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Do Density and Food Availability Influence Survivorship and Reproduction in the Freshwater Bivalve, Sphaerium simile?

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Abstract

The small freshwater bivalve *Sphaerium simile*, like most “fingernail clams” (Sphaeriidae), does not exhibit a planktonic larval stage but instead offspring are brooded inside the valves of the parent. The species is generally regarded as reproductively specialized, or relatively “K” selected along the r-K continuum. Multiple offspring can develop simultaneously, but brooding siblings are commonly at different developmental stages. An experiment was conducted to evaluate the relative success of brooding offspring and adults in different adult densities and feeding regimes. A controlled laboratory experiment examined four treatment groups, with adult density and feeding frequency as independent variables. There were two density treatments [0.07 (low) and 0.35 (high) clams per cm²] and two feeding treatments (continuous and once each week). A parallel field experiment was conducted with high, moderate and low density treatments. In the laboratory experiment, offspring production was highest in treatment groups with high densities and/or more frequent feeding. Mortality of adults was highest among subjects kept at low feeding frequency feeding. In the field experiment, offspring production was highest in high and moderate density treatments. These results provide insight into the life history and stress response of *Sphaerium simile*, a brooding bivalve that remains under studied.
MONTCLAIR STATE UNIVERSITY

Do Density and Food Availability Influence Survivorship and Reproduction in the Freshwater Bivalve, *Sphaerium simile*?

by

Lauren O’Neil

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Do Density and Food Availability Influence Survivorship and Reproduction in the Freshwater Bivalve, *Sphaerium simile*?

A THESIS

Submitted in partial fulfillment of the requirements
For the degree of Masters of Science in Marine Biology and Coastal Studies

By
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Introduction

The phenological traits of organisms form the basis of a dynamic ecosystem (Cadotte et al. 2011). Studying the life history of organisms is critical to understanding ecosystem structure and functioning (Moore et al. 2013). Many studies have explored various aspects of invertebrate life history (Strathmann 1985, Coma et al. 1998, Ramírez Llodra 2002, Olabarria & Ramírez-Llodra, 2004). The r-K continuum, coined by McArthur and Wilson (1967), was developed as a model for natural selection originally based on density dependence (Boyce 1984). This original paradigm has been expanded to include other aspects that influence life history. The variability of the environment and predation are features that influence whether a population evolves more r or K-selected life history traits. Life history traits such as mortality, reproduction strategy, and body size also contribute to the evolution of species along the r-K continuum (Reznick et al. 2002 & Pianka 1970). On one extreme, r-selected species generally produce many offspring with little parental investment, while K-selected species produce relatively few offspring with considerable parental investment in each (Mclain 1991). K-selected species generally persist in low densities while r-selected species are periodically found in high densities (Boyce 1984).

*Sphaerium simile* Say (1817) is a freshwater bivalve mollusc in family Sphaeriidae (pea clams or fingernail clams). They are cosmopolitan and important components of the benthos (Gale & Lowe 1971). Fingernail clams are prey items and nutrient cyclers in the ecosystem (Hornbach et al. 1982), and in some cases, provide substratum for other bivalves such as the invasive zebra mussel, *Dreissena polymorpha* Pallas (1771) (Dreissenidae) (Lauer & McComish 2001). Although clams of this species can survive in a range of conditions, they usually burrow in muddy substrata of clear, fresh, standing water between 16-20°C, with 601-900 mm rainfall at an altitude between 1001-1500 m (De Kock & Wolmarans 2008). Once adults, specimens of *S. simile* can grow as large as 16 mm in length and becomes reproductively mature when individuals are 9 mm in length. The species is capable of filter feeding
directly from the water column as well as deposit feeding from sediments (Zumoff et al. 1973).

Surprisingly, *S. simile* exhibits greater reproductive success in water with low dissolved oxygen ranging from 0.75-0.93 mg/L (Joyner-Matos et al. 2011). Joyner-Matos et al. (2011) suggest that this may be due to the small size of this species, and the absence of free radicals in hypoxic conditions. In *S. simile*, elevated dissolved oxygen increases the frequency of free radicals and may induce more stress when individuals must repair damaged tissue (Joyner-Matos et al. 2011).

Sphaeriidae exhibit characteristics that are intermediate on the *r-K* continuum, thus exhibiting characteristics of both *r* and *K*-selected species. *K*-selected characteristics of sphaeriids include euviviparity, brooding developing offspring internally in marsupial sacs between the parental gills, and release of live young. This is an unusual reproductive strategy for bivalves, as most marine bivalves are oviparous (Korniushin & Glaubrecht 2003). This strategy is more common in freshwater bivalves however it is observed in certain families of marine bivalves (Philobryidae) (Prezant et al. 1992). Typically, species that are more *K*-selected have density dependent mortality and have stable populations. For example, *K*-selected populations of the fingernail clam *Pisidium casertanum* Poli (1971) (Sphaeriidae) inhabit permanent lakes, have longer generations, and exhibit smaller litter sizes than conspecifics in a temporary pond (Bailey & Mackie 1986). Sphaeriids are usually found in high-density populations, with individuals coexisting in crowded biotic conditions. Relatively *r*-selected characteristics of sphaeriids include a short lifespan of only about two years (Avolizi 1976 & Zumoff et al. 1973). They are sexually mature and capable of reproduction after reaching an absolute minimum body size of 9 mm. Offspring ready for release are rarely found in adults less than 13.5 mm in length, and adults are rarely larger than 17mm (Zumoff et al. 1973). Adults can brood up to eight individual offspring simultaneously. Also, there is no post-embryonic parental care for the offspring after release. These clams can reproduce throughout the year across multiple seasons, although production of gametes is most active in the spring or summer. Adults are also simultaneous hermaphrodites, and sequential
brooders often contain multiple offspring in different stages of development. Brooding is asynchronous, and multiple offspring of several sizes can often be found brooding in the adult (Guralnick 2004). *S. simile* populations exhibit two major birthing periods—one in the summer and one in the winter (Zumoff et al. 1973).

Sphaeriids typically inhabit freshwater streams and ponds. One major element of pond ecology is seasonal variability (Verma et al. 2016, Gurleyen & Ustaoglu 2017). Food availability is determined in large part by light intensity and temperature that changes with seasons (Seale 1980). Productivity of common phytoplankton is highest in seasons with maximum light intensity, which stimulates photosynthesis, and warmer temperatures which increases metabolism (Jiménez et al. 2003). High seasonal productivity is associated with major birthing periods of *S. simile*. Competition for food during a major birthing period not only influences adult fitness, but competition between retained siblings as well. (Beekey & Karlson 2004).

Populations of *S. simile* exhibit a number of characteristics unique among bivalve species, and little is known about resource allocation and reproductive strategies among individuals in different densities or with different levels of food availability. One of the factors associated with the r-K continuum is density. Life history plasticity is the ability of an organism to adjust life history traits and strategies in response to external factors. For example, Johansson et al. (2001) illustrated the life history plasticity of the damselfly *Lestes sponsa* Hansemann (1823). The damselfly changed foraging behaviors and experienced a decrease in mass and development rate when exposed to predators. Whether *S. simile* can exhibit life history plasticity along the r-K continuum when living at different population densities is presently unknown. Reproductive effort of *S. striatinum* has been investigated based on food availability (Beekey & Karlson 2003), but a study on *S. simile* with manipulations of both density and food availability has not been conducted. Bivalves are an important component in the food web, and
this study is especially important because biology of freshwater invertebrates remains under studied compared to terrestrial and vertebrate animals (Moore et al. 2013).

Natural populations of S. striatinum are typically found existing in high-densities; anywhere from 65.2-482.2 individuals per m² depending on season (Hornbach et al. 1982) while reproducing during seasons of high food availability. This study investigated potential plasticity in life history traits like growth, reproduction, and survival of S. simile in response to differences in population densities and food availability. The null hypothesis of the present study (H₀) is that there will be no change in reproductive effort, growth rate and survivability of brooders, and quantity or size of retained and released offspring in different density and feeding treatments. Alternatively, (H₁) there may be associations between treatments and reproduction rates, growth rate and/or survivability of adults, and quantity and/or size of released and retained offspring. For example, we might expect a plastic response in certain life history traits, as increased mortality caused by draining water from their habitat has been recorded in the sphaeriids Pisidium moitessierianum Paladihe (1866) and P. supinum Schmidt (1851) (Mouthon 2011) and different food availability treatments have influenced brood size in Sphaerium striatinum Lamarck (1818) (Beekey & Karlson 2003). I predicted that reproduction would be negatively associated with increasing density. This prediction would indicate competition among brooders for the same amount of resources. My second prediction was that increased food availability would be positively correlated with reproduction. This would indicate reduced competition between adults and retained offspring.
Methods

Laboratory experiment

Clams used for this experiment were collected from a pond in Byram Township, New Jersey, USA, in May and June of 2017 (40°59'22.29"N, 74°40'37.06"W). They were transported to the laboratory in large plastic buckets containing pond water and placed in an environmental chamber at 16.7°C and kept on a light-dark schedule of 14L:10D. Collected clams were measured using a digital caliper and were sorted by size in 0.5 mm increments. Only individuals ranging from 11.5-13.5 mm in length were used in the study and were randomly assigned to the treatment groups described hereafter. Individuals were identified by labeling the shell with a number using a sharpie marker. An ingredient in sharpie markers, Propyl Alcohol, has the potential to be toxic to marine life. However, other ingredients such as Butyl Alcohol and Diacetone Alcohol has low toxicity to aquatic invertebrates. Experimental clams were placed in 150 mL glass beakers filled with aged tap water in order to control food supply. They were then placed in an environmental chamber at 20°C and a photoperiod of 14L:10D.

Clam density and feeding frequency were manipulated across four experimental treatment groups. Each manipulated variable had two states (low and high). Density treatments were 0.07 clams (low) and 0.35 clams (high) per cm². Feeding frequencies were once per 7 days (low) and continuous (high). All treatments were replicated with 10 independent sets of individuals for a total of 40 replicates. Clams were fed fish flakes that were ground into powder. Continuously fed clams had 0.1 grams of Omega One Freshwater Fish Flakes™ available to them at all times, and the low treatment groups were fed 0.25 grams for six hours on the designated feeding day(s). Beakers were cleaned with hot water and mechanical scrubbing and received a 100% water change twice each week.

The experiment began on July 7th, 2017 and continued through September 26th, 2017. The length (maximum distance on the anterior–posterior axis) and height (maximum distance on the dorsal–ventral axis, across the shell middle) of every adult individual were recorded as per Gaspar et al. (2002).
once each week of the study period. Adult mortality, week of offspring release, and released offspring size (length and height previously described) and quantity were recorded upon release. If a clam perished during the experiment, it was replaced by another clam of similar size from a stock supply to keep all treatments consistent. Water quality testing for hardness was performed for both pond and tap water using LaMotte Aquaculture Test Kit™.

Upon termination of the experiment, all experimental clams were frozen, weighed and then dissected. Dry weights could not be determined directly since retained offspring were removed from adults upon dissection and tissue loss occurred on petri dishes and scalpels, rendering dry weights inaccurate, and were estimated as per Singh et al. 2012. Quantity of retained offspring in each individual adult was immediately recorded, and size of retained offspring was determined using Image J photo processing software (Schneider 2012).

Field experiment

To examine whether laboratory conditions influenced the outcome of the experiments, a parallel field experiment was conducted from July 14th 2017 to October 20th 2017. Clams 11.5-13.5 mm in length were used in three different population densities in ambient pond conditions at the collection site, with three replicates of each. Each replicate was placed in a 31.75 cm-long, 7 cm-wide 1.5mm plastic mesh pouch made of polyethylene. The pouches were attached with plastic zip ties inside a PCV pipe 31.75 cm long and 5.08 cm in diameter. The PVC pipe was perforated with 5mm holes spaced approximately 1-5.08 cm apart randomly located around the enclosure to facilitate water flow. All nine PVC pipes were connected with string, submerged, and secured to nearby shore vegetation. The enclosures rested atop the benthos since the area available for installment of the field experiment had rocky substrate. Positions may have shifted during the experiment due to naturally occurring variation in water level, flow and settlement.
The enclosures were undisturbed by lab staff until the end of the experimental period. No biofouling preventative measures were taken. Length and height of clams at the start of the experiment and on the day the experiment ended were recorded. Length, height, number of offspring per enclosure, and mortality of adult clams were recorded when the experiment ended.

Upon termination of the experiment, all clams were frozen, weighed and then dissected. Dry weights could not be determined directly because brooding offspring were removed upon dissection which resulted in tissue loss making any dry weights inaccurate. Dry weights were estimated as per Singh et al. (2012). Retained offspring were removed from the adult. Number of offspring in each individual was immediately recorded, and size of retained offspring was later determined using Image J (Schneider 2012) photo processing software.

Statistical Analysis

Statistical analyses for the laboratory experiment were performed in JMP Pro 13 statistical software (SAS Institute, Cary, North Carolina, USA). To analyze growth, length, height, and dry weight were used to estimate biomass using a regression analysis. Because growth data are repeated measures, sizes of individuals in all treatment groups across thirteen weeks were analyzed using a MANOVA.

The remainder of the laboratory analyses were done using a full factorial analysis of all independent variables on all responses using least mean squares approach in JMP with the Tukey HSD or Student’s t-test procedure at $\alpha=0.05$. Density, food availability and the interaction of density and food availability were analyzed as the independent variables and adult mortality, quantity of offspring released, size of offspring released, size of retained offspring, and quantity of retained offspring were analyzed as dependent variables. Only clams that survived the entire duration of the experiment were considered in growth and retained offspring analyses.
Results

In total, 92 clams died during the laboratory experiment and 6 clams died in the field experiment (Figure 1). Thirty five (35) offspring were released in the laboratory and fifteen (15) offspring were released in the field (Figure 2). One hundred and fifty three (153) offspring were found retained in surviving clams in the laboratory and seventy eight (78) offspring were found in surviving clams in the field (Figure 3). There was a grand total of 6, 18, and 30 clams in low, moderate, and high field study density treatments respectively. There was a grand total of 20, 60 and 100 clams in low, moderate and high laboratory study density treatments respectively.

Mortality

Laboratory experiment

Analysis of Variance (ANOVA) revealed a significant main effect \[ F(3, 36) = 5.9638, p<0.0001 \] of treatment on mortality. Effects tests revealed significant main effects of density \( F = 7.98, p=0.0077 \) (Figure 4) and food availability \( F = 8.76, p=0.0054 \) (Figure 5) on mortality. No significant interaction between density and food availability was found \( F = 1.16, p=0.2891 \) (Figure 6). Post-hoc analyses indicated significantly higher mortality in high density treatments and low food availability treatments. There is evidence of high survivorship in low density/high food availability compared to other treatments.

Field experiment

ANOVA revealed no significant overall effect \[ F(2, 6)=1.7505, p=0.2519 \] of density on mortality (Figure 7). There was a pattern of higher mortality in low density treatments than moderate or low density treatments, but the pattern was not statistically significant.

Quantity of offspring released
Laboratory experiment

ANOVA did not reveal a significant overall effect [F(3, 36)=0.5857, p=0.6283]. Effects tests also did not reveal significant main effects of density (F =0.0040, p =0.9501) (Figure 8), food availability (F =1.75, p =1.7492) (Figure 9) or the interaction of density and food availability (F =0.0040, p =0.9501) (Figure 10).

Field experiment

ANOVA did not indicate a significant overall effect [F(2, 6)=2.3108, p=0.1803] (Figure 11). There was a pattern in which high density treatments had more offspring released than moderate or low density treatments, but the pattern was not statistically significant.

Size of offspring released

Lab experiment

ANOVA revealed a significant overall effect [F(3, 15)=6.8159, p=0.0041]. Effects tests revealed a significant main effect of density (F =16.24, p=0.0011) (Figure 12). No significant effect of food availability (F=1.73, p=0.2079) (Figure 13) or the interaction of density and food availability (F=0.012, p=0.8944) (Figure 14) was found. Post-hoc analyses indicate that clams released larger offspring in low density treatments.

Field experiment

ANOVA did not reveal a significant overall effect [F(1, 2)=0.7248, p=0.4842] (Figure 15). There was a pattern in which clams in the high-density treatment released larger offspring than clams in the moderate density treatment, but the pattern was not statistically significant.

Quantity of retained offspring
Laboratory experiment

ANOVA revealed no significant overall effect \( [F(3, 36)=0.1772, p=0.9111] \). No significant main effects of density \( (F=0.36, p=0.5534) \) (Figure 16), food availability \( (F=0.16, p=0.6894) \) (Figure 17) or the interaction between density and food availability \( (F=0.01, p=0.9157) \) (Figure 18) on quantity of retained offspring.

Field experiment

ANOVA revealed no significant overall effect \( [F(2, 6)=2.6453, p=0.1501] \) (Figure 19). There was a pattern in which clams in the low density treatment retained fewer offspring than moderate or high density treatment, but the pattern was not statistically significant.

Size of retained offspring

Laboratory experiment

ANOVA revealed no a significant effect \( [F(3, 26)=0.6361, p=0.5985] \). Effects tests revealed no significant effects of density \( (F=0.18, p=0.6767) \) (Figure 20), food availability \( (F=1.14, p=0.2946) \) (Figure 21) or the interaction between food availability \( (F=1.1, p=0.3046) \) (Figure 22) on size of retained offspring. There was a pattern in which larger offspring were retained in high food availability treatments, but the pattern was not statistically significant.

Field experiment

ANOVA revealed no significant effects \( [F(2, 4)=1.1227, p=0.4102] \) (Figure 23). There was a pattern in which larger retained offspring were observed in high and moderate densities, and smallest retained offspring were observed in low density, but the pattern was not statistically significant.

Growth
**Laboratory experiment**

MANOVA revealed no significant difference of biomass between treatments over time \( [(F(8, 77)=1.1306, p=0.3526) \), but there were significant within-subjects differences in biomass over time [\( F(12, 66)=17.3444, p<0.0001 \)]. All treatments exhibited similar growth patterns over time and changed biomass only vary slightly. The general pattern was that clams in most treatments increased in biomass from start to end with the exception of clams in the low density/low food availability treatment, which decreased over time (Figure 24).

**Field experiment**

MANOVA revealed no significant difference in change of biomass between treatments over time \( [(F(2, 6)=3.6494, p=0.0918) \). All treatments showed similar growth patterns and appear to have grown more than clams in the laboratory experiment. Clams in all treatments increased in biomass from beginning to end of the experiment (Figure 25).

**Water Quality**

pH and nitrite nitrogen were similar in pond and tap water samples. Pond water had higher hardness, alkalinity, chloride and ammonia nitrogen than tap water (Table 1).
Discussion

Effect of treatment on survivorship in the laboratory

As expected, clams in high food availability treatments had lower mortality than clams in low food availability treatments. High food availability treatments probably provided the most nutrients and energy per individual, and those animals were able to sustain basic biological functions and possibly allocate more resources to reproduction. These results are consistent with other studies. For example, the clam *Arctica islandica* Linnaeus (1767) (Arcticidae) exhibited higher mortality under low food availability relative to other feeding treatments (Ballesta-Artero et al. 2017). *A. islandica* can live up to 500 years or longer (Pace et al. 2018) which falls toward *K*-selection on the *r*-*K* continuum. Even though there is a stark contrast in lifespan between *A. islandica* and *S. simile*, a steady supply of food is necessary for survival.

According to Peterson (1982), a hierarchy of effects occurs when density of infaunal bivalves is increased. Under natural conditions, the clam *Protothaca staminea* (Veneridae) in high densities will emigrate from higher density patches to lower density patches. This seems like a purposeful migration which reduces intraspecific competition. If this does not sufficiently reduce density, growth rates and reproductive effort will decrease. If competition remains too high, starvation will likely occur and result in increased mortality. In the current study, *S. simile* exhibited negative density dependence. Clams in high density treatments experienced higher mortality than clams in low density treatments. In the laboratory experiment, high densities were kept constant at all times and individuals were unable to migrate to other populations. Therefore, heightened competition for space and food in high density treatments may have had a greater effect on mortality.

Although mortality of *S. simile* was associated with both density and food availability in the laboratory experiments, there were no significant interactions between the two independent variables.
Clams in the low density/high food availability treatment experienced the least mortality while clams in the high density/low food availability treatment experienced the most mortality, but the combined effects of density and food availability were not greater than each effect considered separately.

Effect of treatment on quantity of offspring released and retained in the laboratory

The quantity of offspring released was similar to quantity of offspring retained among food availability treatments. Individuals in high food availability treatments released more offspring and contained more retained offspring than clams in low food availability treatments. This indicates that adequate food increases the rate of embryo formation and development in S. simile. In the current study, clams in high food availability treatments released about twice as many offspring per capita than clams in low food availability treatments. This is also the pattern exhibited by S. striatium, in which brood size and number of marsupial sacs are positively correlated with high food availability (Beekey & Karlson 2003). It should be noted that Beekey & Karlson (2003) reared juveniles to reproductive maturity, whereas in the present study reproductively mature clams were studied. These similar results between the two studies indicate that density and food availability influence offspring production at several life history stages in sphaeriid clams.

In the present study, patterns in the quantity of released offspring and quantity of retained offspring were not the same under different adult density treatments. Density did not appear to influence the quantity of offspring released. Both high and low density treatments were associated with a similar number of offspring released per clam. Similarly, low density/high food availability and high density/high food availability treatments resulted in almost identical quantities of offspring released while low density/low food availability and low density/high food availability also were associated with almost identical quantities of offspring released.
Although not statistically significant, there appeared to be a pattern in which quantity of retained offspring was marginally associated with density. More offspring were retained in clams in low density treatments than high density treatments. The observation that quantity of retained offspring may be affected by density while quantity of released offspring is not, suggests that the number of developing offspring in marsupial sacs does not influence the rate at which offspring are released. Hence clams in low densities with more offspring retained seem to not prematurely displace larger offspring. This does not seem to be the case in *S. striatinum*, in which competition between retained siblings occurs (Beekey & Karlson 2004). Nutrient availability and density of *S. striatinum* embryos influence offspring development *in vitro* (Beekey & Karlson 2004). However, because that study was conducted *in vitro*, the rate of release of metamorphized offspring (from an embryo to exhibiting adult like features such as a foot, demibranchs and shell) could not be directly determined and compared to the results of the present study.

**Effect of treatment on size of released and retained offspring in the laboratory**

Significantly larger offspring were released from clams in low density treatments than in high density treatments. Although not statistically significant, there was also a pattern in which larger offspring were released in low food availability treatments than high food availability treatments. This pattern seems counterintuitive, because we might expect larger offspring in high food availability treatments, with more resources available for offspring production and growth. A possible explanation for this unexpected pattern may lie in the experimental design of this study: it could not be determined whether replacement clams or clams that survived the entire duration of the experiment were responsible for offspring release. Low food availability treatments had the highest mortality and needed many replacement clams. Stock clams were not maintained in the same treatment regimes. Therefore,
replacement clams did not spend the same amount of time in a treatment as original clams. Hence, larger offspring released in low food availability treatments could be a result of replacement clams due to a high level of mortality in these treatments. As expected, larger offspring were released in low density. Retained offspring in *S. striatinum* have been shown to be competent at a size of 2 mm shell length (Beekey & Karlson 2000). Offspring that are released at a larger size reduce the risk of size dependent mortality. Given that both *S. striatinum* and *S. simile* commonly release offspring no smaller than 4 mm shell length, these species exhibit extended parental care. In the present study, individuals in low densities likely face the least competition for resources and can retain offspring for a longer time until they reach a large size.

Although size of retained offspring was not significantly associated with density treatments, individuals in high food availability treatments retained larger offspring than low food availability treatments as predicted. In general, individuals in low density/high food availability had larger offspring than those in low density/low food availability, suggesting that adequate food and less competition allow for production of larger retained offspring.

*Effect of treatment on mortality in the field*

In contrast to the laboratory experiment, highest mortality in the field was associated with low density, followed by moderate density. One possible explanation for this difference from laboratory observations is that filtration rates of the clams in the field enclosures may have been a function of density: more clams in the same volume of water can potentially circulate more water through the enclosure. The enclosures were open and did not have a fixed volume, therefore the definition of volume used here is as if there were no perforations and ends of the PVC pipe were closed. The maximum filtration rate of another sphaeriid clam, *Musculium transversum* Say (1829), is 15.7 ml/hr.
If filtration rates of *S. simile* are similar, given that the volume of the current study enclosure was 643.52 cm³, clams in high densities would have been able to filter 24.4% of the water inside the enclosure in one hour, while clams in low densities would only have filtered 4.9% of the water in one hour. Clams in high density treatments may therefore have had faster replenishment of pond water containing nutrients and oxygen because the enclosure was receiving more water change every hour. According to a model by Newell (2004) increased abundances of bivalves should reduce turbidity, increase intraspecific competition, and reduce oxygen content in sediments in flow through systems. However, the current study does not align with those predictions, because high density treatments had the least mortality and the highest offspring release, indicating that intraspecific competition did not influence clams in the present field study.

Density dependent mortality of bivalves in field conditions can also be attributed to differences in feeding strategy. For example, competition between a deposit feeder, *Macoma balthica* Linnaeus (1758) (Tellinidae) and a suspension feeder, *Cerastoderma edule* Linnaeus (1758) (Cardiidae) was evaluated by Kamermans et al. (1992). Competition occurred in deposit feeding communities but was not as prevalent in suspension feeding communities. Because *S. simile* is both a deposit feeder and filter feeder, the ability of this species to exploit nutrients in sediments and the water column could presumably reduce competition.

*Effect of density on released and retained offspring in the field*

Quantity of released offspring in field conditions varied by density treatment. Most and largest offspring were released in high density treatments, followed by the moderate density treatment. No offspring were released in low density treatments. Similarly, more retained offspring and larger retained offspring were discovered in high and moderate density treatments than in low density treatments.
These effects were potentially associated with mortality events. High mortality in the treatment with the smallest sample of clams could consequently reduce perceived reproductive effort. Because low density treatments only contained six clams in total, even minimal mortality could notably decrease detected reproduction.

It is also possible that in this case, *S. simile* exhibits positive density dependence in the field because of an increase in filtration rate in high densities already mentioned. It is unlikely that mate limitation was experienced, since in the laboratory density had little influence on number of retained offspring. Another fingernail clam, *Pisidium amnicum* Muller (1774) (Sphaeriidae), is capable of adult self-fertilization as well as cross fertilization (Araujo & Ramos 1997). If this is also the case for *S. simile*, this would help explain why density of adults had only a minimal effect on number of retained offspring.

*Effect of treatments on growth in laboratory and field*

There were also different patterns of growth in the field in comparison to the laboratory experiments. Adult clams did not exhibit substantial growth in laboratory conditions, but grew noticeably larger in the field enclosures over time. This might have been influenced by insufficient calcium in the tap water used in the laboratory experiment. When water hardness was tested, the pond water had 300 ppm while the tap water had 96 ppm. This suggests there was not enough calcium in tap water for the clams to adequately develop and grow their shells. Bivalves use hydrophobic silk gel and acid rich proteins to construct the shell (Addadi et al. 2006). These acidic proteins require calcium in order to form the minerals within the matrix (Worms & Weiner 1986). The first layer of shell to form is the periostracum, followed by, in many molluscs, the prism layer. The periostracum contains amorphous calcium carbonate, while the prism layer contains crystalline calcium carbonate, making calcium
important in multiple stages of shell development (Jacob et al. 2008). If clams in tap water did not have access to sufficient calcium, they would have been unable to continue this process.

In a similar experiment, *S. transversum* (Sphaeriidae) grew rapidly in both field and laboratory conditions (Gale 1977). However, water from the natural river habitat was used in both experiments. Patterns of growth might also be associated with the types of habitat sphaeriids inhabit. Rapid growth is advantageous to animals living in temporary ponds, such as certain populations of *S. transversum*. The population of *S. simile* used in the current study inhabits a permanent pond, which may help explain relatively slow growth.

**Conclusion**

In the laboratory experiment, there were apparent differences in competition between treatments. Heightened competition in high density treatments presumably resulted in lower survivorship and lower offspring production. Treatments with high food availability typically resulted in greater survivorship and offspring production. This expected outcome was not seen in the field, however, possibly because of different nutrient levels and water replacement rates in the animal enclosures. Water in the laboratory experiment lacked hardness and was completely replaced once each week. Pond water was presumably richer in nutrients, but the elongated enclosures may have restricted water flow. Although water flow was not determined quantitatively, anecdotal observation indicates the flows were very slow. Stagnated water may have accumulated in the enclosures.

There appeared to be associations between treatments (combinations of density and food availability) and reproduction rates, growth rate and survivability of adults, and quantity and size of released and retained offspring. The first prediction of negative density-dependence was somewhat supported in the laboratory experiment, but not in the field experiment. In the laboratory, highest
mortality was observed in high density treatments, but offspring production did not show any clear patterns of density dependence. In the field, most mortality was experienced in low densities, and greatest reproductive output was observed in high and moderate densities. The second prediction that food availability would positively influence survivorship and reproductive output was generally supported in the laboratory experiment. Animals in high food availability treatments produced more offspring and had higher survivability compared to the other treatment. Overall, the same patterns of life history plasticity were not observed in the field as in the laboratory. These differences may be due to discrepancies in water flow/quality and error in accounting for released offspring in laboratory treatments. These results indicate that the effects of density and food availability can be interactive, or depend on environmental context, and may therefore be less predictable than general life history might expect.
Literature Cited


Mouthon, J. (2011). Response of bivalve populations to drying disturbance and life history traits of two *Pisidium* species (Bivalvia: Sphaeriidae) in a reservoir of the French Upper Rhone river. *Annales de*


Total mortality was highest in the high density treatments and lowest in the low density treatments. There was a grand total of 6, 18, and 30 clams in low moderate and high field study density treatments respectively. There was a grand total of 20, 60 and 100 clams in low, moderate and high laboratory study density treatments respectively.

Most offspring were released in high density treatments.
Figure 3 FS designates field study treatments. Most retained offspring were found in high density field study and high density/high food availability laboratory treatment. Least amount of offspring were found retained in low density treatments.
Figure 4 Individuals in high density treatments experienced significantly more mortality than individuals in low density treatments.

Figure 5 Individuals in low food availability treatments experienced significantly more mortality than those in high food availability treatments.
Figure 6 Individuals in high density/low food availability treatment experienced the most mortality while individuals in low density/high food availability treatment experienced the least mortality. Regardless of density, clams in low food availability treatments experienced higher mortality than those in high food availability treatments.

Figure 7 Clams in high density treatments experienced no mortality, while clams in the low density treatment experienced the highest mortality.
Figure 8: Individuals in high and low density treatments released similar quantity of offspring per capita.

Figure 9: Individuals in high food availability treatments released a larger quantity of offspring than individuals in low food availability treatments.
Regardless of density, high food availability treatments released more offspring than individuals in the low food availability treatment. High and low densities released similar quantities of offspring.

Clams in the high density treatment released the most offspring, followed by the moderate density treatment. Clams in the low density treatment did not release any offspring.
Figure 12 Clams in low density treatments released larger offspring than those in high density treatments.

Figure 13 Clams in low food availability treatments released offspring larger than those in high food availability treatments.
Figure 1 Clams in low density treatments released larger offspring than those in high density treatments. Overall, clams in low density treatments released larger offspring than those in high density treatments.

Figure 15 Individuals in high density treatments released larger offspring than individuals in moderate densities.
Figure 2 Individuals in low density treatments retained more offspring than those in high density treatments.

Figure 17 Individuals in high food availability treatments retained larger offspring than individuals in low food availability treatments.
Figure 18 Clams in high food availability treatments retained larger offspring than those in low food availability treatments. In general, clams in low density treatments retained larger offspring than those in high density treatments.

Figure 19 Clams in moderate and high density treatments retained more offspring than clams in low density treatments.
Figure 20 Individuals in high density treatments retained larger offspring than those in low density treatments.

Figure 21 Clams in high food availability treatments retained larger offspring than those in low food availability treatments.
Figure 22. Individuals in high density treatments retained offspring of similar sizes regardless of food availability treatment. Clams in low density/high food availability retained larger offspring than those in low density/low food availability.

Figure 23. Individuals in high and moderate density treatments retained larger offspring than those in the low density treatment.
Figure 24 All clams except those in low density/low food availability show similar growth patterns. Low density/low food availability treatment shows declining growth, while the others show very minimal increasing growth.

Figure 25 Clams in all treatments show an increase in biomass from start to end of the field experiment.
<table>
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<th>Pond Water</th>
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Table 1: Water quality testing results. Pond water exhibited more hardness, alkalinity and chloride than tap water.