AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
Aquatic Conserv: Mar. Freshw. Ecosyst. **14**: 327–331 (2004)
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/aqc.632

Editorial

Pheromone signalling in conservation

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Introductions, like extinctions, are forever (Marsden, 1993).

In freshwater ecosystems, non-indigenous species (NIS) are the most significant threat to biodiversity (Sala *et al*., 2000). Despite efforts to control the entry of invading species into countries (e.g. Vásárhelyi and Thomas, 2003), there are relatively few practices in place to control established aquatic invasive species without affecting non-target species or causing environmental damage. Pheromones have been used successfully to control terrestrial insect pests that rely on chemical cues to synchronize behaviours without adverse changes to the environment (Payne *et al*., 1986). I suggest that this strategy used to control terrestrial insects be applied to protect aquatic indigenous species threatened by invaders.

All animals are 'leaky bags' with odours being released from skin, gills, lungs, faeces and urine (Atema, 1996). Just as ornaments or elaborate displays are used by individuals to enhance their mating success, odours represent a strong signal to females and males in sexual selection (Wyatt, 2003). Odour as a cue for males to attract females was recognized by Darwin (1874), who described the strong odours of reproductive male elephants, goats and deer. Historically, commercial fishers often deployed caged reproductive male lampreys to attract females, resulting in abundant catches (Fontaine, 1938). This idea of using pheromones from one sex to attract members of the opposite sex during the breeding season could result in a shift in the operational sex ratio of a species, resulting in severe competition for mates.

Since Doving (1976) suggested that fish may have evolved to release hormones as sex pheromones, researchers have shown that pheromones produced in the final stages of maturation function in the synchronization of mating in many fish. Examples include studies on Petromyzontiformes (sea lamprey, *Petromyzon marinus* (Li *et al*., 2002)), Cypriniformes (e.g. goldfish, *Carassius auratus* (Sorensen and Stacey, 1999)), Siluriformes (African catfish, *Clarias gariepinus* (Van den Hurk and Resink, 1992)), Salmoniformes (e.g. Atlantic salmon, *Salmo salar* (Waring and Moore, 1997); brook trout (Young *et al*., 2003)) and Perciformes (round gobies (Murphy *et al*., 2001; Belanger, 2002)).

It is the application of pheromone signalling in the control of NIS to enhance the conservation of native freshwater species that I wish to address. I will provide examples of two NIS (*P. marinus* and round goby, *Neogobius melanostomus*) that exhibit detrimental effects on native fish in the Laurentian Great Lakes. The

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sea lamprey, a fish parasite, represents an ancient vertebrate lineage that survived over 400 million years during considerable changes on Earth. The round goby is a prolific species that represents a more recent group of teleosts or bony fish. I will also describe a field study in which odours are used to capture non-indigenous crayfish. The preliminary success of pheromone traps to capture invasive crayfish is encouraging to fisheries managers, who hope to use a variety of techniques to control fish invaders that have a devastating effect on indigenous species.

Petromyzon marinus (sea lamprey)

Sea lamprey is an anadromous fish that probably used the Welland Canal to circumvent the natural barrier of Niagara Falls to enter Lake Erie (1921) and subsequently the Upper Great Lakes (Leach, 1995). The adult sea lamprey is a destructive parasite that sucks fluids and often kills such hosts as lake trout, lake whitefish, burbot, walleye and catfish, resulting in severe economic losses to the sport and commercial fishery (Smith and Tibbles, 1980).

In spring, adult male sea lamprey migrate upstream from lakes or oceans into tributaries to build a nest (redd) and spawn with the later arriving females (Bjerselius *et al*., 2000). At the time of upstream migration, adult sea lamprey are sexually immature, but sexual maturation progresses during migration, culminating in spawning activities. Once eggs are fertilized, the adults die. The young hatch and burrow in sediments, where they filter-feed from 3 to 18 yr. Afterwards, larvae metamorphose into the parasitic (juvenile) stage and migrate downstream to the receiving waters to feed on fish. In lakes, sea lamprey feed for about 15 months (increasing 100-fold in biomass), after which they stop feeding and migrate upstream to spawn (Hardisty and Potter, 1971).

The devastating effects of sea lamprey on the decline of native fish, especially lake trout, is considered to be the worst ecological disaster to have occurred in a drainage basin (Smith and Tibbles, 1980). Sea lamprey reduced the Lake Huron and Lake Superior harvest of native lake trout from 7×10^6 to 136 000 kg annually (http://www.glfc.org). Annual monitoring and control costs of sea lamprey (shared between the USA and Canada) of the Great Lakes Fishery Commission (GLFC) amounts to 15 million US$ per year. The primary control method used by the GLFC for sea lamprey to remove larvae from tributaries of the Great Lakes basin has been the application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). Costs for lampricide treatment are enormous (full-year 2002 costs were estimated to be $5 838 000 for treatment operation and chemical costs; www.fluorideaction.org/pesticides/tfm.costs.2002.htm). Clearly, alternative methods of control (sterile male release, barrier dams, pheromonal strategies) are desired for both environmental and economic reasons.

Bjerselius *et al*. (2000) provided evidence that sea lamprey larval odour (bile acids) attracts sexually immature male and females to spawning streams. In addition, Bjerselius *et al*. (2000) showed that, as sea lamprey mature on their upstream migration, they produce sex pheromones. Odours obtained from washings of sexually mature males and females attract sexually mature members of the opposite sex; however, sexually mature adults are no longer attracted to the larval pheromone. Recently, Li *et al*. (2002) identified a potent sex attractant, a bile acid (7α,12α,24-trihydroxy-5α-cholan-3-one 24-sulphate) released by mature male sea lamprey. This bile acid initiates searching behaviour in ovulated female lampreys, which was demonstrated in flumes and in a natural stream. The field trial showed that ovulated radio-tagged females, released 65 m downstream from a divided channel, migrated upstream into the channel where mature spermiating males were held (Li *et al*., 2002). The results of recent field experiments showed that traps baited with spermiating males captured 87% of ovulating females released, whereas traps with non-spermiating males and empty traps did not capture any ovulating females (Johnson *et al*., in press). Clearly, the refinement of pheromone traps using natural bile acids or synthetic pheromones would provide an environmentally benign control technique for this devastating invasive species.

***Neogobius melanostomus* (round goby)**

Native to the Black and Caspian Seas, the round goby was probably introduced to the Laurentian Great Lakes by ballast water in 1990 (Jude *et al*., 1992). The fish spread to all five Great Lakes and has begun to invade the Mississippi River basin (Charlebois *et al*., 2001). Reasons for the proliferation of the round goby include its broad diet and availability of molluscan prey (adults eat mainly exotic dreissenids), aggressiveness, high fecundity, iteroparity (females may spawn up to six times per year; it is speculated that males die after a single reproductive season), and male parental care (MacInnis and Corkum, 2000). This reproductive strategy may be responsible, in part, for the tremendous increase in density and distribution of the round goby in the Laurentian Great Lakes. For example, Lee (2003) estimated that there were more than 10.1 billion round gobies in the western basin of Lake Erie in 2002.

The round goby out-competes native forage fish, such as the mottled sculpin, for food and nest sites (Dubs and Corkum, 1996; Janssen and Jude, 2001). Both juvenile and adult round gobies feed on eggs of several native fish: lake trout (Chotkowski and Marsden, 1999), lake sturgeon (Nichols *et al*., 2003), smallmouth bass (G. Steinhart, Ohio State University, personal communication). Because round goby adults feed on exotic zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*), and gobies are consumed by sport fish (e.g. smallmouth bass and yellow perch), there is concern that round gobies may transfer contaminants through the food web (Morrison *et al*., 2000). Moreover, round goby has been implicated in botulism outbreaks in eastern Lake Erie, where dead waterfowl are exposed to toxins from *Clostridium botulinum* Type E (Domske *et al*., 2002). Because round gobies are the main identifiable prey item in the guts of these birds (D. Campbell, University of Guelph, personal communication), the fish may transfer toxins of anaerobic bacteria associated with decaying dreissenids through the food chain. Although the economic costs of the round goby are unknown, there is great value in reducing the reproductive success of this invasive species.

Pheromone signalling is crucial to mating behaviour in round gobies. In the laboratory, mature round goby females swim directly to odours released by mature males (Corkum, unpublished data). Murphy *et al*. (2001) showed that round gobies increased gill ventilation when exposed to putative pheromonal compounds: 18-, 19-, and 21-carbon steroids, including etiocholanolone (ETIO) and ETIO-glucuronide. This gill ventilation response to steroids, as well as to gonadal extracts from mature females, is dependent upon olfactory sensory input (Belanger *et al*., 2003). Specific fractions of conditioned water (which may contain steroids) obtained from mature male round gobies stimulated olfactory responses in mature females (Belanger, 2003). Recently, colleagues discovered that at least four 5β-reduced androgens (including ETIO) were produced in the islets of steroid-synthesizing glandular tissue of male round goby testes (W. Arbuckle, University of Windsor and A.P. Scott, CEFAS, UK, unpublished data). These results show that pheromones synchronize mating behaviour in the round goby.

Pheromone traps

The application of chemical cues can be used to disrupt reproductive behaviours of exotic species or to trap invading species so that the recruitment of native species is enhanced. Promise for the application of pheromone traps to control sea lamprey and round gobies can be gleaned from the recent test trials of traps in the UK to control the non-indigenous signal crayfish, *Pacifastacus leniusculus* (Stebbing *et al*., 2004). Many species of crayfish were intentionally introduced to Europe to provide food for restaurants. In England, signal crayfish escaped from farm ponds and colonized rivers throughout south-eastern England (Lodge *et al*., 2000), affecting the native white-clawed crayfish *Austopotamobius pallipes*. In signal crayfish, sex pheromones, released in the urine of mature females, stimulate mating behaviour in mature males in the laboratory (Stebbing *et al*., 2003). The results of preliminary field trials show that sex-pheromone-baited traps were attractive to males during the breeding season; food-baited traps captured both sexes (Stebbing *et al*., 2004). These results are particularly promising, since the sex pheromone used in baited crayfish traps was water conditioned from mature females alone (i.e. female washings), since the structure and function of the pheromone has yet to be identified. The conditioned water was freeze-dried and embedded into a gel to use as bait in crayfish traps (Stebbing *et al.*, 2004). Although refinements are still needed, pheromone traps have the potential to prevent the non-indigenous signal crayfish from eliminating the white-clawed crayfish.

Though the preliminary success of aquatic pheromone traps is encouraging, additional research is needed to focus on understanding pheromone function, its production, release, transmission in water and sensory detection. It takes a multidisciplinary team to understand the complexity of pheromone communication. I anticipate that pheromone traps will eventually be used to control both sea lamprey and round gobies so that the recruitment of indigenous species is enhanced. This is a crucial time for research on understanding pheromone structure and function and for its application to control aquatic invaders to conserve species.

ACKNOWLEDGEMENTS

P.D. Stebbing kindly provided reports on pheromone trapping of signal crayfish. I thank my collaborators (Dr W. Li, Michigan State University, Dr A.P. Scott, CEFAS, Weymouth, UK, and Dr B. Zielinski, University of Windsor) for their commitment and contributions to the round goby pheromone research project. This research has been financially supported by the Michigan Great Lakes Protection Fund.

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