Effects of Changes in Egg Size and Larval Food Supply on the Development of Two Species of Seastars

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Effects of Changes in Egg Size and Larval Food Supply on the Development of Two Species of Seastars

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Biology from The College of William and Mary

by

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Abstract

Organisms have limited resources available to invest in reproduction, causing a tradeoff between the number and size of offspring. One consequence of this tradeoff has been the evolution of disparate developmental modes, even among close relatives. Marine invertebrates are well known for their tremendous diversity of developmental patterns, much of which has been attributed to changes in maternal investment. In particular, echinoid echinoderms have been widely used to experimentally manipulate how changes in maternal investment affect development. Here I test the generality of the echinoid results by experimentally reducing maternal investment in the seastars *Pisaster ochraceus* and *Asterias forbesi*. Maternal investment in embryos was halved at the two-cell stage by killing one blastomere with a laser. Larvae from manipulated embryos were compared to unmanipulated sibling embryos to test for effects of reduced maternal investment on larval development and juvenile quality. In one experiment I also varied larval food supply during development of *A. forbesi*. My response variables were time to metamorphosis, spine number, disk diameter, and disk area at metamorphosis. In *P. ochraceus* there were no significant differences between juveniles from manipulated and unmanipulated embryos. In *A. forbesi*, for all response variables, reductions in larval food had a significant and much larger effect than reductions in maternal investment. Reductions in maternal investment significantly reduced disk diameter but had no affect on other metrics. These results indicate that seastar larvae may be capable of compensatory growth in response to reduced maternal investment and that food levels are more important than maternal investment in determining larval and juvenile quality.
Introduction

Determining how organisms allocate limited resources has long been a topic of importance among biologists interested in the evolution of life histories (Levins, 1968; Hirshfield and Tinkle, 1975; Drent and Daan, 1980; Tuomi et al., 1983; Boggs, 1992). The allocation of these limited resources, by their very nature of being finite, leads to tradeoffs, or links between traits that may limit the evolution of other traits (Van Noordwijk and de Jong, 1986). One tradeoff caused by the fundamental limitation of resource levels occurs between the number and size of offspring that can be produced by a female in a single clutch (Sinervo, 1990; Stearns, 1992; Roff, 1992). While larger clutches of smaller eggs have a fecundity advantage over smaller clutches of larger eggs, the smaller eggs often give rise to smaller offspring, which can have negative impacts on their fitness (Godfray et al., 1991). Conversely, there can be a cost to smaller clutches of large, well-provisioned eggs in the form of reduced fecundity (Lack, 1947; Sinervo, 1990). Extensive modeling of the tradeoff between these costs and benefits has led to the prediction of an optimal clutch size for a given species, which has evolved to balance the fecundity costs of small clutches with the fitness costs of small eggs (Vance, 1973; Smith and Fretwell, 1974; Sinervo, 1990; Stearns, 1992; Roff, 1992).

Empirical examples of the tradeoff between the number and size of offspring can be found across a wide variety of taxa including reptiles, amphibians, fish, cladocerans, echinoderms, birds, and plants (Emlet et al., 1987; Sinervo, 1990; Arnold, 1992; Venable, 1992; Bernardo, 1996b; Guisande et al., 1996; Landberg, 2014). One example of the tradeoff between number and size of offspring can be seen in the eggs spawned by teleost fish (Duarte and Alcaraz, 1989; Elgar, 1990). In teleosts, some species spawn larger clutches (over 6 million eggs) of small eggs while others spawn smaller clutches (around 35 eggs) of large eggs, fitting
into a model that there is a tradeoff between clutch and egg size (Elgar, 1990). There seems to be evolutionary pressures that have led to the differences in clutch and egg sizes between species, with pelagic fish tending to spawn many small eggs and demersal fish tending to spawn fewer large eggs (Duarte and Alcaraz, 1989). Outside of animals, the tradeoff between egg size and number can be seen across plant species (Sadras, 2007). Some species, such as wheat and soybean have a heavily selected upon seed size, while others such as sunflower and maize have a heavily selected upon number of seeds (Sadras, 2007). The selection pressure on the seed number or size has led to models that predict a target seed size according to optimality within the tradeoff of seed size and number (Sadras, 2007).

The tradeoff between the number and size of offspring within a single clutch can be affected by environmental resources in both plants and animals. In plants, resources available influence the fitness consequences of seed size and number (Venable, 1992). The influence of resource variability on the number and size of seeds can make the tradeoff between seed number and size difficult to measure (Venable, 1992). In copepods, however, resource availability often drives the tradeoff between egg size and clutch size, with individuals favoring small clutches of large eggs in poor resource conditions and large clutches of small eggs when resources are plentiful (Guisande et al., 1996). By changing the optimal clutch size in different environments, copepods are able to change their reproductive strategy in order to maximize the reproductive output of each clutch (Guisande et al., 1996).

One of the earliest and most well known investigations into the tradeoff between clutch and egg size is David Lack’s (1947) work on clutch sizes among and between species of birds. Lack observed variation in clutch size within bird species across both latitudes and longitudes and determined that both environmental factors and maternal effects can modify clutch size
(Lack, 1947). In birds, maternal food is an important determinant affecting the survival of young within a clutch, however there are also differences in the variability of clutch sizes within and between species (Lack, 1947; Lack, 1954). Larger clutches are fed less often per day than smaller clutches, leading to weak, undernourished young that have lower probabilities of surviving than chicks from a smaller clutch (Lack, 1954). When changes in food concentration have a stronger selection pressure on a species, the optimal clutch size may evolve to be smaller than species under weaker selection pressure (Lack, 1947, Lack, 1954). Lack (1954) hypothesized that clutch size has evolved by natural selection to correspond with the largest number of offspring parents can successfully feed in a single clutch.

While Lack’s (1947; 1954) work investigating optimal clutch sizes within birds was pioneering, it was limited to observational data on the relationship between egg size and number within a single clutch. Subsequently, studies expanding the taxonomic coverage of this tradeoff have been conducted, with many of them directly manipulating clutch sizes, egg sizes, or combinations of the two (Sinervo, 1990; Guisande et al., 1996; McEdward, 1996; Sinervo and McEdward, 1998; Nager et al., 2000; Allen, 2012; Pernet et al., 2012). By manipulating clutch size directly, it is possible to see the effects of increased clutch size without other confounding variables such as egg size or maternal effects (Bernardo, 1991). Direct manipulations of egg sizes separate the roles of non-genetic maternal effects and maternal genetic effects in offspring phenotypes, reducing possible confounding variables (Bernardo, 1991).

Since Lack’s early observations, birds have become one model system for clutch size manipulations. Nager et al. (2000) induced Lesser Black-backed Gulls to lay larger than normal clutches through daily removal of eggs. As clutch size increases in gulls, later eggs are of lower quality with lower probability of chick survival (Nager et al., 2000). Eggs from larger clutches
were not only smaller than eggs from smaller clutches, but the size differences between eggs resulted in energy differences, with large eggs containing more energy than small ones (Nager et al., 2000). While there were declines in egg quality as clutch size increased, there were also costs to foster parents when clutch size was altered; foster parents with reduced broods were better suited for raising chicks (Nager et al., 2000). Among other species, such as the house sparrow, great tit, rook, and collared flycatcher, parents are physiologically able to rear larger clutches to fledgling than they do normally, however brood increases lead to decreased future reproductive output, a direct cost to parents with larger broods (Dijkstra et al., 1990). Females may also vary their clutch sizes away from the most productive yearly clutch size if they are less than optimal nutritional or health states (Price and Liou, 1989). Multiple studies of clutch size manipulations in birds have also demonstrated the influence of parental investment after egg production as a key factor in offspring survival (Dijkstra et al., 1990; Monaghan and Nager, 1997; Sousa and Marini, 2011). The overall trend seen in birds shows smaller eggs that are laid in larger clutches have reduced energy available to the chick, and after they hatch, chicks from large clutches tend to have lower survival (Dijkstra et al., 1990; Monaghan and Nager, 1997; Nager et al., 2000; Sousa and Marini, 2011).

Although birds are useful study organisms in manipulations of clutch size, manipulative experiments of egg size or egg energy in birds are relatively rare (Finkler et al., 1998). Much more is known about the effects of egg energy reductions in lizards, mostly due to the work of Sinervo (1990) on the lizard, Sceloporus occidentalis. Sinervo (1990) investigated the effects of experimentally reduced egg and hatchling size in S. occidentalis and demonstrated a tradeoff between egg size and clutch size, with some individuals laying larger clutches of smaller eggs than others. He also found a physiological coupling of egg size, egg number, and total and
relative clutch mass in lizards (Sinervo and Licht, 1991). In *S. occidentalis*, smaller eggs hatch into smaller juveniles, both in manipulated eggs and unmanipulated controls, with smaller juveniles demonstrating a slower sprint speed (one proxy for fitness), which is one direct cost of smaller egg size (Sinervo, 1990). In this system, however, there is a cost to producing large eggs beyond the fecundity loss associated with producing smaller clutches; large hatchlings do not always exhibit higher survival than smaller hatchlings and size interacts with both season and offspring sex to determine survival probabilities (Sinervo *et al.*, 1992). The various costs and benefits associated with producing either large or small offspring lead to selection on an optimal offspring size, which can affect the optimal clutch size for the species (Sinervo *et al.*, 1992).

The optimal clutch size for lizards was further investigated by Abell (1999) who examined the effects of food, and therefore environmental condition, on the variation in clutch and offspring size in *Sceloporus virgatus*. Most of the females observed did not lay the maximum number of eggs possible for their given clutch mass, instead laying smaller clutches of larger eggs, especially when resources were limiting during extreme drought years (Abell, 1999). Differences in clutch and egg size under different environmental conditions show that an optimal clutch size may be dependent on the environment as well as the allocation of maternal resources (Abell, 1999). The evolution of an optimal clutch size in different environments may also be affected by the heritability of egg size and timing of female reproduction that can affect offspring performance (Sinervo and Doughty, 1996). The timing of reproduction can have significant effects on body size and fecundity of offspring in later clutches, introducing variables besides maternal investment in eggs that can influence the tradeoff observed between egg size and clutch size (Sinervo and Doughty, 1996).
While vertebrates have formed the basis for many studies of the egg size/number tradeoff, they may not be the best study system for this type of research. For example, many vertebrates, including birds and lizards, provide parental care for their eggs before they hatch and mothers often continue to care for offspring after hatching (Bernardo, 1991; Monaghan and Nager, 1997; Mousseau and Fox, 1998). Parental care after the mother has laid eggs is an additional maternal effect that introduces variation into experiments on maternal investment and the tradeoff between egg size and clutch size (Bernardo, 1996a). In this way, marine invertebrates are a better system to study the effects of changes in maternal investment on offspring as once eggs are spawned into the water column, there is no additional parental care (Thorson, 1950). In the absence of any additional parental care after eggs are spawned, it is necessary for offspring to gain the energy for development either through feeding or from an initial maternal investment in egg energy content, both of which can be measured and controlled in the lab (Thorson, 1950; Alcorn and Allen, 2009).

Within marine invertebrates, maternal investment in egg energy content varies widely, even among closely related species (Allen and Podolsky, 2007). Among marine invertebrates, 10% of species produce non-feeding (lecithotrophic) larvae, characterized by a high initial maternal investment, 85% of species produce feeding (planktotrophic) larvae, characterized by a low initial maternal investment, and 5% of species produce larvae with intermediate nutritional modes (Thorson, 1950). In lecithotrophic development, eggs are over-provisioned with more resources than needed to allow larvae to reach metamorphosis. Extra energy is believed to enhance juvenile quality post metamorphosis (Emlet and Hoegh-Guldberg, 1997). Conversely, planktotrophic developers are under-provisioned and not given enough resources to allow the larvae to reach metamorphosis without obtaining food from the external environment (Emlet et
Based on models of egg size optimality and evolution, it is predicted that only the extremes of egg sizes possible should be favored (Vance, 1973).

The tradeoff between egg size and clutch size has been observed in many marine invertebrates, but has been most heavily studied within echinoderms (sea urchins, seastars, etc; Emlet et al., 1987; Strathmann, 1987; Sinervo and McEdward, 1988; McEdward and Janies, 1993; Levitan, 2000). Echinoderms are a useful model for studying the effects of variable offspring provisioning: they demonstrate a variety of nutritional modes among close relatives, lack parental care after offspring have been spawned, and their regulative development allows egg energy content to be directly manipulated (Horstadius, 1973). Echinoderms, and especially echinoids (sea urchins and sand dollars), have been valued in experimental embryology due to the ability to perform blastomere separations in these species (Jenkinson, 1909). Blastomeres at the two-cell stage can be separated into individual cells, leading to a 50% reduction in maternal provisioning (Jenkinson, 1909; Horstadius, 1973). Blastomere separations are possible because echinoderms are radially cleaving deuterostomes with cells within embryos dividing evenly and differentiating after multiple rounds of cell division (Wray, 1998). Additionally, echinoids with planktrophic development (eggs approximately 60 – 300 µm) are energetically able to build a viable feeding larva after blastomeres are separated (Emlet et al., 1987).

Sinervo and McEdward (1988) experimentally manipulated the energy available to a planktrophic larva by conducting blastomere separations in two echinoid species: Strongylocentrotus purpuratus and Strongylocentrotus droebachiensis. Larvae from smaller eggs were smaller, had a simpler body plan, and developed more slowly (Sinervo and McEdward, 1988). In addition to size differences, larvae from eggs with reduced energy took longer to reach metamorphosis, but there were no differences in juvenile size, relative to unmanipulated eggs.
(Sinervo and McEdward, 1988). The results from Sinervo and McEdward (1988) supported previous assumptions that parental investment in offspring directly determines hatchling fitness. In 1995, Hart tested the hypothesis laid out in Sinervo and McEdward’s (1988) work that only larval development time was affected by egg energy reductions in planktotrophic echinoids. In contrast to Sinervo and McEdward (1988), Hart (1995) found blastomere separations only affected juvenile size at metamorphosis and did not affect larval development time. As a result, for many years it remained unclear what the underlying costs of blastomere separations were on planktotrophic echinoids.

Alcorn and Allen (2009) clarified the costs of small egg size in echinoids by demonstrating that blastomere separations and changes in larval food concentration have significant effects on both the age at metamorphosis and juvenile quality in two species of echinoids: Strongylocentrotus droebachiensis and Echinarchnius parma. Egg energy reductions and larval food concentration interacted significantly to determine age at metamorphosis and juvenile quality in both echinoid species (Alcorn and Allen, 2009). Because food concentration and egg energy reductions had a significant interaction, the abundance of food in high food treatments during larval development was not enough to compensate for reductions in maternal investment (Alcorn and Allen, 2009). Similarly, Allen (2012) found effects of larval food concentration and egg energy reductions on the age at metamorphosis and juvenile quality in three additional echinoid species: Arbacia punctulata, S. purpuratus, and Dendraster excentricus. These results demonstrated at least two costs of small eggs among echinoids: longer larval development times and reductions in juvenile size (Alcorn and Allen, 2009; Allen 2012). The costs of smaller eggs are balanced by their benefits such as the fecundity advantage of producing more eggs within a single clutch than possible with larger sizes, leading to the
evolution of the majority of clutches to fall at the extremes of possible clutch sizes (Vance, 1973). Among echinoids, development time significantly increases when egg volume is reduced (Allen, 2012).

These previous studies (Sinervo and McEdward, 1988; Hart, 1995; Alcorn and Allen, 2009; Allen 2012) all investigated the effects of egg energy reductions on species with planktotrophic larvae, however not all species of echinoids exhibit planktotrophic development. For example, the sea urchin, *Heliocidaris erythrogramma* has nonfeeding larvae and lipids can be removed from eggs to investigate the effects of egg energy reductions (Emlet and Hoegh-Guldberg, 1997). Larvae with reduced lipids were smaller at metamorphosis, however they reached metamorphosis at the same time as control larvae (Emlet and Hoegh-Guldberg, 1997). Similarly, the sea biscuit, *Clypeaster rosaceus* does not need to feed as a larva to reach metamorphosis, although as a facultative planktotroph *C. rosaceus* has retained the ability to feed while in the water column (Emlet, 1986). Facultative planktotrophs are at the intersection of lecithotrophic and planktotrophic development, allowing researchers to test whether these species respond to egg energy reductions more similarly to planktotrophs or lecithotrophs (Allen *et al.*, 2006). *Clypeaster rosaceus* showed similar patterns to *H. erythrogramma*, a lecithotrophic species, and a different pattern than that seen in planktotrophic echinoids after blastomere separations. Reductions in egg energy in *C. rosaceus* significantly reduced juvenile size, but did not affect larval development time (Allen *et al.*, 2006). These results indicate egg size may have different functions in feeding and nonfeeding larvae (Emlet and Hoegh-Guldberg, 1997; Allen *et al.*, 2006).

While the work performed on sea urchins is convincing, extrapolating the results from this group to other marine invertebrates (the vast majority of animal phyla) could be problematic.
To begin to address this taxonomic bias, Pernet et al. (2012) used direct experimental manipulation of embryo size to study the effects of changes in maternal investment on the annelid, *Capitella teleta*. Using a laser to delete macromeres from the developing embryo, Pernet et al. (2012) showed a significant reduction in larval length and juvenile size when embryonic energy was reduced. These results were consistent with the previous echinoid results, suggesting that in lecithotrophic species maternal energy is allocated to the formation of large, high quality juveniles (Pernet et al., 2012). My goal is to test whether the patterns seen in echinoids, and now one species of annelid, hold across other echinoderm species by examining the relationship between maternal investment and development in two species of seastars (Echinodermata: Asteroidea).

Like echinoids, asteroids exhibit a wide range of egg sizes between species, ranging from 110 to 3500 μm in diameter (Emlet et al., 1987). Among species, the mean egg energy content is significantly correlated with the mean egg volume, suggesting that egg size is a good predictor of egg energy content (McEdward and Chia, 1991; McEdward and Morgan, 2001). Much like the methods for reducing egg energy in echinoids, blastomere separations can be used to study the effects of reductions in egg energy content in asteroids. Blastomeres isolated from 2, 4, and 8 cell seastar embryos have been shown to develop into morphologically normal larvae, although smaller in size (Dan-Sohkawa and Satoh, 1978). Since isolated cells from seastar larvae will develop, it is possible to reduce the energy available to a larva by removing energy in the form of cells in the developing embryo (Dan-Sohkawa and Satoh, 1978).

In this study, I examined the effects of food concentration and egg energy reductions on the larval and juvenile development of two planktotrophic species of asteroids: *Pisaster ochraceus* and *Asterias forbesi*. *Pisaster ochraceus* eggs are between 150 and 180 μm in
diameter while *A. forbesi* eggs are between 110 and 140 µm in diameter (Emlet et al., 1987). Both *P. ochraceus* and *A. forbesi* eggs are smaller than the average asteroid egg size of 700 µm (Emlet et al., 1987). These species, however, do not have unusually small eggs for asteroids. Among planktotrophic developers, the average egg size is 150 µm, so *P. ochraceus* eggs are slightly larger than the average planktotroph and *A. forbesi* eggs are slightly smaller than the average planktotroph. Examining the effects of egg energy reductions in two asteroid species can help identify if patterns seen in echinoids can be applied to asteroids. Understanding the effects of egg energy reductions can help us to understand why eggs in asteroids evolved to be the size that they are in the context of an optimal egg size within the tradeoff between egg and clutch size.
Methods

In order to test the effects of reductions in egg size on development in seastars I conducted three separate experiments: the first, during the summer of 2014 in Friday Harbor, Washington, examined the effects of egg size reduction on development in *Pisaster ochraceus*; the second, during the spring of 2015 in Williamsburg, Virginia, examined the effects of egg size reductions on development in *Asterias forbesi*; and the third, during the summer of 2015 in Brunswick, Maine, examined the individual and combined effects of egg size reductions and variable food levels on development in *A. forbesi*.

*Pisaster ochraceus*

Adult *P. ochraceus* were collected from the intertidal at Snug Harbor on San Juan Island, Washington (48°34’21 N 123°10’19 W) and transported back to Friday Harbor Laboratories, Friday Harbor, WA. Upon arrival at the labs, adults were kept in a flow through sea table at ambient salinity (22 - 31) and temperature (10 - 16°C). Gamete maturation and release were induced in adult seastars through intracoelomic injection of 9 ml of 100 µM 1-methyladenine (Strathmann, 1987). Gametes were released 1-2 h following injection. Several thousand eggs, enough to form a layer one cell thick on the bottom of a beaker, were placed in glass bowls containing 150 ml of 0.45 µm filtered seawater (FSW). FSW was created by filtering ambient seawater through a 0.45 µm nitrocellulose membrane filter (Millipore Corporation, Albany, NY). In order to fertilize eggs, I added 1 ml of dilute sperm suspension to each bowl and stirred gently. Sperm were visually examined under the microscope prior to addition to bowls for signs of active swimming. Eggs were scored as fertilized when the fertilization envelope was elevated and visible under a compound microscope at 4x magnification, generally within 10-30 minutes.
after the addition of sperm. For each bowl, 50 eggs were examined for signs of fertilization, which was initially > 90%. Eggs were fertilized in batches every 30 minutes for two hours and kept at ambient temperature in flow-through sea tables in which the water temperature ranged from 10-12°C. Fertilizations were conducted for 2 hours after the initial fertilization or until fewer than 50% of the eggs successfully fertilized.

Embryos were allowed to develop in the bowls until the first signs of cleavage were observed, approximately four hours post fertilization. At the first signs of cleavage, 40-50 embryos at the two-cell stage were placed onto glass slides in a minimal volume (< 200 µl) of water through the use of a mouth pipette. Slides containing embryos were then randomly designated as either treatment or control. A footed coverslip was created by adhering fragments of a #1.5 coverslip, which has a thickness of 0.16-0.19 mm, to the edges of a #1.5 coverslip with melted dental wax. The footed coverslip minimized the amount of water between the coverslip and the embryo, but prevented compression of the developing embryo. The coverslip was placed over the embryos in the treatment group and then placed under a microscope with a Hamilton Thorne XYClone infrared laser mounted on a 20x objective. One of the cells in each two-cell embryo was killed by firing the laser at the cell at 100% power for 1000 ms. The cells were killed with the laser such that the fertilization envelope was ruptured and the majority of the cytoplasm from the killed cell leaked out of the envelope. Any embryo not at the two-cell stage was killed through the use of the laser on every cell in the embryo to ensure no further development of the embryo. After all of the embryos on a single slide had been treated with the laser, embryos from the slide were rinsed into 5 cm diameter petri dishes containing approximately 10 ml FSW. Control slides remained on the countertop until the laser treatment was completed, at which point the control embryos were also rinsed into petri dishes containing
FSW. The time on the countertop experienced by the control embryos was designed to simulate time spent on the microscope by the treatment embryos. Once placed in the petri dishes, the embryos were allowed to develop for 24 h at ambient sea temperature in flow-through sea tables. After 24 h, hatched larvae in every petri dish were counted and those containing fewer than 20 larvae were discarded.

Initially, 20 larvae from both treatment and control groups were moved from petri dishes into ten 250 ml beakers containing 225 ml of FSW. Beakers were placed in a flow-through sea table at ambient seawater temperatures. To differentiate between treatment and control larvae, one of the groups in each beaker was stained with the vital stain, Nile Blue Sulfate, which has previously been used in both seastars and urchins (Simon, 1974, Allen et al., 2006, and Alcorn and Allen, 2009). To control for effects of the stain on the larvae, beakers were randomly assigned to one of two stain treatments: either treatment larvae were stained or control larvae were stained. The stained and unstained larvae in each beaker were intended to provide a paired experimental design to eliminate random effects based on the beakers larvae were reared in (Hart, 1995). However, the vital stain initially appeared detrimental to the larvae, and by 25 days old, the larvae that were stained had lost the majority of the pigmentation from the stain and would need to be restained. At this point, due to the detrimental effects of the stain on the development of the larvae and the poor retention of the stain, I decided to remove all stained larvae from the beakers and continue the experiment with only unstained larvae. This eliminated our paired design and introduced random effects associated with each beaker. Use of the stain was removed from the experimental design in subsequent experiments.

Larvae were kept suspended in the beakers using a stirring rack attached to a small motor that moved paddles at a rate of 10 strokes min\(^{-1}\) (Strathmann, 1987). Every other day, from the
time the larvae were placed in the stirring rack until metamorphosis, the beakers were removed from the sea table, cleaned, their water replaced, and algal food added. Water changes were conducted by reverse filtering 50% of the water from the beakers using a 35 µm Nitex mesh, leaving ~100 ml of FSW and larvae remaining. Fresh FSW was then added to the beakers to return them to a total volume of 225 ml. Larvae were fed 7500 cells ml\(^{-1}\) from each of three cultures of algae: *Dunaliella tertiolecta* (UTEX Culture Collection of Algae, Austin TX, Catalog #LB999), *Isochrysis galbana*, (National Center for Marine Algae and Microbiota, West Boothbay Harbor, ME, Catalog #CCMP1323) and *Rhodomonas lens* (National Center for Marine Algae and Microbiota, West Boothbay Harbor, ME, Catalog #CCMP739). Algal densities were counted prior to feeding from each culture using a hemocytometer to determine the appropriate volumes of culture to add per beaker. Algae were centrifuged for 6 min at 12,000 RPM and the supernatant of algal growth medium was removed prior to the re-suspension of the algal pellets in FSW and their introduction to the larval cultures. After water changes and the addition of food, beakers were placed back in the flow-through sea table in the stirring rack. When beakers were replaced in the stirring rack, their position within the rack was rotated in order to control for placement effects within the stirring rack.

Larval development was tracked through observations of the larvae under a dissecting scope. When the first larvae developed brachiolar arms and a juvenile rudiment (Figure 1), a shell from the blue mussel, *Mytilus spp.* was added to each beaker to provide a settlement cue for the larvae. When the mussel shells were added to the beakers, cleaning of the beakers stopped to allow a biofilm to develop on the glass, while water changing and feeding proceeded every other day. The mussel shells were removed from the beakers and placed in small bowls with FSW to check for juvenile settlement every day. The beakers and paddles from the stirring rack were also
examined for signs of metamorphosed juveniles. Settled juveniles were removed using a mouth pipet and placed in petri dishes with all juveniles from a single beaker placed in the same petri dish. The mussel shells were then placed back in the beaker, with shells replaced once a week to provide a fresh cue for the larvae. Two days post-settlement, the disk diameters of the juveniles were measured, the spines were counted, and brightfield photomicrographs were taken on a compound scope for later analysis using a 4x objective. Polarized light photomicrographs were also taken using a 4x and 10x objective. The disk diameters of the juveniles were measured as the longest distance across the juvenile and the length of the juvenile perpendicular to the first disk diameter (Figure 2). After measurements and photographs were taken, juveniles were isolated in 6-well plates with a single juvenile in each well. The wells in the well plates were 3.5 cm in diameter and contained 10 ml of FSW. After 45 days of development post fertilization, all of the juveniles that had settled were placed in centrifuge tubes with 1.5 ml of FSW and shipped overnight in a cooler to the College of William and Mary in Williamsburg, VA. Upon arrival in Williamsburg the juveniles were kept in a cold room at 12°C for 3 d until they were placed in new 6-well plates in a water bath at 17-18 °C.

Between 72 and 73 d post fertilization, juveniles were removed from well plates and placed under a compound microscope to photograph the juveniles at 4x and under polarized light at 4x and 10x. Any juvenile that died before day 72 was not included in photographic measurements. After 108 d post fertilization, surviving juveniles were once again measured, this time on a dissecting scope at a lower magnification. At 138 and 172 d post fertilization, the survival of the juveniles was checked and the number of deceased juveniles recorded. After 172 d, all of the juveniles had died. From all time points, the photographs of the juveniles taken using
a 4x objective were measured in ImageJ64 (http://imagej.nih.gov/ij) and the area of each individual was calculated.

Analysis of the data was conducted in IBM SPSS Statistics (Version 22). A linear mixed model ANOVA with laser treatment as a fixed factor and beaker as a random factor was used to determine if there were differences in spine number, disk diameter, age at settlement, and area of the juveniles from the three measurements between the juveniles from whole and half larvae. The residuals of the data were then tested for normality using a Kolmogorov-Smirnov and Shapiro-Wilk test to determine if the data needed transformations and additional analysis. I used a linear mixed model ANCOVA on the proportion of juveniles alive at each time point from whole and half embryos to determine if laser treatment had a significant effect on the death rate in *P. ochraceus* juveniles. For the ANCOVA, proportions were transformed with the arcsine squareroot transformation. I also conducted a nonparametric alternative, a rank transformation ANCOVA because our data violated assumptions of the parametric ANCOVA (Quade 1967 and Olejnik and Algina, 1984). While the rank transformation ANCOVA is an accepted nonparametric alternative to an ANCOVA, there are concerns with the test’s ability to detect heterogeneity of slopes in ANCOVA designs (Quinn and Keough, 2002). Therefore, I believe it is appropriate to include both the parametric ANCOVA and nonparametric alternative, the rank transformation ANCOVA, in my analysis and results.

*Asterias forbesi*

In my second experiment, *A. forbesi* adults were ordered from the Marine Biological Laboratory, Woods Hole, MA and shipped overnight to Williamsburg, VA. Upon arrival, animals were placed in recirculating seawater tanks at 12-14 °C and 32 ppt. The animals were
fed an *ad libitum* diet consisting of mussels and shrimp until they were used in the experiment.

Gamete maturation and release were induced in adult seastars through intracoelomic injection of 3 ml of 100 µM 1-methyladenine (Strathmann, 1987). Several thousand eggs, enough to form a layer one cell thick on the bottom of a beaker, were placed in glass bowls containing 150 ml of 32 ppt artificial seawater (ASW) made from Instant Ocean (Instant Ocean Spectrum Brands, Blacksburg, VA). Eggs were fertilized with 1 ml of dilute sperm that had been visually inspected for swimming activity and stirred gently. Fertilization was scored out of 50 eggs with a fertilized egg scored as an egg with an elevated fertilization envelope. Eggs were fertilized in batches every 30 minutes and kept in a water bath at 18°C. Fertilizations were conducted over the course of 2 hours, after which time fewer than 50% of the eggs successfully fertilized.

Embryos were allowed to develop in bowls until the first signs of cleavage were observed approximately 2 hours post fertilization. At the first signs of cleavage, *A. forbesi* embryos were treated with a laser in the same manner as the *P. ochraceus* embryos in the previous experiment. After being treated with the laser, embryos were allowed to develop in the petri dishes for 24 hours until embryos hatched as blastulae. In petri dishes with greater than 20 hatched embryos, 20 blastulae were sorted into eighteen unique 250 ml beakers containing 200 ml ASW. For each treatment, 9 beakers were used containing either laser treated embryos or control embryos. Beakers were placed in a water bath at 18°C under a stirring rack to keep larvae suspended in the water column (Strathmann, 1987). Larvae were reared to metamorphosis following the same protocol that was used to rear *P. ochraceus* larvae to metamorphosis. At metamorphosis the juveniles were isolated in 6-well plates after removal from the beakers. Two days post settlement, the spines were counted, the disk diameter of the juvenile was measured, and brightfield photomicrographs were taken for later analysis at 4x and polarized light.
photomicrographs were taken at 4x and 10x. After measurement, juveniles were isolated in 6-well plates with a single juvenile in each well. Juveniles were measured a second time for disk area and spine number with second pictures taken at 4x for later measurement of the area of the juveniles 20 days post settlement.

The 4x pictures from both time points were analyzed with the same methods used to measure the 4x pictures in *P. ochraceus*. The data on spine number, disk diameter, age at settlement, and the area of the juveniles were analyzed using the same methods as in the *P. ochraceus* analysis.

In a third experiment, adult *A. forbesi* were hand collected from intertidal and subtidal habitats at Rockland Breakwater, Rockland, Maine (44º6’47N 69º04’52). Adults were placed in coolers and transported by car to the Bowdoin College Coastal Studies Center on Orr’s Island, Maine after collection. Adult seastars were kept in flow through sea tables experiencing ambient salinity and temperature (11-21ºC). Gamete maturation and release were induced in adult seastars through intracoelomic injection of 3 ml of 100 µM 1-methyladenine. Several thousand eggs, enough to form a layer one cell thick on the bottom of a beaker, were placed in glass bowls containing 150 ml of FSW. Eggs were fertilized with 1 ml of dilute sperm and stirred gently. Fertilization was scored out of 50 eggs, with a fertilized egg scored with the presence of an elevated fertilization envelope. Eggs were fertilized in batches every 30 minutes at ambient temperature in flow-through sea tables. Fertilizations were conducted for 2 hours after the initial fertilization, after which fewer than 50% of the eggs fertilized.

Embryos were allowed to develop in glass bowls to the two-cell stage (about 2 hours post fertilization) at which time they were treated with the laser in the same manner as in previous experiments. After the laser treatment, embryos were allowed to develop in the petri dishes for
24 hours at which point successfully developing embryos hatched as blastulae. In petri dishes with greater than 20 hatched embryos, 20 embryos from each petri dish were placed into 250 ml beakers containing 200 ml FSW each. There were 46 beakers used in this experiment, half of which (n = 23) contained embryos treated with the laser and the other half of which (n = 23) contained control embryos. Sample size was limited by the number of embryos to which the laser treatment could be applied prior to development proceeding past the two-cell stage. Beakers were placed in a flow through sea table and experienced ambient seawater temperatures beneath a stirring rack. Larvae were reared to metamorphosis in the same manner as in previous experiments, however a second feeding treatment was introduced. There were 22 beakers (11 laser treated beakers and 11 control beakers) in the high food treatment, which consisted of feedings of 7,500 cells/ml of each of the three algal species every other day. There were 24 beakers (12 laser treated beakers and 12 control beakers) in the low food treatment, which consisted of feedings of 2,500 cells/ml of each of the three algal species every other day. All other aspects of larval rearing and feeding remained constant between all of the treatments.

Larvae were cultured to metamorphosis at which time they were removed from their respective beakers and placed in 6-well plates. Two days post settlement juveniles were measured for their disk diameters and spine numbers. Brightfield photomicrographs were taken for later analysis at 4x and polarized light photomicrographs were taken at 4x and 10x at this time. After measurement the juveniles were isolated in 6-well plates with a single juvenile in each well. Juveniles were remeasured and photographed 20 days post settlement. The juveniles were placed individually in centrifuge tubes with 1.5 ml of FSW 59 days post fertilization. Centrifuge tubes containing juveniles were placed in coolers and transported by car to Williamsburg, VA where they were removed from the tubes and placed individually in 6-well
plates 13 days later. I then continued measurements of juveniles that were at 20 days post metamorphosis. Juveniles were then tracked until their death, at which point the date they were first observed as deceased was recorded.

The data analysis on juveniles at metamorphosis was identical to all previous analyses, except for the addition of food treatment and the interaction of food and laser treatments as fixed factors in the linear mixed model ANOVA. The data analysis for the rate of death of *A. forbesi* juveniles was conducted using the same methods as *P. ochraceus* analysis, except for the addition of food treatment as fixed factor in the linear mixed model ANCOVA and the Rank Transformation ANCOVA.
Results

*Pisaster ochraceus*

A linear mixed model ANOVA with laser treatment as a fixed factor and beaker as a random factor was conducted to determine if there were differences in spine number, disk diameter, age at settlement, and area of the juveniles from the three measurements between the whole and half embryos. Residuals for all of the response variables were calculated and tested for normality. The residuals of all of the response variables (proportion survival, age at settlement, spine number, disk diameter, and area) for *P. ochraceus* were normally distributed under both a Kolmogorov-Smirnov and a Shapiro-Wilk test (p > 0.05).

*Pisaster ochraceus* juveniles began settling 30 days post fertilization with 76% of all larvae reaching metamorphosis (across all beakers, 75% of whole embryos reached metamorphosis and 77% of half embryos reached metamorphosis). *Pisaster ochraceus* exhibited no differences in larval time, proportion survival, spine number, disk diameter, or area at metamorphosis between offspring from whole and half embryos using a linear mixed model ANOVA (Table 1). There was no difference in the percent of larvae metamorphosing between those from whole and half embryos (p = 0.941; Figure 3; Table 1A). Larvae from whole embryos were 32.6 ± 0.58 days (Mean ± SE) old at metamorphosis while larvae from half embryos were 34.3 ± 0.57 days old at metamorphosis. Larvae from whole embryos metamorphosed 5% sooner on average than larvae from half embryos, however this was not significant (p = 0.075; Figures 4 and 5; Table 1B). Juveniles from whole embryos had 41.4 ± 2.5 spines while juveniles from half embryos had 37.4 ± 3.6 spines. Juveniles from half embryos had on average a 9.7% decrease in spine number compared to larvae from whole embryos, however this was also not significant (p = 0.396; Figure 6; Table 1C). At metamorphosis, juveniles from whole embryos had an average
disk diameter of 445 ± 11.2 µm. Juveniles from half embryos had a disk diameter of 441.1 ± 11.2 µm. There was no significant difference in the disk diameter between juveniles from whole and half embryos (p = 0.811; Figure 7; Table 1D).

Juveniles from whole embryos had an area at metamorphosis of 186343 ± 7014 µm² and juveniles from half embryos had an area at metamorphosis of 198395 ± 10503 µm². There was no significant difference in the area of the juveniles at metamorphosis between juveniles from whole and half embryos (p = 0.328; Figure 8; Table 1E). Juveniles from whole embryos 72-73 days post fertilization had an average area of 252315 ± 15190 µm². Juveniles from half embryos at the same time point had an average area of 266518 ± 15473 µm². There was no significant difference between the areas of whole and half juveniles 72-73 days post fertilization (p = 0.531; Figure 8; Table 1F). Juveniles from whole embryos 108 days post fertilization had an average area of 221643 ± 12050 µm². Juveniles from half embryos at the same time point had an average area of 215577 ± 20087 µm². There was no significant difference between the areas of juveniles from whole and half embryos 108 days post fertilization (p = 0.802; Figure 8; Table 1G).

All juveniles, regardless of laser treatment, were deceased by 172 days post fertilization (Figure 9). According to a linear mixed model ANCOVA, in which data were transformed using an arcsine squareroot transformation, there was no significant difference in the rate of death between juveniles from whole and half embryos (F1,56 = 0.287; p = 0.594). A Rank Transformation ANCOVA also found no significant differences in the rate of death between juveniles from whole and half embryos (F1,58 = 3.023; p = 0.087).
Asterias forbesi

A linear mixed model ANOVA with laser treatment as a fixed factor and beaker as a random factor was conducted to determine if there were differences in spine number, disk diameter, age at settlement, and area of the juveniles at metamorphosis. Residuals for all of the response variables were calculated and tested for normality. The male-female pair of *A. forbesi* in Williamsburg, VA exhibited normally distributed residuals for all response variables (proportion survival, age at settlement, spine number, disk diameter, and area) under both a Kolmogorov-Smirnov and Shapiro-Wilk test (p > 0.05).

*Asterias forbesi* juveniles began settling 22 days post fertilization with 72.5% of all larvae reaching metamorphosis (across all beakers, 78% of half embryos and 67% of whole embryos reached metamorphosis). There was no significant difference in the percent of larvae metamorphosing between those from whole and half embryos (p = 0.075; Figure 10; Table 2A). *Asterias forbesi* exhibited no differences in proportion survival, time to metamorphosis, spine number, disk diameter, and area at metamorphosis between juveniles from whole and half embryos using a linear mixed model ANOVA with a sample size of 9 beakers for each treatment (Table 2). Larvae from whole embryos had an average age at metamorphosis of 29.7 ± 0.67 days (Mean ± SE). Larvae from half embryos had an average age at metamorphosis of 30.1 ± 0.81 days. Larvae from whole and half embryos showed no significant difference in their age at settlement (p = 0.717; Figure 11; Table 2B). At metamorphosis, juveniles from whole embryos had 52.3 ± 1.57 spines while juveniles from half embryos had 51.6 ± 2.93 spines at metamorphosis. Juveniles from whole and half embryos showed no significant difference in their number of spines at metamorphosis (p = 0.826; Figure 12; Table 2C).
Juveniles from whole embryos had an average disk diameter of 824 ± 14.1 µm while juveniles from half embryos had an average disk diameter of 796 ± 14.8 µm. There was no significant difference in the disk diameters of juveniles from whole and half embryos (p = 0.184; Figure 13; Table 2D). Juveniles from whole embryos had an average area of 472589 ± 18445 µm². Juveniles from half embryos had an average area of 460339 ± 17376 µm². Juveniles from whole and half embryos showed no significant difference in their areas at metamorphosis (p = 0.635; Figure 14; Table 2E).

When measured 20 days post metamorphosis, juveniles from whole embryos had 62.6 ± 1.9 spines. Juveniles from half embryos at the same time point had 63.4 ± 2.8. There was no significant difference between spine number in juveniles from whole and half embryos (p = 0.712; Figure 15; Table 2F). Juveniles from whole embryos 20 days post metamorphosis had an average disk diameter of 844.2 ± 14.8 µm. Juveniles from half embryos had an average disk diameter of 834.5 ± 21.5 µm. There was no significant difference in the disk diameter 20 days post metamorphosis between juveniles from whole and half embryos (p = 0.815; Figure 16; Table 2G). I also measured the area of the juveniles 20 days post metamorphosis. Juveniles from whole embryos had an average area of 8547622 ± 21829 µm². Juveniles from half embryos had an average area 20 days post metamorphosis of 8227417 ± 26321 µm². There was no significant difference in the area of the juveniles from whole and half embryos 20 days post metamorphosis (p = 0.535; Figure 17; Table 2H).

In the follow-up experiment on *A. forbesi* in Brunswick, Maine, larvae from both whole and half embryos were exposed to both high and low food treatments. There were sample sizes of 11 beakers of larvae fed high food concentrations from whole embryos, 11 beakers of larvae fed high food concentrations from half embryos, 12 beakers of larvae fed low food
concentrations from whole embryos, and 12 beakers of larvae fed low food concentrations from half embryos. A linear mixed model ANOVA with laser treatment and food treatment as fixed factors and beaker as a random factor was conducted to determine if there were differences in proportion survival, spine number, disk diameter, age at settlement, and area of the juveniles at metamorphosis (Table 3). Residuals for all of the response variables were calculated and tested for normality. The residuals of all of the parameters (proportion survival, age at settlement, spine number, disk diameter, and juvenile area) for *A. forbesi* were all normally distributed under a Kolmogorov-Smirnov and a Shapiro-Wilk test (p > 0.05).

Juveniles began settling 23 days post fertilization, with 65% of all larvae reaching metamorphosis. 67% of larvae fed high food concentrations and from whole embryos reached metamorphosis, 72% of larvae fed high food concentrations from half embryos reached metamorphosis, 67% of larvae fed low food concentrations from whole embryos reached metamorphosis, and 56% of larvae fed low food concentrations from half embryos reached metamorphosis (Figure 18). There was no significant difference in the percent of larvae reaching metamorphosis between larvae fed different food levels (p = 0.146; Figure 18; Table 3A), larvae from whole and half embryos (p = 0.805; Figure 18; Table 3A), or the interaction between food and laser treatments (p = 0.152; Figure 18; Table 3A). Juveniles from the high food, whole embryo treatment had an average age at settlement of 34.6 ± 1.5 days. Juveniles from the high food, half embryo treatment had an average age at settlement of 34.6 ± 1.2 days. Juveniles from the low food, whole embryo treatment had an average age at settlement of 38.4 ± 1.9 days, and juveniles from the low food, half embryo treatment were 38.2 ± 2.3 days old at settlement (Figure 19). There was an 11% increase in settlement age between larvae fed high food concentrations and larvae fed low food concentrations, however these results were not significant.
(p = 0.053; Figure 19; Table 3B). There was no significant difference in settlement age between larvae from whole and half embryos (p = 0.979; Figure 19; Table 3B), nor was there a difference in for the interaction between food treatment and laser treatment (p = 0.951; Figure 19; Table 3B).

At metamorphosis, juveniles from the high food, whole embryo treatment had 62.9 ± 1.5, juveniles from the high food, half embryo treatment had 61.2 ± 1.9 spines, juveniles from the low food, whole embryo treatment had 53.9 ± 2.2, and juveniles from the low food, half embryo treatment had 46.5 ± 2.9 spines (Figure 20). Juveniles from high food treatments had 20% more spines at metamorphosis than juveniles from low food treatments (p < 0.001; Figure 20; Table 3C). There was no significant difference in spine number at metamorphosis between juveniles from whole and half embryos (p = 0.061; Figure 20; Table 3C) or the interaction between food concentration and embryo manipulation (p = 0.196; Figure 20; Table 3C).

At metamorphosis, juveniles from the high food, whole embryo treatment had a mean disk diameter of 795.7 ± 16.8 µm, juveniles from the high food, half embryo treatment had a mean disk diameter of 781.7 ± 17.2 µm, juveniles from the low food, whole embryo treatment had a mean disk diameter of 726.7 ± 13.5 µm, and juveniles from the low food, half embryo treatment had a mean disk diameter of 670.3 ± 12.7 µm (Figure 21). Juveniles from larvae fed high food concentrations had on average a 13% larger disk diameter than juveniles from larvae fed low food concentrations (p < 0.001; Figure 21; Table 3D). Juveniles from whole embryos had 4.6% larger disk diameters on average than juveniles from half embryos (p = 0.024; Figure 21; Table 3D). The interaction between food treatment and embryo manipulation was not significant in determining disk diameter (p = 0.197; Figure 21; Table 3D).
Juveniles from the high food, whole embryo treatment had an average area at metamorphosis of 524260 ± 18438 \( \mu \text{m}^2 \), juveniles from the high food, half embryo treatment had an average area of 521221 ± 21509 \( \mu \text{m}^2 \). Juveniles from the low food, whole embryo treatment had an average area of 427797 ± 14799 \( \mu \text{m}^2 \), and juveniles from the low food, half embryo treatment had an average area of 379602 ± 16167 \( \mu \text{m}^2 \) (Figure 22). Juveniles from larvae fed high food concentrations had a 23% larger area on average than juveniles from larvae fed low food concentrations (\( p < 0.001 \); Figure 22; Table 5E). There was no significant difference in area between juveniles from whole and half embryos (\( p = 0.168 \); Figure 22 Table 5E), nor was there a significant difference between the interaction of food treatment and embryo manipulation on the area of juveniles at metamorphosis (\( p = 0.219 \); Figure 22; Table 5E).

All juveniles, regardless of treatments were deceased by 120 days post metamorphosis (Figure 23). According to both a linear mixed model ANCOVA and a rank transformation ANCOVA, food treatment had a significant effect on the rate of death of \( A. \text{forbesi} \) juveniles (\( p < 0.036 \); Tables 4 and 5). Juveniles from larvae fed low food treatments died at a faster rate than juveniles from larvae fed high food concentrations. There was no significant effect of laser treatment or the interaction of laser treatment and food treatment on the death rate of \( A. \text{forbesi} \) juveniles according to both a linear mixed model ANCOVA and a rank transformation ANCOVA (\( p > 0.05 \); Tables 4 and 5).

The male-female pairs from Williamsburg, VA and Brunswick, ME were combined to perform a linear mixed model ANOVA with laser treatment and food treatment as fixed factors and beaker as a random factor to determine if there were differences in proportion survival, spine number, disk diameter, age at settlement, and area of the juveniles at metamorphosis. Residuals for all of the response variables were calculated and tested for normality. The residuals for all
response variables (proportion survival, age at settlement, spine number, juvenile disk diameter, and juvenile area) were all normally distributed by a Kolmogorov-Smirnov and a Shapiro-Wilk test for normality (p > 0.05).

Of all larvae from the two *A. forbesi* experiments combined, 66% of larvae reached metamorphosis. 67% of larvae fed high food concentrations and from whole embryos reached metamorphosis, 74.8% of larvae fed high food concentrations from half embryos reached metamorphosis, 66.7% of larvae fed low food concentrations from whole embryos reached metamorphosis, and 55.8% of larvae fed low food concentrations from half embryos reached metamorphosis (Figure 24). There was no significant difference in the percent of larvae reaching metamorphosis between larvae fed different food levels (p = 0.053; Figure 24; Table 6A), larvae from whole and half embryos (p = 0.753; Figure 24; Table 6A), or the interaction between food and laser treatments (p = 0.061; Figure 24, Table 6A).

Juveniles from the high food, whole embryo treatment had an average age at settlement of 32.4 ± 1 days. Juveniles from the high food, half embryo treatment had an average age at settlement of 32.6 ± 0.9 days. Juveniles from the low food, whole embryo treatment had an average age at settlement of 38.4 ± 1.9 days, and juveniles from the low food, half embryo treatment were 38.2 ± 2.3 days old at settlement (Figure 25). There was a significant difference in settlement age between larvae from high food and low food treatments (p < 0.001; Figure 25; Table 6B), with larvae fed high food concentrations metamorphosing on average 18% earlier than larvae fed low food concentrations. There was no significant difference in settlement age between larvae from whole and half embryos (p = 0.998; Figure 25; Table 6B), nor was there a difference in for the interaction between food treatment and laser treatment (p = 0.908; Figure 25; Table 6B).
At metamorphosis, juveniles from the high food, whole embryo treatment had 58.2 ± 1.6 spines, juveniles from the high food, half embryo treatment had 57.1 ± 2 spines, juveniles from the low food, whole embryo treatment had 53.9 ± 2.2 spines, and juveniles from the low food, half embryo treatment had 46.5 ± 2.9 spines (Figure 26). Juveniles from high food treatments had 15% more spines at metamorphosis than juveniles from low food treatments (p < 0.001; Figure 26; Table 6C). There was no significant difference in spine number at metamorphosis between juveniles from whole and half embryos (p = 0.058; Figure 26, Table 6C) or the interaction between food concentration and embryo manipulation (p = 0.161; Figure 26, Table 6C).

At metamorphosis, juveniles from the high food, whole embryo treatment had a mean disk diameter of 808.6 ± 11.4 μm, juveniles from the high food, half embryo treatment had a mean disk diameter of 788.1 ± 171.4 μm, juveniles from the low food, whole embryo treatment had a mean disk diameter of 726.7 ± 13.5 μm, and juveniles from the low food, half embryo treatment had a mean disk diameter of 670.3 ± 12.7 μm (Figure 27). Juveniles from larvae fed high food concentrations had on average a 14.3% larger disk diameter than juveniles from larvae fed low food concentrations (p < 0.001; Figure 27; Table 6D). Juveniles from whole embryos had 5.3% larger disk diameters on average than juveniles from half embryos (p = 0.004; Figure 27; Table 6D). The interaction between food treatment and embryo manipulation was not significant in determining disk diameter (p = 0.161; Figure 27; Table 6D).

Juveniles from the high food, whole embryo treatment had an average area at metamorphosis of 501008 ± 14059 μm², juveniles from the high food, half embryo treatment had an average area of 493824 ± 15473 μm². Juveniles from the low food, whole embryo treatment had an average area of 427797 ± 14799 μm², and juveniles from the low food, half embryo
treatment had an average area of $379602 \pm 16167 \, \mu \text{m}^2$ (Figure 28). Juveniles from larvae fed high food concentrations had a 23% larger area on average than juveniles from larvae fed low food concentrations ($p < 0.001$; Figure 28; Table 6E). There was no significant difference in area between juveniles from whole and half embryos ($p = 0.088$; Figure 28; Table 6E), nor was there a significant difference between the interaction of food treatment and embryo manipulation on the area of juveniles at metamorphosis ($p = 0.204$; Figure 28; Table 6E).
Discussion

I found that food concentration during the larval development of planktotrophic asteroid species is much more important in determining age at metamorphosis and juvenile quality (juvenile size and number of spines) than maternal investment. These results contrast with previous results from echinoids, which show that reduced egg size increases development time and decreases juvenile size (Sinervo and McEdward, 1988; Hart, 1995; McEdward, 1996; Alcorn and Allen, 2009; Allen, 2012). Care should be taken in using results from echinoids as a paradigm for marine invertebrate responses to reductions in maternal investment, as my work shows this paradigm does not hold true even in other echinoderm species. My results also have implications for life history theory and egg size evolution. For example, if changes in egg size have minimal effects on development time and juvenile performance, it is likely there are other selective pressures on asteroids that have shaped egg size evolution.

Effects of maternal investment and food supply on pre-metamorphic development

The proportion of larvae completing metamorphosis is one measure of larval quality. In previous experiments, echinoderm larvae reared at lower food concentrations resulted in fewer metamorphs than larvae reared at higher food concentrations (Uthicke et al., 2009; Allen, 2012). My results indicated no such significant differences between seastars fed high and low food concentrations. One explanation for the lack of significant differences in larval success between low and high food treatments is the phenotypic plasticity asteroid species exhibit in limited food environments (George, 1999). In low food environments, seastars grow longer arms that allow them to clear a greater volume of water of food particles than they do in high food environments (George, 1999). Phenotypic plasticity in low food environments has also been observed in other
echinoderms including echinoids and ophiuroids, with organisms increasing the size of their feeding structures when exposed to low food environments (Hart and Strathmann, 1994; Miner, 2007).

One possible explanation for the differences in the responses of echinoids and asteroids to low food concentrations is the differences in their feeding structures. Echinoids and ophiuroids develop as pluteus larvae, with a larval skeleton present during development, while asteroid larval development is characterized by the absence of a larval skeleton during development (McEdward and Miner, 2001). It is possible the larval skeleton is more costly to build than larval arms without skeletal elements. If arms containing skeleton are more costly to build, echinoids and ophiuroids may not have the resources available to increase their feeding structures as effectively as asteroids in low food conditions. Another possible explanation for the differences in the responses of echinoids and asteroids to low food concentrations is the degree of phenotypic plasticity observed in both species. Under low food conditions, echinoids and ophiuroids exhibit a 5-20% increase in the length of their arms and ciliary bands (Hart and Strathmann, 1994; Podolsky and McAlister, 2005). *Pisaster ochraceus* can exhibit up to a 25% increase in larval length and width under low food conditions (George, 1999), while another asteroid species, *Acanthaster planci*, exhibits 15-20% increases in larval length and width under low food conditions (Wolf *et al.*, 2015). Observationally, it appears *A. forbesi* larvae in low food conditions have up to 50% longer arms than those in high food treatments (Trackenberg and Allen, unpublished data). It appears then, that asteroids may exhibit greater phenotypic plasticity in response to low food conditions, which may account for differences in the response of asteroids and echinoids to low food concentrations.
In addition to a correlation between larval food concentration and development, there is a correlation between the size of an egg and the amount of time it takes the larva to reach metamorphosis in echinoderms (Levitan, 2000). Egg size and development time are inversely proportional to each other, and multiple models have been proposed to further describe this relationship (Vance, 1973; Podolsky and Strathmann, 1996; Levitan, 2000). Species with planktotrophic larvae take longer to reach metamorphosis than species with lecithotrophic larvae, and species within each developmental mode with smaller eggs tend to take longer to develop than species with larger eggs (Emlet et al., 1987). Within asteroids, egg size ranges from 110 to 3500 µm in diameter, with an average egg size of 700 µm (Emlet et al., 1987). My study species, *P. ochraceus* and *A. forbesi*, with mean egg diameters of 152 and 136 µm respectively, fall on the lower range of all asteroid egg sizes (Emlet et al., 1987). Among planktotrophic asteroids, the average egg size is 150 µm (Emlet et al., 1987), so *P. ochraceus* eggs are the size of the average planktotroph egg and *A. forbesi* eggs are slightly smaller than the average planktotroph.

Previous investigations on the effects of reductions in egg energy content have utilized planktotrophic echinoids that have similar egg sizes relative to the average for echinoids as my study species do for asteroids (Sinervo and McEdward, 1988; McEdward, 1996; Alcorn and Allen, 2009; Allen, 2012). The reductions in egg energy in these planktotrophic echinoids result in an increase in larval development time (Sinervo and McEdward, 1988; McEdward, 1996; Alcorn and Allen, 2009; Allen, 2012). The increase in larval development time due to egg energy reductions is unsurprising given the inversely proportional relationship between egg size and development time (Vance, 1973; Levitan 2000). Based on results from egg energy reductions on seven species of echinoids, I made predictions using the following equation: percent increase in juvenile time = \(-4.223*(\text{Ln egg volume}) – 16.892\) (Allen, 2012). Based on this equation, I
predicted a 10% increase in development time in *P. ochraceus* and an 11% increase in development time in *A. forbesi*. In contrast to my initial predictions, I found that changes in egg energy content did not affect the age at metamorphosis in either *P. ochraceus* or *A. forbesi*.

Although not significant, egg energy reductions in *P. ochraceus* did increase age at settlement by 5%. It is possible the lack of a significant effect of reducing egg energy on the age at settlement of *P. ochraceus* may be due to low power to detect small differences. Initially, I designed a paired experiment with larvae marked with the vital stain, Nile Blue Sulfate, which has been used successfully in seastars (Simon, 1974), and echinoids (Allen *et al.*, 2006; Alcorn and Allen, 2009). Unfortunately, the stain had strong negative effects on larval development. The stain also became much harder to see within the larvae, requiring a second exposure of the larvae to the stain. Due to the negative developmental effects and poor stain retention, stained larvae were removed from the experiment. This reduced the power of my experimental design, possibly masking differences in the age at settlement between juveniles derived from whole and half embryos. I conducted a power analysis to determine the sample size I would have needed to detect a significant increase in development time in *P. ochraceus* given the means and variances of my treatments. In order to detect significance in the difference in age at metamorphosis in *P. ochraceus*, I would have needed a sample size of 11 beakers, only one replicate higher than what I used in my experiment. If the magnitude of the effects of egg energy reductions in *P. ochraceus* aligned with my original predictions based on echinoids, my experiment had a power of 0.998. It is therefore highly likely if there were large effects of egg energy reductions on age at metamorphosis in *P. ochraceus* these differences would have been detected statistically.

While there were measurable, but non significant, effects of egg energy reductions on the larval development time of *P. ochraceus, A. forbesi* larvae demonstrated no such effects. Based
on a power analysis, I would have needed a sample size of over 400 beakers in our *A. forbesi* experiment in Williamsburg, VA, and a sample size of over 8000 beakers in our *A. forbesi* experiment in Brunswick, ME, to detect any significant effects of egg energy reductions on age at metamorphosis. The sample sizes needed to detect significance in the effect of egg energy reductions on age at metamorphosis are extremely large and are unattainable within my experimental design or within timing limitations during laser ablations. If, in contrast, the magnitude of the effects of egg energy reductions had aligned with my predictions, my experiment on the effects of egg energy reductions in *A. forbesi* in Williamsburg had a power of 0.999 and my experiment in Brunswick, ME had a power of 1. Given the extremely high powers associated with my experiments, if the effects of egg energy reductions had aligned with my predictions, I conclude that there simply does not appear to be an effect of egg energy reductions on larval development time in *A. forbesi*.

The lack of an effect of egg size reductions on development time contrasts with the paradigm that has been shown in other species of echinoderms (Allen, 2012). More specifically, my results show seastar larvae do not respond to reductions in egg energy in the same way that echinoids do (Allen, 2012). It is unclear, however, if other species of asteroids with larger egg sizes and different developmental modes (e.g. lecithotrophy) exhibit the same trend as seen in my study, highlighting the need for further experiments on different species of asteroids with different egg sizes and developmental modes.

**Effects of maternal investment and food supply on juvenile quality**

I estimated juvenile quality by measuring the number of spines, disk diameter, and disk area of juveniles at metamorphosis. A decreased size at metamorphosis can have consequences
for asteroid juveniles, including a weakened attachment to the substrate or a decrease in the body calcification of the juvenile, which makes juveniles more vulnerable to predators (Basch and Pearse, 1996). In the planktotrophic seastar, *Acanthaster planci*, differences in juvenile energy content at metamorphosis, which is correlated with juvenile size, had significant effects on the behavior, growth, and physiology of the juveniles (Zann et al., 1987). Similarly, in a second planktotrophic species, *Asterina miniata*, smaller juvenile size may be indicative of lower lipid storage that can affect the length of time the juvenile can survive in a low food environment post metamorphosis (Basch and Pearse, 1996). While juvenile size is correlated with juvenile quality in planktotrophic asteroids (Zann et al., 1987; Basch and Pearse, 1996), juveniles from *Lepasterias aequalis*, a large egged brooding seastar, juvenile size was not significantly correlated with juvenile survival (Gehman and Bingham, 2010). Since the two species I examined were both planktotrophic developers like *A. miniata* and *A. planci*, I predicted a similar relationship between juvenile size and energy content.

There is a correlation between egg size and juvenile size in both echinoids and asteroids (Emlet et al., 1987), so I therefore predicted decreases in juvenile size when egg energy is reduced. The reason I predicted this effect is because egg energy reductions increase the amount of energy the larva must obtain from exogenous sources of food, resulting in a decreased juvenile size if the larva cannot compensate for the loss of endogenous energy by feeding. Based on results from egg energy reductions on seven species of echinoids, I made predictions of the magnitude of the effect of egg energy reductions using the following equation: percent decrease in juvenile size = 1.251*(Ln egg volume) + 18.450 (Allen, 2012). Based on the equation used, I predicted a 10% decrease in juvenile size in both *P. ochraceus* and *A. forbesi*. In echinoids, reductions of egg energy content result in decreased juvenile size at metamorphosis and
decreased spine numbers in echinoids with both planktotrophic and lecithotrophic development (Sinervo and McEdward, 1988; Hart, 1995; McEdward, 1996; Emlet and Hoegh-Guldberg, 1997; Allen et al., 2006; Alcorn and Allen, 2009; Allen, 2012).

The first aspect of juvenile quality I measured was the number of spines present on a juvenile at metamorphosis. Asteroid spines are used as a form of protection from predation, so an increase in spine numbers may be correlated with greater protection and therefore higher survival (Blake, 1983). Based on results from echinoids (Sinervo and McEdward, 1988; Hart, 1995; McEdward, 1996; Alcorn and Allen, 2009; Allen, 2012), I predicted that both egg energy reductions and changes in larval food concentration would result in decreased juvenile size in both *P. ochraceus* and *A. forbesi*. In our experiments, reductions in egg energy did not significantly affect the number of spines present on a juvenile at metamorphosis in either *P. ochraceus* or *A. forbesi*. I did, however, find that changes in food concentrations significantly affected the number of spines at metamorphosis. Juveniles from larvae fed lower food concentrations had 20% fewer spines than juveniles from larvae fed high food concentrations. My results showing no difference in the number of spines at metamorphosis when egg energy is reduced directly contradict results seen in echinoids, whereas my results showing that decreased larval food concentrations significantly decreases the number of spines at metamorphosis agree with previous results seen in echinoids (Allen, 2012). The paradigm observed in echinoids on the effects of reductions in egg energy may not be applicable to asteroids. Alternatively, the paradigm observed in echinoids on the effects of reductions in larval food concentration does appear to be applicable to asteroids.

Similar to the results for spine length, I found no effects of egg energy reductions on juvenile disk diameter at metamorphosis in *P. ochraceus* or in *A. forbesi* from Williamsburg,
VA. When the sample size was increased in my Brunswick, ME experiments, I found significant effects of egg energy reductions on the disk diameter of *A. forbesi* juveniles at metamorphosis. Reduction of egg energy resulted in a 5% decrease in disk diameter between juveniles from whole and half embryos. While the first two results I found in *P. ochraceus* and *A. forbesi* were surprising based on our predictions, the results seen in *A. forbesi* juveniles from Brunswick, ME aligned with our predictions in direction, however they were much smaller in magnitude than my prediction of a 10% decrease in juvenile size based on echinoid results. It therefore appears that while significant, the effects of egg energy reductions on the disk diameter in *A. forbesi* are much smaller than effects seen across echinoids (Allen, 2012).

In echinoids, much like effects of egg reductions on disk diameter, egg energy reductions significantly decrease the area of a juvenile at metamorphosis (Alcorn and Allen, 2009; Allen, 2012). Surprisingly, I found egg energy reductions had no effect on the juvenile area at metamorphosis in either *P. ochraceus* or *A. forbesi* juveniles, contrasting my results on the effects of egg energy reductions on the juvenile disk diameter. I did, however find food concentration to significantly affect both the disk diameter and area of juveniles at metamorphosis. Juveniles coming from larvae fed low food concentrations had 13% smaller disk diameters and 23% smaller areas than juveniles from larvae fed high food concentrations. My results from egg energy reductions once again contrasted with results seen in previous experiments on echinoids, however my results from food concentration manipulations aligned with my predictions that reductions in larval food concentration would decrease juvenile size.

While there were no differences in juvenile quality at metamorphosis between juvenile from whole and half embryos, juvenile survival is not only dependent upon size at metamorphosis (Miller and Emlet, 1999; Moran and Emlet, 2001). Post metamorphosis, a
juvenile must evade predation, find food, and continue to grow within the environment it settled in (Gosselin and Qian, 1997). Latent effects of egg size manipulations could affect the quality of juveniles post metamorphosis if egg energy reductions significantly affect a juvenile’s growth rate or length of survival in low food environments. For example, *A. forbesi* juveniles are cannibalistic post metamorphosis, with larger juveniles consuming their smaller siblings (Allen and Brocco-French, unpublished data). Therefore, being larger at metamorphosis likely is beneficial, so any differences seen in size between juveniles from whole and half embryos could be biologically important. We did not, however, find any significant effects of egg energy reductions on the rate of death in either *P. ochraceus* or *A. forbesi*, indicating there may not be any latent effects of egg size manipulations in asteroids. My investigation of latent effects of egg energy reductions on juveniles was fairly limited. I limited my study to starved juveniles to ensure all individuals were under the same conditions. If the juveniles had been fed, there could possibly have been differences between juveniles from whole and half embryos.

My study investigated only three potential costs of decreased egg size in asteroids, changes in time to metamorphosis, changes in juvenile size, quantified as juvenile disk diameter, area, and spine number; and changes in the rate of death. It is possible there are other costs of decreasing the egg sizes in these species, including increased predation rates, lower fertilization in smaller eggs, and larvae may be unsuited to heterogenous environments (Hart, 1995). It is possible there may have been differences between larvae from whole and half embryos in these categories, however I did not test for these differences. Future experiments including more treatments during the larval period could look to determine if changing the initial egg size leads to differences in these parameters. Previous work has shown there may be a higher predation on smaller larvae, however it is also possible larger larvae are preferentially consumed by visual
predators (Allen, 2008). Therefore, it is possible there are both costs and benefits to smaller size when predation is considered during larval development. It would be extremely difficult to test the effects of egg size reductions on the fertilization of eggs as the eggs must be fertilized prior to energy reductions performed in the same manner as they were for this experiment. Therefore, I must draw conclusions about fertilization rates based on differences between large and small eggs between and within species. There is evidence for lower rates of fertilization in smaller eggs (Levitan, 1993), however species with smaller eggs may avoid this cost of egg size through accessory structures such as jelly coats (Podolsky, 2001). It thus appears that much like changes in predation rate, smaller eggs may exhibit both benefits and costs to the fertilization rate and that these vary with the physical environment (e.g. current patterns) and biological environment (e.g. sperm density).

In order to investigate if the patterns seen in this study hold true for more species of asteroids, future studies should examine the effects of reductions in egg size across multiple species with both planktotrophic and lecithotrophic development. My results suggest that planktotrophic larvae may compensate for decreases in egg size by increasing their feeding while in the water column. Energy from this extra food may enable larvae from half embryos to reach metamorphosis in the same amount of time and at the same size as larvae from whole embryos. Compensatory growth has been shown in fish larvae, with smaller individuals able to grow quicker to match the size of their larger siblings (El Ghazali et al., 2009). Under high food conditions, fish are able to increase their feeding rate and growth rates to become the same size as other fish that were larger initially (Ali et al., 2003). It appears seastars demonstrate compensatory growth similar to that seen in fish, with smaller individuals able to catch up in size to individuals who were larger. The ability of asteroids to exhibit compensatory growth contrasts
with echinoids that do not show signs of compensatory growth (Alcorn and Allen, 2009; Allen, 2012). In lecithotrophic development, compensatory growth is not possible as these species lack the necessary structures to feed while in the water column, and thus cannot compensate for a smaller size through additional energy gain. Because lecithotrophic species cannot exhibit compensatory growth, it is predicted that when egg energy is reduced, lecithotrophic species will exhibit no differences in larval time, but will be smaller in size at metamorphosis (Allen, 2012). If these predictions are accurate, it may be that lecithotrophic species in both echinoids and asteroids respond to decreases in egg size similarly. This would indicate similar selection pressure on the evolution of egg size across species. If, however, asteroids continue to respond differently to reductions in egg size than echinoids, it would indicate different selection pressures and therefore, different roles of larval time and juvenile size in the evolution of asteroids when compared to echinoids.

Another avenue for further research comes from the bet-hedging strategy of multiple species of seastars (Marshall et al., 2008). Bet-hedging is when an organism produces a range of offspring sizes within a single clutch (Marshall et al., 2008). Recently bet hedging has been shown to be an evolutionarily stable strategy (Olofsson et al., 2009), contrasting with previous models of egg size evolution that predicted an optimal egg size within a clutch (Vance, 1973; Podolsky and Strathmann, 1996; Levitan, 2000). Multiple species of asteroids employ a bet-hedging strategy when allocating energy towards reproduction, including A. forbesi (Blackburn, 2013). Asterias forbesi eggs naturally have extreme variance within clutches, with eggs varying up to three-fold in volume (Blackburn, 2013). Interestingly variation in egg size does not appear to be correlated with variations in egg energy content when comparing eggs within species with lecithotrophic development (McEdward and Coulter, 1987 and McEdward and Carson, 1987).
Rather, the differences in egg size between siblings may be due to imprecision in gametogenesis or an adaptive response to variation in the environment instead of differences in the allocation of resources (McEdward and Carson, 1987). Investigations into the development of small and large *A. forbesi* embryos show a significant increase in development time, but no decrease in juvenile size at metamorphosis, however it is unknown if the differences seen in the size of *A. forbesi* eggs are correlated with changes in energy content (Blackburn, 2013). Further experiments to see if there are changes in egg energy content between these eggs of varying sizes within a clutch can help to determine if there is selection pressure for a standard egg energy content within a species. If the eggs vary in energy content within a species, determining the costs of smaller egg size within a clutch can begin to answer more questions about the tradeoff between egg size and number. Investigations into the interaction of egg size and larval food concentration can also be explored within a highly variable clutch to determine if there is an interaction between egg size and food concentrations. A great deal is still unknown about the evolution of egg size and the effects of changing the initial egg energy content in echinoderms and other marine invertebrates. Experiments similar to mine in taxonomically diverse groups can help to uncover the drivers of egg size evolution in marine invertebrates, and my work suggests that even within a phylum there are likely to be different drivers.
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Guisande, C., J. Sanchez, I. Maneiro, and A. Miranda. 1996. Trade-off between offspring number and offspring size in the marine copepod *Euterpina acutifrons* at different food concentrations. *Marine Ecology Progress Series* 143: 37-44.


**Figure 1:** Late stage brachiolaria larva. Juvenile rudiment (JR) glowing under polarized light. Brachiolar arms (BA) are also visible at the top of the larva. Scale bar = 200 µm.
Figure 2: Diagram of juvenile disk diameter measurements. DD1 denotes longest distance across juvenile (disk diameter in analysis) and DD2 denotes the perpendicular to DD1. Scale Bar = 200 µm.
Figure 3: Proportion of *P. ochraceus* larvae reaching metamorphosis from whole (white) and half (gray) embryos. A linear mixed model ANOVA found no significant difference in the proportion metamorphosing from whole and half embryos ($F_{1,8} = 0.006, p = 0.941$).
Figure 4: Rate of settlement of *P. ochraceus* juveniles from whole (black line) and half (gray line) embryos. Juveniles began settlement 30 days post fertilization. A settlement lag appears to exist between whole and half embryos at the beginning of settlement, however a linear mixed model ANOVA found no significant difference in the average age at settlement between juveniles from whole and half embryos ($F_{1,8} = 4.191, p = 0.075$).
Figure 5: Average age at settlement in \textit{P. ochraceus} juveniles from whole (white) and half (gray) embryos. Although there was a 5\% increase in settlement age in larvae from half embryos compared to larvae from whole embryos, this difference was not significant (linear mixed model ANOVA: F$_{1,8}$ = 4.191, p = 0.075). Error bars denote standard error.
Figure 6: Average number of spines in *P. ochraceus* at metamorphosis for juveniles from whole (white) and half (gray) embryos. There was no significant difference between the average number of spines from whole compared to half embryos (linear mixed model ANOVA: F_{1,8} = 0.806, p = 0.396). Error bars denote standard error.
Figure 7: Average disk diameter in *P. ochraceus* juveniles at metamorphosis from whole (white) and half (gray) embryos. There was no significant difference between the disk diameter in juveniles from whole and half embryos (linear mixed model ANOVA: $F_{1,8} = 0.061$, $p = 0.811$). Error bars denote standard error.
Figure 8: Average area for *P. ochraceus* juveniles from whole (black diamonds) and half (grey squares) embryos at metamorphosis (MM), 72-73 days post fertilization, and 108 days post fertilization. There was no significant difference in the area at metamorphosis (linear mixed model ANOVA: $F_{1,8} = 0.911, p = 0.368$), 72-73 days post fertilization (linear mixed model ANOVA: $F_{1,8} = 0.429, p = 0.531$), or 108 days post fertilization (linear mixed model ANOVA: $F_{1,8} = 0.067, p = 0.802$). Error bars denote standard error.
Figure 9: Proportion of *P. ochraceus* juveniles alive at 0, 68, 71, 100, 138, and 172 days post fertilization from whole (black diamonds) and half (gray squares) embryos. There was no significant difference in the rate of death between whole and half embryos according to a linear mixed model ANCOVA (F$_{1,56}$ = 0.287, p = 0.594) and a Rank Transformation ANCOVA (F$_{1,58}$ = 3.023, p = 0.087). Error bars denote standard error.
Figure 10: Proportion of *A. forbesi* larvae from Williamsburg, VA metamorphosing from whole (white) and half (gray) embryos. There was no significant difference in the proportion metamorphosing from whole and half embryos (linear mixed model ANOVA: $F_{1,16} = 3.628, p = 0.075$). Error bars denote standard error.
Figure 11: Average age at settlement in *A. forbesi* juveniles from Williamsburg, VA from whole (white) and half (gray) embryos. There was no significant difference in the age at settlement between juveniles from whole and half embryos (linear mixed model ANOVA: $F_{1,16} = 0.136$, $p = 0.717$). Error bars denote standard error.
Figure 12: Average number of spines at metamorphosis in *A. forbesi* juveniles from Williamsburg, VA. There was no significant difference in number of spines between juveniles from whole (white) and half (gray) embryos (linear mixed model ANOVA: $F_{1,16} = 0.059$, $p = 0.826$). Error bars denote standard error.
Figure 13: Average disk diameter at metamorphosis in *A. forbesi* juveniles from Williamsburg VA. There was no significant difference in the disk diameter at metamorphosis in juveniles from whole (white) and half (gray) embryos (linear mixed model ANOVA: $F_{1,16} = 1.928, p = 0.184$). Error bars denote standard error.
Figure 14: Average disk area at metamorphosis in *A. forbesi* juveniles from Williamsburg, VA. There was no significant difference in disk area between juveniles from whole (white bar) and half (gray bar) embryos (linear mixed model ANOVA $F_{1,16} = 0.234$, $p = 0.635$). Error bars denote standard error.
Figure 15: Average number of spines 20 days post metamorphosis in *A. forbesi* juveniles from Williamsburg, VA from whole (white) and half (gray) embryos. There was no significant difference in the number of spines 20 days post metamorphosis between juveniles from whole and half embryos (linear mixed model ANOVA: $F_{1,16} = 0.141, p = 0.712$). Error bars denote standard error.
Figure 16: Average disk diameter of *A. forbesi* juveniles from Williamsburg, VA 20 days post metamorphosis from whole (white) and half (gray) embryos. There was no significant difference in the disk diameter 20 days post metamorphosis in juveniles from whole and half embryos (linear mixed model ANOVA: $F_{1,16} = 0.056$, $p = 0.815$). Error bars denote standard error.
Figure 17: Average disk area 20 days post metamorphosis in *A. forbesi* juveniles from Williamsburg, VA. There was no significant difference in disk area between juveniles from whole (white bar) and half (gray bar) embryos (linear mixed model ANOVA $F_{1,16} = 0.402$, $p = 0.535$). Error bars denote standard error.
Figure 18: Proportion of *A. forbesi* larvae from Brunswick, ME that completed metamorphosis. Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. Food treatment, laser treatment, and the interaction between food and laser treatments were all shown to not have significant effects on the proportion of larvae completing metamorphosis (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 19: Average age at settlement in juveniles from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos in *A. forbesi* from Brunswick, ME. Food treatment, laser treatment, and the interaction between food and laser treatments were found to not have significant effects on age at settlement (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 20: Average number of spines at metamorphosis in juveniles from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos in *A. forbesi* from Brunswick, ME. Food treatment had a significant effect on spine number (linear mixed model ANOVA: $F_{1,41} = 27.932, p < 0.001$). Laser treatment and the interaction between food and laser treatments were found to not have a significant effect on spine number (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 21: Average disk diameter at metamorphosis in juveniles from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos in *A. forbesi* from Brunswick, ME. Food and laser treatments were both found to have significant effects on disk diameter (linear mixed model ANOVA, \( p < 0.05 \)). The interaction between food and laser was found to not have a significant effect on disk diameter (linear mixed model ANOVA: \( F_{1,41} = 1.723, p = 0.197 \)). Error bars denote standard error.
Figure 22: Average area at metamorphosis in juveniles from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos in *A. forbesi* from Brunswick, ME. Food treatments was found to have significant effects on area (linear mixed model ANOVA, $F_{1,41} = 42.925$, $p < 0.001$). Laser treatment and the interaction between food and laser were found to not have a significant effect on disk diameter (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 23: Proportion of *A. forbesi* juveniles from Brunswick, ME, alive at 0, 21, 55, 66, 91, and 120 days post metamorphosis in juveniles from high (Hi, black outlines) and low (Lo, gray outlines) food concentrations, and whole (filled circles) and half (empty circles) embryos. Data is offset in the figure for days 21, 55, 66, and 91 for differentiate between treatments due to overlap of the data. Analysis of the rate of death between all treatments was conducted for days 0, 16, 18, 20, 21, 55, 62, 64, 66, 91, and 120 days post metamorphosis and analyzed in a linear mixed model ANCOVA and a Rank Transformation ANCOVA, however for ease of visualization, the data from days 16, 18, 20 and 21 were represented by day 21 data, and the data from days 62, 64, and 66 were represented by day 66 data. There was no effect of laser treatment on the rate of death between juveniles from whole and half embryos in either model (linear mixed model ANCOVA and Rank Transformation ANCOVA, $p > 0.05$). Food treatment was shown to have significant effects on the rate of death between juveniles from larvae fed high and low food treatments in both models (linear mixed model ANCOVA and Rank Transformation ANCOVA, $p < 0.05$).
**Figure 24:** Proportion of larvae that completed metamorphosis after combining both *A. forbesi* male-female pairs (VA and ME experiments). Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. Food treatment, laser treatment, and the interaction between food and laser treatments were found to not have a significant effect on the proportion of larvae metamorphosing (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 25: Average age at settlement for *A. forbesi* juveniles from both male-female pairs combined (VA and ME experiments). Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. Food treatment was shown to have a significant effect on the age at settlement (linear mixed model ANOVA: \( F_{1,60} = 16.595, p < 0.001 \)). Laser treatment and the interaction between food and laser treatments did not have a significant effect on the age at settlement (linear mixed model ANOVA, \( p > 0.05 \)). Error bars denote standard error.
Figure 26: Average number of spines at metamorphosis for A. forbesi juveniles from both male-female pairs combined (VA and ME experiments). Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. Food treatment was shown to have a significant effect on the number of spines at metamorphosis (linear mixed model ANOVA: $F_{1,60} = 11.878, p = 0.001$). Laser treatment and the interaction between food and laser treatments were shown to not have a significant effect on the number of spines at metamorphosis (linear mixed model ANOVA, $p > 0.05$). Error bars denote standard error.
Figure 27: Average disk diameter at metamorphosis for *A. forbesi* juveniles from both male-female pairs combined (VA and ME experiments). Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. Both food and laser treatments were shown to have significant effects on the disk diameter at metamorphosis (linear mixed model ANOVA, *p* < 0.004). The interaction between food and laser treatments was found to not have a significant effect on the disk diameter (linear mixed model ANOVA: $F_{1,60} = 2.017$, *p* = 0.161). Error bars denote standard error.
Figure 28: Average area at metamorphosis for *A. forbesi* juveniles from both male-female pairs combined (VA and ME experiments). Juveniles are from high (white bars) and low (gray bars) food treatments and whole (left bars) and half (right bars) embryos. The food treatment was shown to have a significant effect on the area of the juveniles at metamorphosis (linear mixed model ANOVA: \( F_{1,60} = 34.444, p < 0.001 \)). Laser treatment and the interaction between food and laser treatments were shown to not have a significant effect on the area of the juveniles at metamorphosis (linear mixed model ANOVA, \( p > 0.05 \)). Error bars denote standard error.
Tables

**Table 1:** A linear mixed-model ANOVA on the effects of Laser treatment on proportion metamorphosed, age at settlement, spine number, disk diameter, area, second measured area, and third measured area in *P. ochraceus* juveniles. Beaker was included as a random effect in the model, but is not shown here for clarity.

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<th>Predictor</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F-value</th>
<th>P</th>
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<td></td>
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<tr>
<td><strong>G) Third Measurement</strong></td>
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<td>0.067</td>
<td>0.802</td>
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Table 2: A linear mixed-model ANOVA on the effects of Laser treatment on proportion metamorphosed, age at settlement, spine number, disk diameter, area, spine number 20 days post metamorphosis (MM), disk diameter 20 days post MM, and area 20 days post MM in *A. forbesi*. Beaker was included as a random effect in the model, but is not shown here for clarity.

<table>
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<th>Denominator df</th>
<th>F-value</th>
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<td>B) Age at Settlement</td>
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<tr>
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Table 3: A linear mixed-model ANOVA on the effects of Laser treatment, Food treatment, and the interaction of Laser and Food treatments on proportion metamorphosed, age at settlement, spine number, disk diameter, and area in *A. forbesi*. Beaker was included as a random effect in the model, but is not shown here for clarity.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Numerator df</th>
<th>Denominator df</th>
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<th>P</th>
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<td>Food</td>
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Table 4: A Linear Mixed Model ANCOVA on the effects of Laser treatment, Food treatment, and the interaction of Laser and Food treatments on the rate of death in *A. forbesi*. Data were transformed using an arcsine square root transformation for analysis. Day was included in the model as a covariate and Beaker was included in the model as a random effect, but is not shown here for clarity.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Numerator df</th>
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Table 5: A Rank Transformation ANCOVA table on the effects of Laser treatment, Food treatment, and the interaction of Laser and Food treatments on the rate of death in *A. forbesi*. Day was included in the model as a covariate and Beaker was included in the model as a random effect, but is not shown here for clarity.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F-value</th>
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<td></td>
</tr>
<tr>
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Table 6: A linear mixed-model ANOVA on the effects of Laser treatment, Food treatment, and the interaction of Laser and Food treatments on proportion metamorphosed, age at settlement, spine number, disk diameter, and area in *A. forbesi* juveniles from both experiments combined. Beaker was included as a random effect in the model, but is not shown here for clarity.

<table>
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<th>Denominator df</th>
<th>F-value</th>
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<td><strong>A) Proportion Metamorphosed</strong></td>
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