FACTORS LEADING TO CANNIBALISM IN *LYTECHINUS VARIEGATUS* (ECHINODERMATA: ECHINOIDIA) IN THE LABORATORY

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FACTORS LEADING TO CANNIBALISM IN *LYTECHINUS VARIEGATUS* (ECHINODERMATA: ECHINOIDIA) IN THE LABORATORY

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BIOLOGY

ABSTRACT

Due to increased popularity of consumption of sea urchin gonad (uni) as sushi in recent years and the decline of sea urchin fisheries worldwide, a need for an effective method of farming sea urchins in aquaculture has arisen. An obstacle to optimizing output of sea urchins in aquaculture is cannibalism. Approximately 2000 adult and juvenile *Lytechinus variegatus* (1g-45g) were collected from Port Saint Joseph Peninsula State Park, FL over the course of 5 collection trips between June and September 2009. These urchins were randomly stocked into one of three experiments designed to explore the potential factors leading to cannibalism of *L. variegatus* in the laboratory. The factors investigated were the combination of feed level coupled with density among two size classes of urchins, infliction of various gradations of physical injury, and the consequence of varying size ratios of urchins housed together to determine if any of these factors caused a significant increase in cannibalism. Regarding density coupled with feed level, it was determined that starved, high density conditions contributed to the highest level of cannibalism among small (12-21g) urchins (percent cannibalism = 18.75% over 4 weeks), whereas fed, high density conditions contributed to the highest level of cannibalism among large (32-37g) urchins (percent cannibalism = 18.4% over 4 weeks). Infliction of severe physical injury to urchins (16-24g) resulted in the greatest level of cannibalism (47.1%) over six days in comparison with 8.3% and 0% cannibalized at lesser degrees of injury over the same time course. Urchins were stocked into various size ratio classifications based
upon weight of the urchins (1g-19g); however, based on observations we suggest that differences in size could be a causative factor contributing to cannibalism. From an aquaculture standpoint, it is important to determine those factors that contribute to the incidence of cannibalism in *L. variegatus* so that the appropriate culture conditions can be maintained to reduce the incidence of cannibalism.

Keywords: cannibalism, sea urchin, *Lytechinus variegatus*
DEDICATION

To my family and friends, for their constant patience, guidance,
and unconditional support
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CHAPTER 1
INTRODUCTION

By definition, cannibalism involves one individual of a species consuming part or all of another animal of the same species for food (Polis, 1981). Cannibalism has been noted in more than 1300 species either in the field or laboratory including such groups as insects, fish, amphibians, reptiles, birds, and mammals (Polis and Myers, 1985; Tietje et al., 1986; Rootes and Chabreck, 1993; Boal and Bacorn, 1994; Babbitt and Meshaka, 2000). Interestingly, even though cannibalism has been documented extensively in many invertebrate species, it has only been minimally recognized or described anecdotally in echinoderms. Many articles reference incidental findings, such as a report of a conspecific sea-star found in the gut contents of the sea star *Astropecten articulatus* (Wells et al., 1961), or as an anecdote in a results section of an article explaining loss of animals during the course of an experiment. Although cannibalism is noted in both types of reports, a hypothesis about why it may have occurred or a discussion of potential factors leading to cannibalism were not included.

Several types of cannibalism have been defined in echinoderms and other organisms, including sexual cannibalism, larviphagy, and oophagy. Sexual cannibalism is most well documented in spiders and other arachnids (Elgar, 1992). Larviphagy (cannibalization of larvae by adults) and oophagy (cannibalization of eggs by adults) are documented in many different species including the sea urchin *Dendraster excentricus* (Timko, 1979).
Sometimes there are unusual circumstances that may promote cannibalism. There are reports of intragonadal cannibalism (sometimes referred to as adelphophagy) in the sea-stars *Patiriella vivipara* and *P. parvivipara*. These sea stars develop and metamorphose in the gonads of the parent where they remain until they are large enough to survive on their own. During this post-metamorphic stage, while the juvenile sea stars are still being brooded, their mouths open and the juveniles begin to cannibalize their intragonadal siblings. Usually the larger individuals prey upon the smaller. The cannibals benefit by increased size after maturation. It was noted that sometimes the juveniles can become very large (up to 25-30% of the parent’s total diameter) within the gonad before they are released through the gonopore (Byrne, 1996; Byrne and Cerra, 1996; Hart et al., 1997).

Another unusual causative factor of cannibalism may be the presence of a certain phenotype. Emson and Crump (1976) determined that only individuals of the sea star *Asterina gibbosa* of a certain phenotype and sex were cannibalized in the field. This cannibalism occurred at an increased rate during breeding season.

Typically, cannibalism is most often described in classical predator/prey interactions. The advantages to the predator in a cannibalistic encounter are obvious. They gain essential nutrients while reducing their competition for resources. Population size will be reduced, thus allowing more resources per individual with an increased chance for survival and growth (Fox, 1975; Crump, 1983). The larger they grow, the less vulnerable they may be to attacks by conspecifics, and their added size may allow them to cannibalize smaller individuals more easily. Additionally, it is conceivable that essential nutrients can be obtained by consuming a conspecific individual as compared to other available food sources. Anecdotal observations of cannibalistic events by *Lytechinus variegatus* in
aquaria indicate that the lantern is sometimes preferentially consumed along with the gonads (personal observation). In this case, it may be that a nutritional deficit is resolved by cannibalizing specific organs of a conspecific.

The rate of cannibalism varies considerably among and within species; however, it is dependant, at least in part, on size ratios of the conspecifics, degree of hunger, and density (Fox, 1975; Polis et al., 1989). However, some species display cannibalistic tendencies even in low density and in well-fed situations. Another factor influencing cannibalism is the presence of a vulnerable individual. If an individual is younger, injured, or diseased, it is more susceptible to becoming prey to a larger individual of the same species or another species (Birkeland et al., 1971; Polis, 1985; Tietje et al., 1986; Hagen, 1987; Rootes and Chabreck, 1993; Babbitt and Meshaka, 2000; Witman et al., 2003). In some cases cannibalistic individuals displayed a preference for a distantly-related conspecific rather than one more closely related (Pfennig, 1997). Presumably, the inherent benefit for this behavior would be to perpetuate the existence of its genes above the existence of another individual’s genes leading to an increase in fitness. A possible regulating factor against widespread cannibalism among species that cannibalize is the potential for reduction of the population to the extent that reproductive success is decreased.

Most reports of cannibalism in the Echinodermata are from the class Asteroidea with the sea star genus *Asterias* having the most reports of intraspecific cannibalism in the literature (Kvalvagnes, 1972; Jangoux, 1982; Harris et al., 1998; Campbell et al., 2001). Cannibalism was documented in *A. forbesi* and *A. vulgaris* both in laboratory and field experiments in response to food limitations, size ratios, high density and presence of injury (Witman et al., 2003). *Asterias forbesi* has long been known to cannibalize in la-
boratory-held populations in response to food limitations (Galtsoff and Loosanoff, 1939), and *A. vulgaris* is known to cannibalize in field populations (Lubchenco and Menge, 1978). Fukuyama and Oliver (1985) reported that *A. amurensis* was found cannibalizing in the field at two different locations in two different years, albeit at low rates (1/56 and 1/52 observations).

In addition to *Asterias*, a few other species of sea stars exhibit occasional cannibalism as well. Sloan (1980) compiled a list of observed cannibalism in sea stars in the field and in the laboratory. This list contains 13 species of sea stars from three different orders. Several of these species were observed to cannibalize in the laboratory, including *Oreaster reticulates, Acanthaster planci,* and *Patiriella regularis* (Schiebling, 1982; Moran, 1986; Martin, 1970). Schiebling (1982) found that cannibalism in *Oreaster reticulates* is rare in nature, but occurs in conjunction with food limitation. There were also individuals in the field with scar patches consistent with having survived a cannibalistic encounter. *Coscinasterias calamaaria* was noted to cannibalize in the field (Martin, 1970). Although some genuses of sea stars are specifically mentioned as being known cannibals in the field, it is important to recognize how many species were not on this list. In fact, Wobber (1975) specifically indicated that sea stars from the genuses *Patiria* and *Pycnopodia* did not display cannibalism in the field. It is obvious that sea star cannibalism is a behavior that is occurring in the field at varying levels, but perhaps it is not recognized as such and so is not reported frequently.

In some species, that experience frequent cannibalism, there are reports of a strong escape response upon encountering a conspecific. The sea star *Solaster dawsoni* is a known congeneric predator of *Solaster stimpsoni*, but was found to cannibalize a con-
specific during 1/55 encounters in the field (Mauzey et al., 1968). The frequency of cannibalism in this species is low, which is perhaps due to the strong, highly effective escape response noted when an individual of this species comes into contact with a conspecific. The *S. dawsoni* that is being predated upon (usually they are attacked by the predator moving over its aboral surface) curls its rays above its aboral surface and then pushes upwards and away on its attacker and in a forward direction to facilitate a quick escape. It was shown that laboratory-held *Asterias rubens, Psammachinus miliarus*, and *Echinus esculentus* display an avoidance response, by moving away from the water source containing the predatory stimulus (the gut homogenate of conspecifics) into stimulus-free water. Evidence of cannibalism in *A. rubens* was based on gut content analysis of predatory sea stars (Jangoux, 1982). Additionally, *P. miliarus* and *E. esculentus* also display an avoidance response when exposed to conspecific coelomic fluid and gonad homogenate (Campbell et al., 2001) and that *P. miliarus* and *E. esculentus* may be displaying an avoidance response as a mechanism to avoid predation and perhaps conspecific cannibalism. Some sea star species have developed a strategy for avoiding lethal cannibalism by autotomizing body parts, thus avoiding lethal cannibalism and escaping while autotomized arms are consumed by the predator. *Meyenaster gelatinosus*, a Chilean sea star, was observed autotomizing its rays in response to an encounter with a conspecific, and only cannibalism of the rays was observed (Dayton et al., 1977). This finding is similar to a report for *Luidia clathrata* which displayed the same escape response to an attack by a conspecific in the laboratory (Lawrence et al., 2009). It is evident that some sea star species have developed a strategy for avoiding cannibalism, which may indicate that cannibalism is more prevalent than had been previously considered.
Reports of cannibalism in sea urchins are not as prevalent in the literature as are those for sea stars. This may be a consequence of the relative number of studies reporting on feeding behavior of sea stars compared to sea urchins. Despite the low number of reports, it seems that the cannibalistic process is similar in *Lytechinus variegatus* (personal observation), *Strongylocentrotus droebachiensis* (Himmelman and Steele, 1971), *S. intermedicus* (Yaqing Chang, personal communication), and *Diadema antillarum* (Levitan, 1989). The process of cannibalism begins by all of the spines being consumed (usually on the aboral surface), then a hole is eaten in the aboral surface of the test and the guts and gonads are largely consumed. Occasionally, the lantern is also extracted and consumed. Often, much of the test is discarded. This process has been similarly described in nearly all of the cannibalistic encounters of sea urchins that have been recorded in the literature or by anecdotal observations. In addition to a similar progression of cannibalism, the potential causative factors for cannibalism appear to be the same. Cannibalism has been observed in the sea urchins *D. antillarum* and *S. droebachiensis* in the laboratory as a result of food deprivation (Himmelman and Steele, 1971; Levitan, 1989). Himmelman and Steele also made a note of a specific instance of cannibalism in the field due to deprivation of a suitable food source, sometimes with more than one urchin attacking a conspecific. Serafy and Fell (1985) reported that *S. droebachiensis* adults will eat smaller specimens in aquaria, which may imply size difference as a factor in the rate of cannibalism. In addition, *S. droebachiensis* was observed in the field cannibalizing conspecifics as a result of a parasitic outbreak in 1983 (Hagen, 1987), suggesting that the presence of disease causes an increase in intraspecific cannibalism. Several observations have been made of large aggregations of sea urchins occurring in the field, providing opportunities
of observations of cannibalism. Although densities reported were upwards of 600/m², cannibalism was not noted (Camp et al., 1973; Beddingfield, 1997; Rose et al., 1999). Cannibalism most likely occurs naturally in the wild; however, it is most likely not recorded due to minimal cannibalism in the vast area of habitat observed, or because observed mortalities are being attributed to predation by other organisms.

Sea urchins are an important species in marine habitats around the world. Economically, sea urchins are prized for their gonads (called roe or uni), which are consumed in Asian, Mediterranean and, more recently, in western countries. For this reason, sea urchin aquaculture has received widespread attention in the last several years. If urchins could be raised inland in large numbers then natural populations could recover while maintaining a cultured supply for export and use as food and in research settings. One of the important keys to successful aquaculture of urchins is the ability to culture all life history stages, from larva to adult. In preliminary observations, it was noted that newly-metamorphosed juvenile *L. variegatus* display cannibalism in the laboratory (Richardson and Watts, 2008). These observations suggest cannibalism and the factors causing it should be considered when culturing large populations of urchins in a hatchery. Thus, the objective of this study is to determine those factors that promote cannibalism in these juvenile and adult populations. Factors to be evaluated include density, degree of hunger/satiation, presence of injury, and size ratios. Density and degree of hunger could be examined separately; however, testing them in combination allows for a better understanding of how these two factors interact. We hypothesize that the rate of cannibalism will be highest in high density, starved conditions and that the lowest rate of cannibalism will occur under low density, well-fed conditions. In addition we hypothesize that inflic-
tion of a severe injury will cause an increased level of cannibalism compared to a less severe one. Lastly, we hypothesize that small urchins housed together with larger urchins will have an higher chance of being cannibalized than if they were housed with urchins of a similar size, and that as the size/weight differential between the large and small urchins increases, the rate of cannibalism will increase as well.
CHAPTER 2
EFFECT OF DENSITY AND NUTRITIONAL STATE ON CANNIBALISM IN LYТЕCHINUS VARIEGATUS HELD IN CULTURE

Introduction

Sea urchins are an important species in marine habitats around the world. Economically, sea urchins are prized for their gonads (called roe or uni), which are consumed in Asian, Mediterranean and western countries. For this reason, sea urchin aquaculture has received widespread attention in the last several years. If urchins could be raised inland intensively, then natural populations could recover while maintaining a cultured supply for export and use as food and in research settings.

One of the important keys to successful aquaculture of urchins will be the ability to culture all life history stages, from larva to adult. In preliminary observations, it was noted that newly-metamorphosed juvenile *Lytechinus variegatus* display significant rates of cannibalism in the laboratory (Richardson and Watts, 2008), and that cannibalism decreased as the urchins increased in size (personal observation). These observations suggest cannibalism, and those factors affecting cannibalism, should be considered when culturing intensive populations of urchins in aquaculture.

Among the species that cannibalize, density is hypothesized to be an important contributing factor. This trend is seen across phyla from protozoans and mollusks to echinoderms and mammals (Baur, 1992; Elwood, 1992; Hiraiwa-Hasegawa, 1992; Waddell, 1992; Witman et al., 2003). For sea urchins in intensive culture, as space becomes limited there may be an increased incidence of contact. Although stress cannot be easily
quantified in sea urchins, we hypothesize increased stress caused by increased contact may lead to cannibalism. As more urchins are restricted to a defined area, resources become limited and competition may become a contributing factor to cannibalistic activity. Comparatively, the sea star *Asterias vulgaris* cannibalized conspecifics as well as its congener, *Asterias forbesi* (Harris et al., 1998) both in the field and in the laboratory in response to high density. This occurrence may be a result of a food or space limitation.

Often coupled with density, degree of hunger is important in the rate of cannibalism. The urchins *Eucidaris tribuloides*, *Strongylocentrotus droebachiensis*, and *Diadema antillarum* all cannibalized in the laboratory in response to food limitation (McPherson, 1968; Himmelman and Steele, 1971; Levitan, 1989). In general, the species previously mentioned display an increase in conspecific cannibalism due to a shortage of food, both in the field and in the laboratory. In the present study we evaluated the effects of size, density, and degree of hunger both singularly and in combination, to better understand those factors affecting cannibalism in laboratory-held populations of the urchin *Lytechinus variegatus*. We hypothesize that: (1) the rate of cannibalism will be highest in high density, starved conditions and that the lowest rate of cannibalism will occur under low density, well-fed conditions, and (2) that small urchins will cannibalize with a higher frequency than large urchins.

### Materials and Methods

**Collection of Urchins**

Approximately 2000 adult *L. variegatus* (10g to 45g) were collected over the course of 5 collection trips in June-September 2009 from Port St. Joseph Peninsula State Park, FL (30° N, 85.5° W) and transported to the University of Alabama at Birmingham.
Urchins were placed in a 4000 L recirculating, synthetic seawater aquaria system (Instant Ocean, 32 ± 1 ppt) equipped with a Polygeyser DF-3 biological filter (Aquaculture Systems Technologies, L.L.C., New Orleans, LA), followed in series with a SMART high-output 80 watt UV sterilizer (Emperor Aquatics Inc., Pottstown, PA) and a TF500 double-venturi protein skimmer (Top Fathom, Hudsonville, MI) used for foam fractionation. Urchins were held at a constant photoperiod and temperature (12h light, 12h dark; 22.5 ± 1°C) and were fed three times weekly a formulated feed described to promote growth (Hammer, 2006a). Urchins of the appropriate weights were then stocked into test chambers, at which time they were subjected to either starvation or *ad libitum* feeding for a period of four weeks to investigate the effect of density combined with degree of hunger on the rate of cannibalism in both large (31-38g) and small (11-21g) urchins.

**Experimental System and Design**

After exposure to laboratory conditions, urchins were transferred into one of 16 80L experimental tanks in the same 4000L aquaria system described previously. Each experimental tank was divided into four test chambers using 0.47 cm thickness Plexiglass dividers (11.5 cm length x 30 cm width, Fig. 1). The water level of the test chambers was lowered to 10 cm, resulting in a surface area of 1,175 cm² in each test chamber. Each Plexiglass baffle was raised 0.7 cm off of the tank floor to allow for adequate water movement, and to maintain the water level between compartments at 10 cm. The additional 5 cm height of the dividers prevented urchins from moving from one section to
another. To maintain water flow and oxygen in each compartment, a raised water delivery manifold system was constructed from 1.3 cm Schedule 40 PVC pipe and suspended above the test chambers. Each compartment received water from four drilled holes in the output pipe, two on each side. The holes were evenly spaced along the length of the pipe and were directed towards the wall of the tank to facilitate oxygenation. A total of 12 experimental tanks were divided in this manner, allowing for a total of 48 test chambers.

Fig. 1. Schematic diagram of arrangement of 0.47 cm thick Plexiglass dividers in 80L tank with measurements included (a & b) and of the 1.3 cm Schedule 40 pvc water delivery system (c). This tank design was replicated in 12 tanks.

Each treatment replicate was randomly assigned (using Excel’s random number generator) to a compartment position in one of the 12 tanks. The treatments used were the same for both large and small urchins: a high density treatment and a low density treatment, both fed and non-fed. Each of the four treatments had 8 replicates, and all replicates of each treatment were run concurrently (Fig. 2). The starved treatments were com-
pleted during the first four weeks, and then the fed treatments were completed during the
next four weeks to prevent the potential influence of chemical cues from food leachate in
the water.

Large urchins (34.9g ± 0.08)

By Equivalent Density

By Equivalent Biomass

Small urchins (15.6g ± 0.06)

High Density Fed  Starved

Low Density Fed Starved

High Density Fed Starved

Low Density Fed Starved

Fed  Starved

Fed Starved

Fed Starved

Fed Starved

Fed Starved

Fed Starved

Fig. 2. Experimental design for large and small Lytechinus variegatus cannibalism trials.

Weights are shown as mean ± 1 SE, in grams. Large urchins were held at two densities
(high or low) and fed *ad libitum* or starved. Small urchins were stocked either by equiva-

lent number of urchins or equivalent biomass to large urchins and were fed *ad libitum* or

starved. A total of 12 treatments were tested over an eight-week period.

Temperature, salinity, and water chemistries (ammonia-nitrogen, nitrate-nitrogen,
nitrite, and alkalinity) were monitored daily and kept within normal range (Basuyaux and
Mathieu, 1999). Urchins were checked at least once daily to maintain basic urchin care
(siphoning of each compartment, checking water parameters, and feeding if necessary
depending on treatment), to count individuals, and note if cannibalism was in progress in
any compartment or to remove cannibalized urchins. Only urchins that appeared to be
cannibalized (i.e. having a hole in the aboral surface of the test with the gonad and gut eaten) were removed and each was photographed to record the general progress of cannibalism in *L. variegatus* (length of time, general process).

Treatment 1: Large Urchins

*Starved, varying density.* A subsample of large urchins (33-38g) was randomly selected from a large, recently-collected laboratory population. Individuals were weighed and stocked into 1 of 16 test chambers (position determined using Excel’s random number generator) at either a high (21 urchins per compartment, 8 replicates) or low (5 urchins per compartment, 8 replicates) density. These numbers of urchins were selected based upon surface area coverage of the test chamber with high density equaling ca. 80% surface area coverage and low density equaling 20% surface area coverage. These numbers of urchins are equivalent to approximately 180 urchins/m² and 40 urchins/m², respectively. These densities reflect ranges that might be found in intensive aquaculture (N. Brown, personal communication). Urchins were maintained in these experimental conditions for four weeks and were starved. The stock urchins were starved as well to provide a general large urchin laboratory population maintained under the same feeding conditions. Upon completion of the trial, surviving urchins were weighed and returned to the general large urchin laboratory population and were fed three times weekly *ad libitum* for six weeks before being possibly restocked into the fed trial.

*Fed, varying density.* A subsample of large urchins (31-38g) was randomly selected from a large, recently-collected laboratory population. Individuals were weighed
and stocked into 1 of 16 test chambers at either a high or low density (equivalent to those used in the starved treatment). Urchins were maintained in these experimental conditions for four weeks and were fed *ad libitum* at least four times weekly. Upon completion of the trial, surviving urchins were weighed and returned to the general large urchin laboratory population.

*Treatment 2: Small Urchins*

Two experiments using small urchins (11-21g) were conducted. In one experiment, an equal number of urchins (as described in Trial 1: 21 for high density or 5 for low density) were placed into each replicate test chamber. In a concurrent experiment, the number of urchins equivalent to the biomass of the large urchins in Trial 1 were placed into test chambers. As the biomass of each small urchin was approximately half of the biomass of each large urchin, the number of urchins was doubled (42 for the high density and 10 urchins for the low density treatments).

*Starved, small urchins equivalent by numbers.* A subsample of small urchins was (13-21g) randomly selected from a recently-collected laboratory population. Individuals were weighed and stocked randomly into 1 of 16 test chambers (position determined using Excel’s random number generator) at either a high (21 urchins per test chamber, 8 replicates) or low (5 urchins per test chamber, 8 replicates) density. These numbers of urchins are equivalent to approximately 180 individuals/m² and 40 individuals/m². Urchins were maintained in these experimental conditions for four weeks and were starved. The stock urchins were starved as well to provide a general small urchin laboratory popu-
lation maintained under the same feeding conditions. Upon completion of the trial, surviving urchins were weighed and returned to the general small urchin laboratory population and were fed three times weekly *ad libitum* for six weeks before being possibly restocked into the fed trial.

**Fed, small urchins equivalent by density.** A subsample of small urchins (12 - 21) was randomly selected from a recently-collected laboratory population. Individuals were weighed and stocked randomly into 1 of 16 test chambers (position determined using Excel’s random number generator) at either a high (21 urchins per test chamber, 4 replicates) or low (5 urchins per test chamber, 4 replicates) density. Urchins were maintained in these experimental conditions for 4 weeks and were fed *ad libitum* at least 4 times weekly for the duration. Upon completion of the trial, surviving urchins were weighed and returned to the general small urchin laboratory population.

**Starved, small urchins equivalent by biomass.** A subsample of small urchins (13 - 21) was randomly selected from a recently-collected laboratory population. Individuals were weighed and stocked randomly into 1 of 16 test chambers (position determined using Excel’s random number generator) at either a high (42 urchins per test chamber, 8 replicates) or low (10 urchins per test chamber, 8 replicates) density by biomass. These numbers of urchins are equivalent to approximately 360 individuals/m² and 80 individuals/m². Urchins were maintained in these experimental conditions for four weeks and were starved. The stock urchins were starved as well to provide a general small urchin laboratory population maintained under the same feeding conditions. Upon completion of
the trial, surviving urchins were weighed and returned to the general small urchin laboratory population and were fed three times weekly *ad libitum* for six weeks before being restocked into the fed trial.

*Fed, small urchins equivalent by biomass.* A subsample of small urchins (12 - 21) was randomly selected from a recently-collected laboratory population. Individuals were weighed individually and then stocked randomly into 1 of 16 test chambers (position determined using Excel’s random number generator) at either a high (42 urchins per compartment, 8 replicates) or low (10 urchins per compartment, 8 replicates) density. Urchins were maintained in these experimental conditions for four weeks and were fed *ad libitum* at least four times weekly for the duration. Upon completion of the trial, surviving urchins were weighed and returned to the general small urchin laboratory population.

**Statistical Analyses**

Upon stocking the urchins, the weights of the urchins in each replicate test chambers were determined to be statistically equivalent using a 1-way analysis of variance (ANOVA) (*p>*0.05) and Tukey’s HSD inequality to confirm that any differences noted throughout the course of the experiment could not be attributed to stocking error (v12.0; SPSS, Chicago, IL).

After completion of the experiment, SigmaPlot 11 was used to generate a Kaplan-Meier survival curve for each treatment and each was analyzed against one another using the Log-Rank test for survival analysis (SYSTAT Software, Inc., San Jose, CA). A high statistic and low p-value (*p*<0.05) indicated a significant difference in survival rates that
are unlikely to have occurred by chance. Additionally, final wet weights were recorded and were compared to initial wet weights using a two tailed t-test (p<0.05) to determine whether there was a significant difference in treatment weight at completion of the trial. All weight data were presented as mean ± SE, in grams.

Results

Size, Density, Biomass, & Nutritional State on Rates of Cannibalism

Large urchin, fed vs. starved at high density. The initial weights of the large, high density starved treatment urchins and the large, high density fed treatment urchins were 35.3 ± 0.09 and 34.6 ± 0.14, respectively, with a range from 31 to 38 grams. The percent surface area coverage for these two treatments were 81.5% for the starved treatment and 79.7% for the fed treatment. Standard error was minimal; therefore error bars are obscured by the symbol used in the figure. Fed urchins at high densities cannibalized significantly (18.4%) more than starved urchins (1.8%) over four weeks (LogRank, test statistic = 24.84, df = 1, p<0.001) (Fig. 3).
Fig. 3. Percent survivorship of large urchins (31-38g) held at equivalent high density (21 urchins per test chamber) fed *ad libitum* or starved for four weeks (7 and 8 replicates per treatment, respectively). Values represent mean ± 1 SE. The rate of cannibalism was significantly higher in the fed urchin treatment than in the starved urchin treatment over this time period (LogRank, test statistic = 24.84, df = 1, \( p<0.001 \)).

*Large urchin, fed vs. starved at low density.* The initial weights of the large, low density starved treatment urchins and the large, low density fed treatment urchins were 35.3g ± 0.17 and 34.5g ± 0.25, respectively, with a range from 32-38g. The percent surface area coverage for these two treatments were 19.4% for the starved treatment and 19% for the fed treatment. There was no cannibalism during the four week period regardless of feed level (starved vs. fed *ad libitum*) in large urchins held at equivalent low density (5 urchins per compartment).
Large urchin, starved -- high vs. low density. The initial weights of the large, high density (21 urchins per test chamber) starved treatment urchins and the large, low density (5 urchins per test chamber) starved treatment urchins were 35.3g ± 0.089 and 35.3g ± 0.17, respectively, with a range from 33-38g. The percent surface area coverage for these two treatments were 81.5% for the high density treatment and 19.4% for the low density treatment. Standard error was minimal; therefore error bars are obscured by the symbol used in the figure. These urchins showed no significant difference in amount of cannibalism between high and low density treatments (1.8% vs. 0%) when starved over four weeks (LogRank, test statistic = 0.72, df = 1, p = 0.4) (Fig. 4).

Fig. 4. Percent survivorship of large urchins (33-38g) held at high density (21 urchins per test chamber) and low density (5 urchins per test chamber), starved for four weeks (8 replicates per treatment). Values represent mean ± 1 SE. These urchins showed no signifi-
cant difference in rate of cannibalism between high and low density treatments when starved over four weeks (LogRank, test statistic = 0.72, df = 1, p = 0.4).

*Large urchin, fed – high vs. low density.* The initial weights of the large, high density (21 urchins per test chamber) fed treatment urchins and the large, low density (5 urchins per test chamber) fed treatment urchins were 34.6g ± 0.14 and 34.5g ± 0.25, respectively, with a range from 31-38g. The percent surface area coverage for these two treatments were 79.7% for the high density treatment and 19% for the low density treatment. Standard error was minimal; therefore error bars are obscured by the symbol used in the figure. The amount of cannibalism was significantly higher in the high density urchin treatment (18.4%) than in the low density urchin treatment (0%) over four weeks (LogRank, test statistic = 8.239, df = 1, p = 0.004) (Fig. 5).

Fig. 5. Percent survivorship of large urchins (31-38g) held at high density (21 urchins per test chamber) and low density (5 urchins per test chamber), fed *ad libitum* for four weeks (7 and 8 replicates per treatment, respectively). Values represent mean ± 1 SE. The rate
of cannibalism was significantly higher in the high density urchin treatment than in the low density urchin treatment over this time period (LogRank, test statistic = 8.239, df = 1, p = 0.004).

*Large urchin growth.* Initial wet weights were obtained from large adult urchins (31-38g) as they were stocked into 1 of the 4 treatments and were compared to end wet weights 4 weeks later. Urchins that were starved and maintained at high density (21 urchins per test chamber) lost a significant amount of weight over that time course (t-test, df = 290, p<0.05) (Fig. 6-A). Urchins that were starved and maintained at low density (5 urchins per test chamber) exhibited no significant weight change over 4 weeks. Urchins that were fed *ad libitum* and held at both high (21 urchins per test chamber) and low (5 urchins per test chamber) density gained a significant amount of weight over the course of 4 weeks (t-test, df = 236, p<0.0001; t-test, df = 68, p<0.0001, respectively) (Fig 6-B, C, and D)
Fig. 6. Size-frequency distribution of large urchins (31-38g): (A) large urchins held at high density (21 urchins per test chamber) starved for 4 weeks, (B) large urchins held at low density (5 urchins per test chamber) starved for 4 weeks, (C) large urchins held at high density (21 urchins per test chamber) fed *ad libitum* for 4 weeks, and (D) large urchins held at low density (5 urchins per test chamber) fed *ad libitum* for 4 weeks.

Small urchins equivalent by density. The initial weights of the starved treatment urchins and the fed treatment urchins were as follows: starved urchins held at high density (21 urchins per test chamber) were 16.4g ± 0.15, starved urchins held at low density (5 urchins per test chamber) were 16.8g ± 0.28, urchins held at high density (21 urchins per test chamber) and fed *ad libitum* were 15.03g ± 0.31, and urchins held at low density (5 urchins per test chamber) and fed *ad libitum* were 15.4g ± 0.58 and ranged from 11-21g. The percent surface area coverage was 57.3% for the high density treatment and 13.6% for the low density treatment. Regardless of treatment, no cannibalism resulted over the course of 4 weeks. Urchins that were starved did not experience a change in weight over this time course, whereas urchins fed *ad libitum* gained a significant amount of weight at both high (t-test, df = 139, p<0.0001) and low density (t-test, df = 58, p<.001) (Fig. 7).
Fig. 7. Size-frequency distribution of small urchins equivalent by density to the large urchins (11-21g): (A) small urchins held at high density (21 urchins per test chamber) starved for 4 weeks, (B) small urchins held at low density (5 urchins per test chamber) starved for 4 weeks, (C) small urchins held at high density (21 urchins per test chamber) fed *ad libitum* for 4 weeks, and (D) small urchins held at low density (5 urchins per test chamber) fed *ad libitum* for 4 weeks.

Small urchins equivalent by biomass: fed vs. starved at high density. The initial weights of the small, high density starved treatment urchins and the small, high density fed treatment urchins were 16.7g ± 0.11 and 16.2g ± 0.12, respectively, with a range from 11-21g. The percent surface area coverage for these two treatments was 115.6% for both the starved and fed treatments. There was no significant difference in the frequency of cannibalism between the starved and fed treatments (18.75% vs. 13.7%) over 4 weeks (LogRank, test statistic = 3.75, df = 1, p = 0.053) (Fig. 8).
Fig. 8. Percent survivorship of small urchins (11-21g) held at high density (42 urchins per test chamber) starved or fed *ad libitum* for four weeks (8 replicates per treatment). Values represent mean ± 1 SE. The urchins showed no significant difference in rate of cannibalism between fed and starved treatments over the course of 4 weeks (LogRank, test statistic = 3.75, df = 1, p = 0.053).

Small urchins equivalent by biomass: fed vs. starved at low density. The initial weights of the small, low density starved treatment urchins and the small, low density fed treatment urchins were 19.2g ± 0.15 and 16.7g ± 0.23, respectively, with a range from 11-21g. The percent surface area coverage for these two treatments were 81.5% for the starved treatment and 79.7% for the fed treatment. There was no significant difference between the frequency of cannibalism in the starved and fed urchins (3.75% vs. 1.25%) over 4 weeks (LogRank, test statistic = 1.01, df = 1, p = 0.3) (Fig. 9).
Fig. 9. Percent survivorship of small urchins (11-21g) held at low density (10 urchins per test chamber) starved or fed *ad libitum* for four weeks (8 replicates per treatment). Values represent mean ± 1 SE. The urchins showed no significant difference in rate of cannibalism between fed and starved treatments over the course of 4 weeks (LogRank, test statistic = 1.01, df = 1, p = 0.3).

*Small urchins equivalent by biomass: starved – high vs. low density.* The initial weights of the small, high density (42 urchins per test chamber) starved treatment urchins and the small, low density (10 urchins per test chamber) starved treatment urchins were 17g ± 0.11 and 19.2g ± 0.15, respectively, with a range from 13-21g. The percent surface area coverage for these two treatments were 115.5% for the high density treatment and 27.5% for the low density treatment. Starved urchins at high density cannibalized significantly (18.75%) more than starved urchins held at low density (3.75%) over four weeks (LogRank, test statistic = 10.5, df = 1, p = 0.001) (Fig. 10).
Fig. 10. Percent survivorship of small urchins (11-21g) maintained at high density (42 urchins per test chamber) and low density (10 urchins per test chamber), starved for four weeks (8 replicates per treatment). Values represent mean ± 1 SE. The rate of cannibalism was significantly higher in the high density urchin treatment than in the low density urchin treatment over this time period (LogRank, test statistic = 10.5, df = 1, p = 0.001).

Small urchins equivalent by biomass: fed – high vs. low density. The initial weights of the small, high density (42 urchins per test chamber) fed treatment urchins and the small, low density (10 urchins per test chamber) fed treatment urchins were 16.2g ± 0.12 and 16.7g ± 0.23, respectively, with a range from 11-21g. The percent surface area coverage for these two treatments were 115.5% for the high density treatment and 27.5% for the low density treatment. Fed urchins at high density cannibalized significantly (13.7%) more than fed urchins held at low density (1.25%) over four weeks. (LogRank, test statistic = 9.61, df = 1, p = 0.002) (Fig. 11).
Fig. 11. Percent survivorship of small urchins (11-21g) maintained at high density (42 urchins per test chamber) and low density (10 urchins per test chamber), fed *ad libitum* for four weeks (8 replicates per treatment). Values represent mean ± 1 SE. The rate of cannibalism was significantly higher in the high density urchin treatment than in the low density urchin treatment over this time period (LogRank, test statistic = 9.61, df = 1, p = 0.002).

*Small urchin growth.* Initial wet weights were obtained from small juvenile urchins (11-21g) as they were stocked into 1 of the 4 treatments and were compared to end wet weights 4 weeks later. Urchins that were starved exhibited no significant weight change over 4 weeks regardless of high or low density conditions. (Fig. 12-A, B) Urchins that were fed *ad libitum* and held at both high (42 urchins per test chamber) and low (10 urchins per test chamber) density significantly gained weight over the course of 4 weeks (t-test, df = 570, p<0.0001; t-test, df = 146, p<0.0001, respectively) (Fig. 12-C, D)
Discussion

Reports of cannibalism in sea urchins are not as prevalent in the literature as are those in sea stars. This may be a consequence of the relatively high number of reports of feeding behavior of sea stars compared to sea urchins. Despite the low number of reports, it seems that the method of cannibalism proceeds similarly in *Lytechinus variegatus* (this
study), *Strongylocentrotus droebachiensis* (Himmelman and Steele, 1971), *S. intermedius* (Yaqing Chang, personal communication), and *Diadema antillarum* (Levitan, 1989).

First, a significant percentage of the spines are consumed completely (usually on the aboral surface), along with the tube feet, pedicillaria, and test epithelium in that specific region. It is not uncommon to see > 30% of the area of total spine coverage consumed by the predator sea urchin. After consumption of the spines and surface epithelium, a hole 1-2 cm in diameter is eaten into the aboral surface of the test and the gut and gonads are largely consumed. Occasionally, the Aristotle’s lantern is also extracted and consumed (it is removed via the oral surface after consumption of the peristomial membrane). Often, much of the remaining test is discarded following consumption of these other organs. The procedure of cannibalism appears to involve first the removal of spines, effectively removing any defensive armature of the prey urchin may possess against cannibalism. Following consumption of the spines the predatory urchin can now access the most energy dense organs, which are consumed almost always in their entirety. These organs contain relatively high levels of protein, lipids, and carbohydrates (Hammer et al., 2006b). In general, the entire process of cannibalism takes about 24 hours, but can occur in less than 12 hours under the conditions of this study. As an anecdotal observation, it was observed that in some attempts cannibalistic activity was abandoned in its early stages (before a hole was eaten in the aboral surface) and the predated urchin recovered.
Fig. 13. Photographs of urchins in various stages of cannibalism. Panel A depicts an urchin that has been mostly consumed. In panels B, C, and D the urchins are still alive (active spine movement) with a hole in their aboral surface. Panel E depicts an urchin that has had many of its spines consumed. Panel F shows the Aristotle’s lantern being removed and consumed from a cannibalized urchin.

Effect of Size on Rate of Cannibalism

The urchins used in these study treatments were of similar size (either large or small in respective treatments); consequently, a size advantage could not be easily realized by a potential predator. Therefore, cannibalistic activity most likely results from basic behaviors involving nutrient acquisition. In general, it is likely that small urchins cannibalize one another with greater frequency than large urchins because they are hungrier due to an increased need for nutrients to support somatic growth (Hammer et al., 2004; Hammer et al., 2006c) Small urchins require a greater amount of protein and carbohy-
drate in their diet than large urchins (Hammer et al., 2004; Hammer et al., 2006c), and these and other nutrients are readily available when a predator cannibalizes a conspecific. In addition, high rates of cannibalism in small urchins indicate that there is no intrinsic chemical defense mechanism that is effective against predatory conspecifics. The fact that small urchins will cannibalize more frequently than large urchins in intensive culture may be useful in developing an appropriate husbandry protocol for housing large numbers of sea urchins for commercial growout. We suggest that small urchins should be cultured in habitats that restrict contact or interaction to reduce the incidence of predatory cannibalism.

Effect of Density and Biomass on Rate of Cannibalism

In field populations, sea urchin densities are often quantified in units of individuals/m² or biomass units (e.g., kg/m²). In this experiment, we distinguished between these two types of densities with small sea urchins by referring to density only in number of individuals. Densities of *L. variegatus* can exceed 600 individuals/m² in urchin fronts (Camp et al., 1973; Rose et al., 1999), but typical field populations are much less (Watts et al., 2007). In intensive aquaculture, the economic goal will be to maximize densities and growth rates while maintaining high survival rates. Consequently, those factors that affect survival will ultimately affect production costs. Density was an important contributing factor to cannibalism in both large and small urchins. The increase in cannibalism as space became limiting at high densities directly affected survival. Although stress cannot be easily quantified in sea urchins, we hypothesize increased stress caused by increased contact can lead to increased cannibalism, even when food is not limiting. Our
data suggest that urchins, large and small, held at 80% surface area coverage or greater will cannibalize one another with a much greater frequency than those housed at a lower density (less than 60% surface area coverage). Small urchins continued to cannibalize at greater rates than large urchins even when stocked at equivalent biomass to the large urchins and held at high density. This finding suggests that if urchins are maintained in intensive aquaculture conditions, they should first be graded into size classes and then held at reduced densities. The smaller urchins should be held at lower densities than larger urchins to further reduce cannibalism.

**Effect of Nutritional State on Rate of Cannibalism**

We hypothesized that food availability or nutritional state will affect cannibalism in any species that readily cannibalizes. In general, urchins cannibalize at greater rates when starved than when fed *ad libitum* at both high and low densities. These data indicate that food abundance can affect cannibalistic behavior, and that a satiated urchin may be less likely to engage in this behavior. However, small urchins held at high density cannibalized at a high rate when starved or when fed *ad libitum*. These data suggest that increased physical interactions leading to cannibalism at high densities are the result of the lack of discrimination of the food source, and that urchins in high densities will consume a recognized food source whether that food source is a formulated pellet or a conspecific.

Although the small urchins were initially stocked at a similar weight there was a greater variance in the weights at the end of the study, with some individuals increasing in weight and others decreasing in weight. Urchins that grew larger may have found it easier to cannibalize a smaller conspecific, resulting in a relatively high level of cannibal-
ism despite being fed. In support of this hypothesis, studies have suggested that larger urchins can selectively prey on smaller urchins (Chapter 3). Small urchins fed *ad libitum* at low density did not cannibalize with the frequency of those held at high density, further indicating the interaction between feed availability and density. It is possible that at low density the competition for the available food decreases to the point where there is no need to cannibalize.

A similar trend was seen in large urchins held at high densities. It was unexpected to find that the large urchins fed at high densities had a higher frequency of cannibalism than those that were starved, as it seems more likely that a starved individual would cannibalize a conspecific more readily than a fed individual. We suggest that large urchins lower their metabolic rate and become more sedentary when starved to compensate for food shortage (Fenaux et al., 1977) and, as a consequence, the incidence of physical contact would be reduced. Large urchins that were fed would remain active and the possibility for increased contact and potential cannibalistic interactions would increase. The increased contact among fed individuals may have caused an increased stress level or resulted in accidental injury, which, if detected by another urchin, may incite a predatory reaction (Chapter 3). If true, this supports the hypothesis that physical contact is an important prerequisite for enhanced cannibalistic activity. In species that readily cannibalize conspecifics (including some echinoderm species), an individual that is injured or diseased was cannibalized more frequently than a healthy individual (Kvalvagnes, 1972; Witman et al., 2003; Chapter 3). Like the small urchins, the weight range increased from the beginning to the end of the trial, potentially causing cannibalism based on an increased size differential and not just density or nutritional state.
The rate of cannibalism in *L. variegatus* appears to be largely dependent on density and nutritional state, and less dependent on size of the urchins. In small urchins, cannibalism occurred at a steady rate when starved, but the survival curve developed a concave hyperbolic shape in fed populations. In large urchins this concave hyperbolic curve was more evident than in the small urchins, and much more pronounced in those treatments that were fed. We found that among populations of large and small urchins, when fed, cannibalism will occur at a greater rate in a population once cannibalism has already occurred in that population. This was evident among individual replicates for these treatments, as cannibalism increased rapidly within a replicate population once cannibalism occurred within that specific replicate population (data not shown). It was not known whether most urchins within a specific replicate became engaged in increased cannibalistic activity, or whether one to several urchins were responsible for the observed increases in cannibalistic activity, as individuals could not be traced. It is likely that starved and fed urchins cannibalize for different reasons. Whereas a starved urchin most likely cannibalizes a conspecific out of hunger, a fed urchin may simply be nondiscriminatory when encountering a food source (whether it is a food pellet or a conspecific). An increasing rate of cannibalism with active cannibalism may further suggest the presence of a chemical cue that incites a predatory response when an urchin is cannibalized.

Creating a successful protocol for culturing intensive populations of sea urchins in aquaculture requires considerable knowledge and development of an adequate diet as well as the conditions which will promote the best possible growth. An obstacle to successful culture of urchins in the laboratory is cannibalism, especially in small, juvenile urchins. Knowing that, in general, the sea urchin *Lytechinus variegatus* will cannibalize
less frequently in low density, well fed conditions is an important first step in identifying the conditions that will be most beneficial to raising and housing sea urchins for commercial use.
CHAPTER 3

THE EFFECT OF VARYING SIZE RATIOS OR DEGREE OF INJURY ON CANNIBALISM IN LYTECHINUS VARIEGATUS HELD IN CULTURE

Introduction

One of the important keys to successful aquaculture of sea urchins is the ability to culture intensively all life history stages, from larva to adult. In preliminary observations, it was noted that newly-metamorphosed juvenile Lytechinus variegatus display significant rates of cannibalism in the laboratory (Richardson and Watts, 2008). Size, density, and nutritional state were three factors that strongly influenced the rate of cannibalism in small and large (adult) L. variegatus (Chapter 2), but other potential factors may affect the rate of cannibalism and should also be considered when culturing intensive populations of urchins in aquaculture.

Any predator attempting to attack a conspecific will encounter some inherent risks. The individual being attacked often has the same defensive weapons as the attacker and there is always a risk to the would-be cannibal that its prey may, in fact, become the winner. Therefore, it is common in species that cannibalize that the predator has a distinct advantage over its prey to make the potential risk worthwhile. A common advantage observed is the preference of an individual to prey on another that is smaller or younger than itself or to prey on an injured individual.

A size differential between conspecifics may serve as a motivating factor for cannibalism (Fox, 1975; Polis, 1981; Claesson et al., 2000). A size advantage leads to canni-
balism in black bears and in alligators (Tietje et al., 1986; Rootes and Chabreck, 1993). As cannibalism has been mentioned only a few times in echinoderm literature, it is not surprising that there are even fewer potential causes for cannibalism noted. However, Serafy and Fell (1985) reported that *Strongylocentrotus droebachiensis* adults will eat smaller specimens in aquaria, which may imply size difference as a factor in the rate of cannibalism. In addition, juvenile specimens of *Mediaster aequalis* have also been seen to predate on newly metamorphosing juveniles in the lab, as these newly metamorphosing juveniles provide an easy food source (Birkeland et al., 1971).

The presence of an injury may increase the chance of an individual being cannibalized. Injury has been cited in sea star literature as a known causative factor leading to incidental cannibalism. Noted by Kvalvagnes (1972), *Asterias rubens* cannibalized conspecifics with high frequency (4 of 10 individuals) after having been physically tagged in aquaria despite the fact that they were of fairly even size and fed generously during these experiments. They hypothesized a chemotactic response to the physical injury was initiated by the non-injured sea stars. In addition, we have observed that adult *L. variegatus* being held in aquaria have often been cannibalized following induced spawning (spawning by hypodermic injection of a chemical stimulus) if they are released back into a tank of unspawned individuals. The reaction of the healthy individuals to cannibalize the recently spawned individual may be an indication of attack as a response to a perceived physical injury or weakened physiological state.

Based on these findings, we hypothesize that small urchins cultured together with larger urchins will have a higher chance of being cannibalized than if they were cultured with urchins of a similar size, and that as the size/weight differential between the large
and small urchins increases, the rate of cannibalism will increase. Additionally, we hypothesize that an injury will cause an increased level of cannibalism.

Materials and Methods

Collection of Urchins

Approximately 2000 *L. variegatus* (1g to 45g) were collected over the course of five collection trips in June-September 2009 from Port St. Joseph Peninsula State Park, FL (30° N, 85.5° W) and transported to the University of Alabama at Birmingham. Urchins were placed in a 4000 L recirculating, synthetic seawater aquaria system (Instant Ocean, 32 ± 1 ppt) equipped with a Polygeyser DF-3 biological filter (Aquaculture Systems Technologies L.L.C., New Orleans, LA), followed in series with a SMART high-output 80 watt UV sterilizer (Emperor Aquatics Inc., Pottstown, PA) and a TF500 double-venturi protein skimmer (Top Fathom, Hudsonville, MI, USA) used for foam fractionation. Urchins were held at a constant photoperiod and temperature (12h light, 12h dark; 22.5 ± 1°C) and were fed three times weekly a formulated feed described previously to promote growth (Hammer, 2006a). Urchins of different size classes used in these experiments were held in these conditions for approximately 10 weeks to allow adjustment to laboratory conditions. Urchins of appropriate weights were then stocked into either: (1) one of four experimental treatments representing combinations of various size classes, or (2) were subjected to one of three treatments of increasingly-severe injury and stocked with uninjured urchins.
Effect of Varying Size Ratios

Experimental system and design. After adjustment to laboratory conditions, urchins were transferred into one of three 80L experimental tanks in the same 4000L aquaria system described above. Each experimental tank contained eight plastic Tupperware bowls (Mainstays™ 946 mL capacity round, Bentonville, AR). These bowls had four ~2.5 cm slits cut approximately 4 cm from the top of the rim to allow water to drain and prevent the urchins from escaping from the bowl. Above each slit there was an overflow hole to allow water to drain more rapidly should the water level become higher than the slit allowed. The available surface area in each bowl was 337 cm². To maintain water flow and oxygen in each compartment, a raised water delivery manifold system was constructed from 1.3 cm Schedule 40 PVC pipe and suspended above each bowl. Each bowl received water from four drilled holes in the output pipe, two on each side. The holes were evenly spaced along the length of the pipe and were directed towards the interior of the tank to facilitate oxygenation. A total of three experimental tanks were constructed in this manner, for a total of 24 bowls (Fig. 14).
The urchins were initially weighed and then randomly assigned (using Excel’s random number generator) to a compartment position in one of the 24 bowls. Each treatment had 6 replicate bowls, all of which were run concurrently. Each treatment was assigned a number and the bowls were distributed randomly (using Excel’s random number generator) among the three tanks. Temperature and salinity were monitored daily, and water chemistries (ammonia-nitrogen, nitrate-nitrogen, nitrite, and alkalinity) were monitored three times weekly and kept within normal range (Basuyaux and Mathieu, 1999). Urchins were inspected at least once daily to maintain basic urchin care (siphoning of each compartment and checking water parameters), to count individuals, to note if canni-
Balism was in progress in any compartment, and to remove cannibalized urchins. Only cannibalized urchins (i.e. having a hole in the aboral surface of the test with the gonad and gut eaten) were removed and each was photographed. Upon completion of the four week trial, surviving urchins were weighed and returned to the general laboratory population.

Urchins were stocked into one of four treatments to determine the effect of combinations of various size classes on rate of cannibalism in urchins held in intensive culture. Urchins were distributed into one of two size ratio classes by wet weight, either in a 3:1 weight ratio or a 6:1 weight ratio (Table 1), each with 6 replicates. For each replicate, 1 large urchin was stocked with a number of small urchins. All treatments were stocked to an equivalent biomass; therefore, the number of small urchins required to attain equal biomass varied by treatment. Urchins were starved throughout the trial.

Table 1. Experimental design for size ratios experiment. Number of urchins is the number stocked in a single replicate (6 replicates total). One large urchin was stocked with a number of small urchins, the size of which was indicated in the target weight. Urchins were stocked to equivalent biomass among replicates and treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Target Weight Ratio</th>
<th>Target Weight</th>
<th># of Urchins in Each Replicate</th>
<th>Total Biomass (g) of All Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3:1 ratio</td>
<td>12g:4g</td>
<td>4</td>
<td>154.4</td>
</tr>
<tr>
<td>2</td>
<td>3:1 ratio</td>
<td>9g:3g</td>
<td>6</td>
<td>161.5</td>
</tr>
<tr>
<td>3</td>
<td>6:1 ratio</td>
<td>18g:3g</td>
<td>3</td>
<td>148.4</td>
</tr>
<tr>
<td>4</td>
<td>6:1 ratio</td>
<td>12g:2g</td>
<td>8</td>
<td>158.8</td>
</tr>
</tbody>
</table>
Statistical Analysis

After 4 weeks, percent cannibalism was determined per treatment. Treatment values were compared using a 1-way ANOVA to determine significance (p<0.05).

Effect of Varying Degree of Injury

Experimental system and design. Urchins were removed from the laboratory population and transferred into one of six 80L experimental tanks in the same 4000L aquaria system described above. However, each experimental tank contained three plastic Tupperware bowls (Mainstays™ 1656 mL capacity round, Bentonville, AR). These bowls had four ~2.5cm slits cut approximately 4 cm from the top of the rim to allow water to drain and prevent the urchins from escaping from the bowl. Above each slit there was an overflow hole to allow water to drain more rapidly should the water level become higher than the slit allowed. The available surface area in each bowl was 552 cm². To increase available oxygen to each bowl, the same water delivery system that was used in the size ratios experiment was constructed. Six experimental tanks were constructed in this manner, for a total of 18 bowls (Fig. 15).
Fig. 15. Schematic diagram of arrangement of Tupperware bowls (Mainstays™ 1656 mL capacity round, Bentonville, AR) in the 80L tank and of the 1.3 cm Schedule 40 PVC raised water delivery manifold system. Water entered the manifold at point A and was distributed equally among the replicate bowls (aerial view – L, side view – R). This tank design was replicated in six tanks.

The urchins were initially weighed and then randomly assigned (using Excel’s random number generator) to one of the 18 bowls. The effect of physical injury on rate of cannibalism was examined by either injuring the urchins (1) externally by removing the spines from the test with scissors (the test remained intact) (6 replicates), or (2) drilling a small hole (1.6mm) into the test and coelomic cavity at the ambitus of the urchin (12 replicates), or (3) drilling a larger hole (4mm) into the test and coelomic cavity at the ambitus of the urchins (12 replicates). Each successive treatment represents an apparent increasing degree of injury to the urchins within that respective treatment. All replicates
among each treatment were run concurrently and urchins were starved throughout the 6-day study.

Water quality was maintained and urchins were inspected the same way as they were in the size ratios treatment study. Upon completion of six day trial, surviving urchins were weighed and returned to the general laboratory population.

**Spine removal.** The initial wet weights of urchins stocked in this treatment were 22.1 ± 0.19 (mean ± SE, in grams), with a range from 21-24 grams. Spines were partially removed from one of the five urchins (spines were sheared at the base from ca. 25 percent of the test of the urchin along a continual quarter radius using dissecting scissors). The test remained intact. Immediately after mechanical spine removal, each urchin was placed in its own bowl with four uninjured urchins of equivalent weight and all were starved for six days.

**Test perforation.** The initial wet weights of urchins stocked into these treatments were 19.0 ± 0.23 and 19.1 ± 0.27 (mean ± SE, in grams) (treatments were small hole and large hole, respectively), with a range from 16-24g in weight. In all treatments, each replicate was stocked with five urchins (1 injured and 4 uninjured). A small (1.6 mm) or large (4 mm) hole was drilled through the test (along the ambulacra) into the coelomic cavity with a hand-held drill of one of the five urchins. A small area of spines was carefully removed at their base in this area to allow for easier drilling and to allow better visual inspection of the injury. The placement of the hole was to ensure that the gut and gonad were not damaged in the drilling process, although the water vascular system sus-
tained injury. Immediately after injury, the injured urchins were placed into replicate bowls with four additional uninjured urchins of equivalent weight and were starved for 6 days.

Statistical Analysis

After 6 days, percent cannibalism was determined per treatment. These values were compared using a 1-way ANOVA to determine significance (p<0.05). If significance was indicated then the least significant difference post-hoc comparison was used to determine where the difference between treatments existed.

Results

Effect of Varying Size Ratios

Only one individual in one replicate of Treatment 1 was cannibalized. Although populations in Treatment 1 did not have significantly lower rates of cannibalism than those in other treatments (4% vs. 14%, 17%, and 20.8% in Treatments 2, 3, and 4, respectively) (ANOVA, Table 2), trends for all treatments suggest an effect of size differentials. The highest rate of cannibalism was seen in Treatment 4, having the greatest size differential among individuals. In Treatment 4, small individuals were always consumed by the large individuals. No significant differences were detected between any treatments by ANOVA (p>0.05).
Table 2. Effect of size ratios on percent cannibalism. Columns indicate treatment number, target ratio, actual stocked ratio of grams/grams (g/g), actual stocked ratio of diameter/diameter (d/d), total biomass for all replicates combined, initial number of urchins stocked, and percent cannibalism for each treatment (six replicates per treatment).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Target Ratio</th>
<th>g/g</th>
<th>d/d</th>
<th>Total Biomass per Treatment (g)</th>
<th>Initial #</th>
<th>% Cannibalism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3:1</td>
<td>2.7:1</td>
<td>1.5:1</td>
<td>154.4</td>
<td>24</td>
<td>4%</td>
</tr>
<tr>
<td>2</td>
<td>3:1</td>
<td>2.8:1</td>
<td>1.4:1</td>
<td>161.5</td>
<td>36</td>
<td>14% *¹</td>
</tr>
<tr>
<td>3</td>
<td>6:1</td>
<td>5.1:1</td>
<td>1.8:1</td>
<td>148.4</td>
<td>18</td>
<td>17% *²</td>
</tr>
<tr>
<td>4</td>
<td>6:1</td>
<td>5.5:1</td>
<td>1.9:1</td>
<td>158.8</td>
<td>48</td>
<td>20.8%</td>
</tr>
</tbody>
</table>

*¹ 2 of 5 eaten were the larger urchin  
*² 1 of 3 eaten was the larger urchin

Effect of Varying Degree of Injury

Individuals with 25% of the spines removed were not cannibalized by uninjured urchins (Table 3). Injury resulting from a puncture of the test resulted in higher percentages of cannibalized individuals, with the highest percentage of cannibalism found in those with the more severe injury. Urchins with the most severe injury had 47.1% cannibalism over 6 days, significantly more than either other treatment (ANOVA, p<0.05).
Table 3. Effect of injury on percent cannibalism. Columns indicate injury treatment, average biomass of urchins included in each treatment ± SE (6 replicates for sheared, 12 replicates for both small and large hole), the initial number of urchins injured per treatment, the final number of injured urchins remaining after 6 days, and the total percent cannibalized at the end of the trial. Urchins with the most severe injury (a large hole) displayed significantly more cannibalism than those injured in either of the other treatments (ANOVA, p<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Biomass (g)</th>
<th>Initial #</th>
<th>Final #</th>
<th>% Cannibalized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheared</td>
<td>22.12 ± 0.19</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Small hole</td>
<td>19.97 ± 0.28</td>
<td>12</td>
<td>11</td>
<td>8.3</td>
</tr>
<tr>
<td>Large hole</td>
<td>19.13 ± 0.27</td>
<td>12</td>
<td>4</td>
<td>47.1*</td>
</tr>
</tbody>
</table>

* 5/12 cannibalized, 3/12 died over 6 days
Significantly different than other treatments (ANOVA, p<0.05)

Discussion

Reports of cannibalism in sea urchins are not as prevalent in the literature as are those in sea stars. This may be a consequence of the relatively high number of reports of feeding behavior of sea stars compared to sea urchins. Despite the low number of reports, it seems that the process of cannibalism proceeds similarly in *Lytechinus variegatus* (Chapter 2), *Strongylocentrotus droebachiensis* (Himmelman and Steele, 1971), *S. intermedius* (Yaquing Chang, personal communication), and *Diadema antillarum* (Levitan, 1989).
Effect of Varying Size Ratios

Previous studies have suggested size, density, and nutritional condition as factors affecting cannibalism in *L. variegatus* (Chapter 2). Populations of small individuals cannibalize each other more frequently than populations of large individuals (Richardson and Watts, 2008), but no study has evaluated size effects when small and large urchins are held together. In this study, we suggest that differences in size can be a causative factor contributing to cannibalism. As we hypothesized, larger urchins appear to cannibalize small urchins preferentially, and that the greater the difference in size, the more likely that the larger predator will cannibalize the smaller prey. This finding is consistent with the risk/reward theory; thus, a large size differential will give a larger predator less risk when cannibalizing a significantly smaller prey. However, there was no preference among individuals that were close in size, even when individual weights may vary by a factor of two to three. When compared statistically, these trends were not significantly different. Additional studies evaluating larger populations would provide more conclusive information about size differentials. In facilities where urchins might be cultured intensively, we believe that small urchins housed cultured with larger urchins would have a higher probability of being cannibalized than if they were cultured with urchins of a similar size, and that as the size/weight differential between the large and small urchins increased, the rate of cannibalism would increase as well. These observations provide a rationale for developing a protocol for grading urchins in intensive culture. Urchins should only be cultured with those of equivalent size to reduce the risk of cannibalism.
Effect of Varying Degree of Injury

Injured urchins are more likely to be cannibalized than uninjured urchins, and the incidence of cannibalism increases with the severity of the injury. All urchins whose spines were mechanically removed recovered and their spines began to regenerate without any instance of cannibalism. This finding was surprising because previous observations indicated that shearing (consuming) spines is a common first stage of cannibalism and was presumed to be detrimental to the urchin. However, it may simply be that since cannibalism has to start somewhere, consuming spines may be easier than removing the Aristotle’s lantern to gain access into the coelomic cavity. Mechanical removal of spines did not stimulate a cannibalistic response. Mechanical ablation of spines at the base with scissors, with the test remaining structurally intact, probably does not inflict enough damage for an urchin to be cannibalized because the coelom is not penetrated, and perhaps a chemical cue necessary to promote cannibalism is not released.

Urchins who were injured by drilling a small hole into the ambulacra of the test repaired the wound 11 out of 12 times. We observed that these urchins were able to seal the coelom rapidly, usually with 30 minutes of the injury. The fact that urchins have the ability to repair such a significant injury to the calcified test is remarkable, as urchins lack musculature that could close a wound quickly. Instead, they rely on clotting factors that are present in their coelomic fluid to seal any puncture as quickly as possible (Kindred, 1924; Bookhout and Greenburg, 1940; Endean, 1966). There is, however, a limit to the size of a hole an urchin can repair, and a larger hole can stimulate a cannibalistic act from a non-injured conspecific. In fact, urchins that were injured by drilling the large hole...
were cannibalized 5 of 12 times. When the test is significantly compromised by injury, coelomic fluid is released until the injured area is closed by clotting. We hypothesize that coelomic fluid may contain a chemical cue that is released into the water upon injury and stimulate a cannibalistic response. It is apparent that there is a limit to the amount of damage an urchin can recover from quickly enough to escape cannibalism when held with non-injured urchins. In fact, 3 of 12 urchins died from this injury. Four of twelve urchins were able to successfully repair the large hole and evade cannibalism. It is important to note that individuals who died from the injury were not cannibalized as carrion and were, in fact, avoided by the remaining urchins. We have observed that dead individuals, regardless of the cause of death, are not typically consumed in laboratory populations.

These results may be of practical importance in intensive culture of sea urchins. If an urchin has only experienced an external injury to its spines, it is probably healthy enough to be cultured with other urchins without risk of being cannibalized. However, if an urchin is severely injured, it may either die of its injuries or risk cannibalism.


APPENDIX

IACUC APPROVAL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

**DATE:** June 16, 2009

**TO:** Watts, Stephen A.
CH-375 1170
934.2045

**FROM:** Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee

**SUBJECT:** Title: Sea Urchin Culture for Improved Biomedical/Ecotoxicological Testing
Sponsor: Mississippi-Alabama Sea Grant Consortium
Animal Project Number: 090508135

On June 16, 2009, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

<table>
<thead>
<tr>
<th>Species</th>
<th>Use Category</th>
<th>Number in Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
<td>A</td>
<td>1000</td>
</tr>
</tbody>
</table>

Animal use is scheduled for review one year from May 2009. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

**Please keep this record for your files, and forward the attached letter to the appropriate granting agency.**

Refer to Animal Protocol Number (APN) 090508135 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 904-7052.