Are bivalves susceptible to domestication selection? Using starvation tolerance to test for potential trait changes in eastern oyster larvae

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Short Title: Starvation tolerance in bivalve larvae as a first test for domestication selection

Keywords: *Crassostrea virginica*, growth rate, respiration, domestication, selective breeding, oyster, physiology

Abstract

Conservation efforts are increasingly being challenged by a rapidly changing environment, and for some aquatic species the use of captive rearing or selective breeding is an attractive option. However, captivity itself can impose unintended artificial selection known as domestication selection (adaptation to culture conditions). For most marine species, it is not known to what degree domestication selection affects traits related to fitness in the wild. To test for domestication selection in a marine bivalve, we focused on a fitness-related trait (larval starvation resistance) that could be altered under artificial selection. Using larvae produced from a wild population of *Crassostrea virginica* and a selectively bred, disease-resistant line we measured growth and survival during starvation versus standard algal diet (control) conditions. Larvae from both lineages showed a remarkable resilience to food limitation, possibly mediated by an ability to uptake and utilize dissolved organic matter for somatic maintenance. Water chemistry analysis showed dissolved organic carbon in filtered tank water to be at concentrations similar to natural river water. We observed that survival in larvae produced from the aquaculture line was significantly lower compared to larvae produced from wild broodstock (8 ± 3% and 21 ± 2%, respectively) near the end of a 10-day period with no food (phytoplankton). All larval cohorts had arrested growth during the starvation period and took at least two days to recover once food was reintroduced before resuming growth. Phenotypic differences between the wild and aquaculture lines suggest potential differences in the capacity to sustain extended food limitation, but this work requires replication with multiple selection lines and wild populations to make more general inferences about domestication selection. With this contribution we explore the potential for domestication selection in bivalves, discuss the physiological and fitness implications of reduced starvation tolerance, and aim to inspire further research on the topic.

Keywords: *Crassostrea virginica*, growth rate, respiration, domestication, selective breeding, oyster, physiology

Introduction

For many regions, climate change predictions suggest rising temperatures and dramatic variations in precipitation [1] that will stress estuarine and coastal populations through rapid changes in salinity and the spread and proliferation of disease [2,3]. Given the urgency of conservation needs to maintain population viability under rapid environmental change, the prospects for "assisted evolution" using selective breeding or developmental manipulations have increasingly been discussed and investigated [4]. One concern with selective breeding as part of a population management strategy is that captivity itself can impose unintended artificial selection [5,6]. Evolutionary responses to this "domestication selection" can be swift in captive populations [5,7,8] with the potential for reduced fitness in the wild relative to wild born individuals [9]. The propensity for rapid domestication selection is tied to life history because selection can be especially strong on cohorts of high fecundity organisms with high early mortality (type III survivorship curve). When domestication selection is strong within a single propagation cohort, such as was demonstrated for salmonids [5], then its effects can potentially impact the success of hatchery-based population supplementation. These considerations are of particular relevance to marine bivalves because (1) they express extreme versions of this life history, (2) native bivalve populations are depleted in some areas and receiving hatchery-based population supplementation [10], and (3) selectively bred strains intended for commercial aquaculture have been promoted and used for population supplementation, for example in an attempt to mitigate disease mortality [11]. Unfortunately, there is virtually nothing known about the prevalence of domestication selection in

aquatic organisms other than salmon, or its fitness consequences in the wild when it occurs. Here, we hypothesize that larval starvation tolerance is a likely trait subject to domestication selection and experimentally measure and compare this trait in wild and selected-strain oysters.

Natural populations of the eastern oyster *Crassostrea virginica* are only a fraction of historic densities, and are deemed functionally extinct in some regions of the northeastern U.S. [12]. The great ecological and economic value of oysters has prompted aggressive population supplementation (= "restoration") programs in parts of its native range [13,14] and millions of U.S. dollars have been spent to revive this once thriving ecosystem engineer [15]. Oyster restoration approaches often include the hatchery production and planting of spat (juvenile oysters) on shell to supplement wild recruitment and help jumpstart a population on restored habitat. To reduce genetic bottlenecking and other genetic changes in culture, the Nature Conservancy recommends using fresh wild broodstock when producing spat to be deployed for stock enhancement [16]. In practice, however, selectively-bred eastern oyster strains sometimes get used in population supplementation for several reasons. In some regions, "wild" broodstock (i.e. non-feral oysters) are locally sparse and logistically challenging to collect from remnant populations, or available seed oysters from regional production hatcheries only include domesticated oyster strains intended for aquaculture production. Also, in some cases selectively bred traits such as disease resistance are deemed desirable or necessary for successful restoration [11,17,18]. Genetic assignment tests have generated mixed results documenting spat recruitment from hatchery-produced selected strain cohort plantings [19,20]. Trade-offs between aquaculture traits and fitness in the wild have not been evaluated in bivalves to our knowledge.

Concerns with using artificially selected lines in restoration include the degree to which selective breeding inadvertently alters non-target traits, either because they are genetically

87 correlated with the selected trait, or because culture conditions impose selection (domestication
88 selection). Also, hatchery culture imposes a genetic bottleneck relative to wild cohorts and
89 domestication selection further strengthens genetic drift, reducing genetic diversity to a degree that
90 may have a fitness cost under natural conditions [8,10,14]. Evolution of domestication traits is
91 increasingly under study to understand their rate of change and fitness impacts [5–8,21]. Not
92 surprisingly, phenotype and performance data for oysters is mostly restricted to commercially
93 important traits during grow-out from planted spat to adult (market size). Fitness trade-offs across
94 other parts of the life cycle are relatively unexplored, yet larvae are arguably the most sensitive
95 life stage [22,23]. Fitness trade-offs are extremely challenging to study in species with a complex
96 life cycle that includes pelagic larvae [24,25], but it is a reasonable assumption that traits
97 conferring higher relative fitness in culture, at high population density in a homogeneous
98 environment, will not increase mean population fitness in the wild.

99 For many marine species, including oysters, tremendous fecundity (e.g. millions of eggs
100 per female) and high, non-random early mortality suggests that the larval stage may be under
101 particularly strong selection pressures [23,26,27]. High early mortality (Type-III survivorship)
102 often characterizes these species in culture as well as in the wild [22,23], potentially involving
103 strong selection in culture that could result in directional shifts in the mean value of traits favored
104 by the hatchery environment. In contrast, early mortality due to the expression of a high genetic
105 load may swamp the signal of directional selection on larval traits, or the two mechanisms could
106 interact if the segregation of mildly or strongly deleterious mutations contributes to fitness or
107 growth rate differences among larvae [28–30]. Therefore, understanding the selective forces
108 underlying early mortality in the hatchery is particularly important in the context of supportive

breeding for restoration, but these mechanisms also are relevant for optimization of selective breeding.

Nutrition is the major driver for growth and development during pelagic life stages of bivalve larvae [31,32]. Natural temporal and spatial variation in food quality and quantity can be extreme [32], leaving free swimming larvae vulnerable to periods of insufficient food supply [33]. During the hatchery culture of bivalves, environmental conditions are controlled to support the best growth and survival at a relatively high larval density, including a simple (low diversity) diet [34]. Because of the high variability of food quality and quantity in the natural environment compared to *ad libitum* feeding under hatchery culture, metabolic processes may be under different selection pressures resulting in domestication selection. In a hatchery-based larval culture experiment in the Pacific oyster, Plough [35] showed that rearing with a 3-species algal diet significantly increased larval growth and survival, and reduced the expression of genetic load (measured as genetic inviability) compared to a single species algal diet, highlighting the importance of food quality specifically and genetic by environment interactions more generally.

Oysters produced for the aquaculture industry are often selectively bred for traits that speed up production, such as fast growth, and that improve survival (e.g. disease resistance; [36]. However, it is unclear whether or not other (unintended) traits are evolving due to genetic correlations, adaptation to the artificial environment, or heritable epigenetic changes during hatchery culture. As a first step towards examining the potential effects of domestication selection in oysters, we performed an experiment comparing the starvation resistance of larvae produced from wild (no prior hatchery exposure) and artificially selected (over multiple generations of hatchery propagation) broodstock oysters. The goal of this paper was to measure and compare starvation tolerance of larvae produced from wild (no previous hatchery propagation) and

selectively bred (many generations of hatchery propagation) broodstock as an initial exploration of possible domestication effects. Feeding environment during early development matters a great deal for both aquaculture and population supplementation goals, making starvation resistance an appealing first target among the many traits that could have changed as a result of adaptation to hatchery conditions. Because oyster larvae have a wide variance in growth rates within families [30] and hatchery production often includes the culling of slow growing larvae, we also separated and compared slow and fast early growth larval phenotypes in each line. Separating larvae by early early growth rate provided the opportunity to compare stress responses of physiologically distinct portions of each line. We used growth, survival, and respiration (rate of oxygen depletion) as measures of physiological response to a prolonged (10-day) starvation period between lines and among cohort growth-fractions.

Methods

Broodstock conditioning and spawning

Wild adult oysters were collected from the Choptank River, Maryland in the Chesapeake Bay and two disease-resistant aquaculture lines (Deby (DBY) and DBY-CROSbreed (XB)) were obtained from the Virginia Institute of Marine Science, Aquaculture Genetics and Breeding Technology Center (ABC). All broodstock were held under chilled (20°C) flow-through Choptank River water at the Horn Point Laboratory Oyster Hatchery in Cambridge, Maryland to promote gametogenesis, but prevent spontaneous spawning. Local salinity was 9 – 11 ppt during the conditioning period for Choptank River broodstock. Aquaculture (DBY and XB) oysters were partially conditioned at the ABC at a salinity of 14 - 16 ppt, before being shipped to Horn Point Laboratory where they were held under conditions described above for four weeks prior to

spawning. The DBY and XB lines have been bred over multiple generations with hatchery propagation and intensive selection for disease resistance (both MSX and dermo; [36,37]). Broodstock originated in 1998 from Virginia Institute of Marine Science and the Haskin Shellfish Research Laboratory for DBY and XB, respectively, but have since been interbred with Chesapeake Bay oysters and broodstock from Louisiana known to have naturally acquired dermo resistance [36].

On June 26, 2017, oysters were spawned by raising the water temperature from 20°C to 30°C in individual containers with flow-through seawater at a salinity of 9.9 ppt. When temperature did not induce spawning, heat-killed sperm was added to stimulate spawning. As individuals began to release gametes, the water flow was stopped for that individual, time was noted, and sex determined by assessing the released gametes of each individual microscopically. Oysters were allowed to finish spawning in their individual container to collect and isolate gametes for each individual. A total of six pair-cross fertilizations were completed between two females and three males for each strain type (wild and aquaculture) within one hour of the start of spawning to assure quality of gametes. The DBY and XB selection lines are maintained with methods that limit inbreeding [36], but to eliminate any potential for inbreeding effects here, our experimental aquaculture cohort was created with males from the DBY line and females from the XB line (hereafter referred to as AQF1).

Approximately one-hour post fertilization, developing embryos were enumerated microscopically for each pair cross and the number of embryos from each pair were equalized to give each parent pair equal opportunity to contribute to the cohort. For each line, the pair-cross embryos were pooled, then split in half and reared in duplicate 200-L tanks at a density of 30 larvae mL^{-1} for seven-days using 0.5 µm filtered Choptank River water at 9.7 ± 0.1 ppt and 27°C. Larvae

were fed a diet of 50:50 *Isochrysis galbana* and *Chaetoceros calcitrans* beginning at 10,000 cells mL^{-1} on day one and was increased each day by 10,000 cells mL^{-1}. Water changes were completed on day three using a 25 µm sieve to assure no larvae were lost and re-stocked at a density of 15 larvae mL^{-1} by random size culling. Water changes were completed every other day thereafter with no culling until day seven. All water was pumped from the Choptank River through a sand filtration system down to 2 µm followed by successive string and cartridge filtration to 0.5 µm.

Total organic carbon (TOC) analysis

To gain a better understanding of the potential sources of dissolved organic matter (DOM) that may be available as a food resource to larvae during the starvation period, water samples were collected directly from the Choptank River, from the 0.5 µm river water filter system used to fill tanks, from the 0.5 µm filtered water with additional carbon filtration, and from experimental buckets (fed and starved treatments) during a water change (24 hours after filling) 8 days into the starvation period. Water samples were collected in duplicate and filtered through a 0.2 µm glass-fiber filters and shipped to the University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory for total organic Carbon (TOC) and total dissolved Nitrogen (TDN) quantification.

Starvation challenge

At age 7 days old (July 3, 2017), larvae from each line were separated into fast and slow early growth cohorts by size selecting on an 85 µm sieve. Under normal hatchery rearing, some culling of small larvae (slow growers) is likely to occur inadvertently by age 7 days as a result of increasing sieve size to remove dead shell during water changes. All larvae that were caught on the 85 µm sieve were deemed fast growers and all larvae that went through the sieve, slow growers.

This allowed for an approximately equal split in numbers between the fast and slow early-growth fractions for each cohort, although there was overlap in the resulting size distributions (Fig 1). The slow early-growth group includes larvae that would typically be culled during normal hatchery practice.

Fig 1. Length distributions of larvae at the start of the starvation experiment (T = 0; Age = 7 days). Pink bars represent the fast-growing larvae and blue bars represent slow growing larvae for each line (wild and AQF1). A total of 60 larvae were measured for each cohort (Wild/Fast, Wild/Slow, AQF1/Fast, AQF1/Slow). The purple region indicates the overlap in size between the two groups for each line.

Larvae were stocked at approximately 15 larvae mL^{-1} in 20 L buckets (~300,000 larvae / bucket) using 0.5 µm filtered Choptank River water at ambient salinity (9.4 ± 0.7 ppt) and temperature (27.6 ± 0.4°C). For each cohort (Wild/Fast, Wild/Slow, AQF1/Fast, AQF1/Slow) two treatment conditions were maintained; starved and fed with four replicate buckets for each cohort/treatment (S1 Fig). Fed control buckets were fed according to the Horn Point Oyster Hatchery protocol with an increase in phytoplankton concentration by 10,000 cells mL^{-1} each day and starting at 70,000 cells mL^{-1} on day 1 of the experimental period. A live phytoplankton diet of 50:50 *Isochrysis galbana* and *Chaetoceros calcitrans* was maintained throughout the entire experiment so as to keep conditions constant between fed controls and recovery of the starved treatment. Starting on day 7 post-fertilization, all phytoplankton were withheld from the starved treatment for ten days after which feeding was resumed. The feeding regime re-started as if the oysters were 8 days old (80,000 cells L^{-1}) and increased by 10,000 cells L^{-1} each day thereafter. During the experimental period (starvation and recovery), water changes were completed every 1-3 days at which time live survival counts were completed microscopically using volumetric

222 counts and samples were preserved in formalin for length analysis. To obtain concentrated samples
223 and conduct survival counts, larvae were transferred to 500 mL beakers, mixed well and four
224 replicate samples from each beaker were counted to improve precision. Pictures of preserved
225 larvae were taken using a Nikon Eclipse E600 microscope (Nikon® Instruments, Melville, New
226 York, USA) equipped with an AmScope MU800B digital camera (AmScope© Irving, California,
227 USA) and analyzed for length measurements in the AmScope software. Shell lengths of 30
228 individuals were measured per replicate bucket, except in cases where mortality limited the
229 numbers of measurable shells in later sampling time points. When < 10 larvae were found for
230 length measurements, these replicates were removed from growth analysis to avoid sampling error.
231 Changes in tissue coloration and locomotion were noted during live survival counts, but were not
232 quantitatively measured.

233 # Respiration

234 Respiration rates of larvae were measured using FireStingO_2 fiber-optic oxygen meter
235 (Pyro Science Co., Aachen, Germany) in 5mL closed respirometry chambers. Measurements were
236 made for each of three replicate buckets per cohort/treatment on T = 10, 15 and 18 days after
237 treatment onset (ages 17, 22, 25 days) by transferring 1,000 larvae (volumetric counts) to each vial
238 (200 larvae mL^{-1}). Filtered seawater controls were run simultaneously to account for background
239 respiration and vials were kept in the dark to inhibit photosynthesis of any autotrophic organisms
240 present in the culture media (e.g. phytoplankton in the fed treatments). Oxygen depletion was
241 monitored continuously with measurements recorded every minute. Respiration rates are reported
242 as the slope of the linear regression giving a rate of oxygen depletion during the period of linear
243 decline and over a period of one hour, starting approximately 20 minutes after deployment to the
244 chambers to limit noise just after handling. Rates were normalized by mean shell length to improve

comparability over time and between treatments where mean length varied significantly. Due to high mortality in the starved treatment for the AQF1 line (both fast and slow cohorts), replication was lacking and therefore respiration rates were not measured.

Statistical analysis

A one-way ANOVA was used to test for significant differences in the water chemistry (TOC and TDN) among water sources. Survival and growth were analyzed by two-way ANOVA with length or survival being the response variable, cohort (Wild/Slow, Wild/Fast, AQF1Slow, AQF1/Fast) and day being independent factors to test for differences between lines and growth cohorts over time. Feeding treatment (starved and fed) was also included as an independent factor for analysis of the first seven days of the experiment. However, due to differences in developmental stage, feeding treatments were also analyzed independently to examine differences among line and growth cohorts. Survival data were arcsine square root transformed to meet the assumptions of normality. Respiration rate was analyzed by two-way ANOVA using cohort, feeding treatment, and day as independent factors. When differences were detected, all analyses were followed by a Tukey's HSD post hoc analysis. Statistical analysis was completed in RStudio version 3.5.2 and significance reported when $p \leq 0.05$.

Results

Spawning and initial cohort attributes

Fertilization success was ≥ 95% for all pair crosses. The number of fertilized embryos produced from each pair ranged from $1.4 \times 10^6 - 9.0 \times 10^6$ and averaged 2.0×10^6 for

AQF1pairs and 5.4 × 10^6 for the wild pairs. A standardized count of 1.4 × 10^6 embryos per pair cross were pooled within each line to give each pair equal chance to contribute.

At the start of the starvation treatment (age 7 days old) fast growing larvae in the AQF1 and wild lines averaged 103.6 ± 0.6 µm and 108.8 ± 1.7 µm shell length, and the slow growing larvae were 89.4 ± 1.6 µm and 94.23 ± 1.8 µm, respectively (Fig 1). The coefficient of variation in size was similar across cohorts (ranging from 8 – 11% among cohorts). Wild/Fast larvae were significantly larger than Wild/Slow and AQF1/Slow (One-way ANOVA $F_{3,4} = 14.97$; $p < 0.05 = 0.012$), however AQF1/Fast had a larger overlap in size with AQF1/Slow and were not statistically different ($p = 0.058$) from each other. Larvae from duplicate culture tanks were pooled before counts, and at that time survival (age 3-7 days) was 60% and 67% in AQF1 and wild lines, respectively.

Total organic carbon and dissolved nitrogen

The amount of TOC in the hatchery water was lower than that in the river water in this experiment, however large amounts of carbon remained in the hatchery filtered water (CFS) for potential assimilation by larvae (Fig 2A). The addition of the carbon filter significantly reduced the amount of TOC present in the water ($F_{6, 11} = 331.11$; $p < 0.001$; Post Hoc Tukey's HSD $p < 0.001$; Fig 2A). The carbon filter addition contributed to the removal of TDN from the river water, although the change was subtle. TDN was significantly elevated in the fed treatments compared to starved treatments and the initial concentrations in both the River water and filtered hatchery water ($F_{6, 11} = 14.908$; $p < 0.001$; Post Hoc Tukey's HSD $p < 0.001$; Fig 2B).

Fig 2. Total organic carbon (A) and total dissolved nitrogen (B) in water collected directly from the Choptank River, the Cartridge Filtration System (CFS) used for experiments, and water from

the filtration system with an additional carbon filter (CFS carbon). Additionally, water samples were collected from fed and starved treatments during water changes for comparison. Samples from the buckets were taken after approximately 24 hours of larval culture. Asterisks indicated water samples that are significantly different.

Survival

After only 3 days of starvation (T = 3, age = 10 days), clear distinctions in gut coloration were seen with dark full guts in the fed controls and only light coloration in the starved larvae (S3 Fig). Starved larvae were still actively swimming and casual observations indicated comparable activity to that of the fed controls. After 7 days of starvation (T = 7, age = 14 days), swimming activity was minimal in the starved treatment and guts showed little coloration (S4 Fig). Many larvae in the fed controls developed eye spots by age 14 days, a predictor of settlement competency. By the end of the starvation period (T = 10 days; Age = 17 days) larvae in the starved AQF1 line primarily consisted of dead shell with very few live larvae found in samples for measurements (S5 Fig) and developmental differences had increased between the fed control and the remaining live larvae in the starved treatment. Starved larvae were stunted in the early stages of developing the umbo, while fed larvae had fully developed umbos and many had advanced to develop eye spots. Additionally, settlement was observed on the sides of the fed treatment tanks beginning at age 15 days.

In the fed controls, survival was significantly affected by day ($F_{8, 82} = 7.2417$; $p < 0.05$) and cohort ($F_{3, 82} = 9.326$; $p < 0.05$), but there was no interaction between them. The Wild/Slow cohort had significantly lower survival than all other cohorts (Tukey's HSD $p < 0.01$) throughout the experimental period; however, analysis at age 14 days, the last day before setting was evident,

309 showed no significant difference among the four cohorts (Fig 3A). By age 14 days, larvae in all
310 fed cohorts showed signs of competency (eyespots and extension of the foot in search of substrate)
311 and setting was observed on the sides of the buckets in the days following this observation.
312 Therefore, survival beyond day 14 could not be used to accurately compare cohorts as reduction
313 in larval numbers was in part due to settlement.

314 **Fig 3.** Survival over time for each cohort. The starvation period lasted from age 7 – 17 days
315 (indicated by the blue box on the x-axis) at which time (T = 10 days) food was reintroduced and
316 the recovery period began. Fed controls (top) began setting to the sides of the buckets by 15 days
317 old (T = 8 days) and thus the plot is truncated. Inset shows closer detail of the survival observed
318 for starved larvae at the end of the experimental period (Age 19 – 29 days). By age 22 days,
319 replicates for AQF1/Slow and AQF1/Fast were each pooled due to low survival within each
320 replicate. Error bars represent standard error.

321 Survival in the starved treatment showed a significant interaction between day and cohort
322 ($F_{33,\ 133} = 2.073$; $p < 0.005$). During the first seven days of starvation, survival in the starved
323 treatments was similar to that of the fed controls, however the Wild/Slow starved had significantly
324 lower survival than all other cohorts and treatments ($F_{7,\ 212} = 6.476$; $p < 0.001$; Tukey's HSD $p <$
325 0.05) due to initially high mortality (Fig 3B). Survivorship patterns were otherwise similar between
326 treatments with high early mortality leveling off from age 11 to 14 days. A second drop in survival
327 was observed toward the end of the starvation period (age 15-17 days), especially for the
328 AQF1line. By the end of the starvation period (age 17 days) no statistical difference was observed
329 between the Wild/Slow and Wild/Fast cohorts with 19 ± 5% and 23 ± 11% survival, respectively
330 (Fig 3B). Both AQF1 cohorts showed a large drop in survival from age 15-17 days with
331 AQF1/Slow and AQF1/Fast cohorts finishing the 10-day starvation period with only 11 ± 3% and

4 ± 3% survival, respectively (Fig 3B). Mortality continued even after food was reintroduced with < 10% survival after 4 days of recovery (Age = 22 days) for all starved cohorts (Fig 3B inset). Graphical trends suggest that the higher survival observed for wild cohorts at the end of starvation was maintained during the recovery period. However, AQF1 cohorts were each pooled by age 22 days, therefore statistical comparison among lines was not possible due to a lack of replication. Although overall survival was low in the starved treatments, those that survived were successful in completing metamorphosis and eye spot development was observed as early as age 22 days, just four days into the recovery period.

Growth

For larvae in the fed controls, shell length was significantly impacted by cohort ($F_{3, 93} = 16.497$; $p < 0.001$) and day ($F_{7, 93} = 250.557$; $p < 0.001$) but not their interaction. The significance of cohort as an effect was largely driven by the significantly larger size observed in the Wild/Fast larvae compared to all other cohorts at ages 14 – 19 days (Tukeys HSD $p < 0.001$; Fig 4) due to faster early growth. Signs of settlement competency were evident as early as 14 days pot fertilization, and recently settled oysters were observed on the sides of the buckets from day 15 onward. Thus, after age 14 days, the slope of the growth curves (growth rate) is not interpretable because of a bias from larger individuals settling out of the larval pool. Nonetheless, a steeper decline in size for Wild/Fast for the day 17 - 22 interval relative to Wild/Slow, and subsequent slower increase in size relative to Wild/Slow, indicates that a combination of growth rate and/or settlement differences led to a convergence of size in the Wild/Slow and Wild/Fast fed cohorts by day 25.

353 **Fig 4.** Mean shell length over time for each line by cohort grouping in fed controls and starved
354 treatment. Culture from days 3-7 post fertilization occurred in 200-L larval tanks (prior to feeding)
355 and size-based experiments in 20-L tanks. Shell lengths for fed controls after age 14 days and
356 starved treatments after age 22 days should be interpreted with caution because settlement of large
357 individuals out of the larval pool may have biased the size distribution. Arrows indicate days in
358 which settlement was clearly observed in each treatment. Age 7 – 17 days represent the period of
359 starvation, indicated by the blue box on the x-axis. Error bars represent standard error. Due to low
360 survival in the AQF1 cohorts, length measurements were not possible after age 14 and 22 days for
361 fast and slow growers, respectively. Additionally, from age 17 to 22 days in the AQF1/Slow cohort
362 only one replicate of length measurements was possible.

363 Patterns in shell length differed significantly between the fed controls and starved
364 treatments with significant treatment by day ($F_{1, 71} = 254.39$; $p < 0.001 = 2.2\times10^{-16}$) and cohort by
365 day ($F_{12, 71} = 2.62$; $p < 0.001 = 0.006$) interactions during the first seven days of treatment (before
366 settlement started in fed control; Fig 4). All larvae in the fed treatment continued to show
367 significant growth over the first seven days, while growth in the starved treatment was stunted for
368 all cohorts (Fig 4). Within the starved treatments, a significant cohort by day interaction ($F_{21, 83} =$
369 2.32; $p < 0.001 = 0.004$) also was observed across the duration of the experimental period. Shell
370 length showed no significant increase once food was removed, so fast early-growth cohorts
371 remained larger than slow early-growth cohorts. The exception was AQF1/Fast, which had a
372 growth slow down during the same day 10-14 interval when the fed AQF1/Fast slowed growth.
373 Even after food was reintroduced to the starvation treatment, growth remained stunted for at least
374 two days (age 17-19 days) before starting to increase (age 19-22 days) and showing the fastest
375 growth of the experiment between day 22 and 25 (after which settlement started; Fig 4). Once

376 growth resumed during the recovery period, Wild/Fast and Wild/Slow had no significant
377 difference in shell length (Fig 4). For the AQF1 line, high mortality in the starved treatments
378 prevented growth comparisons after day 14 when less than 10 larvae were found for measurement
379 in all replicates except for one replicate in the AQF1/Slow cohort. A one-way ANOVA with wild
380 larvae endpoint data (age 29 days when both groups are losing larger individuals to settlement)
381 showed no significant difference in shell length between treatments although the trend was for
382 starved treatment larvae to have smaller size (251.0 ± 3.4µm) on average compared to fed controls
383 (267.0 ± 6.2µm).

384 # Respiration

385 Due to low replication, Fast and Slow cohorts for each line were pooled for comparison of
386 respiration rates between lines. Length-normalized respiration rate was significantly impacted by
387 day ($F_{1, 38} = 13.8952$; $p < 0.001$) and increased with age for both fed control and post-starved larvae
388 (Fig. 5). Larvae at age 25 days had significantly higher length-normalized respiration rate than
389 both 17 and 22 days (Tukey's HSD; $p < 0.05$). There was not a significant effect of treatment, but
390 this may be due in part to low replication (N=2) in the starved treatment for measurement at age
391 17 days. Normalized respiration rates at the end of the 10-day starvation period were low compared
392 to fed controls (Fig 5), but within 5 days of the reintroduction of food the Wild/Starved larvae had
393 rates similar to that observed in the fed controls.

394 **Fig 5.** Shell length standardized respiration rates of all cohorts measured at the end of the
395 starvation exposure (T = 10; Age 17 days), and during recovery at 5- and 8-days post
396 reintroduction of food (Age 22 and 25 days, respectively). The number of replicates per
397 timepoint for each cohort is indicated above each bar. Error bars represent standard error.

Discussion

The distribution of genomic and phenotypic changes during domestication are a fundamental question related to breeding practice in aquatic species [5–7], with potential fitness impacts on wild populations if there is interbreeding or deliberate population supplementation with domesticated strains. As a first step to investigating the potential for domestication selection in the eastern oyster, we compared starvation tolerance of larvae produced from wild and selectively bred broodstock. Assuming that selection for this trait is strong in the wild and nonexistent under culture, we predicted that starvation tolerance would be greater in the larval progeny of wild oysters relative to larvae from closed selection lines (multiple generations of larval culture with *ad libitum* feeding). This prediction was informed by the considerable molecular transporter machinery that allows for larval body maintenance via DOM absorption in the absence of algal food [38]. If this transporter machinery is energetically costly, starvation tolerance could weaken in closed selection lines as either a response to release from selection, or to selection for faster growth. Selection for faster growth under *ad libitum* feeding could have selected against expression of these proteins, lowering starvation tolerance. Alternatively, selection for faster larval growth could have generated changes in other metabolic traits with DOC transporter functions maintaining their fitness value due to pleiotropy even under *ad libitum* feeding. These scenarios suggest that selected strain larvae should grow faster than wild larvae under *ad libitum* algal feeding, all else being equal and assuming no inbreeding effects. In the absence of growth rate differences on a live phytoplankton diet, suggesting weak selection on this life history stage in the history of the selected strain, unequal starvation tolerance would imply correlations with traits under stronger selection.

Control larvae with ad *libitum* phytoplankton showed overall similar mortality and growth rates across all cohorts. This contradicts predictions that selectively bred aquaculture strains have greater larval growth rates compared to wild larvae. In fact, the Wild/Fast larvae maintained a greater mean size throughout the developmental period compared to both AQF1 cohorts. At the end of the 10-day starvation period (age 15-17 days), wild larvae showed significantly greater survival than the AQF1 line, consistent with our prediction. Starvation consisted of withholding the preferred food (phytoplankton), but micronutrients in the form of dissolved organic matter (DOM) remained available to the larvae in both the fed and starved treatments, and presumably were utilized to maintain viability. If AQF1 strains lost some of the wild capacity to utilize DOM to maintain body condition under food limitation, it suggests that there is an energetic cost to the molecular transport machinery for providing DOM uptake, and release from selection unlimited provisioning or selection for fast larval growth could lead to selection against those transport mechanisms if they have few other functions. Testing these more specific mechanistic hypotheses, and their impact on fitness, will require further study. Given that this is the first test to understand mechanisms that could be linked to domestication in eastern oysters, that we are aware of, we discuss caveats and implications for these findings in the context of related literature.

DOM as a source of nutrients during starvation

This study focused on a larval trait predicted to affect fitness in the wild and be subject to inadvertent selection in hatchery culture (or release from selection maintaining tolerance). Specifically, we compared starvation tolerance in larvae produced from wild parents and larvae produced from selectively-bred parents. The selectively bred AQF1 line experienced nearly complete mortality during the last three days of starvation, while both fast and slow wild cohorts experienced better survival. Analysis of dissolved organic carbon suggests that although

particulate food (phytoplankton) was withheld, micronutrients (e.g. DOM) were present during starvation. Larvae are capable of utilizing natural sources of DOM [38–41] which may have provided the energy needed to fuel basal metabolism requirements during the "starvation" period. While DOM alone is not expected to sustain growth and development, it might allow for somatic maintenance under food limitation [41] and is expected to represent a large potential energy source for developing larvae under natural conditions [38]. We therefore hypothesize that the ability to absorb, transport, and assimilate micronutrients may be an important source of differentiation between the lines tested and represent promising phenotypes to explore as possible mechanistic changes associated with domestication selection. It is plausible that aquaculture lines can adapt to the hatchery environment in which food is provided in excess thereby limiting their ability to utilize DOM during prolonged starvation events as a result of many generations of hatchery propagation. However, this would require further and more detailed testing to understand the role of DOM under food limitation.

Survival during prolonged starvation

The most dramatic mortality was observed between days 8 and 10 of the starvation (age 15 – 17 days) in the AQF1 line suggesting that a critical point was reached. Blaxter and Hempel [42] described a point of no return, in which the duration of starvation induces an irreversible physiological toll, resulting in death even if a proper food source is restored. This is consistent with the continued mortality we observed during the recovery period. Survival continued to decline through age 22 days (5 days into the recovery), during which growth also remained stunted, suggesting that larvae had not yet rebounded physiologically. When starvation begins at the time of hatch, His and Seaman [43] suggest that the point of no return for *C. gigas* larvae occurs when maternal reserves are depleted (6-8 days post-fertilization), but Moran and Manahan [44] observed

no significant change in mortality rate during a starvation period up to 14 days post fertilization. In our study, both wild and AQF1 lines had lower tolerance than that reported by Moran and Manahan [44], perhaps due to the different age of onset for starvation. When starvation occurs from onset of hatch, larvae can utilize lipid reserves provided by the egg and reduce metabolic rates allowing for long term survival (up to 14 days) [32,44]. However, when food is removed after egg lipid reserves are depleted (6-8 days post hatch), as in our study, mortality can be high even under short periods of starvation (e.g. 4 days) [32].

Larval survival and successful recruitment of subsequent generations following plantings of hatchery produced spat or adult oysters is an essentential component to long term restoration success. If planted oysters from selective breeding programs produce larvae that cannot survive the gaunlet of stressors in the estaurine environment, then long term restoration success is stymied. While we acknowledge that a 10-day period of no phytoplankton availability may be unlikely under natural conditions, patchiness in larval food quantity [33] and nutritional quality [44] are expected. Under these conditions an ability to withstand and recover from periods with limited exogenous energy sources is a critical fitness trait. The lower tolerance to starvation in the selected strain studied here is consistent with this trait being a costly adaptation weakened as a result of selection for fast growth or, more likely, a trait correlated with commercial traits under direct selection. Further comparisons with other eastern oyster selected strains are needed to determine if this larval tolerance difference is a general result of domestication or specific to the tested strains.

Physiological recovery

We observed a two to five-day delay in shell growth once food was reintroduced to the starved treatments. This is in contrast to Moran and Manahan [44] who saw an immediate

488 resumption of physiological rates, including growth, at the onset of delayed feeding of *C. gigas*
489 larvae. The delay in growth we observed during the recovery period may be due in part to a delay
490 in recovering normal feeding behavior [43] and the rebuilding of lost tissue mass and energetic
491 reserves before energy is used for shell growth [46]. We observed reduced respiration rates at the
492 end of the 10-day starvation period, but within 5 days of the recovery period respiration rate was
493 similar to that observed in the fed control groups. Shell growth during the first 5 days of recovery
494 was low suggesting that although there was an observed recovery in respiration, there was likely
495 a priority given to somatic tissue growth prior to the production of new shell. While not quantified,
496 larvae from both lines had a visible loss of tissue mass and an inhibition of locomotion during
497 starvation, suggesting that any active metabolism was only to maintain homeostasis during a
498 depressed metabolic state. However, even after 10-days without food, respiration rates in both wild
499 fast and slow cohorts were still measurable, suggesting that they may be utilizing some exogenous
500 energy source (e.g. DOM; [44]). After eight days of recovery, growth (as measured by mean shell
501 length) in the starved treatments was similar to that observed in the fed controls and starved larvae
502 reached final shell lengths similar to that observed in the fed controls during peak settlement
503 showing a full recovery for those that survived the starvation period.

504 Variation in growth cohorts between lines

505 Starvation tolerance is a complex trait, so we expected there might be interactions with
506 growth rate. Given the development of high size variance early in each line (typical of eastern
507 oysters), we separated each line into fast and slow early-growth cohorts to test for relationships
508 with starvation tolerance. The only study that previously examined oyster larval growth variance
509 [30] did not separate and follow individual size fractions. Even though distinct growth fractions
510 might be better separated later in larval culture, for the purposes of this experiment the separation

was quite early, at day 7 postfertilization. If the fractions represent differences that only affect early larval growth then their subsequent growth trajectories were expected to be parallel and an interaction with starvation tolerance was less likely. Alternatively, if early growth differences marked a persistent phenotypic difference then distinct growth rates were expected for slow and fast fractions of each line. Size distributions in fed treatments are interpretable in terms of growth rate only until initiation of settlement at age 14 days (biasing the size distribution thereafter). For the wild larvae in the fed treatment, the initial size separation between fast and slow growth cohorts was maintained throughout the experimental period, with no difference in growth rate observed. In the fed AQF1 line, the initial size distinction was maintained up to age 10 days (3 days since size separation), but converged by age 14 days between the growth cohorts. The reason for this difference between wild and AQF1 are not known, but both observations are consistent with growth differences being a function of early larval processes, not an enduring difference in rate.

A similar distinction between fast and slow cohorts was maintained in the starved treatments throughout the starvation period. Interestingly, once food was reintroduced, the significant difference in length between growth cohorts disappeared for wild larvae. This is in part due to a slightly faster growth recovery in the Wild/Slow cohort during age 19 to 22 days, during which CV increased for both wild cohorts suggesting that some larvae rebounded more rapidly than others (Fig. S2). The convergence of growth trajectories between fast and slow Wild larvae is suggestive of compensatory growth in the Wild/Slow cohort, however size specific mortality and/or settlement cannot be ruled out as interval mortality was high during this time (30% and 48% for fast and slow, respectively) and settlement was apparent in both cohorts by age 22 days.

Impacts of line history on the experimental design

There are a number of additional factors associated with the design and setup of this experiment that may have influenced the outcome and are important to consider. Inbreeding depression in the AQF1line is an unlikely explanation for their relatively low survival during starvation because two largely independent selected lines were crossed to produce the AQF1 larvae for this experiment. In fact, heterosis effects were a potential outcome of this mating strategy [47], but were not apparent. Differential maternal effects can also impact larval survival and response to stress given the importance of egg lipids to early larval development [48]. However, all broodstock were held under local ambient conditions for four weeks prior to spawning in order to reduce the impact of different environmental conditions on gamete quality. Also, the experimental treatment was delayed until day seven postfertilization to reduce the potential for differences in maternal energy reserves to impact larval tolerance to starvation [43]. Broodstock source salinity is also a potential confounding factor in this experiment. The aquaculture lines were partially conditioned at a higher salinity (14 – 16 ppt) prior to arriving at Horn Point where all broodstock were held for four weeks at the experimental salinity (9.5 ppt) prior to spawning. This did not seem to put the AQF1 line at a developmental disadvantage because growth was similar among the fed controls and survival of AQF1 larvae was similar or better than the Wild line. Also, the aquaculture lines used in this experiment have previously performed well under low salinity conditions (9 – 15 ppt) [37]. Salinity during broodstock gonad conditioning are known to have transgenerational plastic effects on salinity tolerance in the larval offspring [49], so it is possible that the reduced salinity at the end of the conditioning period could impact larval resistance to stress. Lastly, we note that because of extensive restoration in the Chesapeake Bay that includes seeding with hatchery produced spat [13,14], it is conceivable that our wild broodstock oysters could have some ancestry from hatchery produced restoration oysters. However, oysters produced in this region

556 would have been sourced from the Horn Point Oyster Hatchery where wild broodstock are used
557 for supportive breeding. Therefore, broodstock collections used here would at most only have one
558 prior generation of hatchery propagation, compared to many generations in the closed aquaculture
559 lines.

560 Conclusions

561 For oysters, lines artificially selected for aquaculture are serving the farming industry,
562 where lifetime fitness is not a focus, but rather the emphasis is on farm to table production rates in
563 which yield is largely a function of growth rate and survivorship. However, in the context of using
564 hatcheries for stock enhancement, planted oysters must not only grow to maturity, but also
565 successfully reproduce and generate offspring with robust abilities to withstand the many stressors
566 encountered during the pelagic life stage. At this early stage of selective breeding in eastern
567 oysters, the unknown phenotypic impacts from domestication selection may be large or small,
568 depending on the trait. In this initial experiment, we have shown a slightly lower tolerance to
569 prolonged starvation in the AQF1 line intentionally selected for disease resistance over multiple
570 generations of hatchery propagation. Replication of this experiment will be necessary using
571 different aquaculture strains and additional wild oysters to strengthen the inference that reduced
572 larval starvation tolerance is linked to domestication selection generally. Based on these initial
573 results, we suspect bioenergetic processes related to micronutrient uptake and utilization may be
574 promising candidate traits for investigating mechanistic changes as a result of domestication
575 selection, or are genetically correlated with traits under direct selection. The great successes of
576 selective breeding to produce shellfish with improved aquaculture yields is likely to result in an
577 increased reliance on selectively bred lines for oyster farming. With further oyster domestication

578 expected, our work is of importance to understand inadvertent trait evolution as well as the
579 potential impacts domesticated oysters can have on natural populations.

580

581 Data for this study are available at: to be completed after manuscript is accepted for publication.

582

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587

588 # References

589 1. IPCC. Climate Change 2013: the physical science basis. Contribution of working group I
590 to the fifth assessment report of the intergovernmental panel on climate change. Stocker
591 TF, Qin D, Plattner G, Tignor KM, Allen SK, Boschung J, et al., editors. Cambridge,
592 United Kingdom/New York, NY, USA: Cambridge University Press; 2013.

593 2. Ford SE, Smolowitz R. Infection dynamics of an oyster parasite in its newly expanded
594 range. Mar Biol. 2007;151: 119–133. doi:10.1007/s00227-006-0454-6

595 3. Burge CA, Eakin MC, Friedman CS, Froelich B, Hershberger PK, Hofmann EE, et al.
596 Climate change influences on marine infectious diseases: Implications for management
597 and society. Ann Rev Mar Sci. 2014;6: 249–277. doi:10.1146/annurev-marine-010213-

135029

4. van Oppen MJH, Oliver JK, Putnam HM, Gates RD. Building coral reef resilience through assisted evolution. Proc Natl Acad Sci USA. 2015;112: 2307–2313. doi:10.1073/pnas.1422301112

5. Araki H, Berejikian BA, Ford MJ, Blouin MS. Fitness of hatchery-reared salmonids in the wild. Evol Appl. 2008;1: 342–355. doi:10.1111/j.1752-4571.2008.00026.x

6. Waples RS. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Can J Fish Aquat Sci. 1991;48: 124–133. doi:10.1139/f91-311

7. Christie MR, Marine ML, French RA, Blouin MS. Genetic adaptation to captivity can occur in a single generation. Proc Natl Acad Sci USA. 2012;109: 238–242. doi:10.1073/pnas.1111073109

8. Araki H, Cooper B, Blouin MS. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science (80-). 2007;318: 100–103. doi:10.1126/science.1145621

9. Lorenzen K, Beveridge MCM, Mangel M. Cultured fish: Integrative biology and management of domestication and interactions with wild fish. Biol Rev. 2012;87: 639–660. doi:10.1111/j.1469-185X.2011.00215.x

10. Camara MD, Vadopalas B. Genetic aspects of restoring Olympia oysters and other native bivalves: Balancing the need for action, good intentions, and the risks of making things worse. J Shellfish Res. 2009;28: 121–145. doi:Doi 10.2983/035.028.0104

11. Allen SK, Hilbish TJ. Genetic considerations for hatchery-based restoration of oyster

reefs: A summary from the September 21-22, 2000 workshop. 2000.

12. Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, et al. Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience. 2011;61: 107–116. doi:10.1525/bio.2011.61.2.5

13. Damiano MD, Wilberg MJ. Population dynamics of eastern oysters in the Choptank River complex, Maryland during 1989 – 2015. Fish Res. Elsevier; 2019;212: 196–207. doi:10.1016/j.fishres.2018.12.023

14. Hornick KM, Plough LV. Tracking genetic diversity in a large-scale oyster restoration program: effects of hatchery propagation and initial characterization of diversity on restored vs. wild reefs. Heredity. 2019;123: 92–105. doi:10.1038/s41437-019-0202-6

15. Hernández AB, Brumbaugh RD, Frederick P, Grizzle R, Luckenbach MW, Peterson CH, et al. Restoring the eastern oyster: how much progress has been made in 53 years? Front Ecol Environ. 2018;16: 463–471. doi:10.1002/fee.1935

16. Brumbaugh RD, Beck MW, Coen LD, Craig L, Hicks P. A practitioners guide to the design & monitoring of shellfish restoration projects: An ecosystems services approach. Arlington, VA; 2006.

17. Mann R, Powell EN. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. J Shellfish Res. 2007;26: 905–917.

18. Miller LS, La Peyre J, La Peyre M. Suitability of oyster restoration sites along the Louisiana coast: examining site x stock interaction. J Shellfish Res. 2017;36: 341–351. doi:10.2983/35.036.0206

640 19. Hare MP, Allen SK, Bloomer P, Camara MD, Carnegie RB, Murfree J, et al. A genetic
641 test for recruitment enhancement in Chesapeake Bay oysters, *Crassostrea virginica*, after
642 population supplementation with a disease tolerant strain. Conserv Genet. 2006;7: 717–
643 734. doi:10.1007/s10592-005-9108-3

644 20. Carlsson J, Carnegie RB, Cordes JF, Hare MP, Leggett AT, Reece KS, et al. Evaluating
645 recruitment contribution of a selectively bred aquaculture line of the oyster, *Crassostrea*
646 *virginica* used in restoration efforts. J Shellfish Res. 2008;27: 1117–1124.

647 21. Skaala Ø, Besnier F, Borgstrøm R, Barlaup B, Sørvik AG, Normann E, et al. An extensive
648 common-garden study with domesticated and wild Atlantic salmon in nature reveals
649 impact on smolt production and shifts in fitness traits. Evol Appl. 2019; 1–16.
650 doi:10.1111/eva.12777

651 22. Kennedy VS. Biology of Larvae and Spat. In: Kennedy VS, Newell RI, Eble AF, editors.
652 The Eastern Oyster: *Crassostrea virginica*. College Park, Maryland: Maryland Sea Grant;
653 1996. pp. 371–421.

654 23. Plough LV, Shin G, Hedgecock D. Genetic inviability is a major driver of type-III
655 survivorship in experimental families of a highly fecund marine bivalve. Mol Ecol.
656 2016;25: 895–910. doi:10.1111/mec.13524

657 24. Crean AJ, Monro K, Marshall DJ. Fitness consequences of larval traits persist across the
658 metamorphic boundary. Evolution. 2011;65: 3079–3089. doi:10.5061/dryad.574r7

659 25. Emlet RB, Sadro SS. Linking stages of life history: How larval quality translates into
660 juvenile performance for an intertidal barnacle (*Balanus glandula*). Integr Comp Biol.
661 2006;46: 334–346. doi:10.1093/icb/icj023

662 26. Bitter MC, Kapsenberg L, Gattuso J-P, Pfister CA. Cryptic genetic variation underpins
663 rapid adaptation to ocean acidification. bioRxiv. 2019; doi:https://doi.org/10.1101/700526

664 27. Plough LV. Genetic load in marine animals: a review. Curr Zool. 2016;62: 567–579.
665 doi:10.1093/cz/zow096

666 28. Launey S, Hedgecock D. High genetic load in the pacific oyster *Crassostrea gigas*.
667 Genetics. 2001;159: 255–265.

668 29. Plough LV, Hedgecock D. Quantitative trait locus analysis of stage-specific inbreeding
669 depression in the Pacific Oyster *Crassostrea gigas*. Genetics. 2011;189: 1473–1486.
670 doi:10.1534/genetics.111.131854

671 30. Taris N, Batista FM, Boudry P. Evidence of response to unintentional selection for faster
672 development and inbreeding depression in *Crassostrea gigas* larvae. Aquaculture.
673 2007;272: 69–79. doi:10.1016/j.aquaculture.2007.08.010

674 31. Pan T-CF, Applebaum SL, Manahan DT. Genetically determined variation in
675 developmental physiology of bivalve larvae (*Crassostrea gigas*). Physiol Biochem Zool.
676 2015;88: 128–136. doi:10.1086/679656

677 32. Kheder RB, Quéré C, Moal J, Robert R. Effect of nutrition on *Crassostrea gigas* larval
678 development and the evolution of physiological indices. Part A: Quantitative and
679 qualitative diet effects. Aquaculture. 2010;305: 165–173.
680 doi:10.1016/j.aquaculture.2010.04.022

681 33. Olson RR, Olson MH. Food limitation of planktotrophic marine invertebrate larvae: Does
682 it control recruitment success? Annu Rev Ecol Syst. 1989;20: 225–247.

683 34. Helm MM, Bourne N. Hatchery culture of bivalves: A practical manual. FAO Fisheries
684 Technical Paper 471. Rome: Food and Agriculture Organization of the United Nations;
685 2004.

686 35. Plough LV. Environmental stress increases selection against and dominance of deleterious
687 mutations in inbred families of the Pacific oyster *Crassostrea gigas*. Mol Ecol. 2012;21:
688 3974–3987. doi:10.1111/j.1365-294X.2012.05688.x

689 36. Aquaculture Genetics and Breeding Technology Center. The Aquaculture Genetics and
690 Breeding Technology's Oyster Breeding Program. Gloucester Point, VA; 2009.

691 37. Frank-Lawale A, Allen SK, Dégremont L. Breeding and domestication of eastern oyster
692 (*Crassostrea virginica*) lines for culture in the mid-Atlantic, USA: Line development and
693 mass selection for disease resistance. J Shellfish Res. 2014;33: 153–165.
694 doi:10.2983/035.033.0115

695 38. Manahan DT, Crisp DJ. The role of dissolved organic material in the nutrition of pelagic
696 larvae: amino acid uptake by bivalve veligers. Am Zool. 1982;22: 635–646.

697 39. Baldwin BS, Newell RIE. Omnivorous feeding by planktotrophic larvae of the eastern
698 oyster *Crassostrea virginica*. Mar Ecol Prog Ser. 1991;78: 285–301.

699 40. Manahan DT. The uptake and metabolism of dissolved amino acids by bivalve larvae.
700 Biol Bull. 1983;164: 236–250.

701 41. Widdows J. Physiological ecology of mussel larvae. Aquaculture. 1991;94: 147–163.

702 42. Blaxter J, Hempel G. The influence of egg size on herring larvae (*Clupea harengus* L.).
703 ICES J Mar Sci. 1963;28: 211–240.

704 43. His E, Seaman MNL. Effects of temporary starvation on the survival, and on subsequent
705 feeding and growth, of oyster (*Crassostrea gigas*) larvae. Mar Biol. 1992;114: 277–279.
706 doi:10.1007/BF00349530

707 44. Moran AL, Manahan DT. Physiological recovery from prolonged "starvation" in larvae of
708 the Pacific oyster *Crassostrea gigas*. J Exp Mar Bio Ecol. 2004;306: 17–36.
709 doi:10.1016/j.jembe.2003.12.021

710 45. Vanderploeg HA, Liebig JR, Gluck AA. Evaluation of different phytoplankton for
711 supporting development of zebra mussel larvae (*Dreissena polymorpha*): The importance
712 of size and polyunsaturated fatty acid content. J Gt Laje Resour. 1996;22: 36–45.

713 46. Kooijman SALM. Dynamic Energy Budget Theory for Metabolic Organization. Third.
714 Cambridge: Cambridge University Press; 2010.

715 47. Hedgecock D, Mcgoldrick DJ, Manahan DT, Vavrab J, Appelmans N, Bayne BL.
716 Quantitative and molecular genetic analyses of heterosis in bivalve molluscs. J Exp Mar
717 Bio Ecol. 1996;203: 49–59.

718 48. Bayne B. Some effects of stress in the adult on the larval development of *Mytilus edulis*.
719 Nature. 1972;237: 459.

720 49. Davis HC. Survival and growth of clam and oyster larvae at different salinities. Biol Bull.
721 1958;114: 296–307.

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Supporting information

S1 Fig. Schematic showing the experimental design.

S2 Fig. Box plots showing length distributions over time for each treatment cohort. After age 14 days, the starved aquaculture growth fractions have very few measurements and means may be skewed due to the high mortality rates.

S3 Fig. Micrographs of 10-day old larvae in each treatment after three days of starvation.

S4 Fig. Micrographs of 14-day old larvae in each treatment after seven days of starvation.

S5 Fig. Micrographs of 17-day old larvae in each treatment after ten days of starvation.

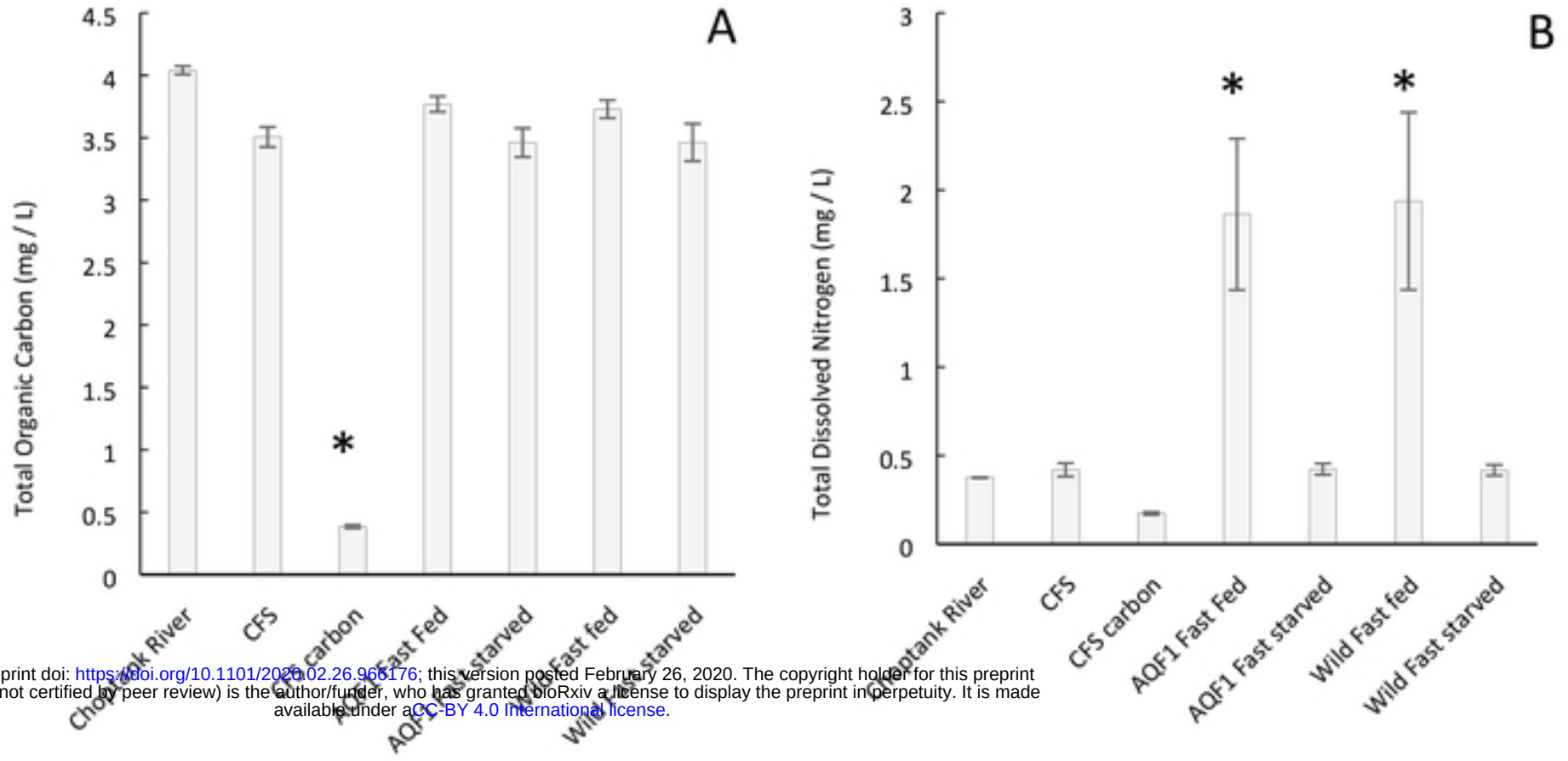
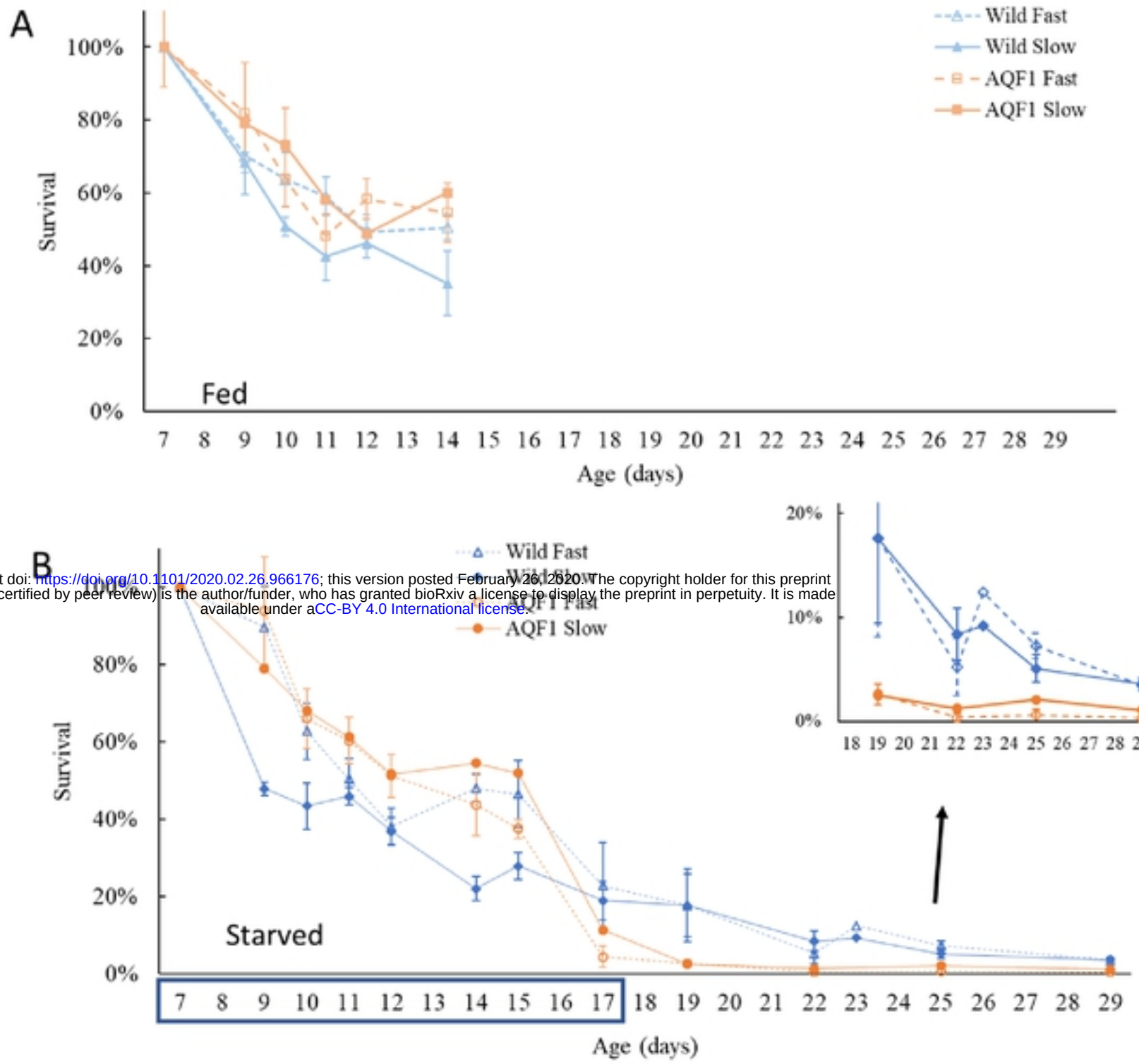


Fig 2



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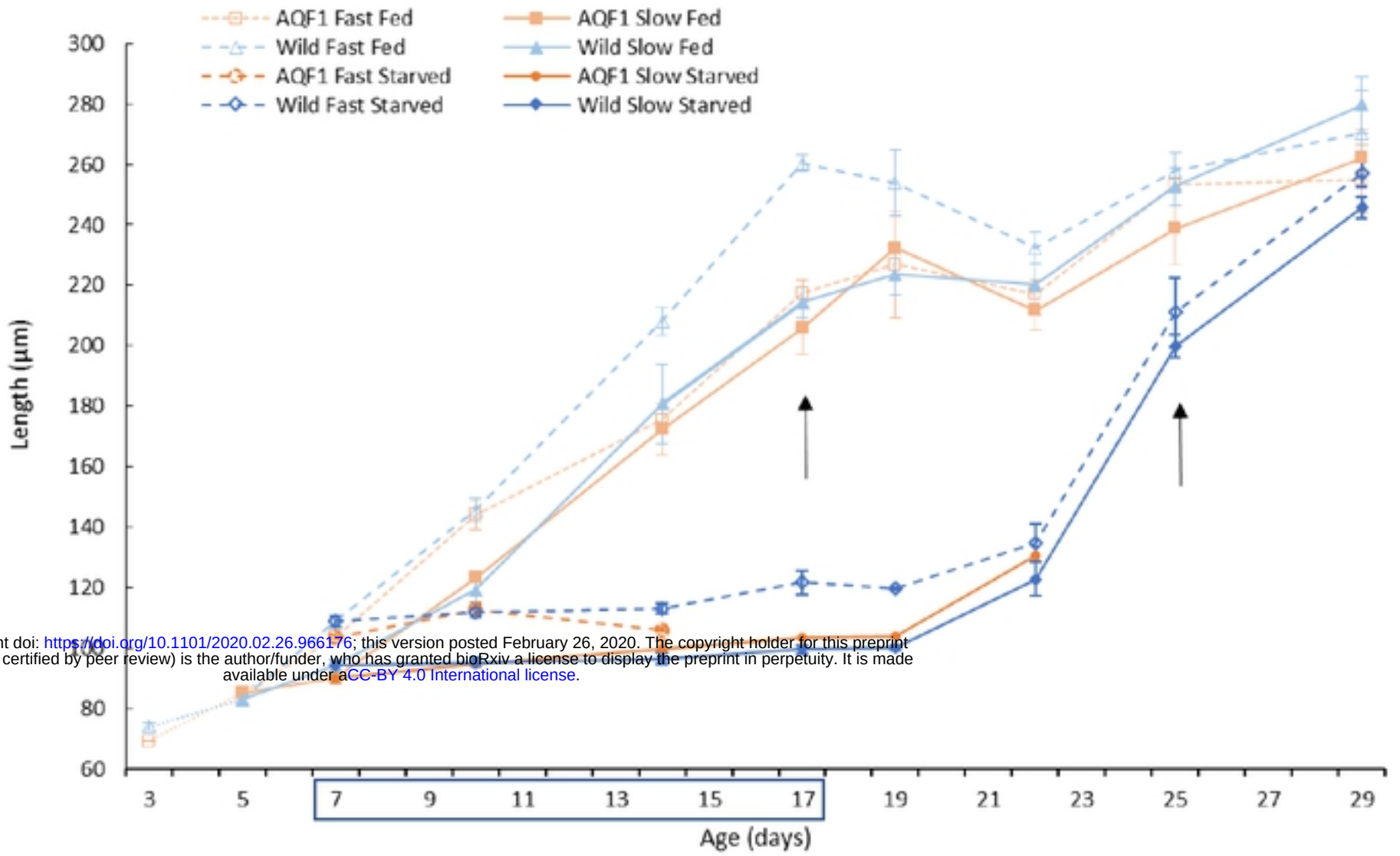


Fig 4

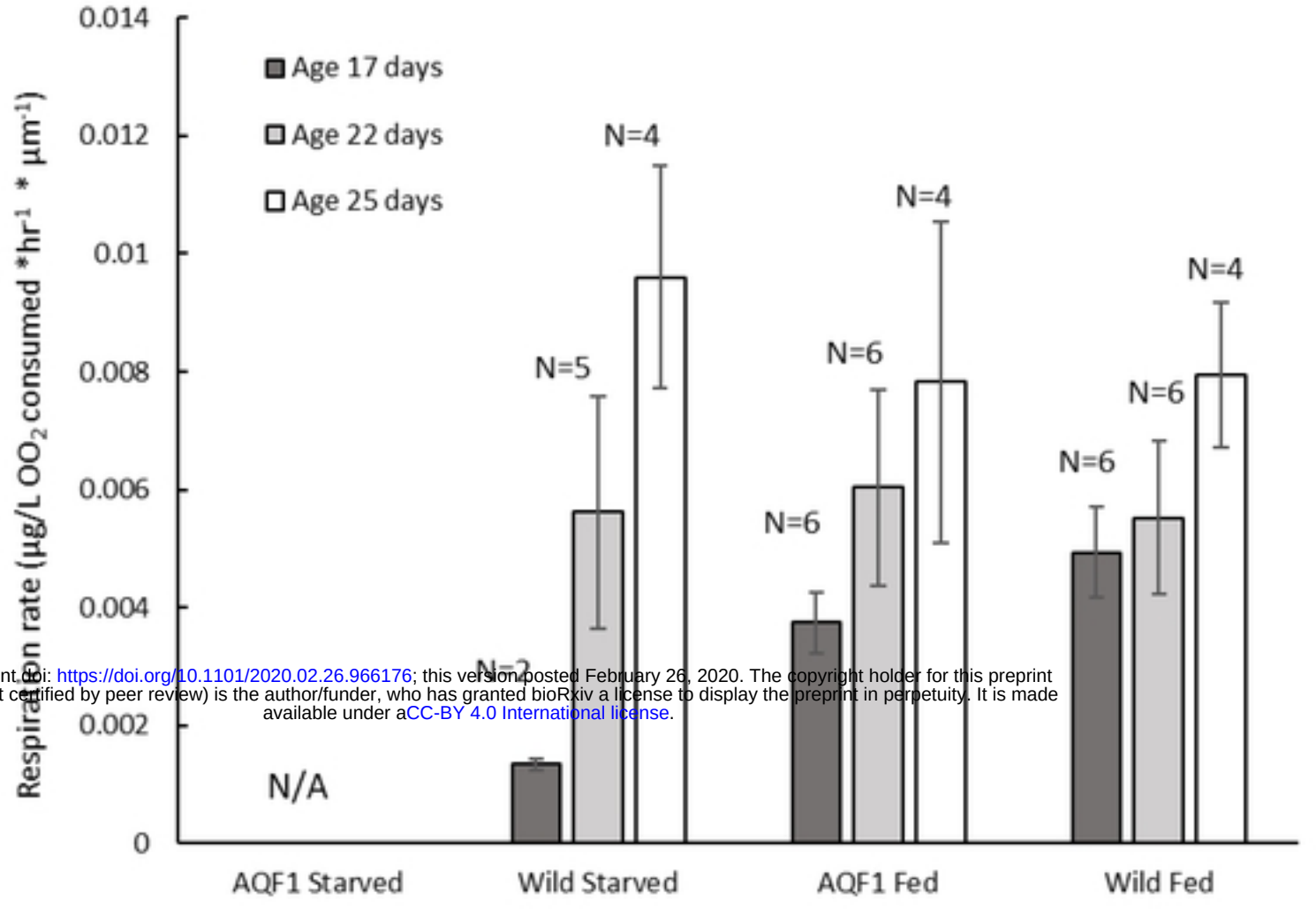
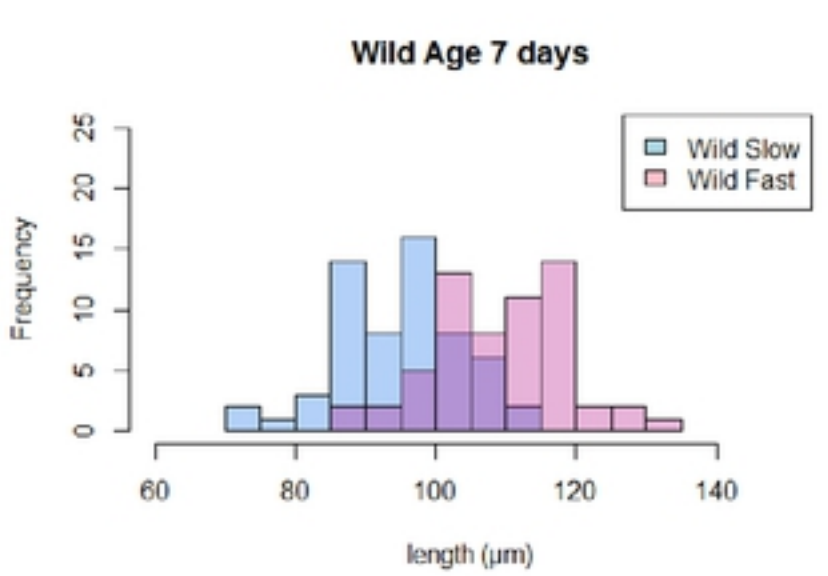


Fig 5



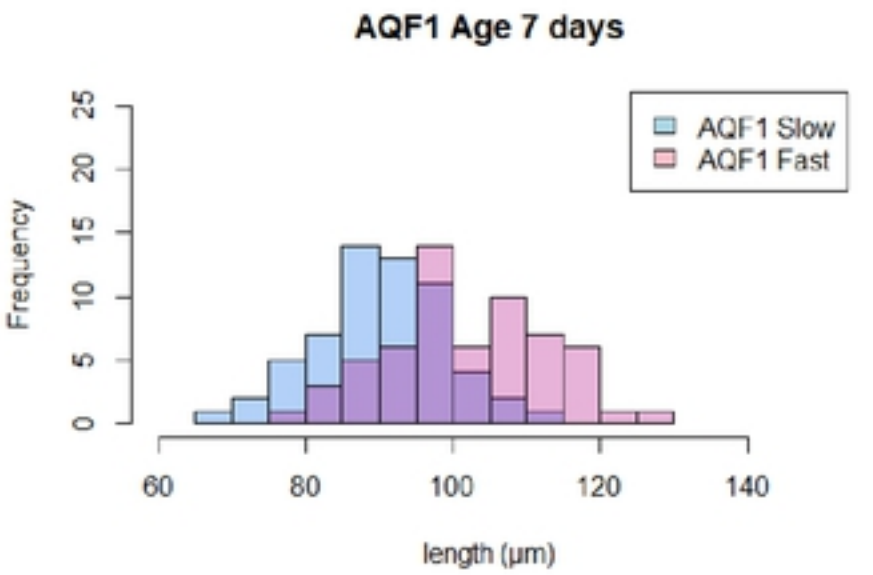


Fig 1