Survival Variation Analyses of Maryland Soft-Shell Clams under Heat Stress

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Introduction

Figure 1 - Adult Soft-Shell Clam. Retrieved from Rudow's FishTalk Magazine[2].

- The soft-shell clam (*Mya arenaria),* also referred to as a " manno" or "steamer", is a filter-feeding icon that was once a leading fishery resource in Maryland, topping U.S. production[1].
- However, populations have declined drastically due to overfishing, disease, predation, and global warming[1]. Given this species' formidable tolerance in low salinity conditions and high economic value, M. arenaria harbors strong potential for aquaculture in Maryland, contributing to diversification and resilience[3,4].
- The PEARL aquaculture team has successfully bred soft-shell clam seeds in hatchery conditions and begun pilot-scale culture trials. However, consistent and elevating temperatures during the summer seasons have constrained large-scale aquaculture projects, hindering survival rates.
- Therefore, PEARL is currently conducting a selective breeding program to improve heat tolerance. Understanding variation in survival under heat stress is a key step in this process.

Goals and Objectives

To investigate survival variation within and between populations and life stages of soft-shell clams under heat stress, heat shock experiments were executed on both adult and juvenile clams. In addition, the following results would identify the lethal temperature threshold of the mentioned clams and provide tissue samples for genome-wide association studies (GWAS) to identify candidate genes and genetic markers related to heat tolerance.

Methods

Adult Heat Shock

- Adult soft-shell clams were collected from three locations in Maryland: Poplar Island, Honga River, and PEARL's broodstock repository (comprised of clams purchased from a local seafood market and raised at our hatchery for several months). The following clams were aliquoted into four indoor flow-through tank with ambient water seawater at the PEARL. Feeding regimens were provided daily, supplementing each tank with fresh algae.

Figure 2 - Map showing the locations of the adult clam populations

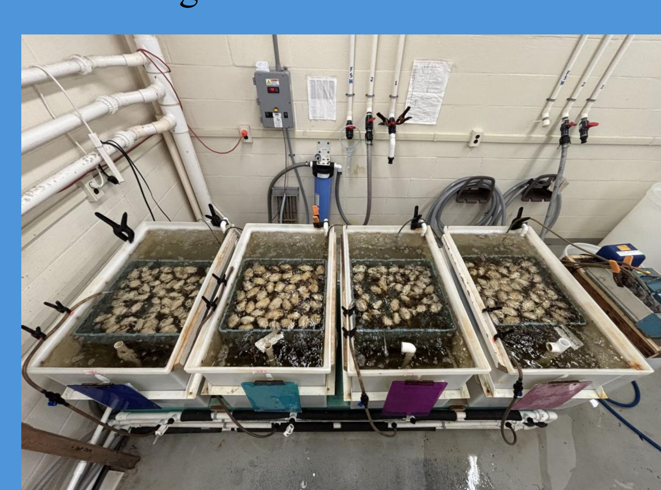


Figure 3 – Heat shock experiment tank set-up for adult clams.

- Mortality was monitored twice daily, and water temperature was recorded continuously using a temperature data logger and verified manually with a YSI probe. Dead clams were preserved in 95% ethanol for DNA extraction. For each clam, the date of death and source of population were recorded to serve as phenotypic data for genome-wide association studies (GWAS). Daily mortality and population-specific survival data were analyzed to evaluate variation within and between populations under heat stress.

Juvenile Heat Shock

- Juvenile clams were produced in April 2025 from two broodstock sources: a heat-tolerant line maintained at PEARL and a wild control line. Seeds were reared in an upweller at PEARL's pier under natural temperature conditions. Water temperature was monitored continuously with a data logger and periodically verified using a YSI probe.
- The experimental design included three replicate silos for the selected line and two for the wild line, with 1000 seeds per replicate. Survival was assessed on July 21, 2025, to compare heat tolerance between the two lines.

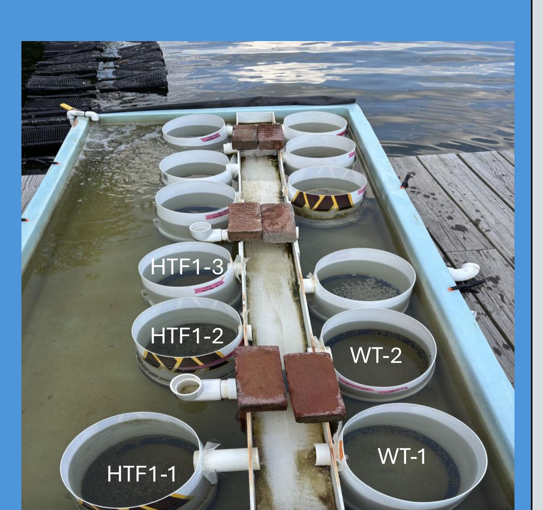
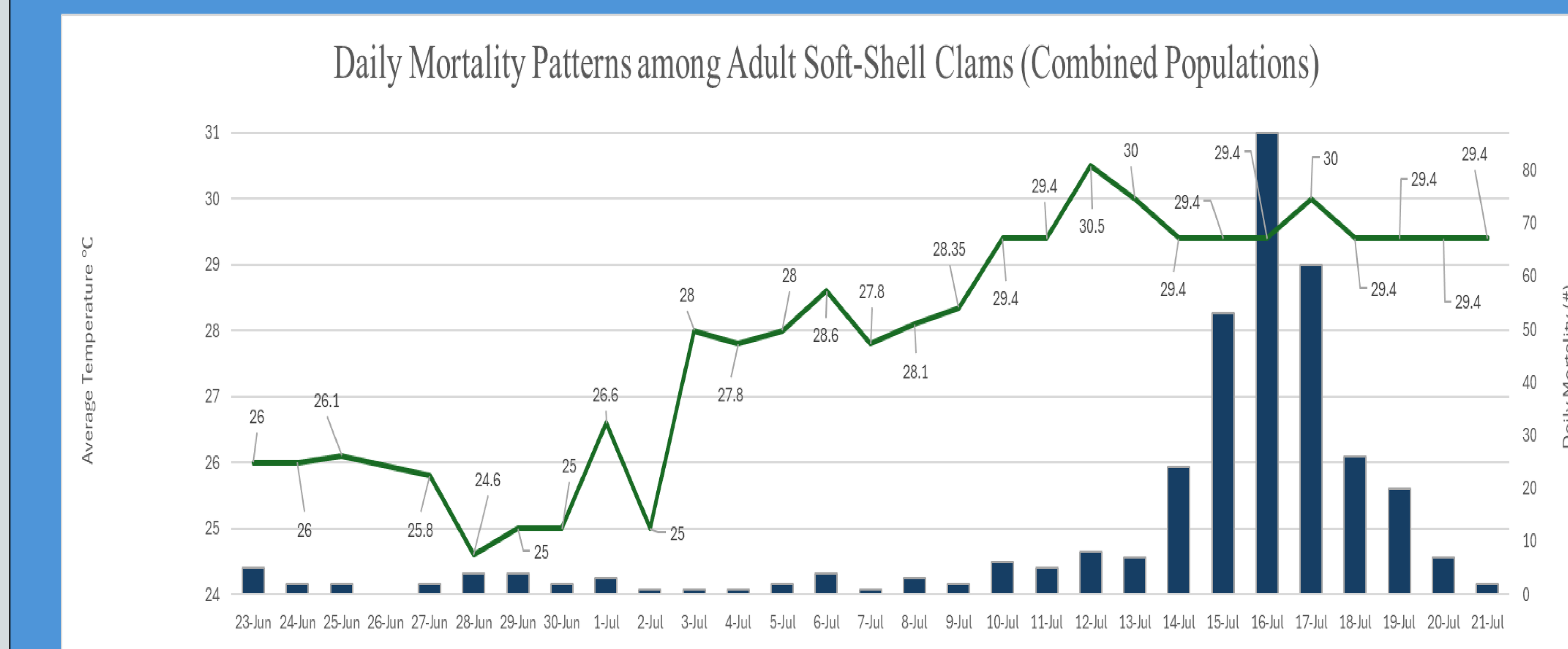


Figure 4 - Heat shock experiment tank set-up for juvenile clams.

Results



	23-Jun	24-Jun	25-Jun	27-Jun	28-Jun	29-Jun	30-Jun	1-Jul	2-Jul	3-Jul	4-Jul	5-Jul	6-Jul	7-Jul	8-Jul	9-Jul	10-Jul	11-Jul	12-Jul	13-Jul	14-Jul	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul
BS#1-4	5	2	2	2	4	4	2	3	1	1	1	2	4	1	3	2	6	5	8	7	24	53	87	62	26	20	7	2
AVG Temperature (°C)	26	26	26.1	25.8	24.6	25	25	26.6	25	28	27.8	28	28.6	27.8	28.1	28.35	29.4	29.4	30.5	30	29.4	29.4	29.4	30	29.4	29.4	29.4	29.4

Figure 5 – Graph: Daily Mortality of Soft-Shell Clams (combined tanks and populations)

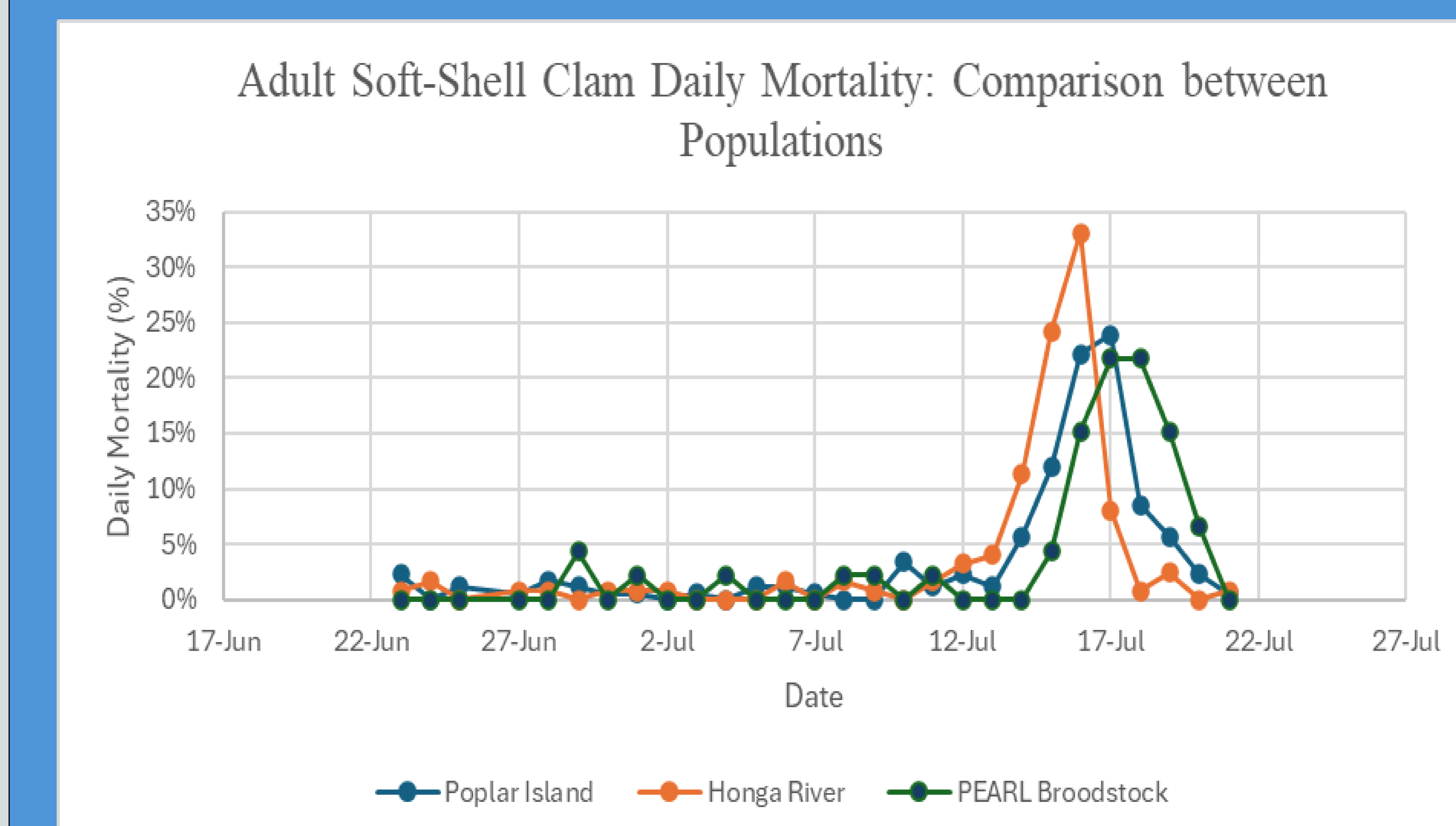


Figure 6 – Graph: Daily Mortality of Adult Soft-Shell Clams between Populations. One-way ANOVA test showing no significant differentiations among populations (p=0.13).

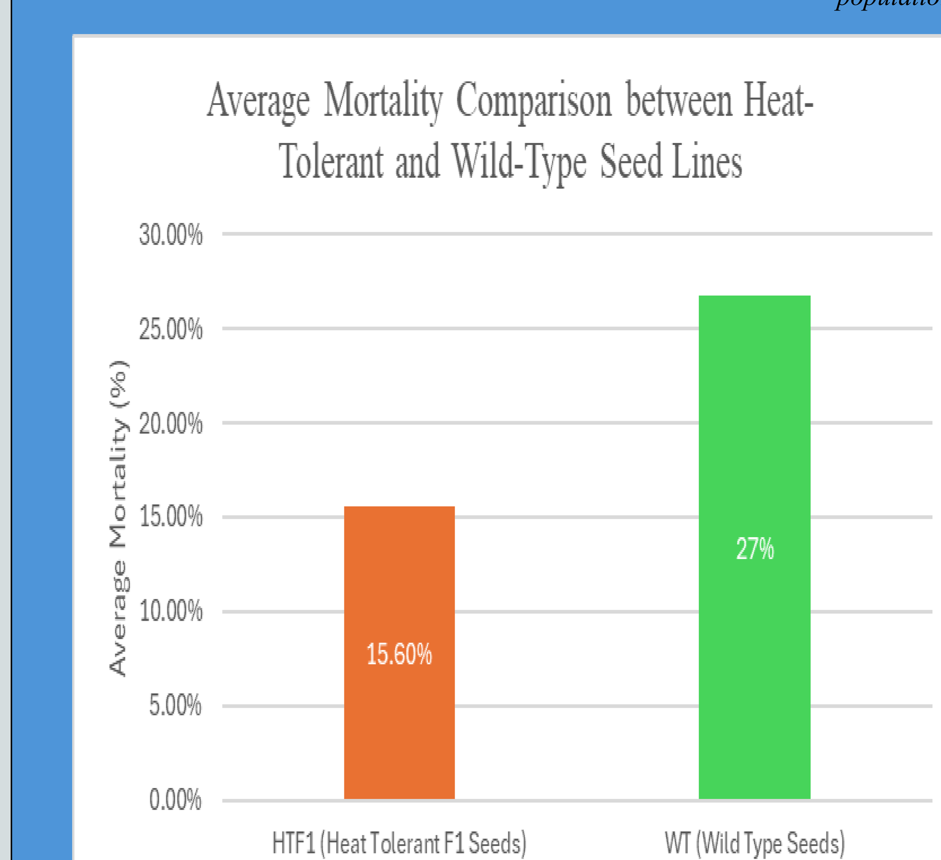


Figure 7 – Bar Chart: Average Overall Mortality of Juvenile Soft-Shell Clams.

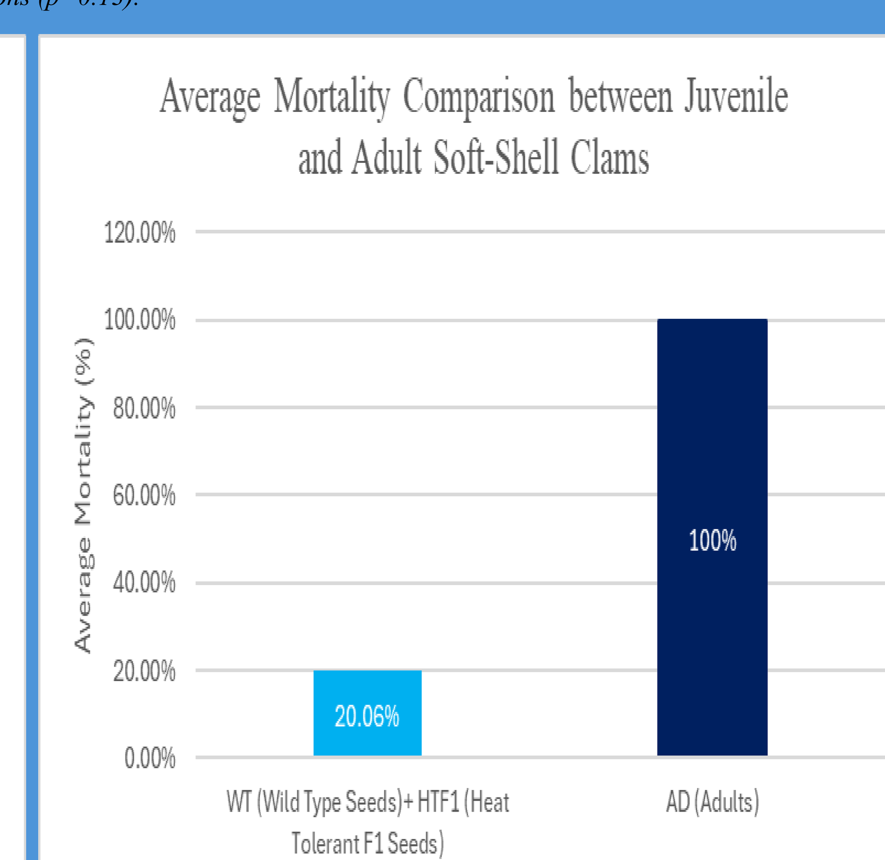


Figure 8 – Bar Chart: Average Overall Mortality between Juvenile and Adult Soft-Shell Clams.

Discussion and Conclusion

Adult Heat Shock

Extreme temperatures resulted in higher mortality

- Notably, daily mortality of individual soft-shell clams from July 10 to July 23rd , where average water temperatures were greater or equal to 29.4°C, was notably higher than daily mortality observed from June 23rd to July 9th where average water temperatures were below 29.0°C. This implies the lethal temperature threshold among adult Maryland soft-shell clams, being approximately 29.0 °C.

No significant difference in daily mortality among clams of varying locations, indicating shared genetic background.

- There was no significant difference in daily mortality between populations of clams from Poplar Island, the Honga River, or PEARL's broodstock. This particular finding assumes that clams within different locations of Maryland share a similar genetic background.

Seed Heat Shock

Slight survival advantage was observed in F1 generations of heat tolerant clams (HTF1), reflecting the effectiveness of selective breeding.

- A slightly lower mortality percentage among the HTF1 clam seeds was observed when compared to the wild type clam seeds (27%); thus, showcasing strong potential of selective breeding for enhanced tolerance.

Mortality among the adult clams were notably higher when compared to clam seeds, indicating higher tolerance among earlier stages.

- The following clam seeds exhibited much higher heat tolerance than the adults, highlighting a novel and important biological difference that warrants further study.

Future Directions

- A total of 346 adult clam samples were collected and preserved, providing critical material for upcoming GWAS to identify genetic markers associated with heat tolerance. Tissue samples were also collected from juvenile and adult clams for transcriptome experiments to investigate the genetic mechanisms accounting for the tolerance differentiations.
- Additionally, among the clam seeds, similar experiments should be enacted indoors to observe the difference of influence of other environmental factors towards mortality. Nonetheless, we contributed to scarce research on soft-shell clams, understanding the difference in survival between individuals, populations, and ages.

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