SfCRC Research Travel Grant: Zooplankton Research Tour and Fish Hatchery/Farm Visits in Taiwan

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AUSTRALIAN
SEAFOOD
COOPERATIVE
RESEARCH CENTRE

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OBJECTIVES OF RESEARCH TRAVEL GRANT

The SARDI Aquaculture Science Program Area is seeking optimal copepod production techniques for the ongoing Australian Seafood CRC Yellowtail Kingfish (YTK) and Southern Bluefin Tuna (SBT) larval rearing projects (2008/746 and 2008/718). This visit provided the opportunity to review international technologies and gain practical knowledge and hands-on experience on seawater zooplankton culture to produce good-quality live food for YTK and SBT larvae. This information will help to establish proper feeding regimes for next season's SBT and YTK larval rearing research at SARDI, and potentially influence procedures used at Clean Seas Tuna (CST) for commercial SBT larval production.

NON TECHNICAL SUMMARY:

Dr Chen undertook a research tour in Taiwan, as previously organised, to investigate the copepod pond production system used for marine larval finfish rearing. This involved capturing information about the system and getting some hands-on experience. The system could possibly be adopted, with modification, by Australian finfish hatcheries, including Clean Seas Tuna Ltd, which is focused on the hatchery production and growout of YTK and SBT. Local issues that need to be addressed include biosecurity issues associated with the growth of a natural live foods in an outdoor situation and the effects on production of the large diurnal and seasonal environmental variation experienced outdoors in many regions of South Australia.

Before the research tour, Dr Chen attended the “AquaSeed 2009” international conference, held in Kaohsiung, Taiwan. As one of the key speakers, Dr Chen gave an oral presentation titled “Prospects of kingfish and tuna larviculture in South Australia”. The conference allowed Dr Chen to network with other key speakers.

After the conference, Dr Chen also visited the Yellowfin Tuna and Cobia propagation research groups located in Tungkang Biotechnology Research Centre, Fisheries Research Institute.

To conclude the trip, Dr Chen visited the Department of Aquaculture, National Taiwan Ocean University in Keelung, to initiate networking and to seek further collaboration between Taiwan and Australia under the Australian Academy of

Technological Sciences and Engineering (ATSE) International Science Linkages Programme.

OUTCOMES ACHIEVED TO DATE

During the research tour, Dr Chen met with a number of scientists and farmers, reviewed the culture system for copepod mass production using outdoor earthen ponds and gained the hands-on experience of copepod pond production techniques developed in Taiwan.

The typical copepod production system is commonly an outdoor earthen pond about 8000 m^2 with a depth of 1 to 1.5 m. Ponds are filled with sea water, and protocols are followed that include control of potential predators and inoculation with non-sulphate purple photo-synthetic bacteria to reduce the effects of undesirable microorganisms. A paddlewheel is used in each pond to generate water current to homogenise food particles/nutrients in the pond and collect copepods. Fish meal and soybean meal are added as feed for the copepods. During feeding, both fish meal and soybean meal are suspended in the water column within fine mesh bags hung downstream of the paddlewheel. Continuous copepod harvesting occurs using a zooplankton net set in the same downstream location. The system could possibly be adopted, with modification, by Australian finfish hatcheries, including Clean Seas Tuna Ltd, which is focused on the hatchery production and growout of yellowtail kingfish and southern bluefin tuna. Local issues that are likely to need to be addressed include biosecurity issues associated with the growth of a natural feed in an outdoor situation and the effects on production of the large diurnal and seasonal environmental variation experienced outdoors in many regions of South Australia.

The techniques of cobia broodstock management/larval rearing and yellowfin tuna broodstock management developed in Taiwan were reviewed by on-site visits and communication with the scientists.

OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT:

- Outdoor pond copepod mass production system developed in Taiwan will be recommended to local industry;
- If accepted by the local industry, a modified copepods mass production system will be developed within the hatchery with regarding to the biosecurity issues and the local ambient conditions in many regions of South Australia;
- Information of hatchery techniques from Taiwan will be presented to the local industry;
- Information of Cobia and Yellowfin Tuna broodstock management and Cobia larviculture developed in Taiwan will be introduced to local industry;
- Potential collaboration opportunities between Australia and Taiwan will be reviewed.

BACKGROUND AND NEED

Good quality live foods are critical for improving survival and growth in marine finfish larval rearing. Current live food feeding regimes use enriched rotifer and *Artemia* nauplii. However, recent trials on SBT larval rearing (Seafood CRC project code 2008/718), have shown that these traditional approaches are inadequate and alternatives must be found. The SARDI Aquaculture Science Program Area is seeking optimal copepod production techniques for the ongoing Australian Seafood CRC yellowtail kingfish (YTK) and southern bluefin tuna (SBT) larval rearing projects (2008/746 and 2008/718). This visit provided the opportunity to review international technologies and gain practical knowledge and hands-on experience on seawater zooplankton culture to produce good quality live food for YTK and SBT larvae. This will help to establish proper feeding regimes for next season's SBT and YTK larval rearing research at SARDI, and potentially influence procedures used at Clean Seas Tuna (CST) for commercial SBT larval production.

Mass production of zooplankton such as copepod for finfish hatchery production has been carried out successfully for many years in Taiwan, unlike elsewhere. As previously organised, Dr Chen conducted a research tour through several commercial farms to investigate the copepod pond production techniques and gain practical knowledge and hands-on experience of zooplankton production for larval fish rearing. During the research tour, Dr Chen met with a number of scientists and farmers, reviewed the culture system for copepod mass production using outdoor earthen ponds and gained the hands-on experience of copepod pond production techniques developed in Taiwan. This visit to Taiwan provided a unique opportunity to better understand copepod production on a commercial scale and the techniques being used for this.

RESULTS

Copepod pond production system

In Taiwan, the finfish hatchery industry relies on pond produced copepods. There are three zooplankton generally found in their local waters: a cladoceran (*Diaphanosoma aspinosum*), and two copepods species (*Apocyclops royi* and *Pseudodiaptomus spp*) (Fig.1). In terms of nutritional profile, the two copepods are considered to be good live foods for finfish larval rearing. They can be harvested from outdoor earthen ponds all year round. As highlighted above, the copepod pond production system was reviewed by on-site visits and communication with the owners/managers of several copepod farms. Hands-on experience was gained through activities at some farms.

Fig. 1. Some local zooplankton species in Taiwan. Photo was taken in Tungkang Biotechnology Research Centre, Fisheries Research Institute.

Outdoor copepod production in Taiwan involved:

- Earthen ponds about 8000 m^2 in area and with a depth of about 1 to 1.5 m. Shrimp culture ponds can be used for producing copepods (Fig. 2 and 3);
- The ponds being filled with sea water and potential predators eradicated;
- The use of a paddlewheel to generate water current to homogenise food particles and nutrients in the pond and collect copepods (Fig. 2, 3 and 4);
- Inoculation of ponds with non-sulphate purple photo-synthetic bacteria to manage undesirable microorganisms;
- It is not necessary to inoculate the copepods;
- Placing fish meal and soybean meal in fine mass bags, which are hung in the water column behind the paddlewheel to allow slow release of food particles and nutrients (Fig. 4);
- Continuous copepod harvesting using a zooplankton net fixed just behind the paddlewheel (Fig 3C and 4) and;
- In the hatchery, after purchase from the copepod producer, placing the copepods in tanks and feeding them microalgae to maintain their nutritional profile (Fig. 5). Usually, the copepods are fed to the fish within 12 hrs. The remaining copepods can be transferred into a new tank and fed microalgae.

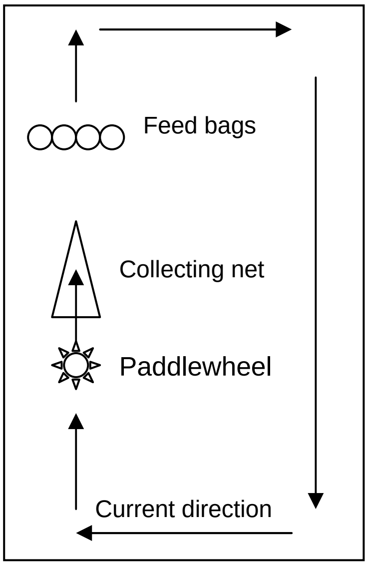

Fig 2 Diagram of a typical operation of copepod production pond in Taiwan. The arrows show the water current in the pond.

Fig. 3. Copepod pond culture system in Taiwan. A) overview of a typical copepod production pond; B) paddlewheel (arrow) and feed bags (arrowhead) hanging in the pond; C) copepod collection net (arrow) just behind the paddlewheel; D) copepod collected from the production pond.

The copepod outdoor earthen pond production system is widely used in Taiwan and benefits finfish hatcheries for a number of species including grouper, cobia and milkfish. Their use reduces production costs by replacing the majority of *Artemia* cysts, as well as improving larval fish survival and growth.

Fig. 4. Copepod production pond, showing the paddlewheel and feeding bags hanging in the pond.

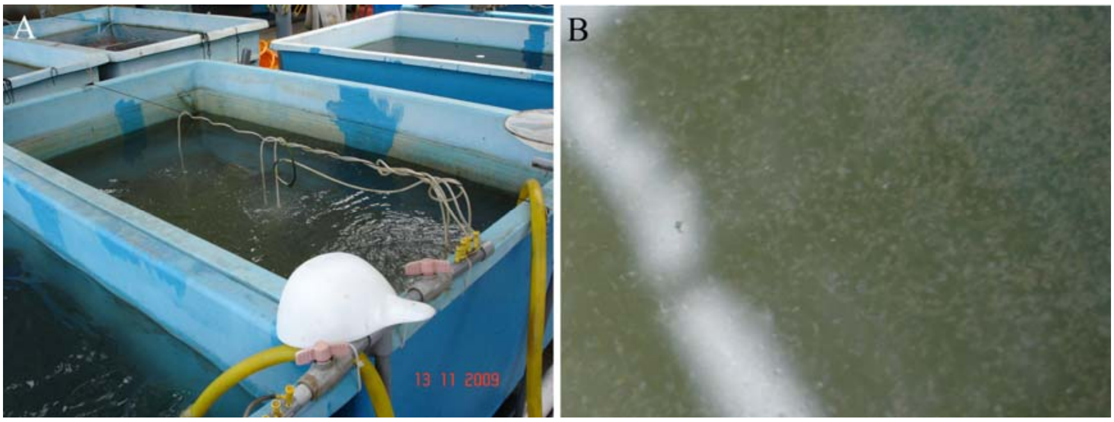

Fig.5. Copepod temporary holding tank (A); and live copepod in the holding tank (B).

Suggestions for industry and research organisations in Australia:

- Copepods are natural live foods for most marine finfish larvae, including YTK and SBT;

- Providing copepods to a certain larval stage will improve larval performance including enhancing growth and increasing survival rate, which if done appropriately may be a cost-effective way to benefit hatchery production;
- A modified on-site pond system for copepod production is recommended, for instance Clean Seas Tuna Ltd could utilise the ponds outside their Arno Bay and Port Augusta hatcheries and;
- Such a system will need to be developed for local conditions so as to address any necessary biosecurity issues and the large diurnal and seasonal environmental variations experienced.

EXTENSION ACTIVITIES

1. AquaSeed 2009 conference

AquaSeed 2009 is a specialist international conference on fish and shrimp larviculture. As one of the invited speakers, Dr Chen gave an oral presentation titled “Prospects of kingfish and tuna larviculture in South Australia”. In the presentation, approved by Clean Seas Tuna Ltd, Dr Chen introduced the current research and hatchery practice on YTK and SBT larval rearing in South Australia. The audience showed great interest on this topic. Suggestions such as using umbrella *Artemia* (early, embryo stage) and on-grown *Artemia* as tuna live foods were given after the presentation. Additional useful information was also gained from the conference:

1.1. Current hatchery techniques in Taiwan:

- Larviculture technology in Taiwan has developed over the last few decades after shrimp larval rearing techniques were successfully established in 1969.
- Finfish larviculture procedures are segmented into separate businesses such as broodstock management and egg collecting, hatching, early stage larval rearing (rotifer feeding stage), late stage larval rearing (*Artemia* and copepod feeding stages) and nursery, which is unique and different from elsewhere.
- Copepods produced from outdoor earthen ponds are commonly used as good quality live foods for fish and shrimp hatchery production.
- There is a strong need to develop hatchery techniques for giant grouper *Epinephelus lanceolatus*, which is known as Queensland grouper in Australia.
- Research on cobia larviculture has been successfully undertaken in Taiwan for many years, however due to low survival from accumulated broodstock inbreeding used in commercial production, there is a strong need to initiate a long term breeding program to establish family lines for selective breeding purposes.

1.2. Disease resistance fish by marker assisted selection (MAS):
Disease resistance is an important trait for production efficiency and profitability of aquaculture species. Marker-assisted breeding (marker-assisted selection, MAS) carried out using DNA markers is a suitable tool for improvement of disease resistance, because allelic information of DNA markers linked to responsible genes for disease resistance can realise an individual’s capacity for disease resistance. This trait is different from traits like growth or colour where it is possible to directly measure phenotypes of candidates for selection. A good example of such work is the

establishment of lymphocystis disease (LD) resistant flounder by MAS that has since resulted in no-outbreaks of LD in Japanese fish farms.

1.3. Umbrella *Artemia* and on-grown *Artemia* for larval fish rearing (potential for tuna larval rearing):

Umbrella-stage *Artemia (early embryo stage)* can be collected during the hatching process of *Artemia* cysts. The idea of using umbrella stage *Artemia*, if successful, can realise several opportunities:

- Simplify the finfish larval rearing protocol by eliminating the use of enriched rotifers due to their smaller size;
- Become an alternative substitution food in case of sudden crashes of rotifer production or shortage of rotifer production;
- Provide a more energy-rich food in comparison with enriched rotifers during larval development and;
- Benefit from extra energy present in cysts that is lost during the hatching process and/or swimming activities of nauplii.

On-grown *Artemia*, when subjected to bioencapsulation, ingest the oil emulsified product and have their digestive tracts partially filled with high nutritional profile oil particles. The completion of bioencapsulation can be achieved within three hours. The nutritional quality of on-grown *Artemia* in term of HUFA content can be enhanced through bioencapsulation. With the bioencapsulation technique, the nutritional quality can be tailored to suit the fish requirement. This technique may have beneficial applications in improving the performance and enhancing the quality of YTK and SBT fingerlings.

1.4. Specific pathogen free (SPF) shrimp and potential application on fish fingerling production:

The supply of SPF shrimp seedlings is common in the Asian-Pacific region. The technology for producing SPF shrimp seedlings has been developed for years. Scientists are working actively to transfer this technology to finfish larval rearing for the following reasons:

- Improved hygiene and sanitation in the facility;
- Disinfection of water supplies and waste water with UV irradiation, ozonisation or electrolysation;
- Selection of pathogen-free broodstock by cell culture isolation and detection of specific antibody with ELISA or detection of viral genes using Polymerace Chain Reaction (PCR) techniques;
- Health monitoring of hatched fry by cell culture isolation, immunological and molecular biological methods;
- Control of normal intestinal flora, for example, by feeding an antiviral intestinal bacteria and;
- Temperature control.

Under these circumstances, hatched fish are healthy and free of specific pathogens, but there is still the possibility they can be subsequently infected after they are moved to pond or netcage growout facilities. So the development of effective vaccines, vaccine injection machines and immunological tools for monitoring vaccination still remain desirable.

2. Cobia larviculture practice

Cobia (*Rachycentron canadum*) has been an important marine finfish species for sea cage farming in Taiwan since the early 1990s. All fry for aquaculture are produced from hatcheries. The information for cobia broodstock management and larval rearing is as follows:

Broodstock and management

- Maturation between 2 and 3 years;
- Typical broodstock tank is a round outdoor concrete tank with diameter about 6 to 8 m and depth about 2 m, usually holding 20 cobia (Fig. 6A and 6B);
- Temperature was around 29 °C at the time of the visit;
- There is a paddlewheel in the tank (Fig. 6B) and;
- Broodstock are fed trash fish.

Fig. 6. Cobia propagation facility located in Tungkang Biotechnology Research Centre, Fisheries Research Institute. A, far view of the cobia broodstock tanks (arrows); B, close view of the cobia broodstock tank; C, cobia larval rearing tank; D & E, cobia larvae on 21 days after hatch.

Cobia larval rearing

- Cobia larval rearing is conducted in similar outdoor concrete tanks with central standpipe as a water outlet (Fig. 6C);
- There is also a paddlewheel in the tank to generate water currents.
- Stocking density is about 20 to 30 larvae per L;
- Temperature was around 29°C at the time of the visit;

- Cobia larvae feed on large strain rotifers at 3 DPH and feed until 7 to 8 DPH. Freshly hatched *Artemia* nauplii are usually provided at 7 DPH and then enriched *Artemia* 2nd and 3rd instar nauplii for another couple of days until 10 DPH, then they are fed on copepod until 30 DPH before weaning (Fig. 7) and;
- Although weaning of cobia can be started as early as 15 DPH, feeding on copepods towards the later stages is considered cost-effective in Taiwan.

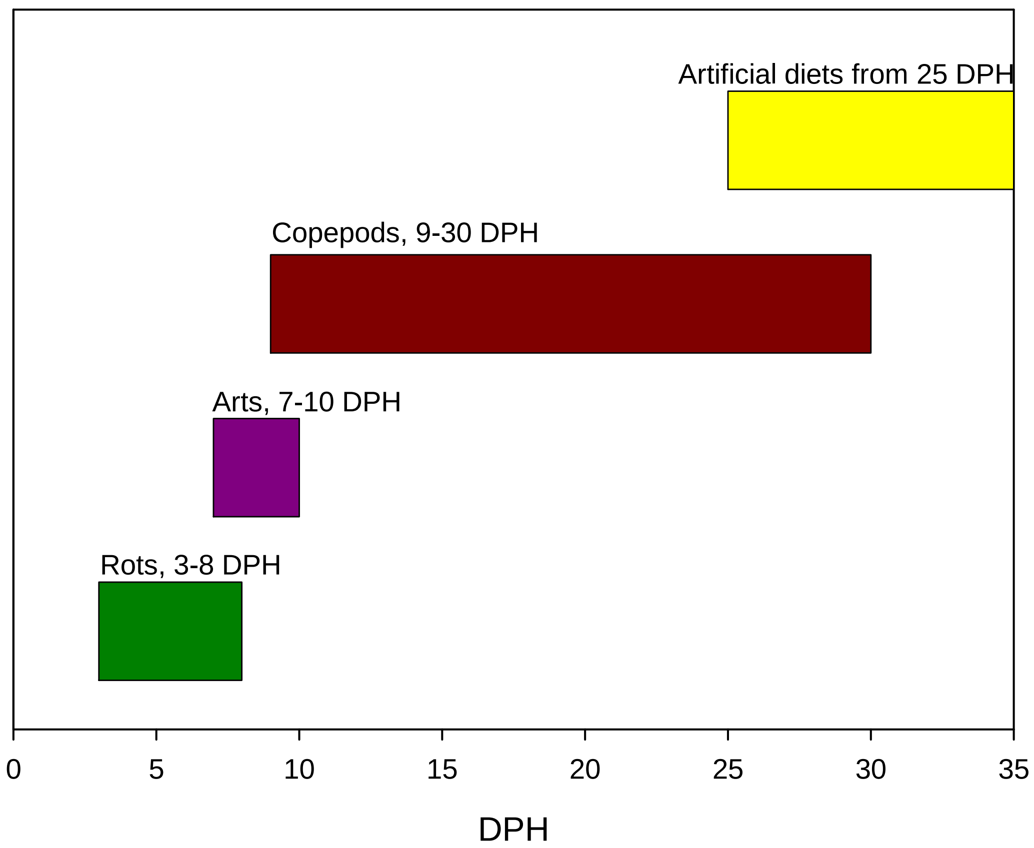

Fig. 7. Cobia larval fish feeding regime which has been used by the hatcheries in Taiwan (temperature was around 29 °C at the time of the visit).

There are some issues with the cobia industry in Taiwan:

- Frequent typhoons that limit available sites for sea cage farming, and require specially designed stronger cage systems;
- Technology for disease resistance fingerlings by marker-assisted selection/breeding need to be established and;
- Research on genetic breeding and family line establishment is required.

Suggestions for the local industry and research organisations in Australia:

- Use copepods as good quality live foods to improve growth and survival rates;
- Develop an on-site copepod pond production system in hatchery to address any biosecurity issues and unstable environmental conditions;
- Develop technologies to produce SPF and disease resistance fingerlings and;
- Genetic breeding and family line establishment.

3. Yellowfin tuna broodstock facility and management

Although the industry of yellowfin tuna farming has not started yet, a research group has been funded in Taiwan for a number of years. Currently, a broodstock facility is established and operating in Tungkang Biotechnology Research Centre, Fisheries Research Institute located in Tungkang (Fig. 8 and 9). At present, the research is still in the early stages of building up broodstock numbers by collecting young, wild, 1 year old fish.

Broodstock facility:

- One round outdoor concrete tank under a roof with diameter of 18 m and depth at 6 m, currently holding 40 yellowfin tuna broodstock age from 1 to 3 years (Fig. 8);
- Water temperature was 29 °C at the time of the visit;
- Water treatment: Sand filter, UV lights, biofilters and two protein skimmers (Fig. 9) are in the broodstock system for water treatment;
- Water exchange: recirculating system with 10% water exchange rate daily and;
- Broodstock are fed on trash fish.

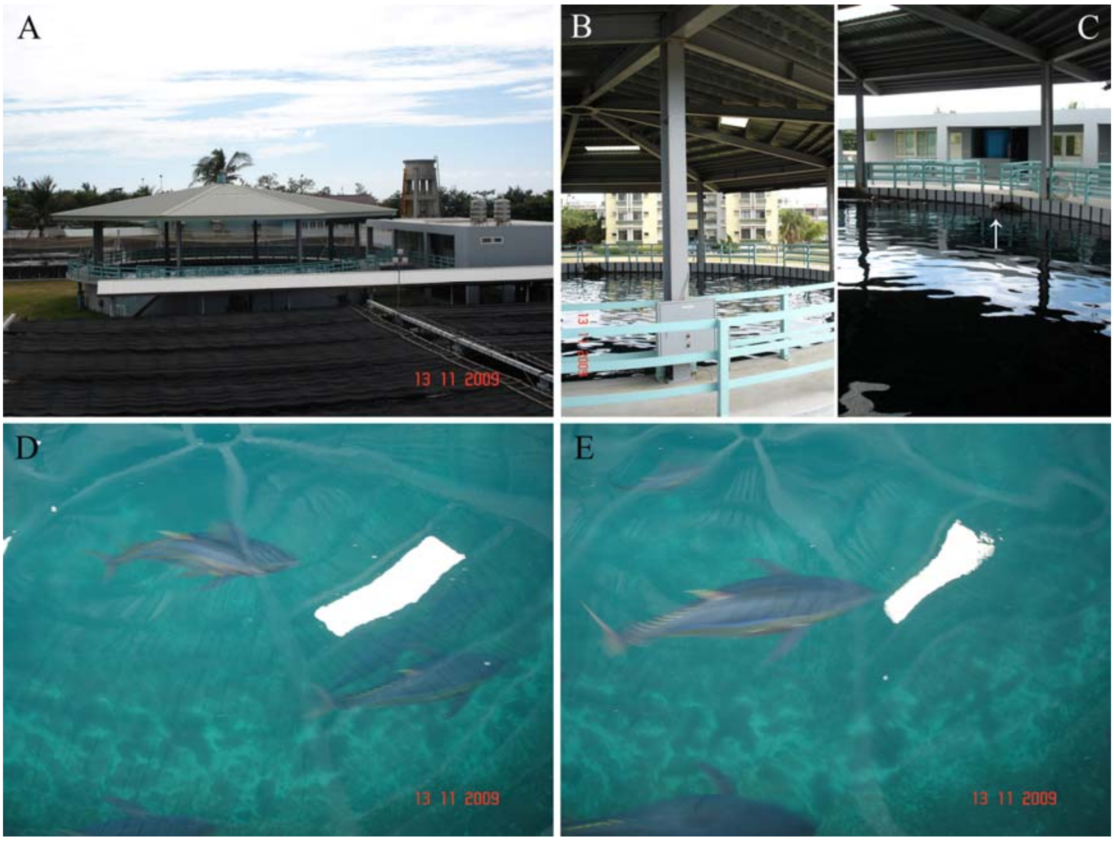

Fig. 8. Yellowfin tuna broodstock facility in Tungkang Biotechnology Research Centre, Fisheries Research Institute. A, overview of the tuna tank and accessories; B & C, close view of the tuna tank, show one of the three water inlets on the tank wall (arrow); D & E, yellowfin tuna broodstock in the tank.

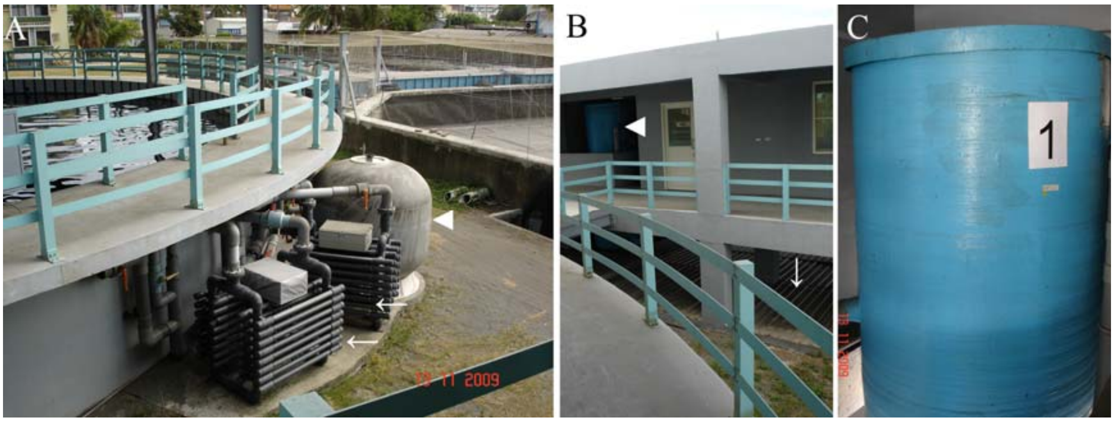

Fig. 9. Water treatment A, showing the sand filter (Arrowhead) and UV lights (Arrows) in the system; B, showing the biofilter (Arrow) and protein skimmers (Arrowhead) in the system; C, one of the two protein skimmers in the system.

Basic advice for tuna catching and handling based on the Taiwanese experience with yellowfin tuna:

- When catching tuna from the wild, choose one-year old juveniles and catch them using a barbless hook;
- Never handle the fish with bare hands and;
- Use a thin plastic liner (with holes in it) to hold and handle fish when transferring (Fig. 10).

These things are either not applicable to southern bluefin tuna or already known.

Fig. 10. Juvenile tuna handling with plastic liner which can avoid injuries to fish when transferring.

Suggestions for the industry and research organisation in Australia:

- it is cost-effective to use young fish (either wild-caught or hatchery reared) to supplement the broodstock group, because they are easy to handle, more easily transported and their survival rate is higher than for older fish;
- Use a plastic liner of the type described above when transferring SBT fry between land based tanks or from land based tanks to sea cages, i.e. using soft thin plastic liner to constrain fish at a corner of larval or nursery tank followed by bucketing them out and moving to the new tank or sea cages.

4. Networking and Collaboration

The following are the suggested potential areas for future collaboration, which would benefit the aquaculture industry in both Australia and Taiwan, under the Australian Academy of Technological Sciences and Engineering (ATSE) International Science Linkages Programme:

4.1. Improvement of larval fish (potentially YTK and SBT) performance by feeding copepods

Currently, the main challenges facing the aquaculture industry in Australia are 1) low survival rate and high deformity rate during the YTK hatchery production; 2) lack of suitable live foods for SBT to enhance larval growth in the early life stages. These issues were also experienced in Taiwan previously in other fish species and have improved substantially since the introduction of feeding copepods in their hatchery feeding regimes. Their experiences might have broader implications because the larval rearing practices are very similar for most finfish species farmed globally.

It is anticipated this project might consist of three steps:

1. evaluation of the performance of local finfish larvae (for example YTK) fed with local copepods caught from the wild,
2. cost/benefit analysis and;
3. development of large scale copepod production technique or evaluation of the techniques developed in Taiwan with Australian local species.

4.2. Establish of a breeding program to improve cobia growth and survival in Taiwan

Cobia aquaculture has been well established in Taiwan for many years (Fig. 11). However, their survival rates at both hatchery stage and after transfer into sea cages have decreased significantly in recent years. Taiwanese scientists believe that the reduction in survival at these stages may result from cumulated inbreeding in their broodstock used in commercial production. Establishing a selective breeding program has been identified as one of the highest priorities to address these issues.

In Australia, cobia aquaculture is in its infancy. Therefore establishing a breeding program would not be a priority. However, there is significant Australian expertise in breeding program design and evaluation, and genetic analyses. The involvement of Australian scientists in the development of a cobia breeding program in Taiwan would obviously help the Taiwanese aquaculture industry. Meanwhile, the experience and knowledge obtained from this involvement would also accelerate the application of a cobia breeding program in Australia when needed.

Fig. 11. Cobia juveniles in tank.

4.3. Giant grouper (*Epinephelus lanceolatus*) larviculture

The giant grouper (Fig. 12), known as Queensland grouper in Australia, is one of the marine finfish species most favoured by the people of Taiwan, where its aquaculture is well established. Due to its fast growing characteristics and good flesh quality, giant grouper would be an ideal candidate for aquaculture development in northern Australia.

Similar to cobia, giant grouper aquaculture is in its infancy in Australia. The experiences from Taiwan suggest that the following technical issues need be addressed first: 1) development of a reliable method to induce broodstock maturation in captivity; 2) development of larval rearing techniques, and 3) development of proper sex reversal techniques to obtain available male broodstock at a younger age.

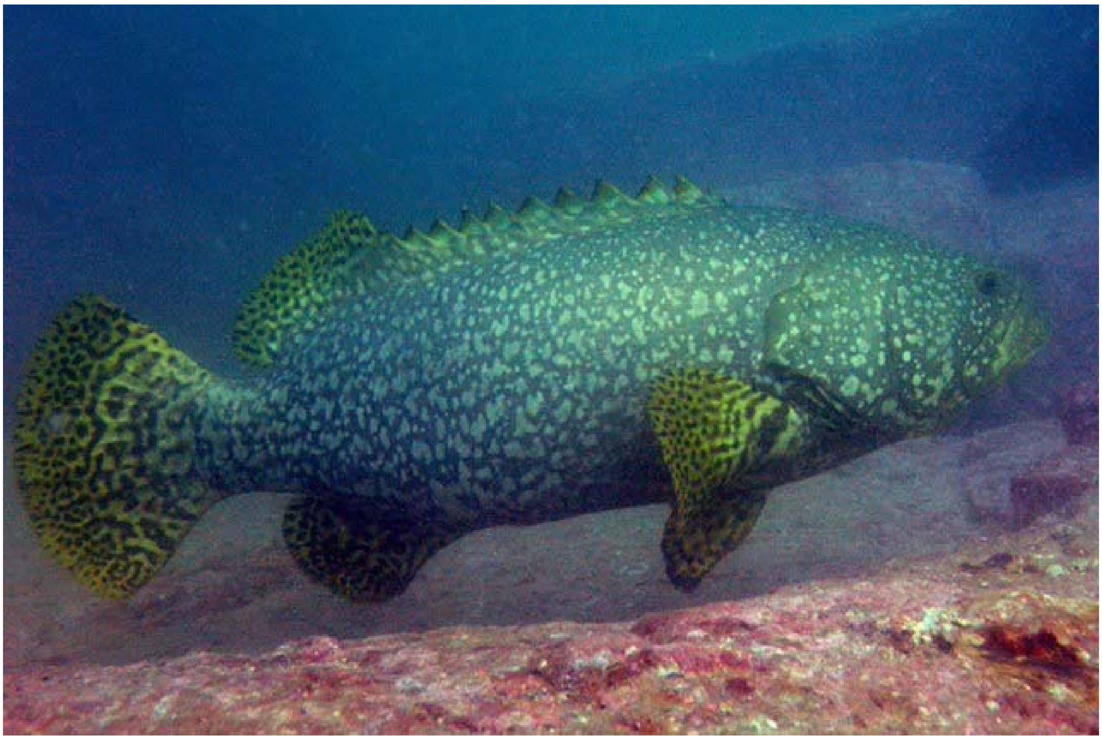

Fig. 12. Giant grouper (Queensland grouper) *Epinephelus lanceolatus*.

4.4. Sea urchin *Hemicentrotus pulcherrimus* (Fig. 13) larviculture/farming

Sea urchin roe is one of most expensive seafoods globally, especially in Japan and China. In recent years interest in sea urchin farming has increased in both Australia and Taiwan. This has largely been due to the need to diversify from species cultured in similar environments, such as abalone. Research on sea urchins has started in Taiwan and Australia on different species. It is expected that the collaboration and/or information exchange would enhance the development of sea urchin aquaculture in both places.

Fig. 13. Sea urchin *Hemicentrotus pulcherrimus*, in Taiwan.

4.5. Disease resistance and selective breeding of abalone

Abalone aquaculture is well established in both Taiwan (*Haliotis diversicolor* – Fig, 14) and Australia. However, the industries on both sides have suffered from viral outbreaks in recent years; in Taiwan many abalone farms have closed. The industries in both Taiwan and Australia desire disease resistant seedlings for carrying out sustainable production. The development of the selective breeding techniques to address disease issues is considered a good solution. A number of research projects have been established in both places to address this issue. Collaboration and information exchange would not only benefit the existing researchers but industry development in both places.

Fig. 14. Small abalone *Haliotis diversicolor* which is farmed in Taiwan.

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

- Practical knowledge and hands-on experience of the copepods outdoor pond production system developed in Taiwan.
- Current status and technologies of hatchery production in Taiwan, such as cobia and yellowfin tuna broodstock management and larval rearing techniques.
- Live copepods as good quality live foods for finfish hatchery production will be considered by either researchers and industries.

SUMMARY OF CHANGE IN INDUSTRY (WHAT IMMEDIATE CHANGES ARE EXPECTED)?

Nil

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

- Adding copepod in cobia, YTK and SBT larval feeding regime in the future.
- Develop a mass copepod pond production system suitable to local cobia and YTK/SBT hatcheries.

FURTHER ACTION REQUIRED IN REGARDS TO COMMUNICATION?

- Presentation provided to the December 2009 Aquaculture Production Hub meeting (Finfish network group).

- Provide additional information about potential collaboration between Taiwan and Australia to SARDI (abalone, sea urchin), the Australian Seafood CRC (cobia, giant grouper) and Clean Seas Tuna (copepod production system);
- Recommendation of a modified copepod pond production system to Clean Seas Tuna;
- Develop and submit a collaborative preliminary project proposal “The effects of feeding copepods on the performance of specific larval rearing stages” to the Australian Academy of Technological Sciences and Engineering (ATSE) International Science Linkages Programme.

OTHER OPPORTUNITIES

Research and develop copepods mass production system, in particular:

- Effects of using live copepods on YTK/SBT survival and growth and advantages;
- Develop a copepods mass production system within hatchery for supplying high quality live foods to fish larvae.

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

Cumulated inbreeding in commercial production

Currently, the cobia larval survival rates in hatchery in Taiwan have decreased significantly in recent years. It is believed that this may result from cumulated inbreeding in their broodstock used in commercial production. Establishing a selective breeding program has been identified as one of the highest priorities to address these issues in Taiwan. This is a good example to be considered by funding agency like CRC and local industries when developing new projects with finfish such as cobia, YTK and SBT.

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