PREDATION AVOIDANCE RESPONSE BEHAVIORS, OVIPOSITION AND DISTRIBUTION OF THE INTERTIDAL GASTROPOD *LIRULARIA SUCCINCTA*

by

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THESIS ABSTRACT

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The small trochid gastropod *Lirularia succincta* occurs in rocky intertidal habitats along the Pacific coast of North America. Strong escape responses of adult *L. succincta* were elicited by the predatory seastars *Leptasterias hexactis* and *Pycnopodia helianthoides* but not by the nonpredatory seastar *Henricia* sp. Escape responses to juvenile *L. hexactis* were not observed in newly-hatched *L. succincta.* The snails exhibited weak avoidance responses to water-borne chemical stimuli from *L. hexactis*. The vertical distribution of a population of *L. succincta* was described, and changes in the size-frequency distribution of the population in the spring and summer were documented. Finally, factors that may affect oviposition in *L. succincta* were investigated in the laboratory. The snails deposit egg masses year round with a peak in reproductive output in the summer. In the laboratory and in the field, egg masses are preferentially deposited in crevices.

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CHAPTER I

GENERAL INTRODUCTION

While in the rocky intertidal late one night during a negative tide, I noticed that on almost all of the rocks that I picked up there were several small, beautiful snails. I collected a few individuals and brought them back to the lab. I identified them as *Lirularia succincta* (Carpenter, 1864), and a quick search showed me that little information on the life history and ecology of the species has been published (e.g. Hadfield and Strathmann, 1990). I decided to investigate different aspects of the ecology of this small (<5 mm) trochid.

Lirularia succincta is commonly found on cobbles and loose rocks throughout the rocky intertidal. Its geographic distribution may be highly patchy; while they are quite common in Cape Arago State Park in Oregon, they are uncommon in the rocky intertidal of Cape Blanco, 40 miles to the south.

Another species commonly found in the same microhabitats as *Lirularia succincta* is the predatory sea star *Leptasterias* spp. While their distributions appear to overlap to some extent, *L. succincta* are not commonly found under the same rocks as *Leptasterias* spp. In fact, when searching in the intertidal for snails for my experiments, I noticed that if I found a *Leptasterias* individual in one of the small tidepools, I wasn't likely to find *L. succincta* in the same pool. A similar observation was made by Bullock (1953) when observing responses of gastropods within a tidepool to water from a *Pycnopodia helianthoides* or *Pisaster ochraceus* that he dripped into the pool. After the sea star-scented water was dripped into the pool, the gastropods increased their activity and soon crawled out of the pool. I wondered if *Leptasterias* spp. individuals were

predators of *L. succincta.* If so, had *L. succincta* evolved defensive behaviors enabling it to evade predators such as *Leptasterias* spp.?

Phillips (1977) described two types of behaviors that snails exhibit in response to predators: escape responses, which are elicited by direct contact with a predator, and avoidance responses, exhibited after a prey species detects a predator from a distance. In both cases, predators emit a chemical cue that is detected by chemoreception, a common method gastropods use for gathering information about their environment (reviewed by Kohn, 1961; Croll, 1983). Escape and avoidance responses have been documented in many different species (Mauzey et al., 1968; Helfman, 1986; Semlitsch and Reyer, 1992; Kusch, 1993), and there is abundant literature documenting escape and avoidance responses of gastropods to predators such as crabs (Jacobsen and Stabell, 1999; Cotton et al., 2004) and sea stars (Bullock, 1953; Fishlyn and Phillips, 1980; Espoz and Castilla, 2000).

In Chapter II of this thesis, I describe and quantify the escape and avoidance responses of *Lirularia succincta* to predatory sea stars. Phillips (1976) and Fishlyn and Phillips (1980) have demonstrated that gastropods exhibit escape or avoidance responses to predatory sea stars such as *Pisaster ochraceus, Pycnopodia helianthoides,* and *Leptasterias* spp. that they encounter in the intertidal, but not to the nonpredatory sea star *Patiria miniata*. In Chapter II, I test the prediction that *L. succincta* would respond to the predatory sea stars *Leptasterias* spp. and *P. helianthoides,* but not to the nonpredatory *Henricia* spp. While escape responses of adult prey species to their predators have been investigated in many gastropods, few studies have investigated escape responses of juveniles of the same species to predators. Rochette et al. (1996) found that juveniles of the whelk *Buccinum undatum* did not exhibit the same intensity of escape responses to the predatory sea star *Leptasterias polaris* as did conspecific adults. Harvey et al. (1987) found that juvenile *B. undatum* exhibited weaker escape responses to *L. polaris* than adult individuals. I

tested the hypothesis that newly-hatched juvenile *L. succincta* do not exhibit the same escape responses to *Leptasterias* spp. as do adult *L. succincta* in Chapter II.

Gendron (1977) documented seasonal changes in the distribution of *Littorina littorea* in the intertidal, and concluded that the changes were the result of the snail's active migration. In Chapter III, I focus on the distribution of *Lirularia succincta* in the rocky intertidal in a wave-protected site and document changes in the distribution of the snail throughout the year. I also look at the size-frequency distributions of L. succincta at four tidal levels through the spring and summer. The tidal height selected by gastropods can vary with size (Gendron, 1977). Paine (1969) described the ontogenetic migration of *Chlorostoma (Tegula) funebralis* into lower parts of the intertidal after 5 or 6 years. From size-frequency distributions, Toyohara et al. (1999) found an increase in the number of new recruits of *Lirularia iridescens* that coincided with a decrease in the number of snails in the larger size class, possibly indicating that the snail has an annual lifespan. Other aspects of *L. succincta* ecology can be inferred and hypotheses made for future studies once the spatial and size-frequency distributions of the snail are known.

One of the factors affecting the distribution of a species is the dispersal of its larvae. *Lirularia succincta* has no larval dispersal because it has direct development, i.e. embryos hatch from an egg mass as fully-metamorphosed juveniles. Encapsulated development ensures less time exposed to planktonic predators in the water column (Rumrill, 1990). Females may select appropriate sites for oviposition that will optimize the survival of embryos in the egg mass and of juveniles after they hatch, ensuring that they start life in suitable habitats (Hendler and Franz, 1971). Oviposition site preference has been shown for amphibians (Caldwell, 1986), insects (Meadows and Campbell, 1972; Wiklund, 1981), and some marine gastropods (Biermann et al., 1992) but the literature on oviposition site selection is scarce compared to the numbers of species that deposit benthic egg masses (Resetarits, 1996). For species that deposit egg masses, the site of oviposition can affect the mortality of embryos within the mass if the mass is deposited in

locations with high physical stress (Biermann et al., 1992). When the species undergoes direct development, the oviposition sites of the females will determine the distribution of the juveniles (Meadows and Campbell, 1972). The timing of oviposition can affect the mortality of the juveniles as well, as food source availability, temperature, and salinity may change throughout the year (Toyohara et al., 1999).

Lirularia succincta is an ideal species to use for studying oviposition behaviors. Throughout the year, adults deposit egg masses soon after being collected and brought into the laboratory. Chapter IV examines some of the conditions under which *L. succincta* will deposit egg masses. This thesis provides a framework for further investigations of a snail that is too large to be studied by microgastropod experts and too small to be noticed by anyone else.

CHAPTER II

ESCAPE AND AVOIDANCE RESPONSES OF *LIRULARIA SUCCINCTA*

Introduction

Predator-prey interactions have long been recognized as capable of structuring natural communities. Interactions with predators can cause animals to develop defensive behaviors, as has been reported for several diverse phylogenetic groups including ciliates (Kusch, 1993), birds (Maloney and McLean, 1995), amphibians (Semlitsch and Reyer, 1992), fish (Helfman, 1986; Magurran, 1990) and marine invertebrates (Mauzey et al., 1968; Feder, 1963). Kats and Dill (1998) have written an extensive review of defensive behavioral responses across many phyla. Defensive behaviors may be learned (Helfman, 1986; Maloney and McLean, 1995; Rochette et al., 1998) or inherited (Semlitsch and Reyer, 1992; Rochette et al., 1996), but even inherited behaviors may be modified in response to encounters with predators (Magurran, 1990; Kusch, 1993; Kats and Dill, 1998).

Prey species that do not exhibit defensive behaviors often make up a larger proportion of a predator's diet than their abundance would suggest (Bullock, 1953; Kusch, 1993) while successful defensive responses can effectively remove an organism from the diet of a predator (Feder, 1963; Mauzey et al., 1968; Fishlyn and Phillips, 1980). Feder (1963) noted that the limpet *Lottia scabra*, which lacks defensive responses to the predator *Pisaster ochraceus,* was not the most abundant limpet in the asteroid's habitat, but was the most prevalent limpet in the predator's diet. The defensive behaviors of prey may also affect the apparent preferences of predators in the field. Phillips (1977) notes the difficulty in discriminating between actual

predator preferences and the apparent predator preferences that result after defensive responses are expressed by prey species. He suggested that prey species exhibiting defensive behaviors may be rare in a predator's diet because of the predator's food preferences. However, these preferences may result from the predator's selection of prey that do not exhibit defensive responses.

Defensive response mechanisms vary as much as the organisms that express them. Responses can be chemical (Fishlyn and Phillips, 1980; Bryan et al., 1997) or physical (Mauzey et al., 1968; Kent, 1981) in nature. Predators can induce changes in morphology (Kusch, 1993) or behavior (Semlitsch and Reyer, 1992; Maloney and McLean, 1995). Responses can be dependent on habitat (Mauzey et al., 1968), on season (Jacobsen and Stabell, 1999) and on previous experiences with predators (Magurran, 1990; Maloney and McLean, 1995; Rochette et al., 1998). Defensive responses also have varying degrees of success (Fishlyn and Phillips, 1980). A diverse array of defensive responses have been observed in marine invertebrates including anemones, urchins and sea stars (Mauzey et al., 1968), crustaceans (Glynn, 1980), scallops and brittle stars (Feder, 1963) and especially in marine gastropods (Kohn, 1961; Feder, 1963; Menge, 1972; Phillips, 1977; Bryan et al., 1997; Jacobsen and Stabell, 1999; Espoz and Castilla, 2000; Cotton et al., 2004). Most marine gastropods exhibit similar avoidance and escape responses. Classic predator-induced responses include "mushrooming," where the snail elevates its shell over its foot (Bullock, 1953; Feder, 1963; Fishlyn and Phillips, 1980), shell rotation or twisting (Feder, 1963; Bryan et al., 1997), tentacle waving (Feder, 1963; Fishlyn and Phillips, 1980) falling off of a vertical surface or narrow surfgrass blade (Bullock, 1953; Fishlyn and Phillips, 1980), negative geotaxis or "crawl out" responses (Bullock, 1953; Jacobsen and Stabell, 1999) and increased general activity. A few gastropods also respond by withdrawing into their shells (Kohn, 1961; Bryan et al., 1997) or becoming immobile (Feder, 1963). The predominant behavioral defense for marine gastropods appears to be flight (Kohn, 1961; Feder, 1963; Fishlyn

and Phillips, 1980; Kent, 1981; Bryan et al., 1997; Cotton et al., 2004). Occasionally, marine gastropods may supplement or replace the defensive behaviors described above with other less common behaviors. For example, after contact with a predator, the whelk *Buccinum undatum* exhibits a leaping behavior following the release of mucus (Rochette et al., 1996). Two small gastropods, *Alia carinata* and *Amphissa columbiana,* strike or "bite" the tube feet or radial nerves of predatory sea stars with their proboscis (Fishlyn and Phillips, 1980; Kent, 1981; Braithwaite et al., 2010). The top shell snail *Calliostoma canaliculatum* releases a chemical defensive substance from its hypobranchial gland (Bryan et al., 1997) and the olive snail *Callianax biplicata* burrows into sand (Phillips, 1977). These behaviors likely contribute to the observation that gastropods with defensive responses are not eaten in the field in proportion to their abundance (Mauzey et al., 1968; Menge, 1972).

In marine ecosystems, defensive behaviors are often triggered by water-borne chemical cues. For gastropods, chemoreception is the primary mode of detecting distant objects including food or prey, mates, or predators (Kohn, 1961; Croll, 1983). Chemosensory cells on the surface of a gastropod's foot and tentacles, and an osphradium near the siphon collect chemical stimuli from its environment (Croll, 1983). Chemical cues allow marine gastropods to obtain information about their environment that can enable predatory gastropods to find nearby prey (Kohn, 1961) and enable prey species to assess predation risk from a distance (Kats and Dill, 1998; Rochette et al., 1998). Using chemoreception to gather information about the risk of predation, including the magnitude of the danger, enables prey species to assess the benefits of exhibiting defensive behaviors and to adjust the intensity of the response (Helfman, 1986; Kats and Dill, 1998). When a predator is detected, the benefits of exhibiting defensive responses are undeniable, as effective defensive behaviors increase chances of survival. Many studies have demonstrated the importance of chemoreception in gastropod escape responses (Bullock, 1953; Kohn, 1961; Croll, 1983; Kats and Dill, 1998; Jacobsen and Stabell, 1999).

Many of the defensive responses described above are elicited when a gastropod comes in contact with chemicals released from the tube feet of predatory sea stars, but contact by a predator is not always necessary to induce a response (Feder, 1963). Phillips (1977) divided chemically-mediated defensive responses into two categories: escape responses, displayed when a gastropod comes in direct contact with a predator; and avoidance responses, elicited by chemicals diffusing through the water from a distant predator (see also Bullock, 1953). For example, if water from a *Pisaster ochraceus* or *Pycnopodia helianthoides* individual drips into a tidepool, gastropods will move out of the pool (Feder, 1963). *Lacuna marmorata* exhibits escape responses including shell rotation and tentacle waving when it is 9 cm away from the predatory sea star *Leptasterias polaris*. In the laboratory, 100% of *L. marmorata* exhibit the same responses in predator-scented water (Fishlyn and Phillips, 1980).

Some species only exhibit escape responses after direct contact with a predator and appear to have no avoidance responses. *Calliostoma canaliculatum* combines escape responses with a form of chemical defense, but appears unable to detect the predator eliciting those responses from a distance (Bryan et al., 1997). *Alia carinata* and *Amphissa columbiana* strike predators with their proboscis while exhibiting escape responses, but the responses are only invoked after the predator has touched the snail's foot. The predator can touch the shell of the snail without inducing a response (Fishlyn and Phillips, 1980; Kent, 1981; Braithwaite et al., 2010). Fishlyn and Phillips (1980) hypothesized that the absence of avoidance responses in some species may be due to the fact that their alternate defensive mechanisms are effective enough to allow them to ignore the predator until escape becomes necessary.

Many gastropods exhibit both escape and avoidance responses to the same species of predatory asteroid (Menge, 1972; Fishlyn and Phillips, 1980; Espoz and Castilla, 2000), but for some of these species, the behaviors exhibited after contact and after detection from a distance differ (Feder, 1963; Phillips, 1976, 1977). For these species, the distance of the gastropod from

the sea star can determine the set of responses that will be employed. For example, the most common escape response of *Lacuna vincta* to *Leptasterias polaris* was falling, while the most common avoidance response was shell rotation and flight (Fishlyn and Phillips, 1980). For species that exhibit both an avoidance and escape response, the risk inherent with living in the same area as a potential predator can be greatly diminished (Phillips, 1976).

Many studies indicate that gastropods are able to differentiate between predatory and non-predatory asteroids; gastropods that exhibit escape responses to predatory sea stars rarely exhibit escape responses to non-predatory sea stars (Phillips, 1977; Fishlyn and Phillips, 1980; Harvey et al., 1987; Rochette et al., 1996; Espoz and Castilla, 2000). For example, the defensive responses of *Lacuna* and *Alia* are generally not elicited by sea stars that don't prey on mollusks (Fishlyn and Phillips, 1980). In some cases, defensive responses may only be elicited by predatory sea stars that are naturally encountered in a gastropod's habitat (Bullock, 1953; Phillips, 1976). Gastropods can also differentiate between the odors of different species of sea stars (Bullock, 1953; Phillips, 1976, 1977), even when they are in the same genus (Phillips, 1976), and the escape or avoidance behaviors can be species-specific (Bryan et al., 1997).

Chemical cues released by injured conspecifics may also elicit avoidance responses in some species, as gastropods may associate these signals with predation (Kohn, 1961; Jacobsen and Stabell, 1999). These cues potentially induce the avoidance responses if they are used by the gastropod to recognize predators that have been feeding on conspecifics (Hadlock, 1980; Jacobsen and Stabell, 1999).

Lirularia succincta is a small (<5 mm) intertidal gastropod found on rocky shores from northern Mexico to the Gulf of Alaska. This gastropod is widely distributed throughout the intertidal, probably foraging on microalgae that cover cobbles, loose rocks and macroalgae. The distribution of *L. succincta* overlaps that of predatory sea stars such as *Pisaster ochraceus*, *Leptasterias* spp. and *Pycnopodia helianthoides.* While all three sea stars may be potential

predators of *L. succincta,* the six-rayed sea stars in the *Leptasterias* species complex (hereafter referred to by the genus name, *Leptasterias*) likely feed on *L. succincta* to the greatest extent. The morphology of *Leptasterias* spp. individuals makes them well-suited for life on cobbles and loose rocks in the intertidal; its flexible rays allow it to move into small crevices and cracks, and its long, agile tube feet wave back and forth as it moves, extending its range of capture (Fishlyn and Phillips, 1980; personal observation). When *Leptasterias hexactis* occurs in the rocky intertidal, it is known to be an important predator of small gastropods and *Balanus* spp., which are its preferred diet (Menge, 1972; Niesen, 1973). Due to its small size, it is likely that *Leptasterias* individuals are more common predators of *L. succincta* than other sea stars with overlapping distributions. Both species are rarely found on the same rocks, even when populations are high, which could be an indication of avoidance or escape responses of *L. succincta* to *Leptasterias* spp. individuals.

The objectives of this study were: (1) to describe the escape response behaviors of *Lirularia succincta* to the predatory sea star *Leptasterias* spp.; (2) to determine the specificity of any exhibited escape responses by observing the responses of *L. succincta* to two other species of sea stars, one predatory (*Pycnopodia helianthoides*) and one non-predatory (*Henricia* sp.); (3) to determine if the escape responses of *L. succincta* to *Leptasterias* spp. differ when the *Leptasterias* individuals is too small to pose a threat to the snail; (4) to determine whether escape responses are exhibited by newly-hatched *L. succincta*; and (5) to determine whether *L. succincta* can detect waterborne odors from a predatory sea star or alarm signals potentially released from injured conspecifics. Both avoidance and escape responses will be described in this study, with primary emphasis on escape responses. The results from these studies on predator-prey interactions between *Leptasterias* and *L. succincta* suggest potential structuring in the intertidal communities to which these species belong.

Materials and Methods

Snail Collection and Maintenance

Lirularia succincta were collected from North Cove, Cape Arago State Park, Oregon (43° 18' 31" N, 124° 23' 55" W) between July of 2010 and February of 2011 (Figure 2.1). *Lirularia succincta* not used immediately for an experiment were kept in 3.7 L jars filled with seawater. The jars were kept in a cold room at temperatures ranging from 9 to 13°C. The seawater was changed every other day. All *L. succincta* were used in experiments within 2 weeks of being brought into the laboratory.

Shell diameters of *L. succincta (*i.e. the maximum width from the lip of the aperture to the opposite body whorl) were measured with dial-type vernier calipers to the nearest 0.1 mm as described by Frank (1975). Adult snails with shell diameters greater than 2 mm were used in most experiments, though one experiment was conducted using newly-hatched snails with diameters of approximately 300 µm.

Sea Star Collection and Maintenance

Leptasterias, members of the *Henricia* species complex (referred to throughout this Chapter as *Henricia*), and *Pycnopodia helianthoides* were also collected from North Cove, Cape Arago State Park, Oregon between July of 2010 and February of 2011 (Figure 2.1). Adult *Leptasterias* or small *Henricia* or *P. helianthoides* (individuals with mean arm lengths > 7 mm) were kept in plastic containers with large openings cut out and screened with plastic mesh. Mean arm length was measured as the mean of the distance from the center of the disc to the tips of the four longest arms. The containers were partly submerged in a seawater table with flowing seawater. *Leptasterias* and *P. helianthoides* were fed once each week to satiation with *L. succincta* or with littorine snails collected from North Cove.

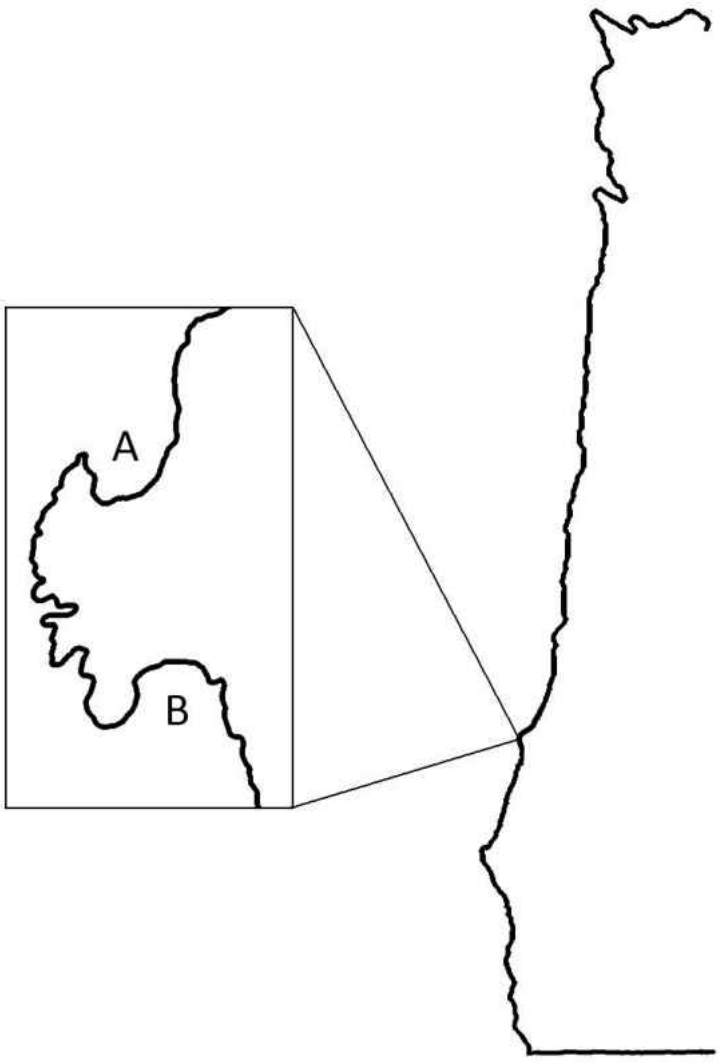


Figure 2.1. Location of study sites on the southern Oregon coast. (A) North Cove, and (B) South Cove of Cape Arago State Park.

Juveniles (defined as individuals with mean arm lengths < 3 mm) of *Leptasterias* and *Henricia* were collected from North Cove between May and July of 2010 and were kept in the laboratory in 3.7 L jars filled with seawater. The jars were kept in a cold room at temperatures ranging from 9 to 13^{o}C, and the seawater was replaced every 2 days. At Cape Arago State Park,

Niesen (1973) reported that *Balanus glandula* was the primary prey for juveniles of *Leptasterias hexactis* (84 to 91% of prey), while *Spirorbis* spp. (4 to 6.6%) and *Littorina scutulata* (2 to 6%) were the next most common prey. Juveniles of *Leptasterias* were also observed in the laboratory feeding on *L. succinct* with diameters less than 3 mm (pers. obs.). The diet of juvenile *Henricia* is unknown, but adult *Henricia leviuscula* feed on bacteria and other small particles, and may feed on sponges and bryozoans (Morris et al., 1980). Therefore, several cobbles from North Cove were placed in jars containing juveniles of *Leptasterias* and *Henricia*. The cobbles selected had barnacles, bryozoans, sponges, or spirorbid worms on them. The cobbles were replaced once or twice during each spring tide. In the autumn and winter, *L. succincta* were added once each week for the *Leptasterias* juveniles to feed on.

Laboratory Feeding Rate of *Leptasterias*

The feeding rate of *Leptasterias* on *Lirularia succincta* was calculated during three monitoring periods in the lab. For the first monitoring period, *Leptasterias* individuals were starved for eight days prior to the study. Eleven *Leptasterias* individuals (mean arm length= 17.4 ± SD 8.1 mm) were placed into eleven separate 250-mL beakers with 150 mL of seawater. Eight *L. succincta* were added to each beaker. The beakers were kept for 12 hours in a cold room at 10^{o}C and checked each hour. When an individual had been eaten and its shell was discarded by the sea star, the shell was removed and replaced with a living snail. After 12 hours, the *Leptasterias* individuals were left in their beakers with eight snails for an additional 10 hours. After 10 hours, the number of snails eaten by the *Leptasterias* individuals was recorded.

For the second monitoring period, the *Leptasterias* individuals were starved for 11 days prior to the feeding experiment. Eight *Leptasterias* individuals (mean arm length= 16.9 ± SD 7.0 mm) were placed into each of eight separate 100 x 80 mm culture dishes (Pyrex #3250) in 250 mL of seawater. Eight *Lirularia succincta* were placed in each dish. The dishes were kept for 26

hours in a cold room at 10°C. The dishes were checked after 4, 8, 10, 24, and 26 hours. When snails had been eaten and the asteroid had discarded the shells, they were removed, measured and replaced with living snails.

For the third monitoring period, the feeding rates of juvenile *Leptasterias* were examined. Six *Leptasterias* juveniles (diameter= 6.0 ± SD 1.1 mm) were starved for eight days prior to the experiment. Each *Leptasterias* juvenile was placed into a separate 150 mL beaker with 100 mL of seawater. Ten *Lirularia succincta* were added to each beaker. When a *L. succincta* individual was eaten and its shell was discarded, the shell was removed, measured and replaced with a living snail of the same size.

For all monitoring periods, the feeding rates were calculated as the number of snails eaten per hour.

Escape Responses of *Lirularia succincta* to Three Sea Stars

I quantified the intensity of responses of actively crawling *Lirularia succincta* to tactile stimuli from two predatory sea stars, *Leptasterias* spp. and *Pycnopodia helianthoides,* and a non-predatory sea star, *Henricia* spp., in five experiments. In each experiment, two stimuli were compared: (1) *Leptasterias* and a control stimulus, (2) *Leptasterias* and *Henricia*, (3) *Henricia* and a control stimulus; (4) *P. helianthoides* and a control stimulus, and (5) *Leptasterias* and *P. helianthoides.* In the controls, a blunt metal probe was touched to the head and cephalic tentacles of *L. succincta* individuals. For each sea star stimulus, the sea star was moved towards the *L. succincta* until the tip of the arm with tube feet touched the head and cephalic tentacles of the snail. In each experiment, all *L. succincta* were exposed to both of the stimuli. For example, *L. succincta* in the first experiment were exposed to direct contact with both *Leptasterias* and a control stimulus.

Each snail in each experiment was randomly assigned to one of two treatments. Half of the snails from the first treatment were exposed to the first stimulus (for example, the tube feet of *Leptasterias*); half of the snails from the second treatment were then exposed to the second stimulus (for example, the control stimulus). This was repeated until all snails from each treatment had been exposed to one of the stimuli. The snails were then exposed to the opposite stimulus. For example, snails in the first treatment were exposed to the control stimulus and snails in the second treatment were exposed to the tube feet of *Leptasterias.* Snails experienced the second stimulus 2 hours after contact with the first stimulus.

For each trial, each snail was placed in a plastic tray (40 x 25 x 6 cm) filled with 2 L of seawater. The snail was allowed to right itself with the foot extended on the bottom and its cephalic tentacles protruding out from under its shell. Once the snail had exhibited sustained movement for 30 seconds, the distance it traveled in 15 seconds was marked in pencil on the bottom of the tray. Immediately after the distance had been marked, the contact stimulus was applied. The behavior of each *Lirularia succincta* was recorded for 30 seconds following the stimulus. Immediately after the stimulus, the distance the snail traveled in 15 seconds was marked on the bottom of the tray. Preliminary studies showed that snails never slowed down until they had crawled for at least 15 seconds.

After the experiment, the plastic tray was dried, and the pencil marks indicating the distances and directions of movement, before and after each stimulus, were traced onto a sheet of acetate film. The length of each path was measured and the speed of the snail before and after the stimulus was calculated. The angle of the change in each snail's direction of movement immediately after the stimulus was also measured. Because I was interested in the magnitude of the angle change, or the amount of turning following the contact stimulus, and not in the final direction of movement, I measured the total angle of change in the snail's direction of movement. Therefore, angular values in all of the following experiments represent total change in direction.

The final values were unbounded (it was possible to have angular values >360^{o}); for example, a snail that turned completely around 1.5 times was given a value of 540^{o}. The difference in a snail's speed before and after the stimulus, and its angle of change in direction, were used to quantify the intensity of each snail's response to each stimulus.

For all experiments, the angle by which the snail's direction of movement was altered was compared for both stimuli between treatments with a two-way repeated measures analysis of variance (ANOVA) with Treatment (the order in which the stimuli were applied to both groups of snails) and Stimulus as factors. Because angular values were unbounded, linear statistics were used for the analysis. The change in speed between treatments for both stimuli was also analyzed with a two-way repeated measures ANOVA with Treatment and Stimulus as factors. If an analysis resulted in an interaction between treatment and stimulus, the responses to the two stimuli were compared within treatments using paired t-tests.

Behavioral responses were classified into categories (Table 2.1). Because a normal distribution of responses was not likely for the behavioral scores, the numbers of responses in each category were compared between stimuli for each experiment using Kruskal-Wallis tests.

Leptasterias

The first experiment investigated responses of *Lirularia succincta* to encounters with *Leptasterias.* The experiment was conducted in September of 2010. Contact trials were performed on 32 individual snails, with each snail exposed to tube feet from *Leptasterias* and a metal probe (control) stimulus. The predator stimulus was applied with a randomly-chosen *Leptasterias* (mean arm length= 26.6 ± SD 6.2 mm; n=3).

Table 2.1. Behavioral response scores used to evaluate the responses of *Lirularia succincta* to contact with adult and juvenile predatory sea stars, non-predatory sea stars and control stimuli.

Response Score	Behavior of *L. succincta*
0	No response
1	Complete retraction; immobile throughout the observation period
2	Pull back or vigorous waving of cephalic and epipodial tentacles
3	Pull back and vigorous tentacle waving
4	Pull back and/or tentacle waving, shell rotation
5	Pull back and tentacle waving, multiple shell rotations

Leptasterias and *Henricia*

The second experiment compared responses of *Lirularia succincta* to tactile stimuli from either a predatory sea star (*Leptasterias*) or a non-predatory sea star (*Henricia*). The *Henricia* sp. individuals used for this experiment (randomly selected from three; mean arm length= 17.4 ± SD 2.3 mm) were similar in size to the *Leptasterias* individuals (randomly selected from three; mean arm length= 23.2 ± SD 1.4 mm). Contact trials were performed on 26 individual snails in September of 2010.

Henricia

The purpose of the third experiment was to compare the responses of *Lirularia succincta* to a non-predatory sea star, *Henricia* (mean arm length= 9.6 ± SD 3.6 mm; n=2), and a control stimulus. There were 16 snails in each treatment. The experiment was conducted in February of 2011.

Pycnopodia helianthoides

The fourth experiment quantified the intensity of the responses of *Lirularia succincta* to encounters with a different predatory sea star, *Pycnopodia helianthoides* (mean arm length= 12.5 ± SD 1.7 mm). Contact trials were performed in February of 2011 on 32 individual snails, with each snail exposed to tube feet from *P. helianthoides* and a control stimulus.

Leptasterias and *Pycnopodia helianthoides*

The fifth experiment compared responses of *Lirularia succincta* to tactile stimuli from two predatory sea stars, *Leptasterias* (randomly selected from 3; mean arm length= 23.2 ± SD 1.4 mm) and *Pycnopodia helianthoides* (randomly selected from 2; mean arm length= 19.0 ± SD 4.7 mm). The experiment was conducted in September of 2010 with contact trials performed on 24 snails.

Escape Responses to Juvenile Sea Stars

The purpose of this experiment was to ascertain if juvenile sea stars elicit escape responses in *Lirularia succincta*. *Lirularia succincta* were randomly assigned to one of three treatments, with individuals in each treatment exposed to a different stimulus. In the first treatment, the head of the snail was gently brushed with the arm of a juvenile *Leptasterias* (randomly selected from four; mean diameter= 3.8 ± SD 1.0 mm) held in front of the snail with forceps. In the second treatment, the head of the snail was gently brushed with the arm of a juvenile of *Henricia* (randomly selected from 2; mean diameter= 3.0 ± SD 0.7 mm), also held in front of the snail with forceps. The heads of *L. succincta* in the third treatment (control) were touched with a metal probe. There were 25 snails in each treatment. In this experiment, individual snails were used only once as test objects and each snail experienced only one stimulus.

Contact experiments were conducted in the same manner as the first experiments. Experiments were conducted in the same plastic trays (40 x 25 x 6 cm) filled with 2 L of seawater. The change in speed and angle of change in its direction of movement of each snail after the stimulus were measured in the manner described above.

The change in speed following the contact stimulus, and the angle by which the snail's direction of movement was altered were compared among treatments with one-way ANOVAs with Bonferroni *post-hoc* comparisons. The angle data were square-root transformed. The data for both the change in angle and speed violated the assumptions of homogeneous variance so I lowered the α to 0.025 (Gamst et al., 2008).

Behavioral responses were classified into the categories described in Table 2.1. The numbers of responses in each category were compared among treatments using a nonparametric Kruskal-Wallis test, followed by nonparametric *post hoc* multiple comparisons if necessary.

Escape Responses of Newly-Hatched *Lirularia succincta*

The next experiment was conducted to describe escape responses, if present, in newly-hatched juvenile *Lirularia succincta* elicited by contact with a juvenile predator, *Leptasterias.* Four egg masses were deposited by *L. succincta* adults in four 60 x 15 mm disposable polystyrene petri dishes in August of 2010. The dishes were filled with filtered sea water (FSW) and kept in a cold room at 10^{o}C. The FSW was changed every three days. After 12 days, the juveniles began hatching from the egg masses. A juvenile *Leptasterias* (diameter= 4mm) was used as the predator stimulus, and the end of a sterile Pasteur pipet was used as the control stimulus. The responses of the newly-hatched juvenile *L. succincta* to the juvenile *Leptasterias* were observed under a dissecting microscope. Using fine-tipped forceps, the juvenile *Leptasterias* was placed into the FSW of the petri dish until its tube feet were extended. A single tube foot was then gently brushed against the anterior region of 15 juveniles crawling in each of

the four dishes (n=60). The response of the juvenile *L. succincta* to the juvenile *Leptasterias* was recorded. After 15 juveniles in a dish had been touched with the tube foot of the juvenile *Leptasterias*, the pipet tip was gently brushed against the anterior region of 15 separate juvenile *L. succincta* crawling in each dish (n=60) and the responses of the *L. succincta* juveniles to the pipet were recorded. The numbers of each type of response were compared between treatments to determine if the frequency of each type of response was independent of treatment. This was analyzed with a chi-squared analysis of a contingency table.

Avoidance Responses to Chemical Stimuli from a Predator

The purpose of this experiment was to document the presence of avoidance behaviors in *Lirularia succincta* as a response to water-borne chemical cues potentially released by the predatory sea star *Leptasterias.* Specimens of *L. succincta* and *Leptasterias* were collected in December of 2010. The experiment was conducted three days after collection.

This experiment was done using a choice chamber similar to that described by Kohn (1959). The chamber (30.5 x 12.5 x 8 cm) was made of Plexiglas with a Plexiglas partition 22 cm in length partially separating the two halves of the chamber (Figure 2.2). Fresh seawater was admitted to the chamber equally through two inflow tubes. Fluorescein dye was used to ensure that there was minimal mixing of water at the end of the chamber before it exited through the valve and to verify that the seawater in both sides was moving at the same velocity of 1.25 $cm \cdot sec^{-1}$. Water moving along the bottom of each side was flowing at a much lower velocity (0.04 $cm \cdot sec^{-1}$).

The bottom of each side of the chamber was subdivided into five equally-sized, numbered sections. Square 5 was closest to the water inflow, upstream of where the *Lirularia succincta* was placed at the beginning of each trial (in Square 3) (Figure 2.2). The *Leptasterias*

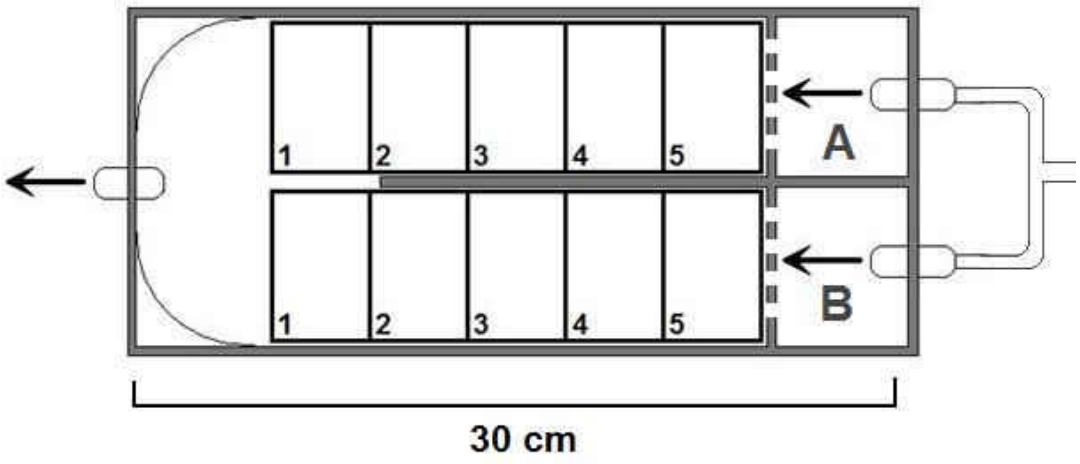


Figure 2.2. Choice chamber for detection of predation avoidance responses of *Lirularia succincta.* Lines were marked on the floor of the chamber as shown. During an experiment, fresh seawater flowed through the chamber through two inflow tubes. Water velocities were the same on both sides of the chamber. The predator was placed into one of the chambers (A or B) while the other chamber was left empty. *L. succincta* were placed into the middle of Square 3 in both arms at the beginning of each trial.

used in the experiment had a mass of 8.03 g (blotted wet weight) and a mean arm length of 5.1 ± SD 0.9 cm. It was placed near the water inflow, separated from the rest of the choice chamber by Plexiglas that had been drilled with numerous holes. An identical area in the opposite side of the choice chamber was empty, serving as the control. The *Leptasterias* was placed in the chamber with the water flowing over it for 20 minutes before beginning the experiment. This was to ensure its scent had traveled the length of the chamber along the bottom where the snails would be crawling, and where the flow was 0.04 $cm \cdot sec^{-1}$. All snails were then randomly assigned to one of two groups: placement into the side with no *Leptasterias* present (n=40 snails), or placement into the side arm with *Leptasterias* present (n=40 snails). One snail from each group was removed from the well plate and was placed into each side of the choice chamber in the center of Square 3. After 4 minutes, the numbered square to which they had moved was

recorded. In preliminary trials, 4 minutes was the time it took for snails to reach the area downstream of Square 1. The height each snail had climbed up the side of the choice chamber was also measured to the nearest mm. Individual snails were used only once as test objects.

After ten trials, the *Leptasterias* was taken out of the small chamber. The walls of both arms were scrubbed in fresh water and rinsed with seawater. The *Leptasterias* was then put into the small chamber in the opposite arm and left for 20 minutes before resuming the experiment. This was repeated after every 10 trials for 40 trials (n=40 for each treatment).

The horizontal position of the snails in both treatments was categorized as their position on the numbered piece of Plexiglas (location in Squares 1 through 5). The number of snails in each square after 4 minutes was compared between treatments using a chi-squared analysis of a contingency table, where the null hypothesis was that the horizontal position of snails was independent of treatment. A chi-squared goodness-of-fit test was used instead of a goodness-of-fit G-test because chi-squared goodness-of-fit tests are less prone to Type I errors (Zar, 2010). The mean height of the snails on the wall of the choice chamber after 4 minutes was compared between treatments using a Kruskal-Wallis test, as the data violated the assumption of normality.

Avoidance Responses to *Leptasterias* and Injured Conspecifics

The purpose of this experiment was to further document avoidance responses of *Lirularia succincta* to *Leptasterias.* Jacobsen and Stabell (1999) found that gastropods can detect chemical alarm substances released by conspecifics, and that the presence of these cues can affect avoidance behaviors that are induced by a predator. To ascertain if such chemicals are released and detected by *L. succincta*, the chemical stimuli used for the experiment consisted of filtered extracts of crushed *L. succincta* in addition to water conditioned by *Leptasterias.* FSW was used as a control stimulus. The stimuli were prepared 24 hours prior to the experiment. To prepare the predator-conditioned water, seven *Leptasterias* individuals (mean arm length= 14.3 ± SD 3.4

mm; mean blotted wet weight= 0.78 ± SD 0.63 g) were rinsed in FSW and placed into a jar containing 300 mL of FSW. An air hose was placed into the jar, which was placed in a cold room and kept at 11°C. After 18 hours, the sea stars were removed from the jar, and the water was filtered through a 125-µm mesh and poured into a clean 100 x 80 mm culture dish. The extract of crushed *L. succincta* was prepared by crushing 35 snails (0.63 g blotted wet weight) in a 150 mL beaker containing 100 mL of FSW, using a pair of clean artery forceps. The beaker was then placed in a cold room at 11°C. After 1 hour, the contents of the beaker were then filtered through a 125-µm nylon mesh into a clean 150 mL beaker. 300 mL of FSW (control) was put into a 100 x 80 mm culture dish and placed in the cold room at 11°C for 1 hour.

The responses to the stimuli were measured in one of three compartments (15 x 8.5 x 3 cm) in a plastic tray. Each compartment was filled with 300 mL of FSW. In a single series, one snail was placed into each of the three compartments of the tray. The three snails were then randomly assigned to receive one of the three stimuli. One snail was exposed to extracts of injured conspecifics, one snail was exposed to water conditioned with *Leptasterias*, and one snail was exposed to FSW. Snails were used only once, and each snail was exposed to only one stimulus. All three stimuli were used in each series. Twenty-five series of testing, with three snails per series, were conducted (n=25 for each of three treatments).

In all experiments, single *Lirularia succincta* were placed at the center of the compartment at the beginning of the trial. Individuals that had not begun moving after 5 minutes were not used in the experiment. Once a snail had begun to move, a pencil was used to mark the distance the snail had traveled for 15 seconds. Then, a 1.5 mL sample of one of the stimuli was squirted approximately 1.5 cm in front of the snail using a 5000 µL variable-volume micropipet. Immediately after the introduction of the stimulus, the distance the snail traveled for 15 seconds was again marked, and the snail's behavior for 30 seconds was observed and recorded. After all

three snails had been exposed to their respective stimuli, their shell diameters were measured. The compartments were rinsed with fresh water followed by FSW after each series of tests.

After the experiment the plastic tray was dried and the paths of each snail from before and after each stimulus were traced onto a sheet of acetate film. The length of each path was measured, as was the angle of change in each snail's direction of movement after the stimulus in the manner described for the first experiments. These measurements were again used to quantify the intensity of the snail's response to each stimulus. The mean change in speed before and after the stimulus and the mean angle of change in the direction of movement for snails in all three treatments were compared using two one-way ANOVAs. The angle data were square root transformed to meet the assumption of normality and six data points were randomly selected from the predator and injured conspecifics treatments to be excluded from the analysis to make even numbers among treatments.

Behavioral responses were classified into the categories used in Table 2.1. The numbers of responses in each category were compared among treatments using a Kruskal-Wallis test with nonparametric multiple comparisons when necessary.

Results

Observations on Anti-Predator Behavior

In the laboratory, both motionless and actively crawling *Lirularia succincta* exhibit a stereotyped gastropod response to a predatory sea star. The responses were quick, occurring immediately after the contact stimulus. During normal locomotion, the snail moves forward with the cephalic and epipodial tentacles extended and waving slightly. Common defensive responses of *L. succincta* evoked by its predator *Leptasterias* include shell rotation, vigorous waving of epipodial and cephalic tentacles, turning more than 90^{o} and increased locomotor activity. The most common escape response was rapid movement away from the point of contact with the

predator. Control stimuli in all experiments generally resulted in a brief retraction of the tentacles followed by a brief pause before normal locomotion resumed. Escape movements were never observed in response to control stimuli or *Henricia*. Occasionally both contact and sea star stimuli caused animals to become immobile for the duration of the behavioral observation period, and this cessation of movement was frequently accompanied by increased tentacle waving. Occasionally, and only when coming in direct contact with a predator stimulus, *L. succincta* exhibited foot contortions causing the snail to roll or somersault away from the predator. After this behavior was expressed, the snail immediately righted itself and increased its crawling rate.

In the laboratory, snails on vertical surfaces were frequently observed falling in response to contact with the tube foot of a *Leptasterias.* As Fishlyn and Phillips (1980) describe this as a common escape response for two species of gastropods, it is likely that this is another behavior in the array of escape responses of *Lirularia succincta* to *Leptasterias.*

Laboratory Feeding Rate of *Leptasterias*

In the first monitoring period, starving *Leptasterias* individuals were able to eat an average of 0.17 ± SD 0.11 *Lirularia succincta* individuals per hour over a 12 hour period. One sea star was able to capture and eat 5 snails in the 12-hour period. Following this experiment each *Leptasterias* was left in its beaker with 8 snails overnight for 10 hours. After 10 hours they had eaten an average of 0.23 ± SD 0.19 snails per hour, with one sea star eating 5 snails in the 10 hour period.

In the second monitoring period when the *Leptasterias* individuals were starved for three more days prior to the experiment and more snails were placed into the jar, the adult *Leptasterias* individuals ate an average of 0.24 ± SD 0.08 *Lirularia succincta* individuals per hour, with 2 individuals eating 9 snails in a 26-hour period. The mean size of *L. succincta* successfully captured and eaten by *Leptasterias* was 3.0 ± SD 0.4mm. Half of the snails eaten had diameters

less than 3mm. There is no correlation between feeding rate and the size of *Leptasterias* (Pearson's r=0.400, p=0.326).

In the third monitoring period, starving *Leptasterias* juveniles ranging from 4.5 to 7.5 mm in diameter were able to eat up to four *Lirularia succincta* individuals in a 48-hour period, with a mean feeding rate of 2.0 ± SD 1.5 snails. 67% of the snails eaten were between 1.7 and 2.2 mm, but the juvenile sea stars were able to eat snails with a diameter of up to 3.5 mm. The average size of *L. succincta* that were successfully captured and eaten by the juvenile *Leptasterias* was 2.2 ± SD 0.7 mm. In the field, a *Leptasterias* individual with a diameter of 7.7 mm has been observed attempting to eat a *L. succincta* individual with a diameter of 3.3 mm.

Escape Responses of *Lirularia succincta* to Three Sea Stars

Lirularia succincta exhibited specificity in their responses to the three species of sea stars; classic escape responses were elicited by *Leptasterias* and *Pycnopodia helianthoides,* but not by *Henricia*, which is not a molluscan predator.

The summary tables for the repeated measures ANOVA for all five experiments comparing the four stimuli are contained in Table 2.2 for measurements of changes in speed and in Table 2.3 for measurements of changes in the angle of direction of movement. The assumption of normality was violated for the analysis of the changes in speed from the first experiment and the changes in angle from the second experiment. The analyses were still conducted, as repeated measures ANOVAs are robust for normality violations. Angle data in the first, third and fourth experiments were square-root transformed to meet the assumptions of equal variance and normality (Table 2.3). From one to five data points in each experiment were randomly selected to be excluded to ensure even numbers for the analyses. Mean responses to the stimuli in each experiment are listed in Table 2.4.

Table 2.2. Repeated measures ANOVA tables testing the change in speed of *Lirularia succincta* after exposure to one of four contact stimuli: the tip of an arm of a *Leptasterias*, the tip of an arm of a small *Henricia*, the tip of an arm of a small *Pycnopodia helianthoides,* or the tip of a blunt metal probe. Treatment refers to the order in which the stimuli were received.

		df	SS	MS	F	P
Leptasterias vs. probe						
Between Subjects	Treatment	1	0.48	0.48	17.98	0.0002
	Residual	30	0.80	0.03		
Within Subjects	Stimulus	1	0.77	0.77	17.61	0.0002
	TreatxStim	1	0.35	0.35	8.05	0.0081
	Residual	30	1.30	0.04		
Leptasterias vs. *Henricia*						
Between Subjects	Treatment	1	0.05	0.05	0.69	0.4144
	Residual	23	1.54	0.07		
Within Subjects	Stimulus	1	3.41	3.41	65.31	<0.0001
	TreatxStim	1	0.19	0.19	3.58	0.0712
	Residual	23	1.20	0.05		
Henricia vs. probe						
Between Subjects	Treatment	1	0.00	0.00	0.00	0.9653
	Residual	24	1.08	0.04		
Within Subjects	Stimulus	1	0.00	0.00	0.13	0.7224
	TreatxStim	1	0.01	0.02	0.24	0.6284
	Residual	30	1.96	0.07		
P. helianthoides vs. probe						
Between Subjects	Treatment	1	0.06	0.06	0.77	0.3858
	Residual	30	2.42	0.08		
Within Subjects	Stimulus	1	1.21	1.21	16.53	0.0003
	TreatxStim	1	0.10	0.10	1.37	0.2511
	Residual	30	2.20	0.07		
Leptasterias vs. *P. helanthoides*						
Between Subjects	Treatment	1	1.34	1.34	9.17	0.0066
	Residual	20	2.91	0.14		
Within Subjects	Stimulus	1	0.04	0.04	0.63	0.4354
	TreatxStim	1	0.05	0.05	0.76	0.3937
	Residual	20	1.40	0.07		

Table 2.3. Repeated measures ANOVA tables testing the change in the angle of the direction of movement of *Lirularia succincta* after exposure to one of four contact stimuli: the tip of an arm of a *Leptasterias*, the tip of an arm of a small *Henricia*, the tip of an arm of a small *Pycnopodia helianthoides,* or the tip of a blunt metal probe. Treatment refers to the order in which the stimuli were received.

		df	SS	MS	F	P
Leptasterias vs. probe*						
Between Subjects	Treatment	1	14.92	14.92	2.89	0.1005
	Residual	27	139.29	5.16		
Within Subjects	Stimulus	1	587.73	587.73	162.18	<.0001
	TreatxStim	1	2.29	2.29	0.63	0.4340
	Residual	27	97.84	3.62		
Leptasterias vs. *Henricia*						
Between Subjects	Treatment	1	9718.52	9718.52	3.59	0.0713
	Residual	22	59521.79	2705.53		
Within Subjects	Stimulus	1	68176.69	6581.25	32.93	<.0001
	TreatxStim	1	3451.02	58.17	1.67	0.2101
	Residual	22	45548.79	2070.40		
Henricia vs. probe*						
Between Subjects	Treatment	1	3.26	3.26	0.29	0.5944
	Residual	24	268.47	11.19		
Within Subjects	Stimulus	1	24.41	24.41	1.32	0.2617
	TreatxStim	1	3.46	3.46	0.19	0.6690
	Residual	24	443.30	18.47		
P. helianthoides vs. probe*						
Between Subjects	Treatment	1	0.11	0.11	0.01	0.9039
	Residual	20	148.44	7.42		
Within Subjects	Stimulus	1	199.25	199.25	15.11	0.0009
	TreatxStim	1	0.82	0.82	0.06	0.8051
	Residual	20	263.79	13.19		
Leptasterias vs. *P. helanthoides*						
Between Subjects	Treatment	1	7700.63	7700.63	6.97	0.0166
	Residual	18	19882.65	1104.59		
Within Subjects	Stimulus	1	75.63	250.00	0.14	0.7156
	TreatxStim	1	5593.23	6708.10	3.68	0.0712
	Residual	18	32837.90	1824.33		

* Data were arcsine transformed

Table 2.4. Mean values for responses of *Lirularia succincta* to four different stimuli: an arm of *Leptasterias,* an arm of a small *Pycnopodia helianthoides,* an arm of a small *Henricia*, or the tip of a blunt metal probe. Two types of responses were measured for each stimulus type: the change in the snail's speed immediately following the stimulus (measured in $mm \cdot sec^{-1}$), and the angle of change in the snail's direction of movement.

	Leptasterias	**Metal probe**	**N**
Angle turned	134.5	27.06	32 snails
SD	47.4	21.14	
Change in speed	0.32	0.10	
SD	0.27	0.15	
	Leptasterias	***Henricia***	**N**
Angle turned	126.6	65.7	26 snails
SD	47.1	70.5	
Change in speed	0.49	-0.67	
SD	0.30	0.17	
	Henricia	**Metal probe**	**N**
Angle turned	69.72	46.66	32 snails
SD	72.25	46.88	
Change in speed	-0.06	-0.04	
SD	0.24	0.21	
	P. helianthoides	**Metal probe**	**N**
Angle turned	133.3	61.6	32 snails
SD	63.5	61.6	
Change in speed	0.18	-0.09	
SD	0.30	0.25	
	Leptasterias	***P. helianthoides***	**N**
Angle turned	118.0	117.63	24 snails
SD	44.8	36.78	
Change in speed	0.39	0.49	
SD	0.88	0.39	

Lirularia succincta turned a significantly greater amount when exposed to the *Leptasterias* than when exposed to the metal probe for snails in both treatments (Table 2.3, Figure 2.3A). The change in speed directly following exposure to the arm of *Leptasterias* was significantly greater than the change in speed following the probe stimulus, but there was a significant Treatment x Stimulus interaction (Table 2.2, Figure 2.3B). In the first treatment, snails were contacted with the tube feet of *Leptasterias* before the control stimulus was applied, and there was not a significant difference in the change of speed following contact with either stimulus ($t = 1.259$, $p = 0.227$). Snails in the second treatment were exposed to the metal probe stimulus first with a resulting mean change in speed that was significantly lower than the change in speed after contact with the arm of *Leptasterias* ($t = 4.178$, $p = <0.001$). When the responses of *L. succincta* to the tube feet of *Leptasterias* were compared between treatments with a one-way ANOVA, there was also a significant difference in the response to *Leptasterias* between treatments ($F=17.580$, $p<0.001$). Overall, the probe stimulus elicited very little response in *L. succincta*, while the tube feet of *Leptasterias* evoked a definite turning response and increased locomotor activity in one of the treatment groups.

Similar results were observed when responses of *Lirularia succincta* were compared between stimuli from *Leptasterias* and *Henricia* (Table 2.4, Figure 2.4). Tube feet from the *Henricia* elicited weak escape responses, if any, in *L. succincta*, while the tube feet of *Leptasterias* induced strong escape responses in both treatments (Table 2.4, Figure 2.4). The mean change in speed after encountering a stimulus from *Leptasterias* was significantly greater than the change in speed of *L. succincta* after experiencing the stimulus from the *Henricia* (Table 2.2, Figure 2.4A). There was also a significant difference in the turning responses between the two stimuli (Table 2.3, Figure 2.4B). *Lirularia succincta* in both treatments responded to the arm of *Leptasterias* by turning on average twice as far as they turned after being exposed to the arm of

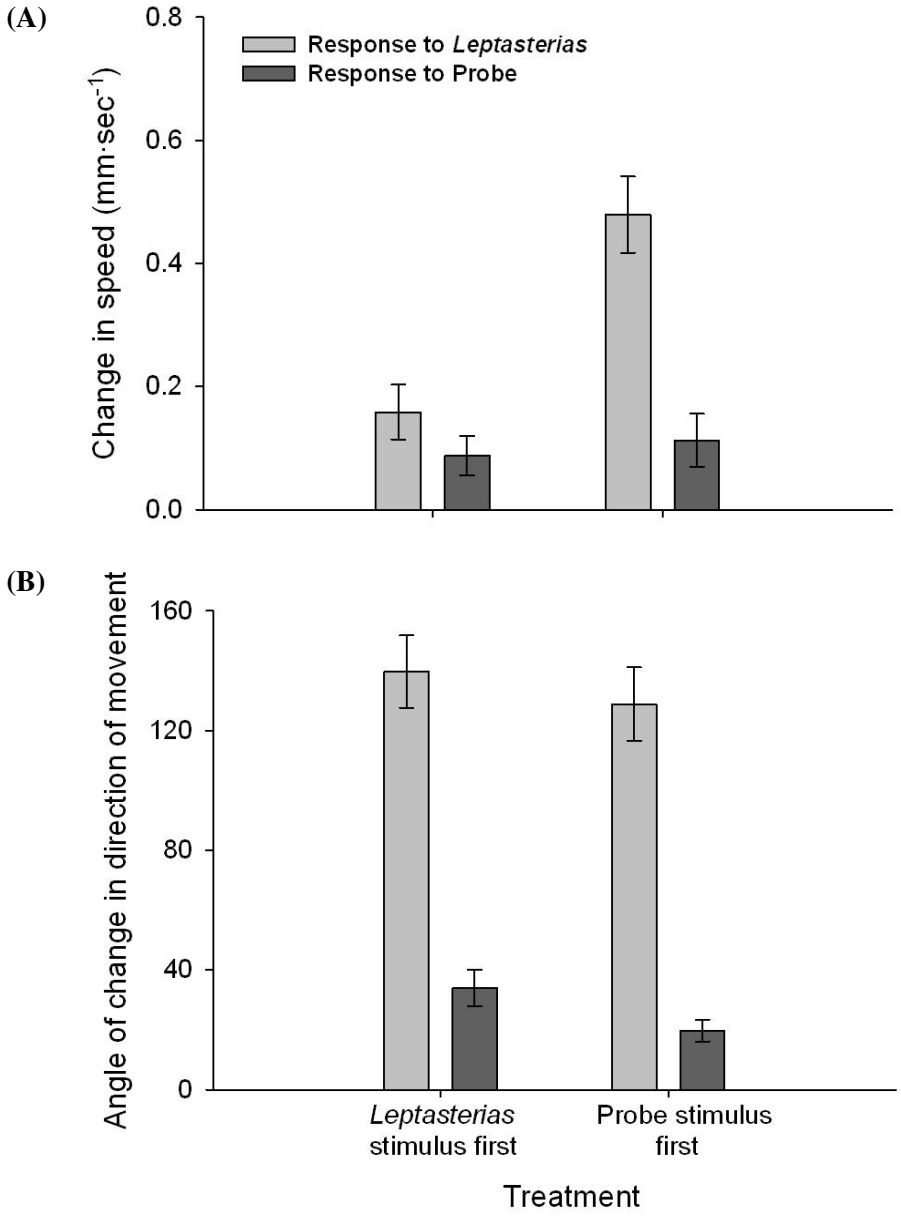


Figure 2.3. Responses of *Lirularia succincta* to an arm of *Leptasterias* and a metal probe. Snails in the first treatment (n=16) received a stimulus from a *Leptasterias* followed by a control stimulus (metal probe). Snails in the second treatment (n=16) were exposed to the probe followed by *Leptasterias*. (A) The change in the speed of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli ($F=17.61$, $p=0.0002$) and there is a significant Stimulus x Treatment interaction ($F=8.05$, $p=0.0081$). (B) The angle of change in the direction of movement of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli ($F=162.18$, $p<0.0001$) and there was no interaction ($F= 0.63$, $p=0.4340$). Error bars represent 1 SE.

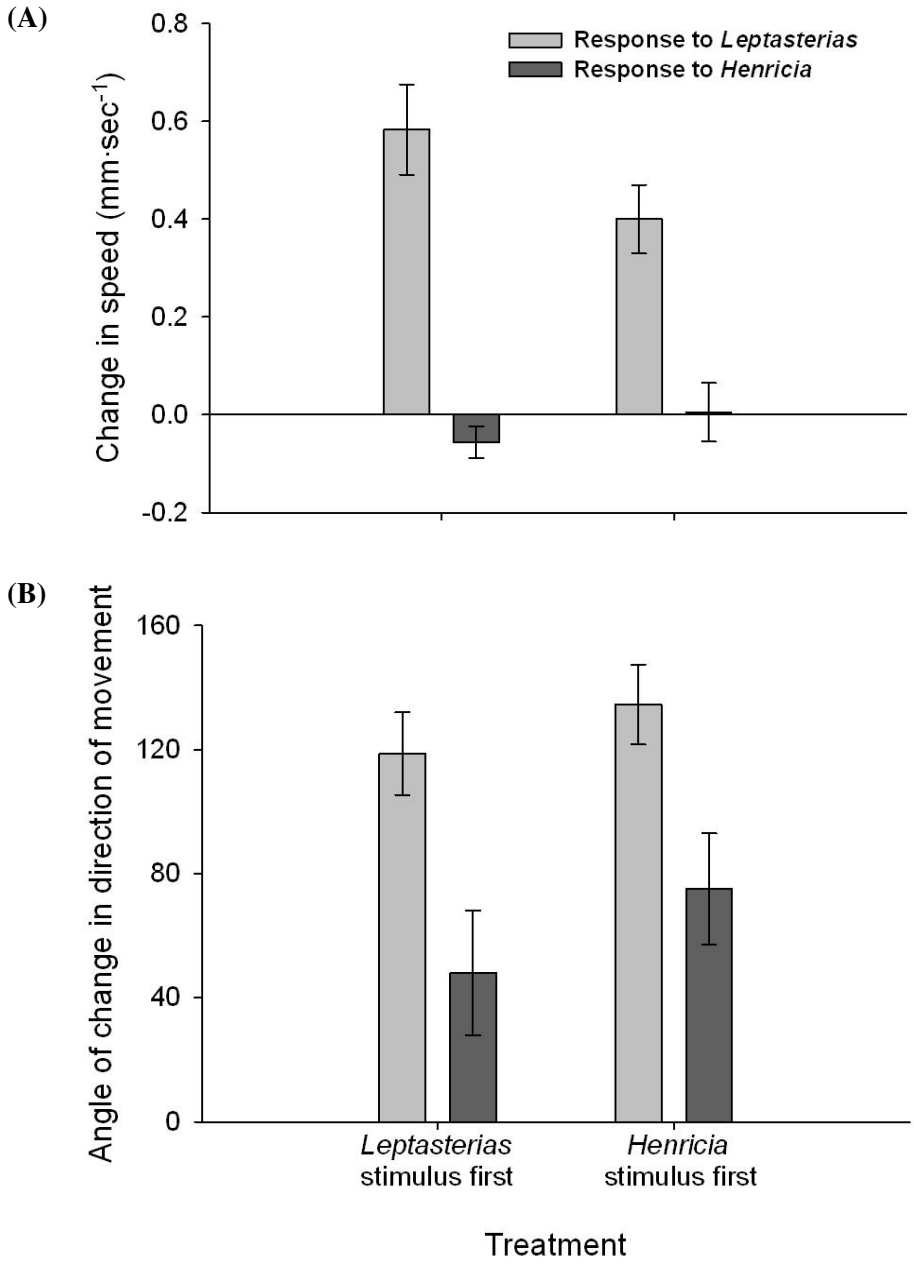


Figure 2.4. Responses of *Lirularia succincta* to an arm of *Leptasterias* and an arm of *Henricia* sp. Snails in the first treatment (n=13) received a contact stimulus from a *Leptasterias* followed by a stimulus from a *Henricia*. Snails in the second treatment (n=13) were exposed to the *Henricia* followed by *Leptasterias*. (A) The change in the speed of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli ($F=65.31$, $p<0.0001$). (B) The angle of change in the direction of movement of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli ($F=32.93$, $p<0.0001$). Error bars represent 1 SE.

a *Henricia* (Figure 2.4B). The differences in the turning responses are statistically significant and there was no significant interaction between stimulus and treatment (Table 2.3).

The mean changes in speed between the control stimulus and the arm of *Henricia* were not significantly different (Table 2.2) and were both negative, indicating decreased locomotion following both stimuli (Table 2.4, Figure 2.5A). The angle of change in the direction of movement was also not significantly different between the two stimuli (Table 2.3, Figure 2.5B).

The mean change in the angle of the direction of movement for snails in both treatments was significantly greater when contacted with the arm of *Pycnopodia helianthoides* than after exposure to the metal probe stimulus (Table 2.3, Figure 2.6A). After contact with the tube feet of *P. helianthoides*, *Lirularia succincta* increased their speed, while the mean response to the probe stimulus was a decrease in speed (Table 2.4). The mean changes in speed following each stimulus were significantly different (Table 2.2, Figure 2.6B).

Escape responses of *Lirularia succincta* to the two predatory sea stars *Leptasterias* and *Pycnopodia helianthoides* indicate that the turning or locomotor responses did not differ between the two asteroid stimuli (Tables 2.2 and 2.3). For both types of responses, however, there was a significant difference between treatments (Tables 2.2 and 2.3). Overall, mean changes in speed for snails exposed to the tactile stimulus from *P. helianthoides* followed by the arm of the *Leptasterias* were significantly greater than when snails were exposed to the stimuli in the reverse order (Table 2.2, Figure 2.7A). When *L. succincta* were exposed to the tube feet of *Leptasterias* first, the turning response elicited by *Leptasterias* was greater than the turning response elicited by *P. helianthoides*, but this difference was not significant ($t=1.218$, $p=0.249$). When *L. succincta* were exposed to the tube feet of *P. helianthoides* first, the response to that asteroid was greater than the response to the *Leptasterias*. This difference was also not significant ($t=-1.241$, $p=0.246$) (Figure 2.7B).

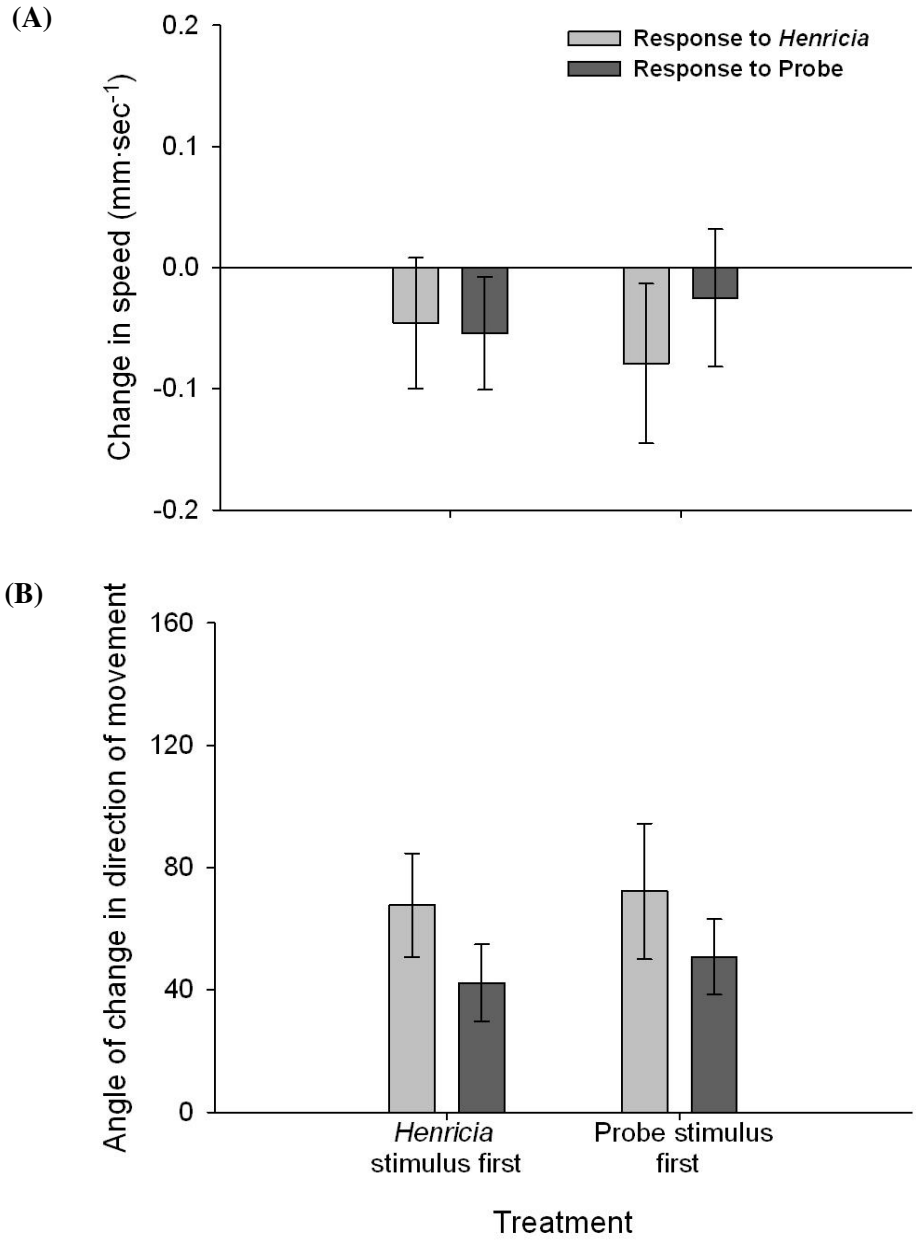


Figure 2.5. Responses of *Lirularia succincta* to an arm of *Henricia* sp. and a metal probe. Snails in the first treatment (n=16) received a contact stimulus from a *Henricia* followed by a stimulus from a metal probe (control). Snails in the second treatment (n=16) were exposed to the metal probe followed by *Henricia*. (A) The change in the speed of *L. succincta* in response to the two stimuli. Values are not significantly different between stimuli (F=0.13, p=0.7224). (B) The angle of change in the direction of movement of *L. succincta* in response to the two stimuli. Values are not significantly different between stimuli (F=1.32, p=0.2617). Error bars represent 1 SE.

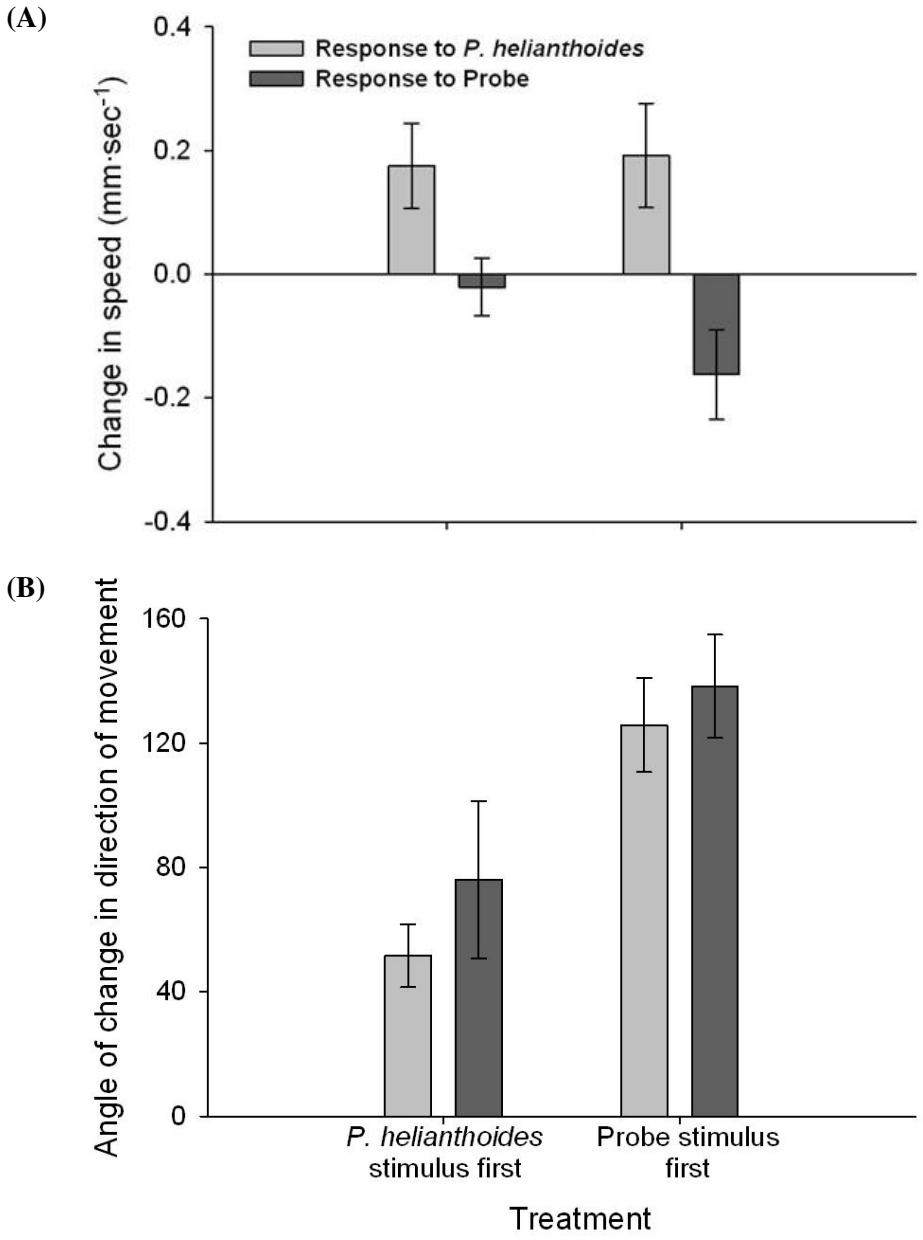


Figure 2.6. Responses of *L. succincta* to an arm of *Pycnopodia helianthoides* and a metal probe. Snails in the first treatment (n=16) received a contact stimulus from a metal probe followed by a stimulus from *P. helianthoides*. Snails in the second treatment (n=16) were exposed to *P. helianthoides* followed by the control stimulus. (A) The change in the speed of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli (F=16.53, p=0.0003). (B) The angle of change in the direction of movement of *L. succincta* in response to the two stimuli. Values are significantly different between stimuli (F=15.11, p=0.0009). Error bars represent 1 SE.

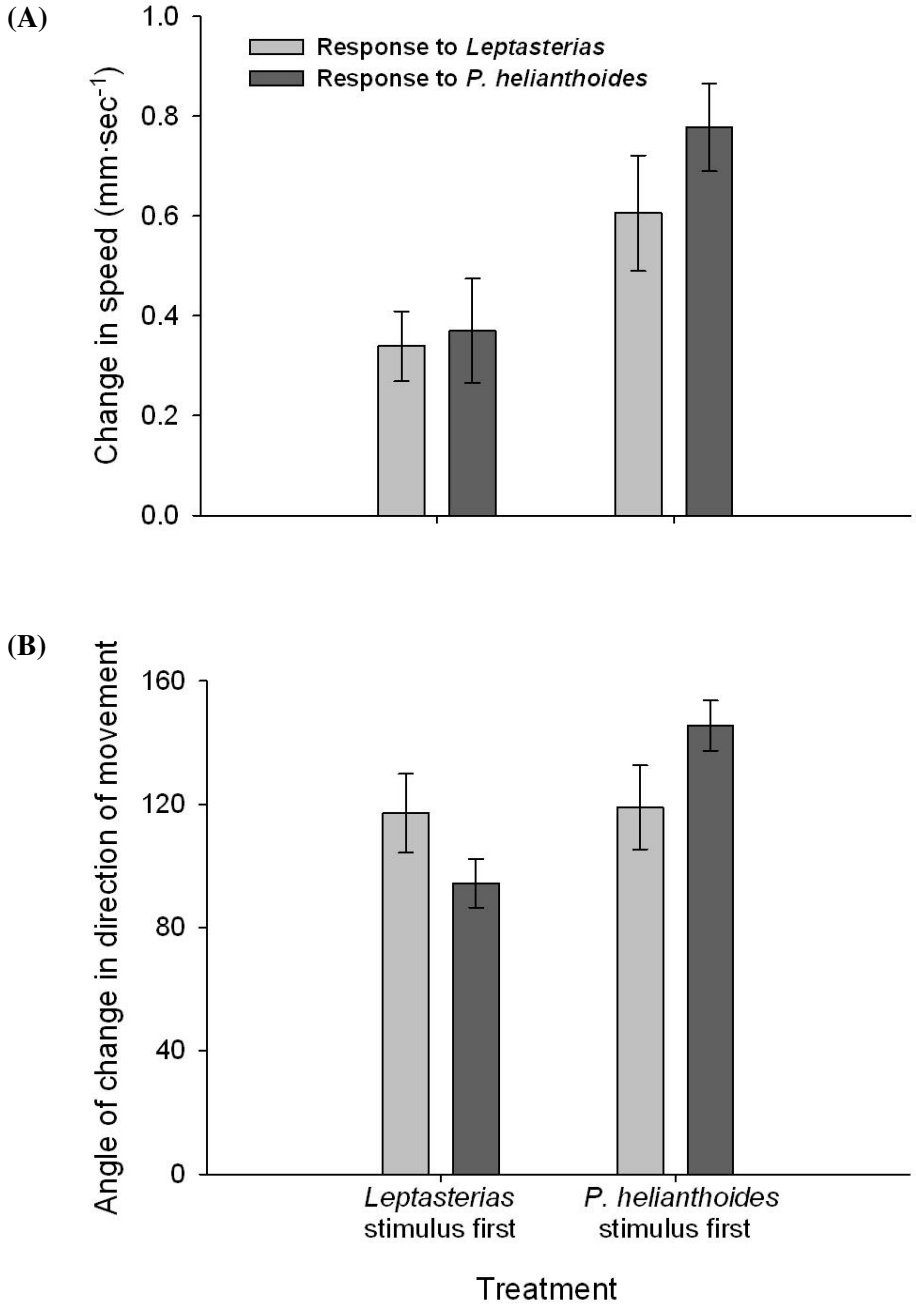


Figure 2.7. Responses of *Lirularia succincta* to an arm of *Leptasterias* and an arm of *Pycnopodia helianthoides*. Snails in the first treatment (n=12) received a contact stimulus from a *Leptasterias* followed by a stimulus from *P. helianthoides*. Snails in the second treatment (n=12) were exposed to *P. helianthoides* followed by *Leptasterias*. (A) The change in the speed of *L. succincta* in response to the two stimuli. Values are not significantly different between stimuli (F=0.63, p=0.4354) but are significantly different between treatments (F=9.17, p=0.0066). (B) The angle of change in the direction of movement of *L. succincta* in response to the two stimuli. Values are significantly different between treatments (F=6.97, p=0.0166) but are not significantly different between stimuli (F= 0.14, p=0.7156). Error bars represent 1 SE.

As described previously, the behaviors of *Lirularia succincta* elicited by the contact stimuli were assigned response scores, with the highest score being the most extreme response (Table 2.1). Responses of *L. succincta* to the predatory sea stars (Figure 2.8) were more vigorous than responses to *Henricia*. (Figure 2.9). Behavioral responses to *Leptasterias* were the most intense; response scores were significantly higher after contact with the tube feet of *Leptasterias* than after contact with the control stimulus ($H=48.556$, $p<0.001$), the small *Henricia* individual ($H=34.769$, $p<0.001$), and the *Pycnopodia helianthoides* ($H=6.671$, $p=0.010$). No significant differences were detected in responses to the *Henricia* and the probe ($H=0.0350$, $p=0.852$). The responses of *L. succincta* to the tube feet of *P. helianthoides* were more intense than the responses of the same snails to the probe ($H=44.985$, $p<0.001$). With the exception of the comparison between the responses elicited by the two predatory sea stars, the results from the comparisons of behavioral responses agreed with the results from the repeated measures ANOVAs testing for change in speed and turning response.

All of the snails tested exhibited some type of escape response after contact with the two predatory sea stars; only 34% (n=58) of snails exhibited a behavioral response following contact with *Henricia*. The primary response to a light touch with the control stimulus in all experiments was to withdraw briefly or retract the cephalic tentacles and then resume normal activity (classified as "no reaction"). Shell rotations were never induced by either the control stimulus or the tube feet of *Henricia*. The most common response to *Leptasterias* was turning followed by accelerated locomotion. Another common response was shell rotation and vigorous tentacle waving as described by Fishlyn and Phillips (1980). This response was always coupled with rapid movement away from the contact site. 71% of *Lirularia succincta* tested exhibited shell rotations in response to *Leptasterias.* Twenty percent and 0% of snails exhibited the same response after contact with *Pycnopodia helianthoides* and *Henricia*, respectively. The most vigorous escape responses involving several shell rotations were only elicited by *Leptasterias.*

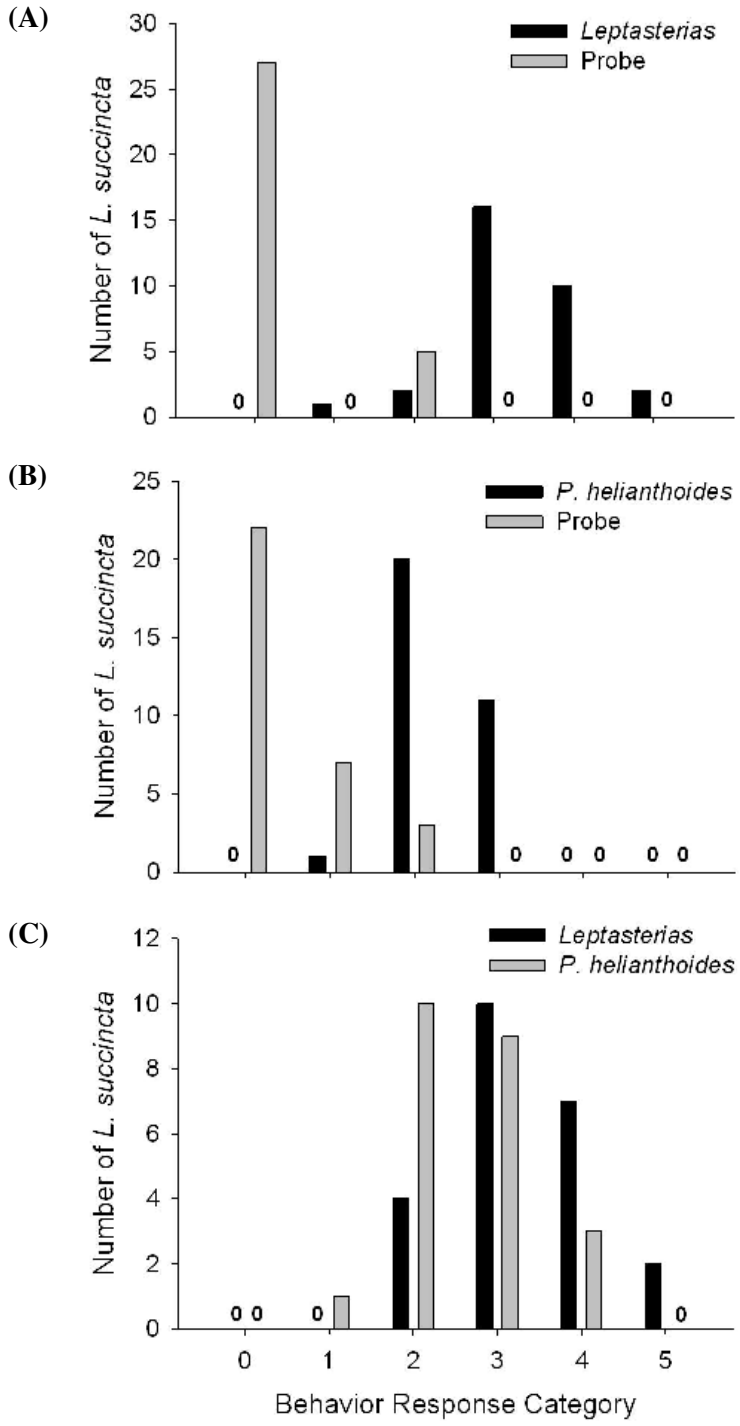


Figure 2.8. Behavioral responses of *Lirularia succincta* to contact with *Leptasterias* and *Pycnopodia helianthoides.* Contact was with (A) the tube feet of *Leptasterias* and a metal probe (control), (B) the tube feet of *P. helianthoides* and a metal probe, and (C) the tube feet of both *Leptasterias* and *P. helianthoides.* Response scores used to quantify *L. succincta* behavior are given in Table 2.1.

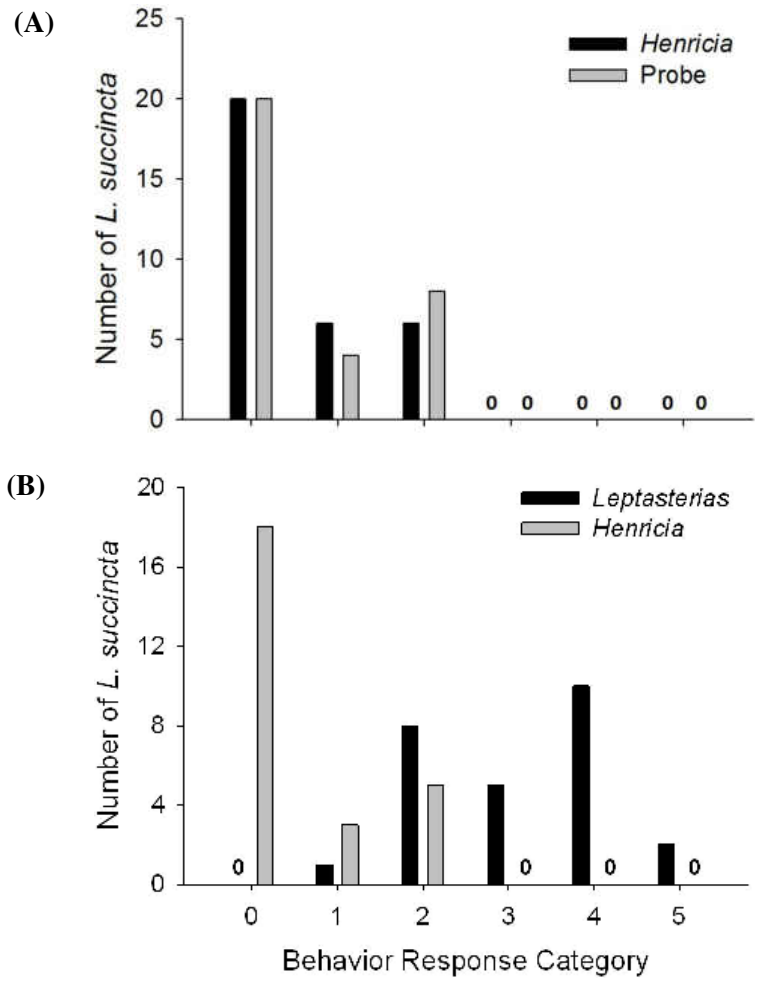


Figure 2.9. Behavioral responses of *Lirularia succincta* to contact with *Henricia* sp. and *Leptasterias.* Contact was with (A) the tube feet of *Henricia* or a metal probe (control), and (B) the tube feet of *Leptasterias* and *Henricia.* Response scores used to quantify *L. succincta* behavior are given in Table 2.1.

Escape Responses to Juvenile Sea Stars

There was a significant difference among treatments in the change in a snail's speed after contact with a juvenile sea star or control stimulus ($F=47.233$, $p<0.001$) (Figure 2.10A). Contact with the arm of a juvenile *Leptasterias* resulted in an average change in speed of 0.74 ±

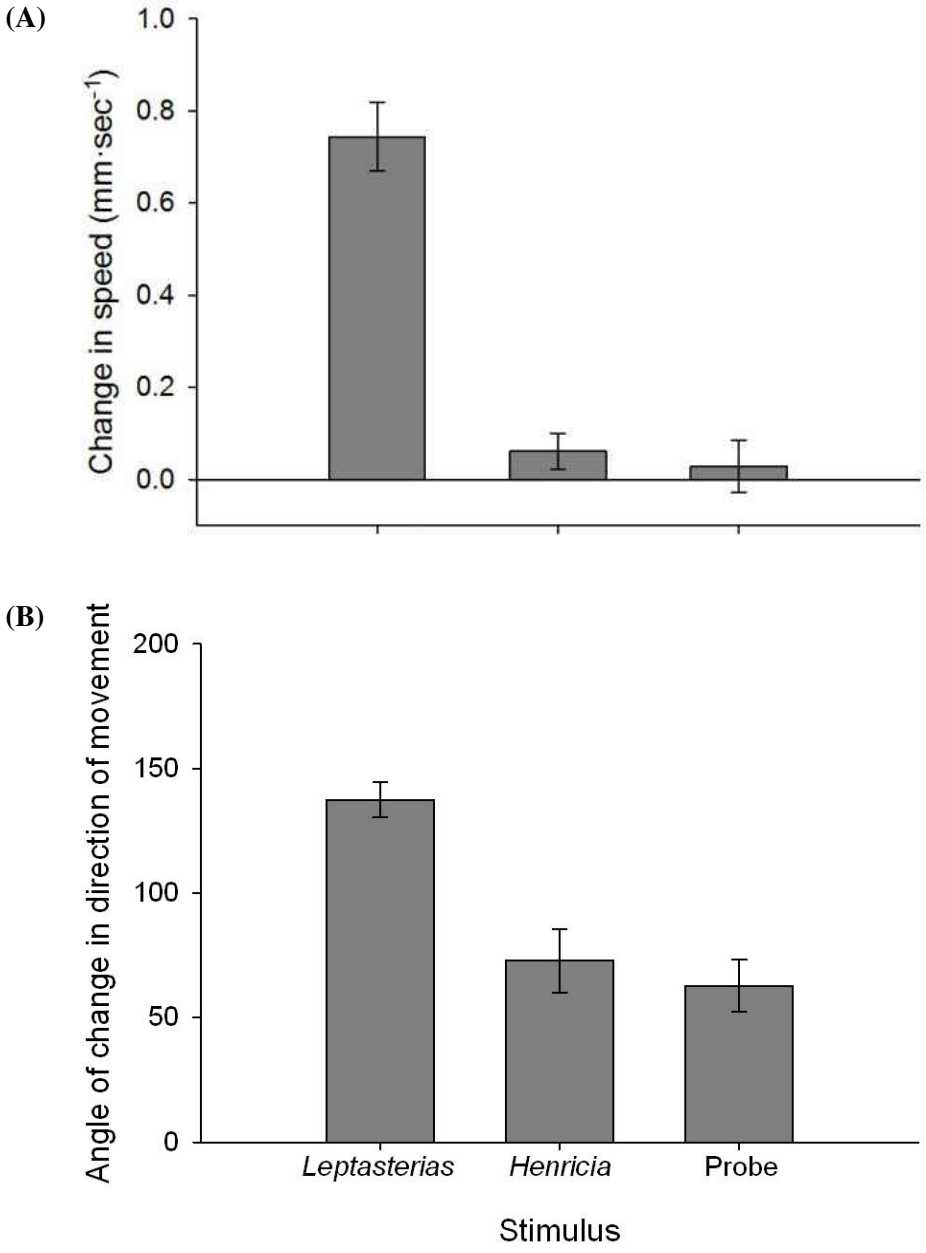


Figure 2.10. The responses of actively moving *Lirularia succincta* to one of three contact stimuli: the tip of an arm of a juvenile *Leptasterias*, the tip of an arm of a juvenile *Henricia*, or a metal probe (control) (n=25 snails per treatment). (A) The change in the speed of *L. succincta* in response to the contact stimuli. Values are significantly different between stimuli ($H=40.738$, $p<0.001$). (B) The angle of change in the direction of movement of *L. succincta* in response to the contact stimuli. Values are significantly different between stimuli ($H=24.210$, $p<0.001$). Error bars represent 1 SE.

SD 0.38 cm·sec^{-1}, which was significantly higher than the change in speed caused by the arm of a juvenile *Henricia* (0.06 ± SD 0.20 mm·sec^{-1}) or by the control (probe) stimulus (0.02 ± SD 0.28 mm·sec^{-1}) (Figure 2.10A). *Post hoc* tests showed significant pair-wise differences between responses of *Leptasterias* and both the probe ($t=8.603$, $p<0.001$) and the *Henricia* ($t=8.218$, $p<0.001$), but not between the probe and *Henricia* ($t=0.385$, $p>0.050$). There was also a significant difference in the turning response between treatments ($F=15.189$, $p<0.001$). The mean angle of change in the direction of movement for *Lirularia succincta* after contact with the arm of a juvenile *Leptasterias* was 137.4 ± SD 36.7^{o}, which was significantly higher than the mean altered direction of movement for snails responding to the control stimulus (62.8 ± SD 52.3^{o}) or to the tip of an arm of a juvenile *Henricia* (72.8 ± SD 63.4^{o}) (Figure 2.10B). *Post hoc* comparisons again showed significant pair-wise differences between responses of *Leptasterias* and both the probe ($t=5.049$, $p<0.001$) and the *Henricia* ($t=4.439$, $p<0.001$), but not between the probe and *Henricia* ($t=0.610$, $p>0.050$). The responses of *L. succincta* to the two juvenile sea star stimuli and to the control stimulus were assigned the response scores in Table 2.1, with the highest score being the most extreme response. More intense escape responses were induced by juvenile *Leptasterias* (Figure 2.11A) than by juvenile *Henricia* (Figure 2.11B). Response scores for *Lirularia succincta* were significantly different among treatments ($H=29.432$, $p<0.001$); *post hoc* comparisons show response scores were significantly higher for snails applied with a predator contact stimulus from the juvenile *Leptasterias* than for those contacting the juvenile *Henricia* or the metal probe, and differences between responses to the juvenile *Henricia* and to the probe were not significant. Overall, the results from the comparisons of behavioral responses agreed with the results from the repeated measures ANOVAs testing for change in speed and turning response for this experiment.

All of the snails exposed to the juvenile *Leptasterias* exhibited an escape response; 44% of snails that were exposed to the arm of a *Henricia* and 56% of the snails exposed to the control

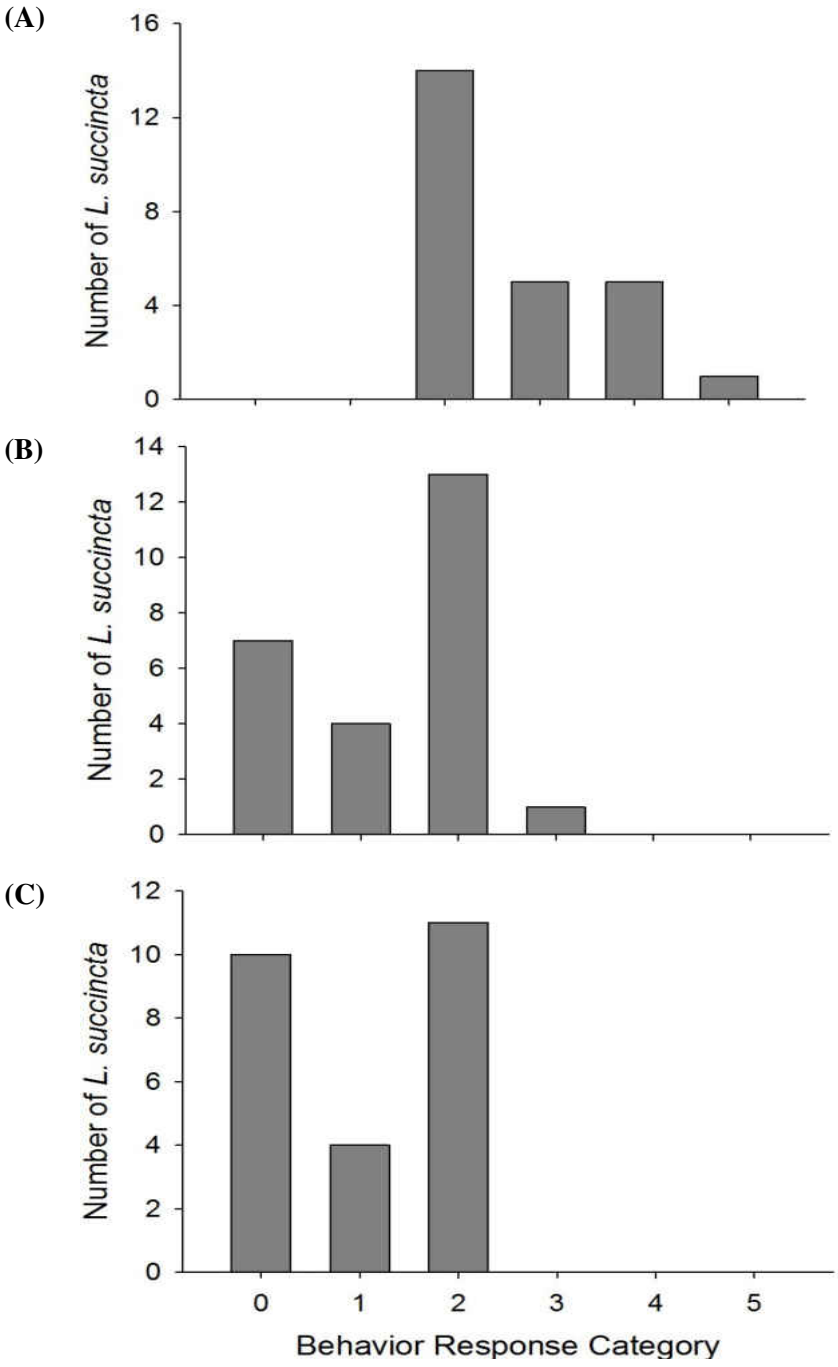


Figure 2.11. Behavioral responses of *Lirularia succincta* to one of three contact stimuli: (A) a juvenile *Leptasterias,* (B) a juvenile *Henricia*, or (C) a metal probe (control stimulus). Response scores used to quantify *L. succincta* behavior are given in Table 2.1.

stimulus either did not respond to the stimulus or remained stationary after the stimulus. Snails exposed to the juvenile *Leptasterias* were the only ones to exhibit any type of shell rotation, which occurred in 24% of the test subjects.

Escape Responses of Newly-Hatched *Lirularia succincta*

When the frequencies of the observed behavioral responses of the newly-hatched *Lirularia succincta* to the tube foot of a juvenile *Leptasterias* were compared with a contingency table, no significant difference in the frequency of responses between treatments was detected (df= 4, χ^2=7.928, p=0.0942)(Table 2.5). If the responses were converted into scores (level 0= no response; level 1= stop; level 2= pull back or turn; level 3= pull back and turn) and the scores between treatments were compared using a two-tailed Mann-Whitney U test (Zar, 2010), the differences in the frequencies of response behaviors elicited by the tactile stimuli of the pipet tip and of the *Leptasterias* were not significant (U=1436, p=0.0561).

Table 2.5. Contingency table used to analyze the responses of recently-hatched juveniles of *Lirularia succincta* to one of two stimuli: the arm of a juvenile *Leptasterias* (diameter= 4 mm), or the tip of a sterile pipet. The null hypothesis was that the frequency of each type of response was independent of treatment. Numbers in parentheses are expected frequencies.

	Response of *L. succincta* juveniles					
Treatment	**No Response**	**Stop**	**Turn**	**Pull Back**	**Pull Back & Turn**	n_1
Juvenile *Leptasterias*	19 *(23.5)*	11 *(10.5)*	14 *(14)*	6 *(5.5)*	10 *(6.5)*	60
Pipet tip (Control)	28 *(23.5)*	10 *(10.5)*	14 *(14)*	5 *(5.5)*	3 *(6.5)*	60
n_2	47	21	28	11	13	**120**

Avoidance Responses to Chemical Stimuli from a Predator

In the choice chamber the numbers of snails ending in the squares upstream from their original position (in Squares 4 and 5) were small in both treatments; 85% of snails in the predator treatment and 88% of snails in the control treatment moved in the same direction as the water was flowing. Seventeen of 40 snails placed into the arm with the *Leptasterias* ended up in the square farthest from the predator after 4 minutes; 8 of 40 snails placed into the testing chamber ended up in the same square in the control arm (Figure 2.12A). The change in the horizontal position of *Lirularia succincta* was significantly greater in the side of the choice chamber containing the *Leptasterias* than in the side of the choice chamber without the *Leptasterias* (df= 4, χ^2=11.667, p=0.020) (Table 2.6). As the frequencies of snails found in Squares 3, 4, and 5 did not appear to be different between treatments, I subdivided the contingency table to test the hypothesis that the frequency of snails found in each of those three squares were independent of treatment, ignoring the data for Squares 1 and 2 (Zar, 2010). The nonsignificant results for that 3x2 table (df= 2, χ^2=1.1443, p=0.5643) supports the null hypothesis of uniform distributions in Squares 3, 4, and 5 in both treatments. When I compared the frequencies of snails found in Square 2 to the frequencies found in all other squares combined in a 2x2 contingency table, I found that the occurrence of snails in Square 2 and in all other squares was independent of treatment (df= 1, χ^2=3.1638, p=0.0752). The occurrence of snails in Square 1, as compared to their occurrence in all other squares combined, was not independent of treatment (df= 14, χ^2=4.7127, p=0.0299). The significant difference in the horizontal distributions of snails in the arms of the choice chamber appears to be due to the greatest extent to differences in the observed number of snails found in Square 1 after 4 minutes in the chamber.

There was a significant difference in the vertical distance off the bottom of the chamber between snails in both sides of the choice chamber (H=11.791, p<0.001). Seventy-five percent of *Lirularia succincta* exposed to the waterborne odors of *Leptasterias* climbed the sides of the

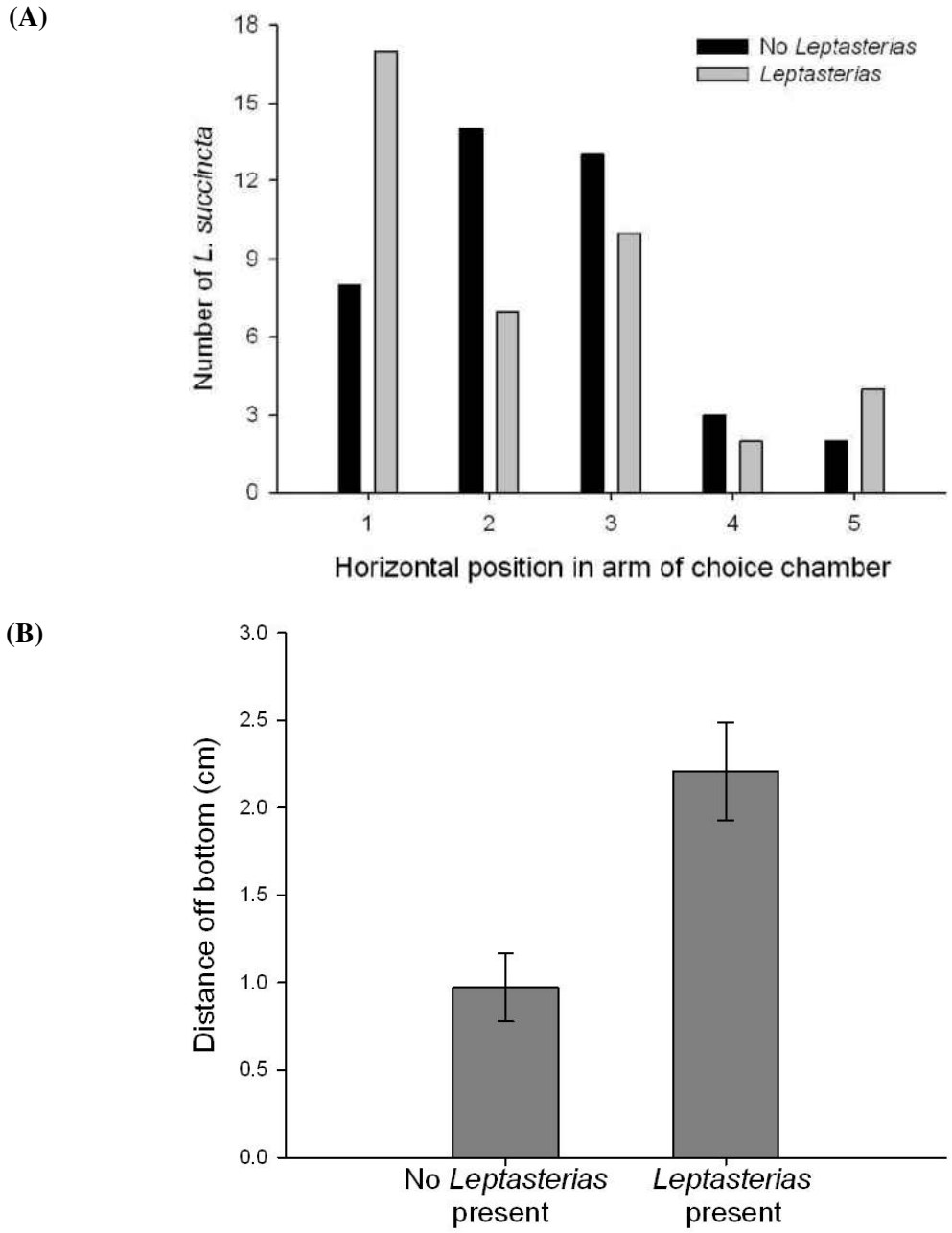


Figure 2.12. Position of *Lirularia succincta* 4 minutes after being placed into the center of one of the sides of a choice chamber (shown in Figure 2.2). In one side, a *Leptasterias* individual was upstream from where the snail was placed; the other side did not have a *Leptasterias* individual. (A) The horizontal position of *L. succincta* in each treatment after 4 minutes. Both arms of the chamber were sectioned into 5 equally-sized squares, with Square 5 upstream from Square 1. Snails in both arms were placed in the center of Square 3 at the beginning of each trial. The distribution of final horizontal positions are significantly different between treatments (χ^2=11.667, p=0.020). (B) The mean upward displacement of *L. succincta* in both treatments. The difference is significant (H=11.791, p<0.001). Error bars represent 1 SE. n= 40 snails on each side.

Table 2.6. Contingency table used to analyze the horizontal positions of *Lirularia succincta* placed into each of two sides of a choice chamber into which seawater was flowing. A *Leptasterias* individual was in one side of the chamber upstream of the snail. A *Leptasterias* individual was not placed in the opposite side of the choice chamber. The bottom of the side on which snails were placed was subdivided into five equally-sized squares and snails were placed in the center of square 3. The current was flowing from Square 5 towards Square 1. Observed frequencies indicate the numbers of snails located on each square after 4 minutes. Expected frequencies (italicized) were calculated based on the null hypothesis that the distribution of snails on the squares after 4 minutes was independent of treatment.

	Position in arm of choice chamber					
Treatment	**1**	**2**	**3**	**4**	**5**	n_1
Leptasterias	17 *(12.5)*	7 *(10.5)*	10 *(11.5)*	2 *(2.5)*	4 *(3.0)*	40
Control	8 *(12.5)*	14 *(10.5)*	13 *(11.5)*	3 *(2.5)*	2 *(3.0)*	40
n_2	25	21	23	5	6	**80**

choice chamber, ending at a mean distance of 2.2 ± SD 1.8 cm off of the bottom of the chamber. 50% of snails exposed only to seawater climbed the sides of the choice chamber, ending at a mean distance of 1.0 ± SD 1.2 cm (Figure 2.12B).

Avoidance Responses to *Leptasterias* and Injured Conspecifics

There was no significant difference in the mean change in speed or angle of change in the direction of movement of *Lirularia succincta* following the stimulus of predator-conditioned water, water conditioned with injured-conspecifcs, or FSW (Table 2.7, Figure 2.13). The behaviors of *L. succincta* following each chemical stimulus were assigned the same response scores as those used in the escape response experiments (listed in Table 2.1) (Figure 2.14). There

Table 2.7. Two ANOVA summary tables comparing the responses of *Lirularia succincta* to chemical stimuli potentially released by a predator (*Leptasterias*) or by injured conspecfics. (A) Response measured was a change in the angle of direction of the snail's movement following the stimulus. Data were square-root transformed. (B) Response measured was the change in the speed of the snail following the stimulus.

(A)

Source of Variation	df	SS	MS	F	P
Treatment	2	8.461	4.230	0.581	0.563
Residual	51	371.503	7.284		

(B)

Source of Variation	df	SS	MS	F	P
Between Groups	2	15.387	7.693	0.656	0.522
Residual	72	844.560	11.730		

was a significant difference in behaviors among the three treatments (H=8.291, p=0.016). *Post hoc* tests showed significant pair-wise differences only between responses to predator-conditioned water and to water conditioned with injured consepcifics. Behaviors elicited by predator-conditioned water do indicate a weak response to water-borne cues from *Leptasterias.* Snails exposed to the predator stimulus were the only snails to exhibit the shell rotation response, but only 20% of snails responded with that behavior. No snails exhibited the most extreme response of multiple shell rotations (Level 5). Snails exposed to seawater conditioned with injured conspecifics had the greatest frequency of "no response" behaviors (64% of snails tested).

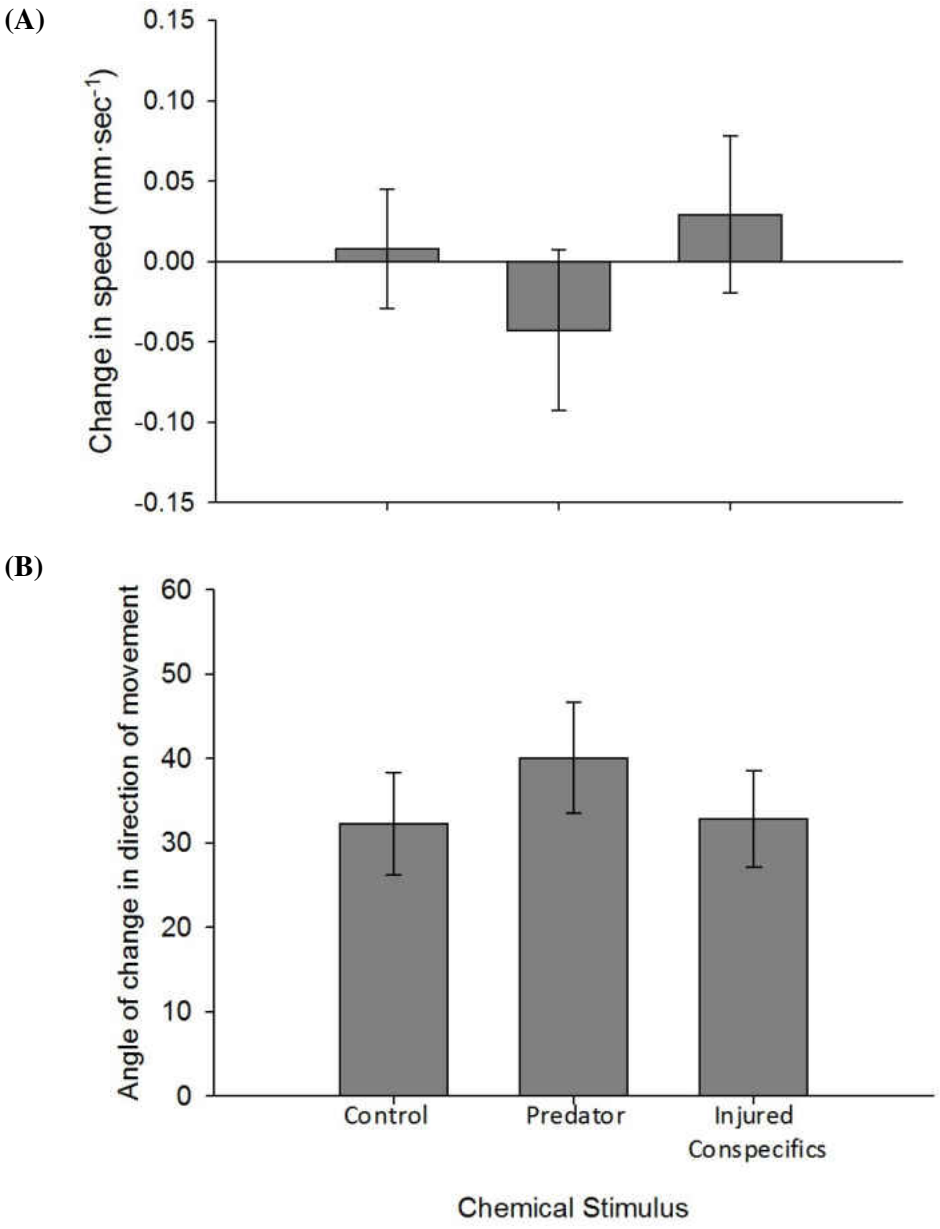


Figure 2.13. The responses of *Lirularia succincta* to one of three chemical stimuli: seawater conditioned with odors from the predator *Leptasterias,* seawater conditioned with odors from injured conspecifics, or FSW. 25 snails were tested in each treatment. (A) The change in the speed of *L. succincta* after exposure to one of the chemical stimuli. Differences in values are not significant (F=0.656, p=0.522). (B) The angle of change in the direction of movement of *L. succincta* in response to the chemical stimuli. Values are not significantly different among treatments (F=0.581, p=0.563). Error bars represent 1 SE.

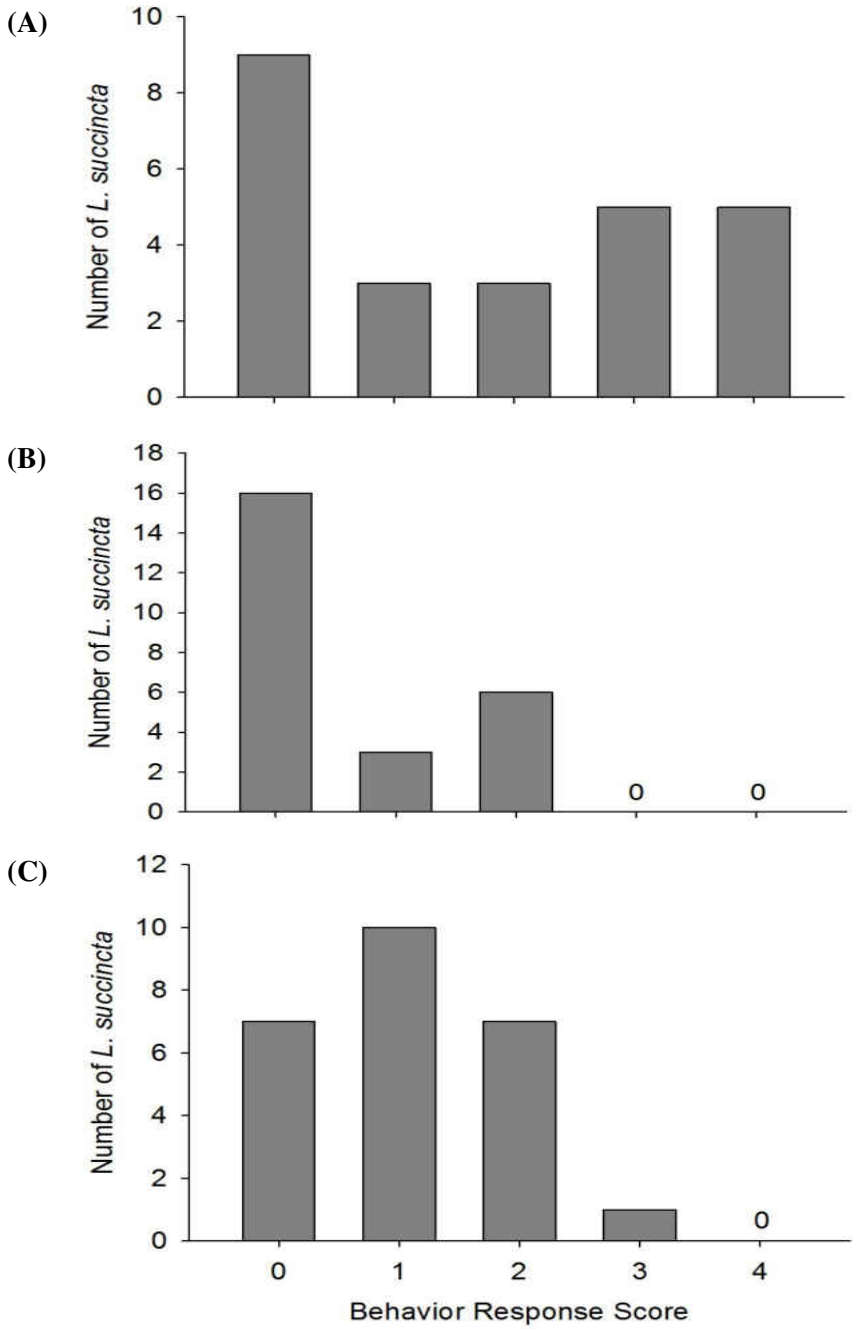


Figure 2.14. Behavioral responses of *Lirularia succincta* to one of three chemical stimuli: (A) seawater conditioned with *Leptasterias,* (B) seawater conditioned with injured conspecifics, or (C) FSW (control stimulus). Response scores used to quantify *L. succincta* behavior are given in Table 2.1.

Discussion

In general, *Lirularia succincta* exhibited strong escape responses and weak avoidance responses as they did appear to be capable of sensing the predator from a distance by means of chemoreception. The behaviors exhibited by *L. succincta* in response to direct contact and to water-borne chemical cues were the same in all laboratory experiments. Escape and avoidance response behaviors included vigorous shell rotations, tentacle waving, and occasionally foot contortions that resulted in the snail rolling away from a contact stimulus. The most common behaviors were a turning response combined with an increased crawling rate. Defensive behaviors may be different for *L. succincta* in the field. Fishlyn and Phillips (1980) observed crawl-out responses, shell rotation and increased crawling rates in response to predator-scented water in the laboratory, while the most frequent escape response observed in response to contact with *Leptasterias polaris* in their natural environment was falling (Fishlyn and Phillips, 1980).

As falling has been observed as an escape response of *Lirularia succincta* under laboratory conditions, it is likely that these escape behaviors would be expressed in the field, especially as the topography of the habitat of both the predator and prey are highly variable, and both can frequently be found on vertical surfaces or attached to the underside of rocks or cobbles. Shell twisting can be an effective escape response, as the rotation can detach the sea star's tube feet if they attach to the shell, allowing the snail to flee. The success of this response has been observed by Fishlyn and Phillips (1980) and Kent (1981).

Contact with inanimate objects (a metal probe or a glass pipet tip) consistently failed to elicit escape responses, affirming that tactile stimuli alone are ineffective in inducing defensive behaviors (Bullock, 1953; Feder, 1963). The responses of *Lirularia succincta* to *Henricia* were not significantly different from responses to the control stimuli; in most cases, the snail resumed its pre-contact speed immediately after the stimulus. This indifference to contact with *Henricia* suggests that *L. succincta* are able to recognize *Henricia* as a non-predatory sea star (Harvey et

al., 1987). While *L. succincta* exhibited immediate escape responses to *Pycnopodia helianthoides,* the responses were not as intense as those exhibited after contact with the tube feet of *Leptasterias*. The turning and locomotor responses between *Leptasterias* and *P. helianthoides* were not significantly different, but differences in the intensity of the behavioral responses were significantly different. Shell rotation was elicited by *Leptasterias* much more than by *P. helianthoides,* and only *Leptasterias* individuals induced the most extreme response behavior of multiple shell rotations. The significant differences in behavior indicate that *Leptasterias* evokes stronger escape responses than *P. helianthoides.* This is not surprising, as *Leptasterias* spp. is found in greater abundance than *P. helianthoides* in the areas from which the *L. succincta* specimens were collected. Overall, when avoidance behaviors are taken into account, *Leptasterias* elicited the strongest escape responses of the three sea stars tested.

The responses of *Lirularia succincta* to the two species of juvenile sea stars were similar to those induced by their larger counterparts in the first set of experiments. The juvenile *Henricia* did not induce the escape responses observed with the juvenile *Leptasterias.* The turning and qualitative behavioral responses of *L. succincta* to the juvenile *Leptasterias* were similar to those responses evoked by the adult *Leptasterias.* The mean change in the speed of *L. succincta* following contact with the juvenile *Leptasterias* (0.74 ± SD 0.38 mm·sec^{-1}; n=25) was much higher than the greatest mean change in speed elicited by an adult *Leptasterias* (49 ± SD 30 mm·sec^{-1}; n=26). The results of this experiment show that the cue or chemical label produced by *Leptasterias* is present when the sea star is very small, that it can be detected by *L. succincta* and that the snails exhibit escape responses to juvenile predatory sea stars that may be more intense than those that are elicited by the adult sea stars. Other gastropods such as *Haliotis* also exhibit escape responses to predators that are smaller than the adult gastropod (Bullock, 1953).

Interestingly, the escape responses of newly-hatched juvenile *Lirularia succincta* to juvenile *Leptasterias* were not similar to those exhibited by adult snails. In contrast to the adults,

juvenile *L. succincta* never exhibited shell rotation. The most vigorous escape response exhibited by the juvenile snails was to pull back from the stimulus and turn, and this response was occasionally exhibited by juveniles exposed to the control (pipet tip) stimulus. Their rate of locomotion never appeared to increase following the stimulus from the juvenile *Leptasterias*, and snails frequently remained stationary for at least 60 seconds following contact with either stimulus. While 100% of adult *L. succincta* responded to adult or juvenile *Leptasterias* in the previous studies, only 68% of juvenile *L. succincta* exhibited an escape response. These results suggest that *L. succincta* may not inherently have the ability to discriminate between stimuli. These behaviors may develop ontogenetically as the juveniles are exposed to contact with predators in the intertidal, as suggested by Rochette et al. (1996). Animals across many phyla, from ciliates to birds, are capable of modifying their behaviors following experiences with predators (Kusch, 1993; Maloney and McLean, 1995; Rochette et al., 1998); these modifications could take the form of sensitization, with an increase in the intensity or frequency of defensive behaviors arising from an increased exposure to predators (Rochette et al., 1996).

While avoidance responses observed in this study were not as strong as escape responses, they did provide evidence that *Lirularia succincta* is able to detect water-borne chemicals released by *Leptasterias.* This was evident in the responses of *L. succincta* in the choice chamber. Snails in both treatments crawled with the current in both treatments; very few snails crawled upstream (towards Squares 4 or 5) and many snails did not move from Square 3, where they were originally placed. Snails in the treatment exposed to chemical cues from the predator ended up significantly farther from the predator than snails in the control treatment to a significantly greater extent than would be expected if the movements of snails in both treatments were random. This suggests that chemical cues detected by *L. succincta* caused the snails to alter their behavior, most likely by increasing their locomotor activity.

The results of the choice chamber experiment do not necessarily indicate that snails crawl away from the source of a water-borne chemical stimulus, or that they are able to determine the direction from which the stimulus originates. The predominant avoidance response to the detection of the predator was likely merely an increase in locomotor activity; *Lirularia succincta* in the control treatment moved downstream as well. The results from the second avoidance response experiment seem to confirm this. Specifically, when water conditioned with the odors of *Leptasterias* was squirted at a *L. succincta* individual, there was not a significant difference between the turning response elicited by this stimulus and the turning response elicited by the control stimulus. This makes sense, as, under natural conditions, *L. succincta* would not be able to detect the direction from which a stimulus originated in the turbulent intertidal. There was not a significant increase in the crawling rate of the *L. succincta* exposed to predator stimulus; this behavior would have been observed if, as predicted, the avoidance responses exhibited in the choice chamber experiment resulted from increased locomotor activity. A failure to detect differences in crawling rate between treatments could be an artifact of the experimental design. Chemical cues from *Leptasterias* were strong enough to be detected; predator-conditioned water was the only stimulus evoking the shell rotation response that is characteristic of encounters of *L. succincta* with chemical cues of *Leptasterias*.

The failure of *Lirularia succincta* to respond to seawater conditioned with extracts from injured conspecifics could be because alarm signals are not released from injured *L. succincta,* or because the snails have no ability to detect them. The injured conspecific stimulus failed to elicit shell rotation in *L. succincta.* This indicates that responses of *L. succincta* to *Leptasterias* are due to chemical cues from the sea star, and are not affected by scents of injured conspecifics that *L. succincta* may have detected had the *Leptasterias* been feeding on them (Hadlock, 1980; Jacobsen and Stabell, 1999).

Shell rotation occurs after the detection of the predator's scent. This may serve to expose more of the snail's sensory receptors, allowing it to detect more chemical cues (Fishlyn and Phillips, 1980). This behavior may also indicate that the snail is anticipating contact with the predator, and a corresponding flight response. Endler (1986) notes a shift from passive to active defensive behaviors in prey species as predation sequences commence. The ability to sense a predator from a distance can prepare for or initiate the flight of a gastropod before the predator is close enough to contact it, which can give the slow-moving gastropods a "head start." The purpose of this advanced increase in speed may be to enable the prey species time to find a refuge (Cotton et al., 2004). The fact that the snails exposed to the predator stimulus experienced greater upward displacement in the choice chamber may indicate their attempts to find a refuge from the predator above the water level (Bullock, 1953; Phillips; 1976).

Menge (1972) indicated that, in the absence of *Pisaster ochraceus, Leptasterias* can strongly affect the composition of an intertidal community. Niesen (1973) conducted a feeding census of prey species consumed by *Leptasterias hexactis* at South and Middle Coves at Cape Arago State Park, and at another intertidal site three miles to the north. The survey indicates that *Balanus* spp. make up from 69 to 85% of the diet of adult *L. hexactis.* The next most abundant prey species, *Littorina scutulata*, comprised from 6 to 9% of the sea star's diet. It is interesting to note that, despite the abundance of gastropod species at these two sites, gastropods only account for 10 to 24% of the sea star's diet. This difference may be accounted for by the differences in the abilities of barnacles and gastropods to exhibit defensive responses to *Leptasterias. Lirularia succincta,* or species that may have been mistaken for *L. succincta*, were never identified as prey species, even though they are common in South Cove. While this may be because the species was only recently introduced to the area, it could also be because the snail's avoidance and escape responses have effectively removed it from the diet of *Leptasterias*. I have only observed three *L. succincta* specimens being eaten by *Leptasterias* spp. individuals in the field. So, while

Leptasterias did feed on *L. succincta* in the laboratory, these experiments only showed the number of snails that could be eaten by the *Leptasterias* under optimum conditions in the laboratory. If *Leptasterias* are able to catch and eat *L. succincta* in the field, their feeding rate on the snail would be much lower than that observed in the laboratory. More time would be spent finding the snail, and the snail's escape and avoidance responses would increase its chance of finding a refuge before it could be eaten. The microhabitats on which both snail and asteroid species are found could provide more refuges for *L. succincta*, especially if falling is used as an escape response. More time would also be spent in locating *L. succincta*, especially as their distribution is characterized by a high degree of spatial patchiness.

The study of Mauzey et al. (1968) describing laboratory preference experiments and field observations of ten sea star species demonstrated that the diet of a sea star can vary locally and that laboratory experiments and field data on any particular species should be compared to determine which species are a sea star's most important natural prey. They emphasized that, when defining a sea star's diet, laboratory observations should not be omitted or exclusively relied upon. Some species that are preferred prey in the laboratory are not eaten in the field, and vice versa (Feder, 1963; Mauzey et al., 1968). The effect of *Leptasterias* on *Lirularia succincta* populations or abundance likely varies seasonally; the feeding rate of *Leptasterias hexactis* decreases markedly in the winter and early spring (Niesen, 1973). Conclusions based on the results of the feeding rate experiments done in this study should therefore be made with caution. The small size of the snail and its low abundance in the diet of *L. hexactis* in the field suggest that the snail is not an energetically important species in the sea star's diet. This could only be confirmed with further field studies

This study has clearly demonstrated that *Lirularia succincta* possess defensive behaviors that they may use in response to the predatory sea stars *Leptasterias* and *Pycnopodia helianthoides*. The snails appear to be eaten only occasionally by *Leptasterias* in the field, even

though *L. succincta* can be abundant. Small *P. helianthoides* are uncommon in the areas in which *L. succincta* are found, so it would be difficult to estimate the importance of *L. succincta* in its diet. It now remains to discover the actual effectiveness of these responses in the field under conditions similar to those in which the defensive behaviors are naturally employed. The defensive behaviors of *L. succincta* were observed under conditions that the snail is not likely to experience in the field, including slow unidirectional flow of water and a smooth substratum. Field experiments could confirm that the alarm behaviors exhibited by *L. succincta* increase its ability to avoid predation under a wider variety of environmental conditions. Hadlock (1980) suggested that knowledge of both the predator's natural feeding behavior and of the type of refuge sought by the gastropods are important in understanding how or why these defensive behaviors work. When Fishlyn and Phillips (1980) were observing the predator-prey interactions of *Lacuna vincta* and *Leptasterias polaris* in the field, they were able to quantitatively determine the effectiveness of the escape response of *L. vincta* by understanding the type of refuge used by the snail. In the field, behaviors can also be confirmed using natural chemical concentrations that are not usually experienced in the laboratory (Phillips, 1976) and experimental designs can allow for the exhibition of many different types of responses (Endler, 1986).

The experiments in this study also raise further interesting questions. Do escape and avoidance responses in *Lirularia succincta* vary by season or by geographic location? Would *Leptasterias* elicit the same defensive behaviors in *L. succincta* from communities in which the sea stars are absent? How do ontogenetic changes or varying amounts of exposure to predators affect the responsiveness of newly-hatched juvenile *L. succincta* to predators including *Leptasterias* spp.? A suite of laboratory and field experiments would enable us to learn more about the predator-prey interactions occurring in, and possibly contributing to the structure of the intertidal community to which these small marine invertebrates belong.

Bridge

Chapter II demonstrated that *Lirularia succincta* exhibits escape and avoidance responses to chemical cues from *Leptasterias.* In the laboratory it was demonstrated that *Leptasterias* spp. individuals are predators of *L. succincta,* but it is not known if they are important predators of *L. succincta* in the field. If *Leptasterias* spp. individuals do eat *L. succincta* to any great extent, then the sea star could be important in determining the lower distribution of the snail in the intertidal (Connell, 1961, 1970). For animals that are present in greater abundance in the low intertidal, predation and other biotic interactions are a greater source of mortality than physical stressors associated with life high in the intertidal (Vermeij, 1972). The vertical distribution of *L. succincta* in its habitat has never been documented, so in Chapter III, I attempt to describe its distribution in a habitat in which it is prevalent.

CHAPTER III

THE SEASONAL AND SPATIAL DISTRIBUTION OF *LIRULARIA SUCCINCTA*

Introduction

All species are found in specific habitats and are often found in varying densities within those habitats. Studying the spatial distributions and densities of populations and the factors that affect each contributes to our knowledge of ecology at the population and community levels. Distribution and abundance can vary for a species on large and fine spatial and temporal scales, and these variations will ultimately affect the reproductive success of the species. Local distributions of species are determined in part by habitat selection which, as described by Meadows and Campbell (1972) is the "relationship between behavior and environment." Animals choose to remain in or return to selected habitats, thereby establishing and maintaining local distributions. These selected habitats can be altered by both biotic and abiotic factors that may also vary widely on spatial or temporal scales (Gendron, 1977). While these patterns apply to many species found in many different habitat types, this paper will focus on the rocky intertidal habitat.

The spatial distribution of a species may be influenced by a number of physical or biological factors. Generally, mortality at high intertidal levels is due to physical factors including wave action and disturbance (Connell, 1970), temperature (Bertness and Schneider, 1976; Johnson et al., 2001), desiccation and osmotic stress (Chow, 1975). These factors can define the upper limit of a species distribution (Connell, 1961; Green and Hobson, 1970; Vermeij, 1972; Chow, 1975). Biotic interactions including competition and predation may serve to

determine the lower limit of the distribution of a species (Connell, 1961, 1970; Green and Hobson, 1970). If the species spends a limited amount of time in the plankton, or has direct development, oviposition site choice may affect local distributions (Benkendorff and Davis, 2004). Gregarious behaviors, if present, may also contribute to patterns of abundance (Meadows and Campbell, 1972), as might the availability of protective micro-habitats (Chow, 1975) and associations with macroflora (Meadows and Campbell, 1972; Nakaoka et al., 2001). On very small spatial scales, recruitment and mortality will affect the distribution of sessile species to a greater extent than species that are mobile and are able to move in response to their environment (Underwood and Chapman, 1996). On very large spatial scales, the mode of dispersal a species employs can affect its geographical distribution. The spatial distribution of species with direct development is more likely to be affected by disturbance and is generally more patchy than the distributions of species with planktonic development (Johnson et al., 2001). For species with direct development, there can be greater variability in the densities of species between sites separated by only a few hundred meters (Johnson et al., 2001).

The distribution of a species throughout the year may vary on a temporal scale with the seasons due to changes in food availability (Toyohara et al., 1999), abiotic environmental factors (Bertness and Schneider, 1976) and predation. The feeding rate of the predatory sea star *Leptasterias hexactis* varies seasonally, with minimum rates from December to April (Niesen, 1973). Menge (1978) found that for some predatory gastropods, predation rates could be affected by algal cover and desiccation; he concluded that predation intensity also depended on the effect of biotic and abiotic environmental conditions within a habitat.

For mobile species, distributions are ultimately established by behavior as individuals avoid areas in which stress leads to mortality (Bertness and Schneider, 1976). In response to varying environmental conditions throughout the year, mobile animals may alter their spatial distributions by migrating vertically through the intertidal. For instance, the limpet *Acmaea*

strigatella exhibits vertical displacement in the winter and in the late spring (Seapy and Hoppe, 1973). Preferred tidal height varied with season in *Littorina littorea* (Gendron, 1977). Nakaoka et al. (2001) noted patterns of seasonal change in abundance for many species living in seagrass beds, although the patterns of seasonal change differed between species. The areas in the intertidal that may cause higher mortality may differ between individuals of the same species. Tolerances to environmental stressors may be affected by the size of the animal exposed to the stress. Chow (1975) found that tolerances to desiccation and osmotic stress were higher for larger individuals. He determined that thermal tolerances were not correlated with size, although Bertness and Schneider (1976) found that thermal tolerances differed between species, and, for one species of whelk, thermal tolerances were greatest in small individuals.

Some predators preferentially eat large prey (Connell, 1970); others are unable to eat prey that are too large (Bertness and Cunningham, 1981). The resulting differential mortality in one size group relative to another can result in size gradients in the distribution of a species, especially for sessile species (Chow, 1975). For species that are mobile and are actively able to select their habitat based on changing environmental conditions, populations may become segregated by size due to the migration of different size groups relative to each other (Vermeij, 1972; Chow, 1975). This migration of animals to different tidal levels will lead to differences in the intensity of competition, predation, food availability, or other causes of mortality to which individuals are exposed (Connell, 1961, 1970; Paine, 1969; Vermeij, 1972). Green and Hobson (1970) noted that size gradients in *Gemma gemma* were probably the direct result of these migrations up or down the shore in response to temperature or competition. Size gradients for a species are therefore the outcomes of or responses to mortality gradients in the intertidal (Vermeij, 1972).

There are many instances of different sizes of organisms occurring in different areas within a habitat that may indicate a change in the preferred habitat of a species at different ages

(Vermeij, 1972; Chow, 1975; Gendron, 1977). In many species of gastropods, zonation patterns vary among size classes (Williams, 1964; Vermeij, 1972). Gendron (1977) found that the preferred tidal height of *Littorina littorea* decreased as snail size increased. In *Gemma gemma*, mortality was greater at higher shore levels for juvenils and at lower shore levels for adults, which established a size gradient for a population studied by Green and Hobson (1970). Paine (1969) observed that *Chlorostoma funebralis* originally settled higher in the intertidal where it experienced less predation but moved into the lower intertidal after it reached sexual maturity. Vermeij (1972) described this occurrence as pre-reproductive snails inhabiting "zones of minimum mortality" and hypothesized that this pattern may be true for many other invertebrate species as well. He observed that for limpets found in the low or mid-intertidal zones, size decreased as tidal level increased. For limpets occurring in the high intertidal, however, the opposite size gradient was apparent. In the family Trochidae, the mean size of individuals often decreases at higher levels in the intertidal (Vermeij, 1972). Size gradients are not exhibited in all gastropod populations, however, and some size gradients may result from differences in growth rate among individuals at different shore levels in the intertidal zone (Connell, 1961; Green and Hobson, 1970; Vermeij, 1972).

If the behavior of the adult or juvenile stages of small gastropods in a population determines the population's spatial distribution in a single area, the abundance of animals at very small scales (1-2 m or less) should vary significantly because, 1) these animals are not capable of dispersing great distances after recruitment, and 2) many gastropods move less than a meter during high tides (Underwood and Chapman, 1996). As described previously, observed distributions are likely affected by behavior as gastropods select habitat in which to remain, but small gastropods encounter the pressures that affect distribution at small scales. Green and Hobson (1970) observed differences in mortality rates and densities between groups of *Gemma gemma* only 6 m apart, while Underwood and Chapman (1996) and Olabarria and Chapman

(2001) found significant variability in patterns of abundance at scales of centimeters to less than 10 m. For many different species, this small-scale variability in abundance accounted for most of the variability among locations. This patchiness can affect the observation and analysis of abundance patterns at large spatial or temporal scales in the intertidal zone (Olabarria and Chapman, 2001, 2002), so scientists attempting to describe patterns of abundance or distribution should know the smallest scales at which there are still predictable patterns in the abundance of a species (Olabarria and Chapman, 2001).

Because seasonal or ontogenetic vertical migration occurs frequently in other mollusks, it is possible that such changes in distribution also occur for *Lirularia succincta*, a small trochid found exclusively in the intertidal zone (Carlton, 2007). Very little is known about the biology or ecology of this snail; nothing has been published about the distribution of this species, either on small or large spatial scales, or about the factors that affect its distribution. The snail is common at two wave-protected sites that are separated by less than 2 km (North Cove and South Cove, Cape Arago State Park, Oregon). Preliminary observations at both sites indicated that *L. succincta* were found more frequently in the lower intertidal. The extent to which *L. succincta* impacts the ecology of the lower intertidal community is uncertain; however, any impacts could vary with season if *L. succincta* exhibit vertical seasonal migrations.

In order to obtain a rudimentary understanding of the population structure of *Lirularia succincta,* I described its intertidal distribution on a seasonal basis at North Cove of Cape Arago State Park. I also tested the hypothesis that the size of *L. succincta* decreases in an upshore direction.

Materials and Methods

Sampling Design

The distribution of *Lirularia succincta* was sampled in North Cove at Cape Arago State Park, south of Charleston, Oregon (43° 18' 31" N, 124° 23' 55" W) (Figure 2.1 in Chapter II of this thesis). North Cove is a north-facing rocky intertidal area protected from strong wave action by an offshore reef. I sampled 4 line transects along 4 different tidal heights at lower low water during spring tides. The "high" transect line, at a height of 0.20 m, was parallel to the shoreline, and the lower three transect lines ran parallel to each other on a rocky shelf within the cove (Figure 3.1). The "mid-high" transect line ran along the top of this shelf at an elevation of

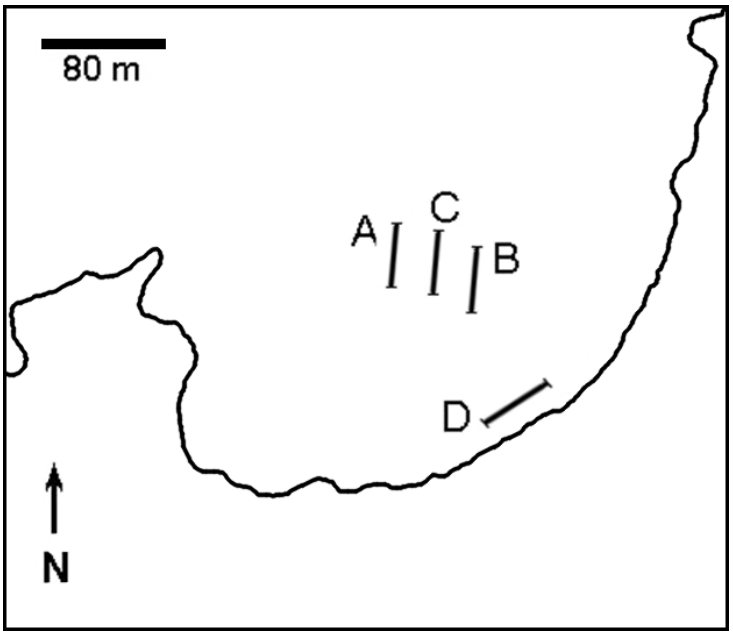


Figure 3.1. Location of transect lines in North Cove. (A) Low tidal level (-0.37 m), (B) Mid-low tidal level (-0.05 m), (C) Mid-high tidal level (0.06 m), and (D) High tidal level (0.20 m).

0.06 m. The "mid-low" transect line, at an elevation of -0.05 m, was 23 m shoreward of the mid-high line, on the eastern edge of the rocky shelf. The "low" transect line was 25 m seaward of the mid-high line on the western side of the rocky shelf at an elevation of -0.37 m. Lines were selected to run nearly continuously through *L. succincta* habitat. Preliminary studies indicated that cobbles, rocks, and gravel are preferred habitat for *L. succincta,* so the lines did not cross any patches of sandy sediment that were devoid of cobbles. Rocks and cobbles along the high line were infrequently covered with up to three inches of wrack, and there was evidence of more sand movement at that tidal level than at the other three levels. The low and mid-high lines were characterized by many different kinds of kelp and algae, including *Egregia menziezii, Laminaria* spp*., Nereocystis leutkeana,* and *Alaria marginata*. The mid-low and high lines had primarily fucoid algae and *Ulva* spp. The low and mid-high lines were exposed to the greatest amount of wave action, while the mid-low and the high lines were protected from waves by the rocky shelf. The mid-low line was the closest transect line to the high line.

Transect lines were 40 m long, and each line was always laid between the same two points. A random number generator was used to select locations along the transect lines to place 100 cm^2 quadrats. Sixteen quadrats were sampled on each transect line. The *Lirularia succincta* on rocks, cobbles, and pieces of gravel within each quadrat were counted. Quadrats that did not lie in areas with cobbles were rejected, as preliminary studies indicated that *L. succincta* were found almost exclusively on loose cobbles and gravel. The population was sampled in this way at least once monthly during negative low tides from January to December of 2009. Differences aomng tidal level and months were compared using a two-way analysis of variance (ANOVA) with Tidal Level and Month as factors. The data violated the assumptions of normality and of homogeneous variance, so the α for the analysis was lowered to 0.010.

After several months of doing transects in the intertidal, it became apparent that there was a great deal of small-scale variability. To determine the degree of variability in the observed

patterns of abundance at small temporal scales, the population was also sampled for four consecutive days in December. Data were analyzed using a two-way ANOVA with Day and Tidal level as factors to determine if the distributions varied significantly each day. The data for this analysis also violated the assumptions of normality and of homogeneous variance, so the α was lowered to 0.010.

Size data for *Lirularia succincta* were also collected on each sampling date from April to August 2009. All individuals found along the transect line at each tidal level were placed into a 50-mL Falcon tube. Tubes containing snails from each tidal level were then brought back to the laboratory, and the diameter of each individual's shell was measured with dial-type vernier calipers to the nearest 0.1 mm. Shell diameter was measured as described in Chapter II of this thesis. The snails were returned to North Cove 24 hours after each collection and replaced at their respective tidal levels. I used the coefficient of variation (CV) to test for differences in size distributions between tidal levels and dates (Ebert and Russell, 1988).

Data on wave height and sea surface temperature for the dates sampled were obtained from the National Data Buoy Center (2011). As there is no buoy offshore of Cape Arago or Coos Bay, wave heights for Cape Arago were estimated by calculating the mean daily wave height for each sampling date at Station 46229 (Umpqua Offshore) and Station 46015 (Port Orford), and averaging the two values. This information was used to see if there was a correlation between the distribution of *L. succincta* and sea surface temperature or wave height.

Results

The distribution of *Lirularia succincta* is highly patchy; 0 to 114 individuals were found in a 100 cm^2 area. It was not uncommon to count 40 individuals in one quadrat, and no individuals in a quadrat 30 cm away. There was no apparent preference for rock type or size, and the snails were found in pools as often as they were found on rocks that were exposed at low

tides. They were often found in small crevices or pits in rocks, but less frequently in large crevices. The average densities of *L. succincta* found at each tidal level were significantly different ($F=101.516$, $p<0.001$), although there was an interaction between tidal level and sampling date ($F=2.956$, $p<0.001$) (Figure 3.2). The highest densities of *L. succincta* occurred at both the low (mean= 16.1 ± SD 16.7 snails/100 cm^2) and the mid-high tidal levels (mean=13.8 ± SD 15.4 snails/100 cm^2). The densities at the mid-low (mean= 6.1 ± SD 7.3 snails/100 cm^2) and high lines (2.6 ± SD 4.2 snails/100 cm^2) were much smaller.

Over the year of study, there did seem to be a seasonal trend of changes in mean density at each tidal level, and the differences in the mean densities among sampling dates are significant ($F=35.400$, $p<0.001$). The highest mean densities of *Lirularia succincta* occurred in September (the low tidal level also had a peak in density in January). The densities of *L. succincta* declined across all tidal levels through spring, reaching the lowest densities at the end of May. The snails all but disappeared from the high intertidal in May. Snails in the summer months were found in high densities on blades of *Ulva* spp. attached to rocks and cobbles; it is likely that they may have moved from cobbles onto boulders that were covered with *Ulva*, but were not sampled.

There was no correlation between the observed densities and wave height (Pearson's $r=0.195$, $p= 0.3960$). There was a correlation between the observed densities and sea surface temperature ($r=0.495$, $p= 0.0225$); however, when the data were plotted, it appeared that the significance of the correlation was due to a single outlier. The highest mean density of *Lirularia succincta* observed (26.0 ± SD 20.3) occurred on the day that had the highest sea surface temperatures (17.62°C, September 19). When this point was removed, the correlation was no longer significant ($r=0.281$, $p= 0.2300$).

Sampling conducted on four consecutive days in December also showed a significant difference in mean densities among tidal levels ($F=36.682$, $p<0.001$), but not among days ($F=1.727$, $p=0.162$), and there was not a significant interaction ($F=0.678$, $p=0.729$) (Figure 3.3).

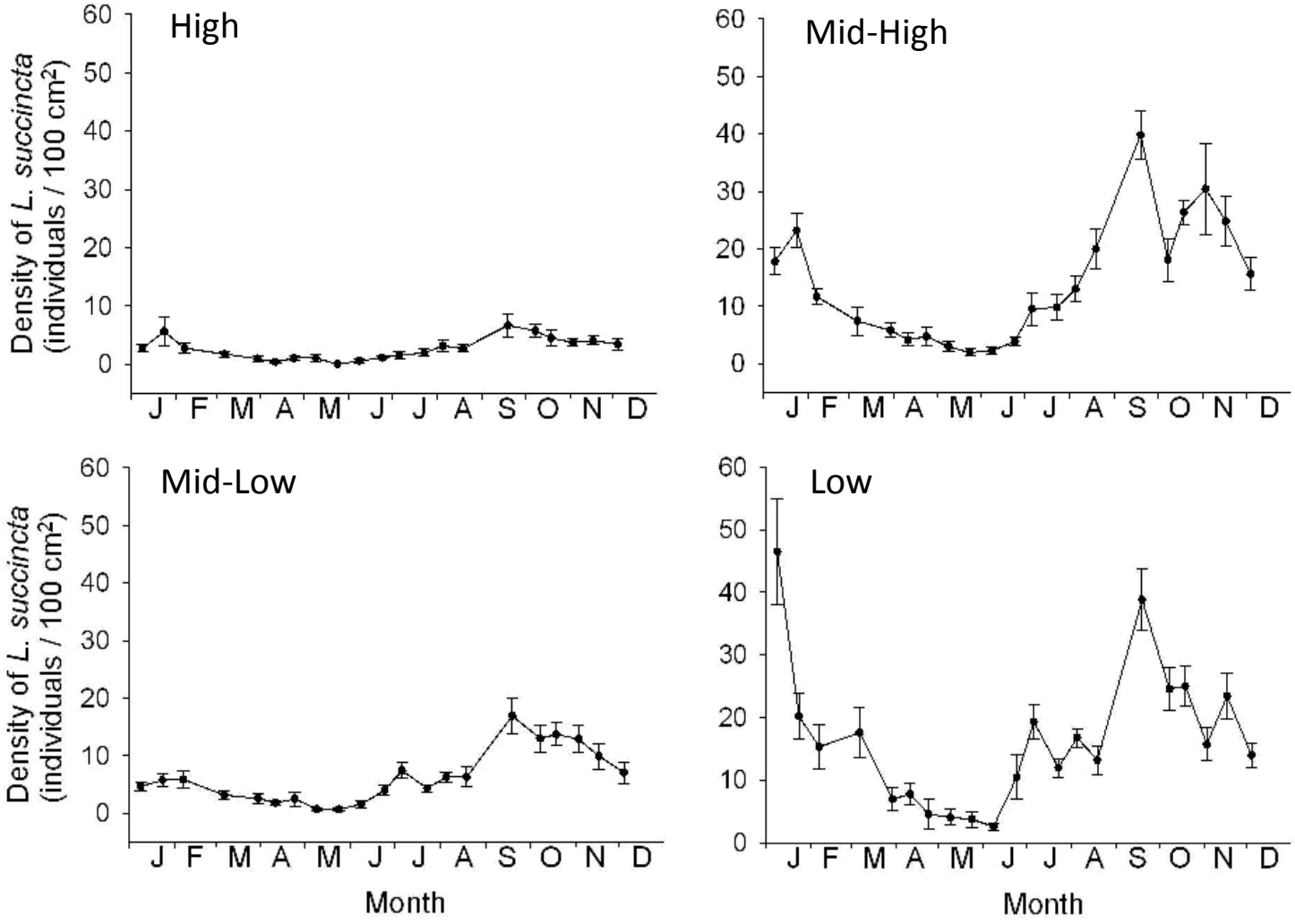


Figure 3.2. Distribution of *Lirularia succincta* at four tidal levels at North Cove. Error bars represent 1 SE.

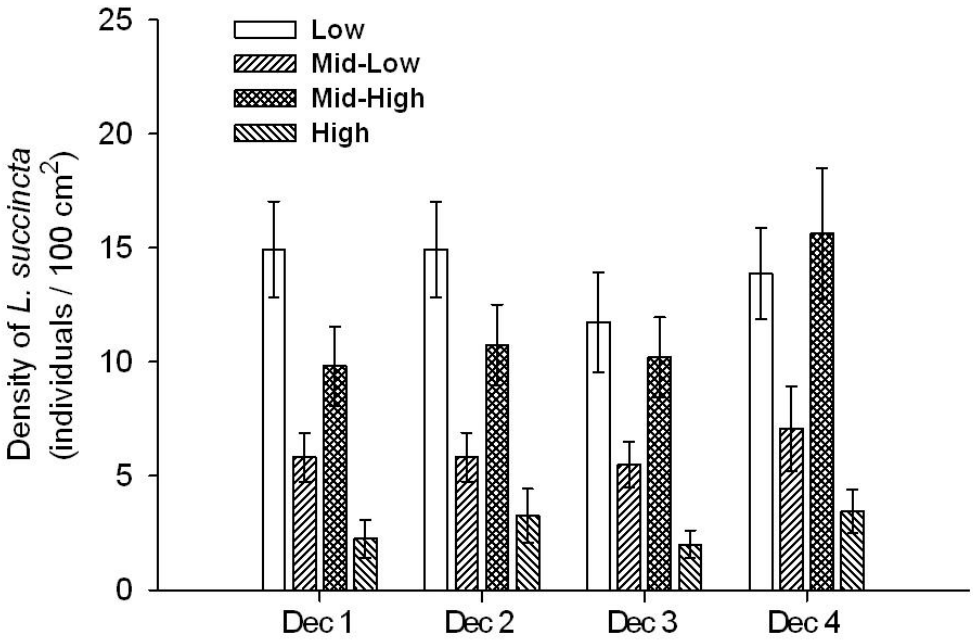


Figure 3.3. *L. succincta* distribution at four tidal levels on four consecutive days in December 2009. Error bars represent 1 SE.

The average shell size and the coefficient of variation for each tidal level on each sampling date are included in Figures 3.4 and 3.5. Shell sizes ranging from 0.7 mm to 4.8 mm were collected from North Cove between April and August. Until the end of May, the largest snails were located in the high intertidal; on April 25, mean snail size in the high intertidal was almost 1 mm larger than in all other tidal levels. After May, the mean size of *Lirularia succincta* was approximately equal at all tidal levels; total mean diameter decreased more than 1 mm, from 3.10 ± SD 0.44 mm to 2.08 ± SD 0.69 mm, until July. After July, the total mean diameter of snails in all tidal levels began to increase, which coincided with an increase in the density of *L. succincta* at each tidal level as well. The coefficients of variation increased steadily from April until July, when they began to decrease, indicating that there

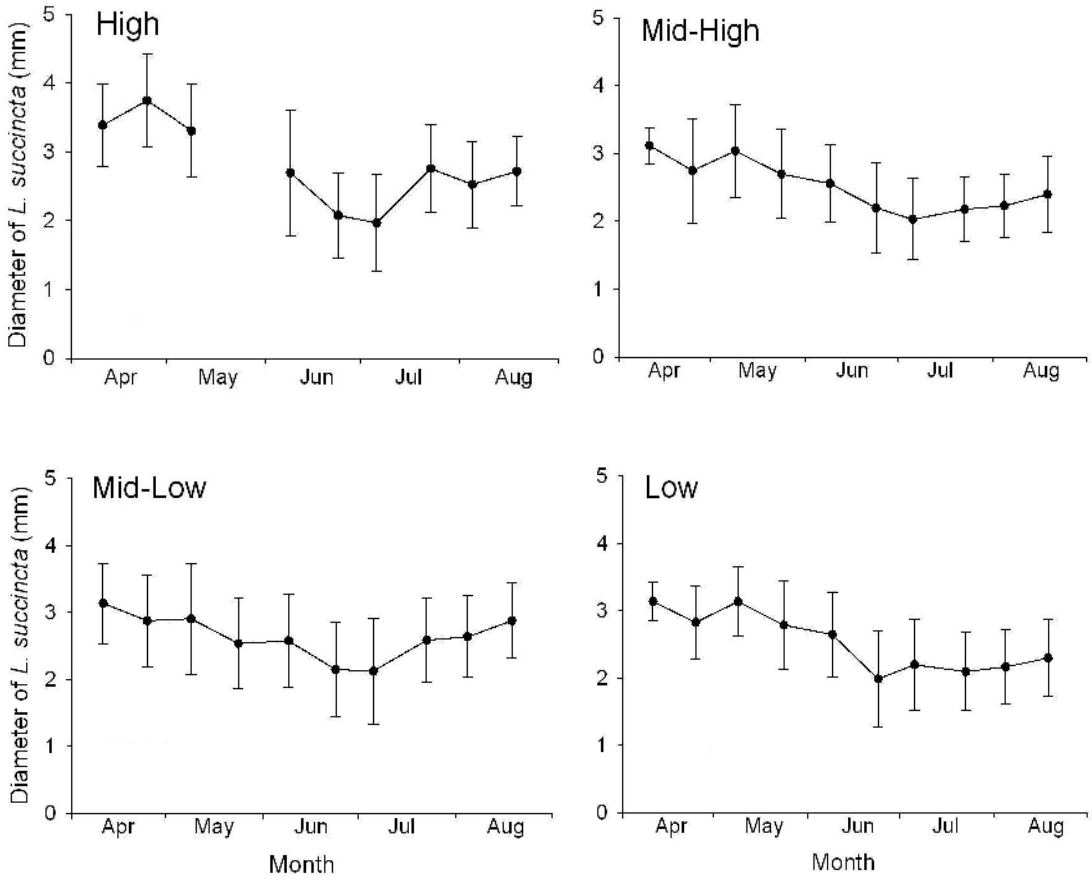


Figure 3.4. Mean sizes of *Lirularia succincta* collected at four tidal levels at North Cove from April through August of 2009. No snails were found in the high intertidal on May 24.

were more size-classes present in the intertidal in the summer, specifically in the low and mid-low tidal levels (Figure 3.5).

Age frequency histograms were generated for each tidal level on each sampling date and are included in Appendix A. The sizes from all tidal levels were combined to show overall size-frequency trends from April through August (Figure 3.6). In the late spring, the peaks were beginning to shift slowly towards smaller size classes until the end of June when

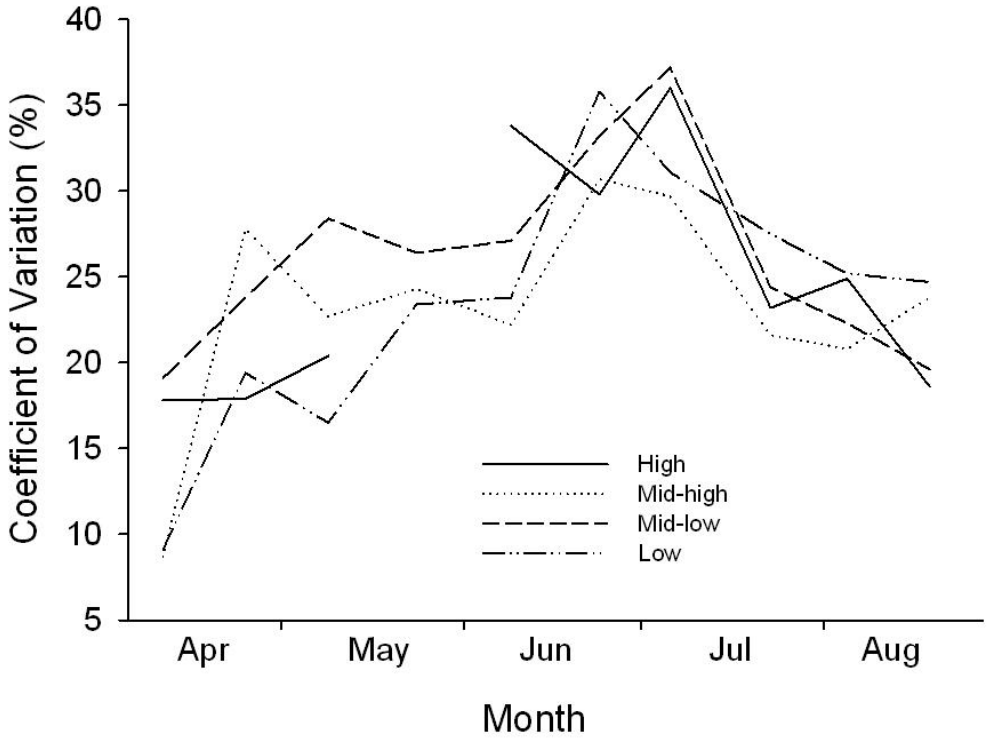


Figure 3.5. Coefficients of variation in size-frequencies of *Lirularia succincta* at each tidal level from April through August of 2009.

there appeared to be an influx of the smallest size classes. From April until June 9, numbers of snails found in the lowest four size classes ranged from 0 to 13; on June 24, there were 109 individuals collected from those classes. After June 24, the numbers in those size classes decreased through August, and the peak was beginning to shift towards larger size classes again. The peaks for the size classes at all four tidal levels shifted from between 3.5 and 4.3 mm in the late spring to less than 2 mm at the end of June. There was a slight decrease in density in June that coincided with this decrease in large size classes. The density of *Lirularia succincta* increased in August, and the peaks in size classes shifted again towards 3 mm, but snails were still smaller than they had been in the spring.

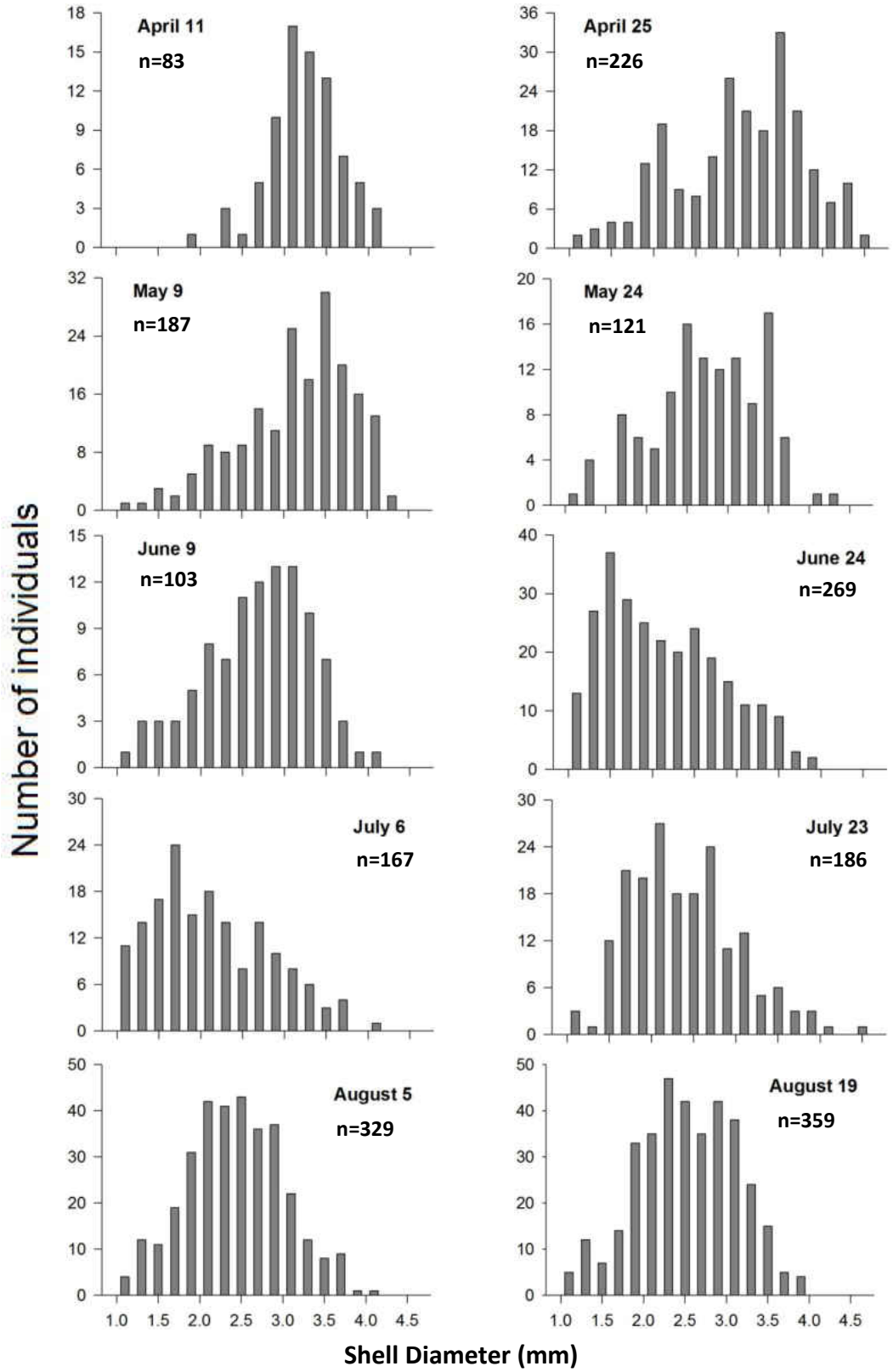


Figure 3.6. Size-frequency histograms of *Lirularia succincta* collected at North Cove from April through August of 2009.

Discussion

Population density for *Lirularia succincta* is difficult to estimate; animals are highly mobile, and are often clumped. Overall, differences in abundance and size frequency within North Cove occurred between tidal levels over a horizontal scale of almost 100 m. Abundances and sizes varied seasonally, but were not correlated with sea surface temperatures or wave height.

Declines in the densities of *Lirularia succincta* in the spring months could have been due to large-scale migration into the subtidal. If this occurred, I would have expected to see either 1) a decrease in the densities of snails at the upper tidal levels and an increase in densities at the lower tidal levels, or 2) a time lag in the decline in densities in the lower tidal levels. Although it is unknown if *L. succincta* is migratory, it does not appear that vertical migration occurs; there was never a shift in the "zone of maximum density" of the snail (Gendron, 1977) or apparent movement from one tidal level to another. There was merely a decrease in abundance across all tidal levels. Throughout the year, the majority of *L. succincta* were usually found in the low intertidal.

Variations in the patterns of distribution may have been caused by migration, but not along the vertical gradient of the shore. In Japan, densities of *Lirularia iridescens* within seagrass beds decreased in May and increased again until August (Nakaoka et al., 2001). Changes were related to surface area of the leaves, rather than to vertical position within the intertidal. A change in habitat preference resulting in the movement of *Lirularia succincta* from one habitat type to another could be indicated in the decline in densities in the spring. Macroalgae including *Ulva* spp. are much more prevalent in the summer. It is possible that snails moved from the cobble substratum onto boulders on which macroalgae was growing. Since the transects were still conducted counting individuals in the cobble habitat, a change in habitat preference could have manifested itself as a decline in snail abundance. Adult *L. succincta* and egg masses were

often found near the base of *Ulva* spp., so this change in habitat preference may have been driven by reproduction.

Olabarria and Chapman (2001) emphasized the importance of determining the scale of spatial replication required to ensure that the measurements of distributions are representative of a species. In an attempt to determine if the observed spatial and temporal variations in abundance were representative of the actual distribution, I conducted transects on four consecutive days in December. Differences in abundance among days were not significant; it is likely that, while the density of *L. succincta* varied to a great extent among the replicate quadrats, particularly when abundances were great, the variations in observed densities among transects did not influence the observed temporal variation. The fact that similar trends were observed throughout the year at each tidal level also supports this conclusion.

Changes in the size-frequency distributions of *Lirularia succincta* in the spring and summer were similar at each tidal level. There did not appear to be a shift in the size frequencies of one tidal level relative to another. It did appear that there was a weak size gradient in April and early May, with larger but fewer individuals high in the intertidal and higher densities of smaller individuals in the low intertidal. After June 24, the abundance of snails high in the intertidal remained low, but the size gradient disappeared. Vermeij (1972) hypothesized that gastropods that are prevalent lower in the intertidal would increase in size towards higher shore levels; this pattern was not observed in *L. succincta.*

Egg masses of *Lirularia succincta* are deposited throughout the intertidal, so the occurrence of small size classes at one tidal level would be the result of active migration. The small size classes were present at all tidal levels. The abundance of snails in the small size classes appeared to diminish after only two weeks; this might be explained if mortality in these size classes were very high, or if the juveniles were growing quickly (at a rate of 0.2 mm each month to account for the shifts in peaks observed in the size-frequency histograms). The growth

rate of juvenile *L. succincta* is unknown; I was unable to keep them alive in the laboratory for more than 10 days. If mortality is high or unpredictable throughout the vertical range of the species, the snails could potentially have higher growth rates or mature earlier (Vermeij, 1972).

Toyohara et al. (1999) found that population densities of *Lirularia iridescens* in Japanese seagrass beds increased rapidly due to recruitment from May to July. As with *Lirularia succincta,* eggs of *L. iridescens* can be found in the intertidal in almost all months, although *L. iridescens* experiences a breeding peak in April; in the laboratory, *L. succincta* increases its rate of oviposition through the summer, with a breeding peak in July (see Chapter IV of this thesis). It is possible that the breeding peak for *L. succincta* is earlier in the field than in the laboratory; if so, then the increase in the number of individuals in the smaller size classes at the end of June could have resulted from a peak in oviposition in the spring. If new recruits were present in the population, I would have expected to see bimodal size-frequency distributions similar to those described by Toyohara et al. (1999). It is possible that such distributions were present in April but did not register because the recruits were too small to be noticed until June. Newly-hatched *L. succincta* individuals are white and have an average size of 293.2 ± SD 26.7µm (Chapter IV of this thesis). The smallest *L. succincta* individual collected and measured was 0.7 mm and had the tan, sculptured shell of an adult. The coefficient of variation (CV) increased through the spring and into summer, and then began to decrease. If my sample sizes were too small to show bimodal distributions, the higher coefficients of variation may indicate incoming small size classes with older large size classes present because *L. succincta* deposits egg masses year-round. After the mortality of both juvenile and adult *L. succincta* individuals, the remaining population of L. succincta consisted of the younger, smaller cohort that was more homogeneous in size, decreasing the CV.

The increase in the abundance of snails in August could be due to migration from the surrounding algae. The fact that the larger individuals mostly disappeared from the distribution

after June 24 could result from a migration into alternate habitats with a different food source, or it could be due to mortality, especially if the species has an annual lifespan. It is interesting to note that after June 24, only 4 snails larger than 4 mm were ever found, and only one was found in almost 700 individuals collected in August. This could indicate that the larger individuals died off in May or June as the new recruits appeared. However, this does not explain the sudden increase in the abundance of intermediate-sized *Lirularia succincta* in August. Unfortunately, because I only had data for 5 months of the year, I cannot infer causes of the size changes.

The observations of the densities and size-frequencies at the high intertidal level were always most similar to those for the mid-low intertidal, which was the closest transect line to the high line. Similarly, the observed densities and size-frequencies of *Lirularia succincta* in the low intertidal were more similar to observations from the mid-high tidal level, which was the closest transect line. This indicates that tidal elevation does not affect the distribution of *L. succincta* as much as other factors that, at North Cove, may vary based on the proximities of the transect lines, such as wave action, biodiversity, and possibly predation.

It is likely that *Lirularia succincta,* like its congener *Lirularia iridescens,* grazes on microalgae. If this is the case, it is probable that the distribution of the species is not influenced by food availability or by competition, either for food or for space. However, the distribution of *L. succincta* may be affected by predation. The sea star *Leptasterias* spp. has been observed on three occasions feeding on *L. succincta* in the field; *Leptasterias* spp. individuals also chase and consume *L. succincta* in the laboratory (see Chapter II of this thesis). Studies done by Niesen (1973) show that the incidence of predation of *L. succincta* in the field is probably low; preferred prey of *Leptasterias hexactis* are *Balanus* spp. and spirorbid worms, although small intertidal gastropods do make up a small part of its diet. Other animals such as juvenile *Pycnopodia helianthoides*, juvenile *Cancer productus* and *Hemigrapsus* spp.have been observed eating *L. succincta* in the laboratory, and the shrimp *Heptacarpus brevirostris* eats the eggs of *L. succincta.*

Although incidences of predation by these animals have been observed, the extent to which the snail population is limited by predation is unknown. To interpret these interactions of predators with *L. succincta,* the density of the predator, and the importance of the species in the predator's diet must be examined (Paine, 1969).

When a species is motile and can respond to its environment, the variability in the patterns of its distribution or abundance are influenced to the greatest extent by biotic interactions, behavior, and small-scale environmental variables (Underwood and Chapman, 1996). Variations in the densities and in the size-frequencies among tidal levels may have resulted from fluctuations in their environment, from interactions with predators, or from changes in habitat preference resulting from the growth of macroalgae in the summer. If *L. succincta* has an annual lifespan, this could account for the decreases in densities and changes in size-frequencies in the spring and early summer. Underwood and Chapman (1996) predicted variability in abundance at very small scales if the behavior of the adults is important in determining distribution patterns. Therefore, I would predict that behavior is important in determining the patterns of abundance for *Lirularia succincta.*

This study merely described the distribution of *Lirularia succincta* in a single location; ultimately, the factors determining the distribution and abundance of *L. succincta* remain unclear. For species such as *L. succincta* found primarily in the lower intertidal, Vermeij (1972) predicted that mortality is probably most intense lower in the intertidal, where biotic interactions such as predation are more likely to affect the abundance of the species (Connell, 1961, 1970). If *L. succincta* is an annual species, this study has served to indicate the time at which the population replaces itself as well. Information gathered from this study can lead to hypotheses regarding preferred habitat and factors affecting their distribution, and can give an indication of their ecological importance in the habitats in which they may be found.

Bridge

Chapter III described the vertical distribution of *Lirularia succincta* in a protected cove. The data indicated that *L. succincta* is more abundant in the low intertidal and that the densities of *L. succincta* decrease across all tidal levels in the early spring. Densities of *L. succincta* increased in June, at the same time that small size-classes began to appear in greater numbers in the population. I had observed *L. succincta* egg masses deposited year-round, but no studies have been done to indicate if *L. succincta,* like its congener *Lirularia iridescens* (Toyohara et al., 1999), exhibits seasonal peaks in its reproductive output. *Lirularia succincta* are direct developers; juvenile *L. succincta* emerge from egg masses after metamorphosis and do not spend time in the plankton. Therefore, oviposition behaviors of female *L. succincta* will define the distribution of the juveniles (Meadows and Campbell, 1972). To fully understand the patterns observed in the intertidal, knowledge of oviposition behaviors are necessary. Previous studies have shown that in direct developers, the sites selected and the conditions under which the embryos develop within the mass can affect the survival of embryos within the mass (Biermann et al., 1992). Chapter IV focuses on determining conditions that may affect oviposition behavior in female *L. succincta.*

CHAPTER IV

FACTORS THAT AFFECT OVIPOSITION IN *LIRULARIA SUCCINCTA*

Introduction

Trochaceans, while being some of the most evolutionarily advanced archaeogastropods, are still among the more primitive of prosobranch mollusks (Hadfield and Strathmann, 1990). They have separate sexes and external fertilization, and their eggs are often covered with jelly, forming gelatinous egg masses (Fretter and Graham, 1977; Strathmann, 1987). These egg masses can be deposited on surfaces (Holyoak, 1988; Hadfield and Strathmann,1990; Toyohara et al., 1999) or dispersed in water currents (Strathmann, 1987; Hadfield and Strathmann, 1990). Developmental mode is variable within the trochaceans, to the extent that it cannot be predicted based on sub-family or genus (Hadfield and Strathmann, 1990). Some trochacean species develop fully within a benthic egg mass, hatching as juveniles (Holyoak, 1988; Hadfield and Strathmann, 1990; Toyohara et al., 1999). In other species, eggs are initially surrounded by jelly when they are spawned, but the jelly disperses, and larvae hatch from individual embryos (Strathmann, 1987; Hadfield and Strathmann, 1990).

Embryo encapsulation is common in plants and is encountered among many animals, including insects, amphibians, fish, polychaetes and marine gastropods (D'Asaro, 1970; Pechenik, 1979; Wiklund, 1981; Caldwell, 1986; Wilson, 1986; Reich and Downes, 2003). In marine gastropods, egg masses may take different forms, including gelatinous egg masses, firm egg capsules, adherent masses of eggs with open interstices between them, and eggs in fluid-filled, thin-walled capsules (D'Asaro, 1970; Pechenik, 1979; Strathmann and Chaffee, 1984;

Rawlings, 1994; Strathmann and Hess, 1999). Egg mass forms, like developmental modes, can differ among families and closely-related species (D'Asaro, 1970; Strathmann 1987; Hadfield and Strathmann, 1990).

There are several potential benefits to depositing egg masses. Depositing embryos in egg masses may retain the embryos at sites deemed by the adults to be favorable (Strathmann, 1985; Pechenik 1986), and may provide them with protection from environmental stress or from planktonic predators (Rumrill, 1990; Woods and DeSilets, 1997). [All pre-metamorphic developmental stages contained within an egg mass will be termed 'embryos', as defined by Giese and Pearse (1974)]. Embryos that remain on the bottom for some part of their development may spend a shorter period drifting in the plankton than embryos released by broadcast spawners. Less time in the plankton limits or prevents dispersal of propagules away from areas that are favorable either for embryonic development or for juvenile growth (Hendler and Franz, 1971). This is especially true if the species exhibits direct development and if the females select sites for oviposition that will be optimal for juvenile survival and growth. The absence of a planktonic larval stage can affect both the gene pool and the distribution of populations within a species because direct developers have limited dispersal and probably receive a large proportion of their recruits from their own population (Kyle and Boulding, 2000). After comparing two littorinid species with direct development to two littorinid species with a planktonic larval stage, Kyle and Boulding (2000) found that the species with direct development had higher levels of genetic variation between populations. In a conservation study of 13 rocky intertidal sites in New South Wales, Australia, Benkendorff and Davis (2004) discovered that species with direct development occurred at fewer sites than species with larvae that spend at least some time in the plankton.

The encapsulation and retention of embryos in the parental habitat can also reduce embryonic mortality by maintaining developing embryos within the egg mass until they are better able to avoid benthic predation or to cope with conditions they might encounter in the plankton

(Pechenik, 1979, 1986). Indeed, Hadfield and Strathmann (1990) have hypothesized that the late intracapsular stages observed in direct developers may result from a delay in embryonic hatching caused by toughened jelly coats inside the mass.

Further protection is provided to the embryos by the very nature of the gelatinous matrix of the egg mass. Gel has been shown to slow the exchange of heat and particles between the mass and its environment (Woods and DeSilets, 1997; Lee and Strathmann, 1998). During periods of emersion or desiccation, this can moderate the change in salinity experienced by the embryos within the mass, enabling encapsulated embryos to survive several hours exposure at low tide, while individual eggs would not be expected to survive (Strathmann and Hess, 1999). The effect of gel on exchange processes within a mass can protect embryos from rapid decreases in salinity as well. By slowing the rate of salinity change, the cellular mechanisms that regulate cellular volume have more time to acclimate (Woods and DeSilets, 1997; Strathmann and Hess, 1999). Capsule walls may also shield embryos from UV radiation (Rawlings, 1996).

Less time in the plankton could also provide the embryos with a refuge from predation in the plankton (Rumrill, 1990; Rawlings, 1994), as the mortality rates of encapsulated embryos appear to be lower than those for planktonic larvae (Strathmann, 1985). Development on the sea floor may not necessarily be safer than development in the plankton, as the safety of embryos deposited in gelatinous masses depends upon a suite of variables, including the parental choice of oviposition site, the presence of benthic egg predators, and the structure of the mass itself. Species experience varied availability of safe benthic oviposition sites (Benkendorff and Davis, 2004; von Dassow and Strathmann, 2005), egg mass predation (Shimek, 1981; Rawlings, 1990, 1994), and differences in the ability to produce masses that provide protection in the adult's range of habitat (Biermann et al., 1992).

Oxygen limitations present another drawback for egg masses. Strathmann and Chaffee (1984) likened a deposited egg mass to "a large mass of tissue without a circulatory system,"

limited in size and form due primarily to oxygen requirements of embryos within the mass. The amount of oxygen reaching each embryo is limited by the gel and by sibling embryos in the mass (Cohen and Strathmann, 1996; Moran and Woods, 2007). Oxygen limitation within the mass can alter the embryos' development time and the size of juveniles upon hatching, and can ultimately lead to embryo mortality (Woods and DeSilets, 1997; Lee and Strathmann, 1998). Cohen and Strathmann (1996) found that the decrease in oxygen available to embryos may be mitigated by the presence of photosynthetic microorganisms associated with egg masses, although Biermann et al. (1992) concluded that these microalgae have a detrimental effect on egg masses. While increasing the volume of gel per embryo in masses can increase the oxygen supply to the embryos, the gel itself is an extra investment by the female (Lee and Strathmann, 1998). The energy expenditure associated with production and deposition of the egg mass could be high for the female, affecting her fecundity. Therefore, an adaptive compromise is required between parental investment and the need for ventilation within the mass that will ultimately affect each embryo's development and mortality (Strathmann and Chaffee, 1984; Lee and Strathmann, 1998).

While the gel surrounding the embryos can provide some degree of protection from solar radiation, desiccation and predation, encapsulated embryos are not immune from these potentially lethal factors (Pechenik, 1986; Biermann et al., 1992; Rawlings, 1994; Przeslawski, 2005). Russell and Phillips (2009) noted that desiccation led to increased mortality in the encapsulated embryos of a species of bubble-shell snails. Solar radiation can kill benthic embryos within an egg mass, especially when the mass is deposited in shallow tide pools, although thick jelly and other embryos can provide protection for the embryos in the inner portions of the mass (Biermann et al., 1992). It is likely that the vulnerability of embryos to environmental stressors is species-specific (Russell and Phillips, 2009). The selection of an oviposition site can mitigate the effects of some environmental stressors on an egg mass. Biermann et al. (1992) noted that egg masses of *Archidoris montereyensis* were preferentially laid in shady spots, and that embryo mortality was

lowest in the shade; they concluded that egg masses were preferentially laid at sites in which survival of embryos was high.

The survival, growth and development of embryos within egg masses are necessarily associated with the oviposition behavior of the parents and so can be temporally or spatially variable (Reich and Downes, 2003). The effect of oviposition behavior on embryo survival and growth can influence the dynamics of populations, especially in direct-developing species. Oviposition behaviors can be influenced by environmental factors and by conspecifics. Environmental factors affecting oviposition behaviors may include season, temperature, water movement, and topography (Caldwell, 1986; Martel and Chia, 1991; Biermann et al., 1992; Benkendorff and Davis, 2004). Many species of marine gastropods are reproductive in certain seasons of the year; others, such as *Lirularia iridescens* and *Margarites marginatus* deposit egg masses year round (Strathmann, 1987; Toyohara et al., 1999). *Lacuna vincta* deposits egg masses year round, but still exhibits periodicity in egg mass deposition in an annual cycle (Martel and Chia, 1991). The seasons in which females deposit egg masses determines the physical stressors to which the embryos and newly-hatched juveniles are exposed. Southern leopard frogs exhibit distinct oviposition patterns that are related to season and to temperature (Caldwell, 1986). Temperature may also affect egg mass deposition in *Lirularia succincta,* as other trochids are known to shed gametes when warmed (Strathmann, 1987). Water movement may also affect a female's choice of deposition sites. Low water velocities around benthic egg masses can arrest or retard development (Cohen and Strathmann, 1996); animals may select sites for the deposition of egg masses in areas with greater water movement. Higher water velocities may also affect the extent to which masses are fouled with microalgae, although the effect of microalgal fouling on the embryos within the mass varies among species (Biermann et al., 1992). The topography of the site selected for oviposition may affect both water movement around the mass and shading of the mass. Egg masses in crevices or shaded areas are less likely to become desiccated, and

mortality rates from exposure to UV radiation in such sites are lower (Biermann et al., 1992). Oviposition for most of the marine gastropods surveyed by Benkendorff and Davis (2004) required specific microhabitats.

While environmental factors may strongly influence oviposition behaviors, those behaviors may also be influenced by other conspecifics. Oviposition behaviors have been shown to be temporally or spatially affected by the formation of breeding aggregations (Kupfermann and Carew, 1974; Jahan-Parwar, 1976). The formation of such aggregations may occur in response to the environmental factors described above (current, topography, temperature) or to the availability of resources (D'Asaro 1970; Croll, 1983). Aggregations may also form due to the accumulation of egg masses deposited by conspecifics or to chemical cues released by mating or egg-laying adults (D'Asaro, 1966, 1970; Kupfermann and Carew, 1974; Audesirk, 1977; Croll, 1983). Jahan-Parwar (1976) noted that one actively depositing *Aplysia* individual attracts other *Aplysia* individuals and elicits further mating and egg-laying within individuals of the aggregation. These behaviors may also be initiated by the introduction of an egg mass into the holding tank of the adults. Kupfermann and Carew (1974) recorded several *Aplysia* individuals depositing egg masses at sites with accumulated egg masses, resulting from either the egg-laying activity of several animals or of the same animals at different times. Breeding aggregations are also found in frogs (Caldwell, 1986) and in insects, where swarming behavior induced by mating can be associated with the selection of a site for oviposition (Reich and Downes, 2003). Such aggregations of breeding individuals allow individuals of both sexes to find mates (D'Asaro, 1970).

As with other members of family Trochidae, sexes of *Lirularia succincta* are separate. Fertilization in *L. succincta* is external, and is facilitated when adults pair during spawning (Hadfield and Strathmann, 1990). At this time, the male sits atop the female and releases sperm as the eggs are deposited onto the substratum by the female. This reproductive behavior has also

been observed in another trochoidean; in the species *Margarites marginatus*, the male sits either on, or close to, the female's shell while the female deposits her egg mass (Holyoak, 1988 (described as *M.* helicinus); Hadfield and Strathmann, 1990). In both species, the sticky gel coating the eggs is apparently penetrable by sperm (Hadfield and Strathmann, 1990). After the gelatinous egg masses are deposited by the females, embryos develop, pass through an intracapsular veliger stage, and then metamorphose and emerge as crawling juveniles from the mass. This developmental strategy is shared by other trochoideans including a congener of *L. succincta, Lirularia iridescens* (Toyohara et al., 1999).

Factors affecting oviposition within the superfamily Trochacea probably vary as much as both egg mass and developmental forms. These factors are rarely studied despite their importance in determining population distributions. Because *Lirularia succincta* are common in the intertidal and deposit egg masses soon after being brought into the laboratory throughout the year, they are an ideal species for studying factors that can affect oviposition. Egg masses are found in the field year-round as well. However, to this date, factors affecting oviposition have not been studied in the subfamily Lirulariinae. In this study I examine several factors that I expected to influence oviposition in *L. succincta.* These factors include season, light, temperature, water movement, topography, and the presence of other masses. This study documents the presence of experimentally-determined patterns in oviposition behavior for *L. succincta.*

Materials and Methods

Snail Collection and Maintenance

Lirularia succincta individuals were collected from South Cove (43^{o} 18' 11" N, 124^{o} 23' 55" W) and North Cove (43^{o} 18' 31" N, 124^{o} 23' 55" W), Cape Arago, Oregon (Figure 2.1 in Chapter II of this thesis). Individuals not used immediately for an experiment were kept in 3.7 L

jars of seawater in a cold room at temperatures ranging from 9 to 13°C. The feeding habits of *L. succincta* are unknown, but the radular morphology of members of the subfamily Lirulariinae may indicate suspension or deposit feeding (Hickman, 1985; Hickman and McLean, 1990). Although it was observed that *L. succincta* can be kept for at least one month in 60 x 15 mm polystyrene petri dishes without food, I placed cobbles from North Cove in the jar with them if they were maintained in the laboratory for longer than two weeks (except for snails used in the Season oviposition experiment). The cobbles were replaced every spring tide. When rocks were placed in the jars, the snails moved onto the rocks, presumably to feed. Snails were not kept for longer than six weeks in the laboratory.

Factors Affecting Oviposition

Season

During one spring tide each month, up to 200 *Lirularia succincta* were collected from North Cove and brought into the laboratory. Two haphazardly selected individuals were placed into each of 100 60 x 15 mm disposable polystyrene petri dishes. The petri dishes were filled with seawater, covered, and placed in a single layer on the bottom of several plastic containers (25 x 37 x 14 cm), which were floated in a sea table with flowing seawater. For three months when it was difficult to find *L. succincta* in the intertidal, fewer than 100 pairs were used (97 pairs in June, 61 pairs in August, and 77 pairs in September).

Seawater was changed in the petri dishes every one to three days. When the water was changed, each dish was examined for egg masses. If an egg mass had been deposited, the dish was removed from its container.

Although *Lirularia succincta* has two separate sexes, an individual's sex cannot be determined unless the snail is dissected. I assumed an equal number of males and females were collected for the experiment, giving me expected values of 50% of the petri dishes with a male-

female pairing, 25% with a female-female pairing, and 25% with a male-male pairing. Every ten days, partners of snails that hadn't laid egg masses were switched to increase the probability that each snail would be paired with a snail of the opposite sex. Since each time that a snail was paired with a new partner, the likelihood of being paired with a snail of the same sex, or of a different sex, was equally probable, after being paired with three different partners, the expected probability that any snail had been paired with a snail of the opposite sex at some point in the experiment was 87.5% (the actual probability of a pairing with a snail of the opposite sex would be less than this value, as it is unlikely that the same number of each sex had been collected initially). All snails were removed from the petri dishes after 27 days. Any adverse effects of oxygen depletion or of a build-up of snail waste in the dishes were expected to be similar in all dishes across all months.

The Presence of a New Partner

In the first few months of the Season oviposition experiment described above, I noticed that if the mating pair of *Lirularia succincta* were not immediately removed from the petri dish in which an egg mass had been laid, another egg mass would often be deposited. An experiment was conducted to determine if individual snails were more likely to deposit additional egg masses if they retained their original partner or if they were paired with a new partner.

This experiment was conducted in May and July of 2010. In May, 30 egg-mass producing mating pairs were used, while 38 pairs were used in July. Each mating pair of snails was randomly assigned to one of two treatments. Mating pairs in the first treatment (n=15 in May, n=19 in July) were removed from the petri dish in which they had been originally placed. The individuals were then separated and placed into a clean new dish with an individual from a different mating pair. Mating pairs in the second treatment (n=15 in May, n=19 in July) were placed with the same partner into a clean petri dish. All dishes from both treatments were then

randomly placed in a single layer on the bottom of two containers (25 x 37 x 14 cm) and floated in a sea table with flowing seawater.

Every three days the water in the dishes was changed and the dishes were examined for egg masses. At that time, snails in the first treatment that had not deposited an egg mass were placed with a new partner in an effort to ensure that each snail was paired at some point with a snail of the opposite sex. When an egg mass was deposited, the number of eggs in the mass was counted. The snails in the first treatment had only an 87.5% chance of being paired with a member of the opposite sex, while all snails in the second treatment were paired with a snail of the opposite sex. For each trial, the number of egg masses deposited by snails in both treatments was compared using a chi-squared goodness-of-fit test with the Yates correction for continuity (Zar, 2010). For the analysis, the expected frequencies of egg masses deposited by snails in the first treatment were 87.5% of the expected frequency of egg masses deposited by snails in the second treatment. The numbers of eggs deposited per egg mass were compared between treatments and between trials using a two-way ANOVA. To ensure even numbers for the analysis, randomly selected egg counts were dropped from the first treatment in Trial 1 and from both treatments in Trial 2. The numbers of eggs in each egg mass were square-root transformed so the data would meet the normality assumption.

Light

Lirularia succincta were collected from South Cove in May of 2010. Five adults were assigned to each of 42 50-mL Falcon tubes. All Falcon tubes were laid horizontally in a cold room at 11^{o}C under constant fluorescent lighting. Each Falcon tube was randomly assigned to one of three treatments. In the first treatment, 14 Falcon tubes were completely covered in three layers of black plastic. These Falcon tubes remained in the dark continuously throughout the experiment. The 14 Falcon tubes in the second treatment experienced a 12:12 light:dark cycle.

The Falcon tubes were completely covered in 3 layers of black plastic for 12 hours. The black plastic was removed, then replaced every 12 hours. In the third treatment, 14 Falcon tubes remained uncovered throughout the experiment. The water in the Falcon tubes was replaced each day. The number of egg masses in each tube was recorded after four days.

The experiment was repeated three weeks later as described above. However, the number of egg masses in each tube was also recorded on the first and second day.

The mean numbers of egg masses deposited per Falcon tube were compared among treatments and between trials using a two-way ANOVA. The mean numbers of egg masses deposited per day for the second trial were analyzed using a two-way repeated measures ANOVA with Time and Treatment as factors.

To determine if *Lirularia succincta* preferentially lay egg masses in crevices, the position of each mass within each Falcon tube was also recorded. The internal surface area of a 50-mL Falcon tube was measured and the percentage of the internal surface area considered to be a 'crevice' was calculated. 'Crevice' areas in the Falcon tube were deemed to be the area near the mouth of the Falcon tube that is covered or shaded by the cap, and the conical end, from the crease to the tip. This overestimation of 'crevice' area within the Falcon tube enabled a more conservative analysis. A goodness-of-fit G-test was performed to determine if the masses were laid in the bottom or under the lid of the Falcon tube (areas similar to rock crevices) more frequently than would be suggested by chance. The numbers of masses deposited in each area of the Falcon tube were compared between treatments using a chi-squared analysis of a contingency table. The null hypothesis for the contingency table was that the egg mass deposition sites in the Falcon tubes were chosen independent of light treatment.

Shading

To test for selective deposition between 'shaded' and 'non-shaded' areas another experiment was conducted. *Lirularia succincta* were collected from North Cove in February of 2011. Six *L. succincta* individuals were placed into each of twenty 60 x 15 mm disposable polystyrene petri dishes. The 20 dishes were then randomly assigned into one of two treatments. In the first treatment, half of the bottom and lid of each dish was covered with black plastic. In the second treatment, the lid and the bottom of the petri dish were divided into halves with a Sharpie line, and one of the halves was marked, to distinguish between the two sides. All of the dishes were then placed in random positions, and at random orientations, on a tray, which was placed on a shelf below a fluorescent light in a cold room, and kept at 10^{o}C for 3 days. Once each day, the location of each snail in each dish was noted and the number of egg masses laid on each side of each dish was counted. At that time, the seawater in each dish was replaced.

The experiment was repeated as described above in March of 2011 with snails collected from South Cove.

The numbers of egg masses deposited on both sides of the dishes in both treatments were compared using a chi-squared goodness-of-fit test for each trial. The null hypothesis was that egg masses were deposited on both sides of the dishes in both treatments with equal frequency.

The frequencies of adults positioned on each side of the dishes in both treatments were compared using two-way repeated measures ANOVAs with Day and Treatment as factors. The frequency data were arcsine transformed before the analysis.

Water Movement

The effect of water movement on oviposition in *Lirularia succincta* was tested using 3 different experiments, with three different methods for generating water movement. In the first experiment, 180 snails were collected from South Cove in March of 2010. Ten snails were

placed into each of twelve 900 mL glass jars that had been filled with 800 mL of seawater. The jars were then randomly assigned to one of two treatments. In the first treatment, six jars were attached to the arms of two plankton wheels (Figure 4.1) with rubber bands. The arms of the plankton wheels form two cylinders, each with a circumference of 62 cm. The arms of the wheels turn around a fixed point at 11 rpm. The plankton wheels were placed in a cold room and kept at an average temperature of 11°C. The six jars in the second treatment were placed horizontally on the floor of the cold room next to the plankton wheel. The seawater in both treatments was changed on the second day. After four days, the number of egg masses in each jar was counted. The mean number of egg masses laid per jar of each treatment was compared using a one-way ANOVA.

Figure 4.1. The plankton wheels used in an experiment designed to test the effect of water movement on oviposition in *Lirularia succincta*.

Sixty more *Lirularia succincta* were collected from South Cove in March of 2010. Five snails were placed into each of twelve 50-mL Falcon tubes that had been filled with 45 mL of seawater. The same procedure as described above was followed, with six Falcon tubes attached to the arms of the plankton wheels with rubber bands, and six Falcon tubes laid horizontally on the floor of the cold room. The seawater in the Falcon tubes was changed every other day. The number of egg masses that had been laid in each tube was recorded on the fourth and eighth days of the experiment. The mean number of egg masses deposited per Falcon tube between treatments was compared using a two-factor repeated measures ANOVA with Day and Treatment as factors.

In the second experiment, a hole was drilled into the side of one of two plastic containers (13 x 32 x 7 cm) that were set into an empty seawater table. A rope was tied through the hole in one of the plastic containers, and was attached to a lever arm on a small rotary motor which turned at a speed of 14 rpm and was set 65 cm above the plastic containers. When in motion, the rotary arm lifted the side of the container to which the rope was attached, and returned it to the surface of the sea table, in an erratic semicircular motion. The container was set next to the wall of the sea table, and would strike the wall of the sea table on its descent. When the container was moving in this fashion, water in plastic beakers set into the container sloshed back and forth.

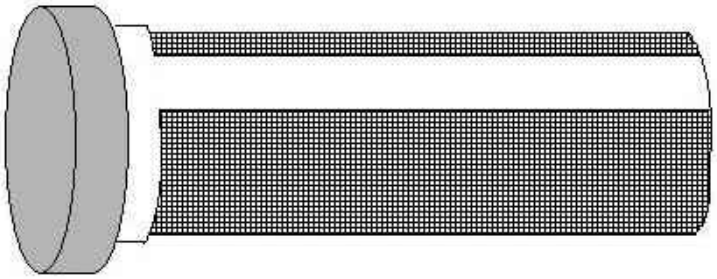
Lirularia succincta were collected from South Cove in March of 2010. Five snails were placed into each of 26 250-mL Nalgene beakers, and 175 mL of seawater was added to each beaker. Beakers were then randomly assigned to one of two treatments. The 13 beakers in the first treatment were placed into the container attached by a rope to the rotary motor. The 13 beakers in the second treatment were placed into an identical container, which was set in the sea table next to the moving container, and remained stationary throughout the experiment. Each afternoon, the seawater in the beakers was replaced. The number of masses in each beaker was

recorded on the third day of the experiment. The mean number of egg masses deposited per beaker between treatments was compared using a one-way ANOVA.

The final experiment examined the effect of unidirectional water flow of varying velocity on the oviposition of *Lirularia succincta.* In this experiment, the conical ends were removed from eight 50-mL Falcon tubes, creating a bottomless, lidded cylinder. Two 4.5 x 6.5 cm rectangles were removed from the sides of 4 of the Falcon tubes, leaving two segments less than 1 cm wide extending from the uncut section of the tube. Eight sections of plastic screen (1/16" mesh) were cut and rolled into cylinders with the same dimensions as the inside of the Falcon tubes (height=9 cm, diameter=2.5 cm). The seams of the screen tubes were glued with hot glue, and one screen tube was inserted into each of the eight Falcon tubes. This made four tubes in which the screens were completely enclosed in the Falcon tube and four tubes in which most of the screens were exposed (Figure 4.2A). The four Falcon tubes with the exposed screen were glued upright along one of the short sides of a 15 x 40 cm piece of Plexiglas. The tubes were spaced 0.5 cm apart. The four Falcon tubes with the enclosed screen were glued in a line perpendicular to the other four tubes on the opposite end of the Plexiglas, and were also spaced 0.5 cm apart (Figure 4.2B).

This experiment was done using a re-circulating flow tank (trough dimensions: 16 x 18 x 110 cm) similar to that described by Vogel and LaBarbera (1978). The Plexiglas was placed into the flow tank and oriented with the exposed Falcon tubes upstream from the enclosed tubes. The flow rate was set to the testing velocity and the water level was adjusted so that it was just below the top of the screen in the exposed Falcon tubes. The water was placed at this level to ensure the snails did not crawl into the lid of the test tube, out of the current. A brick was placed in the flow tank adjacent to the Plexiglas/Falcon tube apparatus on the downstream side. This caused the water level around the enclosed tubes to rise to roughly the same water level as that surrounding the exposed tubes. Fluorescein dye was used (i) to observe water flow, to ensure that water

(A)



(B)

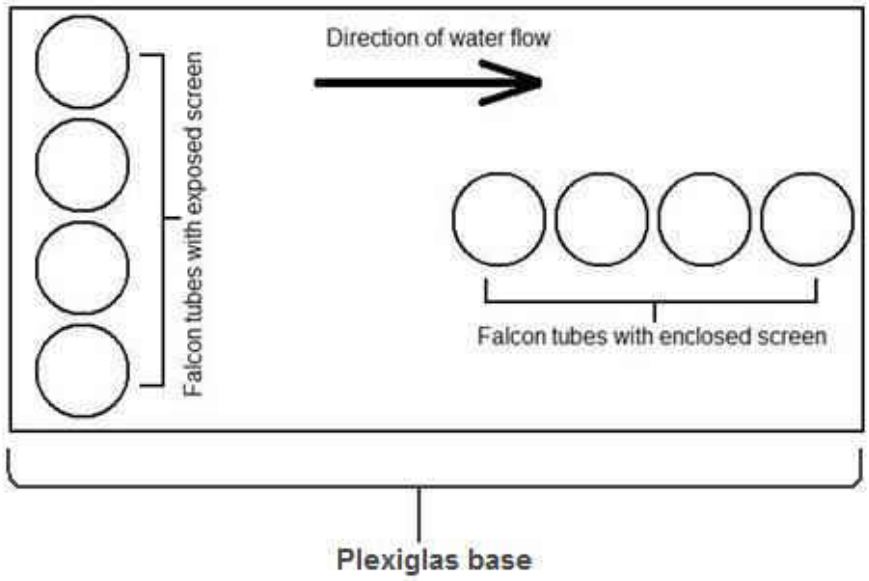


Figure 4.2. Diagrams of (a) an exposed Falcon tube, and (b) the entire Plexiglas/ Falcon tube apparatus. This equipment was used in an experiment designed to test the effect of water flow on *Lirularia succincta* oviposition.

obstructed by the enclosed test tubes and the brick had no noticeable effect on water flow through the exposed test tubes; (ii) to ensure the water travelled through the screen tubes at all velocities used in the experiment; and (iii) to determine the flow rates through the exposed Falcon tubes by timing the movement of the dye across a known distance.

Lirularia succincta were collected from North Cove in January of 2011. Ten snails were randomly assigned to each of the eight Falcon tubes within the flow tank. The experiment was left to run in the flow tank for 48 hours, after which time, the water in the flow tank was replaced, and 80 new *L. succincta* were randomly assigned to the eight Falcon tubes. The flow tank was then set to a new velocity and allowed to run for 48 hours. Every 12 hours the Falcon tubes were checked to see if any egg masses had been laid. At this time, snails that had crawled above the water line were replaced in the water, and the water temperature was measured. Water in the enclosed test tubes was changed every 24 hours. Four water velocities were tested: 25, 30, 35, and 40 $cm \cdot sec^{-1}$. The order in which the velocities were tested was randomly assigned. Because the flow tank was not completely filled when the velocity of the water was at 40 $cm \cdot sec^{-1}$, a vortex was created in the flow tank by the propeller, entraining air bubbles.

The experiment was repeated in February, and again in March of 2011. In the February trials, the water temperature in the flow tank was 4 to 5^{o}C colder than in the previous trial, so an aquarium heater was set into the flow tank to maintain the same temperatures that were present during the first trial.

The Presence of Other Egg Masses

The purpose of the next two experiments was to see if *Lirularia succincta* preferentially deposits egg masses next to masses that are already present. During the Water Movement experiment, egg masses were laid in twenty-three 250-mL Nalgene beakers. Four days after the Water Movement experiment was completed, these 23 beakers were randomly assigned to one of three treatments. In the first treatment, the water in eight of the beakers was replaced with 200 mL of filtered seawater (FSW), and the masses were left intact on the sides of the beakers. The masses were circled on the outside of the beaker with a Sharpie to identify them as 'old' masses. The water in the eight beakers of the second treatment was replaced with 200 mL of FSW. The

masses that had been laid in the beakers were gently scraped off of the walls. The masses were then swirled vigorously but gently in the water, and were left in the beakers (leaving the scent of the masses in the jar). In the third treatment, all of the masses were removed from seven beakers, and the beakers (plus an additional beaker in which no masses had been laid) were thoroughly cleaned with fresh water, rinsed with FSW, and refilled with 200 mL of FSW. Snails were collected from South Cove in March of 2010. Five snails were placed in each beaker, and the beakers were placed in a cold room, where they were kept at 12°C for five days. The beakers were checked for egg masses on the first, third, and fifth day of the experiment. The presence of new egg masses was recorded, as was the proximity of the new masses to 'old' and to other new masses. Water in the beakers was not replaced to avoid losing the scent of the masses in the second treatment. It was assumed that any adverse effects of oxygen depletion or the build-up of snail waste in the beakers were similar in all beakers. After five days in the beakers, the snails were not showing any signs of stress.

For the second experiment, *Lirularia succincta* individuals were collected from South Cove in April of 2010 and brought into the laboratory. Four adults and 40 mL of FSW were placed into each of 25 Falcon tubes and the tubes were placed in a seatable with flowing seawater for 6 days. The water in the Falcon tubes was changed every day. After six days, 20 of the Falcon tubes had at least one egg mass laid in it. The snails were removed, and the 20 Falcon tubes were placed in a cold room at 9°C for three days. Then, the egg masses in the tubes were circled on the outside of the tubes with a Sharpie to identify them as 'old' masses, and the tubes were randomly placed into one of 40 holes in a Styrofoam Falcon tube holder. Twenty clean Falcon tubes were placed in the 20 remaining holes in the Styrofoam. The water in all tubes was replaced with 40 mL of FSW and five newly-collected *L. succincta* were placed into each tube. The Falcon tubes were kept in a cold room at 9°C, and the water in the tubes was changed each

day. On the first, second, and fifth day, the number of new masses deposited in each tube was counted, and their position relative to each other and to the 'old' masses was also recorded.

For both experiments, the mean number of egg masses laid in each Falcon tube was compared between treatments using a two-way repeated measures ANOVA, with Day and Treatment as factors.

Temperature

To determine the effect of temperature on oviposition in *Lirularia succincta,* a temperature gradient block similar to that described by Thomas et al. (1963) was used. It was made of an aluminum block (20 x 60 x 6.5 cm) into which forty 2.7 cm holes had been bored. This design allowed for four replicates at ten different temperatures. Refrigerated and heated water moving through tubes at opposite ends of the aluminum block using circulators (VWR Scientific, Model 1146) maintained the desired temperature gradient over the course of the experiment.

In the first trial, *Lirularia succincta* were collected from South Cove in June of 2010. Four snails were placed into each of 40 scintillation vials that had been filled with FSW. The vials were then randomly assigned to a temperature treatment in the temperature gradient block. Four scintillation vials were in each of ten temperature treatments from 11°C to 22°C. Every 24 hours, the temperature of the water in each vial was measured using a YSI Model 43TD Tele-thermometer temperature monitor (Yellow Springs Instrument Company, Yellow Springs, Ohio). Then, the number of egg masses that had been laid in each vial was recorded, and the FSW in each vial was replaced. The experiment ran for three days.

The experiment was repeated with *Lirularia succincta* collected from North Cove in February of 2011. The temperature treatments ranged from 7°C to 23°C. The experiment ran for four days.

The experiment was repeated with *Lirularia succincta* collected from South Cove in March of 2011. The temperature treatments ranged from 6°C to 21°C. This experiment also ran for four days.

For each trial, the mean number of egg masses laid per vial at each temperature was compared using a two-way repeated measures ANOVA with Day and Temperature as factors.

Tidal Level

To determine if tidal level affects the frequency of egg mass deposition, 20 cages were built out of plastic screen with 1.5 mm mesh. Cages were cylindrical with a height of 2.5 cm and a diameter of 5 cm. The edges and seam of the cage were fastened using hot glue. The bottom of the cage was made of half of a 60 x 15 mm disposable polystyrene petri dish with the edges of the dish cut off. Two of the cages were placed into a re-circulating flow tank (trough dimensions: 16 x 18 x 110 cm) similar to that described by Vogel and LaBarbera (1978). Fluorescein dye was used to ensure that water passed through the cages at speeds up to 45 $cm \cdot sec^{-1}$. Flow rates were determined by timing the movement of fluorescein dye across a known distance.

The experiment was conducted twice, in September and December of 2010. One hundred *Lirularia succincta* were collected from North Cove in September of 2010. Five snails were randomly assigned to each cage. Snails were placed in the cage, which was glued shut with hot glue. Cages were then taken immediately to North Cove.

Ten of the cages were randomly assigned to be placed at -1.23 ft in the low intertidal. Five cages were placed 2 m apart on the landward side of a large rock outcropping, and 5 cages were placed 2 m apart on the seaward side of the outcropping. Ten of the cages were placed at 0.60 ft in the high intertidal. Five cages were placed 2 m apart on the seaward side of a boulder approximately the same size as the rock outcropping, and five cages were placed 2 m apart on the landward side of the boulder. The cages were checked every day to see if they were damaged or

missing. The cages were returned from the field after four days. The tops of the cages were removed, and the cages were inspected under a dissection microscope to see if any egg masses had been laid while the cages were in the field.

The second trial was done in December using the same protocol, except 200 *L. succincta* were collected and 10 snails were randomly assigned to each dish. More snails were used in this trial because the rate of oviposition appeared to be lower in December, using preliminary data from the Season oviposition experiment. The rate of oviposition in November was 8%, compared to 70% in August.

The mean number of egg masses deposited at the high and low tidal levels was compared using a one-way ANOVA for the first trial. Too few were returned after the second trial to be analyzed.

Egg Masses Deposited in Crevices on Cobbles

From 2010 to 2011, each time an egg mass was found on a rock in the field, the rock was brought in to the laboratory. Pictures were taken of the rock from all sides. The crevices on each side of the rock were marked on the pictures. The surface area of the rock and of the areas of the rock deemed to be crevices were measured using Image J to the nearest 1mm. The number of egg masses, either on flat surfaces or in crevices of the rock, was counted. A goodness-of-fit G-test with Yates' correction for continuity was used to determine if *Lirularia succincta* preferentially deposit egg masses in crevices on rocks in the field.

Results

Observations on Oviposition by *Lirularia succincta*

Lirularia succincta individuals deposited transparent gelatinous egg masses on the sides of the containers in which they were kept in the laboratory throughout the year. Snails can

deposit egg masses within a few hours of being placed in a sea table, and can lay egg masses in the laboratory after three weeks in the laboratory. When *L. succincta* adults were isolated individually in Petri dishes, they did not deposit egg masses. Once a female deposited an egg mass, she never laid another egg mass unless a male was present, suggesting that females do not store sperm. In the laboratory, each female deposited up to three egg masses. The second and third egg masses were laid from one to eight days after the first mass was laid. When egg masses were observed under a microscope shortly after having been deposited, sperm appeared to be in the gel throughout the mass. Occasionally, many eggs in a mass remained unfertilized.

The average size of the egg masses of *Lirularia succincta* was 5.4 ± 1.3 SD mm (n=43). The eggs varied in color from beige to the palest green. Masses were generally flat, with embryos laid in a single layer (Figure 4.3A), although masses did have two layers of embryos when they were large or when their shape was constrained (i.e., by the crevices in which they were deposited). Each mass contained an average of 127.6 ± SD 57.4 eggs per mass, with numbers ranging from 30 to 311 eggs per mass (n=136). The mean diameters of 100 newly-laid eggs in nine egg masses were 187.6 ± SD 13.2 µm (range 150.8- 215.7 µm), enclosed in oval envelopes with a mean diameter of 247.1 ± SD 19.0 µm (range 192.9- 297.9 µm) (Figure 4.3B). Cleavage was spiral. Embryos developed into intracapsular veligers (Figure 4.3C), which continued developing within the egg mass until the young hatched as crawl-away juveniles (Figure 4.3D). The time it took for the juveniles to develop and hatch depended upon temperature; juveniles began hatching after seven days at 20^{o}C, and after 14 days at 10^{o}C. Upon hatching, juvenile shells had a mean diameter of 293.2 ± SD 26.7µm.

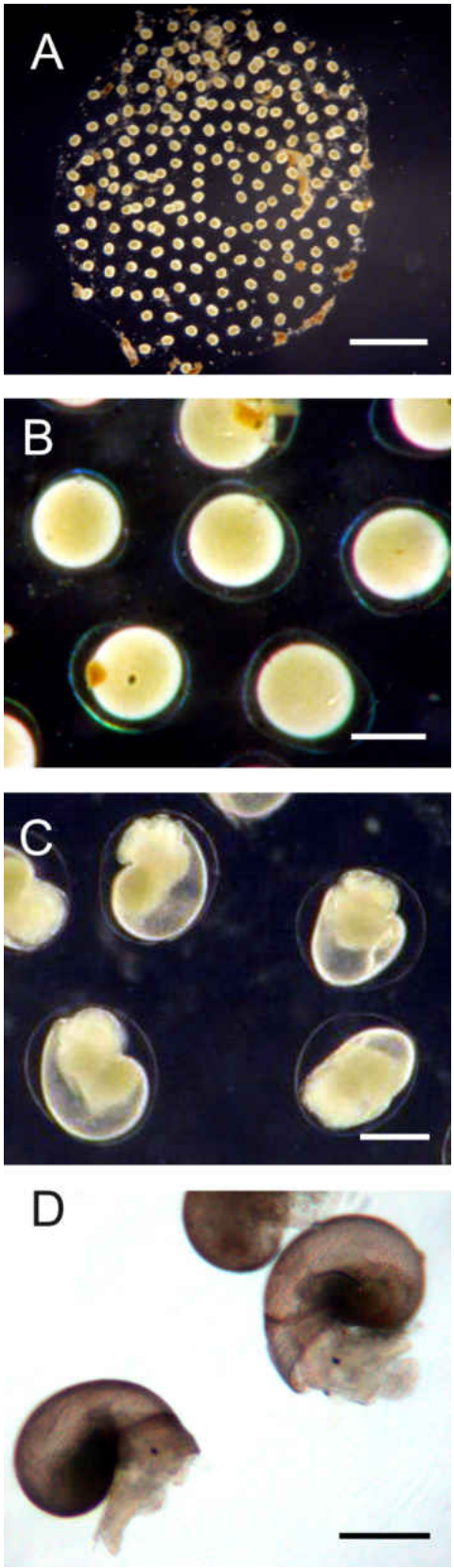


Figure 4.3. (A) An egg mass of *Lirularia succincta.* Scale bar is 1.5 mm. (B) Eggs of *L. succincta* at the 1-cell stage, (C) *L. succincta* as intracapsular veligers, and (D) Newly-hatched juveniles of *L. succincta*. Scale bars for B, C and D are 150 µm.

Factors Affecting Oviposition

Season

While *Lirularia succincta* deposit egg masses year-round in the laboratory, the reproductive output varies throughout the year (Figure 4.4). It appears to peak in July, when 85% of the pairs of *L. succincta* deposited an egg mass, and reaches its lowest point in November, when only 8% of mating pairs deposited an egg mass.

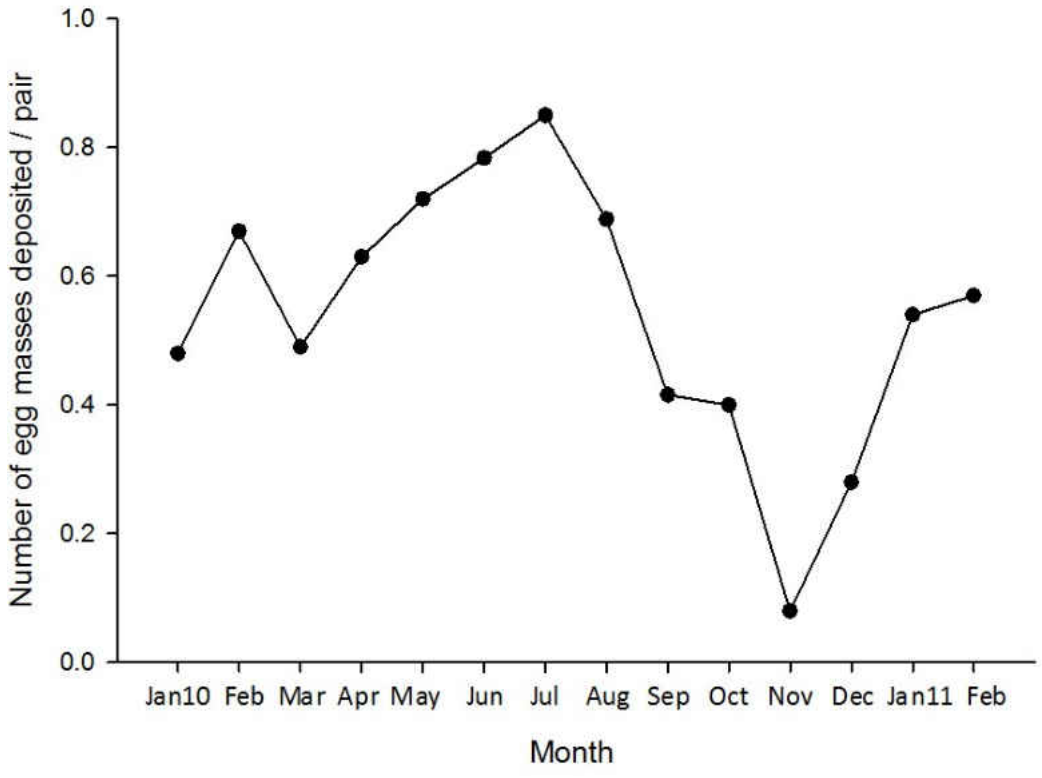


Figure 4.4. Number of egg masses deposited by pairs of *Lirularia succincta* each month over a 14-month period. 100 pairs were used each month except June (97 pairs), August (61 pairs), and September (77 pairs).

The Presence of a New Partner

No significant difference was detected between the number of egg masses deposited by females paired with partners that they had already mated with, and females paired with new partners (Trial 1: df=1, χ^2=0.0444, p=0.8331; Trial 2: df=1, χ^2=0.1473, p=0.7011). Females were more likely to lay a second egg mass in July, when 0.87 ± SD 0.34 masses per mating pair were deposited, than in May, when 0.57 ± SD 0.50 masses per mating pair were deposited. There were significantly more eggs per mass deposited in July (60.5 ± SD 20.5 eggs per mass) than in May (27.7 ± SD 19.6 eggs per mass). However, the number of eggs deposited per mass was not significantly different between treatments for either trial (F=0.572, p=0.455) (Table 4.1; Figure 4.5).

Light

In both trials, there was no statistically significant effect of light on the number of egg masses laid in the Falcon tubes (Figure 4.6A), but there was a significant difference between

Table 4.1. Results of an ANOVA comparing the number of eggs deposited per mass for snails in two treatments (paired with a new partner, or paired with a partner with which the snail had already mated) for two different trials (in May and July).

Source of Variation	df	SS	MS	F	P
PartnerType	1	1.362	1.362	0.572	0.455
Trial	1	43.657	43.657	18.342	<0.001
PartnerType x Trial	1	0.758	0.758	0.318	0.577
Residual	29	69.025	2.380		

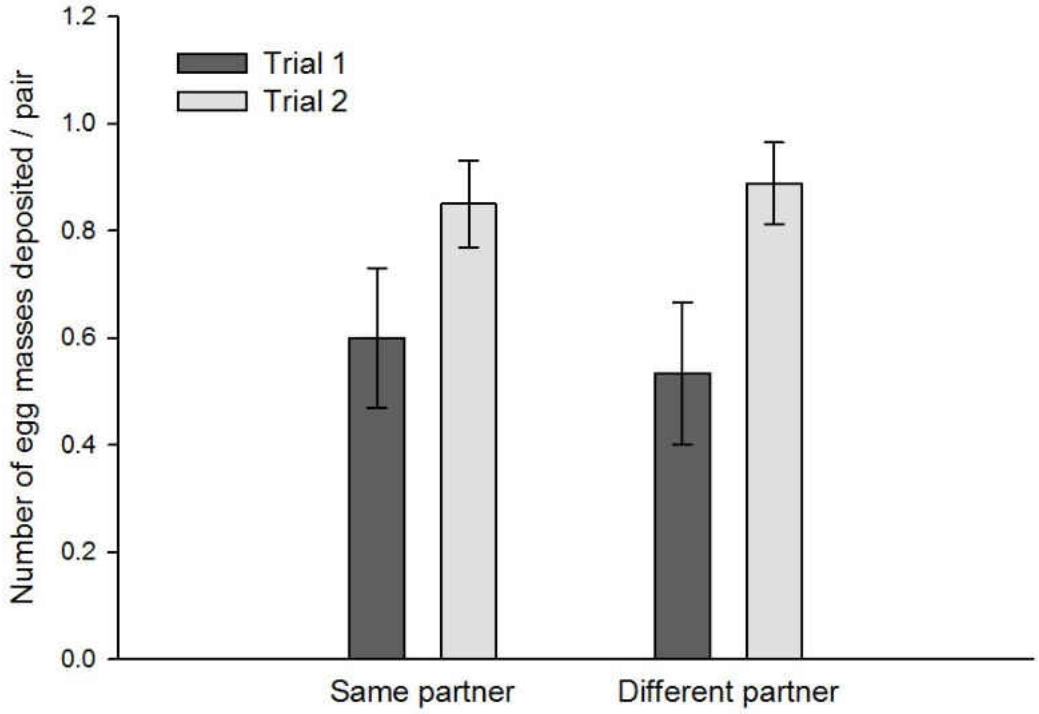


Figure 4.5. Mean number of eggs per mass deposited by a mating pair of snails (n=15 in Trial 1, n=19 in Trial 2). Females had previously deposited an egg mass, either with the same partner, or with a different partner. Error bars represent 1 SE. Values are not significant between treatments ($F=0.572$, $p=0.455$), but are significant between trials ($F=18.342$, $p<0.001$).

trials in the number of egg masses laid (Table 4.2A). At the beginning of May, the average number of egg masses laid in each Falcon tube was 1.5 ± SD 1.3. At the end of May, 2.7 ± SD 1.7 egg masses were laid on average in each Falcon tube. The two-way repeated measures ANOVA violated the assumption of sphericity (Mauchley's $W=0.7807$, $p=0.0091$). The value for the Huynh-Feldt Epsilon was greater than 0.7 ($\varepsilon=0.8952$), so the Huynh-Feldt adjusted df were used for within-subject factors (subjects being the individual Falcon tubes). This analysis verified that there was no significant difference among the three light treatments (Table 4.2B). There was a significant difference in the number of egg masses laid each day within the Falcon tubes, but there was no interaction between Day and Treatment (Table 4.2C). When the days were

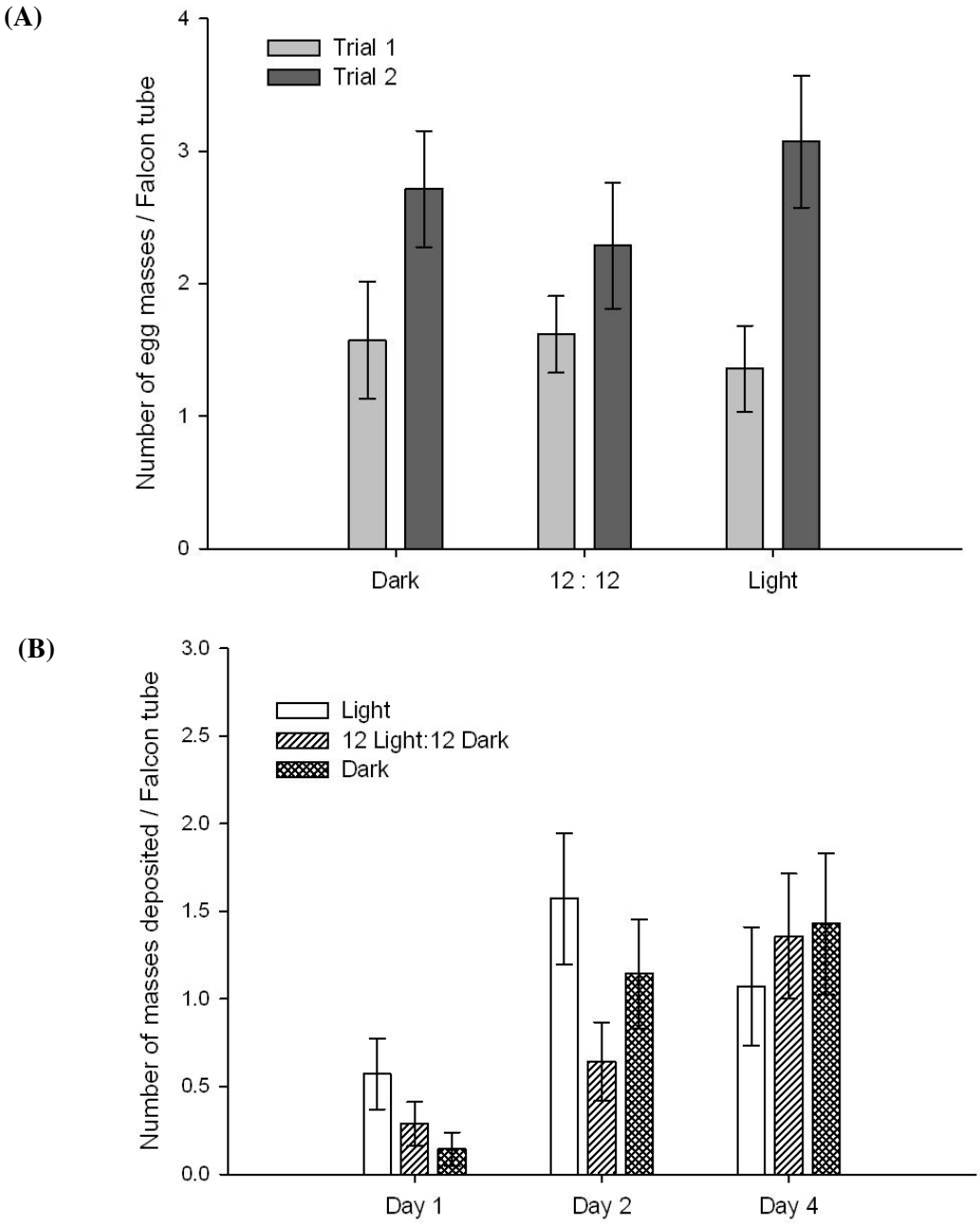


Figure 4.6. (A) Mean number of egg masses deposited per Falcon tube across all light treatments in two trials (n=14 Falcon tubes per treatment). Values are not significant between treatments ($F=0.208$, $p=0.813$), but are significant between trials ($F=11.718$, $p<0.001$). (B) Mean number of egg masses deposited per Falcon tube each day of the second trial. Values are significant for day ($F=8.66$, $p=0.0007$) but not for light treatment ($F=0.98$, $p=0.3836$). Error bars represent 1 SE in both graphs.

Table 4.2. Results of (A) a two-way ANOVA comparing the number of egg masses deposited per Falcon tube in three light treatments (constant light, constant dark, 12 hours of light and 12 hours of dark) for two different trials (at the beginning and end of May); (B) the between-subjects, and (C) the within-subjects tests for a two-way repeated measures ANOVA for the second trial, with Day and Treatment as factors. Subjects were Falcon tubes (n=14 per treatment). P^H is the Huynh-Feldt adjusted P value.

(A)

Source of Variation	df	SS	MS	F	P
Trial	1	28.666	28.666	11.718	<0.001
Treatment	2	1.018	0.509	0.208	0.813
Trial x Treatment	2	3.759	1.879	0.768	0.467
Error	77	188.363	2.446		

(B)

Source of Variation	df	SS	MS	F	P
Treatment	2	2.016	1.008	0.98	0.384
Error	39	40.024	1.026		

(C)

Source of Variation	df	SS	MS	F	P^H
Day	2	21.730	10.865	8.66	<0.001
Day x Treatment	4	6.365	1.591	1.27	0.292
Error (Day)	78	97.905	1.255		

compared using a *post hoc* Bonferroni t-test, the number of egg masses laid on Day 1 was significantly different from the number of masses laid on Day 2 (t= 3.214, p=0.006) and Day 4 (t=3.896, p<0.001). There was not a significant difference between the number of egg masses deposited on Day 2 and Day 4 (t=0.682, p=1.000).

The area under the lid constituted 15.8% of a Falcon tube's internal surface area. This area was shaded by the lid when the tube was lying horizontally. The opposite end of the tube, which is referred to in these experiments as the "conical end," made up 10.2% of the tube's internal surface area. The conical end was deemed to be the entire bottom of the tube, from the crease to the rounded tip. The remaining 73.8% of the Falcon tube's internal surface area is referred to as the "side" of the tube. In the Falcon tubes from the Light experiment, 41.7% of egg masses in all treatments were laid under the lid of the tube, 30.4% were laid in the conical end of the tube, and 27.8% were laid on the side. The frequencies of masses deposited in each of the three areas of the Falcon tubes were significantly different from what would be expected if selection of deposition sites in each tube had been random ($G=106.141$, $p<0.0001$)(Figure 4.7). A goodness-of-fit G-test was used for this analysis because, in 2 cells, the absolute value of the difference between the observed and expected frequencies was much greater than the expected frequency (Zar, 2010).

When the numbers of egg masses laid in various areas of the tube were compared using a chi-squared analysis of the contingency table illustrated in Table 4.3, the null hypothesis was rejected ($df=4$, $\chi^2=16.4695$, $p=0.0024$). The results of the analysis show that the frequencies with which egg masses were deposited in all three areas of the Falcon tube were not independent of light treatment. Snails in the treatment exposed to 24 hours of darkness deposited more egg masses on the side of the Falcon tube than in the lid or in the conical end. Snails in the light treatment that experienced both light and dark preferentially deposited their egg masses under the lid of the Falcon tube, while snails experiencing constant light deposited their egg masses under the lid or in the conical end of the tube twice as frequently as they deposited them onto the side of the tube (Figure 4.8).

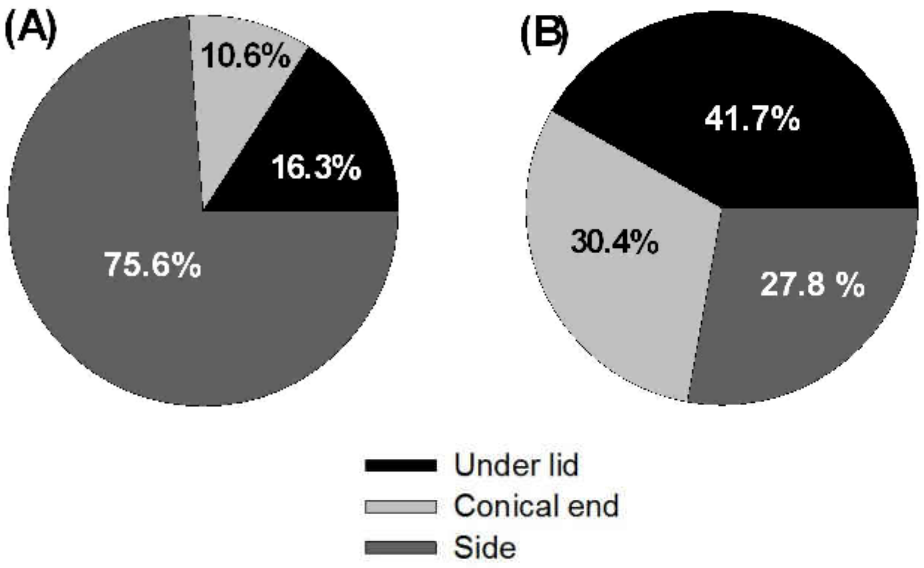


Figure 4.7. (A) Proportion of the internal surface area of a Falcon tube categorized as the area under the lid, the conical end of the tube, or the side of the tube. (B) Proportion of egg masses deposited in each area of the Falcon tube (n=115). Masses were deposited in the conical end or under the lid more frequently than would be suggested by chance (G=106.141, p<0.0001).

Shading

In the first trial, nine masses were laid on the dark side and none were laid on the light side of the dishes in the experimental group, while three masses and one mass were laid on the marked and unmarked sides of the dishes in the control group, respectively. Egg masses were laid on the dark sides of the dishes significantly more than on the light sides or on either side of the dishes in the control group (df=3, χ^2=15.000, p=0.0018). The expected frequencies for the analysis were small (3.25 for all columns); however, chi-squared analyses are robust when testing for uniform distributions (Zar, 2010). By subdividing the chi-square analysis, I found that the frequencies of egg masses deposited on either side of the control dishes and on the light sides of the dishes in the experimental group were uniformly distributed (df=2, χ^2=3.500, p=0.1737).

Table 4.3. Contingency table used to analyze the location of deposition sites of egg masses in Falcon tubes exposed to three different light treatments (constant darkness, constant light, 12 hours of light and 12 hours of dark). Deposition sites in the tube were classified as being under the lid, on the side, or in the conical end. Observed frequencies were the number of egg masses deposited in each area in 14 Falcon tubes per treatment. Expected frequencies (italicized) were based on the null hypothesis that the distribution of egg mass deposition sites in the 3 areas of the Falcon tube was independent of treatment. If the null hypothesis were true, the expected frequency of masses laid in each area of the Falcon tube (columns "lid", "side", and "end") would have the same proportion of egg masses as the final proportion of masses laid per treatment (column n_1), and the proportion of egg masses in each treatment (rows "dark", "12:12", and "light") would be the same as the proportion of egg masses laid in each area overall (row n_2)

	Area of Falcon tube			
Treatment	**Lid**	**Side**	**End**	n_1
Dark	9 *(48 x 0.330)*	16 *(32 x 0.330)*	13 *(35 x 0.330)*	38
12 light: 12 dark	21 *(48 x 0.278)*	7 *(32 x 0.278)*	4 *(35 x 0.278)*	32
Light	18 *(48 x 0.391)*	9 *(32 x 0.391)*	18 *(35 x 0.391)*	45
n_2	48	32	35	**115**

Therefore, the nonconformity of the data to the original hypothesized (uniform) distribution is due to the observed frequency of egg masses deposited on the dark sides of the dishes. In the second trial, the data did not differ significantly from the null hypothesis of a uniform distribution of egg masses on all sides of all dishes (df=3, χ^2=7.500, p=0.0575), although more masses were again deposited on the dark sides of the dishes (eight masses) than on the light sides (one mass) or on either side of the control dishes (one and five masses).

For the first trial comparing the positions of the adults in the dishes, the assumption of sphericity was violated for the repeated measures ANOVA (Mauchley's W=0.4236, p=0.0135), but the Huynh-Feldt Epsilon was greater than 0.7 (ε =0.9018), so the Huynh-Feldt adjusted df

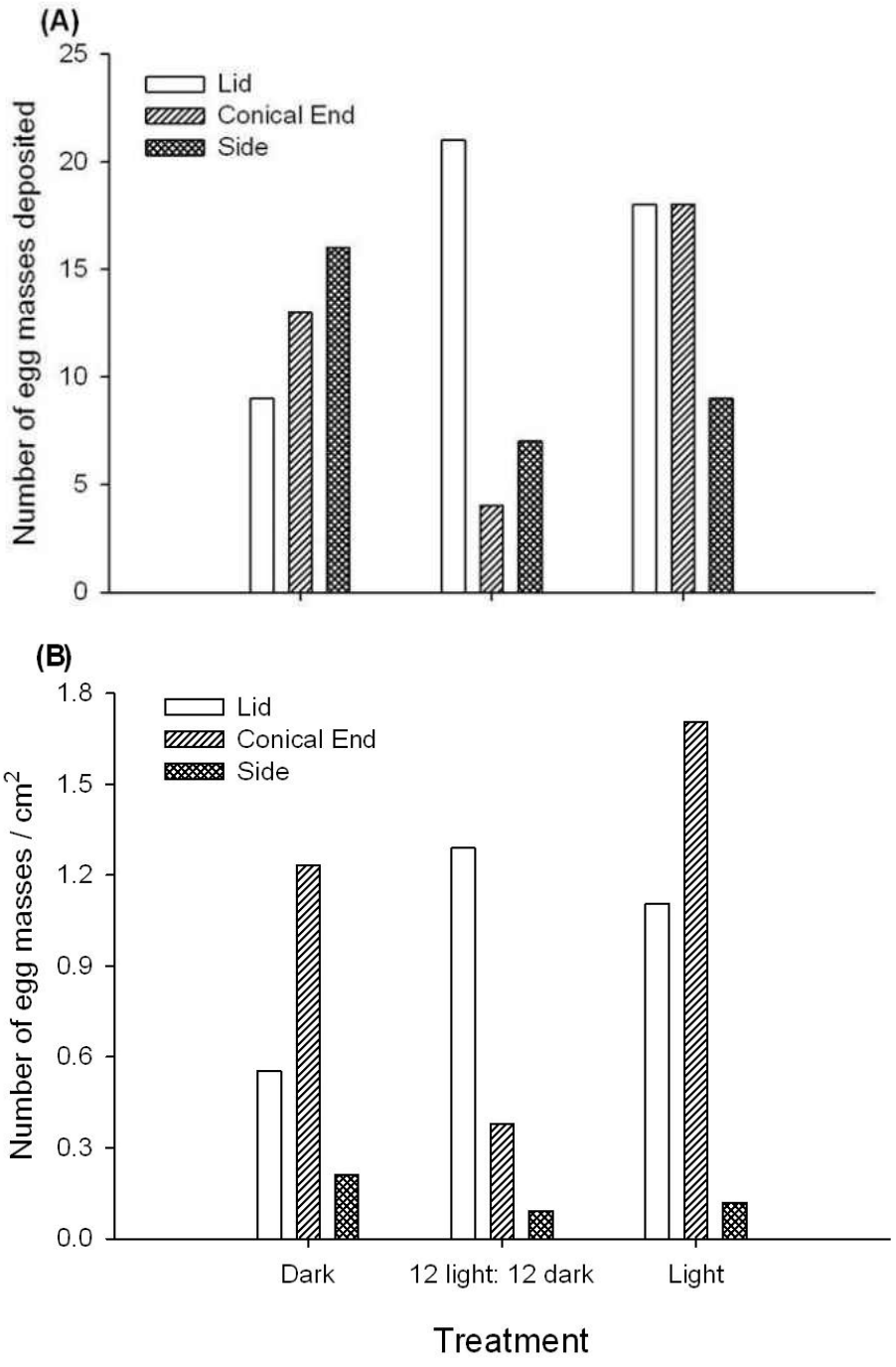


Figure 4.8. Frequency of egg masses deposited under the lid, on the side, or in the conical end of 42 Falcon tubes placed in one of three different light treatments (constant dark, 12:12 light:dark cycle, constant light). (A) Total number of masses deposited in each area, for each treatment. (B) The number of egg masses deposited in each area adjusted for surface area of the inside of the Falcon tube. There was a significant difference in the number of masses laid in each area between treatments (df=4, χ^2=16.4695, p=0.0024).

was used for within-subject factors (subjects being the individual petri dishes). The assumption of sphericity was met for the second trial (Mauchley's W=0.4750, p=0.0693). In both trials, there was a significant difference in the position of adults in the control dishes and the partially-covered dishes (Table 4.4A), but there was not a significant difference between days (Table 4.4B). The frequency with which snails were found on the dark sides of the partially-covered dishes (0.34 ± SD 0.18 in the first trial; 0.22 ± SD 0.17 in the second trial) was significantly lower than the frequency with which snails were found on the marked sides of the control dishes (0.51 ± SD 0.22 in the first trial; 0.46 ± SD 0.27 in the second trial) (Figure 4.9).

Water Movement

In the first three experiments in which different containers were manipulated to simulate water movement, the differences in the mean number of eggs laid in the stationary containers were, in all cases, significantly lower than the mean number of eggs laid in moving water. The stationary jars in the first experiment had an average of 0.7 ± SD 0.8 masses laid in each jar, which was significantly lower than the number of masses laid in the rotating jars (3.8 ± SD 1.2 masses per jar) ($F=29.590$, $p<0.001$). Similarly, the stationary Falcon tubes in the second experiment, having an average of 2.2 ± SD 1.2 masses laid in each tube, had significantly fewer masses deposited in them than in the Falcon tubes attached to the plankton wheel (5.0 ± SD 2.5) ($F=6.202$, $p=0.032$). In the third experiment, the stationary beakers had an average of 1.8 ± SD 1.1 masses laid in them. The beakers in the container attached to the lever arm of the motor had an average of 3.5 ± SD 2.0 masses laid in them. These two means were significantly different ($F=7.100$, $p=0.014$).

The results from the flow tank experiment show that the degree of variability in *Lirularia succincta* oviposition is greater than can be accounted for with this experimental design, which was limited in the number of replicates that could be used (Figure 4.10). The number of egg

Table 4.4. Results of 2 two-way repeated measures ANOVAs testing the effect of "shade" on the position of snails in petri dishes for two trials. The table shows (A) tests for between-subjects effects, and (B) tests for within-subjects effects for both trials. Subjects were petri dishes (n=10 per treatment) bisected by a Sharpie line (control treatment), or half-covered with black plastic (experimental treatment). P^H is the Huynh-Feldt adjusted P value. Data were arcsine transformed.

(A)

	Trial 1					Trial 2				
Source	**df**	**SS**	**MS**	**F**	**P^H**	**df**	**SS**	**MS**	**F**	**P**
Treatment	1	0.938	0.938	25.13	<0.001	1	1.749	1.749	14.41	0.002
Error	18	0.672	0.037			15	1.821	0.121		

(B)

	Trial 1					Trial 2				
Source	**df**	**SS**	**MS**	**F**	**P^H**	**df**	**SS**	**MS**	**F**	**P**
Time	3	0.114	0.038	0.52	0.651	3	0.084	0.028	0.32	0.808
Time x Treatment	3	0.109	0.036	0.49	0.669	3	0.181	0.060	0.69	0.562
Error(Time)	54	3.954	0.073			45	3.931	0.087		

masses deposited was inconsistent at water velocities of 20 to 35 cm·sec^{-1}; however, oviposition never occurred at a water velocity of 40 cm·sec^{-1}.

The Presence of Other Egg Masses

When multiple egg masses were deposited in the laboratory, 62.5% of the masses (n=195) were laid adjacent to other recently laid masses. 63.1% of egg masses (n=48) observed on cobbles in the field were laid adjacent to other masses. The number of isolated and adjacent masses could not be compared using one-way ANOVAs because the assumption of normality was violated, so the non-parametric Kruskal-Wallis one-way ANOVA on ranks was used to determine that the number of masses that were deposited adjacent to other masses was

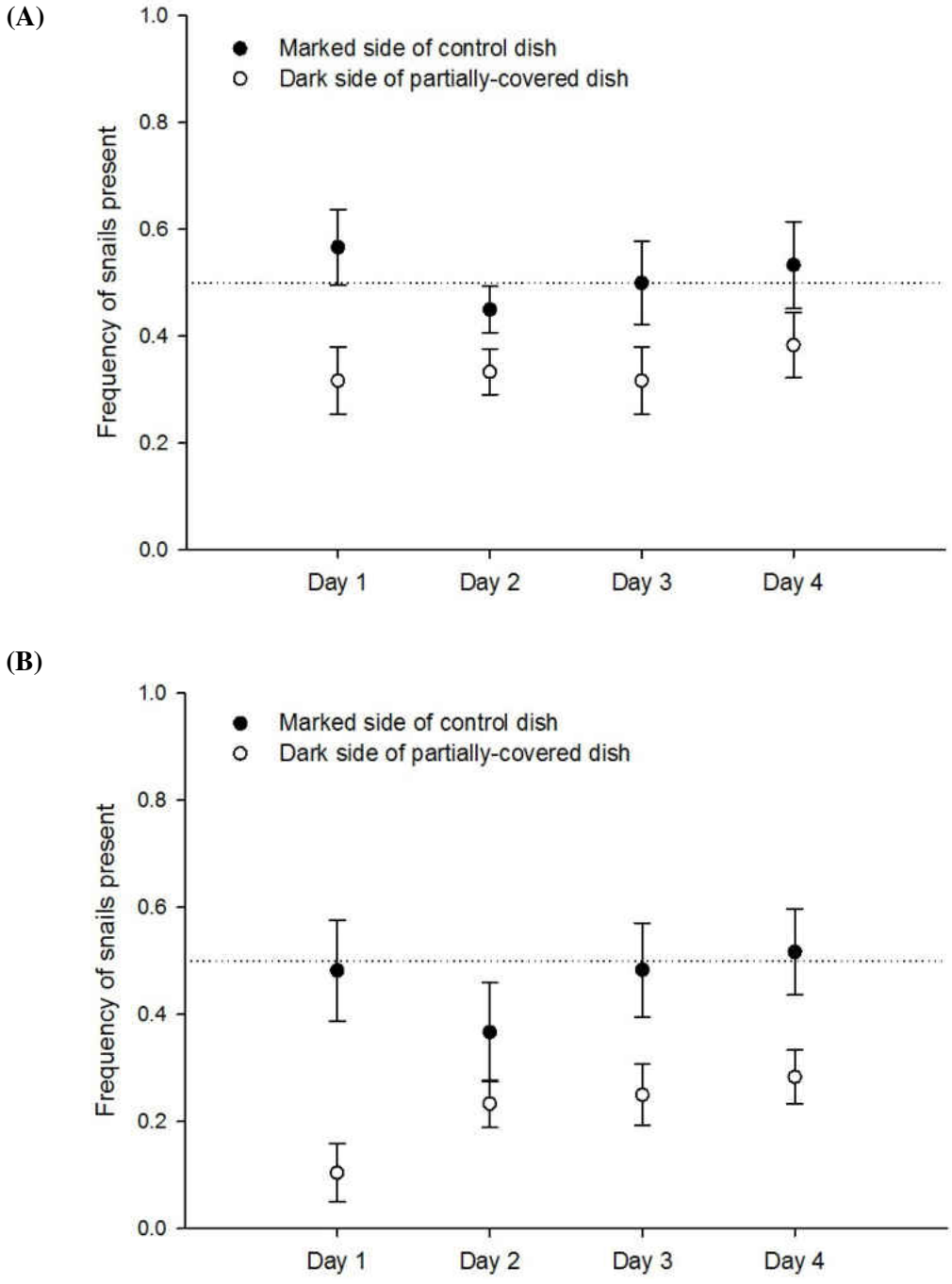


Figure 4.9. Average frequency with which snails were found on the specified side of a petri dish. Each petri dish in the control treatment (closed circles) was bisected by a line, and one side of the dish was marked. Half of each petri dish in the experimental treatment (open circles) was covered with black plastic (A) Trial 1. Values are significant between treatments ($F=25.13$, $p<0.0001$), but are not significant between days ($F=F=0.52$, $p=0.6513$). (B) Trial 2. Values are significant between treatments ($F=14.41$, $p=0.0018$), but are not significant between days ($F=0.32$, $p=0.7793$). Error bars represent 1 SE in both graphs.

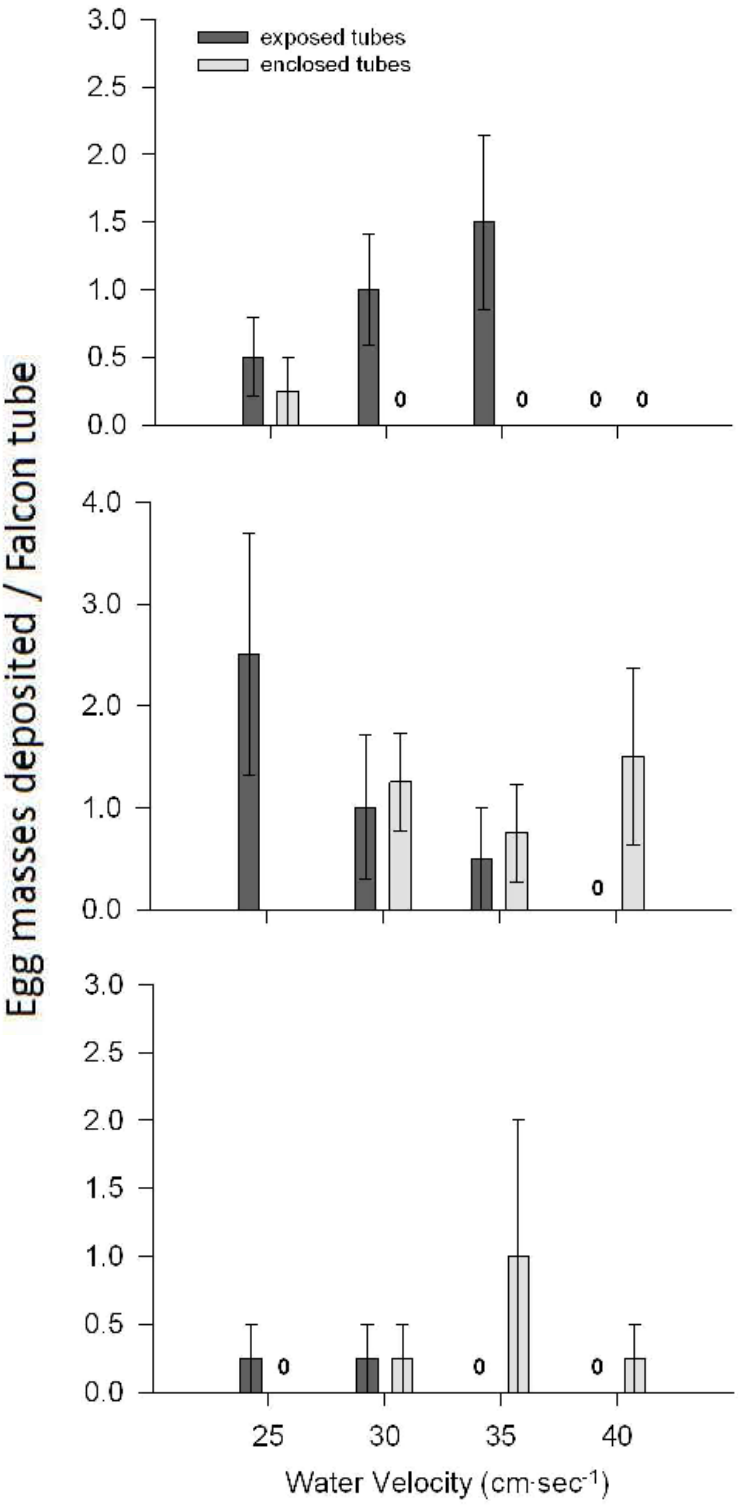


Figure 4.10. Effect of current on oviposition. Four Falcon tubes had mesh sides open to water flow; four tubes were enclosed and not exposed to the current. Ten *Lirularia succincta* individuals were placed in each Falcon tube. The experiment was run in a flow tank where each water velocity was tested for 48 hours. *L. succincta* individuals were replaced between velocity treatments. The experiment was conducted three times. Error bars represent 1 SE.

significantly higher in the laboratory ($H=13.326$, $p<0.001$), but not in the field ($H=0.178$, $p=0.673$). The difference in the ability of the tests to detect significant differences probably resulted from fewer masses being considered in the latter analysis.

When the two-way repeated measures ANOVAs were run, the data from the experiment with the beakers violated the assumption of sphericity (Mauchley's $W=0.6579$, $p=0.0152$). The Huynh-Feldt adjusted df was used for the test of within-subject effects (Huynh-Feldt $\varepsilon = 0.8653$). The assumption of sphericity was met for the data from the experiment with the Falcon tubes (Mauchley's $W= 0.9706$, $p=0.5753$), but the assumption of normality was violated. Repeated measures ANOVAs are robust for violations of this assumption, so I proceeded with the analysis. In both experiments, there was no significant difference in the mean number of egg masses laid per container in each treatment (Table 4.5A). In the beakers, there was a significant difference in the mean number of masses laid each day ($F=11.83$, $p= 0.0002$). An average of 0.33 ± SD 0.64 masses per beaker were deposited on the first day, 0.83 ± SD 0.76 masses per beaker were deposited between the first and third day, and 1.75 ± SD 1.39 masses per beaker were deposited between the third and sixth days, which is not surprising, as more time had lapsed. There was not a significant difference in the mean number of masses laid each day in the Falcon tubes ($F=3.57$, $p=0.0331$) (Table 4.5B). An average of 0.58 ± SD 0.81 masses per tube were deposited on the first day, but only 0.20 ± SD 0.41 masses per tube were deposited on the second day. 0.50 ± SD 0.78 masses per tube were deposited between the second and fifth day.

In the treatments of the experiments that contained previously-deposited egg masses, it was noted that masses were laid next to recently-laid masses more often than they were laid next to masses that were more than four days old. After a new mass was deposited in each beaker or Falcon tube, the females depositing subsequent egg masses had the option of depositing a mass adjacent to a recently-laid mass, adjacent to an old mass, or distant (> 1 cm) from other masses. In the first experiment, one mass was deposited next to an old mass, and eight were deposited

Table 4.5. Results of 2 two-way repeated measures ANOVAs testing the effect that the presence of deposited egg masses had on the oviposition of *Lirularia succincta*. The table shows (A) tests for between-subjects effects, and (B) tests for within-subjects effects for both experiments. Subjects in the first experiment were 250-mL Nalgene beakers (n= 8 per treatment); treatments were clean beakers, beakers containing the scent of deposited egg masses, and beakers containing previously-deposited egg masses. Subjects in the second experiment were Falcon tubes (n= 20 per treatment); treatments were clean Falcon tubes, and Falcon tubes containing previously-deposited egg masses. P^H is the Huynh-Feldt adjusted P value.

(A)

	Experiment 1 (250 mL beakers) Between-Subjects Effects					**Experiment 2 (Falcon tubes) Between-Subjects Effects**				
Source	**df**	**SS**	**MS**	**F**	**P^H**	**df**	**SS**	**MS**	**F**	**P**
Treatment	2	1.194	0.597	0.67	0.523	1	0.075	1.749	0.14	0.711
Error	21	18.750	0.893			38	1.821	0.541		

(B)

	Experiment 1 Within-Subjects Effects					**Experiment 2 Within-Subjects Effects**				
Source	**df**	**SS**	**MS**	**F**	**P^H**	**df**	**SS**	**MS**	**F**	**P**
Day	2	24.778	12.389	11.83	<0.001	2	3.150	1.575	3.57	0.033
Day x Trmt	4	3.222	0.806	0.77	0.536	2	1.950	0.975	2.21	0.117
Error(Day)	42	44.000	1.048			76	33.567	0.442		

next to recently-deposited masses. In the second experiment, one mass was deposited next to an old mass, and five were deposited next to a recently-laid mass. In each of the two experiments, six masses were deposited distant from other masses.

Temperature

There was no difference in the number of egg masses deposited among treatments in any of the trials, despite a trend toward greatest mean numbers of egg masses between 15 and 17°C

(Figure 4.11; see Table 4.6 for all F and p values for all trials). The data sets for all three trials violated the assumption of sphericity but the Huynh-Feldt Epsilon was greater than 0.7 for all 3 trials (Table 4.7) so the Huynh-Feldt adjusted df was used for within-subject factors (subjects being individual scintillation vials). There was a significant difference in the number of egg masses laid each day in Trial 1 ($F=4.59$, $p=0.014$). An average 0.39 ± SD 0.59 of masses were laid in each scintillation vial on Day 1, and an average of 0.36 ± SD 0.54 masses were laid per vial on Day 2, but only 0.09 ± SD 0.30 masses were laid per vial on Day 3.

Tidal Level

When the cages from the first trial were brought in from North Cove, a mean of 0.6 ± SD 1.0 eggs per cage had been laid in the low intertidal, and a mean of 0.2 ± 0.4 eggs per cage had been laid in the high intertidal. Because the assumption of normality was violated, the Kruskal-Wallis test was used to show that a significant difference in the mean number of egg masses laid in the cages at both tidal levels had not been detected ($H=1.076$, $p=0.300$). In the second trial, only four cages were brought back from the low intertidal, and five cages were brought back from the high intertidal. The rest of the cages had been carried away by high wave action. Of the cages that were brought back, there was a mean of 0.5 ± SD 0.6 egg masses per cage laid in the low intertidal, and a mean of 0.6 ± SD 0.9 egg masses deposited in the cages in the high intertidal.

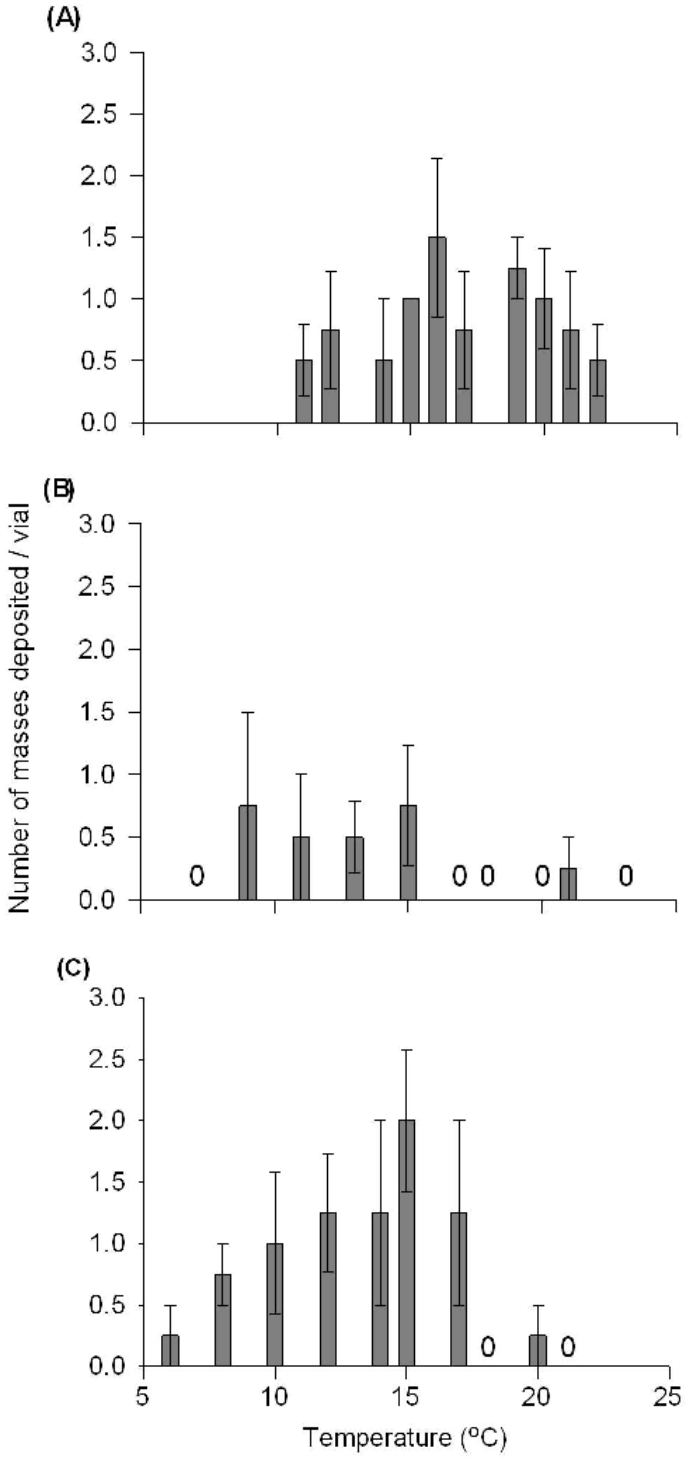


Figure 4.11. Effect of temperature on oviposition. There were ten temperature treatments in each trial, with four scintillation vials per treatment, and four *Lirularia succincta* per vial. The experiment was conducted three times. Values are not significant between treatments (Trial 1: F=0.65, p=0.745; Trial 2: F=0.87, p=0.560; Trial 3: F=2.00, p=0.0749. Error bars represent 1 SE.

Table 4.6. Results of three two-way repeated measures ANOVAs testing the effect of temperature on oviposition of *Lirularia succincta*. The table shows tests for between-subjects effects and tests for within-subjects effects for all three trials. Subjects were scintillation vials with 4 snails per vial (n=4 per treatment). P^{H} is the Huynh-Feldt adjusted P value.

TRIAL 1						
	Source	**df**	**SS**	**MSS**	**F**	**P**
Between Subjects	Temperature	9	1.367	0.152	0.65	0.745
	Error	30	7.000	0.233		
Within Subjects	Day	3	2.067	1.033	4.59	0.014
	Day x Temp	27	6.433	0.357	1.59	0.093
	Error(Day)	90	13.500	0.225		

TRIAL 2						
	Source	**df**	**SS**	**MSS**	**F**	**P**
Between Subjects	Temperature	9	0.931	0.103	0.87	0.560
	Error	30	3.563	0.119		
Within Subjects	Day	3	0.369	0.123	1.65	0.183
	Day x Temp	27	2.694	0.100	1.34	0.152
	Error(Day)	90	6.688	0.074		

TRIAL 3						
	Source	**df**	**SS**	**MSS**	**F**	**P**
Between Subjects	Temperature	9	3.975	0.442	2.00	0.075
	Error	30	6.625	0.221		
Within Subjects	Day	3	1.550	0.517	1.87	0.140
	Day x Temp	27	8.575	0.318	1.15	0.306
	Error(Day)	90	24.875	0.276		

Egg Masses Deposited in Crevices on Cobbles

A chi-squared goodness-of-fit test was used to determine if *Lirularia succincta* preferentially deposit egg masses in crevices on rocks in the field. Of the egg masses deposited on 23 cobbles photographed in the laboratory, 78.5% of the masses (n=65) were deposited in a rock's crevices. Crevices in rocks only accounted for 10.7 ± SD 3.2% of their surface area, so there were significantly more egg masses deposited in crevices than would be accounted for by

Table 4.7. Mauchley's criterion, its associated p-value, and the Huynh-Feldt Epsilon for three trials of an experiment testing the effect of temperature on oviposition. In all three trials, the assumption of sphericity was violated, so the Huynh-Feldt adjusted df were used.

	Mauchley's W	**P**	**Huynh-Feldt ε**
Trial 1	0.7641	0.0202	1.1051
Trial 2	0.4955	0.0012	1.0890
Trial 3	0.6427	0.0264	1.1055

chance ($G=193.408$, $p<0.0001$). An average of 2.8 ± SD 2.9 egg masses per rock were laid in the crevices of the cobbles, and 0.8 ± SD 0.1 masses per rock were deposited on flat surfaces. On one of the cobbles in the latter category, three masses were laid on a flat area of the rock, but were laid at the base of *Ulva.*

Discussion

A few patterns in the oviposition behavior of *Lirularia succincta* emerged from these studies. The first experiment showed a clear trend in reproductive periodicity. Several species of marine gastropods reproduce year-round, but exhibit periods of higher reproductive output (Strathmann, 1987). *Lirularia iridescens,* a congener of *L. succincta* found in Japan*,* deposits egg masses throughout the year with peak oviposition frequencies in April (Toyohara et al.*,* 1999). Reproduction for *L. succincta* is nearly continuous and probably cyclical, as it is for *Lacuna vincta* (Martel and Chia, 1991). While it appears that there is a seasonal trend in the frequency with which egg masses are deposited in the laboratory, variations in the observed frequency of egg mass deposition could be the result of an altered sex ratio in the field when the snails were

collected. If the ratio of females to males differed to any great extent from month to month, the change in the sex ratio would have manifested itself as a reduction in the rate of oviposition in *L. succincta*; however, it is unlikely that the ratio of females to males in the population was skewed enough to lower the oviposition frequency from 85% in July to 8% in November. The observed degree of change in the frequency of oviposition most likely occurred as a result of the peak in reproductive output in the late summer. The differences in fecundity of the adults may also be a reflection of differences in physical stress or resource availability, which is also associated with seasonality. In the summer months, wave action is lower, so the substrata on which *L. succincta* are found is more stable. Sea surface temperatures are higher, approaching the temperatures at which greater mean numbers of egg masses appear to be deposited by *L. succincta* in the laboratory. *Ulva* spp. are present in greater abundance in the intertidal, providing more abundant and diverse microhabitat for the snails, and possibly providing more suitable shaded sites on which to deposit egg masses (see Biermann et al., 1992).

Lirularia succincta egg masses are frequently found adjacent to other *L. succincta* masses. The occurrence of adjacent masses was similar in the laboratory (62.5%) and the field (63.1%). In the laboratory, clusters of 15 to 20 egg masses may be deposited in the corners of the 1-gallon jars in which the snails are kept; interestingly, the masses are all adjacent to each other, but rarely overlap. When aggregations of masses are found in the field, they are also found adjacent to, but not overlapping, each other; when the surface of a crevice is covered with egg masses, other masses are often found deposited in nearby cracks or crevices. This oviposition strategy allows for greater diffusion of oxygen within all egg masses, as the oxygen supply to each embryo within a mass can be limited by the thickness of the mass or mass aggregation (Cohen and Strathmann, 1995; Strathmann and Hess, 1999). There is no way to tell, however, if aggregations of masses in the laboratory or the field were produced by several different females depositing a single mass, or by one or two females depositing multiple masses. Kupfermann and

Carew (1974) found that *Aplysia* may return repeatedly to the same locations to deposit masses. The same could be true of *L. succincta* individuals; clusters of three masses found in the field could all have been deposited by the same female. The experiment testing for the effect of the presence of a new partner on oviposition behavior shows that females are as likely to mate with a male that they have already mated with as they are to mate with a new partner. Lee and Strathmann (1998) suggest that partitioning embryos into smaller clutches would provide an adequate oxygen supply to the embryos with less gel required in the mass, thereby decreasing embryo mortality without requiring an energy expenditure that limits fecundity. It then follows that a viable reproductive strategy would be to lay multiple, smaller egg masses rather than one large mass. For species depositing multiple small masses, the parental investment could still be high if there were a scarcity of suitable oviposition sites available (Lee and Strathmann, 1998). Hence the deposition of multiple small masses could be an effective adaptive compromise for *L. succincta*, as they do not seem to be limited in suitable oviposition sites. The frequent occurrence of adjacent masses in the field and in the laboratory could also result from aggregating behavior exhibited by adults. This behavior was never observed in the laboratory or in the field, although cobbles in the field on which egg masses had been deposited often had three to four times as many individuals on them as on surrounding cobbles.

The results of both experiments testing for the effect of previously-deposited egg masses on oviposition seem to indicate that egg masses were not preferentially deposited in containers when other egg masses were present. It is possible that chemical spawning cues are not produced by *L.* succincta, or, if they are, they are not released from egg masses. The fact that the egg masses were 4 days old could also have led to the observed results; D'Asaro (1966) noted that communal spawning in *Thais haemastoma* did not occur after the embryos within the mass had reached the veliger stage.

The results from the temperature experiment seem to indicate that *Lirularia succincta* preferentially deposit egg masses in temperatures warmer than those likely experienced by the snail in the intertidal. This could simply be an artifact of being warmed in the laboratory, and not an indication of an oviposition behavioral pattern; other gastropods will also shed their gametes if they are warmed after being brought into the laboratory (Strathmann, 1987). It is not likely that this is the temperature range in which they preferentially deposit egg masses in the field, as ocean temperatures near Cape Arago rarely reach 15°C. The variance in the number of egg masses deposited per vial was also high; some vials had four egg masses deposited in them, while others in the same treatment had no egg masses deposited. This high degree of variability could in part account for the results obtained in the second trial (Figure 4.11B), which did not correspond with the results from the first or third trial.

The first three water movement experiments indicate that water movement past or around a snail may stimulate oviposition, but these results were not verified by the three trials of the flow tank experiment. It is possible that water movement may simply stimulate a shedding of gametes in *L. succincta.* It is also possible that, like *Aplysia*, chemical cues released by reproductively active *L. succincta* individuals stimulate oviposition in their conspecifics, and that the chemical cues are dispersed through the water more thoroughly and rapidly in agitated water (Audesirk, 1977).

The most consistent pattern emerging from these investigations was the preference that *Lirularia succincta* females exhibited for depositing egg masses in crevices. All goodness-of-fit tests comparing the placement of egg masses in 'crevice' and 'non-crevice' areas indicated that egg masses were deposited in a non-random manner with respect to crevice surface area. Egg masses in the field were primarily deposited in crevices, even though crevice areas made up a small percentage of the surface area of the rocks on which they were found. The results from the experiments done in the laboratory agreed with these findings, and further indicated that shade

could potentially be the characteristic selected for by females depositing egg masses. More egg masses were deposited on the shaded side of partially-covered petri dishes than on any of the non-shaded sides of the petri dishes. As adults were positioned on the light side of the partially-covered dishes significantly more than they were on the dark side of the dish, the adults either purposefully moved into the dark side to reproduce, or only deposited egg masses if they came in contact with a snail of the opposite sex while they were on the dark side of the dish. The latter scenario is unlikely because snails do not preferentially deposit egg masses in the dark. If the areas deemed 'crevices' inside a Falcon tube are selected as oviposition sites merely because they present tactile cues that resemble the crevices found in the field, then the frequencies in the number of egg masses deposited in each area of the tube should have been similar in all treatments. In all three treatments, I did observe a greater number of egg masses deposited in the conical end of the tube than would be suggested by chance. However, the area under the lid was preferred only in the two treatments experiencing light; the side of the tube was a preferred oviposition site in the treatment experiencing constant dark. For snails experiencing complete darkness, the area under the lid was not 'shaded'. The results from this experiment, and from the experiment with partially-covered dishes, suggest that the shaded areas, or areas physically resembling a crevice, were being selected as appropriate deposition sites by *L. succincta*.

Crevices can provide safe oviposition sites for many gastropods. In the study conducted by Biermann et al. (1992), in 7 of 7 cases, egg mass portions that were in direct sunlight and full current experienced slower development, more microalgal fouling, and lower survival overall. Crevices can provide shade for benthic egg masses, especially when crevices penetrate far into the rock. Shading has been found to decrease embryo mortality (Biermann et al., 1992). Crevices can also provide a refuge from wind, which, combined with the shade, can lower the risk of desiccation. Egg masses of *L. succincta* are particularly vulnerable to desiccation due to their thin shape, which yields a large surface are and little gel per embryo (Strathmann and Hess,

1999). Rawlings (1996) concluded that *Nucella emarginata* preferentially select habitats that provide protection from desiccation and UV radiation. Crevices also may provide protection from dislodgement by abrasive sediment or from crushing by shifting rocks. Algal fronds may also provide the same types of protection to developing masses. In the field, *L. succincta* deposit egg masses on both rocks and *Ulva* spp. The deposition of masses on more than one type of substratum can be uncommon on wave-exposed shores; in Benkendorff and Davis' (2004) survey of 54 marine gastropod species, only eight were found to deposit egg masses on more than one type of substratum. Overall, however, the majority of the egg masses discovered throughout the year at North and South Cove were deposited in cracks or crevices on rocks.

For the remaining factors tested there were no apparent patterns detected in the oviposition behavior of *Lirularia succincta*. This may be due to small sample sizes, as rates of oviposition in these snails can be highly variable. In any case, the non-significant results for these experiments only indicate an absence of oviposition choice; this could be due to an actual lack of preference by *L. succincta*, but it could also result from the inability of *L. succincta* to discriminate between the variables being tested (Resetarits, 1996). For example, having already deposited an egg mass, females will deposit a second or a third egg mass with the same partner or with a new partner with equal likelihood. It is possible that *L. succincta* females are unable to discriminate between different males. Also, a difference in oviposition behavior was not detected between the two different tidal levels. As with the flow tank experiment, this is most likely because the number of replicates was low. My inability to detect significant differences in this case could be because of the high degree of variability within the population, or it could result from the fact that, with less than 1 meter of elevation difference between the two tidal levels, the difference in physical stress experienced by snails at both cage locations was not significant.

This study provides a framework for more sophisticated questions relating to oviposition behavior in *L. succincta* and in other trochids. It is more likely that stronger effects would be

observed when two or more of the factors investigated in these studies interacted. Oviposition behavior, especially oviposition site selection (*i.e.*in crevices) can have a far-reaching influence on structuring populations, and should therefore be studied to a greater extent. "How important is oviposition site choice? A brief survey suggests that when we look for evidence of oviposition site choice, which hasn't been often for most taxa, we often find it" (Resetarits, 1996).

CHAPTER V

CONCLUDING SUMMARY

The purpose of this thesis was to observe defensive behaviors of *Lirularia succincta* to predatory and non-predatory sea stars and to quantify the intensities of the responses, to describe the vertical distribution of the snail in the intertidal, to observe how its distribution changed throughout the year, and to examine the factors that may affect oviposition in this species. The only published literature describing this snail's biology or ecology to any great extent is a paper by Hadfield and Strathmann (1990), in which they describe reproductive behavior and shell morphology. Prior to this study, the snail was described as exclusively intertidal (Carlton, 2007), with no indication of its vertical distribution in the intertidal. Generally, for gastropod species with direct development, distribution is directly affected by oviposition behavior. Distribution can be affected by predation as well (Connell, 1961, 1970; Vermeij, 1972). Thus, studying predation and oviposition preferences in *L. succincta* may provide clues to the population dynamics of this species and offer insight into the ecology of rocky intertidal communities in general.

In Chapter II, I showed that the predatory sea star *Leptasterias* feeds on *Lirularia succincta* in the laboratory. I then described some of the escape responses of *Lirularia succincta* to direct contact with the predatory sea stars *Leptasterias* and *Pycnopodia helianthoides.* The snail did not exhibit escape responses to the non-predatory sea star *Henricia* sp. The primary escape response of *L. succincta* was flight. The snail exhibited a turning response and increased its speed after contact with the tube feet of a predatory sea star. It also frequently exhibited shell

rotation following contact with *Leptasterias.* Juvenile *Leptasterias* elicited escape responses from adult *L. succincta,* but not from newly-hatched juvenile *L. succincta.*

In the next part of Chapter II, I documented the presence of weak avoidance responses of *L. succincta* to water-borne chemical cues from *Leptasterias. Lirularia succincta* placed into a water current downstream from a *Leptasterias* individual will move further downstream than individuals placed into a water current without a *Leptasterias* individual. Water-borne chemical cues from *Leptasterias* may also elicit a climbing response in *L. succincta*, although the climbing response may result from a general increase in the activity of snails exposed to water containing the *Leptasterias* chemical cues. Some snails also exhibit shell rotation when exposed to water conditioned with the scent of *Leptasterias. Lirularia succincta* does not appear to respond to water conditioned with chemical cues leached from injured conspecifics.

In Chapter III, I described the vertical distribution of *Lirularia succincta* in a sheltered cove over the course of a year. The snails were more abundant in the low intertidal than in the high intertidal. Seasonal changes in distribution appeared to follow the same trend for all tidal levels. The densities of the snails decreased through the spring and began to increase in June, with peak densities observed in September. In June, the increase in the abundance of snails at all tidal levels coincided with a shift in the size-frequency distributions of the snails towards smaller size classes.

In Chapter IV, I investigated factors that might affect oviposition of *Lirularia succincta* in the laboratory. *Lirularia succincta* deposited egg masses year-round with the highest rate of oviposition in the summer. In the lab and in the field snails deposited egg masses in crevices significantly more than they deposited them on flat surfaces. Snails also frequently deposited egg masses adjacent to other egg masses. Snails deposited more egg masses in moving water than in stationary water. Oviposition behavior did not appear to be significantly affected by temperature or by the amount of light the snails received each day. Female *L. succincta* did not discriminate

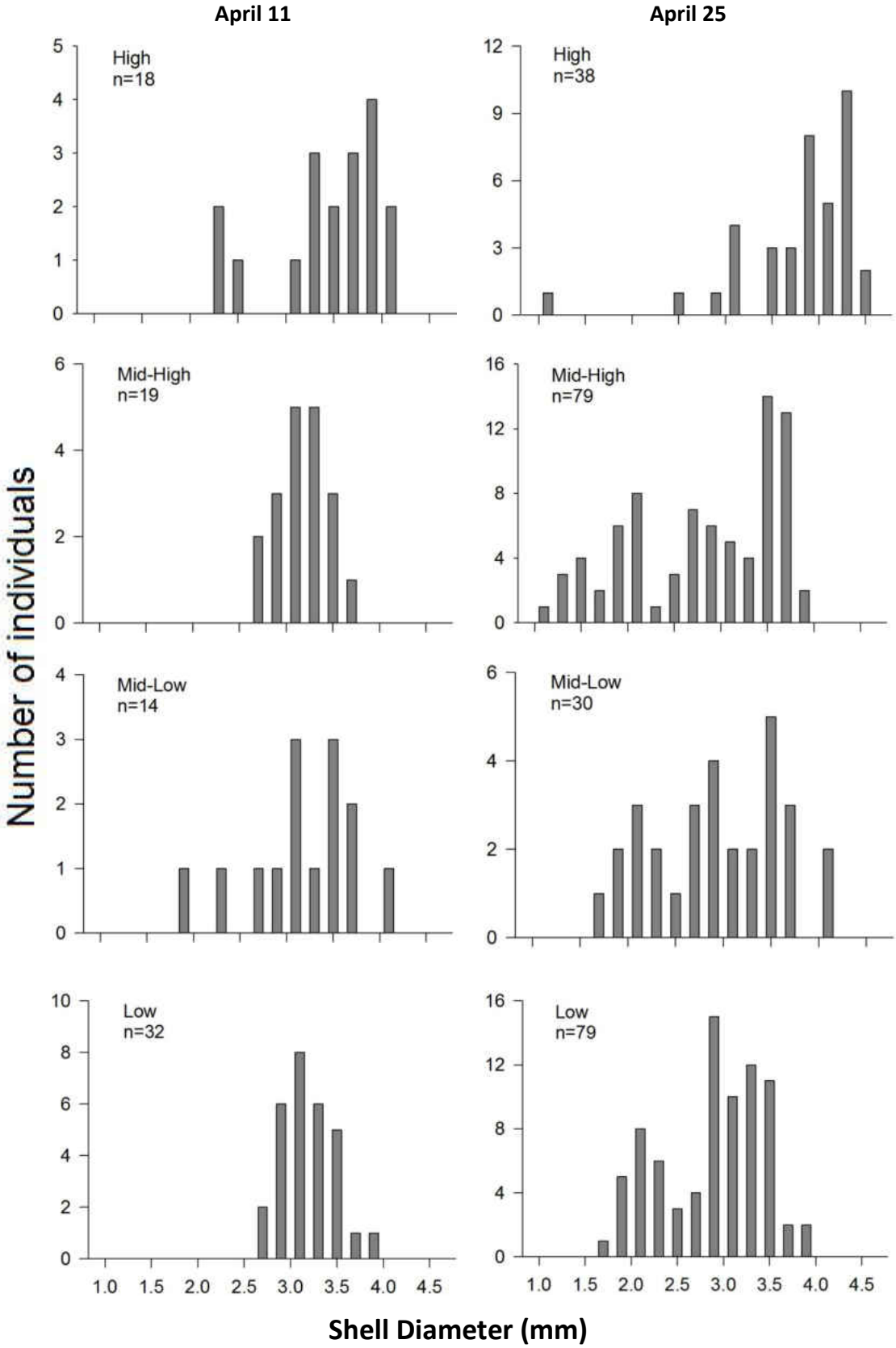
between new partners and partners with which they had already mated. Whether or not they are capable of discriminating between old and new partners is uncertain.

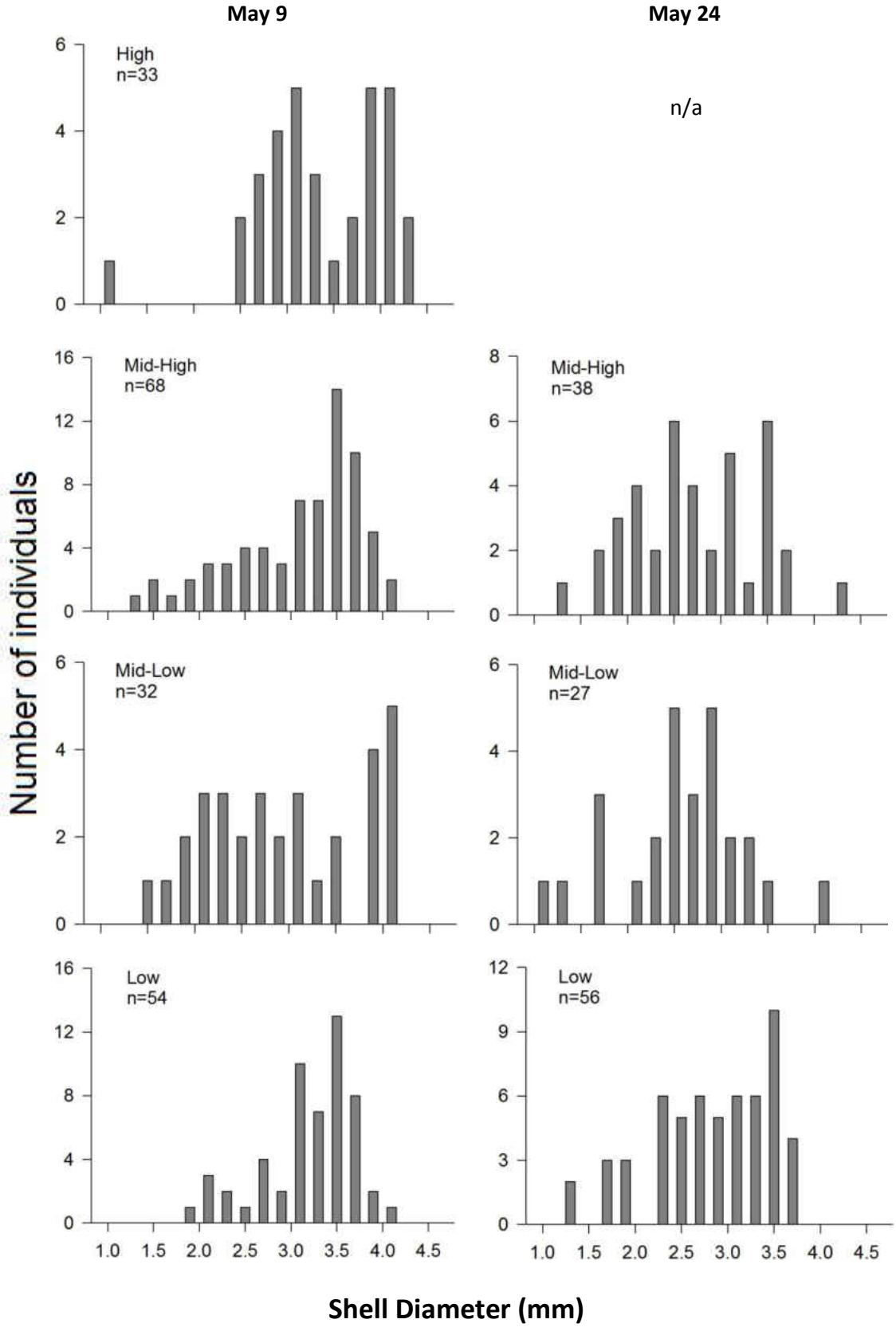
These are the first ecological studies that have been conducted on this small intertidal gastropod. Patterns observed in *L. succincta* appear to follow patterns observed in other gastropods. They exhibit common predator avoidance and escape responses, and they preferentially deposit egg masses under certain conditions. Both of these factors may affect their density and size-frequency distributions in the intertidal and their ecological impact in the rocky intertidal community.

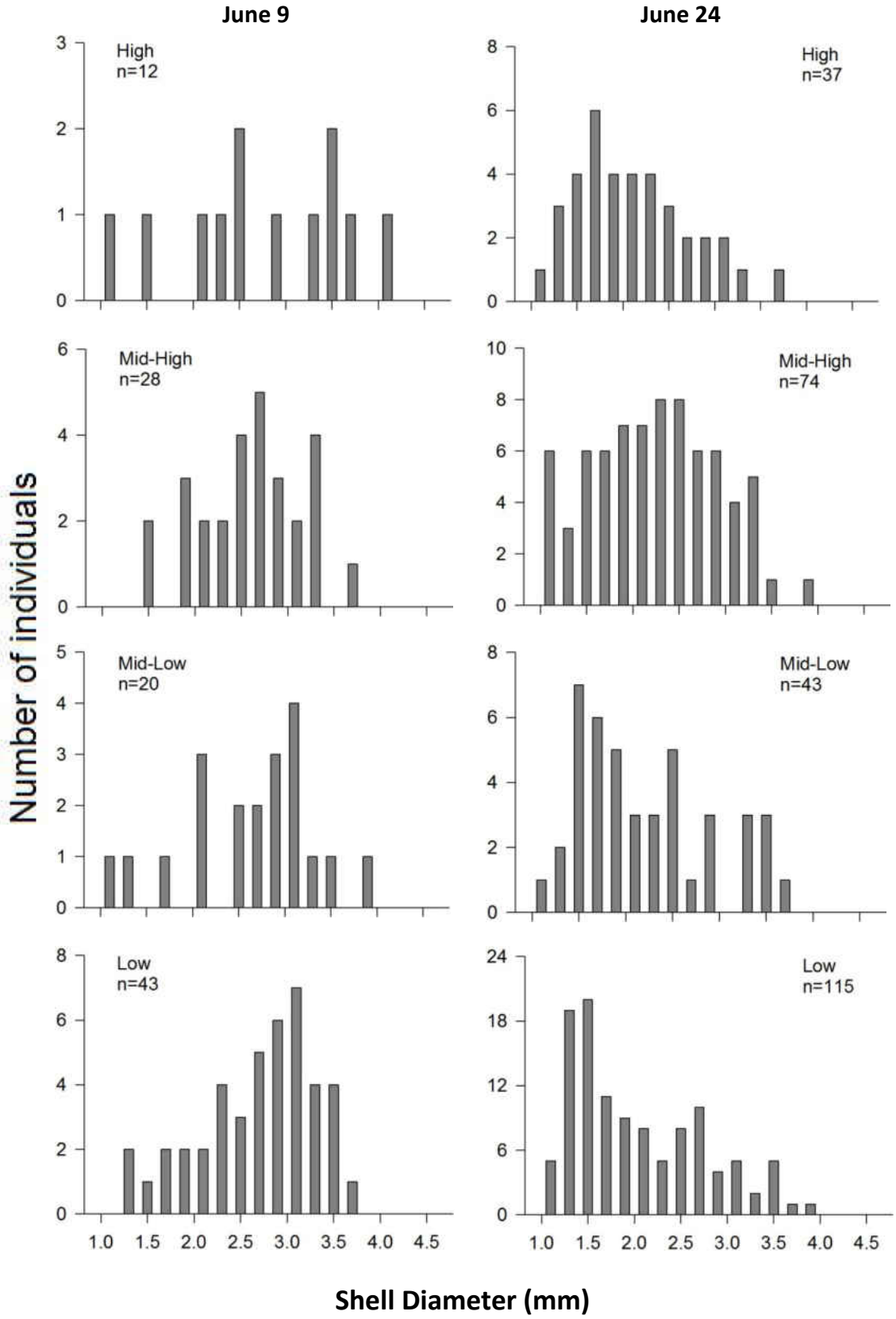
APPENDIX

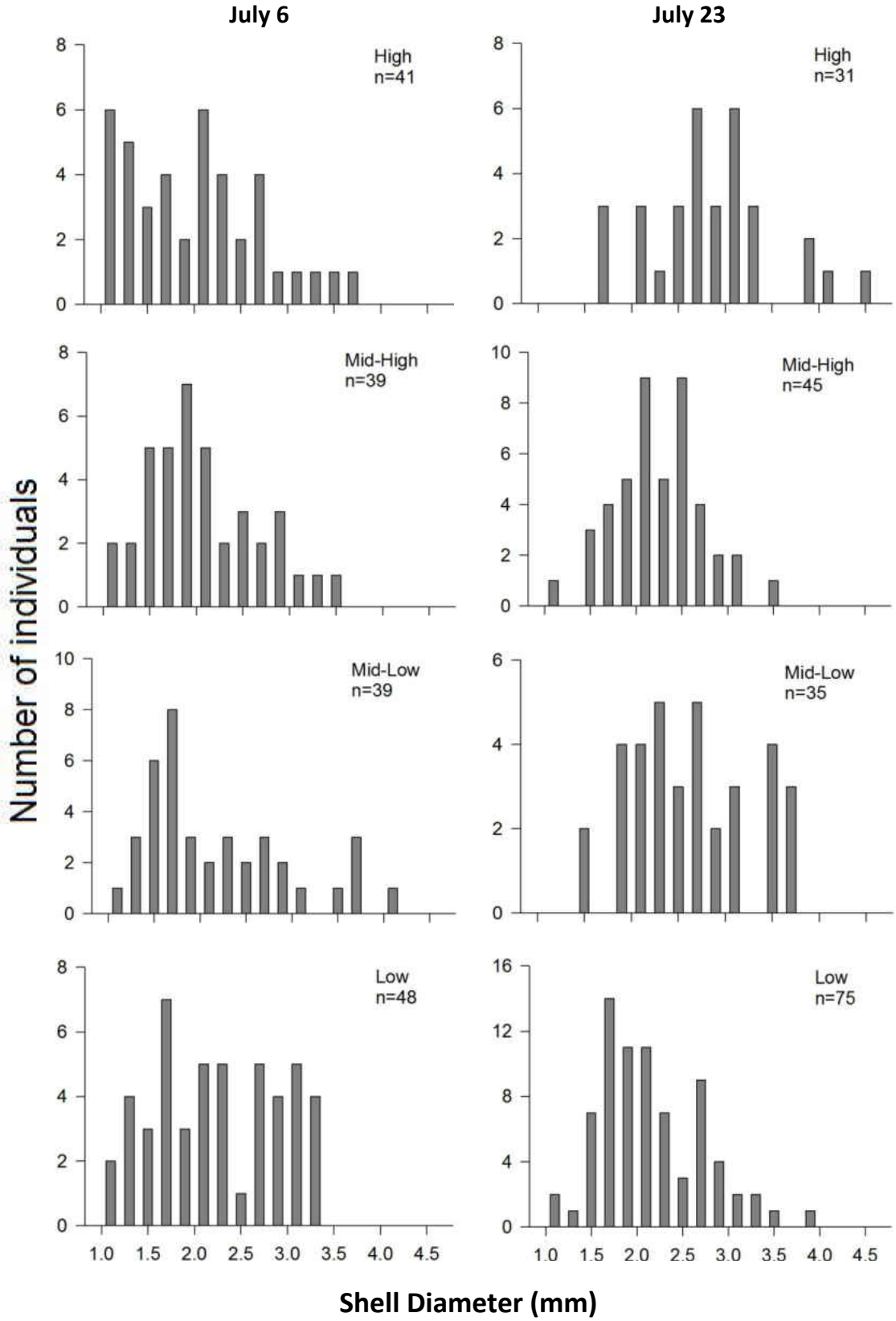
SIZE-FREQUENCY HISTOGRAMS FOR *LIRULARIA SUCCINCTA* BY TIDAL LEVEL

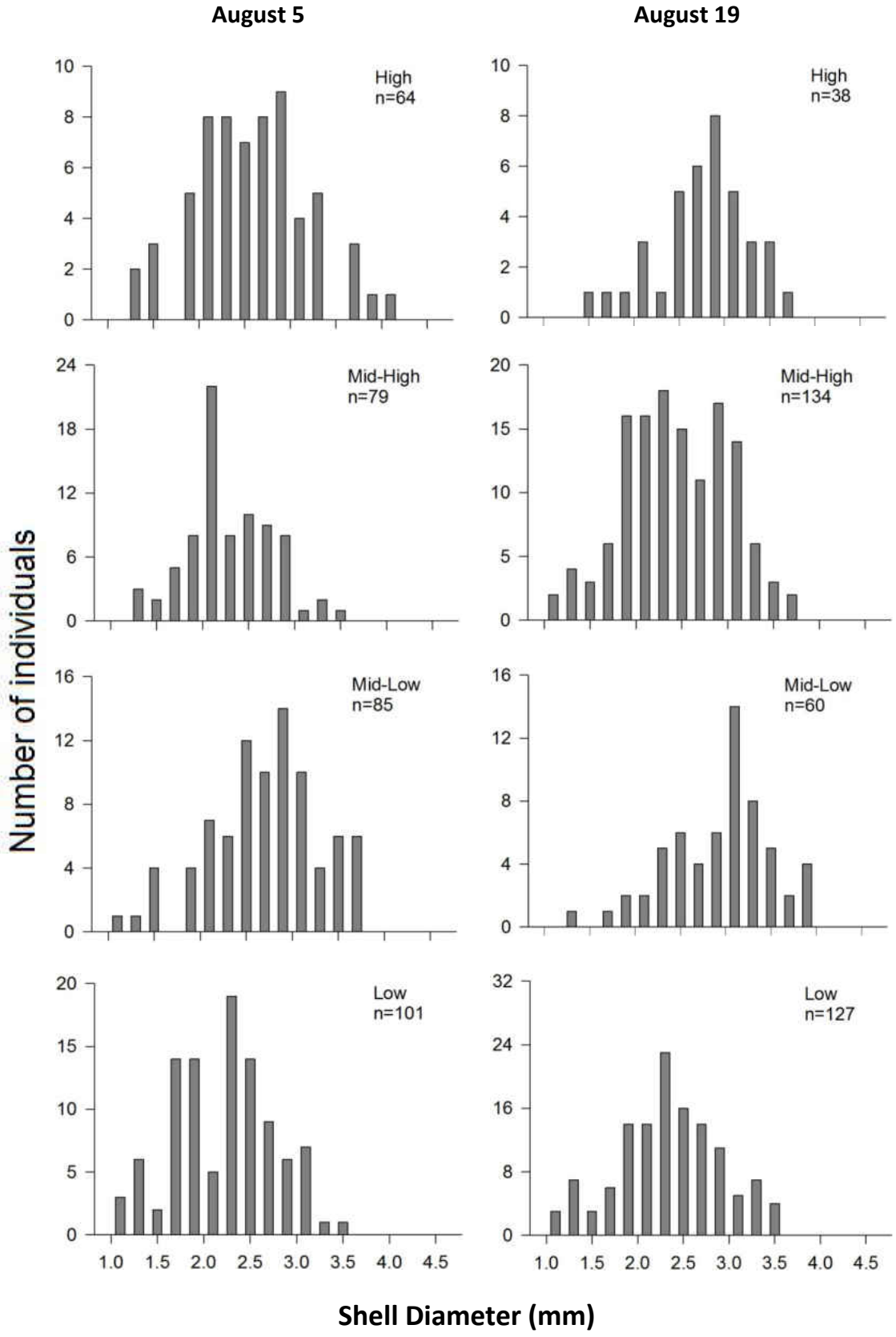
These figures show the size-frequency distributions of *Lirularia succincta* at each tidal level from April through August of 2009. Surveys were conducted twice each month during negative tides. Because the snails are highly motile in the intertidal, the numbers of snails in each size-class were combined for all four tidal levels to create one size-frequency histogram for each date, found in Chapter III. Data show a shift in the size-frequency distributions towards snails in smaller size classes on June 24.


April 11
April 25
High
n=18
High
n=38
Mid-High
n=19
Mid-High
n=79
Mid-Low
n=14
Mid-Low
n=30
Low
n=32
Low
n=79
Number of individuals
Shell Diameter (mm)


May 9
May 24
High
n=33
n/a
Mid-High
n=68
Mid-High
n=38
Mid-Low
n=32
Mid-Low
n=27
Low
n=54
Low
n=56
Number of individuals
Shell Diameter (mm)


June 9
June 24
High
n=12
High
n=37
Mid-High
n=28
Mid-High
n=74
Mid-Low
n=20
Mid-Low
n=43
Low
n=43
Low
n=115
Number of individuals
Shell Diameter (mm)


July 6
July 23
High
n=41
High
n=31
Mid-High
n=39
Mid-High
n=45
Mid-Low
n=39
Mid-Low
n=35
Low
n=48
Low
n=75
Number of individuals
Shell Diameter (mm)


August 5
August 19
High
n=64
High
n=38
Mid-High
n=79
Mid-High
n=134
Mid-Low
n=85
Mid-Low
n=60
Low
n=101
Low
n=127
Number of individuals
Shell Diameter (mm)

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