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### Effects of Salinity on *Physa acuta*, the Intermediate Host for the Parasite *Posthodiplostomum minimum*: Implications for the Translocation of the Protected White Sands Pupfish

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ARTICLE

## Effects of Salinity on *Physa acuta*, the Intermediate Host for the Parasite *Posthodiplostomum minimum*: Implications for the Translocation of the Protected White Sands Pupfish

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### Abstract

We report the salinity tolerance of snails to evaluate how parasite communities with complex life cycles have been altered by translocations of the White Sands pupfish *Cyprinodon tularosa* to habitats with altered salinity levels. Native and introduced pupfish populations co-occur with the gastropods *Physa acuta* and associated white grub parasite, *Posthodiplostomum minimum*, at two brackish springs (Malpais and Mound), but physids are absent from the two saline habitats occupied by White Sands pupfish. We conducted a salinity challenge experiment to test the hypothesis that environmental salinity limits the distribution of physid snails. A 22-d survival experiment with the Malpais Spring physid population indicated that exposure to elevated salinities significantly reduced survival. We also saw sequential declines in survival and reproduction that were proportional to salinity exposure. Salinity of 7‰ was the apparent threshold for these effects. These results have implications for the use of translocation as a conservation tool in the management of fish populations.

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Translocations are widely used in the management and conservation of freshwater fishes (Williams et al. 1988; Minckley 1995; Harig et al. 2000; Stockwell and Leberg 2002; George et al. 2009), yet this species-centric management tool can have important ecological effects, such as altering parasite associations (Griffith et al. 1993; Cunningham 1996; Huspeni and Lafferty 2004). The persistence of parasite–host interactions can be particularly complicated for parasites with complex life cycles (Huspeni and Lafferty 2004; Miura et al. 2006). For instance, the distribution of *Myxobolus cerebralis*, which causes whirling disease in salmonids, is governed by the distribution and habitat requirements of the first intermediate host, the oligochaete *Tubifex tubifex* (Beauchamp et al. 2002; Dubey and Caldwell 2004; Krueger et al. 2006; Hallett and Bartholomew 2008). Similarly, digene trematodes require a first intermediate

host, a second intermediate host, and a definitive host, typically a gastropod, a fish, and a piscivorous bird, respectively (Hoffman 1999). Thus, ecological tolerances of the intermediate host species to environmental factors such as salinity or temperature can mediate the temporal or spatial distribution, or both, of parasites with complex life cycles (Janovy and Hardin 1983; Janovy et al. 1997; Rogowski and Stockwell 2006; Kupferberg et al. 2009).

Physid snails *Physa* spp. are ubiquitous freshwater snails and serve as the first intermediate host to a variety of digene parasites, such as the equally ubiquitous white grub fish parasite, *Posthodiplostomum minimum* (Hoffman 1999). Native and introduced populations of the New Mexico threatened White Sands pupfish *Cyprinodon tularosa* co-occur with *Physa acuta* and their associated white grub parasite *Posthodiplostomum*

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TABLE 1. Distribution of snails and associated parasites for native and introduced populations of White Sands pupfish.

Population <sup>a</sup>	History	Salinity range (‰)	Snail species	Endoparasites
Salt Creek	Native	15–80 <sup>b,c</sup>	<i>Juturnia tularosae</i> <sup>c,d</sup>	<i>Ascocoytl</i> sp.-1 <sup>c,e</sup> <i>Ascocoytl</i> sp.-2 <sup>c,e</sup>
Lost River	Introduced from Salt Creek	13.5–77 <sup>b,f</sup>	NONE <sup>e,g</sup>	NONE <sup>e,g,h</sup>
Mound Spring	Introduced from Salt Creek	1.5–4 <sup>b,g,h</sup>	<i>Physa acuta</i> <sup>g,h</sup>	<i>Posthodiplostomum minimum</i> <sup>e,g,h</sup>
Malpais Spring	Native	3–15 <sup>b,g,h</sup>	<i>Physa acuta</i> <sup>g,h,i</sup>	<i>Posthodiplostomum minimum</i> <sup>e,g,h</sup>

<sup>a</sup>The White Sands pupfish comprises two evolutionarily significant units (ESUs), the Malpais Spring and Salt Creek ESUs. The latter includes the populations derived from Salt Creek at Lost River and Mound Spring (Stockwell et al. 1998).

<sup>b</sup>Stockwell and Mulvey (1998).

<sup>c</sup>Rogowski and Stockwell (2006).

<sup>d</sup>Hershler et al. (2002).

<sup>e</sup>C. A. Stockwell, M. L. Collyer, and J. Janovy, unpublished data.

<sup>f</sup>Rogowski et al. (2006).

<sup>g</sup>Collyer (2000).

<sup>h</sup>Collyer and Stockwell (2004).

<sup>i</sup>Brian Lang, New Mexico Department of Game and Fish, personal communication.

*minimum* at two brackish springs: Malpais Spring and Mound Spring. Notably, physids are absent from the two saline habitats (Salt Creek and Lost River) that are occupied by White Sand pupfish, and thus these populations are not exposed to parasitism by *P. minimum*. This variance in parasite risk is of particular interest because experimental evidence has shown *P. minimum* to be costly to pupfish in terms of survival, fat storage, growth, and altered morphology (Harstad 2003; Collyer and Stockwell 2004).

Limited information exists regarding the salinity tolerance of physids (Kefford and Nuggeoda 2005; Zaluzniak et al. 2009). Here, we describe the salinity tolerance of *Physa acuta* and synthesize these findings with both published and previously unreported parasite data to examine how historic translocations of the White Sands pupfish among divergent habitats have resulted in altered parasite–host associations for this protected fish species.

## METHODS

**Background and summary of parasite–host relationships.**—White Sands pupfish occur in both native and introduced populations that occupy a variety of habitats in terms of environmental salinity (1.5‰ to 80‰; Stockwell and Mulvey 1998; Collyer and Stockwell 2004; Rogowski et al. 2006). Physid snails only occur at brackish sites with relatively low salinity (1.5–3.5‰) such as Malpais Spring, which also harbors one of the two native populations of White Sands pupfish. The other native population of White Sands pupfish occupies Salt Creek and co-occurs with a different gastropod, the endemic hydrobiid snail, *Juturnia tularosae*, and two associated heterophyid parasites: *Ascocoytl* sp.-1 and *Ascocoytl* sp.-2. (Hershler et al. 2002; Rogowski and Stockwell 2006).

Historic translocations that occurred circa 1970 resulted in populations derived from Salt Creek at brackish Mound Spring

(3.0‰) and saline Lost River (13.5–77‰; for population history, see Stockwell et al. 1998 and Pittenger and Springer 1999). Snails are not present in Lost River and thus White Sands pupfish are not parasitized by digene trematodes (Table 1). At Mound Spring, White Sands pupfish co-occur with *P. acuta* (Table 1). Thus, physids co-occur with White Sands pupfish in the brackish springs (native in Malpais Spring and introduced in Mound Spring), but are absent from the saline creeks occupied by White Sands pupfish (native in Salt Creek and introduced in Lost River; Table 1).

**Salinity challenge experiment.**—A pilot study showed that the effects of salinity on survival did not vary among individuals from the physid populations at Mound Spring and Malpais Spring. Here, we report the results of an experiment which used *P. acuta* collected from Malpais Spring to evaluate the effect of environmental salinity on snail survival and reproduction by exposing individuals to osmotic stress for a period of 22 d at the following salinity levels: 3.5, 5, 7, and 9‰. Stock solutions for each salinity level were made with Instant Ocean dissolved in commercially available dechlorinated water. A conductivity meter was used to determine when the targeted salinity levels were reached. Instant Ocean has a similar ionic composition to that observed at Salt Creek in which both are dominated by sodium (~80% of cations) and chloride (69–87% of anions), making it a reasonable proxy for a site where physids are not observed. We also used a control treatment in which snails were exposed to water from Malpais Spring (3.5‰). The stock solution was subsequently divided into aliquot portions of 150-mL that were placed into each of 40 plastic containers for each treatment. The experiment was conducted at a room temperature of approximately 25°C.

Our experiments employed a biologically relevant design whereby snails were spontaneously introduced to arbitrarily selected salinity levels, mimicking the way by which snails can be spontaneously introduced to saline habitats by aquatic birds

(Boag 1986) or through human activities. Owing to the duration of the experiment, snails were provided with fresh iceberg lettuce for food, which was replaced daily. Water was replaced as necessary based on visual evaluation of water quality. Snails were inspected for death at 12-h intervals for a period of 96 h. Death was determined in two ways: (1) the snail was upside down and withdrawn into the shell, and (2) the snail did not respond to probing (Morley et al. 2004; Bernot et al. 2005). Egg masses were visually inspected and the number of eggs in each mass counted. To control for differential survival among treatments, reproductive effort was measured as the number of eggs produced per day.

**Statistical analyses.**—A proportional hazards model was used to evaluate the null hypothesis that survivorship of physid populations was independent of osmotic stress. We chose the proportional hazards model because it is a robust, semiparametric method that does not require the data to fit a specific probability distribution (Cox 1972; Allison 1995). All statistical analyses were conducted in SAS version 9.5 (SAS Institute 2007). We used the PHREG procedure to determine treatment effects on survival time. The LIFETEST procedure was used to estimate the survival distribution function for each salinity treatment level. We evaluated the effect of salinity treatment on the reproductive effort with an analysis of variance (ANOVA). Post hoc pairwise treatment comparisons were made while maintaining an experimental-wise error rate ( $\alpha$ ) at 0.05.

## RESULTS

There were no significant differences between the control and the 3.5‰ treatment levels, suggesting that Instant Ocean provided a suitable ionic comparison for this system. Exposure to elevated salinities significantly reduced survival ( $\chi^2 = 48.89$ ,  $df = 4$ ,  $P < 0.0001$ ; Figure 1).

We found no significant differences between the control and 3.5‰ treatment levels or between the 5‰ and 7‰ treatment levels. Therefore, we reanalyzed the data set using these three treatment levels: low salinity (control and 3.5‰), moderate salinity (5‰ and 7‰), and high salinity (9‰), and again found a highly significant ( $\chi^2 = 77.82$ ,  $df = 2$ ,  $P < 0.001$ ) effect of salinity on survival. Significant differences were observed between the low and moderate salinity treatments ( $\chi^2 = 48.89$ ,  $df = 1$ ,  $P < 0.001$ ) and between the moderate and high salinity treatments ( $\chi^2 = 65.28$ ,  $df = 1$ ,  $P < 0.001$ ; Figure 1).

In addition, we found that there was a significant effect ( $F = 7.93$ ,  $df = 4$ ,  $P < 0.001$ ; Figure 2) of salinity on reproduction. A series of post hoc contrasts showed no significant differences in reproductive effort between the control, 3.5‰, and 5.0‰ treatments (Figure 2). The 7.0‰ treatment group was significantly different from the 3.5‰ ( $F_1 = 13.23$ ,  $P < 0.001$ ) and 5.0‰ ( $F_1 = 8.06$ ,  $P = 0.005$ ) treatments; however, it did not differ significantly from the control and 9.0‰ treatment groups. No individuals in the 9.0‰ treatment group reproduced. In general, we observed decreased reproduction with increased osmotic stress.

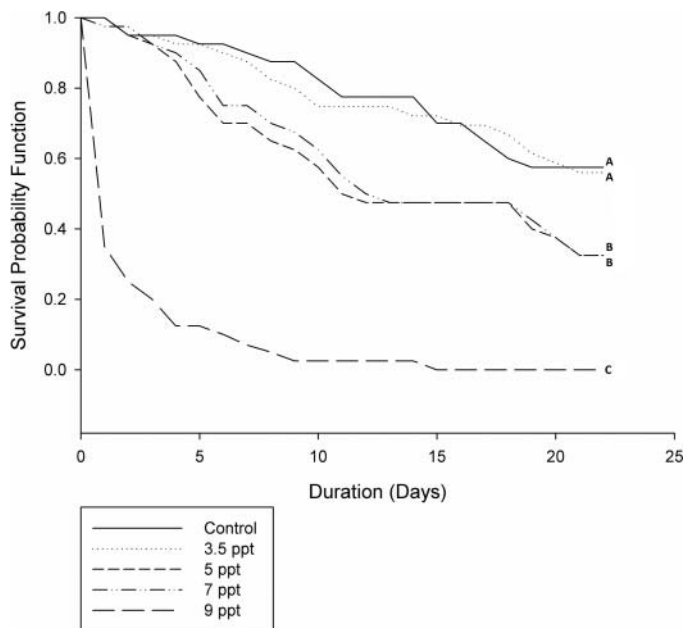


FIGURE 1. Survival probability function for a 22-d salinity challenge experiment with *Physa acuta* from Malpais Spring. Snails were exposed to control conditions (Malpais Spring water, 3.5‰) or one of four salinity treatment solutions: 3.5, 5, 7, and 9‰. Treatments labeled with the same letter did not differ significantly.

## DISCUSSION

Here we have shown *P. acuta* is sensitive to salinity, which is consistent with its absence from the saline habitats occupied by White Sands pupfish. We observed a decline in survival and reproduction at 7‰ salinity and snails did not survive at 9‰. Our experiment provided limited insights as to the importance of ionic composition (but see Zaluzniak et al. 2009). Snail survival and reproduction did not differ between the control (Malpais

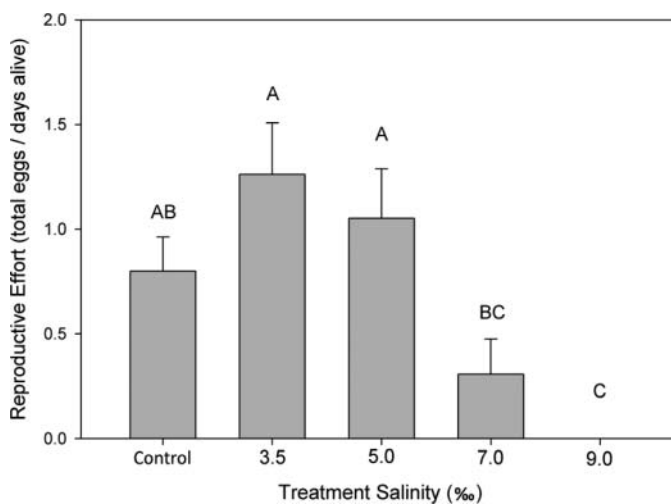


FIGURE 2. Effects of salinity on the reproduction of *Physa acuta*. Reproductive effort was measured as the number of eggs produced per day to control for differential survival. Treatments with the same letter did not differ significantly.

Spring water) and the 3.5‰ treatment, which only differed in ionic composition. The cationic and anionic composition of Instant Ocean is dominated by sodium (83% of cations) and chloride (87% of anions), which is similar to the ionic composition observed at Salt Creek (sodium, 70–85%; chloride, 65–80%) (Carmen 2009).

Thus, our results suggest that salinity is one of the factors that govern the distribution of physid snails in our study system. It is notable that within our study system physid snails only occupy the springs where salinities are typically between 3‰ and 3.5‰. This has important implications for pupfish, because pupfish are euryhaline and thus can occupy habitats unavailable to physid snails and their associated parasites.

Understanding the factors that affect the distribution of parasites is of interest because both field and experimental evidence indicates that parasitism by *Posthodiplostomum minimum* is costly to White Sands pupfish. Evidence of parasitism at both sites shows seasonal patterns in intensity suggesting that parasites may contribute to reduced overwinter survival (Collyer and Stockwell 2004). Further, experimental work showed *P. minimum* to be costly to White Sands pupfish in terms of survival, fat storage, growth, and altered morphology (Harstad 2003; Collyer and Stockwell 2004). Finally, an apparent decline of the Mound Spring White Sands pupfish population in 1995 was associated with a high level of *P. minimum* parasitism (John Pittenger, Blue Earth Ecological Consultants, personal communication).

Our findings are most important when evaluated in the context of how translocations can alter parasite communities. In our study system, translocations altered the parasite community owing to the presence–absence of snails in the new habitat. The ancestral population at Salt Creek co-occurs with the hydrobiid snail, *J. tularosae*, and is exposed to parasitism by heterophyid parasites (Hershler et al. 2002; Rogowski and Stockwell 2006). The Salt Creek-derived pupfish population at Mound Spring has not been parasitized by heterophyid parasites, but now co-occurs with *Physa acuta* and is thus exposed to parasitism by *Posthodiplostomum minimum*. The Lost River White Sands pupfish population, which was also derived from the Salt Creek population, has escaped exposure to parasites with complex life cycles altogether, owing to the absence of snails at this site.

As species are translocated to new habitats, they may face altered parasite communities mediated by the presence/absence of intermediate hosts such as physid snails. These findings are likely to generalize to other parasite–host study systems. For instance, parasitism in the plains killifish *Fundulus zebrinus* is mediated by the temporal variation in physid abundance, which in turn is apparently governed by flow conditions (Janovy and Hardin 1983; Janovy et al. 1997). Finally, the spatial distribution of whirling disease in salmonids appears to be partially determined by the distribution and abundance of *T. tubifex*, the alternate host for *M. cerebralis* (Dubey and Caldwell 2004; Krueger et al. 2006).

Our work suggests that translocation plans should take into account the potential for a translocation to alter historic

parasite–host associations. Such effects may be predicted if the local fauna is well characterized. Our findings take on more relevance in the face of climate change as altered parasite communities are one possible consequence of managers assisting in the colonization of species (Hoegh-Guldberg et al. 2008) to new habitats.

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