigorovich, I. A. & Ojaveer, H. (2002). Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59,** 1209–1228.

Warner, R. R. (1990). Male versus female influences on mating-site determination in a coral reef fish. *Animal Behaviour* **39,** 540–548.

Webb, P. W. (2002). Control of posture, depth, and swimming trajectories of fishes. *Integrative and Comparative Biology* **42,** 94–101.

Wickett, R. G. & Corkum L. D. (1998). You have to get wet: a case study of the nonindigenous Great Lakes fish, round goby. *Fisheries* **23,** 26–27.

Wyatt, T. D. (2003). *Pheromones and Animal Behaviour Communication by Smell and Taste*. Cambridge: Cambridge University Press.