AN ABSTRACT OF THE THESIS OF

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Title: The Effects of Parasites on Host Behavior: Who Benefits?

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Some parasites may modify the behavior of their hosts. Altered behaviors may: 1) benefit the host in that they defend against the pathogen, 2) benefit the pathogen and represent manipulations of the host response, and 3) benefit neither the host or the pathogen and simply be a product of the host response to infection.

In this thesis I examine four host/parasite systems. For each system, I explore host/parasite behavioral interactions, and examine them with regard to selective pressures that may be acting on both the host and the parasite.

I test the Hamilton and Zuk hypothesis in 26 species of lizards. I find an inverse relationship between a lizard species’ brightness and parasite prevalence. My result lend credence to criticisms of the Hamilton and Zuk Hypothesis.

If infection does occur, animals may alter their behavior to impair the growth and reproduction of the parasite. To test this prediction, I examine behavioral
thermoregulation in two strains of the snail *Biomphalaria glabrata*, one resistant to, and one susceptible to, the parasite *Schistosoma mansoni*. The preferred temperature of infected snails drops five weeks after exposure to the parasite.

I propose the hypothesis that pathogen-induced host defense responses result in altered host behaviors and enhanced predation. In particular, I examine the effects of the acute phase response (a physiological response whose symptoms include fever, reduced activity and malaise) on antipredatory behavior in bullfrog (*Rana catesbeiana*) tadpoles. This host response is associated with the preliminary stages of infection with many pathogens yet its behavioral effects have received little attention. I find that the stereotypical effects of the acute phase response can lead to increased predation. I suggest that altered behaviors may afford some parasites a potential pathway to their next host.

I examine the behavioral effects of a yeast, *Candida* spp., a single-host parasite species in its natural host, the red-legged frog (*Rana aurora*). Infected tadpoles exhibit the same behavioral modifications that are noted in bacteria injected bullfrog tadpoles. These results suggest that some altered behaviors may occur due to a host response to infection and not due to parasitic manipulation.
The Effects of Parasites on Host Behavior:  
Who Benefits?  

by  

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THE EFFECTS OF PARASITES ON HOST BEHAVIOR: WHO BENEFITS?

CHAPTER ONE
INTRODUCTION

In the broad sense, parasites compose the majority of species on earth and strongly affect the evolutionary biology, ecology and sexual interactions of the organisms they infect. Parasites, which can include viruses, bacteria, protists, helminths and arthropods (Margolis et al., 1982), can have wide ranging physiological and behavioral effects on their hosts.

Physiological changes induced by pathogens have concerned physicians for thousands of years and physiologists for hundreds. Recently, accounts of behavior alterations in parasitized hosts have attracted much attention in behavioral ecology. Yet behavioral and physiological changes cannot be separated. Behavior stems from physiology, and behaviors can alter physiology. An examination of the interplay of physiology and behavior is key to understanding how parasites may be associated with alterations of host behavior.

Ewald (1980; 1983; 1987) has addressed the problem of how to interpret the altered physiological responses of some parasitized hosts. Although he did not directly consider them, behavioral changes associated with
parasites can be included in his system and tied-in with physiological changes. He states that alterations can be explained in three ways: I) alterations that benefit the host in that they defend against the pathogen, II) alterations that benefit the pathogen and represent manipulations of the host response, and III) alterations that benefit neither the host or the pathogen and are only "side effects" of infection (Ewald, 1980).

In this thesis I examine four host/parasite systems. For each system, I explore the interrelationships between parasites and host behaviors. I also speculate on the selective pressures that may be acting on both the host and the parasite.

BEHAVIORAL ALTERATIONS THAT BENEFIT HOSTS - PARASITE MEDIATED SEXUAL SELECTION

Darwin (1871) proposed that elaborate and apparently deleterious secondary sex characteristics of male animals exist due to sexual selection. The existence of exhaustive bellowing in mammals such as elk, elaborate plumage and complex song in birds, and vivid coloration in many reptiles, amphibians and fishes would therefore be due to female choice for these traits. Energetically expensive and showy traits may evolve due to sexual selection, but this begs the question of why females are
not attracted to dull males (Kirkpatrick, 1987; Clayton, 1991).

Hamilton and Zuk (1982) suggested that, if parasite resistance is a heritable trait, animals may reduce the ravages of parasites on reproductive success by choosing parasite-free (and therefore parasite-resistant) mates. Not only would females avoid contact with directly-transmitted parasites but they also benefit indirectly when their offspring inherit disease-resistant genes. The Hamilton and Zuk hypothesis states that females "discern" parasite resistance by using secondary sexual traits that are only fully expressed when an individual is healthy and parasite-free.

Hamilton and Zuk (1982) examined surveys of blood parasites in 109 species of North American passerine birds. They found weak, but statistically significant, associations between prevalence of infection (five genera of protozoa and one nematode) and showy traits (male "brightness", female "brightness" and male song). To determine a species plumage-brightness and song showiness Hamilton and Zuk used photographs and recordings. Hamilton and Zuk (1982) made two major predictions. First, that within a species, bright males being naturally disease resistant would have fewer parasites than dull males (tested by Borgia, 1986; Houde
and Torio, 1992; Kennedy et al., 1987; Pomiankowski 1989; Milinski and Bakker, 1990; and Zuk, 1987; 1988; 1990). Second, that between species, those species with high parasite burdens would have experienced selective pressure for bright characteristics as an honest indicator of disease resistance.

The intraspecific prediction that parasites alter male showiness has been supported in some insects (Zuk, 1987; 1988), fishes (Houde and Torio; Kennedy, 1987; Milinski and Bakker, 1990) and birds (Pruett-Jones et al., 1990; Clayton, 1990; Moller, 1988; 1990; 1991; Hillgarth, 1990; Weatherhead, 1990; Zuk, 1990). The prediction has also not been supported in some amphibians (Hausfater et al., 1990), reptiles (Schall, 1986; Ressel and Schall, 1989), and birds (Borgia, 1986; Borgia and Collis 1989; 1990; Gibson, 1990).

The interspecific prediction has been supported in some birds (Hamilton and Zuk, 1982; Read, 1987), fish (Ward, 1988; 1989). The hypothesis has also not been supported in some birds (Read and Harvey, 1989a; 1989b; Read and Weary, 1990).

The inconclusive nature of tests of parasite-mediated sexual selection may be due to inherent problems associated with tests of the Hamilton and Zuk hypothesis. These include misidentification of
parasites, inappropriate choice of parasite taxa, inaccurate measurement of parasite load, not controlling for unequal exposure of hosts to parasites, measurements of host showiness by human standards, and the role of parasite manipulation of hosts to augment parasite transmission (Clayton, 1991).

Tests of the Hamilton and Zuk hypothesis in lizards are particularly interesting. Hamilton and Zuk predicted an inverse correlation between male showiness and parasite load. Indeed, as a general trend, sick vertebrates do tend to be more drab than healthy conspecifics (Hamilton and Zuk, 1982). Yet Schall (1986) found that bright blue male Cnemidophorus arubensis lizard were more likely to be infected with a haemogregarine parasite that were dull males. Similarly, Ressel and Schall (1989) found that malaria infected male fence lizards (Sceloporus occidentalis) were significantly more showy than conspecifics of the same age because sub-adult infected males display markings normally restricted to adult males. In this species, adult males develop vivid black coloration on their ventral surface. This black coloration contrasts sharply with parallel blue stripes on their ventral surface. Males engage in "push-up" displays that reveal their venters and are important in intersexual
interactions (Noble, 1934).

Evidence from *Sceloporus occidentalis* contradicts the intraspecific prediction of the Hamilton and Zuk hypothesis since infected males are brightly colored yet physiologically debilitated and suffer lowered reproductive success (Schall, 1983; Schall and Dearing, 1987). Nor does the malarial parasite *Plasmodium mexicanum* benefit from making its host more attractive to females since the parasite is not directly transmitted (such as lice) but is vector borne. Ressel and Schall (1989) suggested that the striking color of infected males may support Zahavi's (1976) handicap principle, i.e., showiness of infected males may be used by females as a mark of quality in that only truly exceptional males could survive with such handicaps (see also Dawkins, 1992).

**BEHAVIORAL ALTERATIONS THAT BENEFIT HOSTS - BEHAVIORS THAT MINIMIZE CONTACT WITH, OR SLOW THE GROWTH OF PARASITES**

Besides choosing parasite-free mates, animals often change their behavior in other ways both to avoid being parasitized and to enhance their ability to fight off infection (reviewed by Hart, 1988; 1990). Many animals preen to remove ectoparasites, avoid reusing nesting
areas, avoid areas where ticks are abundant, and flee from aerial parasites such as botflies that are seeking hosts in which to lay eggs (Hart, 1990; 1992). Endoparasites are avoided by altering foraging strategies. Many ungulates avoid feeding near recently deposited feces (Taylor, 1954; Michel, 1955). Other strategies are to defecate only in specific areas of a territory, to avoid large congregations of possibly disease-carrying conspecifics, to avoid cannibalism, and to change their diet when infected (Hart, 1990; see also Freeland, 1983).

Even if potential hosts are unable to avoid being infected, many are able to use behavioral means to slow the growth of, or to eliminate, a parasite. Infected vertebrates exhibit reductions in food intake which may serve to reduce iron levels (a necessary nutrient for gram negative bacteria, Hart, 1990). Indeed, mice starved for three days have increased survival when challenged with the bacterium *Listeria monocytogenes* (Wing and Young, 1980). Sick mammals often are inactive and lethargic (Hart, 1990). Hart (1990) interprets these behaviors as mechanisms to husband resources to fight off infection. Chernin (1967) reported that snails (*Biomphalaria glabrata*) infected with trematodes (*Schistosoma glabrata*) were sluggish. Infected animals
often seek-out and dwell in warm microhabitats. This raises the temperature of the animal (behavioral fever) and results in increased survivorship during infection for both ectotherms (Kluger, 1975) and endotherms (Dimock et al., 1991). Fever increases survival rate during bacterial infections by lowering blood iron concentrations, mobilizing the immune response, enhancing cytolysis, and exposing some bacteria to lethal temperatures (Kluger, 1990).

To examine behavioral alterations that benefit hosts, I test Hamilton and Zuk's interspecific hypothesis using lizards in chapter two. Lizards are often brightly colored and species vary in parasite loads. Has selective pressure from female choice in heavily infested species resulted in the evolution of showy males as a mark of disease resistance? In chapter three, I examine if trematode infected snails alter thermoregulatory behaviors.

BEHAVIORAL ALTERATIONS THAT BENEFIT PARASITES - PARASITE MANIPULATIONS OF HOST BEHAVIOR AND GROSS PHYSIOLOGY

Host behavior may also be altered due to parasitic manipulations that enhance a parasite's rate of transmission. Bethel and Holmes (1972) proposed three possible strategies that parasites may use to augment
the capture of intermediate hosts by definitive hosts: 1) decreasing host stamina, 2) increasing host conspicuousness, 3) disorienting the host and altering the ability of the host to elude predators.

Decreased host stamina is a common symptom of infection (Hart, 1988). As early as 1906, it was noticed that butterfish (Peprilus triacanthus) with cestodes in their musculature had reduced activity (Linton, 1906). Shinner fish infected with cestodes were also found to be sluggish and inhabited warmer, shallow water (Dence, 1958; Bethel and Holmes, 1972). Hydatid cestode cysts in ungulates have been correlated with severe decreases in stamina and ability to elude capture by predators (Fenstermacher, 1937; Cowan, 1951; Ritcey and Edwards, 1958 and Mech, 1966). Reduced ambulatory activity has been observed in CD-1 mice (Mus musculus) with Trichinosis-encysted muscles (Zohar and Rau, 1986).

Increased conspicuousness is another strategy that parasites use to attract visually stimulated predators (Holmes and Bethel, 1972). Altered pigment patterns in fish are associated with trematode infections (Rothschild, 1962). Hares exhibit inappropriate seasonal color changes when infected with cestodes (Leiby and Dyer, 1971). Coral polyps infected with
trematode metacercaria appear as bright pink swollen nodules that the corals are unable to retract into protective calices (Aeby, 1991). The trematode’s rate of transmission was enhanced as colored corals were consumed by fish-definitive hosts at a higher rate than uninfected corals. The most dramatic example of parasite-induced conspicuousness is that of a snail that when infected by a trematode exhibits pulsating tentacles which attract avian definitive hosts (Hecker and Thomas, 1965).

Disoriented hosts often display inappropriate behaviors, have a reduced fright-response, and may separate from the herd due to neurological damage. Mice artificially infected with nematodes have increased activity levels due to brain lesions (Rau, 1984; Hay et al., 1985; Dolinsky et al., 1985). Lemmings also exhibit less of a fright response when infected with a coccidian parasite (Quinn et al., 1987). Blindness is a common result of trematode infections in the eyes of fish (reviewed by Bethel and Holmes, 1972; Crowden and Broom, 1980). Ants infected with trematode metacercaria exhibit a suite of behaviors that increase their chance of being accidentally ingested by sheep—the trematode’s definitive host (Carney, 1969; see also Curtis, 1990). Some of the above examples illustrate
the exhibition by hosts of behaviors that have no
relation to normal activities, while others are normal
behaviors exhibited at inappropriate times.

Gammarid crustaceans infected with acanthocephalans
have reversed phototaxis and an altered fright response
to predacious birds (Bethel and Holmes, 1972; 1977).
Isopods parasitized by acanthocephalans are
disproportionately found on light colored substrate,
unsheltered areas and in dry areas of their habitat.
These altered behaviors make the isopods more
susceptible to starlings which are the definitive host
of the parasite (Moore, 1983). Indeed, behavioral
alterations are so common among acanthocephalan
intermediate hosts, that Moore (1984) has suggested that
all acanthocephalans may have evolved from an ancestor
that altered the behavior of its intermediate host.
Cestode infected beetles fail to exhibit normal
photophobic behavior (Hurd and Fogo, 1991). *Plasmodium*
infected rodents exhibit reduced ability to fend off
biting mosquito vectors during the period when the
rodents are most infective (Day and Edman, 1983).
Mosquitoes carrying *Plasmodium* have reduced ability to
locate blood vessels. This results in both increased
probing times and an increase in the number of hosts
contacted during a feeding session (Rossignol et al.,
1984). Sand flies infected with *Leishmania* exhibit a response similar to *Plasmodium* infected mosquitoes (Beach, et al., 1985).

**BEHAVIORAL ALTERATIONS THAT BENEFIT PARASITES - PARASITE MANIPULATIONS OF HOST IMMUNE AND HORMONAL FUNCTIONS**

Observations of altered behaviors are common. However, studies that explore humoral and hormonal mechanisms associated with altered behaviors are rare. Changes in the clinging behaviors of gammarids, noticed by Bethel and Holmes (1972; 1977), are due to acanthocephalan-induced changes in serotonin-sensitive pathways (Helluy and Holmes, 1990). Stickleback fish infected with cestodes spent more time at the surface than uninfected controls. Lester (1971) found that this was due to infected fish having higher respiration rates.

To understand how the physiology of an animal is liable to change during infection, it is necessary to examine the changes that occur during infection. During the initial stages of infection tissue macrophages and blood monocytes are activated and they release cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF). These cytokines serve as an early warning system to nearby cells which begins a
cascading response of fever, the acute phase response, and the differentiation and activation of T-cells, B-cells and macrophages. (Titus et al., 1991). Interestingly, mosquito (*Aedes aegypti*) salivary glands contain a substance that inhibits TNF release (Bissonnette et al., 1992).

IL-1 causes the release of IL-6 and TNF. IL-1 reduces appetite (McCarthy et al., 1985), induces general malaise (Bluthe et al., 1992) and slow-wave sleep (Kluger, 1990). IL-6 also induces fever and is released during psychological stress (LeMay et al., 1990).

The acute phase response also occurs during the initial stages of infection and has two components. The first local phase involves hemostasis, inflammation, and kinin generation. The second systemic phase results in pain, leukocytosis, the manufacture of acute phase proteins such as C-reactive protein, increased protein catabolism, increased gluconeogenesis, changes in blood concentrations of heavy metals, increases in cortisol, glucagon, catecholamines, and thyroxin (reviewed by Stadnyk and Gauldie, 1991).

Febrile (i.e. elevated) temperatures are detrimental to the growth of some bacteria and some stages of *Plasmodium*. Yet overproduction of cytokines can also be
detrimental. Brain damaging and fatal temperatures for hosts are usually just a few degrees above febrile temperatures. Furthermore, overproduction of TNF causes the wasting and gauntness of cancer and AIDS patients. TNF's side effects in humans are muscle stiffness, muscle pain, nausea, and vomiting (Clark, 1987). Indeed, the pathology of cerebral malaria may largely be due to TNF (Clark, 1987). During the acute phase response the liver of humans has a reduced oxidase system which results in a decreased ability to detoxify pathogen-derived toxins (Stadnyk and Gauldie, 1991). Nematode infected mice show a similar response (Tekwani et al., 1987).

Paradoxically, the immune system of vertebrates may enhance the transmission of certain parasites. Doenhoff et al. (1978) examined mice infected with the human blood fluke Schistosoma mansoni. In this system, an adult parasite-pair inhabit mesenteric arteries of their mammalian definitive host. Eggs are released and, to be infective, must travel from mesenteric arteries through a thick intestinal wall to the intestinal lumen from where they are eventually excreted. How the nonmotile eggs are able to reach the lumen is unclear. Interestingly, although immunocompetent mice release parasite eggs, immunodeficient mice do not (Doenhoff et
al, 1978). Yet both groups of mice have identical worm burdens with identical fecundity rates. When infected immunodeficient mice were given T-cells, they began to excrete Schistosome eggs (Doenhoff et al., 1985). Baboons with Schistosoma mansoni infections possess intestinal granuloma that contain Schistosome eggs and host derived neutrophils, macrophages, and lymphocytes (Damian et al., 1984). Damian (1987) suggests that although the eggs are nonmotile, immune cells such as T-lymphocytes, B-lymphocytes, plasma cells, eosinophils, neutrophils, fibroblasts and macrophages are motile. He proposes, and provides evidence, that the egg/immune cell granuloma travels as a unit from the mesenteric arteries to the lumen of the intestine. Once in the lumen the granuloma breaks down and the egg is released.

Some parasites may actively cause a host defense response and then benefit from the resulting behavioral changes. The inflammatory response can be utilized by parasites. Mycobacterium and Salmonella utilize traveling monocytes to spread from their initial point of invasion (Parker and Schneider, 1981). Schistosomes and trypanosomes are able to rapidly shed host antigens (Parker and Schneider, 1981), and mammals infected with bacteria often have high levels of bacterial proteins in their blood (Rake, 1933). This may act as "chaff", to
confuse the immune system as to the actual location of the pathogen. Bacterial releases of this nature would result in massive production of cytokines which would result in a cascade of physiological and behavioral effects. Some of these changes in behavior, i.e. drowsiness and altered thermoregulation, may result in the death of the host through predation. Some multi-host parasites may be under selective pressure to induce such changes at a parasite life-history stage that is conducive for transfer to a subsequent host.

In chapter four, I explore the behavioral effects of the host defense response. This response may be available to parasites as a mechanism to alter intermediate-host behavior in ways that lead to the definitive host consuming the intermediate host.

III) BEHAVIORAL ALTERATIONS THAT BENEFIT NEITHER THE HOSTS NOR THE PARASITES

Evolutionary biologists rarely examine host changes that benefit neither the host or the pathogen. Although Ewald (1980) brings attention to these changes, he uses it as a "lump-all" category for alterations of hosts that do not fit into his other two categories. An examination of these "side-effects of infection" is overdue because alterations of hosts are almost always
viewed as adaptive to either the host or the parasite (see also Dobzhansky, 1956; Gould and Lewontin, 1979; Mayr, 1983 and Wright, 1949).

Although unexplainable from the adaptationist viewpoint, altered host behaviors are also associated with parasites that do not gain fitness-benefits from these altered behaviors. For example, mice (Peromyscus maniculatus) are more susceptible to predation by weasels if the mice are infected with two or more bot fly maggots (Smith, 1978). Infected mice are less active. Bot fly larvae die when their mouse host is consumed. Thus, altered behaviors are harmful to both the host and the parasite. Mite infestations of an homopteran insect result in increased predation rates - yet the mite is not transmitted to most predators (LaMunyon and Eisner, 1990). Ectoparasitic copepods are attracted to moving trout (Poulin et al., 1991), yet once a trout is infected its activity levels increase which results in further infestations. The net result is a weak host and fewer resources for each individual parasite. The trout dies and the parasites are thereby deprived of a source of nutrients. Unless the copepods are kin (untested), behavioral alterations that result from infection benefit neither the host nor the parasite. Some of these studies may have examined a
predator that is not the definitive host but other unobserved predators may be (or have been, Connell, 1980) the definitive host. Yet many of the parasites in this category have only a single definitive host and are directly transmitted. The unexplainable nature of these studies deserves closer attention.

In this thesis, I will examine the hypothesis that pathogens cause selective pressure for: 1) hosts to modify their behavior to avoid becoming infected 2) altered host behaviors that, along with physiological changes, reduce the growth and detrimental effect of parasites; 3) altered behaviors that may enhance the growth and reproduction of the pathogen; and 4) altered behaviors that do not benefit the host or the pathogen and are only a byproduct of substances hosts release during infection.

In chapter two, I explore how potential hosts may alter their mating systems to avoid becoming infected. Specifically I examine the predictions of the Hamilton and Zuk hypothesis (1982) using lizards as a model system. The hypothesis predicts that species with high parasite loads should have evolved showy characteristics as a true indicator of parasite resistance. Contrary to the predictions of the hypotheses, I found a significant inverse correlation between a species' parasite load and
its showiness.

In chapter three, I explore how a snail, *Biomphalaria glabrata*, alters its normal thermoregulatory behavior when infected with the trematode *Schistosoma mansoni*. I found that infected snails thermoregulate at lower temperatures than healthy controls. This may be advantageous to the snail because the parasite has impaired development at lower temperatures.

In chapter four, I examine behavioral alterations that occur in tadpoles during the acute phase response. Compared to invertebrates, anurans have sophisticated defense systems, possessing many of the immune system components of mammals, including cell-mediated and humoral immunity. They possess IgM, IgY (IgG-like), and IgX (related to IgA) immunoglobulin. Anurans have T and B cells, lymphoid tissue, histocompatibility complexes, complement system, and phagocytosing cells, and magainins (peptides with antibiotic and antifungal abilities, Tinsley, 1989). Furthermore, they are capable of antibody responses that include immunological memory.

I found that tadpoles with activated defense systems have impaired refuge seeking behavior and suffer increased predation by newts (*Taricha granulosa*).
Manipulations of this response may provide multi-host parasites with a method to augment transition to a definitive host.

In chapter five, I build on my results from chapter four and investigate how infection with a pathogenic yeast alters the behavior of tadpoles. Infection results in many symptoms of the acute phase response, e.g. fever, and results in a similar suite of behaviors that I discovered in chapter four. However, in this system neither the host nor the parasite benefits from behavioral alterations.

Finally, in the summary, I tie together these results and propose a framework to interpret behavioral alterations of parasitized hosts.
CHAPTER TWO

PARASITE LOAD AND BRIGHTNESS IN LIZARDS:
AN INTERSPECIFIC TEST OF THE HAMILTON AND ZUK HYPOTHESIS

by
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and
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ABSTRACT

Hamilton and Zuk (1982) hypothesized a positive correlation between a species sexual showiness and its level of parasitic infection. We tested the hypothesis in 26 species of lizards, members of a class of vertebrates never before used to test the model. The prevalence of parasites was determined using published lists of parasites found in wild lizard populations. An index of showiness (brightness) was derived by scoring photographs of lizards in natural settings. Contrary to expectations of Hamilton and Zuk, we found an inverse correlation between a lizard species' brightness and parasite prevalence. No correlation was found between a species' brightness and the number of parasite genera, species, or percentage of individual infecting parasite taxa. These results are discussed in relation to other interspecific tests of the hypothesis.
INTRODUCTION

Hamilton and Zuk (1982) proposed that there should be a positive association between a species' sexual showiness and its level of parasitic infection. They predicted that interspecifically, highly parasitized species are more showy than those with less of a parasite load, and intraspecifically, that males with more conspicuous secondary sexual characters have a lower parasite load than those with less conspicuous characters. In species where female choice of mates is prevalent, it is assumed that females will choose the showiest males because amplified secondary sexual characters should reflect a relatively low parasite burden. Individuals that choose mates with a lower parasite (or disease) load, would be favored by natural selection if parasite (or disease) resistance is at least partially heritable.

The Hamilton and Zuk (1982) hypothesis has been tested in several species from varying taxonomic groups. The intraspecific aspect of the hypothesis has been tested by Borgia (1986), Kennedy, Endler, Poynton et al. (1987), Milinski and Bakker (1990), Pomiankowski (1989), and Zuk (1987; 1988; 1990). Many studies have also focused on the interspecific element of the hypothesis. Positive correlations of parasite prevalence and showiness have been suggested for birds (Hamilton and
Zuk, 1982; Read, 1987; Read and Harvey, 1989a and Read and Weary, 1990) and fish (Ward, 1988; 1989). However, support for the hypothesis is not conclusive because some analyses of particular data sets seem to support it (Hamilton and Zuk, 1982; Read, 1987; Ward, 1988) whereas others do not (Read and Harvey, 1989a; Read and Weary, 1990). Discrepancies in how data sets are analyzed has added to the confusion. For example, using independently derived plumage scores Read and Harvey (1989a) reexamined Hamilton and Zuk's original data and found no evidence to support the hypothesis.

An Interspecific Test

To further test the interspecific aspect of the Hamilton and Zuk hypothesis, we examined the relationship between sexual showiness (brightness) and parasitic infection among lizard species. Lizards seem to be an ideal group in which to test the Hamilton and Zuk hypothesis. The presence of bright male colors are important in lizard sexual displays (Noble, 1934; Schall and Sarni, 1987; Schall and Dearing, 1987) and lizard color varies with parasite prevalence (Ressel and Schall, 1989). However, Ressel and Schall (1989) found that contrary to expectations of the Hamilton and Zuk hypothesis, male Sceloporus occidentalis lizards
possessed an increased degree of bold black ventral pigment when infected with the malarial pathogen *Plasmodium mexicanum*. This is particularly interesting considering the drastic decrease in male reproductive success when suffering from malaria due to an inability to engage in energetic male/male interactions (Schall, 1983; Schall and Dearing, 1987). The dark venters of sexually mature but young infected males, contrasts sharply with the dull colored venters of young noninfected males. A heavily pigmented ventral surface is normally the sign of an older mature male. Ressel and Schall (1989) thought that to their eyes "... a male *Sceloporus occidentalis* venter accented with copious black is the most impressive color pattern". Similarly, in the lizard *Cnemidophorus arubensis*, bright blue males had higher levels of infection with a haemogregarine parasite (Schall, 1986).

Previous tests (e.g. Hamilton and Zuk, 1982; Read, 1987; Read and Weary, 1990; and Ward, 1988) encompassed potentially confounding variation by examining extremely broad taxonomic groups. Read and Weary (1990) have suggested that interspecific comparisons may suffer from statistically non-independent species points. This could arise if the species examined were not phylogenetically independent. Clusters of closely
related species within a larger data set would be treated as independent points. This could skew the results when these species clusters are compared to other clusters (see also Pagel and Harvey, 1988).

We investigated predictions of the hypothesis in 26 species of lizards from 14 genera and 8 families (Table II.1). Furthermore, we attempted to reduce and equalize the phylogenetic variation between species by concentrating on two families of iguanian lizards, the Phrynosomatidae (eight species examined) and the Polychridae (six species examined, see Frost and Etheridge, 1989 for recent phylogenies). The taxonomic level of family was chosen since it is the most restrictive classification for which an adequate sample size of lizard species and their parasites was available. Key ecological characteristics of the species were also examined to determine their relationship with parasite prevalence and sexual showiness. Since this is the first interspecific study using reptiles to specifically test Hamilton and Zuk's hypothesis, results from our analysis will add to the generalization between parasite prevalence and sexual showiness.
METHODS

To locate studies numerically listing the endoparasites of lizard families containing four or more species of lizards, we examined all studies of iguanian parasites listed in Zoological Record from 1960 - 1989. To our knowledge only the six studies examined meet this criterion. Only families from the Americas were examined since only for these groups were we able to obtain adequate photographs.

We determined the mean number of parasite species and genera in 26 lizard species using lists of endoparasites found in 1580 free ranging lizards that were compiled by Waitz (1961), Telford (1970), Pearce and Tanner (1973), Benes (1985), Lyon (1986) and Bundy et al (1987). The investigators made no attempts to associate parasite prevalence with color. We obtained mean parasite prevalence by summing the individuals infected by each parasite species and dividing by the number of individuals of that lizard species in the study (for explanation of terms see Margolis et al., 1982). Furthermore, to examine if differences in mode of transmission and life cycle stage of the parasites affected the color/parasite prevalence relationships, we derived and examined the percentage of the total number
of parasite species and parasite genera made up of flagellates, ciliates, amoebas, sporozoans, platyhelminths, nematodes, or acanthocephala separately for each lizard species. Because some lizards were infected with more than one parasite species, the total number of infections exceeded the number of lizards (see Hamilton and Zuk, 1982). In the examination of parasite prevalence, all parasites were considered equivalent to each other in pathogenic effects whether they were in their intermediate or definitive host. This may be justified in that the theory predicts only that males must demonstrate overall parasite resistance and not resistance to particular types (Ward, 1988). However, certain parasites such as blood-borne hematozoa can have far greater deleterious effects on reproductive fitness than, for example, intestinal parasites (Schall, 1983; Ewald, 1983). For this reason we investigated the relationship between the parasite taxa examined and a species’ brightness.

As in Read (1987) and Ward (1988), brightness scores for each lizard species were obtained by asking naive associates to score brightness. Six colleagues judged brightness on a ten point scale (1 = dull, 10 = bright and conspicuous) from either Kodachrome slides (collection of R. M. Storm) or pictures of male lizards
from field guides (Behler and King, 1988; Schwartz and Henderson, 1985) for head, ventral body, dorsal body and tail brightness. An average value for each species was derived. All photographs included areas of the body used during displays. Two judges scored the Kodachrome slides and four scored the book pictures. For species exhibiting large morphological variation over their range, more than one photograph was used and the results were averaged. The statistical methods used to determine a species' color score were similar to those of Read (1987) and Ward (1988).

Read and Harvey (1989a; 1989b) used two-tailed statistical tests because they believed that the null hypothesis would be rejected had a significant negative relationship been found. Zuk (1989) has questioned this assumption claiming that a negative correlation would not support the Hamilton and Zuk hypothesis, stating that only one-tailed tests should be used. Given the unresolved nature of this disagreement and the increased tendency of a type I statistical error if one-tailed tests are used, we believe that two-tailed tests should initially be used, followed by one-tailed tests if P-values lie between 0.05 and 0.1. All values reported are two-tailed P-values unless otherwise specified.

As in Read (1987) and Read and Harvey (1989a), we
examined the association between parasite prevalence and a lizard species' brightness scores using Spearman's Rank correlation. We also examined if brightness is correlated with the number of parasite genera or species. Most tests (Hamilton and Zuk, 1982; Read, 1987; Read and Harvey, 1989a; Read and Weary, 1990; Ward, 1988, 1989) of the Hamilton and Zuk hypothesis pooled data from more than one study to determine the parasites load of a vertebrate species. We believe that the literature on the parasites of lizards is inadequate to indicate the parasite prevalence of individual lizard species. For this reason, and to control for differences in methods of data collection between parasite studies (see for example Kuris and Blaustein, 1977), we not only pooled data from separate studies but we also examined data from each study separately, with regard to brightness scores. Furthermore, in an attempt to control for differences between lizard families, studies that included the Phrynosomatidae (Telford 1970) and Polychridae (Bundy et al. 1987), the families with the largest number of lizard species samples, were used to determine if within these two families brightness was correlated with any index of parasite load.

Ecological factors were analyzed using Spearman's Rank correlation to determine if additional variables
might affect parasite prevalence or brightness score. The variables were: (A) food gathering, whether a species is a sit-and-wait predator or an active forager; (B) strata, whether a species is most often found (1) on the ground, (2) between ground level and one meter, or (3) above one meter [for the Anoles of Bundy et al., (1987) the criteria were (1) trunk/ground, (2) twig, (3) trunk/crown]; (C) month of initial breeding; (D) total length of breeding season (weeks); (E) size (total length in mm); and (F) altitude of capture (m). These data were obtained from Behler and King (1988) and Stebbins (1954).
RESULTS

The color scores among judges (Table II.1) were normally distributed and they were correlated [mean Spearman rank coefficient ($r_s = 0.64$, range $0.36 - 0.81$, $P < 0.05$)]. The mean scores of the Kodachrome and the book values were highly correlated ($r_s = 0.89$, $P < 0.05$).

In all of the studies we examined we failed to find a positive correlation between a lizard species' brightness and any measure of parasite load (Table II.2). Results from the single large study used in our analyses (Telford, 1970) indicated a significant (one-tailed) inverse correlation between parasite prevalence and brightness ($r_s = -0.448$, $P = 0.038$). Analyzed using a two-tailed test the correlation would no longer be significant with a $P$-value of 0.076. Telford's study encompassed a taxonomically broad group of seven families, twelve genera and eighteen species, including two subspecies. When we restricted our analyses of the Telford study to the monophyletic Phrynosomatidae ($N = 8$ species), no correlation was found between brightness and any of the measures of parasite load.

The analyses of the pooled studies revealed no correlation between species' brightness and any measure of parasite load. For all studies pooled the altitude
of capture was the only ecological variable that was correlated with overall parasite prevalence \([r_i = -0.87 \ P < 0.01 \ (Table \ II.3)]\). When altitude-of-capture was controlled for (A. Read, pers. comm.), by dividing the lizard species into three separate altitude groups (0 - 600m, 601 - 1000m and 1001+m), none of the studies individually, or pooled, showed any correlation between species' brightness and parasite prevalence, the number of parasite genera, parasite species, or any of the ecological variables.

Using data from each of the smaller studies and the Polychridae study individually, no correlation was found between a species brightness score and either overall parasite prevalence, number of parasite genera, or parasite species. The presence of any particular parasite taxon was not correlated with a species brightness (Table II.4).
DISCUSSION

Our analysis does not support the Hamilton and Zuk hypothesis that highly parasitized species are more showy than those species with reduced parasite prevalences. Among 951 lizards from 18 lizard species in our one large study (Telford, 1970) we actually found an inverse correlation, using one-tailed tests, between species' brightness and parasite prevalence, thus, brighter lizard species had fewer parasites. Our finding supports the contention of Read and Harvey (1989b) that continued female choice of bright parasite resistant males may lead to a negative correlation between parasite load and species brightness. We report this one-tailed value because previous studies of lizard parasites (Schall, 1986; Ressel and Schall, 1989) suggest a negative correlation between species color and parasite load. Reporting the results of one-tailed tests is important because this may indicate a significant trend in the correlation between parasite load and lizard color.

The number of parasite species, genera, or percentages of individual parasite taxa were not correlated with a lizard species' brightness. Nonsignificant results were also found in the five smaller studies, both individually and when we pooled
them with Telford’s (1970) study. Of six ecological attributes examined in relation to parasite prevalence and brightness, only the altitude of capture was correlated, being inversely related to parasite prevalence. When we controlled for altitude-of-capture in Telford’s (1970) study, by dividing the lizards into three separate altitudes groups, no correlation was noted between a lizard species’ brightness and parasite prevalence. We do not feel that this detracts from the significance of the inverse correlation we found in Telford’s study (1970) between species’ brightness and parasite prevalence since the lack of significance could be due to the small number of lizard species (4, 6 and 8) in each of these groups. The fact that only Telford’s study (1970), and not the pooled analyses, yielded a significant correlation between a lizard species’ brightness and parasite prevalence could be due to a number of factors. Telford’s data set is the largest (18 species and 954 animals) and most comprehensive of the studies we examined. Furthermore, widely different methods between studies in examining the lizards for parasites, may render a pooled analysis of limited use and could lead to spurious correlations (see discussion in Kuris and Blaustein, 1977).

Hamilton and Zuk (1982) may be correct that a male’s
brightness advertises health and vigor to females. Yet brightness traits are also under many other selective pressures, largely from male-male interaction, camouflage from predators, and thermoregulatory factors. For example, Norris (1967) showed that desert lizards were darker dorsally in the morning, thereby increasing UV absorption, and lighter at mid-day to facilitate reflection of incident solar radiation.

Bright secondary sexual characteristics are important in lizard social communication (Schall and Sarni, 1987; Schall and Dearing, 1987; Cooper, 1988), yet non-visual cues such as vocalizations or odors, might also be involved. Although the lizards censused in this study are not known to vocalize, the degree and form of chemical signals could be important variables in determining a species' "showiness", as has been suggested for mammals (Blaustein, 1981). Moreover, color may significantly change with behavioral context. For example, an individual may show intense colors when aggressive and be rather drab when not aroused.

An interesting form of showiness has been described in the lizard Dipsosaurus dorsalis (Alberts, 1989). While the color score we obtained for this species was a drab 2.3 (out of 10), Alberts (1989) has shown that D. dorsalis is actually conspicuous compared to its desert
background when viewed in the near ultraviolet (wavelengths the animal is capable of perceiving). If *D. dorsalis* females are using the near ultraviolet to determine brightness then the color score we obtained using the visible spectrum could be incorrect. Thus, it is possible that human investigators may not accurately be assessing color patterns as they are perceived by some animals.

Difficulties are inherent when testing the Hamilton and Zuk hypothesis. If the analyses are conducted among too broad a taxonomic group, then morphological, ecological and historical differences between species can reduce the significance of any parasite/color relation (Read and Harvey, 1989a). Hamilton and Zuk (1982) examined a sampling of species from an entire order (*Passeriformes*). Read (1987) considered a similarly broad group although he attempted to control for taxonomic and behavioral variables. Ward (1988) limited his analyses to comparisons between families, yet his study averaged fewer than three species per family (Table II.5). Using Telford's (1970) broad based study encompassing seven families we found a negative correlation between brightness and parasite prevalence - the reverse of what Hamilton and Zuk (1982) would predict. We also limited part of our study to a narrow
monophyletic group yet we failed to support the hypothesis.

One potential problem with correlational studies testing the Hamilton and Zuk hypothesis (Hamilton and Zuk, 1982; Read, 1987; Ward, 1988; 1989) is that all parasite species are given equal weight regardless of the host's position in the parasite's life cycle, pathogenicity, or mode of transmission. However, Ewald (1987) has shown that modes of transmission are important determinants in parasite pathogenicity. Perhaps it is prudent to differentiate among types of parasites rather than to lump entire taxa under headings such as "blood-borne". Instead of approaching parasites as homogenous units with constant effects, tests of the hypothesis should explore how ecological variables among parasite groups affect host reproductive success. For this reason we investigated not only overall parasite prevalence, but also if any particular parasite taxa was correlated with a species brightness.

Similarly, the accuracy of measuring parasite prevalence differ among parasite taxa. While counting helminths infesting the gut may be an accurate indication of parasite prevalence, data on hematozoa numbers from stained blood smears are less reliable (Cox, 1989).
A further potentially more serious problem with previous studies, and one only partially addressed by our study, is that of measuring parasite prevalence at the species level rather than on a population level. Variation in pathogen resistance among populations is not addressed, nor is it clear if the geographic range of all parasites is coincident with all host populations. Furthermore, brightness may vary among populations. To properly address these issues parasites and brightness should be measured between closely located ecologically similar populations.

By limiting part of our analyses to the family level, historical and ecological variance between species was minimized yet our results failed to find evidence of a positive association between a species’ sexual showiness and its degree of parasitic infection as proposed by Hamilton and Zuk (1982). We found that a negative correlation exists in lizards which lends support to Read and Harvey’s (1989b) interpretation of the hypothesis. Additional studies, testing other vertebrates hosts and their parasites, and addressing the problems listed above, will be necessary before the overall significance of the hypothesis is ascertained.
ACKNOWLEDGEMENTS

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<table>
<thead>
<tr>
<th>Lizard species</th>
<th>No. Individuals/Study</th>
<th>Derived Mean Brightness Score</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anolis grahami</td>
<td>14</td>
<td>6.8</td>
<td>2</td>
</tr>
<tr>
<td>Anolis sargei</td>
<td>12</td>
<td>5.9</td>
<td>2</td>
</tr>
<tr>
<td>Eumeces skiltonianus</td>
<td>21</td>
<td>5.8</td>
<td>5, 6</td>
</tr>
<tr>
<td>Sceloporus magister</td>
<td>115</td>
<td>5.8</td>
<td>1, 4, 5</td>
</tr>
<tr>
<td>Coleonyx variegatus</td>
<td>71</td>
<td>5.4</td>
<td>1, 5</td>
</tr>
<tr>
<td>Sceloporus orcutti</td>
<td>23</td>
<td>5.2</td>
<td>5</td>
</tr>
<tr>
<td>Anolis garmani</td>
<td>4</td>
<td>5.0</td>
<td>2</td>
</tr>
<tr>
<td>Xantusia henshawi</td>
<td>51</td>
<td>5.0</td>
<td>5</td>
</tr>
<tr>
<td>Anolis opalinus</td>
<td>18</td>
<td>4.9</td>
<td>2</td>
</tr>
<tr>
<td>Callisaurus draconoides</td>
<td>19</td>
<td>4.6</td>
<td>1, 5</td>
</tr>
<tr>
<td>Eumeces gilberti</td>
<td>6</td>
<td>4.4</td>
<td>5</td>
</tr>
<tr>
<td>Anolis valencienni</td>
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<td>4.3</td>
<td>2</td>
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<td>Uta steinegeri steinegeri</td>
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<td>4.2</td>
<td>1, 5</td>
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<td>Gambelia wislizenii</td>
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<td>3, 5, 6</td>
</tr>
<tr>
<td>Uta stansburiana hesperis</td>
<td>22</td>
<td>4.1</td>
<td>5, 6</td>
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<tr>
<td>Cnemidophorus tigris</td>
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<td>3.8</td>
<td>1, 3, 5</td>
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<td>Xantusia riversiana</td>
<td>78</td>
<td>3.6</td>
<td>5</td>
</tr>
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<td>Anolis lineatopus</td>
<td>41</td>
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<td>2</td>
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<td>5</td>
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<td>2.9</td>
<td>4</td>
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<td>Sceloporus graciosus</td>
<td>220</td>
<td>2.8</td>
<td>3, 4, 5, 6</td>
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<tr>
<td>Uma notata</td>
<td>24</td>
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<td>5</td>
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<tr>
<td>Phrynosoma solare</td>
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<td>2.5</td>
<td>1</td>
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<td>2.3</td>
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<td>Sauromalus obesus</td>
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<td>Urosaurus graciosus</td>
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<td>1.7</td>
<td>5</td>
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1 = Benes (1985)  
2 = Bundy et al., (1987)  
3 = Lyon (1986)  
4 = Pearce and Tanner (1973)  
5 = Telford (1970)  
6 = Waitz (1961)
<table>
<thead>
<tr>
<th>Study</th>
<th>#Spp</th>
<th>#Liz</th>
<th>Brightness vs.</th>
<th>Parasite Prev.</th>
<th>No. Par. Genera</th>
<th>No. Par. Species</th>
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</thead>
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<tr>
<td>Benes (1985)</td>
<td>6</td>
<td>271</td>
<td></td>
<td>-0.700</td>
<td>0.738</td>
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<td>Bundy et al. (1987) X</td>
<td>6</td>
<td>96</td>
<td></td>
<td>0.257</td>
<td>-0.406</td>
<td>-0.406</td>
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<td>Pearce and Tanner (1973)</td>
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<td>55</td>
<td></td>
<td>-0.200</td>
<td>-0.949</td>
<td>0.633</td>
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<td>Lyon (1986)</td>
<td>4</td>
<td>183</td>
<td></td>
<td>0.800</td>
<td>0.447</td>
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<tr>
<td>Telford (1970)</td>
<td>18</td>
<td>954</td>
<td>-0.488*</td>
<td></td>
<td>0.050</td>
<td>0.040</td>
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<tr>
<td>Waitz (1961)</td>
<td>4</td>
<td>21</td>
<td>0.800</td>
<td></td>
<td>0.258</td>
<td>0.258</td>
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<tr>
<td>Telford (1970) XX</td>
<td>8</td>
<td>679</td>
<td>-0.200</td>
<td></td>
<td>0.400</td>
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</tr>
<tr>
<td>All Studies Pooled</td>
<td>26</td>
<td>1580</td>
<td>-0.316</td>
<td>-0.055</td>
<td>-0.085</td>
<td></td>
</tr>
</tbody>
</table>

P > 0.05 in all tests.
* P = 0.0383 (one-tailed). Two-tailed P = 0.076.
X Polychridae
XX Phrynosomatidae
### TABLE II.3

Relationships ($r_i$) between Ecological Variables and Parasite Prevalence, Number of Parasite Genera and Lizard Species Brightness

<table>
<thead>
<tr>
<th>Variable</th>
<th>correlation with parasite prevalence</th>
<th>correlation with number of par. gen. $r$</th>
<th>correlation with brightness $r_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food gathering</td>
<td>0.358</td>
<td>-0.184</td>
<td>-0.103</td>
</tr>
<tr>
<td>Strata</td>
<td>0.073</td>
<td>-0.755</td>
<td>0.368</td>
</tr>
<tr>
<td>Month of breeding</td>
<td>-0.078</td>
<td>-0.099</td>
<td>0.178</td>
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<tr>
<td>Length of breeding</td>
<td>0.144</td>
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<tr>
<td>Size</td>
<td>0.340</td>
<td>0.054</td>
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<tr>
<td>Altitude</td>
<td>-0.871*</td>
<td>0.381</td>
<td>0.357</td>
</tr>
</tbody>
</table>

* = $P < 0.05$

$P > 0.05$ for all other tests.
TABLE II.4

Relationship ($r_i$) between the Percent of a Parasite Taxon Infecting a Lizard Species and Lizard Species Brightness

<table>
<thead>
<tr>
<th>Parasite Taxa</th>
<th>$r_i$</th>
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</thead>
<tbody>
<tr>
<td>Flagellates</td>
<td>-0.136</td>
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<tr>
<td>Ciliates</td>
<td>0.000</td>
</tr>
<tr>
<td>Amoebas</td>
<td>0.014</td>
</tr>
<tr>
<td>Sporozoans</td>
<td>-0.236</td>
</tr>
<tr>
<td>Platyhelminths</td>
<td>-0.312</td>
</tr>
<tr>
<td>Nematodes</td>
<td>0.037</td>
</tr>
<tr>
<td>Acanthocephalans</td>
<td>0.314</td>
</tr>
</tbody>
</table>

$P > 0.05$ in all tests.
TABLE II.5

Variables and Outcomes of Interspecific Tests of the Hamilton and Zuk (1982) Hypothesis

<table>
<thead>
<tr>
<th>Study</th>
<th>Host Taxa</th>
<th>Taxonomic Level</th>
<th>#Spp.</th>
<th>Parasite Taxa</th>
<th>Measure of Parasitic Infection</th>
<th>Sig.</th>
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<tr>
<td>Present study sauria</td>
<td>suborder</td>
<td>26</td>
<td>1-7</td>
<td>prevalence</td>
<td>n.s.*</td>
<td></td>
</tr>
<tr>
<td>Present study sauria</td>
<td></td>
<td></td>
<td></td>
<td># gen. &amp; spp.</td>
<td>n.s.</td>
<td></td>
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<tr>
<td>Present Study iguanids</td>
<td>family</td>
<td>6 &amp; 8</td>
<td></td>
<td>prevalence</td>
<td>n.s.</td>
<td></td>
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<tr>
<td>Present study</td>
<td></td>
<td></td>
<td></td>
<td># gen. &amp; spp.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Read &amp; Weary, 1990</td>
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<td>order</td>
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1=amoebas, 2=ciliates, 3=flagellates, 4=sporozoans, 5=platyhelminthes, 6=nematodes, 7=acanthocephala.

* Significant as interpreted by Read & Harvey (1989b).
CHAPTER THREE

THERMAL PREFERENCES OF RESISTANT AND SUSCEPTIBLE
STRAINS OF BIOMPHALARIA GLABRATA (GASTROPODA) EXPOSED
TO SCHISTOSOMA MANSONI (TREMATODA)

by

Hugh Lefcort

and

Christopher J. Bayne

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ABSTRACT

The thermal preferences of two strains of the snail Biomphalaria glabrata, one resistant to, and one susceptible to, the parasite Schistosoma mansoni were determined in an aquatic thermal gradient. Snails were tested without exposure to the parasite, and both two hours and five weeks after exposure to trematode miracidia. The mean temperature selected by susceptible strain snails two hours post exposure was 0.5 ±0.3°C lower than that of unexposed controls. In this strain, at five weeks post exposure, the preferred temperature dropped a further 1.9 ±0.5°C. The resistant strain displayed a significant drop of 1.8 ±0.6°C two hours post exposure. These results are consistent with the hypothesis that a drop in mean temperatures selected by snails is due to altered levels of endogenous cytokines such as IL-1 or TNF in association with parasite activation of the snail internal defense system.
INTRODUCTION

Diseased states, including parasitism, have long been associated with alterations in animal body temperatures. In almost all cases, invasion by pathogens result in an elevation of body temperature (Kluger, 1979). Fevers (defined as an elevation of the thermoregulatory set point above the normal set point), and pyrogenic agents that elicit fevers, have been correlated with infectious diseases for over 2000 years (Atkins, 1982). Fevers in endothermic birds or mammals generally are achieved either metabolically or behaviorally. In ectotherms, fevers are achieved only behaviorally, e.g. individuals can seek out and dwell in microhabitats where heat energy can be obtained.

A lowering of temperature set-points has been less commonly associated with disease. However, starvation often leads to a lowering of set-points (Regal, 1966; Lillywhite, Licht and Chelgren, 1973) and impaired renal function is correlated with lowered set-points in rabbits (Kluger et al., 1981; Eiger and Kluger, 1985). Kluger et al. (1981) postulated the existence of an endogenous cryogen that when released into the bloodstream of vertebrates acts to lower set-points. At this time no such substance has been identified (see review by Kluger, 1991).
TEMPERATURE AND HOST/PARASITE GROWTH

The natural aquatic habitats of the snail Biomphalaria glabrata are thermally heterogeneous, providing for possible temperature selection. Small alterations in ambient temperature have strong effects on the biology of snails that are host to trematode parasites of the genus Schistosoma (Shiff, 1964; Shiff and Hustong, 1966; Sturrock and Sturrock, 1972; Sodeman and Dowda, 1974). For example, growth rates were progressively higher for snails reared at 20°C, 25°C and 30°C, and lower for those reared at 35°C (Sturrock and Sturrock, 1972). Fecundity was highest between 25 and 30°C. Indeed, temperatures around 28°C have been found to be the mean selected temperature of B. glabrata (Chernin, 1967).

While higher temperatures favor the growth of B. glabrata, the growth of Schistosoma mansoni in its molluscan host is also greatest at temperatures above 26°C (Stirewalt, 1954). It has also been reported that some snails kept at low temperatures lost their infections (Stirewalt, 1954; see also Webbe and James, 1972).

In a test of the thermoregulatory behavior of B. glabrata, Chernin (1967) found that snails infected with S. mansoni behaved more erratically and had more
lethargic movements than uninfected individuals, but he found no difference in their thermal preference.

With the knowledge that appropriate behavioral changes in thermally heterogeneous habitats could affect the host-parasite balance, and intrigued by Chernin's (1967) mention of erratic behavior, we have further explored the effects of *S. mansoni* on the thermal behavior of *B. glabrata*. Our interest was further piqued by the report of interleukin-1 (IL-1) and tumor necrosis factor (TNF) in molluscs (Hughes et al., 1990); in vertebrates, both of these cytokines are secreted into the blood during immune responses, and influence temperature set-points (Dinarello, 1984; Dinarello et al., 1986; see also Kluger et al., 1990). IL-1 is pyrogenic (Dinarello 1984), as is TNF at pharmacological doses (Dinarello, 1986); TNF is also cryogenic at physiological doses (Long et al., 1990a; 1990b).

We examined the thermal behavior of snails during the two periods when their internal defense systems would be maximally challenged by schistosomes. For resistant snails this occurs immediately following miracidial encounter as the host's encapsulation response leading to parasite death is initiated (Bayne, 1983). In susceptible snails, entering miracidia, and the mother sporocysts into which they quickly transform, do not
elicit an immune response by the host internal defense system (Bayne, 1983). However, when the infection is patent (peak level is approximately at five weeks) and mature cercariae are leaving this host as their first step in locating a final (mammalian) host, the emigration of cercariae can be quite disruptive to the snail, at least as seen in histological examinations (Pan, 1963; 1965; Loker, 1979; van der Knaap and Loker, 1990; see also Sturrock and Sturrock 1970; 1971 for parasite-induced shell abnormalities). In measuring the snail’s thermal preference, Chernin (1967) either randomly assigned snails along a thermal gradient, or placed groups of snails at temperatures well above or well below their thermal preference. In contrast, in our experiments to determine thermal preferences post exposure, we first determined the thermal preference of unstressed unexposed snails of the susceptible strain. Subsequently we placed snails at that temperature, thus forcing them to choose between warmer or cooler water. With this protocol we investigated if differences existed between the thermoregulatory behavior of a) unexposed snail strains known to be either susceptible to, or resistant to, S. mansoni PR1 strain and b) snails of each strain exposed previously to miracidia of PR1 S. mansoni.
METHODS

The PR1 strain of S. mansoni was propagated using male CF-1 Mus musculus mice and susceptible M-line B. glabrata snails. Miracidia were procured from the livers of mice 6 - 8 weeks after they were exposed to 250 to 300 cercariae (Stibbs et al., 1979).

The two snail strains used in this study were M-line animals (80% of which are susceptible in our laboratory to PR1 S. mansoni) and 13-16-R1 animals [90% of which are resistant to PR1 miracidia (Fryer and Bayne, 1990)]. Histological examination of resistant snails three days post-exposure reveal no persistent effects of S. mansoni (Bayne, 1983).

Six treatments were used: 1) two hour unexposed susceptible strain snails, 2) two hour exposed susceptibles, 3) five week unexposed susceptibles, 4) five week exposed susceptibles, 5) two hour unexposed resistants and 6) two hour exposed resistants.

Snails were reared in a 600 L tank containing about 500 individuals. All snails used had a shell diameter of 4 - 5 mm and were four weeks old when first used. Snails were fed ad libitum with romaine lettuce and were kept at a constant 26°C. The laboratory was illuminated between 6:00 and 19:00 PDT.

For exposure to the parasite, individual snails were
confined for two hours in 3 ml plastic wells containing 15 miracidia in 2 ml of artificial spring water at 26°C. To control for the potential stress of such confinement (see Fryer, Dykes-Hoberg and Bayne, 1989) all unexposed animals were placed for two hours, prior to testing, in identical wells containing only artificial spring water. After two hours in these small wells both exposed and sham-exposed snails were either directly tested, or transferred to 8 L tanks for five weeks at which time they too were tested. Prior to testing five-week-exposed and sham-exposed snails were again placed in 3 ml wells containing only artificial spring water.

Experiments were run in a gray epoxy-painted container measuring 273 cm (l) x 27 cm (w) x 46 cm (d). Aged tap water was added to a depth of 1.5 cm. The warm end of a thermal gradient was created by placing three 200 Watt immersion coils in a 2.8 liter beaker of water at one end of the container. At the opposite end a 15 liter glass jar was filled with ice. Snails were isolated from the cold and the hot end of the gradient by fine mesh screen placed 19 cm from the container ends. The resulting length available to the snails was 234 cm. This was divided by faint lines into 24 zones of 9.7 cm length. Temperatures to the nearest tenth of a degree were recorded at the center of each zone with a
K-type thermocouple connected to an Omega HH82 electronic thermometer. No vertical stratification of water temperatures was present. Air stones at either end of the container (behind the screens) served to make an almost linear thermal gradient (Figure III.1). Temperatures in each zone varied by less than two degrees Celsius between trials. All animals within a given zone were assigned the same temperature. Experiments were conducted daily between 13:00 and 16:00 hours. Individual animals were tested only once.

Preliminary experiments confirmed Chernin’s (1967) finding of no difference in thermal preference in illuminated or dark thermal gradients. Consequently for the experiments reported here, the container was evenly illuminated by fluorescent lighting to an intensity of 525 lux.

Data for the movement experiment were analyzed using two two-way ANOVAs with significance at P < 0.05. The experimental design was not orthogonal. In order to avoid a type I error, all ANOVA generated P values were corrected using the Bonferroni multiple comparison procedure (Johnson and Wichern, 1988). The test of exposure x time involved two hour unexposed susceptible strain snails, two hour exposed susceptibles, five week unexposed susceptibles and five week exposed
susceptibles. The test of exposure x resistance involved two hour unexposed susceptibles, two hour exposed susceptibles, two hour unexposed resistants and two hour exposed resistants.

MOVEMENT EXPERIMENT USING SUSCEPTIBLE SNAILS

To identify individual snails, shells were uniquely marked with three drops of enamel nail polish. Twenty-four snails were placed along a line separating zones 12 and 13 at an average temperature of 21.7 ±0.4°C [a temperature below that reported by Chernin (1967) to be the mean selected temperature]. After 30 minutes the zonal position of each snail was recorded. The color markings were not always apparent so all snails were individually lifted from the container, their identity and zonal position recorded, the temperature of the zone noted, and then the snails were replaced in their original position. This process lasted fifteen minutes. Therefore, the following four readings were taken at 45 minute intervals. The total time of the experiment was 210 minutes. Five trials each with 24 unexposed snails and five trials each with 24 snails five weeks past exposure to miracidia were conducted. Snails were not placed in 3 ml infection wells prior to testing.
THERMAL PREFERENCE EXPERIMENT

For each trial ten unmarked snails of a single experimental group were placed at the previously determined thermal preference of the unexposed susceptible strain (zone 20 at an average temperature of 28.7 ±0.4°C). One hour later their positions and the temperature of each zone were recorded and a mean temperature of the group was derived.
RESULTS

CONTROLS

Lengthwise, in the absence of a thermal gradient, no preference was noted for one half of the tank over the other half ($X^2 = 0.78$, d.f. = 1, $P = 0.37$).

The stress of confinement in the 3 ml infection wells for two hours could have influenced thermoregulatory behavior. Uninfected snails confined for two hours in the infection wells tended to select temperatures slightly, but not statistically, lower than unexposed snails taken from a 600 liter tank (31.0 ±0.5 and 31.5 ±0.6°C respectively, $P = 0.103$). As a result of this, confinement regimens were carefully controlled for in all further experiments.

In contrast, the mean temperature selected by snails confined for five weeks to 8 L rearing tanks, followed by two hours in the 3 ml infection wells, was not significantly different from that of snails from the 600 L tank (Tukey test, $P > 0.050$).

MOVEMENT EXPERIMENT

Of the 192 individuals tested, all but 11 snails moved from zone 12/13 (21.7°C) towards zones containing warmer water. At the readings taken at 30, 75, 120, 165, and 210 minutes, the snails harboring five week
infections were consistently located at lower (but not statistically so) temperatures than those of unexposed snails (Figure III.2). The total movement of infected snails (13.2 ±0.6 zones) was less than that of unexposed snails [18.3 ±0.8 zones (P = 0.008)]. Since the mean temperatures at which unexposed and infected snails were located were not significantly different (P > 0.5 at all readings), this indicates that infected snails proceeded more directly to preferred temperature regions while unexposed animals moved back and forth from warmer and cooler waters.

THERMAL PREFERENCE EXPERIMENT

Five weeks after exposure it was determined that greater than 80% of the exposed susceptible strain snails developed patent infections. The mean temperature of each experimental group at the end of one hour is illustrated in Figure III.3.

The mean temperature selected by susceptible strain snails two hours post exposure was 0.5 ±0.3°C. lower than that of unexposed controls. In this strain, at five weeks post exposure, the preferred temperature dropped a further 1.9 ±0.5°C. The resistant strain displayed a significant drop of 1.8 ±0.6°C two hours post exposure.
Exposure to the parasite for two hours resulted in a lowering of selected temperatures for both the susceptible and the resistant strain (two-way ANOVA, $F = 7.012$, d.f. = 1, $P = 0.032$). Resistant snails consistently selected lower temperatures than susceptible snails; both in the presence and the absence of the parasite (two-way ANOVA, $F = 6.941$, d.f. = 1, $P = 0.036$). The temperature, that two hour exposed susceptibles selected, was lower than that of the baseline temperature of unexposed resistant snails.

When comparing two hour exposure versus five week exposure, we found that exposure to the parasite lead to lower temperatures in susceptible snails at both two hours and five weeks (two-way ANOVA, $F = 6.316$, d.f. = 1, $P = 0.034$). The magnitude of the changes in temperature was no different at two hours post-exposure versus five weeks post-exposure (two-way ANOVA, $F = 0.072$, d.f. = 1, $P = 0.793$)
DISCUSSION

Our results imply that molluscs regulate body temperature as part of their response to parasitic infection. The selection of lower temperatures at times of immunological response was unexpected, and the significance of this direction of change warrants evaluation.

The snail *Lymnaea stagnalis appressa* has an increased metabolic rate when infected by trematodes (Hurst and Walker, 1935) and *B. glabrata* infected with *S. mansoni* have higher heart rates than noninfected snails (Lee and Cheng, 1971). The possibility exists that infected snails move to lower temperatures to shed excess heat. However, the high thermal conductivity of water reduces the likelihood that changes of the observed magnitude would be required to achieve this. We therefore propose that the movement of snails to lower temperatures is an evolutionary strategy of snails to lessen the impact of parasitism.

There are data from other snail/parasite systems suggesting that lower temperatures aid the survival of the host relative to the parasite (Webbe and James, 1972; Blankespoor et al., 1989). Using the same snail and parasite as we used, Stirewalt (1954) found that lower temperatures (23 - 25°C) favored the survival of
infected snails, partly because they lost their infections. However, the optimal temperature range for *B. glabrata* infected with *S. mansoni* appears to be relatively narrow and above 25°C. Mortality of the snail increases below 26°C and above 30°C (Standen, 1952). Therefore, while an infected *B. glabrata* may lessen the impact of cercarial shedding by seeking waters cooler than 26°C, this would decrease the survival of the snail.

Much work has been done towards finding mechanisms that raise thermoregulatory set-point (Kluger, 1979), yet little has been done to find mechanisms that result in a lowering of set-points. Two endogenous cryogens have been proposed: Glucocorticoids (Besedovsky et al., 1986) and TNF (Long et al., 1990a; but see also LeMay et al., 1990). It is possible that the lower thermal preference of the resistant strain is due to chronic low level releases of endogenous cryogens. The marked drop in temperature preference (1.8°C) in resistant snails after exposure to miracidia may be caused by larger releases at times of immunological stress (see Kluger et al., 1990). Selection by unexposed resistant snails of a temperature that is lower by 1.5°C than that of unexposed susceptible snails, could, in thermally heterogeneous habitats, contribute to protection against
S. mansoni.

Our results are consistent with the hypothesis that behavioral changes which take certain snails into cooler water are mediated by factors released into the hemolymph, and with the postulate that such factors may be cytokines or stress hormones of host origin. This surprising drop in temperature preference following infection may be beneficial to the host (Stirewalt, 1954). Since most animals exhibit an increase in thermoregulatory set-point following infection (Kluger, 1979), this snail/trematode model may prove to be an excellent system in which to search for the hypothetical (Kluger et al., 1981), yet still undiscovered, endogenous cryogen.
ACKNOWLEDGEMENTS

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Figure III.1  Mean temperatures measured at the centers of zones within the thermal gradient.
FIGURE III.1

WATER TEMPERATURE Deg C

ZONES
Figure III.2 Mean temperatures (±S.E.) of uninfected susceptible snails (n = 96) and five week infected susceptible snails (n = 96) over 210 minutes. The snails were placed at 21.7°C.
Figure III.2

TIME (minutes)

TEMPERATURE Deg C +/- SE

5 Week Infected Susceptible
Unexposed Susceptible
Figure III.3 Mean temperatures (±S.E.) at which snails were located one hour after placement at 28.7°C in a thermal gradient. All snails were placed individually in identical infection wells two hours prior to testing. Susceptible strain snails were either not exposed to *S. mansoni* miracidia (unexp), unexposed and then reared for five weeks in 8 L tanks (5wk unex), exposed for two hours (2hr exp) or exposed for two hours and then reared for five weeks in 8 L tanks (5wk inf). Resistant strain snails were either not exposed (unexp) or exposed for two hours (2hr exp).
FIGURE III.3

EXPERIMENTAL GROUPS

SUSCEPTIBLE

RESISTANT

TEMPERATURE Deg C +/- SE
CHAPTER FOUR
ANTIPREDATORY BEHAVIOR OF FEVERISH TADPOLES:
IMPLICATIONS FOR PATHOGEN TRANSMISSION

by
Hugh Lefcort
and
Steven M. Eiger
ABSTRACT

In this paper we propose the hypothesis that pathogen-induced host defense responses result in altered host behaviors and enhanced predation. In particular we examine the effects of the acute phase response (whose effects include fever, reduced activity and malaise) on antipredatory behavior in bullfrog (Rana catesbeiana) tadpoles. This host response is associated with the preliminary stages of infection with many pathogens yet its behavioral effects have received little attention. Bullfrog tadpoles were injected with alcohol-killed bacteria to induce a response to infection and their ability to detect and avoid capture by predatory salamanders (Taricha granulosa) was explored. We predicted that acute phase responses increase tadpole vulnerability to predation by influencing thermoregulatory behavior and their ability to detect, and avoid capture by, salamanders.

We found that the stereotypical effects of the acute phase response can lead to increased predation. Malaise affected the refuge seeking behavior of the tadpoles in the presence of salamanders. We suggest that for tadpoles provided with refuges, altered behaviors are a liability. This endogenous response may afford some parasites a potential pathway to their next host.
INTRODUCTION

Pathogens, including viruses, bacteria and parasites, have long been known to affect the behavior of their hosts (Bethel and Holmes, 1972; 1977; Camp and Huizanga, 1979; Brassard et al., 1982; Hay and Hutchison, 1983; Rau, 1983; Moore, 1984; Quinn et al., 1987; Lefcort and Bayne, 1991). Altered behavior due to damaged host physiology and neurology have been documented (Livesey, 1980; Minchella, 1985; Helluy and Holmes, 1990; Holmes and Zohar, 1990; Thompson, 1990; Kavaliers and Colwell, 1992). However, few studies have examined the behavioral effects of endogenous substances (e.g. proteins such as the interleukins) released into the bloodstream by infected, but otherwise unimpaired, animals. Because this response occurs in most vertebrates when infected with pathogens, some pathogens may benefit from behavioral changes associated with host sickness, such as behavioral fever and "malaise".

Traditionally, nonmemory host defense responses (e.g. the acute phase response, which typically results in fever, perturbations of trace metal concentrations in blood, and the release of cytokines such as the interleukins), have been viewed as acting as protection against pathogens and not as mechanisms that enhance pathogen success. For example, Kluger et al. (1975)
showed that the lizard *Dipsosaurus dorsalis*, placed in a thermal gradient, chose a higher ambient temperature (a behavioral fever) after being injected with the bacterium *Aeromonas hydrophila*. Infected lizards prevented from raising their body temperature had decreased survival relative to lizards which maintained high temperatures post-injection. Thus, increased body temperature was deleterious to the pathogen.

However, some host defense responses facilitate parasite transmission (Hayunga, 1979; Shinn, 1985a; 1985b; Soulsby, 1987; Kluger, 1990). Damian's (1987) prediction that manipulations of host defenses would either aid pathogen reproduction within the host, or transmission of the pathogen to a subsequent host (see also Ewald, 1986; 1991; Williams and Nesse, 1990), has had some support. For example, some tapeworms employ the intestinal granulomatous (i.e. encapsulation) response of their fish host to ensure a protected environment and a means of attachment (Hayunga, 1979). Similarly, turbellarian flatworms occupying the coelom of sea cucumbers may rely on physiological defense mechanisms of the host to ensure transmission (Shinn, 1985a; 1985b). Furthermore, schistosome egg expulsion from the mouse gut is facilitated by the immune response of the host (*Mus musculus*, Doenhoff et al., 1985). In
these examples, pathogens receive fitness benefits from the physiological effects of an active host defense response.

Additionally, altered host behavior facilitate the transmission of multi-host parasites to secondary hosts by either enhancing predation of primary hosts, or increasing accessibility of hosts to vector (Rossignol et al., 1984; Rossignol and Rossignol, 1988; Kwiatkowski, 1989; Kwiatkowski and Greenwood, 1989).

After infection with acanthocephalan parasites, isopods, (Armadillidium vulgare) are consistently more susceptible to predation by starlings (Moore, 1983). Lemmings infected with the protozoan Sarcocystis rauschorum have increased exploratory activity which can increase their susceptibility to avian predators (Quinn et al., 1987; see also Dolinsky et al., 1985).

Predation of infected hosts could be increased if malaise, a symptom of the host defense response, resulted in a reduced fright response. For example, minnows observed to be "sick" show little or no reaction to alarm substances from conspecifics (von Frisch, 1941) and stickleback fish parasitized by a pseudophyllid cestode recover more quickly from a frightening overhead stimulus than do non-parasitized fish (Giles, 1983; Godin and Sproul, 1988). Malaise may also alter refuge

In this paper we propose the hypothesis that pathogen-induced host defense responses lead to altered host behavior that enhance predation. In particular we examine the effects on antipredator behavior of acute phase responses in bullfrog (Rana catesbeiana) tadpoles. We predict that acute phase responses increase tadpole vulnerability to predation by influencing their ability to detect, and avoid capture by, predatory salamanders (the roughskin newt, Taricha granulosa).
METHODS

Sympatric bullfrogs and rough-skinned newts were collected from two field sites: bullfrog tadpoles were collected at Pisgah Pond (Linn County, Oregon), and newts were collected at Soap Creek Pond (Benton County, Oregon). Bullfrogs are preyed upon by newts at these sites (pers. obs.). Tadpoles (Gosner stages 23–25, i.e. without leg buds, [Gosner, 1960] mass 0.8–1.2 g) were housed at Oregon State University, fed alfalfa pellets ad libitum, and maintained between 18 and 22°C. The laboratory was illuminated between 06:00–19:00 hours. Newts were housed under similar conditions and fed midge fly larvae (Chironomus spp.).

We conducted laboratory experiments between June and October, 1991, and field experiments in September and October, 1991. Experiments were conducted between 13:00–17:00 hours. We tested individual tadpoles only once.

Aeromonas hydrophila, a naturally occurring gram negative bacterium, was chosen to experimentally induce the acute phase response in bullfrog tadpoles (sensu Casterlin and Reynolds, 1977). This bacterium causes the often fatal "red-leg" disease in anurans (Gibbs, 1963). A. hydrophila is not transmitted through predation from bullfrog tadpoles to T. granulosa (Lefcort, Unpub.). To test our hypothesis, the acute
phase response's effect on behavior must be isolated from physical or neurological damage caused by a pathogen or by the products and toxins a pathogen releases. We used alcohol-killed bacteria to induce fever in hosts, rather than a live pathogen, to separate the behavioral effects of the acute phase response from the myriad of physiological changes and tissue damage that may occur during infections with living pathogens.

Lyophilized bacteria (American Type Culture Collection) were grown in nutrient broth, then killed by suspension in ethanol, washed in sterile pyrogen-free saline (0.9% sodium chloride), centrifuged, and resuspended in saline. Plating of bacteria on blood agar produced no growth. A concentration of $1 \times 10^{10}$ dead bacteria/ml was assessed by its turbidity (Difco Laboratories; Kluger, 1977). In all experiments, experimental tadpoles were injected intraperitoneally with 0.1 ml of this solution to induce the acute phase response. Control tadpoles were injected with 0.1 ml of pyrogen-free saline.

To test for significant treatment effects we used Wilcoxin rank sum tests (a nonparametric test), whenever a violation of the assumption of normality prevented the application of Student's t-test and ANOVA parametric
tests. Statistical significance of all tests was
P < 0.05.

I. LABORATORY THERMAL PREFERENCE EXPERIMENT

One indication of the acute phase response in
ectotherms is behavioral fever - i.e. altered
thermoregulatory behavior (Kluger, 1990). We determined
the thermal preferences of control and bacteria-injected
tadpoles in the laboratory. We conducted experiments in
a gray epoxy-painted container measuring 273 cm (l) x 27
cm (w) x 46 cm (d). We divided the container lengthwise
into two lanes by a dark plexiglass partition. We added
dechlorinated tap water to a depth of 1.5 cm. We
created the warm end of a thermal gradient by placing
two 200 W immersion coils in a 2.8 liter beaker of water
at one end of the container. A compressor driven
cooling coil was submerged at the opposite end in a 5.0
liter beaker of water. Test-lanes were isolated from
the extreme cold and the hot ends of the gradient by
fine mesh screen placed 19 cm from the container ends.
The resulting lane length of 234 cm was divided
widthwise by faint pencil lines into 24 zones of 9.7 cm
length. We recorded temperatures to the nearest tenth
of a degree at the center of each zone with a K-type
thermocouple connected to an Omega HH82 electronic
thermometer. By keeping the water depth low, no vertical stratification of water temperatures was detected. Air stones at each end of the container (behind the screens) provided continuous water flow and an essentially linear thermal gradient. Temperature in each zone varied by < 2°C between trials. To determine the instantaneous temperature of a tadpole, a tadpole was assigned the temperature of the zone it occupied. The container was evenly illuminated by diffuse incandescent lighting to an intensity of 320 lux.

We introduced a single tadpole into the central zone (zone 12, mean temperature = 19.9 ±0.6°C) of each lane, and then left them undisturbed for 24 hours. During hour 25, we recorded the positions of the tadpoles every five minutes. We then injected the tadpoles with either dead A. hydrophila (to induce fever) or sterile pyrogen-free saline (control treatment), and we returned the tadpoles to the zones from which they were found. For the next two hours we recorded the positions of the tadpoles every five minutes. Lane assignments of tadpoles (experimental vs. control treatment) were alternated between runs.

II. FIELD THERMAL PREFERENCE EXPERIMENT

To determine if bacteria injections also induced
altered thermal preferences in the field, we conducted the following experiment. Experiments were conducted at Soap Creek Pond, Oregon. We placed 12 tadpoles in each of 12 enclosures (the experimental design was similar to Walls, 1991) constructed of 5 cm wide plywood strips, composing a frame covered on three sides with fiberglass mesh insect screening (mesh size = 1 mm). Enclosures measured 137 x 30 x 30 cm and were placed lengthwise, along the natural slope of the pond bottom. The uncovered top of the enclosure extended above the surface of the water. Enclosures were placed 30 cm apart and were parallel to each other and perpendicular to the pond's edge. Due to the slope of the pond, the end farthest from the pond's edge was situated in "deep" water (20 cm) and the opposite end of the enclosure was situated in "shallow" water (2 cm).

Mean temperatures in the enclosures were: 23.7 ±0.12°C at the shallow end, 21.8 ±0.12°C at the deep end, and 23.4 ±0.14°C 1 cm below the surface of the deep end (air temperature = 22.0°C). Temperatures were measured only at the end of the experiment because preliminary results indicated that wading into the pond to record temperatures stirred-up pond silt, agitated the tadpoles and disturbed any existing thermal gradient. Temperatures at other areas of the pond increased by
less than 0.3°C over the 120 minutes of the experiment. We placed tadpoles to be used in field experiments in a "holding" enclosure identical to the 12 experimental enclosures and allowed them to habituate to the pond for 24 h.

At the start of the experiment, we injected 12 tadpoles with either alcohol-killed *A. hydrophila* bacteria or saline (control treatment) solution and added them to the center of each enclosure. Treatments were interspersed to control for a gradient of temperatures along the pond margin. After 60 minutes (the time at which maximum fevers were observed in the laboratory), the number of animals in both the shallow half of the enclosure and top 1 cm of the deep half were recorded by observers using binoculars at a distance of 4 m. Sixty minutes later (i.e. 120 minutes post-injection, after laboratory fevers subsided), the positions of the tadpoles were again recorded and temperatures were recorded at the geometric center of the shallow end, the geometric center of the deep end, and the water 1 cm below the surface of the deep end. We then terminated the experiment and counted the number of tadpoles in each container to determine if any had escaped. The mean temperature of the tadpoles within a given enclosure was estimated by assigning to the
animals within one of the three zones the temperature of that zone.

III. FIELD EXPERIMENT TO TEST THE EFFECT OF SALAMANDERS ON THERMOREGULATION

This experiment tested how the presence of a predatory salamander affected behavioral thermoregulation of bacteria-injected tadpoles. Prior to the experiment ten tadpoles were allowed to acclimate in a holding enclosure for 24 hours. Each of the 12 enclosures contained 10 tadpoles and received one of the following four treatments: a two by two design had all combinations of tadpoles, injected with either bacteria or saline, crossed with salamanders either present or absent. The salamander was placed in a container constructed of a plastic frame with mesh sides (15 x 15 x 8 cm). This container was attached to the geometric center of each enclosure. Empty containers were placed in enclosures whose treatment did not include a salamander. We carried out observations as in the previous experiment. Mean temperatures in the enclosures were: 17.15 ±0.13°C at the shallow end, 13.92 ±0.15°C at the deep end, and 17.09 ±0.14°C 1 cm below the surface of the deep end (air temperature = 19.6°C).
IV. LABORATORY EXPERIMENT TO TEST THE EFFECT OF REFUGES ON PREDATION BY SALAMANDERS

To determine if bacteria injections affect the use of refuges, we conducted the following experiment. Twenty-four hours before testing we dyed the tadpoles either blue (methylene blue, EM Science Co.) or red (neutral red, Matheson, Coleman & Bell Co). Dyes were used for identification and colors were reversed on alternate trials. We placed tadpoles in seven-liter opaque bowls, filled with five liters of water. Two treatments were used: half of the bowls contained plastic plant refugia and half did not. We added four bacteria-injected and four saline-injected tadpoles to each bowl. We added a starved (24 hours) salamander after 40 minutes. After a further 60 minutes we recorded the number and color of the remaining tadpoles. We tested a total of 320 tadpoles.

V. PREDATOR-CUE EXPERIMENT

This experiment tested the behavioral response of tadpoles to chemical cues present in water that had been in contact with predatory salamanders. The experimental apparatus was a modification of that used by Petranka et al. (1987). We tested tadpoles in a gravitational flow-through system composed of three 25 liter plastic tubs.
The tubs were arrayed at different heights so that water flowed from one to another at 0.5 l/min. The uppermost tub was filled with 23 liters of water. In half the trials, the middle tub contained a salamander, from which water flowed to the lower-most tub, which contained eight tadpoles and was divided lengthwise such that half the tub contained plastic aquarium plants and half did not. The two lower-most tubs had output openings and never contained more than 10 liters of water. Ten minutes after flow was initiated, we recorded the tadpoles' positions every minute for ten minutes. We recorded the number of tadpoles in each half of the enclosure, along with the number of times a tadpole crossed between the two halves of the enclosure (i.e. moves per trial). Four experimental treatments were used: 1) saline-injected tadpoles, salamander absent, 2) saline-injected tadpoles, salamander present, 3) bacteria-injected tadpoles, salamander absent, 4) bacteria-injected tadpoles, salamander present.

Preliminary results indicated that handling and injecting animals had no significant affect on the distribution of tadpoles.
RESULTS

I. LABORATORY THERMAL PREFERENCE EXPERIMENT

From 0 to 60 minutes prior to injection, the mean water temperature (±S.E.) of both bacteria (experimental) and saline-injected (control) animals was 21.6 ±0.71°C (n=31 tadpoles). Control animals (n=13) did not exhibit a rise in temperature after injection (Wilcoxin rank sum test, P >0.05). Experimental animals (n=18) exhibited a mean rise in temperature (in the zones they occupied) of 2.8 ±0.90°C between 30 and 90 minutes after injection (mean rise was calculated from the difference of each animal from its own preinjection mean, Wilcoxin rank sum test, P < 0.05; Figure IV.1). This compares closely to a similar rise of 2.6 - 2.7°C for bullfrog tadpoles found by Casterlin and Reynolds (1977) using a similar dose of the same bacterium. We found that both experimental and control tadpoles selected lower temperatures than those reported by Casterlin and Reynolds (1977). This difference may have been due to either the acclimation of tadpoles to our laboratory conditions affecting their thermoregulatory set-point, or to Casterlin and Reynolds' use of a "shuttle box".
II. FIELD THERMAL PREFERENCE EXPERIMENT

Sixty minutes after injection, experimental animals (n=6 enclosures) were present in water with a mean temperature of 23.2 ±0.23°C and control animals (n=6 enclosures) were in deeper water at a cooler mean temperature of 22.4 ±0.14°C. After 120 minutes post-injection, experimental and control animals had similar temperatures, 22.6 ±0.17°C vs. 22.4 ±0.17°C. There was a significant interaction term between type of injection and the amount of time post-injection (repeated-measure ANOVA, F = 7.184, d.f.= 1, P = 0.014). This suggests that experimental animals were able to "clear" the bacteria from their systems after 120 minutes and had returned to control temperatures.

III. FIELD EXPERIMENT TO TEST THE EFFECT OF SALAMANDERS ON THERMOREGULATION

Sixty minutes after injection, experimental tadpoles were found at higher temperatures than control tadpoles, both in the presence and absence of salamanders (ANOVA, F = 29.98, d.f.= 1, P = 0.006, Table IV.1). The presence of salamanders resulted in lower temperatures for both experimental and control tadpoles (ANOVA, F = 12.63, d.f.= 1, P = 0.008). Control and experimental tadpoles responded to the presence of salamanders in
different ways. In the presence of the salamander, experimental animals were found at temperatures 1.9°C higher than control animals (Student's t-test, \( P = 0.002 \)). Control tadpoles, in the absence of the salamander, were found at temperatures 1.4°C higher than control tadpoles in the presence of a salamander (Student's t-test, \( P = 0.006 \)). Experimental tadpoles were also found at slightly higher temperatures in the absence, than in the presence, of salamanders (0.6°C), but this difference was not statistically significant (Student's t-test, \( P = 0.656 \)).

One-hundred and twenty minutes post-injection the effect of injection was no longer significant (ANOVA, \( F = 2.05 \), d.f. = 1, \( P = 0.190 \)) but tadpoles in the presence of a salamander (both treatment groups examined together) were still found at lower temperatures (ANOVA, \( F = 20.85 \), d.f. = 1, \( P = 0.018 \)).

When the readings at 60 and 120 minutes are analyzed together, bacterial injections resulted in higher selected temperatures (repeated-measure ANOVA, \( F = 20.47 \), d.f. = 1, \( P = 0.002 \)) and the presence of salamanders resulted in lower selected temperatures (repeated-measure ANOVA, \( F = 23.02 \), d.f. = 1, \( P = 0.001 \), Figure IV.2). There were no significant interaction
IV. LABORATORY EXPERIMENT TO TEST THE EFFECT OF REFUGES ON PREDATION BY SALAMANDERS

The presence of plant refuges reduced the number of tadpoles eaten by salamanders, regardless of whether the tadpoles had been injected with bacteria or saline. When plant refuges were absent, salamanders consumed a mean of 1.56 ±0.22 total (bacteria plus saline) tadpoles per trial. When plants were present salamanders consumed significantly fewer tadpoles per trial (0.83 ±0.18, Student’s t-test, P = 0.032).

When no plants were provided, the mean number of experimental tadpoles consumed (0.55 ±0.16) tended to be lower, but was not significantly so, than the mean number of control tadpoles consumed (1.00 ±0.21; Student’s t-test, P = 0.22). In contrast, when refuges were provided, significantly more experimental than control tadpoles were consumed (0.67 ±0.16 and 0.07 ±0.17 respectively; Student’s t-test, P = 0.022).

V. PREDATOR CUE EXPERIMENT

A summary of the differences in the distributions of animals in the lower-most tub is given in Table IV.2.
No significant treatment effects were found for refuge use (% of tadpoles in open vs % among plastic plants, ANOVA, $F = 2.64$, d.f. = 3, $P = 0.073$). Control animals used refuges in greater frequency than all other treatments but this difference was not significant.

Significant treatment effects were found for mobility effects (total number of times tadpoles crossed from one half of the tub to the other half, ANOVA, $F = 17.36$, d.f. = 3, $P < 0.001$). All experimental treatment groups exhibited less movement than control groups. In contrast to experimental animals, control tadpoles exhibited significantly less mobility in the presence of salamander exposed water. Mobility of experimental animals was not significantly altered by the presence of salamander exposed water.
DISCUSSION

We found that the stereotypical effects of a tadpole’s physiological responses to infection (indicated by the presence of behavioral fever, expts. I & II) can affect predation. This response reduced activity and the refuge seeking behavior of tadpoles in the presence of a predator (expts. III, IV and V). In both the laboratory and the field, bacteria-injected experimental tadpoles did not seek shelter as actively as saline-injected controls.

Control tadpoles reduced their activity when exposed to water that had been in contact with predatory salamanders, but experimental tadpoles did not. While some behavioral alterations may be beneficial to hosts, in that lethargic hosts conserve resources (Hart, 1988; 1990), we suggest that behavioral alterations can be costly to hosts because they may lead to higher predation. When plant refuges were available, salamanders consumed a greater number of experimental than control tadpoles. Behavioral fevers in an ectotherm can have wide reaching effects. Thermoregulation may take precedence over predator avoidance. While control tadpoles avoided warm water when a predator was present, experimental tadpoles did
not exhibit this response. Physiological fevers have been documented in a wide range of mammals and birds (D’Alecy and Kluger, 1975; Atkins, 1982) and behavioral fevers have also been found in many species of reptiles, amphibians, fishes and arthropods (Kluger, 1990). Therefore, maladaptive behaviors associated with a host defense response may be widespread among diverse taxa.

Our findings with bacteria are consistent with results we have obtained using red-legged frog tadpoles (Rana aurora infected with live yeast Candida spp., a single-host pathogen, Lefcort, unpub.).

Given the dramatic effects of a host defense response on behavior we suggest that some multi-host parasites may capitalize on normal host defense responses by eliciting acute phase responses at key periods of the parasite’s lifecycle and then benefit from the resulting behavioral changes of the host. A large number of acute phase responses are triggered by pathways dependent on a common mediator, such as interleukin-1 (IL-1). A pathogen may benefit from some of these responses, whereas other responses may remain deleterious to the pathogen. Recently it was discovered that IL-1 enhances the growth of virulent strains of the bacterium Escherichia coli (Porat et al., 1991). If the net effect of the host defense response is harmful to
the invader, then a strategy of avoidance of the host defense system (Damian, 1964; Soulsby, 1987) would be expected. However, if certain elements of the defense response are helpful to the reproductive efficiency of the pathogen, then we would expect detection to occur. In fact, as pathogens undergo changes within their hosts, and their needs change, we expect that the various life stages might alternate between enhanced and suppressed recognition by the host's defense system. Currently we are exploring if altered behaviors also occur in a system where tadpoles are the intermediate host for a parasite that is transmitted through predation.
ACKNOWLEDGEMENTS

Susan Walls provided extensive help in the formation and analyses of the experiments. The critical readings of E. Berlow, A. Blaustein, M. Hixon, R. Huey, M. Kluger, A. Kuris, F. Lefcort, D. Olson, P. Rossignol, S. Walls and H. Wilbur considerably improved earlier drafts of the manuscript. D. Wilson assisted both in the field and laboratory. D. Ingle and K. Hoff provided advice on anuran anti-predator behavior. Financial assistance to H. L. was provided by an Oregon State Univ. Zoology Dept. grant.
### TABLE IV.1

**MEAN TEMPERATURE (°C ±S.E.)**

OF TADPOLES 60 and 120 MINUTES POST-INJECTION

All adjacent pairs of means are significantly different except those labelled "ns" (see text).

<table>
<thead>
<tr>
<th></th>
<th>60 MINUTES</th>
<th>120 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SALAMANDERS</td>
<td>SALAMANDERS</td>
</tr>
<tr>
<td></td>
<td>ABSENT</td>
<td>PRESENT</td>
</tr>
<tr>
<td>SALTINE</td>
<td>16.23 ±0.01</td>
<td>14.86 ±0.19</td>
</tr>
<tr>
<td>INJECTION</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td>BACTERIA</td>
<td>17.30 ±0.48</td>
<td>ns 16.75 ±0.14</td>
</tr>
</tbody>
</table>
**TABLE IV.2**

RESULTS OF PREDATOR-CUE EXPERIMENT

Means that are not significantly different at $P < 0.05$ are identified with similar letters (Tukey test)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% OUTSIDE REFUGES (MEAN ± S.E.)</th>
<th>MOVES/TRIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria-injected, salamander exposed water, n = 8 trials</td>
<td>51.5 ±4.9 a</td>
<td>9.0 ±1.8 b</td>
</tr>
<tr>
<td>Bacteria-injected, control water, n = 6 trials</td>
<td>46.5 ±6.6 a</td>
<td>13.3 ±3.5 b</td>
</tr>
<tr>
<td>Saline-injected, salamander exposed water, n = 8 trials</td>
<td>35.4 ±3.3 a</td>
<td>25.3 ±3.1 c</td>
</tr>
<tr>
<td>Saline-injected, control water, n = 6 trials</td>
<td>47.5 ±2.3 a</td>
<td>37.7 ±3.5 d</td>
</tr>
</tbody>
</table>
Figure IV.1

Mean change in body temperature ±S.E. minus the mean body temperature during of the period 60 minutes pre-injection of alcohol-killed bacteria-injected tadpoles (n=18) and saline-injected tadpoles (n=13) from 60 minutes before injection (at time 0) and 120 minutes post-injection.
Figure IV.2

Mean temperature ±S.E. of tadpoles 60 minutes after injection of alcohol-killed bacteria or saline in either the presence or absence of salamanders. Each treatment represents 3 enclosures of 10 tadpoles.
FIGURE IV.2

TEMPERATURE Deg C +/- SE

SALINE

BACTERIA

EXPERIMENTAL GROUPS

ABSENT

PRESENT

ABSENT

PRESENT

FIGURE IV.2
CHAPTER FIVE

FEVER AND PREDATOR AVOIDANCE OF PARASITIZED TADPOLES

by

Hugh Lefcort

and

Andrew R. Blaustein
ABSTRACT

Many parasites alter the behaviors of their hosts. In most cases that have been studied, predation of the intermediate host by the definitive host is necessary for parasite transmission. Single-host parasites have been studied less frequently and the fitness benefits from altering host behaviors are not obvious. Parasitic infection in vertebrates usually results in a physiological response to infection. The behavioral effects of this response have similarly received little attention. If altered behaviors are a generalized host response to infection, irrespective of the infecting parasite’s life history, i.e. single-host or multiple-host species, then alterations of host behavior may be a general phenomenon of infection. In this paper, we examine the behavioral effects of a yeast, Candida spp., a single-host parasite species in its natural host, the red-legged frog (Rana aurora). Infected tadpoles were found more frequently in warm shallow water, exhibited reduced movement, failed to detect chemical cues from predatory newts (Taricha granulosa), and suffered increased predation by newts, compared to uninfected controls.
INTRODUCTION

Some parasites alter the behavior of their animal hosts in ways that increase the host's susceptibility to predation (e.g. Bethel and Holmes, 1972; Holmes and Zohar, 1990; Thompson, 1990). Many of these parasites exhibit multiple host life-cycles that require the definitive host to consume the intermediate host for transmission to occur (Bethel and Holmes 1977; Brassard et al., 1982; Camp and Huizanga 1979; Giles, 1983; Lester, 1971; Moore, 1984; Quinn et al., 1987). However, the behavioral effects of single-host parasites has received less attention (Baer, 1973; LaMunyon and Eisner, 1990).

Single-host parasites often die when their host dies. Thus, single-host parasites may gain no obvious selective advantage from inducing host behaviors that result in the death of their host. One mechanism by which pathogens alter host behavior is by causing their host to release substances into the bloodstream as part of the infection response (Kavaliers and Colwell, 1992; Lefcort and Eiger, unpub.). When a vertebrate host becomes infected, an initial host defense response results that involves inflammation, increased levels of stress hormones, cytokines (reviewed by Stadnyk and Gauldie, 1991), and a subsequent immune response. Some
cytokines have behavioral effects. For example, interleukin-1 (IL-1) and tumor necrosis factor (TNF) can cause ectothermic vertebrates to behaviorally thermoregulate (i.e. seek out and dwell in warm microhabitats) at higher (feverish) temperatures (Kluger, 1991). Other cytokines causes fatigue and induce sleep in mammals (Shohan et al., 1987; Spiggs et al., 1988). Lefcort and Eiger (unpub. data) elicited a host-defense response in bullfrog tadpoles (Rana catesbeiana). They found that the host-defense response induces behavioral fevers, alters the tadpole’s predator avoidance behavior, and increases the tadpoles susceptibility to predation by newts (Caudata: Taricha granulosa).

Infection of vertebrates with viruses, bacteria and most eukaryotic parasites results in a host defense response (Titus et al., 1991). This response occurs regardless of the parasite’s single or multi-host life-history. If changes in behavior are associated with the host defense response, then alterations of host behavior may be a more widespread phenomenon than is currently believed. To determine if behavioral alterations associated with infection occur in systems where alterations are detrimental to the parasite’s genetic fitness, we examined the behavioral effects of a one-
host parasitic yeast (*Candida* spp., Richards, 1958; 1962; Steinwascher, 1979) in tadpoles of the red-legged frog, *Rana aurora*. Specifically we tested: 1) if infected tadpoles have altered thermal preferences, 2) how the presence of a predator affects thermoregulation, and measure 3) the ability of infected tadpoles to detect and respond to chemical cues from a predator, and 4) the susceptibility of infected tadpoles to predation.
METHODS

We gathered all tadpoles and newts at a pond located eighteen km south of Waldport, Oregon (elev. 20m). Tadpoles were gathered as eggs, housed at Oregon State University, and fed alfalfa pellets *ad libitum*. We maintained the animals between 18 and 22°C. We began tests when tadpoles reached Gosner stages 22 - 25 (i.e. without leg buds, Gosner, 1960, mass 0.8 - 1.2 g). The laboratory was illuminated between 06:00 - 18:00 hours. We conducted experiments in March and April of 1992 between 13:00 - 17:00 hours.

The yeast, *Candida* spp. has a one-host life-cycle and is naturally transmitted among individual tadpoles through water and feces (Richards, 1958). We infected the tadpoles by exposing them for seven days to water and feces from high density (15/liter) groups of *R. aurora* that were already infected when captured. These crowded tadpoles were collected from the same pond as the experimental tadpoles at Gosner (1960) stages 22-25. After the seven day exposure period, experimental tadpoles exhibited signs of wasting and microscopic examination of gut contents revealed colorless, round, vacuolated cells, as described by Richards (1958). These cells were not recovered from control tadpoles kept at a lower density (2/liter). Although *Candida*
Sap. was present in all experimental tadpoles examined, it is possible that other pathogens were also present. Microscopic examination of newts that had fed on infected tadpoles (three days post-exposure) indicated that the yeast was probably not transmitted to newts, although transmission at undetectable low levels could have occurred. Although Candida yeasts, when parasitic, are generally single-host parasites, (Lodder, 1970) i.e. no intermediate and definitive hosts, it is possible that predators other than newts can act as secondary hosts. Tadpoles, seven to nine days post initial exposure, were tested only once. Field experiments were conducted at Soap Creek Pond, located 15 kilometers north of Corvallis, Oregon.

To test for significant treatment effects, paired t-tests, Student’s t-test and two-way analyses of variance (ANOVA) tests were used. Statistical significance of all tests was $P < 0.05$.

EXPERIMENT 1: FIELD THERMAL PREFERENCE

One indication of an initial host defense response in ectotherms is behavioral fever - i.e. altered thermoregulatory behavior (Kluger, 1991). To determine the thermal preferences of control and exposed tadpoles in the field, we used an experimental design similar to
that of Walls (1991). We used 12 enclosures constructed of 5 cm wide plywood strips, composing a frame covered on three sides with fiberglass mesh insect screening (mesh size = 1 mm). Enclosures measured 137 cm (l) x 30 cm (w) x 30 cm (d) and were placed lengthwise, along the natural slope of the pond bottom. The uncovered top of the enclosure extended above the surface of the water. Enclosures were placed 30 cm apart and were parallel to each other and perpendicular to the pond’s edge. Due to the slope of the pond, the end farthest from the pond’s edge was situated in "deep" water (20 cm). The opposite end of the enclosure was situated in "shallow" water (2 cm). We placed aquatic plants in the enclosures.

We placed 15 tadpoles in the center of each enclosure and allowed them to acclimate to the pond. Six enclosures contained uninfected tadpoles and six contained infected tadpoles. After 24 hours, we recorded the number of animals in both the shallow half of the enclosure and the top 1 cm of the deep half by viewing with binoculars at a distance of 4 m. We also recorded water temperatures at the geometric center of the shallow end, the geometric center of the deep end, and 1 cm below the surface of the pond’s deep end. To control for a gradient of temperatures along the pond margin the treatments were alternately interspersed.
The positions of the tadpoles were again recorded 30, 60, and 90 minutes later. At the last reading the temperatures of the three zones were again recorded. We then terminated the experiment and counted the number of tadpoles in each container to determine if any had escaped. Temperatures were not recorded more frequently because preliminary results indicated that wading into the pond to record temperatures stirred-up pond silt, agitated the tadpoles and disturbed any existing thermal gradient.

EXPERIMENT 2: EFFECT OF PREDATORY NEWTS ON THERMOREGULATION OF TADPOLES

This experiment tested how the presence of a predatory newt (*Taricha granulosa*) affected behavioral thermoregulation of infected tadpoles. We used the same plywood and mesh enclosures as experiment 1. We allowed 15 tadpoles to acclimate for 24 hours in each of the enclosures, and we then added a newt satiated on tadpoles to half of the enclosures. Thus, each of three enclosures received one of the following four treatments in a randomized block design:

1) newt present, tadpoles uninfected, 2) newt absent, tadpoles uninfected, 3) newt present, tadpoles infected, or 4) newt present, tadpoles infected.
Observations were carried out as in experiment 1.

EXPERIMENT 3: SUSCEPTIBILITY OF TADPOLES TO PREDATION BY NEWTS

To determine if yeast infections affect the susceptibility of tadpoles to predation we used the same design as the previous two experiments, but with the following treatments: six enclosures contained 10 uninfected tadpoles and six contained 10 infected tadpoles. After a 24 hour acclimation period we added a 24 hour-starved newt to each enclosure. Sixty minutes later, we counted the number of surviving tadpoles.

EXPERIMENT 4: PREDATOR-CUE

This experiment tested the behavioral response of tadpoles to chemical cues present in water that had been in contact with newts. The experimental apparatus was similar to that of Petranka et al. (1987). Tadpoles were tested in a gravitational flow though system composed of three 25-l plastic tubs (51 cm (l) X 37 cm (w) X 21 cm (d)). The tubs were arrayed at different heights so that water flowed from one to another at 0.5-liter/min. The two lower tubs had output openings and never contained more than 10 liters of water. The uppermost tub was filled with 23 liters of water. In
some of the trials (see below) the middle tub was either unoccupied or contained two newts. From the middle tub, water flowed to the lower-most tub, which contained eight tadpoles and was divided across its width such that half the tub contained the input opening and half contained the output opening. Ten minutes after flow was initiated, we recorded the number of tadpoles in each half of the enclosure along with the number of times a tadpole crossed between the two halves of the enclosure. These data were recorded for 10 minutes at 1 minute intervals.

Three treatments were used: 1, infected tadpoles, newt absent; 2, infected tadpoles, newt previously fed insect larvae (midge flies, Chironomus spp.); and 3, infected tadpoles, newt previously fed R. aurora tadpoles. Since R. aurora alter their behavior in response to predators who have fed on conspecifics (Wilson and Lefcort, in press) the newts were maintained on, and fed, either R. aurora tadpoles or insect larvae ad libitum 24 hours prior to testing.
RESULTS

EXPERIMENT 1: FIELD THERMAL PREFERENCE

Mean temperatures in the enclosures at 13:30 were as follows: 17.57 ±0.19°C at the shallow end, 16.06 ±0.24°C at the deep end, and 17.28 ±0.16°C 1 cm below the surface of the deep end (air temperature = 13.4 - 14.5°C). Significantly more infected than uninfected animals were found in the two combined shallow zones (Paired t-test $P < 0.001$).

EXPERIMENT 2: EFFECT OF PREDATORY NEWTS ON THERMOREGULATION OF TADPOLES

Mean temperatures in the enclosures at 14:30 were as follows: 21.57 ±0.14°C at the shallow end, 20.18 ±0.18°C at the deep end, and 21.47 ±0.16°C 1 cm below the surface of the deep end (air temperature = 21.4 - 22.4°C). When the readings at 30, 60 and 90 minutes are analyzed together, infection with the parasite resulted in a slight, but not significant, increase in the number of tadpoles in warm shallow zones (Table V.1, repeated measure ANOVA, $F = 4.04$, d.f. = 1, $P = 0.079$). The presence of newts had no affect on the distribution of tadpoles (repeated measure ANOVA, $F = 0.96$, d.f. = 1, $P = 0.355$, Table V.1). There was no significant interaction
between newt presence and infection (repeated measure ANOVA, F = 0.02, d.f. = 1, P = 0.892).

EXPERIMENT 3: SUSCEPTIBILITY OF TADPOLES TO PREDATION BY NEWTS

Significantly more infected (3.17 ± 0.48) than uninfected (1.33 ± 0.42) tadpoles were consumed (Student’s t-test, P = 0.016)

EXPERIMENT 4: PREDATOR-CUE

When only the three treatments of infected tadpoles were examined, no significant treatments effects were found for the mean number of animals on the side of the tub closest to incoming treated water (ANOVA, F = 0.48, d.f. = 2, P = 0.62, Table V.2). Significant treatment effects were found for the number of moves (total number of times tadpoles crossed from one half of the tub to the other half) made by the tadpoles (F = 13.34, d.f. = 2, P < 0.0001).

When data (Wilson and Lefcort, In Press; identical design and tadpoles) from uninfected tadpoles are included in an examination with infected tadpoles, no differences were found among the six treatments based on the mean number of animals occupying the tub-side near the influx of treated water (P > 0.380 for all
treatments). Significant differences in the mean number of moves were found for the presence of the parasite \( (F = 68.57, \text{ d.f.} = 1, P < 0.001) \) and the diet of the predators \( (F = 34.44, \text{ d.f.} = 1, P < 0.001) \). However, there was also a significant effect for the interaction between parasite and predator-diet \( (F = 12.42, \text{ d.f.} = 1, P < 0.001) \).
DISCUSSION

Physiological responses of *R. aurora* are associated with altered behavior and increased predation. Infected tadpoles were found in warm shallow water. Behavioral fevers have been associated with the release of cytokines such as IL-1 and TNF in anurans (Kluger, 1977; 1991). The behavioral effects of these endogenously produced substances may be related to alterations in the behavior of infected *R. aurora*; i.e., infected tadpoles suffered increased predation by newts. Furthermore, infected tadpoles exhibited less movement than did uninfected tadpoles when exposed to chemical cues from newts fed insect larvae or conspecific tadpoles. However, there was a significant interaction between the presence of the parasite and the predator’s diet, suggesting that the parasite alters the tadpole’s normal response to newts. Interestingly, thermoregulatory behavior of infected and uninfected tadpoles was not altered by the presence of a caged newt.

Theoretical models indicate that, under certain circumstances, two-host parasites have higher genetic fitness if their hosts’ exhibit altered behavior (Dobson, 1988; Freedman, 1990; see also Lafferty, 1992). However, the benefits to a one-host parasite may be more variable. For example, parasites such as the rabies
virus have increased transmission if the infected host becomes more aggressive (i.e., in the form of biting). However, parasites such as Candida may have decreased fitness if their host is eaten since we were unable to recover the yeast from newts that had consumed infected tadpoles.

For hosts, physiological responses to infection have behavioral ramifications. Behavioral effects of the host defense response may provide a mechanism by which behavioral alterations occur in some one and two-host systems. These behavioral alterations may have adaptive value to hosts [e.g. febrile ectothermic lizards have higher survival than nonfebrile lizards (Kluger et al., 1975) or may simply be by-products of the host defense response that lead to increased predation. The host defense response was selected for, and is maintained because it provides a valuable tool for fighting infection. But aspects of the response may have negative effects on host survival. Overproduction of cytokines can be detrimental. Brain damaging and fatal temperatures are usually just a few degrees above feverish temperatures. Furthermore, overproduction of TNF in mammals causes the wasting and gauntness of cancer associated with many chronic diseases (Titus et al., 1991). TNF's side effects in humans are muscle
stiffness, muscle pain, nausea, and vomiting (Clark, 1987). Indeed, the pathology of cerebral malaria may largely be due to TNF (Clark, 1987).

Cytokines, released during almost all vertebrate infections are extremely powerful and have a wide array of physiological (Titus et al., 1991) and, as this study suggests, behavioral effects. Altered host behavior due to the host defense response may occur in many systems (see Damian, 1987). This may result in augmented transmission in some systems (Ewald, 1991) or, as in this system, result in decreased transmission. Adaptive arguments to explain altered host behavior should therefore be made with caution since altered hosts behaviors may be a generalized response to infection that is independent of parasite life-history variables.
ACKNOWLEDGMENTS

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TABLE V.1
MEAN NUMBER OF TADPOLES (±S.E.) IN SHALLOW ZONES

<table>
<thead>
<tr>
<th>INFECTION</th>
<th>NEWTS</th>
<th>( \text{ABSENT} )</th>
<th>( \text{PRESENT} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSENT</td>
<td>(-) 5.00 ±1.87</td>
<td>4.67 ±1.99</td>
<td></td>
</tr>
<tr>
<td>PRESENT</td>
<td>(-) 7.22 ±2.28</td>
<td>6.22 ±1.61</td>
<td></td>
</tr>
<tr>
<td>TREATMENT</td>
<td># TRIALS</td>
<td>MOVES/TRIAL (MEAN ±S.E.)</td>
<td>% TOWARD INCOMING SCENT (MEAN ±S.E.)</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
<td>--------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated Water</td>
<td>21</td>
<td>28.57 ±3.47</td>
<td>39.82 ±2.46</td>
</tr>
<tr>
<td>Insect-fed Newts</td>
<td>23</td>
<td>15.96 ±4.04</td>
<td>43.15 ±3.17</td>
</tr>
<tr>
<td>Tadpole-fed Newts</td>
<td>19</td>
<td>3.89 ±1.03</td>
<td>39.14 ±3.51</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated water</td>
<td>18</td>
<td>46.55 ±5.77</td>
<td>43.13 ±2.18</td>
</tr>
<tr>
<td>Insect-fed newts</td>
<td>16</td>
<td>64.75 ±6.17</td>
<td>40.48 ±2.54</td>
</tr>
<tr>
<td>Tadpole-fed newts</td>
<td>14</td>
<td>23.00 ±4.35</td>
<td>43.03 ±3.29</td>
</tr>
</tbody>
</table>
SUMMARY

Explanations and interpretations of the adaptive significance of parasitic effects on hosts must consider both selective pressures on hosts and on parasites. Host responses involve a change in physiology, and often, behavior. The responses may be due to many factors: host modifications to avoid or to fight off infection, manipulations of hosts by parasites, or alterations that are not adaptive for hosts or parasites and are simply the consequences of the host response to infection.

Hamilton and Zuk (1982) hypothesized that animals alter their mate choosing behavior to avoid a parasitized mate. Support of the hypothesis has been mixed, although it has found general support in fishes and birds. In the first test of the hypothesis in reptiles, I failed to find supportive evidence (chapter two). In fact, contrary to the hypothesis, my results indicate that lizard species with high parasite loads are showy. Therefore, I did not find evidence that lizards have modified their mating behavior to avoid becoming parasitized.

Snails (Biomphalaria glabrata) do seem to modify their behavior in ways that lessen the impact of parasites. The timing of these modifications appears to
be dependent on whether the snail is susceptible to the parasite, or has innate resistance. Naturally resistant-strain snails seek out cooler temperatures (two degrees Celsius) - temperatures that have been correlated with impaired parasite fecundity - hours after contact with the invading parasite (chapter three). The parasite is unable to establish itself and hence the snail is able to quickly return to normal preferred temperatures. Susceptible-strain snails lower their temperature only slightly (0.5 degrees Celsius). This minor change in temperature of the susceptible strain allows the parasite to successfully establish itself. Later, at five weeks post exposure, the parasite begins to release progeny that damage snail tissues. At this point the susceptible snail responds by seeking out cool water temperatures.

In chapter four I explored fever as a mechanism that may be available for parasites to modify host behavior. In chapter five I found that many behavioral effects of parasitism do not aid in the elimination of the parasite (i.e., are not for the host’s benefit) or the increased transmission of the parasite (i.e., for the parasite’s benefit). Tadpoles parasitized with a yeast exhibited much of the same alterations of behavior that tadpoles exhibit when injected with killed bacteria that are not
growing. In both cases, the behavioral alterations resulted in increased rates of predation. The host does not benefit from being killed (although tenuous inclusive fitness arguments could be made), and since the bacterium and yeast die when its host is consumed, the behavioral alterations of the host do not benefit the pathogen.

My findings in chapters four and five extend our understanding of host-parasite interactions. While it is generally accepted that parasites are under selective pressure to maximize growth, development, fecundity and/or their own transition to a subsequent host; the relative success of these "goals" is largely dependent on the physiological and behavioral responses of their host. The host is far more than just a source of nutrients. The host provides a habitat, a controlled environment and a mode of transportation. Host defense systems (i.e., the acute phase response), due to its effects on host behavior, represent an opportunity for parasitic manipulation.

The acute phase response involves the release of cytokines such as IL-1, IL-6 and TNF whose effects are far reaching and result in a cascade of responses that are often detrimental to the elimination of some parasites. Fever mobilizes the immune system and
exposes some bacteria to lethal temperatures but it also
taxes nutrient stores and can lead ectothermic animals
to engage in thermoregulatory behavior that result in
exposure to predators (chapters four and five).
Casterlin and Reynolds (1977) showed that when bullfrogs
are injected with killed bacteria they exhibit
behavioral fevers. This response was later shown to be
due to the release of cytokines (Kluger 1990).
Cytokines induce sleep that help animals to conserve
resources and possibly avoid activities that expose them
to predators (Hart, 1988), but that same lethargic
behavior can make a foraging or behaviorally
thermoregulating animal susceptible to predation.

Part of the host defense system is specific, i.e.,
antibody production, but part is extremely generalized.
Host defenses must be broad enough to counter bacteria,
viruses, fungi, and both single and multi-celled
animals. By its very nature, a generalized response
must include aspects that are not always beneficial in
dealing with all parasites. For example, the release of
histamine by mast cells causes inflammation which limits
the movements of microorganism through epithelial
layers. However, the same response, when misdirected
against harmless plant pollen, can be incapacitating and
lead to asthmatic attacks. Because the acute phase
response is so easily induced - the exposure macrophages
to any foreign antigen - parasites have the potential to
spark the acute phase response and then benefit from the
resulting response.

Each of the systems studied in this thesis involves
associations of parasites and behaviorally modified
hosts. In every chapter adaptive arguments are
presented that may explain why (potential) hosts do or
do not alter their behavior. Yet the effects of
parasites on host behavior cannot be simplified into a
"who gains" question (see Gould and Lewontin, 1979).
Each host/parasite system is unique. While the
behavioral alterations that animals exhibit may seem to
be either for the host or the parasite, many of these
alterations benefit neither, therefore the driving
forces that cause and conserve these alterations must be
examined on a case-by-case basis.
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BIBLIOGRAPHY


Field guide to North American reptiles and amphibians.  


Bissonnette, E. Y., Rossignol, P. A. and Befus, A. D.
Extracts of mosquito salivary gland inhibit tumor
necrosis factor alpha release from mast cells.


Walls, S. 1991. Mechanisms of coexistence in a guild of
Ambystomatid salamanders: the importance of stage-
dependant regulation in species with a complex life
Lafayette Louisiana.

Parasit. 47:51

Ward, P. I. 1988. Sexual dichromatism and parasitism in
36:1210-1215.

Ward, P. I. 1989. Sexual showiness and parasitism in
freshwater fish: combined data from several isolated

Weatherhead, P. J. 1990. Secondary sexual traits,
parasites and polygyny in red-winged blackbirds,

Webbe, G. and James, C. 1972. Host-parasite
relationships of Bulinus globosus and B. truncatus
with strains of Schistosoma haematobium. J. Helm.
46:185-199.


