

Research Progress (February 2016 – January 2017)

Black Gill Disease in Georgia Shrimp: Causes, Distribution and Transmission.

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Research. The primary research goals of this project during the past year have been three-fold; 1) to continue efforts to identify the causative agent of shrimp black gill (sBG), 2) to determine the seasonal distribution of sBG using a recently developed high sensitivity molecular assay and 3) continue investigations of mortality and morbidity due to sBG infections.

1. Identifying the causative agent of shrimp black gill. Molecular taxonomic studies based on the sequencing of the 18S rRNA gene from the sBG ciliate and from other apistome ciliates confirm that the sBG ciliate is an apistome ciliate closely related to *Hyalophysa chattoni*. During the past project period we completed phylogenetic analysis based on the sequences we recovered during the project period. Microscopy-based efforts to identify the sBG ciliate have also progressed and continue to indicate that the sBG ciliate is not *H. chattoni* though confirm that it is an apistome ciliate. Previous microscopy analyses had been unable to confirm the results of genetic studies. During the past project period two notable microscopic observations were made. First, silver staining of the sBG ciliate attached to shrimp gills revealed definitive diagnostic ciliature confirming the molecular evidence that the sBG is an apistome ciliate. Surprisingly, these studies indicated that the life form attached to the gill is not an encysted phorant as previously assumed based on scanning electronic microscopy, but actually a trophont. This behavior is not associated with any described species of apistome ciliate strengthening the conclusion that the sBG ciliate is an undescribed species. A second discovery, based on transmission electron microscopy observations, was evidence that the sBG ciliate can penetrate and invade shrimp gill tissues. This observation strengthens the conclusion that the sBG ciliate is the causative agent of shrimp black gill in Georgia and South Carolina and suggests a pathogenic mechanism. These studies were largely directed by S. Landers at Troy University, Troy, Alabama.
2. Intensive temporal and spatial sampling of shrimp facilitated by close collaboration with the GA CRD and MECA has provided samples to define the seasonal cycle of black gill. Utilizing the recently

developed (supported by GA Sea Grant) molecular (PCR) diagnostic assay for the sBG ciliate. Efforts have focused on 1) identifying whether the sBG ciliate is present throughout the year and if not, when it is present and absent and 2) the timing of the emergence of the sBG ciliate and its relationship to environmental parameters and the appearance of the visual symptoms of shrimp black gill. Based on sampling results from three years (2014 - 2016) we can conclude that black gill does disappear from GA shrimp in the late winter and spring. However, the infestation and the prevalence of the sBG ciliate exhibits inter-annual variability with the sBG ciliate emerging earlier each year since 2014 (Figure 1).

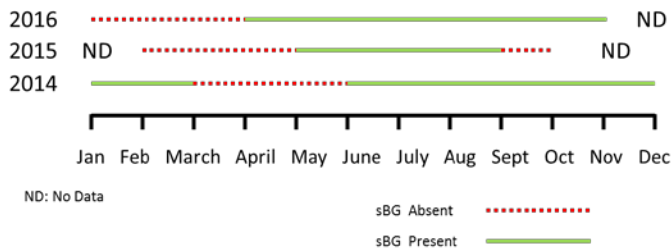


Figure 1. Presence of the sBG ciliate in Georgia identified by molecular diagnostic (PCR) assay.

In 2014 the sBG ciliate was present in Georgia shrimp from May through February and was absent in March through June. In 2015 the sBG ciliate was present from May – August, absent in September and February through April. Due to complications associated with boat availability, we were not able to collect samples in January and October through December. In 2016 the sBG ciliate was detected from April through November and absent from January through March. We were unable to collect samples in December in 2016 due to damages from Hurricane Mathew. These observations raise several important questions including: where does it go? How does it get back into the shrimp? and can the infection cycle be disrupted?

As a component of her MS thesis research Ashleigh Price investigated possible reservoirs of the sBG ciliate. The sBG ciliate, or closely related ciliates, was detected in a broad spectrum of crustaceans including the lesser blue crab (*Callinectes similis*), the spider crab (*Libinia emarginata*), Mantis shrimp (*Squilla empusa*), the seabob shrimp (*Xiphopenaeus kroyeri*) and grass shrimp (*Palaemonetes* spp.). However, because of limited sampling, this is not considered to be an exhaustive catalog of potential sBG carriers. Interestingly, there was an inverse relationship between the abundance of ciliate prevalence in commercial shrimp species and grass shrimp ($r = -0.7, p=0.05$) with peak abundance in grass shrimp occurring through the late fall and spring when prevalence in commercial

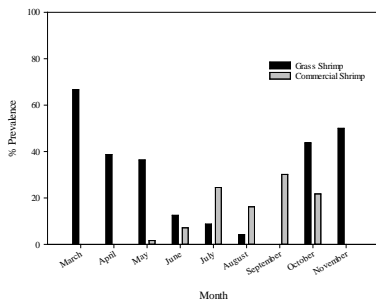


Figure 2. Monthly distribution of sBG ciliate in grass shrimp *Palaemonetes* spp. (N=3-31) collected from Skidaway River, GA and commercial penaeid shrimp (N=36-49) collected from Wassaw Sound estuary system during the Georgia Department of Natural Resources Ecological Monitoring Trawl Survey from March to November in 2015. Commercial shrimp samples were not collected in November. A significant negative correlation was observed ($P<0.05$).

penaeid shrimp was low (Figure 2). These observation suggest that grass shrimp may serve as a winter reservoir for the sBG ciliate. Ashleigh Price completed her thesis and graduated from Savannah State University in December 2016.

3. Mortality and physiological effects. During the previous project year in experimental studies we observed a short period (2-3 weeks) in late August and early September of high shrimp mortality beginning approximately 12 days following maximum seasonal temperatures and presumably associated with black gill as there were no other sources of mortality. Over this period 83% of experimental shrimp died (Figure 3). During the past project period we followed-up these studies by investigating the effect of temperature on shrimp mortality associated with black gill. Significantly lower mortality was observed in shrimp maintained at $\sim 2^{\circ}\text{C}$ lower than ambient temperatures (Figure 4). These studies were conducted at the Skidaway Institute of Oceanography.

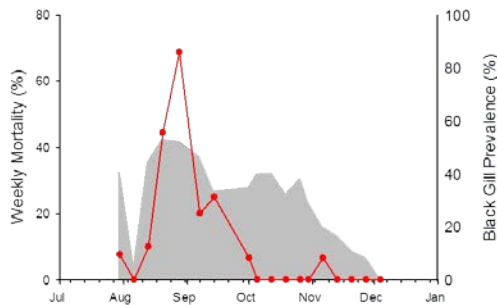


Figure 3. Shrimp mortality associated with black gill infections (2015). Wild shrimp (16) were maintained in individual aerated aquaria isolated from predation and fed daily. Shrimp were replaced approximately monthly so that experimental shrimp reflected the wild populations.

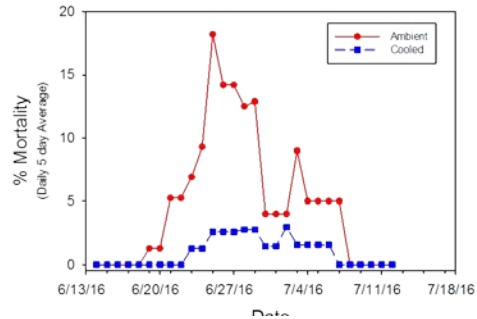


Figure 4. Shrimp mortality associated with black gill infections at ambient (●) and cooled (◻) temperature. Mortality was significantly lower in cooled tanks.

Collaborative investigations with the crustacean research group led by Jeff Brunson, Elizabeth Gooding and Dr. Peter Kingsley-Smith at the South Carolina Marine Resources Institute (SC DNR) and Dr. Amy Fowler (George Mason University) were continued during the past project period. Studies to investigate the impact of black gill on shrimp respiration and behavior were initiated. Principle efforts involved the optimization of an aquatic treadmill respirometer that will allow the quantification of respiration rates (O_2 consumption) of shrimp under standardized conditions. Initial studies involved the calibration of the system and the measurement of resting and active respiration rates of a set of shrimp with (9) and without (9) visible symptoms of black gill. Pilot studies validated the experimental system and confirmed that shrimp with black gill exhibit reduced respiratory capacity. Extended physical activity (60 – 180 minutes) resulted in a significantly greater decline in respiratory capacity of shrimp with black gill versus those without. These results support the hypothesis that shrimp with black gill likely exhaust sooner than those without and are therefore at greater risk of predation (secondary mortality) in nature.

Taking advantage of complimentary support from the Georgia Coastal Incentive Program studies are also being conducted to explore the impact of secondary mortality. During the past project period studies to investigate the effect of black gill on secondary mortality were also initiated. To achieve this objective experimental controlled studies with representative fish predators are planned. These studies are also being conducted in South Carolina taking advantage of the unique facilities on the

Fort Johnson campus. During the past project period, pilot studies were conducted to identify key experimental parameters for these experiments. Specifically, tank size, shrimp stocking density and experiment duration were investigated. Preliminary studies were conducted with flounder and red drum. Based on these pilot studies a final experimental design was developed. Tanks (3.35 m diameter) filled to 0.5 m with seawater containing 20 shrimp and 1 fish predator will be utilized. The duration of each experiment will be 24 hours. Full scale experiments will be conducted during the upcoming summer and fall (2017) when black gill prevalence is at its maximum.

Outreach Progress. This project continues to bridge a broad segment of stakeholders in Georgia and the region. Especially because the results of our studies indicate that the problem of shrimp Black Gill extends along the entire US Atlantic seaboard and likely the Gulf of Mexico, establishing broad research, outreach, industry cooperation and education is critical. To implement this cooperation we have worked closely with Georgia and South Carolina DNR and maintained relationships with the North Carolina Dept. of Marine Fisheries and the Florida Fish and Wildlife Research Institute. Outreach and education activities have been continuous throughout the past project period. Working closely with Bryan Fleuch we hosted an open black gill informational workshop (summit) during early summer prior to the start of the 2016 shrimp season. The summit was well attended (~30 participants), particularly because of the high interest generated by the early emergence of black gill in 2016. During the fall, at the peak of black gill a second all stakeholder activity was held, a research cruise on the R/V Savannah was held. Again, a broad diversity of stakeholders were present to share, discuss and learn. Both the summit and the research cruise were covered by the local and regional press. Information is also available on the web and hosted by the GA Sea Grant and Extension (<http://gacoast.uga.edu/research/major-projects/black-gill/>). Information and relevant web links are regularly updated. A list of news stories produced in associated with this project is included as Appendix I to this report.

A major component of this project is the development of a new smartphone application that can be used by commercial and recreational shrimpers to collect high quality data on the prevalence of black gill. During the past project period the Shrimp Black Gill Tracker app was completed, optimized and training materials developed. The Shrimp Black Gill Tracker is available for download (free) on the Apple App Store and Google's Google Play. The app was developed by coPI Kyle Johnsen and the development of training materials and publicity is being led by Bryan Fleuch (GA Sea Grant/Marine Extension). Efforts are currently underway to recruit and train commercial shrimpers in its use and to recruit commercial shrimpers to participate in a pilot program that would allow shrimpers to contribute quality data on the distribution and prevalence of shrimp black gill. If successful, this data will likely become integral in the management of shrimp black gill and the fishery. We envision that data collected via the smartphone application will be used by the GA DNR and the shrimp advisory panel to make decisions on when to open and close the shrimping season. We anticipate applying for NOAA Cooperative Research Program (CRP) in 2017. If awarded this project would support the engagement of commercial shrimpers and sustain these activities beyond the scope of the current GA Sea Grant funded program.

Other outreach activities have been numerous presentations to local groups and classes. A highlight of the year was our participation in a Georgia legislator's retreat that focused on coastal issues. During this event we were able to discuss the shrimp industry, highlighting GA Sea Grant's investment in black gill

research. We also conducted a quite popular taste test of shrimp with and without black gill. The results of the taste test were convincing to the participants that shrimp black gill does not affect the quality, taste or health safety of always delicious Georgia shrimp.

Education Progress. During the past project period, 1 MS student and 1 summer undergraduate research associate was supported.

Appendix I – 2016 Press Coverage of Shrimp Black Gill

- 6/2/16 -- News release announcing black gill summit (Also posted to Facebook, Skidaway Inst website and Skidaway Inst blog)
- 6/3/16 -- Advance article in Fla Times Union on black gill summit meeting
- 6/13/16 -- WTOC story on black gill advancing black gill summit meeting
- 6/13/16 -- WTGS story on black gill advancing black gill summit meeting t
- 6/13/16 -- WTVM (Columbus) story on black gill advancing black gill summit meeting
- 6/16/16 -- GPB story on black gill / STEM teachers cruise
- 6/22/16-- WTOC story on black gill summit
- 6/22/16-- WTVM story on black gill summit
- 6/22/16 -- WJCL story on black gill summit
- 6/22/16 and multiple dates -- Events announcement in Savannah Morning News for black gill summit
- 6/27/16 -- Savannah Morning News story on black gill update
- 6/27/16 -- Florida Times Union article on black gill update
- 6/29 & 30, 2016 -- WTKS radio Interviews with Marc Frischer on black gill.
- September 2016 -- Article on black gill summit in Skidaway Campus Notes newsletter
- 11/10/16 -- News release on black gill cruise (Also posted to Facebook, Skidaway Inst website and Skidaway Inst blog)
- 11/10/16 - WJCL -- story on black gill update
- 11/17/16 -- Darien News, published Skidaway Institute news release on black gill cruise
- 12/13/16 -- Article in digital Skidaway Campus Notes newsetter on the black gill cruise
- 12/15/16 -- WSAV story on black gill update
- December 2016 -- Southern Tides Magazine -- article on black gill update and tracker app
- Most of the releases and news stories were also copied into the Skidaway Institute blog and Facebook page.