**Title**
The Importance of Spatial Heterogeneity in Organisms with Complex Life Cycles: Analysis of Digenetic Trematodes in a Salt Marsh Community



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**Publication Date**
1996-03-01

Peer reviewed

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UNIVERSITY OF CALIFORNIA
Santa Barbara

# The importance of spatial heterogeneity in organisms with complex life cycles: analysis of digenetic trematodes in a salt marsh community

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Division of Ecology, Evolution and Marine Biology

by

Theresa Stevens

Committee in charge:

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March 1996

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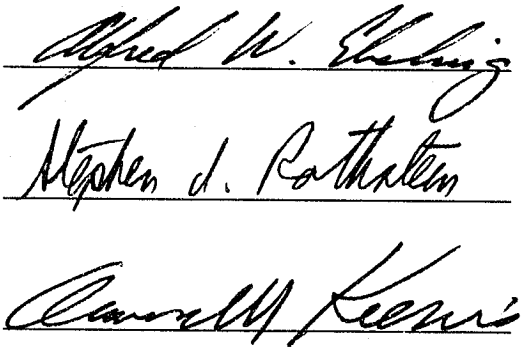

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**Acknowledgments**

This dissertation is dedicated to my father Bill, and sister Denise. Thank you for being my most enthusiastic supporters. I could not have completed this project without your encouragement, understanding, patience, and Denise's French translations. Because of her diligence and professionalism, Denise is now qualified to analyze the role of larval trematodes in the plankton.

Many thanks are also due to my wonderful friends and lab-mates, Carrie Culver, David Kushner, Elzbet Diaz de León, Jeff Shields, Iván Davila-Marcano, Kevin Lafferty, Mark Torchin, Mark Walter, Nancy Smith and Todd Huspeni, who struggled with me to make sense of the complexities of parasitic organisms. Jenny Dugan, Mark Page, Dave Hubbard and Miriam Polne-Fuller provided essential moral support during long weekends at the marine lab. Andy Brooks and his undergraduate volunteers will be remembered for giving us a glimpse at the magnitude of human suffering caused by schistosome dermatitis in a marine system.

Shannon Thomas, Kathleen Sweet and Suzanne Bechtold deserve special recognition for volunteering to move tons of mud, count thousands of estuarine beasts, and for having the determination to learn how to identify larval trematodes.

Richard Hartman helped me keep things in perspective, provided unforgettable flying adventures to Mexico, Alaska and Colorado, and kept me sharp by continually asking me pesky questions about topics in molecular biology and genetics.

Finally, I am indebted to my major advisor, Armand Kuris, whose guidance and encouragement sparked my interest in larval trematodes. Thank you Armand, for giving me a shot at the PhD. Stephen Rothstein and Al Ebeling are responsible for my scholarship and attention to detail. I am grateful to all committee members for providing valuable comments and helpful recommendations on this project and manuscript.

California Sea Grant Graduate Student Research Fellowship, Sigma Xi, and the UC Natural Reserve System Mildred Mathias Graduate Student Fellowship generously sponsored this work.

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## ABSTRACT

**The importance of spatial heterogeneity in organisms with complex life cycles : analysis of digenetic trematodes in a salt marsh community**

**by**

**Theresa Stevens**

Populations of organisms often exhibit a surprisingly heterogeneous spatial distribution. Spatial heterogeneity may result from recruitment variation, habitat variability or post-recruitment processes such as competition, predation or disease. Parasites, particularly those with complex life cycles, can be used to study the generation of spatial heterogeneity and recruitment because the sampling units (their hosts) are biologically meaningful replicates and spatially heterogeneous in their distribution. Furthermore, the parasites must recruit from one host to another to complete the life cycle, and recruitment processes may intensify or diminish spatial variability. Spatial heterogeneity and recruitment of parasites can be studied at several levels; within individual hosts, host populations or host communities. Host behaviors or activities may also affect the distribution of parasites within individuals or populations. Specifically, the movement of a host in its' habitat (host vagility) may effectively diminish spatial heterogeneity of parasites as a host moves between areas of variable exposure risk.

Digenetic trematodes have complex life cycles, which require at least two hosts. In this study, the first intermediate host is the snail,

Cerithidea californica. Second intermediate hosts include the crab, Hemigrapsus oregonensis, two fishes, Gillichthys mirabilis and Fundulus parvipinnus, and the clam, Tagelus californianus. The snail population at Carpinteria Salt Marsh harbors 13 different trematode species. Of these 13 species, two parasitize the crab, two parasitize the fish, G. mirabilis, and one parasitizes the clam. Other larval trematode species infect F. parvipinnus, or don't require a second intermediate host (Austrobilharzia sp.).

The purpose of this study was to investigate processes that affect the spatial distribution abundance of larval trematodes at two host levels. This was accomplished by determining if spatial heterogeneity of larval trematodes in the snail population at Carpinteria Salt Marsh was reflected in second intermediate host populations, and determining the influence of second intermediate host vagility on infection patterns (Chapter 1). I experimentally examined this second issue by determining whether vagile versus restrained crabs had intermediate levels of infection, compared to control groups. A field survey was conducted to investigate spatial and temporal variability in emergence of infective larvae (recruits) from the snail source population (Chapter 2). I also estimated the proportion of infections expected in snails in the absence of intra-molluscan interspecific interactions (Chapter 3). This was done by using the proportion of infected snails in a model developed by Lafferty et al. (1994) which permitted an analysis of the importance of these interactions on the

abundance of larval trematodes. In addition, this analysis evaluated the effect of heterogeneous recruitment of larval trematodes to snails.

These studies confirmed that there was significant spatial heterogeneity of trematode infections in the snail, crab and clam populations, but not in fish. There was also a significant correlation between the density of infected snails and the abundance of trematodes for one of the species in crabs, Himasthla rhigedana; while Probolocoryphe uca showed a positive but non-significant association in crabs. There was no association between density of infected snails and the abundance of Euhaplorchis californiensis in fish, and a slightly negative association for Renicola buchanani. Abundance of Acanthoparyphium spinulosum in clams was not significantly associated with prevalence in snails.

Experimental investigations on vagile versus sedentary (control) sentinel crabs showed that all experimental crabs attained nearly 100% prevalence in a short (22 day) period. Vagile crabs obtained infection prevalences that were similar to control crabs, but infection intensities were generally lower than free-ranging resident individuals.

Emergence of infective larvae from the source snail population was intermittent on a daily basis and seasonally variable. Spatial and temporal heterogeneity was also observed in the diversity and abundance of infective larvae in the water. Temperature most strongly influenced emergence of infective larvae from snails; and there was a highly significant, positive correlation between temperature and daily abundance

in the field. Salinity had no effect on spatial or temporal emergence patterns. There were 9 cercarial species identified in the water samples, all which emerged from C. californica. For half of these species, there was a significant positive association between abundance of infective larvae in the water and abundance in the snail source population.

Interspecific interactions of larval trematodes in C. californica reduced the abundance of most species in the snail population; however, this study reports a greater number of double infections than other comparable studies of C. californica. Furthermore, heterogeneity in recruitment of larval trematodes to snails intensified these interactions rather than isolate species.

## TABLE OF CONTENTS

## LIST OF FIGURES

# CHAPTER 1

## The Importance of Spatial Heterogeneity for Larval Trematodes

### Introduction

Spatial patchiness, a heterogeneous distribution of organisms, is commonly observed. However, how such heterogeneity is generated and/or maintained is in dispute. Spatial variation in the availability of suitable habitat and recruitment of individuals to the available habitat could lead to a patchy distribution of organisms. Temporal variation in recruitment of individuals to the available habitat could further influence spatial patterns by altering the density of individuals in a patch. Within a patch, post-recruitment processes such as differential mortality of individuals or intra- and interspecific interactions among individuals may control populations and influence the spatial distribution of organisms. Furthermore, post-recruitment movement of organisms between patches could also alter spatial patterns. In short, the relative importance of the different mechanisms that generate or maintain spatial heterogeneity is difficult to elucidate because recruitment variation and post-recruitment processes are not mutually exclusive.

There were three primary objectives to this study. Firstly, I investigated spatial patterns of larval trematode populations at 2 host levels. Second, I tested the hypothesis that the abundance of larval trematodes in second intermediate hosts was independent of infection levels measured in the first intermediate host population. Finally, I tested the hypothesis that movement of second intermediate hosts reduced

spatial patchiness in the snail population and thereby increased dispersion of larval trematodes in a second intermediate host population.

***Digenetic Trematodes as Model Systems***

Digenetic trematode populations provide ample opportunity to address basic ecological questions. Their complex life cycles permit investigation of mechanisms that generate or maintain spatial heterogeneity because trematodes recruit via free-living stages to their first and second intermediate host populations. In addition, since hosts are discrete, replicated habitat units (Esch et al. 1990), trematode population size and dispersion can be readily quantified at different host levels.

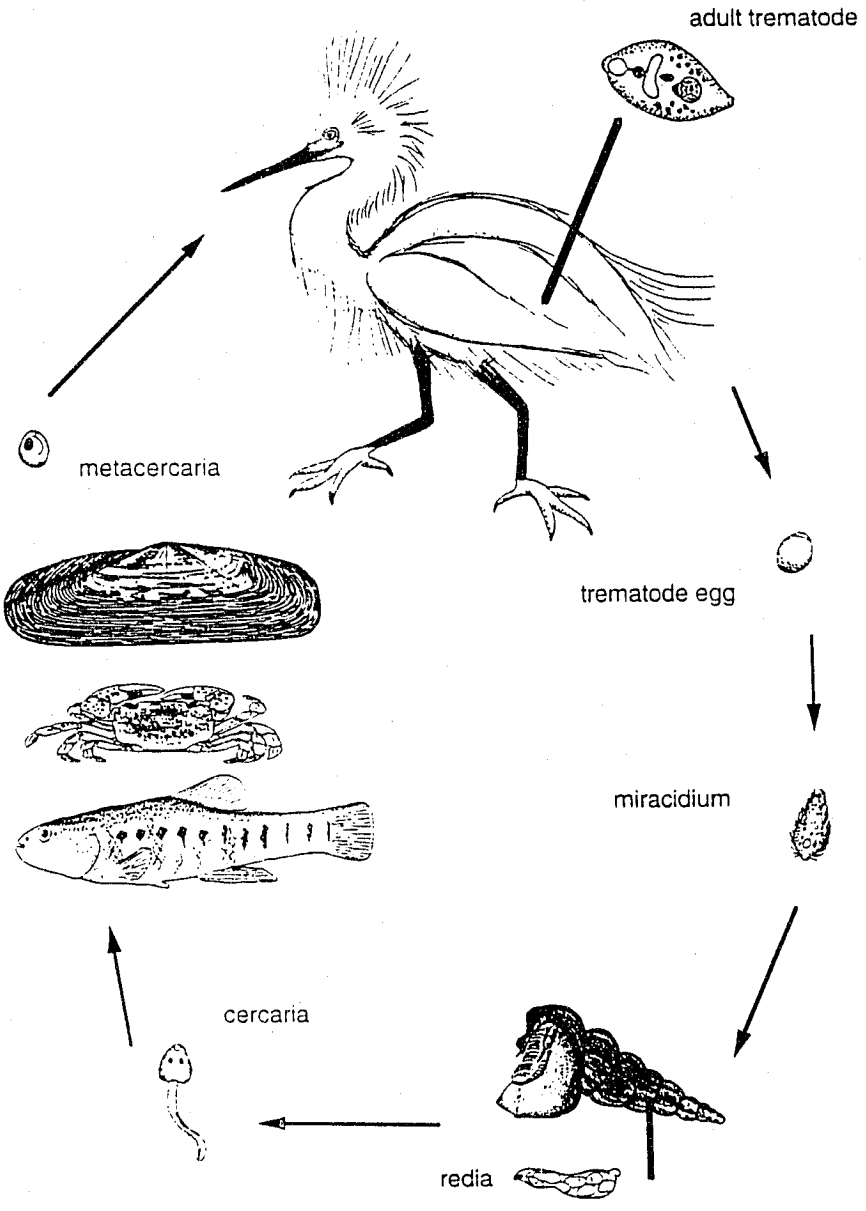
A recent controversy underscores the importance of two very different mechanisms that create spatial heterogeneity of larval trematodes in snail populations. Kuris (1990) showed that interspecific interactions effectively control the prevalence (proportion of infected hosts) of larval trematode species in individual snails, Cerithidea californica. Kuris (1990) concluded that these interactions were important determinants of species abundance in the snail population. Furthermore, Lafferty et al. (1994) and Kuris and Lafferty's (1994) meta-analysis of 62 data sets showed that spatial and temporal variation in recruitment of larval trematodes (i.e. eggs or miracidiae) to snails intensified potential interactions in individual snails. Interspecific interactions among trematode species in individual snails were shown to have the greatest impact on the abundance of subordinate species (see Kuris 1990

dominance hierarchy), were responsible for the low frequency of double infections (Kuris 1990; Lafferty et al. 1994; Kuris and Lafferty 1994), and potentially affected the abundance of transmissive stages (i.e. cercariae) which emerge from snails to infect the next host (Kuris 1990).

In contrast, Sousa (1990) suggested that the complex life cycles of digenetic trematodes (Fig. 1) were responsible for isolating species in the first intermediate host snail, C. californica. Although Sousa (1990) agreed with Kuris' (1990) view that interspecific interactions operate in individual snails, he suggested that the outcome of these interactions was not detectable in the snail population. Spatial patchiness and the prevalence of each trematode species in the snail population was attributed to spatial and temporal variation in abundance of larval trematode recruits (eggs or miracidiae) and susceptible hosts (Sousa 1990). This view was also supported by observations of Fernandez and Esch (1991a, b) in a fresh water pond system. Thus, Sousa (1990) and Fernandez and Esch (1991a, b) concluded that interspecific interactions between larval trematodes in individual snails do not control the prevalence of larval trematode species in snail populations.

In essence, this debate framed the present study because Kuris (1990), Sousa (1990) and Fernandez and Esch (1991a, b) provided evidence that recruitment variation and post-recruitment interactions do not operate independently, in that both interspecific interactions in snails and variable recruitment to the snail population have a direct impact on

Fig. 1. Life cycle of digenetic trematodes from Carpinteria Salt Marsh (CSM, Santa Barbara County, California. Adult trematodes generally live in the small intestine of local and migratory birds. Exceptions include Renicola buchanani which lives in veins of the kidney, and Austrobilharzia sp. which lives in veins of the hepatic portal system. Trematode eggs are passed onto the mudflat or into the water with the bird feces. Snails become infected by ingesting eggs or they are infected by a free-swimming miracidium hatched from an egg. Within the snail, eggs or miracidium metamorphose to worm-like rediae or sac-like sporocysts. These stages can live in the mantle, digestive gland or gonad of the snail, castrating snails when the gonad is occupied. Rediae and sporocysts asexually produce infective cercariae, which emerge from the snail to infect second intermediate host clams, crabs or fish. In these hosts, cercariae lose the tail during the process of encysting as metacercariae. Metacercariae remain in a diapause-like state while encysted on the surface of the host or embedded within tissues of second intermediate hosts. Definitive host birds become infected via predation on infected second intermediate hosts. Digestive enzymes in definitive hosts facilitate excystation of metacercariae, and new adult worms develop and begin egg production in the small intestine, kidneys or hepatic veins of the bird. In contrast to all other species in this system, Austrobilharzia cercariae infect definitive host birds directly after leaving the snail. Drawing adapted from Lafferty 1996 (in press).



production of transmissive stages (i.e. cercariae) and ultimate trematode population size at other host levels.

***Spatial Heterogeneity and Second Intermediate Host Vagility***

Most organisms are vagile and move within or between habitat patches in search of food, shelter or mates. In host-parasite systems, host vagility could influence dispersion of parasites among hosts because vagility increases or decreases the rate of contact between host and parasite. Kuris (1990) proposed that second intermediate host (e.g. crab, clam, fish) vagility integrates spatial patchiness of larval trematode species in the snail population on a local scale. Sousa and Grosholz (1991) alternatively suggested that the spatial distribution of hosts and their parasites would not be closely associated when parasites have widely dispersing, free-living infective stages. When applied to larval trematode systems, their hypothesis suggests that vagility of larval trematode cercariae may be a more important determinant of trematode dispersion in host populations than host movement or behavioral patterns that affect the rate of host contact with free-living cercariae.

As with arguments presented for mechanisms that generate or maintain spatial heterogeneity in snails (recruitment variation versus interspecific interactions), host vagility and parasite dispersal are not mutually exclusive. Thus, the key to understanding trematode population dynamics and flow of parasites through a series of hosts, is to distinguish the contribution of each mechanism (spatial heterogeneity, host vagility,

parasite dispersal, and competitive interactions between parasites) to infection levels in different host populations.

***Objectives***

The main objectives for this study were to investigate spatial heterogeneity of larval trematode species in first and second intermediate host populations, and quantify the impact of second intermediate host vagility on larval trematode dispersion in an experimental population of uninfected second intermediate hosts.

Cerithidea californica is the most abundant snail at Carpinteria Salt Marsh (CSM). This snail population serves as first intermediate host to a well known assemblage of 13 different larval trematode species (Maxon and Pequegnant 1949; Martin 1955; Martin 1972; Yoshino 1975; Sousa 1983, 1990; Lafferty 1991). Furthermore, spatial heterogeneity of larval trematode infections in these snails has been documented at Bolinas Lagoon (Sousa 1990) and at CSM (Kuris 1990; Lafferty et al. 1994). Second intermediate hosts were identified from Upper Newport Bay, California (Martin 1972), and included C. californica, Uca crenulata (crab) Fundulus parvipinnis (fish), and Gillichthys mirabilis (fish).

The issue of spatial heterogeneity of larval trematodes in host populations was addressed at CSM by conducting host surveys and reciprocal transplant experiments. These methods were used to: 1) determine which organisms serve as second intermediate hosts for trematodes that infect C. californica, 2) quantify spatial heterogeneity of larval trematodes in first and second intermediate hosts, 3) determine how

much the prevalence of larval trematodes in the first intermediate host explains or influences spatial patterns of infection and abundance of larval trematodes in second intermediate hosts, and 4) assess the importance of second intermediate host vagility as a mechanism that integrates high and low prevalence patches of larval trematodes.

## Materials and Methods

Host-parasite surveys were conducted during the spring and summer of 1992 and during the winter and spring of 1993. Trematodes in the California salt marsh snail, Cerithidea californica, can be identified using the free-swimming cercarial stages produced by either rediae or sporocysts (Martin 1972). Second intermediate host crabs, clams and fish are infected with metacercariae of some of these trematodes.

This survey was conducted in Carpinteria Salt Marsh (CSM), Santa Barbara County, California. Eight sites (A - H) were randomly chosen by placing a grid over a map of the marsh (Fig. 2). Pairs of numbers were randomly selected until eight sites which fell within or directly next to a channel were obtained. I used a compass and a map marked with the selected points to locate each site in the field. At each site a one meter long piece of PVC pipe was inserted in the mud as a marker and flagged with surveyors' tape.

Host-parasite surveys were completed at each site by using transect lines, quadrats and cores to collect specimens. At each site a 10m transect line was placed along the edge of the pickleweed (Salicornia virginica), bisected by the site marker pipe. Three sampling

Fig. 2. Map of Carpinteria Salt Marsh (CSM). Sites A - H were surveyed for snails, crabs and fish. Infections were quantified for snails and crabs in the summer and winter. Clams were surveyed at sites 1 - 4. Infections in fish and clams were quantified in summer only.

points were randomly selected along the transect line. These three points along the line (meter 3, 5 and 8) were used at each site. Hosts were obtained at each site by counting and collecting individuals from a 30 x 300 cm swath, perpendicular to the transect line at each of the randomly selected points.

***Host Collection and Dissection***

Cerithidea californica density was estimated by counting all snails in 900 $cm^2$ (approximately 0.1 $m^2$) quadrats. Two- to four hundred snails were randomly collected at each site, with about 1/3 taken from each swath. A minimum of 200 snails was sufficient to estimate prevalence at each site [prevalence is the percentage of snails that are infected (Margolis et al. 1982)]. Snails were taken to the laboratory and dissected. Single, double and triple infections were also recorded. Snail length was measured in millimeters from the tip of the spire to the posterior margin of the outer lip of the aperture; snail sex was determined by noting the presence/absence of a female ovipositor (Houbrick 1984); and the trematode(s) were identified (Martin 1972). The density of snails infected with a particular trematode species at each site was estimated by multiplying the proportion infected times the average snail density. Double (and triple) infections were scored such that each species in the combination contributed one infection to the estimated prevalence for that species. After the snails were counted and collected from each quadrat, a

destructive sampling method was used to collect yellow shore crabs, Hemigrapsus oregonensis, and clams.

Hemigrapsus oregonensis was selected for this study because it is abundant, easy to collect, tolerates laboratory conditions, and the distribution of its burrows on the mud-flat occurs beneath the preferred habitat of C. californica. In each quadrat, a garden trowel was used to capture crabs from burrow entrances. At each site, most crabs were collected using this method. This was less destructive than coring or spading, and disturbances to the sites were usually remedied by tidal action after a few weeks. A total of 50-75 crabs from each site were usually available to estimate trematode prevalence and intensity [intensity is the number of metacercariae (or parasites) per infected host; measures of abundance include unifected individuals in calculation of the mean and standard deviation (Margolis et al. 1982)]. Crabs were taken to the laboratory and within one day were sexed, measured (mm carapace width), and dissected. Dissections included examination of the surface of the carapace and each limb; characterization of the molt stage (Poinar and Kuris 1975); and examination of the thoracic ganglion, gills and buccal mass. All of the hepatopancreas tissue was removed from the body cavity and pressed between two microscope slides for quantification of metacercariae embedded in these tissues. Trematode metacercariae were counted and identified using the methods of Stunkard and Cable (1932), Sarkisian (1957), Adams and Martin (1963), and Heard and Sikora (1969). Bush confirmed species identifications using adult specimens

obtained from experimentally infected chicks (personal communication). The prevalence and intensity (number of parasites per host) of other parasites (larval nematodes, Portunion conformis) were recorded.

Clams were collected using a core sampler (10 cm diameter, 78.5 $cm^2$ area). Two 0.5 m deep cores were taken within each quadrat. All clams in the cores were identified and counted. After the core was extracted from the mud, the bore holes were checked for other individuals by probing. Coring usually did not provide enough clams for a sample, so additional individuals were collected by coring from outside the quadrats but within the transect boundary. In this manner, approximately 30 individuals of Tagelus californianus, Protothaca staminea, Macoma nasuta and Laevicardium substriatum were collected from sites where clams were present. Shell length was recorded, and clams were dissected within 3 days to determine if trematode metacercariae were present. Dissections included examination of the shell surface (inner and outer), heart, siphons, digestive gland, foot, and mantle tissue.

Tagelus californianus was the only clam infected with trematode metacercariae, and it was only abundant at site H. Hence, it was also surveyed at three other sites (2 - 4, Fig. 2). Approximately 30 T. californianus were collected at each of the three new sites, as were approximately 200 snails. All animals were taken to the laboratory and dissected. Trematodes were identified and counted within 4 days of collection.

Fish were also surveyed for trematode infections. Seining was attempted at each site, but was ineffective at most sites because it was hard to pull the seine over extremely soft mud. Baited traps were employed as an alternative method, which limited the study to the long jawed mudsucker, Gillichthys mirabilis, because only these fish readily entered the traps. Furthermore, G. mirabilis is known to be infected with at least two trematode species that originate from C. californica (Martin 1950a; Martin and Gregory 1951; Martin 1971; Martin 1972). Traps were baited with pieces of salted mackerel and placed at each site for a series of days until at least twenty fish were collected. All G. mirabilis were taken to the lab and frozen prior to dissection. Fish were thawed, and standard length (in millimeters), sex, prevalence and intensity of trematode infections were recorded. Dissections included examination of the body surface, and internal organs. The primary focus of dissections was the liver and brain tissues because these tissues were known to be infected with larval trematodes. These tissues were pressed between two microscope slides and metacercariae were counted.

***Host Vagility Experimental Design***

Results from the host surveys indicated that the shore crab, Hemigrapsus oregonensis, was the most suitable host for vagility experiments. These crabs are abundant in coastal salt marshes, are easily captured, and can tolerate a range of temperatures. Himasthla rhigedana was the trematode selected for this transplant experiment because it encysts on the carapace of crabs, and because this species

was relatively abundant in snails at 3 of the 6 sites. Sites B and C (Fig. 2) were not included in the transplant experiment because site B had been covered with silt during winter storms and all snails were killed. Site C was not included because it only experienced tidal inundation on extreme high tides, therefore caged experimental crabs probably would have suffered from temperature and desiccation stress. Prevalence of H. rhigedana in snails was determined in a winter 1994 snail survey.

Approximately 200 Hemigrapsus oregonensis were collected from the Morro Bay boat harbor, San Luis Obispo County, California. All crabs had at least a 12 mm carapace width, to reduce the chance of escape from the cages. All crabs collected from Morro Bay were examined prior to caging, to verify that none were infested with H. rhigedana. These crabs were maintained in the laboratory and fed a diet of salted mackerel for one week prior to the start of the experiment. Cages were constructed out of heavy (6.3 mm mesh) Vexar™. Each cage was tube shaped, approximately 75 cm long and 15 cm in diameter. Each end of the tube was covered with a separate piece of Vexar™. The tube and end pieces were lashed with cable ties, and each cage was tethered in the center of the channel with stakes. Each cage housed 25 crabs. Crabs of difference sizes were distributed evenly across cages such that each cage contained approximately the same size distribution. Two cages were placed at each site, and left in the field for 22 days. Cages were checked every other day, and molted exuviae or dead crabs were removed at that time. Small pieces of salted mackerel or anchovy were added to each cage every 5

days. At day 11, one cage from each site was reciprocally transplanted. The other cage was left at the original site, and cages were left in the field for an additional 11 days. Prevalence of H. rhigedana in snails was designated as either high or low (Table 1), and crabs were transplanted from a low to high prevalence site, or vice versa. At the end of the 22 day period, approximately 40 crabs (greater than 12 mm carapace width) from each site were collected using the garden trowel method. All crabs (caged and wild caught) were taken to the lab and frozen. Crabs were dissected according to the procedures described above.

This reciprocal transplant experiment was designed to quantify the effect of artificially imposed host vagility on infection prevalence and intensity, and to test the hypothesis that second intermediate host vagility can reduce spatially patchy infections in snails. This hypothesis predicts that infection intensities in transplanted hosts would be intermediate between infection intnesities of control crabs in either a zone of high prevalence or in a low prevalence zone.

**Table 1** **Prevalence (%) of *Himasthla rhigedana***

| Site and Code | Initial site | Final site |
|---|---|---|
| A - low | 3.8 | 18.1 |
| D - low | 2.0 | 15.4 |
| H - low | 1.3 | 4.4 |
| E - high | 15.4 | 2.0 |
| F - high | 4.4 | 1.3 |
| G - high | 18.1 | 3.8 |

A mark-recapture experiment was conducted to determine natural vagility of H. oregonensis. Approximately 305 crabs were collected from the Santa Barbara Boat Harbor. All crabs were marked with a small spot of waterproof paint on the carapace. Regularly spaced pit traps were placed across the width of the main north-south channel on the east side of the access road in CSM (Fig. 2), for a total linear distance of 25 meters. Crabs were released simultaneously in the center of the pit trap array, and the site was left undisturbed for one week. After one week, pit traps were collected and the entire pit trap area was excavated to one shovel depth.

***Data Analysis***

Data were processed using Microsoft Excel 4.0 and Systat 5.2. Analysis of variance and analysis of covariance (ANOVA, ANCOVA) were used to test for differences in abundance of metacercariae among sites. A heterogeneity Chi-square (G-test) was used to test for differences in prevalence between sites. Prevalence was Arcsin transformed prior to regression analyses among snail sizes, snail density and prevalence. This transformation normalizes the data. Step-wise multiple regression analyses were used to test for associations between infections in snails and second intermediate hosts. For all regression analyses, abundance in second intermediate hosts was log transformed [log (n + 1)] prior to statistical test(s). ANCOVA was used to test for differences in abundance between resident and sentinel crabs in the reciprocal transplant experiment. Analyses on second intermediate hosts were also conducted on the more common measure of intensity (number of parasites per

infected host). Typically, analyses involving variation in intensity exclude hosts that were uninfected, but in this study abundance of metacercariae provided a more meaningful measure of trematode population size in second intermediate hosts, For example, the mean abundance of metacercariae was calculated using data from all hosts, whether they were infected or not. In all cases, regression analyses using abundance as the dependent variable had slightly steeper slopes than similar analyses using intensity, and in no case, was there a statistically significant regression slope for abundance but not intensity.

## Results

### *Prevalence of trematodes in the snail, Cerithidea californica*

A total of 13 larval trematode species were identified from the 2,905 C. californica dissected in the host survey, with Euhaplorchis californiensis being the most common. Overall, trematode prevalence at different sites ranged from 15.8 to 81.7% (Fig. 3), and there was a significant difference in prevalence of larval trematodes between sites (Table 2; $G_H = 515.06$, df = 7, $p < .01$). Prevalence of larval trematodes was positively associated with snail size (Fig. 4, ANOVA: $F = 10.987$, $p = .016$), as size varied significantly among sites (ANOVA: $F = 212.73$, $p < .05$) and accounted for 64.7% of the variation in prevalence. Sites E and G had the largest snails and the highest overall prevalence of larval trematodes, Himasthla rhigedana and E. californiensis being most common at these sites (Table 2). In contrast, site B had the lowest prevalence of larval trematodes (15.8%) and site H had the smallest snails (mean = 20.5 ± .283 s.e.). At

**Table 2.** Prevalence of trematode species in Cerithidea californica at CSM, summer and winter samples pooled. Snails are listed according to the dominance hierarchy of Kuris (1990). Immature infections in snails were not identified to genus or species. N represents the number of snails dissected from each site. Prevalence estimates for individual species include double infections.

**Key to Trematode Species:**

PARO - *Parorchis acanthus*
HIMA - *Himasthla rhigedana*
AUST - *Austrobilharzia* sp.
ECHI - *Echinoparyphium* sp.
ACAN - *Acanthoparyphium spinulosum*
CLOA - *Cloacitrema michiganensis*
EUHA - *Euhaplorchis californiensis*
PHOC - *Phocitremoides ovale*
PYGI - *Pygidiopsoides spindalis*
RENI - *Renicola buchanani*
CATA - *Catatropis johnstoni*
STRI - Strigeid cercaria
PROB - *Probolocoryphe uca*

| Species | Site A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| uninfected | 44.55 | 84.23 | 36.43 | 63.87 | 18.23 | 59.49 | 25.28 | 68.23 |
| PARO | 0.79 | 0 | 0.37 | 0 | 2.03 | 1.90 | 0.83 | 0 |
| HIMA | 3.76 | 1.15 | 4.09 | 2.00 | 15.44 | 4.43 | 18.06 | 1.34 |
| AUST | 0 | 0 | 0 | 0 | 0.51 | 0 | 0.83 | 0 |
| ECHI | 3.37 | 0.38 | 41.3 | 1.00 | 7.09 | 25.95 | 11.94 | 1.34 |
| ACAN | 1.19 | 0 | 2.60 | 1.20 | 3.80 | 2.53 | 3.33 | 0.67 |
| CLOA | 0 | 0 | 0 | 0 | 0 | 0.32 | 0 | 0.33 |
| EUHA | 18.61 | 7.31 | 9.67 | 27.15 | 41.52 | 1.58 | 34.17 | 18.39 |
| PHOC | 0.20 | 0 | 0.37 | 0.20 | 3.29 | 0 | 1.39 | 1.34 |
| PYGI | 0 | 0 | 0.74 | 0.80 | 0.51 | 0.63 | 0 | 0 |
| RENI | 2.57 | 3.08 | 0.74 | 1.20 | 7.59 | 1.58 | 3.33 | 3.01 |
| CATA | 0.79 | 0 | 1.49 | 0 | 0 | 0.32 | 0 | 1.67 |
| STRI | 3.76 | 4.62 | 0.37 | 3.79 | 11.65 | 0 | 2.78 | 2.01 |
| PROB | 0.59 | 0 | 0 | 0.20 | 1.77 | 0 | 1.94 | 0.33 |
| immature | 20.99 | 0 | 3.35 | 2.59 | 1.52 | 1.27 | 2.78 | 4.35 |
| N Snails | 505 | 260 | 269 | 501 | 395 | 316 | 360 | 299 |

Fig. 3. Prevalence of larval trematodes in Cerithidea californica at different sites. See Table 2 for prevalence of individual species.

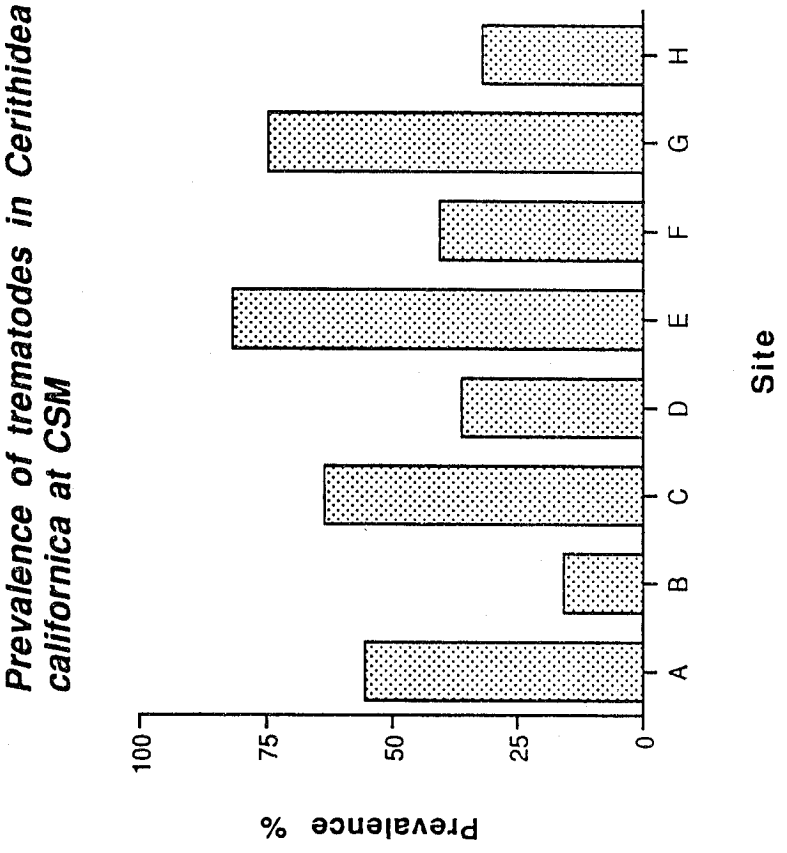
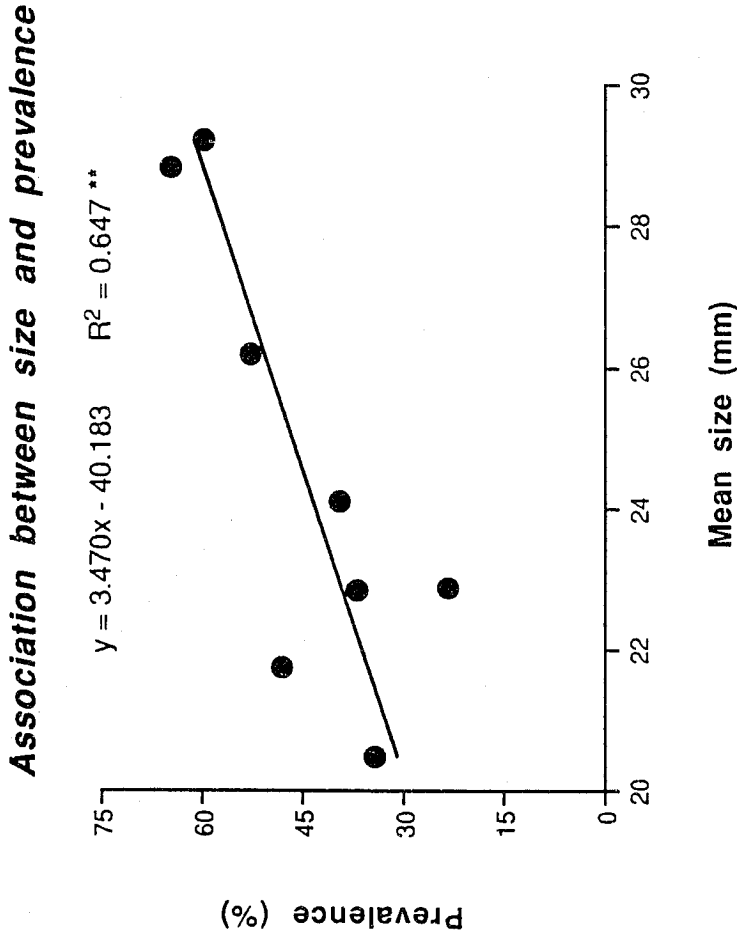


Fig. 4. Association between size and prevalence in C. californica. Each point represents a different site. Prevalence was Arcsin transformed prior to regression analyses.



site F Echinoparyphium sp. was the most abundant larval trematode (25.95%), in contrast to all other sites where E. californiensis was the most abundant larval trematode species in snails (Table 2).

Regression analysis revealed no significant association between snail size and density (Fig. 5, $F = .220$, $p = .656$), nor was there any significant association between snail density and prevalence (Fig. 6, $F = .384$, $p = .558$). However, there were significant differences in snail density among sites (ANOVA: $F = 10.79$, $p < .05$). There was no significant difference in prevalence between males and females among the 2,735 snails that were sexed (Chi square = 1.41, $p > .05$).

Prevalence of Himasthla rhigedana and Probolocoryphe uca in snails was not significantly different between summer and winter samples, so these data were pooled for further analyses involving snails and second intermediate host crabs. There was also no significant seasonal difference in prevalence of Renicola buchanani and Acanthoparyphium spinulosum in snails. In contrast, there were significant seasonal differences in prevalence of Euhaplorchis californiensis in snails ($G_H = 24.9$, $df = 5$, $p < .05$). However, there was no need to pool summer and winter data for R. buchanani, E. californiensis or A. spinulosum because collections of second intermediate host fish and clams were done only in the summer sample period.

Fig. 5. Association between snail density and snail size. Density data were log transformed (n + 1) to account for quadrats which contained no snails. Each point represents a different site.

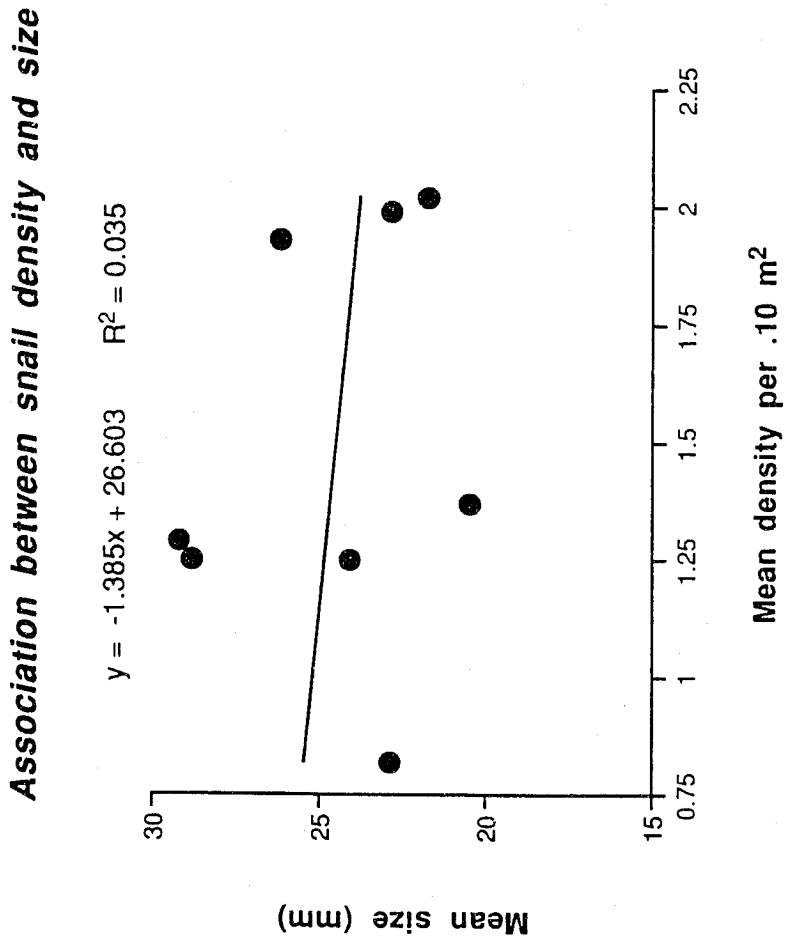
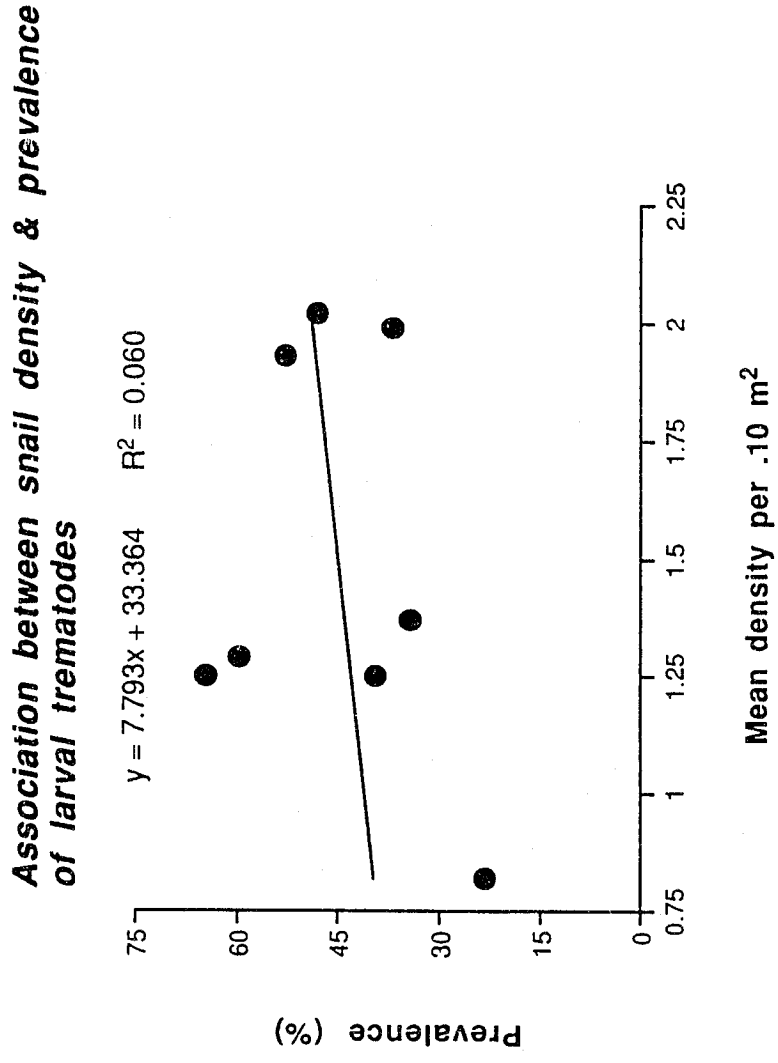


Fig. 6. Association between snail density and total prevalence of larval trematodes. Prevalence was Arcsin transformed prior to regression analysis to normalize the distribution of proportions. Density data were log transformed (n + 1) to account for quadrats which contained no snails. Each point represents a different site.



***Prevalence and abundance in the crab, Hemigrapsus oregonensis***

Of the 13 trematode species identified from C. californica at CSM, metacercariae of Himasthla rhigedana (Echinostomatidae), and Probolocoryphe uca, (Microphallidae) were abundant in the crab and it was common for crabs to be infected with both species; H. rhigedana on the carapace, P. uca in the hepatopancreas. A third, much less common, metacercarial species, Levinseniella charadriformis, was also observed as an aggregated mass beneath the hepatopancreas tissue dorsal to the thoracic ganglion. This trematode utilizes littorinid snails (Littorina scutulata) as its first intermediate host (Ching 1963). Dissection of approximately 100 of L. scutulata and Littorina saxitalis from CSM revealed no infections, and the following analyses on H. oregonensis do not include this species.

Crab size, sex, site, and the density of snails infected with either H. rhigedana or P. uca were found to account for some of the variation in abundance of metacercariae in crabs. Crab size varied significantly among sites (ANOVA: $F = 11.244$, $df = 7$, $p < .05$), and sites C and H had the largest crabs (site C: mean size = 14.76 mm, site H: mean size = 15.27 mm carapace width). Crab size was significantly different between male and female crabs. Male crabs were larger than females (ANOVA: $F = 95.08$, $p < .05$). However, when crab size was held constant there was no significant difference in prevalence (Chi-square = .106, $p < .05$) or abundance (ANCOVA: $F = 1.913$, $p = .167$) of H. rhigedana metacercarie

between sexes. In contrast, there were significant differences in prevalence (Chi-square = 4.53, $p < .05$) and abundance (ANCOVA: $F = 15.2$, $p < .05$) of P. uca metacercariae in males and females. Overall, male crabs harbored approximately P. uca 55% of the infections; but female crabs had higher intensities of P. uca metacercariae than males.

Prevalence of H. rhigedana infestations ranged from 24.2 to 99% (Fig. 7), and abundance ranged from zero to approximately 700 metacercariae. Abundance was positively correlated with crab size for both H. rhigedana and P. uca ($R = .294^*$ and $.362^*$ respectively, $p < .05$). When crab size was held constant, both prevalence and abundance of H. rhigedana varied significantly among sites ($G_H = 64.2$, $df = 7$, $p < .01$; ANCOVA: $F = 116.76$, $p < .05$).

Probolocoryphe uca was also abundant in crabs. Prevalence ranged from 40.6 to 100% (Fig. 7), and abundance from zero to 537 metacercariae. Like H. rhigedana infections, P. uca prevalence and abundance varied significantly among sites when crab size was held constant ($G_H = 116.7$, $df = 7$, $p < .05$; ANCOVA: $F = 64.78$, $p < .05$).

Regression analyses revealed that there was a positive association between the density of infected snails and abundance of metacercariae in second intermediate hosts. This association was significant for H. rhigedana (Fig. 8, $F = 19.01$, $p = .001$) and showed that crabs from sites with a high density of infected snails had significantly more metacercariae than crabs from sites with a low density of infected snails. In contrast, the

Fig. 7. Prevalence of Himasthla rhigedana (HIMA) and Probolocoryphe uca (PROB) in the crab, Hemigrapsus oregonensis.

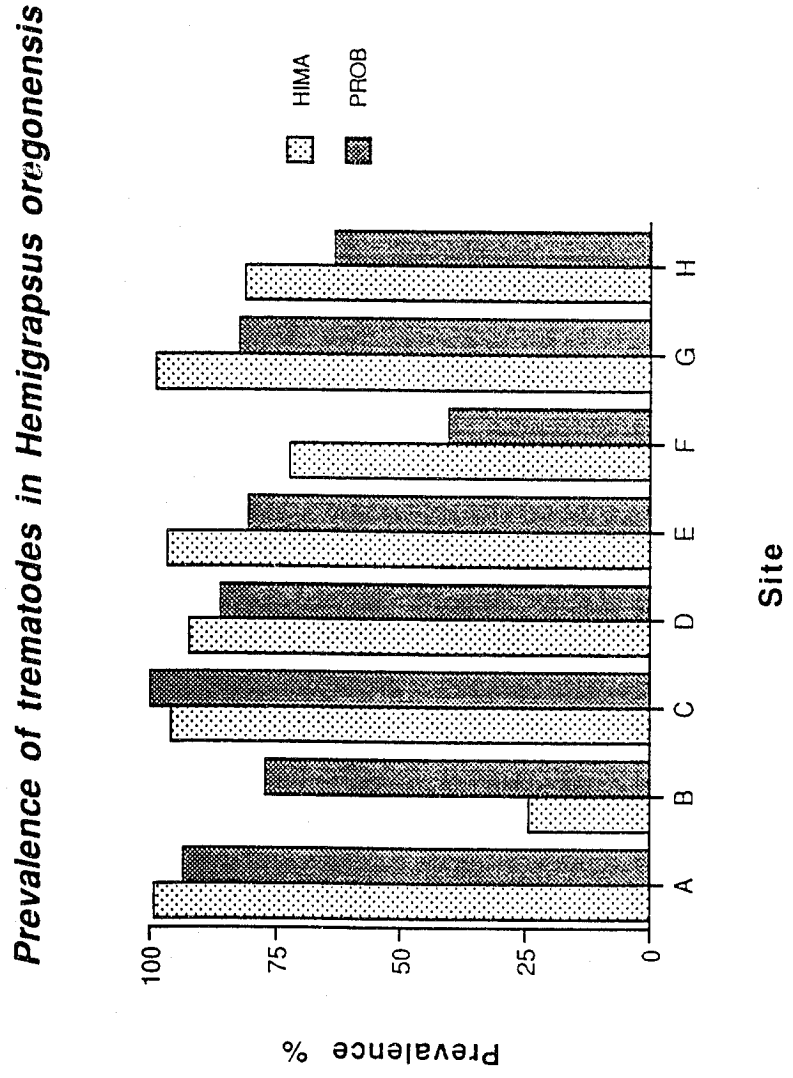


Fig. 8. Regression analysis on abundance of H. rhigedana metacercariae on crabs as a function of density of snails infected with H. rhigedana rediae. Abundance in crabs was log transformed (n + 1) prior to statistical tests. Summer and winter data were pooled for this analysis. Each point represents a different site. * $.01 < p < .05$

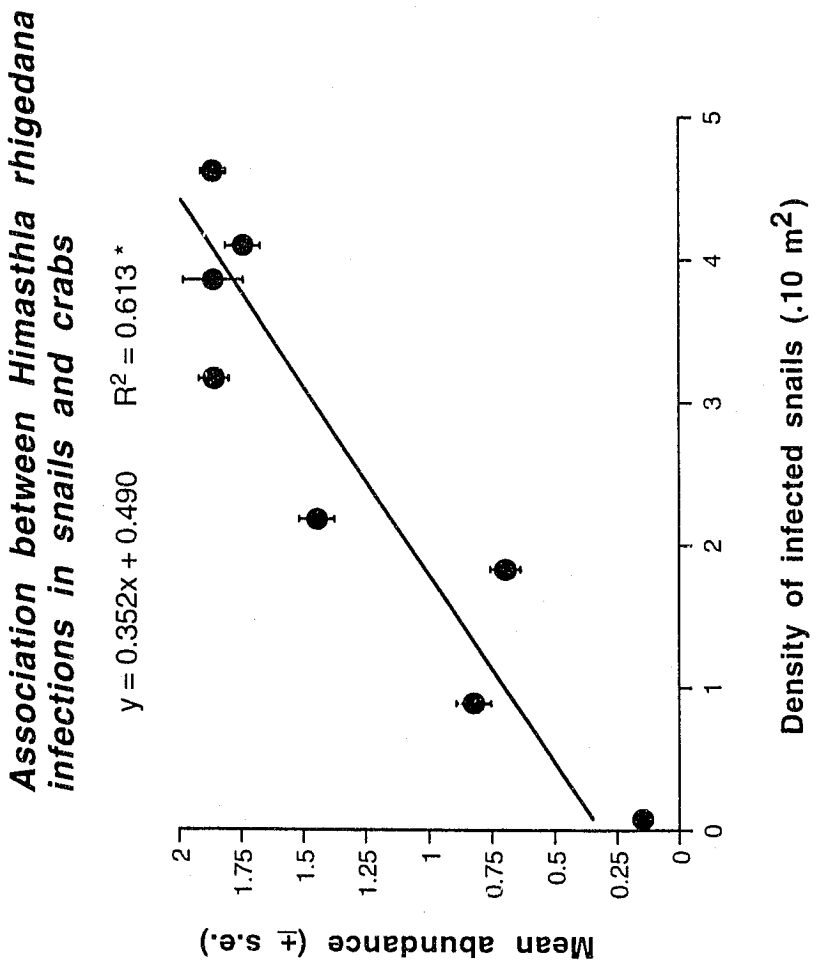


Fig. 9. Regression analysis on abundance of P. uca metacercariae in crabs as a function of density of snails infected with P. uca sporocysts. Abundance in crabs was log transformed (n + 1) prior to statistical tests. Summer and winter data were pooled for this analysis. Each point represents a different site. * $.01 < p < .05$

***Association between Probolocoryphe uca infections in snails and crabs***

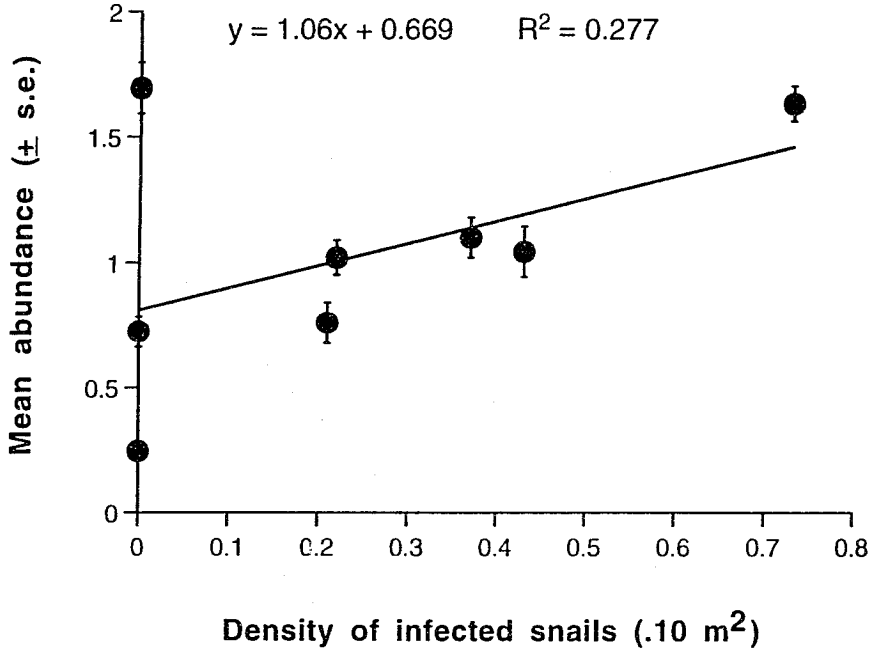


**Table 3a & b**. Multiple regression analysis of crab size and density of infected snails on trematode abundance in the crab, Hemigrapsus oregonensis. Linear regression equations are presented beneath each table. Data were not grouped by site for these analyses. $R^2$ values show the amount of variation explained by each variable. * $.01 < p < .05$.

**Table 3a**

***Himasthla rhigedana* on *Hemigrapsus oregonensis***

| Variable | Coefficient | t - statistic | $R^2$ |
|---|---|---|---|
| Constant | -.715 | -7.312 * | |
| Crab size | .075 | 12.14 * | .086 |
| Density in snails | .379 | 24.39 * | .414 * |
| Density in snails + crab size | | | .500 * |

$$y = .075x_1 + .379x_2 - .715$$

**Table 3b**

***Probolocoryphe uca* in *Hemigrapsus oregonensis***

| Variable | Coefficient | t - statistic | $R^2$ |
|---|---|---|---|
| Constant | -.596 | -5.667 | |
| Crab size | .089 | 12.62 | .131 * |
| Density in snails | 1.36 | 13.11 | .168 * |
| Density in snails + crab size | | | .299 * |

$$y = .089_{x1} + 1.36_{x2} - .596$$

association between infections in snails and crabs was almost significant for P. uca (Fig. 9, F = 4.6, p = .053).

A step-wise multiple regression analysis was used to quantify the effect of crab size and density of infected snails on the abundance of metacercariae in the crab, Hemigrapsus oregonensis. In this model, all crabs were included, and size explained only 8.6% of the variation in abundance of H. rhigedana metacercariae in crabs. When density of H. rhigedana infected snails was added into the model, an additional 41.4% of the variation in H. rhigedana abundance in crabs was accounted for (Table 3a). In contrast, crab size explained 13.1% of the variation in abundance of P. uca metacercariae in crabs (Table 3b). Adding density of infected snails to this model explained an additional 16.8% of the variation in P. uca abundance (Table 3b).

***Prevalence and intensity in Gillichthys mirabilis***

A total of 147 G. mirabilis were dissected in the summer host survey. Prevalence and intensity of metacercariae were recorded for 20-30 fish from each site, except sites B and H, where only 9 and 4 fish were caught. Fish from all sites were parasitized with two larval trematode species. Renicola buchanani (Renicolidae) metacercariae infect the liver (Martin and Gregory 1951; Martin 1972), and Euhaplorchis californiensis (Heterophyidae) metacercariae are found inside the skull (Martin 1950a, 1972; Lafferty and Morris in press).

Fish size did not vary significantly among sites (ANOVA, $R^2$ = .011, F = .181, p = .989). There was no significant difference in prevalence

among sites for R. buchanani (Fig. 10, $G_H = 13.6$, df = 7, $p > .05$), but prevalence of E. californiensis in fish was different among sites (Fig. 10, $G_H = 21.6$, df = 7, $p < .05$). In contrast, there was a significant difference in abundance among sites for both species (R. buchanani: ANOVA: $F = 5.36$, $p < .05$; E. californiensis: ANOVA: $F = 16.55$, $p < .05$). Fish from site D had the highest, and site F the lowest abundance of R. buchanani metacercariae. In contrast, fish at site D had the lowest and site C the highest abundance of E. californiensis metacercariae.

As with crabs, regression analysis was used to determine the proportion of variation in abundance of metacercariae explained by fish size and density of snails infected with either R. buchanani or E. californiensis. Multiple regression analysis was not used on these data because simple regression analyses revealed no significant association between fish size and abundance of metacercariae for either species (R. buchanani: $F = .305$, $p = .582$; E. californiensis: $F = 1.034$, $p = .311$). Furthermore, fish size explained less than 1% of the variation in abundance of trematode metacercariae (R. buchanani - $R^2 = .002$; E. californiensis - $R^2 = .007$). However, for both species there was a negative correlation between abundance of metacercariae in fish and infected snail density; however, this association was not significant for either E. californiensis (Fig. 11, $F = 1.53$, $p = .262$) or R. buchanani (Fig. 12, $F = .608$, $p = .465$).

Fig. 10. Prevalence of Renicola buchanani (RENI) and Euhaplorchis californiensis (EUHA) in the fish, Gillichthys mirabilis. Fish were collected only in the summer survey.

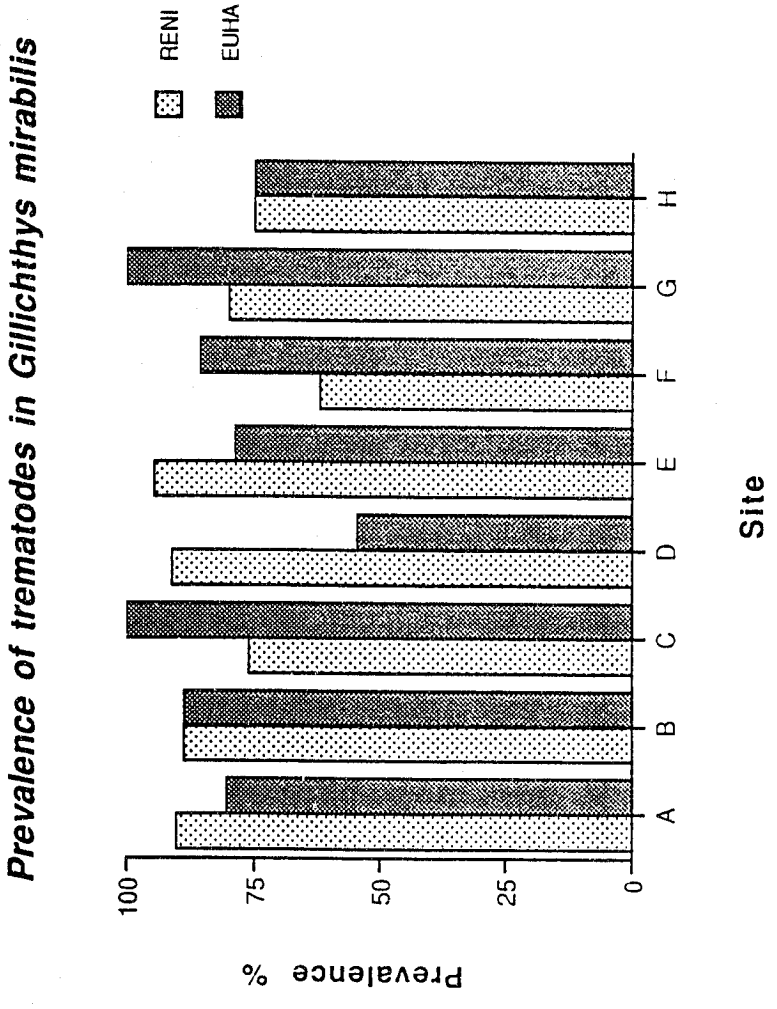


Fig. 11. Regression analysis on abundance of E. californiensis metacercariae in fish as a function of density of snails infected with E. californiensis rediae. Each point represents a different site.

***Association between Euhaplorchis californiensis infections in snails and fish***

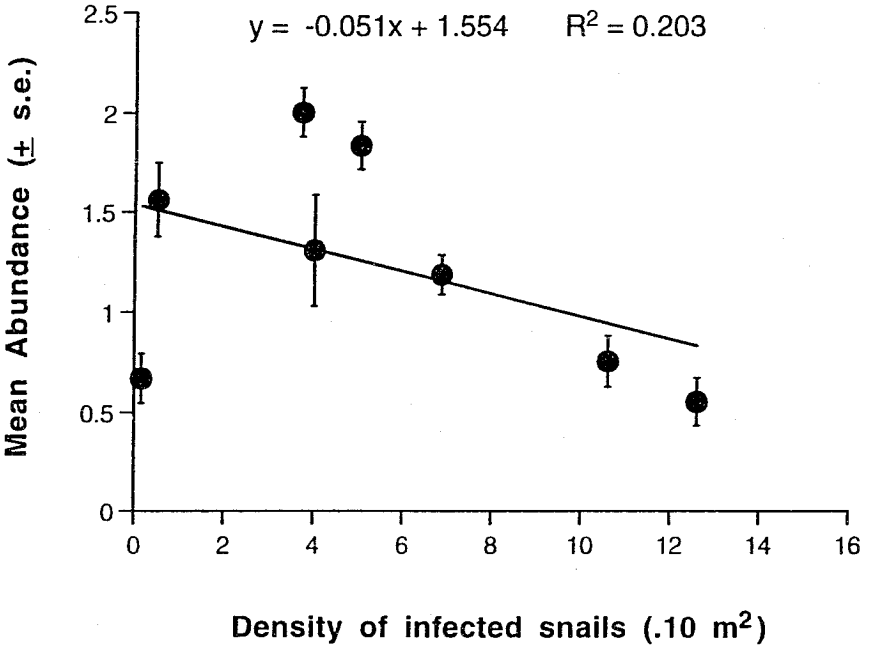


Fig. 12. Regression analysis on abundance of R. buchanani metacercariae in fish as a function of density of snails infected with R. buchanani sporocysts. Each point represents a different site.

***Prevalence and abundance in Tagelus californianus***

A total of 113 clams from 4 sites were dissected during the summer survey. Most clams were infected with Acanthoparyphium spinulosum (Echinostomatidae) metacercariae, and prevalence ranged from 78 to 100%. There were significant differences in clam size among sites (ANOVA: $F = 4.69$, $p = .004$), however there was no significant positive association between clam size and abundance of metacercariae ($y = .009x + 1.043$, $F = 2.26$, $p = .135$). Prevalence and abundance of A. spinulosum metacercariae in clams were significantly different among sites ($G_H = 17.52$, $df = 3$, $p < .05$; Fig. 13, ANOVA: $F = 54.33$, $p < .05$), with site 1 having the least and site 3 the greatest abundance of metacercariae in clams. Similar to the crab H. oregonensis, abundance of A. spinulosum metacercariae in clams was positively associated with prevalence of A. spinulosum in snails (Fig. 14) but regression analyses showed that this association was not significant ($F = .895$, $p = .452$).

***Host vagility and larval trematode abundance***

Sentinel, uninfected H. oregonensis obtained nearly 100% prevalence of Himasthla rhigedana by the end of the experimental period of 22 days (Fig. 15), and there was no significant difference in prevalence in the caged crabs compared to resident crabs. In contrast, analysis of covariance revealed significant variation in H. rhigedana abundance between the transplanted crabs at each pair of sites (Fig. 16a, b, c). There was also significant variation between control crabs at each pair of sites (Fig. 16a, b, c). For each pair of sites, there was one transplanted

Fig. 13. Abundance of Acanthoparyphium spinulosum (ACAN) metacercariae in the clam, Tagelus californianus. Clams were only collected in the summer survey.

Fig. 14. Regression analysis on abundance of A. spinulosum metacercariae in clams as a function of prevalence of A. spinulosum in snails. Each point represents a different site.

***Association between Acanthoparyphium spinulosum infections in clams and snails***

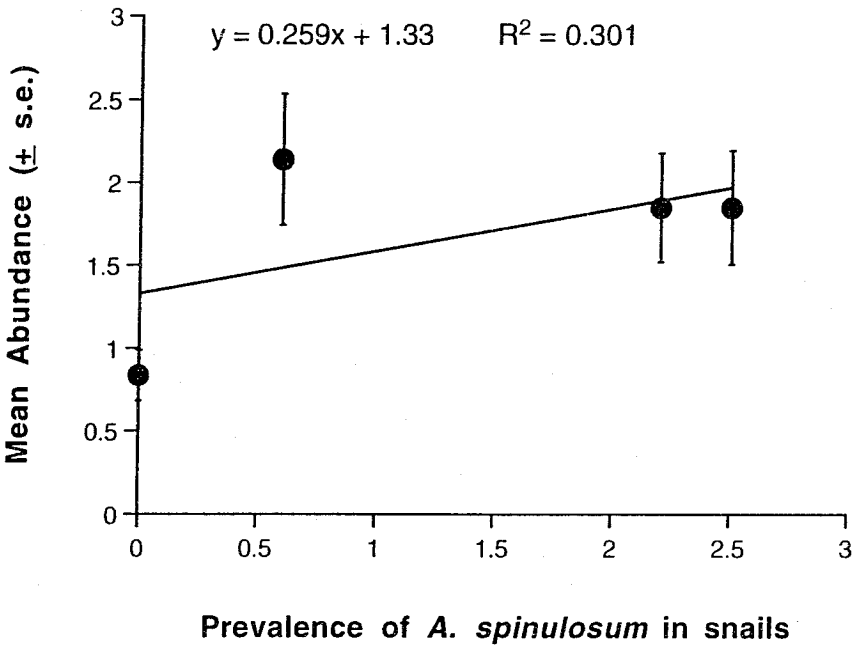


Fig. 15. Prevalence of Himasthla rhigedana in resident and sentinel crabs from the reciprocal transplant experiment.

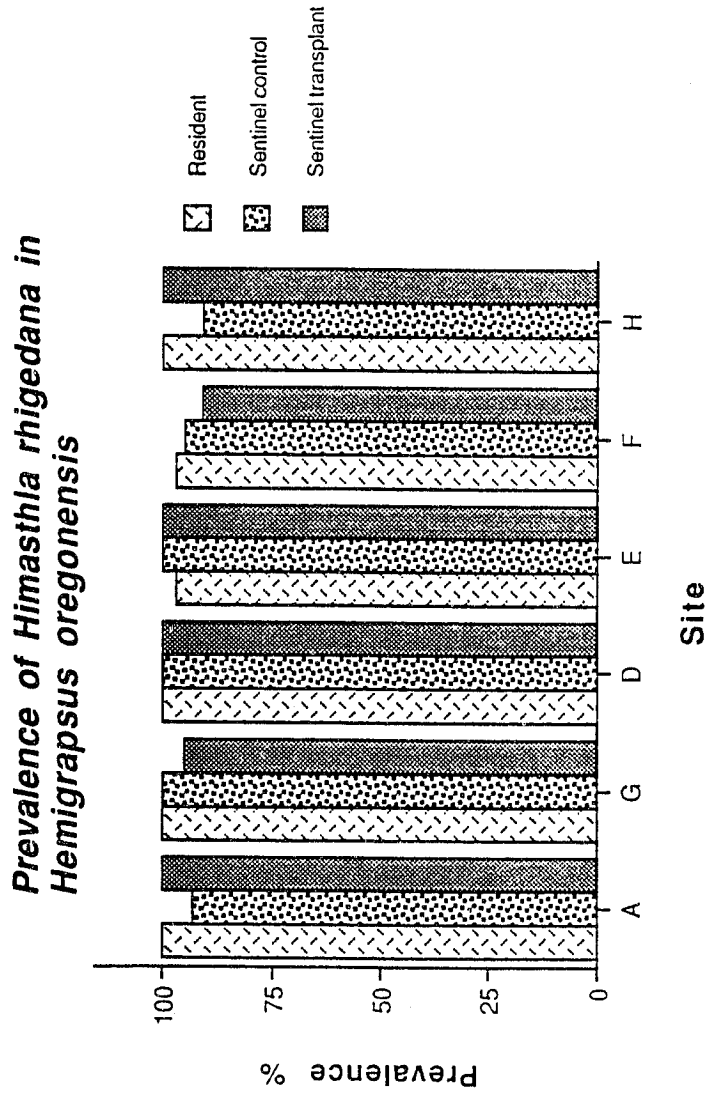


Fig. 16. Abundance of H. rhigedana metacercariae on experimental crabs, Hemigrapsus oregonensis. Control groups remained at a site for the duration of the experiment (22 days). Transplanted crabs were moved after 11 days. Analysis of covariance was used to test for differences between groups, crab size was held constant in these analyses. * - $0.01 < p < 0.05$

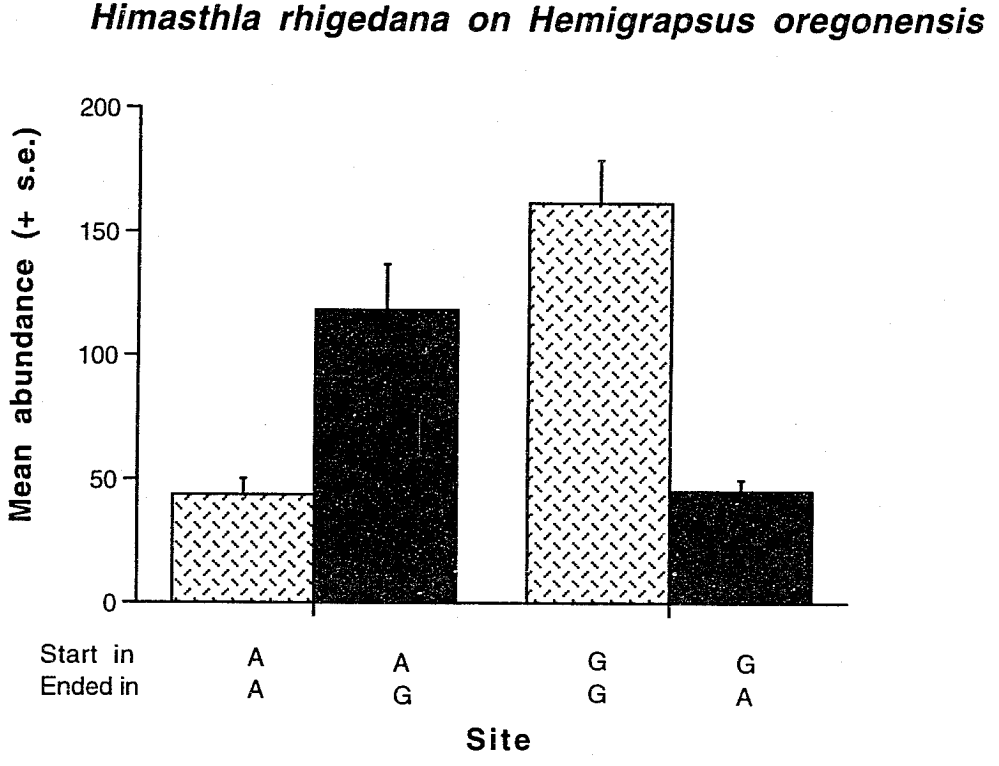



Fig. 16a

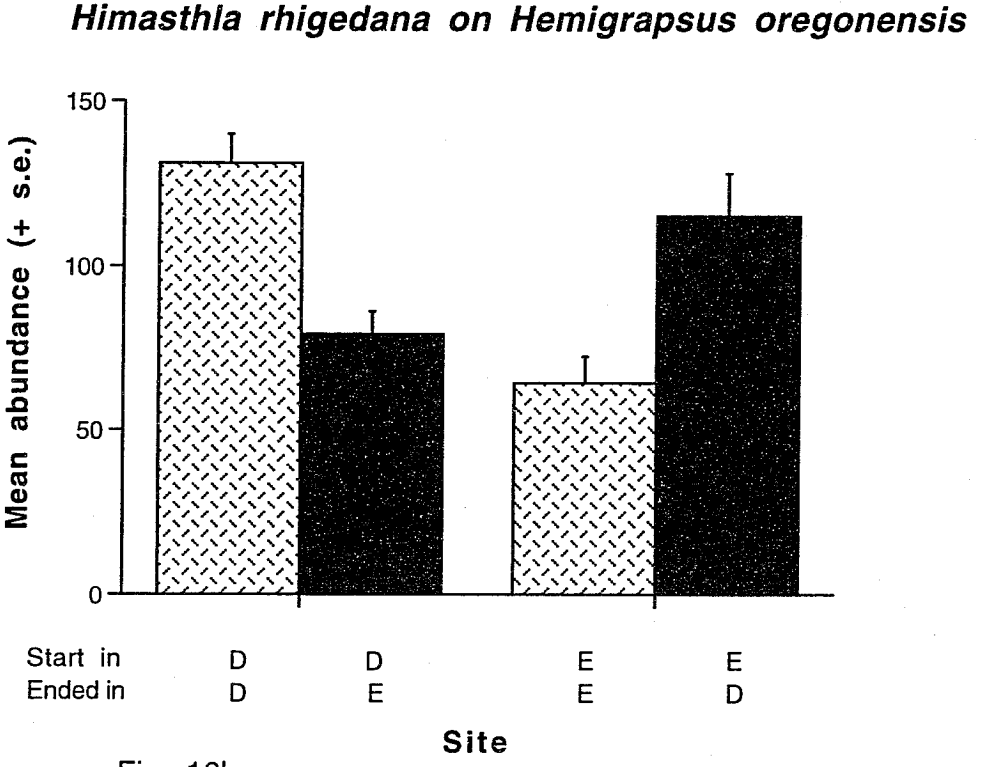



Fig. 16b

Fig. 16c

group that had intensities of H. rhigedana that were intermediate to each respective control group, which supported the prediction that crab vagility would integrate high and low prevalence trematode patches in snails. However, these intermediate levels were not significantly different from at least one of the control groups (Fig. 16a, b, c). In all cases, the average infection intensity of each control group was not significantly different than the group that was transplanted into the site.

## Discussion

This study of trematodes with complex life cycles suggests that a variety of factors influence the dispersion of larval trematodes in second intermediate hosts. A spatially heterogeneous distribution of larval trematodes is common at the first and second intermediate host level, but this heterogeneity can be ameliorated by vagility of second intermediate hosts throughout the marsh. Furthermore, vagility of second intermediate hosts generally increases dispersion of trematodes in the system. The abundance of larval trematodes in second intermediate hosts could also be reduced by biological peculiarities (e.g. molting) of a host, which also temporarily decreases dispersion. Compared to the high specificity for their first intermediate host snail, Cerithidea californica, larval trematodes in this system appear to be generalists in selecting their second intermediate hosts, which greatly increases their dispersion and further increases the likelihood of transfer to definitive host birds.

***Host Vagility and Trematode Dispersion***

An analysis of the association between the local prevalence of larval trematodes in the snail population and the abundance of their metacercariae in second intermediate hosts was used to test the hypothesis that activity (vagility) of second intermediate hosts will increase dispersion as proposed by Kuris (1990). This hypothesis predicts that for trematode species using a highly vagile second intermediate host, the spatial association between density of these trematodes in the snail population and their density in second intermediate host populations will be less (both lower slope and $R^2$) than for species that use less vagile (sessile or sedentary) second intermediate hosts.

Host vagility can also influence dispersion of larval trematodes upon transfer between host levels. The highly significant spatial heterogeneity in prevalence of trematodes in snails was not reflected in their dispersion patterns in second intermediate hosts. In terms of prevalence, nearly all second intermediate hosts were infected so that the range of relatively high prevalence was generally narrow among sites. Although the prevalence of E. californiensis for example, varied significantly from 1.58% to 41.52% in snails, it reached nearly 83% in G. mirabilis (Table 2 and Table 5). Average intensities were also high but displayed a wide range in second intermediate hosts and was quite variable among sites (Fig. 11, 12). Further, this pattern of high prevalence and high intensity was even more apparent for another fish host, Fundulus parvipinnus, in which all fish examined from CSM were heavily parasitized

with E. californiensis (Lafferty and Morris 1995). Thus, upon transfer from first to second intermediate host, dispersion of larval trematodes increased and the pattern appears to be common among a variety of host species.

***Vagility and Associations Between Host Levels***

In general, the association between abundance of trematodes in first versus second intermediate hosts was weakest for the most vagile fish hosts (Fig. 11, 12). A strong association was most evident for H. rhigedana on crabs. This worm encysts on a variety of hard substrates, notably including crab exoskeletons. These substrates are renewed via molting (Kuris 1971) and metacercariae do not continually accumulate on these surfaces, thus molting periodically reduces dispersion and interrupts the integrating effect of host vagility. In contrast, P. uca accumulates over the life span of the shore crab host since they encyst in the hemocoel of the crab. Thus, crab vagility and contact with varying exposure risks should gradually even the average intensity of P. uca in crabs.

**Table 5.** Summary of prevalence, mean abundance (includes all crabs) and mean intensities (includes only infected crabs) for 4 larval trematode species in second intermediate hosts. Prevalence and intensity were transformed (Arcsin transformation, log (n + 1) respectively) prior to statistical analyses. Significant variation (* - .01 < p < .05, ** p < .01) among sites and was determined by ANOVA for abundance and intensities, or a heterogeneity Chi-square (G-test) for prevalences.

| Parasite | Average Prevalence in snails | Host | Average Prevalence (%) | Range (%) | Mean Abundance | Mean Intensity |
|---|---|---|---|---|---|---|
| *H. rhigedana* | 6.3 ** | crab | 82.1 ** | 24-99 | 79.6 * | 95.7 * |
| *P. uca* | 0.6 * | crab | 77.6 * | 41-100 | 43.3 * | 59.2 * |
| *R. buchanani* | 2.9 ** | fish | 82.3 | 62-95 | 114.1 * | 136.5 * |
| *E. californiensis* | 19.8 ** | fish | 82.9 * | 54-100 | 45.4 * | 53.8 * |

Considerable debate centers on the importance of spatially heterogeneous recruitment of trematodes to snails (Robson and Williams 1970; Dronen 1978; Matthews et al. 1985; Brown et al. 1988; Kuris 1990; Sousa 1990; Fernandez and Esch 1991a, 1991b; Sousa 1992, 1993; Kuris and Lafferty 1994; Lafferty et al. 1994; Sousa 1994) as a measured means which isolates species and has been used to explain the rarity of double infections in snails (Robson and Williams 1970; Dronen 1978; Matthews et al. 1985; Brown et al. 1988; Sousa 1990; Fernandez and Esch 1991a, 1991b; Sousa 1992, 1993; Sousa 1994). Alternative views suggest that interspecific interactions account for the low number of double infections and heterogeneous recruitment further intensifies interactions (Kuris 1990; Kuris and Lafferty 1994; Lafferty et al. 1994). Be that as it may, the effects of heterogeneous recruitment to snails are ameliorated to the point that spatial variation in infection intensity for vagile second intermediate hosts such as fish (F. parvipinnus and G. mirabilis) cannot be detected on the scale of the salt marsh. Spatial heterogeneity among marshes cannot of course be integrated by vagility of second intermediate hosts as these hosts don't migrate between these habitats. However, as Kuris (1990) noted, the distribution and abundance of trematodes in snails is likely influenced by the movement between marshes of definitive host birds.

Vagility of hosts potentially integrates spatially patchy infections on a local and regional scale (Kuris 1990). Movement of second intermediate

hosts within or between habitats could facilitate acquisition of parasites from different sites and likely influences infection rates and intensities of metacercariae. On a local scale (i.e. a marsh), movement of infective stages (i.e. cercariae) could also reduce spatial heterogeneity of larval trematodes in snails during transfer between host levels (Sousa and Grosholz 1991). However, the importance of local dispersal of cercariae as an integrating mechanism for larval trematodes is limited by the very short life span and hostile environmental conditions (UV light, high water temperatures, predators) experienced by free-swimming cercariae in the water column (see Chapter 2). Furthermore, transfer from first to second intermediate hosts may be severely reduced by water currents that exceed 1 meter per second (Radke et al. 1961). At CSM, average current speeds were about 0.1 meter per second and approached 0.6 meter per second during 2 ebb tides. This suggests that current speeds measured at CSM rarely reached the threshold required to inhibit transmission from snails to second intermediate host, but that long distance dispersal of cercariae throughout the marsh via tidal currents was limited.

***Empirical Evidence for Host Vagility as an Integrating Mechanism***

The influence of host vagility on prevalence has been experimentally demonstrated in a well studied snail-trematode system. Fernandez (unpublished) and Snyder and Esch (1993) showed that the snail, Physa gyrina, was more vagile than a sympatric population of the snail, Helisoma anceps. Snyder and Esch (1993) documented that P. gyrina had a greater number of double infections compared to H. anceps,

and prevalences of two trematodes (Haematoloechus complexus and Halipegus eccentricus) were considerably higher in P. gyrina than in Helisoma anceps. Prevalence of a third species, Echinostoma trivolvis, was similar for both snail species. Differences in Haem. complexus and Hal. eccentricus prevalence were attributed to host vagility, because infective stages of these trematodes were sessile eggs that were encountered and ingested while snails moved about and grazed (Snyder and Esch 1993). Furthermore, trematode eggs were patchily distributed in shallow water and on leaf litter (Williams and Esch 1991). Thus, the more vagile P. gyrina apparently contacted and ingested the patchy trematode eggs more frequently than did Helisoma anceps and thus had a higher prevalence of infection than did H. anceps (Snyder and Esch 1993).

The evidence presented by Snyder and Esch (1993) showed that host vagility has important consequences for prevalence of larval trematodes in snail populations. However, their experiments did not demonstrate that snail vagility integrated spatial patchiness as proposed by Kuris (1990). Rather, Snyder and Esch (1993) demonstrated that vagility increased the encounter rate of patches of trematode eggs. This led to a higher prevalence of trematodes in P. gyrina compared to the more sedentary H. anceps. Compared to P. gyrina and H. anceps, C. californica at CSM is also relatively sedentary, moving at most a few meters in a random fashion over a 7 day period (Davila-Marcano unpublished). Vagility, or rather the general lack of it in snails, sustains or just slightly reduces the spatial patchiness inherent in the distribution of

trematode eggs or miracidiae. Movement patterns of C. californica also increase encounter rates with patchy trematode eggs; thus, like P. gyrina, overall prevalence of infection in C. californica populations is probably higher than if snails were sessile.

The hypothesis that second intermediate host vagility integrates spatially patchy larval trematode infections in snails at CSM would be supported if vagile hosts harbored infestations of metacercariae that were intermediate in intensity between comparable sessile hosts and highly vagile hosts. The reciprocal transplant experiment showed that experimental second intermediate host crabs acquired H. rhigedana metacercariae rapidly and were as equally susceptible to infestation as were resident crabs. Furthermore, integration of spatially variable prevalence of larval trematodes in snails by crabs operated at each of the three transfer sites whereby the transplanted crabs attained infection intensities intermediate to the control groups (Fig. 16). These results indicated that host vagility has the potential to create more even infection intensities among individuals in a second intermediate host population.

***Host Vagility: Theoretical Considerations***

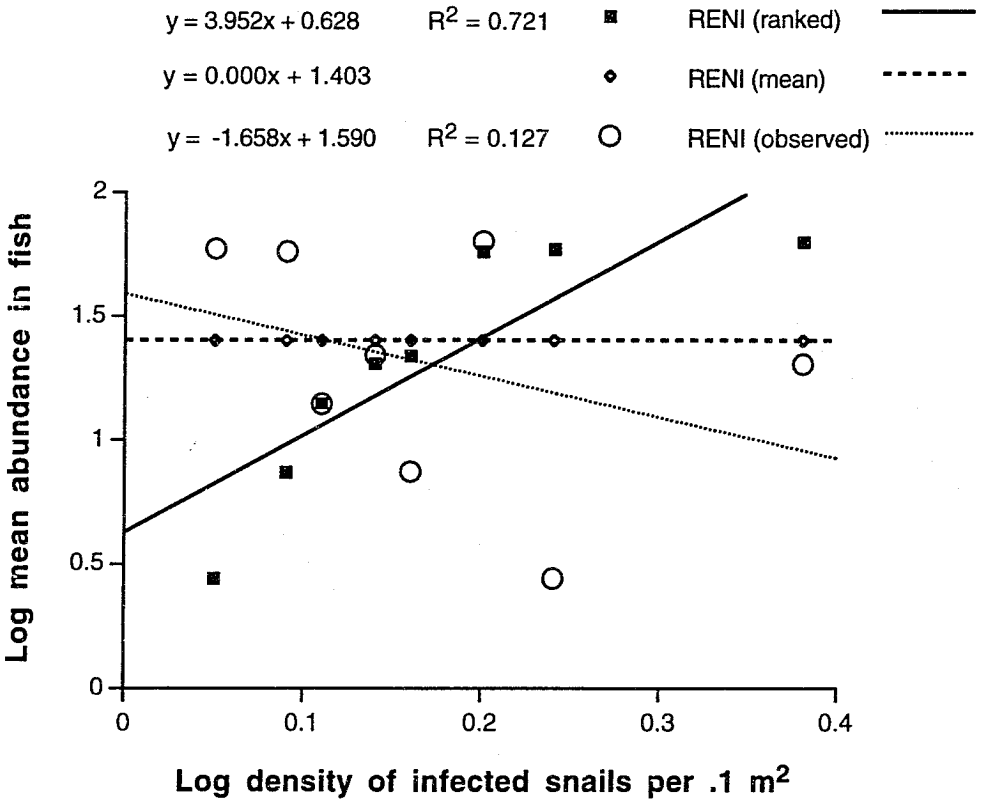
Second intermediate hosts at CSM varied with respect to vagility and differed markedly in their potential ability to integrate the spatially patchy infections in the first intermediate host snails. Based on the range of vagility exhibited by second intermediate hosts and the evidence that the density of infected snails was variable among sites, the relative impact of vagility as an integrating mechanism can be modeled (Fig. 17). At one

extreme, for a sessile or sedentary second intermediate host (vagility is low) there will be a strong positive association ($R = 1$, slope $\geq 1$) between parasite abundance in the first and second intermediate hosts. That is, second intermediate host infection levels will be dependent on the abundance of larval trematodes in snails. This extreme theoretical relation for any parasite species can be shown by rank ordering the actual data (Fig. 17). Alternatively, if vagility of a second intermediate host is high, there will be no association (slope = 0) between infections in first and second intermediate hosts. This theoretical extreme is shown by the constant mean abundance for each parasite species (Fig. 17). Associations in a second intermediate host which exhibits an intermediate level of vagility for example will lie between the theoretical extremes. That is, the slope of the relation between the prevalence of infection in snails and abundance of metacercariae in second intermediate hosts will lie somewhere between $\pm 1.0$ and zero.

Some support for the predicted effect of host vagility on associations between host levels was shown by the distribution of larval trematodes in crabs and fish. There was no association between the density of infected snails and abundance of R. buchanani or E. californiensis metacercariae in highly vagile fish and the regression slope(s) were not significantly different from zero (Fig. 17a, b). This suggests that fish integrated spatial patchiness of larval trematodes in the

Fig. 17a - d. Summary of associations between larval trematodes in snails and second intermediate hosts. Both the density of infected snails and the abundance of metacercariae in second intermediate hosts were log transformed (n+1) to permit comparisons between host species. Data were rank ordered to estimate the maximum slope and $R^2$ (i.e. vagility = 0) which could have been obtained by each data set. The mean for all (y) values shows the slope expected if vagility of the second intermediate host was high. The observed data were not ranked or manipulated other than the log transformation.

**Fig. 17a - *Renicola buchanani***



## Fig. 17b - *Euhaplorchis californiensis*

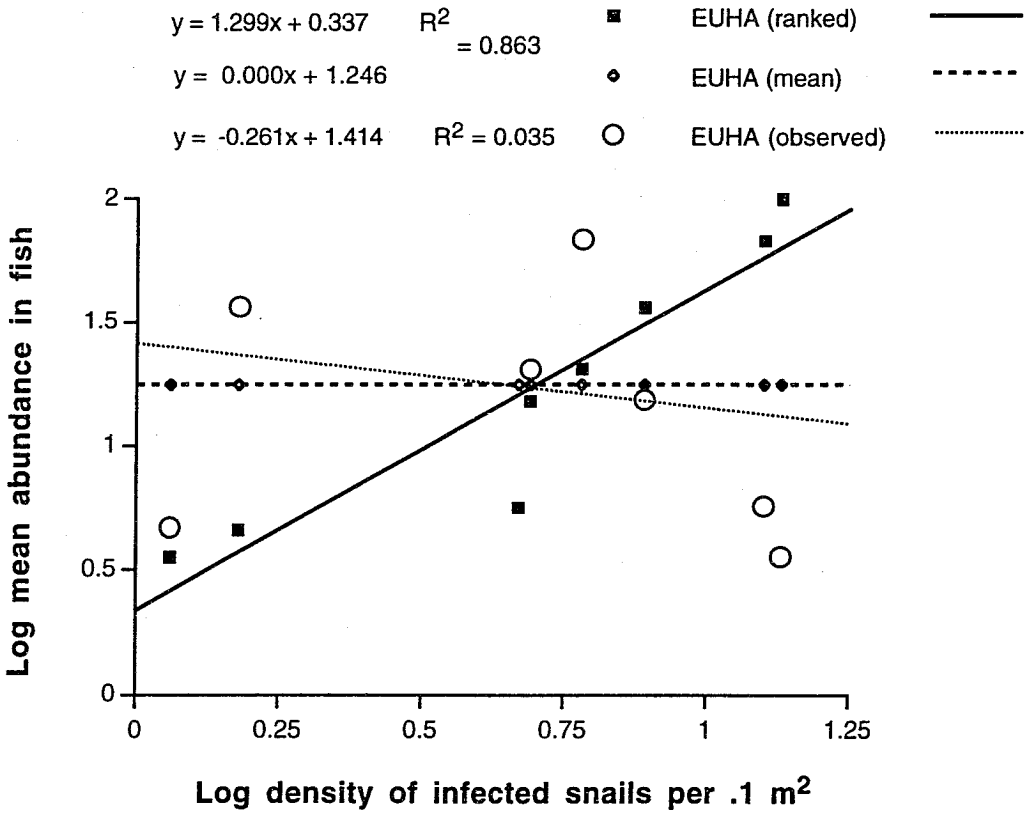
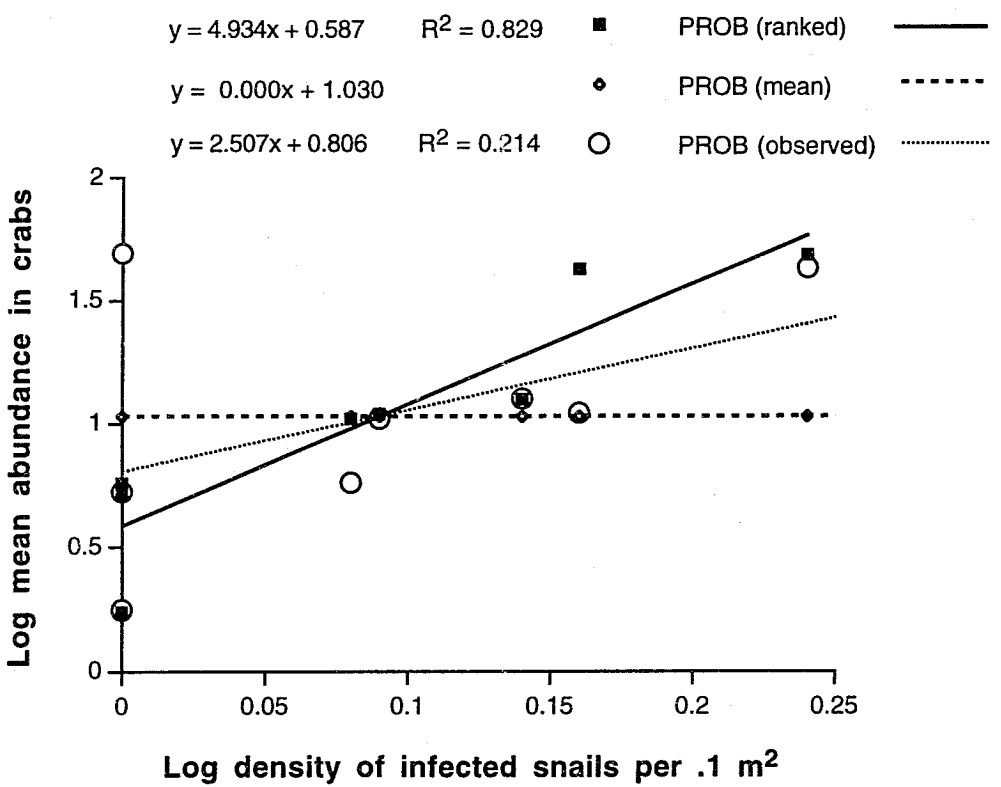
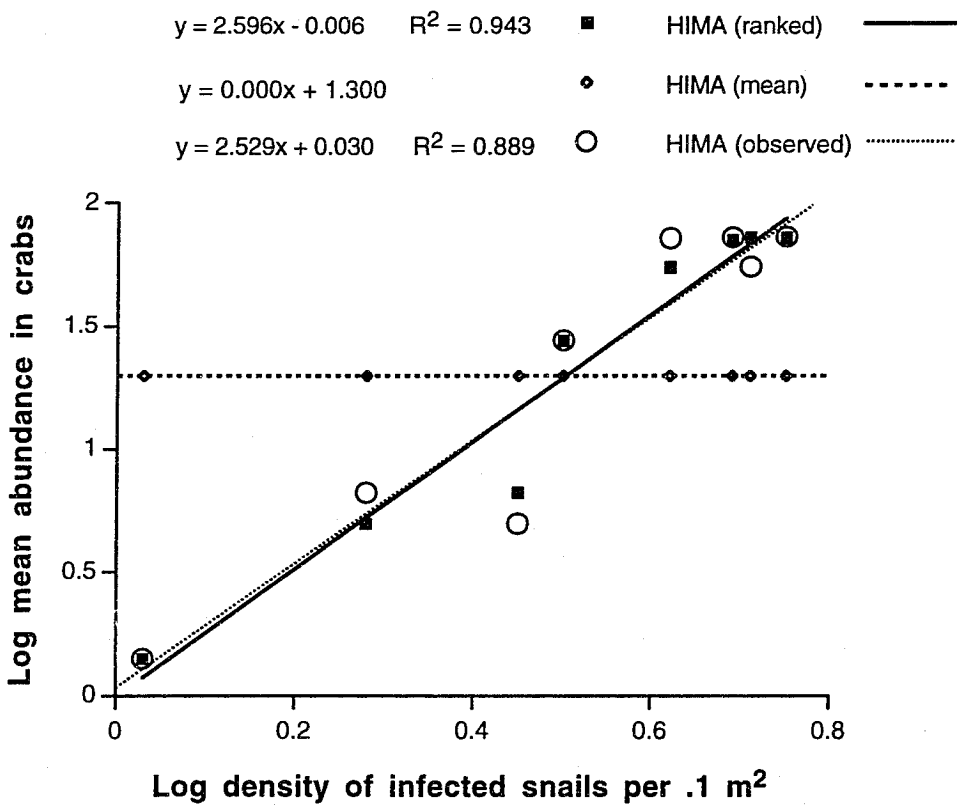


**Fig. 17c - *Probolocoryphe uca***



## Fig. 17d - *Himasthla rhigedana*



snail population as they moved to and from the home channel. The drop in $R^2$ values between the rank ordered data and the observed data suggests that abundance of metacercariae in fish was not dependent on prevalence of larval trematodes in snails. Crabs presented an intermediate level of vagility. The association between both H. rhigedana and P. uca infections in snails and crabs was positive (Fig. 17c, d), the regression slope was significantly greater than zero for H. rhigedana (Fig. 8) and nearly significant for P. uca (Fig. 9, $F = 4.6$, $p = .053$). The similar $R^2$ values shown for the ranked ordered and observed data for H. rhigedana indicates that the abundance of metacercariae in crabs was dependent on the density of infected snails, and that this is a good predictor of metacercarial abundance (Fig. 17d). The decrease in $R^2$ for P. uca (Fig. 17c) was primarily due to abundance at one site being high while prevalence in snails was zero. Elimination of this site from the analysis increased both the slope and $R^2$ for this association so that it approached the significant $R^2$ and slope shown by the rank ordered data.

Associations between H. rhigedana infections in snails and crabs displayed a pattern similar to the theoretical prediction for sessile or sedentary hosts. This suggests that crabs may move only short distances within a channel and may not integrate spatially patchy prevalence in snails to the same degree as highly vagile fish. Clams are capable of some movement, but compared to other second intermediate hosts they are effectively sessile. In contrast to crab hosts, a significant association

between A. spinulosum infections in snails and clams could not be detected although this association had a relatively high $R^2$ value (Fig. 14, $R^2$ = .301). This was probably due to low sample size (4 samples) and the low range of prevalence of A. spinulosum in the snail population (Table 2).

***Patterns of Infection in Second Intermediate Hosts***

Beyond the spatial integration effect, there is another important population consequence upon transfer from first to second intermediate host: prevalence is greatly amplified in second intermediate hosts. Trematodes that were rare in the snail (e.g. R. buchanani and P. uca) became nearly ubiquitous in second intermediate host populations.

Prevalence of metacercariae in second intermediate hosts exhibited a relatively narrow range but was generally high (about 75%) for each trematode species despite the small range and low prevalence measured in first intermediate host snails. This indicates that for all trematode species studied here, only a few snails shedding cercariae can infect most second intermediate hosts. Moreover, a small amount of variation in prevalence among sites (in snails) could produce significant differences in the intensity of metacercariae in the next host if the host was not highly vagile (e.g. Fig. 9 - P. uca), or if biological characteristics of the next host prevented accumulation of parasites over long time periods (e.g. Fig. 8 - H. rhigedana).

High prevalence in second intermediate hosts is commonly observed. A similar amplification in prevalence was demonstrated by Young (1938) and Dronen (1978). Both studies showed unequivocally

that prevalence in second intermediate hosts was consistently high (nearly 100%) in second intermediate hosts when it was low (less than 10%) in the first intermediate host snail(s). This suggests that trematode cercariae find and infect their second intermediate hosts relatively efficiently.

The non-significant associations for trematodes in fish were even observed in fish with "homing" behavior. Brooks (unpublished data) has shown that G. mirabilis returns from deep water repeatedly to the same channel, which suggests that intensities of metacercariae in fish should be associated with the density of particular trematodes in snails in the "home" channel. This lack of association between larval trematodes in snails and fish indicates that fish may become infected continually regardless of density of trematodes in snails in the home channel. Thus, unlike crabs, intensities of metacercariae in fish were not dependent on the prevalence of a particular trematode species in snails.

Preliminary observations on other salt marsh fish species (e.g. Fundulus parvipinnus) that do not return to a "home" channel suggest that vagility by this species also integrates spatial patchiness. Fundulus parvipinnus also exhibits high prevalence and intensity of E. californiensis and R. buchanani metacercariae but intensities of E. californiensis are typically an order of magnitude higher than in G. mirabilis. Although first and second intermediate host associations have not been measured for larval trematodes in F. parvipinnus, the high prevalence and intensities observed in these fish would predict a non-significant association.

Host size is also an important correlate of infection intensity in second intermediate hosts. Large hosts are presumably older and have been exposed to infection for a greater period of time compared to small hosts. Significant positive associations between host size and intensity were observed only for crab second intermediate hosts, but size accounted for less than 15% of the variation in infection intensity for both H. rhigedana and P. uca in crabs (Table 3a, 3b) because there was considerable variation in infection intensity among comparably sized crabs (Appendix 1). In contrast, there was no association between host size and infection intensity in fish or clam hosts. Lack of a significant positive association between size and intensity in these hosts indicates that larger, older, heavily infected hosts may have died from high intensity infections (Lafferty and Morris in press).

***Cercarial Biology and Infection Mechanisms***

Unique biological characteristics of cercariae can influence the likelihood of successful host transfer and subsequent associations between infections in first and second intermediate hosts. Cercariae have short life-spans, which are further reduced by some environmental factors. However, transfer from the first to the second intermediate host is likely aided by host behaviors or activities and presumably adaptive behavioral patterns of cercariae. For example, the clam, T. californianus, is likely infected by A. spinulosum while feeding. Filter-feeding clams draw water into the mantle cavity where it then passes through the gill filaments.

Infective cercariae suspended in the water are drawn into the clam during feeding, and then they probably penetrate and encyst in clam tissues.

Himasthla rhigedana cercariae encyst rapidly (within about 10-20 minutes) after emerging from the snail (Adams and Martin 1963; Martin 1972). My preliminary experimental evidence indicates that cercariae preferentially encysted on crab carapaces rather than on plastic surfaces. This suggests that H. rhigedana cercariae select living substrates which are almost certainly more efficient routes to avian definitive hosts. Probolocoryphe uca and other Microphallidae infect crab hosts primarily via penetration of the crustacean cuticle, facilitated by a piercing stylet located at the anterior end of the cercaria (Heard 1976). Transfer to crabs is aided by respiratory currents which draw water into the branchial chamber where these cercariae penetrate the thin cuticle of the gill filaments or of the dorsal surface of the branchial chamber (Heard 1976).

The larval trematodes that parasitize G. mirabilis also exhibit mechanisms which may promote host transfer. Renicola buchanani cercariae emerge reluctantly from snails in the laboratory (personal observation). However, once in the water column these cercariae form an aggregated mass (termed rattenkönig, Claus 1880) with the proximal portion of the tails intertwined and secured by adhesive secretions (Martin and Gregory 1951; Martin 1971, 1972). The large tails lash and propel the mass through the water. Although the tails are lost upon ingestion, the aggregation may appear to be a food item and if eaten by a susceptible

fish, it could deliver tens or hundreds of infective cercariae to that host. These then transform into metacercariae in the host liver.

In contrast, E. californiensis cercariae appear to lack behaviors or aggregating mechanisms which increase the efficiency of host transfer. However, compared to other larval trematodes in this system, E. californiensis is by far the most abundant species in the snail population and in the water column (Table 2, Appendix 1a & 1b). Therefore, efficient host transfer by this species may be facilitated simply by its ubiquity and abundance in the water so that second intermediate host fish are continually exposed to infective cercariae.

***Host Specificity and New Host Records***

At CSM, all the larval trematodes that develop in C. californica appear to use this snail as their exclusive first intermediate host. In contrast, their use of second intermediate hosts tends to be more generalized, and relatively broad host specificity in second intermediate hosts is commonly observed (Evans et al. 1981; McCarthy and Kanev 1990). At CSM, I recovered R. buchanani and E. californiensis from Gillichthys mirabilis. Martin (1972) also reported these trematode species in the killifish, Fundulus parvipinnus, which is also common in CSM. Probolocoryphe uca was recovered from one crab (Hemigrapsus oregonensis) at CSM. It was previously reported in the fiddler crab, Uca crenulata (Sarkisian 1957; Martin 1972) which occurs but is rare at CSM. A metacercariae similar in shape and morphology to P. uca also infects Pachygrapsus crassipes in high numbers, although the species was not

identified. Acanthoparyphium spinulosum was recovered from only one clam species (Tagelus californianus); however, metacercariae have been previously reported only in the first intermediate host snail, C. californica (Martin and Adams 1961; Martin 1972), and are also present in low numbers in snails at CSM (Olsen, personal communication).

The biology of H. rhigedana metacercariae is anomalous compared to other metacercariae in this study. These metacercariae were recovered from both living (i.e. crab carapace, snail operculum) and inanimate hard substrates. Previous reports (Adams and Martin 1963; Martin 1972) examined these metacercariae exclusively from inanimate surfaces (i.e. glass slides, petri plates) and suggested that second intermediate hosts for this species were not required. However, H. rhigedana cercariae settling on inanimate substrates (i.e. rocks, drift wood) or vegetation, would probably not encounter definitive host birds which are more likely to feed on the crustacean or molluscan hosts of this trematode.

***Conclusions and Summary***

Spatial patterns of larval trematode infections in their first and second intermediate hosts showed that the relation between abundance of metacercariae in second intermediate hosts is sometimes dependent on the prevalence of larval stages in the first intermediate snail host population. This association can be obscured by host vagility among sites in the marsh, whereby vagile second intermediate hosts integrate spatial patchiness of infections observed in the snail population. A continuum of host vagility produced strong associations between host levels when second intermediate hosts were relatively sessile and this association weakened as vagility increased.

## CHAPTER 2

## The Cercarial Community: Factors Influencing Species Composition and Abundance

### Introduction

Parasites contend with a unique suite of environmental conditions. Host defenses function to expel or kill parasites. Within this hostile environment, parasites must produce infective progeny to persist in a host population. Parasites which require more than one host to complete the life cycle (trematodes, nematodes, cestodes) have free living transmissive stages that must deal with hostile environmental conditions both inside and outside the host. Thus, factors which influence the production of transmissive stages and availability of susceptible hosts are key components of parasite biology.

Digenetic trematode cercariae emerge from snails, their first intermediate hosts. They must infect the second intermediate or final host within about a day. From an efficiency perspective, emergence from the snail should be coincident in space and time with the next host in order to maximize the likelihood of transmission. Théron (1984) recognized the ecological and evolutionary importance of circadian emergence rhythms of larval digenetic trematodes, and postulated that periodic emergence of cercariae from snail hosts was adaptive and may have evolved to coincide with the activity patterns or presence of the next host. More recent work

has suggested that emergence patterns are genetically controlled (Therón and Combes 1988; Pages and Théron 1990).

Previous studies have generally focused on single species, have documented periodic cercarial emergence and clarified the role of physical factors (light and temperature). Under laboratory conditions light and temperature influence the daily emergence pattern of cercariae from their intermediate snail hosts (Cort 1922; Giovannola 1936b; Bauman et al. 1948; Rees 1948; Mao et al. 1949; Kendall and McCullough 1951; Komiya and Ishii 1954; Gumble et al. 1957; Webbe 1965; McClelland 1965; Asch 1972; Wagenbach and Alldredge 1974; Mouahid and Théron 1987; Raymond and Probert 1987; Lewis et al. 1989; Shostak and Esch 1990). A few studies have demonstrated seasonal emergence in the field (Webbe 1962a; Schiff et al. 1975; Pitchford and DuToit 1976; Ouma et. al 1989). Surprisingly few investigations have attempted to quantify the association between the density of cercariae or density of intermediate host snails in the field, although this has been attempted for some medically important trematodes (Webbe 1962a; Théron et al. 1978; Ouma et al. 1989). These studies (Théron et al. 1978; Ouma et al. 1989) demonstrated positive associations between the density of cercariae in the water and the prevalence and/or density of infected snails at a site.

In the laboratory, cercarial production is influenced by light/dark cycling and elevated temperatures. Peak shedding usually occurs soon after the light (or dark) period begins (Kuntz 1947; Raymond and Probert 1987). Emergence patterns can be reversed by altering the light/dark

cycle (Giovannola 1936a; Glaudel and Etges 1973). However, these laboratory studies may not reflect the mechanisms that induce shedding in the field because peak shedding occurs an average of 4-5 hours after sunrise (Pitchford and Du Toit 1976; Théron et al. 1978) for some schistosome species.

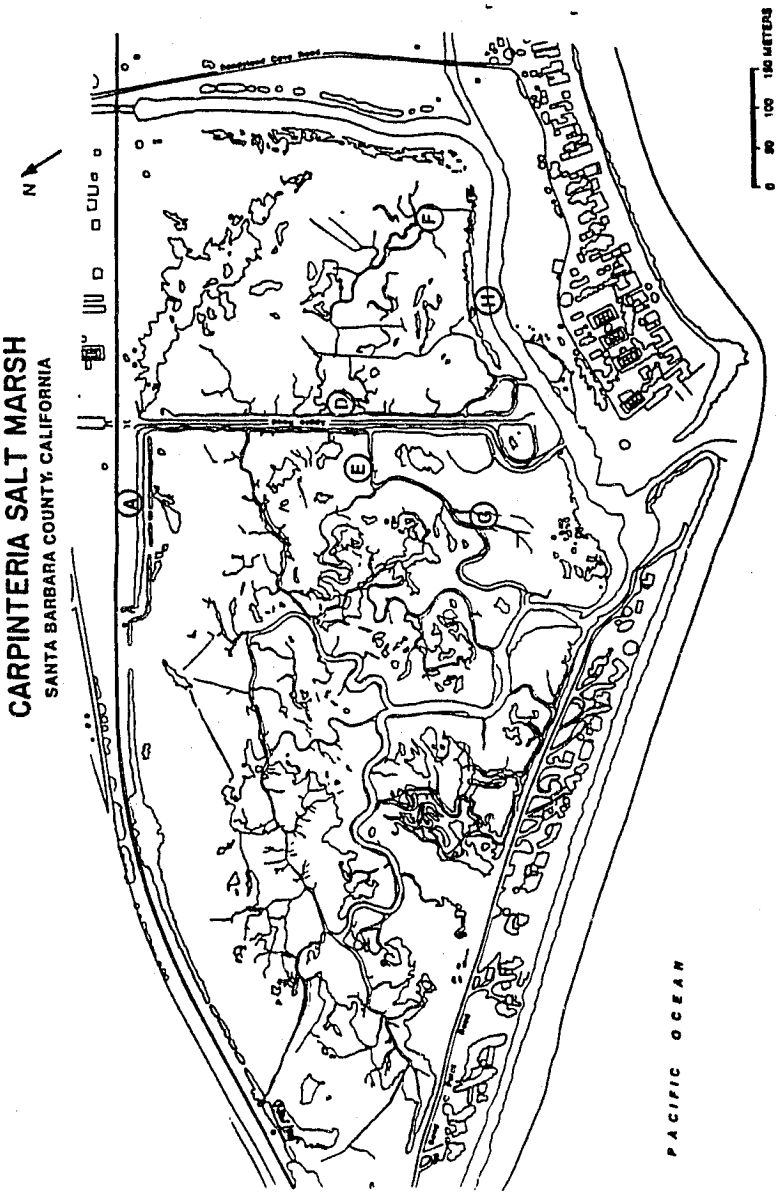
Here I investigated cercarial emergence in both space (within Carpinteria Salt Marsh - CSM) and time (daily and seasonally). The influence of light and temperature on cercarial production was measured in the laboratory, and the association between the density of infected snails (Cerithidea californica) and cercarial densities in the water column were quantified.

The principle findings of this study were that 1) cercarial emergence fluctuated and was variable daily, seasonally and spatially, 2) the combination of elevated temperature and light resulted in the greatest number of cercariae shed in the laboratory, and 3) positive associations between infected snail density and cercarial density were commonly detected in the summer samples when cercariae were abundant.

## Materials and Methods

A diel water sampling program was conducted during the summer of 1993 and the winter (February - early April) of 1994 at six sites in CSM (Fig. 1). Water samples were collected over one full day (dawn to dusk) each week, for 7 weeks (summer) and 5 weeks (winter). A total of ten liters (3 replicates of a 3.3 liter sample) were collected at each site every 3

Fig. 1. Map of cercarial sample sites at Carpinteria Salt Marsh, Santa Barbara County, California.

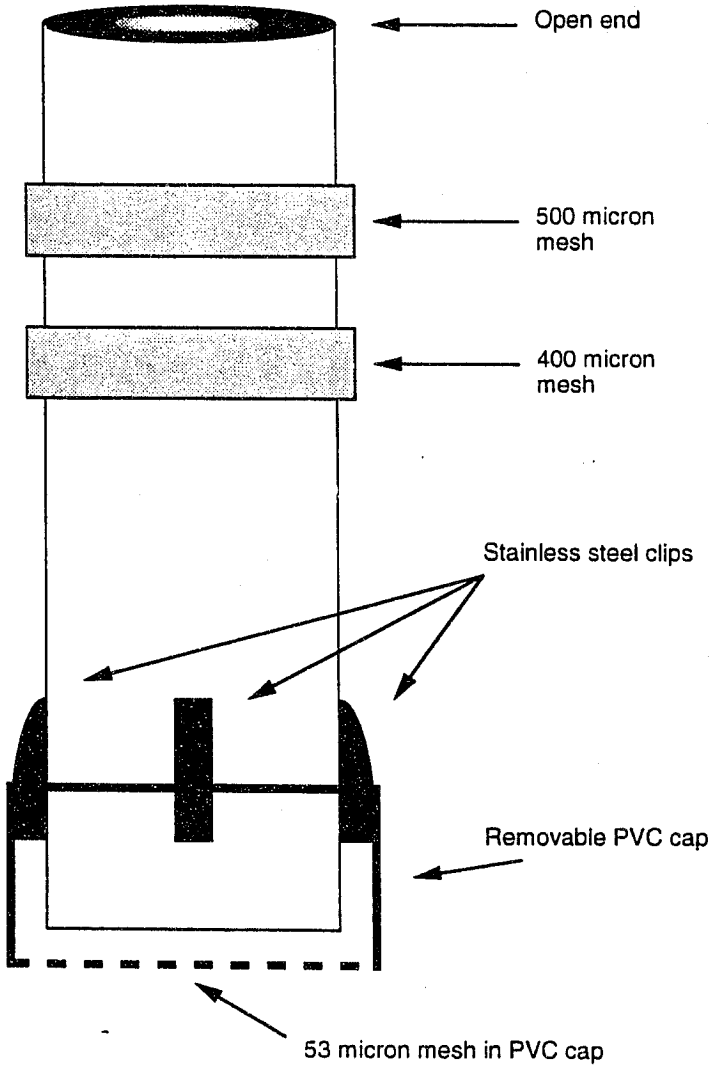


hours. Water temperature, salinity, current speed, depth and tidal cycle were recorded for each sample.

Prior to beginning the summer series, four days were spent on a preliminary study during which water samples were filtered through a coarse mesh (1 mm and 0.5 mm) and then into a fine mesh (40 μm) sieve which trapped the smallest cercariae. The contents from the 40 μm sieve were then rinsed twice into a Büchner funnel attached to a vacuum flask, and filtered again onto filter paper using vacuum filtration with a hand held vacuum pump. Filter papers were removed to a petri dish and were doused with approximately 3 ml of Lugol's iodine solution. All samples were taken to the lab where cercariae trapped on the papers were identified and counted.

Based on the preliminary study, a time-saving cercarial filtration device (Fig. 2) was constructed out of 0.9 meter of PVC pipe, with a diameter of 7.5 cm. The total volume of the tube was 3.3 liters. A graded sieve was built into one end of the PVC pipe. The outermost mesh was 500 μm, and just inside of this there was another section of 400 μm mesh. These were secured in the tube with epoxy. The sections where the tube was cut were reinforced with an outer, larger ring of PVC, also attached with epoxy. At the opposite end of the PVC, a PVC cap was constructed to fit over the end, providing a fine filter for the water samples. Built into the cap was a section of 53 μm mesh, which trapped all the cercariae,

Fig. 2. Sketch of cercarial sample tube design. Refer to Materials and Methods for a description of the size of the tube and appurtenant features.



and a rubber O-ring to provide a good seal and prevent leakage between the pipe and the cap. The capped end was secured during the sampling procedure with three stainless steel clips and was easily removed, rinsed and replaced for subsequent samples.

Each sample was collected by placing the tube horizontally into the water and forcing it down from the water surface to the muddy substrate. It filled as it was pushed through the water. Once at the bottom, the open end of the tube was sealed by hand as it was lifted out of the water. The water in the tube was then drained out through the 53 μm mesh at the capped end. Once the water had drained completely, the cap was removed and the contents trapped on the 53 μm mesh were rinsed twice into a clean petri dish, doused with 3 ml of Lugol's iodine solution and taken to the lab where cercariae were identified and counted. Diversity of other planktonic organisms was also recorded, but these were not identified beyond the ordinal level or quantified. Estimates of cercarial abundance are conservative, as only heads and whole cercariae were counted. Laboratory tests using known numbers of cercariae were conducted to estimate the recovery rate and the percent damaged by the filtering process.

It was often difficult to fill the tube completely each time it was placed in the water, particularly when the tide was out. During these times it was necessary to repeat the filling procedure until 3.3 liters of water was obtained. If the water level in the marsh was so low that the tube wouldn't fill without large quantities of sediment obscuring the sample, the 3.3 liters

were collected with a cup and bucket and then poured through the sampling tube.

Cerithidea californica populations at each site were surveyed before and after the cercarial studies using the procedures outlined in Chapter 1. Between 200-400 snails were collected in the pre-cercariometry survey, and 100-150 were collected in the post-cercariometry survey, taken to the lab, measured, sexed, and trematode infections recorded.

Additional laboratory experiments were conducted to test the influence of light/dark cycling and temperature on cercarial shed. Thirty-six snails of each size class (25 - 30 and 30 - 35 mm) were collected and taken to the lab. Snails were randomly placed in individual cells which contained about 10 ml of sea water (33 ppt). Twelve snails of each size class were then assigned to a particular temperature treatment (cold - 15 C, room - 23 C, warm - 37 C). The experiment was conducted over a period of three days for a total of 3 light and 3 dark cycles. The light dark cycle was 10L:14D. Prior to the ensuing L/D cycle, all snails were transferred to fresh sea water. Freshly shed cercariae were killed and stained with about 2-3 ml of Lugol's iodine, identified and counted. Prior to counting, cercariae within each cell were swirled to break up clumped cercariae and even their distribution in the dish, and then allowed to settle. Once settled, all the cercariae in one field (15x - dissecting microscope) were counted.

Data were analyzed using correlation analysis, analysis of variance (ANOVA) and regression statistics. Correlation and regression analyses were used to determine associations between physical factors (temperature and salinity) and the number of species in the samples. Analysis of variance was used to test for differences in cercarial densities between sites. Regression analyses were conducted on the data rather than the means shown in figures, and were used to test for associations between trematode prevalence in snails and abundance of cercariae in the water column. A two-way analysis of variance was also used to test for the effect of temperature and light/dark treatments in the shedding experiment. Cercariae were identified using Martin (1972), and additional unpublished observations of Stevens, Lafferty and Kuris.

## Results

### *General Patterns*

Species richness and abundance of cercariae released by larval trematode parasites in the snail, Cerithidea californica, increased until the mid-afternoon and then declined in the evening on most summer days (Fig. 3). Water temperature was positively correlated with species richness for all dates during the summer sampling period (Table 1, Fig. 3). There was no correlation between salinity and the number of species observed on each day, nor was there any correlation between temperature and salinity (Table 1).

Water temperature and the influence of light/dark cycling were also investigated in the laboratory. There was a significant difference between

| Date | N samples | Temperature & No. species R | Temperature & No. species $R^2$ | Temperature & density R | Temperature & density $R^2$ | Temperature & salinity R |
|---|---|---|---|---|---|---|
| **Summer 1993** | | | | | | |
| June 7, 9 | 42 | .621 | .385 | .438 | .192 | .062 |
| June 15, 17 | 42 | .473 ** | .223 | .491 | .241 | .237 |
| August 5 | 29 | .629 ** | .375 | .552 | .305 | .036 |
| August 12 | 30 | .440 * | .194 | .401 | .161 | .005 |
| August 19 | 30 | .693 ** | .480 | .585 | .342 | .188 |
| August 26 | 24 | .496 * | .246 | .471 | .222 | .168 |
| September 2 | 30 | .741 ** | .549 | .556 | .309 | .140 |
| **Winter 1994** | | | | | | |
| February 24 | 30 | .258 | .066 | .302 | .091 | .423 * |
| March 3 | 30 | .495 ** | .245 | .425 | .180 | .235 |
| March 10 | 24 | .080 | .006 | .380 | .145 | .510 * |
| March 17 | 30 | ------ | ----- | ----- | ----- | .204 |
| April 7 | 30 | .276 | .0796 | .199 | .040 | .307 |

Table 1. Pearson correlation coefficients, R and $R^2$ from regression analyses, on the association between temperature, salinity, the number of cercarial species, and the density of cercariae sampled from the water column. N equals the number of samples taken for each parameter on each day. There were either 5 or 7 samples per day, and 6 sites. (** - $p < .01$, * - $.01 < p < .05$).

**Table 2.** Two-way analysis of variance (ANOVA) test of differences in cercarial shed in light versus dark condition, and at different temperatures. $p < .05$ indicates significant differences between temperature treatments during the light and dark periods, and a significant interaction between temperature and light regime.

| | LIGHT | DARK |
|---|---|---|
| **LOW (15 C)** | | |
| N (number of snails) | 23 | 23 |
| mean | 165.5 | 12.3 |
| standard deviation | 124.9 | 19.9 |
| standard error | 26.1 | 4.2 |
| **AMBIENT (23 C)** | | |
| N (number of snails) | 23 | 21 |
| mean | 275.1 | 147.7 |
| standard deviation | 221.9 | 175.9 |
| standard error | 46.3 | 38.4 |
| **WARM (37 C)** | | |
| N (number of snails) | 21 | 22 |
| mean | 71 | 104.2 |
| standard deviation | 127.6 | 138.9 |
| standard error | 27.8 | 29.6 |
| **POOLED DATA** | | |
| N | 67 | 66 |
| mean | 173.5 | 86 |
| standard deviation | 183 | 138 |
| standard error | 22.4 | 17 |

| Independent variable | F | P |
|---|---|---|
| Light | 10.29 | .002 |
| Temperature | 10.61 | < .05 |
| Interaction | 5.09 | .007 |

Figure 3, a-g. Summer 1993. Histograms of mean number of cercariae at different times (± s.e.) for each sampling day. Means were calculated by using total numbers of cercariae collected from each site, so at each time period the total volume of water filtered was 60 liters. Note that scales are different in the histograms. Numbers in parentheses indicate time of day and tidal height, as recorded in a tide book.

**Key to Trematode Species:**

EUHA - *Euhaplorchis californiensis*

STRI - Strigeid cercariae

ECHI - *Echinoparyphium* sp.

ACAN - *Acanthoparyphium spinulosum*

RENI - *Renicola buchanani*

PROB - *Probolocoryphe uca*

HIMA - *Himasthla rhigedana*

AUST - *Austrobilharzia* sp.

PARO - *Parorchis acanthus*

UN-ID - Un-identified cercaria (not from C. californica)

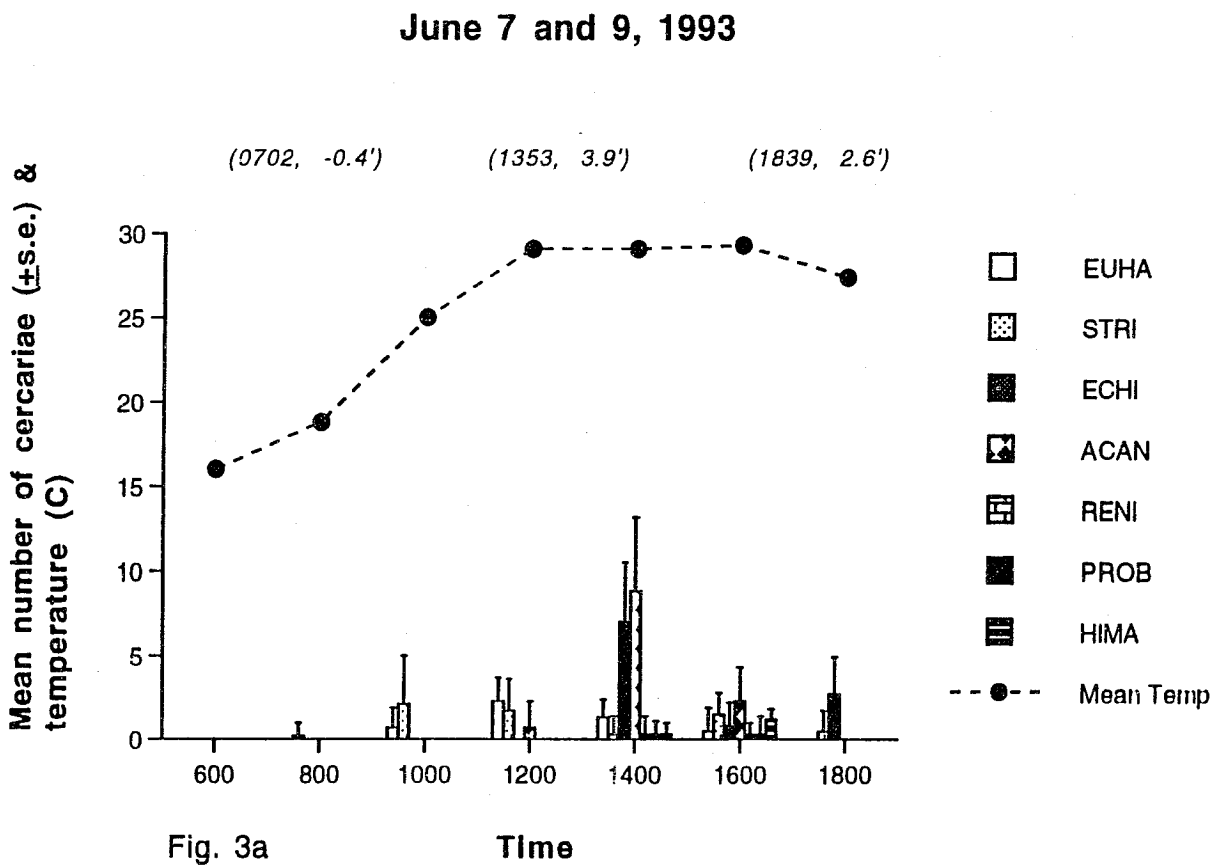



Fig. 3a

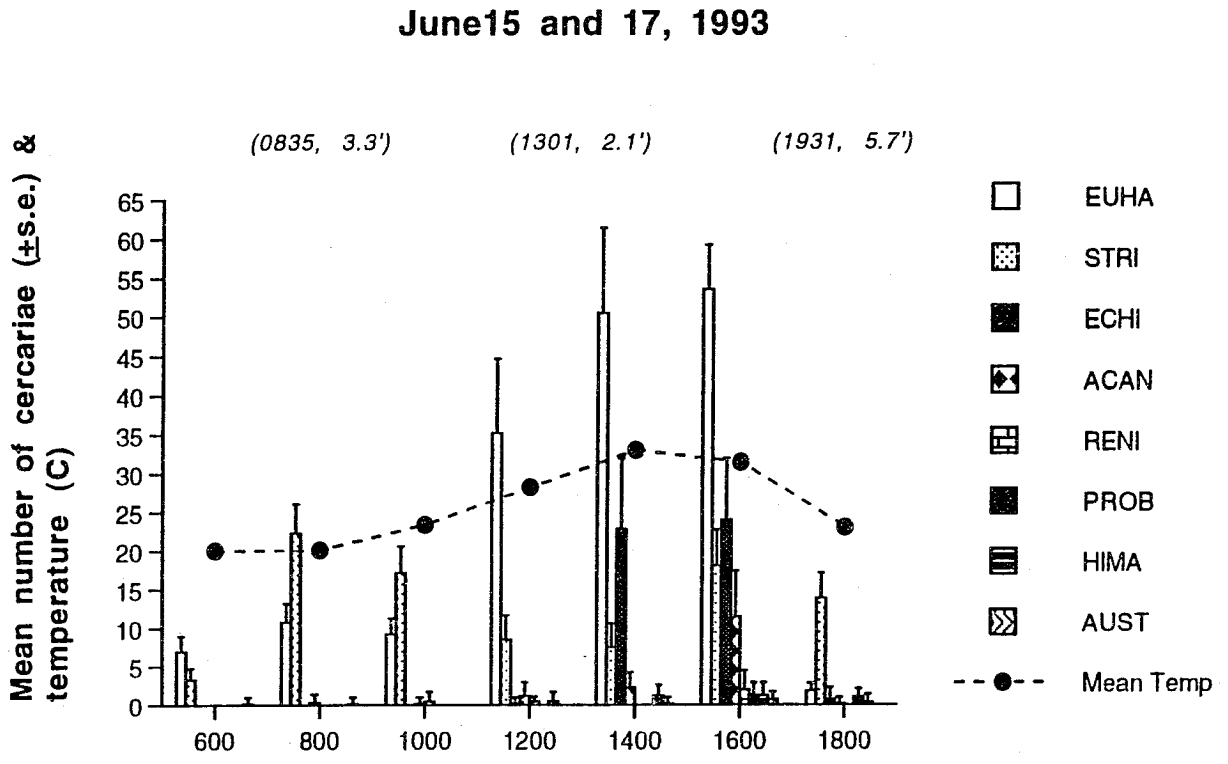



Fig. 3b

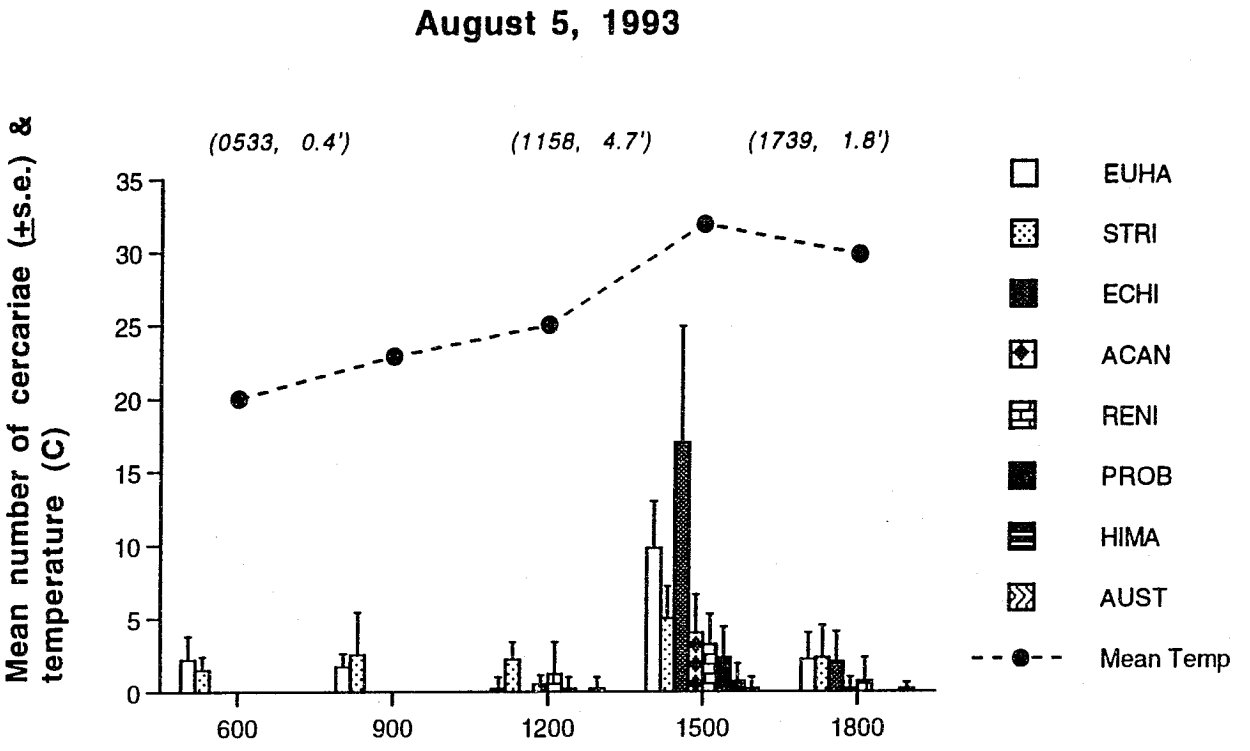



Fig. 3c

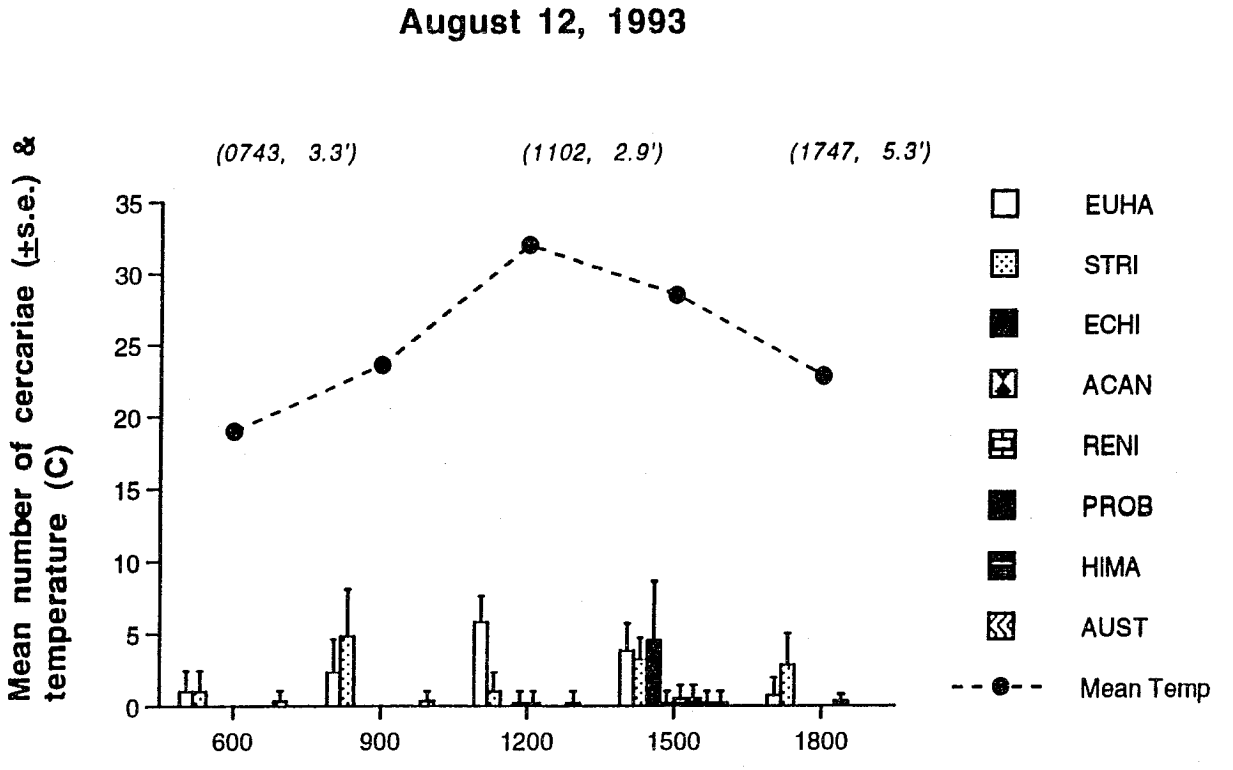



Fig. 3d

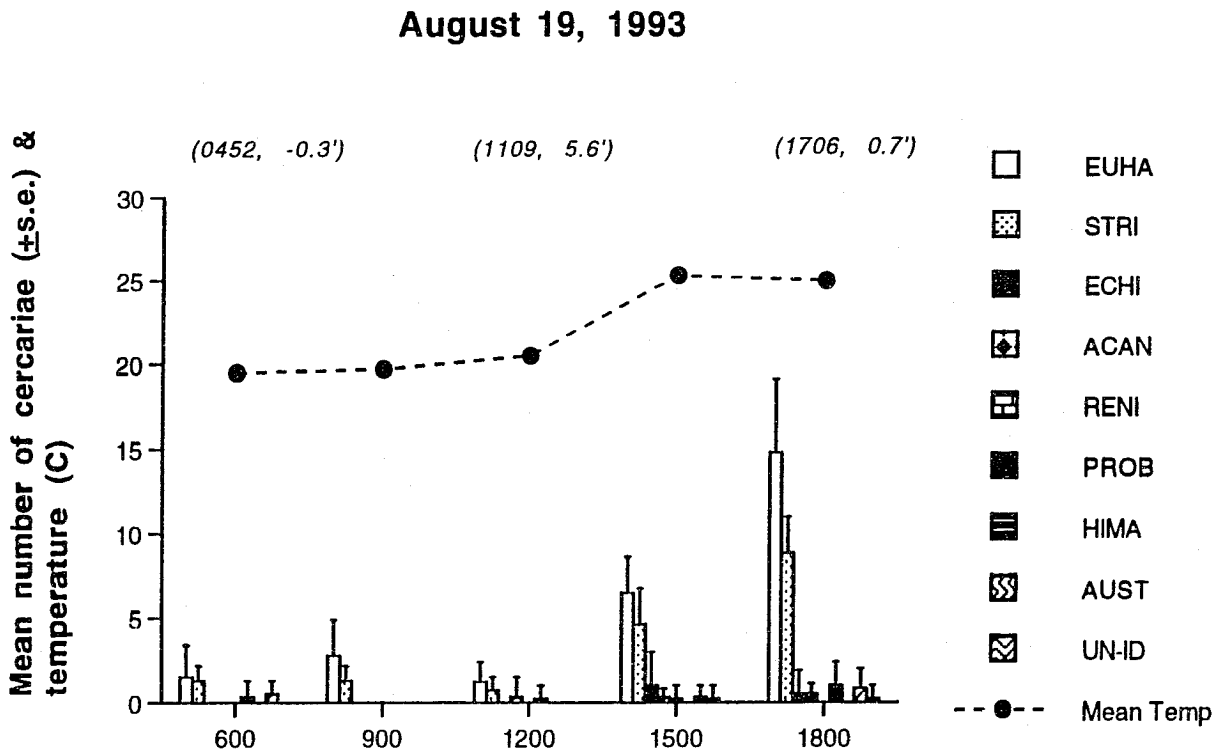



Fig. 3e

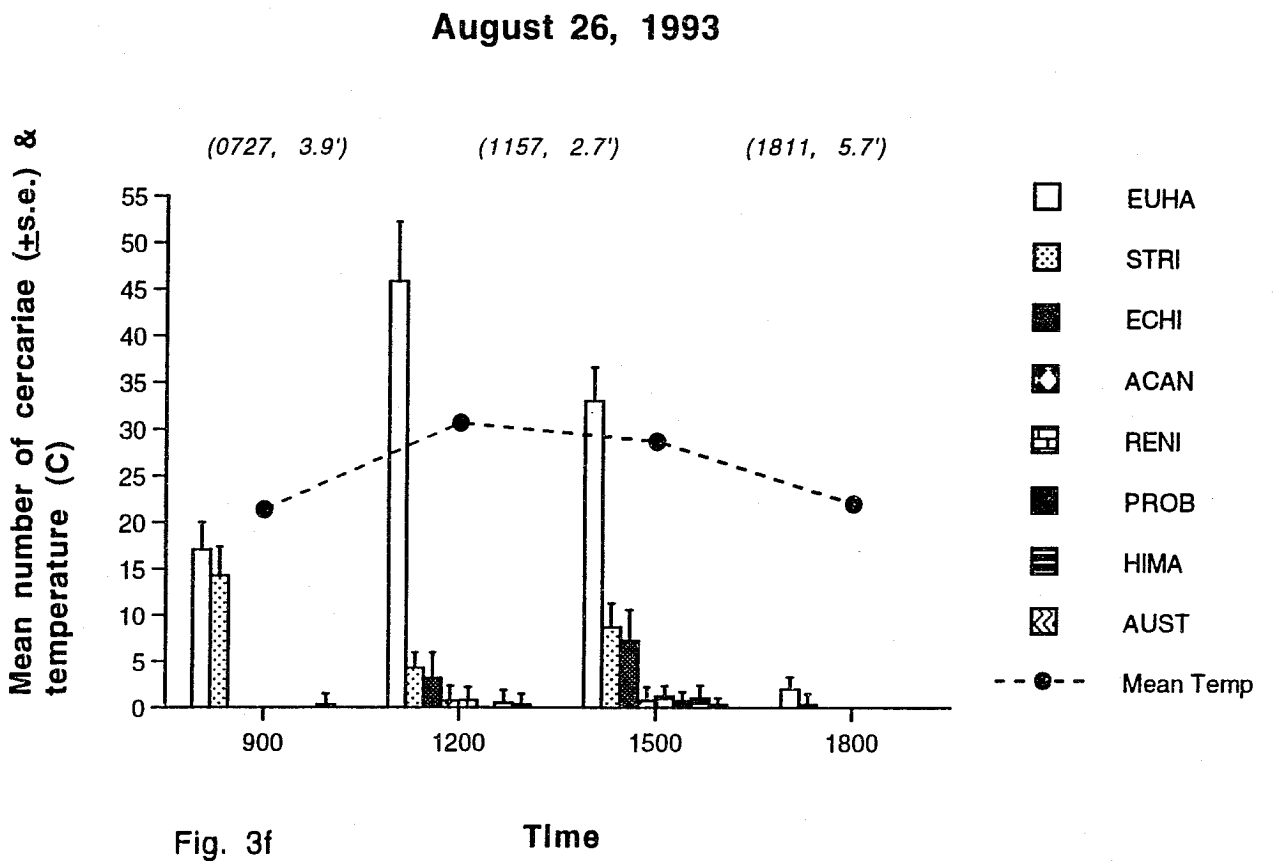



Fig. 3f

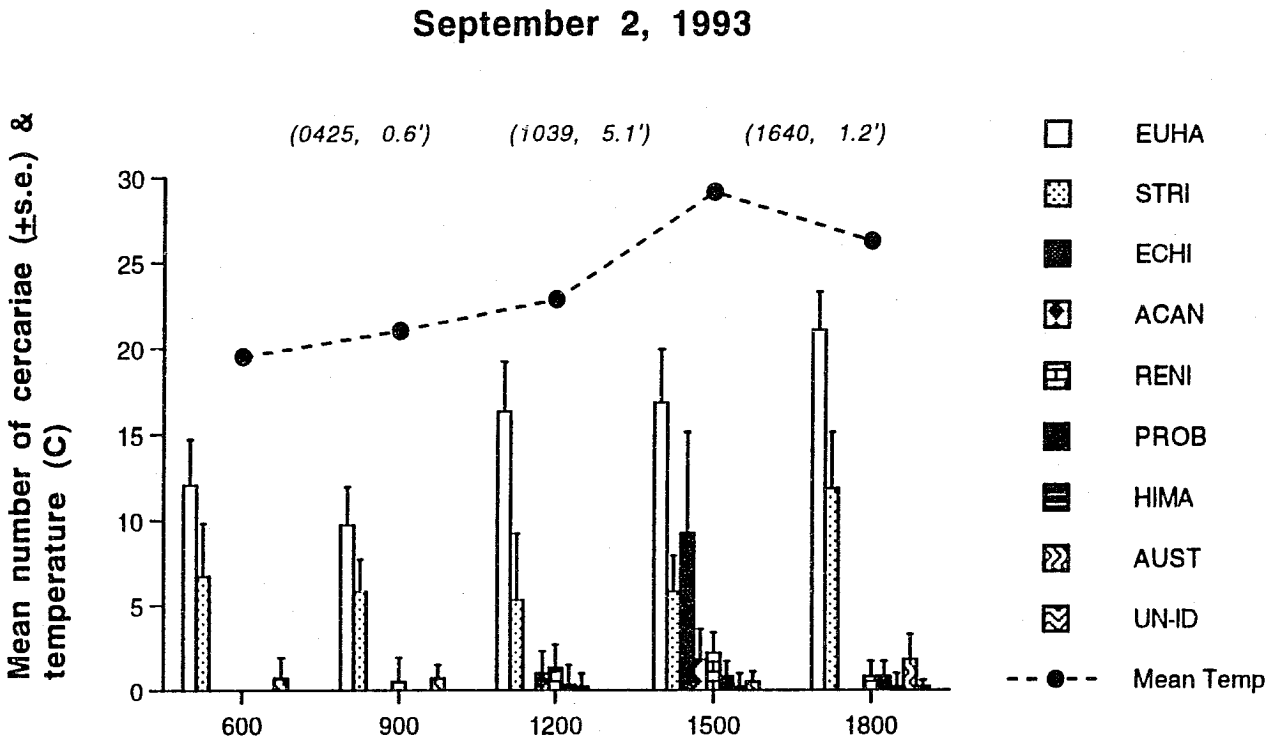



Fig. 3g

temperature treatments (Table 2, Two-way ANOVA, $F = 10.16$, $p < .05$) and the light/dark periods (Table 2, Two-way ANOVA, $F = 10.29$, $p = .002$), and there was a significant interaction between light and temperature (Table 2, Two-way ANOVA, $F = 5.09$, $p = .007$). The warm (37 C) temperature seemed to reduce cercarial output during both the light and dark periods, however there was no significant difference in cercarial abundance between ambient and warm temperature treatments.

Cercarial samples taken during the winter period were depauperate. Water temperatures were about 5 C lower than during the summer. Salinity was lower at 2 sites along channels that conveyed fresh water run-off to the ocean. Abundance was also much lower than during the summer (Fig. 4). There was no consistent correlation between temperature and salinity or salinity and species richness (Table 1). However, temperature and species richness were positively correlated on one sampling date.

***Spatial Patterns***

To detect spatial heterogeneity in the abundance of cercariae among sites, I compared daily totals for each species, pooled over the entire sampling period (Table 3). Analysis of variance was used to test for differences among sites (see Appendix 2a & 2b for cercarial totals used in this analysis). For the summer sampling period, 5 species (strigeid cercariae, Echinoparyphium sp., Acanthoparyphium spinulosum, Renicola buchanani, and Probolocoryphe uca) displayed significant variation in abundance among sites (Table 3; ANOVA, $p < .05$). Abundance of a sixth

| Species | Cercarial Abundance mean ± s.e. | F-ratio | p |
|---|---|---|---|
| **Summer 1993** | | | |
| EUHA | 57.2 ±14.9 | 1.617 | .181 |
| STRI | 28.5 ± 6.2 | 3.011 | .023 |
| ECHI | 14.6 ± 6.5 | 4.368 | .003 |
| ACAN | 5.4 ± 2.1 | 2.832 | .029 |
| RENI | 2.7 ± 0.7 | 3.968 | .006 |
| HIMA | 1.05 ± 0.43 | 2.293 | .066 |
| PROB | 1.5 ± 0.3 | 2.401 | .056 |
| AUST | 1.3 ± 0.32 | 1.879 | .122 |
| **Winter 1994** | | | |
| EUHA | 3.8 ± 2.3 | .687 | .638 |
| STRI | 0.27 ± 0.14 | .489 | .781 |
| ECHI | 0.83 ± 0.54 | 1.111 | .381 |
| ACAN | 0.87 ± 0.36 | 1.715 | .170 |
| RENI | 0.2 ± 0.14 | .800 | .561 |
| HIMA | 0.13 ± 0.08 | 1.760 | .159 |
| PROB | 0.13 ± 0.13 | 1.000 | .439 |
| PARO | 0.17 ± 0.07 | .756 | .590 |

Table 3. Analysis of variance (ANOVA) for differences in abundance of cercariae between sites. There were six sites and seven sample days (N = 42) in the summer, and five sample days (N = 30) in the winter. Cercarial totals at each site, on each day were used in this analysis. Appendix 2a & b shows these cercarial totals for each day. (* - .01 < p < .05).

Figure 4, a-e. Winter 1994. Histograms of mean number of cercariae at different times (± s.e.) for each sampling day. Means were calculated by using total numbers of cercariae collected from each site, so at each time period the total volume of water filtered was 60 liters. Note that scales are different in the histograms. Numbers in parentheses indicate time of day and tidal height, as recorded in a tide book. Refer to Fig. 3 for the Key to trematode species.

Fig. 4a

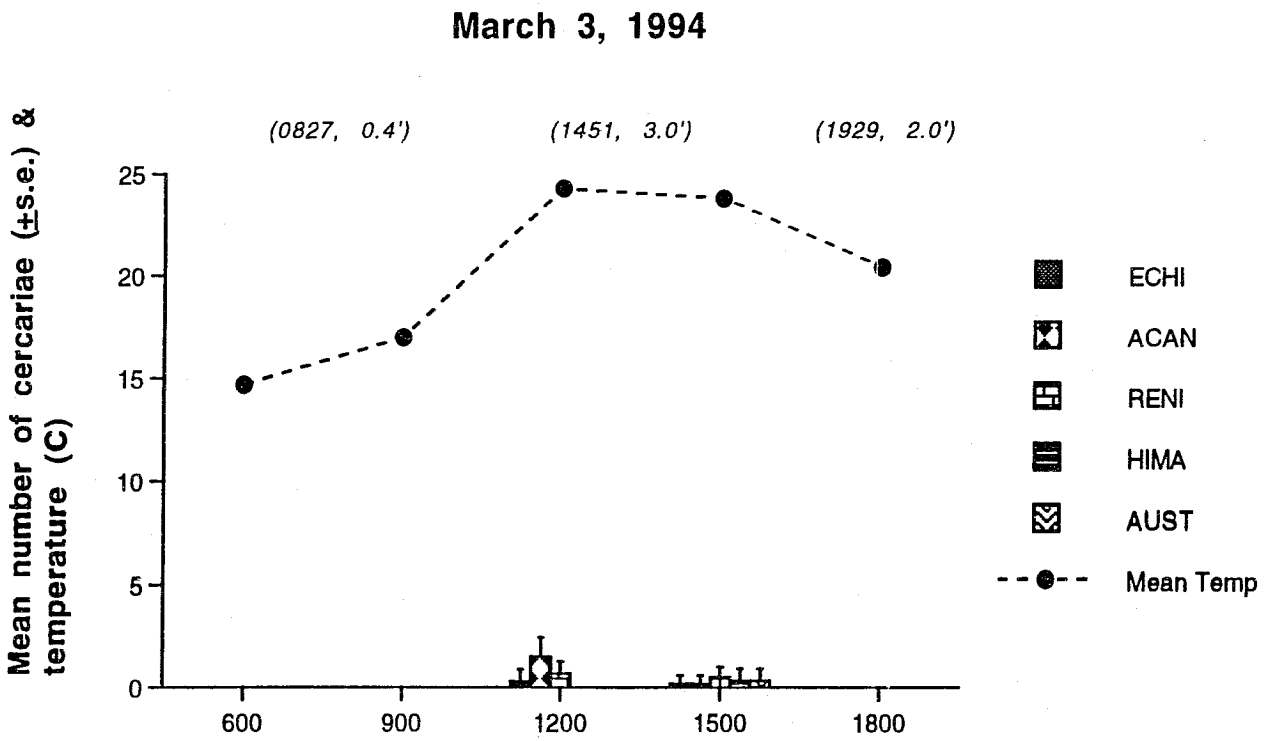



Fig. 4b

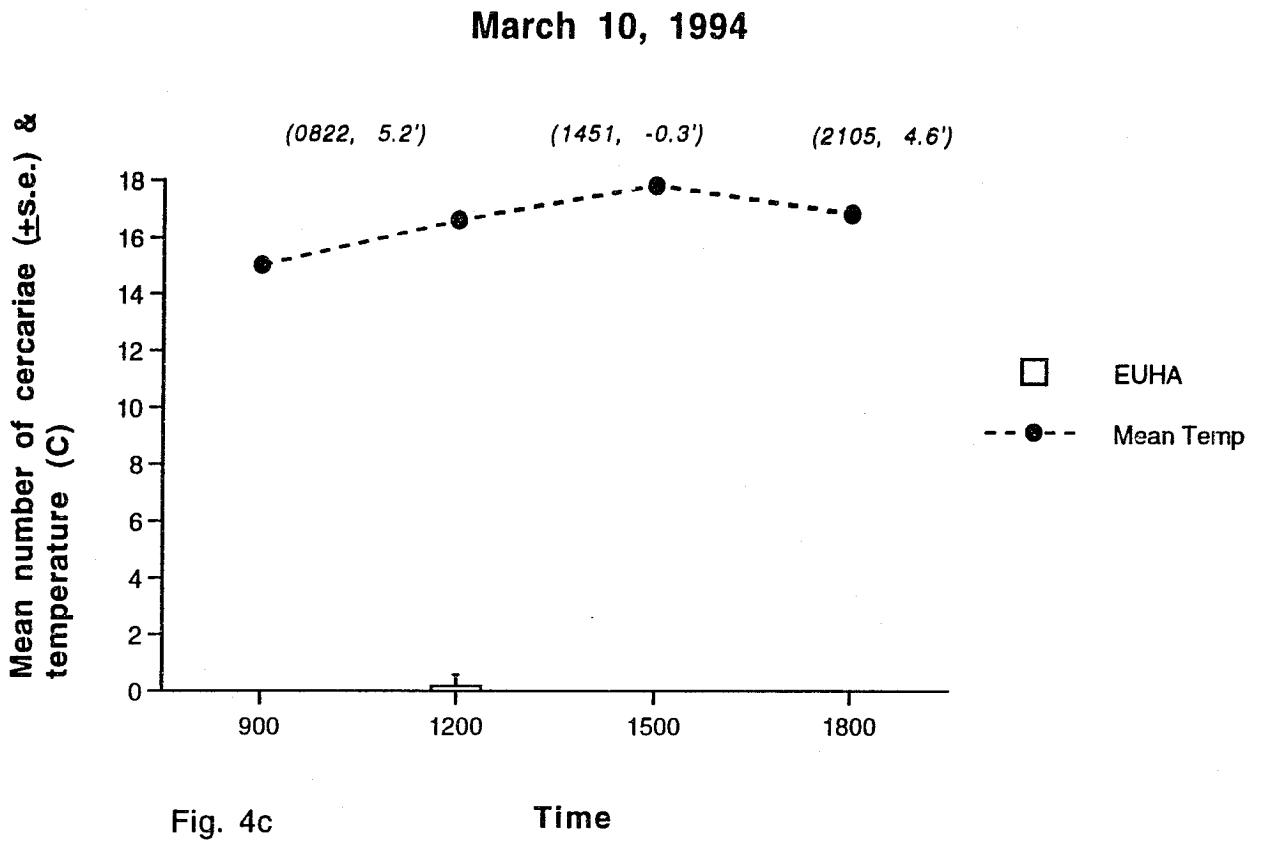



Fig. 4c

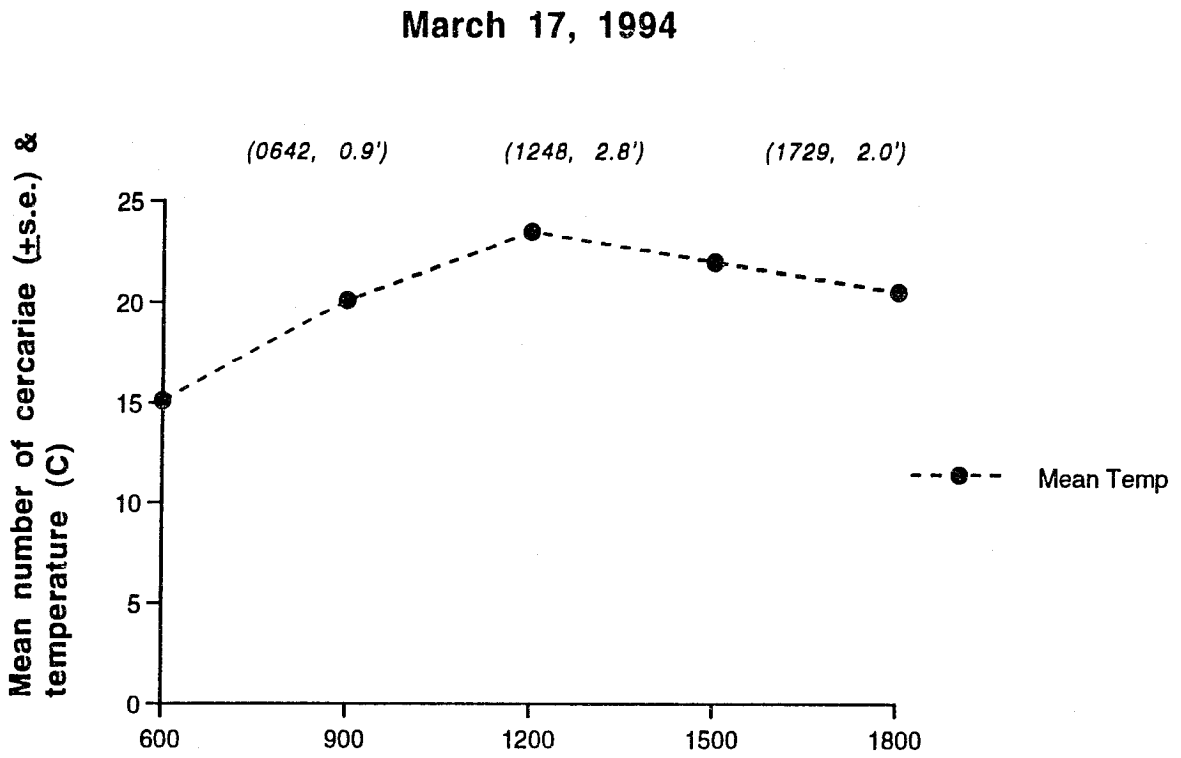



Fig. 4d

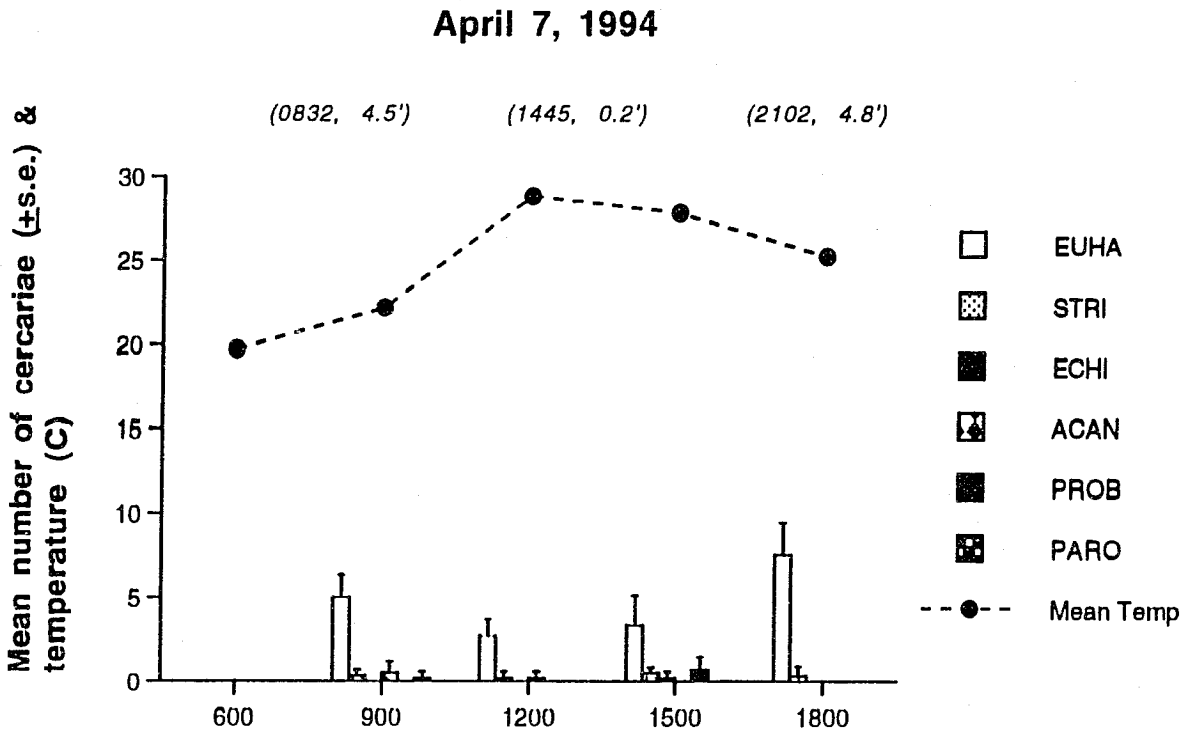



Fig. 4e

species, Himasthla rhigedana, also varied among sites, but the variation was not statistically significant (ANOVA, $p = .066$). Examination of cercarial abundance from the winter samples revealed no significant variation among sites for any species (Table 3; ANOVA, $p > .05$).

***Association between snails and cercariae***

A regression analysis of cercarial density as a function of infected snail density was conducted on log transformed, normalized data because the variance increased with the mean, and variances were not equal among samples. Significant positive associations between infected snail abundance and cercarial abundance were demonstrated for Euhaplorchis californiensis, strigeid cercariae, Echinoparyphium sp., A. spinulosum, and H. rhigedana in the summer samples (Table 4a; Fig. 5 a-h; $p < .05$). A significantly negative association was detected for R. buchanani ($R = -.387$, $m = -.397$, $p < .01$). Regression analyses on the untransformed data yielded similar results with significantly positive associations shown for only 3 species (strigeid cercariae, Echinoparyphium sp., A. spinulosum). Again, R. buchanani cercarial abundance was significantly negatively associated with infected snail abundance. A similar analysis of the winter data revealed no significant positive or negative associations between the density of infected snails and cercarial abundance (Table 4b).

| Summer 1993 Species | N | Max Snail Density ($m^2$) | Slope (m) | Y-intercept (b) | $R^2$ | F-ratio |
|---|---|---|---|---|---|---|
| EUHA | 42 | 114.8 | .067 * | .904 | .15 | 7.17 |
| STRI | 42 | 26.5 | .416 * | .586 | .36 | 22.63 |
| ECHI | 42 | 111.0 | .138 * | .127 | .61 | 61.95 |
| ACAN | 42 | 10.2 | .496 * | .184 | .11 | 4.98 |
| RENI | 42 | 14.0 | -.397 * | .578 | .15 | 7.12 |
| HIMA | 42 | 36.3 | .070 | .047 | .08 | 3.46 |
| PROB | 42 | 4.2 | .457 | .219 | .04 | 1.86 |
| AUST | 42 | 1.5 | .952 | .194 | .05 | 1.99 |

Table 4a. Regression analyses for log cercarial density as a function of infected snail density during the summer. The independent variable is the infected snail density at each site, the dependent variable is the log of the cercarial density at each site. Refer to Fig. 5 for graphical presentation and Fig. 3 for the key to trematode species names. * - $.01 < p < .05$

| Winter 1994 | N | Max Snail Density ($m^2$) | Slope (m) | Y-intercept (b) | $R^2$ | F-ratio |
|---|---|---|---|---|---|---|
| **Species** | | | | | | |
| EUHA | 30 | 100.5 | .016 | .139 | .02 | .46 |
| STRI | 30 | 19.0 | .039 | .045 | .03 | .81 |
| ECHI | 30 | 15.0 | .147 | -.024 | .05 | 1.4 |
| ACAN | 30 | 8.5 | -.109 | .182 | .01 | .38 |
| RENI | 30 | 26.0 | -.046 | .101 | .09 | 2.9 |
| HIMA | 30 | 13.0 | .018 | .021 | .003 | .08 |
| PROB | 30 | 9.6 | -.043 | .033 | .01 | .39 |
| PARO | 30 | 6.3 | -.057 | .059 | .01 | .37 |

Table 4b. Regression analyses for log cercarial density as a function of infected snail density during the winter. The independent variable is the density of infected snails at each site, the dependent variable is the log of the density of cercariae at each site. Refer to Fig. 3 for the key to trematode species names.

Figure 5 a-h. Summer 1993. Regressions of the log transformed density of cercariae in the water column and the mean density of infected snails at six sample sites. The density of infected snails was calculated using average density of snails, prevalence of each trematode species, and average size of snails at each site. Mean cercarial density (log n+1) was calculated after log tranformation. Each point represents a different site. Refer to Table 4a for regression statistics.

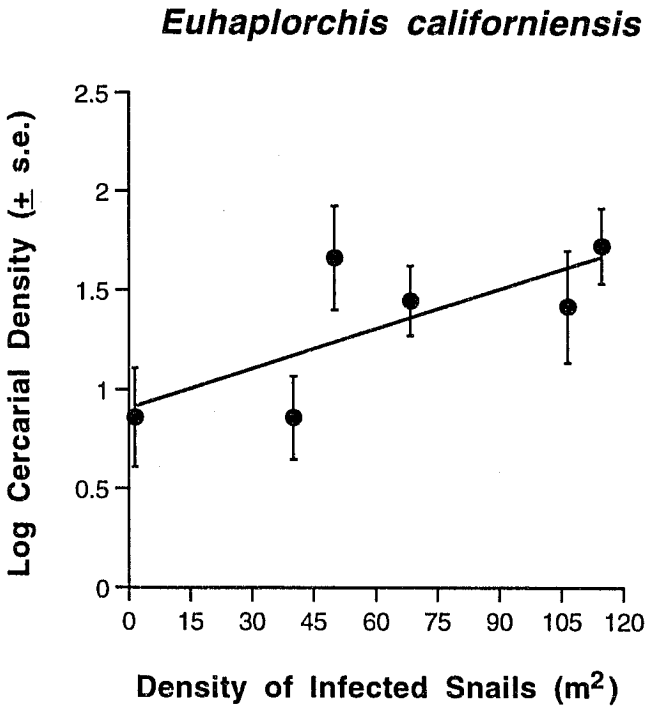



Fig. 5a

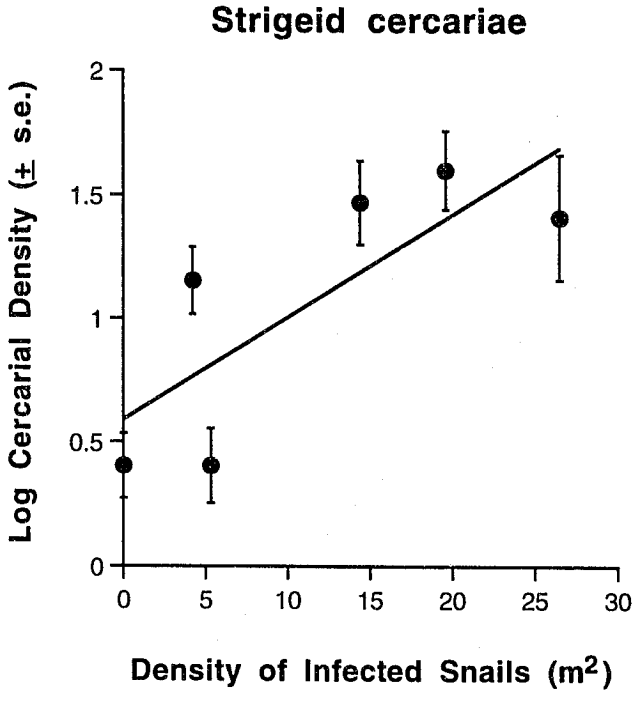



Fig. 5b

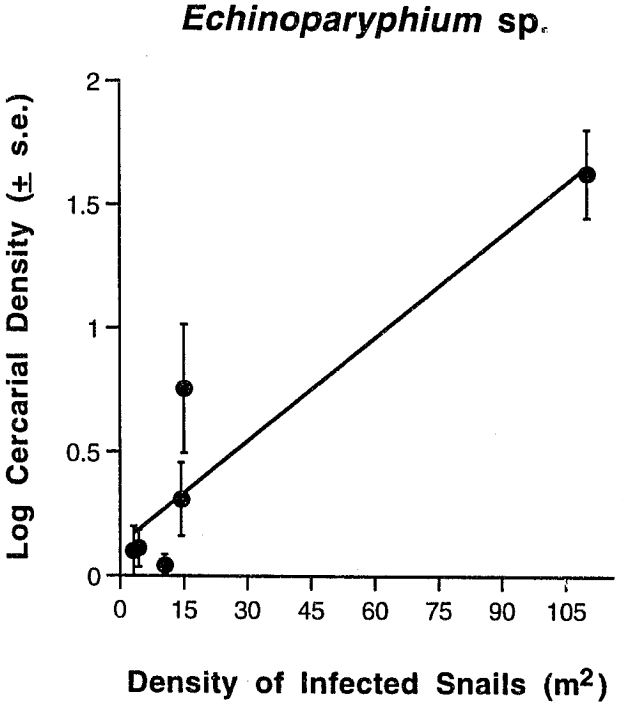



Fig. 5c

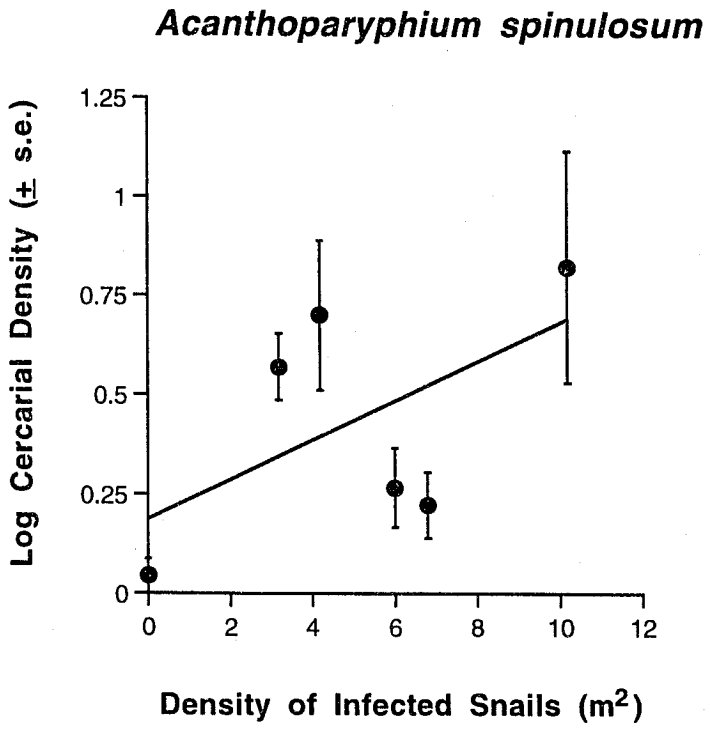



Fig. 5d

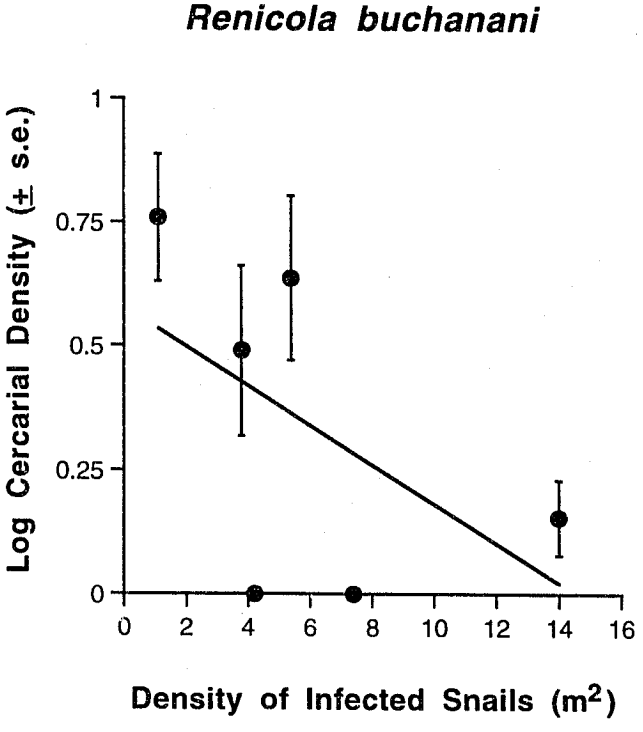



Fig. 5e

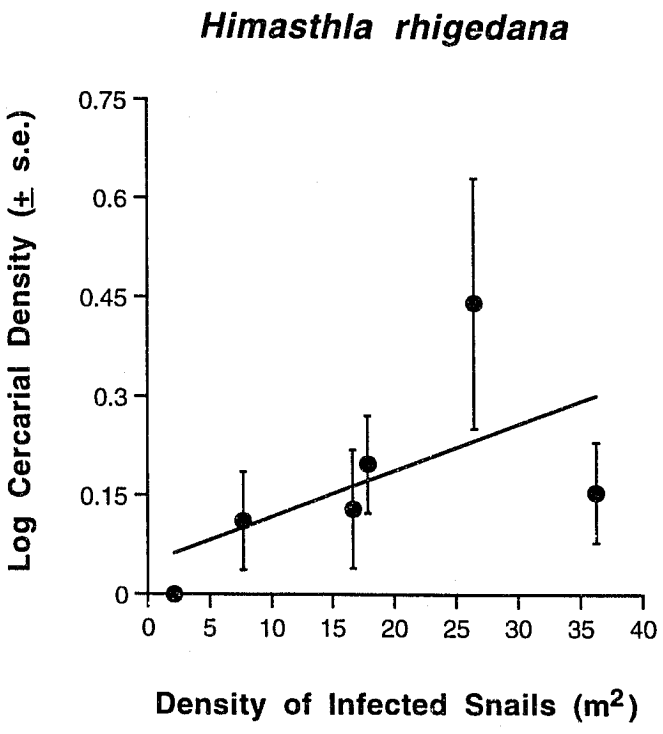



Fig. 5f

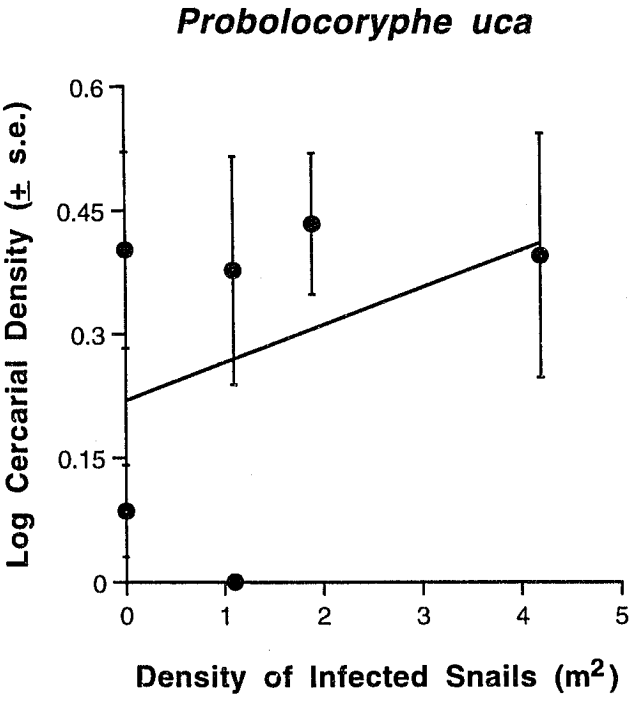



Fig. 5g

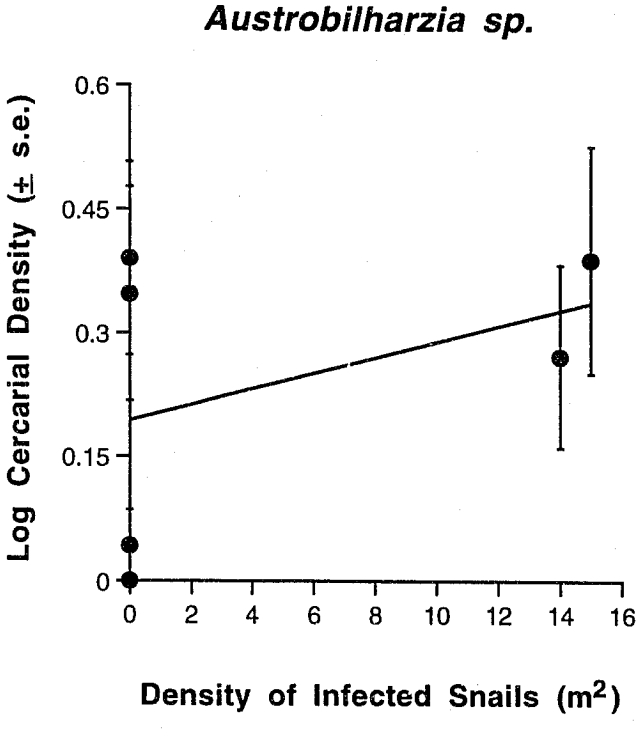



Fig. 5h

## Discussion

This study is the first to report the distribution and abundance of an entire assemblage of planktonic cercariae in an aquatic habitat. These cercariae were readily identified and quantified, and they appear to comprise a substantial fraction of the soft-bodied zooplankton at CSM (unpublished data). Virtually all the species of cercariae collected in the water column were released from parasites of C. californica (Fig. 3).

This species-rich assemblage has a diel and seasonal shedding periodicity. In general, there was a positive association between the abundance of infected snails and cercarial abundance for all but one species (R. buchanani). Community structure and species interactions of the guild of larval trematodes in C. californica have been well studied (Hunter 1942; Martin 1955; Yoshino 1975; Sousa 1983, 1990; Kuris 1990; Sousa 1993; 1994; Lafferty et al. 1994). Analysis of cercarial abundance in the water column permits, for the first time, an assessment of the risk of infection of the next host in their life cycles, and the impact of intra-molluscan trematode interactions on the output of cercariae from these snails (see Appendix 2c). Such interactions appeared to have the most impact on species (e.g. Euhaplorchis californiensis) placed in the middle of Kuris' (1990) and Lafferty et al. (1994) dominance hierarchy.

A number of hypotheses have been proposed to explain periodic emergence of cercariae from snails. Théron (1984) postulated that periodic emergence of cercariae evolved to coincide with activity patterns of the next host in the life cycle, and inferred that intra-specific variation in

Schistosoma mansoni emergence times were driven by activity patterns of definitive hosts, either man or rats. Shostak and Esch (1990) proposed that periodic cercarial emergence could serve as either 1) a dispersal mechanism, or 2) a refuge from predators. Finally, Lowenberger and Rau (1994) proposed that low levels of infectivity upon emergence, coupled with a dispersal phase, might reduce overall infection levels and parasite induced mortality in the next host. They also suggested that a delay in maximum cercarial infectivity could provide time for dispersal.

Each of these hypotheses relies on the assumption that periodic emergence of cercariae is an adaptive strategy which has evolved to increase the likelihood of finding and infecting the next host. Similar arguments for adpative behaviors have appeared in studies of spawning patterns of marine invertebrates (Hahn 1989); however, these studies have failed to demonstrate that conditions which induced gonadal development and are optimal for reproductive adults are equally suitable for their planktonic larvae which are dispersed from the natal area. Furthermore, aquaculture species such as scallops and mussels, are known to spawn reliably when shocked or stressed by changes in femperature or pH (Barber and Blake 1991). Presumably, this is an attempt to reproduce under adverse conditions, and therefore may also be considered adaptive even though reproduction may not be positively associated with chances of future reproductive success.

Two equally plausible alternative explanations for periodic emergence of cercariae are, 1) periodic emergence is a response to

physiological cues experienced by cercariae in the snail host, or 2) cercariae are stressed by elevated temperatures in the snail host and are responding to adverse conditions, thus leaving the snail host when a temperature threshold is reached. Under such circumstances, activities of other hosts or predators would probably have little influence on the emergence pattern of cercariae at CSM because this assemblage of second intermediate hosts typically don't exhibit similar active periods each day (crabs are most active at night, fish inhabit small channels during high tide and move to large deep channels at low tide, clams feed at high tide). Furthermore, it is unlikely that mass cercarial emergence would serve as a refuge from non-host vertebrate or invertebrate predators. Thus, the pattern reported here does not appear to be adaptive for second intermediate host infection at CSM, and raises doubts about hypotheses which suggest that periodic cercarial emergence evolved as a response to activities or behaviors of other organisms.

***Associations Between Snails and Cercariae***

The density of cercariae was positively associated with density of infected snails during the summer. Variation in infected snail density accounted for an average of 20% of the variation in cercarial density. This is consistent with other field studies on schistosome species (Théron et al. 1978; Ouma et al. 1989), where infected snail density accounted for 25% of the variation in cercarial density (Ouma et al. 1989). In these studies, prevalence in snails was locally high (range: 0-41.2%, average: 9.3%) but infected snail densities overall were very low (Théron et al. 1978, average:

1.0 per $m^2$) in comparison with C. californica at CSM (average prevalence 45%, average density of infected snails: 145 per $m^2$). Furthermore, both studies (Théron et al. 1978; Ouma et al. 1989) demonstrated that sites containing many infected snails and abundant cercariae were a result of habitat use by definitive hosts. Habitat areas most intensively used by definitive hosts had correspondingly high prevalence in snails (Théron et al. 1978; Ouma et al. 1989). Similarly at CSM, intensity of habitat use by definitive host birds could influence prevalence of trematodes in snails and subsequent cercarial abundance, but aggregation of cercariae in the water is reduced by tidal action and water flow.

For most of the species studied at CSM, there was a positive association between infected snail and cercarial densities in the summer. In contrast, the density of R. buchanani cercariae was negatively associated with infected snail density. However, the mechanisms which may account for this negative association are not clear.

Rowan (1965) postulated that cercarial predation by non-host fish could drastically reduce cercarial abundance in the water. In that study, he provided empirical evidence that Poecilia reticulata removed large numbers of cercariae from the water column in the field (up to 7000 per hour), and suggested that other fish species could also effectively deplete populations of cercariae. Likewise at CSM, there are a variety of fish (Atherinops affinis-topsmelt, Clevelandia ios-arrow goby) which do not serve as second intermediate hosts (unpublished data) for E. californiensis

but are abundant (Brooks personal communication), and may be an important source of mortality for these and other cercarial species at CSM.

Exposure to sunlight or ultra-violet radiation kills and reduces infectivity of schistosome cercariae (Krakower 1940; Tomberg and Lagrange 1952; McClelland 1965; Ghandour and Webbe 1975; Ariyo and Oyerinde 1990). In particular, Krakower (1940) demonstrated that nearly all schistosome cercariae were dead after about one hour following exposure to cloudless mid-day sunlight. At CSM, exposure to sunlight/ultra-violet radiation may also affect the longevity and infectivity of cercariae. Dead cercariae, recognized by their withered appearance, were rarely collected, and I could not determine that these cercariae died due to exposure from ultra-violet radiation. However, if ultra-violet radiation was an important source of cercarial mortality and caused a distinct appearance, then abundance of dead cercariae in the samples should have been higher than was observed.

High water temperatures may also negatively affect cercarial longevity (Shostak and Esch 1990; Meyrowitsch et al. 1991; Rea and Irwin 1992). They showed that increased temperatures significantly shortened the life span of cercariae, negatively affected the ability of cercariae to encyst (Shostak and Esch 1990), decreased the infective period and infectivity of cercariae (Meyrowitsch et al. 1991), and increased the rate of spontaneous tail loss (Rea and Irwin 1992). At CSM, dissociated cercariae (H. rhigedana, Echinoparyphium sp., A. spinulosum, Euhaplorchis californiensis) were occasionally seen in summer water

samples; however, the cause of the head loss was never determined. In addition, there were two sample days (August 26 and September 2, 1993) where hundreds of E. californiensis cercarial heads and detached tails were collected in the mid-afternoon samples which suggests that cercariae were stressed by higher mid-day temperatures on these days.

***Daily and Seasonal Patterns***

The water sampling program at CSM revealed that cercarial emergence is intermittent daily, and that there were some seasonal differences. The daily pattern suggests that cercarial emergence is influenced by water temperature. Light also appears to be an important shedding cue (Giovannola 1936a; Kuntz 1947; Rees 1948; Asch 1972). However, it is often difficult to separate the effect of light and temperature in the laboratory and field (McClelland 1965; Asch 1972). Investigations using C. californica from CSM which measured cercarial abundance during light and dark periods at various temperatures, demonstrated peak shedding occurred during light periods at elevated temperatures. The field patterns measured at CSM coupled with laboratory experiments on cercarial shedding at different temperatures (Table 2) suggest that light and temperature (23 C and greater) result in the highest number of snails shedding cercariae, greatest abundance of cercariae, and a high diversity of cercarial species that emerge from snails. In this study, increased temperature explained from 19.4% - 54.9% of the variation in the diversity of cercarial species, but explained less of the variation in the total number

of cercariae recovered (16.1% - 34.2%) because it had a relatively small effect on E. californiensis.

Daily salinity changes did not influence abundance or richness of cercariae in either the summer or winter samples. Thus, this assemblage of trematodes, like the host snails, are adapted to estuarine conditions and fluctuating salinity. In contrast, Rees (1948) showed that some marine snails and their trematodes die and/or cease shedding as water becomes increasingly brackish (13 - 52 ppt). Stunkard and Shaw (1931) also showed that marine trematodes are negatively affected by brackish water, and often do not survive more than an hour in fresh water. At CSM, only one of the six sites had consistently low salinity. This site was immediately adjacent to a culvert pipe which conveys freshwater run-off from surrounding agricultural fields and urban areas. If longevity was affected by low salinity, I would have expected to collect more dead cercariae in these water samples. However, this was not the case.

Tidal cycling at CSM probably had some influence on the abundance of cercariae because tidal inundation has three important effects. Firstly, incoming ocean water is generally cooler than shallow marsh water (Fig. 3) during the summer. This may decrease shedding rate. Secondly, incoming tides probably dilute concentrations of cercariae (Fig. 3). Finally, tidal cycling probably disperses cercariae away from the point of release.

Hubbard (unpublished data) has demonstrated a prolonged slack period and a delay in the ebb cycle of approximately 2-3 hours at CSM. In

contrast, the flood cycle is in agreement with NOAA predictions of local tides. Hubbard showed that the delay is due to the elevation of the marsh relative to the mouth of the slough, and the saturation of sediment during high tide periods. Therefore, the lowest measurable tide occurs about 3-4 hours after the time predicted by NOAA tide charts. Furthermore, if the subsequent high tide is less than about +1.3 m, the only channels influenced by the incoming high water are those which are adjacent to the mouth of the slough. This means that most of the slough experiences a low tide throughout the day if the predicted low tide is in the morning and the subsequent high tide is below +1.3 m. An all-day low tide occurred on all sample dates except August 19, 1993 and September 2, 1993, which had flood tides of greater than +4 feet mid-morning. The temperatures on these two dates during the flood tide were not significantly different than those measured earlier in the day. However, cercarial abundance and richness in morning samples was less than in later samples. This suggests that incoming tides dilute cercariae and that this lower temperature water may keep cercarial species from emerging. Because most sample dates experienced all day low tides, cercariae were probably not dispersed very far from the point of release. Therefore, spatial variation in cercarial abundance between sites (Table 3) is probably not a result of tidally influenced dispersal. Rather the source of the variation is due to the differences in prevalence between sites (Chapter 1).

Cercarial abundance was lower in winter samples compared to summer samples. This difference might be due to changes in water

temperature, light intensity, salinity or nutritional state of snail hosts. Winter-time declines in water temperature and light intensity may decrease cercarial emergence. Sunlight is less intense during winter months; however, it is greater than the minimum light level, measured by Asch (1972) as full moon light, required to induce cercarial emergence. Furthermore, winter light intensities should not have inhibited cercarial emergence to the low levels measured in the field, because temperatures and light levels during winter appear to be adequate stimuli to induce some shedding in the lab.

The nutritional state of host snails can affect cercarial production (Anderson et al. 1977), but it was not examined here. In most studies of schistosome cercarial production food was provided to experimental snails as a standard maintenance procedure but none of these studies compared shedding of cercariae in fed versus starved snails (Kuntz 1947; Glaudel and Etges 1973; Sluiters et al. 1980; Moné et al. 1986; Mouahid and Théron 1987). However, Anderson et al. (1977) showed that cercarial production ceased after snails had been starved for 5 weeks, and cercarial production resumed an additional 4 weeks after feeding was resumed. In addition, Lafferty (1993) showed that C. californica has a slower growth rate during fall/winter months at CSM, and suggested that food may be a limiting factor for snails during this time. In addition, Race (1979) observed that snails "hibernate" and appear to stop feeding beginning in the late fall months. Thus, food limitation probably has a negative effect on cercarial production in the winter months because available resources

are probably utilized by the host to maintain a basal metabolic rate. Thus, few resources might be allocated to host reproduction or to trematode cercarial production which replaces snail reproduction in the parasitically castrated host.

***Conclusions and Summary***

The diverse cercarial assemblage of C. californica at Carpinteria Salt Marsh emerged periodically from snails each day. The pattern of emergence was strongly associated with increasing water temperature throughout the day, and peak densities were recorded in the early afternoon. However, there was no evidence that these emergence patterns were adaptive strategies for host transfer or predator avoidance. Seasonal differences in diversity and abundance of cercariae were striking and suggest that a combination of factors (e.g. lower water temperatures, host nutritional condition) may affect cercarial production, maturation and emergence from the snail host.

# CHAPTER 3

## Interspecific Interactions Between Larval Trematodes: Quantification and Species Associations

### Introduction

A variety of factors potentially influence the distribution and abundance of species in an assemblage. Physical factors (e.g. disturbance, temperature, desiccation, salinity), and biotic factors (e.g. recruitment , predation, parasitism and disease) operate simultaneously and affect species composition and abundance of organisms, or the "community structure" of an assemblage. The relevance of interspecific competition as a significant structuring force is uncertain compared to these other factors. Furthermore, the magnitude of competition can be difficult to measure, although competitive effects must be quantified to predict how these interactions influence species composition and abundance.

This study quantified the magnitude of interspecific interactions in an assemblage of larval trematodes, and tested the hypothesis that interspecific antagonism reduces the abundance of larval trematodes in first intermediate host snails. In addition, these data were used to determine whether spatial and temporal heterogeneity intensified potential interspecific interactions or isolated species.

Lafferty et al. (1994) developed formulae to estimate the prevalence at which a trematode species recruits to a snail population (the null prevalence) by using the prevalence measured in nature. Their model

assumes that all trematode species have an equal probability of infection. Further, if there are dominant species in the assemblage, then over time, a proportion of subordinate infections will be lost via antagonistic interactions with dominant species; and this may lead to an accumulation of dominant species in large snails. The null prevalence of dominant species estimated by the model would be the same as that observed in the snail population. In contrast, the null prevalence of subordinate species would be higher than that observed in the snail population. Using their methods, I quantified the expected frequency of infection prior to antagonistic interactions from the observed frequency of infection in snails. These expected frequencies were then used to calculate the expected number of double infections in order to assess the importance of interspecific competition as a function of spatial and temporal heterogeneity in larval trematode recruitment.

Pioneering studies of interspecific antagonism between trematode species within first intermediate snail hosts (Lie 1966; Lim and Heyneman 1972) revealed that some species are considered subordinate as they were 1) unable to persist and were eventually eliminated in the first intermediate host when challenged with a second "dominant" species; 2) were unlikely to establish in snails previously infected with dominant species; and 3) had their cercarial production drastically reduced in double infection combinations with dominants prior to their complete elimination from the snail host (Lim and Heyneman 1972; DeCoursey and Vernberg 1974; Curtis and Hubbard 1992). Interspecific competition between larval

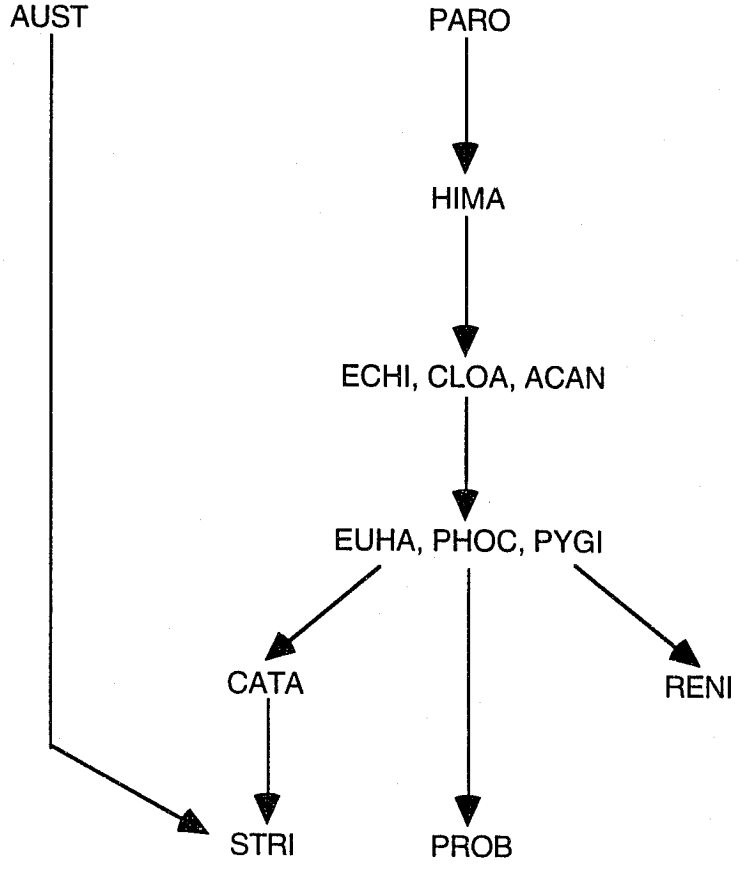
trematodes has been inferred statistically from the frequency of double infections (Heyneman and Umathevy 1968; Robson and Williams 1970; Kuris 1990; Sousa 1993) in systems where dominant species, such as those in the family Echinostomatidae or Philophthalmidae, are known to be relatively common members of an assemblage.

Kuris (1990) proposed a dominance hierarchy for larval trematodes that utilize Cerithidea californica as the first intermediate host (Fig. 1). Dominance relationships were inferred from mark-recapture studies showing that dominant species eliminated subordinate species in double infections, dominants became established in snails previously infected by subordinate species and thus had a higher prevalence in larger snails, and dominant rediae ingested larval stages of subordinate species in the snail (Kuris 1990; Sousa 1990). Other indirect evidence (morphology of larval stages, taxonomic affinities, sizes of larval stages, site specificity in the host, site displacement in the host) also supported these conclusions. Recent *in vitro* studies (Sousa 1993; Olsen unpublished data) directly demonstrated the ability of rediae of dominant species to attack and consume rediae, sporocyst and cercariae of subordinate species.

**Fig. 1**. Dominance hierarchy of larval trematodes in the snail, C. californica at Carpinteria Salt Marsh, California. Adapted from Kuris (1990) and Lafferty et al. (1994). Austrobilharzia is set apart from the group because it is considered by Kuris (1990) as a co-dominant with P. acanthus and H. rhigedana. Species are separated according to their trematode family classification.

PARO - *Parorchis acanthus* (Philophthalmidae)
HIMA - *Himasthla rhigedana* (Echinostomatidae)
ECHI - *Echinoparyphium* sp. (Echinostomatidae)

ACAN - *Acanthoparyphium spinulosum* (Echinostomatidae)
CLOA - *Cloacitrema michiganensis* (Philophthalmidae)
EUHA - *Euhaplorchis californiensis* (Heterophyidae)
PHOC - *Phocitremoides ovale* (Heterophyidae)
PYGI - *Pygidiopsoides spindalis* (Heterophyidae)
CATA - *Catatropis johnstoni* (Notocotylidae)
RENI - *Renicola buchanani* (Renicolidae)
STRI - Strigeid (Strigeidae)
PROB - *Probolocoryphe uca* (Microphallidae)
AUST - *Austrobilharzia* sp. (Schistosomatidae)



Kuris (1990, using Martin's 1955 data) and Lafferty et al. (1994) have shown that within the snail, C. californica, a large proportion of larval trematode infections are lost due to intra-molluscan interspecific competitive effects by dominant species. Lafferty et al. (1994) further suggested that subordinate species could maintain themselves in the host community through several adaptations. Subordinates could 1) preferentially infect small snails which are more likely to be uninfected (Kuris 1990; Sousa 1990); 2) develop or possess novel searching stages or strategies that increase the efficiency of finding the next host in the life cycle; 3) have a wide host distribution or be host generalists in second intermediate or definitive hosts (see Chapter 1), which would likely increase the opportunity for encountering a suitable host and advancing the life cycle. Finally, subordinates could have a longer active period or life span during free-living stages (i.e. miracidiae or cercariae) in which to locate the next host.

Here I use the methods developed by Lafferty et al. (1994) to test the null hypothesis that interspecific competition has no effect on the abundance of species in a larval trematode assemblage. This hypothesis predicts that observed frequencies of larval trematode species in a snail population will not change if competitors are introduced into the system, and that interspecific interactions do not control larval trematode populations in snails. I also determined the effect of both spatial and temporal heterogeneity in trematode recruitment on the expected

frequency of double infections in C. californica, and showed that dominant species were more prevalent in the largest snails.

## Materials and Methods

Between 200-400 C. californica were collected from each of six randomly selected sites at Carpinteria Salt Marsh (hereafter CSM, see map in Chapter 2). Snails were collected during the summer of 1992, and approximately 100 additional snails were collected from these sites in the winter of 1993-94. In contrast to Lafferty et al. (1994), whose snail sizes were restricted to 25-30mm, all snail sizes were collected and examined from each site. Snail length, snail sex and infection status (single or double infection and trematode species) were recorded. The expected prevalence ($e_i$) of each trematode species was calculated using the following equation developed by Lafferty et al. (1994):

$$e_i = (p_i - o_{id}) / (1 - p_d),$$

where ($p_i$) = the observed prevalence of each species, ($o_{id}$) = the observed prevalence of double infections between species (i) and all species dominant species (i), and ($p_d$) = the observed prevalence of all species dominant to species (i). The observed prevalences were weighted prior to calculation of ($p_i$) and ($e_i$) to account for variation in sample size. This was done using the following formula:

$$\text{Weighted Prevalence} = (F * X \text{ snails per sample})/N$$

In this calculation, F = frequency of each larval trematode species in the sample and N = total number of snails sampled. Prevalence represents the proportion of hosts infected with a particular trematode species, and is

expressed as a percentage (Margolis et al. 1982). Chi-square tests of the differences between ($p_i$) and ($e_i$) permitted an estimate of the proportion of infections lost due to interspecific interactions in snails.

The expected number of double infections was calculated as: $N(e_i)(e_j)$, for each possible double infection combination, where N = the number of snails. Each double infection matrix produced one value for the expected number of double infections: $\sum N(e_i)(e_j)$.

Effects of spatial heterogeneity in trematode recruitment were determined by comparing the sum of expected number of double infections from the 6 sites with the expected number of double infections from the pooled sample. Effects of temporal heterogeneity in recruitment were determined by comparing the sum of expected number of double infections from the 2 seasons with the expected number of double infections from the pooled sample. If $\sum N(e_i)(e_j)_{site}$ was significantly greater than $\sum N(e_i)(e_j)_{pooled}$ then spatial heterogeneity intensified potential interspecific interactions among larval trematodes in snails. In addition, if $\sum N(e_i)(e_j)_{season}$ was significantly greater than $\sum N(e_i)(e_j)_{pooled}$ then temporal heterogeneity also intensified potential interspecific interactions. If $\sum N(e_i)(e_j)_{season}$ or $N(e_i)(e_j)_{site}$ were significantly less than $\sum N(e_i)(e_j)_{pooled}$, then trematode species would be at least partially isolated in space and time (Lafferty et al. 1994). Further, according to Lafferty et al. (1994) if the summed values of $N(e_i)(e_j)$ are not significantly different from the pooled value of $N(e_i)(e_j)$, then spatial and

temporal heterogeneity in larval trematode recruitment does not influence community structure.

Snail size is also an important correlate of trematode prevalence and could explain single and double infection patterns in snails. In order to determine the importance of snail size, snails were grouped into three size classes and the prevalence of each trematode species was recorded. Mean snail size for each size class was calculated for infected and uninfected snails separately. Prevalence comparisons were made between the two largest size classes only because prevalence in the smallest snails was often zero, and thus violated statistical requirements of the heterogeneity Chi-square ($G_H$-test).

Data were analyzed using 95% confidence intervals to compare $N(e_i)(e_j)$ between different samples. A Mann-Whitney U-test was used to test for differences in snail size between infected and uninfected snails. Chi-square was used to test for differences between weighted observed ($p_i$) and expected ($e_i$) prevalences. A heterogeneity Chi-square ($G_H$-test) was used to test for differences in the overall observed prevalence between sites, seasons and size classes.

**Results**

Snail size was variable among sites. Snails ranging from 20-29.9mm were the most abundant size class in the salt marsh. Sites E and G had the largest snails and site H the smallest snails (Table 1). At five of the six sites, infected snails were larger than uninfected snails (Table 1, Mann-Whitney U-test, $p < .05$), and E. californiensis was the most

common larval trematode. However, E. californiensis infections were not restricted to a particular size class (Fig. 2). Prevalence increased significantly between size classes for dominant trematode species (P. acanthus, H. rhigedana, Echinoparyphium sp., A. spinulosum), the most common species (Euhaplorchis californiensis) and the proposed co-dominant, Austrobilharzia sp. ($G_H > 3.841$, df = 1, $p < .05$). In contrast, there was no significant decrease in prevalence of subordinate species (R. buchanani, P. uca, strigeids) in the largest snails ($G_H < 3.841$, df = 1, $p > .05$). Overall, prevalence of larval trematodes was significantly heterogeneous among sites (Table 1, $G_H = 348.6$, df = 5, $p < .05$), with Euhaplorchis californiensis being the most abundant trematode species in the snail population at CSM (Table 2).

**Table 1**. Summary statistics for Cerithidea californica at CSM. Data for summer and winter samples were pooled. Medians, and (means) are shown for purposes of the Mann-Whitney U-test for differences between size of infected and uninfected snails. * - $.01 < p < .05$.

| Site | N snails infected | N snails uninfected | Median snail size (mean) infected | Median snail size (mean) uninfected | Total Prevalence (%) |
|---|---|---|---|---|---|
| A | 280 | 225 | 21.9 (21.8) | 22.2 (21.7) | 55.5 |
| D | 181 | 320 | 25.9 (25.7) * | 22.4 (21.6) | 36.1 |
| E | 323 | 72 | 29.7 (29.9) * | 26.2 (24.1) | 81.8 |
| F | 128 | 188 | 28.0 (28.6) * | 21.6 (21.1) | 40.5 |
| G | 269 | 91 | 31.2 (30.9) * | 26.0 (24.3) | 74.7 |
| H | 95 | 204 | 25.2 (24.9) * | 19.7 (18.4) | 31.8 |

Fig. 2. Prevalence of larval trematodes in Cerithidea californica at CSM. Summer and winter samples were pooled. Size class 1 = 10-19.9mm, 2 = 20-29.9mm, and 3 = 30-39.9mm. All trematode species listed on the legend were included in the total for each size class, although they may not appear as separate species if their prevalence was low.

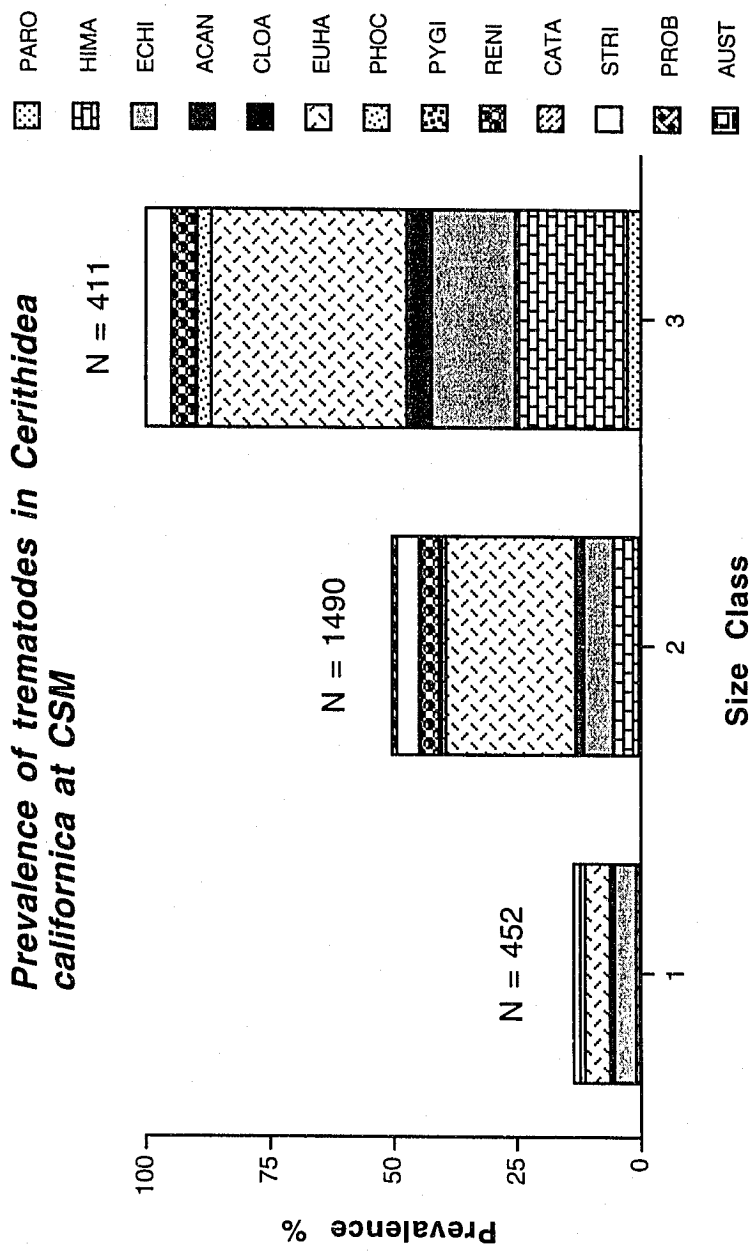


**Table 2.** Observed, weighted ($p_i$) and expected ($e_i$) prevalence of each trematode species at each site. Weighted and expected prevalences were calculated using methods developed by Lafferty et al. (1994). Data for summer and winter were combined for this table. The % difference shows the impact of interspecific interactions on each species. The % difference was calculated as follows: (1 - ($e_i/p_i$)) * 100% from unrounded values.

† - denotes that the weighted prevalence ($p_i$) of a particular trematode species was higher than expected ($e_i$) based on the Lafferty et al. (1994) model.

* - indicates ($p_i$) was significantly different than ($e_i$), Chi-square test, df = 1, $p < .05$.

| Species | Observed prevalence | $p_i$ | $e_i$ | Difference between $p_i$ and $e_i$ (%) |
|---|---|---|---|---|
| **Site A** | | | | |
| PARO | 0.79 | 1.21 | 1.21 | 0 |
| HIMA | 3.76 | 3.79 | 3.84 | -1.32 |
| ECHI | 3.37 | 3.54 | 3.73 | -5.4 |
| ACAN | 1.19 | 0.75 | 0.79 | -5.3 |
| CLOA | 0 | 0 | 0 | 0 |
| EUHA | 18.6 | 23.5 | 25.9 | -9.3 |
| PHOC | 0.2 | 0.48 | 0.53 | -9.3 |
| PYGI | 0 | 0 | 0 | 0 |
| RENI | 2.57 | 4.47 | 3.81 | 14.8 † |
| CATA | 0.59 | 0.73 | 1.06 | -31.3 |
| STRI | 3.76 | 4.51 | 7.1 | -63.5 |
| PROB | 0.59 | 1.44 | 2.27 | -36.5 |
| AUST | 0 | 0 | 0 | 0 |
| **Site D** | | | | |
| PARO | 0 | 0 | 0 | 0 |
| HIMA | 2 | 1.96 | 1.96 | 0 |
| ECHI | 1 | 0.98 | 1 | -1.96 |
| ACAN | 1.2 | 0.76 | 0.77 | -1.96 |
| CLOA | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| EUHA | 27.1 | 23.8 | 24.7 | -3.69 |
| PHOC | 0.2 | 0.47 | 0.49 | -3.69 |
| PYGI | 0.8 | 0.51 | 0.53 | -3.69 |
| RENI | 1.2 | 2.49 | 1.5 | 39.8 † |
| CATA | 0 | 0 | 0 | 0 |
| STRI | 3.79 | 2.41 | 0.36 | 85.1 †* |
| PROB | 0.2 | 0.13 | 0.17 | -27.4 |
| AUST | 0 | 0 | 0 | 0 |
| **Site E** | | | | |
| PARO | 2.03 | 2.43 | 2.43 | 0 |
| HIMA | 15.2 | 14.0 | 14.3 | -2.43 |
| ECHI | 7.09 | 9.77 | 11.7 | -16.4 |
| ACAN | 3.8 | 5.05 | 6.04 | -16.4 |
| CLOA | 0 | 0 | 0 | 0 |
| EUHA | 41.5 | 34.6 | 50.3 | -45.4 * |
| PHOC | 3.29 | 5.09 | 7.4 | -31.2 |
| PYGI | 0.51 | 0.33 | 0.48 | -31.2 |
| RENI | 7.59 | 9.01 | 13.7 | -65.5 |
| CATA | 0 | 0 | 0 | 0 |
| STRI | 11.6 | 11.3 | 14.6 | -77.4 |
| PROB | 1.77 | 1.53 | 2.04 | -25.2 |
| AUST | 0.51 | 0.33 | 0.81 | -58.9 |
| **Site F** | | | | |
| PARO | 1.9 | 1.39 | 1.39 | 0 |
| HIMA | 4.43 | 3.51 | 3.56 | -1.39 |
| ECHI | 25.9 | 20.9 | 21.9 | -4.9 |
| ACAN | 2.53 | 2.12 | 2.23 | -4.9 |
| CLOA | 0.32 | 0.23 | 0.24 | -4.9 |
| EUHA | 1.58 | 2.23 | 3.1 | -28.1 |
| PHOC | 0 | 0 | 0 | 0 |
| PYGI | 0.63 | 0.73 | 1.02 | -28.1 |
| RENI | 1.58 | 1.16 | 1.68 | -68.9 |
| CATA | 0.32 | 0.5 | 0.73 | -31.1 |
| STRI | 0 | 0 | 0 | 0 |
| PROB | 0 | 0 | 0 | 0 |
| AUST | 0 | 0 | 0 | 0 |
| **Site G** | | | | |
| PARO | 0.83 | 0.89 | 0.89 | 0 |
| HIMA | 18.1 | 15.3 | 15.4 | -0.89 |
| ECHI | 11.9 | 11.7 | 14 | -16.2 |

| | | | | |
|---|---|---|---|---|
| ACAN | 3.33 | 3.25 | 3.87 | -16.2 |
| CLOA | 0 | 0 | 0 | 0 |
| EUHA | 34.2 | 28.9 | 42 | -45.3 * |
| PHOC | 1.39 | 2.55 | 3.7 | -31.1 |
| PYGI | 0 | 0 | 0 | 0 |
| RENI | 3.33 | 3.57 | 4.26 | -16.2 |
| CATA | 0 | 0 | 0 | 0 |
| STRI | 2.78 | 2.23 | 0.53 | 76.2 †* |
| PROB | 1.94 | 2.29 | 2.7 | -14.9 |
| AUST | 0.83 | 0.57 | 1.65 | -65.4 |
| **Site H** | | | | |
| PARO | 0 | 0 | 0 | 0 |
| HIMA | 1.34 | 1.5 | 1.5 | 0 |
| ECHI | 1.34 | 1.25 | 1.27 | -1.5 |
| ACAN | 0.67 | 1 | 1.02 | -1.5 |
| CLOA | 0.33 | 0.5 | 0.51 | -1.5 |
| EUHA | 18.4 | 18 | 18.6 | -2.9 |
| PHOC | 1.34 | 1.5 | 1.57 | -4.0 |
| PYGI | 0 | 0 | 0 | 0 |
| RENI | 3.01 | 3.51 | 3.27 | 6.8 † |
| CATA | 1.67 | 2.5 | 3.23 | -22.6 |
| STRI | 2.01 | 1.76 | 1.05 | 40.3 † |
| PROB | 0.33 | 0.25 | 0.35 | -27.6 |
| AUST | 0 | 0 | 0 | 0 |

Methods developed by Lafferty et al. (1994) to estimate the null prevalence of larval trematodes revealed that ($p_i$) was less than ($e_i$) for most species at most sites (Table 2). There were significant differences between ($p_i$) and ($e_i$) only for E. californiensis and strigeid infections in snails. The weighted prevalence ($p_i$) of E. californiensis was significantly less than ($e_i$) only at sites E and G (Table 2, Chi-square = 4.9 and 4.1 respectively, df = 1, $p < .05$). In contrast, some species had a weighted

prevalence which was greater than the expected prevalence ($p_i > e_i$). Weighted prevalence ($p_i$) was significantly greater than ($e_i$) at sites D and G for strigeid infections in snails (Table 2, Chi-square = 11.6 and 5.5 respectively, df = 1, $p < .025$). Renicola buchanani also had ($p_i$) greater than ($e_i$) at sites A, D and H, but differences were not significant (Table 2, Chi-square = .114, .653 and .018 respectively, df = 1, $p > .05$).

Double infections were common in the samples. The most abundant pairs were EUHA/STRI and EUHA/RENI (Table 3, see Fig. 1 for species codes). Site F had no double infections in either the summer or winter samples. In contrast, Site G had the greatest diversity of double infection combinations. Site E had more double infections than any other site, the majority were EUHA/STRI and EUHA/RENI.

**Table 3.** Frequency of double infections. These values are weighted according to methods developed by Lafferty et al. (1994). Summer and winter data were pooled in this table. Totals refer to the sum of all weighted double infections at each site. $\Sigma N p_i p_j = 106.8$.

| Species | Site | | | | | |
|---|---|---|---|---|---|---|
| **Combination** | **A** | **D** | **E** | **F** | **G** | **H** |
| Euha/Reni | 7.6 | 5.6 | 13.7 | 0 | 7.1 | 3.9 |
| Euha/Stri | 0 | 8.5 | 30.4 | 0 | 8.1 | 3.9 |
| Euha/Prob | 0 | 0 | 0.66 | 0 | 0.76 | 0 |
| Euha/Aust | 0 | 0 | 0 | 0 | 0.76 | 0 |
| Echi/Euha | 0 | 0 | 0 | 0 | 0 | 0.99 |
| Echi/Prob | 0 | 0 | 2.8 | 0 | 0.76 | 0 |
| Acan/Prob | 0 | 0 | 0 | 0 | 0.76 | 0 |
| Phoc/Prob | 0 | 0 | 0 | 0 | 2.0 | 0 |
| Reni/Hima | 0 | 0 | 0 | 0 | 0.76 | 0 |
| Reni/Phoc | 0 | 0 | 6.3 | 0 | 0 | 0 |
| Reni/Prob | 0 | 0 | 0 | 0 | 0.76 | 0 |
| Aust/Hima | 0 | 0 | 0.66 | 0 | 0 | 0 |
| Total | 7.6 | 14.1 | 54.5 | 0 | 21.8 | 8.8 |

Analyses of the pooled data also showed that ($p_i$) was less than ($e_i$) for most larval trematodes. Similar to data for sites A, D, G and H, R. buchanani and strigeids were more abundant than expected ($p_i > e_i$) (Table 4). However, there were no significant differences between ($p_i$) and ($e_i$) for any species (Chi-square < 3.841 for each species, df = 1, $p > .05$).

**Table 4.** Observed, weighted ($p_i$) and expected ($e_i$) prevalence of larval trematodes at CSM. Data were pooled over the six sites and two seasons. Observed prevalence ($p_i$) of RENI and STRI (denoted by †) was higher than the expected prevalence ($e_i$), because of the high number of double infections observed for these species. The expected number of double infections generated by this data set was, $Ne_ie_j$ pooled = 277. Chi-square tests showed that differences between ($p_i$) and ($e_i$) were not statistically significant for any trematode species.

† - denotes that the observed prevalence ($p_i$) of a particular trematode species was higher than expected ($e_i$) based on the Lafferty et al. (1994) model.

| Species | pi | ei | % change |
|---|---|---|---|
| PARO | 0.99 | 0.99 | 0 |
| HIMA | 6.67 | 6.74 | -0.99 |
| ECHI | 8.02 | 8.69 | -7.66 |
| ACAN | 2.15 | 2.33 | -7.66 |
| CLOA | 0.12 | 0.13 | -7.66 |
| EUHA | 21.8 | 26.6 | -17.8 |
| PHOC | 1.68 | 2.05 | -17.9 |
| PYGI | 0.26 | 0.32 | -17.9 |
| RENI | 4.03 | 3.67 | 8.9 † |
| CATA | 0.62 | 1.03 | -39.8 |
| STRI | 3.7 | 2.8 | 24.3 † |
| PROB | 0.94 | 1.01 | -7.11 |
| AUST | 0.15 | 0.17 | -11.8 |
| Total | 51.1 | 56.5 | |

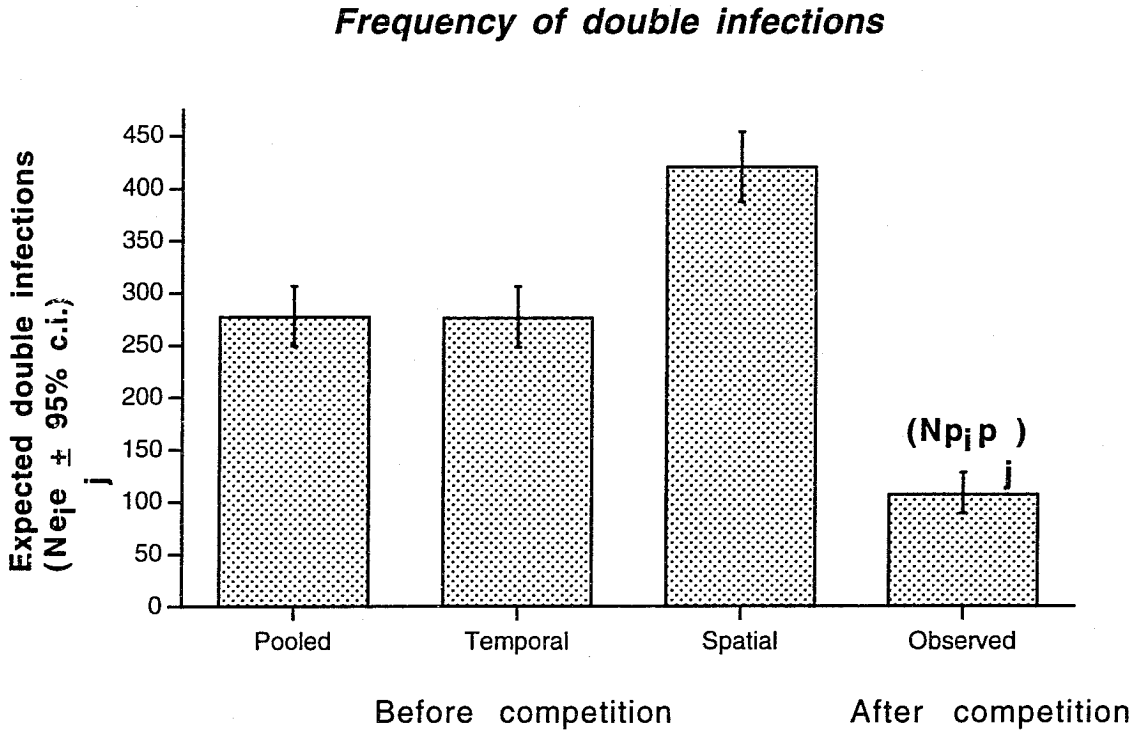
Comparisons of $N(e_i)(e_j)$ and $N(p_i)(p_j)$ revealed that interspecific interactions occurred in snails. The observed number of double infections $N(p_i)(p_j)$ was significantly less than expected $N(e_i)(e_j)_{\text{pooled}}$ (Fig. 3). Spatial heterogeneity in recruitment of larval trematodes significantly intensified the number of interactions $N(e_i)(e_j)_{\text{pooled}} < N(e_i)(e_j)_{\text{spatial}}$. In contrast, temporal heterogeneity in larval trematode recruitment neither isolated or intensified interactions between larval trematodes $N(e_i)(e_j)_{\text{pooled}} = N(e_i)(e_j)_{\text{season}}$.

## Discussion

Spatial and temporal heterogeneity in larval trematode recruitment is largely driven by seasonal fluctuations in the abundance and diversity of definitive host birds which deliver infective eggs and miracidiae to snails (Sousa 1990; N. Smith personal communication). A spatially heterogeneous distribution of larval trematodes in snails may be further affected by post-recruitment competitive interactions between larval trematodes in snails, which modifies the initial prevalence of trematodes by eliminating a relatively high proportion of established infections of subordinate trematodes from the snail population.

The impact of heterogeneous recruitment and subsequent competitive interactions was investigated using methods developed by Lafferty et al. (1994) to test the hypothesis that spatial and temporal heterogeneity in larval trematode recruitment isolates species and thus

Fig. 3. Frequency of double infections. $Ne_ie_j$ was calculated from double infection matrices. $Ne_ie_j$ pooled represents the number of double infections expected if recruitment of larval trematodes were homogeneous in space and time. $Ne_ie_j$ temporal represents the number of double infections expected if recruitment of larval trematodes were heterogeneous in time. $Ne_ie_j$ spatial represents the number of double infections expected if recruitment of larval trematodes were heterogeneous in space. $Np_ip_j$ is the observed number of double infections in the pooled sample and was calculated using weighted frequencies (see Table 3).



explains the low frequency of double infections in snails. These methods also permitted an estimate of the number of larval trematodes adversely affected by competitive interactions in snails that were sampled at CSM. I expanded on their model by including all snail sizes and a temporal component (2 seasons) in the analysis.

***General Findings***

Most larval trematode species were less abundant than predicted by a random assortment model. Further, spatial heterogeneity in larval trematode recruitment intensified interspecific interactions in snails while temporal heterogeneity had no significant effect on the intensity of interspecific interactions. The significant increase in prevalence of dominant species in the largest size class of snails suggests that these species may be eliminating subordinate species over time. Further, as time passes and snails grow, there are more opportunities for dominants to replace subordinates.

Euhaplorchis californiensis was the most abundant larval trematode in the C. californica population at CSM. Double infections were common and two combinations (E. californiensis/strigeid and E. californiensis/R. buchanani) were proportionally more abundant in snails at CSM in this study (Table 5) than reported in previous studies from Upper Newport Bay (Martin 1955), Goleta Slough (Yoshino 1975), Bolinas Lagoon (Sousa 1993), and CSM (Lafferty et al. 1994). Further, in all studies, except Sousa (1993), E. californiensis had the highest prevalence in C. californica. At two sites in Bolinas Lagoon, Echinostoma sp. and

Parorchis acanthus were more abundant than most other species at both sites (Sousa 1993).

**Table 5.** Prevalence of double infections between E. californiensis, strigeids and R. buchanani from 5 different studies of C. californica.

| | | Prevalence (%) | |
|---|---|---|---|
| **Study** | **N snails** | **EUHA/STRI** | **EUHA/RENI** |
| Martin 1955 | 12,995 | 0.7 | 0.05 |
| Yoshino 1975 | 2,910 | 2.1 | 0.83 |
| Sousa 1993 | 24,252 | 0 | 0.008 |
| Lafferty et al. 1994 | 849 | 0.35 | 0.12 |
| **This study** | **2,376** | **2.7** | **1.3** |

***Dominance Associations and Double Infections***

A pattern of frequent double infections could be explained by a variety of mechanisms which may be exogenous or endogenous with respect to the snail host. Such mechanisms may include delayed elimination of subordinate species by dominant species, the sequence of infection, equal competitive ability among species in an assemblage, site specificity or preference within the host snail, and the presence of some species that are obligate secondary invaders.

Empirical evidence from studies of larval echinostomes and schistosomes revealed that elimination of schistosome sporocysts by echinostome rediae can be delayed by almost 2 months (Lie 1967, 1969; Lie et al. 1973; Lim and Heyneman 1972) if the dominant rediae establish an infection after subordinate sporocysts have matured (e.g. produced

cercariae) in the snail host. Furthermore, this appears to be a fairly widespread phenomenon and has also been reported for species pairs that involved only echinostomes (Lie et al. 1968). Because competitive exclusion by larval trematodes in snails is not instantaneous, high numbers of double infections could indicate a recent recruitment event of the dominant species. In C. californica, mature infections of R. buchanani, strigeids and E. californiensis were identified by their cercariae (i.e. infections were mature in snails). Thus, abundant double infections reported in this study suggest that I measured a recruitment pulse of E. californiensis even though I was unable to detect significant temporal effects using the model. A weak recruitment pulse of larval trematodes would likely be damped by the weighting procedures employed in the model; however, these temporal events may have been detected by the model if samples were collected more frequently or if the samples were taken through subsequent years.

The sequence of infection by subordinate species is a primary determinant of the potential for future cercarial production. However, competitive exclusion of subordinate sporocysts may be further slowed by the ability of sporocysts to resist or recover from an initial attack. Resistance by sac-like sporocysts (no mouth) to attack by mouthed rediae may take two forms. Firstly, sporocysts of strigeid trematodes and of R. buchanani are much larger than mature rediae of E. californiensis. Therefore, size may provide a refuge from predation. Lie (1969c) reported that immature rediae caused the majority of attacks on subordinate

sporocysts, and that large numbers of these rediae were required to effectively reduce the number of subordinates in a snail (Lie 1966, 1967). Cercarial production continued even in damaged sporocysts and sporocyst size appeared to provide a temporary refuge from attack because sporocysts were not consumed whole. In addition, Lie (1967) documented that the tegument of schistosome sporocysts was somewhat resistant to attack by rediae and that wounds inflicted by rediae healed. However, neither large size nor tissue repair provided absolute protection and schistosome sporocysts were eventually eliminated from snails by echinostome rediae.

Competitive dominance of larval trematodes has been shown experimentally for medically important schistosomes and echinostomes in studies of potential bio-control agents (Lim and Heyneman 1972). However, dominance relationships for most other larval trematodes are largely inferential. Thus, it is possible that in double infections, larval strigeid and R. buchanani sporocysts may be competitively equal (or nearly so) to E. californiensis rediae rather than subordinate as proposed by Kuris (1990), Sousa (1990) and Lafferty et al. (1994). Kuris (1990) suggested that a primary determinant of dominance was the presence or absence of a mouth. Lacking mouths, strigeid and R. buchanani sporocysts are likely to be susceptible to predation by E. californiensis rediae, therefore subordinate according to Kuris' (1990) dominance hierarchy.

Site specificity of larval trematodes within snails has received little attention in recent studies of larval trematode communities. A preference for particular sites or organs in the host snail may allow species to co-exist in snails because different organs may provide refugia from predation or access to additional host resources. Most larval trematode species occupy the gonad or ovotestis of the snail host and deplete these resources during the asexual build-up of rediae or sporocysts. When two (or more) trematode species occupy the gonad, competition for the host resource is direct and one species typically excludes the other. However, if species prefer (or require) different organs in the host, competition for gonadal resources would likely be indirect. Renicola buchanani sporocysts are found in the mantle cavity of C. californica while E. californiensis rediae reside in the gonad of the snail, which suggests that sporocysts may enjoy a refuge from attack by E. californiensis rediae. Whether R. buchanani sporocysts compete for gonadal resources is unknown and until further studies are conducted on this species, it is best to assume that it is probably subordinate to E. californiensis rediae based on the indirect evidence provided by Kuris (1990).

Finally, some species of larval trematodes require previously infected snails in order to establish an initial infection. This has been reported for Austrobilharzia terrigalensis, whose sporocysts were found almost exclusively in double infections with other trematodes, and doubly infected snails did not lose the A. terrigalensis infection over time (Walker 1979; Appleton 1983). Empirical evidence for other genera or species of

secondary invaders is scant, but is considered as an alternative explanation for the relatively high frequency of double infections involving R. buchanani and strigeid sporocysts with E. californiensis (Table 5). Although strigeid and R. buchanani sporocysts were abundant in C. californica, single infections were common, unlike Austrobilharzia (Walker 1979; Appleton 1983). Thus, these trematodes are probably not obligate secondary invaders, and it is likely that the double infections reported here represent a slowly resolving recruitment pulse of E. californiensis.

***Recruitment Heterogeneity and Species Comparisons***

Several data sets for larval trematode assemblages in Cerithidea spp. permit assessment of the impact of environmental heterogeneity on larval trematode recruitment (Bush et al. 1993; Sousa 1993; Lafferty et al. 1994), and whether interspecific competition between larval trematodes in snails significantly reduces the abundance of subordinate trematode species in snail populations (Martin 1955; Epstein 1972; Wardle 1974; Yoshino 1975; McNeff 1978; Bush et al. 1993; Sousa 1993; Lafferty et al. 1994). These issues were investigated by Kuris and Lafferty (1994) using aforementioned data sets for 9 different snail genera including three Cerithidea species: C. californica, C. pliculosa and C. scalariformis. They showed that at Bolinas Lagoon, CSM and 3 sites in Florida (Ramrod Key, Cedar Key, Anclote River), spatial heterogeneity intensified the likelihood of interspecific interactions by focusing recruitment of several trematode species at relatively few sites (data from Bush et al. 1993; Sousa 1993; Lafferty et al. 1994). This was also confirmed in the present data set (Fig.

3). In addition, although Kuris and Lafferty's (1994) re-analysis of Bolinas Lagoon data (Sousa 1993) showed that the significant temporal heterogeneity in recruitment intensified potential interaction among trematodes, I could not detect this effect at CSM among seasons for one year (Fig. 1). However, this lack of significant temporal influence may have been due to a lack of statistical power with samples at only 2 periods compared to the 7 available for Bolinas Lagoon (Sousa 1993). Further, seasonal or annual differences in the abundance and/or diversity of definitive host birds could also account for between year differences or intensify potential interactions at certain times of the year. However, neither this nor Sousa's study correlated bird abundance or diversity with changes in infection patterns in snails.

Environmental heterogeneity generally intensified potential interspecific interactions between larval trematodes in Cerithidea species. Thus, it is not surprising that these interactions had a significant negative impact on the frequency of most pairs of trematodes in double infections in C. californica at CSM (also true for Lafferty et al. 1994). This pattern appears to be widespread and was also detected in C. californica at Upper Newport Bay, Goleta Slough, Bolinas Lagoon, and in C. pliculosa from Dauphin Island, Alabama and Galveston Bay, Texas (Kuris and Lafferty 1994 Appendix).

The importance of interspecific competition as a determinant of the frequency of double infections in C. scalariformis from Florida (Bush et al. 1993), differed notably from that in other Cerithidea species, since this

frequency did not differ significantly from that expected by a random assortment model (Kuris and Lafferty 1994). In contrast, interspecific competition was a significant structuring factor in all assemblages of larval trematode in other Cerithidea species. These differences may be due to 1) the life history of C. scalariformis, 2) the recruitment sequence of trematodes to C. scalariformis populations, or 3) the overall prevalence of dominant trematode species in the Florida system.

Cerithidea scalariformis is short-lived (1-2 year life-span, Houbrick 1984) compared to C. californica (8-10 year life-span, Sousa 1983). Immature snails are abundant in the late fall and mature by the following summer when second-year adults die off (Houbrick 1984). It is not known whether trematodes recruit to immature C. scalariformis. However, if mature snails are required, then the short life span of mature snails probably decreases the overall prevalence of larval trematodes in C. scalariformis. Furthermore, the relatively short exposure window (summer and fall) would likely reduce the frequency of replacement by dominant trematode species and limit accumulation of trematodes in the largest snails over time (N. Smith personal communication). This would also intensify potential interactions in snails because the recruitment period is short and intense due to seasonally high bird abundance (N. Smith personal communication).

Probolocoryphe sp. (a subordinate species per Kuris 1990) appears in the C. scalariformis snail population in the summer prior to other larval trematode species. Its infections are mature by the time other trematode

species recruit to these snail populations (N. Smith, personal communication). This species also had the highest prevalence at Cedar Key, and Cedar Key was the only site where double and triple infections existed. Compared to other sites in Florida, dominant trematode species (Euhaplorchis sp. and an un-identified philophthalmid) were rare in the snail population at Cedar Key (Bush et al. 1993). Thus, the low prevalence of dominants in the system may have permitted subordinates like Probolocoryphe, to become established and persist in snails.

Some trematode species may be more likely than others to establish double infections in C. scalariformis. Bush et al. (1993) reported that the majority of observed combinations were of Probolocoryphe sp. and Catatropis sp. (second highest prevalence at Cedar Key). Probolocoryphe uca occupies the gonad while Catatropis johnstoni is found under the mantle near the head of C. californica. These organs are also occupied by congeners in C. scalariformis (N. Smith, personal communication). Different site usage suggests that double infections between these species might be more common than expected, like E. californiensis and R. buchanani (in C. californica) which also exhibited site differences. Thus, the short life span of C. scalariformis, the sequence of larval trematode recruitment, low prevalence of dominant trematode species and differential site specificity within snails increase the likelihood that double infections will be observed.

***Conclusions and Summary***

Larval trematode assemblages in Cerithidea californica were significantly influenced by interspecific competitive interactions which caused most species to be less abundant than expected. These interactions were also intensified by spatially heterogeneous recruitment, contrary to other studies which predicted that heterogeneous recruitment of larval trematodes would isolate trematode species in space and time. Competitive interactions among larval trematodes, as demonstrated by Kuris and Lafferty's (1994) meta-analysis, were evident in nearly all Cerithidea species except C. scalariformis which possesses a notably different life history than other well known Cerithidea species. Biological characteristics particular to the host snail (e.g. life history, activity patterns) or the trematode species (e.g. site in host) have often been overlooked. It is likely that these attributes are important because they probably explain the distribution of larval trematodes in snails, overall prevalence and the frequency of species co-occurrence.

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**Appendix 1a and 1b.** Total number of cercariae collected from water samples on each sample day at each site during the summer (a) of 1993 and winter of 1994 (b). These data were used to determine difference between sites, for each species, using Analysis of variance (ANOVA, Table 3).

**Summer 1993** **Appendix 1a**

| | Sample day | | | | | | |
|---|---|---|---|---|---|---|---|
| **EUHA** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| A | 13 | 51 | 5 | 12 | 28 | 65 | 126 |
| D | 10 | 166 | 26 | 14 | 76 | 162 | 131 |
| E | 0 | 181 | 30 | 11 | 18 | 73 | 90 |
| F | 1 | 35 | 2 | 1 | 1 | 59 | 19 |
| G | 5 | 556 | 13 | 27 | 20 | 227 | 68 |
| H | 0 | 20 | 1 | 17 | 13 | 3 | 23 |
| **STRI** | | | | | | | |
| A | 8 | 126 | 7 | 21 | 28 | 41 | 77 |
| D | 26 | 168 | 26 | 8 | 26 | 58 | 88 |
| E | 0 | 152 | 38 | 30 | 25 | 43 | 33 |
| F | 0 | 6 | 3 | 1 | 0 | 1 | 5 |
| G | 4 | 64 | 7 | 14 | 12 | 22 | 9 |
| H | 0 | 13 | 0 | 3 | 2 | 1 | 1 |
| **ECHI** | | | | | | | |
| A | 11 | 0 | 2 | 0 | 0 | 1 | 1 |
| D | 1 | 0 | 0 | 0 | 0 | 2 | 0 |
| E | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| F | 32 | 250 | 108 | 26 | 9 | 18 | 55 |
| G | 13 | 31 | 4 | 1 | 0 | 42 | 0 |
| H | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| **ACAN** | | | | | | | |
| A | 0 | 1 | 2 | 0 | 2 | 0 | 1 |
| D | 7 | 4 | 2 | 1 | 3 | 1 | 4 |
| E | 0 | 2 | 3 | 0 | 1 | 0 | 2 |
| F | 54 | 71 | 17 | 1 | 1 | 0 | 1 |
| G | 10 | 15 | 4 | 0 | 0 | 8 | 9 |
| H | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **RENI** | | | | | | | |
| A | 1 | 4 | 15 | 2 | 0 | 3 | 14 |
| D | 2 | 11 | 14 | 2 | 1 | 6 | 8 |
| E | 0 | 2 | 1 | 0 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| F | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 7 | 1 | 1 | 0 | 0 | 13 | 5 |
| H | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **HIMA** | | | | | | | |
| A | 0 | 0 | 3 | 0 | 0 | 0 | 1 |
| D | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| E | 1 | 2 | 0 | 0 | 1 | 0 | 0 |
| F | 0 | 2 | 1 | 0 | 1 | 0 | 1 |
| G | 8 | 16 | 0 | 1 | 0 | 3 | 0 |
| H | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **PROB** | | | | | | | |
| A | 0 | 2 | 7 | 2 | 2 | 2 | 0 |
| D | 2 | 5 | 3 | 0 | 0 | 0 | 5 |
| E | 0 | 6 | 3 | 0 | 6 | 0 | 2 |
| F | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| G | 2 | 1 | 2 | 3 | 0 | 2 | 4 |
| H | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **AUST** | | | | | | | |
| A | 0 | 4 | 0 | 2 | 0 | 5 | 2 |
| D | 0 | 0 | 1 | 2 | 4 | 2 | 5 |
| E | 0 | 3 | 2 | 1 | 1 | 0 | 10 |
| F | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | 0 | 0 | 1 | 3 | 1 | 4 |
| H | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

**Winter 1994** **Appendix 1b**

| | Sample day | | | | |
|---|---|---|---|---|---|
| **EUHA** | 1 | 2 | 3 | 4 | 5 |
| A | 2 | 0 | 0 | 0 | 21 |
| D | 0 | 0 | 0 | 0 | 63 |
| E | 0 | 0 | 0 | 0 | 20 |
| F | 0 | 0 | 0 | 0 | 5 |
| G | 0 | 0 | 0 | 0 | 2 |
| H | 0 | 0 | 1 | 0 | 0 |
| **STRI** | | | | | |
| A | 0 | 0 | 0 | 0 | 3 |
| D | 0 | 0 | 0 | 0 | 2 |
| E | 0 | 0 | 0 | 0 | 1 |
| F | 0 | 0 | 0 | 0 | 0 |
| G | 0 | 0 | 0 | 0 | 2 |
| H | 0 | 0 | 0 | 0 | 0 |
| **ECHI** | | | | | |
| A | 0 | 1 | 0 | 0 | 0 |
| D | 0 | 0 | 0 | 0 | 0 |
| E | 3 | 0 | 0 | 0 | 0 |
| F | 2 | 0 | 0 | 0 | 1 |
| G | 16 | 2 | 0 | 0 | 0 |
| H | 0 | 0 | 0 | 0 | 0 |
| **ACAN** | | | | | |
| A | 0 | 1 | 0 | 0 | 3 |
| D | 0 | 0 | 0 | 0 | 1 |
| E | 2 | 2 | 0 | 0 | 0 |
| F | 2 | 0 | 0 | 0 | 0 |
| G | 8 | 7 | 0 | 0 | 0 |
| H | 0 | 0 | 0 | 0 | 0 |
| **RENI** | | | | | |
| A | 0 | 0 | 0 | 0 | 0 |
| D | 0 | 0 | 0 | 0 | 0 |
| E | 0 | 0 | 0 | 0 | 0 |
| F | 0 | 3 | 0 | 0 | 0 |
| G | 0 | 3 | 0 | 0 | 0 |
| H | 0 | 0 | 0 | 0 | 0 |
| **PARO** | | | | | |
| A | 0 | 0 | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| D | 0 | 0 | 0 | 0 | 0 |
| E | 1 | 0 | 0 | 0 | 1 |
| F | 1 | 0 | 0 | 0 | 0 |
| G | 1 | 0 | 0 | 0 | 0 |
| H | 1 | 0 | 0 | 0 | 0 |
| **PROB** | | | | | |
| A | 0 | 0 | 0 | 0 | 0 |
| D | 0 | 0 | 0 | 0 | 0 |
| E | 0 | 0 | 0 | 0 | 0 |
| F | 0 | 0 | 0 | 0 | 0 |
| G | 0 | 0 | 0 | 0 | 0 |
| H | 0 | 0 | 0 | 0 | 4 |