# 8th Annual Workshop on Physiology and Aquaculture of Pelagics with Emphasis on Reproduction and Early Developmental Stages of Yellowfin Tuna, Thunnus albacares held at the Achotines Laboratory, Republic of Panama, Central America, 7-19 June 2010

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Fig 1. Achotines Laboratory

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**Objectives of Research Travel Grant.**

The purpose of attending the workshop was to learn culture methodologies applied to yellowfin tuna, which can then be transferred to species relevant to Australian aquaculture, as well as establishing professional relationships with academic and industry peers. I was fortunate enough to be one of three Australian representatives to attend the 8$^{th}$ Annual Workshop for Tuna Culture in Panama. As this is the first year of my PhD attendance at the workshop exposed me to the latest trends in tuna aquaculture which I can directly apply to my research program.

My PhD study is investigating the visual development and feeding success of Southern Bluefin Tuna (SBT). Very little is known about SBT larval development as successful spawning has only been achieved in recent years. This lack of knowledge means that larviculture methodologies that maximize survival, growth and health are still unclear. SBT experience high, early larval mortality, which limits the time available for experimental work. Many of the feeding experiments I conducted at first-feeding cannot be replicated past 4 dph due to the number of larvae required for trails. The ability to culture significant numbers of larvae to metamorphosis would allow a much larger number of experiments to be undertaken over a wide range of ages. Technical and theoretical information gained from attendance of the workshop will be shared with Clean Seas Tuna management and technical staff and staff and students at TAFI Marine Research Laboratories in Hobart.

**Non Technical Summary:**

Southern Bluefin Tuna is a major contributor to the Australian aquaculture industry, however at the moment it is reliant on the on-growing of wild-caught juveniles to market size. The recent captive-spawning of SBT by CST has opened up the potential for the aquaculture of this high market species. Early attempts at production of SBT have identified a number of culture bottlenecks, predominantly associated with larval culture. The Seafood CRC has developed an active research plan to aid in commercialisation of this species. Part of this initiative is to sponsor PhD programs to aid in the culture of SBT**.** Elucidation of the visual system and factors that promote first feeding success in SBT are critical to gain a thorough understanding of culture strategies that will work for this species. Due to the novelty and the limited spawning season of SBT one of the most effective tools to use to rapidly develop culture methods is to gain knowledge of successful strategies with comparative animals. This is the case with yellowfin tuna (YFT). A large amount of knowledge is available due to the Achotines Laboratory having a domesticated spawning population of YFT releasing viable gametes every day of the year. Attendance of this workshop provided invaluable exposure to leading researchers and professionals in the tuna field.

**Outcomes Achieved to date:**

A number of outcomes were achieved as a result of attendance at the workshop.

Discussions with academic and industry peers attending the workshop dealt with relevant theoretical and practical issues associated with tuna culture. Through these discussions and practical sessions a document was developed of recommended best-practice protocols to be used in the culture of successive generations of yellowfin tuna (see attached).

In addition to the development of culture strategies, the opportunity to work alongside peers in the tuna community provided a unique opportunity to establish

relationships that will promote future discussions and exchanges of technical information

**Outputs developed as a result of travel grant.**

The major output developed as a result of this travel grant was the production of the revised "**2010 edition of the tuna workshop UM – IATTC Suggested topics to further research and perfect technology to improve survival of tuna larvae during early developmental stages**" (see attached). This edition included two new experiments dealing principally with stocking density;

a) The effect of stocking density on growth and survival of early developmental stage (1-5 dph) and,
b) The effect of stocking density on growth and survival of flexion and post-flexion stages (9-10 to 15-16 dph)

In addition to these experiments workshop participants also investigated the use of oxygen supplementation with larviculture, the use of copepods as a live feed source and a preliminary investigation into reducing cannibalistic behaviour through the use of laminar currents and the addition of artificial feeds at 14dph (see attached).

**Background and Need:**

Increasing fishing pressure has resulted in serious stock depletions of many of the commercially important tuna species worldwide. The demand for high quality tuna meat however is not declining. The ability to provide sufficient quantities of tuna to the market while maintaining adequate wild populations of tuna has meant that tuna sourced from aquaculture must now fill this gap.

The ability to culture tuna and complete the egg-to-egg cycle however has encountered many technical difficulties. Many of the species i.e. Pacific Bluefin, Yellowfin and Southern Bluefin Tuna appear to have very similar larval biology and similar bottlenecks in culture are experienced between all species. For this reason discussion and information exchange with peers, is relevant across the species.

The Achotines Laboratory is the only facility with access to viable tuna eggs 365 days a year. The ability to meet with industry peers while also having access to viable eggs (allowing practical investigation), provides a unique opportunity to investigate the larviculture of tuna.

**Results**

A large component of the workshop was a "hands-on" approach to understanding the larviculture of YFT. This resulted in participants working daily on the larviculture tanks with strong encouragement to try new techniques to expand the knowledge of what biotic and abiotic factors affect the survival of these larvae.

Fig 2&3. Workshop participants collecting newly hatched Yellowfin Tuna larvae for stocking in larviculture tanks. Photograph of a 15 days post hatch Yellowfin Tuna larvae.

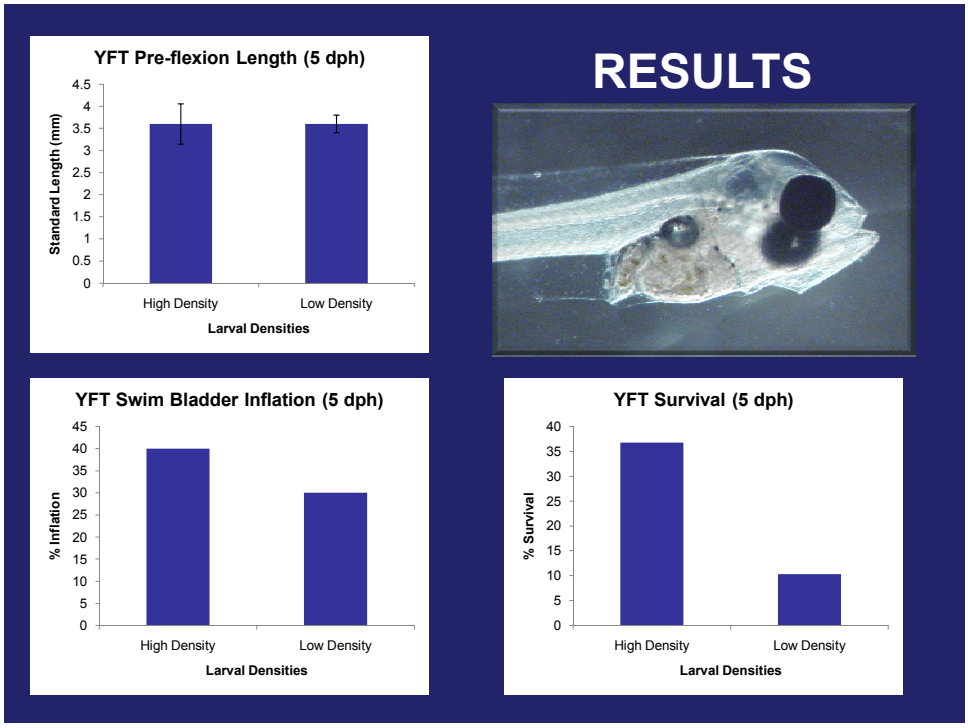
Workshop participant had the ability to work on two experiments. The first was the effect of stocking density on growth and survival of early developmental stage (1-5 dph) and the second was the effect of stocking density on growth and survival of flexion and post-flexion stages (9-10 to 15-16 dph).

It should be noted, that these were un-replicated experiments and should be interpreted with caution due to lack of replication. The trials where designed to provide the basis for the design of future experiments.

**Experiment 1. Effect of stocking density on growth and survival of early developmental stage (1-5 dph)**

- Yolk-sac larvae were stocked at 20/L and 100/L in two 700-L tanks.
- Standard protocols were used (see attached).
- Tanks were kept under 24-light constant light @ 3,000 Lux starting at first feeding; daily water exchange rate was set at 150%; aeration and pure oxygen were provided to maintain D.O. levels at or above saturation levels (100-120%)
- *Nannochloropsis granulata* was added from first feeding to maintain “green water” @ 0.5-1M cells/mL at all times
- Enriched (Ori-Go Green) rotifers were fed and maintained at 5/mL concentrations
- Yolk-sac and first feeding larvae (1-5 dph) were randomly sampled daily and measured to determine length, swim bladder inflation, % fullness and gut content
- Temperature, D.O., pH and salinity were recorded coinciding with feeding 5 times a day
- At the end of the trial, larvae distribution was homogenized by increasing aeration, 6 random - 1L samples and 3 vertical samples were obtained to enumerate the larval survival

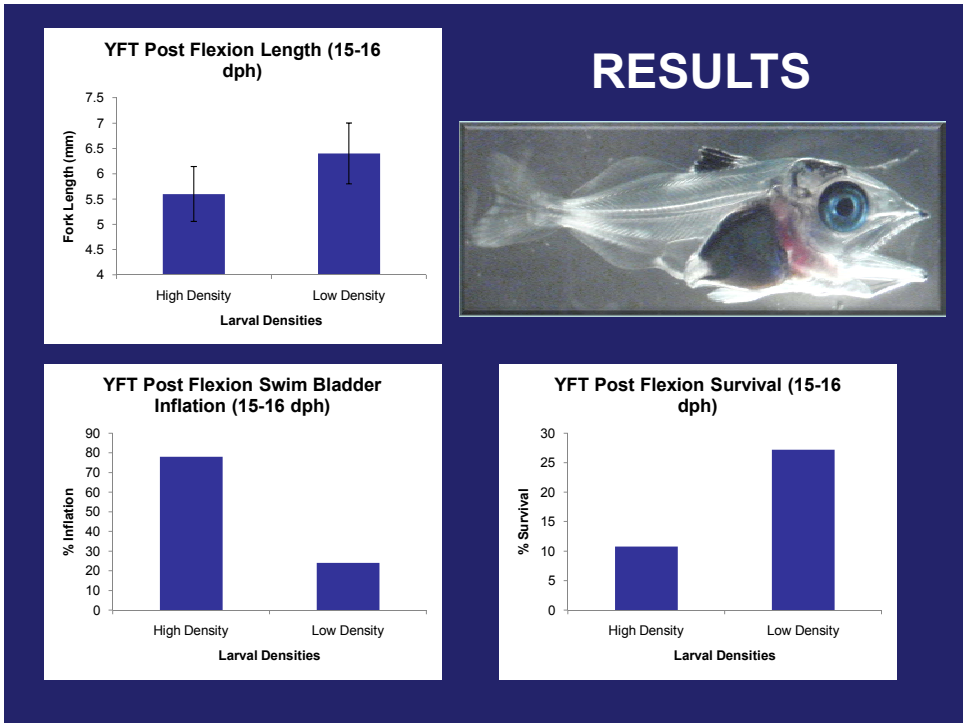
- Results suggest that there may be a positive effect of higher stocking density on survival of yellow fin tuna larvae through 5 dph
- Additionally swim bladder inflation was more prominent in larvae stocked at higher density.




**Experiment 2. Effect of stocking density on growth and survival of flexion and post-flexion stages**
**(9-10 to 15-16 dph)**

- 9-10 dph larvae at the onset of flexion were transferred and stocked at 1/L and 6/L in two 700-L tanks; 50% transfer mortality assumed (Minkoff, Margulies, Scholey, pers. com. 2010)
- Standard protocols were used (see attached).
- Tanks were kept under 24-light constant light @ 3,000 Lux; daily water exchange rate was set at 150-300%; aeration and pure oxygen were provided to maintain D.O. levels at or above saturation levels (100-120%)
- *Nannochloropsis granulata* was added to maintain “green water” @ 0.5-1M cells/mL at all times
- Enriched rotifers were replaced by wild copepods (cyclopoids, *Oithona sp)* and rotifers collected at the Achotunas settling ponds to maintain 5/mL concentrations; ratio rotifer:copepodes was approximately 60:40.
- Flexion and post-flexion larvae were randomly sampled daily and measured to determine length, swim bladder inflation, % fullness and gut content
- Temperature, D.O., pH and salinity were recorded coinciding with feeding 5 times a day
- At the end of the trial, all remaining larvae were counted and a random sample of 20-30 larvae were measured and parameters evaluated

- Results suggest that there may be a negative effect of higher stocking density on growth and survival of yellow fin tuna larvae through the post-flexion stage
- However swim bladder inflation was more prominent in larvae stocked at higher density.




Observations

Through these trails a number of observations were made that may make significant improvements to tuna survival.

- Tuna larvae experience high early mortality and it was for this reason that high initial stocking densities were investigated. The reasoning behind this initiative was a significant mortality will be experienced within the first few days of hatch. If the initial number is high enough then the percentage survival will give adequate larval numbers for the remaining culture period. Studies conducted on the early life history of YFT (dph 1-5) showed no difference in growth when larvae where cultured at high densities, but interestingly, %survival (i.e. % surviving larvae from initial stocking) improved with increasing stocking density. It should be noted that increasing the biological load on a larviculture system (for example increasing the stocking density) can cause a rapid increase in bacterial levels that in the majority of cases has a detrimental effect on survival. This could not be tested as the experiment was terminated at dph5 prior to any effect of the increased biological load being experienced.
- Oxygen supplementation maintained the level of dissolved oxygen at a saturation level between 100 - 120%. Initial trends indicated a positive larval response to higher than ambient dissolved oxygen levels which was less

than100% saturation. Of particular note was the improved transfer survival rate when supplemental oxygen was used.

- Wild-caught zooplankton (predominantly copepods) were used as a live feed source in addition to rotifers. The general consensus was a superior growth and survival response was achieved as compared to previous larviculture trials using rotifers and artemia.
- A very small number of post-flexion fish were exposed to a laminar flow and the introduction of artificial weaning diet in an attempt to combat the high cannibalism experienced at this stage. Due to low numbers the effect on cannibalism could not be accurately assessed however 87% of larvae exposed to the weaning diet had accepted the diet after ~60 hours.

Once again it should be noted that these are preliminary, un-replicated results but they do indicate areas that warrant further investigation.

In addition to practical components of the workshop, current world trends in larviculture methodologies where also discussed. A major issue in all aquaculture larviculture facilities is the ability to maintain a healthy tank environment throughout the larviculture cycle. Mortalities are often experienced due to increasing levels of pathogenic bacteria that build up during the larviculture procedure. Three approaches to the same problem where discussed in detail.

1. The first approach works on the principle of maintaining very low bacterial levels in the system while constantly reinforcing tank health with the addition of “healthy water”. This is achieved by the use of very low larval and live prey densities. The tank is fed with influent water that is passed directly from a biofilter, which has the effect of introducing “probiotics” (i.e. healthy bacteria) into the tank. Live, mixed -algal populations are maintained in the culture tank to maintain balance in microbial populations and copepods are used as a food source.
2. The second approach involves the use of an antimicrobial agent e.g. formalin every three days to eliminate all pathogenic microbial populations present within the tank .After each prophylactic treatment live food was added that had been enriched with probiotics.
3. The third approach dealt with the passive transfer of larvae from “dirty” to “clean” tanks every fifth day. This approach does not allow pathogenic bacterial populations to increase to detrimental levels within the tank.

**Extension Activities**

Extension activities undertaken while attending the workshop included capture and acclimation of wild, yellowfin tuna broodstock and a visit to an off-shore cobia farm.

Broodstock capture:

Workshop participants had the opportunity to actively participate in the capture of wild, yellowfin tuna broodstock. Quarantine protocols, prophylactic treatments and transfer techniques were demonstrated.


Fig 4. Luke Vanderburg, Luke Cheviot, Patrick Dunaway, Pollyanna Hilder and Zack Daugherty preparing to go broodstock fishing.

Open Blue cobia farm.

While attending the workshop in Panama participants had the opportunity to visit Open Blue, a commercial aquaculture venture on-growing cobia, *Rachycentron caladium* in the Caribbean. Brian O'Hanlon is the founder and president of this open-ocean, commercial aquaculture facility situated 12km off shore where pristine waters and strong ocean currents maintain optimal growth conditions. Due to its exposed position this site utilizes submerged sea cages (Ocean Spar®).


Fig 5. Submerged sea cage (Ocean Spar®) being raised to allow inspection.

Workshop participants also had the opportunity to visit the land-based hatchery where juveniles (sourced from the University of Miami) are on-grown until they reach sufficient size to be moved to the sea cages.

Fig 6. Juvenile Cobia in land based tanks.

The initial stage of acclimation to off-shore sea cage conditions is the transfer to a cage situated in the bay just in front of the hatchery. Fish are then transferred to the

one of four sites situated 12km off-shore. Workshop participants had the opportunity to snorkel on both bay and off-shore cages to observe sea cage configuration, fish behaviour, feeding techniques and cage maintenance. This was an excellent opportunity to observe off shore aquaculture first hand and a high point with all workshop participants.

Fig 7. Visiting the sea cages.

**Project Outcomes (that initiated change in industry):**

This workshop produced a number of outcomes (see results). It is hoped that a number of these suggestions can be trialled in the impending SBT larviculture season. If these protocols can make a significant improvement in the growth and survival it would be expected that these protocols would be incorporated into commercial rearing techniques.

**Summary of change in industry (what immediate changes might be expected for business/industry)**

No immediate change will be made as these suggestions first have to be trialled in a SBT rearing application.

**What future and ongoing changes are expected?**

It is hoped that techniques gained in YFT larviculture in Panama can be directly applied to the larviculture of SBT. Ideally these techniques will promote improved survival and growth which would have a direct impact on improving larviculture methodologies.

In a research application this would have the direct effect of allowing me to expand my experimental programme by allowing me to rear older larvae for experimentation.

In a commercial application it would be hoped that these methodologies may aid in the successful commercialisation of this species

**How will you communicate and share what you learnt with other CRC members?**

Communication with other CRC members will be managed in four ways.

1. Submission of this report will provide written documentation detailing all aspects of the workshop.
2. An oral presentation has been delivered to University of Tasmania staff and students where many of the attendees have CRC affiliations.
3. It is expected that relationships formed with other CRC-funded workshop participants will continue and discussions through subsequent culture seasons will continue to develop and refine SBT larviculture methodologies.
4. Knowledge and skills gained in Panama can be discussed and utilised with the staff of Clean Seas Tuna for the impending larviculture season.

**Further action required in regards to commercialisation? (Ip protection, licensing, sales, revenue etc)**

Not applicable to this workshop

**Lessons learned and recommended improvements**.

Lessons learned have been outlined in the results section. It is now appropriate to trial these methods directly on SBT to evaluate the degree of success.

**Acknowledgements**

I would like to acknowledge the Australian Seafood Co-operative Research Centre, The NSW Government, Industry and Investment, Dr Daniel Benetti, Dr Vernon Scholey and the staff of the Achotines Laboratory, the workshop participants Luke Cheviot, Luke Vanderburg, Zack Daugherty, Patrick Dunaway, Dr Gavin Partridge, John Steiglitz and Dr Bent Urup. I would also like to acknowledge Dr Jenny Cobcroft and Assoc Prof Stephen Battaglene for supporting my travel application.

**Describe your experiences with the workshop and how it contributed to the development of your personal skills as well as your PhD.**

This workshop provided me with the most incredible experiences and I am so thankful for this opportunity. The workshop has expanded my theoretical and practical knowledge base while providing memories that I cherish. The intensity of the workshop due to the amount of practical and theoretical content covered, the successes achieved and the dynamics of the workshop team made this a very unique experience. It was fantastic to be working larviculture tanks with so many different people all with so much to contribute. The exchange of information was wonderful. Besides the workshop, exposure to an offshore, submerged sea-cage cobia farm was brilliant. It is always good to see large-scale successful aquaculture particularly when new technologies are being applied. This workshop has aided my PhD in so many ways. Not only do I have a better understanding of tuna culture, I now have a set of culture techniques that I can apply to tuna (or any other larval fish species). This workshop has expanded my ability in both applied and theoretical techniques and I hope the degree of knowledge I have gained is reflected in my scientific output.

**Contacts made/networks**.

The workshop was conducted between June 7-19. This was an intensive two -week period that often saw participants working in excess of 15 hours per day, every day of the week. This provided a unique opportunity to forge strong friendships with each other. It was noted by all participants and workshop providers what a cohesive team we were.

Contacts have been made that can now be readily called upon. These include (Left to right): Luke Vanderburg, Dr Bent Urup, Luke Cheviot, Dr Vernon Scholey, John Steiglitz, Dr Gavin Partridge, Patrick Dunaway, Zack Daugherty, Pollyanna Hilder and Dr Daniel Benetti. In addition to people present at the workshop, contacts were also made with staff at Open Blue sea farms, primarily Brian O'Hanlon.

**Describe new technologies/methodologies/knowledge learnt.**

See results and extension activities.