**Discussion of Chapter 1: Evolution and implications of differing reproductive life history adaptations on diversification rates in Nassariidae: Gastropoda**

**Initial goal of the chapter:**

To relate maternal care adaptations (ex: larval type) to evolutionary trends (ex: physiological constraints) in a clade. I essentially wanted this chapter to relate the next two chapters to larger evolutionary patterns and get a sense of why these maternal cost differences may or may not matter to an individual parent and to the species overall.

In my search to solidify this chapter’s hypothesis and switch the subject to Gastropoda, which have a more physically resilient larval shell, I came across an interesting study:

Summary: While planktotrophic species are often predicted to have lower speciation rates, a study of Sacoglossan slugs by Krug et al. (2015) using ancestral state reconstruction and estimation of diversification rates, found that both speciation rates were higher and extinction rates were lower in planktotrophic lineages. They suggest that the pattern of lecithotrophic larvae being an evolutionary dead end was not identified in early paleontological studies, because the switch between the character states, from planktotrophic to lecithotrophic, was not counted as a planktotrophic speciation event (Krug et al., 2015). Additionally, while no gastropod adult trait has been previously correlated with a gain in lecithotrophy, they found a correlation in a gain of lecithotrophy with the development of an extra-capsular yolk, analogous to nurse eggs, producing larger larvae from a given size of egg (Krug et al., 2015).

Candidate study system:

The family Nassariidae have species with planktotrophic and non-planktotrophic larvae, are primarily marine, shallow water (0-1,000 m, but mostly 0-300m), mostly tropical, and have had a recent five marker (3 mitochondrial and 2 nuclear) phylogeny done on 218 species in the family, with approximately 350 currently described (Gili, 2015; Galindo et al., 2016). Additionally, nurse eggs are an adaptation within the family (Averbuj, 2015).

Idea A: Krug et al.’s larger finding of higher diversification rates in planktotrophic species could be retested in a clade with a shell fossil record, with a gain of lecithotrophy counted as a planktotrophic speciation. Then additional species traits, such as max body size, latitude, depth, and geographic range could be used to suggest why these evolutionary trends occur.

Issue: This idea may not end up connecting well with the next two chapters.

Idea B (possibly pilot to A): Examine if nurse eggs produce larger larvae in Nassariidae and the connection between nurse eggs and the sizes of protoconch 1 and protoconch 2. Is the signature of maternal care provisioning preserved in the shell?

References

Averbuj, A., Rocha, M.N. and Zabala, S., 2014. Embryonic development and reproductive seasonality of Buccinanops globulosus (Nassariidae)(Kiener, 1834) in Patagonia, Argentina. *Invertebrate Reproduction & Development*, *58*(2), pp.138-147.

Galindo, L.A., Puillandre, N., Utge, J., Lozouet, P. and Bouchet, P., 2016. The phylogeny and systematics of the Nassariidae revisited (Gastropoda, Buccinoidea). *Molecular Phylogenetics and Evolution*, *99*, pp.337-353.

Gili, C., 2015. Revision of the Nassariidae (Gastropoda, Neogastropoda) of the malacological collection of the Museu de Ciències Naturals de Barcelona. *Arxius de Miscel·lània Zoològica*, 13, pp.1-24.

Krug, P.J., Vendetti, J.E., Ellingson, R.A., Trowbridge, C.D., Hirano, Y.M., Trathen, D.Y., Rodriguez, A.K., Swennen, C., Wilson, N.G. and Valdés, Á.A., 2015. Species selection favors dispersive life histories in sea slugs, but higher per-offspring investment drives shifts to short-lived larvae. *Systematic Biology*, *64*(6), pp.983-999.

**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, including fluid and solid excretion. 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.

*XXX*

***TO DO***

*Determine the exact statistical tests to utilize for this study, with consideration as to the assumptions of those tests as I progress through the Stat 220 course.*

References

Allen, J.D. and Pernet, B., 2007. Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy. *Evolution & development*, *9*(6), pp.643-653.

Bouchet, P., 1989. A review of poecilogony in gastropods. *Journal of Molluscan Studies*, *55*(1), pp.67-78.

Jablonski, D. and Lutz, R.A., 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, *58*(1), pp.21-89.

Krug, P.J., 1998. Poecilogony in an estuarine opisthobranch: planktotrophy, lecithotrophy, and mixed clutches in a population of the ascoglossan *Alderia modesta*. *Marine Biology*, *132*(3), pp.483-494.

Krug, P.J., 2001. Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. *Marine Ecology Progress Series*, *213*, pp.177-192.

Krug, P.J., Ellingson, R.A., Burton, R. and Valdés, Á., 2007. A new poecilogonous species of sea slug (Opisthobranchia: Sacoglossa) from California: comparison with the planktotrophic congener *Alderia modesta* (Lovén, 1844). *Journal of Molluscan Studies*, 73(1), pp.29-38.

Krug, P.J., Gordon, D. and Romero, M.R., 2012. Seasonal polyphenism in larval type: rearing environment influences the development mode expressed by adults in the sea slug *Alderia willowi*. *I*ntegrative and Comparative Biology, 52(1), pp. 161–172.

Parker, G.A., Ramm, S.A., Lehtonen, J. and Henshaw, J.M., 2018. The evolution of gonad expenditure and gonadosomatic index (GSI) in male and female broadcast‐spawning invertebrates. *Biological Reviews*, *93*(2), pp.693-753.

Smolensky, N., Romero, M.R. and Krug, P.J., 2009. Evidence for costs of mating and self-fertilization in a simultaneous hermaphrodite with hypodermic insemination, the opisthobranch *Alderia willowi*. *The Biological Bulletin*, *216*(2), pp.188-199.



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, yolk size or other nutritional support to the young, larvae size, 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. 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, eggs can then form planktotrophic or non-planktonic larvae, which can take the form of ‘lecithotrophic’ or brooded larvae (Jablonski & Lutz, 1983; Ituarte, 2009). 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. 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.

Unionida 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). 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.

The Unionida bivalve clades will be utilized in this study. Unionida females producing glochidia will be compared to evaluate the impact of 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, yolk size, and larvae 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 Unionida, 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 just before spawning, 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 in Unionida?

**Methods**

The goal is a comparison of twenty species in Unionida. 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, yolk size, and larvae 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 before spawning 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, with corrections for volume of the shell and shell cavity at each year of growth. 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. Akaike information criterion (AIC) analyses will be used to relate variables of maternal input include how many eggs females produce annually, yolk size, larvae size, sexually divergent GSI values, as well as length of brooding time and the type of host attraction strategy used, where applicable. Comparisons will be corrected for phylogenetic distance and the two continents sampled. 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. 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***

***XXX TO DO*** *This will be data from the pilot project when available.*

*XXX* ***TO DO*** *Additional considerations and predictions not yet incorporated:*

*The comparison of parasitic and nonparasitic as an assumed view of if the species can shift maternal cost may be too simplistic. Need to consider the survivorship and ecology, plasticity of these strategies.*

*Can I capture organic matter in the shell?*

*Chosen variables and what I predict will be the most important, with prediction of correlation to increased differential somatic growth between the sexes. For example, a positive prediction indicates I think a higher value in this variable for a species, will correspond with females being hindered in somatic growth after reproductive maturity.*

Brooding time length (+)

Parasitic or non-parasitic (-, non-parasitic species having > hindered female growth)

Sexually divergent GSI values (+)

Type of host attraction strategy used (+, more active strategies having > hindered female growth)

How many eggs females produce annually (+)

Yolk size (+)

Larvae size (+)

*The phylogeny of the chosen species is not fully fleshed out, so I may need to add a phylogenetic analyses (CO1 and 28S) to the study (8 of the candidate genera and 15 candidate species)*

References

Barnhart, M.C., Haag, W.R. and Roston, W.N., 2008. Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society*, *27*(2), pp.370-394.

Corey, C.A., Dowling, R. and Strayer, D.L., 2006. Display behavior of *Ligumia* (Bivalvia: Unionidae). *Northeastern Naturalist*, *13*(3), pp.319-332.

Cummings, K. S. and Graf, D. L., 2010. Mollusca: Bivalvia. Thorp, J. & Covich, A. (Eds.) *Ecology and classification of North American freshwater invertebrates* (3rd ed.; pp. 309-384) London, UK: Academic Press.

Darling, E.S., Alvarez‐Filip, L., Oliver, T.A., McClanahan, T.R. and Côté, I.M., 2012. Evaluating life‐history strategies of reef corals from species traits. *Ecology Letters*, *15*(12), pp.1378-1386.

Dettman, D.L., Reische, A.K. and Lohmann, K.C., 1999. Controls on the stable isotope composition of seasonal growth bands in aragonitic fresh-water bivalves (Unionidae). *Geochimica et Cosmochimica Acta*, *63*(7-8), pp.1049-1057.

*GBIF: Global Biodiversity Information Facility.* February, 2019. https://www.gbif.org/

Goewert, A., Surge, D., Carpenter, S.J. and Downing, J., 2007. Oxygen and carbon isotope ratios of Lampsilis cardium (Unionidae) from two streams in agricultural watersheds of Iowa, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *252*(3-4), pp.637-648.

Haag, W. R., and Commens-Carson, A. M., 2008. Testing the assumption of annual shell ring deposition in freshwater mussels. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(3), pp.493-508.

Ituarte, C., 2009. Unusual modes of oogenesis and brooding in bivalves: the case of Gaimardia trapesina (Mollusca: Gaimardiidae). *Invertebrate biology*, *128*(3), pp.243-251.

IUCN, 2015. The IUCN Red List of Threatened Species. Version 2015-4. <http://www.iucnredlist.org>.

Jablonski, D. and Lutz, R.A., 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, *58*(1), pp.21-89.

Miyahira, I.C., Santos, S.B.D. and Mansur, M.C.D., 2017. Freshwater mussels from South America: state of the art of Unionida, specially Rhipidodontini. *Biota Neotropica*, *17*(4).

Olderbak, S., Gladden, P., Wolf, P.S.A. and Figueredo, A.J., 2014. Comparison of life history strategy measures. *Personality and Individual Differences*, *58*, pp.82-88.

Parker, G.A., Ramm, S.A., Lehtonen, J. and Henshaw, J.M., 2018. The evolution of gonad expenditure and gonadosomatic index (GSI) in male and female broadcast‐spawning invertebrates. *Biological Reviews*, *93*(2), pp.693-753.

Watters, G. T., Hoggarth, M. A., and Stansbery, D. H., 2009. *The freshwater mussels of Ohio*. Columbus, Ohio: Ohio State University Press.

Zanatta, D.T. and Murphy, R.W., 2006. Evolution of active host-attraction strategies in the freshwater mussel tribe Lampsilini (Bivalvia: Unionidae). *Molecular Phylogenetics and Evolution*, *41*(1), pp.195-208.

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* ***TO DO*** *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 |
| *Pyganodon grandis* | Mucus suspended | Brood August to May | Iroquois River in Illinois |
| *Lasmigona complanata* | Conglutinates | Brood September to May | Midwest USA |
| *Utterbackia imbecillis* | Mucus suspended, but may also direct develop | Brood April to September | Midwest USA |
| *Strophitus undulatus* | Conglutinates | Brood July to May | Midwest USA |
| *Amblema plicata* | Mucus suspended | Brood June to August | Upper Mississippi River in Wisconsin |
| *Leptodea fragilis* | Conglutinates | Brood September to July | Midwest USA |
| *Obliquaria reflexa* | Conglutinates | Brood June to August | Mississippi River in Illinois |
| *Actinonaias ligamentina* | Conglutinates | Brood September to following May-August | Midwest USA |
| *Truncilla truncata* | Conglutinates | Brood April to July | Mississippi River in Illinois |
| *Truncilla donaciformis* | Conglutinates | Brood July to August | 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 |
| *Fusconaia flava* | Conglutinates | Brood from June to August | Upper Mississippi River in Wisconsin |
| *Quadrula pustulosa* | Conglutinates | Brood May to July | Iroquois River in Illinois |
| *Quadrula quadrula* | Conglutinates | Brood July to August | Upper Mississippi River in Wisconsin |
| *Megalonaias nervosa* | Conglutinates and mucus suspended | Brood from September to April | Upper Mississippi River in Wisconsin |
| *Margaritifera margaritifera* | Released into water column | - | Eastern Canada |

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.