Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 147 (2007) 77–83
CBP
www.elsevier.com/locate/cbpa

Differential behavioral responses by reproductive and non-reproductive male round gobies (*Neogobius melanostomus*) to the putative pheromone estrone

Rachelle M. Belanger [a,b,*], Lynda D. Corkum [a], Barbara S. Zielinski [a]

[a] *Department of Biological Sciences, University of Windsor, Windsor, ON, Canada N9B 3P4*
[b] *Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA*

Received 24 July 2006; received in revised form 20 November 2006; accepted 20 November 2006
Available online 24 November 2006

Abstract

Previous studies have shown that the frequency of gill ventilation during exposure to estrone and gonadal extracts in the round goby (*Neogobius melanostomus*) is linked to olfactory sensory input. Control over gill ventilation may be a regulatory mechanism used for odorant sampling during reproductive periods. In this study, we examined changes in gill ventilation in osmic and anosmic (nasal occluded), reproductive and non-reproductive male round gobies to a putative steroidal pheromone estrone (1,3,5(10)-estratrien-3-ol-17-one). We tested 5 different concentrations of estrone (10^{-12} to 10^{-8} M) and showed that the response threshold for estrone varied with the male's reproductive status; it was 10^{-11} M in reproductive males, and rose to 10^{-9} M in non-reproductive males. However, anosmic reproductive and non-reproductive males did not respond to estrone. These findings suggest that olfactory responses to putative pheromones may change depending on the reproductive status of the fish.

Keywords: Estrone; Fish; Gill ventilation; Nasal occlusion; Round goby; Reproductive status

1. Introduction

Chemical communication is exhibited in many types of animal behavior, including aggregation, mate attraction, and predator–prey interactions (Wyatt, 2003). Pheromones have been defined as substances, or mixtures of substances, that are released to the outside by individuals into the environment where they induce specific, adaptive and largely innate biological responses in conspecifics that are mutually beneficial to both the sender and receiver (Karlson and Lüscher, 1959; Sorensen et al., 2000; Meredith, 2001). Fish commonly use hormonal pheromones for mating and reproductive activities (for reviews see: Stacey and Cardwell, 1995; Stacey et al., 1994) and gonadal steroids can act as reproductive pheromones (Moore and Scott, 1991). These pheromones, or chemical messengers, are released in minute amounts by the sender into the water either by the gills or urine (Scott and Vermeirssen, 1994; Vermeirssen and Scott, 1996). Pheromones are detected by highly specific and sensitive receptors on the olfactory epithelium (Sorensen et al., 1992), resulting in diverse physiological and behavioral effects in fish (Stacey and Sorensen, 1986).

Extracellular field potentials, recorded from the surface of the olfactory epithelium (electro-olfactograms; EOGs) and behavioral assays can be used to examine the effects that pheromones or putative pheromones have on fish. Several EOG studies have shown that the response magnitude and/or threshold to conditioned water from reproductive stages or putative pheromones (steroids and prostaglandins), varies with sexual maturity in cyprinids (Irvine and Sorensen, 1993; Cardwell et al., 1995), salmonids (Moore and Scott, 1991; Moore and Waring, 1996), and perciforms (Frade et al., 2002; Belanger et al., 2004). Changes in behavior also are known to be associated with reproductive onset in perciformes. In the black goby (*Gobius jozo*), only ovulated females were attracted to the

* Corresponding author. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA. Tel.: +1 419 372 3845; fax: +1 419 372 2024.
E-mail address: belangr@bgsu.edu (R.M. Belanger).

1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.11.008

putative pheromone etiocholanolone glucuronide (Colombo et al., 1980) and in the round goby (*Neogobius melanostomus*), only reproductive females were attracted to a reproductive male conditioned water source (Belanger et al., 2004).

The putative pheromone estrone (1,3,5(10)-estratrien-3-ol-17-one) has also been shown to induce both physiological and behavioral changes in round gobies (Murphy et al., 2001; Murphy and Stacey, 2002; Belanger et al., 2006). For male round gobies, Murphy et al. (2001) showed that estrone elicited an EOG threshold response at concentrations of 10^{-9} M and the threshold concentration for eliciting a gill ventilation increase was 10^{-10} M; however, reproductive status of the fish was not determined. Recently, gill ventilation increases in response to estrone have been shown to be an olfactory mediated behavior in the round goby (Belanger et al., 2006). Because it has been previously shown that gobiids demonstrate differential behaviors, which depend on sexual maturity (Colombo et al., 1980; Belanger et al., 2004), differences in concentration of putative pheromones could lead to varying ventilation rates in reproductive and non-reproductive fish. Specifically, are ventilation responses to estrone at different concentrations a function of reproductive status?

Researchers have hypothesized that olfactory sensitivities can be modulated due to changes in the number of receptors and/or varying sensitivity of the receptors (Creese and Sibley, 1981; Habibi et al., 1989). Findings by Alekseyenko et al. (2006) support the first hypothesis by showing that in mice, gonadal hormones (e.g. testosterone) play a role in modulating the expression of vomeronasal receptors, thus altering the animals' behavioral responses to pheromones. A similar idea was suggested by Irvine and Sorensen (1993) for sensitivity differences visualized between mature and juvenile common carp (*Cyprinus carpio*) and by Cardwell et al. (1995) for androgen treated Southeast Asian cyprinids (*Puntis schwanenfeldi* and *P. gonionotus*). Additionally, Schreibman et al. (1984) demonstrated morphological changes in the peripheral olfactory organ when fish transitions from a prepubertal to adult stage. A large population of mucous-secreting goblet cells and increases in olfactory epithelial surface area were found at the onset of sexual maturity in platyfish (*Xiphophorus maculatus*).

In our study, we examine gill ventilation rates to test the hypothesis that there is a change in behavioral response, related to sexual maturity, in the sensitivity of the olfactory system to putative pheromones. We used osmic and anosmic (nasal occluded), reproductive and non-reproductive male round gobies to examine ventilation responses to a putative pheromone estrone, which had been previously shown to evoke ventilation and EOG responses in this fish (Murphy et al., 2001; Murphy and Stacey, 2002; Belanger et al., 2006). This study will link behavioral responses to sexual maturity and olfactory sensitivity.

2. Materials and methods

2.1. Experimental animals

Round gobies (*N. melanostomus*) were collected by angling in the upper Detroit River and by trawling in the western basin of Lake Erie during the spring, summer, and fall (2001 to 2002). Fish were sexed by the shape of the genital papilla (Miller, 1984). Males and females were housed separately in the laboratory under a constant photoperiod (16L:8D) in holding tanks (205 L) with aerated 18 °C flow-through dechlorinated tap water, gravel, and PVC shelters. We collected fish during both the reproductive and non-reproductive seasons. Each fish was housed in the laboratory for less than 3 weeks before being used. Fish were maintained on a diet of Nutrafin® fish flakes and zebra mussels, *Dreissena polymorpha*. All animals were maintained in accordance with Canadian Council on Animal Care and the Ontario Animals for Research Act guidelines.

Males with secondary sexual characteristics (black body and swollen cheeks) were classified as reproductive and males lacking these traits were classified as non-reproductive (MacInnis and Corkum, 2000). Male reproductive status was confirmed using the gonadosomatic index (GSI) (Strange, 1996). Mean (±standard error, S.E.) GSI values were calculated following behavioral bioassays and were found to be 1.54±0.19% for reproductive males and 0.24±0.03% for non-reproductive males. Reproductive males had significantly larger GSI values than non-reproductive males ($t_{(0.05,\ 195)}=6.790$; $P<0.0001$). Male round gobies were more robust during the reproductive season with respect to mean (±S.E.) total length and mass (117.1±2.3 mm; 22.92±1.53 g, $n=97$) than non-reproductive males (100.6±1.4 mm; 12.92±0.24 g, $n=100$). We tested increases in ventilation rates of untreated (given a procedural control) and nasal occluded male round gobies to estrone (E1; E1274, Sigma-Aldrich, Wisconsin, Milwaukee, WI). Reproductive males were tested in May and June 2002 (peak spawning season) and non-reproductive males were tested between September 2001 and January 2002, with each fish only being used once.

2.2. Gill ventilation assay

The gill ventilation assay followed the protocol described in Belanger et al. (2006). For each trial ($N=10$ per treatment), a male round goby was placed in an 8 L aquarium containing 5.5 L dechlorinated water (18 °C), an air stone, and a "nest". The nest was made with ceramic tile (10 cm wide×15 cm long×5 cm deep) and a single Plexiglas® transparent side, facilitating our ability to videotape gill ventilation by the fish. The aquarium was surrounded by Styrofoam®, with an opening on one side where a camera lens was positioned. Video images of fish ventilation were obtained using a Panasonic WVBL604 high-resolution video camera and Panasonic AG6050 time-lapse video recorder. Opaque barriers surrounding both the nest and the aquarium prevented the test animals being disturbed. The test odorant was added over the air stone, which was located behind the nest (adapted from Murphy et al., 2001; Gammon et al., 2005; Belanger et al., 2006).

Behavioral assays were conducted with the fish being placed in a nest. Each fish was acclimated to the tank overnight and the assays were conducted the following day. Gill ventilation was recorded over a 15-min pre-test period before the odorant was added, and for a 30-min test period after the odorant was added.

The values for percentage change in basal ventilation were determined by comparing ventilation rate before and after the application of the odorant. Basal ventilation rates are stable in the round goby (Murphy et al., 2001; Murphy and Stacey, 2002). In this study, we compared the average basal ventilation rates at 5, 10, and 15 min prior to odorant application with the percent increase in basal ventilation at 4-min post-odorant application. The selection of the 4-min sampling period was based on dye diffusion tests, and on the ventilation responses observed by Murphy et al. (2001, Fig. 4), Murphy and Stacey (2002, Fig. 1), and Belanger et al. (2006, Figs. 2 and 3).

2.3. Nasal occlusion

Having previously demonstrated that nasal occlusion prevents odorant access to OSNs (Belanger et al., 2006), we repeated the protocol for this study. As before, we used hydrophilic vinyl polysiloxane, a nontoxic dental impression material (Reprosil®, DENTSPLY International Inc., Milford, Delaware) to block the nasal cavity. Briefly, after anesthetizing a fish with 0.5% MS-222 (Finquel, Argent Chemical, Fisheries Chemical Division, Redmond, Washington), Reprosil® was injected into the posterior nares until the nasal cavity was filled (Belanger et al., 2003). These fish were designated anosmic. A procedural control, including all of the steps for plugging the nose, excluding the Reprosil® injection, was performed on fish. Control fish were designated as untreated, osmic animals.

2.4. Concentration effects of estrone on the gill ventilation responses in reproductive and non-reproductive males

Osmic and anosmic, reproductive and non-reproductive, male round gobies were tested for gill ventilation responses to 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12} M final concentration of estrone (using a 10^{-3} and 10^{-6} M stock solution dissolved in 95% ethanol diluted to the test concentration in dechlorinated tap water). The preparation and delivery are described in detail in Belanger et al. (2006). We used a 4-way ANOVA test (on untransformed data) to determine if mean percent basal ventilation rate differed among treatments (reproductive status, concentration of estrone, osmic or anosmic status, and pre and post odor delivery times). As reported in Belanger et al. (2006), responses to the carrier (containing ethanol) were conducted and evaluated similarly to the test odors; no ventilation increases were observed ($p<0.05$).

3. Results

Results of the 4-way ANOVA test showed mean ventilation rate differed significantly with respect to osmic and anosmic status ($F=30.29$, $P<0.001$), estrone concentration ($F=3.22$, $P<0.05$), and pre-and post-odorant delivery times ($F=40.27$, $P<0.001$), but not season (Fig. 1). There was a significant difference between basal ventilation rates and ventilation rates for osmic reproductive males recorded at 4-min post-odorant

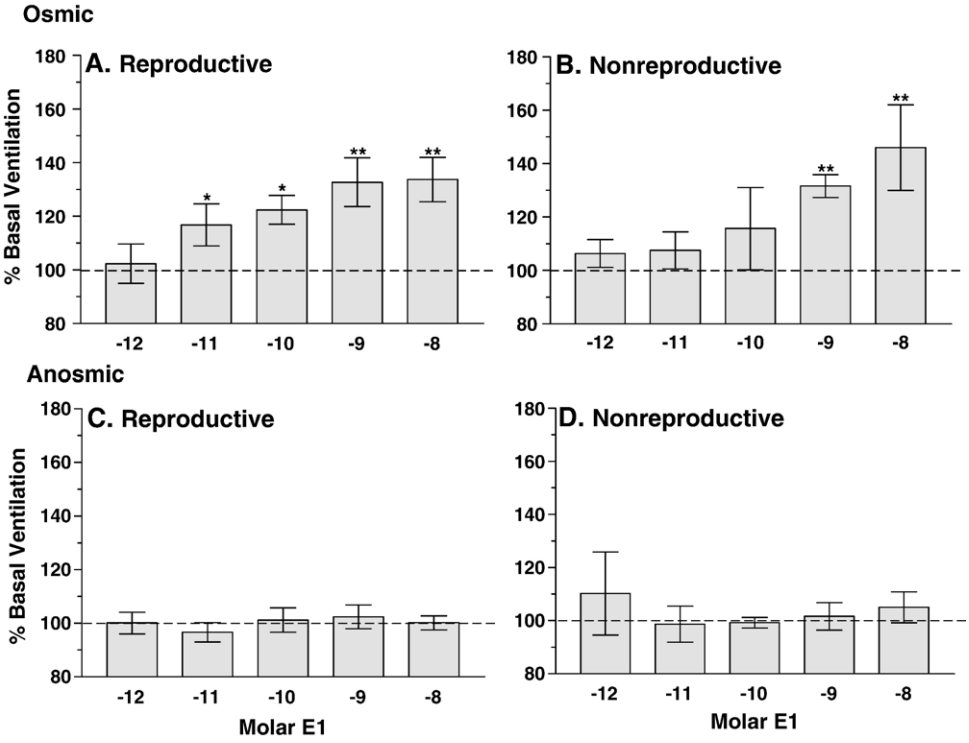

Fig. 1. The percentage change in basal ventilation (mean±S.E.) in osmic reproductive (A) and nonreproductive (B) male round gobies 4 min after the addition of 5 different concentrations of estrone (E1, 10^{-12} to 10^{-8} M). Similarly, the effect of nasal occlusion (anosmic males) on the ventilation response to estrone is shown for reproductive (C) and nonreproductive (D) male round gobies. The dashed line represents the mean basal ventilation of both osmic and anosmic fish. The symbols, *($P<0.05$) and **($P<0.01$), indicate that there were significant differences between mean basal ventilation and ventilation recorded at 4 min after the introduction of estrone in osmic fish (4-way ANOVA).

delivery at four concentrations of estrone (10^{-11} M, $P=0.041$; 10^{-10} M, $P=0.019$; 10^{-9} M, $P<0.001$; 10^{-8} M, $P<0.001$). These findings indicate that gill ventilation showed a response threshold at 10^{-11} M estrone in osmic reproductive males (Fig. 1A). Because these responses were absent following nasal occlusion ($P>0.05$ for all concentrations; Fig. 1C), nasal chemoreception accounts for the gill ventilation responses over this broad concentration range. Nasal chemosensory processes also were necessary for the gill ventilation responses of osmic non-reproductive males (Fig. 1B). In osmic non-reproductive males, significant differences between basal ventilation rates and 4-min post-odorant delivery occurred in response to 10^{-9} M ($P<0.001$) and 10^{-8} M ($P<0.001$) estrone only. The gill ventilation responses of osmic, non-reproductive males at 10^{-10}, 10^{-11}, and 10^{-12} M estrone did not differ from the mean basal ventilation rates. Following nasal occlusion, the basal ventilation rate remained unchanged after application of estrone (10^{-12} M to 10^{-8} M) in non-reproductive males (Fig. 1D). Overall, the response threshold for osmic non-reproductive males was 10^{-9} M, while it was 100 fold higher for osmic reproductive males (10^{-11} M). In addition to the significant main effects (concentration of estrone, osmic or anosmic status, and pre and post odor delivery times), the following interaction terms were significant: concentration of estrone * pre and post odor delivery times, $P<0.01$; concentration of estrone * osmic or anosmic status, $P<0.05$; pre and post odor delivery times * osmic or anosmic status, $P<0.001$.

4. Discussion

Recently, Belanger et al. (2006) showed that male round gobies increased gill ventilation in response to 10^{-8} M estrone. Belanger et al. (2006) also showed that gill ventilation responses were mediated by the olfactory system by testing osmic and anosmic fish. Earlier, Murphy et al. (2001) had alluded to the importance of olfaction in determining ventilation responses by round gobies to steroids. The present study uses the gill ventilation assay to show that reproductive male round gobies exhibit a lower threshold response than non-reproductive males to the putative pheromone estrone. Specifically, gill ventilation, which is tightly correlated to EOG responses in the round goby (Murphy et al., 2001), increased significantly when reproductive males were exposed to 10^{-8} through 10^{-11} M estrone. This differed from non-reproductive males, where gill ventilation responses occurred at 10^{-8} and 10^{-9} M. Therefore, the 100 fold molar difference in the sensitivity to this odorant was dependent upon the sexual maturity of the fish. To our knowledge, this is the first study comparing concentration thresholds for behavioral responses to putative pheromones between reproductive and non-reproductive fish.

Using extracellular field recordings from the olfactory epithelium (EOG recordings), researchers have shown that fishes display differences in response thresholds and magnitudes to putative pheromones or conditioned water when reproductive and non-reproductive teleosts are compared (e.g. Bjerselius and Olsén, 1993; Irvine and Sorensen, 1993; Moore and Scott, 1991; Moore and Waring, 1996; Belanger et al., 2004); however, not all species of fish demonstrate these differences (e.g. Sveinsson and Hara, 2000). These differences may be attributed to the complex pheromonal systems in teleost fishes and variation in response among different species (Sveinsson and Hara, 2000). Likewise, behavioral responses by individuals to conspecific odours, conditioned water or putative pheromones may change depending on reproductive status of the fish or olfactory sensory deprivation (Table 1). Sorensen et al. (1988) showed that 10^{-8} M and 10^{-10} M prostaglandin $F_{2\alpha}$ and 15-keto-prostaglandin $F_{2\alpha}$ were required to initiate a reproductive behavior (chasing) in goldfish (*Carassius auratus*); however they did not determine if these behaviors were absent in non-reproductive fish.

The responses of reproductive male round gobies to estrone were similar to the threshold of other reproductive fish to putative pheromones tested using EOG recordings (e.g. Sorensen et al., 1988; Resink et al., 1989b; Cardwell et al., 1992; Kitamura et al., 1994; Lower et al., 2004). Differences in EOG response threshold, and thus olfactory sensitivity, also were demonstrated when responses by reproductive and non-reproductive males were compared in the crucian carp *Carassius carassius* (Bjerselius and Olsén, 1993) and Atlantic salmon *Salmo salar* (Moore and Scott, 1991; Moore and Waring, 1996). One hundred-fold increases in response threshold were also seen in male crucian carp (Bjerselius and Olsén, 1993) and male Atlantic salmon (Moore and Waring, 1996). Spermiating crucian carp were shown to be more sensitive than immature crucian carp to prostaglandin $F_{2\alpha}$ (Bjerselius and Olsén, 1993). Bjerselius and Olsén (1993) observed that spermiating crucian carp show a typical sexual behaviour (chasing and nudging) towards prostaglandin $F_{2\alpha}$-injected females, while immature fish do not. Round goby males and females do not respond to prostaglandins (Murphy et al., 2001).

The temporal changes in olfactory sensitivity to both prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ in Atlantic salmon, observed by Moore and Waring (1996), are similar to those reported previously for testosterone (Moore and Scott, 1991), where both species of fish demonstrated 100 fold increases in sensitivity to the odorants tested. This suggests that sensitivity to pheromones and other olfactory odorants may be strongly dependent upon the maturation stage of the fish and possibly the particular period in the spawning season (Moore and Waring, 1996). Support for this idea also comes from a study of juvenile male Southeast Asian cyprinids, *Puntius schwanenfeldi* and *P. gonionotus*, with increased EOG sensitivity to 15-keto-prostaglandin $F_{2\alpha}$ following androgen treatment (Cardwell et al., 1995). Androgen-implanted fish showed a 100 fold molar increase (10^{-10} to 10^{-12} M) in EOG responses (Cardwell et al., 1995), similar to the changes in sensitivity found in this study.

Differential behavioral responses to chemical cues occur when reproductive and non-reproductive male and female fish are examined. In gobiids (*G. jozo* and *N. melanostomus*), non-reproductive females were not attracted to a putative pheromone or reproductive male conditioned water source (Colombo et al., 1980; Belanger et al., 2004). This is similar to results obtained in this study for male round gobies, where the behavioral response to odorants was diminished when the fish were not in

Table 1
A summary of the behavioral responses by reproductive and non-reproductive, osmic and anosmic teleost fishes (subdivision Euteleostei)

Order / Family / Genus species	Sex	Substance Tested	Behavioral Response by Reproductive/ Osmic Fish	Behavioral Response by Non-reproductive/ Anosmic Fish	Reference
Cypriniformes					
Cyprinidae *Carassius auratus*	M	Ovulated female	Courtship, fertilization	No courtship during anosmia	Van den Hurk and Lambert (1983)
	M	Prostaglandin $F_{2\alpha}$	Chasing at 10^{-8} M	N.A.	Sorensen et al. (1988)
		15-keto-prostaglandin $F_{2\alpha}$	Chasing at 10^{-10} M	N.A.	
Cobitidae *Misgurnus anguillicaudatus*	M	Females	Increased courtship toward ovulated females	No courtship by non-reproductive or anosmic males	Kitamura et al. (1994)
Siluriformes					
Clariidae *Clarias gariepinus*	F	Male conspecifics	Attracted ovulated females	Unovulated and ovulated anosmic females not attracted	Resink et al. (1987)
	F	Seminal vesicle fluid	Attracted ovulated females	N.A.	Resink et al. (1989a)
Salmoniformes					
Salmonidae *Salmo trutta*	M	Nesting ovulated female	Attracted osmic parr	Less attraction by anosmic males	Olsén et al. (1998)
Perciformes					
Cichlidae *Oreochromis mossambicus*	M	Pre-ovulatory female-conditioned water	Osmic males increased urination	Anosmic males did not increase urination. Post-ovulatory female odors did not affect osmic males	Miranda et al. (2005)
Gobiidae *Gobius jozo*	F	Etiocholanolone glucuronide	Attracted ovulated females at $<10^{-6}$ M	Little/not responsive	Colombo et al. (1980)
Neogobius melanostomus	M	1,3,5(10)-estratrien-3-ol-17-one	Increased ventilation at 10^{-11}M threshold	Increased ventilation at 10^{-9}M threshold	This study
	F	Reproductive male conditioned water	Attracted reproductive females	Non-reproductive females were not attracted	Belanger et al. (2004)

The taxonomic classification scheme follows Nelson et al. (2004). Genus and species names are listed alphabetically within a family. N.A. = Not applicable/Not examined.

the reproductive state. Also, as shown in Belanger et al. (2006), no enhanced ventilation responses occurred when anosmic fish were tested with the putative pheromone estrone or conspecific odors. Elimination of the gill ventilation response after sensory deprivation demonstrates that the peripheral olfactory organ is vital to the perception and identification of interspecific pheromones as previously stated by Sorensen et al. (1992).

Other researchers have found that the behavior of a reproductive fish is altered when the fish is olfactory sensory deprived (e.g. Van den Hurk and Lambert, 1983; Resink et al., 1987; Kitamura et al., 1994; Olsén et al., 1998; Miranda et al., 2005; Belanger et al., 2006). Olsén et al. (1998) have shown that olfactory occlusion resulted in decreased time spent with an ovulated nesting female in precocious male Atlantic salmon. Olfactory sensory deprivation also blocked frequent male urination in the presence of pre-ovulatory females by male tilapia, *Oreochromis mossambicus* (Miranda et al., 2005). Similar results were found by Kitamura et al. (1994), who showed that male cobitide loach (*Misgurnus anguillicaudatus*) displayed courtship behaviors to ovulated females, but scarcely responded to unovulated females. Responses to ovulated females were eliminated after olfactory sensory deprivation.

Our findings are in agreement with many others, who demonstrated the effects of maturity on olfactory responses to putative pheromones (e.g. Sorensen et al., 1987, 1990; Sorensen and Goetz, 1993; Bjerselius and Olsén, 1993; Irvine and Sorensen, 1993). Resink et al. (1987, 1989a) showed that the behavioral responses of female African catfish (*Clarius gariepinus*) to conspecific males and steroid glucuronides were dependent on ovulatory state. Colombo et al. (1980) showed that female black gobies were not responsive to glucuronides outside of the interval between ovulation and oviposition. Cardwell et al. (1995) also demonstrated that the hormonal state of male *P. schwanenfeldi* can influence their olfactory response to prostaglandin $F_{2\alpha}$.

Reasons for increases in olfactory sensitivity during the reproductive season have been proposed by several authors (Creese and Sibley, 1981; Sorensen et al., 1987; Habibi et al., 1989; Moore and Scott, 1992) and most believe that the sensitivity of the olfactory epithelium to certain olfactory mediated signals is controlled by the endocrine system (Yamazaki and Watanabe, 1979; Yamazaki, 1990; Irvine and Sorensen, 1993). Cardwell et al. (1995) provided evidence for this by showing that androgens, specifically 17α-methyltestosterone, affect peripheral olfactory responses. After implanting two cyprinid species with androgens, increased EOG responses to a putative sex pheromone were demonstrated. Also, it has been shown that in Atlantic salmon, olfactory sensitivity to testosterone appears only during their brief spawning period and immature fish do not respond at any time (Moore and Scott, 1991; Moore and Waring, 1996). Taken together, these studies indicate that olfactory sensitivity to putative pheromones may be linked to the reproductive state of the fish.

In vertebrates, behavioral differences that occur during the reproductive season likely result when gonadal hormones change the expression of olfactory receptors (Alekseyenko et al., 2006).

Hormonal changes may also lead to morphological changes in olfactory epithelium at the onset of sexual maturity (Schreibman et al., 1984). Our findings support this idea by showing that fish use olfaction to respond to putative pheromones and that sensitivity to these pheromones is a function of reproductive status. Further research is needed to understand how olfactory sensitivities are moderated during reproductive and non-reproductive seasons.

Acknowledgements

This project was supported by NSERC, the Michigan Great Lakes Protection Fund, and the University of Windsor, Faculty of Graduate Studies. We thank Dr. T. Johnson (Ontario Ministry of Natural Resources) for providing round gobies for this study, Dr. P. Moore (Bowling Green State University) for assistance with statistical analysis, and many others for assistance with fish collection and laboratory work.

References

Alekseyenko, O.V., Baum, M.J., Cherry, J.A., 2006. Sex and gonadal steroid modulation of pheromone gene expression in the mouse vomeronasal organ. Neuroscience 140, 1349–1357.

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

Belanger, A.J., Arbuckle, W.J., Corkum, L.D., Gammon, D.B., Li, W., Scott, A.P., Zielinski, B.S., 2004. Behavioural and electrophysiological responses by reproductive female *Neogobius melanostomus* to odours released by conspecific males. J. Fish Biol. 65, 933–946.

Belanger, R.M., Corkum, L.D., Li, W.M., Zielinski, B.S., 2006. Olfactory sensory input increases gill ventilation in male round gobies (*Neogobius melanostomus*) during exposure to steroids. Comp. Biochem. Physiol. A 144, 196–202.

Bjerselius, R., Olsén, K.H., 1993. A study of the olfactory sensitivity of crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*) to 17α,20β-dihydroxy-4-pregnen-3-one and prostaglandin $F_{2\alpha}$. Chem. Senses 18, 427–436.

Cardwell, J.R., Dulka, J.G., Stacey, N.E., 1992. Acute olfactory sensitivity to prostaglandins but not to gonadal steroids in two sympatric species of *Catostomus* (Pisces: Cypriniformes). Can. J. Zool. 70, 1897–1903.

Cardwell, J.R., Stacey, N.E., Tan, E.S.P., McAdam, D.S.O., Lang, S.L.C., 1995. Androgen increases olfactory receptor response to a vertebrate sex pheromone. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 176, 55–61.

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. Boll. Zool. 47, 355–364.

Creese, I., Sibley, D.R., 1981. Receptor adaptations to centrally acting drugs. Annu. Rev. Pharmacol. Toxicol. 21, 357–391.

Frade, P., Hubbard, P.C., Barata, E.N., Canário, A.V.M., 2002. Olfactory sensitivity of the Mozambique tilapia to conspecific odours. J. Fish Biol. 61, 1239–1254.

Gammon, D.B., Li, W., Scott, A.P., Zielinski, B.S., Corkum, L.D., 2005. Behavioural responses of female *Neogobius melanostomus* to odours of conspecifics. J. Fish Biol. 67, 615–626.

Habibi, H.R., de Leeuw, R., Nahorniak, C.S., Goos, H.J.Th., Peter, R.E., 1989. Pituitary gonadotropin-releasing hormone (GnRH) receptor activity in goldfish and catfish: seasonal and gonadal effects. Fish Physiol. Biochem. 7, 109–118.

Irvine, I.A.S., Sorensen, P.W., 1993. Acute olfactory sensitivity of wild common carp, *Cyprinus carpio*, to goldfish hormonal sex-pheromones is influenced by gonadal maturity. Can. J. Zool. 71, 2199–2210.

Karlson, P., Lüscher, M., 1959. 'Pheromones': a new term for a class of biologically active substances. Nature 385, 725–729.

Kitamura, S., Ogata, H., Takashima, F., 1994. Activities of F-type prostaglandins as releaser sex pheromones in cobitide loach, *Misgurnus anguillicaudatus*. Comp. Biochem. Physiol. 107A, 161–169.

Lower, N., Scott, A.P., Moore, A., 2004. Release of sex steroids into the water by roach. J. Fish Biol. 64, 16–33.

MacInnis, A.J., Corkum, L.D., 2000. Fecundity and reproductive season of the round goby *Neogobius melanostomus* in the Upper Detroit River. Trans. Am. Fish. Soc. 129, 136–144.

Meredith, M., 2001. Human vomeronasal function: A critical review of the best and worst cases. Chem. Senses 26, 433–445.

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

Miranda, A., Almeida, O.G., Hubbard, P.C., Barata, E.N., Canário, A.V.M., 2005. Olfactory discrimination of female reproductive status by male tilapia (*Oreochromis mossambicus*). J. Exp. Biol. 208, 2037–2043.

Moore, A., Scott, A.P., 1991. Testosterone is a potent odorant in precocious male Atlantic salmon (*Salmo salar* L.) parr. Philos. Trans. R. Soc. Lond., B 332, 241–244.

Moore, A., Scott, A.P., 1992. 17α,20β-dihydroxy-4-pregnen-3-one 20-sulfate is a potent odorant in precocious male Atlantic salmon (*Salmo salar* L.) parr which have been pre-exposed to the urine of ovulated females. Proc. R. Soc. Lond. B 249, 205–209.

Moore, A., Waring, C.P., 1996. Electrophysiological and endocrinological evidence that F-series prostaglandins function as priming pheromones in mature Atlantic salmon (*Salmo salar*) parr. J. Exp. Biol. 199, 2307–2316.

Murphy, C.A., Stacey, N.E., 2002. Methyl-testosterone induces male-typical ventilatory behavior in response to putative steroidal pheromones in female round gobies (*Neogobius melanostomus*). Horm. Behav. 42, 109–115.

Murphy, C.A., Stacey, N.E., Corkum, L.D., 2001. Putative steroidal pheromones in the round goby, *Neogobius melanostomus*: olfactory and behavioral responses. J. Chem. Ecol. 27, 443–470.

Nelson, J.S., Crossman, E.J., Espinosa-Pérez, H., Findley, L.T., Gilbert, L.T., Lea, C.R., Williams, R.N., 2004. Common and scientific names of fishes from the Unites States, Canada, and Mexico. American Fisheries Society, Special Publication 29, Bethesda, Maryland.

Olsén, K.H., Järvi, J.T., Mayer, I., Petersson, E., Kroon, F., 1998. Spawning behavior and sex hormone levels in adults and precocious brown trout (*Salmo trutta* L.) males and the effect of anosmia. Chemoecology 8, 9–17.

Resink, J.W., Van den Hurk, R., Groeninx van Zoelen, R.F.O., Huisman, E.A., 1987. The seminal vesicle as source of sex attracting substances in the African catfish, *Clarias gariepinus*. Aquaculture 63, 115–127.

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., 1989a. The chemical nature of sex attracting pheromones from the seminal vesicle of the African catfish, *Clarias gariepinus*. Aquaculture 83, 137–151.

Resink, J.W., Voorthuis, P.K., Van den Hurk, R., Peters, R.C., Van Oordt, P.G.W.J., 1989b. Steroid glucuronides of the seminal vesicle as olfactory stimuli in African catfish, *Clarias gariepinus*. Aquaculture 83, 153–166.

Schreibman, M.P., Margolis-Kazan, H., Halpern-Sebold, L., O'Neil, P.A., Silverman, R.C., 1984. Structural and functional links between olfactory and reproductive systems: puberty related changes in olfactory epithelium. Brain Res. 302, 180–183.

Scott, A.P., Vermeirssen, E.L.M., 1994. Production of conjugated steroids by teleost gonads and their role as pheromones. In: Davey, K.G., Peter, R.I., Tobe, S.S. (Eds.), Perspectives in Comparative Endocrinology. National Research Council of Canada, Ottawa, pp. 645–654.

Sorensen, P.W., Goetz, F.W., 1993. Pheromonal and reproductive function of F prostaglandins and their metabolites in teleost fish. J. Lipid Mediat. 6, 385–393.

Sorensen, P.W., Hara, T.J., Stacey, N.E., 1987. Extreme olfactory sensitivity of mature and gonadally-regressed goldfish to a potent steroidal pheromone, 17α,20β-dihydroxy-4-pregnen-3-one. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 160, 305–313.

Sorensen, P.W., Hara, T.J., Stacey, N.E., Goetz, F.W., 1988. F-prostaglandins function as potent olfactory stimulants that comprise the postovulatory female sex pheromone in goldfish. Biol. Reprod. 39, 1039–1050.

Sorensen, P.W., Hara, T.J., Stacey, N.E., Dulka, J.G., 1990. Extreme olfactory specificity of male goldfish to the preovulatory steroidal pheromone 17α,20β-dihydroxy-4-pregnen-3-one. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 166, 373–383.

Sorensen, P.W., Irvine, I.A.S., Scott, A.P., Stacey, N.E., 1992. Electrophysiological measures of olfactory sensitivity suggest that the goldfish and other fish use species-specific mixtures of hormones and their metabolites as pheromones. In: Doty, R., Muller-Schwarze, D. (Eds.), Chemical Signals in Vertebrates, vol. VI. Plenum Press, New York, pp. 357–364.

Sorensen, P.W., Scott, A.P., Kihslinger, R.L., 2000. How common hormonal metabolites function as relatively specific pheromonal signals in the goldfish. In: Norberg, B., Kjesbu, O.S., Taranger, G.L., Andersson, E., Stefansson, S.O. (Eds.), Proceedings of the 6th International Symposium on Reproductive Physiology of Fish. Bergen, Norway, pp. 125–128.

Stacey, N.E., Cardwell, J.R., 1995. Hormones as sex pheromones in fish: widespread distribution among freshwater species. In: Goetz, F.W., Thomas, P. (Eds.), Proceedings of the fifth international symposium on the reproductive physiology of fish. Fish Symposium 95, Austin, TX, pp. 244–248.

Stacey, N.E., Sorensen, P.W., 1986. 17α,20β-dihydroxy-4-pregnen-3-one: a steroidal primer pheromone which increases milt volume in goldfish, *Carassius auratus*. Can. J. Zool. 64, 2412–2417.

Stacey, N.E., Cardwell, J.R., Liley, N.R., Scott, A.P., Sorensen, P.W., 1994. Hormones as sex pheromones in fish. In: Davey, K.G., Peter, R.I., Tobe, S.S. (Eds.), Perspectives in Comparative Endocrinology. National Research Council of Canada, Ottawa, pp. 438–448.

Strange, R.J., 1996. Field examination of fishes, In: Willis, B.E., Willis, D.W. (Eds.), Fisheries Techniques, 2nd ed. American Fisheries Society, Bethesda, pp. 433–446.

Sveinsson, T., Hara, T.J., 2000. Olfactory sensitivity and specificity of Arctic char, *Salvelinus alpinus*, to a putative male pheromone, prostaglandin $F_{2\alpha}$. Physiol. Behav. 69, 301–307.

Van den Hurk, R., Lambert, J.G.D., 1983. Ovarian steroid glucuronides as sex pheromones for male zebrafish, *Brachydanio rerio*. Can. J. Zool. 61, 2381–2387.

Vermeirssen, E.L.M., Scott, A.P., 1996. Excretion of free and conjugated steroids in rainbow trout (*Onchorhynchus mykiss*): evidence for branchial excretion of the maturation-inducing steroid, 17,20β-dihydroxy-4-pregnen-3-one. Gen. Comp. Endocrinol. 101, 180–194.

Wyatt, T.D., 2003. Pheromones and animal behaviour: communication by smell and taste. Cambridge University Press, Cambridge, UK.

Yamazaki, F., 1990. The role of urine in sex discrimination in the goldfish, *Carassius auratus*. Bull. Fac. Fish., Hokkaido Univ. 41, 155–161.

Yamazaki, F., Watanabe, K., 1979. The role of steroid hormones in sex recognition during spawning behaviour in the goldfish, *Carassius auratus*, L. Proc. Indian Natl. Sci. Acad., B Biol. Sci. 45, 505–511.