

## Fisheries Resource Grant Final Report:

# Development of Preventive Measures to Reduce Mortalities for Holding Live Wild-caught Flounder in Recirculating Aquaculture Systems

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## Abstract

A substantial high-value market exists for wild-caught live summer flounder (*Paralichthys dentatus*). An important component of accessing this market is the ability to hold wild-caught flounder in land based recirculating aquaculture systems (RAS). A major constraint to holding summer flounder in land-based RAS is fish mortalities associated with the ectoparasite argulus spp. Very little information exists that provides a live flounder holding facility with FDA approved treatment options to prevent introduction of argulus from wild caught fish into holding systems. The project objective was to test the available FDA approved chemotherapeutics for treatment of ectoparasite infections in summer flounder. To validate treatment efficacy, 8 infected individuals were treated with bath treatments according to maximum recommended doses (250ppm formalin for 60 m; 200 ppm hydrogen peroxide for 30 m; and freshwater for 20 m) and then transferred to separate RAS to monitor for two weeks. Daily observations were made to determine if treatments were lethal to the attached argulus. Following the two week monitoring period, fish from all treatments showed no sign of a reduction in attached argulus. According to the findings of this study, there are currently no FDA approved treatments for argulus infections on summer flounder. It is recommended that a live wild flounder holding facility visually inspect all incoming fish for the presence of argulus and maintain fish in a quarantine system prior to holding in a RAS.

## Introduction and methods

Mid-Atlantic Aquatic Technology (MAAT) is currently working with local watermen to continue the development of a value added fishery in the Eastern Shore of Virginia. Summer flounder are purchased live from the fishery for a premium price. The fish are then held in recirculating aquaculture systems (RAS) for later sale to high value sushi markets which require a high quality live fish. Initial work was temporarily stopped due to an episode of heavy losses in the RAS. MAAT began working on this venture in the fall of 2009 and the mortalities were attributed to the presence of an ectoparasite which was identified to be argulus. Holding fish in RAS has the advantage of being able to hold fish through the winter through temperature control, however these holding systems are also more susceptible to the introduction of diseases from wild caught fish. The objectives of this study were to compare the current available treatments for ectoparasites for effect on fish health and prevention of parasite infestation in the RAS holding systems. A series of experiments were carried out to determine the effect of treatments on fish health and argulus infestation in RAS. Below is a summary of the experimental treatments.

- Prophylactic treatment of wild caught flounder to prevent argulus infestation.
  - o Compare short-term bath treatments
    - Formalin (formaldehyde)- 250 ppm for 1 hr
    - Fresh water – 20 min
    - Hydrogen peroxide – 200 ppm for 30 min
    - Control – no treatment of infected fish

To conduct the trials, three independent 1,000 gal RAS were constructed consisting of a bead filter, ultra violet filter, and a recirculation pump. These components maintained healthy water conditions during the monitoring periods. Five fish were first subjected to the treatments to insure that treatment dose has no negative effect on fish health. Treated fish were monitored in three separate RAS for 5 days post treatment.

For experimental trials, animals were obtained by hook and line capture during the 2010 season and held in a RAS. Once infected fish were obtained from the wild population, they were maintained in a separate RAS to provide the trials with infected fish. Eight infected fish were treated with each of the corresponding treatments. For trial one, eight infected fish were treated in a 1 h formalin bath at a concentration of 250 ppm. Eight separate infected fish were concurrently treated in a 30 m hydrogen peroxide bath at a concentration of 200 ppm. Immediately following treatments, fish were inspected visually for the presence of argulus and then monitored for two weeks in two separate recirculating systems. Argulus were counted on the fish before treatments. Following treatment, fish from each experimental treatment were maintained in separate RAS and monitored for 14 days post treatment to determine the effect of treatments on fish lice infection. For trial two, eight infected fish were treated in a 20 m freshwater bath. Eight additional infected fish were also dipped in a 30 m sea water bath to serve

as a control treatment. No immediate effect on attached argulus was noticed for either the fresh water treatment or the control. Fish were then placed in their respective monitoring system to observe for two weeks following treatments.

## Results and discussion

Collection of flounder began April 5, 2010 and initial fish collected showed no signs of argulus infection. The first sign of argulus was noticed on June 9, 2010 on a captured fish that had a preexisting wound. That fish was transferred to a separate quarantine tank and maintained with 5 additional fish to inoculate the infection tanks. The infection tank was monitored daily for signs of a reproducing argulus infection characterized by egg casings attached to the tank walls. On September 8, 2010, two flounder mortalities were noticed in the infection tank and argulus egg casings were seen on the tank walls indicating a reproducing population of argulus. Sixteen fish were then used from this tank to begin trial 1. The average number of argulus on the experimental fish used for trial 1 were 19 and 20 for the hydrogen peroxide and formalin treatments respectively.

For trial 1, no effects on attached argulus were noticed following the formalin or hydrogen peroxide baths. Argulus infection was still present following the two week monitoring period in both treatments with no noticeable effect between treatments. Additionally, two mortalities were observed 4 d following the formalin treatment. These mortalities were most likely due to the argulus infection.

For trial two, eight infected fish with an average of 23 argulus per fish were treated in a 20 m freshwater bath. Eight additional infected fish with an average of 20 argulus per fish were also dipped in a 30 m sea water bath to serve as a control treatment. No immediate effect on attached argulus was noticed for either the fresh water treatment or the control. Fish were then placed in their respective monitoring system to observe for two weeks following treatments. Following the two week observation period, there was no noticeable effect on the argulus infections between treatments.

During treatment procedures, particularly the hydrogen peroxide treatment, several other parasites were noticed on the bottom of the treatment bath. One particular parasite was identified to be another type of parasitic copepod, caligus spp. which has not been determined to be problematic with flounder held in RAS. There may be advantages to treatment of the flounder before introduction into the RAS holding system through overall parasite load reduction, however there does not appear to be an FDA approved dose to effectively eliminate introduction of argulus to a RAS holding system. There have been several recommended treatments for argulus that are not permitted for use with food fish, however have been shown to be effective for broodstock flounder. Copper sulfate has been used by flounder spawning facilities as an off-label treatment for eradication of argulus from an infected RAS and has been determined to be effective, in addition a "hot dip" of formalin for a 1 min duration has also been used by

broodstock facilities to detach argulus, allowing for mechanical removal. For prevention of argulus infection in a wild flounder holding facility, it is imperative that all incoming fish are carefully inspected for presence of argulus. It is also advisable that all fish be quarantined in a separate holding system prior to introduction to a main holding system.