Stoeckel Lab Crayfish Research Updates for the 2022 Alabama Mollusk and Crayfish Working Group Annual Meeting

For more information, contact Dr. Jim Stoeckel, jimstoeckel@auburn.edu

First detection of white spot syndrome virus in Alabama crayfish: a case study on a research station in Auburn, Alabama

Nicole Tripp, Nicholas Barnes, Courtney Harrison, Abdulmalik Oladipupo, Timothy Bruce, and James Stoeckel: Auburn University, Shell Fisheries Research Center, AL-147, Auburn, AL 36830

White spot syndrome virus (WSSV) is a pathogen of concern for aquatic crustaceans. WSSV infection of crayfish in the United States has been reported in Louisiana crayfish farms since 2007 but has not been previously reported in Alabama. In the spring of 2022, *Procambarus clarkii* in laboratory raceways at the E.W. Shell Fisheries Center in Auburn, AL exhibited patterns of lethargy and mortality indicative of WSSV. Subsequent qPCR assays confirmed WSSV, the first report of this disease in Alabama. Crayfish surveys of ponds, streams, and experimental systems were conducted from spring through fall 2022 to determine spatial and temporal variability of WSSV.

Map of Locations Sampled for WSSV on E.W. Shell Fisheries Center

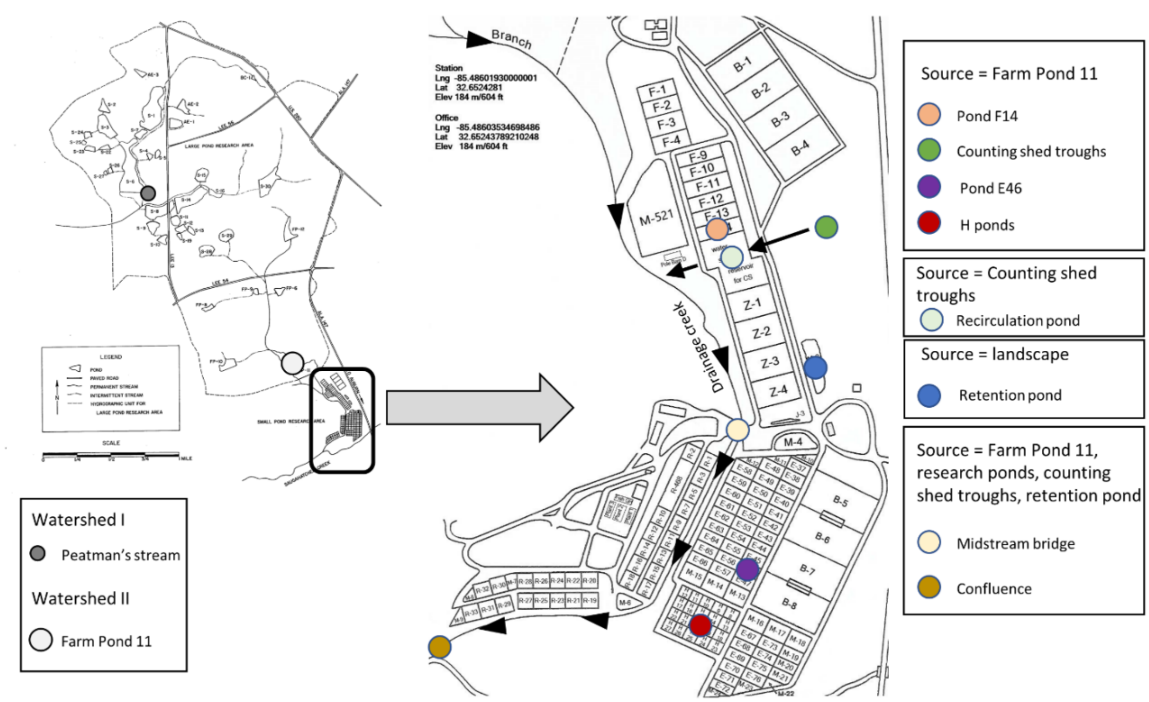

Figure 1 Locations where crayfish were sampled for the white spot virus. Map key indicates the water source for each respective site.

Results showed that WSSV was already widespread in wild *P. clarkii* populations as of May/June 2022, with peaks in Spring and Fall, but low to negative results in mid to late summer. Previous research has suggested an optimal temperature range of 19-30 ± 1℃ for WSSV, which may explain why WSSV prevalence in the wild was low in summer when temperatures regularly exceeded 30℃. However, high temperatures were insufficient to mitigate WSSV. Crayfish that were initially quarantined tested below detectable limits, but eventually tested positive even though they were held at 30℃. Despite carrying the virus, these crayfish exhibited no mortality even though the insertion of internal pit tags had stressed them as part of an ongoing study. Thus, the presence of WSSV does not necessarily cause virulence, even in presumably stressed animals. The widespread occurrence of WSSV in streams and ponds of the E.W. Shell Fisheries Research Center (1600 acres, two watersheds) suggests that WSSV is already endemic in parts of Alabama. Widespread surveys are needed to determine the geographic extent of the disease and prevalence in wild populations. Additional research is also necessary to discern factors that affect the degree of virulence in infected populations. We are currently working with the USDA Animal and Plant Health Inspection Service (APHIS) to officially document the occurrence of WSSV in Alabama and discuss biosecurity issues. Collaborative studies with other agencies also detected the presence of WSSV in shipments of red swamp crayfish to Wisconsin from a leading biological supply company. It is possible that WSSV is currently widespread in many states due to shipments of contaminated crayfish from commercial suppliers for crawfish boils and academic research.

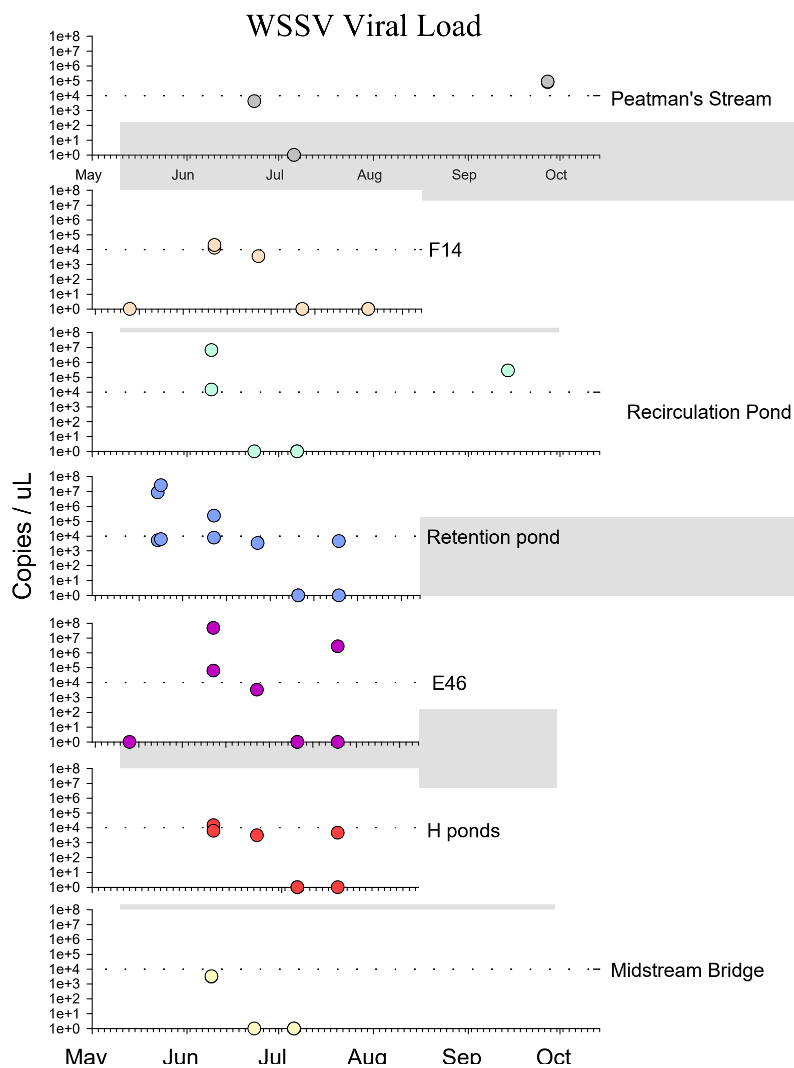

Figure 2 Seasonal patterns in copies per µL of white spot syndrome virus DNA from crayfish sampled at multiple sites on the research station. Dashed line represents the threshold for borderline positive results.

Figure 3 Image shows potential discoloration due to White Spot Syndrome Virus. Crayfish from this trough tested positive for the virus.

Evaluation of Control Techniques for Invasive Red Swamp Crayfish in Burrows

Benjamin Bates, Nicole Tripp, Nicholas Barnes, and James Stoeckel

Auburn University, Shell Fisheries Research Center, AL-147, Auburn, AL 36830

The red swamp crayfish (RSC; *Procambarus clarkii*) is native to the southeast United States but it has successfully invaded nearly every continent around the world. Although physical, biological, and chemical control are employed to reduce or eliminate populations in open-water systems, terrestrial burrows provide a potential refuge from aquatic control treatments. We conducted natural burrow trials to test whether two physical blocker treatments would kill RSC in their burrows. Benseal® (a pond and levee sealing clay) or Great Stuff™ (an insulating foam sealant) were each applied to 37 crayfish burrows with 36 additional burrows used as controls (i.e.,110 total burrows). Burrows were excavated 48 hours after the application of the physical blockers to assess crayfish status. There was 71% mortality in crayfish removed from occupied burrows treated with Benseal®, 62% in burrows treated with Great Stuff™, and 5% mortality in burrows treated as controls. Benseal® and Great Stuff™ appear to be strong candidates as control agents for invasive crayfish populations in burrows. Advantages of Benseal® (bentonite clay) include it is relatively inexpensive and simple to apply; may be used to mitigate damage to levees and shorelines while reducing invasive population; does not require federal and state permitting for its use; and can be used with other control agents such as pesticides. The use of physical blockers may be limited at field sites that have burrows with complex structure. We believe the use of Benseal® to control invasive crayfish in burrows will provide resource managers with an effective tool for their integrative pest management programs.

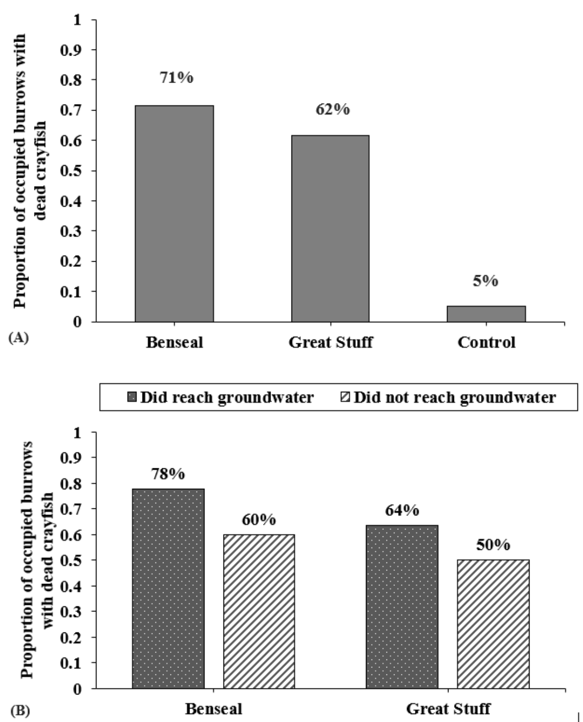

Figure 1 Proportion of occupied burrows with dead crayfish in each treatment (A) and proportion of occupied burrows with dead crayfish when treatments did or did not reach groundwater (B).

Ongoing studies are also examining the use of hot water for control of RSC in burrows. Preliminary lab studies used 10 individuals in each group and exposed them to different temperature water, starting at control of 30 ºC, then 50 ºC, 65º C, and 80 ºC as treatment groups. 100% of individuals exposed to 50 ºC water died, with the animals dying within 60 seconds of exposure. There was 100% mortality in all treatment groups beyond 50 ºC. To conduct field studies, 200 RSC were added to 40'x40' earthen ponds which were subsequently drained over 5 days to promote burrowing. Water was heated to 65 ºC using a propane crayfish boil kit and transferred to a battery powered sprayer. Burrow temperature before treatment was taken, then water was added until the burrow temperature remained above 50 ºC for 5 minutes. Temperatures were taken at 15-minute increments, and after 60 minutes the burrow was dug to check for mortality. Of the 9 burrows treated, only 33% of crayfish were experienced mortality after 60 minutes. In three of the trials, water was reapplied after 30 minutes to try to keep the burrow temperature elevated, but those individuals survived. To effectively use hot water without manually heating and transferring water with no heat loss or significant risk of injury, it requires a heated pressure washer with its own water storage tank and spray line that can be taken into the field without requiring electrical or water supply lines. The Easy-Kleen Stationary commercial hot water gas pressure cleaning system holds > 100 gallons, has a gas-powered sprayer, and oil powered burner capable of heating water to boiling. Using this, we will be able to safely ramp up water temperatures during field experiments to find the most effective temperature required to kill crayfish in their burrows without requiring a blocker or chemical be applied.

Figure 2 Easy-Kleen Stationary commercial hot water gas pressure cleaning system that will be used for future research evaluating effective temperatures for eradicating invasive crayfish within burrows without the addition of physical or chemical blockers.

Additional experiments are being designed to better understand the burrowing behavior of RSC using artificial burrowing chambers. These chambers allow greater control over water level, substrate, and light cycles. Understanding the optimal conditions under which these individuals burrow will give greater insight into natural activities within ponds and streams. Parameters being looked at include drainage rates, ground water levels, light cycles, and air and water temperatures. Current attempts to promote natural RSC burrows in drained, earthen ponds have only a 10-20% success rate, which limits sample sizes.

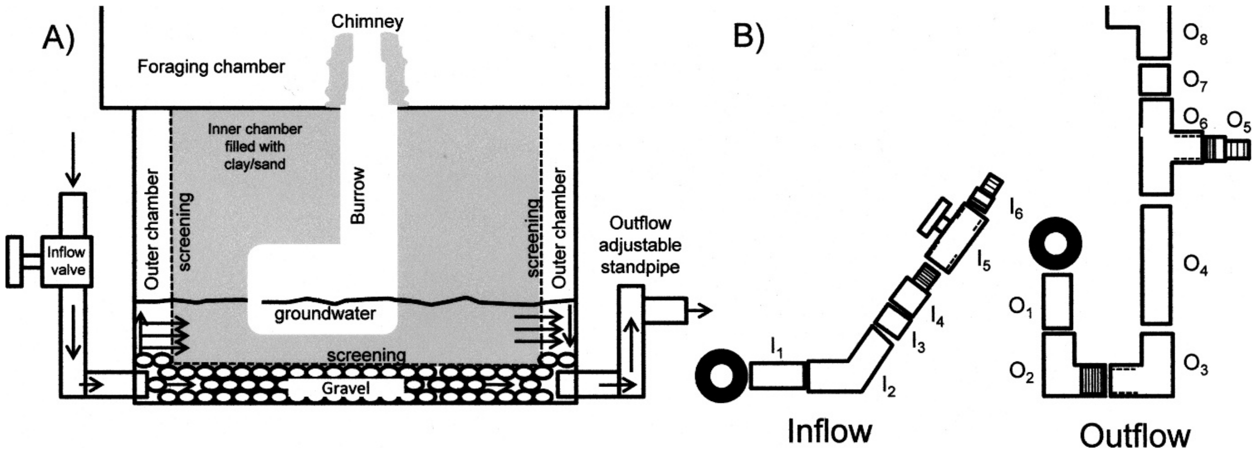

Figure 3 Schematic of burrowing chamber design (A) and exploded diagram of inflow and outflow plumbing (B). Letters and numbers next to each part correspond to the parts list in the appendix. Black circles represent uniseals through which the inflow and outflow pipes enter the burrowing chamber. Arrows indicate direction of water flow. Figure is from Stoeckel, Helms, and Cash 2011 and this original burrowing chamber design is being modified for present-day burrowing chamber studies with Procambarus clarkii.

Comparison of Thermal Tolerance Among Primary and Secondary Burrowing Species

Kaelyn Fogelman[1], Kayla Boyd[1], Chester Figiel[2], and James Stoeckel[1]

[1]*Auburn University, E.W. Shell Fisheries Research Center, Auburn, AL 36830*
[2]*United States Fish and Wildlife, Warm Springs, Georgia, USA*

Conservation of threatened aquatic taxa is impeded by a lack of understanding of the relationships between physiology and thermal tolerance and the ability to obtain sufficient numbers of study animals that are protected or hard to obtain (i.e., primary burrowing crayfish). In this study, we measured physiological responses and tolerances of three common, secondary-burrowing crayfish [*Procambarus clarkii* (Girard 1852), *Cambarus latimanus* (LeConte 1856), and *C. striatus* (Hay 1902)], and one state-threatened primary burrower [*C. harti* (Hobbs 1981)], to acute thermal stress. Our objectives were to: 1) test for differences in critical thermal maxima (CTmax) and upper thermal limits (UTL) between species; and 2) trace physiological responses to thermal stress across multiple levels of organization (cellular to organismal). Crayfish were acclimated to 25°C and then exposed to increasing temperature (2°C/h) until they reached CTmax (loss of equilibrium) and UTL (lack of response to probing). Thermal performance curves were developed for respiratory enzyme activity (ETS), organismal respiration rate (MO_2) and absolute aerobic scope (AAS: ETS minus MO_2). For all species, as temperatures increased maximum AAS was the first endpoint observed and was followed by maximum ETS activity, maximum MO_2, then CTmax, and finally UTL. All crayfish experienced CTmax at temperatures ranging from 35-39 °C with *C. latimanus* being the most sensitive and *P. clarkii* being the most tolerant. For all species, UTL occurred <5 °C after CTmax and UTL ranged from 39.4-39.8 °C with the UTL of *C. harti* significantly lower than that of *C. latimanus*, but the difference was less than 0.5°C. All species reached CTmax when, MO_2, ETS activity, and AAS had declined to 31-91%, 73-96%, and 70-95% of the maximum rates, respectively. This translated to species reaching CTmax when temperature at maximum rates had been exceeded by 1-4°C, 4-11°C, and 5-11°C for MO_2, ETS activity, and AAS respectively. All species reached UTL when MO_2, ETS activity, and AAS had declined to 9-37%, 69-81%, and 69-87% of their maximum rates, respectively. This translated to species reaching UTL when temperature at a maximum rate had been exceeded by 3-7 °C, 8-11 °C, and 9-12 °C for MO_2, ETS activity, and AAS respectively.

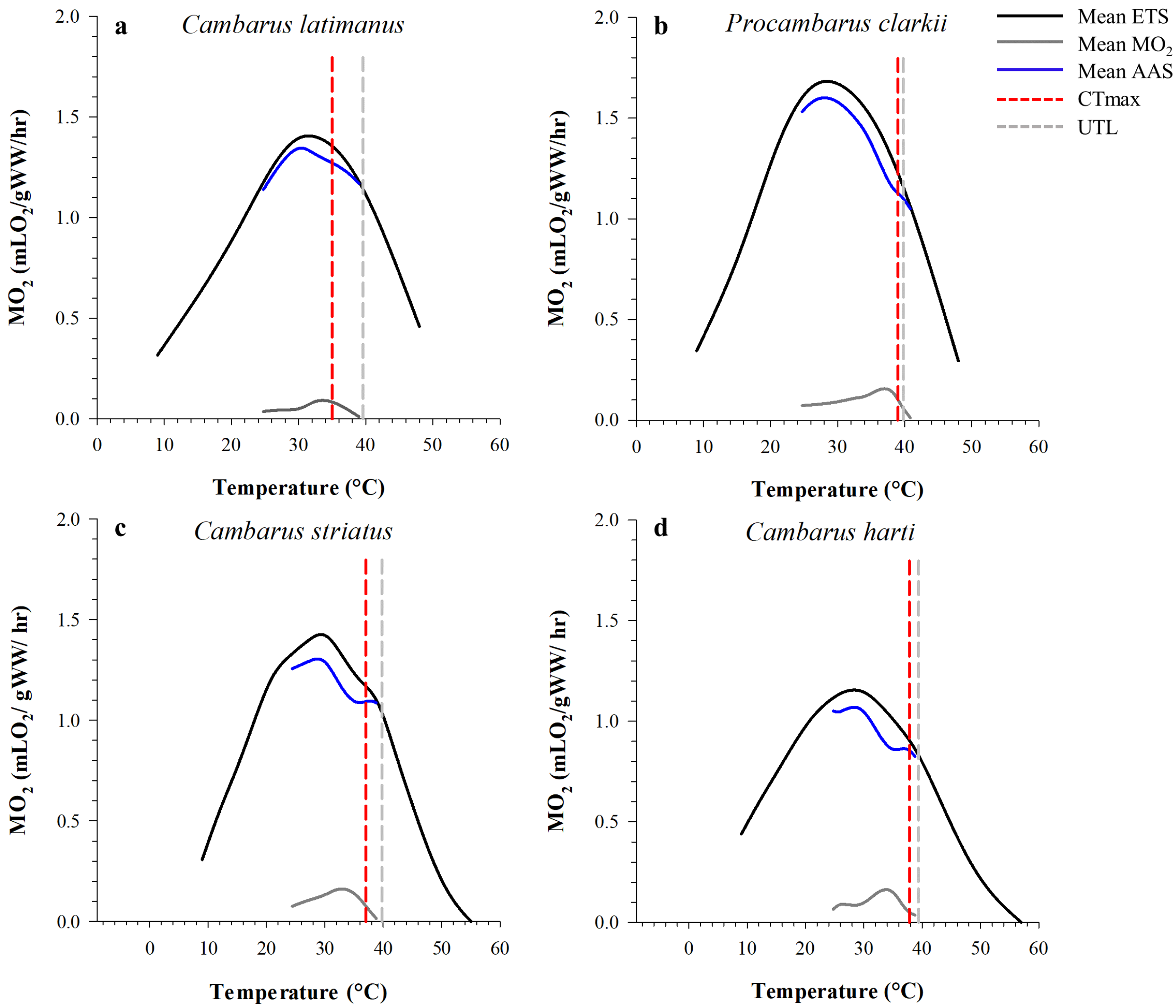

Figure 1 Relationships between electron transport system (ETS) activity, metabolic rate (MO2), absolute aerobic scope (AAS) and temperature for Cambarus latimanus (a), Procambarus clarkii (b), Cambarus striatus (c), and Cambarus harti (d). The vertical red dashed line represents the median temperature of critical thermal maximum (CTmax) and the vertical grey dashed line represents the median temperature of upper thermal limits (UTL).

Results provide sublethal and lethal acute temperature thresholds for focal taxa and suggest that acute thermal thresholds protective of common species will be equally protective of a rare primary burrower. Linkages between physiological responses and acute thermal stress will be valuable for future modelling of adverse outcome pathways (AOPs) for crayfish subjected to warming temperatures. Development of accurate AOP models may ultimately reduce the number of individuals and assays required for determining effects of acute thermal stress on species of interest.