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Ecology of a Host-Parasite System in the Rocky Intertidal Zone

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Ecology of a Host-Parasite System in the Rocky Intertidal Zone

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by

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Thank you to Armand Kuris and Bob Warner for taking a chance on me.

Always stay gracious best revenge is your paper

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Fong, CR and Fong P. Press versus pulse nutrient subsidy regimes and community context impact macroalgal performance: 2 case studies. *In review* at *Oikos*

Fong CR, Posner H, and Kuris AM. Fear and feeding: risk of infection predicts parasite avoidance behavior. *In review* at *Internation Journal of Parasitology*

Fong CR, Sura SA, Fong P. Macroalgal predictability, quantity, and species assemblage affect estimates of herbivory rate and herbivore selectivity on coral reefs. *In review* at *Marine Ecological Progress Series*

ACCEPTED MANUSCRIPTS

Bergman JL, Dang BN, Tabatabaee M, McGowan MM, **Fong CR**, Bittick SJ, Fong P. Nutrients induce and herbivores maintain thallus toughness, a structural antiherbivory defense in *Turbinaria ornate*. *In press* at *Marine Ecological Progress Series*

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Fong P, Fong JJ, and **Fong CR**. 2004. Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. *Aquatic Botany* 78:83-95.

SYNERGISTIC ACTIVITIES

RMP Research Mentorship Program (3 years, 11 students). The RMP program brings high school students from across the country to UCSB for an immersive 6-week research program. 40% of students are funded by scholarships collected from private and corporate donations. Through this program, I have mentored 11 students and just submitted a revision to Proceedings of the Royal Society B with a former mentee.

ScienceLine (3 years). ScienceLine is an online platform for disseminating information to K-12 students. This is an NSF funded program that focuses on local schools, though through popularity has become worldwide. Students submit science questions to a panel of researchers, and answers are returned weekly. For the past 3 years, I have served as a designated responder for this program and answered dozens of questions asked directly by young students.

Center for Ocean Sciences Education Excellence (COSEE) (1 year). This NSF funded program linked researchers to high school teachers. The goal of this program was to incorporate an active research program into a high school curriculum. I provided an overview of my research and answered direct questions about both my research and ecology in general from high school teachers.

Condor-Tech (1 year, 4 students). Condor-Tech brings STEM students from Oxnard Community College to UCSB for an intensive summer internship program. This program provides the first hands-on research experience to these students, and provides students with information on research-based careers. This program, Project ASCENSIÓN, is part of the Department of Education Title V STEM grant

Cooke's Bridge (1 year, 3 students). This program brings STEM students from community colleges across California to UCSB for an intensive summer internship program, similar to the Condor-Tech program. The Jack Kent Cooke Foundation privately funds this program.

RISE Research Internships in Science and Engineering (1 year, 1 student). Through this 10-week program, I mentored a young woman from Jackson State University. The student conducted an independent research project and at the end, wrote a paper, gave a talk, and participated in a poster session. This NSF is funded through the Materials Research Science and Education Center (MRSEC) and aims to increase access to research for students that attend schools without a rigorous and active research program.

CAMP California Alliance for Minority Participation (1 year, 1 student). Through this 20-week program, I mentored a student through an independent research project where at the end, he wrote a paper, gave a talk, and participated in 2 poster sessions. This program is supported through the NSF Louis Stokes for Minority Participation (LSAMP) program and is part of the CAMP Statewide alliance.

SIMS Summer Institute in Math and Science (1 year, 4 students). I mentored incoming freshman at UCSB through an immersive research program. The purpose of this program is to introduce underrepresented groups to research and research opportunities early in their college career and expose students to research based careers.

TEACHING EXPERIENCE

Fall 2011 LS1 at UCLA, Introduction to Ecology, Evolution and Biodiversity
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ABSTRACT

Ecology of a Host-Parasite System in the Rocky Intertidal Zone

by

Caitlin Ryan Fong

The field of ecology seeks to understand and predict the patterns and processes shaping the distribution and abundance of populations, the structure and organization of communities, and the dynamics and energetics of ecosystems structure. Parasitism is understudied as an interaction driving these patterns and processes in spite of research demonstrating that parasitism is the most common type of consumer interaction and numerous case studies clearly documenting that parasitism impacts populations, communities, and has a role in ecosystem energetics.

Ecologists have used the rocky intertidal zone as a model for understanding basic ecological processes and theories; thus, elucidating the role of infectious processes in this system will provide researchers with novel insights that may translate into broad structuring principles in the field. However, there is very little research on infectious processes in the rocky intertidal zone. Thus, in spite of decades of ecological research in this system, parasitic interactions remain unexplored and research may yet reveal a strong structuring role of infections in this system.

Barnacles are infected by a protandrous, semelparous, castrator that affects only female function; this parasite is largely unexplored but potentially strongly impacts barnacle

ecology. In this thesis, I addressed basic questions concerning the ecology of this host-parasite system.

First, I explored behavioral defenses of the barnacle host, *Chthamalus fissus*, versus its isopod parasite, *Hemioniscus balani*. I found when infectious stages were present, barnacles decreased filtration rate by ~ 50% when they were at high risk of infection. Barnacles not at risk did not respond to the presence of an infectious stage. The difference in response based on barnacle condition implies a high cost of reduced feeding as a parasite avoidance behavior.

Second, I explored how barnacle sex allocation varied with size, and how this differential allocation affected patterns of parasitism. The barnacle host is hermaphroditic, where gender is not fixed and individuals allocate variable energy to male or female functions. Since the parasite requires ovarian fluid, only barnacles with female reproductive function should be appropriate hosts. We documented a unimodal relationship between barnacle size and female reproductive function. This female function-size relationship was mirrored by patterns of parasitism. Further, we found within the subset of suitable hosts, parasitism increased with size.

Third, I explored spatial patterns of parasitism in the field as mediated by parasite predators. I investigated whether a sea anemone, protects an associated barnacles from parasitism. Barnacles associated with anemones had reduced parasitism and higher reproductive productivity than did barnacles remote from sea anemones. In the laboratory, anemones readily consumed the transmission stage cryptoniscus larvae of the parasite. Hence, anemone consumption of parasite transmission stages may provide a mechanism by which community context regulates, and in this case reduces, parasitism at a local scale.

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CHAPTER 1: Fear and feeding: risk of infection predicts parasite avoidance behavior

ABSTRACT

Animals have a nested sequence of defenses to guard against parasitism. Behavior is often the first line of defense. Parasitism and parasite avoidance behaviors are costly; thus, the strength of parasite avoidance behavior should reflect the uncertain risk of infection and the likely cost of such an infection. We experimentally evaluate the parasite avoidance behavior of a barnacle, *Chthamalus fissus*, versus its isopod parasite, *Hemioniscus balani*. *H. balani* is an ephemeral semelparous parasitic castrator, a single parasite prevents its host from producing eggs. Thus, the cost of infection is high. We conducted experimental observations in a laboratory setting of *C. fissus* to quantify the effects of infection status and reproduction status on filtration rates in the presence of an infectious stage of *H. balani*. When infectious stages were present, barnacles decreased filtration rate by ~ 50% when they were uninfected and were non-reproductive, conditions associated with high risk of infection. Infected and reproductive barnacles did not respond to the presence of an infectious stage. The difference in response based on barnacle condition implies a high cost of reduced feeding as a parasite avoidance behavior.

INTRODUCTION

To minimize fitness reduction due to parasitism organisms have a sequential defensive strategy to reduce encounter with infectious stages, and if this does not succeed mitigate the compatibility of the parasite regarding immune defenses and its nutritional costs (a paradigm developed by Combes 2001). Encounter with a parasite can be eliminated or reduced if the host does not live in the same place as the parasite or the host avoids contact with the parasite due to behavior (Combes 2001). Compatibility with a parasite can be eliminated or reduced if the host does not have sufficient resources for the parasite or the host is able to defend against parasitism (for review see Combes 2001). Implementations of these tactics range broadly and have been heavily investigated by parasite ecologists.

Avoidance of encounter by parasites must generally be the first line of defense. Such behaviors take a diversity of forms at multiple scales that result in physical avoidance and minimal encounter with infectious stages (reviewed by Hart 1990). For example, migration, a population-level relocation, may minimize encounter with infections. Migration allows animals to leave infected areas and effectively reduces prevalence in a population by culling infected individuals unable to withstand the rigors of travel (for review see Altizer et al. 2011). In some cases, human activity has disrupted migration and eliminated this benefit. For instance, sedentary populations of monarch butterflies have higher prevalence of a specialist protozoan parasite (Satterfield et al. 2015), underscoring the importance of this parasite avoidance behavior. Herding may be another behavioral response to minimize encounter with infectious stages. A 'selfish herd' dilutes the per-capita risk of parasitism (Fauchald et al. 2007). At a smaller scale, some birds may change roosting sites regularly to avoid encounter with parasites (Rohner et al. 2000). Similarly, pelagic marine larval dispersal may avoid localized infection risk (Strathmann et al. 2002). Additionally, research has demonstrated that selective foraging by herbivores minimizes contact with infectious stages of gastrointestinal parasites present in feces (Cooper et al. 2000, Fleurance et al. 2007, Ezenwa 2004). While a diversity of parasite avoidance behaviors have been documented across a range of taxonomically diverse animals, the condition of the individual host and subsequent impact on individual risk remains an open question.

Both parasitism and parasite avoidance behaviors are energetically costly; thus, display of parasite avoidance behavior should balance (i) the cost of parasite avoidance, (ii) the cost of parasitism, and (iii) the risk of parasitism. (i) Parasite avoidance behavior costs include increased energy expenditure, increased risk of predation, and reduced reproductive success (for review see Hart 1990, Rigby et al. 2002). Given these costs, we expect the strength of display to be contingent on (ii) the cost of parasitism and (iii) the risk of parasitism. (ii) The cost of parasitism depends on both the parasite's trophic strategy and host condition. Parasites have a diversity of trophic strategies that vary in cost to the host (Lafferty and Kuris 2002). For example, typical macroparasites (such as adult trematodes) impact the host in an intensity-dependent manner— more parasites means greater cost (Lafferty and Kuris 2002). For this reason, host condition may play a regulatory role on the cost of infection if an additional infection bears greater cost to already infected hosts than to uninfected hosts. Other infectious consumer strategies act in an intensity-independent fashion, such as parasitoids, pathogens, and parasitic castrators (Lafferty and Kuris 2002, Lafferty and Kuris 2009). In this case, a single infection produces the totality of the cost, a complete loss of fitness for a parasitoid or a castrator. Thus, the cost of a single infection varies widely based on the type of parasite, where infection by a single macroparasite bears relatively little cost compared to infection by a single parasitoid or castrator.

The risk of parasitism can vary within a population based on individual characteristics such as size (Bell et al. 2005) or age (He and Wang 2006). Additionally, certain host behaviors can also increase risk of infection. For example, *Daphnia* with higher feeding rates are more likely to become infected by ingesting more infective bacterial spores (Hall et al. 2007). Thus, the cost of parasite avoidance behavior, cost of parasitism, and risk of parasitism vary among individuals in a population. Given the requisite value of feeding to the fitness of an organism, and the inherent risk of infection caused by feeding, there is a lack of investigations of behavior to modulate feeding in the context of risk of infection.

Here we experimentally investigate the influence of risk of infection on avoidance behavior of a barnacle, *Chthamalus fissus* (Darwin 1854) to *Hemioniscus balani* (Buchholz 1866), an isopod parasite that infects at least 11 species of barnacles. *C. fissus* is the most frequently infected host on the California coast of the United States (Crisp 1968, Blower and Roughgarden 1988). *H. balani* enters the mantle cavity of the barnacle, attaches to cuticle near the ovaries, draining ovarian fluid and rendering the barnacle unable to reproduce as a female. After the isopod matures, it releases its offspring, and then dies. Hence, *H. balani* is an ephemeral, semelparous, parasitic castrator. This parasite has a cosmopolitan distribution and is recorded from the East and West Coast of the United States, Western Europe, and South Africa (Crisp 1968).

The *H. balani*- *C. fissus* system is ideal for evaluating the relationships among the cost of parasite avoidance behavior, the cost of parasitism to host fitness, and the risk of parasitism because we can quantify the behavior, we know the cost of parasitism, and we can directly assess the risk of parasitism. Because parasites enter through the barnacle's scutum and tergum, on first principles, we assume barnacles with a closed aperture can avoid encounter with a parasite. Thus, barnacles can avoid parasites by reducing filtration rate, in essence reducing their encounter probability with parasitic infectious stages, despite the intrinsic cost due to cessation of feeding. Hosts infected by a single *H. balani* are no longer able to produce eggs. Thus, the fitness cost of infection is high and intensity-independent (Lafferty and Kuris 2009). Risk of infection should vary among individual barnacles based on both infection status and reproductive condition. Previously infected barnacles are not at risk of a further cost of infection since a single *H. balani* blocks host reproduction (Kuris 1974). Barnacles with ovoposited eggs, or with ripe ova, are also not at risk for infection only occurs in unripe barnacles. Thus, barnacles without eggs are at risk of parasitism because they have ovarian fluid for the parasite to consume. Here we conducted experiments to evaluate how host infection and reproductive condition affect parasite avoidance behavior.

METHODS

Experimental Design

To obtain specimens, we collected barnacles from rocks at low tide in Santa Barbara County, CA, USA from June 2014 to March 2015. Barnacles were isolated in individual wells filled with seawater and returned to the laboratory, where they were placed in a plastic cup with 20 mL of seawater and acclimated to light levels and water temperature for 5 minutes. After the acclimation period, we observed filtration through a dissecting microscope. A barnacle was recorded as filtering when its cirri emerged and swept the water. We counted the number of times a barnacle filtered in five 20-second intervals, averaging them. In experimental treatments we added an infectious cryptoniscus larva to the water with forceps. In the control treatment we disturbed the water with forceps to mimic disturbance associated with transferring a cryptoniscus into the cup with forceps. After a 30 min. to one-hour acclimation we averaged filtration rates for the five 20-second intervals. Change in filtration rate was average final filtration rate minus the average initial filtration rate. Negative values indicated a reduction in filtration rate while positive values were an increased rate.

We measured each barnacle to the nearest ¼ mm and assessed their reproductive and infection status. To control for size-specific variability in filtration rate, we only used barnacles 2.5 mm to 4 mm long. For reproductive status we reported the presence of oviposited eggs or ovaries with ripe (mature) ova, or unripe ovaries. For infection status, we inspected the mantle cavity for *H. balani*. Thus, we had three types of classified barnacles, infected (I), uninfected/non-reproductive (UI/NR), or uninfected/reproductive (UI/R).

Baseline filtration rates

To establish a baseline filtration rate, we collected barnacles from Miramar Beach in Santa Barbara County CA, USA and measured filtration rate of infected, uninfected/reproductive, and uninfected/non-reproductive barnacles (n=69). We conducted a 1-Factor ANOVA followed by a Tukey HSD post hoc analysis to analyze the difference in baseline filtration rate among I, UI/R, UI/NR barnacles.

Infected versus Uninfected/Non-Reproductive Barnacles

To quantify the effect of the presence of an infective cryptoniscus larva on filtration rate, we introduced an infectious cryptoniscus larva to a compartment with a barnacle and quantified the change in filtration rate. We used a fully crossed experimental design, varying infection status (I or UI/NR) and introduction of a cryptoniscus larva stage (+/- Parasite) (n=26). The proportion of uninfected/reproductive barnacles at Miramar Beach was too low to obtain sufficient specimens to include this treatment (prevalence of *H. balani* at Miramar Beach can reach 90% for barnacles between 2.5 and 4 mm CRF, personal observation of >6000 individuals). We randomly assigned individuals to a +/- Parasite treatment and measured change in filtration rate. These data were analyzed with a 2- Factor ANOVA where the first factor was category (I or UI/NR) and the second factor was parasite addition (+/- Parasite).

Uninfected/Reproductive versus Uninfected/Non-Reproductive Barnacles

To explore the effect of reproductive status on filtration rate, we collected barnacles from Campus Point, Santa Barbara County, CA, USA. We used this location because only ~4% of barnacles were infected by *H. balani* compared to 90% of barnacles at Miramar Beach in the size classes examined. We used a fully crossed experimental design, varying reproductive status (UI/R or UI/NR) and introduction of a cryptoniscus larva (+/- Parasite) and measured change in filtration rate (n=46). These data were analyzed with a 2- Factor ANOVA where the first factor was category (UI/R or UI/NR) and the second factor was parasite addition (+/- Parasite).

RESULTS

Both actively reproductive and infected barnacles had significantly lower rates of filtration than did barnacles that were not in an active reproductive state (ANOVA, F= 3.819, df= 2, p= 0.027, Tukey HSD). On average, both infection and reproduction had ~50% reduced filtration rate (Fig. 1).

The response of a barnacle to the presence of a cryptoniscus larva was dependent on whether or not the barnacle was already infected (2-F ANOVA, F=3.4105, df=3, interaction p=0.022). Absent an infectious larva, neither infected nor uninfected/non-reproductive barnacles changed their mean filtration rate from the initial measurement (Fig 2). When an infectious stage was present, filtration rate of the already infected barnacles did not change, while filtration rate of the uninfected/non-reproductive barnacle was reduced an average of 5.2+/- 1.4 SE per 20 seconds, a 50% reduction in filtration rate from baseline.

The response of a barnacle to the presence of a cryptoniscus larvae also depended on whether or not the barnacle was reproductive (2-F ANOVA, F=3.0213, df= 3, interaction p=0.0402). Absent an infectious larva, neither reproductive nor non-reproductive barnacles changed their mean rate of filtration (Fig 3). In contrast, in the presence of an infectious cryptoniscus stage, the filtration rate of the barnacle depended on its reproductive status. Reproductive barnacles did not respond to the presence of a cryptoniscus larva whereas non-reproductive barnacles reduced their filtration rate an average of 4.7+/- 2.1 SE per 20 seconds, a reduction of ~40% from the baseline rate.

DISCUSSION

At risk barnacles substantially reduced their filtration rate when in the presence of an infectious parasite. This is likely a strong, direct fitness cost 40-50% less energy consumed when at risk of infection. In other systems hosts have been shown to avoid parasitism. However, these costs are not as direct as is diminished food consumption. For example, selective foraging by herbivores (Cooper et al. 2000, Fleurance et al. 2007, Ezenwa 2004) avoids discrete, small patches of contaminated plants, but presumably bears a low cost compared to a direct reduction in feeding rate. Frog tadpoles expend energy to swim away from parasites, but this movement simultaneously results in physical separation of the tadpole from the parasite (Daly and Johnson 2011, Koprivnikar et al. 2006). Migration is costly, but this behavior also physically removes individuals from parasites and migration costs are also borne by requirements to migrate for better resources or appropriate breeding conditions (examples reviewed in Altizer et al. 2011). One possible reason for the high cost of the parasite avoidance behavior lies in the parasite's trophic strategy. *H. balani* is a parasitic castrator, so infection by one parasite eliminates the barnacle's ability to produce eggs. When contrasted with the high cost of infection, a 40-50% reduction in energy intake appears to be cost effective. An additional, and not mutually exclusive, explanation is that reduced feeding is the only solution for a sessile marine animal. Selective foraging, swimming away, and migration are not options. Associated with this problem, the hypothesis that pelagic larval dispersal evolved to escape local natural enemies may be particularly applicable to sessile marine animals (Strathmann et al. 2002, McCallum et al. 2004).

We found only at-risk barnacles reduced filtration rate in the presence of cryptoniscus larvae, which underscores the cost of reducing food intake. Risk of infection is not even among individual barnacles, and the specificity of the reduced filtration response highlights this heterogeneity. Parasites with mobile infectious stages capable of detecting variation in host quality can select hosts based on factors that maximize its reproductive success (Bell et al. 2005, He and Wang 2006, Liu et al. 2011). This has received most attention in studies of parasitoid selectivity. The parasite in this study has a highly mobile infectious stage, and castrators share many characteristics with parasitoids (Kuris 1974). For castrators and parasitoids, the entirety of their fitness depends on the quality of the host; how much host energy the castrator can extract and convert to the production of parasite offspring. From this perspective, such parasites should display high selectivity in host selection because the impact of host quality on parasite fitness is so high (Bell et al. 2005, He and Wang 2006, Liu et al. 2011). This is particularly true for *H. balani* because it is ephemeral, making the quality of the host upon infection crucial to parasite fitness.

Lost feeding opportunity to avoid parasites should result in slower-growing, less reproductive barnacles. Barnacles with greater access to food grow more rapidly (for review see Crisp & Bourget 1985), and larger barnacles have larger broods (Hines 1978). Additionally, barnacle populations supplied with higher doses of food have a higher fraction of brooding individuals and more broods than those with lower doses of food. Barnacles with restricted food rations by 1/3 were half as likely to be reproductive (Hines 1978). This effect translates to the field, where barnacles have a higher fraction of individuals brooding eggs at sites with higher near shore productivity (Leslie et al. 2005, Bertness et al. 1991). Thus, parasite avoidance behavior directly reducing feeding likely substantially impacts reduces barnacle fitness.

As a baseline, barnacles that were are infected or uninfected/reproductive had reduced filtration rates compared to uninfected/non-reproductive barnacles. The effects of infection on feeding rates are diverse and complex. Anorexia, or the reduction in feeding, is common in infected animals across a range of host and parasite taxa (for review see Symons 1985). Multiple functional explanations have been proposed to explain how anorexia may benefit either host or parasite (for review see Kyriazakis et al. 1998). For example, reduced feeding can be an anti-parasite defense; mice experimentally infected with bacteria reduced feeding rates, effectively driving resources down too low and killing the infection (Murray and Murray 1979). The reduced performance of parasitized hosts is largely due to anorexia (Coop and Holmes 1996), making this an active area of research. In some cases, animals increase feeding rates to offset the energetic effects of infection and mounting an immune defense. For example, damselflies infected with bacteria compensated by increasing feeding rate (González-Tokman et al. 2011). Blue tit parents increase feeding rates to heavily infected chicks to offset the cost of parasitism by blowflies (Hurtrez-Boussès et al. 1998). While we do not know the underlying cause, anorexia is common in parasitized organisms.

Additionally, reproductive barnacles had reduced filtration rates. Brood protection is a common phenomenon that can result in reduced feeding rates (Yanagisawa and Ochi 1991, Fernald and Hirata 1979, Schürch and Taborsky 2005, Groscolas and Robin 2001). Based on the data presented here, we are unable to discriminate if the reduction in filtration rate of infected barnacles results from infection or is a property of having a full brood cavity.

Behavior is necessarily the first line of defense against parasites, making this a model system for understand the interactions between risk of infection, cost of infection, and cost of parasite avoidance behavior. We find the display of parasite avoidance behavior of *C. fissus* against *H. balani* is directly related to the risk of infection. Additionally, the specificity of the behavior underscores the implicit cost of avoiding parasites in this system.

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Figure 1 a.) Mean baseline filtration rate +/- standard error of infected (white), uninfected/reproductive (hatched) and uninfected/non-reproductive (black) barnacles per 20 seconds. Letters represent significantly different groups based on Tukey's HSD (n=69). b.) Mean change in filtration rates +/- standard error of infected (white) and uninfected/non-reproductive (black) barnacles per 20 seconds when infectious stages were not present (- Parasite) or present (+ Parasite) (n=21). Zero values indicate no change in filtration rate, positive values and increase in filtration rate, negative values are a decrease in filtration rate. c.) Mean change in filtration rates +/- standard error of uninfected/reproductive (hatched) and uninfected/non-reproductive (black) barnacles per 20 seconds when infectious stages were not present (- Parasite) or present (+ Parasite) (n=26). Zero values indicate no change in filtration rate, positive values and increase in filtration rate, negative values are a decrease in filtration rate.

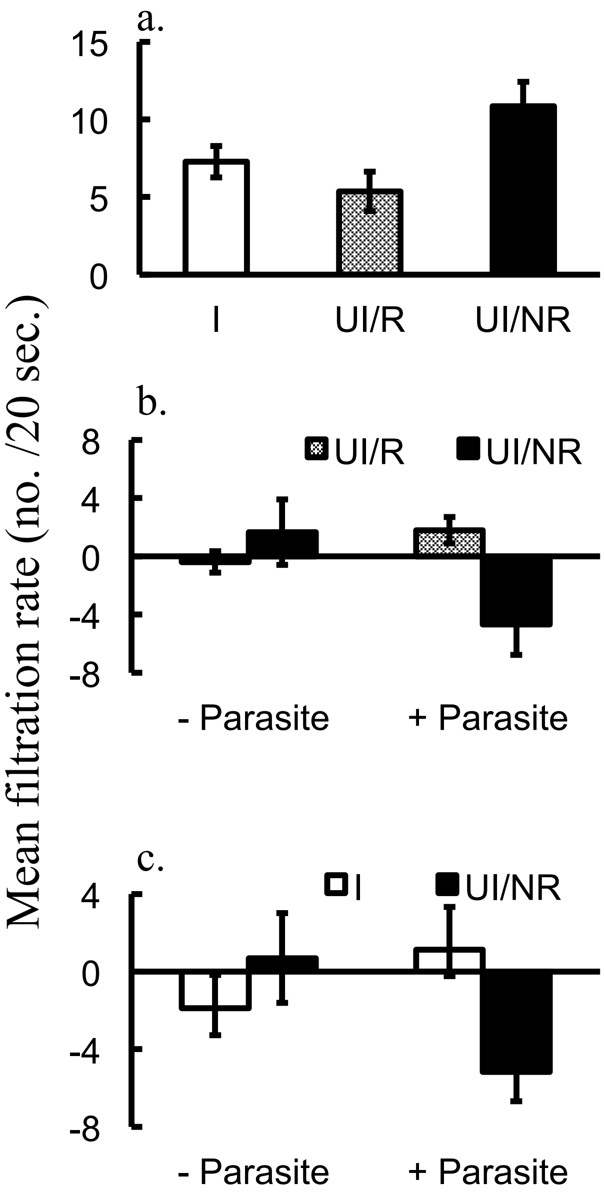
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Figure 1

CHAPTER 2: Hermaphrodites and parasitism: size-specific female reproduction drives infection by an ephemeral parasitic castrator

ABSTRACT

Gender can influence patterns of parasitism because males and females can differ regarding both encounter and susceptibility. We investigate an isopod parasite (*Hemioniscus balani*) that consumes ovarian fluid, blocking reproduction of its host barnacle (*Chthamalus fissus*), a simultaneous hermaphrodite. As a hermaphroditic species, where gender is not fixed and individuals allocate variable energy to male or female functions, *C. fissus* may be able to allocate energy differentially to male versus female reproduction. Since *H. balani* requires ovarian fluid, only barnacles with female reproductive function should be appropriate hosts. We surveyed 24 populations spanning roughly 400 km of coastline of southern California and documented a unimodal relationship between barnacle size and female reproductive function. This female function-size relationship was mirrored by patterns of parasitism by *H. balani*. This was consistent with the hypotheses that the parasite can only infect barnacles actively functioning as females, and that the distribution of female reproductive function among individuals primarily dictates patterns of infection within the entire barnacle population. Further, we found within the subset of suitable hosts, parasitism increased with size. We suggest physiological compatibility (female reproductive function) and host choice (for larger susceptible hosts) drive the documented patterns of parasitism.

INTRODUCTION

Host gender can drive patterns of parasitism in populations for two overarching reasons. First, males and females can vary in how much they encounter parasite transmission stages. Differential exposure could, for instance, be driven by gender differences in behavior (e.g. Tinsley 1989, Poole et al. 1983, Wilson et al. 2001). Second, males and females can differ in how compatible they are as hosts after encounter. For example, males generally have weaker immune systems than do females and are often more parasitized (Klien 2004). These sorts of gender differences in parasitism may be associated with other factors such as body size. For instance, if males and females differ in size or in spatial distribution, differential parasitism of males and females could drive size-related or spatial patterns of parasitism within host populations. An interesting twist to this theme occurs for species where gender is not fixed. In hermaphroditic species, individuals allocate variable energy to male or female function (e.g. Charnov 1982). In such cases, an individual can vary in gender-based differences in parasitism over a single lifetime. Here, we examine barnacle hosts that can switch from being male to being simultaneous hermaphrodites. The distribution of female function in these hosts influences patterns of infection by a parasite that specializes on female reproductive tissue.

Hemioniscus balani is an isopod parasite reported to infect and block reproduction in at least 14 barnacle species (Crisp 1968, Goudeau 1970, Blower and Roughgarden 1988). Infection by a single parasitic castrator eliminates host reproduction for the duration of the infection (Lafferty and Kuris 2009). *Hemioniscus balani* infects the barnacle with a highly mobile stage, the cryptoniscus larva, and attaches to the ovaries, draining ovarian fluid and rendering the barnacle unable to reproduce as a female. After the isopod matures, it releases its offspring and then dies, permitting the host to recover its female reproductive capability. Hence, this isopod is an ephemeral, semelparous parasitic castrator. Male reproductive function is retained (Goudeau 1972, Blower and Roughgarden 1988). This is a distinctive parasitic trophic strategy, with no other examples known to us (other parasitic castrators are long-lived and iteroparous, see lists in Kuris 1974, Lafferty and Kuris 2009). This unusual parasitic strategy is even more interesting given the complex sexuality of its barnacle hosts.

Barnacles have diverse sexual systems (reviewed in Darwin 1854, Yusa et al. 2013, Yamaguchi et al. 2008). Many species are hermaphroditic, and individuals beginning their lives as males (Yamaguchi et al. 2008, Hines 1987). With increasing age and size, individuals allocate increasing amounts of energy to female function and become simultaneous hermaphrodites (Yamaguchi et al. 2008, Hines 1987). Additionally, barnacle sex allocation can be context dependent. For instance, though empirical evidence is limited and mixed (Raimondi and Martin 1991, Hoch 2009, Hoch and Levinton 2012, Yusa et al. 2013), theoretical models predict increased allocation to male function when barnacles find themselves in larger mating groups (Charnov 1982). Hence, the amount of energy barnacles allocate to female function can vary with age, size, and extent of aggregation.

We hypothesized that variation in female function in barnacles would be mirrored by patterns of infection by *H. balani* because this parasite is an ovarian specialist. We examine the acorn barnacle, *Chthamalus fissus*, a host for *H. balani* (Blower and Roughgarden 1988). We first quantified how allocation to female function varies with body size in *C. fissus*. As for other hermaphroditic barnacle species (e.g., Raimondi and Martin 1991, but see Hoch and Levington 2012), we expected variable investment in male versus female function. We then tested the hypothesis that the documented relationship between size and female function mirrors the relationship between size and probability of being infected, because only barnacles with active female function should be suitable hosts. We hypothesized that the distribution of suitable hosts primarily dictates patterns of infection within the *entire* barnacle population. However, within the *subset* of suitable hosts, we hypothesized that the probability of being infected increases with body size because larger hosts are larger targets. This could result in higher infection rates in larger barnacles even under random encounter scenarios. Also, parasites might prefer and actively infect larger hosts, because, consistent with other parasitic castrators (e.g., Kuris 1974, Muñoz and George-Nascimento 1999, Kuris and Lafferty 2000, Hechinger et al. 2009), the size and fecundity of *H. balani* increases with host size (Fong, *unpubished data*).

To test these hypotheses, we surveyed 12 populations of *C. fissus* for parasitism by *H. balani* from 6 localities along the Southern California Bight. We measured barnacle size, female reproductive function, and infection status. We then documented the relationship between female function, parasitism, and barnacle size.

METHODS

Over a three-day period (16-18 Sep 2013), we collected barnacles from 2 habitat types (1 natural rock and one pier piling) at each of 6 localities spread throughout the Southern California Bight (Fig. 1, 12 sites in total). We sampled 2 habitat types because Sites were chosen based on accessibility and because they had both habitat types. To minimize tidal differences and differences in encounter rate within a site, barnacles were collected in a stratified random design from the lower 10 cm of their elevational range. We collected all barnacles encountered in 10 haphazardly placed circular 11.34 cm^2 cores. Barnacles were frozen immediately after collection, remaining frozen until dissection.

At the laboratory, all barnacles were thawed and dissected. Barnacle length was measured as the widest shell diameter to the nearest 0.25 mm. Barnacles were recorded as infected or uninfected, and as “non-reproductive” or “reproductive,” based on female reproductive function, where reproductive individuals had ripe ovaries (as indicated by yellow/orange fluid within the ovary), developing eggs, or oviposited eggs. We only included barnacles ≥ 1 mm, thereby avoiding barnacles that are typically pre-reproductive (Hines 1978, Fong, *personal observations*).

We determined whether the size frequency distribution of infected hosts was a non-random subset of the size frequency distribution characterizing the entire barnacle population using a Kolmogorov-Smirnoff test.

We examined the influence of size, habitat type, and locality on the probability of female reproductive function and infection using logistic regression and AICc model selection. We first estimated the probability of female reproductive function only among uninfected barnacles. However, disproportionate infection of barnacles with female function would cause us to underestimate this probability. For instance, in the extreme case of 100% infection of barnacles with female function, there would be zero probability of female reproductive function. Hence, we also calculated the probability of female reproductive function by counting infected barnacles as reproductive females. This is sensible given the specialization of the parasites on female ovaries, which necessarily implies that the barnacle was a functional female. The probability curves from both analyses were generally very similar as expected given the typical low prevalence of infection. Because the curves were similar, in the main text we present the results counting infected individuals as barnacles with female reproductive function, because that likely provided the best representation of the pattern. We present a comparison of the two probability curves (Fig. 4). Because the size with the predicted maximum probability of female reproduction varied between sites (see results), we used a regression approach to determine the relationship between the size of the largest individual at a site and the size with the predicted maximum probability of female reproduction at a site (Fig. 5).

We used the regression equations from the favored models to extract the host sizes corresponding to the maximum probabilities of female reproductive function and infection for each site. We then compared the size of maximum probability of female reproductive function for each population to the maximum probability of infection at each site using a 1-sample t-test because the maximum probability of infection was statistically invariant (see results).

Additionally, we examined the influence of size, habitat type, and locality on the probability individuals in the susceptible class were infected using logistic regression and AICc model selection. Because *H. balani* infection blocks female reproduction, susceptible barnacles were those that were uninfected/reproductive plus infected barnacles.

RESULTS

We dissected 6,381 barnacles, of which 362 were infected, a regional prevalence, percentage infected, of 5.67% [5.12, 6.26 95% CI] (Table 1). However, prevalence varied substantially between sites and ranged from 0 to 23.9%. The size frequency distribution of infected barnacles was significantly different from uninfected barnacles at all sites at which there were at least 10 infections (Table 1). Thus, the distribution of infected individuals is not a random subset of the population of hosts.

The model for predicting female reproductive function with the lowest AICc score included size of the barnacle host, habitat type, and locality (Table 2). The probability of female reproduction varied non-linearly with size (size*size $p<0.0001$) reflecting the unimodal nature of the predicted probability distribution. Barnacles of intermediate size always had the highest probability of being reproductive females (Fig. 2). However, the size of barnacle with the predicted maximum probability of being such a female varied among localities and habitat types, ranging from 3.00-8.00 mm (locality * size * size interaction $p<0.0001$, habitat type * size * size interaction $p<0.0001$) (Fig. 3). While the size-specific probability of female reproductive function differed between natural rock and pier habitats, one habitat type did not consistently have higher probabilities of female reproductive function (locality * habitat type interaction $p<0.0001$). However, among populations, the size of barnacles most likely to be reproducing as females was linearly related to the largest size of barnacle at that site ($y=0.4x+1.3$, $R^2= 0.67$, Fig. 5).

The model predicting the probability of infection with the lowest AICc score included barnacle host size, habitat type, and locality (Table 3). Similar to the probability of active female reproduction, the probability of infection responded non-linearly to host size (size * size interaction $p<0.0001$), such that 4 mm barnacles always had the highest probability of infection, irrespective of site (i.e., size never interacted with habitat or locality) (Fig. 2) (Table 3). However, the magnitude of the probability of infection varied among sites, with the maximum probability ranging from ~0-0.60. While sites had different maximum probabilities of infection, differences between natural rock and pier habitats were not consistent and varied among localities (habitat*locality interaction $p<0.0001$).

We observed no *H. balani* infections above 5mm, even though there are appropriate hosts (18 of 50 large barnacles were actively reproducing as females). This was significantly different from the prevalence observed within 4.75 mm barnacles, the next smallest size class (binomial test, $p=0.002$).

The size with the maximum probability of infection did not differ significantly from the size having the peak proportion of reproducing females (1-sample t-test, p=0.22) (Fig. 3). Across all sites, the average maximum probability of active reproduction was 4.2 ± SE 0.38 mm while the maximum probability of infection was 4.0 mm.

The model best predicting the probability of infection within the class of susceptible barnacles included site, habitat, and size. In general, we found that the probability of infection increased with size (Fig. 2), though the shape of these curves varied among localities (locality * size, p=0.002), but not consistently among habitat types (locality*habitat, p<0.0001) (Table 4).

DISCUSSION

We first discuss our findings concerning an unexpected, unimodal, size-specific distribution of reproductive females, which is counter to models of barnacle reproductive allocation (Charnov 1982, Yusa et al. 2013, Yamaguchi et al. 2012, Yusa et al. 2013). We then focus on how that distribution intersected with patterns of infection.

Patterns of female reproductive function

The proportion of reproducing female *Chthamalus fissus* reached a unimodal peak at a body size less than the maximum barnacle size in the sampled populations. This conflicts with theoretical models of hermaphroditic barnacle reproductive allocation (Charnov 1982, Yusa et al. 2013, Yamaguchi et al. 2012, Yusa et al. 2013), in which the largest barnacles simultaneous allocate resources to both to male and female functions.

Intermediate-sized barnacles were most likely to be actively functioning as females. There is nothing surprising about the left side of the curve, as small barnacles likely have not yet reached female reproductive maturity (Hines 1978, Yamaguchi et al. 2012). However, what is the explanation for the larger barnacles to be less likely to be actively reproducing as females?

We posit that it is advantageous for some barnacles, including *C. fissus*, to disproportionally invest in male function when large. Barnacle penis length scales with barnacle size, and limits a barnacle's mating success as a male (Hoch 2009). Hence, it may be favorable for an individual to be predominantly male when it is the largest member of a mating group and can dominate sperm competition. While the mechanism varies, there are other hermaphroditic mating systems that show such a pattern where large individuals have increased reproductive success as males due to dominance (e.g. Warner 1988). The results are clear: intermediate-sized barnacles had the greatest chance to allocate substantial resources to female function across 24 populations spanning roughly 400 km of the southern California coast, a pattern we are the first to document.

The specific maximum size of barnacles reproducing as females varied among populations. This maximum was not correlated with mean barnacle size. Hence, there was no general tendency for the female function vs. size curve to simply track overall shifts in the population size-frequency distributions. However, size of maximum probability of female function was correlated with the size of the largest individual at each site. These largest individuals were invariably males, and were usually scarce. These males could therefore set a social environment that favors increased allocation to female function in the surrounding, smaller, barnacles, which would not be competitive as males. This explains the shift in size of the barnacles most likely to actively allocate resources to the female function in each barnacle population.

The peak probability of female reproduction also varied among sites. One possible explanation for this pattern is among-site differences in food availability. Increased food availability leads directly to increased barnacle female reproductive productivity (Hines 1978). For example, the fraction of barnacles brooding eggs can be 5 times higher at sites with higher near-shore primary productivity (Leslie et al. 2005). While we do not have evidence for differential productivity across our sites, we suggest bottom-up forcing may have increased female reproduction and been a source of variation among sites.

Relationship between female function and infection risk

The size-specific relationship for reproductive females was related to the size-specific pattern of infection of *Hemioniscus balani*. Intermediate-sized barnacles were most likely to be actively functioning as females and also to be infected. However, in contrast to the maximum probability of female function, which varied among sites, infection risk appeared to peak at a constant barnacle size throughout the entire Southern California Bight. *C. fissus* barnacles of 4 mm are most likely to be infected. These findings are consistent with our hypotheses that the parasite can only infect barnacles actively functioning as females, and that the distribution of female reproductive function among individuals primarily dictates patterns of infection within the entire barnacle population. The data were further consistent with our hypothesis concerning infection being most likely in suitable hosts of larger size.

As *H. balani* consumes on ovarian fluid, the distribution of parasites within the population should reflect the distribution of appropriate hosts (females with ovarian fluid). While the sub-maximum size of reproductive females in all populations was unexpected, the distribution of parasites was not unexpected because it mirrored the availability of appropriate hosts.

Although the parasite has a nutritional requirement that appears to explain its size-specific patterns of infection in barnacle populations, there are alternative explanations for a unimodal pattern. These include differences among host individuals in the likelihood that parasites can encounter them, and in aspects of compatibility, other than meeting the nutritional requirement of ripe ovaries, such as host immunological defenses and genetic aspects of compatibility. Variable encounter can drive patterns of parasitism (see Combes 2001 for examples). However, barnacles are sessile and our samples were all taken from a single tidal height at each site, reducing differences in exposure risk among hosts of different sizes. Hence, differential exposure to infectious propagules is an unlikely factor in this study. It is plausible that smaller and larger barnacles are less likely to be parasitized for reasons independent of, but covarying with, female function. Could smaller and larger barnacles be less suitable hosts due to aspects of immune defense or to structural aspects of resistance? This explanation would require different reasoning for the large and small sized barnacles and thus seems unlikely. Small barnacles may be less apparent to the searching cryptoniscus larvae and they may not be of sufficient size to house a developing parasite. Searching isopod larvae may be able to detect and avoid such unsuitable hosts since, for barnacles, there is a high probability that a suitably sized host is nearby. The largest barnacles may be better defended against these parasites as they are likely to have a stronger cellular immune response. But, the extent to which a large barnacle can mount a cellular defense against a parasite whose body is actually in the mantle cavity seems unlikely. A hypothesis based on genetic differences among barnacle size classes is unlikely, as it would require that smaller and larger barnacles at the 12 different localities consistently originated from a resistant recruitment stock. In sum, we favor the parsimonious, “bottom-up” explanation as the driver for a unimodal pattern of infection in the host populations. This general explanation is also consistent with the observation that while the modal peak in barnacle host reproduction varies among populations, the peak of barnacle infective success is not significantly different across host geography and habitat types. In other words, it appears that the parasite is under selective pressure at a large geographic scale to maximize its infection success across a range of host sizes that vary locally, but are close to the optimum host size for the parasite.

Patterns of infection among reproductive females

In contrast to the unimodal distribution of infection risk with size throughout entire barnacle populations, among appropriate hosts (reproductive females), the probability of infection generally increased with size. There are several plausible explanations for this pattern. First, prevalence of parasitism often increases with host size because the cumulative risk of infection is higher for older (larger) individuals (Wilson et al. 2001). This is unlikely here because *H. balani* is short-lived, the host loses the semelparous parasite after its short reproductive period. Thus, all infections were recent, precluding accumulation over time. Second, parasitism could be more likely in larger hosts because those hosts are larger targets. Finally, parasites may actively target larger hosts. Generally, larger hosts result in increased body size or reproductive output for parasitic castrators (reviewed in Kuris 1974). This appears to be the case for *H. balani*, as their body size and fecundity are larger in larger hosts (Fong, *unpublished data*). *H. balani* has a highly mobile searching stage that appears to have physical and behavioral capabilities to select among hosts. Actively searching stages of other parasitic species can also be highly selective; for example, adult female wasps are capable of selecting hosts for their parasitoid offspring based on a range of host characteristics (e.g. Liu et al. 2011, Bell et al. 2005, He and Wang 2006). Hence, the probable fitness gains and the searching capability of *H. balani* larvae to select among hosts suggest host selection as the most likely mechanism for the increased risk of infection of relatively large but not the largest, susceptible hosts.

Conclusion

The barnacle, *C. fissus*, exhibits a unimodal relationship between size and female reproductive function with maxima at intermediate sizes among individuals in the 12 barnacle populations surveyed in this study. This is counter to the widely accepted model of barnacle sexual allocation that predicts maximum female reproduction at the maximum barnacle size. The risk of infection by *H. balani*, an ephemeral, semelparous, parasitic castrator largely mirrors the probability of female reproductive function. The relationship of sexual allocation and parasitism in a simultaneously hermaphroditic host indicates that both the host and this parasite are able to maximize their respective reproductive success although these outcomes are in conflict within and among populations.

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TABLE 1 Sites sampled, number of *Chthamalus fissus* barnacles examined (N), number infected by of *Hemioniscus balani* and parasitism prevalence. Kolmogorov-Smirnoff (KS) test probabilities comparing the size frequency distribution of infected and uninfected barnacles at each site. N also indicates barnacle density (number in 10 randomly placed circular 11.34 cm^2 cores).

SITE	KS P-VALUE	# INFECTED	N	PREVALENCE (%)
Gaviota Rock	0.9973	2	629	0.3 [0.1,1.2 95% CI]
Gaviota Pier	**<0.0001**	75	536	14.0 [11.3,17.2 95% CI]
Goleta Rock	**0.0005**	25	615	4.1 [2.8, 5.9 95% CI]
Goleta Pier	**0.0003**	31	201	15.4 [7.3,14.2 95% CI]
Santa Barbara Rock	**<0.0001**	143	598	23.9 [20.1, 27.5 95% CI]
Santa Barbara Pier	**<0.0001**	52	614	8.5 [6.5, 10.9 95% CI]
Ventura Rock	**0.0003**	10	743	1.3 [0.7, 2.5 95% CI]
Ventura Pier	0.4317	4	410	1.0 [0.4, 2.5 95% CI]
San Clemente Rock	0.4846	8	308	2.6 [1.3, 5.0 95% CI]
San Clemente Pier	**0.0198**	10	572	1.7 [1.0, 3.2 95% CI]
Scripps Rock	0.8701	2	413	0.5 [0.1, 1.7 95% CI]
Scripps Pier	---	0	642	0.0 [0, 0.6 95% CI]

TABLE 2 Results of the logistic regression with reproductive function as the response variable.

SOURCE	DF	L-R CHISQUARE	P-VALUE
Locality	5	68.35	<.0001
Habitat Type	1	0.09294	0.7605
Locality*Habitat Type	5	195.1	<.0001
Size	1	862.3	<.0001
Locality*Size	5	14.12	0.0149
Habitat Type*Size	1	6.208	0.0127
Locality*Habitat Type*Size	5	38.21	**<.0001**
Size*Size	1	251.0	**<.0001**
Locality*Size*Size	5	46.23	**<.0001**
Habitat Type*Size*Size	1	18.14	**<.0001**

TABLE 3 Results of the logistic regression with infection status as the response variable.

SOURCE	DF	L-R CHISQUARE	P-VALUE
Size	1	218.1	**<0.0001**
Size*Size	1	62.88	**<0.0001**
Locality	5	305.1	**<0.0001**
Habitat Type	1	0.176	0.6746
Habitat Type* Locality	5	272.2	**<0.0001**

TABLE 4 Results of the logistic regression for the probability of being infected within the susceptible class.

SOURCE	DF	L-R CHISQUARE	P-VALUE
Site	5	376.2	**<.0001**
Habitat Type	1	0.3645	0.546
Site*Habitat Type	5	217.6	**<.0001**
Size	1	0.8590	0.354
Site*Size	5	18.69	**0.0022**

Figure 1 Locations of the 12 survey sites, which were at six localities spread throughout the Southern California Bight. Localities included Gaviota (a,b), Goleta (c,d), Santa Barbara (e,f), Ventura (g,h), San Clemente (i,j), and La Jolla (k,l).

Figure 2. Size-frequency distributions, probabilities of reproduction, and probabilities of infection for barnacles at the 12 study sites located throughout the Southern California Bight. Bars indicate number of barnacles while curves indicate predicted probabilities from the logistic regressions Natural rock habitats are on the left, while pier habitats are on the right. Sites are ordered from north to south: Gaviota (a,b), Goleta (c,d), Santa Barbara (e,f), Ventura (g,h), San Clemente (i,j), and La Jolla (k,l).

Figure 3 Line graph indicating the barnacle size (basal diameter) with the maximum probability of active reproduction for each of the 12 sites (mean =4.2 ± 0.38 mm SE). Red arrow signifies the average of 4 mm among all 12 sites, which corresponds to the maximum infection risk at 4 mm.

Figure 4 Probabilities of reproduction when excluding infected barnacles (solid curves) and including infected barnacles as reproductive females (dashed curves, which are the same as those in Figure 2) for *Chthamalus fissus* at the 12 study sites located throughout the Southern California Bight. Natural rock habitats are on the left (panels a, c, e, g, i, k), while pier habitats are on the right (panels b, d, f, h, j, l). Sites are ordered from north to south: Gaviota (a,b), Goleta (c,d), Santa Barbara (e,f), Ventura (g,h), San Clemente (i,j), and La Jolla (k,l).

Figure 5 Maximum sized barnacle (mm) compared to the size of barnacle with the highest probability of female reproductive function (mm) for each site.

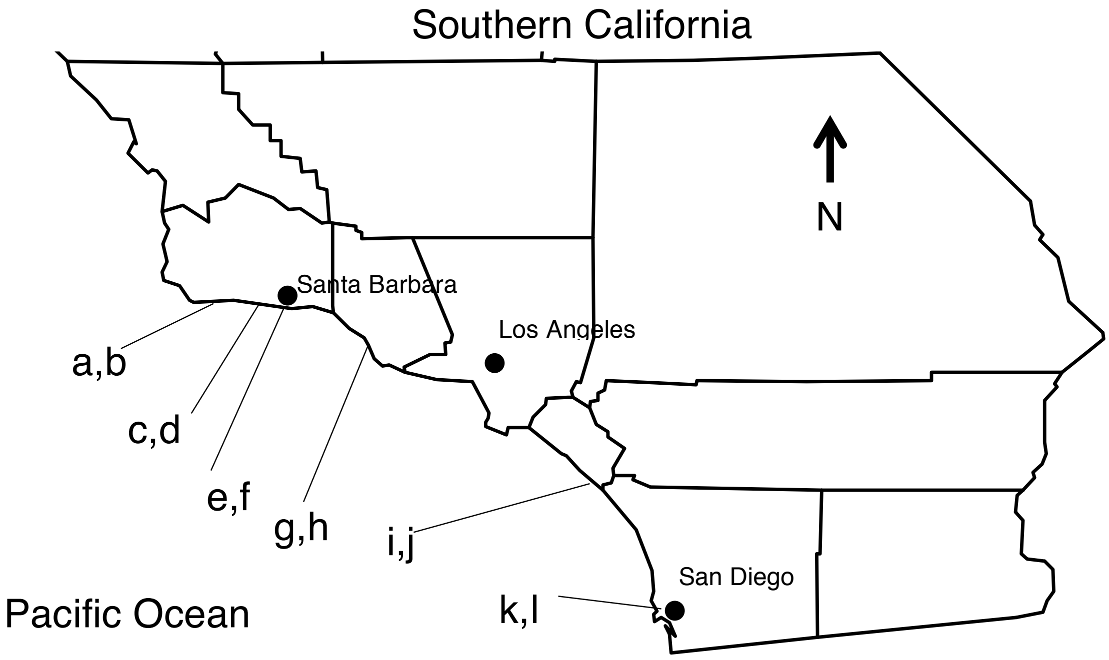

Southern California
N
Santa Barbara
Los Angeles
San Diego
a,b
c,d
e,f
g,h
i,j
k,l
Pacific Ocean

Figure 1

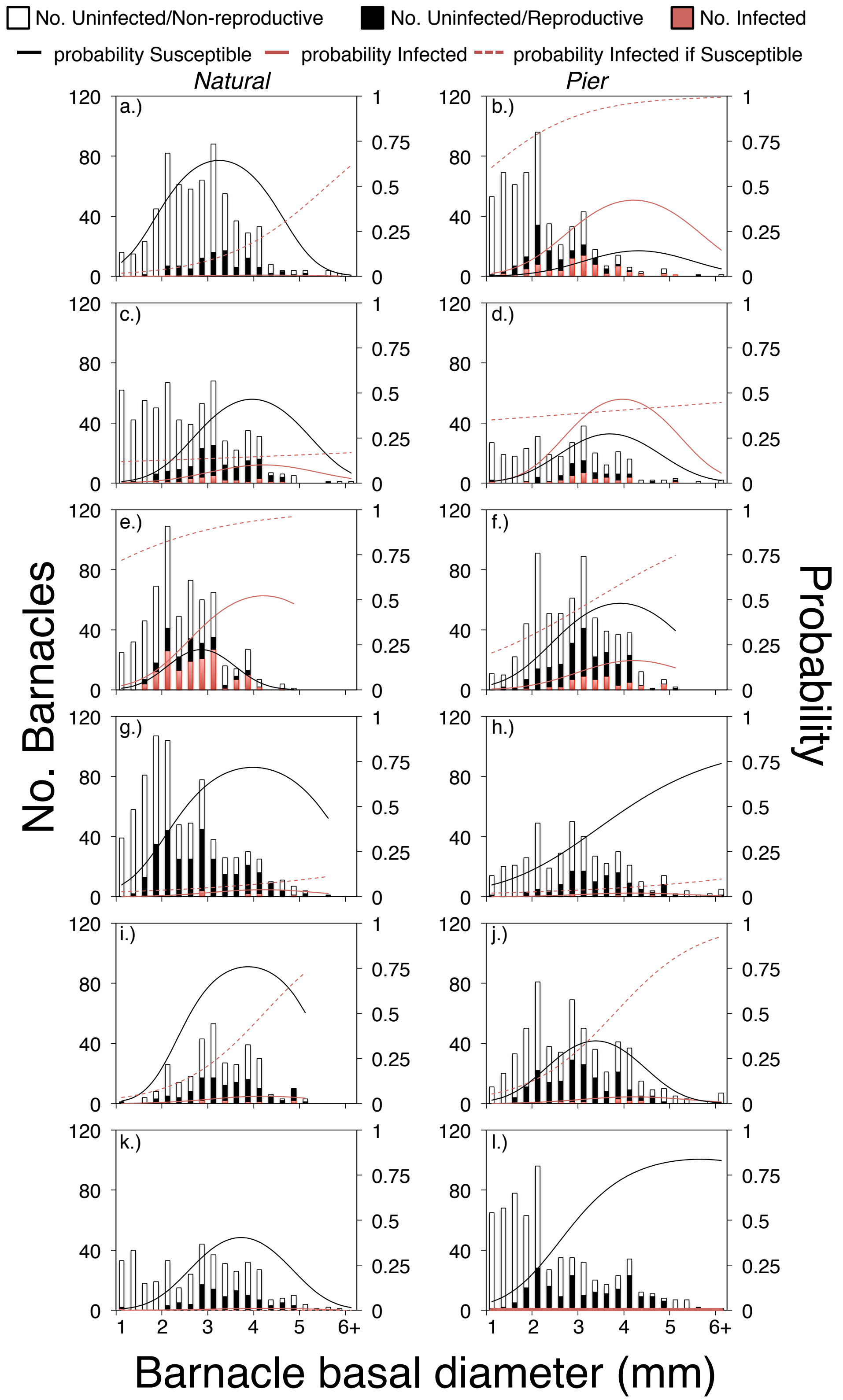

No. Uninfected/Non-reproductive
No. Uninfected/Reproductive
No. Infected
probability Susceptible
probability Infected
probability Infected if Susceptible
Natural
Pier
a.)
b.)
c.)
d.)
e.)
f.)
g.)
h.)
i.)
j.)
k.)
l.)
No. Barnacles
Probability
0
40
80
120
0
0.25
0.5
0.75
1
1
2
3
4
5
6+
Barnacle basal diameter (mm)

Figure 2

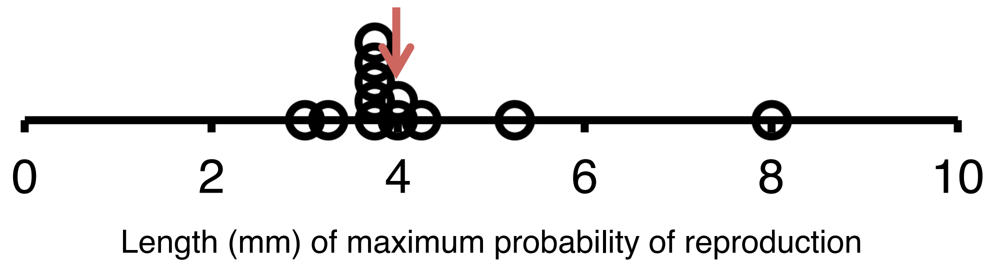
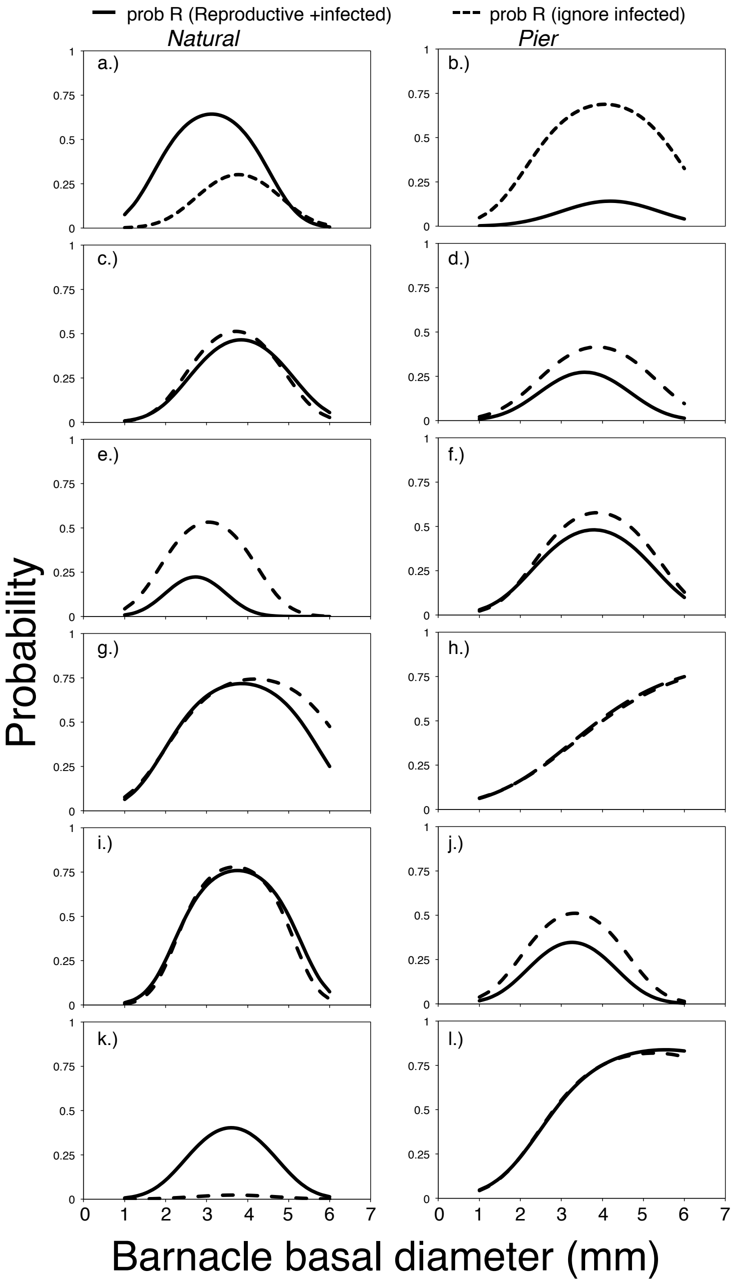


Figure 3



prob R (Reproductive +infected)
prob R (ignore infected)
Natural
Pier
a.)
b.)
c.)
d.)
e.)
f.)
g.)
h.)
i.)
j.)
k.)
l.)
0
0.25
0.5
0.75
1
0 1 2 3 4 5 6 7
Probability
Barnacle basal diameter (mm)

Figure 4

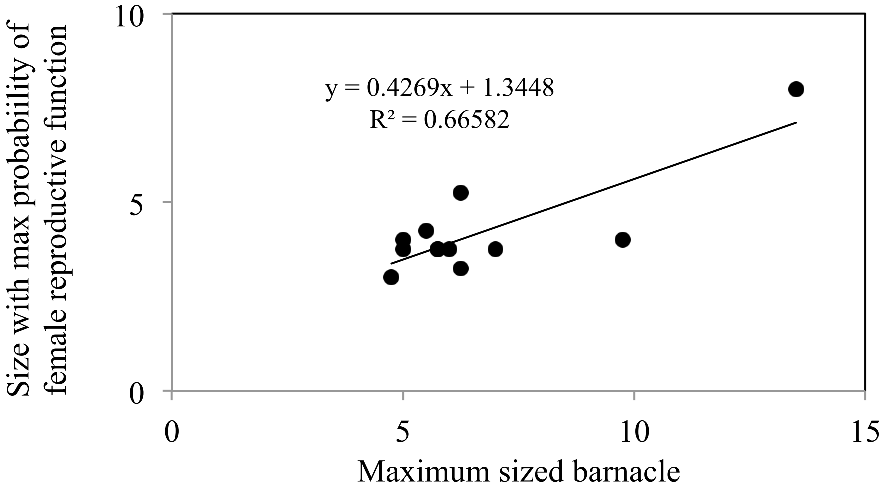

y = 0.4269x + 1.3448
R² = 0.66582
Size with max probability of female reproductive function
Maximum sized barnacle
0
5
10
0
5
10
15

Figure 5

CHAPTER 3: Predation on transmission stages reduces parasitism: sea anemones consume larval transmission stages of a parasitic castrator of barnacles

ABSTRACT

While parasites may serve as prey, does the spatial distribution of predators on parasites provide transmission control and influence patterns of parasitism? Because many of its organisms are sessile the rocky intertidal zone is a valuable but little used system to understand spatial patterns of parasitism and elucidate the underlying mechanisms driving these patterns. Sea anemones and barnacles are important space competitors in the upper rocky intertidal zone along the Pacific coast of North America. Anemones are voracious, indiscriminate, sit-and-wait predators; thus, they may intercept infectious stages of parasites before they reach a host. Here, we investigate whether a sea anemone, *Anthopleura elegantissima*, protects an associated barnacle, *Chthamalus fissus*, from parasitism by *Hemioniscus balani*, an isopod parasitic castrator. At Coal Oil Point in Santa Barbara, California USA, 29% of barnacles were within 1 cm from an anemone at the surveyed tidal height. Barnacles associated with anemones had reduced parasitism and higher reproductive productivity than did barnacles remote from sea anemones. In the laboratory, anemones readily consumed the transmission stage cryptoniscus larvae of the parasite. Hence, anemone consumption of parasite transmission stages may provide a mechanism by which community context regulates, and in this case reduces, parasitism at a local scale. Thus, our results suggest predation may be an important process providing parasite transmission control in nature.

INTRODUCTION

Predation on parasites may be a key mechanism by which community context can regulate the impact of parasitism. This may have important implications for diseases of public health and commercial importance. Predation can be a major source of mortality for adult parasites, can reduce parasite burden in hosts, and can reduce transmission stages in the environment and thus transmission rates. Predation on parasites may be common in ecological communities; for example, Lafferty et al. (2006) showed that 44% of food web links in a Southern California estuary comprised predation on parasites. Increasing predation on parasites may be a useful approach for disease control, incorporating top-down control on transmission dynamics. For example, predation on hosts has been investigated as a management strategy to control transmission of human infectious diseases. Mkoji et al. (1999) and Sokolow et al. (2015) found support for controlling schistosomiasis in human population by controlling transmission; in these cases, crayfish and prawns consumed snails (intermediate hosts), which shed transmission stages into the water where they come in contact with humans. In these studies, reducing snail densities could reduce the number of transmission stages in the water, reducing schistosomiasis in local human population. Thus, consumption of parasites may interrupt transmission dynamics, highlighting the potential importance of studying parasitism within the context of a community (Johnson et al. 2010).

Multiple laboratory studies document examples of predators of parasite transmission stages across a diverse group of animals. In Southern California estuaries, predation on free-living transmission stages comprises 7% of food web links (Hechinger et al. 2011). Sea anemones (Hopper et al. 2008, Prinz et al. 2009), barnacles (Prinz et al. 2009), bivalves (Faust et al. 2009), crabs (Thieltges et al. 2008), fishes (Kaplan et al. 2009), and shrimp (Thieltges et al. 2008) can all be predators on parasite transmission stages; however, all of these studies measure predation with laboratory feeding trials (but see Kaplan et al. 2009 for gut content) without directly correlating predator abundance to parasitism in the field. Thus, direct consumption of transmission stages of parasites has been theorized to reduce parasitism (Johnson et al. 2010), quantification in the field remains unknown. While some research has documented predation on parasite transmission stages in the field (Kaplan et al. 2009), researcher have not related this predation to reduced parasitism in the field. Thus, it remains unknown how natural community contexts shape predation on parasite transmission stages and drives spatial patterns of parasitism and there is a lack of studies providing an estimate, based on field data, for the role of predation on transmission. Here, we use a survey approach in the field to assess the effect of association with a parasite predator on parasitism in a barnacle host.

Spatial epidemiology—the description, quantification, and explanation for spatial differences in diseases—is a burgeoning area of research; however, local predation on parasite transmission stages has yet to be considered in this area of research. Recently, epidemiology models have begun to consider the effects of spatial patterns of transmission on parasitism. Regionally, transmission dynamics can be strongly influenced by abiotic factors such as climate (e.g. Brooker et al. 2006, Brooker 2007, Mordecai et al. 2013), which have been incorporated into models of spatial epidemiology. However, these have all been large-scale spatial investigations, unable to reveal the mechanisms whereby these patterns are regulated. Locally, the distribution of parasite predators may control spatial patterns in transmission and may provide a control for parasites. Further support for the role of proximity to parasite predators is offered by Mouritsen and Poulin (2003), who found increased density of anemones in tidal mudflats reduced parasitism of the bivalves by larval trematode parasites.

Here we investigate the relationship between risk of parasitism and association with an anemone, *Anthopleura elegantissima*, on infection of a rocky intertidal barnacle, *Chthamalus fissus* by an isopod parasite, *Hemioniscus balani*. Barnacles in the rocky intertidal zone are a classic system for studying basic ecological processes with a long history of study (e.g., Cranwele and Moore 1938, Connell 1961, Hooper et al. 2016). *H. balani* is an isopod parasite that infects at least 14 species of barnacles and *C. fissus* is the most frequently infected host on the California coast of the United States (Crisp 1968, Goudeau 1970, Blower and Roughgarden 1988). The isopod enters the mantle cavity of the barnacle and attaches to cuticle near the ovaries. The parasite then drains the ovarian fluid, rendering the barnacle unable to reproduce as a female (Goudeau 1972). After the parasite matures, it releases its offspring, and then dies. Hence, *H. balani* is an ephemeral, semelparous, parasitic castrator. In this study, we test whether association with a non-host species, *Anthopleura elegantissima*, protects the barnacle host from infection by consuming cryptonisci, the transmission stage of the parasite. This anemone reproduces clonally and can reach high densities in the rocky intertidal zone (54.4±34 individuals per m^2, Dayton 1971). It is an abundant and generalist predator, largely of zooplantkton (Zamer 1986).

Both host and anemone are sessile occupiers of limited hard substrate space in the rocky intertidal zone (Dayton 1971). We show that associations between barnacles and anemones are common and we test the hypotheses that parasitism is reduced close to anemones and that anemones actively consume parasites.

METHODS

To determine the frequency with which barnacles were associated with anemones, we surveyed the natural populations at Coal Oil Point, Santa Barbara County, California at a single tidal height, at which organisms are submerged an estimated 64% of the time. We ran five 1m transects parallel to shore and sampled five random 5x5 cm quadrats per transect (n = 25). *Chthalamus fissus* barnacles were considered associated with anemones if they were ≤ 1 cm from an anemone, which approximated the length of the anemone's tentacles. We counted the total number of barnacles in each quadrat associated with an anemone or not associated with an anemone. We then calculated the average density of barnacles and the percentage of barnacles associated with anemones and not associated with anemones within each quadrat.

To quantify the relationship between association with an anemone and parasitism, we hapahazardly collected 125 barnacles associated (≤1 cm away) and unassociated (>10 cm away) with anemones (total n=250) (Fig. 1). All barnacles sampled were >2 mm, as smaller barnacles are infrequently infected (Hines 1978, Fong et al. *in prep*). Barnacles were placed into individual wells in the field, returned to lab, and immediately processed. Each barnacle was measured to the nearest ¼ mm, assessed for the presence of *Hemioniscus balani*, and scored as either reprodutive as females (with ripe ovaries or with eggs in the mantle cavity), or not. Because *H. balani* blocks reproduction, we were able to categorize barnacles as infected, uninfected/reproductive, or uninfected/non-reproductive. Data met assumptions of normality and homogeneity, and we used a t-test to determine if barnacle size varied between the two groups. To test whether parasitism and reproduction varied with respect to association with anemones, we analyzed infection and reproduction data and whether double infections varied with association with anemones with Fisher's Exact Test.

By multiplying host density (survey data) and parasite prevalence (the percentage of infected hosts), we estimated the number of barnacles actively reproducing in each quadrat. We then compared the estimated number of barnacles actively reproducing that were associated with anemones versus the number of such barnacles not associated with anemones using a t-test.

To determine whether anemones were capable of consuming parasites, we collected 3 anemones from the field and isolated them in individual finger bowls with 150 mL of seawater and allowed the anemones to acclimate for 30 min. We collected the cryptoniscus larva mobile transmission stage of *H. balani* from the field by chiseling off sections of rock from the field, rinsing off the rock in seawater in the laboratory, and collecting free-living parasites from the water. After the anemone had acclimated, we added 10 cryptoniscus larvae in 25 mL of seawater to the finger bowl. As a control, we also placed 10 cryptoniscus larvae in 25 mL of seawater to 3 finger bowls without anemones. After 30 min., we counted the number of cryptoniscus larvae remaining in the water and calculated a consumption rate (no./ hr.). We then averaged individual anemone consumption rates for a mean larval consumption rate. Because no larvae were lost in the controls, we performed a one sample t-test comparing our anemone predation rates to zero to determine if the anemones consumed significantly more than zero cryptonisci on average.

RESULTS

We surveyed a total of 1262 barnacles at Coal Oil Point. Their average density was 50.5 ± SE 10.4 barnacles per 25 cm^2. Of all barnacles surveyed at the site (n=370), 29% were ≤ 1 cm away from an anemone and thus associated with the anemone. On average, 39.7 ± SE 7.8% of the barnacles within the 25 cm^2 quadrat were ≤ 1 cm away from an anemone.

Barnacles associated with anemones were similar in size to barnacles not associated with anemones (t-test, p=0.25).

Barnacles associated with anemones were significantly less likely to be infected (Fisher's Exact Test, p<0.0001). Whereas 69.6% (87/125) of the barnacles not associated with anemones were infected, only 28.0% (35/125) of barnacles associated with anemones were infected. Thus, barnacles not associated with anemones were 2.5 times more likely to be infected (Fig. 2). Additionally, double infections differed with association with anemones (Fisher's Exact Test, p=0.0393). Near anemones, 2.9% of barnacles were infected by more than 1 parasite, compared to 22.5% of barnacles distant from anemones (n=1, n=16 respectively). Thus, multiple infections increased 7.7 times away from anemones, and double infections increased with increased prevalence.

When associated with anemones, barnacles were more likely to be actively reproducing (Fisher's Exact Test, p=0.0007). 23.2% of barnacles associated with anemones were actively reproducing (n=29) compared to only 7.2% of barnacles not associated with anemones (n=9). Thus, barnacles near anemones were 3.2 times more likely to be actively reproducing (Figure 2). However, among uninfected barnacles, the same fraction of individuals were actively reproducing, when adjacent or away from anemones (Fisher's Exact Test, p=0.4005).

The density of actively reproducing barnacles that were associated with anemones was not significantly different from barnacles that were not associated with anemones (t-test, p=0.46). Hence, while only 30% of the barnacles were associated with an anemone in the 25 cm^2 quadrats, the number of reproductive barnacles reproducing was equivalent between the two groups.

In laboratory trials, anemones consumed parasite transmission stages. On average, under these conditions, anemones consumed cryptoniscus larvae at a rate of 8.7 ± SE 1.3 per hour (n=3), while in the control treatment, no cryptonisci were lost (1 sample t-test, p=0.0136).

DISCUSSION

Barnacles associated with anemones had reduced parasitism and this resulted in higher reproduction, presumably due to predation on parasite transmission stages. In a community context this factor reduces parasitism at a local scale. Thus, anemones provide a spatial refuge for barnacles from parasites. It is possible that other factors result in the spatial patterns of parasitism we documented in the field. For example, it is possible that anemones settle in microhabitats inaccessible to parasite transmission stages. Similarly, it is possible that parasite transmission stages actively avoid areas colonized by anemones. However, parasites are delivered to hosts by water and we collected all barnacles from a narrow tidal height in the rocky intertidal zone with no obvious microhabitats. Further, because parasitism was reduced and not eliminated near anemones, at least some parasites do reach barnacles near anemones. It is also possible that parasitism was reduced near anemones because barnacles near anemones were of lower quality. For example, if barnacles adjacent to anemones were less likely to be actively reproducing as females, possibly due to competition with the anemone for food, they would be inappropriate hosts for a parasite that consumes ovarian fluid. However, we found increased reproduction in barnacles adjacent to anemones; thus, barnacles adjacent to anemones were compatible hosts, making reduced host quality an unlikely explanation. Further, barnacles next to anemones were similar in size to barnacles away from anemones, making size-based differences in host quality or compatibility unlikely. While genetic differences can drive patterns of resistance (e.g. Little and Ebert 2000) we find it unlikely that barnacles collected from a single site and a single tidal height are genetically distinct, though we can not exclude this possibility. Finally, barnacle density could be lower adjacent to anemones, reducing any signaling cue required by the parasite to find the host. However, previous work has demonstrated parasitism does not increase with density or aggregation of this barnacle host in the field (Fong 2016). Thus, we find predation by anemones to be the simplest explanation for the documented spatial patterns of parasitism.

Similarly to our results, Mouritsen and Poulin (2003) showed that anemones attached to bivalves in New Zealand tidal flats reduced infection of the bivalves by larval trematode parasites, presumably because the anemone consumed cercaria transmission stages, though this was not demonstrated. While only two field studies relate predator and parasite distributions (this and Mouritsen and Poulin 2003), laboratory trials generally corroborate our assessment that predation on parasites may control parasite transmission. Kaplan et al. (2009) found predation on trematode cercariae in the laboratory and detected this consumption in the field. Also, in laboratory trials, Prinz et al. (2009) found that barnacles were able to consume cercariae that infect bivalves, Schotthoefer et al. (2007) showed multiple species of stream invertebrates to consume cercariae that infect frogs, and Thieltges et al. (2008) used mesocosm experiments to determine the presence of a crab and shrimp species reduced parasitism in a bivalve host, and attributed this effect to the directly observed predation. Finally, Faust et al. (2009) found that in mesocosms, bivalves were able to filter avian influenza virions and reduce transmission of the virus to ducks. Thus, while there was evidence predators can consume parasite infectious stages, we are the first to provide evidence that predation may translate to fine-scale spatial patterns of parasitism in nature.

Multiple infections were substantially higher away from anemones, suggesting anemones may be quite effective at locally reducing parasite abundance and providing protection to the associated barnacles. A single castrator consumes all of the reproductive energy of the host; thus, multiple infections necessarily result in competition between the parasites (for review see Lafferty and Kuris 2009). As for parasitoids and predators, parasitic castrators face severe resource limitation with increasing prevalence and can approach saturation of a host population as uninfected hosts become unavailable. Kuris et al. (1980) found that multiple infections by an entoniscid isopod, a parasitic castrator of crabs, only became common at sites where >70% of the hosts were infected. The high incidence of multiple infections in the barnacles not associated with anemones suggests that the parasite may approach saturation of the available susceptible hosts at this particular site.

Barnacle reproduction varies substantially in space, and many studies have sought explanations for this spatial variation (e.g. Hines 1978, Leslie et al. 2005, Berger 2009, Freuchet et al. 2015). Despite their lower abundance near anemones, those barnacles appear to contribute reproductive productivity equivalent to the more numerous barnacles in the sampled quadrats that were relatively distant from anemones. Food availability (Leslie et al. 2005, Hines 1978), salinity gradient (Berger 2009), and temperature stress (Freuchet et al. 2015) all influence barnacle reproduction, both at regional and local scales. We show that parasitism can contribute strongly to these spatial patterns since *Anthopleura elegantissima* and *Chthamalus fissus* are both widespread, abundant and often co-occur at a fine scale in the rocky intertidal zone along the Pacific coast of North America. Thus, we suggest future studies examining factors influencing barnacle reproduction should consider parasitism. Additional factors that potentially influence the success of barnacles include the possible reduction in plankton available to barnacles near anemones, reducing growth or reproduction, and the possible differential predation on barnacle nauplii released from barnacles adjacent to or more distant from the anemones.

Additionally, the effects of diversity on disease are likely strongly depend on transmission and life history (Wood et al. 2014) and any emergent effect is likely a composite of multiple complex interactions. However, work like this study is crucial to arriving at generalizable patterns, such as a role for anemones, and potentially other generalist predators, in reducing disease by consuming infectious parasite stages and disrupting transmission.

Spatial epidemiology focuses on the description, quantification, and explanation for spatial differences in diseases. The rocky intertidal zone is a classic ecological system with high biodiversity. We suggest that this offers a powerful opportunity to study of spatial epidemiology. In many environments, movement of the host can decouple risk of infection from the subsequently observed patterns of infection (e.g. Byers et al. 2015). The rocky intertidal zone with its array of sessile organisms makes it an ideal system to evaluate drivers of spatial epidemiology. These relationships are durable due to the longevity of many of the space competitors, which will allow researchers to directly test the effects of these spatial associations on parasitism. Here, we initiate exploration of parasitism in this ecosystem and were able to correlate association with an anemone on parasitism, host reproduction, and hence, the potential fitness of the barnacles.

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Figure 1 Photo of barnacles and anemones at Coal Oil Point, Santa Barbara County, California at low tide. A group of anemones are outlined while the two arrows point out examples of barnacles considered to be associated with anemones (i.e., those <1cm away from an anemone).

Figure 2 Number of barnacles infected (I), uninfected but not actively reproducing (UI/NR), and uninfected and actively reproducing (UI/R) when associated (+A) or not associated (-A) with an anemone.

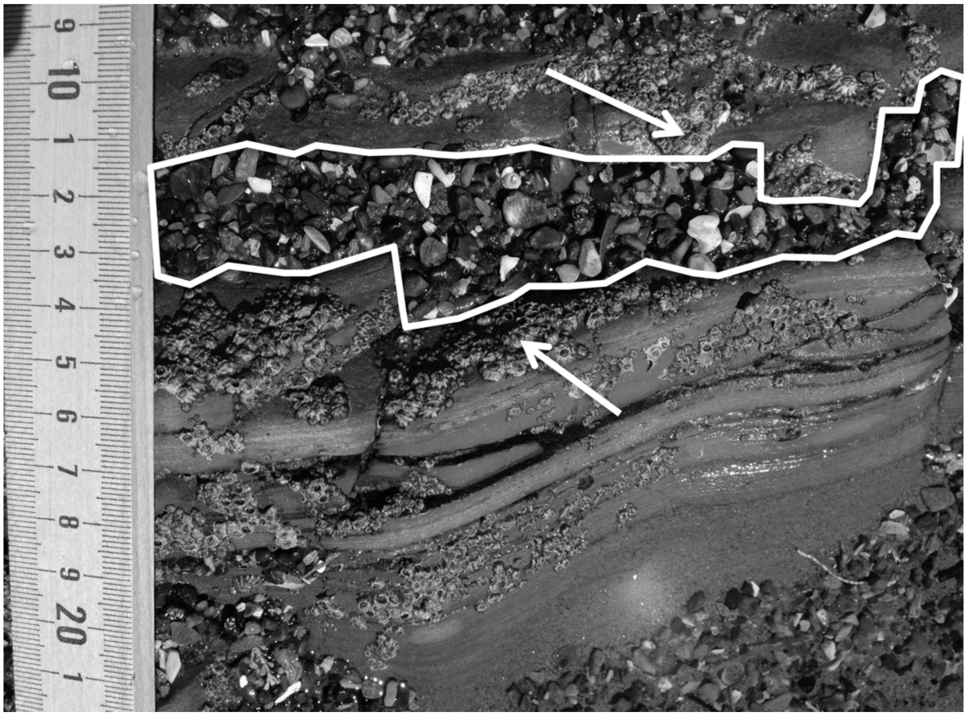

Figure 1

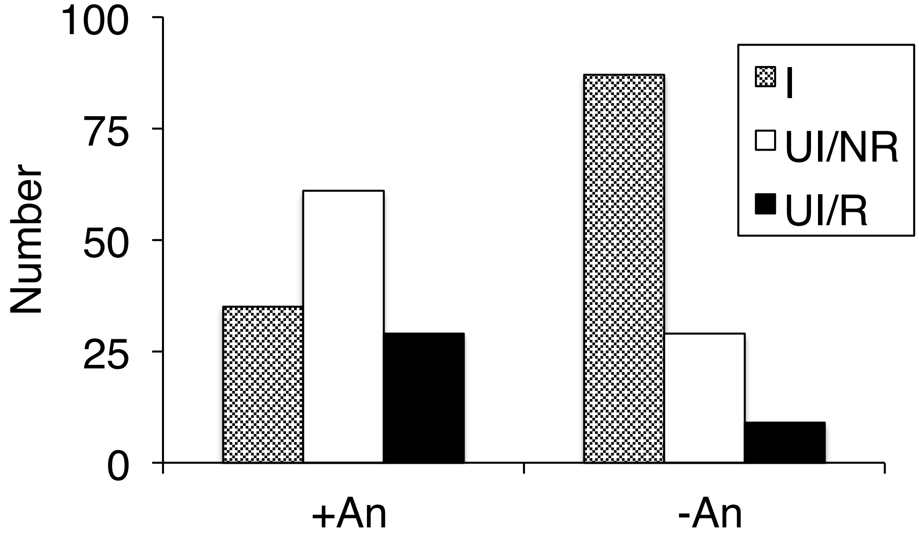

Figure 2