The 2nd Global COE Program Symposium of Kinki University, 2009

"Sustainable Aquaculture of the Bluefin and Yellowfin Tuna-- *Closing the Life Cycle for Commercial Production*"

PROCEEDINGS

Kinki University Global COE Program

International education and research center for aquaculture science of bluefin tuna and other cultured fish

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**"Sustainable Aquaculture of the Bluefin and Yellowfin Tuna –
Closing the Life Cycle for Commercial Production"**

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PREFACE

It is my immense pleasure to welcome all participants to the 2nd Global COE Program Symposium of Kinki University "Sustainable Aquaculture of the Bluefin and Yellowfin Tuna—Closing the Life Cycle for Commercial Production".

The recent problems with avian influenza have encouraged people to increase their consumption of fish to reduce health risks. Moreover, the high meat quality and great taste of bluefin tuna make this species among the most important fish in the world. Consequently, the demand for this species has increased sharply during the last decades. To cope with the increasing demand, a cage culture industry, based on the collection of juveniles from the wild, has developed in different countries. However, this industry increases the risk of resource depletion and adds pressure to the fragile ecosystem on which tuna depend. Therefore, the world is now very eager to develop technologies for increasing bluefin tuna resources and produce fingerlings for culture purposes. Kinki University achieved a full culture cycle of the Pacific bluefin tuna for the first time in the world in 2002. In addition, research collaboration between Kinki University and Clean Seas Tuna Limited has seen the successful completion of fingerling production of Southern bluefin tuna for the first time in the world. It is believed that the tuna industry will benefit from these golden achievements with the Pacific and Southern bluefin tuna.

This Symposium will cover talks on different aspects of Pacific and Southern bluefin tuna as well as yellowfin tuna. We hope that this symposium will meet all your expectations and provide you with the opportunity to learn about the latest advances in bluefin and yellowfin tuna aquaculture.

Apart from the Global COE Program of Kinki University, we believe that this symposium would not have been possible without the enthusiastic support from Clean Seas Tuna Limited, South Australian Research and Development Institute (SARDI), Australian Seafood Cooperative Research Center, Industry and Investment NSW, and the Australian Fisheries Research and Development Cooperation. We thank all these sponsors as well as the Japanese and Australian organizing committees for their tireless and excellent contribution and support to make this symposium a great success. We also thank the editors of the Symposium Proceedings for their efforts in finalizing in time this high quality publication.

Finally, a special thanks to you, the participants, for making this meeting a success.

Hidemi Kumai
Professor and Trustee of Kinki University
The Leader of Kinki University Global COE Program
"International Education and Research Center for Aquaculture
Science of Bluefin Tuna and Other Cultivated Fish"

PREFACE

It is my great pleasure to join with Professor Hidemi Kumai from Kinki University and welcome all participants to this International Symposium on "Sustainable Aquaculture of the Bluefin and Yellowfin Tuna – Closing the Life Cycle for Commercial Production".

Tuna aquaculture based on hatchery produced fingerlings has been called an unobtainable dream but the great advances Clean Seas Tuna Ltd has made in successfully breeding Southern Bluefin Tuna, in addition to those made by Kinki University with the Pacific Bluefin Tuna, have punctured that assessment. Times Magazine ranked "tank-bred tuna" number two in their list of the 50 Best Inventions of the Year for 2009. These advances have come at a critical time. The international quota for Southern Bluefin Tuna has been cut by 20% and quotas for other tuna species have also been reduced. Recent achievements have showed what can be achieved through commercial and scientific collaboration and offer hope for aquaculture producers and consumers of tuna everywhere.

Clean Seas Tuna wishes to acknowledge their appreciation for the contributions made to our endeavours by the following organisations, Ausindustry, Seafood CRC, Fisheries Research and Development Corporation, University of Sunshine Coast, South Australian Research and Development Institute, Flinders University, Industry and Investment NSW, Northern Territory Department of Regional Development / Primary Industry / Fisheries and Resources, Tasmanian Aquaculture and Fisheries Institute, Kinki University, Hellenic Center for Marine Research, Heinrich-Heine Universität, University of Maryland, EU research consortiums SELFDOTT and REPRODOTT, Inter American Tropical Tuna Commission and the Tuna Research and Conservation Center.

This Symposium brings together experts on tuna breeding and aquaculture from throughout the world. I am very grateful to all those who share their knowledge and who are so willing to collaborate in this great endeavour of tuna aquaculture. I'd also like to thank the Steering Committee members who have organised the conference, Mike Thomson, Amal Biswas, Geoff Allan, Helena Heasman, Mark Booth, Steven Clarke, Wayne Hutchinson, Graham Mair, Maria Jedensjo and Emily Downes.

Mr Hagen Stehr AO
Chairman, Clean Seas Tuna
Chairman, Stehr Group of Companies

INTRODUCTION OF SELECTIVE BREEDING OF PACIFIC BLUEFIN TUNA *Thunnus orientalis* AND ITS MOLECULAR BIOLOGICAL APPROACHES

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1. Introduction

The Fisheries Laboratory of Kinki University has been active in research on the aquaculture of Pacific Bluefin Tuna (PBT) since 1970. This research effort has generated a lot of knowledge of the biology of PBT, which has contributed to the successful production of fingerlings on a practical scale this summer. In addition, we have succeeded in the production of PBT, and now some tens of artificially hatched fish are distributed to the market weekly.

When these fish are harvested, body measurements and, for mature fish, sex are recorded for each fish. We also collect blood and gonad samples from these fish frequently. Using these data and samples, we are establishing a DNA bank of our fish for their future selective breeding, also analyzing their genetic features by molecular biological approaches to find DNA markers that associate some phenotypes of the fish such as body size (rapid growth), meat quality, disease resistance, sex etc. These are the useful procedures for the future management of production and longer term objectives for breed improvement of PBT.

Microsatellite loci have proven to be extremely valuable in constructing high-density maps of eukaryotic chromosomes. Microsatellites are polymorphic tandem repeats of sequence only two to five nucleotide pairs long, which present in the highly repetitive DNA of eukaryotes. In most vertebrates, microsatellite sequences composed of polymorphic tandem repeats of the di-nucleotide sequences AC/TG (AC in one strand; TG in the complementary strand) have the potential to be useful markers to find gene loci associated with desirable traits. In addition to microsatellite marker, we performed randomly amplified polymorphic DNA (RAPD) assay to find DNA polymorphism of PBT. RAPD assays can detect the polymorphism of DNA, which produces amplified DNA fragment without the whole genome information (Williams et al. 1990). Here, we introduce the preliminary results of molecular identification of DNA markers of PBT.

2. Materials and Methods

Data base search and identification of microsatellite: We analyzed end sequence of 1,536 independent BAC clones of our PBT (Yagishita et al. 2006). Read sequences were trimmed manually then BLAST searched to DNA Data Bank of Japan, DDBJ. Di-nucleotide repeats were manually confirmed using sequence viewer, 4 peaks software and DNASIS (HITACHI software, Japan).

Tuna and genomic DNA: Whole blood was collected in the harvest of F2 tunas that had been bred at the Ohshima station. Genomic DNA was purified depending on the urea-proteinaseK treatment method (Ashida et al. 1996).

RAPD-PCR: Random 10 mer primers were purchased from LifeTechnologies Japan (Japan). PCRs were carried out in a total volume of 20 μl.

3. Results

Identification of new microsatellite sequence of PBT.

Microsatellite sequences of *Thunnus* have been reported. To date, 23 microsatellites were reported in *Thunnus orientalis* (Morshima et al. 2009), 36 in *Thunnus thynnus* (McDowell *et al*. 2002) and 12 in *Thunnus albacares* (Dammannagoda *et al*. 2007 unpublished data). Genus *Thunnus* has 24 chromosome pairs, therefore these numbers of microsatellite markers are not sufficient to make a high-density map of *Thunnus orientalis.* Therefore, we decided to find more DNA markers of PBT especially in our cultured fish. We analyzed 1,536 BAC end sequence of PBT, and found that 71 of them had long di-nucleotide repeated sequences (Table 1), they had more than 10 di-nucleotides repeats. We are determining our PBT individually whether 71 newly identified sequences show polymorphism. BAC end sequencing is technically difficult, because we should prepare large circular DNA (more than 100kb) before sequencing. Although we succeeded in preparing BAC DNA, if the end sequence consists of poly G, C or CG rich repeat, sequence reading is sometimes unsuccessful. During the analysis of end sequences, we found many genes, which have strong homology to other species (data not shown).

Table 1 - Summary of BAC end sequencing result

long di-nucleotide repeat	weakly or strongly homo-logus to known sequence	sequence read failure	total
71	1093	372	1536

Identification of randomly amplified polymorphic DNA (RAPD).

In addition to microtsatellite markers, RAPD markers are also valuable for establishing a genetic map of PBT. We performed RAPD analysis to find polymorphic marker of PBT. We tested 33 primers to find RAPD markers and found that many DNA fragments were polymorphic in individual PBT (Figure 1). RAPD assay produces many fragments at once. Therefore it is valuable to find polymorphic fragment of PBT. However, we did not find any RAPD resultant fragments, which correspond to sex phenotype in 33 primer tests.

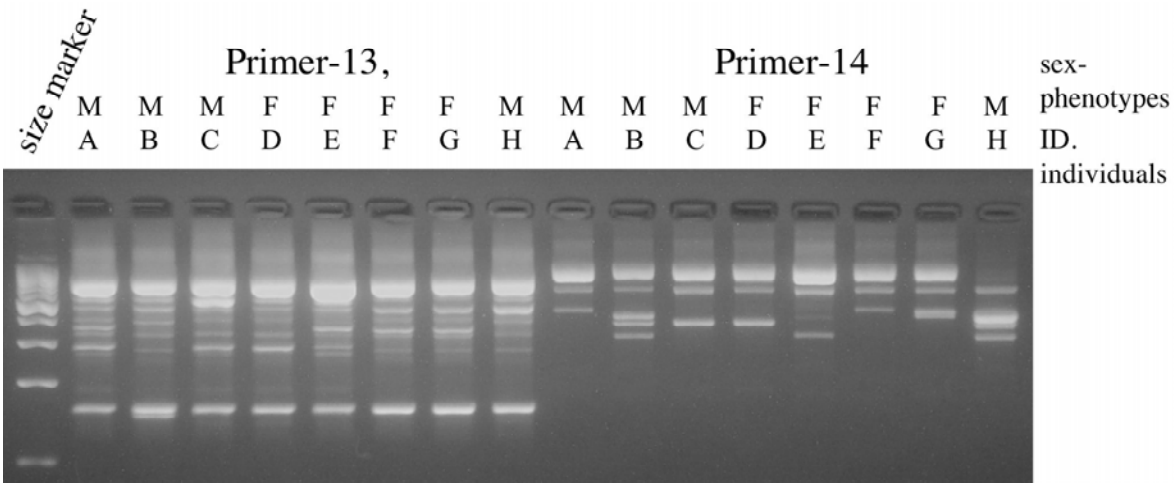

Fig. 1 - DNA fragment pattern of RAPD-PCR products. M indicates male sex phenotype, F is female. A to H represents individual ID.

4. Discussion

We identified 71 sequences containing long di-nucleotide repeat. We should confirm whether these fragments show polymorphism to use them as the microsatellite markers. We have now 93 microsatellite markers together with 23 markers previously reported (Morishima et al. 2009). Therefore, we can roughly estimate that 4 markers per one chromosome had been obtained, considering that *Thunnus orientalis* has 24 pairs of chromosomes. However, the number of microsatellite was not sufficient to find desired traits or to make a high-density genetic map of PBT. In the case of European sea bass, 174 microsatellite markers were mapped to 25 linkage groups. However, even with such number of microsatellites, sex-determining loci were not identified (Chistiakov et al. 2005). Therefore we need to find many more markers of PBT by using microsatellite isolation, RAPD, AFLP and other techniques.

In aquaculture, the use of DNA markers could be a useful tool for marker assisted selection in breeding, and improve the traceability of products after shipment. DNA markers that strongly associate with advantageous phenotypes may make it possible to select individuals with advantageous phenotypes by biopsy, and to help plan brood-stock management strategies using those fish. The offspring from the selected parents could have the possibility of fast growth, high quality of meat, high disease resistance etc. In the case of Atlantic salmon, QTL analysis and following marker assisted selection was successful to obtain ISA virus resistant strain (Moen et al. 2007). Therefore, it has the possibility to add value to traditional breed improvement in aquaculture. There is another advantage of marker-assisted selection in breeding of fishes of large size that are hard to handle. Tunas grow to the large size and grown up tunas are difficult to handle in the procedure of biopsy or body measurements when alive. This is a large obstacle in organizing the brood stock cohort when they are grown up to the market sized fish or adults exhibiting the phenotypic traits which can be the criteria in selection. However, a small piece of fins or a scale is sufficient to detect the DNA markers of fish, it is possible to obtain their genetic information when they are juveniles and are easy to handle with although this approach will only have potential benefit if the genetic markers are strongly associated with the development of commercially important traits in market sized fish.

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DEVELOPMENTS IN BLACKFIN TUNA *Thunnus atlanticus* AQUACULTURE

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Tuna (*Thunnus spp*.) have emerged as one of the most promising candidates for commercial aquaculture in recent decades. Members of this genus are in high demand as food fish and are under heavy fishing pressure throughout the world. However, hatchery technology for all tuna species has been slow to develop, due to various biological and technical limitations, as continued problems with captive spawning, system management, microbial control, diseases / parasites, feeds, feeding, and cannibalism continue to limit expansion of the industry. Blackfin tuna (*Thunnus atlanticus*) represent an ideal alternative model species for tuna aquaculture research because they are smaller and can be kept in captivity without the need for extensive and costly infrastructure. Additionally, blackfin tuna are congeners of other better known tuna species and share a number of physiological and behavioral traits with their larger cousins, making them a useful model species for study. Obtaining large numbers of eggs throughout extended periods of time is a requisite for the successful development of hatchery techniques of any species. Because blackfin tuna mature and begin spawning at a relatively small size (> 3.5 kg), it is anticipated that it will be easier to obtain more numerous volitional spawns from this species in captivity than it would be from their larger counterparts. The University of Miami Experimental Hatchery (UMEH) is developing a captive broodstock population of blackfin tuna, with the aim of developing viable hatchery technology for the reliable spawning, larval rearing and fingerling production of this species.

We describe the first attempts of developing blackfin tuna hatchery methods, including capture, transport, acclimation, prophylaxis, handling, transferring, initiation of feeding and maintenance of blackfin tuna in an environmentally controlled broodstock conditioning system. We are targeting individuals at the juvenile and sub-adult stages for ease of capture, handling, transporting and acclimation. Nonetheless, some individuals in the range of 3.26 kg to 14.44 kg, large enough to be mature, were also captured. Thirty-four blackfin tuna ranging in size from 0.19 – 14.44 kg have been captured off the coast of southeast Florida throughout the year, with larger fish generally being captured in the spring and early summer using live bait fishing techniques (including kites to suspend and keep the bait near or at the surface), and smaller fish caught in the late summer and fall on trolling feathers rigged with barbless hooks. To find clues on their dietary preferences to assist in the onset of their feeding in captivity, we examined the stomach contents of blackfin tuna caught in the Atlantic Ocean waters off the coast of southeast Florida, U.S.A. and found the presence of squid and fish. Indeed, squid was successfully used to initiate

feeding of all individuals. Preliminary data indicates a gonadal somatic index (GSI) ranging from 1.5% mean GSI for males to 2.5% mean GSI for females captured in the spring and early summer seasons (Figure 1), with oocyte sizes at the second and tertiary developmental stages ranging from 347 – 694µm in the female tuna.

Fig. 1 - Blackfin tuna (*Thunnus atlanticus*) captured in the Atlantic Ocean off the coast of southeast Florida, U.S.A. exhibited gonadal somatic indexes (GSI) ranging from 1.5% to 2.5% and oocytes at the secondary and tertiary stages measuring 347 – 694 µm. This female's stomach content was exclusively squid, a hint that proved useful for the onset of their feeding in captivity.

Different methods of fish transport at sea have been used, including custom-built tuna tube systems and more traditional cylindrical transport tanks equipped with supplemental oxygen and directional flow. The tuna have been quarantined in 12,000 and 15,000-L flow through tanks for 1-4 weeks and then transferred to an 80,000-L recirculating broodstock maturation tank.

The tuna broodstock maturation system at UMEH consists of an 80,000-L cylindrical insulated fiberglass tank (7.6 m diameter x 1.8 m deep) with attached 500-L cylindrical egg collector. Flow rates provided by the recirculating system allow four water exchanges per day, and new seawater use in the system averages 10–20% of the tank volume per day. Water temperature is controlled using a 10 HP heating/chilling unit. Other key components of the system include a 2 HP pump, large-solids broken glass filter, UV filter, a 500-L capacity trickling biofilter and a 100-L foam fractionator (protein skimmer). Water is effectively degassed, oxygenated and denitrified following passage through the trickling biofilter and foam fractionator. The biofilter is filled with plastic bioballs of three different sizes and shapes, increasing the surface area for bacteria substrate. In addition, 3 airlifts evenly distributed around the main tank provide aeration while generating a circular current. Supplemental oxygen is provided to maintain dissolved oxygen levels at or above saturation (7-9 mg/L at 26-28 ^{o}C). Blackfin tuna have adapted well to this broodstock conditioning system and full control of environmental parameters is anticipated to allow for effective conditioned volitional spawning of this species in the fall of 2010.

Approximately 20% of the total number of blackfin tuna have adapted well and began eating in captivity. Mortalities have been observed as a result of transport injuries,

secondary bacterial infection and starvation. Left untreated, injuries have been invariably leading to bacterial infections and mortalities. For this reason, tuna exhibiting even the smallest sign of injuries have been treated with baths of oxytetracycline at 60 ppm for 3 hours for 5-8 consecutive days and severe cases have been treated with injections of oxytetracycline at 50 mg/kg of body weight. In most cases, these treatments are proving effective both as prophylaxis and as a cure for diseases. A few of the fish captured developed "puffy snout" syndrome and did not eat in captivity resulting in eventual mortality. One mature tuna weighing 7 kg lived 6 weeks without eating while exhibiting continuous swimming throughout the tank, illustrating some of their unique physiological attributes. From our observations, considering the average swimming velocity of this tuna and the time elapsed, this individual could have conceivably swum approximately 2,000 km without "fuel" (i.e., any food consumption) before it finally perished.

The knowledge gained as a result of this research may be transferrable to the aquaculture of other *Thunnus* species, most notably the bluefin (*Thunnus thynnus*), bigeye (*Thunnus obesus*) and yellowfin (*Thunnus albacares*), which make up the majority of the high grade tuna sold on the global market. In addition to their potential value as a model species, blackfin tuna are a desirable food fish with a flesh quality similar to that of the better known species of tuna. The market potential for these fish combined with the previously mentioned life history traits make blackfin tuna an ideal candidate species for dedicated research to benefit the U.S. aquaculture industry. Currently, all blackfin tuna at UMEH are eating frozen squid, silversides and sardines and in good health in the maturation system, with water temperatures maintained at an average of 25^{o}C. We continue to capture and quarantine blackfin tuna of all sizes to stock in the maturation tank aiming at obtaining a population of 12 fish with a sex ratio of 1:1 at a maximum stocking density of 0.75 kg/m^3 – which we believe to be adequate. We anticipate that fully acclimated young adults will begin spawning in the fall of 2010. Larval rearing trials using both "pulse-feeding" and "microcosm" methods will be conducted in our attempts to raise newly-hatched blackfin tuna larvae and to eventually mass produce post-metamorphic and fingerling stages. We will be drawing from our experience and from technology successfully developed for a number of other marine pelagics such as mahi-mahi or dolphin fish (*Coryphaena hippurus*), yellowtail jacks (*Seriola spp)*, cobia (*Rachycentron canadum*), and goggle eye (*Selar crumenopthalmus*). Both larval rearing methods are basically intensive but differ radically in their principles and practices. These will be presented and discussed with respect to the advantages and disadvantages of their utilization in larval rearing of blackfin tuna based on previous successes and failures achieved with other species of pelagic teleosts, including the yellowfin tuna (*Thunnus albacares*).

TOWARDS THE ESTABLISHMENT OF FORMULATED DIETS FOR PACIFIC BLUEFIN TUNA (*Thunnus orientalis*)

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1. Introduction

Pacific bluefin tuna (PBT) is regarded as one of the most valuable fish in the world because of its high meat quality and great taste. It is extremely popular in Japan, especially in the 'Sashimi' and 'Sushi' cuisine. Research on captive PBT commenced at Kinki University in 1970 and the first spontaneous spawning was recorded in 1979. However, it took almost another 32 years to successfully close the life cycle of hatchery reared PBT (Sawada et al., 2005). During this long journey, live prey such as rotifers (*Brachionus plicatilis*) and *Artemia* nauplii, yolk sac larvae and minced fish meat such as sand lance have been used as feeds during the larval and juvenile stages. However, these feeding practices are difficult, expensive, laborious, time consuming and inefficient. In addition, these strategies were not providing juveniles with a nutritionally balanced diet suitable for the mass culture of this species (Seoka et al., 2008). To address this problem, a series of experiments have been carried out to establish the basic nutritional requirements for PBT juvenile. This paper provides a brief review on the search for new protein sources and determination of the optimum dietary levels of protein, lipid, carbohydrate and vitamin C in a nutritionally balanced diet for PBT juveniles.

2. Search for protein sources

Previous studies with PBT have demonstrated that while there are no major problems with the amino acid balance of normal Chilean fish meal (FM), the digestibility of nutrients and energy from this protein source is low. To remove concerns over low digestibility of FM, different kinds of enzyme treated fish meal were investigated in order to determine their suitability for use in the diet of PBT juveniles.

Two growth trials were conducted. In trial 1, PBT were fed diets containing 63% FM, 63% enzyme treated FM (EFM) or 63% enzyme treated Peruvian fish meal (EPM) and compared to PBT fed on 100% sand lance (SL; control diet). Forty fish with mean body weight ca. 1.48 g were stocked into one of two replicate 1.4 m^3 tanks for each dietary treatment. Fish were fed 6 times daily for 7 days. In trial 2, the growth performance of PBT fed EFM was compared to the growth performance of fish fed SL using large sized tanks (15 m^3) for two weeks. Water temperature for both trials was maintained at 26.5ºC. The formulation of diets used in both trials is presented in Table 1. Oneway ANOVA was used to assess treatment effects and means were compared using Tukey's multiple comparison procedure at $P<0.05$.

In trial 1 there was no significant difference between EFM and SL, but both these treatments recorded significantly higher mean final body weight than the FM and EPM treatments.

Significantly lower feed intake per fish and higher feed conversion efficiency (FCE) were observed in PBT fed the EFM diet compared to PBT fed on other diets (Table 2). The significantly higher growth performance of fish fed the EFM than that of fish fed the FM suggested that the enzyme pretreatment of FM made it more digestible and easier for PBT juveniles to absorb. This promising result was further investigated using larger tanks and a longer rearing duration in trial 2.

Table 1 Feed formula and proximate composition

Ingredients	FM	EFM	EPM	SL
FM	63.0			
EFM		63.0		
EPM			63.0	
SL				100.0
Others*	37.0	37.0	37.0	
Proximate composition (%)				
CP	51.5	54.5	50.9	67.2
CL	19.8	24.7	18.3	21.0
CS	8.3	5.6	7.7	0.0

FM, Chinlean fish meal; EFM, enzyme treated Chilean fish meal; EPM, enzyme treated Peruvian fish meal; SL, sand lance; CP, crude protein, CL, crude lipid; CS, crude sugar

*wheat gluten, 10.0%; bonito oil, 10.0%; α-starch, 3.0%; vitamin mix, 5.0%; mineral mix, 4.5%; soybean lecithin, 2.0%; taurine, 2.0% and feeding stimulants, 0.5%

Source: Ji et al. 2008

Table 2 Growth performance of PBT juvenile

Treatments	FW	SGR (%)[1]	FI (G)[2]	FCE (%)[3]
Trial 1				
FM	3.9[b]	13.9[b]	2.6[b]	74.9[b]
EFM	5.0[a]	17.2[a]	2.1[a]	126.0[a]
EPM	3.2[b]	10.9[c]	2.4[b]	60.7[b]
SL	5.4[a]	18.6[a]	3.9[c]	84.6[b]
Trial 2				
EFM	19.9	18.6	11.8	95.7
SL	24.2	20.2	12.7	89.9

Values in a column with different letters are significantly different (P <0.05).

FW, final mean body weight.

[1]SGR, specific growth rate (%) = 100 × (ln final weight - ln initial weight) / days.

[2]FI, feed intake per fish (g) = [feed intake / {(initial number of fish + final number of fish)/2}]

[3]FCE, feed conversion efficiency (%) = 100 × weight gain / feed intake

In trial 2, final mean body weight of fish reared on EFM was around 80% of that of fish reared on the SL treatment, however, there was no significant difference between final weight, SGR, feed intake or FCE in this trial (Table 2). Feed intake per fish was slightly higher in fish fed the SL but FCE was higher in fish fed the EFM (Table 2). The results from trial 2 further demonstrated the suitability of EFM as a protein source for PBT juveniles when they are reared in 15 m^3 tanks for two weeks.

3. Optimum levels of protein and lipid in diet for PBT juvenile

Protein and lipid requirements are species-specific. Therefore, it is very important to determine the optimum level of protein and lipid for PBT juvenile in order to establish a cost effective diet. Our previous trials revealed that EFM could be used as protein source in PBT juvenile diet (Ji et al., 2008). Other trials revealed that salmon row oil (SRO) can be used as lipid source as it is enriched with phospholipids (Seoka et al., 2008). Therefore, EFM and SRO were used in this experiment as the major protein and lipid sources respectively.

Table 3 Feed formula and proximate composition

Ingredien	$P_{73}L_9$	$P_{67}L_{15}$	$P_{62}L_{18}$	$P_{57}L_{22}$	$P_{53}L_{27}$	SL
EFM	80.0	74.0	68.0	62.0	56.0	
SRO	0.0	4.0	8.0	12.0	16.0	
Cellulose	0.0	2.0	4.0	6.0	8.0	
SL	0.0	0.0	0.0	0.0	0.0	100.0
Others*	20.0	20.0	20.0	20.0	20.0	
Proximate composition (%)						
CP	72.8	66.8	61.9	57.2	53.3	73.4
CL	9.2	14.8	17.9	21.9	27.0	6.3
CS	5.9	5.7	5.8	5.7	5.8	0.0

For abbreviation, refers to Table 1.

*wheat gluten, 2.4%; α-starch, 3.0%; APM, 0.1%; vitamin mix, 5.0%; mineral mix, 5.0%; soybean lecithin, 2.0%; taurine, 2.0% and feeding stimulants, 0.5%

Source: Biswas et al. 2009

Table 4 Growth performance of PBT juvenile

Diets	FW	SGR (%)	FCE (%)	PRE(%)[1]	ERE (%)[1]
$P_{73}L_9$	2.2^{d}	24.1^{b}	97.8^{b}	21.4^{c}	20.1^{c}
$P_{67}L_{15}$	2.6^{ab}	25.9^{ab}	116.2^{a}	26.3^{b}	22.8^{bc}
$P_{62}L_{18}$	2.9^{a}	26.8^{a}	121.2^{a}	30.4^{a}	26.5^{a}
$P_{57}L_{22}$	2.7^{ab}	26.1^{ab}	119.6^{a}	32.5^{a}	24.7^{a}
$P_{53}L_{27}$	2.5^{ab}	25.1^{ab}	114.9^{a}	32.1^{a}	24.2^{a}
SL	2.4^{ab}	24.9^{ab}	72.0^{c}	14.0^{d}	13.2^{d}

Values in a column with different letters are significantly different (P <0.05).

FW, final mean body weight.

[1]protein (PRE) and energy (ERE) retention efficiency (%) = 100 × protein or energy gain / protein or energy intake

In order to determine the optimum level of protein and lipid in the diet of PBT juveniles, five experimental diets were formulated with different levels of protein/lipid; 73/9, 67/15, 62/18, 57/22 or 53/27, which are hereafter denoted $P_{73}L_9$, $P_{67}L_{15}$, $P_{62}L_{18}$, $P_{57}L_{22}$ and $P_{53}L_{27}$, respectively (Table 3). Sand lance was used as reference diet. Two hundred PBT juveniles of mean body weight 0.26 g were stocked into one of two replicate 15 m^3 tanks and reared for 10 days. Fish were fed six times daily until apparent satiation, and water temperature and DO were 26.6ºC and 6.6 mg/l, respectively.

Survival ranged from 61-70% without significant difference between the treatments. With the exception of fish fed the $P_{73}L_9$ treatment, there was no significant difference in growth performance among dietary treatments (Table 4) Fish fed diet $P_{62}L_{18}$ recorded higher mean body weight, SGR and FCE than fish fed on the other test diets (Table 4).

Table 5 Feed formula and proximate composition

Ingredients	CH7	CH13	CH19	CH25
EFM	68.0	63.8	59.6	55.3
SRO	8.0	7.5	7.0	6.5
α-Starch	3.0	8.0	13.0	18.0
S. Ln.	2.0	1.9	1.8	1.6
Cellulose	4.0	3.9	3.9	4.0
WG	2.4	2.3	2.1	2.0
Others*	12.6	12.6	12.6	12.6

S.Ln., soybean lecithin; WG, wheat gluten

For other abbreviations, refers to Table 1.

*vitamin mix, 5.0%; mineral mix, 5.0; APM, 0.1%; taurine, 2.0% and feeding stimulants, 0.5%.

Source: Biswas et al. 2009b

Table 6 Growth performance of PBT juvenile

Diets	CH7	CH13	CH19	CH25
Diet proximate composition				
CP	63.5	60.1	55.9	50.6
CL	17.0	15.8	14.7	13.7
CS	7.0	12.8	18.5	24.8
Growth parameters				
FW	7.8^{a}	7.8^{a}	7.2^{b}	6.7^{c}
SGR (%)	19.3^{a}	19.3^{a}	18.5^{b}	17.5^{c}
FCE (%)	127.4	135.2	122.7	119.5
PRE (%)	36.5^{b}	42.7^{a}	38.7^{ab}	41.6^{ab}
LRE (%)	22.6^{b}	26.9^{a}	23.0^{b}	18.9^{c}

Values in a row with different letters are significantly different (P <0.05).

Protein and energy retention efficiency were significantly higher in PBT fed $P_{62}L_{18}$ compared to PBT fed on SL (i.e. control diet). These results suggest that the optimum level of protein and lipid for PBT juvenile will be ca.. 62% and 18%, respectively. This protein requirement for PBT is higher compared to other fish species.

4. Optimum level of carbohydrate in diet for PBT juvenile

Carbohydrate is well known as one of the important nutrients and least expensive ingredients for human and domestic animals, but relatively low utility has been demonstrated for many fish.

However, there is considerable evidence that different species utilize and metabolize carbohydrate differently (Wilson 1994). For this reason we conducted an experiment to determine the optimum level of carbohydrate in diets for PBT

A modified formulation of the best performing diet from our previous experiment (i.e. protein 62%; lipid 18%) was used to make 4 test diets containing 3, 8, 13 or 18% α-starch, respectively. These diets were named CH_7, CH_{13}, CH_{19} and CH_{25} according to the amount of dietary carbohydrate content each of them contained (Table 5). One hundred and fifty PBT juveniles (36 days old, mean body weight 1.6 g) were distributed into one of 3 replicate 15 m^3 tanks per dietary treatment. Fish were fed 6 times daily for 8 days, and water temperature and DO were 26.8ºC and 7.7 mg/l, respectively.

Final body weight and SGR was similar for PBT fed the CH_7 and CH_{13} diets and significantly higher than PBT fed the CH_{19} and CH_{25} diets (Table 6). However, protein and lipid retention efficiency were higher in fish fed the CH_{13} diet. Therefore, the optimum dietary carbohydrate level for juvenile PBT appears to be around 13%. We assumed that the carbohydrate requirements for PBT juvenile would be low as it is considered to be an obligate carnivorous species. However, the carbohydrate requirement estimated from this trial was found to be reasonably high and similar to that of other carnivorous species (Takii et al. 1997). Combined with earlier information presented in this paper the dietary feed formulation for juvenile PBT will be EFM 63.8%, SRO 7.5% and α-starch 8% which will provide protein 60%, lipid 16% and carbohydrate 13%, respectively.

5. Optimum level of vitamin C in diet for PBT juvenile

Vitamin C (AsA) has been proved to have numerous important biological functions but most fish species cannot synthesize it due to the absence of of the L-gulonolactone oxidase enzyme which is necessary to convert L-gulonic acid to AsA (Dabrowski 1990). For this reason it is important that sufficient amounts of vitamin C are included in the diet. Recommendations on the optimal levels of water soluble vitamins have been made for salmonids (Halver 1957), however these are likely to be different to that of PBT. Therefore an experiment was conducted to identify the optimum dietary level of AsA for juvenile PBT

In this study we used L-Ascorbyl-2-monophosphate Mg^{+2} salt (APM) as a stable derivative of AsA. Different amounts of APM were then added to a control diet composed of EFM 63.8%, SRO 7.5% and α-starch 8%. APM was included at 0, 400, 800, 1200 and 1600 mg/kg diet and diets were coded C0, C286,

Table 7 Feed formula and proximate composition

Ingredients	C0	C286	C466	C668	C856	SL
EFM	63.8	63.8	63.8	63.8	63.8	
Cellulose	4.0	3.9	3.9	3.9	3.8	
APM (ppm)	0	400	800	1200	1600	
Others*	32.2	32.2	32.2	32.2	32.2	
Proximate composition (%)						
CP	59.3	59.3	59.0	59.1	59.2	76.3
CL	16.7	16.7	16.3	16.6	16.8	10.2
AsA (ppm)	0	286	466	668	856	86

For abbreviations, refers to Table 1.

*vitamin mix, 5.0%; mineral mix, 5.0; SRO, 7.54%; soy-bean lecithin, 1.89%; taurine, 2.0%; feeding stimulants, 0.5% and wheat gluten, 2.26%.

Table 8 Growth performance of PBT juvenile

Diets	C0	C286	C466	C668	C856	SL
FW	1.9^{b}	6.8^{a}	6.5^{a}	6.9^{a}	6.6^{a}	7.1^{a}
SGR (%)	16.1^{b}	23.0^{a}	22.7^{a}	23.1^{a}	22.8^{a}	23.3^{a}
FCE (%)	117^{b}	142^{a}	143^{a}	147^{a}	143^{a}	118^{b}
PRE (%)	19.6^{c}	30.5^{b}	35.1^{a}	33.6^{a}	31.1^{b}	13.2^{d}
LRE (%)	18.3^{b}	21.3^{ab}	24.9^{a}	23.2^{a}	22.7^{a}	12.1^{c}
AsA content (ppm)						
Brain	194^{e}	295^{d}	753^{b}	922^{a}	888^{a}	414^{c}
Liver	nd	59^{d}	117^{b}	234^{a}	255^{a}	95^{c}

Values in a row with different letters are significantly different (P <0.05).

C466, C668 and C856, respectively according to their active AsA content (Table 7). Sand lance was also used in this study as a reference diet. Three hundred (25 day old) juveniles with mean body weight ca. 0.27 g were distributed into each of twelve 15 m^3 tank in duplicate. Fish were fed 6 times daily for 14 days, and water temperature and DO were 26.5ºC and 7.5 mg/l, respectively.

Juveniles PBT fed the C0 diet devoid of AsA recorded the lowest growth rate and exhibited deficiency syndromes from the 5th day of the trial. Only 20% of fish reared on this diet survived at day 12. However, final weight and SGR were statistically similar in PBT fed all other diets containing AsA and for fish fed on sand lance (Table 8). Among the supplemented diets, protein retention efficiency in PBT was significantly higher in fish fed diet C446 and diet C668, but there was no difference in lipid retention. AsA content in the brain was numerically higher in PBT reared on diet C668, but was not significantly different from the level recorded in fish fed diet C856. AsA content of the liver was also highest in PBT fed these two treatments (Table 8). Based on peaks in protein retention and the amount of AsA deposited in brain and liver tissue we suggest that diets for juvenile PBT should contain approximately 668 mg AsA per kg. This level is higher than recommendations made by Halver (1957) and suggests that PBT juveniles have a higher AsA requirement than most other fish species.

In conclusion, the aforementioned experiments have led to a greater understanding of the nutrient requirements of juvenile PBT. Based on our results we recommend that diets for juvenile PBT be formulated to include 59.1% crude protein 16.6% crude lipid, 11.9% carbohydrate and 668.3 mg AsA/kg, respectively. This diet formulation will continue to contribute to further developments in the mass culture of juvenile PBT.

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BROODSTOCK MONITORING, HANDLING AND INDUCTION TECHNIQUES – RECENT ADVANCES IN ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) REPRODUCTION

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1. Introduction

Monitoring broodstock and their environment are crucial to obtaining reliable quantities of viable gametes. An important limitation in the broodstock management of BFT is the extreme difficulty in anesthetizing or immobilizing the fish for the purpose of measuring or weighing, administering identification tags or hormonal therapies, or obtaining muscle or ovarian biopsies. These are necessary for sex and genetic identification and reproductive stage evaluation.

It is therefore necessary to resort to underwater sampling-administration methods, which require the use of a diver and remote administration devices (jab stick or spear gun). Some success has been accomplished over the time of the REPRODOTT project (Mylonas et al., 2007), but further improvements in the technology are necessary in order to (a) determine the exact window of opportunity for artificial induction, b) observe the behavior of individual fish under different environmental conditions, c) anaesthetize fish for routine monitoring, and d) increase hormonal administration success to 100%, in order to take full advantage of the reproductive potential of the reared broodstock. The present paper will look at developments over the last two years during the SELFDOTT project of these four applications in our laboratories and during field experiments with SELFDOTT and ALLOTUNA partners.

2. Cage Monitoring and "Optimum Window of Opportunity"

The optimum temperature for spawning in bluefin tuna has been reported to be 24°C (Schaefer, 2001; Rooker et al., 2007). Within the REPRODOTT project and also at the beginning of SELFDOTT, a special software tool developed in IN-EXFISH has been used to access satellite sea surface temperature data to calculate a probable window of opportunity for artificial hormone induction of spawning. Using the data for Spain and Malta a window of opportunity was calculated for the beginning of July.

In 2008 a trial run was made using HOBO data-loggers for temperature measurements and a fuller trial in 2009 attached them to the cage superstructure (Figs. 1. A and B). These were placed at three different depths at each of the four points of the compass (Fig. 1 C) and measured temperature and light at 20 minute intervals. The results for the Spanish cages in Cartagena for 2009 are shown in figure 2. The fish were induced

on the 26th and 27th of June and the maximum spawning was observed on the 3rd of July with 35 million eggs. A period of three days with surface water at 24°C was deemed to be a trigger in this case, although there is new evidence, from towing cages, that egg release may take place at lower temperatures (Gordoa, 2009).

3. Fish Observations

Using purpose designed buoyancy aids it has been possible to implant long-term data-loggers for temperature and depth on individual tuna broodstock . These can be programmed to pop off using suture material which disintegrates with time, thereby releasing the logger to the surface for recovery. The data-loggers are provided by Star-Odi and the new versions (DST-F) can record for a period of two and half years. Simple programming is possible thereby enabling power saving and intensive recordings during spawning season. These have been successfully deployed in REPRODOTT and also SELFDOTT and the ALLOTUNA program.

4. Anaesthetics and their use in tuna

A number of trials have now been carried out using various combinations of commercially available anaesthetics for intramuscular injection. During SELDOTT, over 30 fish were given various doses using a DAN-Inject device. Most of these injections did not harm the fish or cause any measurable anaesthesia. The main problem involved here is that too high an injection pressure causes a backpressure which voids the anaesthetic via the injection opening. Slow injecting, self sealing darts are the method of choice although special multiport needles have now been produced and we have also had some success with inter-peritoneal injections with specialized needles.

5. Induction techniques

Figure 3 shows the relevant details for implanting induction tag in BFT. These tags can be administered by a spear gun, or a custom-made jab-stick. There are numerous adapters for induction or also for tagging and obtaining muscle biopsies. In the REPRODOTT project polypropylene sub-assemblies were used for protecting and holding the GnRHa implants. In the SELFDOTT program, stainless steel and now new titanium implant mounts have been produced which increase protection and reliability. If desired, direct injection of the implant can also be carried out underwater.

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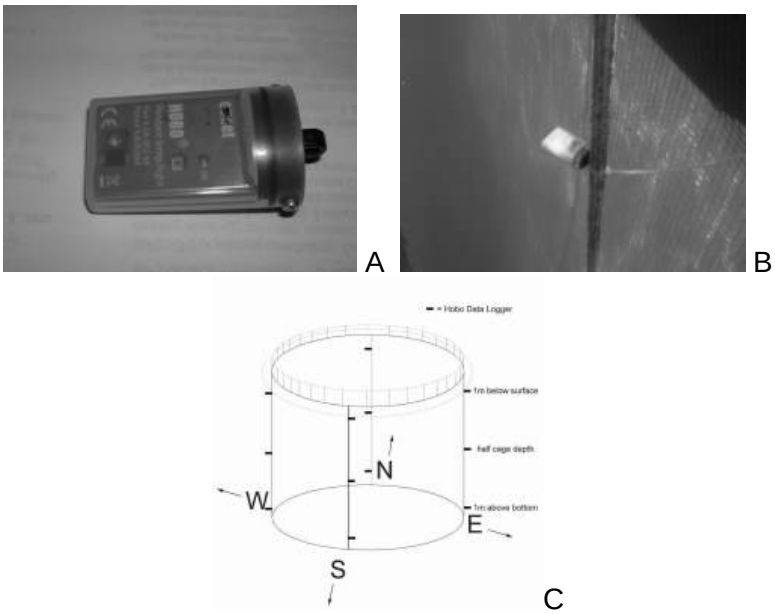

Fig. 1 - The rechargeable HOBO pendant data logger (A), which were deployed in Spanish and Maltese broodstock cages (B) at 3 depths and the four points of the compass (C)

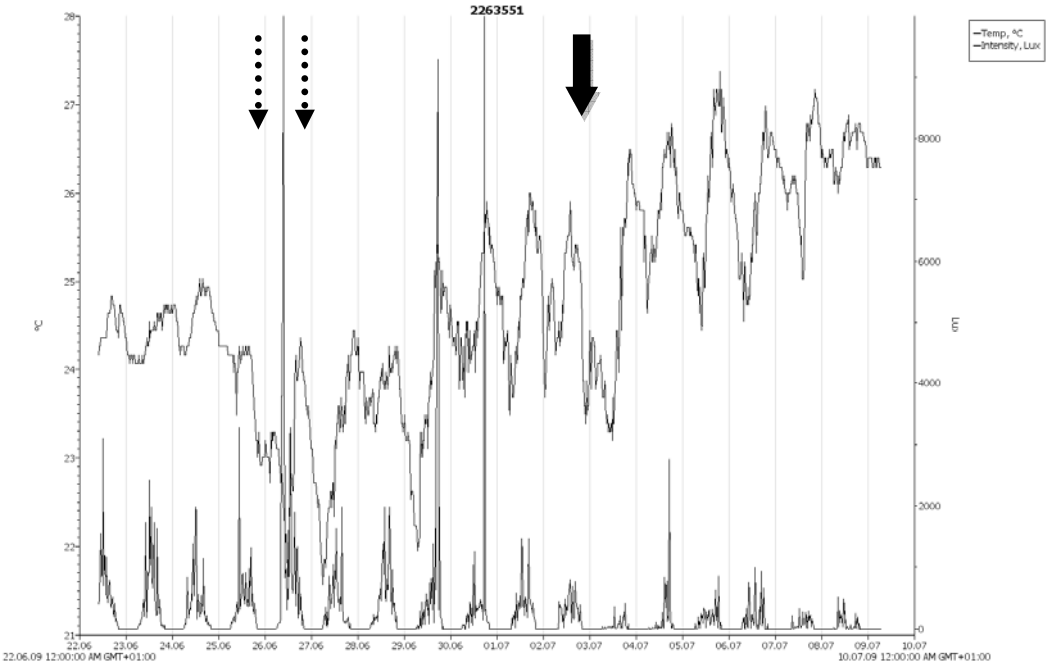

Fig. 2 - Sub-surface (1m) water temperature and light in Spanish broodstock cages in 2009. Dotted lines indicate induction and solid line maximum spawning with 35 million eggs.

Fig. 3 - Spawning induction in Spain. (A) Implanting device with arrowhead-implant-tag assembly. (B) Divers load the spear-gun with the GnRHa implant assembly. (C) A successfully implanted BFT.

RECENT DEVELOPMENTS IN LARVAL AND JUVENILE REARING OF ATLANTIC BLUEFIN TUNA *Thunnus thynnus*

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1. Introduction

Wild stocks of Atlantic bluefin tuna (ABFT) are in decline due to the intense fishing pressure that, in turn, is stimulated by the sushi and sashimi market demand. For this reason the International Commission for the Conservation of Atlantic Tunas (ICCAT) is implementing a program of gradual reduction of the Total Allowable Catch (TAC) for Eastern Atlantic and Mediterranean bluefin tuna. Furthermore, in the last decade, intense research has been carried out in an effort to domesticate bluefin tuna and develop a self-sustained aquaculture industry with the specific aim of alleviating the fishing pressure on the wild population (FAO, 2005).

The present study reports the results obtained in 2008 and 2009 in the framework of the Italian Regional project ALLOTUNA funded by the Regional Government of Apulia with European Union Structural Funds. The main aim of this project was to improve the reproduction control of this species in captive conditions, to develop a system for egg collection in the rearing cages, to test a suitable transport system for eggs and larvae and to establish optimal rearing conditions for bluefin tuna larvae in the commercial sea bream and sea bass hatchery of Panittica Pugliese (Torre Canne di Fasano, Brindisi, Italy), a private company participating in the project with its own funds.

2. Materials and Methods

BFT Broodstock

About 70-90 Atlantic bluefin tuna (estimated body weight 50-60 kg), caught by purse seine from spawning grounds around the Aeolian Islands during the fishing campaigns of 2007, were reared in captivity for two years in a 22 m diameter 13 m in depth floating sea-cage within the Marenostro farm near the coast of Vibo Marina, Italy. They were fed once a day with a raw fish diet made of Pacific mackerel (*Scomber japonicus*) and herring (*Clupea harengus*) at a ratio of 9:1.

Hormonal Induction

During two consecutive reproductive seasons (2008 -2009), when the surface temperatures were consistently above 24ºC, some of the ABFT broodfish were implanted randomly with a GnRHa sustained-release delivery system, as described by Mylonas *et al*. (2007). Implants were administered underwater into the muscle using an *ad hoc* designed spear-gun.

The day after the hormonal induction, a 3 meter-deep PVC curtain running around the perimeter of the cage was installed to contain the floating viable eggs within the circumference of the cage until collected by manual skimming of the surface with 500 µm plankton nets.

Egg collection

On the 28th June, 2008, 3 days after hormonal treatment of 8 broodfish, a first natural spawning event was observed. Over four consecutive days a total of 20 million eggs were collected.

A total of 20 fish were implanted with GnRHa in 2009; 13 on the 27th of June and 7 on the 2nd of July. A total of approximately 37 million eggs were then collected over 13 consecutive days.

The eggs were transferred to the hatchery of Panittica Pugliese, located 400 km from the tuna farm. Small egg quantities (1 million or less) were transported by car, using 20 l PET tanks filled with sterilized sea water. Larger egg batches were shipped by a truck equipped with insulated and oxygenated sea water tanks. All the eggs collected were disinfected with 100 ppm iodine for 10 minutes before incubation.

Larval rearing methods

In 2008 eggs were initially incubated in cylindroconical shaped tanks with a volume of 2 m^3 in almost dark conditions and were supplied with filtered and UV treated sea water. One day post-hatching, larvae were transferred to rearing tanks (volume 4 m^3) with buckets. Since hatched larvae showed a sinking behaviour, the incubation of subsequent egg batches was mainly performed directly inside rearing tanks. In 2009, most of the eggs were incubated directly into rearing tanks.

The different technical parameters used in the 2 rearing seasons are reported in Table 1. Algae and rotifers were offered upon mouth opening, and subsequently fish were fed with *Artemia* nauplii, newly hatched sea bream larvae, and only in 2009 also with moist pellet and minced sardines.

3. Results and Discussion

In 2008 the estimated fertilization rate was about 80% for the first two egg collections, but decreased to about 30% on the third and fourth days. In 2009 all eggs showed excellent hatching rates estimated at around 98%. Fifteen million eggs were used for rearing trials.

Larvae were able to ingest rotifers 60 hours after hatching at 25°C, and 100% of larvae showed 1 to 20 mastax in the stomach after 86 h post hatching. The first functional swimbladder was detected at 4 days post hatch (dph), but, at 10 dph only 20% of the larvae showed an inflated swimbladder. Massive mortality occurred between 4 and 10 dph with only about 1% overall survival rate at 8 dph. Mortalities were probably caused by insufficient nutritional value of rotifers and larval sinking behaviour. In 2009, different upwelling systems, 24 hours light regime and more intensive rotifer enrichments were tested. These changes improved survival to about 1% at 15 days and increased final survival of the fingerling from 63 dph in 2008 (87 mm length and 7.2 g weight) to 110 dph in 2009 (164 mm length and 55.9 g weight).

Feeding regime, larval and fingerling growth rate are reported in Figure 1 for the season 2008 and in Figure 2 for 2009. Growth rate was higher in 2009 than in 2008, especially for the first 30 days of larval rearing. For this reason, in 2009 rotifers were provided for about 15-17 days less than in 2009, while the administration of *Artemia* nauplii was the same in the two years. In 2008, from 25 dph sea bream larvae (1 dph) were offered. Because weaning was difficult, post larvae fifty days old were supplied to tuna fingerlings from 50 dph. In 2009, tuna were fed sea bream larvae from 20 to 37 dph.

Intense cannibalism occurred around 25 dph in 2008. In 2009, it was observed 10 days earlier because of the faster growth. In order to prevent this phenomenon, attempts to separate larger from smaller individuals, using plastic beaker, were performed both in 2008 and 2009. These operations induced high mortality in larvae below 6-7 mm total length, while for larger larvae a lower mortality was observed, but only for very short transfers.

In 2009, minced sardines and artificial moist diet (paste) were fed at 30-32 dph, at average total length of 45.2 ± 11.1 mm. At that time, about 1000 juveniles (survival rate of 0.006% during nursery culture) were reared mostly in 10-18 m^3 tanks. Further studies are necessary in order to understand more about the nutritional requirements of fingerlings and to improve weaning with an artificial moist diet. Actually, a dramatic massive mortality occurred between day 44 and 49 ph without warning; fish became dark and started to swim slowly and showing one side up. The dead fingerlings did not show any external lesions. Histological, parasitological, bacteriological analyses and RT-PCR for Betanodavirus are under development in the Fish Disease Laboratory at the Department of Veterinary Public Health and Animal Pathology, University of Bologna (Italy).

4. Conclusions

A reliable technology for ABFT reproduction in captivity, egg collection and transport with a very high percentage of hatching has been established. In the second year of trials we improved consistently the days of survival and average weight of fish. Further research is required in order to achieve ABFT fry production and, in particular, ways of preventing sinking behaviour, increasing swim bladder inflation, reducing cannibalism and meeting larval energetic requirements. Feed quality improvement should also decrease skeletal anomalies mainly observed on opercula and cranial ossification.

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Finally special thanks to Panittica Pugliese hatchery workers: G. Bianco, G. Loparco, N. Argese, E. Giannoccaro, L. D'Errico, R. Lomartire, S. Loparco, F. Palmisano, G.Colucci

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Table 1 - Technical parameters of bluefin tuna larval rearing experiments

	2008	**2009**
Tank shape	Cylindrical-conic	Cylindrical-conic; Cylindrical
Water volume	2-4 m^3	2-4-10-20 m^3
Egg/Larval density	50-70-100/l	100-150/l
Temperature	23.5-24.5°C	23.5-25.5°C
pH	7.9-8.2	7.9-8.2
Photoperiod (D/L)	8/16	0/24 - 8/16
Light Intensity	50-600 Lux	> 400 Lux
Water renewal	100-300 %/d	100-300 %/d
Water conditioning	Green or clear water	Green or clear water

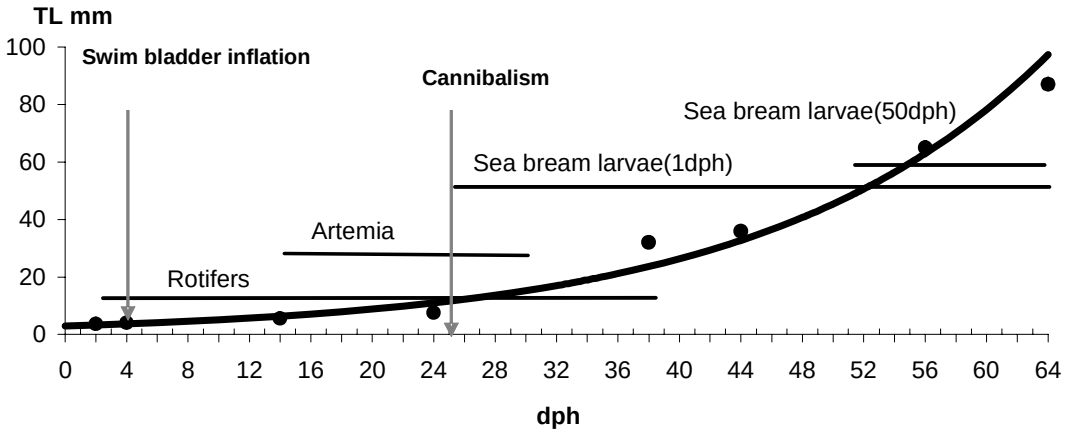

Fig. 1 - Feeding regime and growth during 2008 larval rearing experiments.

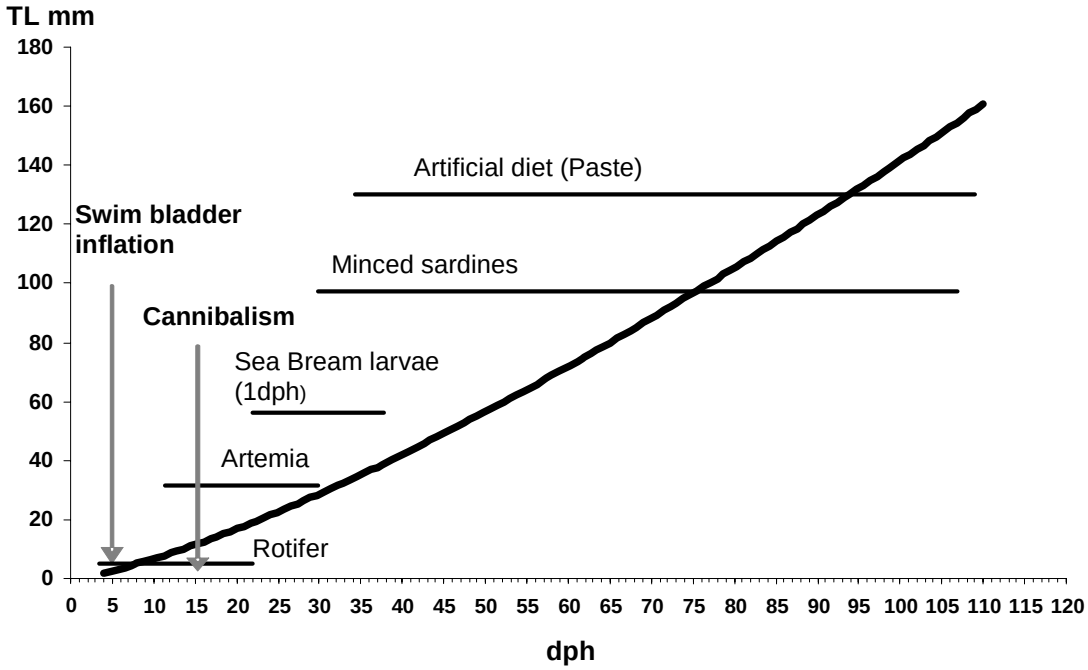

Fig. 2 - Feeding regime and growth during 2009 larval rearing experiments

STRATEGIES TO CONTROL REPRODUCTION IN SOUTHERN BLUEFIN TUNA (*Thunnus maccoyii*) IN SOUTH AUSTRALIA.

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A three prong approach is being applied to control reproduction of the southern bluefin tuna (SBT), *Thunnus. maccoyii*. The first approach involved the study of reproductive development of SBT broodstock in the cages at Arno Bay and at the on-shore broodstock facility using histological studies of gonadal tissue. These results revealed that the SBT broodstock can reach sexual maturity in captive conditions.

A polymer based slow release drug delivery system was then used to administer LHRHa to induce spawning in the SBT broodstock. The poly ethylene-vinyl acetate (EVAc) implants were loaded with the LHRH agonist Des-Gly10,D-Ala6,Pro-NHEt9-L (Bachem) as described in Zohar, (1996), Zohar and Mylonas (2001) and Mylonas et al., (2007). SBT were implanted with about 40ug/kg LHRHa and once spawning commenced, it continued for a period of 33 days. The fish spawned 2-3 times throughout each day, with over 50 million fertilized eggs collected during the 33 days. The spawning dynamics of the fish were determined by genotyping individual larvae and fertilized eggs using published microsatellite markers developed originally for *T thunnus* and adapted for *T maccoyii*.

The second approach aims to advance the age of pubertal development in SBT through the manipulation of the KISS system. In mammals, the KISS1 peptide, together with its receptor, are considered to be the gatekeepers of puberty (reviewed by Navarro and Tena-Sempere, 2008). The KISS system has now been identified in a number of fish species (Reviewed by Elizur, 2009). The genes encoding for the SBT and yellow tail kingfish (YTK, *Seriola lalandi*) KISS peptides and KISS receptor were isolated from brain and gonad tissues. As found in other fish, both SBT and YTK have 2 KISS genes encoding for the KISS1 and KISS 2 forms. The KISS receptor sequences were isolated from SBT and YTK, and appear to be similar to that observed in other fish species.

The deduced SBT KISS1 and KISS2 decapeptides were synthesized and their biological activity tested as described in Fig. 1. We tested whether SBT KISS1 peptide and SBT KISS2 peptide differentially activate the SBT KISS1 receptor. The SRE-Luc reporter plasmid follows PKC activation (A) while the CRE-Luc reporter plasmid follows the PKA pathway (B). SBT KISS2 was more potent then KISS1, eliciting a 3-fold and a more then 3.5-fold increase in the SRE and CRE response respectively, whereas the activity elicited by SBT KISS1 peptide was approximately a 2.5-fold increase for both signaling pathways.

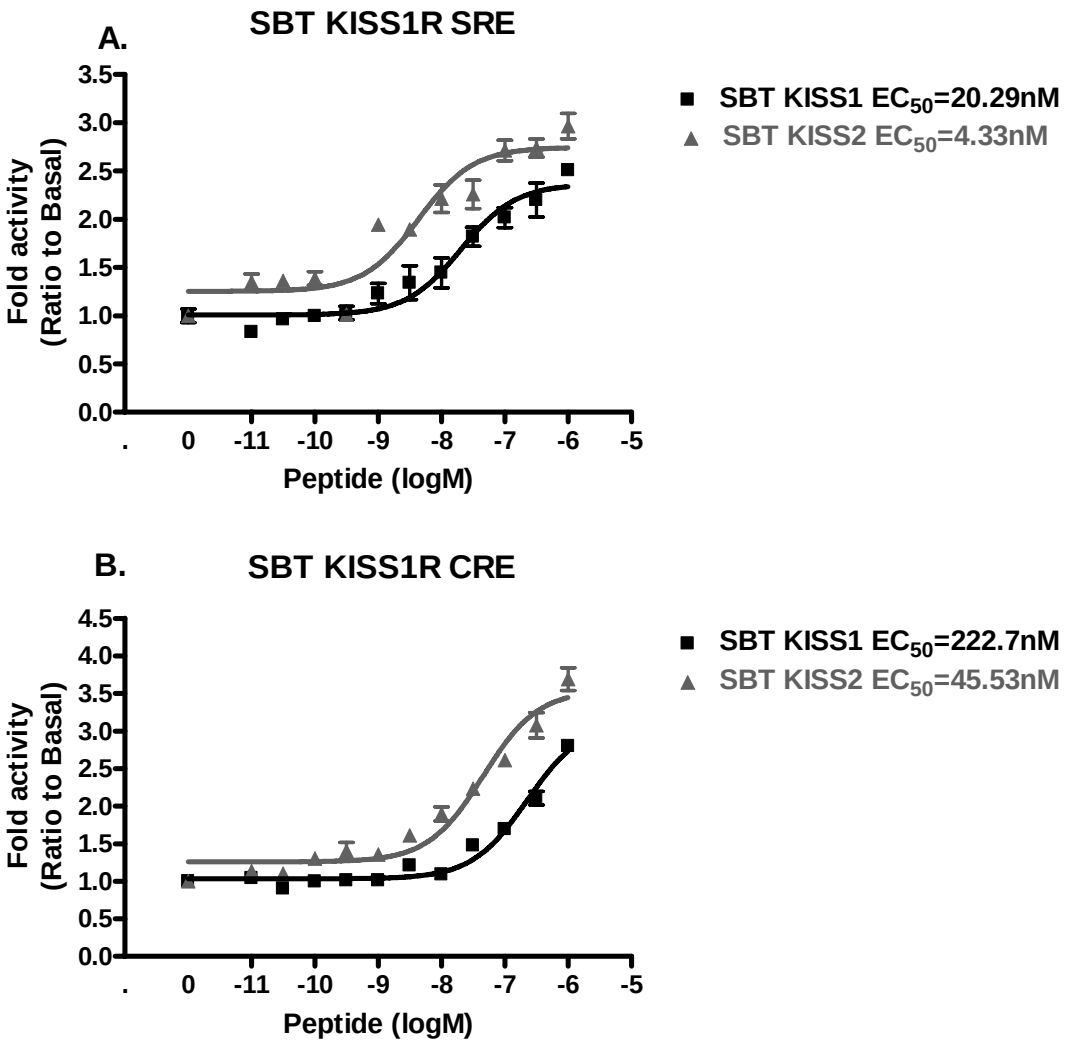

Fig. 1 - KISSPEPTIDE-induced SRE-derived (**A**) or CRE-derived (**B**) transcriptional activity. COS-7 cells were transfected with SBT KISS1 receptor (KISS1R) together with luciferase (LUC) reporter gene transcriptionally regulated by a serum response element (SRE) or cyclic AMP (cAMP) response element (CRE). The cells were treated with various concentrations of SBT KISS1 or SBT KISS2 decapeptides. The data are expressed as the change in luciferase activity over basal activity and are from a single experiment, representative of a total of three such experiments. Each point was determined in triplicate and is given as a mean ± SEM.

The KISS1 and KISS2 peptides have now been administered (using slow release EVAC implants) to pre-pubertal 1 year old YTK to examine their effect on pubertal development. If KISS manipulation is found to be effective in inducing pubertal

development in YTK, SBT of different ages will be administered with KISS peptides with the aim of advancing puberty to reduce the age and size at maturity of the SBT broodstock. The ability of such gonadal development to produce viable gametes would then have to be tested.

The third approach towards controlling reproduction is through the use of surrogate technology. Germ cell transplantation has been successfully used to create surrogate fish using both salmon (Takeuchi et al., 2003) and Nibe croaker (Takeuchi et al., 2009). In a collaborative project with Prof. Yoshizaki we are examining the feasibility of using YTK as a host for SBT germ cells. YTK larvae are being histologically examined to establish the precise timing of primodial germ cell (PGC) migration and SBT testis from fish at different age groups are being analysed to determine the size and age of SBT where the highest proportion of A-type spermatogonia can be found. The aim of the study is to develop YTK as surrogates for SBT germ line: if successful it will overcome many of the hurdles in maintaining and handling large SBT broodstock, as well as facilitating the introduction of a genetic selection program for SBT.

We wish to acknowledge and thank Clean Seas Tuna Ltd, the Australian Seafood CRC and the Fisheries Research and Development Cooperation for their support of this work.

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ACHIEVEMENTS AND BOTTLENECKS FOR YELLOWFIN TUNA, *Thunnus albacares* PROPOGATION AT THE GONDOL RESEARCH INSTITUTE FOR MARICULTURE, BALI, INDONESIA

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1. Introduction.

Many species of tuna migrate through Indonesian waters and about 66% of the total tuna catch in Indonesia consists of yellowfin tuna. It is necessary for Indonesia to have good resource management and also to develop the technology for propagation of yellowfin tuna to establish a responsible fishery and to initiate tuna culture. To achieve these objective, Indonesia together with the Japanese government, constructed a land-based broodstock holding facility at the Gondol Research Institute for Mariculture (GRIM), Gondol, Bali. At this facility, yellowfin tuna propagation was undertaken from 2003 until 2006. The main target was to collect biological data on broodstock and early stage larvae required to establish good resource management and also to develop tuna culture based on hatchery bred juveniles. Since 2008, this project has been supported by an Australian Centre for International Agricultural Research (ACIAR) project being conducted by GRIM in collaboration with the South Australian Research and Development Institute (SARDI), Adelaide, South Australia and Challenger Institute for Technology, Fremantle, Western Australia.

2. Materials and Methods

The project was started by collecting candidate broodstock of yellowfin tuna from the wild. Hand lines were chosen as the most effective fishing method as the fishing grounds are around fish aggregating devices (FAD's) where nets are not used. Fish transportation methods were developed using 1 m^3 fiberglass or canvas onboard tanks with continuous water exchange supplied by a water pump when stationary and a water scoop system when motoring.

Those fish which were successfully transported back to GRIM were treated in a bath of 'erubazu' (Sodium Nifurstirenate-NFS) at 50-100 mg L^{-1} for 1 hour then flushed by running water for between 12 and 24 hours in a 12 m^3 (4 m dia.; 1 m depth). After this treatment, all fish in good condition were transferred to one of two 235 m^3 (10 m dia.; 3 m depth) acclimation tanks. The acclimation tanks operate as a semi-closed system in which 50 % of the flow is fresh filtered sea water and 50 % is pumped from the tank to a sand filter and then back again into the same tank.

Prior to transfer, all fish were measured for fork length, one finlet clipped for DNA analysis and a microchip tag inserted to identify each individual fish. Those fish transferred were reared for between 3 and 5 months in the acclimation tanks. Fish

were fed using scad mackerel (*Decapterus* sp.) and squid (*Loligo* sp.) and a vitamin mix added to the feed just before feeding. Feeding was supplied twice a day.

When fish in the acclimation tanks reached 5 kg, they were transferred into a 1500 m^3 (18 m dia.; 6 m depth) broodstock tank. Here, feed was supplied once a day in the morning. Feed were scad mackerel and squid supplemented with vitamins (vitamin mix and vitamin C and E) which were added as capsules inserted into the feed.

Water quality in the tanks was measured everyday in the morning. Main parameters checked were water temperature, pH, salinity and dissolved oxygen. Ammonia and nitrite were only checked if dissolved oxygen saturation was less than 85%.

All fish that died in the acclimation and broodstock tank were checked, measured, weighed, and external and internal observations conducted to determine the cause of death. Spawning data recorded included the time of spawning, number of eggs, hatching rate and survival activity index. Larval experiments were conducted in 12 fiberglass tanks with volume of 1 m^3 each in order to study the early life history of the larvae.

3. Results and Discussion

On the basis of the survival rate during transportation, and in captivity, and daily growth rate, the optimum size for the candidate broodstock was 2-3 kg and more than 50 cm in fork length (Hutapea and Permana, 2007). Daily growth rate of fish more than 50 cm in fork length was more than 40 g per day. The biggest problem encountered in rearing yellowfin tuna broodstock in concrete tanks was deaths due to wall strikes. This problem imposes a high cost due to the need for continuous replacement. No cause of wall strikes has been identified although the majority occurs between midnight and 6:00 am (Fig. 1).

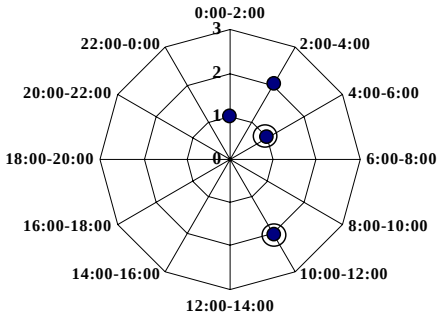

Fig. 1 - Estimated time of day of wall strikes based on the muscle condition when the fish was removed from the tank.

After 2 years in captivity, fish became mature with a body weight of 25-35 kg. In 2004, the first spawning occurred and based on DNA analysis, only one broodstock pair was spawning out of 15-20 broodstock. In 2005, spawning started in August and it was predicted that 2-3 broodstock joined the spawning. Spawning started in the afternoon between 16.00–17.00 hours. Water temperature at spawning time ranged from 28.0 -30.0^{o}C (Figure 2). Egg diameters ranged from 840-990 µm, but mainly ranged from 900-950 µm with an oil globule size ranging from 170-250 µm. Ratio

between oil globule and egg diameter ranged from 24-26% and this data showed that egg quality was good.

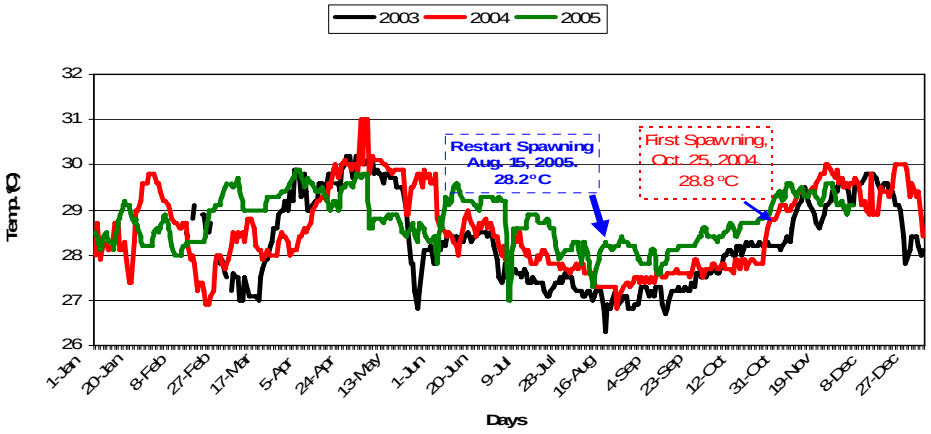

Fig. 2 - Profile of water temperature in the broodstock tank and time and temperature when broodstock spawn at first and second season.

Based on all spawning data collected between 2004 and 2008, the highest productivity of fish was between 2005 and 2006 and productivity declined sharply in 2008. On this basis, yellowfin tuna broodstock in tanks should only be raised for between 3 and 4 years, and should then be replaced.

High hatching rate of eggs gave high survival activity index of larvae even though the relationship was not always positive. Survival activity index of larvae declined after the 40th spawning event, even though the hatching rate was still high. This was due to endoparasite (*Ichthyodinium chabelardi*) infection (Yuasa *et al*., 2006). Permana, *et al.* (2006) found this endoparasite infected horizontally from water to the eggs (Figure 3) and (Zafran *et al.* 2006) suggested that to prevent eggs from infection, it is better to harvest the eggs as soon as fish spawn and treat them with 25-100 mg L^{-1} formalin.

4. Conclusion

Optimum size for the candidate broodstock is 2-3 kg at capture, with no more than 50 cm fork length, which gives higher survival rate during transportation and in captivity and higher daily growth rate. Fish transportation methods still need to be improved. The biggest problem in rearing yellowfin tuna broodstock in concrete tanks is wall strikes, which necessitates high numbers of replacement broodstock and the cost this entails. Size of fish at first spawning in the tank was estimated to 25-35 kg/fish at the second year in the tank. Highest productivity of fish was in 2005-2006 (3-4 year in the tank) and productivity declined sharply in 2008.

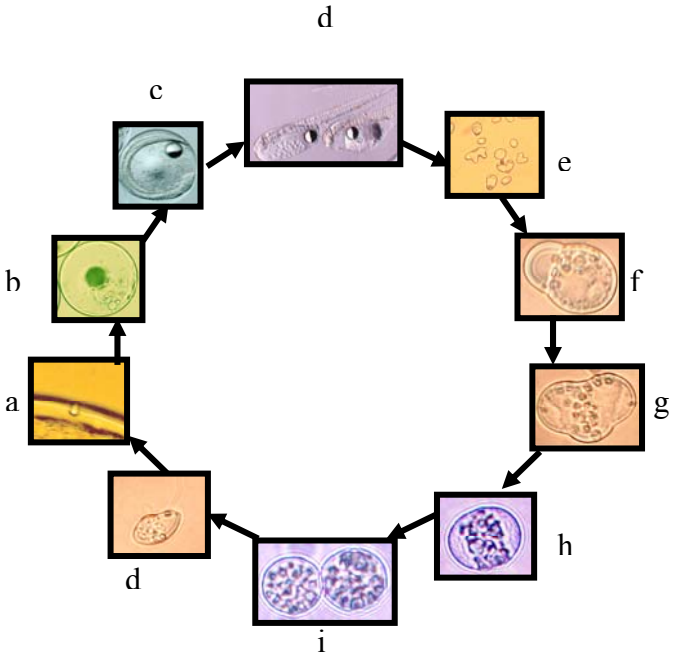

Fig. 3 - Life cycle of endoparasite (*Ichthyodinium chabelardi*), infected yellowfin tuna eggs (*Thunnus albacares*),.***a***.egg infected;***b-d***.cells division in yolk sac; ***e-g***. Cell division in the water; ***h-i***. Final cell division and moving cell and ***j***. Cell with a pairs of flagella.

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SOUTHERN BLUEFIN TUNA (*Thunnus maccoyii*) LARVAL REARING ADVANCES AT THE SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE AND COLLABORATING INSTITUTIONS

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1. Introduction

Propagation of Southern bluefin tuna (*Thunnus maccoyii*, SBT) is being undertaken through an Australian Seafood Co-operative Research Centre (AS CRC) funded project involving Clean Seas Tuna Ltd (CST), the South Australian Research and Development Institute (SARDI), the Port Stephens Fisheries Institute (PSFI) and the Darwin Aquaculture Centre (DAC).

Review of available information on larval rearing of Pacific bluefin (*Thunnus orientalis*) and other tuna species (Takashi *et al*, 2006; Nakagawa *et al*, 2007; Seoka *et al*, 2007) suggests that three periods of high mortality can be anticipated, specifically:

1. High mortality during the first 10 days of culture including losses due to surface adhesion
2. From flexion there is typically a high mortality due to cannibalism
3. High mortality caused by collision with tank walls

Captive SBT broodstock were induced to spawn in the controlled environment holding facilities of CST at Arno Bay, South Australia. Spawning extended from 12 March 2009 until 16 April 2009 (35 days) when at least 50 million fertilised eggs were produced from which SARDI received four batches of SBT eggs (total 1.96 million). As a first objective towards development of a successful larval rearing protocol for SBT, larvae were used to conduct trials to investigate methods and parameters required to achieve high survival during the first 10 days of larval rearing. In addition, approximately 350,000 SBT eggs were transported to PSFI to investigate the possibility of culturing larvae in extensive outdoor ponds. No SBT eggs were provided to DAC as supply from broodstock stopped at the time that this was to occur.

2. Materials and Methods

Trial 1. Effect of photoperiod and light source on growth and survival of SBT larvae

The first SBT larval rearing trial was conducted in 12 fibreglass tanks (1700 L each) at the South Australian Aquatic Sciences Centre (SAASC). The trial comprised 4 different lighting treatments replicated in 3 tanks for each treatment. Treatments were:

1. 13 hour light: 11 hr dark – natural light
2. 13 hour light: 11 hr dark – artificial light (industry standard)
3. 18 hour light: 6 hr dark – artificial light
4. 24 hour light – artificial light

Each of the tanks with artificial light condition were covered with 'Panda' plastic that had a black external surface and white internal surface to reflect light. Each tank was illuminated by a pond light (50 W, 12 Volt) suspended 80cm above the water surface. There were 4 air-stones placed in each tank and one air ring at the base of the central screen pipe. The tanks under natural light conditions were uncovered and exposed to natural light transmitted through fibreglass roof panels and a north facing translucent wall. All larval rearing tanks were stocked at a density of 18.4 larvae L^{-1} (2 days post hatch (DPH)).

Trial 2. Effect of photoperiod and light source on growth and survival of SBT larvae

In response to the adverse impact of aeration in the first trial, SARDI designed and installed an up-welling system within each larval rearing tank that kept SBT larvae supported in the water column. The same treatments were investigated as in the first trial with all larval rearing tanks stocked at a density of 15.2 larvae L^{-1} (2 DPH).

Trial 3. Comparison of survival of SBT larvae at different salinities

A short duration trial was carried out using 12 x 172 L tanks to investigate the effect of salinity on survival of SBT larval during the early stage of development. Salinity was adjusted to 31, 34, 37 or 40 g L^{-1} by addition of an artificial sea salt formulation or distilled water to provide 3 replicates of each salinity treatment. An up-welling water system was installed in each tank. Each tank was stocked with 2,720 SBT larvae (15.8 larvae L^{-1}) at 3 DPH. Microalgae and rotifers were added two times each day to maintain a density of 15 L^{-1}. Mortality was recorded daily and the trial was terminated at 8 DPH when all live larvae were counted. Growth was not measured in this short-term trial.

Trial 4. Effect of stocking density on growth and survival of SBT larvae.

This trial was planned to compare different SBT larval stocking densities (20 larvae L^{-1}, 40 larvae L^{-1} and 60 larvae L^{-1}). Each treatment was to have 4 replicate tanks with one replicate for each treatment exposed to natural light. Availability of SBT eggs declined at this time and numbers of hatched larvae were only sufficient to stock three tanks at the desired stocking densities. The three tanks stocked had an up-welling system installed and were exposed to natural light and photoperiod. Larvae readily consumed L-stain rotifers from 3 DPH and *Artemia* nauplii from 13 DPH. Live microalgae (*Nannochloropsis oculata* and *Isochrysis* strain T. Iso) was maintained in tanks until *Artemia* feeding was established.

Port Stephens Fisheries Institute

SBT eggs were transported by airfreight from the CST hatchery facilities at Arno Bay in South Australia to the PSFI in New South Wales. Eggs were stocked into one 10,000L tank (120,000 eggs) and two 2,000L tanks (60,000 eggs per tank). Rotifers

were added at 2 DPH when larvae were 3.8mm total length. Larval rearing tanks were operated using tank design, water management and larval feeding schedule as advised by CST.

3. Results

Almost total mortality of larvae occurred during between 3 DPH and 4 DPH of Trial 1. Mortality was observed as large numbers of larvae attached to the tank wall. Dead larvae were also found at the top of the mesh of the central screen pipe, just above the water level where air bubbles appeared to have deposited them. Due to high levels of mortality the trial was terminated at 4 DPH.

The up-welling tank system employed in Trial 2 at SARDI minimised mortality of larvae during the first feeding phase (3 – 4 DPH). However, from 6 DPH mortality was high in some of the plastic covered tanks receiving artificial illumination and at 8 DPH these tanks were emptied. Although low growth was observed, survival of larvae was higher in the three tanks exposed to natural light. High mortality (Fig. 1) was recorded in these tanks between 7 DPH and 9 DPH. Some larvae survived until 15 DPH when the trial was terminated. Trial 3 showed that there was no significant difference ($P > 0.05$) in survival of SBT larvae cultured from 3 DPH until 8 DPH in salinities ranging from 31 g L^{-1} to 40 g L^{-1}.

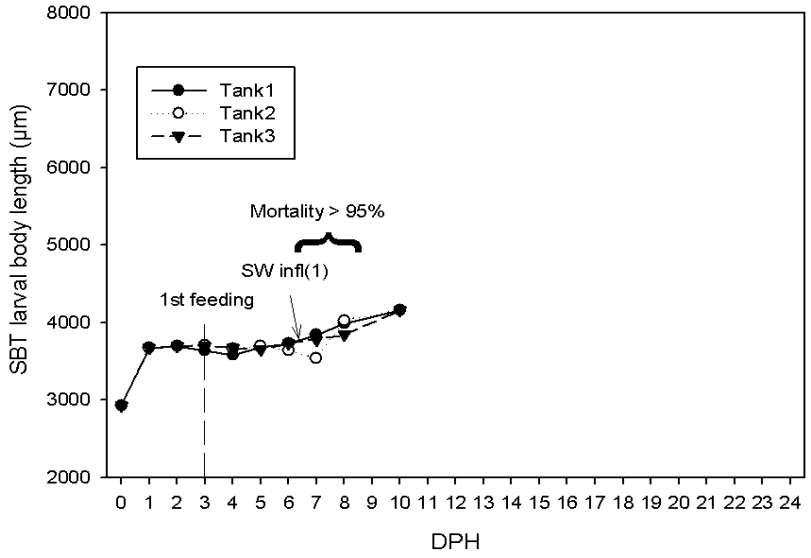

Fig. 1 - Growth, developmental events and feeding protocol used during SARDI Trial 2 for SBT larvae cultured from 1 DPH until 15 DPH.

During Trial 4, slow growth of SBT larvae was recorded until 8 DPH (Fig. 2). This was follwed by an increase in growth that coincided with primary swimbladder inflation. A low level of larval mortality was observed throughout the trial and it was estimated that at least 40% of larvae survived in each tank until 13 DPH. However, significant mortality occurred from 14 DPH until 16 DPH. It is estimated that during this period about 95% of the remaining larvae in each tank were lost. The last SBT larvae were sampled at 24 DPH.

At 1 DPH, all larvae in the 10,000L tank at PSFI had died. The likely reason was stripping of the larvae from the water column due to introduction of micro-bubbles into the tank water as a result of an external pump sucking air. Significant mortality

occurred overnight on 3 DPH and by 4 DPH all larvae in one 2000L tank had died while a few thousand were alive and feeding in the second 2000L tank. On 7 DPH, 100% of larvae sampled (n=10) from this tank had inflated swimbladders. However, the number of SBT larvae remaining at 8 DPH was not large enough to justify transferring larvae to extensive outdoor rearing ponds as planned. The survival of larvae continued to decline daily until Day 11, when no larvae remained.

4. Discussion

Significant improvement in larval survival during this period was achieved for each successive batch of larvae and high survival of SBT larvae until 10 DPH was achieved. The trials conducted during the 2009 larval rearing period confirm the importance of tank hydrodynamics during the early stages of SBT larval rearing. In each trial slow early growth of SBT was recorded, but acceptable survival until 10 DPH was achieved in the final trial

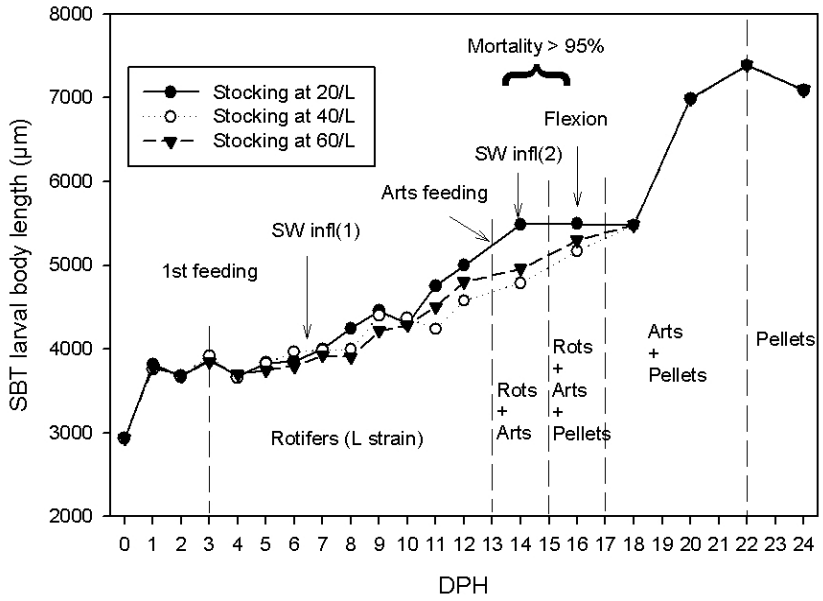

Fig. 2 - Growth, developmental events and feeding protocol used during SARDI Trial 4 for SBT larvae cultured at three different stocking densities from 1 DPH until 24 DPH.

(Trial 4). In this trial the major period of mortality in all three tanks followed the onset of *Artemia* feeding from 13 DPH. After the onset of *Artemia*, urinary calculi were frequently observed in sampled larvae. High mortality and the increased presence of urinary calculi may indicate inappropriate nutrition (Nowak and Battaglene 1996) suggesting that the previous nutrition provided during rotifer feeding and the addition of enriched *Artemia*, might not be a suitable feeding protocol for SBT larvae. SARDI had no access to a supply of fish larvae to feed SBT larvae that would typically be fed to other tuna species from this stage of development (Kaji, 2003). Consequently further trials with SBT larvae may require provision of a 'fish larvae reference' diet to progress larval rearing studies on SBT.

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RESEARCH ON THE REPRODUCTIVE BIOLOGY AND REARING OF LARVAE AND JUVENILES OF YELLOWFIN TUNA (*Thunnus albacares*) AT THE IATTC's ACHOTINES LABORATORY, REPUBLIC OF PANAMA

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1. Introduction

The Inter-American Tropical Tuna Commission (IATTC) conducts research on the reproductive biology and early life history of yellowfin tuna *Thunnus albacares* at the Achotines Laboratory, Republic of Panama. Yellowfin broodstock have been spawning at near-daily intervals since 1996, which represents the only sustained spawning of yellowfin tuna in landbased facilities in the world.

The Achotines Laboratory was developed to support research on the pre-recruit life stages (egg, larval, juvenile) and reproductive biology of tropical tunas. Research at the Laboratory involves laboratory and field studies designed to investigate the effects of physical and biological factors on the pre-recruit survival of tunas. From 1985 to1996, research focused on the early life history of tropical, coastal species of tunas. Beginning in 1996, yellowfin broodstock were established at the Laboratory and the experimental program shifted to laboratory studies of the reproductive biology and early life history of yellowfin. This paper summarizes advances in the management of yellowfin broodstock and describes recent research on larval development and larval and juvenile rearing techniques for this species.

2. Materials and Methods

Yellowfin broodstock are held in an in-ground concrete tank, 17 m diameter and 6 m depth (1,300 m^3). Wild yellowfin are caught by trolling with barbless hooks in coastal waters adjacent to the Laboratory. Each fish is tagged with a microchip implant tag, weighed, measured and injected with oxytetracycline before stocking in temporary reserve holding tanks. After 1-3 months in the reserve tanks healthy individuals are transferred to the large broodstock tank. The growth, survival, feeding and energy requirements of the broodstock were previously described by Wexler et al. (2003). Genetic monitoring of the broodstock has been conducted by comparing mitochondrial DNA (mtDNA) variation of spawning females with those of their eggs and larvae (Niwa et al., 2003). Margulies et al. (2007) described the courtship and spawning behaviors of broodstock, spawning periodicity, the influence of physical and biological factors on spawning and hatching and development of the egg and early larval stages. Multiple generations of broodstock have been utilized from 1996 to the present and we continue to monitor the growth and health of the broodstock as well as the daily egg production and egg viability from each daily spawn.
Yellowfin larvae are studied in a variety of laboratory investigations to determine the effects of selected environmental and biological factors on growth, physiology and

survival. Replicated experimental trials are conducted in circular, 700 l fiberglass tanks using single or multi-factor approaches to assess the effects of different variables on vital rates of larvae.

Early-juvenile yellowfin are routinely reared to 1 to 2 months of age for experimental purposes in circular, 4,000 -12,000 l fiberglass tanks. Experimental studies of juveniles have included investigations of growth, nutrition, behavior and weaning diet acceptance. Juveniles have been reared up to 100 days after hatching.

3. Results

A diet of 50% squid and 50% fish seems to provide adequate nutrition for broodstock yellowfin and fuels almost continuous spawning. Growth in weight was estimated at 9 to 26 kg/yr during 1996-2001 and 1 to 25 kg/yr during 1999-2006. Broodstock fish spawn as long as they receive adequate daily food rations and water temperature is > 23.3°C. Water temperature appears to be the main exogenous factor controlling the occurrence and timing of spawning. Courtship and spawning behaviors are ritualized and yellowfin adjust the time of day of spawning in relation to water temperature, resulting in a narrow range for the time of day at hatching. The age at first spawning for female yellowfin in captivity was estimated at 1.3 to 2.8 years, averaging slightly less than 2.0 years. Water temperature is significantly, inversely related to egg size, egg stage duration, larval size at hatch, and yolk-sac larval duration.

In the laboratory, yellowfin larvae are fed a sequential diet of enriched rotifers, enriched *Artemia*, and newly-hatched yellowfin larvae. Early-larval growth (the first week) is exponential in length and weight. Growth rates increase significantly during the late-larval and early-juvenile stages. Growth increases significantly in the laboratory during the late-larval stage (> 7 mm SL), when yellowfin switch from a zooplankton diet to a fish diet. Early juveniles (older than 3 weeks of age) reared on a fish diet exhibit growth rates > 1 mm/d and >50% dry weight/d. Yellowfin larvae and early juveniles exhibit density-dependent growth during the first 4 weeks of feeding.

Several physical factors, including water temperature, dissolved oxygen, light intensity and microturbulence have the potential to influence larval growth and survival. Microturbulence, in particular, has strong and predictable effects on larval survival. Intermediate levels of microturbulence support survival rates after one week of feeding that are nearly 3 times higher than those at lower or higher turbulence levels. Visual sensitivity of larvae and juveniles has been described based on microspectrophotometry analysis (Loew et al., 2002). Larvae have 3 peaks in spectral sensitivity in the violet, blue-green, and green ranges. The green sensitivity of yellowfin larvae is probably the basis for successful feeding and growth in green water.

Early-juvenile growth in the laboratory is rapid and variable among individuals. Cannibalism, which is strongest at fish lengths of 7 to 20 mm SL begins to subside during the early-juvenile stage. In the laboratory, early-juveniles are sensitive to changes in light intensity and require sufficient time intervals to adapt from scotopic to photopic vision. Juveniles must be physically constrained from contact with tank wall surfaces to prevent developmental problems and secondary bacterial infections. Preliminary weaning diet trials with larvae and early juveniles have indicated greater weaning success with early juveniles.

4. Discussion

The stable environment of onshore tanks seems to promote good health and sustained spawning of yellowfin tuna. Yellowfin collected at 1 year of age in Panamanian waters can be reared to reproductive size in 6-9 months. Broodstock yellowfin can be studied to develop quicker and more sophisticated genetic analyses such as real-time monitoring of genetic variation and spawning profiles for individual females.
Much of the research success of the IATTC's experimental program with the early life history of yellowfin tuna will assist the development of tuna mariculture. Larval and early-juvenile yellowfin exhibit rapid growth in the laboratory. Survival rates to 1 month of age are fairly low. Improved rearing protocols are necessary to increase survival if rearing programs are to be expanded to mariculture levels. Weaning diets have not been extensively studied for yellowfin larvae and juveniles, but improved weaning diets obviously hold the key to successful mariculture efforts with yellowfin tuna.

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THE EFFECT OF A 24-HOUR PHOTOPERIOD ON THE GROWTH AND SURVIVAL OF PRE-FLEXION YELLOWFIN TUNA (*Thunnus albacares*) LARVAE

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Abstract

Low survival of hatchery reared tuna larvae currently constrains the growth of tuna farming worldwide. Survival rates of <0.5% to weaned juveniles are typical and high mortality during the first 10 days has been identified as one of the major limiting factors to their mass culture.

In an attempt to improve the early survival of tuna larvae, a series of three trials investigating the effects of extended photoperiods on growth and survival of pre-flexion yellowfin tuna (*Thunnus albacares*) larvae were conducted at the Inter-American Tropical Tuna Commission, Achotines Laboratory in Panama. During the first two trials, treatment tanks were illuminated with 150 lux for 12 hours followed by a 12 hour phase, during which the light intensity was reduced to 44 lux. Survival of larvae under this regime was 2-3 times higher than those in the control treatment of 12L:12D, however the differences were not significant. There was also no significant difference in larval growth between treatments. A third trial subsequently compared the growth and survival of larvae reared under a 24 hour photoperiod with a light intensity of 150 lux maintained throughout the night, against those exposed to the control photoperiod of 12L:12D. Larvae reared under a 24 hour photoperiod achieved a 9-fold and significant improvement in survival and were significantly larger (22%) than those reared under the control lighting regime. Results suggest that the success of a continuous photoperiod is due to the extended foraging time combined with the prevention of mortality caused by night-time sinking.

YELLOWFIN TUNA (*Thunnus albacares*) AS A CANDIDATE FOR AQUACULTURE IN WESTERN AUSTRALIA

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1. Introduction

The state of Western Australia (WA) is the largest in Australia, with over 12,000 km of coastline and a climate ranging from cool temperate to tropical. Despite such a long coastline, appropriate sites for marine fish culture (both land-based and near-shore) are limited due to a range of factors including isolation or competing interests, large pumping heads and exposed coastlines. For these and other reasons, including onerous government licensing and site permitting processes, marine finfish aquaculture in this state has been slow to develop.

In recent years, the WA Government has been taking steps to provide more certainty in the approvals processes involved in establishing aquaculture ventures and pioneering industry groups have succeeded in identifying suitable sites and commenced production of marine finfish. Two industry groups have demonstrated an interest in the culture of yellowfin tuna, due to its very rapid growth rate and being highly regarded as a sashimi fish. This interest is shared and supported by the WA Government, which has invested in acquiring the knowledge required for developing off-shore cage culture and hatchery-based technology for this species.

2. Industry

Latitude Fisheries

Latitude Fisheries is a Geraldton-based, family fishing company that has been fishing for rock lobster, prawns, demersal fish, tuna and swordfish for the past 30 years. In recent years Latitude Fisheries has expanded into pearl oyster and finfish aquaculture. In 2004, in partnership with a second local fishing company, WT Newbold, it secured an 800 ha marine finfish site in the Zeewijk Channel of the Abrolhos Islands, an established base for the rock lobster fishery 70 km off the coast of Geraldton (Fig. 1). This site was originally issued with an aquaculture licence for yellowfin tuna.

After an extensive environmental impact assessment, the proponent was granted a one-year trial licence from the Environmental Protection Authority (EPA). During this period, 200 tonnes of yellowfin tuna were to be ranched using a similar model to that used in Port Lincoln (South Australia) for Southern bluefin tuna. Up to 200 tonnes of fish were to be purse-seined at the fishing grounds off Exmouth and towed approximately 750 km to the site before being transferred to sea pens where they would be conditioned for up to seven months. During this trial, environmental impacts

were to be carefully monitored and used as the basis for assessing future commercial expansion.

Fig. 1 - Permitted aquaculture lease area in the Abrolhos Islands.

Despite EPA approval, the trial did not proceed due to various economic factors, which made the cost and risks of purse-seining the initial biomass too high. The site licence has since been modified to include southern bluefin tuna and other marine finfish that can be produced in the hatchery, including mulloway and yellowtail kingfish. Mulloway are currently being used as test candidates for both assessing the environmental impact and investigating the logistics of operating a marine fish farm at the Abrolhos Islands.

Marine Produce Australia

Marine Produce Australia (MPA) has been producing saltwater barramundi at its 699 ha site in Cone Bay off the northwest coast of Australia since 2005. Production in 2010 and 2011 is set for 1000 tonnes and 1800 tonnes, respectively. The company is applying for a further 300 ha of lease area within two other nearby bays and considering expanding into an open ocean site to the west of Broome. Given the suitably warm water temperatures in this region, MPA is very interested in pursuing yellowfin tuna aquaculture and has also supported the WA Government's initiatives to acquire the necessary skills to enable commercial hatchery production of juvenile yellowfin tuna.

Both companies believe that, for a sustainable tuna aquaculture industry to develop, knowledge must be acquired to enable juveniles to be produced from a hatchery. Consequently, they support WA Government initiatives being undertaken to obtain this knowledge.

3. Western Australian Government

Aquaculture Development Council

The Aquaculture Development Council (ADC) is an advisory body to the Western Australian Minister for Fisheries. It has been instrumental in improving the licensing and permitting processes for finfish aquaculture and taken several other initiatives to

encourage the development of marine finfish aquaculture industry in the State. For example, due to the aforementioned shortage of suitable near-shore sites, the ADC has actively investigated the economic feasibility for development of an offshore aquaculture industry. Economic modelling has suggested that such an industry would be profitable based on those species for which hatchery-produced juveniles are available, but the ADC also acknowledges the culture of faster growing and more valuable species, such as yellowfin tuna, would further increase the profitability of such an industry. As such the ADC, along with the Department of Education, Employment and Workplace Relations (DEEWR) and the Aquaculture Development Unit (ADU), have invested in obtaining the skills required to achieve the goal of hatchery production.

Aquaculture Development Unit, Challenger Institute of Technology

The Aquaculture Development Unit (ADU) is a Government training and R&D facility. The ADU has a large marine finfish hatchery and undertakes research and development projects for WA industry and the state. Currently operating as the WA hatchery for mulloway, yellowtail kingfish, snapper and emerging species for restocking, it has infrastructure and personnel capability for the future culture of yellowfin tuna.

International Specialised Skills Institute (ISSI)

In 2008, Dr Partridge was awarded a fellowship from the ISSI, sponsored by the Department of Education, Employment and Workplace Relations. The aims of this fellowship were to acquire the skills necessary to develop a hatchery capability for yellowfin tuna in Australia and identify the current bottlenecks to commercial scale production of the juveniles of the species. To achieve this goal an international tour was undertaken to Panama and the United States of America. A detailed report on the findings of this fellowship can be obtained from Dr Partridge.

Australian Centre for International Agricultural Research

In collaboration with the South Australian Research and Development Institute (SARDI), the Aquaculture Development Unit and Western Australian industry participants are involved with the yellowfin tuna propagation project at the Gondol Research Institute for Mariculture (GRIM) in Indonesia, funded by the Australian Centre for International Agricultural Research (ACIAR). ACIAR commissions agricultural research between Australia and developing countries for mutual benefit. Australian industry participants interested in tuna propagation fully endorse this collaboration with GRIM as it provides them access to the data, techniques and lessons learnt by Australian and Indonesian scientists participating in this project and will allow the refinement and further development of valuable hatchery techniques without the high cost of developing a similar yellowfin tuna hatchery in Australia. The outcomes from the ISSI Fellowship will be implemented in this collaborative project with the aim of transferring the techniques to the Australian industry. Success in large-scale larval rearing at GRIM would provide a high degree of confidence that a similar yellowfin tuna facility would work in Australia.

COMPARISON OF LIPID AND FATTY ACID COMPOSITIONS IN DIFFERENT FLESH CUTS OF FARMED FED, FARMED FAST AND WILD PACIFIC BLUEFIN TUNA (*Thunnus orientalis*)

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1. Introduction

The technology for tuna aquaculture continues to develop and it is evident that tuna species are likely to be important globally because of their high market demand and high price which are partly due to their high lipid content. There is some evidence in aquaculture that farmed fish tend to be fattier and softer than wild ones (Skog et al., 2003) and this could be attributed to high lipid content in farmed fish (Grigorakis et al., 2002). Currently, fish lipids attract consumer attention because of the fatty acid profile especially high contents of docosahexaenoic acid (DHA, $C_{22:6n-3}$) and eicosapentaenoic acid (EPA, $C_{20:5n-3}$) and their beneficial association with human health (Ruxton et al., 2004). Consumption of fish has been associated with the prevention of cardiovascular diseases and other health benefits (Barre, 2007; Breslow, 2006). The edible muscular tissue of tuna is frequently marketed as three distinct cuts *akami* (lean meat), *chu-toro* (fatty meat), *o-toro* (very fatty meat) identifiable mainly by lipid content and color (Balshaw et al., 2008). Unlike wild caught fish, farmed fish can be reared and harvested under controlled conditions, enabling the targeting of the timing of end product delivery and the manipulation of the culture environment to optimize the quality products preferred by consumers. An example of this is the known effect of fasting on product quality on sea bream (Grigorakis and Alexis, 2005). It is of considerable importance that the tuna farming industry and consumers of its products are aware of their lipid and fatty acid (FA) composition and any positive nutritional differences between farmed and wild fish. The main objective of this study was to characterize, compare and contrast the lipid and fatty acid profiles of farmed fed, farmed fasted and wild Pacific Bluefin Tuna (PBT) in their different flesh cuts.

2. Materials and Methods

Farmed fed PBT (average weight; 24.7 ± 3.0 kg) and farmed fasted PBT (average weight; 26.1 ± 2.1 kg), were cultured in a net cage in the Fisheries Laboratory of Kinki University, Oshima experimental station, Wakayama, Japan. Frozen thawed wild sesame mackerel (*Scomber australasicus*) was used to feed them to satiation. The farmed fasted fish were fasted for 6 days before harvest. The wild PBT (average weight; 35.7 ± 0.3 kg) were caught in the Japan Sea by round haul net and were purchased from a wholesale market of Sakai-Minato city, Japan. The *cephalal* part of the tuna bodies were cut transversely into slices and the slices were divided into different cuts, namely *wakaremi*, dorsal *chu-toro*, dorsal *akami*, dark, ventral *chu-toro*, ventral *akami* and *o-toro* by observing the lipid content, color and direction of

myotomes (see Figure. 1). Lipid was extracted from the sampled cuts by the Folch method (Folch et al., 1957). FA methyl esters were produced from aliquots of total lipids. FA methyl esters were analyzed by gas-liquid chromatography. The data were presented as mean ± standard error of three specimens (one cut from one fish) in each group of PBT. Significance of any differences in lipid content and FA profiles were tested by one-way ANOVA (Tukey test).

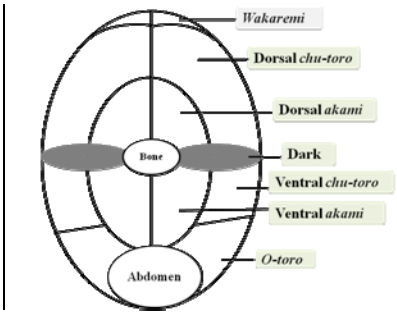

Fig. 1 - Schematic diagram of the cross section of PBT, indicating the different flesh cuts.

3. Results and Discussion

Lipid content

The lipid contents in the different flesh cuts of farmed fed, farmed fasted and wild caught PBTs are shown in Table 1. The lipid content was significantly higher in farmed (fed and fasted) than in wild PBT in dorsal *akami*, *wakaremi* and *o-toro* cuts. Lipid content of dorsal *chu-toro*, *wakaremi*, ventral *akami*, ventral *chu-toro* and dark cuts were not significantly different among PBT groups, although the values in farmed fed and fasted PBT were two or three times higher than in the wild PBT fish. The higher lipid content in farmed fish can be explained as a result of the regular accessible diets and reduced activity in small net cages whilst the lipid content of wild fish was influenced by the prey type and availability (Haard, 1992; Nakamura et al., 2007).The lipid content did not differ significantly between farmed fed and fasted PBTs in all cuts. It seems that 6 days fasting is not effective in lowering lipid content in PBT. The lipid content of wild *o-toro* cuts is very similar to the dorsal *chu-toro, wakaremi,* ventral *chu-toro* cuts of farmed (fed and fast) PBT. This means that almost the whole flesh of farmed PBT can be considered as *o-toro*, when compared with wild PBT *o-toro*, if the lipids content in its different cuts is considered.

Fatty acids content

The FA contents in the different flesh cuts of farmed fed, farmed fasted and wild PBTs are also shown in Table 1. All flesh cuts of farmed fed, farmed fasted and wild PBT contained $C_{16:0}$, $C_{18:1}$ and $C_{22:6n-3}$ FAs abundantly, in agreement with the findings of other studies (Álvarez et al., 2009; Saito et al., 1999).

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Table 1 - Lipid (g/100g wet meat) and fatty acids composition (g/100g lipid) in different flesh cuts of farmed fed, farmed fasted and wild PBT

Components	Groups	Dorsal *akami*	Dorsal *chu-toro*	*Wakaremi*	Ventral *akami*	Ventral *chu-toro*	*O-toro*	Dark
Lipid	Fed	9.5±0.9 a,r	17.1±6.7 a,q,r	22.1±4.2 a,q	9.9±3.0 a,r	30.1±4.4 a,p	41.5±1.8 a,p	10.9±3.2 a,q,r
	Fasted	6.9±2.8 a,r	20.7±5.5 a,q,r	21.0±4.7 a,b,q,r	7.2±3.3 a,r	28.6±7.7 a,q	47.5±5.6 a,p	7.1±1.5 a,r
	Wild	0.6±0.1 b,r	6.5±0.9 a,q,r	6.2±0.8 b,q,r	1.2±0.3 a,q,r	9.4±2.5 a,q	21.7±5.0 b,p	4.5±0.2 a,q,r
SFA	Fed	28.1±0.3 a,p,q	28.3±0.4 a,p	28.0±0.3 a,p,q,r	27.9±0.6 a,p,q,r	25.8±1.4 a,q,r	25.9±0.9 a,r	30.1±1.1 a,p
	Fasted	28.4±0.1 a,p	27.9±0.1 a,p	27.9±0.2 a,p	28.2±0.3 a,p	27.3±0.4 a,p,q	26.4±0.3 a,q	28.1±0.9 a,p
	Wild	29.7±2.6 a,p	25.8±0.5 a,q,r	28.5±1.2 a,p,q	26.3±0.4 a,p,q,r	24.7±0.7 a,q,r	24.0±1.0 a,r	24.3±0.4 a,q,r
MUFA	Fed	29.6±0.2 a,p	29.2±0.4 a,p	29.7±0.2 a,p	29.2±0.4 a,p	28.4±1.0 a,p	28.6±0.7 a,p	28.6±0.6 a,p
	Fasted	30.0±0.1 a,p	29.8±0.3 a,p	30.0±0.3 a,p	29.7±0.1 a,p	29.7±0.3 a,p	29.2±0.2 a,p	27.0±0.5 a,q
	Wild	20.2±2.1 b,p,q,r	23.1±0.7 b,p	22.3±2.3 b,p,q	18.7±1.2 b,q	22.1±0.3 b,p,q	22.2±0.2 b,p,q	17.4±0.6 b,r
PUFA	Fed	33.8±0.2 a,p,q	34.2±0.2 b,p,q	34.3±0.4 a,p,q	34.3±0.5 b,p,q	34.7±0.2 b,p,q	35.6±0.3 b,p	32.3±2.1 b,q
	Fasted	32.8±0.3 a,r	33.8±0.1 b,p,r	33.2±0.4 a,q,r	32.9±0.2 b,r	34.9±0.7 b,p,q	35.8±0.5 b,p	35.5±1.3 b,p
	Wild	40.0±5.1 a,q	39.9±1.0 a,q	39.3±3.0 a,q	45.1±1.1 a,p,q	43.3±0.8 a,p,q	44.3±1.1 a,p,q	48.5±0.1 a,p
C20:5n-3 (EPA)	Fed	8.1±0.1 a,q	8.4±0.0 a,p,q	8.6±0.1 a,p	8.4±0.1 a,p,q	8.3±0.1 a,q	8.8±0.1 a,p	6.9±0.2 a,r
	Fasted	7.7±0.1 a,q	8.3±0.1 a,p	8.2±0.1 a,p	7.6±0.2 a,b,q	8.4±0.2 a,p	8.7±0.2 a,p	6.0±0.1 b,r
	Wild	6.1±0.2 b,r	7.6±0.1 b,p	7.7±0.1 b,q	6.6±0.2 b,q,r	8.7±0.7 a,p	8.8±0.5 a,p	6.2±0.5a b,p
C22:6n-3 (DHA)	Fed	14.6±0.2 a,p	14.7±0.1 b,p	14.6±0.2 a,p	14.9±0.1 b,p	15.0±0.0 b,p	15.2±0.2 b,p	14.8±2.1 b,p
	Fasted	14.2±0.3 a,q	14.7±0.2 b,q	14.2±0.4 a,q	14.6±0.2 b,q	15.3±0.5 b,q	15.6±0.5 b,q	19.2±0.9 b,p
	Wild	23.9±4.9 a,p,q,r	18.8±0.6 a,r	18.8±2.5 a,r	28.6±1.4 a,p,q	20.9±0.4 a,r	22.3±0.7 a,q,r	31.8±0.7 a,p
∑n-3 PUFA	Fed	26.8±0.1 a,q	27.4±0.1 b,p,q	27.5±0.3 a,p,q	27.6±0.2 b,p,q	27.9±0.2 b,p,q	28.7±0.1 b,p	25.8±2.2 b,q
	Fasted	25.9±0.6 a,r	27.2±0.4 b,p,q,r	26.5±0.6 a,q,r	26.2±0.1 b,r	28.0±0.9 b,p,q,r	28.9±0.9 b,p,q	29.1±1.0 b,p
	Wild	32.4±5.7 a,q	31.0±0.4 a,q	30.8±2.8 a,q	37.7±1.1 a,p,q	34.7±1.1 a,p,q	36.2±1.8 a,p,q	41.5±0.1 a,p

Values are Mean ± S.E.

Mean values denoted with a, b, c in the same column for a flesh cut are significantly different for a component in fed, fasted and wild PBT groups (p<0.05)

Mean values denoted with p, q, r in the same row for different flesh cuts are significantly different in same PBT group (p<0.05)

No significant difference was observed in total contents of SFA in all cuts among PBT groups but significantly higher (p<0.05) MUFA content in farmed fed and farmed fasted PBTs than in wild PBT. Wild PBT contained significantly higher (p<0.05) PUFA in all flesh cuts except dorsal *akami* and *wakaremi*. The result agrees with a previous report on wild and farmed sea bass (Erdem et al., 2009). They observed that SFA and MUFA were significantly higher and PUFA were significantly lower in farmed sea bass than in wild sea bass. A significantly higher (p<0.05) DHA ($C_{22:6n-3}$) content was observed in all flesh cuts of wild PBT than in the farmed counterparts except in dorsal *akami* and *wakaremi*, which increases the value of wild tuna lipid compared to farmed (Chen et al., 1995). With regard to FA profile in fasted PBT, levels of DHA were maintained at similar levels to the fed fish. This species seems to be able to use its energy reserves in order to counterbalance the nutrient shortage arising from short periods of fasting. The EPA content was significantly higher (p<0.05) in farmed fed and fasted groups than in wild in dorsal *akami*, dorsal *chu-toro*, *wakaremi*. The wild PBT group showed significantly higher (p<0.05) n-3 FAs content in dorsal *chu-toro*, ventral *akami*, *o-toro* and dark cuts than farmed fed and farmed fasted. It may be that wild fish are better sources of n-3 FAs than their cultured counterparts (Van and Katan, 1990) or that the marine environment provides an excellent source of n-3 rich foods (Alasalvar et al., 2002) for the wild PBTs.

4. Conclusions

Most farmed PBT whole flesh can be considered *o-toro* when compared with the lipid content of wild PBT *o-toro*. Furthermore, the total SFA, MUFA, PUFA, DHA and EPA amounts showed considerable differences between wild PBT and farmed (fed and fast) PBT in different cuts of flesh. However, farmed (fed and fasted) PBT had more valuable flesh quality than wild PBT in terms of FA content and total lipids in distinct cuts. The lipid and FA composition of farmed fed and farmed fasted PBT proved that a short period of fasting before harvesting was not effective in lowering lipid and FA contents.

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ENHANCEMENT OF SURVIVAL RATE OF PACIFIC BLUEFIN TUNA (*Thunnus orientalis*) larvae BY FLOW CONTROL IN REARING TANKS

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1. Introduction

Mass mortality of Pacific bluefin tuna (PBT), *Thunnus orientalis* larvae, needs to be addressed as soon as possible to allow reliable, mass-production of bluefin tuna fingerlings. There are two different causes of initial mass mortality: surfacing death and sinking death of larvae. The former is seen from 0 to 4 days after hatch (DAH). The latter is mainly caused when larvae contact the rearing tank bottom, and is seen between 4 and 10 DAH. Spreading a thin oil film on the surface is an effective method of reducing surfacing death. The high mortality due to sinking is a phenomenon believed to be caused when the larvae come in contact with the tank bottom because they are unable to swim upward against the gravitational physical sinking force. The sinking death is seen mainly in night time. During the day, larvae are actively swimming to forage and feed and are able to maintain their position within the water column. However, during the night, larvae do not feed and they swim less actively. Consequently, at night there is a tendency for larvae to sink gradually. Usually, normal developing larvae inflate their swim bladder at night to increase buoyancy and decrease sinking speed. During the day, larvae deflate their swim bladder to decrease the drag coefficient to facilitate smooth swimming for foraging. The sinking speed of a larva (V_f) needs to be determined and this is a factor of body density and swim bladder volume. Swim bladder control (deflating and inflating) is closely correlated with mass mortality in early stage during 4 and 10 DAH.

If sinking speed of larvae can be reduced by means of physical flow control at night, sinking death should also be reduced. The vertical circulatory flow in the rearing tank is affected by aeration. If the aerator is located on the central part of the bottom, a pair of symmetrical circles are formed in vertical plane (x, z), as shown in Fig.1. At first, air bubbles rise dragging water upward until the bubbles arrive at the surface and burst. At this point, at the surface, flow direction changes from vertical to horizontal. The horizontal component of flow (x) is maintained until the flow reaches to the side wall, thereafter flow changes its direction to downward along the wall (z), and finally horizontal flow appears along the bottom from wall side to the aerator (center of rearing tank). The stream pattern along the fastest circulatory flow is approximated by stream function (Ψ). Vertical upward flow speed (V_w) is necessary to keep greater than sinking speed of larvae. The aim of this study was to measure larval survival under different aeration regimes.

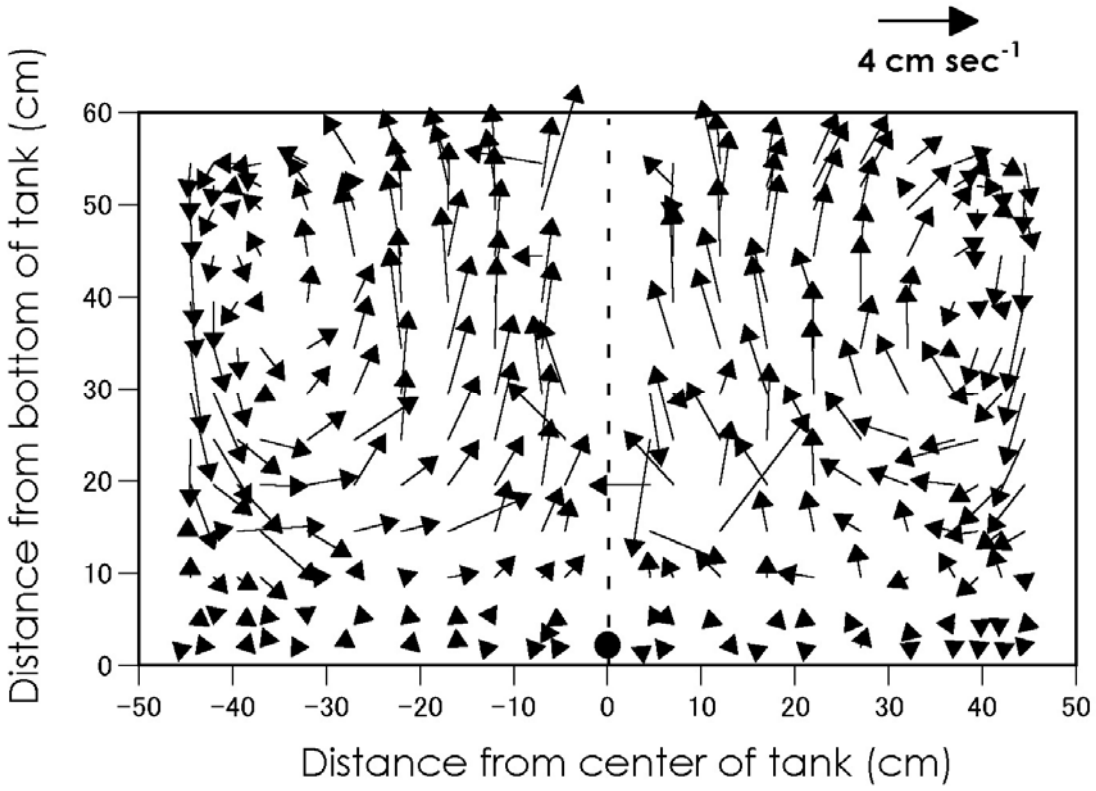

Fig.1 - Vertical section of flow at 1500mL/min aeration in the rearing tank

2 . Materials and methods

Pacific bluefin tuna eggs were spawned and obtained from broodstock fish that were maintained in captivity at the Fisheries Laboratory of Kinki University, Amami Island, Japan. The eggs were transported to the Shirahama Laboratory and incubated in 1000-L tank at 27°C. The larvae were reared until 10 DAH, under a 12 h light/dark photoperiod using 500lx fluorescent light. The larvae opened their mouths at night on 2 DAH. Larvae that were anaesthetized using FA100 were carefully released into upper layer by using a glass pipette. We used 30 larvae for larval density measurement.

The PBT eggs were obtained in a net cage at Amami Station, National Fisheries Agency, Japan and Amami Fisheries Laboratory, Kinki University. Three rearing experiments were conducted up to 10 DAH from 10 to 21 July 2007 (exp.1), from 29 July to 9 August 2007 (exp.2) and from 3 to 14 July 2008 (exp.3). Fertilized eggs were introduced into 500-L cylindrical polycarbonate tanks. An estimated 6,000 eggs were introduced in exp.1 and exp. 2, 8,200 eggs in exp.3.

The density of PBT larvae (ρ_f) was measured according to the method devised by Sakamoto et al. (2005) and Takashi et al. (2006). It is shown using following equation in a two layered water column with different water densities; upper is lighter (suffix is 1), water density (ρ_1, ρ_2), viscosity (η_1, η_2), and sinking speed (V_{f1}, V_{f2}) while in lower is heavier than upper (suffix is 2).

$$\rho_f = (V_{f2}\eta_2\rho_1 - V_{f1}\eta_1\rho_2) / (V_{f2}\eta_2 - V_{f1}\eta_1) \qquad 1)$$

The swim bladder volume (S) is calculated

$$S = 4/3\ \pi \times a \times b^2 \qquad 2)$$

where a is half the maximum bladder length and b is half maximum width (Hunter and Sanchez, 1976).

The stream function is shown in

$$\Psi = \Psi_0 \sin\pi x/H \sin\pi z/H$$

,where Ψ_0 is initial flow condition generated by aeration, H is a radius of rearing tank, x, z is horizontal and vertical length from center to wall side and surface to bottom (Okubo, 1975). The vertical flow component of Ψ is shown;

$$V_w = \partial\Psi/\partial x = -\ \pi/L\ \Psi_0 \cos\pi x/H \sin\pi z/H$$

$$-V_w < V_f \qquad 3)$$

We can show the estimated dangerous zone (EDZ) in the rearing tank in eq.3. However out of the EDZ, as the vertical flow speed is greater than sinking speed of larvae, they are kept in mid layer in circulation. We tried to control flow speed by changing the volume of air supply from 300 mL/min to 1500 mL/min .

3. Results and discussion

Larval density change and swim bladder in each DAH are shown in Fig.2.

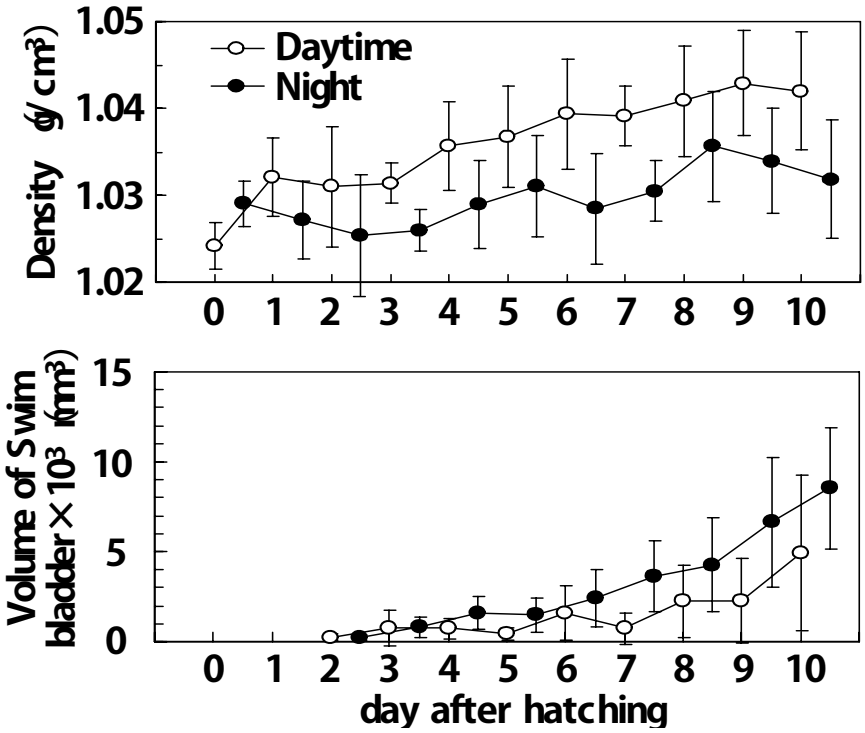

Fig.2 - Density and swim bladder change of bluefin tuna larvae

The individuals were counted in each rearing tank on 10 DAH (Table 1). The maximum survival rate was obtained; 300 mL/min: 24.2 ± 11.1(%), 900 mL/min: 48.6 ± 4.2(%), 1500 mL : 45.6 ± 16.0(%) as shown in Table 1. When we set up in high aeration in the rearing tank greater than 900mL/min, we could obtain high survival rate with low variance in the 500 L rearing tank.

Table1 - Correlation between air supply (mL/min) and survival rate (%). Each experimental series was carried out by 3 rearing tanks with 6000 eggs (exp.1 and 2) and 8200 eggs (exp.3)

Flow rate	Exp.1	Exp.2	Exp.3
300mL/min :	17.6±24.5 %	18.2±13.1%	24.2±11.1%
900mL/min :	22.7±6.5%	48.6±4.2%	20.1±17.2%
1500mL/min	-	-	5.6±1

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A WORLD FIRST FOR TUNA: FORMULATED FEED SUCCESS FOR SOUTHERN BLUEFIN TUNA, *Thunnus maccoyii*

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1. Introduction

Southern bluefin tuna (SBT) is an important aquaculture species in Australia. To date SBT have been farmed using ranching techniques, where juvenile fish (≈ 15kg) are caught in the Southern Ocean and towed backed to the relatively protected waters close to the South Australian coast. Traditionally, SBT are fattened on pilchards or sardines which results in good growth and condition but not surprisingly a high FCR. In addition, the large quantity of frozen pilchards needed to feed a relatively small farm results in considerable logistic issues and costs. This and the drive for a sustainable future have driven the requirement for a commercially manufactured feed.

To date, wild caught tuna have been reluctant to feed on commercially prepared diets and moist or semi moist diets, like traditional feeds need to be stabilised or frozen. In addition, as the major component of these diets was water, this resulted in a high FCR due to limitations on energy content in the feed. The poor acceptance and/or significant mortality of fish fed these 'research diets' has also meant the SBT industry has been reluctant to switch from feeding industrial fish to pelleted diets. However, the pressure to develop a new, highly acceptable diet for SBT has recently increased due to exciting developments within this industry in South Australia; most notably the closing of the life cycle of SBT by Cleanseas Tuna (CST). The challenge for a formulated tuna diet is to have good acceptance and mouth feel, produce superior growth and condition with reduced mortality, and most importantly, produce fish that are well accepted by the Japanese market.

This difficult challenge has been undertaken by two premier feed companies operating in Australia and Japan, respectively; Ridley Aqua-Feed Pty. Ltd. and Hayashikane Sangyo Feed Company. Hayashikane Sangyo had successfully fed Northern Blue Fin Tuna a similar formulated diet in Japan and fish fed this diet were well accepted on the market floor in Japan; the goal of the two companies was to develop a diet to suit SBT farmed under local Australian conditions. This collaboration has resulted in a new diet formulation designed specifically for SBT which is nutritionally complete and shelf stable. This paper describes new commercial scale research designed to validate this new formulation against traditional feeding methods and outlines future work that will take place.

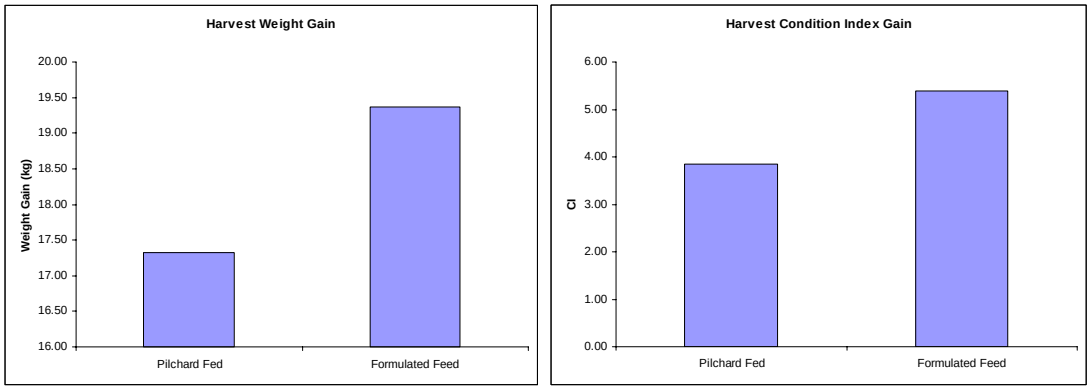
2. Materials and Methods

Initial research on a pelleted diet for SBT commenced in 2008 with small, commercial scale trials in sea cages owned and operated by Cleanseas Tuna (CST). That research was supported by the Australian Seafood CRC. Those early trials proved to be

extremely beneficial and focused the design and implementation of further research. Commercial scale trials commenced in 2009 and involved feeding the 'newly formulated' pelleted diet to SBT in the form of soft, 'sausage like' product. For operational reasons, CST could only provide two commercial sea-cages. As such, one cage was assigned to feeding on the new pelleted diet and the other cage assigned to feeding on pilchards (i.e. traditional feeding practice). Both products were fed by hand shoveling until visual satiation was observed. Each of the sea-cages was stocked in January 2009 with 2,000 SBT each weighing approximately 15-20 kg. At stocking, 50 fish from each cage were weighed, tagged and released for later identification. The feeding trial was run from January 2009 until July 2009, during which time the fish in each cage were physically sampled on 4 occasions using hook and line capture techniques (n=10-20) and these fish recoded for weight, length and condition, tagged and then released. In addition, an electronic monitoring system (AQ1 System AM100) was employed to monitor fish size (length) and number during the entire experiment. At harvest, the SBT that were tagged at the beginning of the trial were recovered and weighed. In the pellet-fed cage, 37 tags were recovered while 28 tags were recovered from the pilchard-fed cage. At the conclusion of the trial the following performance characteristics were evaluated and compared; weight gain, FCR, survival and condition index.

3. Results

The results of the tagged fish trial up to one month before harvest showed a significant improvement in FCR, weight gain and condition for fish fed the formulated pelleted diet compared with those fed pilchards. These results were borne out in the final harvest data with an increase in weight of 2.04kg, an increase in length of 1.44cm and an increase in condition index of 1.53 with a 4 fold reduction in FCR.



In addition to the improved growth performance, the formulated pelleted diet contained high levels of vitamin E and C. Previous data has show addition of these vitamins improves the shelf-life of tuna post harvest and the formulated diet is an excellent vehicle for the delivery of these products. The impact of the shelf life of 'pellet' fed tuna has still to be determined, but it is hoped that this data will be presented in the future.

Results from trials on Northern bluefin Tuna resulted is much higher levels of both E and C in the flesh of the tuna which results in improved maintenance of colour and shelf life in the finished flesh. Results from the first trial with SBT also showed a reduction of heavy metals in the finished flesh of the fish, a result supported by Hayashikane's experience with Northern Blue Fin tuna.

Initial trials using automated feed delivery systems have proved promising. This has demonstrated that fish can be fed from a hopper which is operated remotely on shore or boat. This alone will have huge implications for the industry. In addition, the trial using the automated feed delivery systems was conducted in cool water and fed to SBT >25kg that had only previously been fed on pilchards. The results demonstrated that the formulated pellets were rapidly accepted by large SBT previously fed pilchards at low water temperatures. These data, the implications to the industry and future dietary developments trials will be discussed.

TREND OF BLUEFIN TUNA CATCH, REGULATION AND THE PRICE IN THE JAPANESE MARKET

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1. Purpose and Background

Catch controls on bluefin tuna are being tightened globally due to the very low resource levels of these species, which are heavily targeted for fisheries and aquaculture purposes. Since the International Commission for the Conservation of Atlantic Tunas (ICCAT) applied much reduced catch quota levels on Atlantic bluefin tuna, the Western and Central Pacific Fisheries Commission (WCPFC) have applied catch quotas on bigeye tuna in the Pacific Ocean as well as a tentative catch regulations on bluefin tuna. Therefore, commercialization of tuna farming based on propagation is of great interest, with its feasibility increased by any product price increases caused by reduced quota levels. This paper analyses to what extent the price of bluefin tuna will rise due to a supply shortage of wild tuna and increased availability of juvenile farmed tuna by applying an econometric price determination model.

2. General Trend of Tuna Economy in Japan

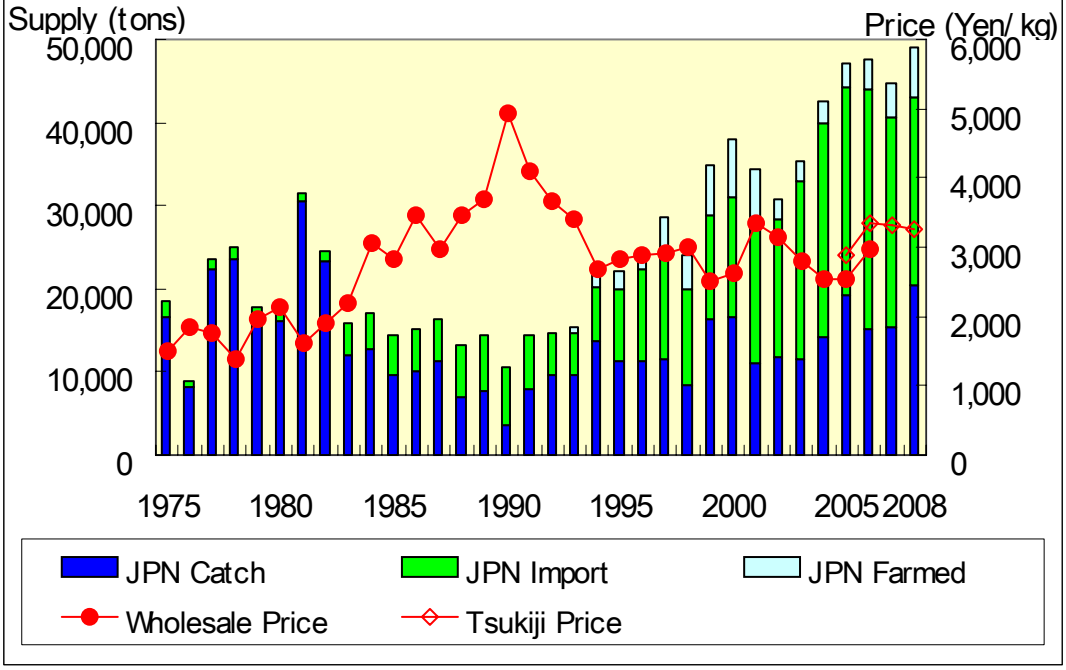
Supply of bluefin tuna to the Japanese market

Supply of bluefin tuna to the Japanese market is composed of three parts; local landing, import and local farmed tuna. As mentioned in Figure 1, most of the consumption used to be supplied by locally landed tuna before the 1980s, but the amount of imported tuna began to increase due to the decline in tuna catch in the Pacific Ocean because of over fishing and/or environmental changes. In this period, the supply of imported bluefin tuna and bigeye tuna increased substantially, which moderated the increase in the price of bluefin tuna. In the Japanese economic boom of the 1980s, the increased supply of bigeye tuna, a substitute for bluefin, had a moderating effect on the price of bluefin. After 1990, the import of wild caught bluefin tuna has been gradually replaced by farmed supply, from Japan as well as other countries.

Tuna farming in Japan is different from farming in the Mediterranean Sea in terms of the length of the farming period. Juvenile tuna in Japan are farmed for a period of 2-3 years. The production is managed by both private enterprises and groups of fishers, and in 2009 had expanded to a size of nearly 10,000 tons per annum, mainly from the *Amami Island* region. Kinki University succeeded in developing the reproductive

farming technology, and the production of bluefin tuna based on this propagation technology is increasing.

Figure 1. Supply and Price of Bluefin Tuna in Japan



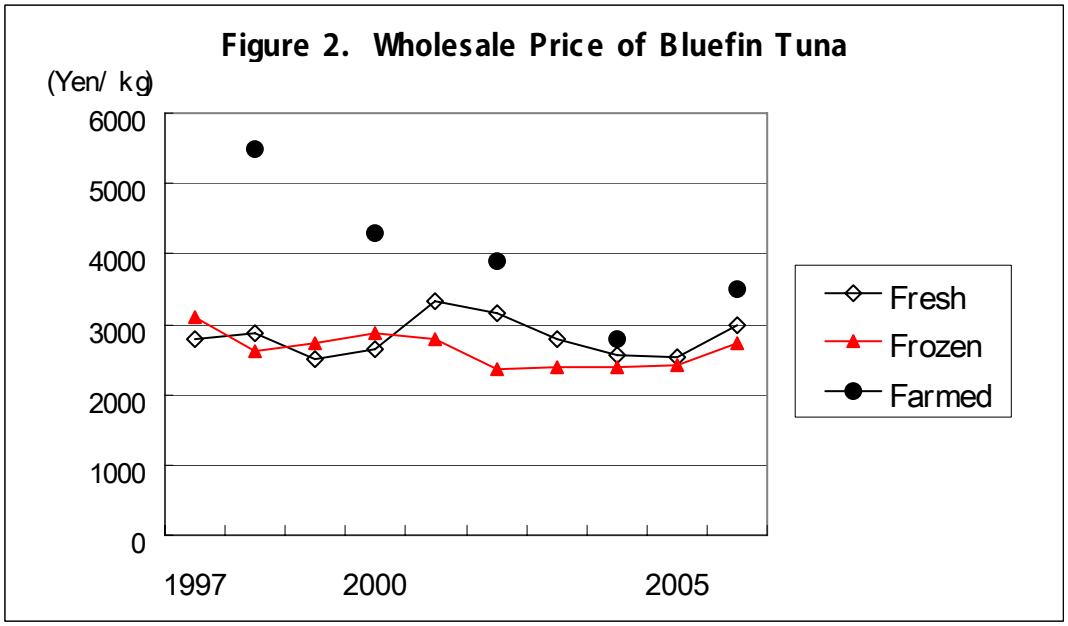
Regulations on each component of the supply

The quantity of imported bluefin tuna, which has a dominant market share in the Japanese market at this time, is likely to decrease as a result of changes to quota resulting from stock depletion issues. ICCAT adopted a strict catch regulation on bluefin tuna that will reduce the amount caught from 28,500 tons in 2008 to 18,500 in 2011. At the same time, the Commission for the Conservation on Southern Bluefin Tuna (CCSBT) has decided to reduce the 2010 catch quota on southern bluefin tuna by 20%.

Most of the local landed bluefin tuna was caught in the Pacific Ocean, where WCPFC has adopted a catch quota on bigeye tuna and a tentative regulation on bluefin tuna for 2010 to protect juveniles, especially from purse seine fleets. Based on this agreement, the catch quota on bigeye tuna will be reduced by 30% from 2009.

Price Trend

At this time, the price of bluefin tuna is relatively low at around 3,000 yen per Kg in the Tsukiji wholesale market. The price is low due to overproduction and the global economic recession. As is seen in Figure 2, there used to be a wide gap between wild and farmed bluefin tuna prices, but the gap has now narrowed. The supply, however, is expected to decrease sharply due to severe catch regulations on natural bluefin and bigeye tunas and as such it is expected that the price of tuna will rise sharply if the current economic recession eases.



Data: "Price of farmed tuna", Torii (2008)

3. The Model of Price Determination and Results

In order to predict the profitability of bluefin tuna farming and fisheries, we constructed an econometric model in which the dependent variable is the wholesale price of bluefin and bigeye tuna in Japan, and the independent variables are the global catch amounts of these species and southern bluefin tuna, the ratio of bluefin to bigeye tuna catches, Japanese GDP per capita. The model assumes that Japan is the dominant country consuming tuna, and that most of the three species are consumed there as sashimi.

In applying the model for price forecasting, we prepared two alternative scenarios that are compared with what actually occurred in 2006. Scenario A assumes current catch regulation, and Scenario B assumes stricter regulation of Atlantic bluefin tuna and Pacific bigeye tuna as presented in Table 2.

Based on Scenario A, our model predicts that bluefin and bigeye tuna price will rise approximately by 20% and 8% respectively against the actual price in 1996. This wide difference in rise in price is due to the assumed production difference; bluefin tuna 17% and bigeye 10%.

Table 2 - Scenarios of Tuna Catch and the Price Forecast

		2006	Scenario A	Scenario B
Bluefin Tuna Catch	Pacific	8,135	8,135	8,135
	Atlantic	32,275	18,500	14,250
	Total	40,410	26,635	22,385
Farmed Bluefin Tuna	Japan	3,500	10,000	10,000
Bluefin Tuna	Total	43,910	36,635	32,385
Southern Bluefin Tuna	Total	12,572	9,449	9,449
Bigeye Tuna catch	Pacific	246,837	205,100	177,300
	Indian	111,611	111,611	111,611
	Atlantic	66,251	66,251	66,251
	total	424,699	382,962	355,162
Bluefin Tuna Price (Yen/ kg)	Japan	2,972	3,570	3,975
Bigeye Tuna Price (Yen/ kg)	Japan	915	985	1,040

4. Conclusion

Catch controls on bluefin tuna are being tightened globally due to the very low abundance levels of the species targeted for fisheries and aquaculture purposes, and as a result commercialization of tuna farming based on propagated tuna is anticipated eagerly. In order to estimate the price level when the current catch regulations are fully implemented, we constructed an econometric price determination model for bluefin and bigeye tuna. The model predicts that the wholesale price of bluefin tuna in the Japanese market will rise by 17% from the recent price of 2,972 yen per Kg to 3,570 yen per Kg. This rise in price provides a significant incentive for further developing tuna farming based on propagated tuna.

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USE OF DIFFERENT TYPES OF SOYBEAN MEAL AS ALTERNATIVE PROTEIN SOURCES FOR JUVENILE PACIFIC BLUEFIN TUNA, *Thunnus orientalis*.

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1. Introduction

The Pacific bluefin tuna (PBT) is a highly prized fish in demand all over the world (Tičina et al., 2004). Demand for PBT has increased sharply (Nakahara, 2004) and is being fuelled by advances in cage culture technology of this species. At present, the cage culture of PBT depends entirely on wild caught juveniles, however the supply and availability of wild juveniles has been steadily declining over the last few decades posing serious problems for the future sustainability of the PBT industry (Kumai, 1997; Sawada and Kumai, 2000). In order to sustain the development of this industry it is necessary to improve the technologies supporting production of hatchery reared seedlings. Of major importance is development of artificial diets for seedlings that provide adequate nutrition for growth and development. Therefore, we recently conducted a series of experiments in which we successfully formulated diets for juvenile PBT using enzyme treated fish meal (EFM) (Biswas et al., 2009a and 2009b). We have also shown that EFM can be replaced by 20% soybean meal (Biswas et al. unpublished data). These encouraging results drove our laboratory to investigate the use of other soybean meal products in diets for juvenile PBT. Therefore, the aim of this study was to verify whether soybean meal (SM), soya protein concentrate (SPC) or full fat soybean meal (FFS) could be used to partially replace EFM in the diet of juvenile PBT without hampering their growth performance.

2. Materials and Methods

Four experimental diets were formulated using EFM; a control diet in which EFM was the primary protein source and 3 other diets that replaced 20% of the EFM in the control diet with the same amount of SM, SPC or FFS, respectively (Table 1). These diets were fed in triplicate to 25 day old PBT (stocking weight 0.49 g) to apparent satiety, 6 times daily (05:30, 08:00, 11:00, 14:00, 1600 and 18:00 h) for 12 days under continuous light. Each of the experiment tanks (15 m^3) was stocked with 300 juvenile PBT and each tank was provided with filtered seawater that was introduced at a flow rate of 30 l min^{-1} during the feeding trial.

Table 1. Dietary formula and proxumate composition of experimental diets

	Diets			
	EFM	SM	SPC	FFS
Fish meal	65.84	45.84	40.84	49.84
Soybean meal	0.00	20.00	20.00	20.00
Fish oil	8.00	8.00	13.00	4.00
α- starch	8.00	8.00	8.00	8.00
Vitamin mixture [1]	5.00	5.00	5.00	5.00
Mineral mixture [2]	5.00	5.00	5.00	5.00
Soybean lecithin	1.50	1.50	1.50	1.50
Cellulose	4.00	4.00	4.00	4.00
Wheat gluten	2.50	2.50	2.50	2.50
APM (ppm)	1200	1200	1200	1200
Vitamin E (ppm)	400	400	400	400
Proximate analysis (% of dry matter basis)				
Crude protein	57.77	50.75	57.58	52.46
Crude lipid	16.30	14.61	18.94	14.98
Crude ash	8.15	7.98	6.32	8.09
Crude sugar	10.99	16.96	11.53	15.53
Energy (kJ/g)	22.89	22.55	23.61	22.30

[1] Halver, 1957 (without AsA)

[2] Halver, 1957

The temperature and DO of the experiment tanks was maintained around 27.5°C and 6.5 mg l^{-1}, respectively. Initial and final fish samples were taken for comparisons of whole body proximate composition. All samples were kept at -80˚C until analyzed. Data were analyzed by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 12.0, Chicago, IL, USA). Data were expressed as the means ± standard error of mean (SE) of triplicates. When the factor was detected to be significant, the means among the treatments were compared using Tukey's test of multiple comparison with a 95% level of significance.

3. Results and Discussion

The growth performance of juvenile PBT fed the four experimental diets is presented in Table 2. Final mean body weight was significantly higher in PBT fed the control diet (EFM), similar for PBT fed the SPC and SM diets and lowest in PBT fed the FFS diet. A similar statistical response was recorded for relative weight gain (%) and specific growth rate (SGR; Table 2). There was no significant difference between the feed intake of PBT fed the EFM, SM or SPC diets, however feed intake on the FSS diet was significantly lower. Feed efficiency was best in PBT juveniles fed the control diet (EFM) and worst in PBT fed the diet containing FFS (Table 2). Although the condition factor was similar among the experimental groups, the survival rate for PBT fed the FFS diet was significantly lower.

Table 2. Growth performance of fish fed the experimental diets

Parameters	Initial	Diets			
		EFM	SM	SPC	FFS
Initial body weight (g)	0.49				
Initial body length (cm)	3.56				
Final body weight (g)		3.0±0.0[a]	2.5±0.1[b]	2.6±0.1[b]	1.9±0.2[c]
Final body length (cm)		6.6±0.3[a]	6.2±0.1[b]	6.2±0.1[b]	5.6±0.1[c]
Weight gain (%)		389.1±12.3[a]	308.8±1.3[b]	333.9±14.5[b]	129.2±30.3[c]
SGR (%)		15.2±0.1[a]	13.6±0.2[b]	14.1±0.2[b]	11.2±0.7[c]
Feed consumed (g)*		478.7±41.2[a]	460.8±21.5[a]	510.2±10.7[a]	287.1±14.8[b]
FCE (%)*		91.5±1.3[a]	77.1±2.0[b]	74.3±0.8[ab]	41.3±7.6[c]
CF		1.1±0.0	1.1±0.0	1.1±0.0	1.1±0.0
Survival rate (%)		56.7±4.2[a]	57.4±1.9[a]	60.1±3.3[a]	28.0±6.1[b]

Values in a raw with different letters are significantly different (P <0.05).

* Dry basis

The whole body proximate composition of PBT sampled at the beginning and end of the feeding trial is presented in Table 3, however only data on the harvest composition of PBT has been compared. A similar moisture content was observed among the treatments, however; lipid content was significantly higher (P<0.05) in PBT fed diet EFM, SM and SPC than diet FFS. Protein content was significantly higher (P<0.05) in PBT fed the SPC diet compared to PBT fed the other dietary treatments. Crude ash content was significantly lower in fish fed the SPC diet compared with other dietary treatments.

Table 3. Carcass proximate composition

Ingredients	Initial	EFM	SM	SPC	FFS
Moisture (%)	79.8±0.2	81.0±0.3[a]	80.7±0.8[ab]	79.4±0.5[b]	80.4±0.5[ab]
Crude protein (%)	13.1±0.2	13.2±0.3[c]	13.9±0.2[b]	14.7±0.5[a]	14.0±0.4[b]
Crude lipid (%)	1.2±0.1	1.9±0.1[a]	1.9±0.1[a]	1.9±0.1[a]	1.4±0.1[b]
Crude ash (%)	3.0±0.1	3.3±0.2[a]	3.4±0.2[a]	3.0±0.1[b]	3.5±0.1[a]

Values in a raw with different letters are significantly different (P <0.05).

Results from this feeding trial demonstrated that the majority of measured growth parameters were significantly lower in PBT fed the FFS diet compared to PBT reared on the fishmeal control (EFM) or diets containing 20% SM or SPC, respectively. The reasons for the poor performance of PBT reared on the diet containing 20% FSS were not identified in this study, but it could be related to the presence of anti-nutritional factors in FFS which might have inhibited the digestion and absorption of nutrients or affected palatability of this diet. A similar observation was reported in Atlantic salmon and rainbow trout (Kaushik et al., 1995; Hardy, 1996; Bakke-McKellep et al., 2000). On the other hand, the diets containing 20% SM or SPC promoted better growth and feed efficiency. Both SM and SPC are processed in different ways using specialized production techniques. These processing techniques have likely removed or deactivated anti-nutritional factors and or reduced the level of soluble carbohydrates and fiber in these products. In addition, the protein content of SPC is much higher than that of SM and FFS and it is reported to have a relatively balanced amino acid profile (Bureau et al., 1998; Storebakken et al., 2000).

Fish fed on the SPC diet had the highest protein content in their carcass. Encouraging results with survival and condition factor indicate potential for further research with protein substitutes such as SPC and SM in diets for juvenile Pacific bluefin tuna.

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THE CHALLENGE OF DOMESTICATION OF BLUEFIN TUNA *Thunnus thynnus* – HIGHLIGHTS OF THE SELFDOTT PROJECT FROM 2008-2009

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1. Overview

The 5th European Union´s FP research programme, REPRODOTT, was the first ever study of the reproductive biology of the Atlantic bluefin tuna in captivity. During this project, a hormonal method for the induction of spawning was developed. The SELFDOTT project (from capture-based to self sustained aquaculture and domestication of Bluefin tuna, *Thunnus thynnus*) is implementing the knowledge on the reproduction of bluefin tuna in captivity and establishing the knowledge-base required for controlled development of eggs, larvae and suitable and environmentally-performing feeds.

2. Background

Tunas constitute the most valuable fishery worldwide with global catches of 4 million tonnes and a value of US$ 6 billion. Fuelled by the increasing demand for this unique fish by the sashimi-sushi market in Japan, Europe and the United States, a “capture-based" aquaculture industry has developed in the Mediterranean Sea over the last decade, which involves the capture of migrating wild fish and their fattening in floating cages for periods ranging from 2 months to 2 years. The dramatic expansion of this industry is considered a threat to the now heavily over-fished wild stock.

In order to alleviate the pressure on the wild fishery for bluefin tuna and aid in its conservation, the domestication of this fish and the development of a sustainable aquaculture industry is necessary. This includes the propagation of this species in captive conditions, through rearing of the larvae and production of fingerlings for

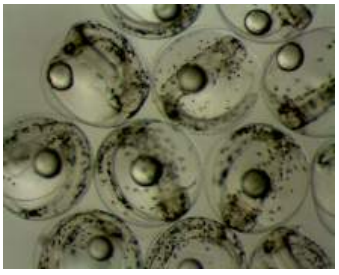
further grow-out on suitable, scientifically-formulated and environmentally-performing feeds, as it is done successfully in the EU for species such as salmon, sea bass and sea bream. Therefore, there is a great interest in developing captive bluefin tuna broodstock and larval rearing methods to support the sustainable development of an aquaculture industry. Studying the reproductive biology and larval rearing of this species in captivity will also result in a better understanding of its life history, which is necessary for management of the wild stocks.

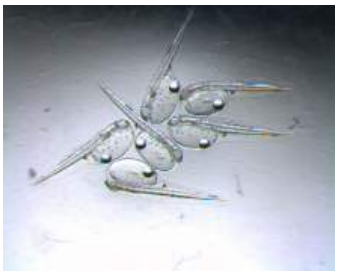
3. The project

The SELFDOTT project was launched in January 2008, funded under the 7th FP Cooperation Work Programme: Food, Agriculture and Fisheries, and Biotechnology and is coordinated by the IEO, Instituto Español de Oceanografía (SPAIN). The other members of the consortium include the Hellenic Centre for Marine Research (Greece); the Institut Français d'Exploitation de la Mer (France); the Heinrich-Heine University of Düsseldorf, (Germany); the National Centre for Mariculture, (Israel); the University of Cádiz, (Spain); the University of Bari, (Italy); the Malta Centre for Fisheries Sciences, (Malta); the Centre National pour la Recherche Scientifique, (France) and the Université de Montpellier II, (France). The tuna are maintained at the facilities of Tuna Graso, S.A., (Spain) and Malta Fish Farming (Malta), who are the industrial partners of the consortium with the Skretting Aquaculture Research Centre, one of the most important fish feed manufacturing companies in the world.

4. Highlights

Wild juvenile and mature bluefin tuna are being reared in floating cages at El Gorguel (Spain) and Marsaxlokk Bay (Malta). A third group of broodstock is placed in Vibo Marina (Italy) and belongs to a regional research consortium (ALLOTUNA) funded by European Union Structural Funds through the region of Puglia (Italy). The two projects have some common partners and have signed a collaboration agreement.



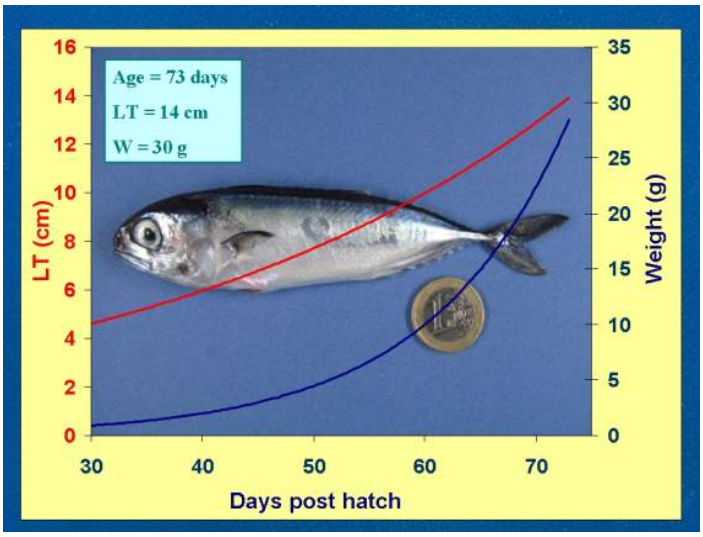


Wild juveniles were captured alive by hook-and-line and adapted to captivity in floating cages in order to establish the knowledge-base required for the development of suitable and environmentally-performing feeds, develop weaning feeds and develop formulated dry feed.

The broodstocks cages have been used to study gametogenesis, and the influence of diet on reproductive maturation and gamete quality. Mature fish have been induced to spawn using hormone implants and the eggs were collected using devices designed specifically for sea cages.

Beginning on June 29th 2009, captive-reared Atlantic bluefin tuna, maintained at El Gorguel (Spain), began spawning after being implanted with a reproductive hormone delivery-system. The fish spawned daily afterwards, producing a total of 140 million eggs, with a daily maximum of 34 million eggs. The Italian broodstock began spawning 3 days after hormone delivery system implantation (30th June), producing a total of 46 million eggs over the course of two weeks.

The eggs produced at the two sites were sent to research hatcheries in Spain, France, Italy, Malta, Greece and Israel to commence, for the first time, research on larval rearing of this magnificent and unique marine fish. The results from larval rearing have been encouraging but have also high-lighted the problems involved.



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THE ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) SPAWNING IN CAPTIVITY

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1. Introduction

During the last decade intense research has been carried out in the Mediterranean region to reproduce the Atlantic bluefin tuna (*Thunnus thynnus*, L.) in captivity (Corriero et al., 2007, 2009; Mylonas et al., 2007). Reproduction control in captivity represents the first step towards the conversion of the "capture-based" bluefin tuna industry that has developed in the Mediterranean Sea over the last 15 years (FAO, 2006) into a true, self-sustained, aquaculture industry. When reared in captivity, many fishes exhibit some degree of reproductive dysfunction (Zohar and Mylonas, 2001). Females fail to undergo oocyte maturation at the completion of vitellogenesis and, thus, do not spawn (Zohar and Mylonas, 2001), while in males a qualitative and/or quantitative decrease in milt production is observed (Billard *et al*., 1986; Billard, 1989). The research project "Set up of an integrated system for bluefin tuna farming" (ALLOTUNA) started in 2007. This project was based on the research work carried out in the framework of the E.U. project "Reproduction of the bluefin tuna in captivity - a feasibility study for the domestication of *Thunnus thynnus*" (REPRODOTT) that allowed the successful fertilisation of eggs after spawning induction with an ad hoc designed GnRHa delivery system (Mylonas et al., 2007).

2. Material and Methods

About 70 Atlantic bluefin tuna, caught by purse seine from spawning grounds around the Aeolian Islands (South Tyrrhenian Sea) during the fishing campaign of 2007, were reared in captivity for two years in a 22 m diameter floating sea-cage within the Marenostro farm near the coast of Vibo Marina, Calabria, Italy. When captured, the fish had an estimated body weight between 50 and 60 Kg and they were fed once a day with *Scomber japonicus* in 2008 and with a mixture of *Scomber japonicus* and *Ilex coindetii* in 2009.

Since temperatures above 24°C are considered essential for tuna spawning (Schaefer, 2001), water surface temperature was recorded in the rearing cage at least two times a week from the beginning of April (Fig. 1).

On 25 June 2008, once the assumed optimal temperature value was reached, 8 broodstock fish were stimulated randomly with a GnRHa implant as described by Mylonas *et al*. (2007). During the spawning season 2009, 20 fish were implanted using the same method of the previous year, 13 of them on 27 June and 7 on 2 July.

The day after the hormonal induction, a 3 m deep PVC curtain was installed around the perimeter of the cage on the surface (Fig. 2) in order to trap any floating viable eggs within the circumference of the cage until collection. The curtain was attached to the inside of the net with the top 30 cm of PVC curtain above sea level.

The experimental cage was checked for eggs at sunrise every day after hormone treatment by skimming the surface with a 500 μm plankton net.

3. Results and conclusions

A total of approximately 20 and 37 million eggs were collected in 2008 and 2009, respectively. The eggs were around 1 mm in diameter with a 250 μm oil droplet, in agreement with a previous study (Mylonas et al., 2007). The fertilized eggs (Fig. 3) were transferred to a commercial hatchery (Panittica Pugliese, Torre Canne di Fasano, Brindisi, Italy), using 20 L PET tanks filled with sterilized oxygenated seawater or a truck equipped with thermally insulated oxygenated seawater tanks. The fertilization ranged between 30% and 80% in 2008 and it was around 100% in 2009. All eggs showed excellent hatching rates estimated at around 98%. Various light intensities were tested as well as several regimes of water renewal, tank volume and aeration. Different rotifer enrichments were also tested while the photoperiod was kept at 8 to 16 hr light in 2008 and at 24 hr light in 2009. Algae and rotifers were offered upon mouth opening (60 hr), *Artemia nauplii* were introduced 13 days post hatching (dph) and newly hatched sea bream larvae were introduced 25 dph in 2008 and 20 dph in 2009. Mortalities were very high during the first stages when an estimated 99% of the larvae died and, in 2008, the last fingerling died 63 dph. In 2009, the last fingerling died 110 dph. Fig. 4 shows a bluefin tuna larva 11 dph and figure 5 shows a juvenile bluefin tuna 44 dph.

Following the successful induction of maturation and spawning in bluefin tuna using a GnRHa implant specifically design for this species (Mylonas et al., 2007), the two-year successful collection of a considerable quantity of Atlantic bluefin tuna fertilized eggs represented the starting point to carry out trials on the larval rearing of this species.

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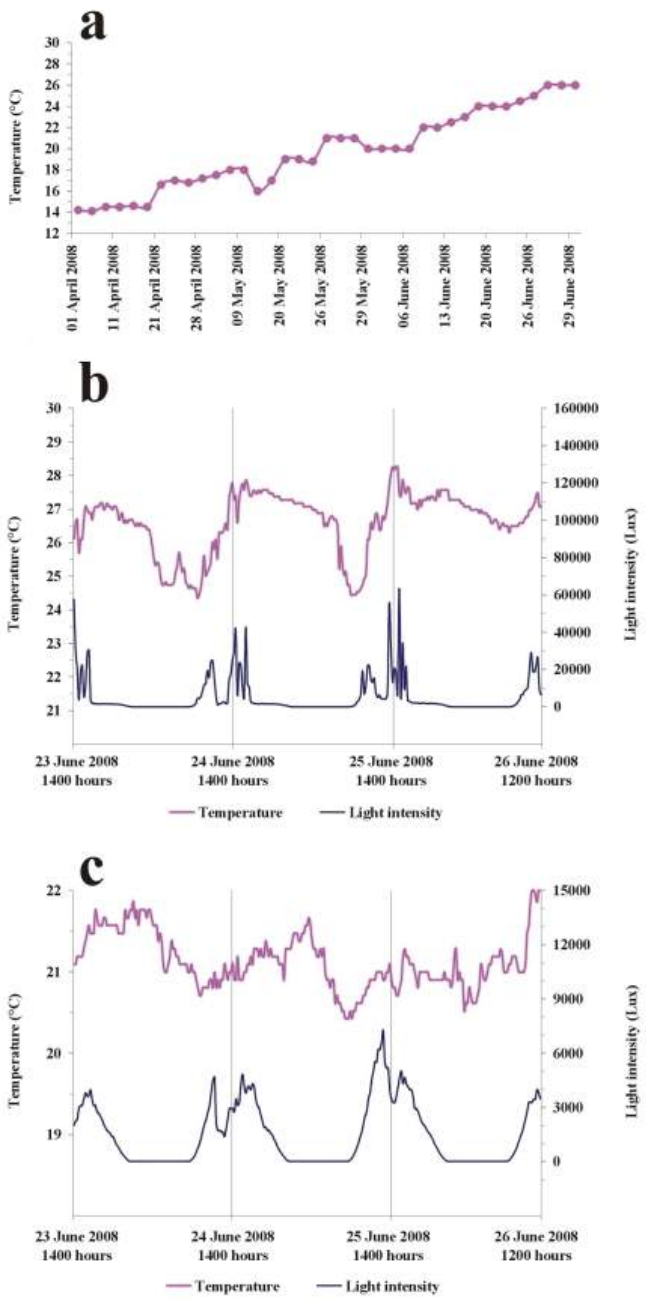

Fig. 1 - (a) Sea water surface temperatures recorded inside the Atlantic bluefin tuna broodstock cage on a twice weekly basis from April to June 2008. (b) Sea water temperatures (^{o}C) and light intensity (Lux) recording of a Hobo Pendant data logger placed 1 m below the water surface just outside the cage. (c) Sea water temperatures (^{o}C) and light intensity (Lux) recording of a Hobo Pendant data logger placed 1 m above the bottom of the cage at approximately 12 m of depth (c).

Fig. 2 - Spawning cage of the bluefin tuna broodstock, with the plastic curtain placed around the circumference of the internal side of the cage to prevent egg escape.

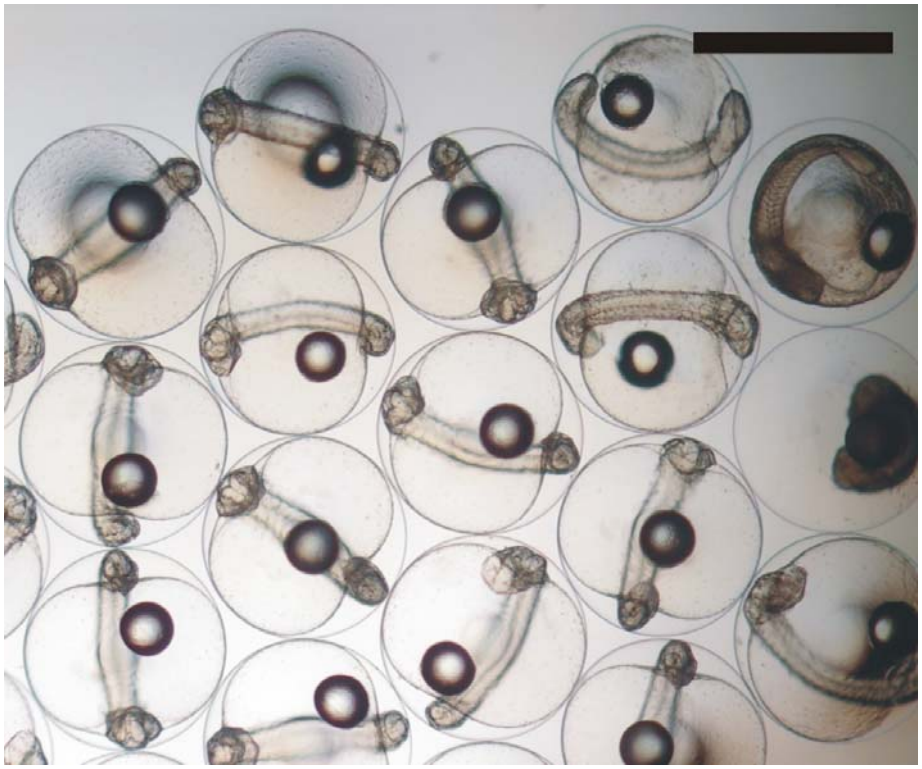

Fig. 3 - Fertilized bluefin tuna eggs collected on 28 June 2008. Magnification bar represents 1 mm.

Fig. 4 - Photograph of bluefin tuna larva at 11 days post hatching and 5,3 mm in TL. Magnification bar represents 1 mm.

Fig. 5 - Photograph of bluefin tuna fingerling at 44 days post hatching and 73 mm in TL. Magnification bar represents 10 mm.

BIOSECURITY PROTOCOLS FOR TRANSLOCATION OF SOUTHERN BLUEFIN TUNA (*Thunnus macoyii*) EGGS FROM SOUTH AUSTRALIA TO NEW SOUTH WALES, PORT STEPHENS FISHERIES INSTITUTE

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Spawning of viable eggs from captive Southern bluefin tuna (SBT) broodstock was achieved for the first time by Clean Seas Tuna (CST) during the summer of 2008/09. Culture of SBT larvae had never previously been attempted and based on the difficulty of rearing other related species including Pacific bluefin and yellowfin tuna, a research program was initiated to include several Australian marine fish hatcheries in order to optimise the potential success of SBT larval rearing. The hatcheries were situated in South Australia (SA; CleanSeas at Arno Bay and SARDI at West Beach), Northern Territory (NT; Darwin Aquaculture Centre) and New South Wales (NSW; Port Stephens Fisheries Institute) and each hatchery operated with different types of facilities (clearwater and greenwater intensive tanks from 2000 to 50,000L, extensive, outdoor ponds) and rearing methods.

Although SBT is endemic to NSW, a rigorous biosecurity protocol was implemented to minimise the risk of introducing any infectious diseases from SA into NSW. The protocol included components associated with both egg handling and translocation, managed by CST, and larval rearing, managed by PSFI. Results from 2009 are reviewed.

Briefly, the protocol for 2009 included the following conditions:

1. eggs were obtained from clinically healthy SBT broodstock requiring issue of a health certificate from a veterinary surgeon
2. eggs were cleaned of contamination and disinfected with ozonated seawater at a CT (concentration ozone [mg/L] x time [min]) of 1
3. seawater used for transporting eggs was filtered to <20um and disinfected with 20mg/L of active chlorine for 12h, then dechlorinated with vigorous aeration
4. a sample of 7 ml of eggs was supplied to Australian Animal Health Laboratory (AAHL) for testing of virus infection by virus isolation using a range of fish cell lines EPC, FHM, Grunt Fin, LC brain, LC kidney, RTG-2, BF-2, CHSE which are routinely used for finfish virology
5. a sample of 3 ml of eggs was also supplied to the Elizabeth Macarthur Agricultural Institute (EMAI) for nodavirus screening using a specific nodavirus Real-time PCR assay and general microbiology for aquatic bacterial pathogens.
6. all egg handling and treatment steps were observed by an independent scientist from Primary Industries and Resources South Australia (PIRSA)
7. on arrival at the PSFI hatchery, eggs and larvae were held in isolation and quarantined until laboratory test results were received

8. following negative laboratory results, larvae were approved for stocking into rearing facilities other than closed, indoor tanks
9. all seawater and waste discharged from SBT rearing facilities was retained in tanks and disinfected with 20mg/L active chlorine for 12h before discharge
10. all equipment used for SBT larval rearing was disinfected with 20mg/L active chlorine for 12 h prior to alternate use

All virology and bacteriology tests were negative. Samples of SBT eggs for virus isolation were processed using two different methods, mortar and pestle ground and MagNa lysed, and then inoculated onto fish cell lines described in condition 4 above in an attempt to isolate virus. No viral cytopathic effect developed and no viruses were isolated. Nodavirus was not detected in SBT egg samples and bacteriology did not detect presence of *Aeromonas salmonicida, Streptococcus iniae* or *Vibrio spp*..

Transportation of SBT eggs from the CST hatchery to PSFI hatchery was successful. Approximately 350,000 fertilised eggs were treated at the CST hatchery according to the biosecurity protocol and translocated to PSFI. On arrival at PSFI, approximately 70% of the eggs remained viable and were stocked into intensive, greenwater larval tanks.

The biosecurity protocol that was implemented involved some costs, including staff time and costs associated with laboratory testing. Biosecurity protocols for future translocations of SBT eggs from CST will continue to be reviewed and refined in consultation with aquatic animal health experts and the I&I NSW Principal Director Biosecurity to ensure that the risk of transfer of pathogens in association with tuna eggs is minimised in a practical and achievable manner.

SKRETTING'S COMMITMENT TO BLUEFIN TUNA

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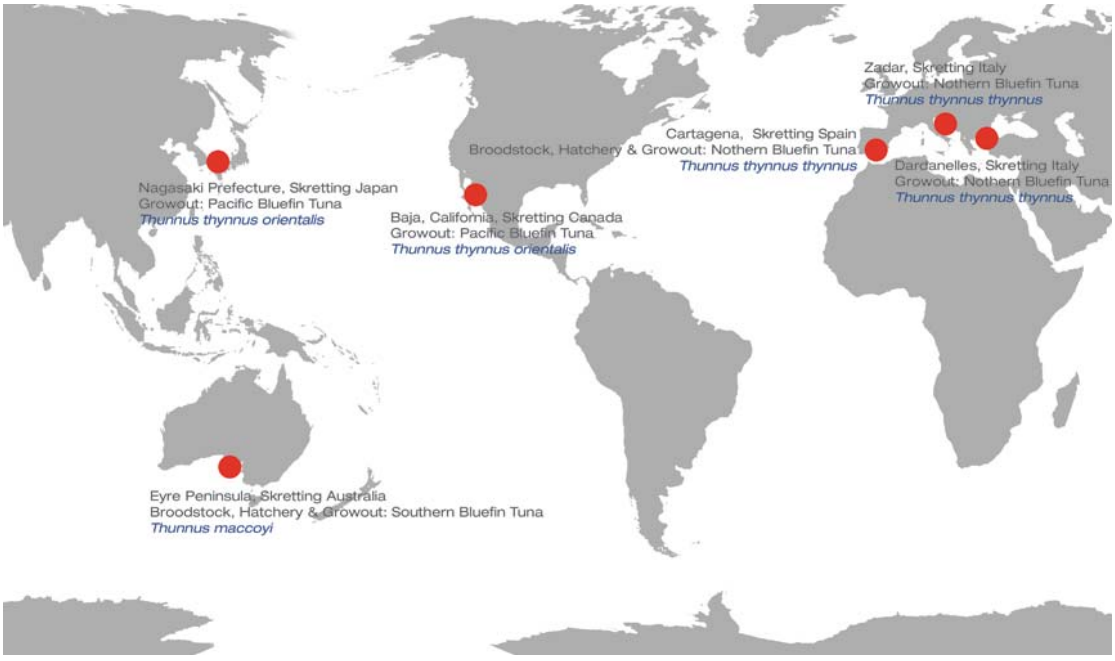
Skretting is the world leader in the production and supply of feed for farmed fish. Total annual production of high quality feeds is more than one million tonnes and provides nutrition suited for over 50 species of farmed fish, such as salmon, trout, sea bream, sea bass, yellowtail, turbot, halibut, cod, eel and bluefin tuna.

For bluefin tuna growout, feed production takes place in dedicated manufacturing facilities in Australia, Canada, Italy, Japan and Spain. Our bluefin tuna feed, Aquasoft Tuna, uses a patented manufacturing process to produce a large, soft pellet with a specifically tailored nutritional content. The highly palatable pellet ensures an ease in weaning, thus maximising growth and reducing feed conversion ratios. Additionally, the combination of a unique nutritional profile and enhanced feed intake in Aquasoft Tuna results in bluefin tuna that are healthy, with superior flesh quality characteristics at harvest that are maintained through to the consumer.

Skretting maintains its position at the forefront of the rapidly developing bluefin tuna industry with the support of the Skretting Aquaculture Research Centre. This internationally renowned institution researches and develops feed formulations and production technology, with a special focus on consumer safety, fish health and productivity, improved feed management and sustainability in feed production, all of which are attributes of AquaSoft Tuna.



AGE AND GROWTH OF FATTENING BLUEFIN TUNA (*Thunnus thynnus* L., 1758) IN THE EASTERN MEDITERRANEAN SEA

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Abstract
A total of 188 specimens of bluefin tuna were sampled from the Greek Bluefin Tuna farm, in the Ionian Sea, between December 2007 and January 2008. The samples ranged from 127 to 272 cm in fork length and from 43 to 475 kg in round weight. Their age was estimated from 5 to 16 years, using caudal vertebrae and the length-weight relationship revealed a positive allometric growth.

Keywords: Eastern Mediterranean, Thunnus thynnus, Age, Growth, Caudal vertebrae

1. Introduction

Bluefin tuna (*Thunnus thynnus* L., 1758) is a large pelagic fish with a highly migratory behavior and trans-oceanic movement. It is found in the Atlantic Ocean including the Mediterranean Sea and sustains important recreational and commercial fisheries as well as the aquaculture industry Block et al, 2005; Tzoumas et al., 2009). The aim of this study is to estimate the age of fattening bluefin tuna using caudal vertebrae and to determine the length-weight relationship.

2. Materials and Methods

Fork length (FL) and round weight (RW) measurements were taken from 188 bluefin tuna specimens sampled from the Greek Bluefin Tuna farm, in the Ionian Sea, from December 2007 to January 2008. Length measurements were taken to the nearest centimeter (cm) and weight to the nearest gram (g) and the length-weight relationship was calculated using the equation $RW=a*FL^b$.

A total of 99 caudal vertebrae were used to estimate age by counting the annual growth zones observed on the inner surface of the cones of the whole vertebrae. One ridge and one groove were interpreted as one annulus. Mean lengths at age and the precision of the ageing method were calculated.

3. Results and Discussion

Fork length and round weight data ranged from 127 to 272 cm and 43 to 475 kg, respectively. The more frequent length classes were between 220 and 240 cm and the more frequent weight classes were between 240 and 270 kg. The slope of the length-weight relationship was bigger than 3 indicating positive allometric growth (Fig. 1).

However, the length-weight relationships for the wild individuals present mostly negative allometric growth (Tzoumas et al, 2009).

Several studies have estimated the age and growth of wild bluefin tuna using calcified structures, but no one has estimated the age of fattening bluefin tuna. The range of the estimated ages was from 5 to 16 years with mean fork lengths 139 cm and 267 cm, respectively (Fig. 2). The age group 10 was dominant. From the samplings we had no young specimens, because at the aquaculture there are only older, from 5 years and over, specimens. A comparison of our results with similar studies revealed that the fattening bluefin tuna present higher mean lengths at age values than these of the wild bluefin tuna (Rodriguez-Marin et al., 2007).

The Average Percent Error (APE), the Coefficient of Variation (CV) and the Index D were 2.21%, 2.89% and 1.67%, respectively. The values of precision estimated keep up with the values of the existing bibliography (Rodriguez-Marin et al., 2007).

The present study revealed the difficulty in distinguishing between the closely spaced increments on the centrum margin of the vertebra that becomes severe at the age of 8 years and onwards. The same difficulty has also been noticed in previous studies (Rodriguez-Marin et al., 2007).This probably constitutes the major disadvantage of the vertebra method and it is likely to underestimate the age of older fish.

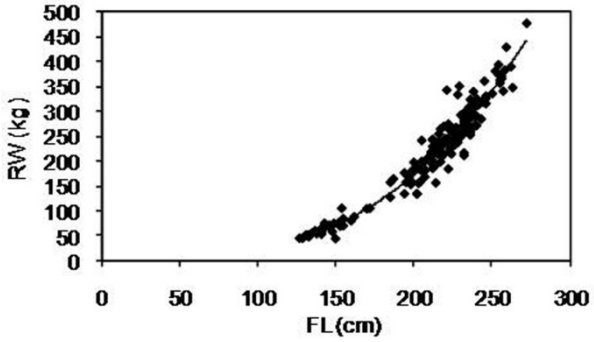

Fig. 1 - Fork length-round weight relationship of fattening bluefin tuna, *Thunnus thynnus* ($RW=1.3*10^{-5}*FL^{3.09}$, $R^2=0.96$, n=188).

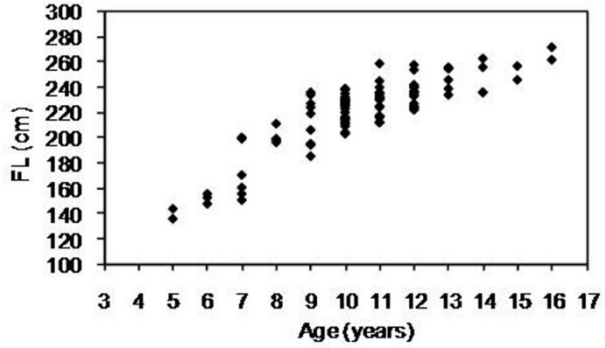

Fig. 2 - Fork length versus age estimates from caudal vertebrae band counts of fattening bluefin tuna, *Thunnus thynnus* (n=99).

4. Conclusion

The length-weight relationships for the wild and fattening individuals present mostly negative and positive allometric growth, respectively.

Vertebrae tend to underestimate age probably because of the crowded banding at the edge of the centrum at the age of 8 years and onwards.

Acknowledgments
We thank Mr A. Tzoumas, Mr K. Papas, Mr B. Zikos and Japanese commercial fishermen, who co-operated during the samplings, at the Bluefin Tuna farm in the Ionian Sea.

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INFECTION AND PARASITIC NEMATODES IN AUSTRALIAN MARINE FISH: PUBLIC HEALTH IMPORTANCE

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1. Introduction

Globally, anisakid nematodes can infect a wide range of invertebrates and vertebrates during their life cycles (Anderson, 2000) and cause significant disease known as anisakidosis (Audicana et al., 2002; Nieuwenhuizen et al., 2006, 2009; Lopata, 2007; Lopata and Lehrer, 2009). In spite of their importance, many fundamental aspects of their biology, epidemiology and systematics remain unknown, mainly because it is not possible to identify larval stages and females to species level morphologically (Adroher et al., 2004; Shamsi et al., 2008, 2009 ab). It is obvious that the right identification of any pathogen is central to establish preventive strategies to control it. Therefore, the aims of the present study were:

- To combine the use of morphological and molecular methods (using genetic markers in nuclear rDNA) for the specific identification of anisakid nematodes.
- To investigate the genetic make-up of anisakid nematodes in a range of different (definitive and intermediate) host species in Australia.
- To determine the incidence and prevalence of these parasites in various fish species in Australia.
- To determine the occurrence of allergens in *Anisakis* and related parasites causing allergic response in sensitised consumer.

2. Materials and Methods:

Since April 2009, 12 species of marine fish from Victoria and South Australia (Table 1) were examined for parasitic infection. Parasitic nematodes were cut in the mid-part (which is not important for morphological examinations) for DNA extraction and ITS-1 and ITS-2 were amplified and characterised (Shamsi et al., 2008, 2009ab). Anterior and posterior parts of the nematodes were examined under microscope to verify morphotypes (Cannon, 1977).

3. Results and Discussion:

Except for sand flathead and Tommy rough, 100% of all other fish were infected with larvae of anisakid nematodes. Nematode larvae were classified into at least four distinct morphotypes, including *Anisakis, Contracaecum, Hysterothylacium* and *Terranova.* Molecular studies showed that *Anisakis* type larvae in Australia, comprise at least four species including *A. pegreffii*, *A. simplex* C, *A. typica* and *A. brevispiculata.* The first three parasites become mature in dolphins and the latter in pigmy sperm whale, respectively. Larvae of *A. pegreffii*, found in the present study, in particular are very important as they are responsible for more than 90% of the cases of human infections worldwide (Lopata and Lehrer, 2009). In addition, it was shown that *Contracaecum* type larvae comprise at least four distinct species including *C. multipapillatum, C. pyripapillatum, C. rudolphii* genotypes D and E and *C. ogmorhini*. The first two species, infect the Australian pelican. *C. rudolphii* are found in cormorants and *C. ogmorhini* infect Australian fur seal. It is known that *Contracaecum* type larvae can cause disease in human. However, it is still not known which species is involved in human anisakidosis. *Hysterothylacium* larvae were the most diverse anisakid larvae found and except for one larvae which identified as *H. pelagicum* occurring in Marlene, the identity of about 13 morphotypes of this genus have to be identified. Only one *Terranova* type larvae were found both morphologically and genetically, but no species identification has been confirmed. The same is true for *Contracaecum* type larvae, of which human cases of infection with *Hysterothylacium* type larvae have been reported, the implication of additional parasite species in infection and allergic reactions has yet to be confirmed.

Funding: This project has been financially supported by the Australian Biological Resource Study (ABRS).

Table 1 - Occurrence and prevalence of anisakid nematodes in the present study.

Fish species	No	Infection rate	No of parasite	Geographic location
Eastern Australian Salmon	2	100%	2-10	South Australia
King George whiting	7	100%	2-15	South Australia
Red snapper	5	100%	20-75	South Australia
Sand flathead	10	20%	1-2	Victoria
Snapper	5	100%	25-93	South Australia
Southern garfish	4	100%	3-7	South Australia
Swallowtail	5	100%	5-27	South Australia
Tiger flathead	25	100%	41-2000	Victoria
Tommy rough	9	84%	3-12	South Australia
Western Australian salmon	24	73%	2-10	South Australia
Yellow eye mullet	5	100%	1-31	South Australia
Yellow fin tuna	1	100%	10	New South Wales
Yellow fin whiting	5	100%	10-102	South Australia

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HATCHING SUCCESS AND EARLY LARVAL DEVELOPMENT OF SOUTHERN BLUEFIN TUNA (*Thunnus maccoyii*)

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1. Introduction

Southern bluefin tuna (SBT, *Thunnus maccoyii*) is a new species to aquaculture that will have a high impact on the industry if its life cycle is closed. The demand for high quality tuna meat makes it one of the highest priced seafood species in the world. SBT is widely distributed between the Atlantic, Indian and Southern Oceans between 30 and 50 ° latitude (Lu et al., 2008). SBT is caught in the Great Australian Bight off the south eastern coast of Australia. The catch for the 2007-08 season was valued at approximately AUD $210 million (ABARE, 2008).

The first successful spawning and hatching of eggs from captive SBT broodstock occurred at Clean Seas Tuna Ltd., Arno Bay, South Australia, in March 2008 and the first significant spawning occurred in the following summer.

Embryonic development, survival and yolk utilization of larvae is affected by environmental conditions, particularly temperature and salinity (Morehead & Hart, 2003; Cook et al., 2005). In order to achieve the goal of successful hatchery production of tuna larvae, the salinity and temperature ranges must be narrowed to maximize hatching rate and larval survival. The current study aimed to examine the embryonic development of captive bred SBT larvae and explore the impact of incubating temperature and salinity on hatching success.

2. Material and Methods

SBT eggs were collected from the water surface of the indoor broodstock tank (Clean Seas Tuna Ltd., Arno Bay tuna broodstock facility) soon after fertilisation at the 2-cell embryonic stage. In the temperature experiment, one hundred viable eggs were placed in each 250 ml Erlenmeyer flask. Replicate flasks (n = 4) were then distributed among four water baths set at 23, 25, 27 and 29 °C. In the salinity experiment, four salinities (25, 30, 32 and 37 ‰), were made up by adding distilled water or artificial sea salt (Instant Ocean®) to seawater (~ 32 ‰) in 250 ml Erlenmeyer flasks. One hundred viable eggs were placed into the salinity treatment flasks without acclimation and four replicate flasks were randomly distributed among four water baths set at 25 °C. Each beaker was aerated and a 24-hour photoperiod was maintained in the lab throughout both experiments.

After 20 hours incubation the number of hatched larvae were sampled from each beaker at 2 hour intervals. The newly hatched larvae were collected and anesthetised with Aqui-S (Aqui-S, New Zealand) prior to measuring the length using a calibrated ocular micrometer on a dissecting microscope.

The developmental stages of embryos and larvae were described from fertilized eggs incubated in 700 l tanks at 25 °C and newly hatched larvae stocked in 2000 l tanks. Light regimes were under natural light conditions 13 L: 11D (~10 000 lux at midday).

3. Results

SBT egg development was rapid and was temperature-dependent (Fig. 1a). Hatching time significantly decreased with an increase in temperature (r^2 = 0.998). Time to hatch was as low as 20 hours when eggs were incubated at 29 °C. Time to hatch increased with an increase in salinity, taking up to 31 hours to hatch at 37 ‰ (Fig. 1b).

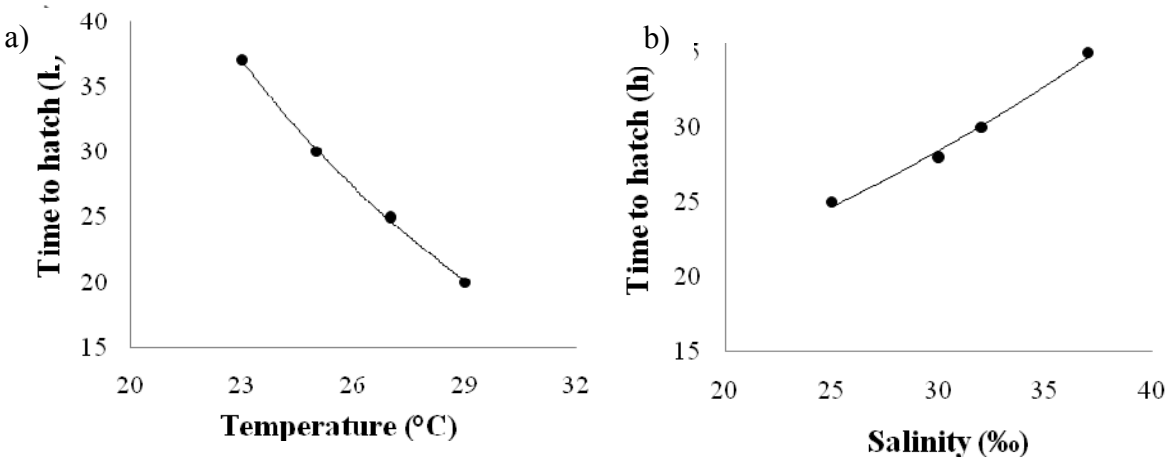

Fig. 1 - a) Mean time taken for *T. maccoyii* eggs to hatch at different temperatures (23–29 °C); curves fitted are exponential, $Y\ (hour) = 381.0T^{-0.10}$, $r^2 = 0.998$ ($n = 16$), where T is water temperature (°C). b) Mean time taken for *T. maccoyii* eggs to hatch at different salinities (25– 7 ‰); $Y\ (hour) = 12.21S^{0.028}$, $r^2 = 0.991$ ($n = 16$), where S is salinity (‰).

The length of newly hatched larvae incubated at different temperatures were significantly different (Table 1). The eggs incubated at higher temperatures (27 and 29 °C) resulted in longer larvae than those incubated at lower temperatures (23 and 25 °C). Hatch rate declined with increasing temperature from 79% to 56% but the differences were not significant.

The length of the larvae incubated at different salinities were not significantly different, but there was a significant reduction in the hatching rate when the eggs were incubated at a salinity of 37 ‰.

Table 1 - Effects of temperature and salinity on fish length and hatch rate in *T. maccoyii*. Values (mean ± SE) within a column with different superscripts are significantly different (ANOVA, $P < 0.05$).

Treatment	Length (mm)	Hatch rate (%)
	Temperature (°C)	
23	2.49 ± 0.018[a]	79 ± 8.62[a]
25	2.46 ± 0.019[a]	71 ± 6.06[a]
27	2.57 ± 0.020[b]	68 ± 6.93[a]
29	2.56 ± 0.020[b]	56 ± 6.96[a]
	Salinity (‰)	
25	2.54 ± 0.020[a]	78 ± 9.39[a]
30	2.47 ± 0.019[a]	64 ± 4.62[a]
32	2.50 ± 0.015[a]	72 ± 4.26[a]
37	2.51 ± 0.022[a]	47 ± 2.40[b]

The development duration from fertilization to hatching was approximately 26 h at 25 °C (Fig. 2). The first cell cleavage (2-cell stage) occurred about 0.30 h after fertilization, gastrulation occurred about 6 h after fertilization and the neurula stage, head developed, occurred about 11.30 h after fertilization. The advanced embryo or tail-free stage occurred 24 h after fertilization and the larvae hatched about 26 h after fertilization. The eggs were positively buoyant after fertilization, rising to the surface of the broodstock tank. The specific gravity of the eggs changed just before hatching, becoming negatively buoyant.

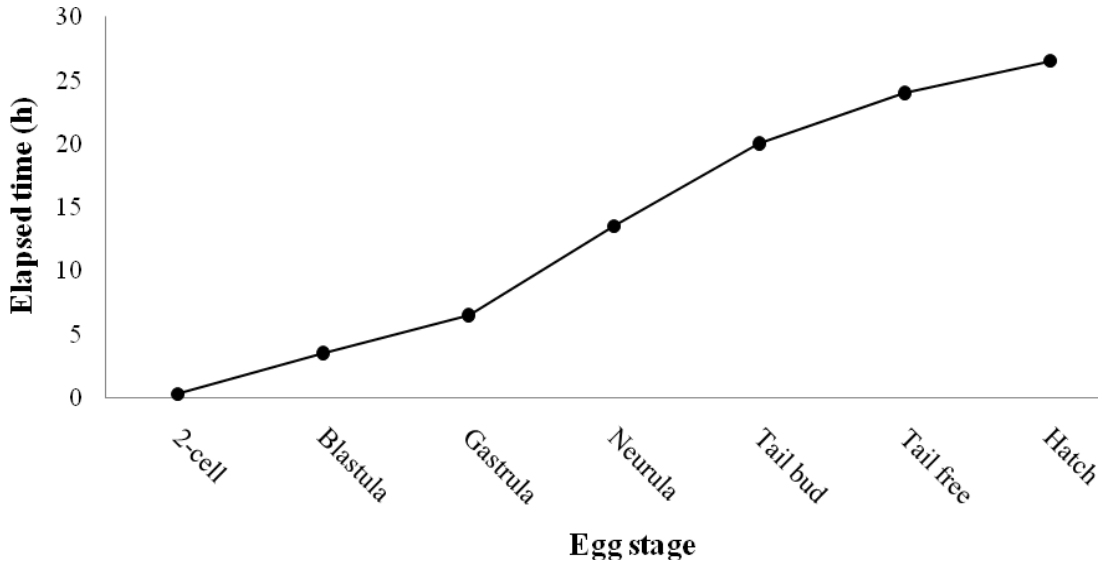

Fig. 2 - The developmental stages of *T. maccoyii* eggs from fertilization to hatch incubated at 25 °C.

Newly hatched larvae were 2.5 ± 0.02 mm in length. Larvae were unpigmented and had no developed mouth or digestive tract after hatching. The larvae had a large oval-shaped yolk sac (372 ± 9.7 μm) with a single oil globule (228 ± 5.1 μm) and a prominent fin-fold keeping the larvae positively buoyant. Within 3 days post hatch

(dph) the yolk sac and oil globule were almost fully absorbed and at the same time exogenous feeding began. By 4 dph the digestive tract had bent and was filled with rotifers. The pectoral fins developed and the otoliths were distinct with three partly ossified semicircular canals. Swimbladder inflation occurred at 7 dph with a mean bladder volume of 0.0014 mm^3.

4. Discussion

Time from fertilization to hatch was decreased to just 20 hours when eggs were incubated at 29 °C but hatching rate was lowered to 56 %. Temperature directly influences developmental rates and development is faster at increasing temperatures (Das et al., 2006). Many studies have shown that incubation temperatures influence hatched larvae size, with larvae generally being smaller in size when incubated at higher temperatures (Bermudes & Ritar, 1999; Morehead & Hart 2003). However, this study did not show this effect as the newly hatched larvae incubated at higher temperatures were larger in length than those incubated at lower temperatures.

Hatching rates were unaffected by salinity from 25 to 32 ‰. Increased salinity did not affect the size of fish larvae at hatching, but hatching rates were significantly reduced in the 37 ‰ salinity treatment. Salinities between 32 and 37 ‰ were not included in the hatching trial. However, as the salinity at the natural spawning ground of SBT is 35 ‰, it is necessary to refine the salinity range in future studies for SBT hatching.
The morphology of SBT eggs and newly hatched larvae are typical of other marine piscivore species. The eggs (>1 mm in diameter) and hatched larvae size (2.5 mm) are simililar to those recorded for yellowfin tuna (Margulies et al., 2007) and Pacific bluefin tuna (Kawamura et al., 2003). Margulies et al. (2007) reported a similar pattern of specific gravity change during the egg development of yellowfin tuna as we discovered in SBT. The negative buoyancy occurs just before hatching when the chorion of the eggs breaks down and more water diffuses into the egg. Therefore, when designing a larval rearing facility, the negative buoyancy of SBT in the early developmental stage needs to be considered.

In conclusion, SBT eggs take 26 hours to hatch at 25 °C. The average hatching rate was 71 % at salinities between 25-32 ‰. The embryonic development of SBT is similar to yellowfin tuna and Pacific bluefin tuna, suggesting the rearing technology developed for other tuna species may be adapted to SBT larval rearing.

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