

ORIGINAL ARTICLE

Adaptation as a potential response to sea-level rise: a genetic basis for salinity tolerance in populations of a coastal marsh fish

Kevin M. Purcell, Alan T. Hitch,* Paul L. Klerks and Paul L. Leberg

Department of Biology, University of Louisiana at Lafayette, Lafayette, LA, USA

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Correspondence

Kevin M. Purcell, Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504, USA. Tel.: +1 337 482 5823; fax: +1 337 482 5660; e-mail: kmp1063@louisiana.edu

*Present address: School of Forestry and Wildlife Sciences, Auburn University, 602 Duncan Dr, Auburn, AL 36849-5814, USA.

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Abstract

Relative sea-level rise is resulting in the intrusion of saline waters into marshes historically dominated by fresh water. Saltwater intrusions can potentially affect resident marsh species, especially when storm-related tidal surges cause rapid changes in salinity. We examined the role of historical salinity exposure on the survival of *Gambusia affinis* from two locations in coastal Louisiana. At each location, we sampled fish populations from fresh, intermediate and brackish marshes. Individuals were then exposed to a salinity of 25‰ and survival time was measured. We found that fish from brackish and intermediate marshes had an increased tolerance to salinity stress relative to fish from freshwater environments. We then tested the descendants of fish from the fresh and brackish marshes, reared for two generations in fresh water, to determine if there was a genetic basis for differential survival. We found that descendants of individuals from brackish marshes showed elevated survivals relative to the descendants of fish with no historical exposure to salinity. The most reasonable mechanism to account for the differences in survival relative to historical exposure is genetic adaptation, suggesting that natural selection may play a role in the responses of resident marsh fishes to future increases in salinity.

Introduction

The coastal marshes of Louisiana are experiencing sea-level rise at rates of approximately 10–11 mm/year; these rates are 10 times the global averages (Penland and Ramsey 1990; Gornitz 1995). During the last 50 years, Louisiana has lost coastal lands at a rate of over 130 km² per year. This results in stands of marsh vegetation separated by open water (Sasser et al. 1986; Rozas and Reed 1993). As marsh areas are converted to shallow open water areas, saline waters can penetrate farther up the estuary because there is less resistance from the marsh to up-estuary flows from tidal flux and storm surge.

Fish inhabiting these deteriorating coastal marshes may be especially susceptible to salinity stress when hurricanes and other storms push salt water into lower salinity marshes. Three nonexclusive mechanisms may govern the ability of fish to survive salinity spikes, or more perma-

nent salinity increases caused by sea-level rise and canalization, and thus maintain viable populations. First, coastal marsh residents may be euryhaline, having an ability to tolerate a wide salinity range. Second, acclimatization to brackish conditions may make fish less susceptible to salinity increases than fish acclimatized to fresh water. Finally, fish living in brackish conditions may acquire genetic adaptations through natural selection for higher individual salinity tolerance than fish from freshwater environments where there has been no selection for salinity tolerance.

Based on previous sampling, the most abundant fish species in coastal freshwater marshes of Louisiana is the Western mosquitofish *Gambusia affinis* (Baird & Girard, 1853). This marsh resident also occurs in brackish marshes, although in reduced numbers (Meffe and Snelson 1989). Because of the broad range of salinity across which this species occurs, we chose it as a model to

evaluate the impact of saltwater intrusion on coastal marsh fish populations. *Gambusia affinis* is a small, sexually dimorphic Poeciliid with a maximum size of 40–50 mm standard length; females are generally larger than males (Constantz 1989).

Salinity tolerance of *G. affinis* and its congener *Gambusia holbrooki* have been examined extensively in previous studies (Foskett et al. 1981; Chervinski 1983; Kandl and Thompson 1996; Nordlie and Mirandi 1996). These studies clearly show that *Gambusia* has a broad physiological tolerance for salinity and that laboratory acclimation to increased salinity is possible. There is also evidence that frequencies of allozyme genotypes differ between fresh and more saline conditions (Congdon 1994; Kandl and Thompson 1996), suggesting that genetic adaptation to different levels of salinity is possible. In spite of this large body of research, it is not clear whether field populations are adapted to increased salinity levels in coastal marshes, or if fish survival is more dependent on having a wide physiological tolerance. The latter would be an advantage in the dynamic environment of coastal marshes where salinities can change rapidly and unpredictably. Rapid changes in salinities would make acclimatization difficult and would be even less favorable to adaptation that might require many generations. The possibility that individuals may move across salinity gradients, resulting in gene flow, would further work against adaptation to localized salinities (Stearns and Sage 1980).

Our objective was to determine if populations of *G. affinis* with a history of exposure to brackish water would display a different level of survival relative to populations without a history of salinity exposure. In addition, we examined if the saltwater intrusion in coastal marshes could serve as a mechanism driving selection for genetic adaptations to increased salinity tolerance in a common marsh resident. The mechanisms through which fish tolerate increases in salinity have implications for the ability of populations to survive short-term changes in salinity due to storm surges, as well as longer term changes due to relative sea-level rise.

Methods and materials

Salinity varies temporally in coastal marshes; so, salinity measured at any point in time may not reflect long-term conditions at a site. We selected sample sites based on vegetation classification (Visser et al. 1998) because the different marsh types in the classification system reflect average salinity conditions that occurred in the recent past. Three marsh types were chosen for fish collections: fresh (0–1‰), intermediate (3–5‰) and brackish (8–12‰). Collections were made along a salinity gradient in Cameron Parish in south-western Louisiana and

another gradient in Terrebonne Parish in south-eastern Louisiana. These collection sites were located approximately 333 km apart in an attempt to see if the relationship between salinity tolerance and past exposure history were similar across space, or a function of local conditions.

Collected fish were returned to the laboratory where source populations were placed in separate 2800-L freshwater tanks (Spencer et al. 2000). All fish were acclimated to freshwater for a period of 1 week to ensure any effect due to past salinity exposure was not due to salinity experienced immediately prior to the experimental exposure (Shikano and Fujio 1998b).

From the acclimation tanks, we selected 100 individuals from each marsh type in both south-western and south-eastern Louisiana (total $n = 600$). For the exposure experiment, each of the 600 fish was placed in a separate 450-mL chamber. These chambers were large enough to prevent oxygen depletion or ammonia toxicity for at least 8 h (K. M. Purcell, unpublished data). A 50% water change was performed every 8 h during the study to prevent stress from anoxia or ammonia toxicity. All chambers were covered with screen and maintained at a temperature of 22.7°C.

The water in chambers of 70 individuals from each of the source populations was spiked with 40 ml of a concentrated artificial salt solution to increase the water salinity to 25‰. The other 30 individuals from each source population were held in control chambers and were spiked with equal volumes of fresh water. The exposure began following inoculation of all experimental chambers. Fatalities were counted at 30 min intervals and death was defined as a lack of opercular movement and no response to three contacts with a blunt instrument (Diamond et al. 1989). Dead individuals were removed from their chamber; time of death, sex and standard length were recorded.

To determine if there was a genetic basis for differences in salinity resistance based on past exposure, we evaluated tolerance using descendents of fish from fresh and brackish marshes from each location. Fish from the same fresh and brackish marshes sampled in the earlier trials were maintained in freshwater conditions and allowed to reproduce. Their descendents were reared in freshwater for a minimum two generations. Therefore, we were able to assess salinity resistance for individuals that had never experienced saline conditions and whose parents had never experienced such conditions. By using fish reared for two generations in a 'common garden', we prevented the effect of salinity acclimatization and the effect of maternal exposure to salinity (Shikano and Fujio 1998a,b), from influencing salinity resistance. Any differences in resistance associated with historical salinity

exposure would be best explained through adaptation of field populations to local conditions. The procedures of these salinity exposure trials were the same as those described earlier using fish sampled from the coastal marshes.

All statistical analyses were conducted using SAS software (SAS 2000). The null hypotheses were that there is no difference in the time to death of individuals collected from different salinities, or their descendents, when exposed to salinity stress. A proportional hazards model was chosen for these analyses because it is a robust, semi-parametric method that does not require data to fit a specific probability distribution (Allison 1995). We assessed the effects of sex and standard length of individuals on survival time as covariates in all trials. These covariates may be collinear, because females obtain greater lengths than males. Therefore, the effect of each covariate was assessed with and without the inclusion of the other covariate in the analysis. The PHREG procedure (Allison 1995) was used to determine the effects of source population, sex and length on survival time. The LIFETEST procedure (Allison 1995) was used to estimate the survival distribution function for each of the source populations.

Results

In the comparison of fish from Cameron Parish exposed to a salinity of 25‰, the survival time of individuals from freshwater marshes was significantly less than fish from marshes with a history of salinity exposure ($\chi_1^2 = 26.16$, $P < 0.0001$; Fig. 1). This difference appeared to be due to reduced survival times of fish from freshwater marshes; the survival times of fish from brackish and intermediate marshes were similar. The survival time increased proportionally with increases in the standard length of individuals ($\chi_1^2 = 7.38$, $P = 0.0066$; Fig. 2). For the Cameron Parish fish, sex did not have a significant effect on survival, regardless of whether standard length was included in the analysis.

For fish from Terrebonne Parish, survival time differed significantly among the three populations with different historical salinity exposure ($\chi_1^2 = 40.85$, $P < 0.0001$; Fig. 1). Individuals from freshwater marsh had the least salinity tolerance; those from saline marshes were the most tolerant (Fig. 1). In this comparison, the standard length of individuals was not found to have a significant effect on the survival time, regardless of whether sex was included in the analysis. Sex, however, was found to have a significant effect on survival time ($\chi_1^2 = 12.91$, $P = 0.0003$); females had a higher mean survival time than males (Fig. 2).

The descendents of fish from fresh and brackish marshes in Cameron Parish had significantly different survival times ($\chi_1^2 = 14.65$, $P < 0.0001$; Fig. 3). The descendents of indi-

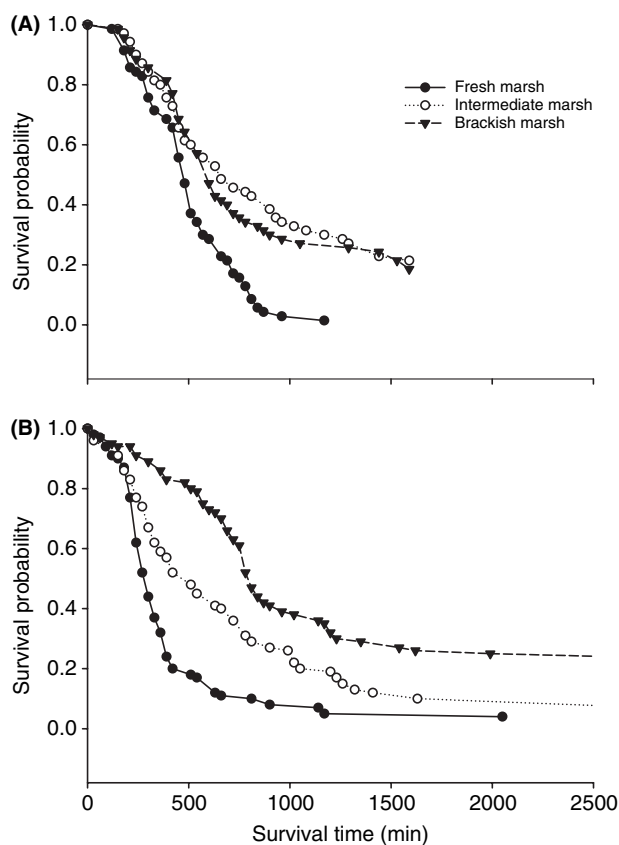


Figure 1 (A) Survival distribution functions for *Gambusia affinis*, collected from Cameron Parish in south-western coastal Louisiana, USA. Fish from three marsh types with different historical salinity regimes (fresh 0–1‰, intermediate 3–5‰ and brackish 8–12‰) were exposed to a salinity of 25‰. (B) Survival distribution functions for three populations of *Gambusia affinis* collected from Terrebonne parish in south-eastern coastal Louisiana, USA. Populations collected from three marsh types with similar historical salinity regimes (fresh 0–1‰, intermediate 3–5‰ and brackish 8–12‰) were exposed to a salinity of 25‰.

viduals from freshwater marsh again displayed reduced survival time relative to fish from brackish marshes when exposed to 25‰ (Fig. 3). As in the parental generation survival time was significantly affected by standard length ($\chi_1^2 = 11.04$, $P < 0.0009$). Increases in the standard length of individuals were positively related to increased survival under stress. The covariate sex did not have a significant impact on survival time in this trial regardless of the inclusion of other covariates.

When comparing the fish from the Terrebonne Parish sites, we found that there was a significant difference between individuals descended from the fresh and brackish populations when exposed to salinity stress ($\chi_1^2 = 29.65$, $P < 0.0001$; Fig. 3). The descendents of fish from the freshwater marsh had a considerably reduced survival time relative to those descended from fish from

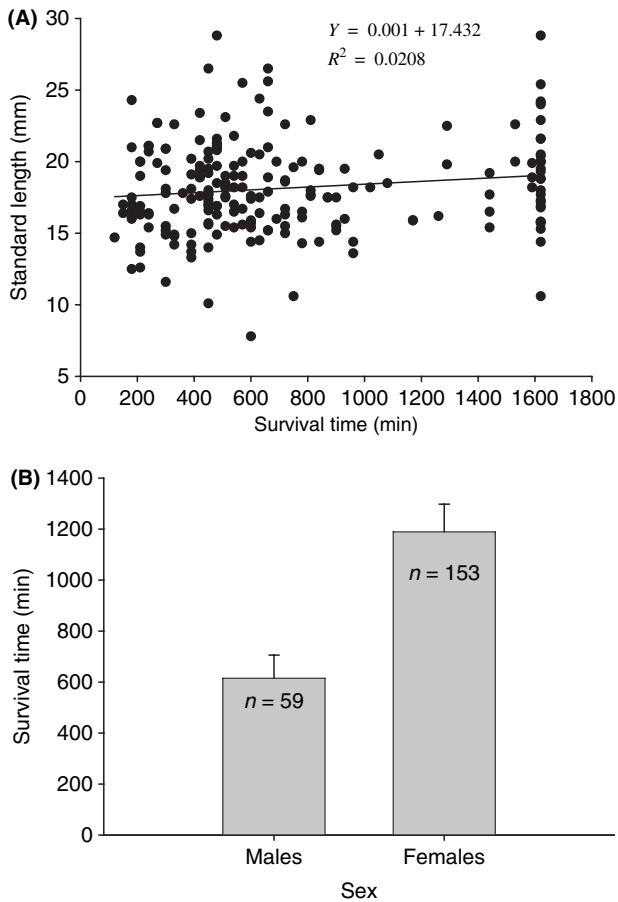


Figure 2 (A) Relationship between survival time and the standard length of *Gambusia affinis*, from the Cameron Parish populations, when exposed to a salinity of 25‰. (B) Comparison of the mean survival time of male and female *Gambusia*, from Terrebonne Parish, when exposed to a salinity of 25‰. Error bars represent the standard error.

brackish marsh (Fig. 3). In this trial, neither of the covariates had any significant effects on the survival time of individuals.

Discussion

Individuals from populations of *G. affinis* with historical exposure to salinity experienced increased survival during elevated salinities. Fish collected from brackish marsh habitats had significantly greater survival than those collected from habitats without historical salinity exposure when treated with a salinity stress of 25‰. Clearly, the wide range of salinity tolerance known to occur in marsh residents like *Gambusia* (Al-Daham and Bhatti 1977; Chervinski 1983; Meffe and Snellson 1989) is influenced strongly by historical salinity exposure.

There would appear to be only three possible explanations for this outcome in field-collected fish. First,

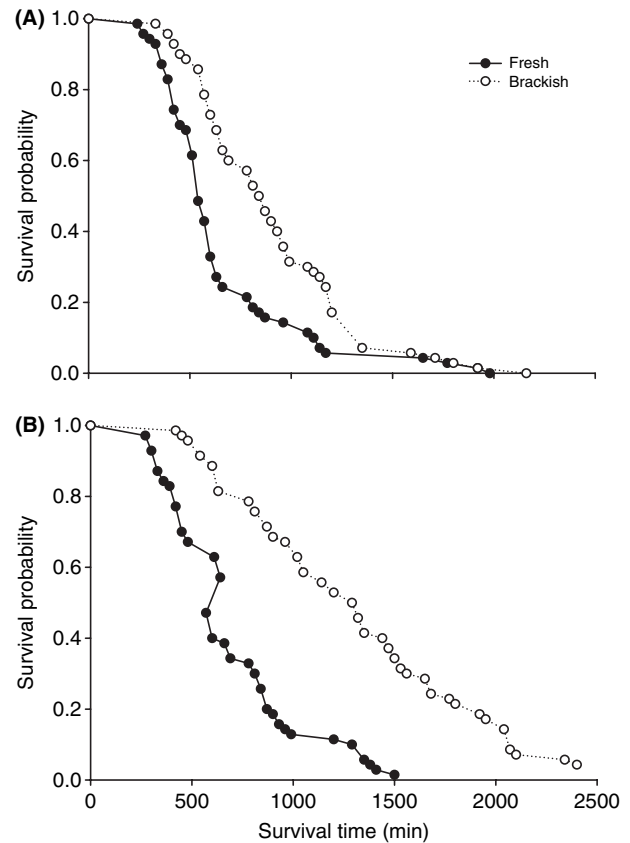


Figure 3 (A) Survival distribution functions for the descendants of *Gambusia affinis* collected from fresh and brackish environments in Cameron Parish in south-western Louisiana, USA. Fish of these marsh types were maintained in a freshwater environment for a minimum of two generations and were then exposed to a salinity of 25‰. (B) Survival distribution functions for the descendants of *Gambusia affinis* collected from fresh and brackish environments in Terrebonne Parish in south-eastern Louisiana, USA. Fish of these marsh types were maintained in a freshwater environment for a minimum of two generations and were then exposed to a salinity of 25‰.

individuals were acclimatized to the salinity of their capture site. Such acclimatization is typically thought to be reversible; so, exposing the fish to freshwater for a period of 1 week prior to experimental exposure should have minimized the effect of acclimatization. In another poeciliid, *Poecilia reticulata*, saltwater tolerance was the same for fish raised at high salinities (acclimatization) as for fish acclimated to high salinities for only a short time (Shikano and Fujio 1998a). Thus, if acclimatization is responsible for our result, it is less reversible in *Gambusia* than in other fish.

Second, it is possible that fish from the different marsh types varied in their sex or size composition that would result in differences in salinity tolerances. We attempted to control for this possibility in field-collected fish by including two covariates, sex and standard length, in our analyses. The sexual dimorphism of the model species *G. affinis*

would suggest that any relationship of these two variables with salinity tolerance would be collinear, with females being larger than males (Constantz 1989). We hypothesized that as an individual fish increases in size, its tolerance for saline environments also increases due mainly to its increased mass; similar relationships have been seen in other studies of *Gambusia* tolerance to stress (Chervinski 1983; Diamond et al. 1989; Nordlie and Mirandi 1996). The covariates had a different effect on survival time depending on the location of the collection. This result indicated that neither sex nor standard length could be used as a reliable estimator of survival following a salinity stress event. The only consistent factor across locations was that past exposure to salinity increased tolerance; the effects of exposure could not be explained by variation in size or sex structure of fish collected from the field sites.

A third possible explanation is that individuals collected from populations with a history of salinity exposure have adaptations that promote survival in high-salinity environments. Genetic adaptation to salinity tolerance has been documented in laboratory strains of *P. reticulata* (Shikano and Fujio 1998c; Shikano et al. 1998, 2000, 2001). Here, we use the term 'adaptation' to refer to a tolerance advantage with a genetic basis resulting from a population being exposed to elevated environmental salinities for several generations. The evidence for adaptations in salinity is not clear for natural populations of *Gambusia*. Studies of *G. holbrooki* under salinity stress showed differential survival among allozyme genotypes (Congdon 1994) and genotypic variation might be related to performance in mesocosms under salinity stress (Kandl and Thompson 1996); however, these studies did not demonstrate local adaptation to salinity levels. Stearns and Sage (1980) conclude that a population of *G. affinis* from a small freshwater stream might have been maladapted for those conditions because it apparently was heavily influenced by gene flow from fish from a large population in a brackish marsh. They saw no evidence that selection for local salinity levels had resulted in local adaptation and we are aware of no investigations of adaptation to localized salinity levels in *Gambusia*.

Our results indicate that individuals that are the descendents of populations with historical salinity exposure, reared in fresh water, are more resistant to salinity stress than the offspring of populations without historical exposure. This difference in salinity tolerance among individuals that had never experienced elevated salinity strongly suggest a genetic basis for the differences observed between fish from brackish and freshwater marshes. This genetic basis for increases in salinity tolerance to stress events suggests that at least some resident marsh fishes have the capacity to respond to saltwater intrusion through natural selection.

Assuming the presence of genetic variation, the most important factor-limiting development of local adaptation to salinity in coastal environments would be gene flow. Gene flow plays an important role in the contemporary evolution of populations (Stockwell et al. 2003); however, it also has the potential to prevent adaptation to localized conditions along gradients such as those existing in coastal marshes. Lenormand (2002) reviews of both theoretical and empirical studies demonstrating how gene flow into populations alters gene frequencies and can limit a population's ability for adaptation. Closer to our study, Stearns and Sage (1980) documented an example in which gene flow from a large population of *G. affinis* prevented a much smaller population from adapting to local salinity conditions. The populations in our study were separated by greater distances than those reported in Stearns and Sage, and were in some cases separated by physical barriers such as levees, weirs and open water with no aquatic vegetation. These barriers may have restricted gene flow sufficiently to allow for local adaptation in the *G. affinis* populations we examined. It is an open question as to whether gene flow is sufficiently high in other marsh residents to prevent local adaptation to increasing salinity.

The adaptation of populations in contemporary time frames to local conditions is of growing interest to ecologists (Stockwell et al. 2003; Bell et al. 2004; Streebman et al. 2004). It is interesting that we see evidence for a genetic basis for adaptations along two salinity gradients separated by 330 km, a distance that would require genes many generations to transverse. Furthermore, these sample sites are isolated by a major barrier, the Atchafalaya River. This large river would make direct gene flow between the two populations experiencing brackish conditions impossible; populations along the river would most likely be adapted to freshwater conditions. Although this freshwater barrier and great distance suggests that salinity tolerance may have evolved due to the local conditions of the sample sites, available evidence provides no insight into whether the adaptations to increased salinity are the same in these two coastal marshes. The degree to which populations can adapt to changing salinities, will dictate how coastal fish communities respond to both short-term phenomena like storm surges from hurricanes, as well as long-term changes due to relative sea-level rise.

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