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Notes

Response to putative round goby (*Neogobius melanostomus*) pheromones by centrarchid and percid fish species in the Laurentian Great Lakes

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ABSTRACT

Pheromone trapping is an increasingly viable strategy to reduce invasive fish populations, largely due to the pheromones' function of evoking behavioral responses among conspecifics. Prior to attempting such population control techniques, the pheromones must be identified and their possible influences on non-target species addressed. The round goby (*Neogobius melanostomus*) is a species invasive to the Great Lakes region, and negatively impacts the ecosystem by interfering with local fish populations. At least two 5β-reduced 3α-hydroxyl steroids released by reproductive *N. melanostomus* (11-O-ETIO and 11-O-ETIO-3s) evoke olfactory sensory responses from the olfactory epithelium of conspecifics, and water conditioned by reproductive males (containing these steroids) attracts female round gobies. In this study, we examined whether these putative pheromones, along with simultaneously-released 11-O-ETIO-17s, stimulate olfactory sensory responses from alternative fish species sharing the same ecosystem as *N. melanostomus* in the Great Lakes region. Rock bass (*Ambloplites rupestris*), bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*Lepomis gibbosus*), smallmouth bass (*Micropterus dolomieu*), and yellow perch (*Perca flavescens*) were the targets of an electro-olfactogram experiment designed to record responses to odors. When compared to round goby responses from previous studies, amino acids and the bile acid consistently elicited electro-olfactogram responses across all species, but only round gobies showed a response to the putative pheromones. This study supports the concept of conducting a pheromone trapping trial in the field without adversely affecting the olfactory responses of non-target fish in the area.

Introduction

Introduced from Eurasia in 1990, the round goby (*Neogobius melanostomus*) has since expanded its range to all Laurentian Great Lakes (Charlesbois et al., 2001), and into the Lakes' lower tributaries (Poos et al., 2010). The success of *N. melanostomus* as an invasive species has been detrimental to local ecosystems where they aggressively interact with and/or prey on eggs, fry, and small fishes and aquatic invertebrates affecting biodiversity and production of resident taxa (Kornis et al., 2012; Poos et al., 2010). Although the proliferation of invasive fishes can be approached with aggressive management strategies including sterilization techniques, piscicide use, barriers, and traps, all but the former may result in the loss or disruption of heterospecific species inhabiting the targeted area (Benstead et al., 1999; Marks et al., 2010; Schreier et al., 2000; Siefkes et al., 2003).

More targeted approaches to isolating and capturing invasive species include pheromone trapping — a strategy under development in the Great Lakes region for the invasive sea lamprey (*Petromyzon marinus*) (Johnson et al., 2009; Luehring et al., 2011; Twohey et al., 2003), and is also under consideration by governing bodies to control round goby and Asian carp populations in the Great Lakes (T.B. Johnson, Ontario Ministry of Natural Resources, pers. comm.). Pheromones are species-specific chemical signals that facilitate communication among individuals (Wyatt, 2003), and sex pheromones specifically contribute to breeding behaviors (Sorensen and Stacey, 2004). While these reproductive pheromones evoke a behavioral response from a single species, the hormonal composition of these cues can stimulate olfactory activity across species (Bjerselius and Olsen, 1993; Lim and Sorensen, 2011). Thus the hypothesis that non-target species may respond to *N. melanostomus* pheromones is not improbable, and

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stresses the importance of investigating the species-specificity of a lure to mitigate heterospecific disruption during field application.

Reproductive male *N. melanostomus* have the ability to synthesize, release and smell steroids that have a 5β-reduced and 3α-hydroxyl configuration, including of 3α-hydroxy-5β-androstane-11,17-dione (11-oxo-etiocholanolone; 11-O-ETIO) and 11-O-ETIO-3s (11-oxo-etiocholanolone-3-sulfate) (Arbuckle et al., 2005; Katare et al., 2011; Laframboise and Zielinski, 2011). Furthermore, behavioral responses have been observed when conspecifics encounter conditioned water containing these released steroids (Bélanger et al., 2004; Gammon et al., 2005; Tierney et al., in press). Numerous fish species show habitat overlap with *N. melanostomus* in littoral and benthic regions of the Great Lakes and their tributaries, including centrarchids (Steinhart et al., 2004) and percids (Duncan et al., 2011); however, little is known about the chemical communication systems among these fishes. These steroids released by *N. melanostomus* presented an ideal model to explore the possible impact of pheromone trapping on heterospecific species sharing the same habitat.

Physiological responses to odors can be tested using an electro-olfactogram (EOG), a technique that measures responses at the cellular level by recording changes in the summed generator potential (field potentials) following the application of a test odor onto the olfactory epithelium (e.g. Sveinsson and Hara, 2000). Specifically, we investigated the selectiveness of the putative pheromones 5β-reduced and 3α-hydroxyl steroids released by *N. melanostomus* by testing the olfactory sensory responses of non-target fish species. These data were compared to *N. melanostomus* responses collected on the same EOG apparatus during the breeding seasons in 2009 and 2010.

Materials and methods

Experimental fishes

All fish collection, husbandry and experimental protocols were reviewed and accepted by the University of Windsor Animal Care Committee and were in accordance with the Canadian Council on Animal Care guidelines. Rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*) were seined from the Detroit River (Windsor, ON, Canada) and angled from Lake Erie (Erieau, ON, Canada) during the breeding season of 2011 to represent the non-target group. We retained any fish pertinent to this study using both fishing techniques, both of which are used to capture fish species overlapping with the *N. melanostomus*. The *N. melanostomus* used for this study were captured using the same techniques and during the same period in 2009 and 2010 (Laframboise and Zielinski, 2011). The aforementioned non-target species represent the majority of by-catch when targeting *N. melanostomus* in these areas (personal observation), and phylogenetically represent families *Centrarchidae* (bass and sunfish) and *Percidae* (yellow perch). All non-gobiidae species caught and tested for this study weighed approximately 2 g and were approximately 5 cm in total length. Because these individuals were caught concurrently with breeding *N. melanostomus*, these fish are expected to represent the age-class that may be impacted by pheromone lures. Fish were housed in the University of Windsor Animal Quarters in a 113 L aquarium containing dechlorinated municipal tap water. When multiple individuals were captured during a single outing, they were housed in the same aquarium. The water temperature was maintained at 16 °C (± 2 °C) and the photoperiod was 16 h light:8 h dark. The aquarium was aerated and enhanced with gravel as lining and plants for shelter. Nutrafin fish flakes were provided as food ad libitum. Each fish was acclimated for at least 24 h, but for no more than 3 days after capture. The 2009 and 2010 *N. melanostomus* was cared for under the same conditions (Laframboise and Zielinski, 2011).

Electro-olfactogram recording

Odor potency was tested using an EOG to measure changes in field potentials after application of the odor to the olfactory epithelium. We recorded olfactory sensory responses to the canonical fish odors, amino acids and bile acids (reviewed by Zielinski and Hara, 2006), and to the 5β-reduced and 3α-hydroxyl steroids released by *N. melanostomus* (Katare et al., 2011). The stimulated olfactory sensory response of *N. melanostomus* to released steroids 11-O-ETIO and 11-O-ETIO-3s has been established (Laframboise and Zielinski, 2011). The EOG recording methods reflect protocols developed and honed in *N. melanostomus* (Bélanger et al., 2004; Laframboise and Zielinski, 2011; Murphy et al., 2001) and in *P. flavescens* (Mirza et al., 2009) (Fig. 1). Immediately before this procedure, the fish was transferred into the anesthetic tricaine methanesulfonate dissolved in dechlorinated water (MS-222; Finquel, Argent Chemical Laboratories, Redmond, WA, USA; 100 mg/L) until the fish lost equilibrium and opercular movement slowed. To prevent involuntary muscle twitches, the fish was injected intramuscularly with the paralytic gallamine triethiodide (Flaxedil, Sigma-Aldrich, Oakville, ON, Canada; 150 mg/kg). The fish was wrapped in wet tissue to retain moisture, and placed in a trough with a low volume of water exposing only the dorsal half of the fish, including its olfactory region,

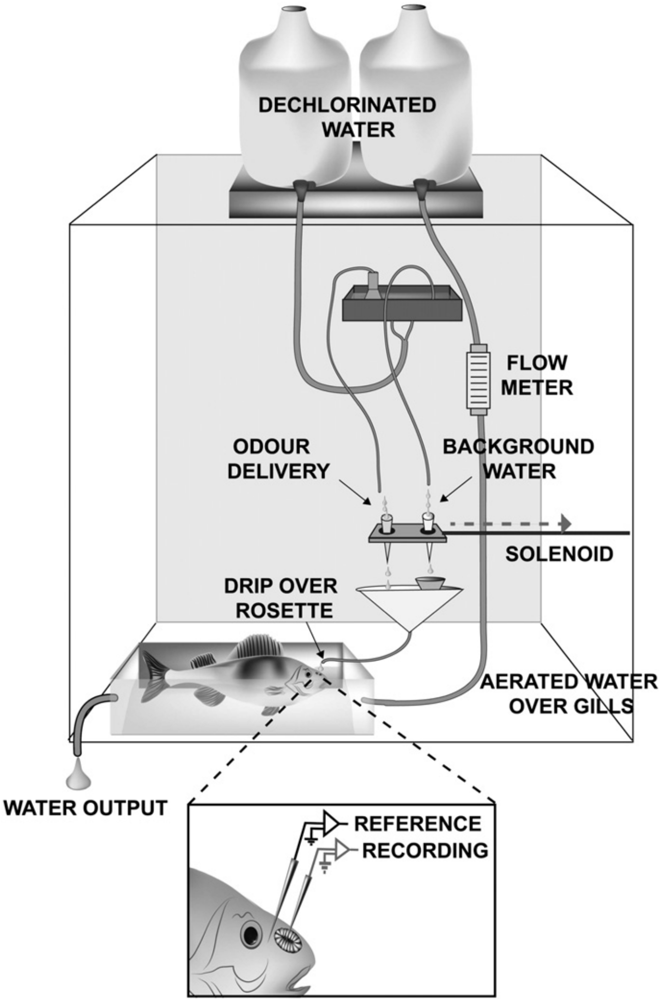

Fig 1. A schematic diagram of the electro-olfactogram. The in vivo gravity-fed experimental setup supports the anesthetized fish in a trough supplied with aerated dechlorinated water. The nares are irrigated with a dechlorinated water drip between odor deliveries controlled by a solenoid-driven mechanism. Any measurable change in field potential values between the reference electrode (placed on the skin) or recording electrode (placed on the olfactory epithelium) are amplified and digitized into a measurable waveform.