**Chapter 1: Evolution and implications of differing reproductive life history adaptations in the context of potential physiological constraints**

**Introduction**

It has been said that evolution functions as a tinker, instead of an engineer (Jacob, 1977). The numerous adaptations we see in the world around us are the result of random mutations that may or may not benefit an individual, population, or species. These adaptations change over time and are limited by their physiological constraints. A classic example is that the lengthening of a cheetah’s leg may allow for faster running, until it is so long that it becomes dangerously susceptible to breaking. When observing the adaptations in of the modern world, it can be difficult to initially determine if the characteristics of present day organisms evolved due to benefits they provided or as a result of unavoidable constraints. The evolutionary history and fossil record can be used to test this distinction, by looking at a lineage’s progression over time and what physiological thresholds historically existed.

One spectrum of adaptations are the types of larvae produced in the class Bivalvia. In most bivalve families, fertilization of eggs occurs externally (Ackerman et al., 1994). Eggs can then form either planktotrophic or non-planktonic, lecithotrophic larvae (Jablonski & Lutz, 1983). Planktotrophic larvae are characterized by little or no egg yolk, high egg production, and minimum energy expenditure in the creation of individual offspring. They are plankton-feeding and stay suspended in the water column for relatively long periods of time, up to several months, experiencing high rates of mortality. In contrast, lecithotrophic (yolk-feeding) larvae are defined by lower egg production, a large egg yolk, and a higher energy expenditure in the creation of individual offspring; they stay in the water column for only a few hours or days, before settling to the benthos. Direct developing larvae, such as brooded larvae, are an additional form of non-planktonic larvae (Jablonski & Lutz, 1983). It was previously hypothesized that larval mode was a strong predictor of speciation and extinction rate in a species, but further analysis revealed that geographic range size was a stronger predictor of those rates (Jablonski & Hunt, 2006). If a species larval type is not a strong predictor of speciation or extinction rates, could the evolutionary history of larval type be the result of a physiological constraint? Are there lineages where larval production type appears to be an evolutionary sink, where in others it appears to be a chance for diversification?

One suggested physiological constraint of larval type possible for a species is maximum adult size (Jablonski & Lutz, 1983). Specifically, the size of the adult females in dioecious species or parent in monecious species, which I will refer to as the larvae-producing adult. The planktotrophic life history mode requires a large production of eggs, as larvae are susceptible to predation in their extended pelagic life stage (Jablonski & Lutz, 1983). Therefore, it is hypothesized that planktotrophic producing species cannot have small larvae-producing adults (Jablonski & Lutz, 1983). The hypothesis of a switch from planktotrophic larvae production to lecithotrophic, as a result of a species growing to a progressively smaller body size has been suggested (Jablonski & Lutz, 1983). Perhaps lecithotrophic and brooding larval production is the result of a physiological constraint of smaller body sizes in larvae-producing adults. How constraining is a minimum adult size against planktotrophic larval production and how common is it to find lecithotrophic and brooding species larger than expected? Further analysis is needed to determine how consistently maximum size of larvae-producing adults corresponds with the type of larvae-produced, suggesting a physiological constraint, and how potential exceptions to this trend get around this constraint. Do these potential exceptions have higher diversification rates?

Adult shells of contemporary animals, as well as fossilized specimens, can be used to determine the larval type of the individual. The prodissoconch, or larval shell gives a record of the size of the adult as a larvae. This study will explore the pattern of evolution of prodissoconch size, to determine if it represents stasis, random walks, or directional evolution in punctuations and shifts (Hunt et al., 2015), as well as explore how these shifts track maximum adult size. The class Bivalvia not only has a lot of contemporary diversity, representing 113 extant families (Combosch et al., 2017), but also an extensive fossil record. The goal of this study is the look at the ancient lineages leading to contemporary families to determine the evolutionary origins and changes of larval mode produced in deep time. These changes will be viewed in the context of the maximum size of the species’ larvae-producing adults, to determine if this physiological trait is a constraint on larval type produced. The null hypothesis is that lineages show no pattern between larval type produced and maximum adult size. This would suggest other costs, benefits, or constraints are acting on these evolutionary changes. The alternate hypothesis is that maximum size of larvae-producing adults corresponds, in least in general, to changes in larval type produced. Then, if specific lineages are able to overcome major physiological constraints found in most lineages based on maximum size, those lineages will be examined more closely to look for potential ways the clade was able to circumvent an otherwise limiting threshold. Their diversification rates will also be compared to see how the evolutionary pathways translate to adaptive change.

**Target Question:** How has the types of bivalve larvae produced evolved in lineages over time and does the maximum size of the larvae-producing parent correspond in predictable ways to changes in these evolutionary path?

**Methods**

The goal of this study is to have all bivalve families represented, with at least three species when possible. In more detail, very diverse families and those with lots of variation in larval type produced will be emphasized by including a higher number of species. Fossils representing ancestors to these modern lineages will also be studied to look at the change of size of the prodissoconchs and maximum size over time. Prodissoconchs I and II will be measured in their maximum length as well as their external surface area to distinguish between planktotrophic, lecithotrophic, and brooded larval development. External surface area of the prodissoconch will be captured and measured using 3-D scans performed with an optical scanner. The size of the prodissoconch in a lineage will be tracked through time.

The changes in prodissoconch size will be compared to evolutionary models to determine if it best represents stasis, random walks, or directional evolution (Hunt et al., 2015). To better understand if the type of evolutionary path changed through time, a path will be tested to with multiple shift in evolutionary patterns possible, to better account for complex lineage histories (Hunt et al., 2015). Model support will be tested using AICc.

The adult maximum size will be measured by the maximum length of the shell, the internal volume of the cavity, and the external surface area, measured using 3-D scans. These measurements will provide information on the changes of the lineage over time. The larvae exist within the “environment” of the larvae-producing adult. This study will determine how a lineage’s evolution of larval type, represented by prodissoconch size, tracks the maximum adult size of that species. How well maximum adult size predicts each type of larva in a lineage will suggest if this physiological trait is acting as a constraint. If a general threshold with notable exceptions is found, the species diversification rates of lineages where maximum adult size appears to constrain larval development type will be compared with lineages that do not.

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**Chapter 2: Differential parental investment in two reproductive life history adaptations utilized within a poecilogonous species, *Alderia willowi* (Gastropoda: Sacoglossa: Limapontiidae)**

**Introduction**

There are many examples of different reproductive life history adaptations. Marine invertebrates exemplify a diversity of egg and larval types, created by different species. Two major categories of marine invertebrate eggs are planktotrophic and non-planktonic, lecithotrophic larvae (Jablonski & Lutz, 1983). Planktotrophic larvae are characterized by little egg yolk, high egg production, and minimum energy expenditure in the creation of individual offspring. They are plankton-feeding and stay suspended in the water column for relatively long periods of time, up to several months, experiencing high rates of mortality. In contrast, lecithotrophic (yolk-feeding) larvae are defined by lower egg production, a large egg yolk, and a higher energy expenditure in the creation of individual offspring; they stay in the water column for only a few hours or days, before settling to the benthos (Jablonski & Lutz, 1983). While intermediate forms between these seemingly dichotomous larval types exist, especially based upon how the categories are delineated along a single spectrum (Allen & Pernet, 2007), they are less common. It is expected that gonadal investment occurs at the cost of somatic growth and potentially individual longevity (Parker et al., 2017). The parental costs and advantages differ based on the type of larvae produced, but appear to provide some amount of trade-off between initial parental input per egg and the number of eggs produced in this larval spectrum. However, it is not clear if these different life history strategies represent equivalent sacrifices of somatic growth for the parent.

Species will typically be adapted to create one form of larvae, but a species able to make multiple forms of larvae present an opportunity to better understand the cost of these differing adaptations. Poecilogonous species have adults that appear the same, but create different forms of larvae, which occurs in several gastropod families (Bouchet, 1989). A poecilogonous species producing planktotrophic and lecithotrophic larva would allow for a within species comparison of energy expenditure between these larval types. *Alderia willowi* (Krug et al., 2007) is a species of hermaphroditic, sacoglossan marine slug with seasonal poecilogony, where most individuals produce lecithotrophic larvae in the summer, around May to September, and a variable percentage of adults lay planktotrophic larvae in the winter, around October to April (Krug et al., 2007). Lecithotrophic eggs are 105 ± 5 μm diameter, within 247 ± 31 μm diameter capsules and a total of 32 ± 12 eggs per egg mass (Krug, 1998; Krug, 2001). Planktotrophic eggs are 68 ± 4 μm diameter, within 121 ± 12 μm diameter capsules and a total of 311 ± 134 eggs per egg mass (Krug, 1998). In the field, eggs representing a “mixed” size condition from lecithotrophic to planktotrophic sizes represented less than 5% of slugs and only in months where planktotrophic egg mass production occurred (Krug et al., 2012). In lab-reared animals, “mixed” egg clutches were uncommon and occurred when individuals were transitioning between reproductive modes (Smolensky et al., 2009). The temperature and salinity lab conditions that cause an individual to produce lecithotrophic, versus planktotrophic, egg masses, appeared to be determined by differing individual environmental thresholds (Krug et al., 2012). Therefore, at a common temperature and salinity condition, some individuals from a population will produce lecithotrophic and others planktotrophic.

This study will utilize *Alderia willowi* specimens collected from egg masses in the same environment and raised in the same laboratory conditions to compare the somatic and resource costs of larval type to the individual parents. These comparisons will be made with laboratory conditions of temperature and salinity levels consistent with that of intermediate seasons of their environment, where some individuals produce planktotrophic larvae and others produce lecithotrophic larvae. In the lab environment, individuals will be raised to adults. This experiment will monitor their intake, the number and type of eggs they produce, the resource costs of the eggs, and the growth and lifespan of the individuals. These comparisons will reveal the impact of parental costs, due to life history adaptations utilized, on parentally influenced growth rates and life spans. Another goal is to determine the differential resource allocation between creating planktotrophic egg masses versus and lecithotrophic egg masses. The null hypothesis predicts that species utilizing different life history strategies will not differ in parentally influenced growth rates and longevity, as well as the resource allocation per egg mass: a lack of significant difference will support a trade-off hypothesis between life history strategies. The alternate hypothesis is that species utilizing different life history strategies will differ in parentally influenced growth rates and longevity, as well as the resource allocation egg mass: a significant difference will suggest that certain individuals have invested more than others in the care of their young. Either outcome will provide a deeper understanding of these life history strategies and more context into why the species *A. willowi* employs these two dichotomous larval production adaptations. To my knowledge this will be the most detailed comparison of parental costs and life history strategies of planktotrophic and lecithotrophic ever conducted.

**Target Question**: Is the parental cost of producing planktotrophic versus lecithotrophic eggs significantly different within *Alderia willowi*? If so, which egg type is more energetically expensive to produce?

**Methods**

*Alderia willowi* lecithotrophic larvae egg masses will be collected wild from estuaries of Southern California. The lecithotrophic eggs are expected to hatch within two days and will be reared for 7-10 days, to allow metamorphosis. Individuals will be allowed to mate in a group and then isolated into individual rearing petri dishes, kept at 16° C and 32 parts per thousand salinity, to encourage divergent egg production in different individuals (Krug et al., 2012).

Time dependent growth will be evaluated over the course of the experiment between individuals producing lecithotrophic and planktotrophic eggs. For both groups, 240 individuals will be raised. Every two weeks, 60 individuals will be randomly chosen. The wet, dry, and ash-free dry weight (to the nearest 0.01 mg) of these individuals will be compared to test for significant differences between the two groups over the course of eight weeks.

For a smaller set of individuals the egg production, diet, longevity, and mass of *Alderia willowi* will be monitored over the course of each individual’s life. Up to sixty individuals producing planktotrophic and up to sixty individuals producing lecithotrophic will be monitored closely. The wet weight of individuals will be tracked over the course of the individual’s life, by blotting slugs dry and weighing to the nearest 0.01 mg. The amount of host algae, *Vaucheria sp.* in loose filament form, consumed and excreted by individuals will be also monitored. To determine the organic matter going into and out of the specimens, the average wet, dry, and ash-free dry weight of the algae, as well as their excrement will be analyzed. The egg masses produced by monitored individuals, number of eggs produced, egg yolk size, and the type of eggs produced will be monitored over the course of each individual’s life. Egg number and average egg yolk size will be determined using dissection microscopes and photography. These collections and analyses will continue for two months. ANOVA analyses will be used to compare the egg production, diet, longevity, and mass variables of *Alderia willowi* specimens producing lecithotrophic versus planktotrophic larvae.

The two types of egg masses will be compared, based on the total and component resource allocation they represent. The two types of egg masses will be collected over the course of the experiment. They will be sectioned into the components of egg mass casing, connective strands, and fluid filled egg capsules. Additional egg masses will be collected and analyzed whole, after the egg number has been counted. Collected egg masses and egg mass components will be weighed, dehydrated, and stored in a -20 °C freezer, to allow the individually small samples to be amassed. These will be analyzed by their wet, dry, and ash-free weight. This will determine the average organic content of lecithotrophic versus planktotrophic eggs masses, per egg, for comparison.

**Expected Results**

The null hypothesis predicts that species utilizing different life history strategies will not differ in parentally influenced growth rates and longevity, as well as the resource costs per egg mass: a lack of significant difference will support a trade-off hypothesis between life history strategies. The alternate hypothesis is that species utilizing different life history strategies will differ in parentally influenced growth rates and longevity, as well as the resource costs per egg mass: a significant difference will suggest that certain individuals have invested more than others in the care of their young.

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B.

A.

Figure 1. Adult morphology of *Alderia willowi*, with scale bar = 200 μm (A; Krug et al. 2007). Comparison of lecithotrophic eggs (top) and planktotrophic eggs (bottom) of *Alderia willowi* with scale bar 1 = mm(B; Krug et al. 2007).

**Chapter 3: Somatic Growth of Females between Related Species as a Function of Variation of Reproductive Life History Adaptations**

**Introduction**

The life history of a sexual species begins with the production and fusion of parental gametes, and continues through parental care, if any, of the resulting young. This reproductive effort of spending energy to produce genetically related offspring, has two parts (Olderbak et al., 2014). The first component of reproductive effort is mating effort, i.e. the energy input necessary to create the eventual zygote, and the second is the parental (nepotistic) effort toward helping the resulting offspring survive (Olderbak et al., 2014). Because females have to balance their survival and somatic growth with the fitness of their offspring, species can differ in the adaptations of these early life stages and how much maternal care that young receive. The variables of maternal input include how many eggs females produce in their lives, egg morphology, yolk size or other nutritional support to the young, and how long females directly shelter and care for their young. These aspects of maternal input can be used to define the type of life history strategy a species is thought to employ. Species can exhibit a spectrum of life history modes in numerous categories beyond the simple *r-K* life history models (Darling et al., 2012). If life history strategies differ among species in a clade, it raises the questions of how these adaptations affect somatic growth in females. Do differing life history strategies have differing levels of impact on a female’s somatic growth or do they represent a trade-off of costs, being equally taxing on female somatic growth?

Molluscan bivalves offer a unique perspective into these questions. This class comprises 113 extant families across a spectrum of marine, brackish, and freshwater habitats (Combosch et al., 2017). While all species are aquatic and the majority are either filter or deposit feeders, they employ a wide range of life history strategies. In most bivalve families, fertilization of eggs occurs externally (Ackerman et al., 1994). Eggs can then form either planktotrophic or non-planktonic, ‘lecithotrophic’ larvae (Jablonski & Lutz, 1983). Planktotrophic larvae are characterized by little egg yolk, high egg production, and minimum energy expenditure in the creation of individual offspring. They are plankton-feeding and stay suspended in the water column for relatively long periods of time, up to several months, experiencing high rates of mortality. In contrast, lecithotrophic (yolk-feeding) larvae are defined by lower egg production, a large egg yolk, and a higher energy expenditure in the creation of individual offspring; they stay in the water column for only a few hours or days, before settling to the benthos (Jablonski & Lutz, 1983). A third alternative is for the females to brood their larvae internally (Ituarte, 2009), providing shelter after fertilization. All three of these common life history strategies result in juvenile bivalves that grow into adults once the larvae have settled to the benthos. The maternal costs and advantages differ based on the type of larvae produced, but appear to provide some amount of trade-off between initial maternal input per egg and the number of eggs produced. However, it is not clear if these different life history strategies represent equivalent sacrifices of somatic growth for the females.

The bivalve story is further complicated by a fourth life history strategy that is limited to members of the freshwater bivalves order Unionida, a diverse group including the families Unionidae, Margaritiferidae, and Hyriidae. These bivalves are characterized by a parasitic larval state called glochidia. This adaptation differs from other described bivalve life history adaptations. The process begins when the eggs, held in the “marsupial” female gill, are internally fertilized by sperm that the males have released into the water column (Cummings & Graf, 2010). Females brood these fertilized eggs, allowing them to develop into glochidia larvae before transferring them to a host, usually a fish. The length of the brooding process varies between species (Cummings & Graf, 2010). In parasitizing a host for their larvae, the brooding females effectively transfer maternal cost onto hosts, which support and protect their glochidia. The relationship between glochidia and fish is considered parasitic, but the host is primarily used for dispersal while glochidia metamorphose into juvenile mussels and then drop off the host and sink into the substrate (Barnhart et al., 2008; Cummings & Graf, 2010). This parasitic relationship allows these mussels to disperse in a novel way, with a chance of being deposited in an upstream habitat. Freshwater Unionida females use a variety of methods to attract and infect hosts, ranging from passive mucus nets to more active strategies, such as making glochidia packages (conglutinates), lures disguised as food items, or trapping the mouths of hosts (Corey et al., 2006; Zanatta & Murphy, 2006; Barnhart et al., 2008). These different host attraction strategies may represent different levels of cost to the female, beyond the maternal input of brooding time, and therefore represent a unique effect on somatic growth to compare. Additionally, in the family Hyriidae, there are species that produce parasitic, hooked glochidia, while others brood their hookless larvae and release them as juveniles (Miyahira et al., 2017). This dichotomy will facilitate the comparison of brooded-direct-developed-larvae production versus brooded-then-parasitic-larvae within and between related bivalve families. Bivalve females overall have thus evolved planktonic and non-planktonic larval production, non-brooding and brooding strategies, and free-living and parasitic larvae, with different host attraction strategies.

Bivalves represent a spectrum of maternal input per offspring and different maternal adaptations to evaluate. Their shells also provide the means for unique insight into the impact these costs have on the females. Individual bivalves grow by laying down new layers of shell, retaining a record of growth over an individual’s lifetime, which is typically multiple years. One of the major causes of strong growth lines in these shells is the slowdown in growth that occurs annually in temperate areas (winter), producing the thickest growth lines (Dettman et al., 1999; Goewert et al., 2007; Haag & Commens-Carson, 2008). In marine species, which may not experience the seasonal temperature differences impacting temperate, freshwater bivalves, Oxygen-13 isotopic analyses may be necessary to confirm which growth lines are annual (Jones & Quitmyer, 1996). The somatic growth, measured as the increase in shell volume over successive years, and after a female sexually matures will give an indication of the way reproduction is hindering somatic growth. This will be accomplished by comparing the ontogenetic somatic growth of females to conspecific male ontogenetic somatic growth. Somatic growth rate differences between females and males can then be viewed in the context of a species’ life history strategies and adaptations to model how strongly reproduction hinders female growth.

Two bivalve clades will be utilized in this study. The family Arcidae will be studied to compare species with planktotrophic, lecithotrophic, and brooded larvae (Huber, 2010). Also, Unionida females producing glochidia will be compared to evaluate the impact of this larvae type based on brooding time length and host attraction strategy (Watters et al., 2009), those that have nonparasitic glochidia will allow further insight into these adaptations (Miyahira et al., 2017). Variables of maternal input include how many eggs females produce annually, egg morphology, and yolk size, as well as, length of brooding time and the type of host attraction strategy used, where applicable. These reproductive life history adaptations will be used to give context to potential differences in somatic growth rates, compared between related species.

I will evaluate multiple species within the chosen bivalve clades, searching for evidence of a difference in female-based growth hindrance among species having different life history adaptations. Environmental effects on morphology will be controlled for by limiting comparisons of males and females of a species to shells taken from a single collecting event. Care will be taken to ensure between species comparisons will focus on species taken from analogous habitats. The dissertation will also analyze gonadosomatic index (GSI) to test for differential energy allocation to gonad production between females and males, with females being potentially hindered by their maternal costs (Parker et al., 2018). For example, a larger male growth rate in comparison to female growth rate is predicted if the maternal cost is high. The analysis will compare the average rate of growth for females and males of a given species in the context of the species life history adaptations. These comparisons will reveal the impact of maternal costs, due to species level life history adaptations, on maternally influenced growth rates. The null hypothesis predicts that species with different life history strategies will not differ in maternally influenced growth rates: a lack of significant difference will support a trade-off hypothesis between life history strategies. The alternate hypothesis is that species with different life history strategies will differ in maternally influenced growth rates: a significant difference will suggest that certain species have invested more than others in the care of their young. Either outcome will provide a deeper understanding of these life history strategies and the evolution of parasitic, glochidia larvae. To my knowledge this will be the broadest and most detailed exploration of maternal costs and life history strategies in bivalves ever conducted.

**Target Question:** Does the hindrance of somatic growth of females, in relation to conspecific males, between related species vary as a function of variation of reproductive life history adaptations?

**Methods**

The goal is a comparison of twenty species in each of the two clades. This study will utilize species that are sexually dioecious, survive multiple years after reaching sexual maturity, be comparable sizes, and represent a spectrum of reproductive life history adaptations for their group. Each species will be collected from the field, with the exception of museum collections being used for *Lampsilis cardium*, *Lampsilis* siliquoidea, and *Epioblasma triquetra* (Table 1)*.* Field specimens will provide information related to how many eggs females produce annually, egg morphology, and yolk size, as well as length of brooding time and the type of host attraction strategy used, where applicable and not known from previous research. The GSI of females and males of each species will also be analyzed using wet weight, dry weight, and average caloric content of tissues. A collection for a species represents the left valve of up to 30 female and 30 male shell specimens from one location. Shells from a given species will be collected from the same location at the same time, to avoid environmentally derived morphological variation.

Comparisons between the growth rates of males and females will be limited to differences within a species collection, to determine the trajectory of sexually dimorphic growth differentials. Specimens will be analyzed morphologically, with simulations of their growth rates over time. Each specimen will have a high resolution photograph taken, using image stacking technology. Each specimen will also be 3-D scanned in an optical scanner to capture a 3-D shape of the individual. The chosen species will also lack extreme ornamentation, to allow the optical scanner to better capture the external shell surface area. The individual’s shell will then be sectioned (Figure 1; Haag & Commens-Carson, 2008), in order to identify the annual growth lines and track changes in the shell thickness over the lifetime of the individual. In marine specimens, confirmation that the growth lines are in fact annual, Oxygen-13 isotope analyses sensitive to seasonal changes in environmental water temperature will be conducted on a subset of shells for each species (Jones & Quitmyer, 1996). These methods will detect whether semi-annual growth lines are present and inform the annual growth line determination of all specimens from that collection region.

The placement of the annual growth lines will be cross-referenced to the 3-D scan of the individual to track the increase in external shell area with each successive year. The thickness of the growth lines along each cross-section will be used to model the shell volume and the internal cavity volume with each successive year. This simulation will assume that the shell thins evenly between cross-sections and toward the edges of the growth lines. Therefore, the cross-sectional information will show the progression of increasing shell thickness over the animal’s life, while the 3-D scan will reveal the increase in external shell area. The combination of both the 2-D and 3-D data will model the ontogenetic growth in volume of the shell, as well as the volume of the shell cavity that reflects the space available for the animal’s softbody.

Females and males of a given species will be compared for each successive year of life. For example, the change in growth rate and shell shape of the average three-year old female *Lampsilis siliquoidea* from its two-year old shell will be compared with counterpart male *L. siliquoidea*, with both taken from the same collection event. To my knowledge, these 3-D methods, as well as the combination of 2-D and 3-D data, have never been used to model bivalve ontogenetic growth. The accuracy of these modeling methods will be assessed, by a detailed study of *Lampsilis cardium*. *Lampsilis cardium* specimens of successive ages, from museum collections, will undergo the same procedures as above to confirm the accuracy from modeling the shell volume and shell cavity volume increase over successive years.

Comparisons based on the sexually dimorphic growth differentials, in relation to reproductive life history adaptations, will be limited to within the phylogenetic bivalve groups chosen. For example, comparisons between species with brooding, lecithotrophic, or planktotrophic larvae will be limited to species in the Arcidae family. Akaike information criterion (AIC) analyses will be used to relate these variables of maternal input include how many eggs females produce annually, egg morphology, and yolk size, sexually divergent GSI values, as well as length of brooding time and the type of host attraction strategy used, where applicable. These reproductive life history adaptations will be used to give context to potential differences in somatic growth rates, compared between related species.

**Broader Impacts**

This analysis will have broader implications in addition to the novel evaluation of maternal costs among diverse life history strategies. Freshwater Unionida species are considered one of the most threatened freshwater groups in the world (IUCN, 2015). This pilot study and dissertation will provide a deeper understanding of their life history from the perspective of the female, as well as potentially identifying which reproductive strategies are the most costly. This information will help us understand the reproductive vulnerability of each species, based on which mothers have their growth more taxed by reproduction, and better inform conservation efforts. For the commercially important species examined among the Arcidae, this study presents a unique way of understanding their life cycles by comparing them to other bivalves. Additionally, the methods proposed to measure 3-D ontogenetic change over the course of a bivalve’s life are also novel. The methods proposed to measure 3-D ontogenetic change over the course of a bivalve’s life could be applied in other studies, such as those examining the ontogenetic impact of environmental or human-caused changes to bivalves or situations where only a few shells from an environment are available.

**Preliminary Data**

**XXXThis will be data from the pilot project when available.**

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Table 1: Candidate species, along with the phylogenetic group they represent, larval type, reproductive information, species size, and proposed location of collection (Watters et al., 2009; Miyahira et al., 2017; GBIF, 2019)

XXX There are a few pieces of information in these tables I am still looking into.

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Host attraction strategy | Glochidia brooding months | Location of collection |
| *Diplodon hylaeus* | Direct development | - | Mid- South America along the east coast |
| *Diplodon paranensis* | Direct development | - | Mid- South America along the east coast |
| *Diplodon delodontus* | - | - | Mid- South America along the east coast |
| *Diplodon parallelopipedon* | - | - | Mid- South America along the east coast |
| *Margaritifera margaritifera* | Released into water column | - | Eastern Canada |
| *Pyganodon grandis* | Mucus suspended | Brood August to May | Midwest USA |
| *Lasmigona complanata* | Conglutinates | Brood September to May | Midwest USA |
| *Tritogonia verrucosa* | Conglutinates | Brood from May to July | Midwest USA |
| *Quadrula quadrula* | Conglutinates | Brood July to August | Midwest USA |
| *Quadrula pustulosa* | Conglutinates | Brood May to July | Midwest USA |
| *Amblema plicata* | Mucus suspended | Brood June to August | Midwest USA |
| *Fusconaia flava* | Conglutinates | Brood from June to August | Midwest USA |
| *Elliptio dilatata* | Conglutinates | Two brooding periods  Regionally timed | Midwest USA |
| *Leptodea fragilis* | Conglutinates | September to July | Midwest USA |
| *Actinonaias ligamentina* | Conglutinates | Brood September to following May-August | Midwest USA |
| *Truncilla truncata* | Conglutinates | Brood April to July | Midwest USA |
| *Potamilus ohiensis* | Conglutinates | Gravid females found year-round | Midwest USA |
| *Lampsilis cardium* | Active lure | First brood July-October  Second brood May-July | Museum collections and shells from Grand River in Lyons, Michigan |
| *Lampsilis siliquoidea* | Active lure | Glochidia released year-round, especially June through August | Museum collections |
| *Epioblasma triquetra* | Females trap snout of host | Brood Mid-September to April and May | Shells from Grand River in Lyons, Michigan |

Table 2. Specimens currently being analyzed from museum collections, courtesy of the Illinois Natural History Survey. The collection location, museum catalog number, and number of specimens being used for this study are provided.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Location | Catalog Number | Number of Males | Number of Females |
| *Lampsilis cardium* | Embarras River | 2781 | 30 | 23 |
| Sangamon River | 7905 | 30 | 20 |
| *Lampsilis siliquoidea* | DuPage River | 9626 | 30 | 30 |
| North Fork Vermilion River | 8578 | 30 | 21 |



3

2

1

Beak and hinge line

Beak and hinge line

Maximum extent of beak

Midpoint

Midpoint

Midpoint

Pallial line and

adductor scar

Pallial line and

adductor scar

Maximum length of shell

Figure 1. Planned methods for cross-sectioning 0.3 millimeters (mm) sections in a given bivalve shell. The first cross-section (1) will be centered on the maximum extent of the beak and intersecting the midpoint of the maximum width of the shell. The second cross section (2) will represent the line created by the anterior point of intersection of the beak with the hinge line and the midpoint between the first cross-section and the intersection of the pallial line with the anterior muscle scar, parallel to the maximum width of the shell. The third cross section (3) will be the line created by the posterior point of intersection of the beak with the hinge line and the midpoint between the first cross-section and the intersection of the pallial line with the posterior muscle scar, parallel to the maximum width of the shell. This example shell is a *Lampsilis cardium* specimen.