

**Shooting Blanks: Mate choice and determination in a
parasitically castrated snail, *Littorina littorea***

Honors Thesis
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Abstract:

The trematode parasite *Cryptocotyle lingua* completely castrates both male and female *Littorina littorea* snails that it infects. Selection should favor males who distinguish between fertile and parasitically-castrated females when choosing mates. I addressed this question by sampling mating pairs in the intertidal and those snails in their immediate vicinity. I compared the frequency of infection in females in mating pairs with that of ambient non-mating females to determine if the frequency of infection was significantly lower in mating females. In addition, to test the assumption that infected males were physiologically incapable of initiating copulatory behavior, I compared the infection prevalence of mating males with that of non-mating ambient males. I found that there was no significant difference in the infection prevalence between mating and non-mating females, indicating that males were not distinguishing between infected and uninfected females. This may be the case due to high levels of gene flow between highly infected and unexposed populations, insignificant costs associated with wasted mating attempts for males, or low heritability of the means for detecting infection in females. Infection prevalence in mating males was nearly significantly lower than that of non-mating males. However, the surprising frequency of infected males mating (15%) suggests that gonad health is not a pre-requisite for initiating copulatory behavior.

Introduction

Infections of the digenetic trematode *Cryptocotyle lingua* in the common periwinkle *Littorina littorea* persist in the gonad of the snail (and parts of the digestive gland), often resulting in complete castration (Stunkand 1930). Gonad infection is well-conserved across trematode species that inhabit mollusks and other taxa, and has been interpreted to be a response to selection for using host tissues that are nutritious and non-essential for survival (Baudoin 1975). Decreased survivorship of infected snails has been documented (Huxham et. al 1993, Lafferty 1993, Lauckner 1984), suggesting that the physiological effects of parasitism may extend beyond the reproductive system. Infections persist for the lifetime of the snail; the probability of infection increases with the size of the snail, presumably reflecting increased probability of exposure with increasing age (Hughes and Answer 1982, Pohley 1976). The castration of larger individuals results in the removal of the most fecund members of the population from the mating pool (Hughes and Answer 1982), thus potentially altering the

structure and output of the mating population in high-infection areas dramatically (Sindermann and Farrin 1962). The potential for selection on mating snails to discern between infected and uninfected individuals is theoretically high, and yet there has been very little direct investigation of behavioral modification in response to this selection pressure.

In New England, three common species of trematode parasite infect the mollusk *Littorina littorea*, with *Cryptocotyle lingua* being the most prevalent (Pohley 1976). *Cryptocotyle lingua* is a three-host parasite. Snails (*Littorina littorea*, *L. saxatilis*, and *L. obtusata*) ingest the host eggs, which hatch into rediae larvae in the digestive gland, which then migrate to the gonad and give rise to cercariae larvae. The cercariae are periodically shed into the water column where they infect their secondary fish host, forming metacercarial cysts on the skin around the fins. When the fish is ingested by a gull or other seabird, the sexual stage is released into the bird's gastrointestinal tract and becomes embedded. The eggs of the parasite are shed constantly in the feces of the bird, where they may later be ingested by the primary snail host, completing the cycle (Hughes and Answer 1982). Infection prevalence by *C. lingua* ranges from 0.7% to 47.4% in New England (Byers et. al 2006).; Prevalence is closely correlated to the abundance of gulls, which deposit feces in the intertidal zone (Galaktionov and Bustness 1999)

There are many theoretical costs associated with infection in addition to the direct cost of castration. While choosing larger females in areas of low infection prevalence would normally optimize male fitness, in areas of high infection prevalence larger females are more likely to be infertile. For any uninfected male or female, the potential costs of mating with an infected individual include the lost opportunity to mate with a fertile individual, lost foraging time, and risk of acquiring sexually transmitted diseases. As the "initiators" of copulation events, mate choice in response to these costs falls on the male, rather than the female (Saur 1990, Erlandsson

and Kostylev 1994). The strength of natural selection for mate choice will be proportional to the effects of these costs on fitness. However, whether selection will lead to avoidance of infected individuals depends on the spatial distribution of trematodes and the amount of gene flow among populations varying in trematode prevalence.

Evidence suggests that *Littorina littorea* is able to maximize fitness in areas of high trematode prevalence by altering trail-following behavior; during the mating season, males followed trails produced by uninfected females more often than trails of highly parasitized females (Erlandsson and Kostylev 1995). Davies and Knowles (2001) found the presence of trematode cercariae in trails repulsive to *L. littorea*. The guano of birds was also found to be repulsive, suggesting that behavioral modifications are present to avoid acquisition of the parasite through ingestion. These results are argued to be a signal that sensory adaptations have occurred for permitting infection avoidance: the ability to detect infection in a potential mate is therefore plausible. Male *L. littorina* spent more time copulating with larger or uninfected females than with smaller or infected females (respectively) in no-choice laboratory pairings, supporting possible mate preference through duration (Saur 1990). The method used here, in which nearby individuals are systematically collected around mating pairs, serves as a proxy for choice by allowing for a description of the size, gender, and infection status of all possible mates.

Previous field studies of reproductive behavior use observation of the male penis to verify copulation (Saur 1990, Paterson et al 2001). This fails to account for the fact that infected males often show a significant reduction in penis size when compared to their uninfected conspecifics (Sindermann and Farrin 1962) and could be overlooking many mating attempts by infected males. During collection of mating pairs for my experiment, no attempt was made to locate a penis; the mating position described by Patterson et al (2001) and the reluctance of the

top individual to release the foot were the only two criteria used to determine mating status. This was done to test the assumption that infected males are incapable of initiating copulatory behavior (Huxham et al 1993). It is my hypothesis that these assumptions are, at least in part, false.

Methods

This study was conducted June - August 2006 at the Shoals Marine Laboratory on Appledore Island in the Isles of Shoals, 10 km off the coast of Portsmouth, NH, USA. All collection sites were located on the NE shore of the island, which consists of protected rocky shores. I selected 12 intertidal pools just below the *Ascophylum* zone for my collection sites. On each of five collection dates, I sampled all pools using a standard collection protocol. I examined each pool from the south side to the north side marking the location of each mating pair. Each pair was picked up and examined to ensure that the two individuals were copulating. Pairs were considered copulating if males were observed in a stationary position, forward, and the right side of the female's shell with the shell-lip and mantle touching (Patterson et al 2001). Mating pairs were individually numbered, and the pool from which they originated was recorded.

A circular quadrat with a 10cm radius was centered over the location of each mating pair, and all *L. littorea* having a shell height greater than 15mm were collected. This size was chosen to ensure that all sampled snails were sexually mature, which occurs at a shell height of 12mm (Williams 1964). If more than one mating pair was present within a 0.1m radius of one another, I centered the quadrat over a point equidistant from all included mating pairs, and collected the non-mating individuals in the same manner as above. Sampled snails were held in labeled containers in seawater tables until they could be processed in the laboratory. Some snail mortality (~15%) occurred during the holding period. Only quadrats for which the mating

individuals and at least 80% of non-mating individuals were alive were included in the study (N=73). Quadrats whose data were used for population-level statistics were only represented once in the data set to avoid pseudoreplication. For gender specific mating behavior analysis, each mating individual had to be associated with their quadrat-level ambient snails to extrapolate correlative conclusions. For quadrats featuring multiple mating pairs, the ambient snail data was consequently included more than once.

Each snail was measured (shell height: Reid 1996) and dissected to determine sex and possible infection with *Cryptocotyle lingua* or other trematodes. Snails were sexed by identifying either a penis (males) or capsule gland (females). The gonad was separated from other tissues and homogenized in salt water solution. I examined the homogenate under a stereomicroscope and searched for cercariae and rediae of trematode parasites. If neither could be found, I assumed the snail was uninfected. If rediae or cercariae were found, I qualitatively categorized the extent of infection (minor or major) and determined whether the infection resulted from *Cryptocotyle lingua* or another species of trematode based on the diagnostic “eye spots” used in (see Huxham 1993).

For each mating individual, size, sex, and infection status (Y/N) were recorded along with the same information for its mate and all non-mating individuals within the same quadrat. This latter information was used to calculate local (quadrat-level) infection prevalence and density of each sex. Quadrats for which non-mating (ambient) individuals of the gender of interest totaled six or fewer were excluded from the data set, as were their mating partners. This was used as an arbitrary standard for accurate calculation of ambient infection prevalence. Following the above data regime, there were 51 mated females and 33 mated males for whom ambient infection prevalence could be accurately calculated.

A nominal logistic regression was used to determine whether a mating individual's infection status could be predicted by the ambient infection prevalence within a quadrat. Included in this model were several parameters in addition to the ambient infection prevalence of individuals of the same gender. The mating individual's shell height, that individual's shell height rank compared to other individuals in the quadrat, the density of other individuals of the same gender in the quadrat and total density within the quadrat, the month of collection, and the shell height of the mate were included as effects. In the female model, the infection status of the mate was also included in the model. This parameter was excluded in the male model because of strong co-variance between female shell height and infection status, resulting in non-convergence of results. After the models were analyzed, all effects with p-values greater than 0.4 were eliminated and the models were then reanalyzed. The final models included shell height of both individuals, the density of non-mating individuals and mating individuals, and the month of collection. A Fisher's exact test was used in addition to the nominal logistic regression to examine whether there was a significant difference in the number of mating individuals that were infected and uninfected. This test was performed separately for each gender using the same data set used for the logistic regression analysis. Jmp statistical computing software (v 5.1.2) was used for all statistical analyses, excluding chi-squares which were performed using an online chi-square calculator (<http://www.graphpad.com/quickcalcs/>).

Results

Population Patterns

The *Littorina littorea* sample included 160 mating and 735 non-mating individuals representing 71 quadrats. For quadrats that had at least 6 individuals and 80% survival rate, the mean total density was 13 snails per 0.62m² quadrat ($\bar{\sigma} = 0.98$), the average number of males per

quadrat was 4.44 ($\bar{\sigma} = 0.33$), and the average number of females was 8.18 per quadrat ($\bar{\sigma} = 0.76$). Prevalence of infection with *Cryptocotyle lingua* was 25.6% overall and differed almost significantly between mating (19.37%) and non-mating (26.9%) subpopulations (Fisher's Exact Test $p = 0.0571$). The infection prevalence in mating males was 13.2% and in mating females was 29.2%. Average male infection prevalence in a quadrat was 18% ($\bar{\sigma} = 2.66$) and for females was 29% ($\bar{\sigma} = 2.67$). A Fisher's Exact Test was used to determine whether there was a gender bias in infection prevalence, it showed that male infection prevalence (18.7%) was significantly lower than female infection prevalence (29.3%, $p = 0.0005$). A regression model was used to determine whether males were exhibiting positive assortative mating based on shell size. Indeed, the larger a mated male's shell height, the larger his female mate. (Figure 1, $p=0.0086$ $R^2=0.06$ $y = 0.24x + 16.04$).

There were five male-male copulation events and three female-female copulation events. The frequency of same-sex copulations for males was 17.9% and for females was 6.3%. A chi-square test was used to determine whether there was a difference in the infection status of same sex individuals compared to those pairing with the opposite sex. A chi-square test was also used to determine whether or not same-sex pairings were gender biased. Neither of these showed any significant difference ($\chi^2 = 0.7522$, and 2.5297 respectively).

Male Mating Patterns

There were 33 mating pairs that had at least six non-mating males within their quadrats. For these quadrats, there were 127 ambient non-mating males, at an average density of 14 snails/quadrat ($\bar{\sigma} = 6.14$). Average male density was 12.4 per quadrat and average female density was 19.0 per quadrat ($\bar{\sigma} = 4.5$ and 9.0 respectively). Of the 33 mating males, 5 (15%) were infected with *C. lingua*, non-mating males had an overall infection prevalence of 18.9%. For all sampled

males (n=315), there was a near significant difference in prevalence of infection between mating and non-mating individuals (Figure 2, Fisher's exact test; $p = 0.0783$). The infection status of the mating male was significantly predicted by quadrat infection prevalence in males ($p=0.001$), shell height (logistic regression: $p=0.048$), and mate shell height ($p=0.04$). A logistic regression model that included only the quadrat infection prevalence in males as an effect predicted the infection status of mating individuals with a p-value of 0.01.

Female Mating Patterns:

There were 51 mating pairs that had at least six non-mating females within their quadrats. There were 432 ambient snails in these quadrats, at an average density of 30/quadrat ($\bar{\sigma} = 13.2$); average density of ambient females was 20.4 per quadrat ($\bar{\sigma} = 9.0$) and males was 8.7 ($\bar{\sigma} = 3.9$). 28.1% of mating females were infected, 29.5% of non-mating females were infected. There was no significant difference between the infection prevalence of mating females and that of non-mating females (Figure 2, Fisher's exact test, $p = 0.8899$). Infection status of mating females was significantly predicted by quadrat infection prevalence in females ($p=0.0039$) and shell height ($p=0.0003$). A logistic regression model that included only quadrat infection prevalence in females predicted the infection status of mating females with a p-value of 0.0086.

Discussion

Female initiations were inferred from five female-female couples, indicating that the mounting behavior is not exclusively performed by males, though clearly this behavior serves little reproductive function given the natural physiology of the snails. The relationship between male size and female size agrees with the prevailing opinion that males mate assortatively according to size with a positive male and female size correlation (Hughes and Answer 1982, Saur 1990). The difference in infection prevalence between males and females is likely an effect

of differences in growth rates between the two genders which must be facilitated by differences in feeding rates, thus increasing female risk of infection (Mouritsen 1999). Infected males were seen attempting copulation in 5/33 mating events; all of these males were fully infected with no sign of a functional gonadal tissue. Infection prevalence in mating males was nearly significantly lower than that of the remainder of the population, indicating that trematode infection does reduce the frequency of mating attempts by males. However, infected males were involved in a surprising number of mating pairs. The apparent ability of infected males to initiate copulation argues that whatever physiological mechanism drives male mating behavior is still functional in parasitized individuals, though operating at a reduced capacity.

The hypothesis that males choose uninfected females to mate with was not supported by the data gathered here. If males are choosing to mate with uninfected females, it would be expected that trematode prevalence in mating females would be lower than the prevalence in non-mating females. Our data showed no difference in infection prevalence between mating and non-mating females, regardless of whether we used the local (quadrat) or whole-study prevalence. If males are not choosing, then the frequency of infected females mating would be a function of the frequency of infected females in some measure of population, be it a quadrat or pool. Infection prevalence in the snails surrounding a mating pair was not significantly different from the infection prevalence of mating females by two different scale measures and analyses. These results suggest that males are not choosing uninfected females as mates, but are instead mating indiscriminately.

This conclusion supports the hypothesis that local selection cannot lead to local adaptation in instances of planktonic dispersal and frequent gene flow between populations (Burton 1983, Curtis 1992, Curtis 1993, Hedgecock 1986, Lafferty 1993). *Littorina littorea* has

planktonic larvae that allow for wide dispersal and regular gene flow between populations (Gooch et al 1972, Berger 1977), making local adaptation unlikely (Curtis 1992, Burton 1983, Hedgecock 1986, Bertness and Gaines 1993). Trematode-snail interactions are so spatially complex that within a single island the infection prevalence of *C. lingua* within local populations of *Littorina littorea* can range from a high of nearly 50% to a low of 0.7% (Byers et. al unpublished). The strength of selection to avoid mating with infected individuals would be predicted to vary in with levels of infection prevalence. However, our results support the hypothesis that widely dispersing larvae would overcome local differences in selection pressure (Burton 1983, Berger 1977, Bertness 1993, Curtis 1992, Lively 1989).

Another possible explanation of these patterns could be that the opportunity costs and disease risk associated with mating with an infected individual are not large enough to impose strong selection pressure. The avoidance of cercariae in slime trails (Davies and Knowles 2001) and the shortened copulation period with infected females reported by Saur (1990) may be behavioral adaptations unrelated to mate choice, and instead could be attributed to infection risk avoidance or physiological barriers respectively. Differentiating between these two hypotheses will require measuring the costs associated with mating with infected individuals in the field. In the New Zealand mud snail (*Potamopyrgus antipodarum*), which becomes infected with castrating trematodes, it was also observed that males do not distinguish between infected and uninfected females (Neiman and Lively 2005). Those authors cite the possibility that the ability to detect infected females may not be a heritable trait. This and other unknowns (e.g., gene flow between populations) are difficult questions to answer, but pose interesting theoretical problems for biological modeling

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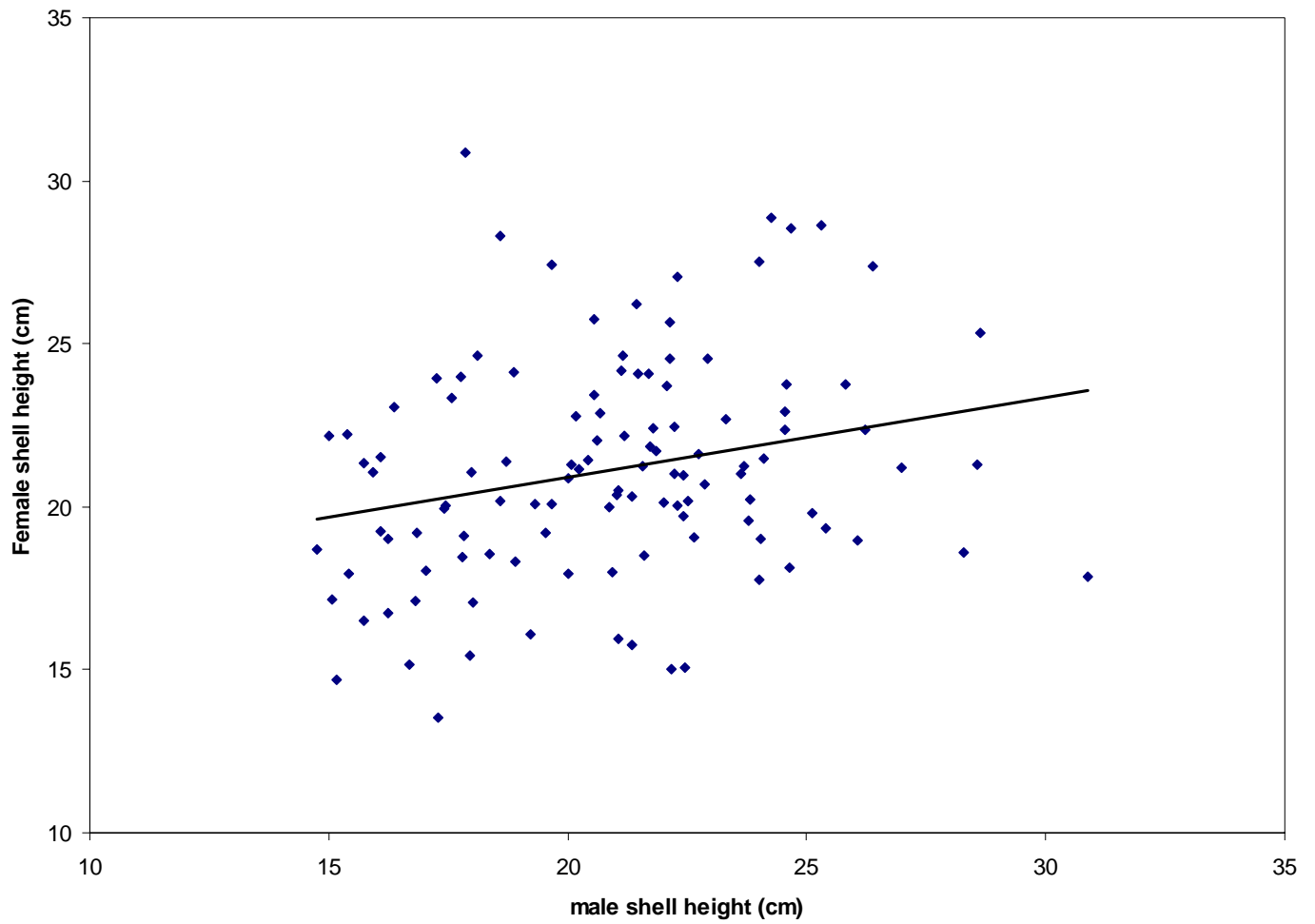


Figure 1: A regression showing the association between male and female shell height in mating pairs. $y = 0.2438x + 16.038$ $p=0.0086$ $R^2=0.06$

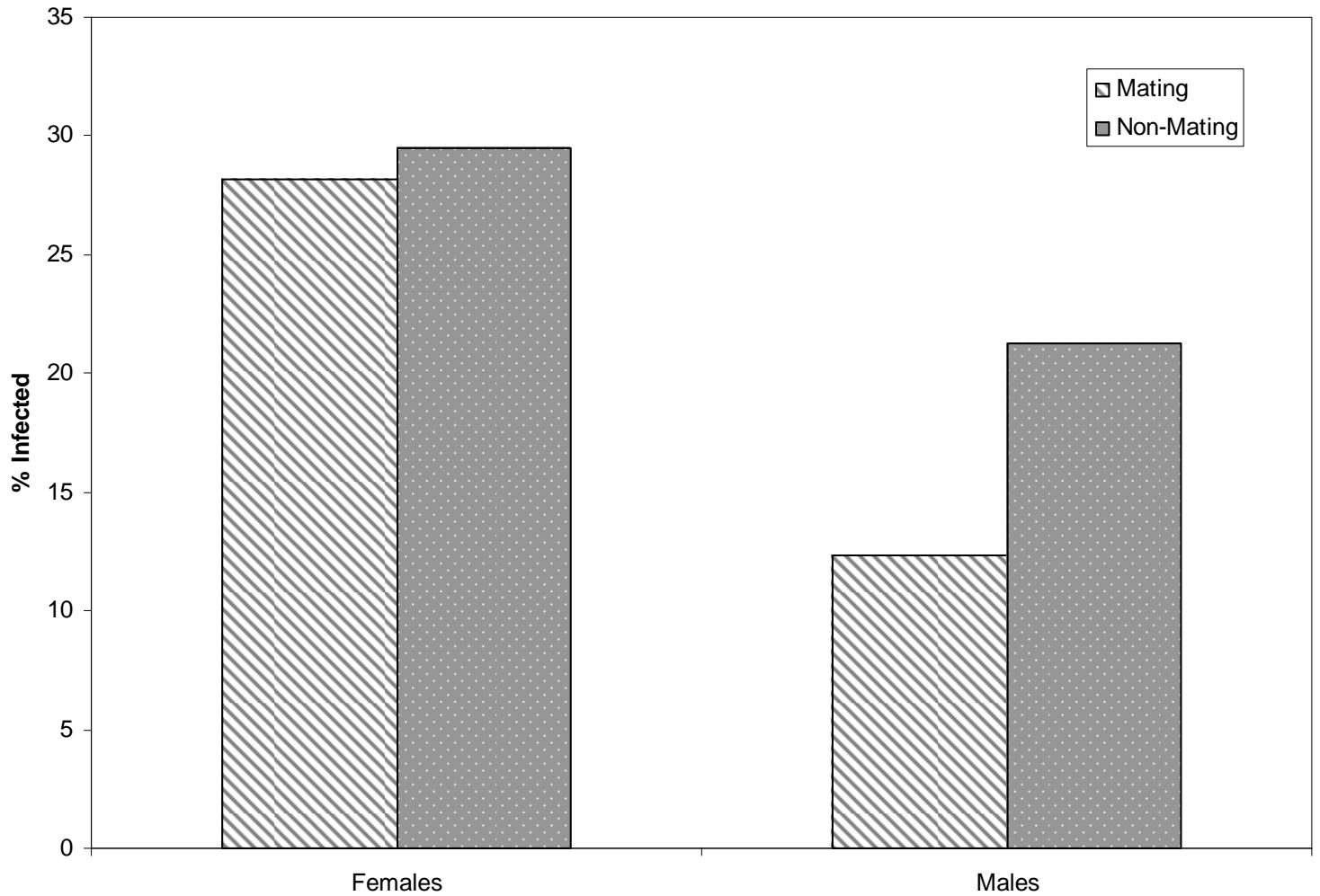


Figure 2: Infection prevalence of mating and non-mating *Litorina littorea* sorted by gender. The infection prevalence of mating and non-mating females was not significantly different ($p=0.8899$), but was nearly significantly different in males ($p=0.0783$).