THE 9TH ANNUAL WORKSHOP ON THE REPRODUCTION AND EARLY DEVELOPMENT OF YELLOWFIN TUNA, ACHOTINES LABORATORY PANAMA, CENTRAL AMERICA, 6 – 16 JULY 2011.

Project No.: 2011/707

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OBJECTIVES OF RESEARCH TRAVEL GRANT

The objectives of the workshop were to allow me the opportunity to learn more about the global trends and major issues in tuna larviculture and future research directions.
The specific objectives of the workshop were to investigate current rearing practices which may improve survival of yellowfin tuna. The trials investigated new yellowfin tuna larval rearing technologies that are hoped to improve growth and survival and thus aid the successful commercialization of southern bluefin tuna in Australia. It is anticipated that the results of the trials and outcomes of the workshop will provide valuable information which can be added to the existing knowledge on tuna larval rearing and used to augment the research activities on southern bluefin tuna larval rearing in South Australia. Achotines laboratory is unique in that it has viable yellowfin tuna eggs available nearly all year round. Larval rearing trials were conducted at Achotines investigating the latest developments in larviculture and building on existing knowledge developed in past workshops. Personally, one key objective was to establish a contacts and network list of scientists involved in tuna propagation. The relationship formed with the workshop organizers and participants is an invaluable source and will allow for future discussions into tuna larviculture.

ISBN: 978-1-925982-90-9

NON TECHNICAL SUMMARY

Currently, wild caught fisheries are under pressure and progressively declining and over 50 % of consumed seafood currently comes from cultured finfish species. One of the most economically valuable and sought after wild caught species is tuna, which has led to their vulnerability to overfishing and rapid population decline. This has led to increasing interest in the propagation of highly valuable species, particularly northern and southern bluefin tuna. Commercialization of tuna is at the forefront of aquaculture worldwide. Although there are many issues that hinder the success of tuna propagation, including reliable spawning in land-based tanks and the high mortality rates that occur through all the culturing stages.

In South Australia, Clean Seas Tuna (Pty) Ltd. (CST) is the leading company in southern bluefin tuna (SBT, *Thunnus maccoyii*) propagation. Tuna are pioneering species to aquaculture and will have a high impact on the industry if the life-cycle is closed. The CST sexually mature, parental fish (broodstock) have successfully spawned in the past three years. However, reliable availability of viable eggs and the larval rearing stages are currently the major bottlenecks in the propagation of SBT. Scientific research on yellowfin tuna (YFT, *Thunnus albacares*) is far more advanced. Yellowfin tuna is considered a valuable surrogate species for research, including larval rearing, which could contribute to the future research on the more valuable SBT species.

The field trip was part of the annual workshop that is organized by the University of Miami and the Inter-American Tropical Tuna Commission (IATTC), conducted at the Achotines Research Laboratory in Panama. Yellowfin tuna broodstock have successfully spawned at this facility for many years. The research laboratory provided facilities to conduct scientific trials on the larval rearing of YFT.

OUTCOMES ACHIEVED TO DATE

The knowledge gained at this workshop will be shared with the project's industry partners, CST. Clean Seas Tuna currently leads Australian tuna propagation and it is hoped that direct technology transfer between aspects of YFT and SBT rearing will be possible. This will aid industry in refining their rearing protocols for SBT. Better larval rearing protocols will allow higher survival and growth and thus aid the successful commercialization of SBT in Australia.

During the workshop I met a group of scientists that are actively involved in developing tuna culture. The discussions about aquaculture within this group highlighted similar areas and stumbling blocks that appear to affect most researchers. This will have a direct effect on my research, allowing me to better develop my research strategies for future trials on both SBT and yellowtail kingfish (YTK) in Australia.

OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY

The major output developed at the workshop was the 'Protocols used for larval rearing of yellowfin tuna by the workshop participants in July 2011 at the Achotines Laboratory in Panama' included an addition to the "2007-2011 suggested topics to future research and perfect technology to improve survival of tuna larvae during the early development stages" document (see attached).

The additions include the two larval rearing experiments trialling 'grey-water culture' and a successful weaning trial undertaken on YFT juveniles (see Results).

BACKGROUND AND NEED

As SBT is a pioneering aquaculture species very little is known about their early larval development and conditions in which they should be reared. YFT is considered a valuable surrogate species for research, including larval rearing, which could contribute to the future research on the more valuable SBT species. The aquaculture of YFT has been more advanced as the captive spawning of YFT broodstock was achieved in the late 1990s. At Achotines spawning of YFT occurs regularly, almost daily. The abundance of viable eggs allows for frequent larval rearing trials to be conducted.

RESULTS

The workshop was designed in such a way that participants are involved in experiments investigating the early larval development of YFT.

Larval Rearing

Limited research exists on the use of grey-water culture, i.e. inorganic clay-water mixture, in marine finfish larval rearing. Although the larval culture of California yellowtail (*Seriola rivoliana*) has begun to implement this strategy in an aquaculture setting. The use of suspended clay increases water turbidity, providing a high contrast between live prey and the water. This contrast allows larvae to more readily capture live prey. Clay binds to proteins and effectively removes pathogens from culture water. This is thought to have antimicrobial properties, inhibiting pathogenic microbial growth and bacteria.

The aim of this year's preliminary trials was to determine if there was an increase in survival in YFT larvae using 'grey-water culture' compared to traditional 'green-water culture'.

Experiment 1

Larval rearing

- Larvae were stocked into six 800 L tanks at a density of 20 larvae L^{-1} at 2 days post hatch (dph).
- A photoperiod of 12 h light: 12 h dark was used and the rearing temperature was maintained at a constant 28 °C.
- Larvae were fed rotifers from 3 dph, 3 times day^{-1} to maintain a target density of 10 rotifers mL^{-1}.

Experimental treatments

1. Live algae –' green-water culture' provided by a mixture of *Nannochloropsis and Tetraselmis* algae at a density of approximately 1 x 10^6 cells mL^{-1}
2. Clay (OM # 4 brand) – 'grey-water culture'
3. Skretting 'Neptune' water conditioner - mixture of clay and freeze dried algae, currently being trialled by Skretting.

- Two replicate tanks per treatment.
- The clay and Neptune were added every 2 h at a dosage proposed by Zach Daugherty, a PhD candidate at the University of Miami, and maintained to occlude vision of the tank bottom.

Outcomes

- The Neptune treatment required more rigorous mixing than the clay treatment to create the slurry and as a consequence heavy foam was produced and was difficult to remove from the slurry.
- The Neptune created sufficient green-water background within the tanks, but an oily layer formed at the water surface and intensive skimming was needed to remove the debris (Fig. 2.).
- This oily layer also trapped larvae at the surface.
- The clay and algae treatments created the desired background and light contrast for optimal larval feeding.
- By 4 dph all sampled larvae were found to have rotifers present in their gut.
- There was high mortality in the larvae held under the Neptune treatment at 4 dph and mass mortality occurred across all treatments by 5 dph. The trial was terminated on 5 dph.
- The high mortality was presumed to be caused by high rates of sinking larvae due to the lack of upwelling current or light stimulus to keep larvae actively swimming.
- The Neptune product is not recommended at this time for delicate species such as YFT.

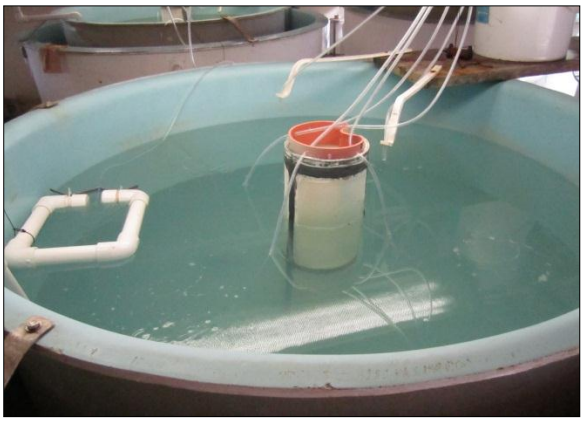


Figure 1. Clay treatment (left) and algae treatment (right) tanks.

Figure 2. Oily surface produced when using 'Neptune' clay in tanks.

<u>Experiment 2</u>

Larval rearing

- Larvae were stocked into four 800 L tanks at a density of 20 larvae L^{-1}.
- In Experiment 2, a 24 h light photoperiod was used as the experiments during the 2010 workshop revealed 24 h light reduced larval sinking deaths.
- Larvae were fed rotifers on 3 dph, 6 times day^{-1} to maintain a target density of 10 rotifers mL^{-1}.

Treatments

1. Live algae - green-water culture provided by a mixture of *Nannochloropsis and Tetraselmis* algae at a density of approximately 1 x 10^6 cells mL^{-1}
2. Clay (OM # 4 brand) – grey-water culture

- Two replicate tanks per treatment.
- The algae and clay dosages were identical to those used in Experiment 1.

Outcomes

- All sampled larvae were feeding on 4 dph. Larvae fed well under both the clay and algae treatments.
- Swimbladder inflation began on 5dph, with mean inflation rates of 42 and 45 % in larvae held under the algae and clay treatments respectively. There was no significant difference between treatments (*t*-test: *P* = 0.5, Fig 3).
- Survival was higher in Experiment 2 than Experiment 1 at 5 dph. The trial was terminated on 5 dph and all surviving larvae were counted from each tank.
- Larvae in the algae treatment had higher survival (20.4 ± 13.9 %) than those in the clay treatment (2.7 ± 0.7 %) but survival was not significant (Mann – Whitney test: *P* = 0.333, Fig 3). The difference in the median values between the two groups was not great enough to exclude the possibility that the difference was due to random sampling variability.
- The algae treatment resulted in high survival but the clay treatment is worth investigating further.

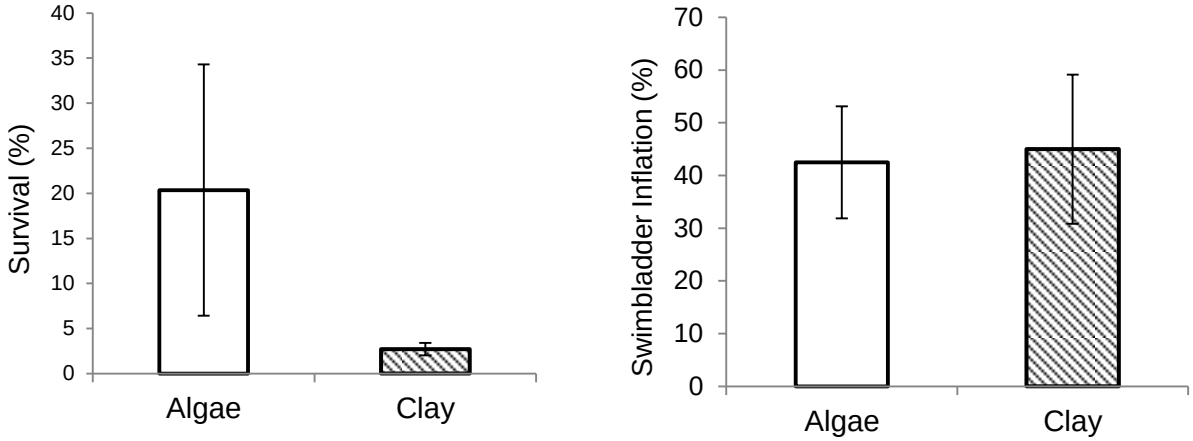

Figure 3. Survival and swimbladder inflation of yellowfin tuna larvae at 5 days post hatch reared under green-water (algae) and grey-water (clay) cultures (*P* = 0.333 and *P* = 0.5, respectively).

Figure 4. Yellowfin tuna at 5 days post hatch with successful swimbladder inflation.

Weaning Trial

A weaning experiment was conducted on YFT juveniles at 28 dph. At this stage juveniles were fed only YFT yolk-sac larvae. On the first day juveniles were fed finely chopped sardines fillets and a formulated pelleted diet (Otohime C2 pellets, Japan). Juveniles were fed every 2 h from 7 AM – 6 PM. Flow rates were increased to keep pelleted feed within suspension and juveniles swimming against a current. On the first few days of weaning yolk-sac larvae where restricted at early feeds and only chopped sardine fillets and Otohime C2 pellets soaked in 'sardine juice' were offered. The 'sardine juice' was prepared by blending fillets and screening through 100 µm mesh. The water surface was agitated before feeding to train juveniles. Over the weaning period the amount of pellet feed was increased as the number of yolk-sac larvae decreased. By 7 days juveniles were weaned and fed solely on dry pellets. Survival was estimated at 20 %.

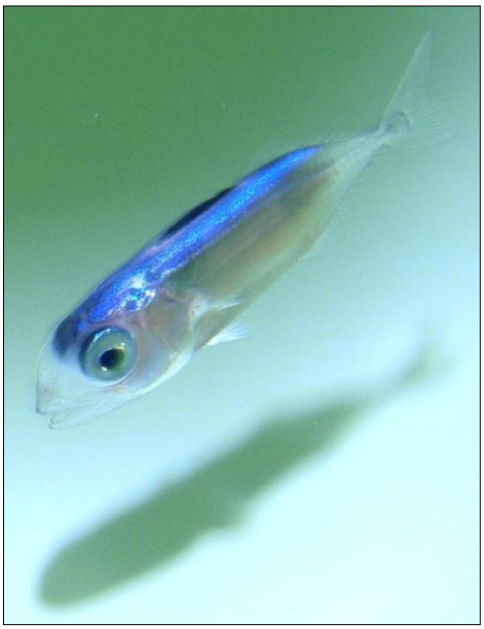

Figure 5. Yellowfin tuna at 30 days post hatch successfully weaned onto artificial pellets (Photo courtesy of Dr. Daniel Benetti).

Broodstock capture

On five occasions staff at the Achotines laboratory caught wild YFT broodstock from the reef surrounding the Achotines Bay. The fish brought in into the lab were treated with oxytetracycline at 50 mg kg^{-1} of body weight and held in a quarantine tank for up to five days. Fish were then bathed in nitrofurazone at 50 ppm for 3 minutes and transferred to a reserve tank (9 x 3 m) before ultimately being moved to a larger maturation tank (18 x 6 m).

Fish remain in the reserve tank for an extended duration, therefore it was suggested that the quarantine period be removed from the capture protocol. The reserve and maturation tanks are on separate water supply lines and the reserve tank is essentially an isolated

quarantine tank. This also minimises stress on the fish due to handling as one less transfer between tanks would be needed.

Figure 6. Capture of wild adult yellowfin tuna.

Figure 7. Quarantine and transfer of yellowfin tuna at Achotines laboratory.

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

The workshop further developed the YFT larval rearing protocols and knowledge base. This can be used for future research on SBT larval protocols.

SUMMARY OF CHANGE IN INDUSTRY

There are no immediate changes expected to occur within industry at present. However, there is much interest to trial grey-water culture on other species. Trials involving less delicate species such as YTK could prove worthwhile. Clay has beneficial properties that are worth investigating further. Also, technologies used in the larviculture of SBT, such as upwelling currents, may be more favourable to grey-water culture as the clay would be kept in suspension better.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

This workshop runs every year trialling new research avenues. Future workshops will use the knowledge gained from this year and previous years to improve rearing protocols and or research topics.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

As there are many parties involved in this area of research there are always various research priorities to be considered. Limited information is available on tuna propagation and protocols for improved survival and growth are still imprecise. Therefore, considerable research is needed in all aspects of rearing making it necessary to determine which areas are key priorities and will yield the highest improvements in survival.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

Changes to the rearing protocols may occur in the next workshop depending on research topics and priorities. The results of this workshop will be conveyed to the industry partners, CST, and some of the research trials may adopt the protocols or products tested during this year's workshop.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

If trials on SBT larvae following the practices used at the workshop prove successful, either through increased survival or growth, adoption of the protocols may be tested at CST through larger scale proof of concept experiments. As such little knowledge exists on the propagation of SBT any advances in larval rearing will be beneficial to the industry.

WHAT BARRIERS ARE THERE TO ADOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

The biggest obstacle is availability of viable SBT eggs to run research trials. The SBT spawning season is very brief, sporadic and uncertain. This limits the implementation of suggested changes to rearing protocols. More time and research is needed to reach the stage where new protocols can be developed.

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

The major output is the '2007-2011 suggested topics to future research and perfect technology to improve survival of tuna larvae during the early development stages' document developed during the workshop. This includes the results on grey-water culture in tuna larviculture and describes the key research areas and topics for further trials.

WHO IS/ARE THE TARGET AUDIENCE/S?

Industry partners such as Clean Seas Tuna (CST) and other stakeholders involved or interested in tuna propagation research, i.e. shareholders and research scientists.

WHAT ARE THE KEY MESSAGES?

The key messages from this year's workshop are the result of the larval rearing trials. Larviculture of YFT using green-water culture resulted in higher survival compared to grey-water culture. The Neptune product needs further development before it can be adequately testing in rearing trials with any confidence. The grey-water culture may prove be more beneficial to other cultured species in Australia.

WHAT IS THE CALL TO ACTION?

This research may be directly applied to SBT technologies to improve survival at the larviculture stage.

COMMUNICATION CHANNELS

Channel	*Who by*	*When*
Larval rearing meetings with industry partners	***Lindsey Woolley***	***Meetings set up to determine research priorities at the beginning and during the next SBT season.***

WHAT IS YOUR FEEDBACK?

The opportunity to work with so many researchers discussing ideas and all contributing to the running of larval rearing trials was a very unique experience. The workshop gave me a far greater insight into tuna physiology and propagation. The volume of knowledge produced through the workshop is quite evident from the success achieved at the Achotines laboratory.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

Not applicable to this workshop.

ACKNOWLEDGEMENTS

I would like to thank the Australian Seafood CRC and Flinders University for funding my travel to the workshop. I would also like thank Dr. Daniel Benetti and Mr Vernon Scholey, the organizers of the workshop. I would like to acknowledge the other workshop participants Dr. Terry Bradley, Dr. Paul Polin, Dr. German Merino, Mario Palma and Roberto Vargas as well as the students from the University of Miami who made the workshop such an enjoyable experience.