

David L. Rogowski · Craig A. Stockwell

## Parasites and salinity: costly tradeoffs in a threatened species

Received: 24 November 2004 / Accepted: 12 July 2005  
© Springer-Verlag 2005

**Abstract** Parasites and environmental conditions can have direct and indirect effects on individuals. We explore the relationship between salinity and parasites in an endemic New Mexico State threatened fish, the White Sands pupfish (*Cyprinodon tularosa*). Spatial variation in salinity limits the distribution of the endemic springsnail (*Juturnia tularosae*) within Salt Creek, a small desert stream. The springsnail is the presumed intermediate host for trematodes that infect the White Sands pupfish, and trematode prevalence and intensity in pupfish are positively associated with the springsnail. Salinity and parasites both have negative impacts on pupfish, but in areas of high salinity, pupfish can effectively escape parasites. Pupfish trematodes were absent from sites lacking snails. At the upstream site, the absence of parasites and lower variance in salinity were correlated with larger pupfish that were in better condition than pupfish at either the middle or lower sites. Springsnails were present in the middle section, an area with moderate salinity, and all pupfish had trematodes (median abundance 847 trematodes/fish). Lipid levels and condition were lowest in fish from the middle site. Additionally, fewer older fish indicated an increased mortality rate. At the lower site, springsnails were absent due to high salinity; pupfish trematode abundance was much lower (six trematodes/fish), and fish condition was intermediate. An additional experiment revealed that snail activity and survival were significantly reduced at

high salinities commonly present at the lower site. Although both high salinity and parasites significantly affect pupfish, parasites might be more detrimental.

**Keywords** Salinity · Trematodes · *Cyprinodon tularosa* · *Juturnia tularosae* · Lipids

### Introduction

Threatened and endangered species are exposed to a number of threats, primarily from anthropogenic impacts such as habitat loss and invasive species (Wilson 1992; Wilcove et al. 1998), and they also face natural threats due to environmental factors such as parasites and disease (McCallum and Dobson 1995). This is especially true for desert aquatic systems where threatened or endangered species are particularly at risk due to high environmental variation (Williams et al. 1985; Sheldon 1988). For instance, desert streams show considerable spatial and temporal variation in temperature, salinity, and flow (Miller 1948, 1981; Meffe and Minckley 1997; Stockwell and Mulvey 1998) which, in turn, limit the distribution of resident organisms (Por 1980; Herbst 2001). In fact, communities are often structured around salinity gradients (Hylleberg 1986; Barnes 1999; Williams 1998; Wolfram 1999; Costil et al. 2001). Despite low biodiversity and the high metabolic costs associated with high salinity (Withers 1992), these systems can be highly productive and organisms able to tolerate extreme salinities are frequently very abundant (Cole 1994).

The pupfishes (Cyprinodonts) are well known for their environmental tolerance (Brown and Feldmeth 1971), having been recorded in waters with salinities of up to four times that of seawater and occurring at temperatures above 40°C (Miller 1948; Barlow 1958; Bennett and Beitinger 1997; Beitinger et al. 2000). They are poor competitors, however, and in areas where they co-occur with other species, pupfishes are often limited to areas of high salinity (Echelle et al. 1972; Martin

---

Communicated by Carla Caceres

D. L. Rogowski · C. A. Stockwell  
Department of Biological Sciences, North Dakota State University,  
Fargo, ND 58105-5517, USA

*Present address:* D. L. Rogowski (✉)  
Center for Aquatic Ecology and Conservation,  
Illinois Natural History Survey, 607 E. Peabody Dr.,  
Champaign, IL 61820, USA  
E-mail: drogowski@uiuc.edu  
Tel.: +1-217-2445123  
Fax: +1-217-3336294

1972; Kodric-Brown and Mazzolini, 1992). High salinity may also structure communities such that pupfishes escape parasitism. For instance, complex life cycle parasites will be excluded from systems where any of the appropriate hosts are absent (Lafferty and Kuris 1999). Many fish are infected with larval trematodes, which depend on the presence of three different hosts: snails, fish, and fish-eating birds (Hoffman 1999). Factors limiting snail distributions, therefore, can indirectly structure fish parasite communities. Snail communities are often structured around salinity (Hylleberg 1986; Barnes 1999; Berger and Gorbushin 2001; Costil et al. 2001), thus the salinity tolerance of snails can indirectly determine the prevalence and intensity of trematodes in pupfish. High salinity can reduce the costs of competition and parasitism for pupfish, but there is evidence of higher physiological costs at elevated salinities (Kinne 1960; Haney et al. 1999).

We report on the interactions of the New Mexico state threatened White Sands pupfish (*Cyprinodon tularosa*), pupfish trematodes, a springsnail (*Juturnia tularosae*), and salinity in a desert creek. Within Salt Creek there is spatial and temporal variance in salinity, as well as spatial variance in the prevalence and intensity of pupfish parasites. These two factors (parasites and salinity) can both be detrimental to pupfish. However, in areas with high salinity, pupfishes have few parasites. We tested the hypotheses that parasites are detrimental to pupfish, and that environmental salinity limits the distribution of the springsnail, which, in turn, limits the distribution of trematodes. This limitation results in a considerable spatial variation in parasitism for the second intermediate host, the White Sands pupfish. Further, we show differences in life history traits among sites that are related to parasite intensity.

---

## Background

The White Sands pupfish is a state threatened species endemic to the Tularosa Basin of southern New Mexico and occurs in four isolated desert aquatic systems (Miller and Echelle 1975; Pittenger and Springer 1999). Fish trematodes and their associated intermediate snail hosts differ among these systems (Stockwell 2002; Rogowski 2004). Salt Creek is a saline riverine system with spatial and temporal variation in salinity and temperature, with levels of both increasing with distance from the springheads (Stockwell and Mulvey 1998, Rogowski 2004). Salinity ranges from 7 to >88 (limit of our instrument) with specific conductance values of 12.7–119.3 mS/cm (Rogowski 2004). Water temperatures range from 2.7 to 37.0°C. Salt Creek can be divided into three segments (upper, middle, and lower) based on two barriers that block upstream fish movement, one, a natural waterfall about 2 m in height, and the other a road crossing with associated culvert, about 0.5–1 m in

height. Neither one restricts fish movement downstream. The fish may be able to move upstream past the culvert in a side channel during and subsequent to high water events (J. Pittenger, NMDGF, personal communication).

The White Sands pupfish is the only fish present in Salt Creek. While the pupfish occurs throughout Salt Creek, an endemic springsnail occurs only in a limited section (*J. tularosae*, Hershler et al. 2002). Pupfish, as the White Sands pupfish is infected with two trematodes, provisionally identified as *Ascocoytl* sp. (Bill Font, personal communication), one that infects gill tissues, and other that infects mesenteric and liver tissues. These two trematodes are hereafter referred to as gill and body cavity trematodes, respectively.

Trematode life cycles can be divided into three stages, each requiring a different host (snail, fish, and bird) (Hoffman 1999). Trematodes generally reach sexual maturity in piscivorous birds and their eggs are released in the host's feces. Intermediate snail hosts become infected either by ingesting the eggs, or by free-swimming miracidia (after hatching) that seek out snails to infect. Once inside a snail host, larval trematodes reproduce asexually and periodically release cercariae from the snail. Cercariae are free-swimming larvae that seek out a second intermediate host, usually a fish. Cercariae transform and encyst within fish, becoming metacercaria, a quiescent stage between the cercaria and adult forms (Hoffman 1999). Larval trematodes reside as metacercaria in fish until eaten by a bird, thus completing the life cycle.

---

## Materials and methods

### Salinity and temperature

Salt Creek is located in a remote area of the White Sands Missile Range, a controlled military installation with limited access. Salinity measurements were compiled from data collected from 1998 to 2002 by Craig A. Stockwell and associates at North Dakota State University (Rogowski 2004). A portable salinity instrument (Yellow Springs Instrument-YSI 85) was used to measure salinity, conductivity and temperature. Salinity measurements are reported in the practical salinity scale, a unit-less number based on a conversion from electrical conductivity readings (Clescerl et al. 1999). Salinity measurements were collected at numerous times throughout the year, with the majority obtained from May to August. Minimum salinity at our sampling sites is not known since measurements were not recorded immediately following rain events. However, we do believe we have an adequate representation of maximum salinity, as sampling often occurred when flows were low or nonexistent. At each site automatic data loggers recorded temperature every 4 h from April 25 to August 13, 2001.

## Pupfish collections and parasite enumerations

Pupfishes were collected from three sites (upper, middle and lower) in Salt Creek in July, and August in 2000, and in June and July 2001. Fish were collected using three unbaited minnow traps (3 mm mesh size) per site, set during daylight for at least 2 h. A minimum of 175 fishes were sexed and measured per sample each month. Subsets of female fish of a range of sizes (20–54 mm standard length) were retained for dissection and parasite enumerations. Due to logistical difficulties and time constraints fish were not collected from the upper site in 2001. Wet mass of fish was determined to the nearest 0.01 g using a digital balance, and standard length was measured to the nearest 0.01 mm using digital calipers. A relative condition factor ( $\alpha$ ) was determined for all fishes in Salt Creek using a nonlinear allometric growth model ( $\text{mass} = \alpha(\text{length})^\beta$ ) (Quinn and Deriso 1999).

Dry mass was obtained after drying fish at 56°C for 36 h, followed by 24 h in a dessicator. Water content was determined by subtracting dry mass from wet mass. Lipids were extracted from somatic tissue using anhydrous ether. Fish carcasses were soaked in ether for 24 h and replaced with fresh ether for another 24 h. Carcasses were subsequently dried for another 24 h before weighing. Lipid and percent water results were arcsin-transformed prior to statistical analyses.

Fish age was determined by scale annuli. Fish without annuli were designated age 0. Fish were examined for macroparasites in gills, eyes, liver, and body cavity tissues using a stereo dissecting scope at a magnification of 40×. Tissues were removed, wet mounted on a slide, pressed, and examined for parasites. Parasite terminology follows that of Bush et al. (1997), with parasite prevalence reflecting the percentage of fish infected with at least one parasite. Intensity refers to the number of parasites within an infected fish, and median abundance refers to the median number of parasites within fish across all individuals, whether infected or not (Bush et al. 1997).

## Snail tolerance experiment

Approximately 400 snails, ranging in size from 0.93 to 3.99 mm, were collected on 22 June 2001 from a small plunge pool in the middle section of Salt Creek. Snails were maintained in a clear plastic container with native vegetation for 2 days prior to the start of the experiment to acclimate to lab conditions. The tolerance experiment was conducted from 24 June 2001 to 15 July 2001. A 16 h:8 h light–dark cycle was maintained and air temperature ranged from 22 to 31°C.

Experimental water was collected directly from Salt Creek at two locations: snail collection site (salinity of 28), and at a section of the lower stream area (salinity  $\geq$  80). Salt Creek water was diluted with distilled water to create experimental salinity treatments of 5, 15, 25, 35, 45, and 55. No contaminants of concern have been detected in Salt Creek (Ortiz et al. 1999 2000).

Twenty-four 12-well plates (maximum volume 10 ml) were used for the experiment. Each plate was randomly assigned a salinity treatment. Water from the snail collection site was placed in each well 48 h prior to the start of the experiment to allow periphyton establishment. Forty-eight snails were used per treatment ( $N=288$ ), and the snails were haphazardly selected from the holding container and placed into individual wells. No supplemental food was provided during the experiment. Water was replaced every 48 h, using the same salinity dilution batch.

Three variables were measured: activity, mortality, and reproduction. Snail activity was scored once a day during daylight hours under a dissecting scope (40×), following Hurst (1927) (Table 1). Individual wells were also examined daily for the presence of cercaria. Mortality was confirmed by the loss of the operculum and/or a swarm of microorganisms surrounding and feeding on the snail. At the end of the experiment the number of offspring per snail was recorded.

## Statistical analyses

Salinity differences among sites were examined using a randomization technique, as sample sizes were unequal and not all were collected at the same time (Manly 1997). We used specific conductance instead of salinity for analyses as salinity exceeded the maximum detection capability of our instrument. We conducted comparisons of the mean difference in specific conductance between sites. Observed values were randomly shuffled without replacement among sites keeping sample size constant for 9,999 iterations. A difference value was generated for each iteration, creating a distribution of potential differences. Our observed difference was compared to the probability distribution generated from the randomization procedure to obtain a probability value.

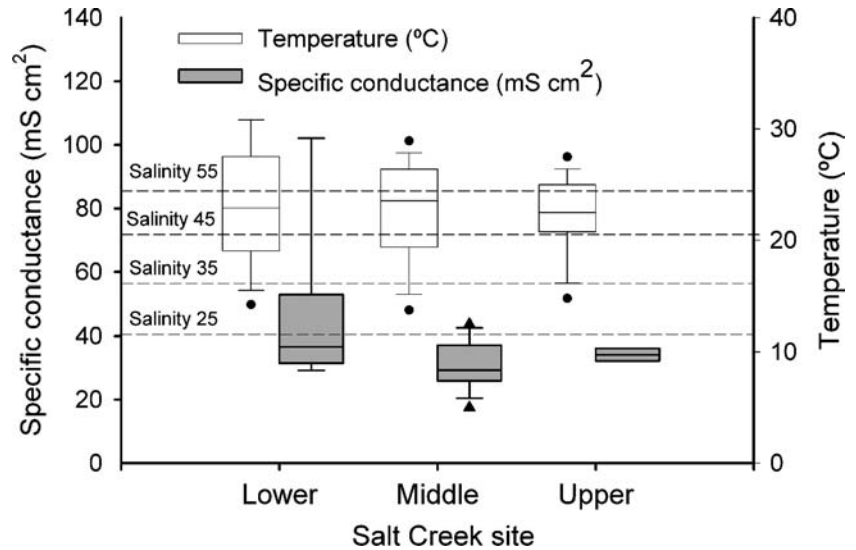
As temperature measurements were correlated temporally and spatially, a paired approach was used for investigating differences among sites. Sites were paired to account for non-independence of samples, and differences in temperatures were calculated to determine if there were significant deviations from a mean of zero.

Length comparisons were conducted separately from life history analyses as these data were collected separately. To maintain relatively equal sample sizes to meet

**Table 1** Snail (*Juturnia tularosae*) activity scores based on work by Hurst (1927)

Score	Activity
0	Dead
1	Snail inactive, operculum completely closed
2	Snail inactive, operculum not completely closed
3	Snail foot out, not actively moving or only sporadically active
4	Moving and/or feeding at a reduced activity level
5	Actively moving and or feeding in a normal manner

**Fig. 1** Box plot of specific conductance, and temperature for three sampling sites (*Lower, Middle, Upper*) in Salt Creek. Horizontal lines represent salinity levels (practical salinity scale) used in snail tolerance experiment



statistical assumptions only length data from 2000 were used. As pupfish parasite distributions differed among sites, relationships between pupfish life history traits and parasites were investigated separately by site. Due to few fish aged 3 and 4, we used three age classes (0, 1, and 2+) for comparisons.

For snail analyses, the unit of measure was the well plate, resulting in  $N=4$  for each treatment. Differences in snail activity, mortality, and reproduction were tested using an analysis of variance (ANOVA). For multiple comparisons among treatments we used the Tukey–Kramer HSD (honestly significant difference) test to maintain an overall alpha level of 0.05. Snail activity was assessed using the mean activity sums by plate of snails surviving the 20 days of the experiment. To assess mortality, the mean number of days snails were alive by plate was used as a metric. Reproduction was assessed using the sum of offspring by plate as our reproductive measure.

Statistical analyses were conducted using SYSTAT 7.0.1. (SPSS Inc. 1997), JMP 5.0.1a (SAS 1989–2002), and Pop Tools 2.6 (a Microsoft Excel add-in program used for the randomization tests) (Hood 2004).

## Results

### Salinity and temperature

Specific conductance values (Fig. 1) were less at the middle site compared to the lower site ( $P=0.0021$ ). There was no difference in specific conductance between the middle and upper sites ( $P=0.2640$ ) or between the upper and lower sites ( $P=0.2169$ ). Temperature differed significantly among sites (mean difference between sampling sites is equal to zero, [equivalent to a paired  $T$ -test], low vs. middle,  $T$ -ratio = 3.754,  $P=0.0002$ ; low vs. upper,  $T$ -ratio = 4.999,  $P<0.0001$ , middle vs. upper,  $T$ -ratio = 3.152,  $P=0.0017$ ).

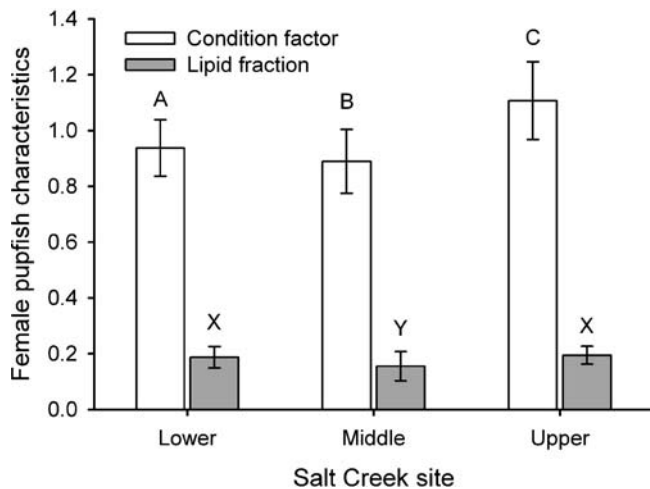
### Pupfish life history traits and parasites

There were significant differences in pupfish life history traits among sites (Table 2). Most pupfish life history traits were greater in fish from the upper site. Condition (Fig. 2) and length (Fig. 3) were greater in fish from the upper site, while fish from the middle site had the lowest condition levels, lowest percent lipids (Fig. 2), and the greatest percent water weight. Age of pupfish also differed among sites (likelihood ratio Chi square = 10.256,  $P=0.0363$ ), with fewer older fish at the middle site. Only 9% of fish at the middle site were 2 years of age or greater compared to 22, and 26% at the lower and upper sites, respectively.

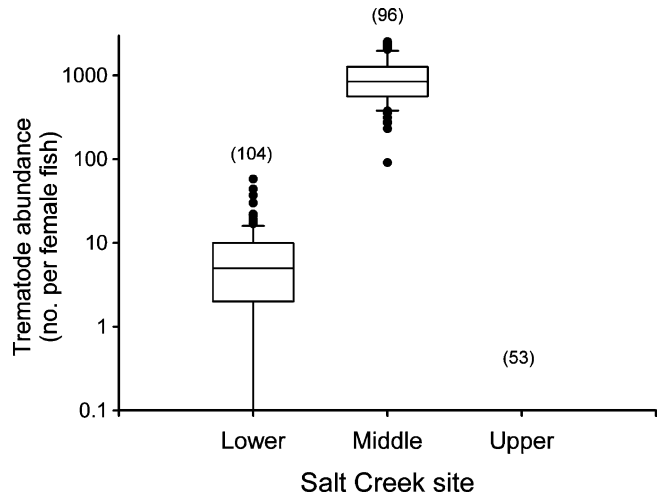
Trematodes (of both gill and body cavity) were found in pupfish from the middle and lower section of Salt Creek, but not in fish from the upper section (Fig. 4). There was a significant difference in mean parasite intensity among sites (ANOVA;  $SS=198$ ,  $F_{1, 182}=2,200$ ,  $P<0.0001$ ), with pupfish from the middle site having significantly more parasites (Fig. 4). Average trematode prevalence was 85% at the lower section, 100% at the middle section and 0% at the upper section, with respective median abundances of 6, 847, and 0. There was no significant difference in prevalence by month (Pearson Chi square 3.193,  $P=0.2026$ ), but there was a difference between the years (Pearson Chi square = 6.233,  $P=0.0119$ ). Trematode prevalence in

**Table 2** Summary ANOVA statistics of pupfish life history trait differences by site

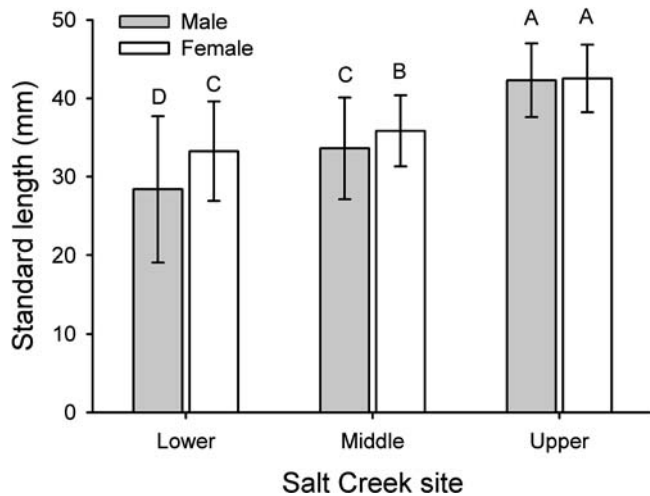
	SS	DF (model, error)	F	P
Length	8803	5, 1122	98.70	<0.0001
Condition	1.667	2, 250	63.00	<0.0001
% lipid	0.147	2, 250	20.32	<0.0001
% water	0.318	2, 247	21.97	<0.0001



**Fig. 2** Condition factor ( $\alpha = \text{mass}/(\text{length})^\beta$ , where  $\beta = 3.386$ ) and mean lipid fraction of female White Sands pupfish in Salt Creek sampling sites (*Lower, Middle, Upper*). Error bars represent standard deviations; different letters represent significant differences (Tukey-Kramer HSD test with overall  $\alpha = 0.05$ )



**Fig. 4** Box plot of total trematode abundance in female White Sands pupfish (*C. yprinodon tularosa*) from Salt Creek sampling sites (*Lower, Middle, Upper*). No trematodes were detected in upper Salt Creek fish. Number of female fish sampled is shown above each plot



**Fig. 3** Standard length (mean  $\pm$  SD) of male and female White Sands pupfish in Salt Creek sites: *Lower, Middle, and Upper*. Different letters represent significant differences (Tukey-Kramer HSD test with overall  $\alpha = 0.05$ )

July and August 2000 were 90 and 94%, respectively, while for June and July 2001 they were 76 and 71%, respectively. Gill trematodes were more prevalent and at higher abundances than those within the body cavity.

As parasite intensity and variance in parasites differed greatly among sites, regressions were conducted separately by site. At the lower site, parasite intensity (parasites/infected fish) in female pupfish was significantly related to percent lipids ( $R^2 = 0.111$ ,  $SS = 0.027$ ,  $F_{1,86} = 10.769$ ,  $P = 0.0015$ ), but not to fish condition ( $R^2 = 0.0185$ ;  $SS = 0.0175$ ,  $F_{1,86} = 1.624$ ,  $P = 0.206$ ), or percent water weight ( $R^2 = 0.00015$ ;  $SS = 0.00011$ ,  $F_{1,86} = 0.135$ ,  $P = 0.715$ ). At the middle site, parasite intensity in female pupfish was significantly related to

fish condition ( $R^2 = 0.0997$ ;  $SS = 0.0997$ ,  $F_{1,94} = 8.192$ ,  $P = 0.0052$ ), and percent water weight ( $R^2 = 0.0797$ ,  $SS = 0.00709$ ,  $F_{1,91} = 7.885$ ,  $P = 0.0061$ ) but not to percent lipids ( $R^2 = 0.0242$ ,  $SS = 0.0133$ ,  $F_{1,91} = 2.304$ ,  $P = 0.1325$ ).

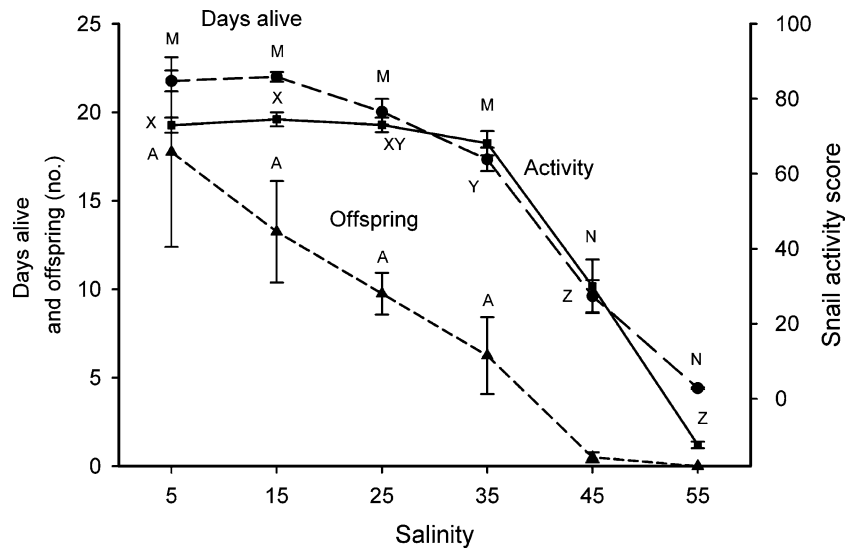
#### Snail salinity experiment

Snail length did not differ among treatments (ANOVA;  $SS = 0.0749$ ,  $F_{5,18} = 0.518$ ,  $P = 0.759$ ), consequently snail length was not used in statistical comparisons. For the snails surviving the 20-day experiment, snail activity declined with increasing salinity (ANOVA;  $SS = 3648$ ,  $F_{4,18} = 22.87$ ,  $P < 0.0001$ ) (Fig. 5). Snail survival was reduced at higher salinities (ANOVA;  $SS = 1124$ ,  $F_{5,18} = 225$ ,  $P < 0.0001$ ) (Fig. 5). No snail survived longer than 3 days in the highest salinity of 55.

The number of offspring per surviving snail ranged from 1 to 7. Due to high mortality in the higher salinities, treatments 45 and 55 were dropped from reproductive analyses. Larger snails had more offspring ( $n = 16$ ,  $R^2 = 0.318$ ,  $P < 0.0228$ ). Salinity did not significantly affect snail reproduction (ANOVA weighted by snail length;  $SS = 689$ ,  $F_{3,12} = 2.34$ ,  $P = 0.125$ ), although there was a clear trend of fewer offspring with increased salinity (Fig. 5). No cercaria were observed during the experiment.

#### Discussion

Difference in pupfish condition, length, and age among sites within Salt Creek can be attributed to salinity and parasites. Environmental barriers to the presumed first intermediate host, a springsnail (*J. tularosae*), indirectly



**Fig. 5** Springsnail (*Juturnia tularosae*) days alive (filled circle) and sum of offspring (filled triangle) at the end of a 20-day salinity tolerance experiment (means  $\pm$  SE). The sum of daily activity scores (filled square) for snails that survived the duration of the experiment (maximum activity score possible=100) is shown on the secondary axis (mean  $\pm$  SE). Different letters indicate significant differences between salinity levels (Tukey-Kramer HSD test with overall  $\alpha=0.05$ ). Salinity treatment levels of 45 and 55 were dropped from statistical analyses of offspring numbers due to low snail survival and little to no reproduction. Salinity levels are based on the practical salinity scale

regulate parasitism in the White Sands pupfish. Salinity limits the downstream occurrence of the springsnail, while a waterfall restricts upstream movement.

Pupfish in high salinity areas can effectively escape the affects of parasites. High salinity, however, is not without costs. In general pupfish are larger and in greater condition in areas of lower salinity. This pattern was also observed in White Sands pupfish within Lost River, a similar system with spatial variation in salinity (Rogowski 2004). If pupfish length and condition were determined solely by salinity, we would expect pupfish from the middle Salt Creek site to be similar to the upper site, as there was no significant difference in salinity between these two sites. However, pupfish length at the middle site was similar to that at the lower site, and fish condition was the lowest of the three sites.

Pupfish from the middle site differed in other metrics, the most obvious being high parasite prevalence and intensity. Lipid content was lowest at the middle site and there were fewer older fish, suggesting an increased mortality rate, presumably due to heavy parasitism (Johnson and Dick 2001). At the individual level, trematode intensity was related to reduced condition and increased water weight in pupfish from the middle site. Increased water weight may be an inflammatory response to trematodes (edema).

Most of the differences in pupfish metrics among sites appeared to be related primarily to parasites and secondarily to salinity. Pupfish from the lower site were

more similar to fish from the upper site (lipid content, water weight, and the age distribution of fish) despite a great difference in salinity. Mean condition of pupfish from the lower site was intermediate, as was trematode prevalence and intensity. At the lower site trematode intensity was related to reduced lipid content in pupfish, this was not observed in pupfish from the middle site. Pupfish from the middle site had the lowest percent lipids and the highest parasite intensity; perhaps these fish are at or near a physiological minimum lipid level (Biro et al. 2004).

Factors other than salinity and parasites may account for the greater size and condition observed in fish from the upper site. Lower variability in temperature and specific conductance may indicate greater groundwater inflow and permanence of water, which may result in a higher probability of pupfish survival. Intermittent flows in the lower section regularly isolate fish in pools that later dry up. Age distributions of pupfish from the upper and lower sites are very similar, suggesting that the differences observed in pupfish from the upper site are not entirely explained by water permanence, or pupfish age, but instead are due to a lower salinity as well as a lack of parasites.

The lack of snails at the upper site cannot be explained by salinity tolerance. Although salinity measurements upstream were sporadic and limited, we have never recorded salinities in the upper section greater than 35. As none of the fish sampled above the waterfall were parasitized, either the presumed first intermediate (springsnail) or the definitive hosts (piscivorous birds) were absent. We have routinely observed great blue herons and their tracks at the upper site, but have never observed any snails. We speculate that the velocity of water over the waterfall, and periodic high flows prevent the snails from migrating upstream. Salt Creek is subject to flash floods (Ortiz et al. 2000) and others have reported that water velocity can control snail occurrence and/or density (Pimentel and White 1959; Jobin and Ippen 1964; Fisher et al. 1982). In fact, we have only found the snails in areas with little to no current.

Snails can moderate their exposure to variation in salinity by withdrawing and closing their operculum (Berger and Kharazova 1997). This was observed in snails in the higher salinity treatments, as most snails spent the majority of time enclosed. In Salt Creek this response may be of limited value if salinities exceed 55 for 3 days or more. Snails may be washed downstream during flood events, but fail to establish due to the higher salinities that are commonly present in lower Salt Creek.

The limitation of the snails to the mid-section of Salt Creek correlates with parasitism risk for the pupfish. In the middle section of Salt Creek all fish were heavily infected with trematodes. At the lower site, no snails were observed, however, fish were lightly infected with trematodes. Two possible explanations could account for this: (1) cercariae are drifting downstream and infecting fish, or (2) infected fish from the middle section are moving downstream (this does not exclude fish temporarily moving into the middle section from lower areas). Median abundance of parasites observed in fish from the lower site was relatively low (six trematodes/fish), which might be more consistent with the cercariae drift hypothesis. However, cercariae generally do not survive longer than 24 h (Toledo et al. 1999; Munoz-Antoli et al. 2002), and high salinity may reduce cercariae survival and infectivity (Pietroock and Marcogliese 2003).

The springsnail, *J. tularosae* has not been conclusively identified as the first intermediate host for the trematodes observed in White Sands pupfish from Salt Creek, but in extensive searches we have not found any other mollusc. Therefore, at the moment, the springsnail is the only plausible host candidate in Salt Creek. Possible explanations for why snails were not shedding cercariae, include low parasite prevalence and time of year. It is not uncommon for parasite prevalence in snails to be around 1%, or to have only 1% of collected snails shed cercariae (Hunter and Wigington 1972). To investigate the probability of observing no cercaria, we conducted a randomization test assuming that 1 percent of the snails were infected and shedding cercariae. The probability of observing no infected snails in a random sample of 288 snails (as in our study) was 0.0536 (4,999 permutations). Based on this analysis we should have observed an infected snail but it is possible that infected snails simply were not shedding at this time.

In summary, high salinity and parasites are both detrimental to pupfish. The springsnail, *J. tularosae*, is the presumed first intermediate host for trematodes that infect pupfish. High salinity prevents the springsnail from occurring in the lower reaches of Salt Creek. As a result of the springsnail's limitation, pupfish can effectively escape the effects of parasitism in high salinity areas. However, high salinity is not without costs; pupfish in high salinity areas have reduced condition and are smaller. Trematodes may be more costly to pupfish than high salinity, as pupfish at the middle site (high trematode intensities) had significantly lower condition and percent lipids. An additional cost of parasitism may be

an increased mortality rate, as we observed fewer older fish at the middle site.

The White Sands pupfish is listed as threatened by the state of New Mexico. The springsnail, *J. tularosae* has a narrower salinity tolerance and a more restricted range than the White Sands pupfish; as a consequence, it probably should receive similar protection. In addition, the trematodes may also be endemic if they are host specific. The limited distribution of *J. tularosae* might argue for translocation as a conservation tool, and salinity levels in Salt Creek above the waterfall are adequate for *J. tularosae*. However, an introduction of this springsnail above the waterfall would expose local pupfish to potentially novel parasites and could be detrimental to the already threatened fish. We would strongly advise against expanding the range of the springsnail until more is known about its role in the trematode life cycles.

**Acknowledgements** This work was part of a thesis submitted in partial fulfillment of requirements for a doctor of philosophy degree in Zoology at North Dakota State University, Fargo, ND. We thank Hildy Reiser, Holloman Air Force Base, for logistical support, B. Warren for help in recording snail activity, N. Renteria, D. Moen, S. Ralston, T. Eppler, and A. Moore for help in fish dissections. Vincente Lombrana of New Mexico State University, Alamogordo, provided logistical support and laboratory space. The New Mexico Alliance for Minority Participation program funded N. Renteria. We thank Robert Myers for advice and for facilitating access to the White Sands Missile Range (WSMR) (Approved for public release by WSMR; distribution unlimited. OPSEC review completed 11 August 2003). D. Harstad and B. Font provided help in identifying parasite species. We thank two anonymous reviewers for comments on earlier drafts of this document. Organisms were collected under Permit#2887 to CAS from the New Mexico Department of Game and Fish. These experiments were in compliance with the current laws of the USA. This work was supported in part by Department of Defense Legacy Resource to CAS (Grant# DACA87-00-H-0014) administered by M. Hildegard Reiser, 49 CES/CEV, Environmental Flight, HAFB).

## References

- Barlow GW (1958) High salinity mortality of desert pupfish, *Cyprinodon macularius*. *Copeia* 1958:231–232
- Barnes RSK (1999) What determines the distribution of coastal mudsnails within North-Western Europe? *Mar Ecol* 20:97–110
- Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ Biol Fish* 58:237–275
- Bennett WA, Beitinger TL (1997) Temperature tolerance of sheepshead minnow, *Cyprinodon variegatus*. *Copeia* 1997:77–87
- Berger VY, Gorbushin AM (2001) Tolerance and resistance in gastropod mollusks *Hydrobia ulvae* and *H. ventrosa* from the White Sea to abiotic environmental factors. *Russ J Mar Biol* 27:314–319
- Berger VJ, Kharazova AD (1997) Mechanisms of salinity adaptations in marine molluscs. *Hydrobiologia* 355:115–126
- Biro PA, Morton AE, Post JR, Parkinson EA (2004) Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 61:1513–1519
- Brown JH, Feldmeth CR (1971) Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (*Cyprinodon*). *Evolution* 25:390–398
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 83:575–583

- Clescerl LS, Greenberg AE, Eaton AD (1999) Standard Methods for Examination of Wastewater, 20th edn. American Public Health Assn
- Cole GA (1994) Textbook of limnology, 4 edn. Waveland Press Inc., Prospect Heights
- Costil K, Dussart GBJ, Daguzan J (2001) Biodiversity of aquatic gastropods in the Mont St. Michel basin (France) in relation to salinity and drying of habitats. *Biodivers Conserv* 10:1–18
- Echelle AA, Echelle AF, Hill LG (1972) Interspecific interactions and limiting factors of abundance and distribution in the red river pupfish, *Cyprinodon rubrofluvialis*. *Am Nat* 88:109–130
- Fisher SG, Gray LJ, Grimm NB, Busch DE (1982) Temporal succession in a desert stream ecosystem following flash flooding. *Ecolog Monogr* 52:93–110
- Haney DC, Nordlie FG, Binell J (1999) Influence of simulated tidal changes in ambient salinity on routine metabolic rate in *Cyprinodon variegatus*. *Copeia* 1999:509–514
- Herbst DB (2001) Gradients of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland salt waters. *Hydrobiologia* 466:209–219
- Hershler R, Liu H-P, Stockwell CA (2002) A new genus and species of aquatic gastropods (Rissooidea: Hydrobiidae) from the North American Southwest: phylogenetic relationship and biogeography. *P Biol Soc Wash* 115:171–188
- Hoffman GL (1999) Parasites of North American Fresh Water Fish, 2<sup>nd</sup> edn. Cornell University Press, Ithaca
- Hood G (2004) Pop tools version 2.6. <http://www.cse.csiro.au/poptools/>
- Hunter GW III, Wigington EE (1972) Ecological observations on the emergence of cercariae from *Goniobasis floridensis* Reeve from the Wekiva River, Florida. *Ecology* 53:901–907
- Hurst CT (1927) Structural and functional changes produced in the gastropod mollusk, *Physa occidentalis*, in the case of parasitism by the larvae of *Echinostoma revolutum*. *UC Publ Zool* 29:322–405
- Hylleberg J (1986) Distribution of hydrobiid snails in relation to salinity, with emphasis on shell size and co-existence of the species. *Ophelia Suppl* 4:85–100
- Jobin WR, Ippen JT (1964) Ecological design of irrigation canals for snail control. *Sci New Ser* 145:1324–1326
- Johnson MW, Dick TA (2001) Parasite effects on the survival, growth, and reproductive potential of yellow perch (*Perca flavescens* Mitchell) in Canadian Shield lakes. *Can J Zool* 79:1980–1992
- Kinne O (1960) Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiol Zool* 33:288–317
- Kodric-Brown A, Mazzolini P (1992) The breeding system of pupfish, *Cyprinodon pecosensis*: effects of density and interspecific interactions with the killifish, *Fundulus zebrinus*. *Environ Biol Fish* 35:169–176
- Lafferty KD, Kuris AM (1999) How environmental stress affects the impacts of parasites. *Limnol Oceanogr* 44:925–931
- Manly BFJ (1997) Randomization, Bootstrap and Monte Carlo Methods in Biology, 2nd edn. CRC Press Boca Raton
- Martin FD (1972) Factors influencing local distribution of *Cyprinodon variegatus* (Pisces: Cyprinodontidae). *T Am Fish Soc* 1972:89–93
- McCallum H, Dobson A (1995) Detecting disease and parasite threats to endangered species and ecosystems. *Trends Ecol Evol* 10:190–194
- Meffe GK, Minckley WL (1997) Persistence and stability of fish and invertebrate assemblages in a repeatedly disturbed Sonoran Desert stream. *Am Midl Nat* 117:177–191
- Miller RR (1948) The cyprinodont fishes of the Death Valley system of Eastern California and Southwestern Nevada. Miscellaneous Publications of the Museum of Zoology. University of Michigan, No.68
- Miller RR (1981) Coevolution of Deserts and Pupfishes (Genus *Cyprinodon*) in the American southwest. In: Naiman RJ, Soltz DL (eds) Fishes in North American Deserts. Wiley, New York, pp 39–94
- Miller RR, Echelle AA (1975) *Cyprinodon tularosa*, a new cyprinodontid fish from the Tularosa Basin, New Mexico. *SW Nat* 19:365–377
- Munoz-Antoli C, Trelis M, Espert A, Toledo R, Esteban JG (2002) Survival and infectivity of *Echinostoma friedi* (Trematoda: Echinostomatidae) miracidia and cercariae under experimental conditions. *Helminthologia* 39:149–154
- Ortiz D, Lange KM, Beal LV (1999) Water resources data, New Mexico, water year 1998, vol 1. the Rio Grande Basin, the Mimbres River Basin, and the Tularosa Valley Basin: U.S. Geological Survey Water-Data Report NM-98-1
- Ortiz D, Lange KM, Beal LV (2000) Water resources data, New Mexico, water year 1999, vol 1. the Rio Grande Basin, the Mimbres River Basin, and the Tularosa Valley Basin: U.S. Geological Survey Water-Data Report NM-99-1
- Pietrocc M, Marcogliese DJ (2003) Free-living endohelminth stages: at the mercy of environmental conditions. *Trends Parasitol* 19:293–299
- Pimentel D, White JPC (1959) Physicochemical environment of *Australorbis glabratus*, the snail intermediate host of *Schistosoma mansoni* in Puerto Rico. *Ecology* 40:533–541
- Pittenger JS, Springer CL (1999) Native range and conservation of the White Sands pupfish (*Cyprinodon tularosa*). *Southwest Nat* 44:157–165
- Por FD (1980) A classification of hypersaline waters, based on trophic criteria *Mar Ecol-PSZNI* 1:121–131
- Quinn TJ II, Deriso RB (1999) Quantitative fish dynamics, Oxford University Press Inc, New York
- Rogowski DL (2004) Direct, indirect, and potential effects of salinity on the White Sands pupfish (*Cyprinodon tularosa*). PhD Dissertation, North Dakota State University, Fargo, ND
- SAS (2002) JMP Release, 5.0.1a. SAS Institute Inc. Cary, NC, USA
- Sheldon AL (1988) Conservation of stream fishes: patterns of diversity, rarity, and risk. *Conserv Biol* 2:149–156
- SPSS Inc. (1997) SYSTAT Version 7.0.1. SPSS Inc., Chicago
- Stockwell CA (2002) Threatened fishes of the world: *Cyprinodon tularosa* Miller and Echelle, 1975 (Cyprinodontidae). *Env Biol Fish* 63:404
- Stockwell CA, Mulvey M (1998) Phosphogluconate dehydrogenase polymorphism and salinity in the White Sands pupfish. *Evolution* 52:1856–1860
- Toledo R, Munoz-Antoli C, Perez M, Esteban JG (1999) Survival and infectivity of *Hypoderaeum conoideum* and *Euparyphium albuferensis* cercariae under laboratory conditions. *J Helminthol* 73(2):177–182
- Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *BioScience* 48:607–616
- Williams WD (1998) Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* 381:191–201
- Williams JE, Bowman DD, Brooks JE, Echelle AA, Edwards RJ, Hendrickson DA, Landye JJ (1985) Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. *J Arizona -Nevada Acad Sci* 20:1–62
- Wilson EO (1992) The diversity of life, 1st edn. Belknap Press, Cambridge
- Withers PC (1992) Comparative animal physiology, Saunders College Publishing, Orlando
- Wolfram G (1999) The zoobenthic community of shallow salt pans in Austria – preliminary results on phenology and the impact of salinity on benthic invertebrates. *Hydrobiologia* 408:193–202