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Experimental evidence for costs of parasitism for a threatened species, White Sands pupfish (*Cyprinodon tularosa*)

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Summary

1. We used field and experimental data to test if white grub parasites (Diplostomatidae) are costly to White Sands pupfish (*Cyprinodon tularosa*), a threatened species restricted to four sites in the Chihuahuan desert, New Mexico.
2. Of the four populations of *C. tularosa*, two are native and two are introduced. The two native populations (Malpais Spring and Salt Creek) are genetically distinct and have been isolated historically in dissimilar aquatic habitats (brackish spring and saline river, respectively). Two populations were established *c*. 1970 from translocation of Salt Creek fish to another saline river (Lost River) and another brackish spring (Mound Spring).
3. Physid snails (Physidae) occur in the two brackish spring habitats but not the saline river habitats. These snails are first intermediate hosts for white grubs (Diplostomatidae). Therefore, the two freshwater populations are infected by diplostomatids. For the Mound Spring population, the ecological relationship of *C. tularosa* and diplostomatids has only recently occurred.
4. In 1995, a population crash occurred for *C. tularosa* at Mound Spring, associated with a parasite outbreak. Diplostomatids were the presumptive cause of this crash, but this was inferred from observation of infection in collected fish.
5. Two years of seasonal sampling of the two populations revealed that all collected fish were infected. Parasite intensities were significantly lower in winter compared to summer, suggesting that heavily infected fish were lost from the population on a seasonal basis.
6. We conducted an artificial infection experiment to assess the costs of parasitism for previously uninfected *C. tularosa* females for various life-history traits. Under experimental conditions, diplostomatid infection caused increases in *C. tularosa* mortality and decreases in growth and fat storage. Individual-level costs of parasitism may translate to population-level patterns of parasitism for *C. tularosa* populations. Results from this study suggest that parasites may impact host overwinter survival, which is consistent with lower parasite intensities found during winters in wild populations.

Key-words: Diplostomatidae, endangered species, life history, parasite, translocation.

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Introduction

Recent studies (e.g. Hedrick, Kim & Parker 2001) have suggested that infection by novel pathogens or parasites may have strong negative impacts on hosts species introduced to new environments, such as endangered species, where refuge populations are established as a conservation strategy. Furthermore, most theoretical models regarding parasite–host associations assume that parasites are costly to their hosts. This assumption is central to models that suggest that parasites play important roles in regulation of host populations (Anderson & May 1978; May & Anderson 1978; Scott & Dobson 1989), evolution of sex (Lively, Craddock & Vrijenhoek 1990; Howard & Lively 1994), mediation of competition (Price 1980; Clayton & Moore 1997), host sexual selection (Hamilton & Zuk 1982), manipulation of host behaviour (Moore 1983, 1984; Lafferty & Morris 1996; but see Edelaar, Drent & Goeij 2003), evolution of

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host immunity and life-history trade-offs (Lochmiller & Deerenberg 2000; Moret & Schmid-Hempel 2000; Zuk & Stoehr 2002), and the coevolution of host resistance and parasite virulence (Gandon 2002; Gandon, Jansen & van Baalen 2002; Gandon, van Baalen & Jansen 2002). It seems intuitive to argue that parasites are costly because they extract resources from their hosts that could be used for growth, reproduction and survival; however, the assumption of parasitic costs is not always tested or found (Møller 1997; Collyer 2000).

Recent literature reviews revealed parasites often have no detectable impact on their hosts (Møller 1997; Collyer 2000). These findings could indicate a true lack of costs or an inability to measure costs. Furthermore, costs may be difficult to measure when parasite intensity varies in space and time. Nevertheless, assessments of parasite costs to hosts should be conducted before models of parasite–host associations are considered for a specific system.

Costs of parasitism may be particularly important for actively managed populations such as rare or endangered species (Viggers, Lindenmayer & Spratt 1993; McCallum & Dobson 1995; Daszak, Cunningham & Hyatt 2000). Associated parasite communities are often altered as a consequence of active management (Viggers *et al*. 1993; McCallum & Dobson 1995; Gompper & Williams 1998; Daszak *et al*. 2000; Stockwell & Leberg 2002). If costly, parasite effects may manifest at the population level, leading to decimation of the host population (Scott 1988). Scott & Dobson (1989) suggest that detailed ecological information is necessary to demonstrate that parasites impact host populations.

Here we examine the costs of parasitism by white grubs (Diplostomatidae) for the White Sands pupfish (*Cyprinodon tularosa*, Miller & Echelle 1975). Historic translocations of *C. tularosa c*. 1970 resulted in altered parasite communities for two new populations (see Stockwell, Mulvey & Jones 1998). One of these introduced populations experienced a population crash in 1995, which was associated with a parasite outbreak (Pittenger 1996), suggesting that diplostomatid infection may have caused *C. tularosa* mortality. In the current study, we incorporate 2 years of parasite surveys with experimental data to determine if diplostomatids are costly to *C. tularosa*. These observations are especially important because translocations are currently being considered as a management strategy in the conservation of *C. tularosa*, a New Mexico state-listed threatened species (Stockwell *et al*. 1998; Pittenger & Springer 1999; Stockwell 2002).

STUDY SYSTEM

C. tularosa provides an interesting system for examining the costs of parasitism because parasite communities vary among habitats. Following the desiccation of the Pleistocene Lake Otero, *C. tularosa* were apparently isolated in two remnant habitats where populations still occur: Malpais Spring and Salt Creek (Miller & Echelle 1975; Pittenger & Springer 1999). Based on the genetic distinction of these populations, and their isolation in ecologically dissimilar habitats, Stockwell *et al*. (1998) proposed that they should be managed as separate evolutionarily significant units (ESUs). In addition, non-native populations of *C. tularosa* were established by the unauthorized translocation of *fish* from Salt Creek to habitats at Lost River and Mound Spring between 1967 and 1973 (Stockwell *et al*. 1998; Pittenger & Springer 1999). The current conservation plan for *C. tularosa* contains a component for the creation of refuge populations of the two ESUs. Thus, the implications of population translocations and parasite exposure can be considered with research involving the introduced populations of *C. tularosa*.

Divergent ecological conditions among the four habitats (Stockwell & Mulvey 1998) have important implications for the distribution of physid snails (*Physa* sp.) and diplostomatid parasites. Salinity is relatively high at Salt Creek and Lost River as compared to the habitats at Malpais Spring and Mound Spring (Stockwell & Mulvey 1998). The high salinity at the former two sites precludes the occurrence of physid snails, which only tolerate salinities below 9‰ (Stockwell, unpublished data). Physid snails are first intermediate hosts to diplostomatids, whereas fish are second intermediate hosts and piscivorous birds such as herons are definitive hosts (Hoffman 1958, 1967; Spall & Summerfelt 1970). Free swimming cercariae shed from physid snails subsequently burrow into fish hosts and migrate to various tissues where they encyst during a 20-day metamorphic period (Hoffman 1958). Diplostomatids encyst within the eyes, visceral and parietal peritoneum, mesentery and viscera of *C. tularosa* (Collyer 2000). In this system, the parasite species has been identified as *Posthodiplostomum minimum* (J. Janovy, University of Nebraska-Lincoln, personal communication); however, diplostomatid neascii (encysted metacercariae) are difficult to differentiate at the species level based on morphology (Hendrickson 1986). Only fish at Malpais Spring and Mound Spring are exposed to these parasites whereas fish at Salt Creek and Lost River are not exposed because physid snails only occur at the former two sites. Therefore, introduction of *C. tularosa* to Mound Spring exposed these Salt Creek ESU fish to novel parasites. The current study was conducted to determine if a diplostomatid outbreak may have been responsible for the *C. tularosa* population crash.

Materials and methods

FIELD COLLECTIONS

Fish were collected with minnow traps and beach seines at Malpais Spring and Mound Spring on a seasonal basis: July 1998, January 1999, August 1999 and February 2000. Samples of 20–48 fish were captured at each location and shipped live to the research laboratory

for examination. Live fish were measured for standard length (SL) to the nearest 0·01 mm, sacrificed by pithing and necropsied for determination of parasite infection. The eyes and all contents of the visceral cavity were removed and pressed between two glass slides for enumeration of parasites under magnification (10–60×). Parasite prevalence was defined as the percentage of infected individuals in each collection, and parasite intensity was determined as the number of cysts in each individual fish host (Margolis *et al*. 1982).

EXPERIMENTAL INFECTIONS

Approximately 200 physid snails were hand-collected from Malpais Spring in May 1999 and shipped live to the research laboratory (Fargo, ND, USA). Snails were subsequently isolated into scintillation vials filled with approximately 20 mL of distilled water mixed with Instant Ocean® to maintain a salinity of 3·5‰ (approximating the salinity at Malpais Spring and Mound Spring). Vials were checked daily for shedding of cercariae. Fifty-five per cent of the snails collected shed cercarial diplostomatids. Approximately 20 snails that actively shed cercariae were used for artificial infections of experimental fish.

In May 1999, fish were collected from Lost River using minnow traps, beach seines and dip nets. Lost River fish are not naturally infected by diplostomatids. Lost River, like the Mound Spring population, was established by translocation of fish from Salt Creek (Stockwell *et al*. 1998; Pittenger & Springer 1999). Therefore, using Lost River fish presents an opportunity to examine costs of parasitism for an introduced population exposed to novel parasites. Of the fish collected, 150 small (< 40 mm) females were chosen arbitrarily and transported live to the research laboratory. Fish were partitioned five fish per 40 litre aquarium, acclimated to 25 °C and salinity equal to 3·5‰, and fed Tetra® brand flake food *ad libitum* over a 1-week period. Spectrum fluorescent lights were used with each aquarium on a timer adjusted for a light/dark cycle of 14 h : 10 h. Fish were maintained with these conditions for up to 4 weeks.

DETERMINING LIFE HISTORY COSTS

We assessed experimentally the effects of parasitism by diplostomatids on *C. tularosa* survival and several life-history traits over a 60-day period. Fifty experimental replicates of female fish (size range = 18·22–36·77 mm, standard length) were introduced to experimental aquaria (one aquarium per experimental replicate) maintained with the same conditions described previously. Each experimental replicate contained two females matched arbitrarily for size: one female was selected randomly as the treatment fish and the other female served as a non-infected control. Each female was given a diagnostic caudal fin clip for identification. Aquaria were distributed in a randomized block design of five blocks of 10 aquaria. The 60-day experiment was temporally staggered with block 5 starting and ending 4 days after block 1.

Artificial infections were administered by placing fish into a 1-L aquarium for 24 h with cercariae collected from snails. Based on empirical evidence from preliminary experimental parasite inoculations (Collyer 2000), we chose a parasite level of 500 cercariae for artificial infections. Control fish were also isolated in a 1-L aquarium but were not exposed to cercariae. Following exposure, fish were returned to aquaria and maintained for 60 days. Standard length (SL) (measured to the nearest 0·01 mm) and mass (measured to the nearest 0·01 g) were measured prior to infection and again at days 10, 20, 30, 45 and 60. Tetra® brand flake food was fed to fish daily except 24 h prior to length and mass measurements. The amount of food was adjusted to 5·0 ± 0·5% mass of both fish.

If a fish died during the experiment, the other fish in the replicate was sacrificed in 500 mg L^{-1} tricane methanesufonate (MS-222) and both fish were fixed in 10% buffered formalin to maintain balanced data. Four replicates were not considered for data analyses because it was determined that at least one fish was a male for two replicates and in two other cases, one fish died within 5 days of parasite exposure. At this early stage, parasites had not encysted and were difficult to enumerate. On day 60, surviving fish were sacrificed and fixed as described above. Survival probability is expected to improve for infected fish after parasites have encysted (approximately 20 days) (Spall & Summerfelt 1970). Therefore, survival differences were considered for both the encystment and postencystment stages of the parasites, days 1–20 and 21–60, respectively.

We also measured the effects of parasitism on life-history traits for all experimental fish. These life-history traits included growth, fat content and reproductive allocation. Assessments of reproductive allocation and fat were obtained following the procedures of Reznick & Braun (1987). Preserved fish were dissected and somatic and reproductive tissues were oven-dried at 56 °C for 48 h. Samples were weighed to the nearest 0·1 mg (gross weight). To determine fat content, somatic tissue was subjected subsequently to fat extraction. Fish carcasses were placed into scintillation vials and filled to approximately 20 mL with anhydrous ether. Ether was discarded and replaced with fresh ether after 24 h. Fish carcasses were removed from ether after 24 h, oven-dried for an additional 24 h and reweighed (net weight). Fat content was determined as the difference between gross and net dry weights. Reproductive allocation and fat content were standardized as the percentage of total dry mass and somatic mass, respectively.

STATISTICAL ANALYSES

All data were analysed using the SYSTAT 7·0 statistical package (SPSS, Inc. 1997). Parasite count data from field collections were analysed using the general linear

model (GLM) procedure. Effects of population, sampling period (sampling event), sex, size (SL) and all interactions were tested for the response variable, parasite intensity. We found no significant interactions of SL and other factors (i.e. the association of parasite intensity and fish size did not significantly vary between populations, sampling periods, or sexes); therefore, SL was considered a covariate in the model. Parasite count data were $\log_{10}$ transformed to meet the normality assumption of analysis of variance (ANOVA). *Post-hoc* pairwise comparisons were made between sampling periods and populations for least-square means of parasite intensity using the Bonferroni pair-wise procedure (SPSS, Inc. 1997).

We used several statistical approaches to consider the experimental effect of parasitism on *C. tularosa* life-history traits. Kaplan–Meier survival analysis was used to determine if there was a difference in survival before and after completion of parasite encystment for infected fish. Survival data most closely fitted a Weibull distribution. Fish that died during the experiment were removed before 60 days; thus, the length of time fish were in the experiment (hereafter, referred to as survivorship) could have an effect on the final life-history calculations.

We used multivariate analysis of variance (MANOVA) to test for the effects of treatment, initial size (SL) and survivorship (length of time, in days, in experiment) on the response variables, dry mass (i.e. growth), fat content and reproductive allocation. There was no significant interaction of size or survivorship with treatment so they were treated as covariates with 1 d.f. each. Reproductive allocation data were arcsine transformed to meet the assumption of normality for ANOVA (Zar 1984). Initial SL was highly correlated with initial wet mass ($r = 0{\cdot}96$, $P < 0{\cdot}0001$), thus indicating that either variable could be used as a measure of initial size. However, we used dry mass as a measure of response variable, final size, for three reasons: (1) because fish could possibly lose mass but not lose length during the experiment; (2) it was measured in the same process as fat content and reproductive allocation; and (3) there was evidence of increased oedema for parasitized fish (Collyer 2000), which could possibly confound wet mass measures.

Because we assume that differences in mass were attributed to different growth rates, we performed univariate ANOVA on initial wet mass and SL. ANOVA indicated that there were no significant differences in initial wet mass ($F_{1,90} = 0{\cdot}242$, $P > 0{\cdot}6$) or initial SL ($F_{1,90} = 0{\cdot}010$, $P > 0{\cdot}9$). This finding illustrates that infected and control fish were matched adequately for initial size; therefore, differences in final dry mass could be interpreted as differences in growth. MANOVA was performed with the GLM procedure of the SYSTAT 7·0 statistical package (SPSS, Inc. 1997).

Generalized Mahalanobis (1936) distances (D) were calculated as a metric of life-history trait difference for multivariate pairwise comparisons (Manly 1996; Legendre & Legendre 1998), using the pooled within-group covariance matrix calculated from MANOVA. In addition to comparing control and infected fish, we also compared survivors to non-survivors within treatments, surviving control fish to surviving infected fish, and non-surviving control fish to non-surviving infected fish. Significance of D statistics was considered with a randomization procedure. For each permutation of the data, individual fish were assigned randomly to either treatment and D statistics were recalculated. The probability of finding a greater D by chance (P_{rand}) was assessed from a distribution of test statistics for 5000 permutations (observed values accounted for one permutation).

A canonical variates analysis (CVA) was used with between-group and pooled within-group covariance matrices from MANOVA to calculate canonical scores from the life history data. CVA involves projecting original data onto the eigenvectors of the matrix of between-group variation relative to within-group variation (Legendre & Legendre 1998). Thus, canonical scores represent linear combinations of original variables along major axes of between-group variation. Visual representation of life history costs was considered by plots of canonical scores vs. survivorship. Pearson correlation analyses were performed with original variables and canonical scores to determine which variables were most associated with life history variation (Manly 1996; Legendre & Legendre 1998).

Results

FIELD COLLECTIONS

Parasite prevalence was 100% for all collections of fish. Parasite intensities varied from two to 1028 cysts per fish (Table 1). There was a significant positive association of parasite intensity and fish size, SL ($r = 0{\cdot}57$; $P < 0{\cdot}0001$). Population, sampling period and their interaction significantly explained variation in parasite intensity (Table 2). However, neither sex nor any interaction with sex significantly explained variation. Parasite intensities were highest in summer samples and lowest in winter samples for both populations (Table 1). Although the population by sampling period interaction was significant, the only pairwise comparison that was significant between populations for any sampling period was in the summer of 1998, when Mound Spring fish were parasitized more heavily than Malpais Spring fish (Table 1).

EXPERIMENTAL INFECTIONS

All inoculated fish were parasitized and survival was significantly reduced for infected fish over the 60-day experiment ($\chi^2_{1\text{d.f.}} = 13{\cdot}67$, $P < 0{\cdot}001$). Thirteen infected fish (27%) died during the experiment compared to none of the control fish. Twelve of 13 non-surviving fish died by day 23 (parasite encystment lasts approximately

Table 1. Comparative standard length (SL) and parasite intensity (cysts/fish) of natural populations of *C. tularosa*. Minimum values, maximum values, means, medians and sample sizes (*n*) are reported for non-transformed data, separated by population and sampling period

	July 1998		February 1999		August 1999		February 2000	
	SL (mm)	Cysts/fish	SL (mm)	Cysts/fish	SL (mm)	Cysts/fish	SL (mm)	Cysts/fish
Malpais Spring								
Min.	21·77	10	18·43	2	20·88	40	22·42	12
Max.	41·66	487	39·61	342	34·45	1028	34·61	437
Mean	30·30	106·48[a]	30·34	40·70[b]	27·56	259·70[c]	28·17	72·07[a]
Median	29·89	87	30·63	26·50	28·32	198·50	27·87	46
n	48		40		20		30	
Mound spring								
Min.	25·69	72	17·39	2	20·19	42	19·09	13
Max.	41·95	886	35·87	314	36·71	806	36·24	371
Mean	31·44	322·37[c]	24·16	68·75[b]	30·52	292·50[c]	26·36	94·94[a]
Median	30·85	281	23·47	17·50	30·86	244·50	25·73	37·50
n	27		28		20		22	

Means sharing the same letter are not significantly different after Bonferroni multiple comparisons of least-square $\log_{10}$ transformed means for $\alpha = 0{\cdot}05$. ANCOVA results are presented in Table 2.

Table 2. Analysis of variance for the $\log_{10}$ transformed dependent variable, parasite intensity, for the effects of population, sex, sampling period (SP) and their interactions. Main effects were fixed and size (SL) was used as a covariate. The model explained 64·3% of the variation in parasite intensity

	Analysis of variance				
Source	Sum-of-squares	d.f.	Mean square	*F*	*P*
Population	1·967	1	1·967	16·664	< 0·0001
Sex	0·026	1	0·026	0·223	0·6374
SP	11·621	3	3·874	32·833	< 0·0001
Population × Sex	0·070	1	0·070	0·595	0·4410
Population × SP	2·749	3	0·917	7·768	< 0·0001
Sex × SP	0·316	3	0·105	0·892	0·4459
Pop. × Sex × Samp. period	0·316	3	0·105	0·893	0·4454
SL	13·503	1	13·503	114·448	< 0·0001
Error	25·720	218	0·118		

20 days; Hoffman 1958) (Fig. 1a). Survival was significantly reduced for infected fish during parasite encystment compared to after encystment (Wilcoxon's $\chi^2_{1d.f.} = 61{\cdot}07$, $P < 0{\cdot}0001$; Fig. 1a). Parasite intensity was significantly different between surviving and non-surviving infected fish (ANOVA: $F_{1,44} = 25{\cdot}63$, $P < 0{\cdot}0001$). Mean parasite intensity was 182·9 (standard error of the mean, SE = 15·3) cysts per fish for non-surviving fish compared to 95·4 (SE = 8·06) cysts per fish for surviving infected fish. Further, all non-surviving fish had at least 100 cysts.

Both surviving and non-surviving infected fish experienced morphological consequences from parasitism by diplostomatids. Within 1 day after parasite exposure, the caudal region darkened for every infected fish. Over the next 10 days, the dark colouration spread across the entire body. Dark body colouration was not observed for any of the control fish. In addition, eye size increased noticeably for infected fish and persisted to day 30. After 30 days, swelling decreased but eyes of infected fish remained large compared to uninfected fish. Infected fish assumed normal colouration between 30 and 45 days. In extreme cases, infected fish developed spinal curvature (Fig. 1b).

Treatment, initial standard length and survivorship significantly explained variance in the MANOVA for life-history data (Table 3). Initial standard length was only significantly associated with dry mass ($r = 0{\cdot}74$; $P < 0{\cdot}0001$ and Table 4). Survivorship was significantly associated with dry mass ($r = 0{\cdot}71$; $P < 0{\cdot}0001$) and fat content ($r = 0{\cdot}70$; $P < 0{\cdot}0001$ and Table 4). Comparison of adjusted least-square means (Table 5) suggested 17·6 and 23·3% lower responses for mass and fat content, respectively, for the infected treatment. We did not detect any significant differences in reproductive allocation between the two treatments but mature eggs were not observed for any control or infected fish. Back-transformed (Zar 1984) mean reproductive allocation was 10·7% and 9·5% for control and infected fish, respectively.

Control and infected fish were significantly differentiated ($D = 0{\cdot}78$; $P_{rand} = 0{\cdot}0032$) for the life-history traits measured (Fig. 2). When considering only fish that

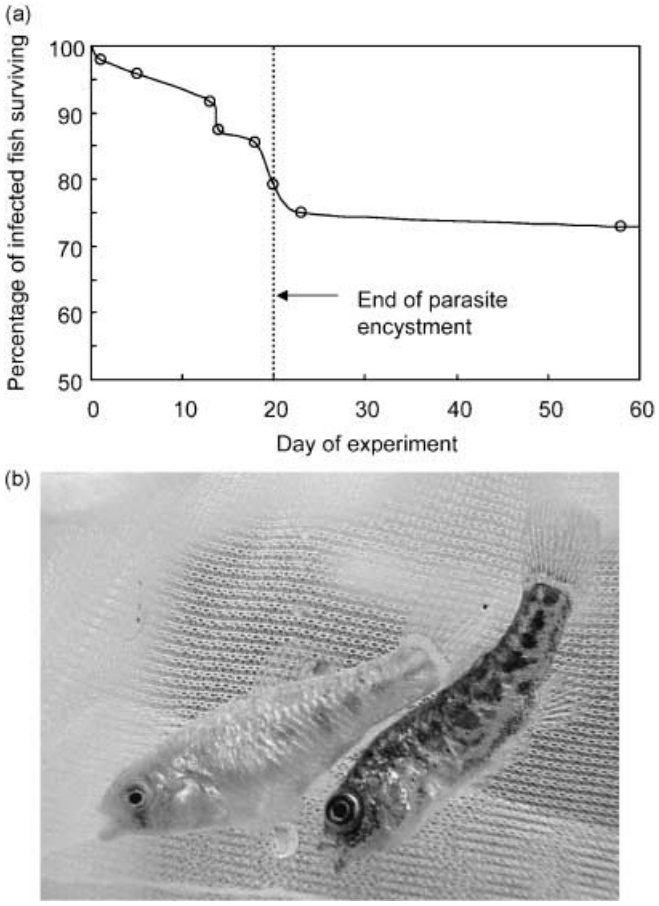

Fig. 1. (a) Survivorship of experimentally infected *Cyprinodon tularosa* females. There was no mortality of uninfected control fish during the 60-day experiment. Survivorship was significantly reduced for infected fish over 60 days ($\chi^2_{1d.f.} = 13{\cdot}67$, $P < 0{\cdot}001$) and during the first 20 days characterized by parasite encystment (Wilcoxon's $\chi^2_{1d.f.} = 61{\cdot}07$, $P < 0{\cdot}0001$). (b) Morphological consequences of parasitism for *C. tularosa* by diplostomatids. This photograph was taken 27 days after infection. The infected fish (right) had enlarged eyes, spinal curvature and dark colouration compared to the uninfected, control fish.

Table 3. Multivariate analysis of variance for life-history traits from experimental *C. tularosa* females. Life history traits include total dry mass, fat content (FC), and reproductive allocation (RA)

Source	Wilks's Λ	d.f. 1, d.f. 2	*P*
Treatment	0·747	3, 86	< 0·0001
Initial SL	0·360	3, 86	< 0·0001
Survivorship	0·472	3, 86	< 0·0001

were removed from the experiment (because the infected fish died), the difference was greater ($D = 1{\cdot}87$; $P_{rand} = 0{\cdot}0002$). However, for surviving fish there was no significant difference between treatments ($D = 0{\cdot}51$; $P_{rand} = 0{\cdot}20$). There were also significant life-history differences between surviving and non-surviving fish within treatments for control ($D = 1{\cdot}67$; $P_{rand} = 0{\cdot}0004$) and infected ($D = 2{\cdot}46$; $P_{rand} = 0{\cdot}0002$) fish; surviving fish had greater growth and higher fat content (see Fig. 2 and below).

Table 4. Univariate test statistics from the multivariate analysis of variance performed on *C. tularosa* life history traits

Source	Response	SS	d.f. 1, d.f. 2	MS	*F*	*P*
Treatment	Dry mass	0·0794	1, 88	0·0794	14·044	0·0003
	FC	0·1140	1, 88	0·1140	27·738	< 0·0001
	RA	0·0028	1, 88	0·0028	0·483	0·4887
Initial SL	Dry mass	0·3809	1, 88	0·3809	67·344	< 0·0001
	FC	0·0001	1, 88	0·0001	0·018	0·8921
	RA	0·0013	1, 88	0·0013	0·214	0·6443
Survivorship	Dry mass	0·3164	1, 88	0·3164	55·945	< 0·0001
	FC	0·3644	1, 88	0·3644	88·698	< 0·0001
	RA	0·0183	1, 88	0·0183	3·101	0·0817

Table 5. Adjusted least-square means (standard error)* from the multivariate analysis of variance performed on *C. tularosa* life history traits

	n	Dry mass	FC	RA†
Control	46	0·334 (0·011)	0·304 (0·009)	0·191 (0·011)
Infected	46	0·275 (0·011)	0·233 (0·009)	0·180 (0·011)

*Units for mass are grams, fat content represents proportion of total somatic mass, and reproductive allocation is proportion of total mass. †Data for reproductive allocation were arcsine transformed (Zar 1984).

The CVA performed on life-history data explained the cumulative dispersion of individual values with one canonical axis. Average scores were 0·394 and −0·394 for control and infected fish, respectively. Canonical scores were significantly and positively correlated with total mass ($r = 0{\cdot}56$; $P < 0{\cdot}0001$) and fat content ($r = 0{\cdot}94$; $P < 0{\cdot}0001$), but not reproductive allocation ($r = 0{\cdot}20$; $P = 0{\cdot}38$). In addition, fat content was significantly correlated with total dry mass ($r = 0{\cdot}80$; $P < 0{\cdot}0001$). Consistent with univariate ANOVA statistics (Table 4), differences in growth and fat content demonstrate significant negative impacts for the experimental parasite infection.

Discussion

Both field and experimental data suggest that white grub parasitism is costly to *C. tularosa*. The frequency of heavily parasitized fish was higher for both populations in summer samples. Parasite exposure is likely to occur during the spring or summer when water temperatures reach 20 °C, the temperature empirically found to initiate cercariae emergence from physid snails (Hoffman 1958; Spall & Summerfelt 1970). Documented evidence on parasite-induced host mortality in field studies suggests that changes in the frequency distributions of parasite intensity may indicate removal of heavily infected individuals from host populations (Gordon & Rau 1982; Rousset *et al*. 1996). The reduced levels of parasitism in winter suggest that heavily parasitized fish were removed from the population sometime between the summer and mid-winter (see Spall & Summerfelt 1970; Lemly & Esch 1984). An alternative explanation is that parasites were lost. However, Lemly & Esch (1984) found that metacercarial cysts of trematodes persist in fish during winter and Hoffman (1967) reported that white grub metacercariae survive as long as 4 years in fish at 12 °C.

The experimental data show that diplostomatids can induce host mortality for female fish. Parasites caused substantial mortality in infected fish (27%) compared to uninfected control fish (0%). The mean parasite intensity of lethally infected fish was at least 50% lower than mean intensities observed at Mound Spring during summer. Thus, it is likely that parasites directly induce mortality in the wild. The vast majority of parasite-induced mortality occurred during or immediately following the period of parasite encystment. This same pattern has also been reported for wild populations of centrarchids (Spall & Summerfelt 1970). The presumptive causes of host mortality are haemorrhaging due to the burrowing action of the parasite and visceral tissue damage due to parasite encystment (Spall & Summerfelt 1970). Therefore, after parasites have encysted, hosts may recover. We found that surviving fish were similar for life-history traits, irrespective of treatment (Fig. 2), suggesting that recovery may have occurred for infected fish.

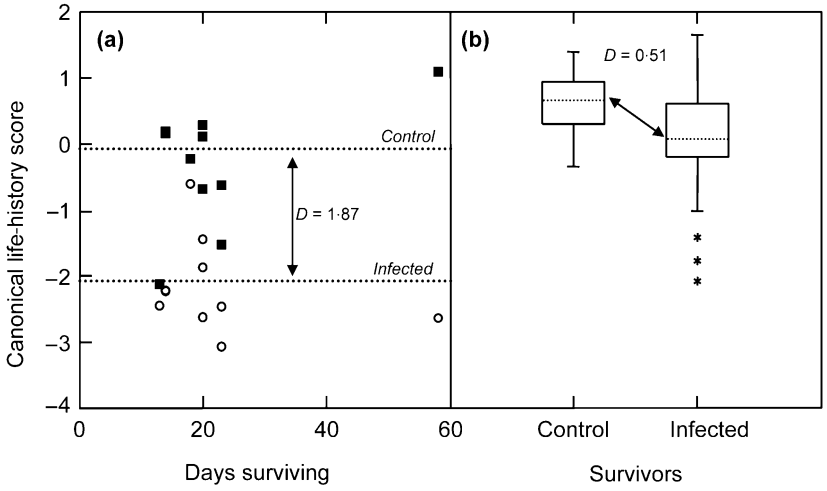

Fig. 2. Life-history canonical scores for (a) non-surviving and (b) surviving experimental *Cyprinodon. tularosa* females. Infected (open circle) and control (filled square) scores are plotted against the length of time fish remained in the experiment (survivorship) for non-surviving fish. Dotted lines represent median values for both control and infected fish. Survivors are represented by box plots of canonical scores. Boxes represent medians and 25% quartiles (50% of the dispersion of data). Bars represent 1·5 times the interquartile range. Values outside this range are represented with asterisks. Positive canonical scores are associated with larger values for fat content and dry mass (see Table 5). Double-headed arrows illustrate the generalized Mahalanobis distances (D) between group means in canonical space. Distances between surviving and non-surviving controls (1·67) and surviving and non-surviving infected fish (2·46) are not shown.

Our data also suggest that a threshold for parasite-induced host mortality exists for *C. tularosa*. Surviving infected fish had a mean parasite intensity of 95·4 cysts per host compared to 182·9 cysts per host for non-surviving fish. These results are striking when compared to winter field parasite intensities. For both wild populations, mean parasite intensities decreased to 94 parasites per host or lower during winter. This indicates that parasite loads must be sufficiently high to cause host mortality.

Parasites may also impact important life history traits associated with over-winter survival. Parasite infection can impact allocation of host resources (see e.g. Sandland & Minchella 2003) through direct competition for energy (Coop & Holmes 1996) or from increased demand for host immunity (Moret & Schmid-Hempel 2000). Parasites caused substantial reductions in growth and fat content for experimental fish. The 23% difference in fat mass suggests that infected fish are less efficient at fat storage. Reduced fat stores are likely to impact over-winter survival (see Reznick & Braun 1987). The lack of an impact on reproductive allocation could suggest a tradeoff, but is due most probably to the fact that most fish were not reproductively active during the experiment.

The possibility exists that our experimental conditions did not match environmental conditions adequately for natural populations in freshwater springs. The experiment was conducted under relatively benign conditions compared to conditions in the wild. Water temperature was held constant at 25 °C and diet was maintained at 5% body mass. Experimental conditions probably influenced fish growth and fat storage as both surviving control and infected fish had increased life history responses compared to fish removed early from the experiment (Fig. 2). In natural habitats water temperature varies greatly (Stockwell & Mulvey 1998) and food availability is probably inconsistent. Parasite exposure risk in the wild is difficult to measure and may differ from the exposure regime used in this experiment. One possibility is that our experimental treatment of *C. tularosa* was severe, and our estimates of costs of parasitism were extreme. We believe that this is probably not the case because we have observed parasite intensities five times greater for fish in natural populations (Table 1) than the maximum level from our experiment. Furthermore, given the high infection rate among snails (55%), parasite exposure risk in the wild may be exceptionally high in this system. Another possibility is that we underestimated costs of parasitism. Other authors have shown that the effects of parasitism are exacerbated by variable environments (Lemly & Esch 1984; Coleman 1993).

Because of their costly nature, white grub parasites may play an important role in the evolutionary or ecological dynamics of *C. tularosa* populations. Here we consider two theoretical models of parasite–host associations that may be relevant to this particular system. First, diplostomatids may influence *C. tularosa* population dynamics. Other authors have demonstrated that parasites may reduce their host populations by inducing host mortality (Anderson & May 1978; Scott & Anderson 1984; Scott & Dobson 1989). Scott & Dobson (1989) posited that indirect life-cycle parasites are unlikely to act in a density dependent manner to regulate host populations because parasite abundance is influenced by a variety of factors not related directly to the density of each host. Our data suggest that white grub parasites may indeed influence the population dynamics of *C. tularosa*. The population decline of the Mound Spring population in 1995 was associated with an outbreak of diplostomatid infection (Pittenger 1996). The results of the current study support the hypothesis that diplostomatids could cause such a population decline.

Secondly, the altered morphology of infected fish may increase vulnerability to predation by piscivorous birds. Infected fish were darker in colour and also suffered from vertebral abnormalities that may also influence swimming performance. Lafferty & Morris (1996) showed that such physical deviations can lead to increased risk of predation. *C. tularosa* populations are clearly impacted by parasites, but whether these morphological changes increase the vulnerability of fish to predation remains to be tested. Further, our observations were made with Salt Creek ESU fish (from Lost River). More research is needed to contrast the difference in infection susceptibility and altered morphology for both ESUs.

In addition to the theoretical implications, our findings also have important relevance to the conservation of *C. tularosa*. The conservation plans of *C. tularosa* calls for the establishment of refuge populations for existing native populations (Stockwell *et al*. 1998; Pittenger & Springer 1999). The results of this study suggest that Mound Spring may not serve as an adequate refuge for Salt Creek fish, because ecological conditions are dissimilar to the native habitat at Salt Creek. Salt Creek fish may not be locally adapted to the ecological conditions, especially parasitism, at Mound Spring. Future translocation efforts should attempt to avoid introducing *C. tularosa* to habitats with novel pathogens.

These results may also have implications for other actively managed species. Translocations are used commonly in the conservation of rare and endangered species (Griffith *et al*. 1989; Hendrickson & Brooks 1991; Stockwell *et al*. 1996). Others have argued that such active management may alter parasite communities with serious implications for host species (Viggers *et al*. 1993; Daszak *et al*. 2000; Stockwell & Leberg 2002). Thus, it is prudent to ask if a proposed translocation may place the targeted taxon at risk to costly pathogens.

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