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Salinity tolerance of the invasive round goby: Experimental implications for seawater ballast exchange and spread to North American estuaries

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ABSTRACT

The Eurasian round goby (*Neogobius melanostomus*) invaded the freshwater North American Great Lakes in ~1990 via accidental introduction from ballast water discharge. Its genotypes in the Great Lakes traced to estuaries in the northern Black Sea, where the round goby flourishes in a variety of salinities to 22 parts per thousand (ppt). To prevent further introductions, U.S. and Canadian Coast Guard regulations now require that vessels exchange ballast water at sea before entering the Great Lakes. Since salinity tolerance of the invasive round goby population is poorly understood, we tested 230 laboratory-acclimated fish in three experimental scenarios: (1) rapid salinity increases (0–40 ppt), simulating ballast water exchange, (2) step-wise salinity increases, as during estuarine tidal fluxes or migration from fresh to saltwater, and (3) long-term survivorship and growth (to 4 months) at acclimated salinities. Almost all gobies survived experiments at 0–20 ppt, whereas none survived ≥30 ppt, and at 25 ppt only 15% withstood rapid changes and 30% survived step-wise increases. Ventilation frequencies were lowest at 10–15 ppt in step-wise experiments, in conditions that were near isotonic with fish internal plasma concentrations, reflecting lower energy expenditure for osmoregulation. Growth rates appeared greatest at 5–10 ppt, congruent with the larger sizes reached by gobies in Eurasian brackish waters. Thus, we predict that the Great Lakes round goby would thrive in brackish water estuaries along North American coasts, if introduced. However, oceanic salinities appear fatal to the invasive round goby, which likely cannot withstand complete seawater ballast exchanges or oceanic habitats.

Introduction

Exotic introductions and the round goby's distribution

An estimated 73% of the 186 aquatic invasive species (AIS) in the freshwater Laurentian Great Lakes arrived via ballast water discharged from oceanic vessels (Holeck et al., 2004; Kelly et al., 2009; Ricciardi, 2001), with 70% from 1985 to 2000 tracing to the Eurasian Ponto-Caspian region (Ricciardi and MacIsaac, 2000). Prior to 2006, an average of 1–2 new AIS was reported in the Great Lakes per year (GLANSIS, 2011). The Eurasian round goby *Neogobius melanostomus* (Teleostei: Gobiidae) first was discovered in the St. Clair River of the Great Lakes in 1990 (Jude et al., 1992); genetic relationships showed that the invasion originated from the Black Sea port in the southern Dnieper River liman (estuary) near Kherson Ukraine, with additional contribution from a southern Danube River source (Brown and Stepien, 2009). The round goby spread rapidly throughout the Great Lakes and associated tributaries, and is one of the most abundant benthic fishes in the lower Lakes (Charlebois et al., 2001; Irons et al., 2006; Ray and Corkum, 2001).

The round goby occupies a diversity of habitats and salinities across its Eurasian range; its native Ponto-Caspian distribution encompasses the Black Sea (reaching salinities of 22 parts per thousand (ppt)), Azov Sea (to 12 ppt), and Caspian Sea (to 13 ppt), along with their associated estuaries and freshwater tributaries (Chotkowski and Marsden, 1999; Reid and Orlova, 2002). Anthropogenic factors, including construction of canals, bait transport, and ballast water introductions, have greatly increased its range in Eurasia during the past decades (summarized by Stepien and Tumeo, 2006; Brown and Stepien, 2008). Its successful invasion of the Baltic Sea in ~1990 had a separate genetic origin from the Great Lakes introduction (Brown and Stepien, 2008; Stepien and Tumeo, 2006; Stepien et al., 2005). The round goby reaches a maximum size of ~250 mm TL (total length) at 13–22 ppt in the Black and Baltic seas (Brown and Stepien, 2008; Sokolowska and Fey, 2011; Svetovidov 1964), but generally attains only ~110–180 mm TL in the freshwater Great Lakes (Ghedotti et al., 1995; Sokolowska and Fey, 2011).

Most round goby genotypes in the Great Lakes also are common in northern Black Sea estuaries, suggesting that the species likely would flourish in North American coastal brackish water habitats (Brown and Stepien, 2008, 2009; Stepien and Tumeo, 2006). Several of its

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close Ponto-Caspian relatives – including the monkey goby *Neogobius fluviatilis*, racer goby *Babka gymnocephalus*, and bighead goby *Ponticola kessleri* – also are invasive in Europe, similarly occupy wide ranges of habitats and salinities, and have been predicted to become future invaders of the Great Lakes (GLANSIS, 2011; Neilson and Stepien, 2009).

Ballast water exchange regulations

To combat further AIS introductions, U.S. Coast Guard control regulations now mandate that ships carrying ballast water to the Great Lakes must have a ballast water management plan (U.S. Coast Guard, 2004). As of this writing, two ballast water management methods are approved: ballast water exchange ≥200 nautical miles from shore, or ballast water retention. Exchange may use either the empty/refill method or the flow through method; in the latter seawater enters at the bottom of the tank and freshwater overflows through the hatches and vents at the top, which must be performed three times. Military ships, domestic oil tankers (such as those traveling between Alaska and other states), and ships that do not leave U.S. waters are exempt from these regulations. Moreover, ballast water exchange is not mandatory under circumstances that might jeopardize the safety of the crew, including: adverse weather, vessel design limitations, equipment failure, and security concerns (U.S. Coast Guard, 2004).

Prior to 2006–8, "No Ballast on Board" (NOBOB) ships were exempt from ballast management regulations, as their tanks were considered empty and originally not considered a risk for transporting AIS; today, >90% of ships entering the Great Lakes are NOBOBs (Colautti et al., 2003). NOBOB vessels carry an average of ~60 metric tons of residual ballast (water and sediment), in which investigations found appreciable numbers of AIS (Bailey et al., 2005; Colautti et al., 2003; Duggan et al., 2005). Since 2006, Canadian legislation has required NOBOB ships to flush their tanks with open-ocean seawater (Canada Shipping Act, 2006); these regulations then were adopted in 2008 for ships entering U.S. waters (Ballast Water Working Group, 2009, 2011).

Regulations now require that all ballast water must be at ≥30 ppt to enter the Great Lakes (Ballast Water Working Group, 2011). These measures are believed to have significantly reduced the risk of AIS introductions to the Great Lakes, as reports of new AIS have declined (GLANSIS, 2011).

Osmoregulation and the round goby's habitats

Most teleost fishes have plasma solute concentrations that are ~10.5 ppt (~30% of seawater); therefore, they are hyperosmotic to freshwater but hypoosmotic to oceanic concentrations. Either hyposaline or hypersaline external water conditions can adversely affect fish physiology, leading to distress and death (Boeuf and Payan, 2001; Deane and Woo, 2009). However, the process of osmoregulation allows euryhaline fishes to maintain relatively consistent internal ionic concentrations in their plasma and tissues despite changing external salinity conditions (Holmes and Donaldson, 1969; Kinne, 1964). Notably, fishes may absorb or actively pump out ions through their gills (e.g., the former in freshwater and the latter in saline habitats), or may excrete or conserve water in their kidneys (the former in freshwater and the latter in saltwater).

Osmoregulation is energy-demanding, estimated at ~10 to >50% of a fish's overall energy expenditure, depending on the species and its internal and environmental osmotic concentrations (Boeuf and Payan, 2001). Even gradual changes in salinity usually cause fish to use more energy to osmoregulate, potentially affecting metabolism, growth, and reproductive success (Boeuf and Payan, 2001). For example, Maxime et al. (1991) discerned significant increase in oxygen consumption by rainbow trout *Oncorhynchus mykiss* in the first 24 h of seawater acclimation. Rainbow trout (Rao, 1968) and tilapia *Oreochromis aureus* (Farmer and Beamish, 1969; Febry and Lutz, 1987) consumed ~27% more oxygen in seawater conditions (35 ppt) than at isosmotic salinities (10.5–12 ppt).

Growth of euryhaline fishes tends to be highest at isotonic salinities, as less energy is used for osmoregulation and metabolism (Boeuf and Payan, 2001). However, euryhaline species often are subject to varying or opposing suites of osmoregulatory challenges over the time frame of hours or days, due to salinity changes in their habitats (reviewed by Evans, 1993), as experimentally evaluated here for the round goby.

In addition to being euryhaline, the round goby is eurythermic, surviving temperatures −1 to +30 °C in the Black Sea (Moskal'kova, 1996) and a critical thermal maximum of ~33.4 °C in the Great Lakes (Cross and Rawding, 2008). Its growth rates may be optimal at salinities and temperatures nearest to those found in its original native estuaries, unless adaptation to freshwater environments has altered this capability in areas such as the Great Lakes, as tested here.

Objectives and hypotheses

Ellis and MacIsaac (2009) examined survival of several Great Lakes AIS during rapid immersion at 30 ppt for 4 h; all of their round gobies died. Here, our more comprehensive experimental regime evaluated short and long-term abilities of the round goby to survive and acclimate in various salinities. Our rapid salinity change experiments tested response to immediate immersion at 0–40 ppt, simulating ballast water exchange with seawater or their release into ports of various salinities. We compared those results to gradual step-wise salinity increase experiments, which mimicked conditions during migrations from fresh to seawater (via currents or swimming) or during tidal flushing in coastal habitats. Our long-term experiments tested survivorship and growth at acclimated salinities, approximating the round goby's potential colonization of oceanic coastal estuaries and bays.

We tested survival, ventilation frequencies, and growth of laboratory-acclimated individuals from Lake Erie in salinities from 0 to 40 ppt. Fish ventilation frequency was determined from mean number of opercular beats/minute (see Gibson and Mathis, 2006; James et al., 2003; Sneddon, 2003) following the approach used by Cross and Rawding (2008) to test respiration rate with water temperature for the Great Lakes round goby; this measurement however, can vary under conditions of stress and exercise, which we strove to avoid. We also measured growth at various salinities, as a possible indicator of long-term adaptability to various habitat regimes (see Boeuf and Payan, 2001).

The following hypotheses were tested (with the null hypothesis as no difference, and the alternative hypothesis as a significant difference): (A) round goby survival varied with salinity during rapid change experiments, (B) survival differed between the rapid change and step-wise (gradual acclimation) salinity experiments, (C) long-term survival (to 4 months) differed among acclimated salinity groups, (D) fish ventilation frequency varied with salinity, and (E) fish growth differed with salinity.

Materials and methods

Fish collection and experimental design

Round gobies (~250) were collected in kick seines from Maumee Bay, Lake Erie (0 ppt) along Bayshore Road, Oregon, OH (41°41′23.61″N, 83°23′59.31″W) under Ohio Collection Permit No. 11-214. Fish were acclimated for one week to laboratory conditions in two side-by-side 946 liter (L) aerated holding tanks under ambient light, at room temperature, and 1 ppt (Instant Ocean, Spectrum Brands