Australian Government

Australian Centre for International Agricultural Research

Final report

project

Achieving consistent spawning of captive yellowfin tuna (*Thunnus albacares*) broodstock at Gondol Research Institute for Mariculture, Bali, Indonesia

project number	FIS/2006/140
date published	July 2012
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final report number	FR2012-20
ISBN	978 1 921962 90 5
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

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1 Acknowledgments

The project participants acknowledge the efforts of Dr Mike Rimmer (formerly Research Fellow at James Cook University), Dr Ketut Sugama (Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries, Republic of Indonesia) and Barney Smith (ACIAR) who initiated discussions and assisted preparation of applications for this project. Dr I. N. Adiasmara Giri (Head of Fisheries Research and Development Centre Cultivation, Ministry of Marine Affairs and Fisheries) provided ongoing support to maintain infrastructure, operational costs and staffing of the project at Research Institute for Mariculture (RIM), Gondol. The RIM Gondol yellowfin tuna propagation project team Gede Sumiarsa, I G. Ngurah Permana, Ananto Setiadi, Gunawan, Ketut Sutaryasa, Jafar Shodiq and Putu A. Sudarsana provided ongoing dedicated scientific and technical support. The insightful advice and ongoing positive support from Professor Dan Benetti, Rosentiel School of Marine and Atmospheric Science, University of Miami is also acknowledged as are the efforts of Rob Michael, Australian Institute of Applied Aquaculture, Challenger TAFE who provided assistance with demonstrating improved broodstock capture methods.

2 Executive summary

Yellowfin tuna, *Thunnus albacares*, aquaculture research has been conducted at the Research Institute for Mariculture (RIM), Gondol, Bali, since February 2003 when construction of an onshore tank system for holding tuna broodstock was completed as part of the Overseas Fishery Cooperation Foundation (Japan) and Ministry of Marine Affairs and Fisheries (Republic of Indonesia) joint "Project for the research on propagation of tuna species". The objective of this project was to develop techniques for tuna species resource enhancement. First spawning of captive broodstock occurred in October 2004 and 150 metamorphosed fingerlings (50 days post hatch) were produced in 2005. The most productive spawning period extended over a 274 day period from 25 August 2005 until 24 May 2006 during which the fish spawned on 259 occasions producing a total of 187.7 million eggs.

In Australia there is interest in commercial aquaculture of yellowfin tuna and it is expected that successful spawning and larval rearing of this species at RIM Gondol will provide the confidence required for commercial investment. In addition, yellowfin tuna is considered to be a potential surrogate species for a range of research activities that could contribute to development of propagated southern bluefin tuna, *Thunnus maccoyii.*

An initial objective was to attempt to capture greater numbers of larger yellowfin tuna within a 30 day period so that these fish would attain maturity within the duration of the project. Consequently, during November 2008 the 40 gross tonne tuna long lining vessel 'Kapal' was chartered to capture between 40 and 60 replacement broodstock greater than 5.0 kg. Although 84 fish were captured the average size (2.7 kg) of fish was less than the target size and survival of fish following transportation was low with only 13 fish transferred into land based holding tanks. This approach was discontinued due to the limited results achieved for the amount of logistical planning and the high cost.

During 2009 a total of 149 fish were caught using local line fishing methods. A high proportion of these were small fish less than 0.5 kg. High mortality of these small fish occurred following transfer into the acclimation tank. The four fish that survived until stocking into the main broodstock tank had an initial body weight greater than 2 kg and had grown to between 5 kg and 9 kg when they were transferred into the main spawning tank in February 2010.

In January 2010 a number of the recommendations were developed by the project participants that have since been implemented to improve the success of broodstock capture. As a result of these changes the average weight of fish captured increased from 0.3 kg to 7.45 kg between 2009 and 2010. A high proportion (93%) of fish landed now survive holding and transfer to RIM Gondol and most (90%) of these fish also survive holding during acclimation prior to stocking into the main spawning tank. The larger initial size of these fish means that they will also reach maturity within a shorter period of captivity.

In September 2009 three large broodstock (2 x 86 kg, 1 x 125 kg) remaining in the main broodstock tank from before this project were removed and all were male. To overcome issues of sexing yellowfin tuna broodstock, samples of blood, fin clips and a biopsy of white dorsal muscle were taken to assess the suitability of using a commercial enzyme linked immunoassay kit for 11 – ketotestosterone (11-KT) to determine sex. Positive results for 11-KT matching the sex of fish from post mortem examination were obtained from the least invasive method (i.e. fin clips).

Samples were also taken from six smaller fish (6.6 to 12.0 kg) to determine if meaningful 11-KT results could be obtained from these immature fish. 11-KT assay on fin clips suggested that five of the six were males. Four of these fish have subsequently died and for each fish their sex determined by autopsy has matched the sex determined by 11-KT assay of fin clip samples. The smallest fish from which a positive 11-KT test was obtained at transfer was 6.6 kg.

During October 2010 the main tuna broodstock tank at RIM Gondol was holding 25 recently stocked fish. These broodstock spawned three times between 24 October and 26 October 2010. A total of only 34,000 eggs (11,300 per spawning) was produced and fertilization rate was very low (1 %).

Comparison of the conditions prevailing in the broodstock tank during the extended spawning period from 25 August 2005 until 24 May 2006 with those prevailing during 2010 suggest that number of fish, sex ratio and water temperature may be significant factors affecting spawning success for captive yellowfin tuna broodstock. During the best spawning period the number of broodstock in the spawning tank ranged from 15 to 27 fish while the average sex ratio was 1.14 ± 0.2. Comparison of the water temperature prevailing in the holding tank in the most successful spawning years shows that temperatures were significantly higher during the period approaching the expected start of spawning in 2010 and to a lesser extent 2009. It is suggested that the higher than normal water temperatures prevailing during the 2010 season may not have provided the environmental signal required to initiate gonad recrudescence and subsequent spawning of recently established first maturing broodstock.

Together these developments have allowed establishment of a potentially productive population of captive yellowfin tuna broodstock at RIM Gondol that given exposure to normal seasonal conditions, should provide a supply of eggs during 2011. The tools developed by this project will allow researchers at RIM Gondol to manage the ongoing need to replace broodstock to maintain a desirable sex ratio to optimise spawning success.

During some years yellowfin tuna eggs and larvae spawned at RIM Gondol became infected with the endoparasite *Ichthyodium chabelardi*. During 2005 spawning started in August and the endoparasite was first detected on 20 October 2005 after broodstock had spawned on 40 occasions. The prevalence of the infection reached 100 % within one week and continued until spawning ceased June 2006. PCR testing with a primer set designed from the 18s rRNA sequence of this parasite showed that eggs and larvae of the parasite were found in the water from the broodstock tank and it was concluded that the endoparasite infected tuna eggs horizontally (Permana et al., 2007; Yuasa et al., 2007). The life cycles of this endoparasite has been study by Hutapea and Permana, (2007) who found that the endoparasite could survive for about 3 days during which time tuna eggs spawned in the main tank could be infected.

Investigations conducted at RIM Gondol on options to control infection by the endoparasite concluded that infection could be prevented by harvesting eggs just after fertilization and bathing these with formalin at 25-100 mg L^{-1}. This treatment was only effective against the free swimming stage of the endoparasite (Zafran *et al.,* 2006). The method was of limited value because only small numbers of recently spawned eggs could be harvested. It is considered that the most efficient water treatment would be to install a UV system to disinfect all incoming seawater since the endoparasite showed horizontal infection. Another suggestion is to increase the water exchange rate within the broodstock holding system to flush a significant proportion of endoparasites from the tank between spawning events.

Hatchery production of tuna species requires large, dedicated facilities and highly trained staff; it is a very long term and comparatively high-risk undertaking; but the

potential benefits are very significant. These facilities and capabilities are available at RIM Gondol providing a significant opportunity for this institute to be at the forefront of International R&D being undertaken to commercialise yellowfin tuna aquaculture. Future engagement will to an extent depend on the Indonesian Government's assessment of the benefit-cost in continuing the program on tuna reproduction at RIM Gondol. The potential to undertake cost effective R&D on tuna larval rearing at an established facility in close proximity remains as a potential benefit to propagated tuna aquaculture in Australia.

3 Background

Yellowfin tuna, *Thunnus albacares*, is a commercially important tuna species that is widely distributed in tropical and subtropical waters (Kaji et al., 1999; Marguiles et al., 2007). Yellowfin tuna has a fast growth rate and wide market acceptance and is considered to be a promising species for tuna aquaculture (Kaji et al., 1999). Investigations on the larval rearing of yellowfin tuna started in 1970 at the Shirahama Fisheries Laboratory of Kinki University and in 1993 the first spontaneous spawning occurred from captive broodstock held in floating cages at the Yaeyama station of the Japan Sea-Farming Association (Kaji et al., 1999). Since 1996, spawning has occurred in yellowfin tuna held in an onshore tank system at the Inter-American Tropical Tuna Commission (IATTC) Achotines Laboratory, Los Santos Province, Republic of Panama (Marguiles et al., 2007). Larval rearing of yellowfin tuna is routinely achieved at this facility and these are primarily used to investigate behaviour of larvae and effects of environmental factors to develop a better understanding of processes effecting recruitment of larvae to support wild stocks of tuna.

Yellowfin tuna aquaculture research has been conducted at the Research Institute for Mariculture (RIM), Gondol, Bali, since October 2003 when construction of an onshore tank system for holding broodstock was completed as part of the "Project for the research on propagation of tuna species in the republic of Indonesia". This project was conducted under a Memorandum of Understanding between the Overseas Fishery Cooperative Foundation (OFCF) of Japan and the Ministry of Marine Affairs and Fisheries (MMAF) of the Republic of Indonesia. This project funded the design and construction of an onshore broodstock holding system (Figures 1a and 1b), equipped laboratories and provided scientific expertise and operating costs. Capture of yellowfin tuna broodstock commenced in 2003 after commissioning of the onshore holding facility and first spawning occurred in October 2004. Larval rearing investigations were conducted and 150 metamorphosed fingerlings (50 days post hatch) were produced in 2005. The OFCF funded yellowfin tuna project at RIM ended in 2006.

Fig. 1. a. Building housing the yellowfin tuna broodstock holding system at RIM, Gondol

b. Main spawning tank and acclimation tank of the yellowfin tuna broodstock holding tanks at RIM Gondol.

During 2006 the Australian Centre for International Agricultural Research (ACIAR) initiated consultation between Indonesia and Australia regarding a collaborative R&D program to enhance the yellowfin tuna aquaculture project being conducted at RIM Gondol. From these discussions it was proposed that the South Australian Research and Development Institute (SARDI) work with researchers at RIM to re-establish consistent spawning of yellowfin tuna broodstock. Subsequently, the project was amended to include expertise of researchers at the Australian Centre for Applied Aquaculture, Challenger TAFE, Fremantle, as they are investigating the potential to culture this species in Western Australia. In Australian there is interest in the commercial aquaculture of yellowfin tuna and it is expected that successful spawning and larval rearing of this species at RIM Gondol would provide considerable confidence for commercial investment.. In addition, yellowfin tuna is considered to be a potential surrogate species for a range of research activities that could contribute to development of propagated southern bluefin tuna, *Thunnus maccoyii* (SBT), which is currently being investigated in South Australia. In July 2008 the collaborative project "Achieving consistent spawning of captive yellowfin tuna, *Thunnus albacares*, broodstock at the Research Institute for Mariculture, Gondol, Bali, Indonesia", commenced and the results of the project are presented in this report.

4 Objectives

1. Regular spawning of high quality parasite free eggs from captive YFT broodstock at GRIM.

2. R&D collaborations between SARDI, GRIM and commercial companies that have an interest in tuna propagation in Australia.

3. Final report including broodstock spawning data (environmental and biological), review of parasite treatment adopted, and evaluation of the economic viability of YFT farming.

5 Methodology

Broodstock holding system

Construction of the onshore holding system for yellowfin tuna broodstock at RIM Gondol (Figure 2) was started in 2002 and was completed in February 2003. The facility comprises three holding tanks (Table 1) used to acclimate recently captured fish and ongrow these to a size suitable for spawning.

A seawater supply and recirculated seawater treatment system maintains water quality within the holding tanks. Seawater is drawn from an intake point 250 m off the shore at a depth of approximately 15 m. Seawater is pumped through high pressure sand filters and then to an elevated header tank from where it is directed to the three tanks of the holding facility. New filtered seawater can be supplied to the broodstock system by 2 pumps that each has a capacity of 1 m^3 min^{-1} providing a total volume of 2,880 m^3 day^{-1} (i.e. 192% of the volume of the main spawning tank day^{-1}).

In addition, water from the holding tanks is recirculated through a treatment system comprised of three pumps, pressure sand filters, a degassing unit and biological filtration. Depending on the number of pumps operated, the water treatment capacity of this system is between 120 m^3 hr^{-1} and 150 m^3 hr^{-1}, or between 2,880 m^3 day^{-1} and 3,600 m^3 day^{-1} (i.e. 192 % - 240% volume of main tank day^{-1}).

Table 1.

Specifications of the yellowfin tuna broodstock holding tanks at RIM.

Tank	Number	Diameter (m)	Depth (m)	Volume (m^3)
Acclimation tank	2	8	3	135
Main holding tank	1	18	6	1,500

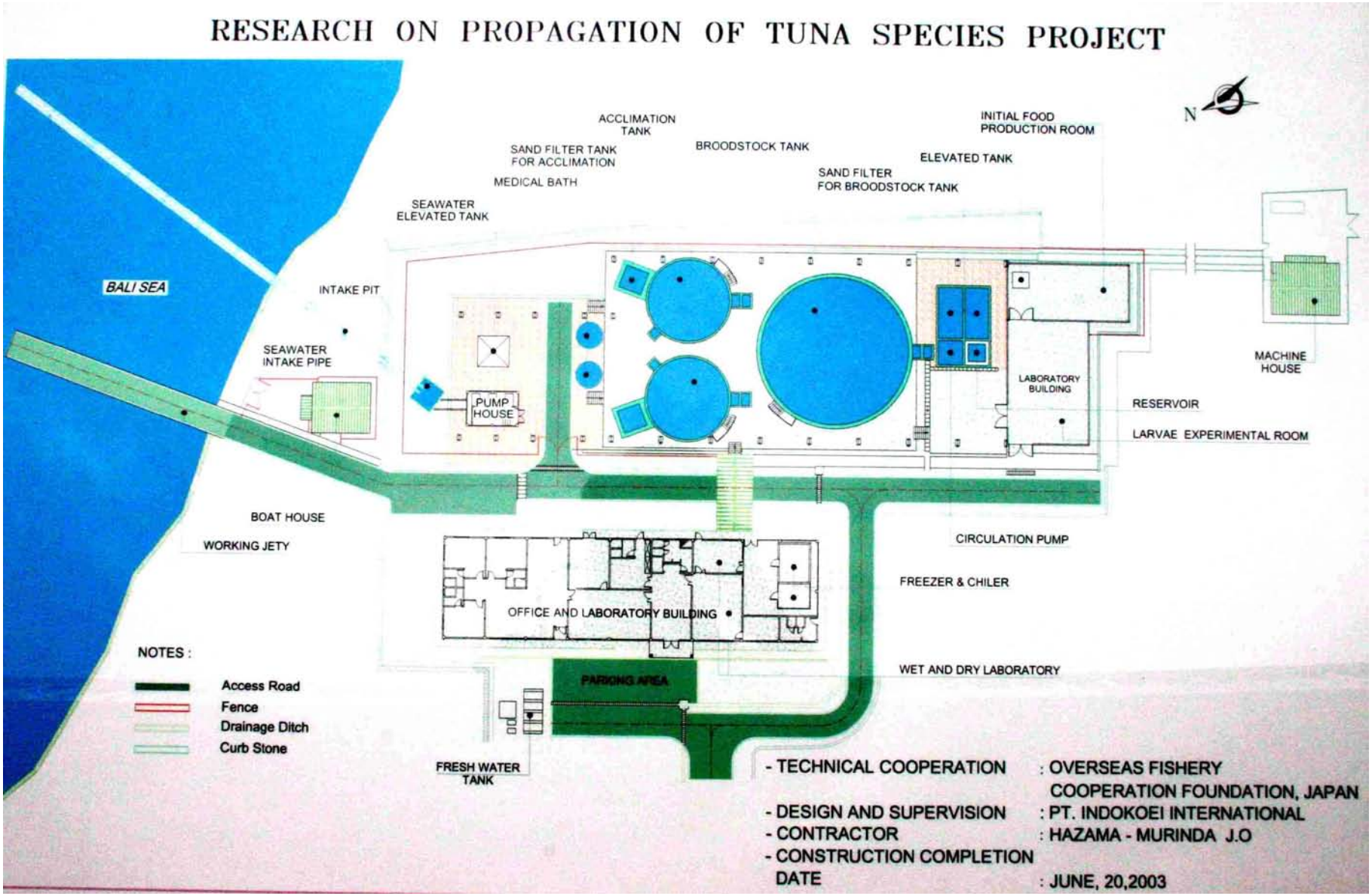

Fig. 2. Layout of the major components of the OFCF funded onshore holding system for yellowfin tuna broodstock constructed at RIM Gondol.

Broodstock capture

Between 2003 and 2008 fishing was generally conducted near local fish aggregating devices (FAD's) constructed of bamboo and palm fronds anchored at sea (Figure 3a). Fishing and transportation of live yellowfin tuna was undertaken from the open deck vessel "Benkunis" (Yamaha, 12m x 3m, 2 x 85 hp) (Figure 3b) supplied by OFCF. The vessel is equipped with a 2,000 L tank on deck with water supplied by a bilge pump when stationary and a scoop over the side when motoring. Fishing was conducted using traditional baited hook and line for fish at depths of between 20-100 m; and trawling using simple lures (Figure 4a and 4b) when schooling tuna were located at the surface. During this period there was a tendency to capture small yellowfin tuna between 1.0 and 3.0 kg as survival during capture and transport was better for this size of fish and the smaller yellowfin tuna were more abundant than bigger size fish. Conversely it was observed that after transfer to the onshore facility the bigger fish survived better in the tank compare to smaller yellowfin tuna.

Fig. 3. a. Fish aggregating device targeted for capture of yellowfin tuna near RIM Gondol.

b. RIM Gondol vessel "Bengkunis" used for capture and transfer of live yellowfin tuna.

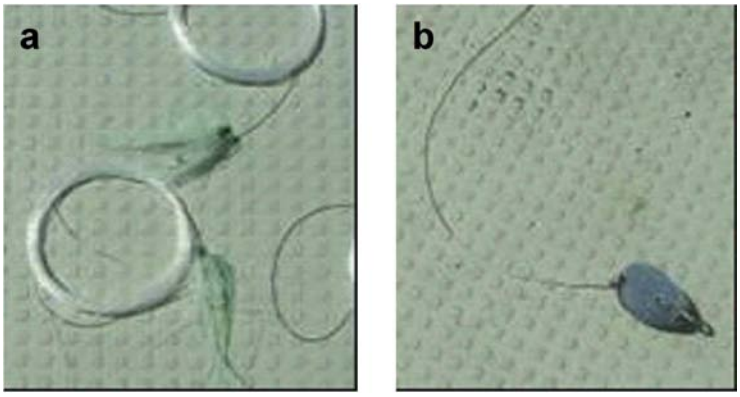

Fig. 4. Lures made to fish for yellowfin tuna near the surface.

a. Handmade bristle lures b. Handmade metal lure.

At the start of the ACIAR project in July 2008 there were 7 broodstock remaining in the main broodstock holding tank at RIM. These fish were estimated to be between 60 and 100 kg. Due to the relatively short 18 month duration of the project, it was considered to be a priority to capture between 40 and 60 broodstock of a size greater than 5 kg so that these fish could and reach a mature size and commence spawning within the time available. The typical fishing methods used at RIM until 2008 provided an average of only 0.3 fish successfully captured, transported and transferred into the acclimation tank from each fishing day. Hence to capture between 40 and 60 new acclimated broodstock would require in the order of 120 to 180 fishing days. Consequently an alternative approach was proposed investigated in which a larger tuna fishing vessel was chartered to allow larger numbers of fish to be captured and maintained at sea before returning to RIM Gondol. The objective of this approach was to capture the desired number of replacement broodstock within 30 days.

A number of fishing vessels were inspected by staff of the RIM Gondol to evaluate their suitability for capture and holding of broodstock. During November 2008 the 40 gross tonne tuna long lining vessel 'Kapal' was chartered for 25 days from a fishing company based in Benoa, Bali. This vessel was equipped with five onboard tanks (2m x 2m x 2m) that were each considered suitable for extended live holding of 3 - 4 tuna up to 10 -12 kg. Prior to fishing some modifications were made to the tanks by RIM staff, including installation of smooth plastic panels to create rounded corners and supply of oxygen to each tank. During the charter period, the vessel was operated as a 'mother' ship with fishing conducted from the smaller 'tender' vessel "Bengkulis" operated by RIM staff. When up to 3 fish were captured these were transferred to the larger vessel allowing fishing to continue until 10 - 12 fish had been captured before returning to RIM.

In February 2009, project collaborators reviewed the progress of the project and identified changes to improve fishing and fish transportation methods. Following this a staff member of the AIAA visited GRIM for 5 days in May 2009 to trial different fishing methods to improve broodstock capture. Until September 2009 project funding for RIM was limited due to changes being made to financial administration processes within the Ministry of Marine Affairs and Fisheries (MMAF), Indonesia. Consequently limited fishing for new broodstock could be undertaken.

In January 2010 the project sponsored Professor Daniel Benetti (University of Miami, Rosentiel School of Marine and Atmospheric Science) to RIM Gondol for discussion between local research staff and project collaborators to identify further changes that could be made to improve capture, handling and holding of yellowfin tuna. During this visit RIM staff captured an additional six fish providing the opportunity to observe transportation, transfer and acclimation methods. From these observations and discussions the following suggestions were proposed;

- Buy fish from local fishermen at the time of capture and pay a premium price.
- Target only a maximum of two tuna per fishing trip. Once the first tuna is caught and determined to be in good condition, fish for additional fish for only another 15 - 30 minutes before heading back.
- Fill up transport tank and turn oxygen on prior to bringing fish into the boat. Use pure oxygen to maintain dissolved oxygen (DO) at >100%. Newly caught fish are in oxygen deficit and will benefit from high levels of oxygen.
- To minimise line damage caused by slow retrieval, test the strong braid or other leader line to allow rapid capture.
- To minimise line damage, use a fishing rod in order to keep the line off the head and body and enable fish to be retrieved faster.
- Cast heavy lures into surface schools of tuna.

- Provide further fishing support from Australia to help achieve improvements in fishing methods.
- Use a purpose built sling to transfer fish from the water directly into the transport tank. De-hook the fish prior to releasing from the sling.
- Avoid rapid changes of light intensity. Shade the transport tank for return to RIM Gondol.
- Use a sling to transfer fish from boat to treatment/medical tank for prophylaxis.
- Continue to maintain high levels of DO in the medical tank, particularly during the static bath period.
- Provide a high rate of water flow through the acclimation tank to ensure optimum water quality and low bacteria numbers.
- Consider formalin baths in the medical tank if further skin parasites are found.
- Consider a preliminary antibiotic injection followed by in-feed gelatin capsules containing an antibiotic if mortalities due to bacterial infection occur.

Sex determination

In September 2009 it was decided to remove three large broodstock and restock the main spawning tank with a larger number of smaller fish nearing maturity. Samples of blood, fin clips and white dorsal muscle were taken from the three large (2 x 86 kg, 1 x 125 kg) broodstock removed. These samples were used to assess the suitability of a commercial 11 – ketotestosterone (11-KT) enzyme immunoassay (EIA) kit (Caymen Chemical Company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA, Catalogue No. 582751) to determine sex of fish based upon colour change following extraction and incubation. Plasma was separated from red blood cells via centrifugation prior to analysis. White muscle samples were taken with both a large biopsy sampler and using a Genetag biopsy (modified Genetag hook, Genetag Research Project Fisheries, Department of Resources, GPO Box 3000, Darwin, Northern Territory, 0801, Australia). Steroids were extracted from fin clips and white muscle using diethyl ether before reconstituting into EIA buffer. Steroids in plasma were measured directly and in samples diluted with EIA buffer at ratios of 10:490, 50:450 and 100:400.

On the same day, six smaller fish ranging in size from 6.6 to 12.0 kg were transferred from an acclimation tank to the main broodstock spawning tank. From each fish, a fin clip and a small muscle biopsy was taken using a Genetag biopsy device to determine if meaningful 11-KT results could be obtained from these immature fish.

Parasite control

During some years yellowfin tuna eggs and larvae spawned at RIM Gondol have become infected with the endoparasite *Ichthyodium chabelardi*. No occurrence of endoparasites were detected during 2004. However, in 2005 spawning started in August and endoparasite was first detected on 20 October 2005 after broodstock had spawned on 40 occasions. The prevalence of the infection reached 100% within one week and continued until spawning ceased in June 2006. The infected eggs displayed opaque colour of yolk similar to the appearance reported from infected yolk sack larvae of Atlantic sardine, *Sardina pilchardus* (Yuasa *et al*., 2007). To confirm this, PCR with a primer set designed from the 18s rRNA sequence of this parasite was conducted that showed 98.1% similarity (completely matched). To understand the route of infection, two female and male broodstock (50 and 60 kg) were sacrificed, and the gonads were removed and samples taken. The gonads of squid and scad mackerel being used as feed were also sampled. In addition, samples were taken from the surface and bottom water of the broodstock tank. All samples were tested for the endoparasite following the same PCR protocol used for eggs and larvae.

6 Achievements against activities and outputs/milestones

Objective 1: Regular spawning of high quality parasite free eggs from captive YFT broodstock at GRIM.

no.	activity	outputs/ milestones	completion date	comments
1.1	Initiate improvements to re-establish spawning of existing broodstock in August – October 2008.	Achieved	October 2010	During October 2010 three small batches of eggs were spawned from a new population of broodstock established. It is likely that high water temperatures prevailing throughout 2010 have adversely effected reproductive development for this season. With favourable environmental conditions developing in 2011 it is expected that consistent and prolonged spawning of new broodstock will be achieved.
1.2	Trial improved broodstock capture methods to be developed to increase the number of broodstock to 40 fish.	Achieved	June 2010	Charter of a larger vessel with onboard holding tanks was trialled resulting in capture of 84 fish within 25 days. However, survival was low and the size of fish was smaller than required. This approach was discontinued. Broodstock capture and holding methods used at RIM Gondol were reviewed. A number of suggested improvements adopted have provided significant improvements in survival of fish until stocking into the main spawning tank. In addition much larger fish can now be captured and acclimated allowing effective broodstock replenishment using bigger fish that will reach maturity following a shorter period of captivity.
1.3	Sex determination	Achieved	September 2009	It was demonstrated that a commercial 11-KT EIA kit was able to discriminate sex of yellowfin tuna from fin clip samples. This provides an effective method to manage sex ratio of broodstock within the spawning tank

PC = partner country, A = Australia

Objective 2: R&D collaborations between SARDI, GRIM, the ADU, Professor Dan Benetti, Latitude Fisheries and other commercial companies that have an interest in tuna propagation in Australia.

no.	activity	outputs/ milestones	completion date	comments
2.1	Attendance of RIM staff at the YFT culture workshop (July 2008) conducted at the IATTC Tuna Research Facility, Achotines, Panama	Achieved	July 2009	RIM Gondol scientist, Mr John Hutapea, attended the YFT culture workshop (July 2009) conducted at the IATTC Tuna Research Facility, Achotines, Panama. In addition Mr Hutapea attended and presented a paper on the RIM Gondol YFT propagation program at the 2nd Global Centre of Excellence Tuna Aquaculture Symposium in Adelaide, December 2009.
2.2	Continued operation and improvement of the YFT propagation program at RIM.	Achieved	Ongoing	Capture methods developed by this project have allowed establishment of a broodstock population likely to spawn in 2011. These methods in combination with sex determination will allow broodstock replacement to maintain a managed spawning population.
2.3	SARDI collaboration with the YFT propagation program at RIM through regular visits.	Achieved	October 2010	Between June 2008 and October 2010, SARDI and AIAA scientists have visited RIM Gondol at approximately 6 monthly intervals to assist with all aspects of this project.
	Information transfer to commercial companies in Australia involved in propagation of SBT or other tuna species (i.e. YFT).	Achieved	October 2010	A representative from Latitude Fisheries visited RIM with ADU and SARDI researchers to discuss potential for future collaborations at this facility. SARDI and AIAA scientists informed Australian tuna companies interested in propagation of the activities and progress on YFT propagation at the RIM Gondol.
	A visit to Port Lincoln, South Australia by key RIM staff to inspect tuna farming operations and discuss future R&D collaborations with the commercial company involved in tuna propagation.	Achieved	December 2009	RIM Gondol scientist, Mr John Hutapea, visited aquaculture facilities in Port Lincoln and R&D facilities in Fremantle following the 2nd Global centre of Excellence Tuna Aquaculture Symposium in Adelaide, December 2009.

PC = partner country, A = Australia

Objective 3: Final report including broodstock spawning data (environmental and biological), review of parasite treatment adopted and evaluation of the economic viability of YFT farming.

no.	activity	outputs/ milestones	completion date	comments
1.1	Broodstock spawning data (environmental and biological)	Achieved	June 2010	Only three small batches of eggs were spawned from the new broodstock population established by the project at RIM Gondol. It is likely that high water temperatures prevailing throughout 2010 have adversely effected reproductive development for this season. A summary of broodstock spawning data is provided in this report. All relevant data is maintained by project scientists at RIM Gondol.
1.2	Review parasite treatment options	Achieved	September 2009	No significant or prolonged spawning was achieved during this project and parasite issues were not confronted. Previous scientific investigation of the life cycle and likely mode of transmission conducted by RIM Gondol tuna aquaculture scientists have suggested methods to reduce the impact of any future occurrence. The most likely effective method will require additional funds to install additional water treatment equipment.
1.3	Desk-top evaluation of the economic viability of YFT farming by SARDI, CRIA and JCU staff using farm-level bioeconomic models developed under FIS/2002/077.	Not achieved	Feb 2011	A potential provider agreed to complete this component. Following and series of ongoing delays the cost for the service was significantly increased and a decision was made in consultation with ACIAR to remove this activity from the project. It is expected that any future consideration of yellowfin tuna R&D will need to complete this activity.

7 Key results and discussion

Broodstock capture

The use of a large charter vessel allowed capture of the desired number of future yellowfin tuna broodstock within a 25 day period. However, although 84 fish were captured (Table 2) the average size of fish was less than the target size (>5.0 kg) and survival of fish following transportation was low with only 13 fish transferred into land based holding tanks. In addition travel warnings did not allow researchers from Australia to assist during this period. Consequently the objective to establish between 40 and 60 new yellowfin tuna broodstock within a short period was not achieved and the approach was discontinued due to the limited results achieved for the high amount of logistical planning and cost involved.

Table 2.

Fishing records for yellowfin tuna broodstock capture and transport using the chartered commercial tuna long-line vessel 'Kapal' during November 2008.

Year	Transportation method	Total No. fish Captured	Ave. weight (kg)	Load capacity (kg/trip)	Survival to land (number)	Survival into acclimation tank (number)
2008	Long line vessel with modified onboard holding tank with continuous oxygen	84	2.7 kg	5 -10	59.5 % (50)	15.5 % (13)

During 2009 a total of 149 fish were caught using local line fishing methods. A high proportion of these were small fish less than 0.5 kg (Table 3). High mortality of these small fish occurred following transfer into the acclimation tank because it was difficult to observe injured fish. A second mortality period was observed for fish that where feeding well and growing in the acclimation tanks. This was attributed to the very fast swimming speed of these small fish contributing to some fish colliding during feeding resulting in mortality after several days due to eye injury and abrasion on the side of the fish. Ultimately almost all of the small fish captured died. The four fish that survived until stocking into the main broodstock tank had an initial body weight at capture that was greater than 2 kg and grew to between 5 kg and 9 kg when they were transfer into the main spawning tank in February 2010.

A number of the recommendations developed by the project participants in January 2010 have since been implemented for fish capture, specifically:

- Fishing using rod and reel with braid line to retrieve fish faster.
- Use a vinyl cradle to land fish and transfer them to the onboard holding tank (Figure 5a and 5b).
- Installation of a fibreglass tank (2 m^3) onboard with a lid to reduce light during transportation (Figure 6a and 6b).

- Use of pure oxygen to maintain dissolved oxygen between 100-120% saturation in the onboard holding tank.
- Return to RIM Gondol no longer than 30 minutes after capture of the first fish.
- Use of a vinyl sling to transfer fish from the boat to the onshore treatment tank (Figure 7a and 7b).
- Use of pure oxygen and increased water flow while fish are held in the "medical" tank for during prophylactic treatments prior to transfer into the acclimation tank (Figure 8a and 8b).

Table 3.

Summary of broodstock capture operations conducted by RIM staff during 2009.

Year	Total no.fishing trips (successful)	Live holding method on vessel	Total No.fish	Survival to land base (number)	Mean weight (kg)	Average load (kg/trip) and size range (kg)	Survival in acclimation tank (number)
2009	27 (19)	PVC Tube, canvas tank 2.5 m3, no oxygen addition	149	67 % (100)	0.3	3.67 (0.5 - 8.5)	4 % (4)

Implementation of these changes resulted in significant improvement to the number of fish surviving until transfer into the main spawning tank. In particular it is suggested that following capture, fish in oxygen deficit recover faster in water that is fully saturated with oxygen helping to minimise a significant physiological stress that has previously contributed to subsequent mortality. These improvements have been significant in allowing larger fish to be captured, transferred and successfully established as broodstock at RIM Gondol (Table 4). The average weight of fish captured has increased from 0.3 kg to 7.45 kg between 2009 and 2010. A high proportion (93%) of fish landed now survive holding and transfer to RIM Gondol and most of these fish also survive holding during acclimation prior to stocking into the main spawning tank. The larger initial size of these fish means that they will also reach maturity within a shorter period of captivity.

Table 4.

Summary of broodstock capture operations conducted by RIM staff during 2010.

Year	Total no. fishing trips (successful)	Live holding method on vessel	Total No.fish caught	Survival to land base (number)	Ave. weight (kg)	Load capacity (kg/trip)	Survival in the acclimation tank (%)
2010	40 (21)	Canvas tank (2.5 m^3) or oval fibreglass tank (2.1 m^3) with continuous water flow and oxygen supplied	42	93% (39)	7.45	3-20	90% (35)

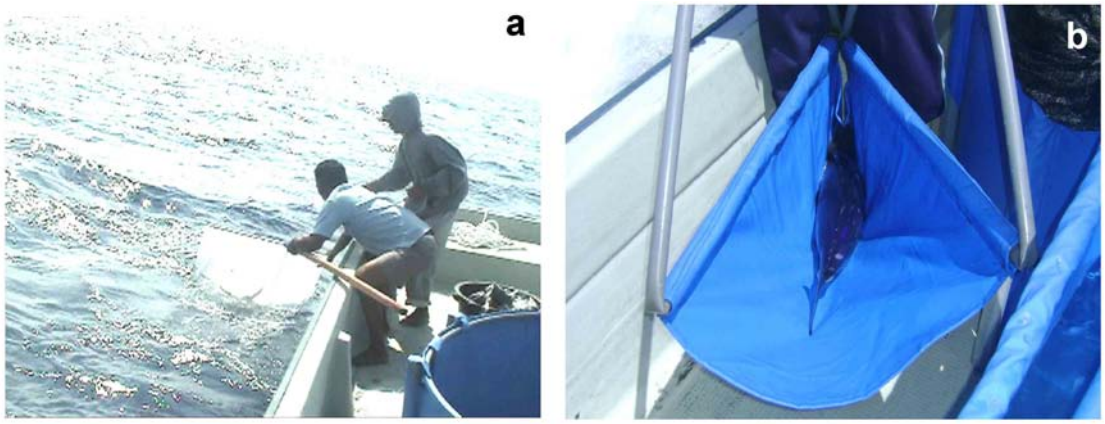

Fig. 5. a. Plastic lined net used previously to transfer fish to the holding tank.
b. Vinyl cradle used in 2010 to transfer captured fish to the holding tank

Fig. 6. a. Vinyl on-board holding tank (2 m^3) used until 2010.
b. Covered fibreglass holding tank (2 m^3) used with oxygen in 2010.

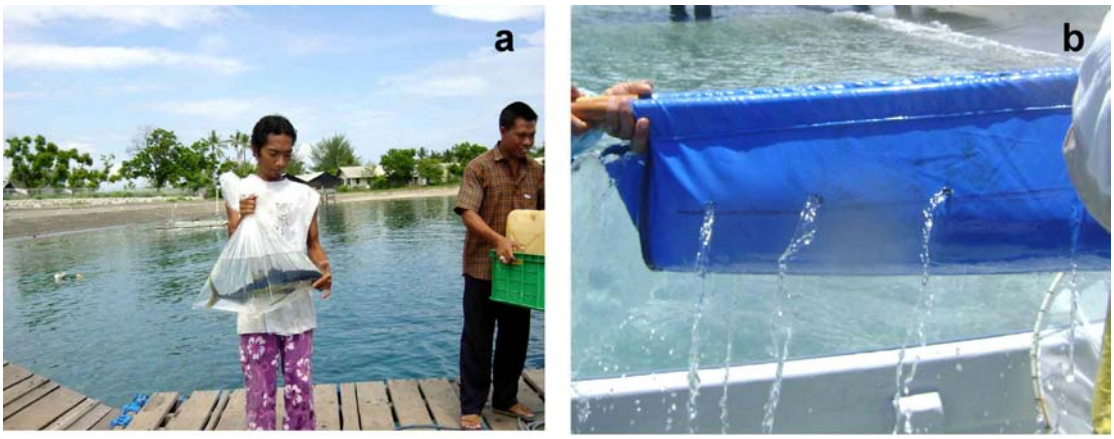

Fig. 7. a. Tuna in water within a plastic bag for transfer onshore from boat.
b. Vinyl sling used in 2010 to transfer tuna onshore from a boat.

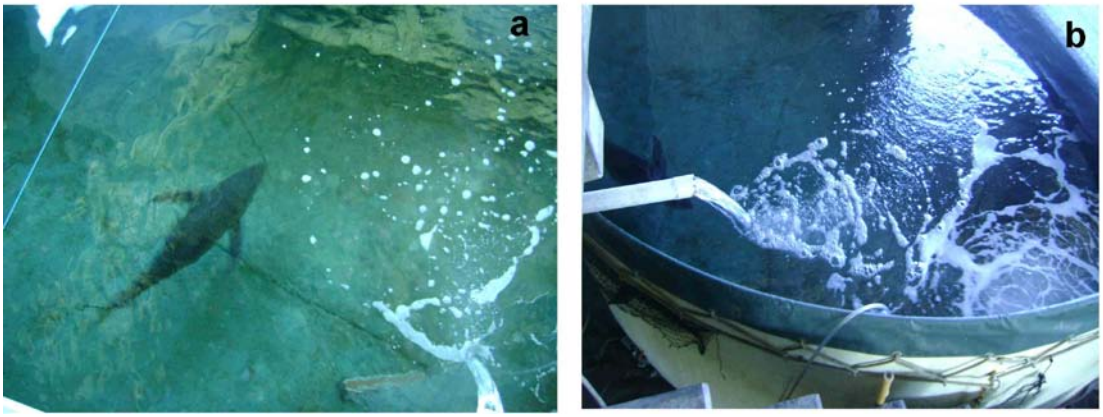

Fig. 8. a. Recently capture yellowfin tuna in 10 m^3 acclimation tank

b. Acclimation tank operated in 2010 with additional water flow and oxygenation.

Sex Determination

Post-mortem analysis of the three large fish removed from the main tank showed they were all mature males. Results of the 11-KT test on large muscle biopsies and fin clips agreed with these findings. Following incubation the colour change of samples was used to determine if samples were male or female (Figure 9). The concentration of 11-KT in the muscle samples taken with the Genetag biopsy was low, suggesting the amount of muscle taken via this method was not sufficient to for this assay. Undiluted plasma samples and those diluted down to 50:450 also had high levels of 11-KT, but those diluted to 10:490 were too dilute to detect 11-KT. The positive results for 11-KT obtained from the least invasive method (i.e. fin clips) was very encouraging. Unfortunately no known females were sampled at this time to compare 11-KT concentrations between mature male and female fish and given that all males were mature and running ripe did not give us an indication on the suitability of the test for small, immature fish.

Of the six immature fish transferred from the acclimation tank, positive 11-KT tests on fin clips suggested that five of the six were males. As was the case with the large fish, samples of muscle taken with the Genetag biopsy did not yield positive results for 11-KT. One of the fish that fin clip testing indicated to be male died within 24 hours of transfer and another 1 month later. Both of these fish were confirmed as males via autopsy and subsequent histology. The smallest fish from which a positive 11-KT test was obtained at transfer was 6.6 kg. This fish died eight months later in May 2010 and an autopsy and subsequent histology confirmed that this fish was a male. The only fish transferred from the acclimation tank in September 2009 which 11-KT testing suggested to be female weighed 8.6 kg. When this fish died in January 2010 it had increased to 16.6 kg and autopsy and histology confirmed that is was a female. The other two fish transferred on this day remain alive in the main tank. All other fish subsequently captured as immature fish had fin clips taken during the transfer from the acclimation tank to the main tank, at the same time they were implanted with a passive implantable transponder (PIT) tag. Assessment of the sex of these fish if they die will provide a greater data set on which to make more definitive conclusions regarding the minimum size at which fish can be accurately sexed using 11-KT assay of fin clip samples.

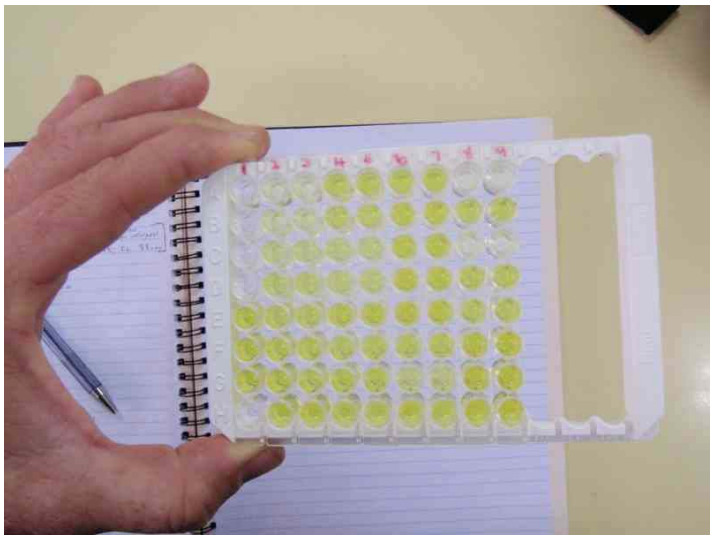

Fig. 9. 96 well 11-Ketotestosterone EIA plate used to determine sex of yellowfin tuna broodstock at RIM Gondol.

Broodstock spawning

The first spawning from captive yellowfin tuna broodstock at RIM Gondol started on 15 October 2004 and continued until 1 November 2004 with eggs collected from 10 spawning events during this period. The most productive spawning period extended over a 274 day period from 25 August 2005 until 24 May 2006 during which the fish spawned on 259 occasions (Table 5). Since this time only small spawning events have been recorded, with few viable eggs collected attributed primarily to a lack of financial support for the project after 2006 followed by a decline in the number of broodstock and less capacity to maintain high quality conditions to hold tuna broodstock from this time.

Table 5.

Summary of spawning periods of yellowfin tuna broodstock in the main holding tank at RIM between 15 October 2004 and 26 November 2010.

Start date	End date	No. of spawns	Average no. eggs per spawn	Total number eggs spawned
15 Oct 2004	1 Nov 2004	10	55,000 ± 46,000	552,000
25 Aug 2005	24 May 2006	259	724,000 ± 435,000	187,773,000
14 Feb 2007	01 Jun 2007	6	36,000 ± 34,000	214,000
24 Nov 2010	26 Nov 2010	3	11,300 ± 1,000	34,000

During October 2010 the main tuna broodstock tank at RIM Gondol was holding 25 recently stocked fish. The majority of these fish had been held in this tank for between 6 and 9 months and were estimated to weigh between 20 kg and 30 kg.. These broodstock spawned three times between 24 October and 26 October 2010.

Preceding spawning one female was chased by 2-3 males. The size of the female was about 60 kg while males were smaller. Time of spawning was near 15:00 hr similar to the time observed in past years. A total of only 34,000 eggs (11,300 per spawning) was produced and fertilization rate was very low (1 %).

Comparison of the conditions prevailing in the broodstock tank during the extended spawning period from 25 August 2005 until 24 May 2006 with those prevailing during 2010 suggest that number of fish, sex ratio and water temperature may be significant factors affecting spawning success for captive yellowfin tuna broodstock. In October 2010 there were 25 broodstock in the main spawning tank at RIM Gondol. Based upon 11-KT assay 14 of the fish being held at RIM are male and 11 are female providing a male to female ratio of 1.27:1. During the best spawning period the number of broodstock in the spawning tank ranged from 27 to 15 fish while the average sex ratio was 1.14 ± 0.2:1 (Fig. 10) which was higher than the period before this when the ratio was generally less than 1:1. Sun et al., (2005) sampled 1,613 yellowfin tuna from central and western Pacific ocean and found that M:F ratio was 1.27:1. This indicates that it may be beneficial to maintain more male fish than female fish in the spawning tank to promote spawning behaviour and optimise fertilisation of eggs.

All of the large broodstock remaining from the previous project removed from the main spawning tank during October 2009 were males. Sun et al., (2005) observed an obvious increase in the number males compared to females as fish grew larger (>138 cm FL) as has been previously reported for yellowfin tuna and other species of tuna. This suggests that there is likely to be a need to replace large fish that are most likely to be males, with smaller fish in order to maintain the desired sex ratio and number of fish in the spawning population.

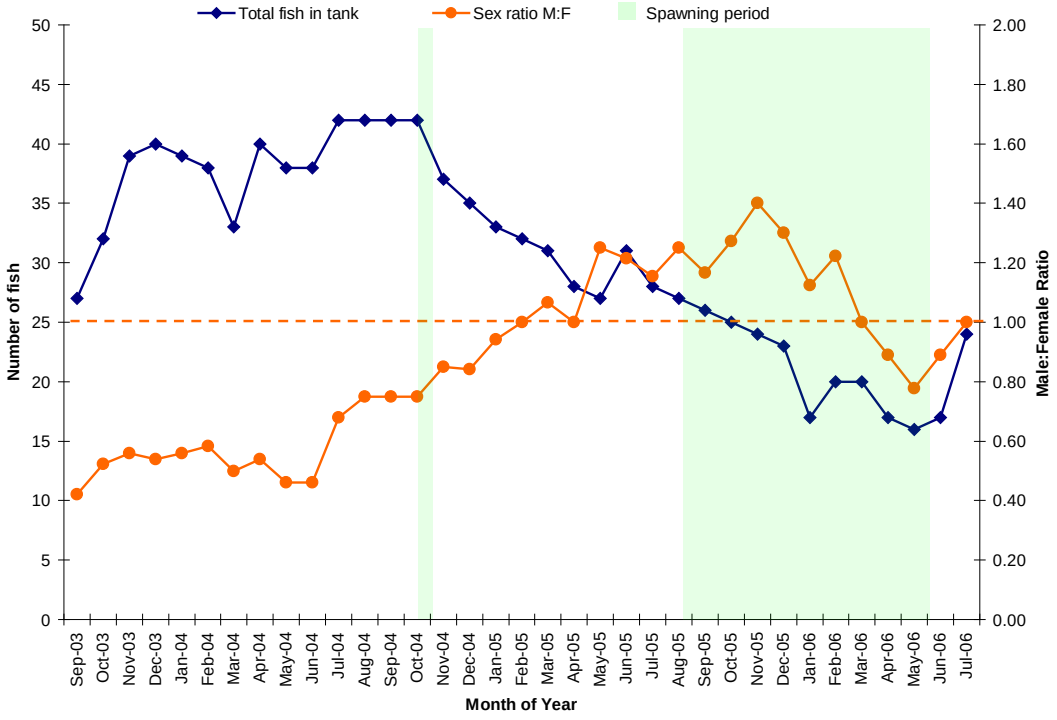

Fig. 10. Comparison of number of fish and ratio of male and female fish in the broodstock holding tank at RIM Gondol during the 2004 and 2006 spawning periods.

Daily records of water temperature and egg production maintained since the tuna holding tanks at RIM Gondol (Fig. 11) show that spawning commences with rising water temperature soon after the minimum annual water temperature has been reached. These records also reveal an annual bimodal cycle that typically includes a peak of highest water temperatures between November and December with minimum water temperatures between August and September. Within these extremes water temperature is also high between April and May and low between January and February (Fig. 11).

Comparison of the water temperature prevailing in the holding tank in the most successful spawning years 2004 and 2005 shows that water temperatures were significantly higher during the period approaching the expected start of spawning in 2010 and to a lesser extent 2009 (Fig. 10 and Fig. 11). It is possible that the atypically elevated water temperatures prevailing during 2010, and to a lesser extent 2009, affected the spawning of yellowfin tuna broodstock held at RIM Gondol. At the IATTC Achotines Laboratory spawning of captive yellowfin tuna broodstock occurs when water temperature is between 23.3^{o}C and 29.7^{o}C (average 27.7^{o}C) although only twice has spawning occurred when water temperature was below 24.0^{o}C (Margulies et al., 2007). In the most successful spawning year recorded at RIM Gondol the water temperature was observed to decline to a minimum of 27.3^{o}C preceding spawning. This has not been the case in 2010 when the minimum water temperature experienced by the recently established broodstock was 28.4^{o}C. It is suggested that the higher than normal water temperatures prevailing during the 2010 season may not have provided the environmental signal required to initiate gonad recrudescence and subsequent maturation and spawning of recently established first maturing broodstock.

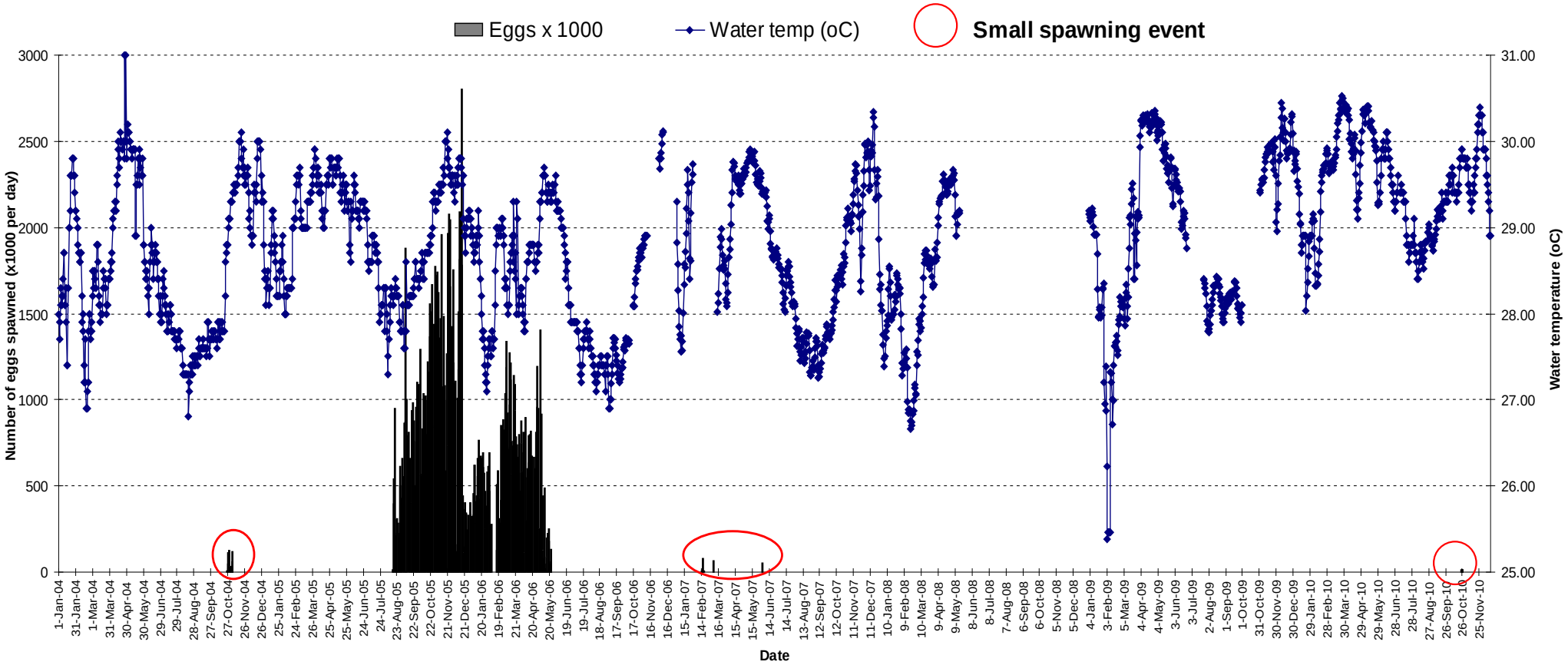

Fig. 10. Daily water temperature and number of eggs collected from each spawning of yellowfin tuna broodstock held at RIM Gondol between 1 January 2004 and 26 November 2010.

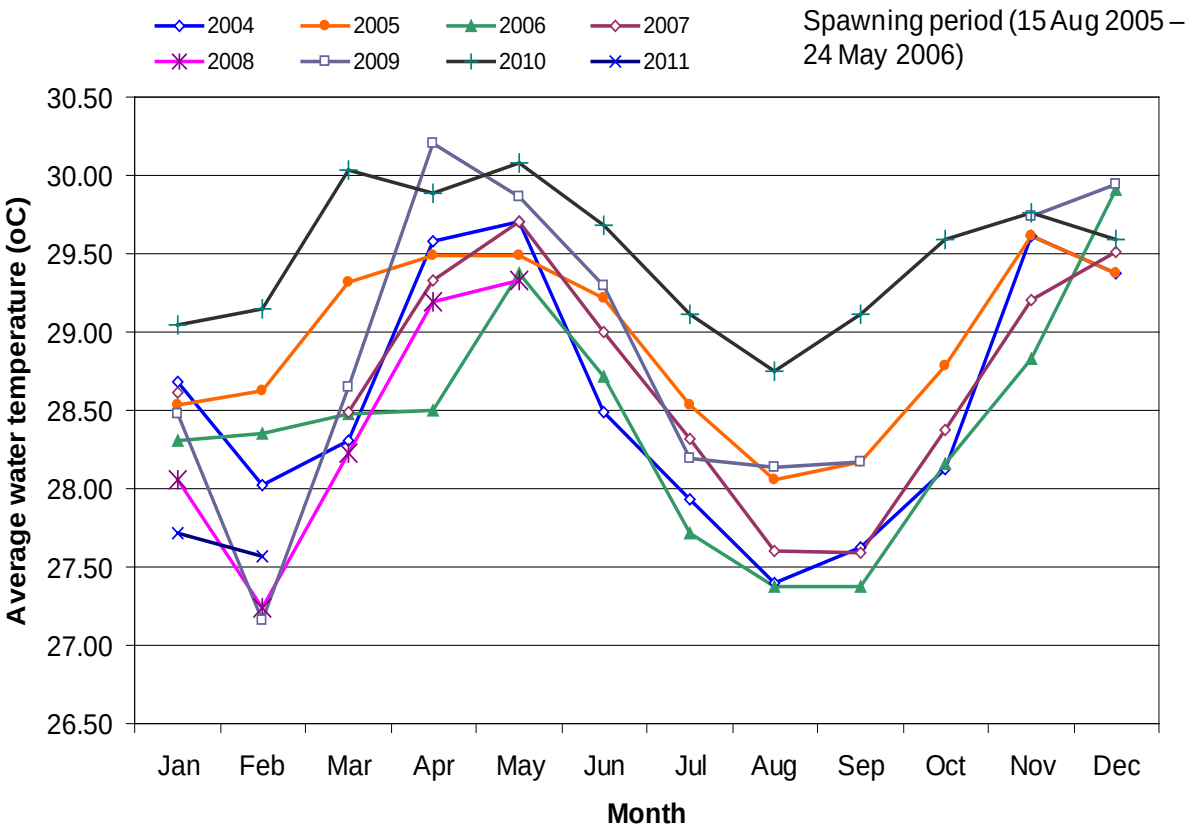

Fig. 11. The average monthly water temperatures (^{o}C) for years 2004 until 2011 in relation to the duration of the best spawning period recorded for captive yellowfin tuna broodstock at RIM Gondol between 15 August 2005 and 24 May 2006.

Parasite control

The results of previous investigation of the parasite of yellowfin tuna at RIM Gondol showed that eggs and larvae of the endoparasite were not found in the gonads of broodstock or in feed, but was found in the water from the broodstock tank. Based on this result, it was concluded that the endoparasite infected eggs horizontally (Permana et al., 2006; Yuasa et al., 2007). Infection could be prevented by harvesting eggs just after fertilization and bathing these with formalin at 25-100 mg L^{-1}. This treatment was only effective against the free swimming stage of the endoparasite (Zafran et al., 2006) and had no effect for spawned yellowfin tuna eggs that were already infected by the parasite. The method was of limited benefit because only small numbers of recently spawned eggs could be harvested. Considering the life cycle identified in these previous studies, it is considered that the most efficient treatment that could be used is a UV light system to disinfect all incoming seawater since the endoparasite showed horizontally infection. This approach would require significant funding to install and maintain this system. The life cycle of this endoparasite has been studied by Hutapea and Permana (2007) who found that the endoparasite infected fertilized eggs and develop by cell division during embryonic development, then burst from the yolk about 4-6 hours after the yellowfin tuna larvae hatched. The endoparasite then changed to a free swimming stage in order to find new host larvae. This stage could survive for about 3 days during which time tuna eggs spawned in the main tank could be infected. Consequently another suggestion is to increase the water exchange rate within the broodstock holding system to flush a significant proportion of endoparasites from the system between spawning events.

8 Outcomes and Impacts

8.1 Scientific impacts – now and in 5 years

Prof. Dan Benetti (Professor and Director of Aquaculture, Rosensteil School of Marine and Atmospheric Science, University of Miami, Florida, USA) visited RIM Gondol between 25 and 29 January 2010 to provide suggestions on methods to improve broodstock survival during capture, transportation and acclimation. Discussions were also held with Prof. Benetti to investigate future research collaborations and the establishment of an international tuna propagation R&D network. Currently there is R&D being conducted on tuna propagation in Japan, South Korea, Taiwan, Spain, Italy, Croatia, Panama and Australia. It is expected that this R&D will make significant advancements towards commercial production of propagated tuna over the next 5 years. As interest in tuna aquaculture continues to grow, RIM Gondol will have opportunities to collaborate with a range of international agencies conducting R&D to propagate tuna species increasing possibilities for commercial tuna production in Indonesia and Australia.

8.2 Capacity impacts – now and in 5 years

Project staff at the RIM Gondol have improved their handling and husbandry abilities resulting in increased survival of yellowfin tuna broodstock following capture, transportation, transfer and acclimation procedures. These improvements will minimise the number of fish needed as broodstock and reduced the ongoing effort required for fishing operations. In addition, the capacity to successfully catch and acclimatise larger fish will shorten the holding time required before broodstock attain mature size and initiate spawning within the holding system at RIM Gondol.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

There were no economic impacts from the project.

8.3.2 Social impacts

No social impacts are envisaged from the project in the foreseeable future

8.3.3 Environmental impacts

No environmental benefits are likely in the near future. However, if controlled reproduction and rearing of any species of tuna becomes a reality in Indonesia, and if this eventually results in decreased pressure on wild stocks, the environmental benefits will be considerable.

8.4 Communication and dissemination activities

From 8th until 20th June 2009, RIM project scientist, John Hutapea attended the '7th Annual Workshop on Physiology and Aquaculture of Pelagic Fishes, with emphasis on reproduction and early developmental stages of yellowfin tuna, *Thunnus albacares*', held at the Inter-American Tropical Tuna Commission, Achotines Laboratory, Los Santos Province, Republic of Panama. During this workshop he assisted Dr Gavin Partridge (AIAA) with yellowfin tuna larval rearing trials and participated in discussions to identify possible improvements to assist broodstock capture, transport and husbandry at RIM Gondol.

John Hutapea gave the presentation “Achievements and bottlenecks for yellowfin tuna, *Thunnus albacares*, propagation at the Research Institute for Mariculture, Gondol, Bali Indonesia” at the Second Global Centre of Excellence Symposium of Kinki University “ Sustainable Aquaculture of the Bluefin Tuna and Yellowfin Tuna – Closing the Life Cycle for Commercial Production”, 1-2 December 2009, SARDI, Adelaide, Australia. Following this symposium John Hutapea inspected kingfish and southern bluefin aquaculture facilities in Port Lincoln and aquaculture R&D facilities in Fremantle.

ACIAR Project reporting

ACIAR Project Profile, 2010

ACIAR Annual Report, May 2009

Project presentation - ACIAR Fisheries Program Meeting, Cronulla, May 2009

9 Conclusions and recommendations

9.1 Conclusions

The project has developed greatly improved methods for capture and holding of new yellowfin tuna broodstock. Much higher survival of fish is now achieved and these methods allow capture of larger fish that also survive better during acclimation and attain maturity within a shorter period of captive holding. The benefits of this advance are significant as there is a continuing need to replace tuna broodstock lost periodically due to wall strikes.

The 11-KT assay of fin clip samples taken during tank transfers was able to discriminate sex of yellowfin tuna as small as 6.6 kg. This procedure will allow staff at RIM Gondol to identify male and female fish to better manage the sex ratio of the yellowfin tuna broodstock in the main tank to promote spawning.

Together these developments have allowed establishment of a potentially productive population of captive yellowfin tuna broodstock at RIM Gondol that, given exposure to normal seasonal conditions, should provide an enhanced supply of eggs during 2011. The tools developed by this project will allow researchers at RIM Gondol to manage the ongoing need to replace broodstock to maintain a desirable sex ratio to optimise spawning success.

The potential to undertake cost effective R&D on tuna larval rearing at an established facility in close proximity remains as a potential benefit to propagated tuna aquaculture in Australia.

9.2 Recommendations

It is recommended that methods for capture and transfer of yellowfin tuna broodstock developed during this project be used and further refined, including:

- Fishing using rod and reel with braid line to retrieve fish faster.
- Use a vinyl cradle to land fish and transfer to the onboard holding tank
- Installation of a fibreglass tank (2 m^3) onboard with a lid to reduce light during transportation.
- Use of pure oxygen to maintain dissolved oxygen between 100-120% saturation in the onboard holding tank.
- Return to RIM Gondol no longer than 30 minutes after capture of the first fish.
- Use of a vinyl sling to transfer fish from the boat to the onshore treatment tank
- Use of pure oxygen and increased water flow while fish are held in the “medical” tank during prophylactic treatments prior to transfer into the acclimation tank

Fishing for replacement broodstock should be conducted at times when fish are plentiful and should target fish greater than 5.0kg. Fin clip samples, in combination with PIT tag identification, should be taken from all new fish stocked into acclimation tanks in order to determine sex of each individual fish. This will allow selection of fish to establish and maintain the desired sex ratio within the main spawning tank at RIM Gondol.

To ensure more reliable and controlled spawning of captive broodstock, future investment in yellowfin tuna propagation at the RIM, Gondol should consider installation of additional system components, including:

1. UV disinfection of incoming seawater to secure the broodstock system against introduction of the lethal endoparasite *Ichthyodium chabelardi.*

2. Water cooling capacity to reduce the water temperature within the spawning tank by 1.0 – 1.5^{o}C below the minimum ambient water temperature if required. This component would only need to operate for 2 – 4 weeks in seasons when abnormally high water temperature conditions prevail.

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10.2 List of publications produced by project

Partridge, G.J., Benetti, D.D., Hutapea, J., McIntyre, A., Chen, B,. Hutchinson, W.G., Scholey, V.P., Margulies, D. The effect of a 24-hour photoperiod on the survival, growth and swim bladder inflation of pre-flexion yellowfin tuna (*Thunnus albacares*) larvae. (Submitted 2010, Aquaculture)

Hutapea, J.H., Gunawan, A.S., Permana, G.N. 2010. Improvements for handling of candidate broodstock of yellowfin tuna (*Thunnus albacares*) post captured and in medical bath tank. Proceedings of the Forum for Aquaculture Technology Innovation in Indonesia (In press).

Partridge, G. J. 2009. Hatchery production of yellowfin tuna. International Specialist Skills Institute, Melbourne, pp 36.