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

Susanne I. Karsiotis, Lindsey R. Pierce, Joshua E. Brown¹, Carol A. Stepien*

The Great Lakes Genetics Laboratory, Lake Erie Center and the Department of Environmental Sciences, The University of Toledo, 6200 Bayshore Road, Toledo, OH 43616, USA

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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.

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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

* Corresponding author. Tel.: +1 419 530 8362 (office).

E-mail address: Carol.Stepien@utoledo.edu (C.A. Stepien).

¹ Present Address: National Oceanic and Atmospheric Administration, National Sea Grant College Program, 1315 East West Highway, Silver Spring, MD 20910, USA.

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 ($\sim 30\%$ 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; Febrly and Lutz, 1987) consumed $\sim 27\%$ 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

Inc., Atlanta, GA, USA, mixed with dechlorinated tap water). We used 1 ppt salinity as a precaution against freshwater parasites/pathogens during laboratory acclimation, which is a common procedure in freshwater fish maintenance (see Harms 1996; Mifsud and Rowland, 2008). Gobies were not fed during acclimation.

After acclimation, gobies were measured (mm, SL (standard length)) and fish within the size range of 25–106 mm were distributed randomly among 38 L glass aquaria. ANOVA tests (Analysis of Variance) were used to verify that fish sizes did not differ among the aquaria and salinity treatments. Aquaria were filled to 30 of 38 L capacity, with 0 ppt being the control, and experimental treatments differing by 5 ppt, encompassing 5 to 40 ppt. Aquaria were covered with aluminum foil to minimize evaporation; their salinities were checked daily with a Vital Sine™ SR-6 Salinity Refractometer (Rhineland, WI, USA) and adjusted as needed. Aquaria were kept at room temperature under identical ambient light conditions in the same room as the larger acclimation tanks. Each aquarium contained two PVC pipes as shelters (25 mm in diameter × 85 mm long and 45 mm in diameter × 120 mm long). In the step-wise and long-term experiments, 1/3 of the water was changed every 3 days.

We conducted three series of salinity experiments: (1) rapid salinity change via immediate immersion at 0 (the control), 5, 10, 15, 20, 25, 30, 35, or 40 ppt, (2) step-wise salinity increase by 5 ppt every 3 days (beginning at 0 and ending at 30 ppt), and (3) long-term survivorship and growth over 4 months, during which the gobies from the first experiment were maintained at their end-point salinities (0–30 ppt). Gobies were observed twice daily, and individuals were removed if distressed (i.e., showed muscle contractions, loss of color, erratic swimming behavior, lack of movement with prodding) or deceased; both of these categories were recorded as non-survivors. Individuals exhibiting distress were euthanized with a lethal dose of tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA) (25 mg/mL) under University of Toledo IACUC 106247. Gobies in step-wise and long-term experiments were fed bloodworms and mysis shrimp to satiation daily, and excess food was removed within minutes. In the three-day rapid salinity change experiments, fish were not fed.

Survival and ventilation frequency during rapid salinity increases (1–3 days)

Rapid salinity change experiments tested 200 gobies (25–106 mm, SL) in 20 aquaria (10 fish each) at salinities of 0 (the control), 5, 10, 15, 20, 25, 30, 35, and 40 ppt. The control group totaled 40 gobies (10 each in 4 aquaria), and each experimental salinity group totaled 20 fish (10 in 2 aquaria, 5–40 ppt). On day 2, we recorded the mean number of opercular beats/minute (three recordings/individual) for 10 gobies/salinity, as a surrogate for respiration (see Cross and Rawding, 2008; James et al., 2003).

Survival and ventilation frequency during gradual step-wise salinity increases

Step-wise experiments tested 30 laboratory-acclimated gobies (30–78 mm, SL) that were not used in the previous experiment, with the control (0 ppt) and experimental groups each containing 20 gobies (5 each in 4 aquaria). Salinity of the experimental group was increased step-wise by 5 ppt every 3 days, beginning at 0 ppt and ending at 30 ppt, at which point all fish died (see Results). Ventilation frequency (as mean number of opercular beats/minute) was recorded for all individuals before each water change on day 3.

Long-term survivorship and growth at salinity (to 4 months)

Long-term experiments tested survivorship and growth of round gobies (25–93 mm, SL) acclimated at salinities 0–25 ppt, for a period

of 4 months. Survivors from the three-day rapid salinity change experiment were re-measured and then maintained in aquaria at that same end-point salinity. The control (0 ppt; $N=20$) and experimental groups (5, 10, 15, 20, and 25 ppt; $N=53$) were housed among 20 aquaria (4–5 individuals/aquarium). Fish size (mm, SL) did not differ among the aquaria or salinity treatments at the beginning of the experiment ($p=0.88$). Gobies were re-measured at 1 and 4 months, and mortality was recorded daily.

Data analyses

Contingency tests evaluated the numbers of fish surviving and dying per salinity treatment in all experiments, with probabilities adjusted using Bonferroni correction for multiple post-hoc tests (Sokal and Rohlf, 1995). ANOVA in JMP v.8 (SAS Institute; www.sas.com) was used to test for differences in ventilation frequency (mean number of opercular beats/minute) and relative growth (mm, SL) among salinity treatments. Tukey's post-hoc tests evaluated the source(s) of any significant difference(s) found in the ANOVA tests ($p<0.05$). Student's *t*-tests were used to determine differences between experiments at the same salinity.

Results

Survival during rapid salinity increases (1–3 days)

Almost all gobies survived immediate immersion in salinities of 5 ppt (95%) and 10 ppt (100%), similar to the number of controls surviving at 0 ppt (95%; Fig. 1A). Those at 15 and 20 ppt had slightly lower survivorship (80% and 70%, respectively), but did not significantly differ from 0 to 10 ppt. Daily survivorship did not vary across the three day course of the experiment, except at 25 ppt: 70% of the fish at 25 ppt survived day 1, decreasing to 15% by day 3 ($\chi^2=13.7$, 2 df, $p=0.0002$). Only 10% of the individuals survived on day one at 30 ppt, after which they all died. No gobies survived for more than a few hours at 35 and 40 ppt. Overall, statistically similar numbers of fish survived salinities of 0–20 and 30–40 ppt, respectively. Contingency tests revealed differences in survival among gobies at 0–20, 25, and 30–40 ppt ($\chi^2=141.5$, 2 df, $p<0.0001$). Survival at 0–20 ppt was significantly greater than at 25 ppt ($\chi^2=56.1$, 1 df, $p<0.0001$) and 30–40 ppt ($\chi^2=93.3$, 1 df, $p<0.0001$); the latter two also differed ($\chi^2=4.8$, 1 df, $p<0.03$). Our results thus reject null hypothesis A, finding that significantly more round gobies survived rapid change experiments (Fig. 2A) at 5–20 ppt than at higher salinities ≥ 25 ppt.

Survival during gradual step-wise salinity increases

Step-wise salinity increase experiments showed that most gobies survived salinities 0–15 ppt (95–100%; Fig. 1B). At 20 ppt, 75% survived, 30% survived at 25 ppt, and none survived at 30 ppt. Pairwise comparisons revealed no significant differences in survivorship at 0–20 ppt. More fish survived at 0–20 ppt than at 25 ppt ($\chi^2=42.8$, 1 df, $p<0.0001$) or 30 ppt ($\chi^2=79.0$, 1 df, $p<0.0001$); survivorship at 25 ppt was significantly greater than at 30 ppt ($\chi^2=7.1$, 1 df, $p<0.0001$). There were no significant differences between the rapid versus step-wise experiments; results were the same with and without acclimation. Thus, our findings did not reject null hypothesis B.

Long-term survivorship at salinity (to 4 months)

Overall survivorship of round gobies did not change among salinities 5–15 ppt across the course of the four-month experiment (Fig. 1C). Notably, 75% survived at 5 and 10 ppt, and 70% at 15 ppt. At 20 ppt, only 25% survived for 1 month, 10% at 2 months, and 5% for 4 months. Similar numbers of fish survived at 0 and 20 ppt, and

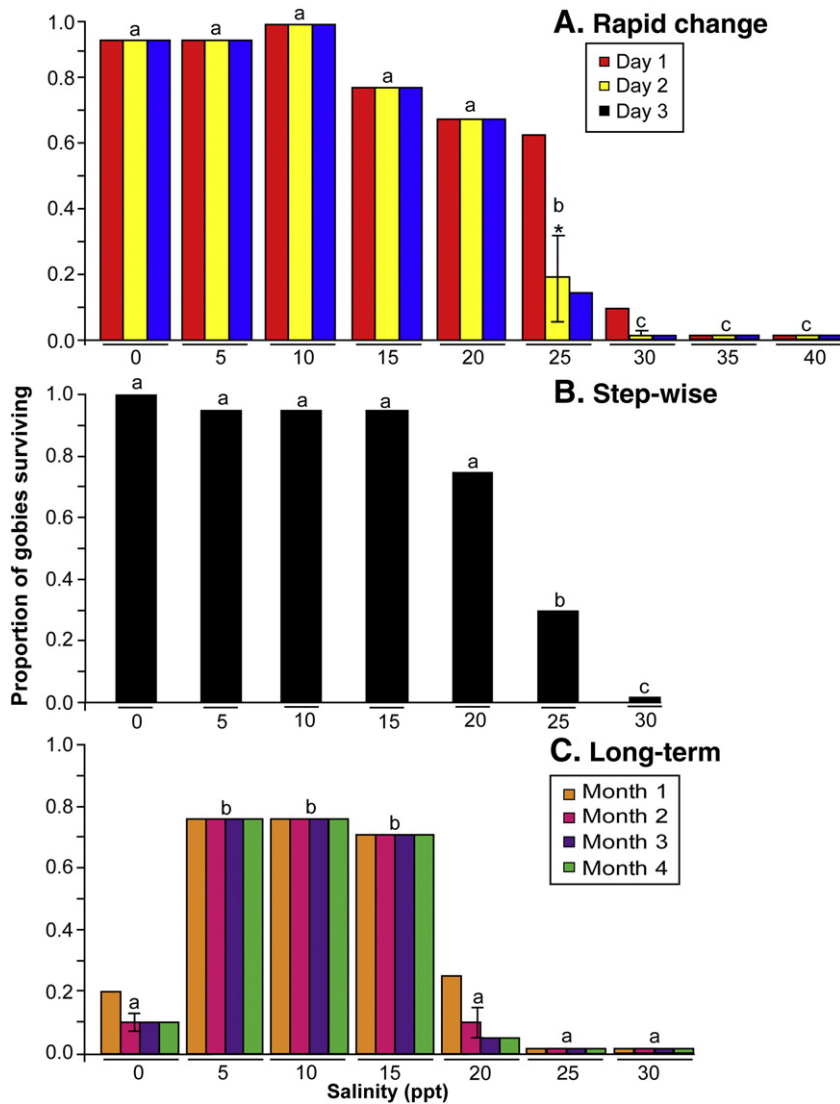


Fig. 1. Proportion of gobies surviving the three types of salinity change experiments: (A) rapid change salinity increases (measured over 1–3 days), (B) step-wise salinity increases (by 5 ppt every 3 days), and (C) long-term experiments at acclimated salinities (over 1–4 months). Salinity groups that statistically differed in survivorship are designated by different lower-case letters, those with identical letters did not differ (A: $\chi^2 = 95.1$, 2 df, $p < 0.0001$; B: $\chi^2 = 85.0$, 2 df, $p < 0.0001$; C: $\chi^2 = 89.4$, 1 df, $p < 0.0001$). Standard error bars depict variation across the time of the experiments (A: days, C: months); the sole significant difference (denoted by an asterisk) occurred in A at 25 ppt ($\chi^2 = 13.7$, 2 df, $p = 0.0002$), all others were not significant. No significant differences in survivorship occurred between the rapid (A) and step-wise experiments (B) at each salinity.

most died at ≥ 30 ppt. Tests showed significantly greater survival of fish at 5–15 ppt than those at 0 and 20 ppt ($\chi^2 = 82.4$, 1 df, $p < 0.0001$). The control fish (0 ppt) experienced greater mortality than did the experimental fish at 5–15 ppt ($\chi^2 = 16.2$, 1 df, $p < 0.0001$). Examination implicated a fungal infection that was not observed at other salinities. Long-term results thus rejected null hypothesis C, with survivorship differing among salinity treatments and being greatest at intermediate salinities of 5–15 ppt.

Ventilation frequencies at salinity

ANOVA showed that fish ventilation frequencies did not vary across salinities of 0–25 ppt during the rapid change experiments, shown in Fig. 2A. Fish at 30 ppt were omitted from these tests due to low survivorship and small sample size. In the step-wise salinity change experiments (Fig. 2B), no significant differences were discerned between salinities of 0 and 5 or between 10 and 15; results at 20 and 25 ppt did not differ from each other or from either group. Ventilation frequency at 0 and 5 ppt differed significantly from that recorded at 10 and 15 ppt ($t = 4.0$, 92 df, $p = 0.0002$),

with the latter being lower. Ventilation frequencies significantly differed between 10 ($t = 2.4$, 28 df, $p = 0.03$) and 15 ppt ($t = 3.1$, 27 df, $p = 0.005$). Results rejected null hypothesis D, with ventilation frequencies lowest at 10–15 ppt in acclimated gobies.

Long-term growth at salinity (to 4 months)

Fish size did not significantly differ among the salinity treatments (0–15 ppt) at the start of the experiment. Gobies at 20 ppt were excluded from the comparisons due to small sample size. After one month (Fig. 3), mean growth rates (mm, SL) over the duration of the experiment were similar among the salinities, although values at 15 ppt were somewhat lower. After four months, fish sizes significantly differed ($F = 24.8$, 3 df, $p < 0.0001$; Fig. 3). Gobies at 5 and 10 ppt grew significantly larger than those at 0 ppt ($t = 6.7$, 14 df, $p < 0.001$) and 15 ppt ($t = 4.4$, 9 df, $p = 0.02$). Growth at 15 ppt was less than at 0 ppt ($t = 3.6$, 10 df, $p = 0.005$). Results indicated that invasive round gobies from Lake Erie appeared to grow best in salinities of 5–10 ppt, rejecting null hypothesis E.

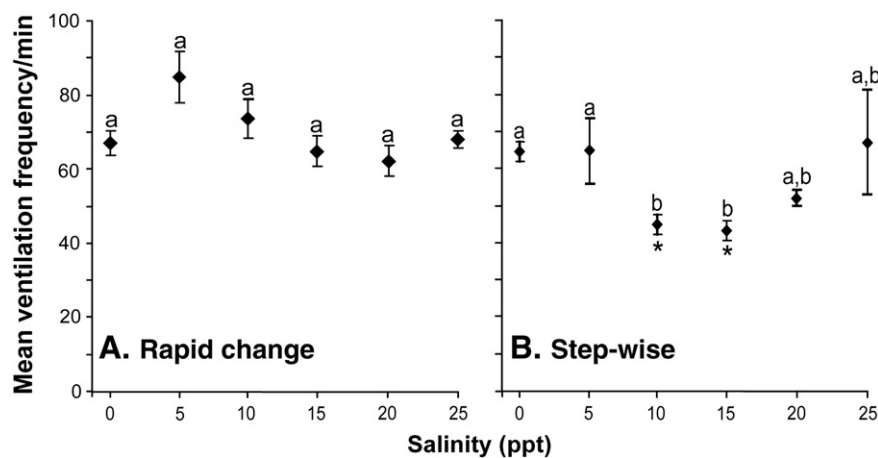


Fig. 2. Ventilation frequencies (as number of opercular beats/minute \pm s.e.) of round gobies at various salinities for: (A) Rapid salinity change experiments and (B) Gradual step-wise salinity increase experiments. Salinity groups whose frequencies significantly differed are designated by different lower-case letters ($t = 4.0$, 92 df, $p = 0.0002$); those with identical letters did not differ. Asterisks denote significant differences between the results of experiments A and B, with ventilation frequencies of gobies at 10 ($t = 2.4$, 28 df, $p = 0.03$) and 15 ppt ($t = 3.1$, 27 df, $p = 0.005$) being significantly less in experiment B.

Discussion

Wide salinity tolerance of the round goby

Results of the rapid salinity change experiment demonstrate that round gobies from the freshwater invasive population in Lake Erie survive appreciable salinities for a short time (up to 30 ppt for 24 h) but do not survive oceanic conditions (35 ppt). Similar results were found in step-wise salinity change experiments, with most fish surviving lower salinities (0–20 ppt) and dying in higher ones (70% mortality at 25 ppt, and 100% at 30 ppt). In their more limited short-term study, Ellis and MacIsaac (2009) likewise found that no round gobies survived 30 ppt. Our findings thus show that if ballast water exchange at sea is not complete – reaching 30 ppt or greater – some round gobies might survive.

The round goby has been reported from high salinities in the northern Caspian Sea's Kaidak Bay, which reaches ~40 ppt (Moskal'kova, 1996). Similarly, the round goby in the Aral Sea (introduced from the Caspian Sea) was one of the last fish species to survive as the water became hypersaline (Aladin, 1995), but eventually

became extirpated (Charlebois et al., 1997; Skora, 1996). Those most likely comprise different genotypes from the round gobies that colonized the Great Lakes, as Brown and Stepien (2008, 2009) discerned no Caspian Sea genotypes in North America. Round goby populations from the Black and Caspian Seas diverged by pronounced genetic differences (Brown and Stepien, 2008, 2009), and the two groups may differ in upper salinity tolerances and osmoregulatory capabilities. If a few round goby individuals temporarily survived higher salinities, their reproduction would be inhibited, since normal egg development ceases in Black Sea populations ≥ 19 ppt (Moskal'kova, 1996). Moreover, reproductive results in various salinities might differ among locally-adapted populations, meriting experimental testing.

Optimal salinity for long-term survival and growth of the round goby

Most of the round gobies we tested survived salinities to 15 ppt and grew best at 5–10 ppt. This salinity range appears to match conditions in the native northern Black Sea estuaries and drowned river valleys to which the Great Lakes' introduction has been genetically traced (Brown and Stepien, 2008, 2009). Our results showed higher mortality at 0 and 20 ppt than occurred at 5, 10, and 15 ppt; this was initially unanticipated because our samples were collected from 0 ppt in Lake Erie, whose population has existed for many years. Visual examination of the fish implicated a fungal infection that was confined to some of the 0 ppt tanks alone. Those control tanks were not adjacent to each other, and adjacent tanks at other salinities were not affected.

Round gobies that remained in our holding tank at 1 ppt survived the 4-month duration of our experiments, free of any signs of infection. This may have been due to their maintenance salinity at 1 ppt, which was designed to deter freshwater parasites and pathogens, and may have aided long-term survival. Several months after the experiment ended, other round gobies in our laboratory survived at 0 ppt for 6 months, then died overnight from a fungal infection that appeared identical to the experimental results here. We only observed the fungus at 0 ppt, and fish at ≥ 1 ppt appeared fungus-free. We note that aquarium mortalities at 0 ppt may not be indicative of overall goby survivorship in the Great Lakes.

Surveys of the round goby population from the Great Lakes Detroit River by MacInnis and Corkum (2000) found that numbers of age 2 individuals were only half those of age 1, and almost none were age 3. Age 4 and older round gobies have not been reported in the Great Lakes. In contrast, invasive round gobies found in brackish waters of the Baltic Sea (7–12 ppt; Omstedt and Axell, 1998) have been

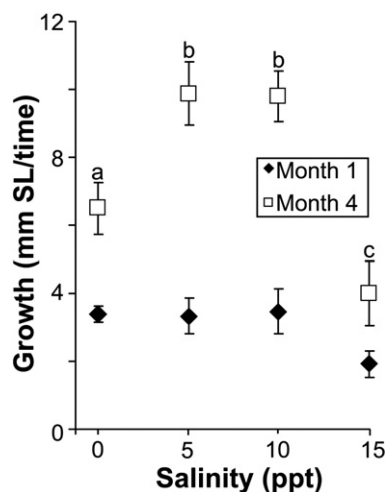


Fig. 3. Mean growth (mm, SL \pm s.e.) of round gobies at various salinities during long-term experiments, recorded at one month (diamonds) and four months (squares). Significant differences in growth are designated with different lower-case letters (a versus b: $t = 6.7$, 14 df, $p < 0.001$; a, c: $t = 3.6$, 10 df, $p = 0.005$; b, c: $t = 4.4$, 9 df, $p = 0.02$); those with identical letters did not differ. At month 1, no significant differences occurred. At month 4, gobies at 5–10 ppt were larger ($F = 24.8, 3$ df, $p < 0.0001$).

reported to reach larger sizes of 160–250 mm and live to 6 years (Sokolowska and Fey, 2011). The occurrences of larger sized gobies in brackish waters, together with our findings, suggest that isosmotic conditions may enhance round goby survivorship and growth. Although the round goby apparently does best at moderate salinities, its considerable physiological and ecological plasticity allows it to thrive in other habitats, including European freshwater tributaries and canals, and introductions to the Baltic Sea.

Laboratory osmoregulation studies of the euryhaline flounder *Paralichthys orbignyanus* from coastal estuarine waters of the south-western Atlantic Ocean discerned no overall survival differences in salinities from 0 to 40 ppt for 90 days; however, individuals at moderate salinities grew larger, which was attributed to lower energy expenditure for osmoregulation (Sampaio and Bianchini, 2002). Increased energy expenditure in freshwater conditions was associated with higher bronchial enzyme activity to maintain plasma osmoregulatory balance. Those findings appeared similar to our results, which showed increased growth of round gobies at 5–10 ppt, matching their native conditions in northern Black Sea estuaries. Optimal round goby growth occurred at lower salinities than found for the flounder, which likely reflects their differential upper salinity tolerances. Salinities of ~5–10 ppt coincide with the highest densities of round gobies observed in our sampling (by C.A.S. and colleagues) from native Black Sea estuaries, including the sources of the Great Lakes introductions (Brown and Stepien, 2008, 2009; Stepien and Tumeo, 2006; Stepien et al., 2005).

Laboratory stress tests of Mozambique tilapia (*Oreochromis mossambicus*) (Cataldi et al., 2005) and juvenile turbot (*Scophthalmus maximus*) (Van Ham et al., 2003) showed increased ionic concentrations in blood plasma at 35 ppt, no change at iso-osmotic salinity, and a loss of ions at 0 ppt. Measurements by Cataldi et al. (2005) also found increased blood cortisol levels, indicating stress, at 0 and 35 ppt as compared with iso-osmotic conditions. Physiological studies of round goby salinity acclimation would provide valuable comparison to those studies of other teleosts.

Laboratory experiments by Plaut (1998) involving two Mediterranean Sea congeners, the freshwater blenny *Salaria fluviatilis* and the marine *Salaria pavo*, revealed that the former has retained high salinity tolerance despite its geographic isolation from salt water for >1 Ma. In contrast, *S. pavo* showed less ability to osmoregulate in freshwater and did not maintain constant extracellular fluid concentration (Plaut, 1998). By comparison, the Great Lakes-adapted round goby appears to survive and grow best in intermediate salinities, despite the fact that it has readily expanded its populations throughout the freshwater Great Lakes. Other environmental variables and interactions, such as temperature, habitat, predators, parasites, pathogens, competitors, and prey also likely are important in the round goby's population success; the interplay among these factors remains to be evaluated.

Our results show that round gobies acclimated to 10–15 ppt had lower ventilation frequencies than did those at 0–5 ppt. This difference was not observed in the rapid salinity change experiments, which may have stressed fish at all salinities. Differences in results between the rapid and step-wise change experiments were attributed to acclimation effects in the latter. An initial rise in plasma sodium and chloride, and total osmolality, is typical of the early acclimation period of euryhaline fish to seawater (Bystrainsky et al., 2007; Gordon, 1959) and may underlie our experimental observations. During initial seawater acclimation, fish struggle to osmoregulate as they have not yet adapted to excrete excess salts from the plasma efficiently. Notably, plasma ion and osmolality levels in the Arctic char *Salvelinus alpinus* appeared to plateau between 48 and 96 h, suggesting acclimation to seawater and improved osmoregulatory capacity (Bystrainsky et al., 2007). We observed lower ventilation frequencies in the round goby at intermediate salinities following acclimation, as well as increased growth, indicating that ~5–10 ppt may represent the optimal salinity for Great Lakes-adapted round gobies.

Ballast water exchange and implications for population introductions

Ballast tanks contain various supports and partitions, leaving sections that may not flush efficiently or completely. On average, mid-ocean exchanges replace 88% (range 76–98%) of the water in ballast tanks, bringing tank salinity to 31–34.5 ppt (Ruiz and Smith, 2005). Ships unable to reach at least 30 ppt during ballast water exchange are prohibited from entering the U.S. (Ruiz and Reid, 2007). As shown by our data, the round goby can survive at 20 ppt for extended periods of time, with select individuals persisting for months, and some individuals survive 30 ppt for up to 24 h. Our study results indicate that ballast water exchange to ≥ 30 ppt for ≥ 24 h appears necessary to eliminate the possibility of introducing the round goby to other ecosystems. Concentrated effort towards achieving oceanic salinity in ballast tanks for an extended period thus appears important to avoid further spread and introductions of the round goby (and likely its congeners and relatives; see Stepien and Tumeo, 2006; Neilson and Stepien 2009, 2011).

Besides oceanic shipping, other possible vectors for round goby transfer include domestic water traffic (Lavoie et al., 1999) and “bait bucket” transport, as it is a popular baitfish that often is released by fishers (Jude, 2001). Domestic water traffic may be problematic because ships traveling from the Great Lakes to other U.S. freshwater ports are not required to exchange ballast water (Lavoie et al., 1999). Bait bucket transport poses a potentially serious risk for introductions to new areas where the round goby may thrive, including estuarine coastal environments.

Implications for round goby survival in North American brackish estuaries

The intermediate salinity tolerance of the round goby implies its likely success for colonizing coastal estuarine habitats. The round goby has not yet been found in North American brackish water habitats, but likely would do well if introduced. Many of those habitats contain abundant *Mytilus* mussel prey, which is one of the goby's preferred native prey items in the Black Sea (Stepien and Tumeo, 2006).

Similarly, the round goby's success in the Great Lakes has been attributed to the co-establishment of its native dreissenid mussel prey, constituting over 50% of the diet (Ghedotti et al., 1995; Ray and Corkum, 1997). This type of relationship, whereby an invader's success may have been enhanced by the presence of another coevolved invader from its native range, has been termed “ecological facilitation” (see Ricciardi, 2001; Simberloff and Von Holle, 1999).

Other factors that may enhance the round goby's adaptability and success in new habitats include its wide temperature tolerance from –1 to +30 C (Ilj'in, 1949; Moskal'kova, 1996), relative lack of parasites 20 years post-introduction supporting the “enemy release hypothesis” (Kvach and Stepien, 2008), high fecundity, and parental care (Corkum et al., 1998). The round goby's adaptedness to a broad spectrum of environmental conditions, including a wide realm of salinities and temperatures, likely underlies its invasive success in North America and Europe. Our results thus predict that, given the transport opportunity, the round goby likely will encounter suitable habitats across much of North America's estuarine coastlines, and that measures should be taken to avoid its transport.

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