Evaluating Reproductive Outputs in Laboratory- and Wild-Mated Dwarf Seahorses (*Hippocampus zosterae*) and Assessing Growth Rates and Survivorship of Laboratory-Born Offspring

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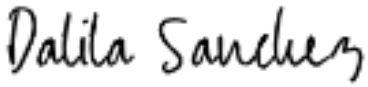

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ABSTRACT

Dwarf seahorses (*Hippocampus zosterae,* Jordan & Gilbert, 1882) are one of the smallest species of seahorse found in coastal seagrass ecosystems in the Gulf of Mexico, Atlantic coast of Florida, and the Caribbean. This thesis addressed the current gaps in life history information for this species by conducting experiments for three main aims to: 1) determine reproductive outputs of laboratory- and wild-mated adult dwarf seahorses, 2) assess morphometrics of juveniles at birth and 100 days of age, and 3) analyze potential impacts of animal handling techniques on survivorship and growth rates of juveniles. Reproductive output and initial offspring sizes of broods from 41 laboratory- and 20 wild-mated males were measured. To evaluate the effects of animal handling on juvenile seahorses during mass and morphometric measurements, offspring from 25 laboratory-mated broods were divided equally into three measurement treatments: photo, weight, and a control group. Average brood size was 33.3 ± 2.1 offspring and ranged from 7 to 71 across the 61 broods, with no difference between laboratory- or wild-mated males. At birth, average offspring length and mass were 9.902 ± 0.024 mm and 0.0019 ± 0.00002 g (n = 61 broods). Final offspring lengths ranged from 18.144 to 39.954 mm, while masses ranged from 0.0105 to 0.1377 g at day 100 (n = 13 broods). Lengths and masses of offspring were positively correlated at both day 0 and day 100. At day 100, all treatments reported around 20% survival (n = 25 broods), with no differences between the animal handling treatments. These results validate the methods of measuring juvenile dwarf seahorses used in this thesis and allow for future studies to measure both variables without affecting offspring survivorship. Overall, this study advanced the knowledge of dwarf seahorse life history traits to enhance conservation efforts for this species, while also providing potential insight for smaller, more vulnerable seahorse species.

INTRODUCTION

Life history trait information is crucial to understanding population dynamics in all species (Cole, 1954; De Roos et al., 2003; Waples et al., 2013). Life history traits, or factors that determine and influence an individual's fitness, consist of reproductive outputs, survivorship, size, and growth that are interconnected with each other (De Roos et al., 2003; Stearns, 1976; Stott et al., 2024). Variables regarding reproduction, such as size at birth compared to size or age at sexual maturity, and lifespan all contribute to an organism's ability to pass on their genes in the next generation. The number of successful matings and offspring throughout their lifetime results in their reproductive success, called fitness. This fitness, and variation between individuals, drives population dynamics and can be an important aspect of conservation biology (Cole, 1954; Waples et al., 2013). There are various experimental approaches that can be conducted in wild and laboratory settings to understand and determine life history traits of species, which can in turn be used to determine baseline densities and fluctuations in populations for conservation efforts (Deevey, 1947; Stearns, 1977).

The family *Syngnathidae,* including pipefish, seahorses, and seadragons, consists of teleost fish with unique life history traits. These fishes have novel morphology compared to other teleosts, with varying shapes, absence of teeth and stomachs, and male pregnancy. They have short life spans relative to other teleost fish, which can generally live 2 to 30 years and over 100 years for some species (Das, 1994). In fact, the longest surviving syngnathid found in the wild was an 8-year-old seadragon (Sanchez-Camara et al., 2011). Syngnathids also have varying mating systems, from monogamy in most seahorses (Foster & Vincent, 2004) to polygynandry in some species of pipefish (*Syngnathus floridae,* Jones & Avise, 1997; *Syngnathus typhle*, Jones et al., 1999), and their reproductive outputs differ from other fish because of the aspect of male

pregnancy. Many teleost fish can spawn great numbers, averaging almost 2 million eggs per brood across 97 species (Duarte & Alcaraz, 1989), or up to 6 million eggs per brood in King Mackerel (Elgar, 1990). Comparatively, syngnathids carry developing embryos within a male brooding area, with a limited carrying capacity (Whittington & Friesen, 2020). One of the largest brood sizes of syngnathids is in seahorses, with 2000 young per brood (*H. ingens*, Foster & Vincent, 2004).

Seahorses, entirely encompassed in the genus *Hippocampus,* include over 40 species across the globe, which vary in size, but have bony plates instead of scales, a horse-shaped head, and a lack of tail fins (Lourie et al., 2016). Seahorses also have a completely enclosed male brood pouch with a single opening for females to deposit eggs into (Whittington & Friesen, 2020). Research across a range of species has indicated that seahorses have social and genetic monogamous mating systems and display conventional sex-roles where the male competes for access to the female (Vincent, 1994). Additionally, like many syngnathids, their life history traits make them vulnerable in their threatened environments (Foster & Vincent, 2004). The entire genus *Hippocampus* was included in Appendix II of the Convention for the International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 2004. Being added to this Appendix implies that seahorses are or will become threatened by human trading (Kuo & Vincent, 2018). It is important to understand the life history information of these species to better improve their conservation efforts.

Adult size is one life history trait of seahorses that has been well documented and varies widely across species. Since masses change for both males and females at differing stages of reproduction, morphometrics of adult size are generally measured in lengths for comparisons across species (Foster & Vincent, 2004). The majority of seahorse species have a size range of over 48 mm (*H. zosterae,* Rose et al., 2019) to

over 300 mm (*H. abdominalis*) in length (Lourie et al., 2004). Seahorses that are smaller than *H. zosterae* are considered pygmy seahorses and may be as small as 13 mm in length (*H. denise,* Lourie & Kuiter, 2008).

Reproductive rates influence population dynamics and have been found to vary greatly across seahorse species (Foster & Vincent, 2004; Lourie et al., 2004). The largest known brood of a seahorse species is from *H. ingens* of approximately 2,000 young (Foster & Vincent, 2004). However, maximum brood size and maximum height across seahorse species are positively related (Foster & Vincent, 2004), and one pygmy seahorse species has been recorded to have 12 to 65 offspring per brood (*H. bargibanti,* Shepherd et al., 2017). Within species, these reproductive outputs are known to vary based on parental sizes (*H. erectus,* Teixeira & Musick, 2001; *H. whitei,* Vincent & Giles, 2003). Additionally, numbers of offspring have been found to vary between wild and captive males, with males in captivity typically giving birth to fewer offspring than those in the wild for species such as *H. guttalatus* (Faleiro & Narciso, 2013).

The first stages of development for seahorses begin in the brood pouch. Eggs with nutritious yolks are deposited into a male brood pouch from a female, and the paternal adult fertilizes and facilitates osmoregulation in the pouch (Kornienko, 2001; Wetzel & Wourms, 2004). Within the brood pouch, offspring undergo multiple developmental stages before being released (Sommer et al., 2012). This includes a stage of egg hatching, after which young remain in the brood pouch to develop (Wetzel & Wourms, 2004). Gestation across seahorse species ranges from 9 to 45 days (Foster & Vincent, 2004), and offspring are then released as free-swimming young (Wetzel & Wourms, 2004).

Once released, many seahorse species have similar sized offspring at birth (Lourie et al., 2004). Most seahorses have average lengths around 7 to 12 mm at birth,

across maximum adult sizes of 100 mm (*H. breviceps*, average size 8.9 mm at birth) to 310 mm (*H. ingens,* average size 8.5 mm at birth) (Foster & Vincent, 2004; Lourie et al., 2004). The outliers include *H. abdominalis,* the largest seahorse*,* known to have a maximum adult size of 350 mm and average lengths around 16 mm at birth, and the smallest pygmy seahorses, including *H. bargibanti* with maximum adult size around 24 mm and average lengths around 2 mm at birth (Lourie et al., 2004). Within species, it has been found that the size of parent seahorses can impact size at birth (*H. kuda,* Dzyuba et al., 2006) and larger brood sizes generally produce smaller offspring sizes (*H. guttulatus,* Faleiro et al., 2016; *H. erectus,* Lin et al., 2012).

Masses of offspring are not as well documented compared to the studies using lengths, making this variable more challenging to comparable across species. Very few studies of seahorse juveniles measure both mass and length to understand correlations or differences between these two variables. Three studies that investigated this relationship found nonlinear correlations between masses and lengths of juvenile seahorses (*H. kuda,* Choo & Liew, 2006; *H. kuda,* Dzyuba et al., 2006; *H. ingens,* Ortega-Salas & Reyes-Bustamante, 2006). Other studies that include both offspring mass and length data focus on effects of certain variables on growth and survivorship rather than the relationship between mass and length.

Growth rates and survivorship are two important life history traits that have been looked at in many seahorse species, mainly through studies that aim to determine the impacts of different environmental stressors. Although many laboratory studies use *Artemia* as a standard food source throughout treatments (*H. kuda,* Hilomen-Garcia et al., 2003; *H. erectus,* Lin et al., 2010; *H. abdominalis,* Martinez-Cardenas & Purser 2007, 2011, 2012, 2016), increased growth and survival rates are commonly seen in juvenile seahorse studies with more nutritious food sources (*H. subelongatus,* Payne &

Rippingale, 2000; *H. whitei,* Wong & Benzie, 2003). Although optimal ranges of variables such as salinity differ between species due to their varying natural habitats, very low salinities have been observed to decrease survival rates across species (*H. kuda,* Hilomen-Garcia et al., 2003; *H. reidi,* Hora et al., 2016). This is consistent even for species inhabiting estuarine systems, such as *H. abdominalis,* which experience fluctuations in salinities (Martinez-Cardenas & Purser, 2016). Temperatures are also determined by species' home ranges, though multiple studies found that higher temperatures (within natural ranges) lead to higher growth rates and higher survivorship in multiple species (*H. erectus,* Lin et al., 2010; *H. abdominalis,* Martinez-Cardenas & Purser, 2011). Across species, photoperiods of 16 or 18 hours of light have yielded higher survivorship and growth rates (*H. reidi,* Hora et al., 2017; *H. abdominalis,* Martinez-Cardenas & Purser, 2012).

Seahorse survivorship has been found to vary across studies in multiple species, not just in relation to controlled stressors. Several studies have reported drastic drops in survivorship and high mortality at young ages (*H. erectus,* Lin et al., 2008; *H. abdominalis,* Martinez-Cardenas & Purser, 2012; *H. reidi,* Pham & Lin, 2013; *H. abdominalis,* Woods, 2000). These deaths are mainly unexplained, and published data of high survivorship after the first few months seem to be rare (Woods, 2000). The few survivorship studies that maintain high survivorship throughout the experiments are only conducted over the course of a few days or weeks (*H. reidi,* Hora et al., 2016; *H. erectus,* Zhang et al., 2015a), or use juveniles that have already survived multiple weeks before starting experiments (*H. kuda,* Hilomen-Garcia et al., 2003; *H. reidi,* Tseng et al., 2020; *H. whitei,* Wong & Benzie, 2003; *H. abdominalis,* Woods, 2003; Woods & Valentino, 2003). Two studies on 3-day-old *H. abdominalis* found lower survivorship in one study (around 60% or lower for all treatments) (Martinez-Cardenas & Purser, 2012)

than the other (all treatments above 66%) (Martinez-Cardenas & Purser, 2007) at the end of 6-week experiments, with laboratory design parameters, such as food type, salinity, light period, and temperature overlapping in one or more treatments. Martinez-Cardenas and Purser explain that this may be due to variance between broods, as the 2007 study only utilized juveniles from the same brood for the survivorship analysis. Overall, this early juvenile survivorship decline continues to be the trend seen across the majority of studies of newborn seahorses (Foster & Vincent, 2004).

One limitation with the current literature for life history traits of seahorses is that most studies were conducted on the larger species in the genus. However, there are at least 7 recognized pygmy seahorses that are also vulnerable in their environments and whose life history traits are important to understand for conservation efforts (Lourie & Kuiter, 2008). The small sizes and cryptic coloration of pygmy seahorses make them challenging to find in the wild, hence causing difficulties studying them *in situ,* while their unique environmental conditions and dependency on host species make them difficult to keep in captivity (Shepherd et al., 2017). Although it is necessary to understand pygmy seahorses' population sizes and dynamics, the difficulties studying them have caused many to be listed as "Data Deficient" on the IUCN Red List (IUCN, 2025). Information collected on other small species of seahorse who are less vulnerable and easier to study could allow for estimations of pygmy seahorse life history traits to enhance conservation efforts.

The dwarf seahorse (*Hippocampus zosterae*, Jordan & Gilbert, 1882) is an excellent model system for investigating life history traits of a smaller species of seahorse as it is considered "least vulnerable" on the IUCN Redlist (Masonjones et al., 2017), has wild populations which have been well monitored seasonally (Masonjones et al., 2010; Masonjones et al., 2019; Rose et al., 2019), and has been successfully bred

and maintained in the laboratory setting (Masonjones, 2001; Masonjones & Lewis, 1996, 2000; Masonjones & Rose, 2019). With adult sizes ranging from 24 mm to over 48 mm in wild populations (Rose et al., 2019), the dwarf seahorse is one of the smallest species of seahorse but is not a pygmy seahorse. Dwarf seahorses have been found to live around 1 year in the wild (Strawn, 1958) and up to 3 years in aquaria (Abbott, 2003). This species has been shown to be socially (Masonjones & Lewis, 1996) and genetically monogamous (Rose et al., 2014). They are also found to be iteroparous throughout the breeding season and will continuously mate with their pair bond (Masonjones & Lewis, 1996, 2000; Rose et al., 2014). Wild populations in Tampa Bay, Florida have been observed as female-biased in sex-ratios (Rose et al., 2019). In the laboratory environment, increasing stocking density and creating male-biased sex-ratios has led to increased competition where males ejected entire broods due to competition (Masonjones & Rose, 2019). When males carry their brood to full term, the gestation period of dwarf seahorses is around 12 days (Masonjones & Lewis, 2000). Brood sizes of the dwarf seahorse range from 3 to 16 offspring in a lab environment (Masonjones & Lewis, 1996), while a maximum of 69 eggs were found in female ovaries and 55 eggs were discovered in a wild-caught male brood pouch (Strawn, 1958).

While there is a growing literature about dwarf seahorse population monitoring and behaviors, there are still gaps in the life history table for this species. For example, the most recent growth and survivorship study of juvenile *H. zosterae* was on a brood born June 11, 1951 (Strawn, 1958). The study measured height of seahorses (from top of crown to tip of tail) and found that newborns ranged from 7 to 9 mm and grew to 18 mm over 17 days (Strawn, 1958). These fish were raised at 85°F with constant light and fed newly hatched *Artemia* (Strawn, 1958). This was the only study found to have any

data on juvenile growth of this species, but it mainly focuses on the reproductive ecology of the adults rather than juvenile growth or survivorship.

The three aims of this thesis are to determine 1) reproductive outputs of wild-mated and laboratory-mated adult males, 2) morphometrics of juveniles at birth and 100 days of age, and 3) whether handling and measuring techniques have an effect of survivorship and growth for juveniles in the dwarf seahorses. Hypotheses include that the laboratory mating setting may have a negative effect on reproductive outputs (Faleiro & Narciso, 2013) due to the stress of the laboratory environment. Further, larger paternal sizes will positively influence brood sizes and body sizes of juveniles (Dzyuba et al., 2006; Strawn, 1958; Vincent & Giles, 2003). A tradeoff between larger broods and smaller offspring is hypothesized, as seen in other seahorse and teleost species (Falerio et al., 2016; Lin et al., 2012). Additionally, if survivorship and growth do not differ between handling treatments, this will support that the animal handling techniques used in the study for measuring seahorses are viable and repeatable.

METHODS

Laboratory Design

Adult seahorses were collected from Tampa Bay, Florida during the summers of 2021 and 2022 and brought to Valdosta State University for mating trails under Florida Fish and Wildlife Conservation Commission's Special Activities Licenses SAL-21-2319-SR and SAL-22-2319-SR. All experiments took place in the aquatic facility at Valdosta State University in accordance with Valdosta State University's Animal Use Protocol AUP-00081-2021 (Appendix A). The lab was kept at 80°F with a 12h/12h light cycle, and all tanks were maintained at 26 ppt salinity throughout all trials. All fish were fed newly hatched *Artemia* brine shrimp daily to satiation, and water changes were performed every eight days to maintain water conditions.

Adult males collected for the mating trials were chosen based on their size and pregnancy status. All males were pregnant when caught in the wild to indicate they were sexually mature. These wild-mated pregnant males were allowed to give birth before they were able to be used in the mating trials. Males that became pregnant from the mating trails were deemed laboratory-mated.

A total of 61 broods were used in this study (Figure 1). Twenty of these broods came from wild-caught pregnant males, and 41 broods were from laboratory-mated males that became pregnant in the laboratory-based mating trials. The 20 wild-caught pregnant males included the first 10 to give birth in the lab after both the June and July 2022 collection trips. The first 10 males to give birth from each collection trip were chosen to minimize the length of time they were housed in captivity and limit any effects of the laboratory settings on pregnancy for the wild-mated males.

All pregnant males, both laboratory-mated (n = 41) and wild-mated (n = 20), used in this study were placed individually into 2.5-gallon tanks and allowed to give birth. A

flowchart shown in Figure 1 outlines how broods were allocated for each experiment. Within 24 hours after the birthing process was complete, all fathers were weighed, brood size was recorded, and offspring were divided into their respective experiments (Figure 1). Group A (broods from 16 laboratory-mated and 20 wild-mated males) were all euthanized using MS-222 immediately after birth to measure initial morphometric patterns. Offspring in Group B (broods from 25 laboratory-mated males) were divided into three treatments, which included photo, weight, or control, to conduct the survivorship and growth experiments. Juveniles for the survivorship trials were housed in either a 2.5-, 5-, or 10-gallon tank, depending on brood size. Tank densities were kept standardized at 2 liters of salt water per 1 fish throughout the experiment. The final Group C offspring consisted of the remaining 13 laboratory-mated broods, a subset of Group B, that were euthanized using MS-222 at the end of the study on day 100 for final morphometrics.

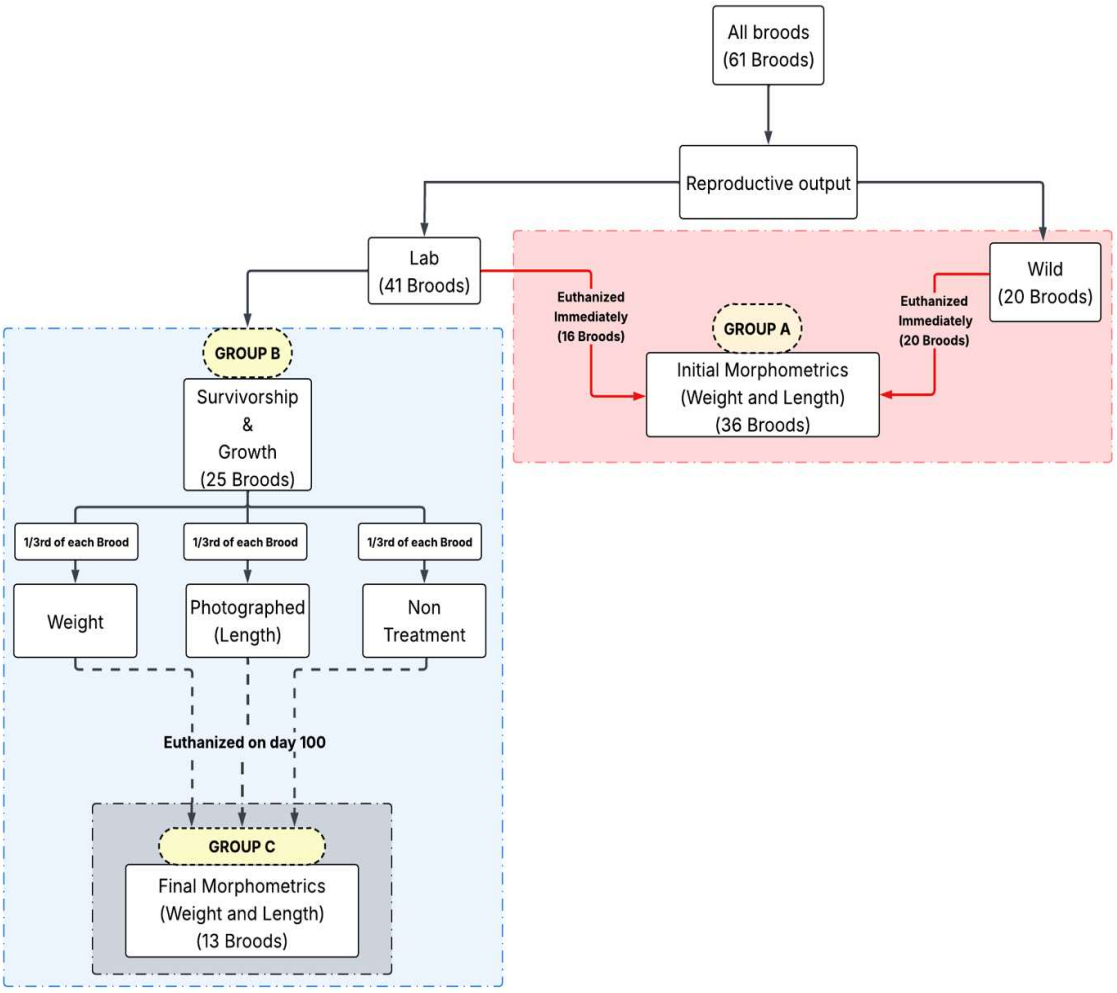

Fig. 1 Flowchart of broods included in study.

For wild-mated males, variation in size was incorporated as much as possible to represent the wild population's natural variation. However, paternal sizes from the laboratory-mated trials were limited due to the size assorted pairings with females required for mating trials conducted for a separate behavioral study. Since maternal sizes were unknown for wild broods, only paternal sizes (ranges provided in Results) were used in this study.

Paternal masses were only measured after males gave birth to reduce the stress of handling on the wild-mated males during their pregnancy. To determine masses of seahorses, males and offspring were individually patted dry on a paper towel before being placed in a weigh boat filled with salt water on the scale, using a Mettler Toledo AG104 Analytical Balance. Masses were taken to the nearest 0.0001 g.

To measure lengths of seahorses, juveniles were individually placed on a petri dish filled with saltwater containing a scale and photographed, using an Olympus Tough TG-6 camera. Photos were analyzed using the ImageJ program (Schneider et al., 2012) to determine standard body lengths according to Lourie (2003). Length measurements included head length [a straight-line measurement from the tip of the snout to the mid-point of the operculum], trunk length [a curved-line measurement from the mid-point of the operculum to the midpoint of the body at the urogenital opening], and tail length [a curved-line measurement from the urogenital opening to the tip of the tail]. Standard length was defined as the sum of all three measurements. Length measurements were taken to the nearest 0.001 mm.

Statistical Analysis for Linear Models

All linear mixed effect models used in this study were run in R (version 4.5.0; R Core Team, 2025) with the same preparation steps. First, the model was assessed for normality of residuals, heteroskedasticity, collinearity, and overdispersion with the Performance package (version 0.13.0; Lüdecke et al., 2021). The lmerTest (version 3.1.3; Kuznetsova et al., 2017) package using Satterthwaite's method provided degrees of freedom and p-values for all linear mixed effects models used. Additionally, backward model selection to evaluate the best models through stepwise removal of non-significant variables (Zuur et al., 2009) and model selection through the dredge function of the

MuMIn package (version 1.48.11; Bartoń, 2025) using AICc scores were used to verify results (model selection and score ranking provided in Appendix B).

Reproductive Outputs

All 61 broods were included in the assessments of reproductive outputs to identify potential differences between laboratory-mated or wild-mated broods and any effect of paternal mass on brood sizes. A linear mixed model was run using the lme4 package (version 1.1.37; Bates et al., 2015) in R (version 4.5.0; R Core Team, 2025) with the “lmer” function. This model used mating location (laboratory-mated or wild-mated) and paternal mass as fixed effects to assess their influence on brood size, with time of birth (month and year as one factor, for a total of 5 factors) accounted for as a random effect. After finding heteroskedasticity in this model, brood size was transformed using a square root transformation.

Initial Day 0 Morphometrics

Offspring lengths and masses were separately assessed across all broods at birth to determine any effects of mating location, paternal mass, and brood size relative to these two independent morphometric parameters. Since all offspring from broods in Group A were weighed and measured for length, compared to Group B that only had one-third of broods measured for each of the variables, the Group A samples were randomly divided into thirds to account for differences in the proportion of broods with each variable measured. These one-third subsections from Group A were randomly selected within R (version 4.5.0; R Core Team, 2025) using the slice_sample function from the dplyr package (version 1.1.4; Wickham et al., 2025) to remove 33% from the initial brood, then 50% of the remaining brood. The first subsection was randomly assigned to mass analysis, and the second subsection was used for length analysis. Due to non-normality of residuals even after transformations, two generalized linear

mixed models, one for offspring length and the other for mass at birth, were made using the lme4 package (version 1.1.37; Bates et al., 2015) with the glmer function including mating location, paternal mass, and brood size as fixed effects with brood identity nested within time as a random effect. The Gamma family with the “log” link was used. Normality of residuals was assessed using visual plots. P-values were taken from summary of the main models. Due to the relationship between brood size and paternal mass, the author would like to note that no collinearity was found in either model for these variables, so both fixed effects were kept in both models.

An analysis of the relationship between lengths and masses utilized the offspring from the 20 wild-caught pregnant males and a subset of 16 broods from laboratory-mated males (Group A, Figure 1) for which all offspring were euthanized at birth to measure both morphometric parameters for each individual. Offspring length and mass at birth were assessed for normality using a Shapiro-Wilk test in R (version 4.5.0; R Core Team, 2025). As a result of the data not achieving normality (length $p < 0.0001$; mass $p < 0.0001$), individual length and mass data were assessed for correlation using a Spearman’s Rank Correlation Analysis run in R (version 4.5.0; R Core Team, 2025).

Final Day 100 Morphometrics

For final morphometric analyses, 13 surviving broods (Group C, Figure 1) of the 25 broods from Group B were analyzed. During 2022, all surviving juveniles were euthanized on day 100 using MS-222, and every individual was weighed and photographed, regardless of treatment. Individuals from this protocol provided the opportunity to determine if the two variables were correlated at day 100 because both length and mass data were collected for all individual offspring.

Lengths and masses were separately analyzed to determine effects of treatment, percentage surviving in brood, paternal mass, and brood size. Linear mixed effect

models were run for each of the morphometric parameters using R (version 4.5.0; R Core Team, 2025) with the function lmer from lme4 package (version 1.1.37; Bates et al., 2015). Brood identity was used as a random effect for both length and mass models. After heteroskedasticity was found with the performance package (version 0.13.0; Lüdecke et al., 2021), the offspring mass was log transformed for its model.

Final offspring length and mass were tested for normality using the Shapiro-Wilk Normality Test in R (version 4.5.0; R Core Team, 2025), and due to non-normality (length $p = 0.967$; mass $p < 0.0001$), offspring length and mass relationships were also assessed for correlation using a Spearman's Rank Correlation Analysis in R (version 4.5.0; R Core Team, 2025).

Survivorship

The Group B subset of 25 broods from the 41 laboratory-mated males were used to assess survivorship and growth rates (Figure 1). All offspring from each paternal male within Group B were divided equally at birth into three treatments: weight, photo, and a control non-handling treatment. At birth and every eight days following, the individuals in the weight treatment were weighed, and the individuals in the photo treatment were photographed. The individuals in the control treatment were not measured or handled. Every four days, the number of surviving offspring was recorded in each treatment. Surviving juveniles were euthanized at the end of the study using MS-222 after 100 days**.**

Survivorship was compared between the three treatments: photo, weight, and control across the 100 days. The survival package (version 3.8.3; Therneau, 2024a; Therneau & Grambsch, 2000) was used to generate survivorship curves. The surv function was used to create the data frame based on day and outcome data. Outcomes were either survival or death, given as 0 or 1, respectively. Each individual offspring was

designated for the model to have a single outcome, either death at the day recorded from survivorship counts every four days or survival at day 100. The survfit function with Kaplan Meier estimations was used to determine the probability of survival over time. Curves were made for each of the three treatments using the survfit function to determine any difference in Kaplan Meier estimations, based on weights of death using G-rho family of Harrington and Fleming (1982) for each of the three treatments by interpreting the χ^2 output for significance.

A Cox mixed effects model was also fitted to the survivorship data to account for differences across replications using the coxme package (version 2.2.22; Therneau, 2024b), as the Kaplan Meier estimations did not account for brood identity across treatments. The Cox model used a hazard ratio, rather than weighted death estimations to determine survivorship, based on the same data frame made by the surv function from the survival package (version 3.8.3; Therneau, 2024a; Therneau & Grambsch, 2000) that the Kaplan Meier estimations used. The hazard ratio interprets the risks of death at each timepoint, unlike the Kaplan Meier estimation. Analysis of differences in survivorship due to treatments, accounting for brood identity as a random effect, was done based on the hazard ratio differences using the output of the coxme function of the coxme package (version 2.2.22; Therneau, 2024b) from likelihood-ratio tests.

The potential effects of treatment on the percentage of offspring surviving were analyzed for days 48 and 96, to identify any potential differences at the midpoint and end of the study. The linear mixed model in R (version 4.5.0; R Core Team, 2025) using the lmer function of the lme4 package was conducted (version 1.1.37; Bates et al., 2015), using treatment as a fixed effect and accounting for brood identity as a random effect, for day 48 and 96 separately.

Growth

Growth curves were created for both length and mass measured over 96 days for offspring from Group B via the AquaticLifeHistory package (version 1.0.5; Smart, 2023; Smart et al., 2016). The Estimate_Growth function was used to create three models of growth: von Bertalanffy, Gompertz, and logistic (Smart et al., 2016). The best fitting model was chosen for both measurements based on AICc values provided by the package. The von Bertalanffy curve was the best fitting model for both morphometric variables. This package used the equation $L_a = L_0+(L_\infty-L_0)(1-e^{-ka})$ to determine von Bertalanffy growth curves (Smart et al., 2016). It gives K, a growth coefficient, which was used in comparing the growth trajectories, as this coefficient is not slope or rate, only a descriptor of how quickly max size (asymptotic length = L_∞) is reached. Only sizes from offspring in this study were input into the model, meaning the max size is solely based on offspring data, not any sizes from sexually mature adults. This is an important designation for this study because these offspring did not reach sexual maturity by day 100, therefore the growth coefficients are being used to determine differences in juvenile growth. I do not infer that these coefficients are true after day 100 when sexual maturity occurs.

As these von Bertalanffy growth curves are based on length and not mass, additional growth analyses were done using break points across the 96 days of growth for both lengths and masses. Break points were defined as a change in the growth trajectory in a regression model at which there was a significant difference in the slope, often representing a decline in growth as organisms reach their transitions between developmental stages or maximum sizes. The regression model used was a linear model using the lm function from the nlme package (version 3.1.168; Pinhero & Bates, 2000; Pinhero et al., 2025) in R (as lme4 was not supported with the package used to

determine break points) (version 4.5.0; R Core Team, 2025). To determine significance and find break points, the package segmented (version 2.1.4; Muggeo, 2008) was used. The pscore.test function of the segmented package (version 2.1.4; Muggeo, 2008) was used to determine significance of change in slope, by testing against a null hypothesis of no break point. Both mass ($p < 0.0001$) and length ($p < 0.0001$) slopes showed significance against the null, so the segmented function was used to determine break points.

RESULTS

Reproductive Output

Laboratory-mated male sizes ranged from 0.0970 g to 0.3192 g (mean ± SE = 0.1912 ± 0.0102 g, n = 41), while wild-mated males ranged from 0.1152 g to 0.3785 g (0.2295 ± 0.0144 g, n = 20).

Of all 61 broods, the largest brood size was 71 offspring, and the smallest was 7 offspring. Brood sizes for laboratory-mated males (29.7 ± 2.3 offspring, n = 41 broods) did not differ from wild-mated males (39.5 ± 3.9 offspring, n = 20 broods) (Table 1), with all broods averaging at 33.3 ± 2.1 offspring per brood (n = 61 broods). Paternal mass had an effect on brood size (Table 1) with a moderate positive relationship (Figure 2).

Table 1. Summary of linear mixed effects model run to determine effects on brood size. Significance values are from lmerTest (version 3.1.3; Kuznetsova et al., 2017) package using Satterthwaite's method.

Model	Parameter	Estimate ± SE	$t\text{-value}_{(df)}$	p-value
lmer(sqrt(Brood Size)~Paternal Mass + Mating Location + (1|Time of Birth))	Intercept	3.96 ± 0.610	$6.490_{(26.8)}$	<0.0001
	Paternal Mass	7.25 ± 2.58	$2.81_{(52.2)}$	0.00697
	Mating Location- Wild	0.267 ± 0.435	$0.614_{(28.6)}$	0.544

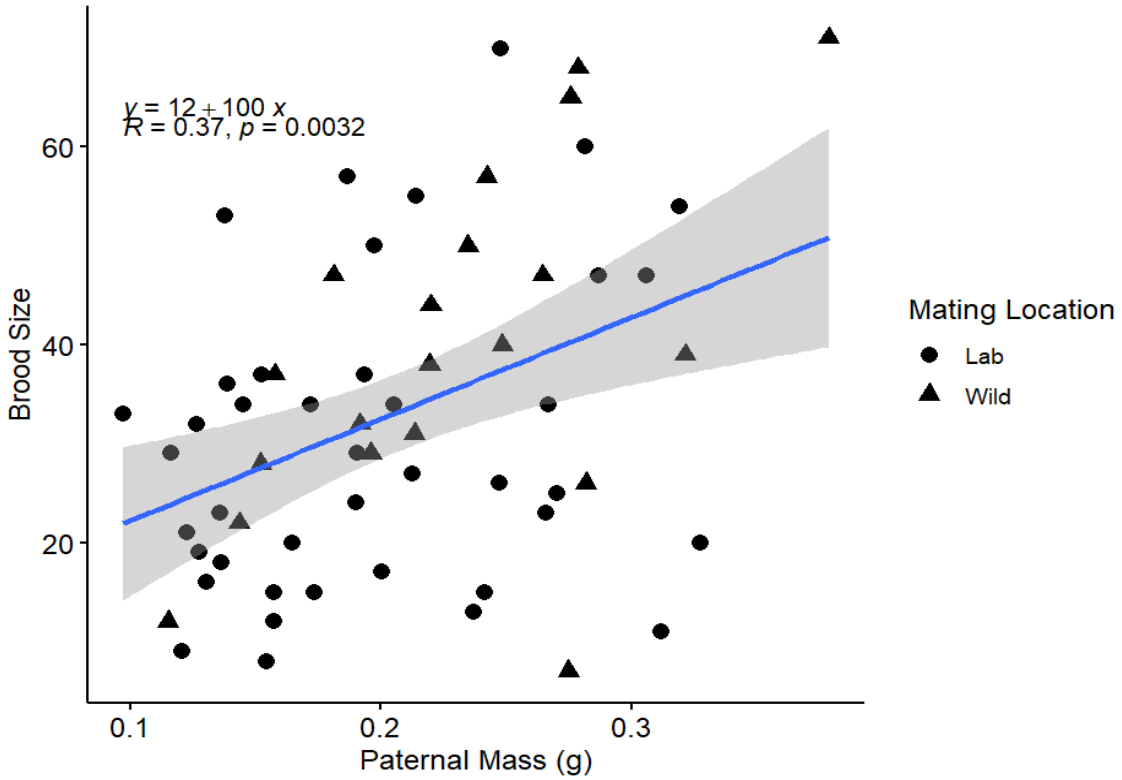

Fig. 2 Regression of paternal mass (g) on brood size in blue with 95% confidence intervals in gray. Shapes indicate mating location with circles for lab-mated males and triangles for wild-mated males. There is a significant positive relationship between paternal mass and brood size (Spearman's Rank Correlation, $r_s(59) = 0.37$, $p = 0.0032$).

Initial Morphometrics

Lengths for offspring at birth ranged from 5.611 mm to 12.783 mm, averaging 9.902 ± 0.024 mm (n = 61 broods, 1,457 offspring), while masses at birth ranged from 0.0001 g to 0.0041 g, averaging 0.0019 ± 0.00002 (n = 61 broods, 1,454 offspring). Mating location, paternal mass, and brood size had no effect on initial offspring masses (Table 2) or lengths (Table 3). Offspring lengths and masses at birth were positively correlated (Spearman's Rank Correlation, $r_s(1,211) = .75$, $p < 0.0001$) (Figure 3).

Table 2. Summary of generalized linear mixed effects model run to determine effects on initial offspring masses. Significance values from summary of model.

Model	Parameter	Estimate ± SE	t-value	*p*-value
glmer((Offspring Initial Mass)~Paternal Mass + Brood Size + Mating Location + (1|Time/Brood Identity))	Intercept	-6.29 ± 0.197	-31.9	<0.000
	Paternal Mass	0.477 ± 0.685	0.696	0.487
	Brood Size	-0.00106 ± 0.00320	-0.332	0.740
	Mating Location-Wild	0.207 ± 0.148	1.40	0.163

Table 3. Summary of generalized linear mixed effects model run to determine effects on initial offspring lengths. Significance values from summary of model.

Model	Parameter	Estimate ± SE	t-value	*p*-value
glmer((Offspring Initial Length)~Paternal Mass + Brood Size + Mating Location + (1|Time/Brood Identity))	Intercept	2.30 ± 0.0424	54.2	<0.0001
	Paternal Mass	0.265 ± 0.209	1.270	0.204
	Brood Size	-0.00117 ±0.000930	-1.255	0.210
	Mating Location-Wild	-0.0192 ± 0.0310	-0.62	0.535

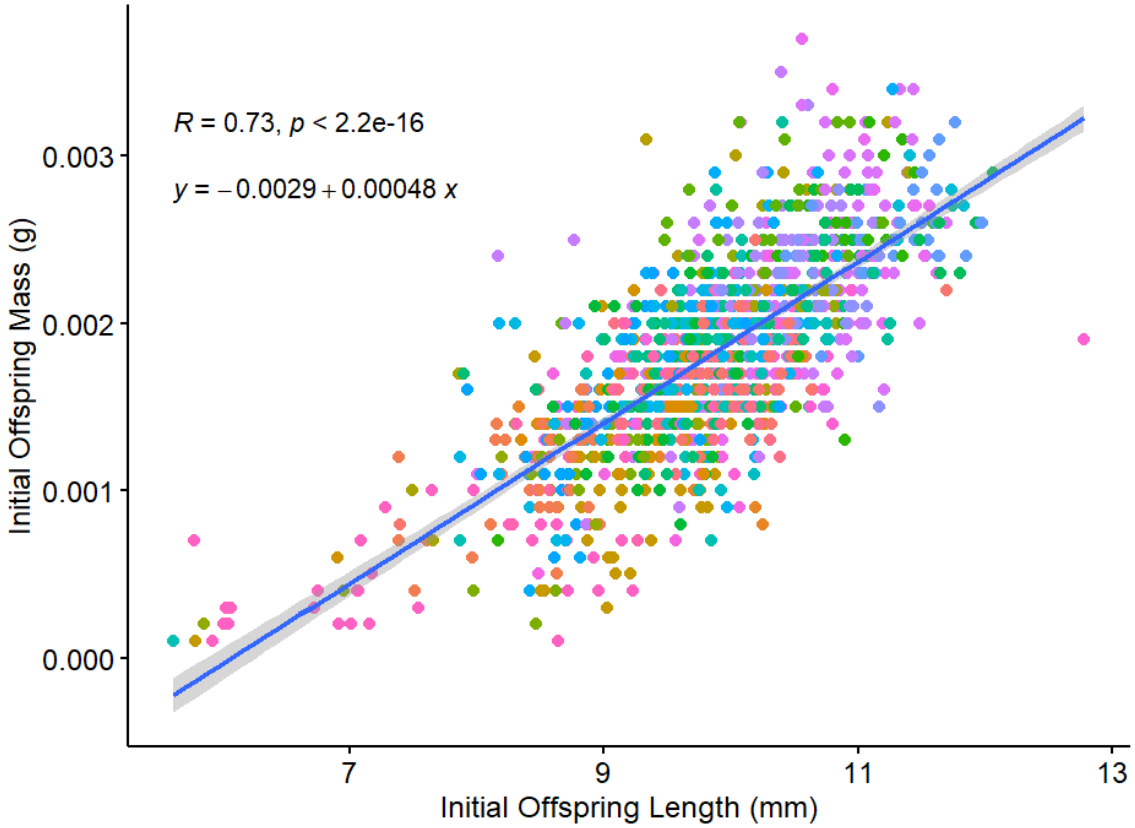

Fig. 3 Regression between mass and length of individual offspring at birth in blue with 95% confidence intervals in gray. There is a significant positive relationship between initial offspring length and mass (Spearman's Rank Correlation, $r_s(1,211) = 0.73$, $p < 0.0001$). The points are individuals (n = 1,213 offspring) with the color representing brood identity (n = 36 broods).

Final Morphometrics

Offspring lengths at day 100 ranged from 18.144 mm to 39.954 mm (27.526 ± 0.412 mm, n = 13 broods, 102 offspring), while masses at day 100 ranged from 0.0105 g to 0.1377 g, (0.0380 ± 0.0018 g, n = 13 broods, 102 offspring).

Final offspring masses (Table 4) and lengths (Table 5) were not influenced by treatment, paternal mass, or brood size. The percent of offspring surviving in each brood at day 100 did have an effect on final offspring masses (Table 4, Figure 4) and lengths (Table 5, Figure 5). Final offspring lengths and masses were positively correlated (Spearman's Rank Correlation, $r_s(100) = .57$, $p < 0.0001$) (Figure 6).

Table 4. Summary of the linear mixed effects model run to determine effects on final offspring masses. Significance values are from lmerTest package (version 3.1.3; Kuznetsova et al., 2017) using Satterthwaite's method.

Model	Parameter	Estimate ± SE	t-value$_{(df)}$	*p*-value
lmer(log(Offspring Final Mass)~Paternal Mass + Treatment + Brood Size + Percent Surviving + (1|Brood Identity))	Intercept	-3.71 ± 0.226	-16.4$_{(12.4)}$	<0.0001
	Paternal Mass	-8.99 ± 0.936	-0.012$_{(9.8)}$	0.99
	Treatment- Photo	-0.030 ± 0.092	-0.327$_{(88.3)}$	0.74
	Treatment- Weight	0.034 ± 0.103	0.334$_{(101)}$	0.74
	Brood Size	-0.000348 ± 0.00388	-0.09$_{(8.35)}$	0.93
	Percent Surviving	0.967 ± 0.0289	3.34$_{(2.84)}$	0.03

Table 5. Summary of the linear mixed effects model run to determine effects on final offspring lengths. Significance values are from lmerTest package (version 3.1.3; Kuznetsova et al., 2017) using Satterthwaite's method.

Model	Parameter	Estimate ± SE	t-value$_{(df)}$	*p*-value
lmer((Offspring Final Length)~Paternal Mass + Treatment + Brood Size + Percent Surviving + (1|Brood Identity))	Intercept	25.3 ± 1.70	14.9$_{(102)}$	<0.0001
	Paternal Mass	-7.28 ± 6.85	-1.06$_{(102)}$	0.29
	Treatment- Photo	0.102 ± 0.864	0.118$_{(102)}$	0.91
	Treatment- Weight	-1.39 ± 0.943	-1.47$_{(102)}$	0.14
	Brood Size	0.0278 ± 0.0273	1.02$_{(102)}$	0.31
	Percent Surviving	8.19 ± 1.61	5.07$_{(102)}$	<0.0001

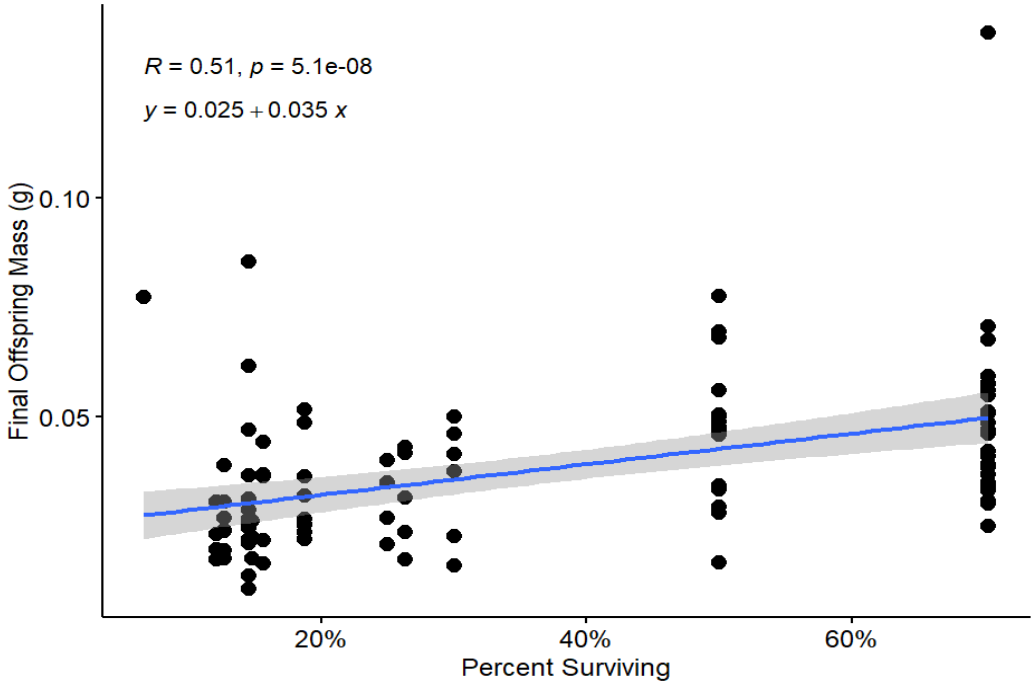

Fig. 4 Regression of percent surviving in each brood at day 100 on offspring mass (g) in blue with 95% confidence intervals in gray. There is a significant positive relationship between the percent surviving and final offspring mass (Spearman's Rank Correlation, $r_s(100) = 0.51$, $p < 0.0001$).

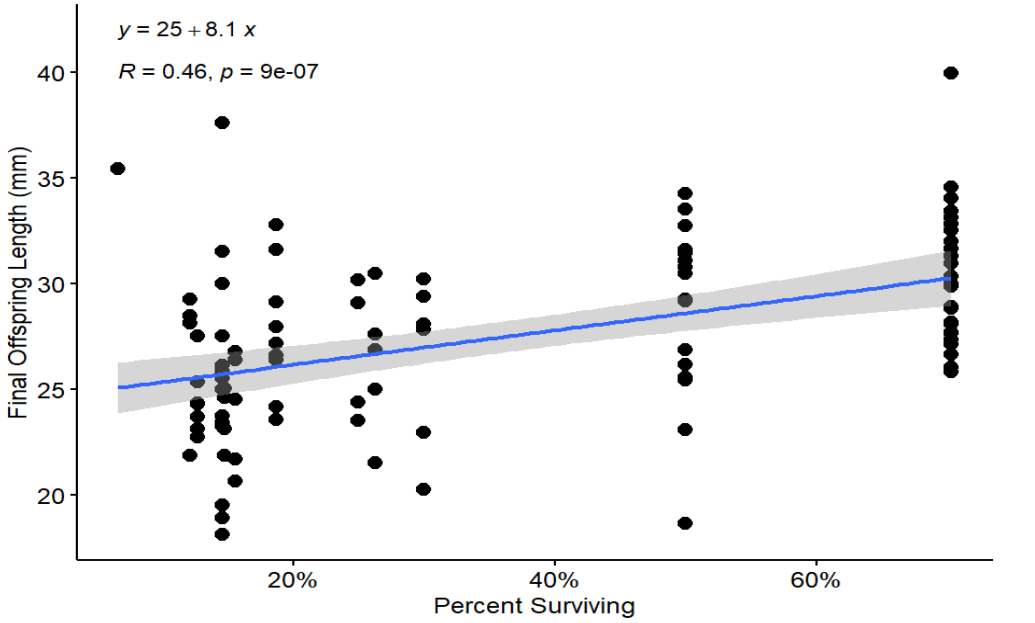

Fig. 5 Regression of percent surviving in each brood at day 100 on offspring length (mm) in blue with 95% confidence intervals in gray. There is a significant positive relationship between the percent surviving and final offspring length (Spearman's Rank Correlation, $r_s(100) = 0.46$, $p < 0.0001$).

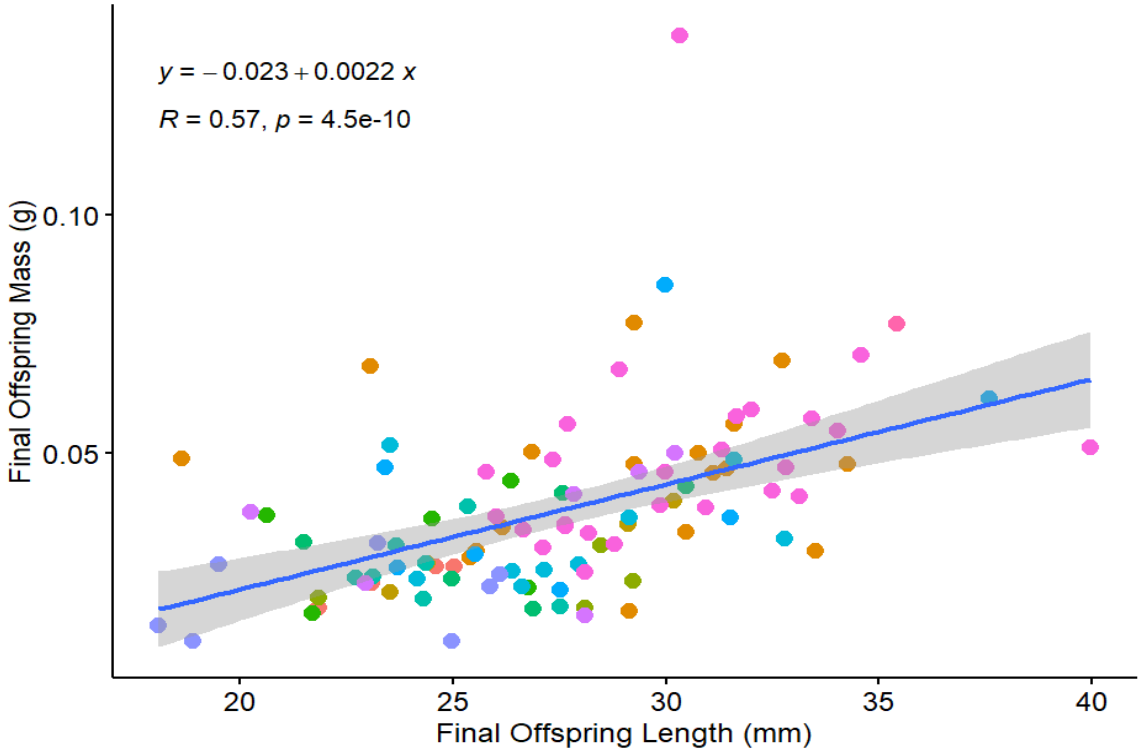

Fig. 6 Regression between final mass and length of individual offspring at day 100 in blue with 95% confidence intervals. The points represent individuals (n = 102 offspring) and color represents brood identity (n = 13 broods). There was a significant positive relationship between offspring length and mass (Spearman's Rank Correlation, $r_s(100) = 0.57$, $p < 0.0001$).

Survivorship

Survivorship curves indicate no difference between treatments based on Kaplan Meier estimations ($\chi^2(2) = 0.7$, $p = 0.7$) or Cox Hazard Ratio estimations (Ratio-Likelihood Tests, Photo- Control: $p = 0.89$, Weight- Control: $p = 0.18$) (Figure 7). All treatments have less than 50% survival by day 50 and around 20% survival by day 100. There was no effect of treatment on percent of surviving offspring at day 48 (Table 6) or day 100 (Table 7).

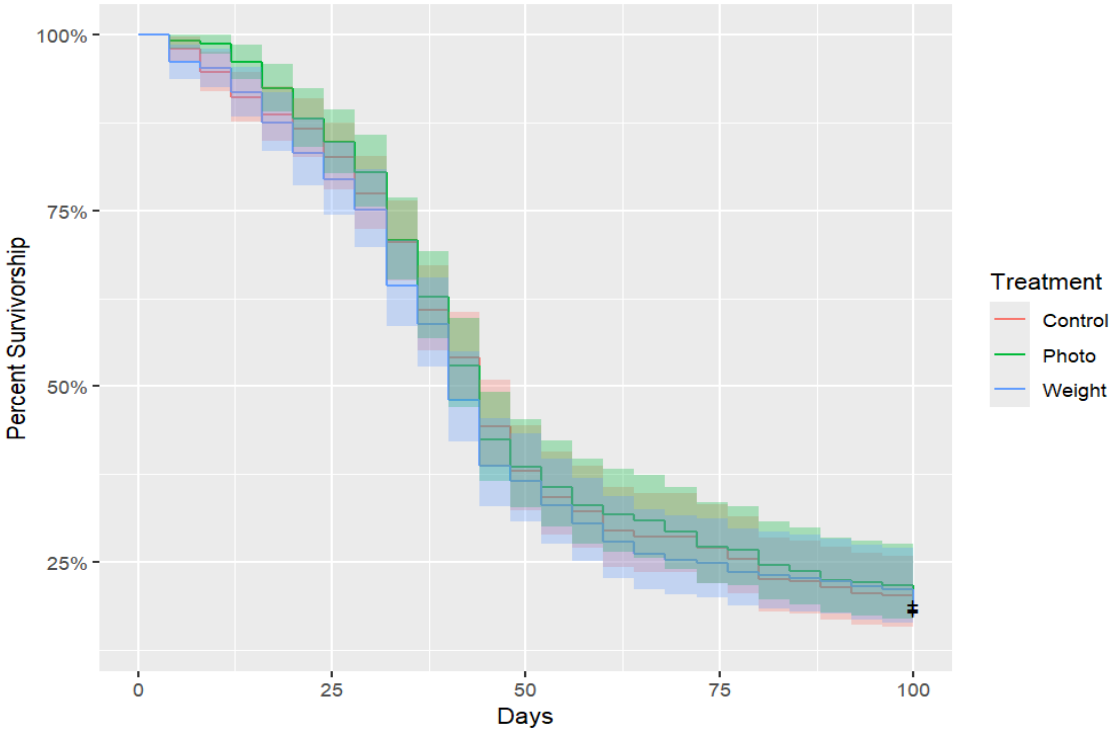

Fig. 7 Survivorship curves created from Kaplan Meier estimations for the three treatments: weight, photo, and control. No difference was found between treatments from Kaplan Meier estimates ($\chi^2(2) = 0.7$, $p = 0.7$) or Cox Mixed Effect Model (Ratio Likelihood Tests, Photo- Control: $p = 0.89$, Weight- Control: $p = 0.18$).

Table 6. Summary of the linear mixed effects model ran to determine effects of treatment on survivorship at day 48. Significance values from lmerTest package (version 3.1.3; Kuznetsova et al., 2017) using Satterthwaite's method.

Model	Parameter	Estimate ± SE	t-value$_{(df)}$	*p*-value
lmer(sqrt(Percent Surviving at Day 48)~Treatment + (1|Brood Identity))	Intercept	0.532 ± 0.0693	7.68$_{(54.2)}$	<0.0001
	Treatment- Photo	-0.0888 ± 0.0748	-1.19$_{(47.0)}$	0.242
	Treatment- Weight	-0.0885 ± 0.0748	-1.18$_{(47.0)}$	0.243

Table 7. Summary of linear mixed effects model run to determine effects of treatment on survivorship at day 100. Significance values from lmerTest package (version 3.1.3; Kuznetsova et al., 2017) using Satterthwaite's method.

Model	Parameter	Estimate ± SE	t-value$_{(df)}$	*p*-value
lmer(sqrt(Percent Surviving at Day 100)~Treatment + (1|Brood Identity))	Intercept	0.372 ± 0.0582	$6.39_{(56.1)}$	<0.0001
	Treatment- Photo	-0.0654 ± 0.0642	$-1.02_{(47.2)}$	0.314
	Treatment- Weight	-0.0672 ± 0.0642	$-1.05_{(47.2)}$	0.300

Growth

Growth curves best followed the von Bertalanffy model for both length and mass measurements (Length AICc = 7998.97, Mass AICc = -11600.60) (Figure 8). Offspring length had an estimated growth coefficient, K, of 0.02598 (± 0.0011), while offspring mass had a K of 0.01697 (± 0.0015).

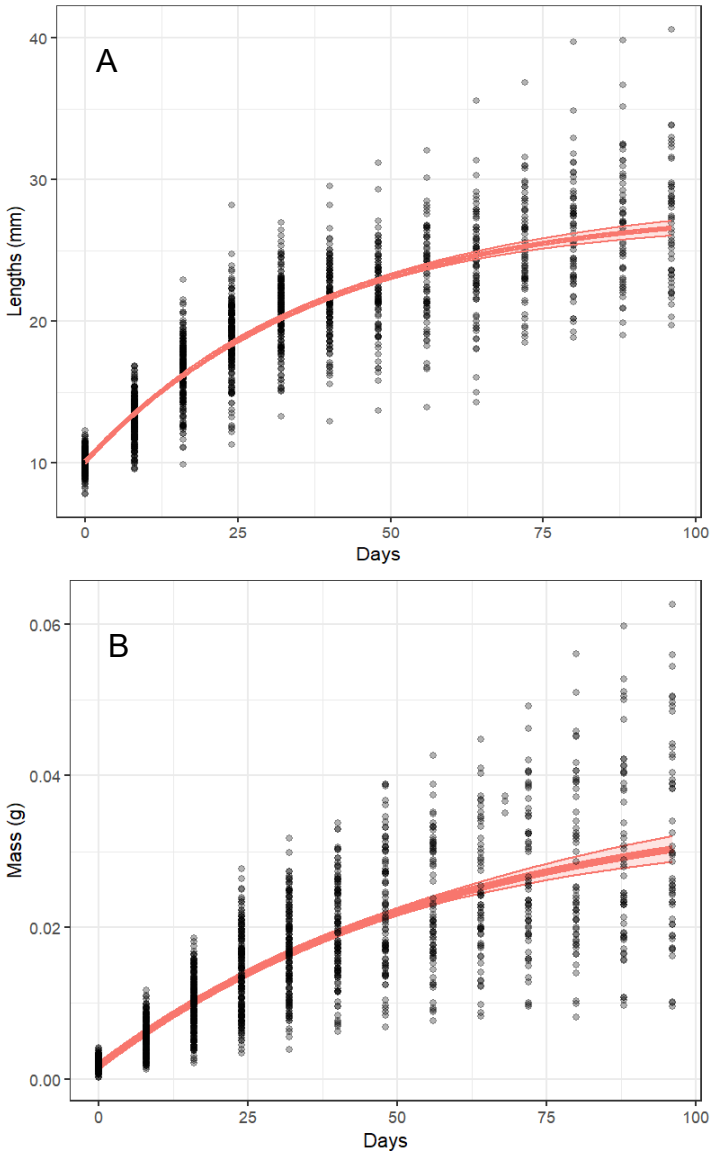

Fig. 8 Growth curves for length (A) and mass (B). Von Bertalanffy growth modeled using equation $L_a = L_0+(L_\infty-L_0)\ (1-e^{-ka})$ for estimations to make curves. Points represent individuals (length n = 244 offspring at birth, 1,686 data points; mass n = 241 offspring at birth, 1,589 data points).

Break points were found in slopes of both masses and lengths. The significant change in slope for masses was found around day 35 and on day 26 for lengths (Figure 9). Both growth curves displayed a decrease in slope after their respective break points.

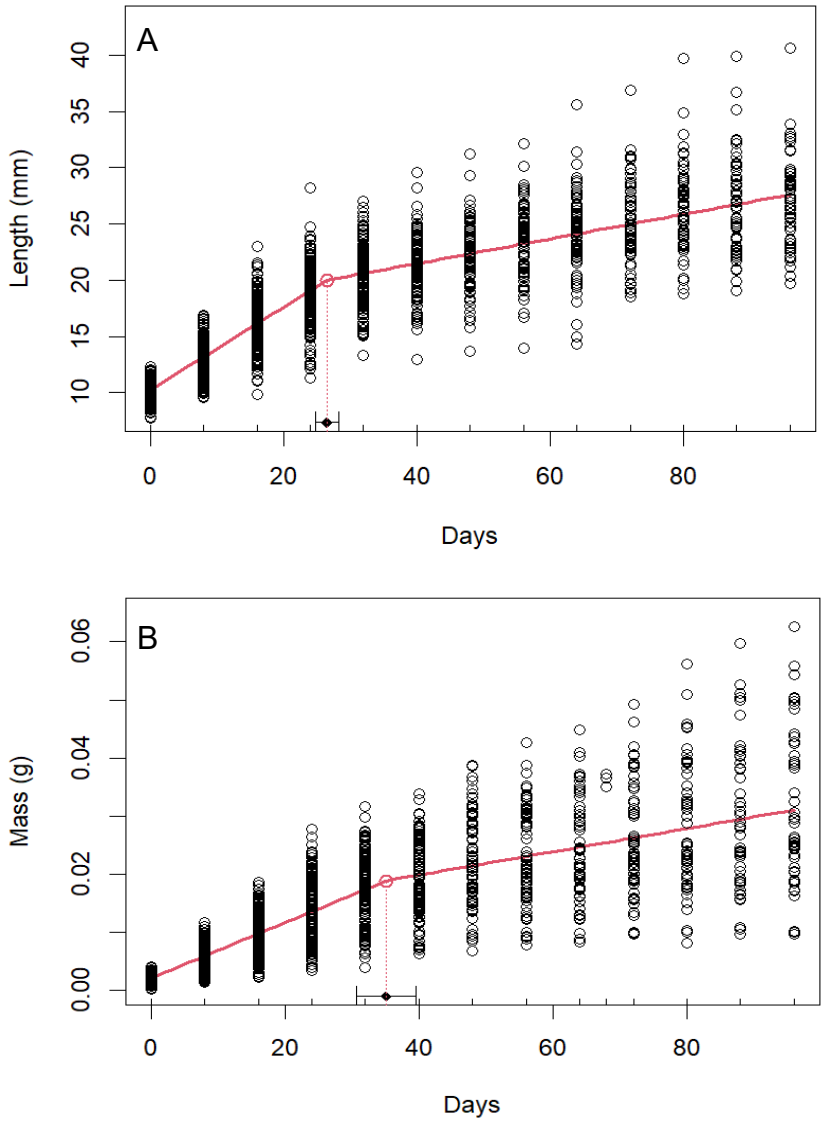

Fig. 9 Growth curves for length (A) and mass (B) of individual offspring with break points. Slopes were created with the segmented package (version 2.1.4; Muggeo, 2008). Points represent individual offspring (length n = 244 offspring at birth, 1,686 data points; mass n = 241 offspring at birth, 1,589 data points).

DISCUSSION

This thesis addressed three main goals: to analyze 1) reproductive outputs of laboratory- and wild-mated males, 2) morphological aspects of juveniles at birth and at 100 days of age, and 3) effects of animal handling techniques of measuring survivorship and growth of juvenile seahorses. Results found larger reproductive outputs than previously described for the dwarf seahorse, correlations between juvenile lengths and masses, and survivorship rates comparable to other seahorse studies with no effect across handling treatment for morphological measurements. The results validate and support previous growth studies using similar measurement techniques and advance the limited knowledge of life history traits for small seahorse species.

The first aim examined the reproductive outputs of wild- and laboratory-mated male dwarf seahorses. It is notable that the range of brood sizes, between 7 and 71, differ from previous studies of the dwarf seahorse (Lourie et al., 2004; Strawn, 1958), and overlap with one of the species of pymgy seahorse, *H. bargibanti,* with 12 to 65 offspring per brood (Shepherd et al., 2017). These results contribute additional information to dwarf seahorse life history and provide evidence that dwarf seahorse reproductive outputs are comparable to pygmy seahorses. The largest broods of 70 and 71 offspring were found in the laboratory and wild setting, respectively. In fact, the mating location of the paternal male seahorses did not influence brood sizes in this study. These results vary from previous studies of other fishes where the captive environment generally had a negative impact on gonadal development (Amberjack (*Seriola dumerilii),* Micale et al., 1999; Zupa et al., 2017; Sharpsnout Seabream (*Diplodus puntazzo),* Micale et al., 1996), as well as offspring numbers and sizes for seahorses (*H. guttalatus,* Faleiro & Narciso, 2013). In the current study, no difference was observed in number of offspring from the wild-caught males or males mated in the

laboratory, suggesting that the captive conditions of the laboratory environment did not disrupt their reproductive outputs during their short-term removal from the wild.

With both males mated in the laboratory and wild settings included, brood sizes were significantly affected by paternal mass, with larger males giving birth to larger broods. This trend has been suggested in a previous study of dwarf seahorses (Strawn, 1958), as well as in *H. erectus* (Teixeira & Musick, 2001). Larger males have been shown to have larger brood pouches (*H. guttalatus,* Faleiro et al., 2016), which could increase the number of offspring that can be incubated. Brood pouches have been found to be a limiting factor in brood size, with smaller brood pouches not able to accommodate the same number of offspring as larger pouches, even when the maternal seahorses had excess eggs to transfer (Teixeira & Musick, 2001). Results here suggest that the trend remains true for dwarf seahorses, though additional measurements of the male brood pouches would allow further analysis into the relationship between brood pouch size and number of offspring.

Paternal mass did not predict initial or final offspring size and there were no significant differences between the handling treatments. In other seahorses, male size had a positive, though in some cases non-significant, role on initial offspring sizes (*H. guttulatus,* Faleiro et al., 2016; *H. erectus,* Teixeira & Musick, 2001; *H. whitei,* Vincent & Giles, 2003). Specifically, in dwarf seahorses, males have been found to invest heavily in offspring (Masonjones & Lewis, 2000). The absence of a relationship between paternal mass and offspring size in this study suggests that other factors may have had a greater influence on the size of offspring.

Interestingly, brood size also had no effect on initial or final offspring sizes in this study. However, in other teleosts (Elgar, 1990) and across several seahorse species (*H. guttulatus,* Faleiro et al., 2016; *H. erectus,* Lin et al., 2012), larger brood sizes typically

lead to smaller offspring. This is a common ecological tradeoff between quantity and quality of offspring (Stearns, 1989). As brood size increases, parents are forced to allocate more energy to produce a greater number of offspring, which would assume that the energy cannot be used to rear larger, more robust offspring and results in larger broods with smaller sized offspring. This tradeoff was not reported in the results of this study. Although the brood sizes did not influence the offspring's body sizes, one aspect that has not been investigated in this study is the potential influence of maternal contributions. In other fish species, the age and size of the female can affect the amount of tradeoff that happens between the quality and quantity of eggs (Lasne et al., 2018). In fact, in other fish species (Barneche et al., 2018) as well as other seahorse species (*H. guttulatus,* Faleiro et al., 2016; *H. whitei,* Vincent & Giles, 2003), maternal size has been shown to affect brood sizes or offspring sizes. Generally, larger females can have larger broods and larger offspring. Unfortunately, for this study, we could not determine the maternal contribution from the wild-mated broods, so we are unable to determine if the size of the maternal female has any additional effect on brood size or offspring size. Future studies may take these complex reproductive relationships into account.

The final factor examined in this study was the percentage of offspring surviving from each brood at day 100. Results indicate that broods with higher survivorship were positively correlated to larger sized offspring at day 100. This implies that there is a quality aspect of the broods that is not determined by brood size or paternal mass. It has been found that the length of gestation and age of adults can affect the quality of broods in *H. kuda* (Dzyuba et al., 2006; Zhang et al., 2015b). Though the age of the paternal seahorses was not determined in this study, future investigations into gestation time may determine if this species exhibits age effects on gestation. In a previous study on *H. kuda*, juveniles that were identified to be higher quality offspring had better survivorship

than those that were weaker and smaller (Zhang et al., 2015b). Since quality of juveniles in previous studies were determined from parameters at birth (*H. kuda,* Dzyuba et al., 2006; Zhang et al., 2015b), future investigation into the offspring sizes both at birth and day 100 relative to survivorship in the current study will allow insight into if higher quality offspring had higher survivorship.

Survivorship rates of laboratory-mated offspring were used to assess the impact of animal handling techniques used for measuring morphometric parameters of the juveniles. Across all handling treatments, there was no difference in survivorship when comparing the photo and weighing portions of each brood with their control group that was not handled. Across all treatments, there was a large decline in survival rates between days 25 and 50. This decline in survivorship has been reported for several species of seahorses (*H. erectus,* Lin et al., 2008; *H. abdominalis,* Martinez-Cardenas & Purser, 2012; *H. reidi,* Pham & Lin, 2013; *H. abdominalis,* Woods, 2000), and survivorship has been found to be highly variable, even within a single species (*H. abdominalis,* Martinez-Cardenas & Purser, 2012). These rapid declines in survivorship remain largely unexplained (Woods, 2000), and in the current study, no evidence of stress or disease was observed, which has been identified as a factor impacting survivorship in previous work (Koldewey & Martin-Smith, 2010). By day 100, all treatments maintained around 20% survivorship. There was also no effect of treatment on final offspring size, indicating that throughout all treatments, offspring were able to survive and grow equally. These results suggest that these animal handling techniques are non-stressful compared to the control treatment of non-handling.

To the author's knowledge, this is the first study of seahorse growth and survivorship that has analyzed the impacts of animal handling techniques. Other studies have used similar techniques to measure growth and survivorship without considering if

there are underlying effects from measuring. Some studies that anticipated impacts of handling techniques have chosen to sacrifice samples of individuals at certain days (Hora & Joyeux, 2009), which is only possible for species with large brood sizes. However, euthanizing large subsets of offspring during each sampling period is not viable for studies examining seahorse species with smaller brood sizes, as is seen here in the dwarf seahorse, or in pygmy seahorses, such as *H. bargibanti* (Shepherd et al., 2017). Since repeated sampling of juveniles is required for tracking changes in allometry and measuring growth rates, the sampling methodology used needs to be tested to determine if there are any additional effects on rates of survivorship. The results of this study indicate that these animal handling techniques are effective, unimpactful ways to take measurements, allowing confidence that there are no underlying effects of measurement techniques in results.

One interesting aspect of the growth patterns was that juveniles differed in their change in length compared to mass over time. This difference is highlighted in the slightly lower correlation coefficient of length and mass at day 100 than at day 0. Offspring were able to grow to a max length faster than they were able to grow to a max mass, according to the growth coefficients from the von Bertalanffy growth curves. The decline in rate of growth in length occurred prior to the decline for the growth of mass, suggesting offspring may have a trade-off, for at least ten days, where they focus energy on gaining more mass than length. The combination of these results suggests that either length or mass may be a better indication of condition. As offspring were able to grow faster to larger sizes in length, it implies that in this laboratory environment seahorse juveniles were able to allocate energy for increasing their length more rapidly than in mass.

One reason offspring may not have been able to gain mass continuously is the limited dietary options and lack of multiple prey items. One study on *H. kuda* has identified that there are morphological stages in seahorse offspring growth which coincide with changes in diet (Choo & Liew, 2006). The break point for growth rates often indicates an ontogenetic shift in feeding patterns, therefore the lack of larger prey in this study could be the reason for a decline in growth. Other studies have also determined that enrichment and addition of food sources increase growth rates for seahorses (*H. subelongatus,* Payne & Rippingale, 2000; *H. reidi,* Olivotto et al., 2008; *H. whitei,* Wong & Benzie, 2003). In this study, *Artemia* brine shrimp was the sole food source for the duration of the survivorship study. The rationale behind only providing *Artemia* brine shrimp was to have a consistent food source throughout the study, whereas cultures of copepods and rotifers are more difficult to have consistent abundances available due to their breeding cycles (Wilson & Vincent, 2000). Controlling a single food source throughout this study was necessary in order to ensure that the results seen were only due to the differences in treatment, rather than any change in diet. Although providing a variety food sources as offspring went through different morphological stages of growth may have allowed juvenile mass to grow more efficiently, this would have been more challenging to replicate across broods throughout the summers if the stock of the prey were unreliable.

Although this study was not able to complete the full life cycle of dwarf seahorses in a laboratory setting, there were important advances in the gaps of life history knowledge for this species. To gain a better understanding of sexual development in dwarf seahorses, future studies will need to implement a feeding regiment that more closely aligns with the dietary changes of juveniles in the wild. Previous data on the dwarf seahorse suggest sexual maturity at 3 months (Strawn, 1958). However, at 100

days in this study, few juveniles showed some evidence of sexual maturity with little evidence of a brood pouch, and no offspring showed visible external evidence of maturing as a female. The brood pouches on a few offspring did not appear fully developed, and because no offspring showed close resemblance to a mature adult, further exploration to identify sexes was not taken. In seahorses, it has been suggested that males and females have different rates of growth due to brood pouch and egg development (*H. reidi,* Hora & Joyeux, 2009; *H. erectus,* Lin et al., 2009), which may also contribute to the variation in growth rates at the later stages in this study. Future studies should work to complete the life cycle to determine the age of maturity and analyze differences of growth between sexes for this species.

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APPENDIX A

Institutional Animal Care and Use Committee (IACUC) Approval

VALDOSTA STATE UNIVERSITY

Institutional Animal Care and Use Committee (IACUC)

Animal Use Protocol Approval

June 17, 2021

Dr. Emily Rose
Department of Biology
Valdosta State University

Dear Dr. Rose;

Animal Use Protocol (AUP) *"The effects of water turbidity on seahorse mating behaviors and reproduction."* (AUP-00081-2021) has been approved by the Institutional Animal Care and Use Committee (IACUC). This approval is from 06.17.2021 – 06.17.2024. Each year, an animal report must be submitted to the IACUC to keep your protocol active. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the annual report is due.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Compliance Specialist, 229.253.2947, email tmwright@valdosta.edu, or IACUC Alias @ iacuc@valdosta.edu.

Sincerely,

Elizabeth W. Olphie

Elizabeth "Ann" Olphie
IACUC Administrator

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APPENDIX B

Supplemental Linear Mixed Effects Model Selection

Appendix B. 1. Model selection for the LMM run to determine effects of paternal mass and mating location on brood size. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
lmer(sqrt(Brood Size)~Paternal Mass +(1|Time of Birth))	**216.4 (0.694)**
lmer(sqrt(Brood Size)~Paternal Mass+ Mating Location +(1|Time of Birth))	218.5 (0.246)
lmer(sqrt(Brood Size)~(1|Time of Birth))	222.1 (0.040)
lmer(sqrt(Brood Size)~Mating Location +(1|Time of Birth))	223.4 (0.020)

Appendix B. 2. Model selection for the GLMM run to determine effects of paternal mass, brood size, and mating location on initial offspring mass. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
glmer((Offspring Initial Mass)~(1|Time/Brood Identity))	**-7726.7 (0.251)**
glmer((Offspring Initial Mass)~Mating Location +(1|Time/Brood Identity))	-7726.6 (0.249)
glmer((Offspring Initial Mass)~ Paternal Mass +(1|Time/Brood Identity))	-7725.2 (0.121)
glmer((Offspring Initial Mass)~Paternal Mass + Mating Location +(1|Time/Brood Identity))	-7725.0 (0.109)
glmer((Offspring Initial Mass)~Brood Size +(1|Time/Brood Identity))	-7724.7 (0.092)
glmer((Offspring Initial Mass)~Brood Size + Mating Location +(1|Time/Brood Identity))	-7724.6 (0.090)
glmer((Offspring Initial Mass)~Paternal Mass + Brood Size +(1|Time/Brood Identity))	-7723.3 (0.048)
glmer((Offspring Initial Mass)~Paternal Mass + Brood Size + Mating Location +(1|Time/Brood Identity))	-7723.1 (0.041)

Appendix B. 3. Model selection for the GLMM run to determine effects of paternal mass, brood size, and mating location on initial offspring length. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
glmer((Offspring Initial Length)~(1|Time/Brood Identity))	**1189.5 (0.295)**
glmer((Offspring Initial Length)~ Paternal Mass +(1|Time/Brood Identity))	1190.5 (0.178)
glmer((Offspring Initial Length)~Brood Size +(1|Time/Brood Identity))	1190.9 (0.146)
glmer((Offspring Initial Length)~Paternal Mass + Brood Size +(1|Time/Brood Identity))	1191.5 (0.108)
glmer((Offspring Initial Length)~Mating Location +(1|Time/Brood Identity))	1191.5 (0.107)
glmer((Offspring Initial Length)~Paternal Mass + Mating Location +(1|Time/Brood Identity))	1192.5 (0.064)
glmer((Offspring Initial Length)~Brood Size + Mating Location +(1|Time/Brood Identity))	1192.8 (0.057)
glmer((Offspring Initial Length)~Paternal Mass + Brood Size + Mating Location +(1|Time/Brood Identity))	1193.2 (0.045)

Appendix B. 4. Model selection for the LMM run to determine effects of paternal mass, brood size, treatment, and percent surviving on final offspring mass. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
lmer(log(Offspring Final Mass)~Percent Surviving +(1|Brood Identity))	**108.8 (0.440)**
lmer(log(Offspring Final Mass)~Paternal Mass + Percent Surviving +(1|Brood Identity))	111.0 (0.147)
lmer(log(Offspring Final Mass)~Brood Size + Percent Surviving +(1|Brood Identity))	111.0 (0.146)
lmer(log(Offspring Final Mass)~ Treatment + Percent Surviving +(1|Brood Identity))	112.9 (0.058)
lmer(log(Offspring Final Mass)~(1|Brood Identity))	113.1 (0.052)
lmer(log(Offspring Final Mass)~Paternal Mass + Brood Size + Percent Surviving +(1|Brood Identity))	113.3 (0.048)
lmer(log(Offspring Final Mass)~Brood Size +(1|Brood Identity))	114.7 (0.023)
lmer(log(Offspring Final Mass)~Treatment + Brood Size + Percent Surviving +(1|Brood Identity))	115.2 (0.018)
lmer(log(Offspring Final Mass)~Paternal Mass + Treatment + Percent Surviving +(1|Brood Identity))	115.2 (0.018)
lmer(log(Offspring Final Mass)~Paternal Mass +(1|Brood Identity))	115.2 (0.018)
lmer(log(Offspring Final Mass)~Treatment +(1|Brood Identity))	116.5 (0.010)
lmer(log(Offspring Final Mass)~Paternal Mass + Brood Size +(1|Brood Identity))	116.9 (0.008)
lmer(log(Offspring Final Mass)~Paternal Mass + Treatment + Brood Size + Percent Surviving +(1|Brood Identity))	117.5 (0.006)
lmer(log(Offspring Final Mass)~ Treatment+ Brood Size +(1|Brood Identity))	118.2 (0.004)
lmer(log(Offspring Final Mass)~Paternal Mass + Treatment +(1|Brood Identity))	118.7 (0.003)
lmer(log(Offspring Final Mass)~Paternal Mass + Treatment + Brood Size +(1|Brood Identity))	120.5 (0.001)

Appendix B. 5. Model selection for the LMM run to determine effects of paternal mass, brood size, treatment, and percent surviving on final offspring length. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
lmer((Offspring Final Length)~Percent Surviving +(1|Brood Identity))	**563.9 (0.317)**
lmer((Offspring Final Length)~ Treatment + Percent Surviving +(1|Brood Identity))	565.7 (0.124)
lmer((Offspring Final Length)~Paternal Mass + Percent Surviving +(1|Brood Identity))	565.8 (0.122)
lmer((Offspring Final Length)~ Brood Size + Percent Surviving +(1|Brood Identity))	565.9 (0.114)
lmer((Offspring Final Length)~(1|Brood Identity))	567.3 (0.056)
lmer((Offspring Final Length)~Paternal Mass + Brood Size + Percent Surviving +(1|Brood Identity))	567.4 (0.054)
lmer((Offspring Final Length)~Paternal Mass + Treatment + Percent Surviving +(1|Brood Identity))	567.6 (0.049)
lmer((Offspring Final Length)~Treatment + Brood Size + Percent Surviving +(1|Brood Identity))	567.7 (0.046)
lmer((Offspring Final Length)~Paternal Mass + Treatment + Brood Size + Percent Surviving +(1|Brood Identity))	568.9 (0.025)
lmer((Offspring Final Length)~Paternal Mass +(1|Brood Identity))	569.2 (0.022)
lmer((Offspring Final Length)~Treatment +(1|Brood Identity))	569.2 (0.022)
lmer((Offspring Final Length)~Brood Size +(1|Brood Identity))	569.3 (0.021)
lmer((Offspring Final Length)~Paternal Mass + Treatment +(1|Brood Identity))	571.1 (0.009)
lmer((Offspring Final Length)~ Treatment + Brood Size +(1|Brood Identity))	571.2 (0.008)
lmer((Offspring Final Length)~Paternal Mass + Brood Size +(1|Brood Identity))	571.3 (0.008)
lmer((Offspring Final Length)~Paternal Mass + Treatment + Brood Size +(1|Brood Identity))	573.3 (0.003)

Appendix B. 6. Model selection for the LMM run to determine effects of treatment on percent surviving at day 48. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
lmer(sqrt(Percent Surviving at Day 48)~(1|Brood Identity))	**43.1 (0.797)**
lmer(sqrt(Percent Surviving at Day 48)~Treatment +(1|Brood Identity))	45.8 (0.203)

Appendix B. 7. Model selection for the LMM run to determine effects of treatment on percent surviving at day 100. AICc scores from dredge function of the MuMin package (Bartoń, 2025). Bold indicates selected model from backward model selection (Zuur et al., 2009).

Model	AICc from Dredge (Weight)
lmer(sqrt(Percent Surviving at Day 100)~(1|Brood Identity))	**19.2 (0.83)**
lmer(sqrt(Percent Surviving at Day 100)~Treatment +(1|Brood Identity))	22.3 (0.17)