Oecologia (1989) 80:456–464

Oecologia

Does parasitic infection compromise host survival under extreme environmental conditions? The case for *Cerithidea californica* (Gastropoda: Prosobranchia)

Wayne P. Sousa and Mary Gleason
Department of Zoology, University of California, Berkeley, CA 94720, USA

Summary. This laboratory study examined the influence of parasitic infection by larval trematodes on the survival of extreme environmental conditions by the salt marsh snail, *Cerithidea californica*. Experimental treatments simulated the durations, combinations, and levels of potentially lethal environmental extremes to which the snail is exposed in its natural habitat, as determined from long-term field measurements. No significant difference was found in the rates of mortality suffered by infected and uninfected snails when exposed to simulated, natural extremes of water temperature, water salinity, or exposure in air. Exposure to low levels of dissolved oxygen was the only treatment that caused differential mortality: infected snails died at higher rates than uninfected. This differential mortality was accentuated by high water temperature, and varied with the species of infecting parasite. The potential impact of this interaction between parasitism and anoxia on snail survival and population dynamics is discussed.

Key words: *Cerithidea californica* – Digenean trematode – Host population – Parasitism – Physiological stress

Parasites have a variety of deleterious effects on individual hosts and, in some instances, a dramatic impact on host population size and dynamics (e.g. Anderson 1979; Anderson and May 1982; Holmes 1982; Augspurger 1984; Lessios et al. 1984; Dobson and Hudson 1986; Short et al. 1987). Parasites may affect the sizes of host populations by reducing rates of host reproduction and by increasing rates of host mortality.

Cerithidea californica, the salt marsh snail examined in this study, is first intermediate host to 15 species of castrating, larval trematodes in the study area (Sousa 1983, 1989), and at least 18 species in California (Martin 1955, 1972; Yoshino 1975). The results of previous studies of the influence of larval trematode infections on host longevity (see Discussion) suggest that the degree of differential mortality suffered by infected molluscs depends not only on the particular host-parasite association under study, but also on the kind and level of coincident abiotic stress experienced by the host. The purpose of this laboratory study was to determine whether parasitism by larval trematodes influences the rate of mortality suffered by *Cerithidea californica* when it is exposed to extreme physical conditions. The results allow us to evaluate the potential effect of differential mortality due to parasitism on host population dynamics.

Experimental treatments were designed to simulate as closely as possible, the durations, combinations, and levels of potentially lethal, environmental extremes to which the snail is exposed in its natural habitat. Test conditions were based on long-term, on-site measurements of selected physical variables. The study period encompassed a variety of extreme climatic conditions including a series of severe winter storms in 1982–1983 associated with a strong, Pacific El Niño (Cane 1983; Rasmusson and Wallace 1983), an unsually wet winter in 1985–1986, and a drought in 1987–1988, so our measurements probably span much of the range of abiotic conditions that could influence the impact of parasitism on the survival of *Cerithidea* at our study site.

Methods

Experimental design

Naturally infected and uninfected snails were exposed to laboratory conditions that mimicked the following environmental stresses: 1) desiccation in warm air, 2) immersion in water of high salinity and temperature, 3) immersion in water of low salinity and temperature, and 4) immersion in oxygen-poor water. In each of these separate experiments, controls with more moderate conditions were run simultaneously.

The chosen values of air temperature, water temperature, and water salinity (and combinations thereof) were based on field measurements made over 4.5–7 years at the Pine Gulch Creek (PGC) site in Bolinas Lagoon, Marin County, California (37°55′N, 122°41′W). Snails used in the experiments were collected from this site. The study site, as well as the biology of *Cerithidea* and its trematode parasites are described in Sousa (1983).

Maximum-minimum air temperatures were measured with a thermometer permanently positioned about 1 m above a stand of pickleweed (*Salicornia virginica*), 20 m landward of the lower limit of marsh vegetation, and at a tidal height of approximately 2.7 m above Mean Lower Low Water (MLLW). Recorded air temperatures were read

Offprint requests to: W.P. Sousa

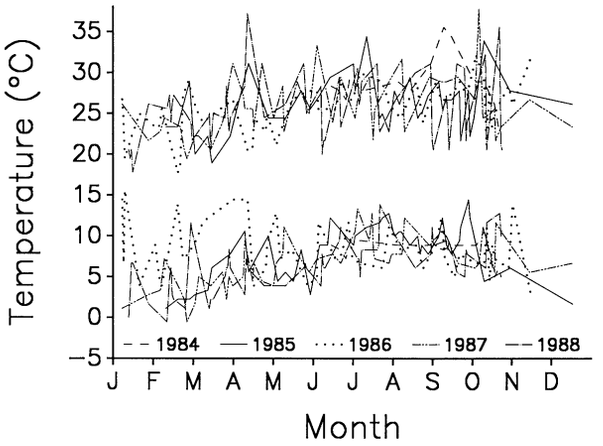

Fig. 1. Maximum-minimum air temperatures (1984–1988) in the salt marsh at the Pine Gulch Creek study site

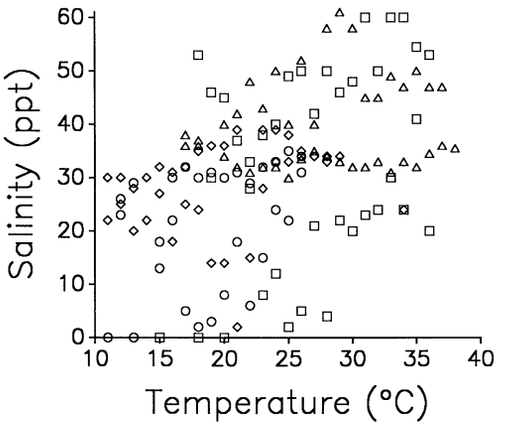

Fig. 2. Salinity and temperature of standing water in salt marsh tidepools at the Pine Gulch Creek study site during daytime low tides in eight years (1981–1988). A total of 670 measurements were made. Plotted points are the maximum and minimum salinities observed at each degree of water temperature recorded in a particular season. Symbols indicate the seasonal quarter of measurement: 22 December–21 March (*circle*), 22 March–21 June (*square*), 22 June–21 September (*triangle*), 22 September–21 December (diamond)

at each visit to the study site from 14 June 1984 to 23 October 1988 (Fig. 1). Temperatures and salinities of standing water in the tidepools inhabited by the snails (Sousa 1983) were measured several times each seasonal quarter during daytime visits (0923–1830 h) at low tide, from 13 June 1981 to 29 March 1988 (Fig. 2). Minimum water temperatures experienced by the snails probably occur at night, and thus are not accurately estimated by our daytime measurements. In shallow tidepools (<10 cm deep), minimum, or nighttime, water temperatures are likely to approach the minimum air temperatures. We therefore used minimum air temperatures as estimates of minimum water temperatures. The particular conditions used in each experiment (Table 1) are described and justified below. Our aim is to tie experimental protocols to natural regimes of abiotic stress in the study area.

Desiccation. During most of the year on the northern coast of California at least one high tide a day covers the +1.3–1.6 m MLLW tidal range (Tide tables, United States Department of Commerce) inhabited by the bulk of the *Cerithidea* population (W. Sousa, unpubl. data), relieving the desiccation stress on snails left stranded outside of tidepools during the intervening period of low water. In the months of March and April, however, there are periods of up to 6 days in which the maximum tidal height does not exceed +1.4–1.5 m MLLW. At these times, the standing water in many of the tidepools drains or evaporates completely, leaving the snails exposed to the air on the dried surface of the mud. Experimental treatments were intended to simulate 5 and 12 day periods of such dry conditions, and were run in an environmental chamber at day-night temperatures approximating maximum-minimum air temperatures recorded in April (Fig. 1, Table 1). Experimental snails were maintained on dry paper towels in 30 cm × 16 cm × 8.5 cm clear plastic boxes, while control snails were kept in similar boxes on paper towels that were resaturated with fresh seawater daily.

High salinity and temperature. At low tide on sunny days in late spring and summer, water in the tidepools may warm to more than 35° C (Fig. 2). Coincident evaporation raises the salinity of the water to over 60 ppt, nearly twice that of seawater. These extreme conditions rarely persist for more than about 4–6 h, because either the sun sets or the site is flooded by an incoming tide. Rainfall rarely occurs during this time of year (W. Sousa, unpubl. data) and, therefore, does not ameliorate high salinity conditions. The experimental treatment simulated 6 h of daylight immersion in such hot, salty water (Table 1). Two controls were run, both with a lower salinity approximately equal to that of seawater: one at the same high water temperature as the experimental treatment; the other at room temperature. The experimental containers were 250 ml glass beakers; plastic mesh restrained the snails below the water surface. The treatment and warm-water control were maintained in a heated water bath. The room temperature control was run on the adjacent lab bench top. All beakers were exposed to ambient, daytime light from a north-facing window.

Low salinity and temperature. Most of the yearly rainfall at the study site occurs from October to April (W. Sousa, unpubl. data). Heavy rains and associated flooding of Pine Gulch Creek, which empties into the lagoon at the study site, can cause sharp reductions in the salinity of standing water in tidepools at ebb tide and in surface waters during flood tide. Snails can experience salinities of 0.0 ppt (Fig. 2) for several days at a time depending on the tidal amplitude, weather conditions, and degree of flooding. From October to late March, no more than 3 successive days pass without a high tide covering the vertical range of the snails (Tide tables, United States Department of Commerce) raising the salinity of the water around them.

To simulate the above conditions, experimental treatments and controls were run for 3 days. Six combinations of water salinity and temperature were evaluated (Table 1): three salinities corresponding to fresh, brackish, and seawater at two water temperatures (approximate winter-spring minimum (Fig. 1) and room temperature control). The experimental procedures were identical to those employed in the high salinity-high temperature trials, except that temper-

Table 1. Designs of environmental stress experiments. See text for methods and reasons for selecting tabulated values

Experiment	Treatment/ control	Duration (h)	Light[a] (Day:Night h)	Temperature (Day:Night °C)	Salinity (ppt)	Dissolved O_2 (ppm)
Desiccation	1	120	12:12	28.4: 8.9	dry in air	
	2	120	12:12	28.4: 8.9	moist in air	
	3	288	12:12	28.4: 8.9	dry in air	
	4	288	12:12	28.4: 8.9	moist in air	
High salinity and temperature	1	6	6:–	38.5:–	60.0	[b]
	2	6	6:–	38.5:–	34.0	[b]
	3	6	6:–	22.0:–	34.0	[b]
Low salinity and temperature	1	72	12:12	5.0: 5.0	0.0	[b]
	2	72	12:12	20.0:20.0	0.0	[b]
	3	72	12:12	5.0: 5.0	20.0	[b]
	4	72	12:12	20.0:20.0	20.0	[b]
	5	72	12:12	5.0: 5.0	35.0	[b]
	6	72	12:12	20.0:20.0	35.0	[b]
Anoxia	1	193	15:9	30.0:18.0	35.0	0.3–2.8
	2	193	15:9	30.0:18.0	35.0	2.5–8.7
	3	534	11:14	20.0:10.0	35.0	0.3–2.5
	4	534	11:14	20.0:10.0	35.0	3.3–8.7

[a] Fluorescent lamps, 20 watts
[b] Not monitored, water was aerated immediately prior to start of experiment

atures (and the light regime) were maintained by environmental chambers rather than water baths. The water in each beaker was replaced daily with aerated water of the appropriate salinity.

Anoxia. There are two circumstances when snails may experience stressful anoxic conditions. The first occurs during the summer and fall after attached mats of green algae (primarily *Enteromorpha* and *Ulva*) and large quantities of drifting algal wrack have accumulated on the mudflats and in high pools inhabited by *Cerithidea*. These algae eventually die and decay, generating anaerobic conditions in surface sediments (evidenced by their blackening) and probably in the near-bottom water of the pools.

Snails may also experience anoxic conditions during the winter when they burrow 1–3 cm beneath the mud surface. Once buried, they remain dormant until warmer spring conditions bring them to the surface to feed and mate (Sousa 1983). This dormancy is occasionally broken in mid-winter on sunny, warm days when snails will return to the surface and feed, only to reburrow with the onset of colder, nighttime temperatures.

We did not attempt to measure the concentrations of dissolved O_2 experienced by snails in the two situations described above. Other studies, however, have shown that, in the absence of aerating bioturbation, the concentration of O_2 in the interstitial water of estuarine sediments falls to 0.0 ppm 1–7 mm below the surface of the sediment (e.g. Sorensen et al. 1979; Revsbech et al. 1980; Baillie 1986). In Sorensen et al's (1979) study, the concentration of O_2 at the sediment surface was found to vary from 7.5 ppm in summer to 11.3 ppm in winter. Everett (1988, pers. comm.) recorded an average O_2 concentration of 2.3 ppm in surface sediments immediately beneath decaying algal mats in Bodega Bay, California, with a minimum value of 1.0 ppm (samples taken in October). At concentrations of 1.0–1.7 ppm, he detected a strong odor of hydrogen sulfide. In contrast, the average concentration of dissolved O_2 in the water immediately overlying the algal mats was 9.9 ppm.

Two experimental treatments were employed: one mimicked an anoxic event in summer/fall and the other, exposure to low O_2 concentrations in winter. Low O_2 concentrations were achieved by bubbling N_2 gas into a 76 l aquarium filled with seawater until the concentration of dissolved O_2 fell to 0.3 ppm. O_2 concentrations were measured with a Nestor model # 8500 portable dissolved oxygen/B.O.D. meter. Experimental snails were introduced into 250 ml flasks that had previously been submerged in the aquarium. Each flask was stoppered underwater to reduce the introduction of air. The top of each flask was then covered with Parafilm held tightly in place with a rubber band. Inevitably, some leakage of air into the flasks occurred causing O_2 concentrations to rise over the course of the experiment to maximum concentrations of 2.5–2.8 ppm. Flasks containing control snails were filled with aerated seawater (8.7 ppm O_2) and stoppered and sealed in the same manner as the low oxygen flasks. This water was replaced at least once daily, but respiration by the enclosed snails over the intervening 13–28 h reduced O_2 concentrations to minimum levels of 2.5–3.3 ppm (summer-winter conditions). As expected, the drop in O_2 concentration was greater in the warm temperature controls. In an effort to reduce the confounding effects of epizoic algal photosynthesis and respiration, the shells of all snails were scrubbed with a brush prior to the experiment to remove as much algae as possible. Experimental temperature and light cycles (Table 1) roughly matched average conditions during these two seasons and were maintained by environmental chambers. The environmental regime in each control was identical to that of the respective treatment.

The two conditions of anoxia described above may last

anywhere from a few hours to many days. Therefore, the durations of the treatments were roughly 1 and 3 weeks (Table 1), which we believe realistically approximate the average length of exposure to anoxia in summer and winter, respectively.

General procedures

Approximately two weeks prior to each experiment, large samples of snails were collected from 2–3 tidepools at the PGC site that were known from prior sampling to have relatively high infection rates. These snails were screened in the laboratory for the presence of nature infections by inducing cercarial larvae to shed (see Sousa 1983). Snails were maintained at a constant temperature of 14° C except when being screened for infections. They were held in closed plastic boxes on paper towels that were regularly dampened with seawater, and were not fed. To reduce the complicating influence of seasonal acclimation (Vernberg and Vernberg 1967; Tallmark and Norrgren 1976), each experiment was run during the season in which the stress it mimicked naturally occurs. Since juvenile *Cerithidea* are rarely infected (Sousa 1983), the question of differential mortality due to parasitism was examined for adult snails only.

Replicates were run for all treatments and controls. Depending on the experiment and the sample size for a particular parasite, each replicate contained about 5–10 individuals. Visual inspection indicated little variation in the rate of mortality among replicates, so we pooled the data (i.e. counts of dead and surviving snails) across replicates to obtain an adequate sample size for statistical analysis. The resultant multidimensional contingency tables were analyzed with log-linear models (Fienberg 1970; Bishop et al. 1975).

The distributions of infections by different species of larval trematodes among size-classes of *Cerithidea* are not homogeneous (Sousa 1983, unpubl. data). Consequently, it was not possible to perfectly match the sizes of snails infected by the different species of parasites tested in the experiments, and some significant differences remained (Appendices 1 and 2). In all cases, however, there was considerable overlap in the size ranges used. The lengths of non-shedding snails used in each trial were matched as closely as possible to those of the infected individuals. The cercarial shedding procedure cannot identify snails that carry immature infections. All non-shedding individuals, dead or alive, were therefore dissected at the end of each experiment to determine if they were indeed uninfected and if so, what sex they were. The sex of an infected individual is often impossible to determine from visual inspection (Sousa 1983) and was not considered in this study.

To distinguish dead from surviving snails at the end of each trial, all individuals were placed in 1 cm deep, freshly aerated seawater for 24 h before final inspection. Just prior to inspection, the water was warmed with a lamp to stimulate the snails. Survivors actively crawled and rapidly retracted their bodies into the shell when touched with a probe. Moribund individuals retracted very slowly and often incompletely, and could not crawl. It was judged that these individuals would probably succumb under natural conditions and they were considered to have been killed by the treatment. Dead animals were immobile and did not retract when touched.

Results

Very few snails died, whether infected or uninfected, in the desiccation, high salinity and temperature, or low salinity and temperature experiments (Table 2). A single snail (infected by the cyathocotylid) died in the control for the 288 h desiccation trial, and no snails died in any of the high salinity and temperature treatments. Mortality was slightly higher in the low salinity and temperature experiment, but less than 5% of the snails died in any of the treatments. Only 11 snails died out of 432 placed in freshwater for 3 days. Snails responded to freshwater by withdrawing tightly into their shells for the duration of the experiment. The numbers of deaths in these experiments were too small to allow statistical comparison among specific categories of infection, or even between pooled categories of infected versus uninfected individuals. It is clear, however, that infected snails did not suffer differential mortality under any of these environmental conditions.

Further, incidental observations and preliminary experiments revealed no evidence of differential mortality due to parasitism under durations of desiccation and low salinity that exceed those measured during our seven years of field monitoring. No snails, uninfected ($n=27$) or infected ($n=43$), died when left in an open plastic box lined with dry paper towels by a south-facing window (21–24° C) for 192 h. Eight out of 12 infected snails accidentally left in a similar dry condition in a 14° C coldroom survived for 3 mo., as did their parasites. Only 7.4% of 27 uninfected snails and 2.3% of 43 infected snails died during a 170 h immersion in freshwater at 20° C. A 260 h freshwater immersion at the same temperature killed 33.3% of uninfected ($n=30$) and 45.0% of infected snails ($n=40$). In neither case, was the difference between the percentages of uninfected and infected snails killed statistically significant (170 h: $G=1.01$, d.f. $=1$, $p=0.315$; 260 h: $G=0.98$, d.f. $=1$, $P=0.322$).

In marked contrast, anoxia caused high mortality at both experimental temperatures (Table 2). Almost no snails, infected or uninfected, died in either of the controls with higher O_2 concentrations. Low expected values for the number of dead snails in these high oxygen conditions precluded analysis of the experiment as a four-way contingency table. Instead, we restricted the analysis to the low oxygen treatments, examining two different three-way tables. The first analysis examined the survival of infected versus uninfected snails, pooled over parasite species and sex of snail (for uninfected snails), as a function of temperature. There was a significant three-way interaction between the variables (Table 3): under low oxygen conditions, infected snails died at a higher rate than uninfected ones, and this differential mortality was enhanced at high temperatures. This influence of temperature is even more striking given that snails in the low temperature treatment were exposed to anoxic conditions for almost three times as long as those in the high temperature treatment. Separate analyses of survival as a function of parasitism at each temperature confirm the strong differential mortality suffered by infected snails (low temperature: $G=43.16$, d.f. $=1$, $P<0.001$; high temperature: $G=98.08$, d.f. $=1$, $P<0.001$).

Analysis of a similar three-way table comparing rates of host mortality under low oxygen conditions caused by each of the four most abundant parasites tested shows that the rate of mortality varied with the species of infecting

Table 2. Percent mortality of parasitized and unparasitized *Cerithidea* in the four experiments. Numbers in parentheses are the sample sizes for each snail condition; sample sizes for parasites are total infections, i.e. the sum of shedding and non-shedding infections (see text for details). See Table 1 for descriptions of treatments and Appendix 1 for key to parasite species codes

Expt./ Treat. #	Snail condition													
	Uninfected		Infected by										Totals	
	M	F	As	Cj	Cy	Ep	Ec	Hr	Mi	Pa	Re	AH	UI	I
Desiccation														
1	0.0	0.0	0.0	–	0.0	0.0	–	–	–	0.0	–	–	0.0	0.0
	(8)	(16)	(7)		(30)	(1)				(10)			(24)	(48)
2	0.0	0.0	0.0	–	0.0	–	–	0.0	–	0.0	0.0	–	0.0	0.0
	(5)	(9)	(7)		(27)			(5)		(14)	(5)		(14)	(58)
3	0.0	0.0	0.0	0.0	0.0	0.0	–	0.0	0.0	0.0	0.0	–	0.0	0.0
	(5)	(10)	(8)	(1)	(32)	(1)		(2)	(1)	(10)	(2)		(15)	(57)
4	0.0	0.0	0.0	–	3.2	0.0	–	–	–	0.0	0.0	–	0.0	1.9
	(10)	(9)	(7)		(31)	(1)				(12)	(2)		(19)	(53)
High salinity and temperature														
1	0.0	0.0	0.0	–	0.0	0.0	0.0	0.0	–	0.0	0.0	–	0.0	0.0
	(10)	(11)	(14)		(31)	(15)	(6)	(16)		(16)	(4)		(21)	(102)
2	0.0	0.0	–	–	0.0	0.0	–	0.0	–	0.0	0.0	–	0.0	0.0
	(10)	(13)			(30)	(1)		(17)		(17)	(1)		(23)	(66)
3	0.0	0.0	0.0	–	0.0	0.0	–	0.0	–	0.0	0.0	–	0.0	0.0
	(8)	(12)	(1)		(34)	(3)		(16)		(15)	(1)		(20)	(70)
Low salinity and temperature														
1	0.0	3.3	0.0	0.0	2.3	0.0	–	0.0	–	4.8	0.0	–	1.9	2.4
	(22)	(30)	(16)	(1)	(43)	(29)		(16)		(62)	(1)		(52)	(168)
2	0.0	0.0	0.0	100.0	4.6	0.0	0.0	0.0	–	4.8	0.0	–	0.0	3.6
	(13)	(32)	(8)	(1)	(44)	(29)	(1)	(16)		(63)	(5)		(45)	(167)
3	0.0	4.2	0.0	50.0	2.1	0.0	–	6.3	–	0.0	0.0	–	2.2	1.8
	(21)	(24)	(9)	(2)	(47)	(30)		(16)		(62)	(1)		(45)	(167)
4	0.0	0.0	0.0	50.0	0.0	0.0	–	6.3	–	3.2	0.0	0.0	0.0	2.4
	(20)	(30)	(9)	(2)	(45)	(29)		(16)		(62)	(2)	(1)	(50)	(166)
5	0.0	0.0	0.0	0.0	0.0	0.0	–	0.0	–	2.2	0.0	–	0.0	0.7
	(11)	(23)	(9)	(1)	(46)	(31)		(16)		(45)	(2)		(34)	(150)
6	11.1	0.0	0.0	–	0.0	0.0	0.0	5.3	0.0	2.3	0.0	–	2.9	1.3
	(9)	(25)	(8)		(45)	(30)	(1)	(19)	(1)	(43)	(2)		(34)	(149)
Anoxia														
1	5.9	0.0	–	–	90.9	63.6	–	100.0	–	87.0	–	–	2.3	86.1
	(17)	(27)			(22)	(21)		(20)		(23)			(44)	(86)
2	0.0	0.0	0.0	–	0.0	0.0	–	5.3	–	0.0	0.0	–	0.0	1.1
	(20)	(19)	(1)		(27)	(20)		(19)		(22)	(2)		(39)	(91)
3	0.0	22.7	0.0	–	80.0	38.1	–	100.0	–	60.0	0.0	–	10.6	67.5
	(25)	(22)	(1)		(20)	(21)		(20)		(20)	(1)		(47)	(83)
4	0.0	0.0	–	–	0.0	0.0	–	0.0	–	4.8	0.0	–	0.0	1.2
	(21)	(26)			(20)	(20)		(21)		(21)	(1)		(47)	(83)

parasite (i.e. significant species × survival interaction, Table 3). As in the first analysis, the mortality caused by the four species of parasites increased significantly with temperature (i.e. significant temperature × survival interaction, Table 3), however, the relative impacts of the four species on host survival did not vary with temperature (i.e. no significant three-way interaction, Table 3). A posteriori comparisons of the percent mortality suffered by the different categories of infected snails revealed that those parasitized by *Himasthla* suffered the highest mortality, and those infected by *Echinoparyphium*, the least (Table 4). Snails infected by the cyathocotylid or *Parorchis* died at intermediate rates.

Discussion

Our laboratory experiments indicate that parasitized *Cerithidea* do not suffer differential mortality under realistic extremes of desiccation, salinity and water temperature. Further, parasitized or not, adult *Cerithidea* exhibit a remarkable physiological tolerance of, and are rarely killed by, such extremes (see also Scott and Cass 1977; McCloy 1979; and Race 1981). Therefore, mortality of neither infected nor uninfected adults due to these particular environmental conditions is likely to be an important contributing factor to changes in snail population density, except under unusual, "catastrophic" circumstances (see below).

How do *Cerithidea's* responses to the above physical stresses compare to those of other mollusc-trematode associations? As we found for *Cerithidea*, parasitic infections do not appear to compromise greatly the physiological responses of other species of marine molluscs to variations in salinity (e.g. sporocyst and redial infections of the snail, *Nassarius obsoletus*: Sindermann and Rosenfield 1957; Kasschau 1975 (but see caveat of Lauckner 1980, p. 372),

Table 3. Results of multidimensional contingency analyses of survival rates in low oxygen treatments of the anoxia experiment (see Table 2). The reported values of the test statistic G are those associated with the deletion of the indicated terms. In other words, they test whether the removal of a term significantly reduces the fit of the model. Factors are water temperature regime (temp), presence of parasites (infect), species of parasite (species), and survival (surv)

Analysis 1: Survival as a function of parasitic infection and regime of water temperature

Interaction	*df*	*G*
temp × infect × surv	1	7.19**

Analysis 2: Survival as a function of species of infecting parasite and regime of water temperature

Interaction[a]	*df*	*G*
temp × species × surv	3	0.55^{ns}
species × surv	3	30.56***
temp × surv	1	7.60**

** $P<0.01$, *** $P<0.001$, ^{ns} $P>0.05$

[a] The temp × species interaction was not tested because it was fixed by the experimental design

Table 4. Statistical comparison of mortality suffered by different categories of infected snails in the low oxygen treatments of the anoxia experiment. A simultaneous test procedure for unplanned comparisons of proportions (Sokal and Rohlf 1981, p. 728) was applied to pooled data from treatments 1 and 3 (see Tables 1 and 2). Lines connect categories with equal rates of mortality ($P>0.05$). See Appendix 1 for parasite species codes. Size of pooled sample in parentheses

Infecting parasite	Ep	Pa	Cy	Hr
% mortality	52.4 (42)	74.4 (43)	85.7 (42)	100.0 (40)

Riel 1975; metacercarial infections of the clam, *Cardium edule*: Lauckner 1983). On the other hand, our finding that parasitized *Cerithidea* did not suffer increased mortality under high temperature conditions is unusual. Larval trematode infections have been shown experimentally to cause increased mortality at high water temperatures in a wide variety of molluscan hosts, including the marine snails *Nassarius obsoletus* (Vernberg and Vernberg 1963, but see opposite result of Riel 1975), *N. reticulatus* (Tallmark and Norrgren 1976), and *Littorina littorea* (McDaniel 1969; Lauckner 1980), the freshwater snail, *Biomphalaria glabrata* (Lee and Cheng 1971), and the marine clam, *Cardium edule* (Lauckner 1983). We know of no previous experimental studies of the effect of trematode parasitism on a molluscan host's ability to survive desiccation stress.

The coastal lagoons of central and southern California occasionally experience natural disturbances that alter physical conditions to such a degree that even the broad tolerances of *Cerithidea* are exceeded. One such disturbance is the closure of a lagoon mouth by oceanic deposition of beach sand (Scott and Cass 1977; Zedler et al. 1986; Onuf 1987). The impounded water may be fresh to brackish in winter and spring months due to high rainfall and terrestrial runoff, but turn hypersaline in summer due to evaporation. In addition, as evaporation proceeds and the level of the impounded water drops, large areas of the mudflat may be exposed to the air for weeks or months at a time, with no relief by tidal inundation.

Meandering shifts in the directions and positions of creek channels can also cause extreme alterations of the local lagoon environment. Such changes in creek morphometry are generated by the high stream flow associated with large winter rainstorms, and the resulting deposition of eroded sediments. They produce sharp, semipermanent reductions in the salinity of sites in or near the new course of the diverted stream (W. Sousa, pers. obs.). Both of the above processes can expose snails to prolonged periods of extremely harsh conditions that exceed their physiological tolerances, and can cause the extinction or near extinction of local snail populations (Zedler and Nordby 1986; W. Sousa, unpubl. data). In such circumstances, the question of differential mortality due to parasitism is moot. All snails, infected or uninfected, are killed.

Anoxia was the only environmental stress we tested that caused differential mortality of parasitized *Cerithidea*. This differential mortality was greater under a warm, summer temperature regime than a colder, winter one. We are aware of only one previous study that has tested for such an effect. Using a similar experimental design, Olivier et al. (1953) found that the freshwater snail *Australorbis glabratus* (*Biophalaria glabrata*) suffers no mortality when exposed to anaerobic conditions for 6 h whether infected by *Schistosoma mansoni* or uninfected, but 16 h of anoxia caused a marked differential mortality of infected snails.

An interacting stress associated with anoxic conditions in both our laboratory experiment and in nature (Theede et al. 1969; Fenchel and Riedl 1970; Newell 1979) is exposure to potentially toxic concentrations of hydrogen sulfide, an endproduct of anaerobic decomposition (sulfate reduction) by bacteria. The seawater of our low oxygen treatments turned a murky gray to black over the course of the experiment and smelled strongly of hydrogen sulfide. Although we did not measure its concentration in the experimental flasks, hydrogen sulfide poisoning probably contributed to the differential mortality of infected snails.

A likely explanation for our finding that infected *Cerithidea* survived the low oxygen treatment better under cooler, winter conditions, is that physiological stresses associated with anaerobic conditions are reduced at low temperatures due to the concomitant decline in snail and parasite metabolic rates. Theede et al. (1969) found a similar temperature-dependent response to both anoxia and hydrogen sulfide in several species of benthic marine invertebrates; however, these authors did not examine the influence of parasitism on this response.

Several studies have demonstrated that the impact of infection on host metabolism and/or survival varies with the species of trematode involved (e.g. Lunetta and Vernberg 1971; Robson and Williams 1971; Watts 1971; Cheng et al. 1983). Such species-specific effects may explain the differential mortality suffered under anoxic conditions by *Cerithidea* infected with different parasites. A definitive answer to this and other questions concerning the mechanisms responsible for the observed differential mortality of infected snails under anoxic conditions awaits detailed physiological studies of *Cerithidea* and its trematode parasites.

At present, we can only speculate about the frequency and intensity with which anoxic conditions cause differential mortality of infected snails in nature. Colder winter temperatures and the associated reduction in snail and parasite metabolisms may ameliorate low oxygen stress in buried, aestivating snails. In addition, this stress is relieved when snails return to the surface on warmer, sunny days. Therefore, infected individuals may not suffer strong disproportionate mortality during this time of year. In contrast, warmer temperatures of summer and fall accelerate the decomposition of dying algal mats, as well as the metabolic rates of snails and parasites, accentuating low oxygen and hydrogen sulfide stress on infected snails. In estuarine environments, the rate of sulfate reduction and resultant production of hydrogen sulfide is higher, and the process occurs at shallower sediment depths, in warm summer months (Sorensen et al. 1979). Thus, the risk of mortality due to anoxia is probably greatest in summer and fall. Snails may be able to avoid these stresses by moving to pool microhabitats where oxygen concentrations are higher, i.e. shallow edges of pools and the adjacent, moist, emergent substratum. Snails commonly exhibit this pattern of local distribution in summer (W. Sousa, pers. obs.) possibly reflecting such avoidance behavior. McCloy (1979) demonstrated that snails that become trapped beneath algal mats often suffer high mortality. He did not note, however, whether this mortality fell disproportionately on infected individuals. We plan to test for this pattern in future field experiments.

Quantifying differential mortality of infected snails under natural anoxic conditions will not be easy. Its occurrence is likely to be very patchy in space and time, reflecting the highly variable distribution and persistence of attached and drifting algal mats (Everett 1988). Its intensity and frequency will also depend on spatial and temporal patterns of infection within the snail population, including the species composition of the parasite guild. These patterns are highly variable (W. Sousa, unpubl. data). Finally, unlike some sources of snail mortality such as crab predation, death due to anoxia leaves no distinctive trace on the shell of the deceased. We cannot, therefore, distinguish snails that are killed by anoxia from those that were killed by other agents that also do not damage the shell. Despite these difficulties, anoxic conditions remain a potentially important cause of mortality in *Cerithidea* populations, along with predation by crabs and disturbance by storm flooding (McCloy 1979; W. Sousa, unpubl. data). Our findings indicate that trematode parasitism exacerbates the negative impact of anoxic conditions on snail survival and population density. Under more moderate environmental conditions, however, the castrating effect of larval trematode infections (Sousa 1983) is likely to have a greater negative impact on snail population density than any reduction these parasites cause in snail longevity.

Acknowledgements. We thank S. Allison, T. Grosholz, P. Kotanen, A. Kuris, B. Mitchell, S. Naeem, G. Roderick, and an anonymous reviewer for helpful critical comments on earlier drafts of this manuscript. E. Adams and P. Kotanen provided valuable statistical and computing consultation. Parasite species identifications were confirmed by H. Ching. The results of a preliminary study by N. Comfort guided the design of our investigation. Access to the field site was kindly allowed by the Marin County Department of Parks and Recreation. This research was supported by National Science Foundation grants DEB-8004192, BSR-8300082, BSR-8505871, and BSR-8516522 to the first author.

Appendix 1. Median, minimum, and maximum lengths (mm, aperture to apex) of snails used in experimental trails. Sample sizes appear in respective tables of results. A median without an associated min-max value indicates a sample size of one

Snail condition	Code[a]	Experiment							
		Desiccation		High salinity and temperature		Low salinity and temperature		Anoxia	
		median	min–max	median	min–max	median	min–max	median	min–max
Uninfected									
All		26.3	23.1–29.8	26.0	21.5–30.1	23.7	17.0–30.6	24.3	18.4–29.4
Male	UI-M	26.7	23.6–29.4	25.9	23.7–28.5	23.0	17.5–30.6	24.0	19.8–29.4
Female	UI-F	26.1	23.1–29.8	26.4	21.5–30.1	24.3	17.0–30.1	24.4	18.4–28.2
Infected by									
Acanthoparyphium spinulosum	As	25.8	23.3–29.7	27.0	22.5–34.1	26.4	10.0–34.0	26.8	20.9–32.1
Catatropis johnstoni	Cj	27.6	–	–	–	22.0	21.0–25.8	–	–
Unidentified cyathocotylid	Cy	29.1	24.0–33.9	29.1	22.1–35.6	27.8	16.3–34.2	29.1	21.2–33.4
Echinoparyphium sp.	Ep	27.8	26.0–31.5	27.9	22.6–33.3	24.2	17.0–35.8	24.1	17.9–32.2
Euhaplorchis californiensis	Ec	–	–	32.7	29.0–36.8	26.2	24.8–32.9	–	–
Himasthla rhigedana	Hr	26.7	25.8–29.7	30.3	25.5–39.6	28.3	21.3–36.6	29.8	23.6–35.4
Unidentified microphallid	Mi	30.0	–	–	–	23.6	–	–	–
Parorchis acanthus	Pa	28.7	23.4–36.2	30.7	23.0–38.7	26.6	17.2–38.9	27.1	20.9–35.7
Unidentified renicolid	Re	27.0	25.1–29.6	27.7	25.5–29.0	26.4	22.0–31.0	24.8	23.4–25.6
Double infection: *Austrobilharzia* sp. and *H. rhigedana*	AH	–	–	–	–	35.3	–	–	–

[a] Same codes are used in Tables 2 and 4, and Appendix 2

Appendix 2. Statistical comparisons for each experiment of lengths of snails in each condition listed in Table 1, for which the sample size was greater than one. Nonparametric Kruskal-Wallis tests and Dunn's method of a posteriori multiple comparisons were employed because large variation in sample size resulted in heterogeneous variances. Data were non-normal in a number of cases. Assumptions of parametric statistical tests could not be met with transformations. Lines connect conditions with the same distributions of snail lengths ($P > 0.05$)

	Kruskal-Wallis statistic H	Dunn's multiple comparisons								
Experiment		*Snail condition*[a]								
Desiccation	88.8***	As	UI	Hr	Re	Ep	Pa	Cy		
High salinity and temperature	132.1***	UI	As	Ep	Re	Cy	Hr	Pa	Ec	
Low salinity and temperature	256.8***	UI[b]	Ep	Cj	As	Re	Ec	Pa	Hr	Cy
Anoxia	137.8***	UI	Ep	Re	As	Pa	Hr	Cy[c]		

*** $P < 0.001$

[a] See Appendix 1 for key to codes

[b] Lengths of uninfected female snails were significantly greater than those of uninfected male snails in this experiment ($H = 23.8$, $P < 0.001$). In the other experiments, they were not significantly different ($P > 0.05$)

[c] Pa < Cy, Cy = Hr = Ec = Re

References

Anderson RM (1979) The influence of parasitic infection on the dynamics of host population growth. In: Anderson RM, Turner BD, Taylor LR (eds) Population dynamics. Blackwell Sci Publ, Oxford, pp 245–281

Anderson RM, May RM (eds) (1982) Population biology of infectious diseases. Dahlem Workshop Rep: Life Sci Res Rep no 25. Springer, Berlin Heidelberg New York

Augspurger CK (1984) Seedling survival of tropical tree species: interactions of dispersal distance, light gaps, and pathogens. Ecology 65:1705–1712

Baillie PW (1986) Oxygenation of intertidal estuarine sediments by benthic microalgal photosynthesis. Est Coast Shelf Sci 22:143–159

Bishop YMM, Fienberg SE, Holland PW (1975) Discrete multivariate analysis: theory and practice. MIT Press, Cambridge, Mass

Cane MA (1983) Oceanographic events during El Niño. Science 222:1189–1195

Cheng TC, Howland KH, Moran HJ, Sullivan JT (1983) Studies on parasitic castration: aminopeptidase activity levels and protein concentrations in *Ilyanassa obsoleta* (Mollusca) parasitized by larval trematodes. J Invertebr Pathol 42:42–50

Dobson AP, Hudson PJ (1986) Parasites, disease and the structure of ecological communities. Trends Ecol Evol 1:11–15

Everett R (1988) The role of a seasonal macroalga in determining patterns of distribution and abundance in an intertidal infaunal assemblage. PhD dissertation, University of California, Berkeley

Fenchel TM, Riedl RJ (1970) The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. Mar Biol 7:255–268

Fienberg SE (1970) The analysis of multidimensional contingency tables. Ecology 51:419–433

Holmes JC (1982) Impact of infectious disease agents on the population growth and geographical distribution of animals. In: Anderson RM, May RM (eds) Population biology of infectious diseases, Dahlem Workshop Rep: Life Sci Res Rep, no 25. Springer, Berlin Heidelberg New York, pp 37–51

Kasschau MR (1975) Changes in concentrations of free amino acids in larval stages of the trematode, *Himasthla quissetensis* and its intermediate host, *Nassarius obsoletus*. Comp Biochem Physiol B 51:273–280

Lauckner G (1980) Diseases of mollusca: gastropoda. In: Kinne O (ed) Diseases of marine animals, vol I, John Wiley & Sons, New York, pp 311–424

Lauckner G (1983) Diseases of mollusca: bivalvia. In: Kinne O (ed) Diseases of marine animals, vol II, Biologische Anstalt Helgoland, Hamburg, pp 477–961

Lee FO, Cheng TC (1971) *Schistosoma mansoni* infection in *Biomphalaria glabrata:* alterations in heart rate and thermal tolerance in the host. J Invertebr Pathol 18:412–418

Lessios HA, Robertson DR, Cubit JD (1984) Spread of *Diadema* mass mortality through the Caribbean. Science 226:335–337

Lunetta JE, Vernberg WB (1971) Fatty acid composition of parasitized and nonparasitized tissue of the mud-flat snail, *Nassarius obsoleta* (Say). Exp Parasitol 30:244–248

Martin WE (1955) Seasonal infections of the snail *Cerithidea californica* Haldeman, with larval trematodes. In: Essays in the Natural Sciences in Honor of Captain Allan Hancock. University of Southern California Press, Los Angeles, pp 203–210

Martin WE (1972) An annotated key to the cercariae that develop in the snail *Cerithidea californica*. Bull South Cal Acad Sci 71:39–43

McCloy MJ (1979) Population regulation in the deposit-feeding mesogastropod *Cerithidea californica* as it occurs in a San Diego salt marsh habitat. MA thesis, California State University, San Diego

McDaniel SJ (1969) *Littorina littorea:* lowered heat tolerance due to *Cryptocotyle lingua*. Exp Parasitol 25:13–15

Newell RC (1979) Biology of intertidal animals. Marine Ecological Surveys Ltd, Faversham, Kent

Olivier L, von Brand T, Mehlman B (1953) The influence of lack of oxygen on *Schistosoma mansoni* cercariae and on infected *Australorbis glabratus*. Exp Parasitol 2:258–270

Onuf CP (1987) The ecology of Mugu Lagoon, California: an estuarine profile. US Fish and Wildl Serv Biol Rep no 85 (7.15)

Race MS (1981) Field ecology and natural history of *Cerithidea californica* (Gastropoda: Prosobranchia) in San Francisco Bay. Veliger 24:18–27

Rasmusson EM, Wallace JM (1983) Meteorological aspects of the El Niño/Southern Oscillation. Science 222:1195–1202

Revsbech NP, Sorensen J, Blackburn TH, Lomholt JP (1980) Distribution of oxygen in marine sediments measured with microelectrodes. Limnol Oceanogr 25:403–411

Riel A (1975) Effect of trematodes on survival of *Nassarius obsoletus* (Say). Proc Malac Soc Lond 41:527–528

Robson EM, Williams IC (1971) Relationship of some species of Digenea with the marine prosobranch *Littorina littorea* (L) II. The effect of larval Digenea on the reproductive biology of *L. littorea*. J Helminthol 45:145–159

Scott DB, Cass TL (1977) Response of *Cerithidea californica* (Haldeman) to lowered salinities and its paleoecological implications. Bull South Calif Acad Sci 76:60–63

Short FT, Muehlstein LK, Porter D (1987) Eelgrass wasting disease: cause and recurrence of a marine epidemic. Biol Bull (Woods Hole, Mass) 173:557–562

Sindermann C, Rosenfield A (1957) The ecology of marine dermatitis-producing schistosomes III. Oxygen consumption of normal and parasitized *Nassarius obsoletus* (*Nassa obsoleta*) under varying conditions of salinity. J Parasitol 43 [S]:28

Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed, WH Freeman & Co, San Francisco

Sorensen J, Jorgensen BB, Revsbech NP (1979) A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. Microb Ecol 5:105–115

Sousa WP (1983) Host life history and the effect of parasitic castration on growth: a field study of *Cerithidea californica* Haldeman (Gastropoda: Prosobranchia) and its trematode parasites. J Exp Mar Biol Ecol 73:273–296

Sousa WP (1989) Spatial scale and the processes structuring a guild of larval trematode parasites. In: Esch GW, Aho JM, Bush AO, Kennedy CR (eds) Patterns and Processes in Parasite Communities. Chapman and Hall, London (in press)

Tallmark B, Norrgren G (1976) The influence of parasitic trematodes on the ecology of *Nassarius reticulatus* (L) in Gullmar Fjord (Sweden). Zoon 4:149–154

Theede H, Ponat A, Hiroki K, Schlieper C (1969) Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. Mar Biol 2:325–337

Vernberg WB, Vernberg FJ (1963) Influence of parasitism on thermal resistance of the mud-flat snail, *Nassarius obsoletus* Say. Exp Parasitol 14:330–332

Vernberg WB, Vernberg FJ (1967) Interrelationships between parasites and their hosts. III. Effect of larval trematodes on the thermal metabolic response of their molluscan host. Exp Parasitol 20:225–231

Watts SDM (1971) Effects of larval Digenea on the free amino acid pool of *Littorina littorea* (L). Parasitology 62:361–366

Yoshino TP (1975) A seasonal and histologic study of larval Digenea infecting *Cerithidea californica* (Gastropoda: Prosobranchia) from Goleta Slough, Santa Barbara County, California. Veliger 18:156–161

Zedler JB, Nordby C (1986) The ecology of Tijuana Estuary: an estuarine profile. US Fish and Wildl Serv Biol Rep no 85 (7.5)

Zedler JB, Covin J, Nordby C, Williams P, Boland J (1986) Catastrophic events reveal the dynamic nature of salt-marsh vegetation in Southern California. Estuaries 9:75–80

Received April 26, 1988