Exploring the feasibility of selectively breeding farmed Atlantic surfclams (*Spisula solidissima*) for greater heat tolerance

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Abstract

Bivalve aquaculture is an important and rapidly expanding sector in global food production, yet climate change presents numerous challenges to its continued expansion. The Atlantic surfclam (Spisula solidissima) is emerging as an attractive alternate species by aquaculturists across the northeastern United States, since it is native, grows rapidly, and complements the region's established farming framework. However, the species is vulnerable to prolonged high temperatures conditions, an issue that will be exacerbated by rising ocean temperatures and particularly problematic on shallow coastal farms. In this study, we evaluated the response of adult farmed surfclams to heat stress after juvenile exposure and the ability for heat tolerance to

be passed to subsequent generations. We found that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent heat stress for significantly longer than individuals not exposed to lethal temperatures as juveniles. We also found that selective breeding enhanced heat tolerance in first-generation surfclam progeny. Moreover, growth of the heat-selected progeny was not significantly different from that of control clams. Although more research on this topic is necessary, this work suggests selective breeding may be a viable strategy for enhancing survival of cultivated bivalves vulnerable to heat stress.

Introduction - The world's oceans have absorbed more than 90% of the heat trapped by anthropogenic greenhouse gas emissions (IPCC 2014), and recent research suggests that ocean warming exceeds previous estimates. For instance, Resplandy and colleagues (2018) measured the outgassing of O_2 and CO_2 from the world's oceans, or the amount of gas the oceans release as they warm, and found that every year since 1991, humans put 60% more energy into the oceans than previously thought. Local warming of the continental shelf along the northeastern United States has been documented for decades (Scavia et al. 2002). Recent estimates of the Northeast's sea surface temperatures suggest that this region is warming two- to three-times faster than the global average (Saba et al. 2016; Pershing et al. 2015). A plethora of marine species have already begun shifting their distributions in response to the temperature change (Sunday et al. 2012; Kleisner et al. 2017; Free et al. 2019; Morson et al. 2019). Among them is the Atlantic surfclam (Spisula solidissima), whose northward range shift is well documented (Munroe et al. 2013; 2016; Powell et al. 2016; Hennen et al. 2018; Hofmann et al. 2018).

As a temperate species, surfclams are known to be vulnerable to high temperature conditions (Goldberg and Walker, 1990; Weinberg 2005; Hornstein et al. 2018). This issue is expected to be exacerbated as the region's waters continue to warm (Narváez et al. 2015; Munroe et al. 2016), and one that may already be problematic on shallow coastal farms. Historically, the habitat for wild populations of surfclams off the coast of New Jersey extended from shallow beaches along barrier islands and the mouths of estuaries to a depth of 60 m on the continental shelf (Jacobson and Weinberg 2006). Recently, the range of wild surfclams off the coast of New Jersey has shifted towards deeper, cooler water (Weinberg et al. 2002; Weinberg et al. 2005; Weinberg 2005; Timbs et al. 2019).

Temperature greatly influences surfclam growth and performance, and their ability to cope with warm water temperatures is in part related to their size (Cerrato and Keith 1992). Large-bodied individuals caught in the surfclam fishery (shell length >120 mm) cannot survive prolonged exposure to temperatures greater than 21˚C (Munroe et al. 2013; Weinberg 2005). However, juveniles and smaller individuals seem to have a wider thermal tolerance, succumbing to mortality when temperatures above 26˚C are sustained for days or weeks (Acquafredda et al. 2019). During an experimental evaluation of nursery rearing temperatures of early post-metamorphic juvenile surfclams (shell length ~0.7–3.0 mm), surfclam seed under heat stress (~26˚C) survived less than half as well as those reared under cooler (≤ 20˚C) conditions (Acquafredda et al. 2019). Notably, surfclams produced from different parent stock responded differently to temperature during these trials, suggesting that thermal tolerance may be a heritable trait (Acquafredda et al. 2019).

The surfclam has strong potential to benefit farmers who are eager to build diversity and resiliency into their farm plans (Acquafredda and Munroe 2020). However, we anticipate this species' vulnerability to heat stress will be one of the greatest limitations to further surfclam aquaculture development across the Northeast. Selective breeding for greater heat tolerance might be a viable strategy for enhancing survival of cultivated surfclams. Selective breeding programs have been the foundation of viable Eastern oyster production along the east coast of the United States (Haskin and Ford 1979). As such, similar programs that produce heat-tolerant surfclam stocks and lead to improved and consistent annual yields would provide stability to farmers and facilitate industrial scale production.

Here, we present observations on the response of adult farmed surfclams to heat stress after juvenile exposure and explore the feasibility of selectively breeding surfclams for greater heat tolerance. In Experiment 1, we examined whether surfclams that survived a month-long heat stress as early juveniles would be more resilient than control clams when re-exposed to similarly stressful conditions as adults. In Experiment 2, we sought to determine whether surfclams could be selectively bred for greater heat tolerance. To address this, surfclam broodstock that survived a lethal heat shock were bred, and the growth, survival, and heat tolerance of their first-generation progeny were compared to the progeny of control broodstock.

<A>METHODS

<C> Assessing heat tolerance of adult farmed surfclams after juvenile exposure to heat stress.– The Atlantic surfclams (Spisula solidissima) used in Experiment I were generated during a previous study (Acquafredda et al. 2019). A brief explanation is provided here. In June and July 2016, a controlled temperature tolerance experiment was conducted at the New Jersey Aquaculture Innovation Center (AIC) in North Cape May, NJ. In this study, juvenile surfclams (initial shell length ~0.7 mm) from three replicate cohorts were assigned to temperature treatments two weeks after metamorphosis. One treatment consisted of a continuous month-long exposure of ~26˚C, which caused a selection event where approximately 79% of clams died. While clams in this study most likely succumbed to heat-induced mortality, it is plausible that a bacterial infection, borne of the high temperature conditions, contributed to or exacerbated the mortality attributed to heat stress alone. However, no latent mortality was observed after the heat-exposure concluded and once the clams were returned to control conditions (≤ 20˚C). The survivors of this treatment were pooled, and the group was designated heat-selected 2016 (HS-16). Another treatment in that study consisted of control conditions (≤ 20˚C) where clams did not experience stress. This group was designated non-selected 2016 (NS-16). Prior to this experiment, both groups were exposed to the same larval conditions in the hatchery (see Acquafredda et al. 2019). Likewise, both groups also experienced the same environmental conditions after the study. For the three months immediately following the selection event, the groups were reared in flow-through upwelling conditions at the AIC (Acquafredda et al. 2019). When the mean shell length reached approximately 13 mm, the NS-16 and HS-16 clams were outplanted at a shallow subtidal farm in southern Barnegat Bay, NJ.

In September and October 2018, a fully-crossed controlled experiment (Experiment 1) was conducted using the NS-16 and HS-16 clams to determine whether prior exposure to heat stress conferred protection during a subsequent exposure to high-temperature conditions (Figure 1). The experiment took place at the Haskin Shellfish Research Laboratory in Port Norris, NJ, and occurred when the clams were approximately 2.5 years old. The NS-16 and HS-16 clams had a mean shell length of 45.00 mm (SD, 4.46) and 48.67 mm (SD, 2.78), respectively. For 12 days, surfclams were exposed to control temperatures between 9 and 11˚C (mean ± SD, 10.2 ± 0.6˚C) or a lethal heat challenge at temperatures between 28 and 30˚C (mean ± SD, 29.4 ± 0.7˚C). The experiment occurred after a one-week acclimation period where the water temperature was slowly adjusted from 16˚C, the conditions of the field from which the clams

were retrieved. The control conditions were adjusted below field conditions due to equipment and space constraints at the laboratory. The heat challenge temperatures used in this and subsequent experiments presented here were chosen based on preliminary studies and previous work (Acquafredda et al. 2019). Due to the number of available clams harvested, four and seven replicate buckets were used for HS-16 and NS-16, respectively, for each temperature treatment. Each replicate consisted of six clams placed in a bucket containing 15-L of treated seawater (TSW), which had been 1-µm filtered and UV-sterilized.

Replicate buckets in the heat challenge treatment shared a common water bath, which was heated with multiple aquarium heaters (300–400W Aqueon) that were controlled by a single-stage digital temperature controller (Aqua Logic, Inc.). Buckets in the control treatment were maintained in a temperature-controlled room set to the target temperature. Continuous water temperature data were logged with SBE 56 (Seabird Scientific) devices using a 600 second sampling frequency. Point temperature, salinity, and nitrogen waste (NH_4^+, NO_2^-, NO_3) data were collected daily with an analog thermometer, refractometer, and API® test kits, respectively. Across all experimental units, the mean salinity was 30.4 ppt (SD, 1.1). Water changes occurred daily or when ammonia, nitrite, or nitrate levels exceeded 0.5 ppm. Buckets were continuously aerated, contained a preconditioned biofilter, and were fed 3% dry weight daily of Shellfish Diet 1800 (Reed Mariculture, Instant Algae), as per manufacturer's instructions. Clam survival was monitored daily, and dead clams were immediately removed from buckets. Survival was monitored until all individuals in the heat challenge suffered mortality.

<C> Selecting heat-selected and non-selected surfclam broodstock groups.– Experiment II began with generating a heat-tolerant surfclam broodstock group. In December 2018, 21-month old farmed-raised surfclams were collected from a farm in Barnegat Bay and transferred to the AIC. The surfclams used in Experiment II were from a separate cohort, distinct from those used in Experiment I (Figure 1). These surfclams had a mean wet tissue weight of 2.5 g (SD, 0.7), a mean dry tissue weight of 0.3 g (SD, 0.08), and a mean shell length of 36.55 mm (SD, 2.46). A random sample of 500 clams was selected as the control broodstock, designated here as the non-selected group (NS-17). The NS-17 group was placed in 900-L of 50-µm filtered and UV-sterilized seawater, which was maintained at a mean temperature of 11.6˚C (SD, 0.6) (Figure 1). The tank was set up as a recirculating system, which was continuously aerated and contained a

preconditioned biofilter. The clams were fed a ration of 3% dry weight per day of Shellfish Diet 1800 (Reed Mariculture, Instant Algae).

The remaining clams were separated into two tanks, each containing 555 individuals. These clams were maintained in identical conditions to the control tank, except the temperature was incrementally increased by 2˚C per day until the clams were exposed to an acute heat shock. The acute heat shock consisted of a continuous exposure to temperatures between 27.5 and 30˚C (mean ± SD, 28.3 ± 2.1˚C) for approximately five days (Figure 1). Throughout the broodstock selection processes, clam survival was monitored daily; dead clams were immediately removed from the tanks. At the end of the heat shock, 53.8% (SD, 6.2) of clams suffered mortality. Immediately following the heat shock, the temperature was decreased to 20˚C and subsequently lowered over several days to match the conditions of the control tank. However, latent mortality continued to occur for approximately one month after the clams were returned to favorable conditions. This occurred even though the feeding ration for these clams was increased to 6% dry weight per day following the heat shock. Due to uneven latent mortality, the final selection differential, or the overall percentage of clam suffering mortality from the selection event, was 74.8% (SD, 13.9). The surviving clams were pooled and designated the heat-selected (HS-17) broodstock group. No NS-17 clams were lost during that period.

Throughout the broodstock selection process, continuous temperature data were logged with SBE 56 (Seabird Scientific) devices using a 600 second sampling frequency. Point temperature, salinity, and nitrogen waste (NH_4^+, NO_2^-, NO_3) data were also collected daily with an analog thermometer, refractometer, and API® test kits, respectively. Across all tanks, the mean salinity was 28.8 ppt (SD, 1.1). Water changes occurred daily or when ammonia levels exceeded 0.5 ppm. Nitrite and nitrate levels did not exceed 0 ppm. Approximately six weeks after the selection event, both broodstock groups were moved back into the Barnegat Bay, so they could become naturally conditioned to spawn.

<C> Spawning broodstock and rearing progeny.– Experiment II continued in May 2019, when ripe surfclam broodstock were transferred from the Barnegat Bay conditioning site to the AIC (Figure 1). Spawning was induced using thermal manipulation (Loosanoff and Davis 1963; Jones et al. 1993). Two males and two females from each broodstock group contributed to their respective progeny groups. NSF1-19 refers to the F1 progeny of the non-selected broodstock

group (NS-17). HSF1-19 refers to the F1 progeny of the heat-selected broodstock group (HS-17). The small number of parents used to produce each progeny group was not intentional, but rather an unfortunate consequence of the limited number of broodstock that had ripe gametes at the time of spawning.

The larvae and juvenile clams from both progeny groups were reared identically using established culture methods (Jones et al. 1993; Acquafredda et al. 2019). Larvae were reared in static 200-L tanks containing TSW. The larval stocking density was reduced from 14 to 2 larvae/mL by metamorphosis (Jones et al. 1993). At metamorphosis, larvae were set in downwelling silos, which were composed of fiberglass cylinders (diameter x height, 60.96 x 60.96-cm) and mesh screen (Nitex) and reared in TSW. Incrementally, clams were moved into new rearing silos with larger mesh screen, from 125 to 150 to 180 to 200-µm. The initial stocking density for post-metamorphic juveniles was 185 clams/cm^2. While the larval and juvenile clams were reared in TSW, the mean temperature and mean salinity were maintained at 20.9˚C (SD, 0.7) and 30.2 ppt (SD, 0.4), respectively. Larvae and juveniles were fed a mixed diet of Tisochrysis lutea and Pavlova pinguis. The feeding ration was incrementally increased from $1.0*10^4$ cells/mL on day 1 to $8.5*10^4$ cells/mL on day 21.

By day 23, the rearing silos were moved to flow-through raceways (length x width, 7.1 x 0.58-m) supplied with unfiltered (raw) seawater from the Cape May Canal. The flow was controlled with a bell siphon, which produced continuous ebb and flow conditions that cycled the height of the raceway between 33 cm and 5 cm approximately every 27 minutes. On day 37, the rearing silos were moved to upwelling conditions, which experienced flow rates between 45 and 55 L/min. Incrementally, clams were moved into new rearing silos (diameter x height, 45.72 x 45.72-cm) with larger mesh screen, from 400 to 600 to 750 to 1000-µm. Concurrently, the stocking density was reduced in accordance with established culture methods, and by the end of the nursery phase, the clams were stocked at 0.38 clams/cm^2 (Jones et al. 1993). During the period when these clams were reared in raw seawater, they experienced mean daily temperatures that ranged from 18.1 to 25.4˚C (mean ± SD, 22.6 ± 1.6˚C). Likewise, the clams experienced mean daily salinities that ranged from 24.2 to 30.3 (mean ± SD, 28.6 ± 1.3).

Survival of the surfclam progeny was assessed with repeated volumetric abundance estimates (Acquafredda et al. 2019). Growth of the surfclam progeny was determined by recording shell length (anteroposterior axis) in proportion to the abundance of each determined

size class. Larvae and juvenile clams less than 3.0 mm in shell length were measured by placing individuals onto a Sedgewick-Rafter slide and measuring each with an ocular micrometer on a VWR compound microscope (100X or 25X). Clams larger than 3.0 mm were measured with digital calipers (Mitutoyo Absolute™ Digimatic).

<C> Assessing heat tolerance of heat-selected and non-selected surfclam progeny.– Experiment II continued in September 2019 at the Haskin Shellfish Research Laboratory to determine whether selectively bred surfclam progeny had greater heat tolerance than non-selected control progeny (Figure 1). This controlled heat tolerance assessment was conducted in the same manner outlined above for Experiment I, except for the following modifications. The temperature of the heat challenge was maintained between 27 and 30˚C (mean ± SD, 28.9 ± 1.2˚C), while the control conditions were kept between 9 and 15˚C (mean ± SD, 11.2 ± 2.4˚C). Before the exposure, the clams underwent an acclimation period wherein the water temperature was slowly adjusted over several days from 23.6˚C, the raw seawater temperature the clams had previously been experiencing. Across all experimental units, the mean salinity was 32.3 ppt (SD, 0.9). Water changes occurred daily or when ammonia levels exceeded 0.5 ppm. Nitrite and nitrate levels did not exceed 0 ppm. Three replicate buckets were established for each progeny group, and each replicate bucket initially contained 100 clams. This approximated the biomass of the six adult clams used per replicate in Experiment I. The clams used in this experiment had a mean shell length of 9.65 mm (SD, 0.72).

<C> Statistical analyses.– All data were analyzed with R (Version 4.0.2 © 2020-06-22 The R Foundation) using RStudio (Version 1.3.1056 © 2009–2020 RStudio, Inc.). Normality and homoscedasticity of all growth data were confirmed using the Shapiro-Wilk Test and Levene's Test, respectively. Measures of dispersion presented in this paper are reported as ± standard deviations (SD) or 95% confidence intervals (CI), wherever noted. To determine the significance of the survival of clams during the heat challenge experiments, generalized linear mixed models fit by maximum likelihood were fit to the data. Experimental day and group were fixed effects, while replicate bucket was a random effect. Due to the linearity of the data, ANCOVA was used to determine the significance of the survival of clams experiencing the control conditions during these experiments. Similarly, ANCOVA was used to determine whether breeding group had a

significant effect on the growth rates of larval and juvenile surfclam progeny. Average daily growth rates were determined using the formula described by Acquafredda & Munroe (2020):

$$X_{GR} = \frac{X_t - X_{t_0}}{\Delta t} \qquad (1)$$

Here, X represents the mean value of a particular growth variable (shell length), collected on the first and last day of the study, and Δt represents the number of days of the study. A Student's t-test was used to compare the final size of surfclam progeny at the end of their nursery phase (day 142).

<A> RESULTS

<B> Experiment I – Response of Adult Farmed Surfclams to Heat Stress after Juvenile Exposure

No clams from either group, HS-16 or NS-16, died when held at the control conditions (Figure 2A). When exposed to the heat challenge (28–30˚C), both experimental day and group were significant predictors of surfclam survival ($P < 0.001$). Mortality of NS-16 clams was first observed on day 4, while mortality of HS-16 clams was not observed until day 5 (Figure 2B). The largest difference in survival between the groups was observed on day 7, where mean survival was 36% (CI, 18) for NS-16 and 71% (CI, 16) for HS-16. All NS-16 clams died by day 9, while complete mortality of HS-16 clams was not observed until day 12 (Figure 2B).

<B> Experiment II – Selective Breeding for Greater Heat Tolerance

<C> Rearing heat-selected and non-selected surfclam progeny.– Overall, no notable differences in larval or juvenile growth were observed between the HSF1-19 and NSF1-19 surfclam progeny groups (Figure 3A, B). Progeny group had no effect on the growth rate of larval clams (Figure 3A, ANCOVA (Progeny Group), $F(1,5) = 0.50$, $P = 0.51$). The average daily larval growth rates observed for HSF1-19 and NSF1-19 clams were 12.7 and 11.4 µm/d, respectively. All larvae metamorphosed between day 16 and 23.

Larvae spawned in early May 2019 had grown to a size ready for deployment on clam farms by September. At that time (142 days post-fertilization), HSF1-19 and NSF1-19 clams had a mean shell length of 14.14 mm (CI, 0.16) and 14.02 mm (CI, 0.17), respectively. There was no significant difference in size (Figure 3B; t-test, $t = -0.9$, $P = 0.35$). Similarly, progeny group had no effect on the growth rate of juvenile clams (Figure 3B; ANCOVA (Progeny Group), $F(1,23)$

= 0.27, P = 0.61). The average daily juvenile growth rates observed for HSF1-19 and NSF1-19 clams were 0.115 and 0.114 mm/d, respectively.

Survival varied between the two progeny groups over the larval and nursery rearing phases; however, since only one cohort of each progeny group was produced, statistical inference is inhibited (Figure 3C). During the larval phase (day 0–16), the survival of the NSF1-19 group was more than double that of the HSF1-19 group (Figure 3C). During the juvenile phase (day 23–142), the survival of the HSF1-19 group was 26% greater that the NSF1-19 group (Figure 3C). Measured over the entire study period (day 0–142), the survival of the NSF1-19 group was 2.68-times greater than that of the HSF1-19 group (Figure 3C).

<C> Heat tolerance of heat-selected and non-selected surfclam progeny.– When exposed to control conditions between 9 and 15˚C, surfclam mortality was negligible for both progeny groups; each progeny group only lost a single clam (Figure 4A). In these conditions, there was no significant difference in survival between the NSF1-19 and HSF1-19 progeny groups (ANCOVA (Progeny Group), $F(1,50) = 0.12$, $P = 0.73$).

When exposed to the heat challenge (28–30˚C), again both experimental day and group were significant predictors of surfclam survival ($P < 0.001$). NSF1-19 clams began to die as early as day 2. HSF1-19 clams did not begin to die until day 4 (Figure 4B). The largest difference in survival between the progeny groups was observed on day 6. On this day, mean survival was 48.0% (CI, 13.7) for NSF1-19 and 78.7% (CI, 12.4) for HSF1-19. However, on day 7, the mean survival of NSF1-19 and HSF1-19 clams was much more similar at 2.7% (CI, 3.3) and 8.3% (CI, 10.7), respectively. All clams from NSF1-19 died by day 8, but clams from the HSF1-19 persisted slightly longer, until day 9 (Figure 4B).

<A> DISCUSSION

This study represents a small yet promising first step towards developing heat-tolerant Atlantic surfclams (Spisula solidissima), which in turn may facilitate more resilient aquaculture production of this species in a warming climate. We found that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent thermal stress for significantly longer than individuals that did not experience an earlier exposure. Moreover, we found that through selective breeding, heat tolerance was improved in first-generation surfclam progeny. Together, the results from these experiments suggest that heat-

induced selection can identify clams genetically predisposed to withstanding high temperature conditions.

Although the manner of selection differed between Experiment I and II, the selection differentials achieved in both experiments (HS-16 = 79%; HS-17 = 75%) were similar, and both had the desired effect of identifying surfclams predisposed to withstand heat stress. The HS-16 surfclams were generated with a chronic stress (26˚C for several weeks), while the HS-17 surfclams were selected using an acute heat shock (27–30˚C for several days). The age of the clams when the selection pressure was administered also varied; HS-16 clams were selected as early juveniles, while HS-17 clams were selected as adults. No latent mortality was observed among HS-16 juveniles after the chronic stress. In contrast, the acute stress used to generate the HS-17 group caused substantial carry-over mortality, despite the adults being quickly returned to favorable temperatures and provided with high food availability. This observation aligns with findings from earlier work which indicate that a surfclam's capacity to cope with heat stress is dependent on multiple factors, such as age, size, duration of the heat stress, and the intensity of the heat stress (Narváez et al. 2015; Acquafredda et al. 2019).

No differences in larval or juvenile growth rate were observed between the heat-selected and non-selected progeny groups. Yet across different developmental stages, survival did vary between the progeny groups. Larvae of the control parents (NSF1-19) exhibited substantially greater survival than larvae from heat-selected parents (HSF1-19). We suspect that the difference in larval survival observed in our study may be related to the lipid reserves these progeny groups received from their parents. Bivalves provision their eggs with polyunsaturated fatty acids and other lipids they ingest from their diet, and the quantity and quality of lipid reserves available to embryos can influence subsequent larval growth and survival (Utting and Millican 1997). We hypothesize that while our heat-selected broodstock (HS-17) were capable of developing gonad over the five-month period between the selection event and spawning, they may not have adequately rebuilt their lipid stores and thus, insufficiently provisioned their eggs. However, if the difference in larval survival was indeed a maternal effect, it was largely mitigated after metamorphosis. The survival of the two progeny groups was similar when measured over just the juvenile or nursery phase of development; in fact, juvenile survival was slightly higher in the offspring of heat-selected parents (HSF1-19). An alternative explanation for the observed

variation in survival could be due to random chance since only one cohort of each progeny group was produced in this study.

After a single generation of selection, heat-selected surfclam progeny had significantly greater survival during continuous exposure to lethal temperatures compared to control progeny. It should be noted that the extreme lethal temperature exposure used in this experiment (~29ºC continuous exposure) is more severe than what would currently be observed at typical subtidal farm sites; however, temperatures can occasionally reach that high on intertidal surfclam farms in Massachusetts (unpublished data). Although the progeny of heat-selected parents (HSF1-19) were more tolerant to heat stress, with the greatest difference in survival occurring after six days of exposure, after eight days, the survival of both progeny groups was comparable. We predict that this heat-tolerant phenotype could be enhanced through additional rounds of selection, further differentiating the heat-selected line from non-selected controls. While this study did not address whether the growth rates of the heat-selected and non-selected progeny groups differed under elevated temperature conditions, the findings of Experiment II suggest that selective breeding for greater heat tolerance in farmed surfclams is possible without compromising growth rate in ambient conditions. However, it must be noted that this study is constrained by the fact that each progeny group was produced from merely four parents in a single spawning event. Undoubtedly, the limited genetic diversity and lack of spawning replication are notable shortcomings of this study. Critically, future studies must use a greater diversity of broodstock and spawning replication when conducting surfclam breeding experiments.

Selective breeding is a critical tool for adapting food systems to the changing climate. Climate change will exacerbate food insecurity, particularly in regions already facing instability, limited food access, and undernutrition (Wheeler and von Braun 2013). For decades, bivalves have been selectively bred to enhance traits relevant to improving aquaculture production (Newkirk 1980; Guo 2004; Abdelrahman et al. 2017; Mizuta and Wikfors 2018). Much attention has been paid to improving fundamental performance measures such as survival and growth for numerous commercially-important species (Manzi et al. 1991; Utting et al. 1996; Zheng et al. 2006; Deng et al. 2009; Li et al. 2011; Dove and Connor 2012; Vu-Van Sang et al. 2019). Additionally, genetic improvements have been made to enhance species' resistance or tolerance to diseases (Haskin and Ford 1979; Guo et al. 2003; Ragone Calvo et al. 2003; Proestou et al. 2016). However, only recently have bivalve breeding efforts begun to explicitly focus on traits

that confer protection against climate change-induced stressors, such as acidification (Fitzer et al. 2019) and thermal stress (Lang et al. 2009; Nie et al. 2017; Tan et al. 2020). For instance, a Manila clam selective breeding program was initiated in China in part to improve stocks that had been suffering devasting summer mortalities (Yan et al. 2005; Zhang and Yan 2010). Since bivalve farming produces fewer greenhouse gas emissions than nearly all other forms of animal protein production (Hilborn et al. 2018), the resilience and growth of bivalve farming is crucial for increasing the sustainability of food systems.

Our work suggests that farmed surfclams may have the capacity to cope with some degree of warming. However, this species remains at risk from the continued rise in ocean temperatures, particularly on shallow coastal farms that currently contain suitable surfclam habitat but occasionally reach temperatures at or near the species' lethal limit (Narváez et al. 2015; Timbs et al. 2019). Another plausible route for improving the heat tolerance of surfclams farmed in the northeastern United States is to cross it with its southern subspecies, S. solidissima similis. Superior survival and growth due to hybrid vigor, or heterosis, have been observed across several cultured bivalve species (Zhang et al. 2007; Yan et al. 2008; 2009; Wang et al. 2010; Mlouka et al. 2020). The range of the southern surfclam extends from the Gulf of Mexico to Cape Hatteras, with patchy populations found as far north as Long Island Sound (Hare et al. 2010), yet neither wild nor laboratory-produced hybrids are documented. Therefore, investigations into whether crossing the northern and southern subspecies will produce a superior clam with a greater propensity for enduring heat stress should be carefully explored. Finally, more research should be devoted to understanding the genetic underpinnings of the surfclam's response to heat stress. To that end, candidate alleles could be identified and could facilitate marker-assisted selection. Furthermore, other studies have documented enhanced heat tolerance in bivalves following sublethal exposure, with the sustained expression (days to weeks) of heat shock proteins mediating induced thermal tolerance (Shamseldin et al. 1997; Clegg et al. 1998; Sung et al. 2011). Studying factors that may modulate thermal tolerance, like heat shock protein expression or epigenetic modifications, will complement the on-going efforts that a.re using genetic techniques to generate heat-tolerant surfclams. Collectively, these alternate approaches may expedite the process of breeding Atlantic surfclams for greater heat tolerance. Ultimately, more research into selectively breeding surfclams is warranted and necessary in order to ensure

399 improved and consistent annual yields of farmed Atlantic surfclams in a warming climate.

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FIGURE 1. Schematic of experimental design. In Experiment I, selection pressure was applied when the clams were early juveniles, two to six weeks post-metamorphosis (Acquafredda et al. 2019). In Experiment II, selection pressure was applied when the clams were 21-month old adults. For the heat tolerance assessments, circles represent replicate buckets, which shared a common water bath (heat challenge) or shared a temperature-controlled room (control

conditions). In the Experiment I heat tolerance assessment, each bucket initially contained six adult HS-16 or NS-16 clams, ~2.5 years old. In the Experiment II heat tolerance assessment, each bucket initially contained 100 HSF1-19 or NSF1-19 juvenile clams, ~5 months old.

FIGURE 2. Survival of heat-selected and non-selected surfclams at control temperatures (A) and under severe heat stress (B). Points represent the survival of replicate buckets of heat-selected surfclams (HS-16, black squares, N = 4) and non-selected surfclams (NS-16, gray circles, N = 7). Each bucket initially contained six adult clams. (A) Control conditions: percent survival (%S) was 100% for both groups for the entirety of the experiment. (B) Heat challenge: lines of best fit were generated using generalized linear mixed models. Models take the form $\%S=(1/(1+\exp(b_0+b_1*t)))*100$, where %S is percent survival, t is the time in days, and b_0 and b_1 represent the model intercept and slope, respectively.

FIGURE 3. Growth (A, B) and survival (C) of heat-selected and non-selected surfclam progeny. NSF1-19 clams are F1 progeny spawned from non-selected surfclam broodstock (gray circles/ bars). HSF1-19 clams are F1 progeny spawned from heat-selected surfclam broodstock (black squares/ bars). Each progeny group was produced from a single spawning event. Points represent the mean shell length and error bars represent 95% confidence intervals. (A) Larval phase growth, N = 25 clams each day. (B) Nursery phase growth, N = 50 clams each day, except day 37 (N = 60) and day 142 (N = 300). (C) Bars show the survival of the two groups through development (N = 1 cohort per progeny group).

FIGURE 4. Survival of heat-selected and non-selected surfclam progeny at control temperatures (A) and under severe heat stress (B). NSF1-19 clams were spawned from non-selected surfclams (gray circles). HSF1-19 clams were spawned from heat-selected surfclams (black squares). Points represent the survival of replicate buckets (N = 3 per progeny group), with each bucket initially containing 100 juvenile clams. (A) Control conditions: linear regressions were used to determine lines of best fit for each curve. Models take the form %S=mt+b, where %S is percent survival, t is the time in days, and b and m represent the model intercept and slope, respectively. (B) Heat challenge: lines of best fit were generated using generalized linear mixed models.

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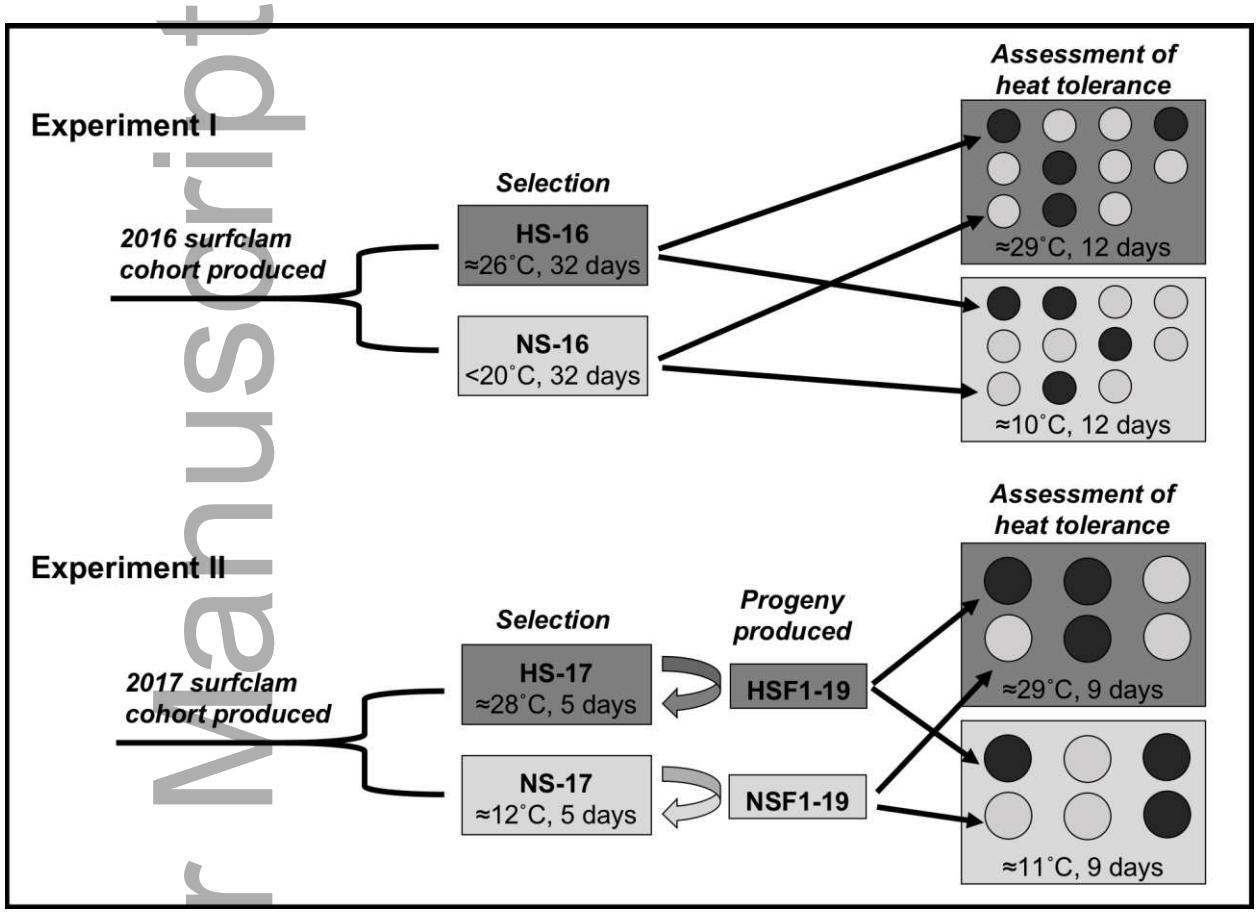

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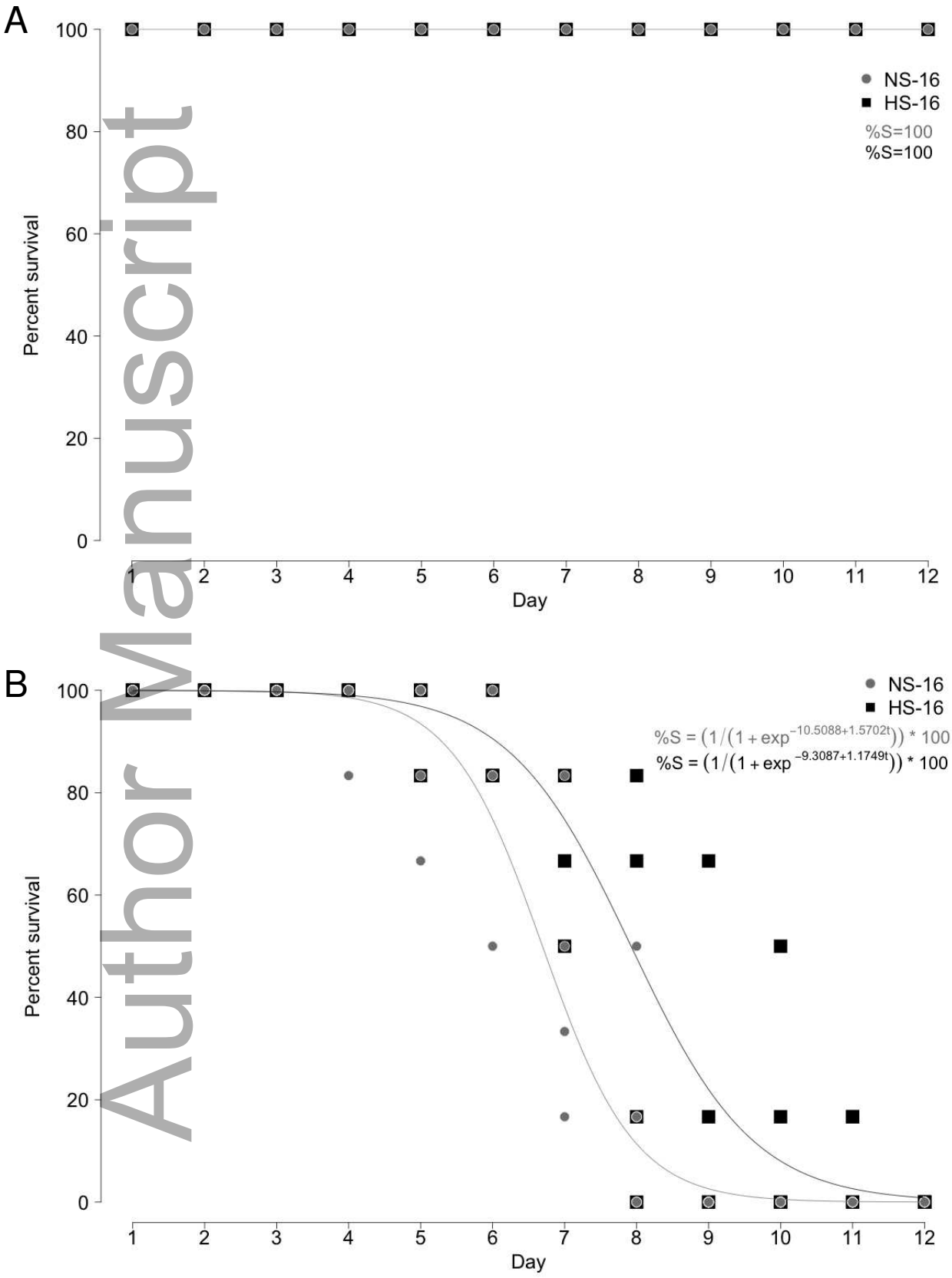

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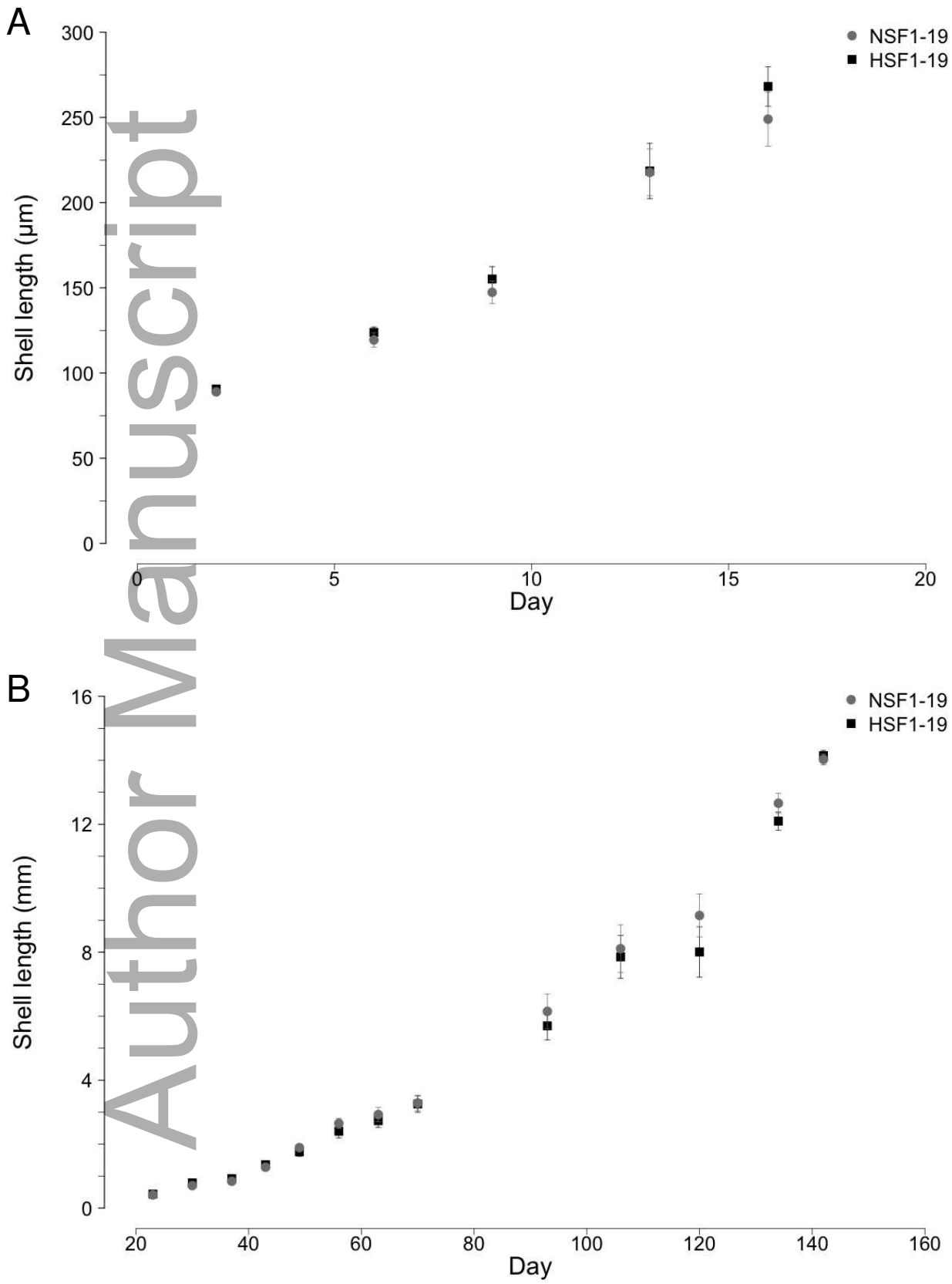

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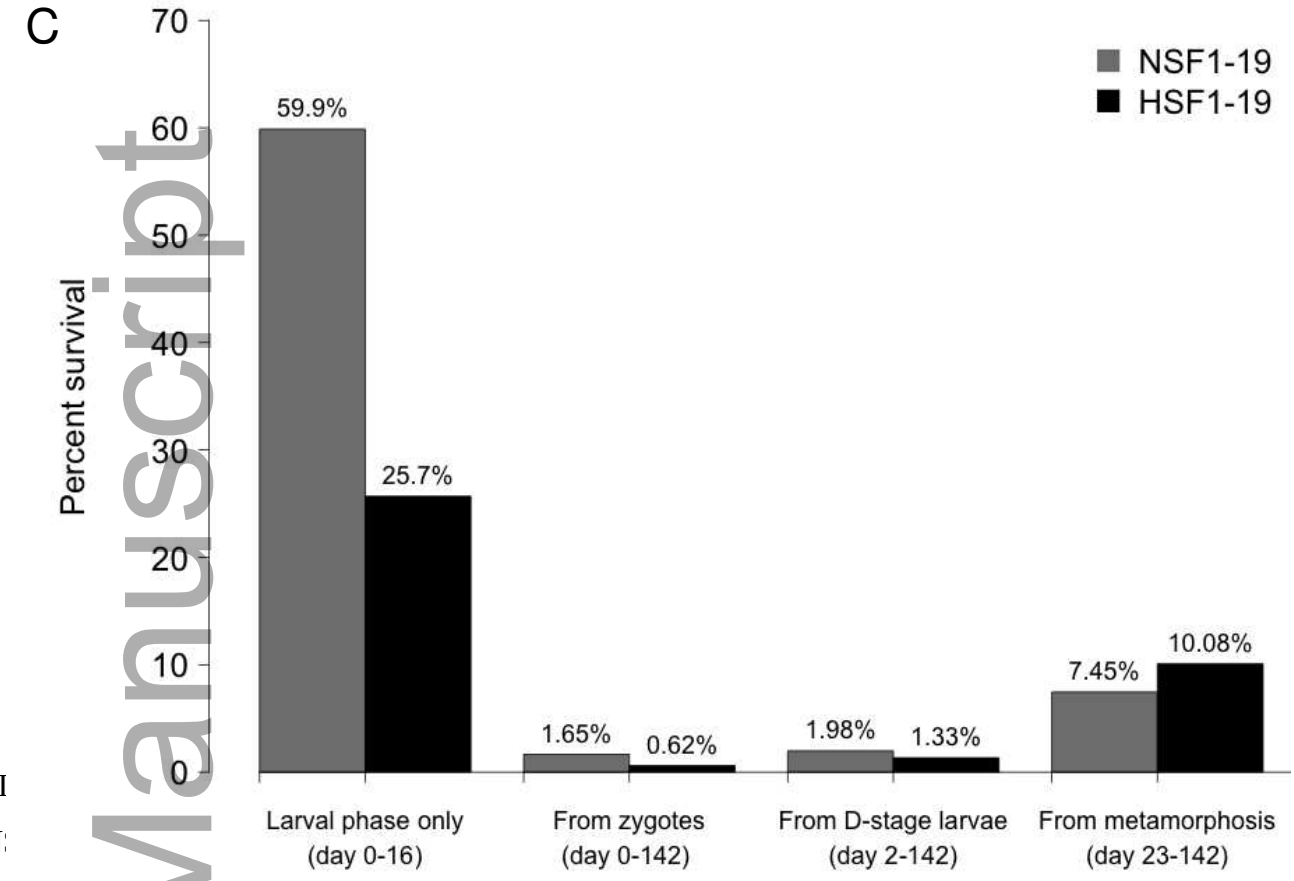

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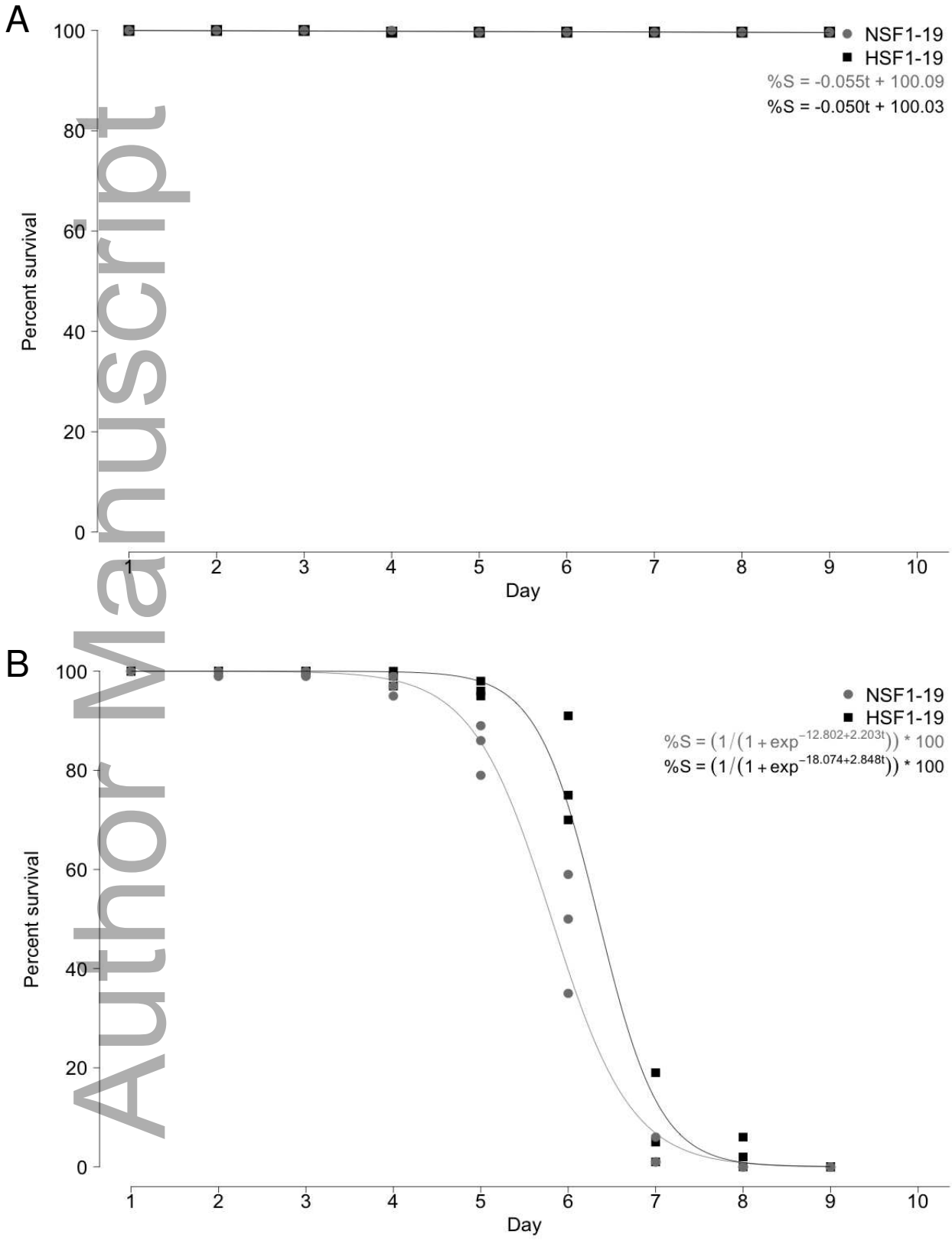

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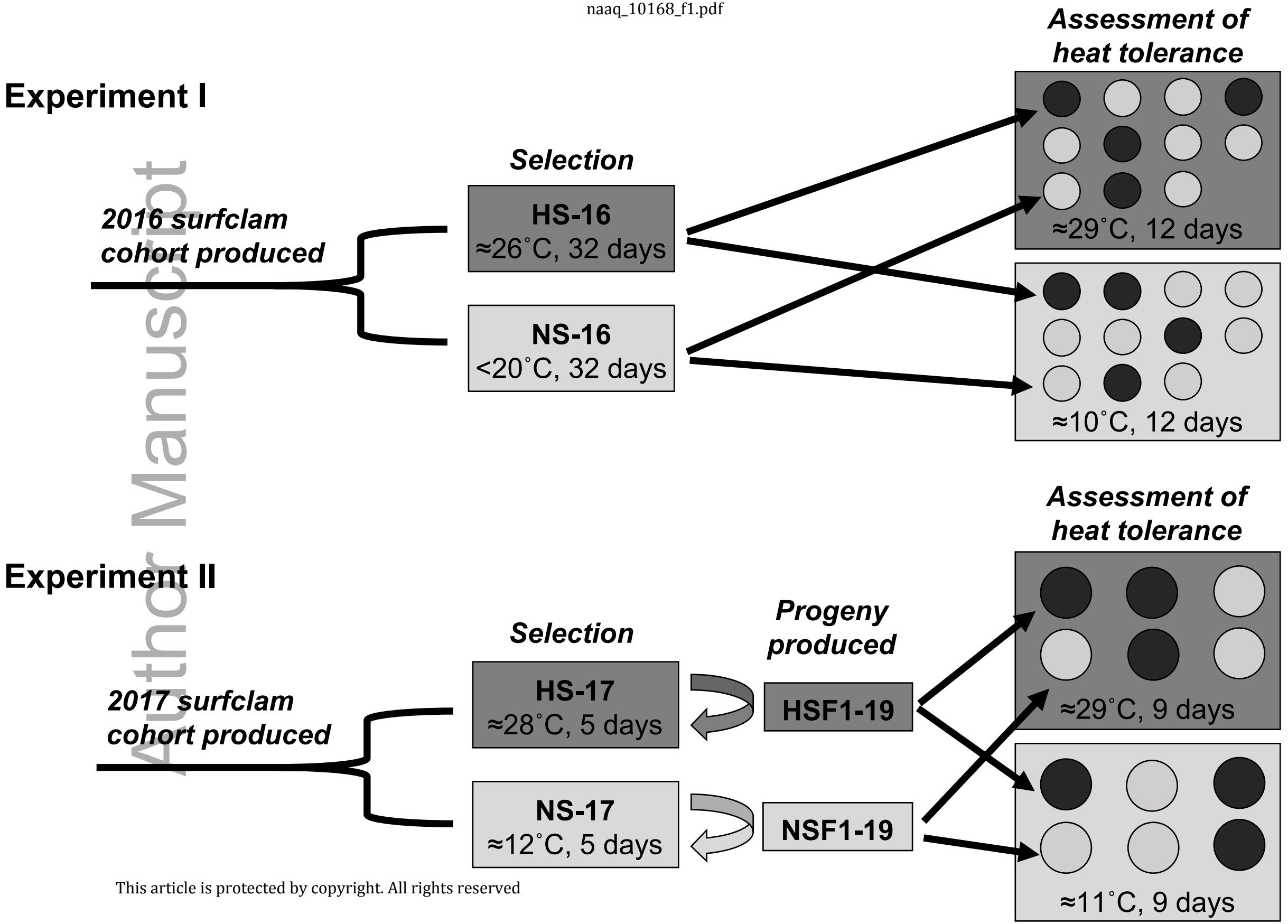

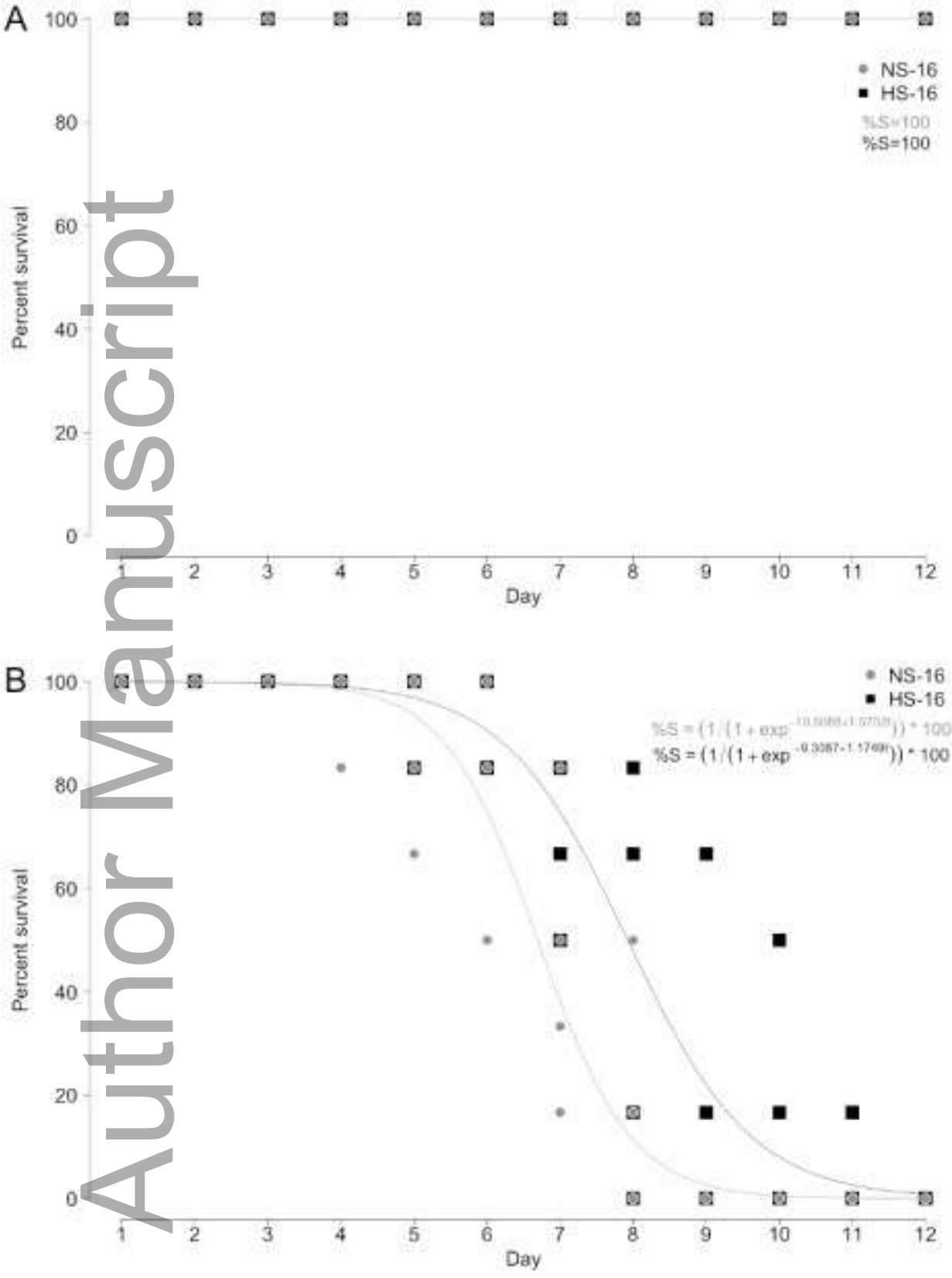

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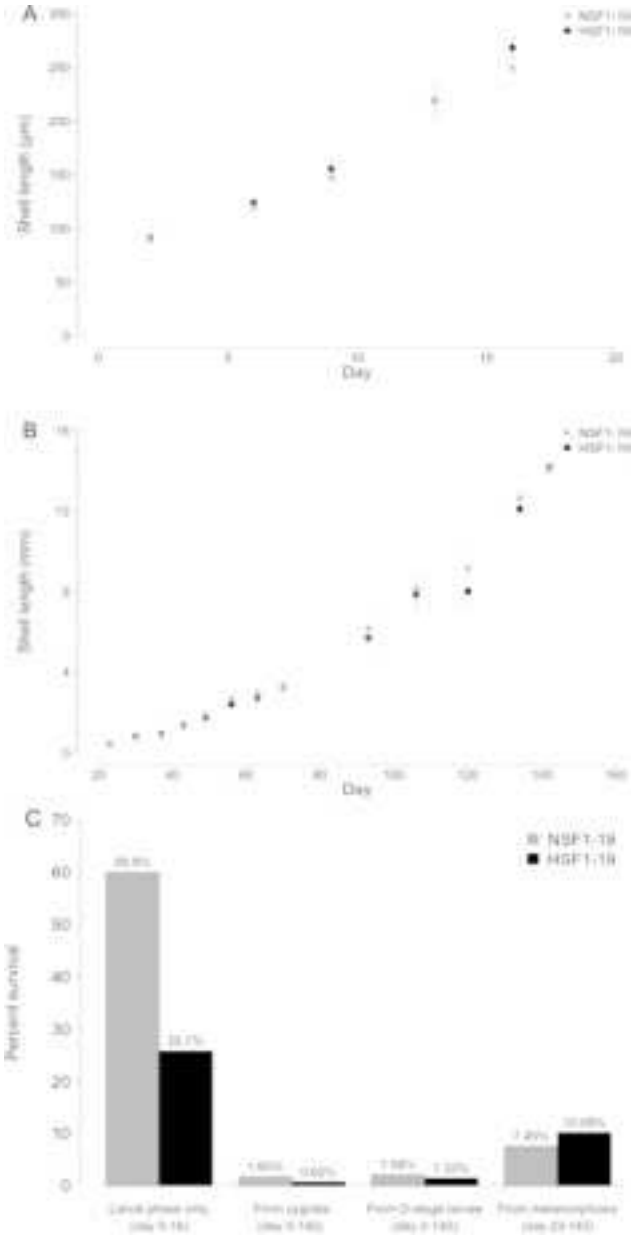

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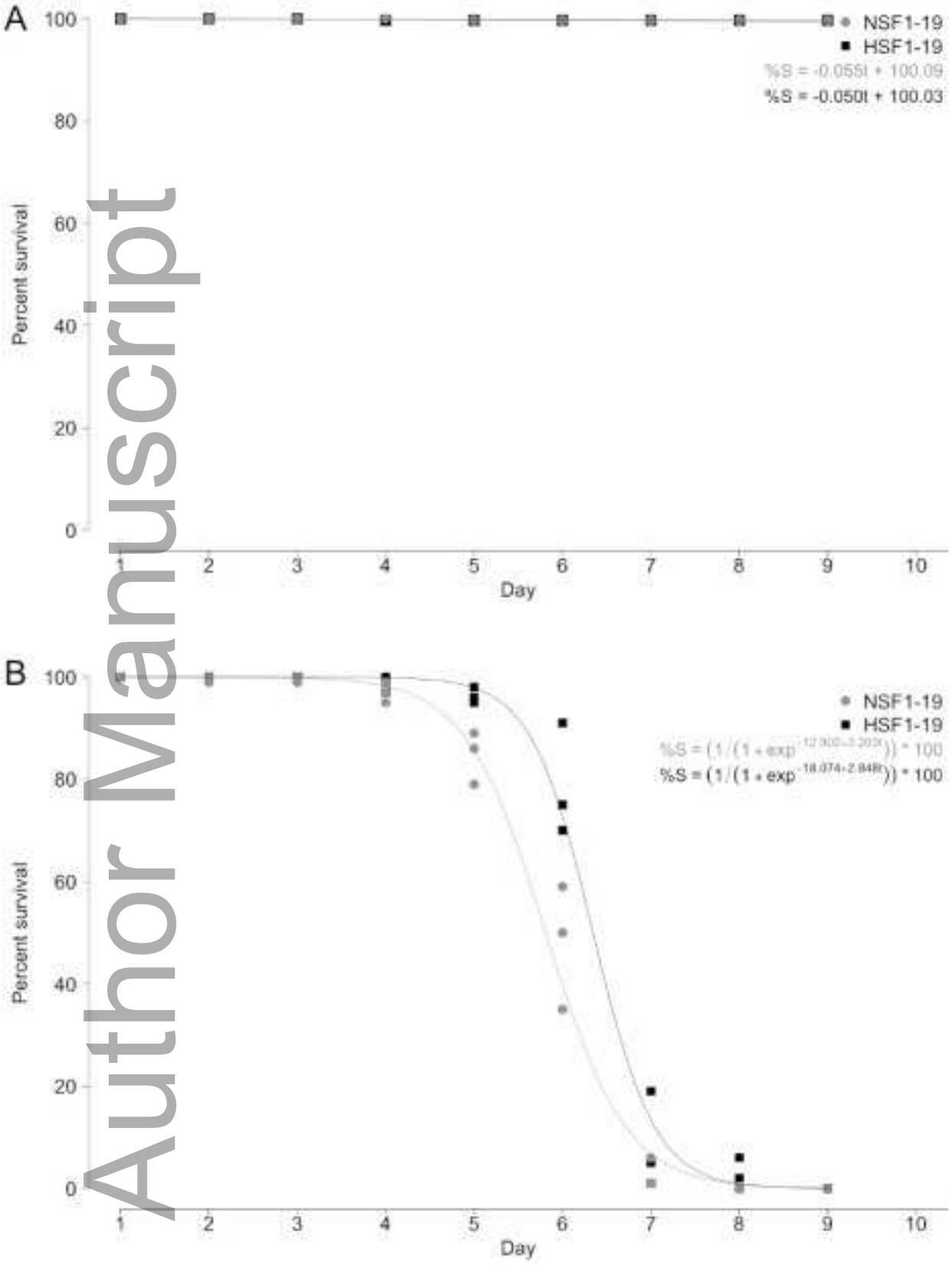

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