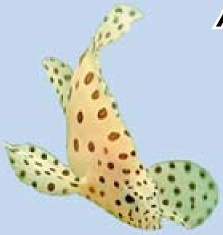

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Advances in the seed production of Cobia *Rachycentron canadum* in Vietnam

By Le Xan

Research Institute for Aquaculture No 1.

Cobia culture is expanding throughout the world, notably in China and Vietnam. Cobia have an extensive natural distribution, grow quickly, and can feed on artificial diets. Under culture conditions, Cobia can reach 3–4 kg in body weight in one year and 8–10 kg in two years. Products from Vietnamese Cobia are exported to the US, Taiwan Province of China and local markets. The market price of one-year farmed Cobia are around US$ 4–6 kg in Vietnam. Research on seed production and grow out culture of cobia in Vietnam began in 1997-1998.

Adult cobia, Rachycentron canadum. These two were on the menu!

Broodstock and spawning

Broodstock can be acquired by purchasing wild fish or by collecting dominant individuals from grow-out operations (selecting broodstock from different parental lines to avoid inbreeding). Most fish more than two years in age have fully developed ovaries, but it is best to collect three-year old broodstock if possible. In Vietnam, cobia spawn twice per year during April to May and September to October. Conditioning of broodstock usually starts some 3-4 months before anticipated spawning, by feeding with trash fish, squid and swimming crab supplemented with mineral vitamins and 17α-methyltestosterone. The amount of trash fish fed is about 4 – 5%/body weight per day.

Mature fish are spawned in dedicated spawning tanks or sometimes in floating net cages. Spawning tanks are $60m^3$ in volume with a depth of 2.5m. Female broodstock are administered with an injection of LRH-e or LRH-a at a dosage of 20 µg/kg female,

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APEC

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Contact
Asia-Pacific Marine Finfish Aquaculture Network
PO Box 1040
Kasetsart Post Office
Bangkok 10903, Thailand
Tel +66-2 561 1728 (ext 120)
Fax +66-2 561 1727
Email grouper@enaca.org
Website http://www.enaca.org/marinefish

Hatchery-reared juvenile cobia.

with males receiving half of this dose. There isn't a need to inject all females but only one or two pairs. Spawning of cobia usually takes place at night, although it occasionally also happens during the day. After spawning, fertilized eggs are separated out and collected using seawater at 35–36‰. Sinking eggs should be discarded.

Eggs are stocked in the incubation tank at a density of 2000–3000 eggs/litre. The incubation tank is 500m^3 in volume maintained with light aeration. Water exchange is carried out at 200-300% per day, using an input and overflow pipe system.

Larval rearing

Cobia larvae are reared in cement ponds, composite tanks or earthen ponds. A suitable pond size is 400-500m^3 in volume with an average depth of 1–1.2 metres. Rearing ponds are fertilized to stimulate production of natural live feed before stocking with larvae. Live feed density needs to be checked frequently, and if low, must be supplemented with correctly sized live feeds (rotifer or copepod) to suit the larvae as they grow. After 22 – 25 days, larvae can be fed with mixed food or artificial diets. However, there may be a need to transfer larvae to a larval rearing tank where they can be trained to accept the new food and receive proper care.

A suitable size for larval rearing tanks is 3–10m^3 in volume. The optimal temperature for rearing the larvae is in the range 24–30^{O}C, with a salinity of 28–32‰,pH 7.5–8.5 and light intensity about 500 lux. Larvae of cobia that must be weaned can be reared in salinity of 20 – 22‰. The microalgae *N. ocullata*, *Chlorella* or *I. galabana* should be supplied and maintained at a density of around 40,000–60,000 cells/ml in the rearing tanks. We have found that dark coloured larval rearing tanks (green or black) tend to give better larval survival.

Density

The optimal density for larvae in rearing tanks varies with their age as follows:

- 1–10 days larvae density at 70–80 individuals/litre
- 11–20 days larvae density at 20–30 individuals/litre
- 21–30 days larvae density less than 10 individuals/litre.

In the earthen ponds, stocking density is 1,500-2,000 individuals/m^2.

Water exchange

Daily water exchange rates are:
- Between days 0–10, 0–10% of tank water is exchanged.
- Between days 11–20, 30–50% of tank water is exchanged using natural flow.
- After day 20, 100–200% of tank water is exchanged daily. We use a simple biofilter, but the electricity cost can be quite high.

Grading

Grading is very important to reduce cannibalism. By day 25, larvae harvested from rearing tanks should be graded into small and large size groups, and maintained separately with their own rearing regimes.

Feeding

First larval feeding is with rotifer *B. plicatilis* at a density of 15 individuals per ml until 12 days after hatching. *Artemia* nauplii can be given from 7–20 day old larvae. Artificial feeds can be introduced from day 17–18, but it typically takes around 3-4 days to train the larvae to accept them.

In feeding experiments using enriched rotifers and *Artemia* nauplii we found that the enriched live feeds give better results than unenriched feeds.

The composition of artificial diets we use are as follow:
- Fresh tunny meat minced: 47%
- Mixed fish meal (45% protein): 25%
- Soybean meal, rice bran meal: 15%
- Vitamins, mineral meal: 3%

All compositions are mixed; crushed and sieved to a size suitable for the mouth of larvae. Artificial diets should be made daily.

Metamorphosis in cobia requires around 25 days to complete at a temperature of 26–28^{O}C with adequate feed. After day 25, larvae can be weaned completely onto artificial diets.

In Vietnam, some hatcheries involved in rearing cobia larvae with the regime above achieve a survival rate of 15–20% (from day 0–day 25), and 40–50% from day 25 to 50, after which fry are around 7.5-8.5 cm in length.

Australian success with barramundi cod

Dr Shannon McBride

Technical Manager Good Fortune Bay Fisheries Ltd.

Good Fortune Bay Fisheries Ltd hatchery at Bowen, Queensland, Australia, has successfully produced 100,000 juvenile barramundi cod (*Cromileptes altivelis*) since January 2005.

The GFB Fisheries Ltd facility is a saltwater aquaculture site incorporating substantial broodstock, hatchery, nursery and grow-out facilities. The company produces saltwater barramundi (*Lates calcarifer*) and intends to further expand its production into reef fish species. High quality seawater is pumped directly from the ocean and is utilized in land-based raceways for grow-out operations. The site is adjacent to the Great Barrier Reef Marine Park and all operations are performed under strict environmental guidelines.

The broodstock are held in 50 m^3 temperature controlled tanks and husbandry conditions ensure a regular supply of high quality fertilized eggs. The hatchery has continued to build on the success of previous years and plans to double the production of barramundi cod this season.

The success in barramundi cod production has been assisted by information and technology made available through ACIAR and the Asia-Pacific Marine Finfish Aquaculture Network.

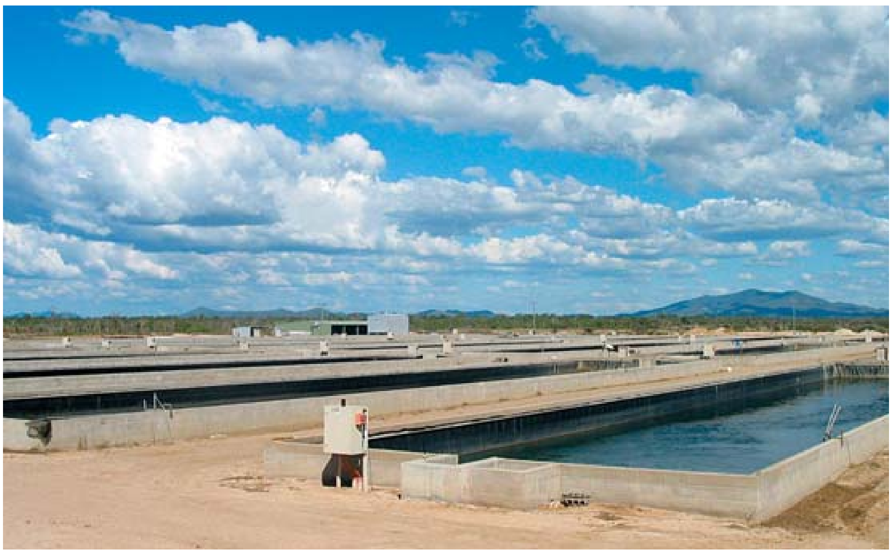
Research and development

GFB Fisheries Ltd is collaborating with the Northern Fisheries Centre in Cairns to assess the feasibility of industrial scale production of copepods as live feed for larval rearing in reef fish aquaculture. The use of copepods will be assessed by improved survival of barramundi cod in the hatchery and by expanding production to include coral trout (*Plectropomus* spp.).

As the number of juvenile barramundi cod produced at the site continues to increase, the company is looking towards the development of appropriate nursery and grow-out diets in conjunction with Ridley Aqua-Feed (Australia). These specific diets would minimize wastes, particularly nitrogen, and also optimize growth.

Future

GFB Fisheries Ltd. continues to develop its expertise in the production of barramundi cod, a reef fish highly valued by international markets. This is an exciting and challenging period for GFB Fisheries Ltd. as a leading Australian company in the development of reef fish aquaculture.



Grow-out raceways at the Good Fortune Bay facilities, Bowen, Australia.

Brief overview of recent grouper breeding developments in Thailand

Sih-Yang Sim[1], Hassanai Kongkeo[1], and Mike Rimmer[2]

1. Network of Aquaculture Centre in Asia-Pacific, Bangkok, Thailand;
2. Northern Fisheries Centre, Department of Primary Industries and Fisheries, Queensland, Australia.

Juvenile coral trout (P. leopardus) produced at Trad Coastal Aquaculture Station.

Thailand's success in breeding grouper species dates back to 1984-85 when the Phuket Coastal Fisheries Research and Development Center (Phuket CFRDC) and Satul Coastal Fisheries Research and Development Center succeeded in breeding *Epinephelus tauvina* (possibly misidentified *E. coioides*)[1,2]. The Phuket CFRDC also achieved the first successful grouper larval rearing during September 1984 to February 1985, when some 130,000 fry aged 45 days were produced[3].

In October 1998, the National Institute of Coastal Aquaculture (NICA) based in Songkhla successfully produced giant grouper *Epinephelus lanceolatus* by artificial propagation, but the survival rate was very low. In September 1999, NICA had another success in giant grouper breeding using preserved milt to fertilise freshly stripped eggs[4]. Since that time, work at NICA has focused on shrimp aquaculture, while other coastal research stations in Thailand have continued to develop marine finfish aquaculture.

In 2002 the Krabi Coastal Fisheries Research and Development Centre (Krabi CFRDC), reported its first success in breeding and larviculture of tiger grouper (*Epinephelus fuscoguttatus*) with a survival rate of 2% to 70 day-old juveniles[5]. The Krabi centre has also succeeded in producing *E. coioides* fingerlings for some years and now provides 100,000 – 200,000 fingerlings per year to Thai farmers. With the recent worldwide interest in ornamental fish, thanks to the film 'Finding Nemo', it is notable that Krabi centre has been able to produce seven varieties of clownfish (anemone fish) native to Thailand[6].

After several trials in October 2003 the Trad Coastal Aquaculture Station (Trad CAS) in eastern Thailand successfully managed to produce its first batch of coral trout *Plectropomus leopardus* fingerlings[7], which it has been consistently producing in small numbers ever since. As of 16 June 2005 there were some 12,000 coral trout larvae at 31 days of age. Trad CAS also holds broodstock of *P. maculatus* (island or bar-cheek trout) but these have not yet spawned.

Mr. Thawat Sriveerachai, Chief of Trad CAS, said the key factor for success of coral trout breeding in Trad is water quality management. As Trad is subject to heavy rainfall throughout the year, it is important to protect the water quality in broodstock tanks from heavy variation, particularly in salinity. Trad station utilises recirculation systems and biological water treatment for coral trout broodstock, as well as other species. The recirculation system is a combination of traditional biological filtration plus bioremediation using shrimp, molluscs, sea urchins, swim-

Close up of a one inch coral trout (P. leopardus) fingerling in larval rearing tanks, Trad CAS.

ming crabs and fish. The water in the broodstock tanks is changed only once per year.

The recirculation systems used at the Trad centre are low cost and relatively robust. In contrast, many of the more sophisticated recirculation systems available in the market today may not suitable for marine finfish species, are expensive, costly to maintain and problematic.

Trad CAS researchers have noticed that larval quality and survival is improved by enhancing the fatty acid composition of the live feeds used in larval rearing. Larvae fed nutritionally enhanced live feeds are usually of better quality and do not show the same 'shock' behaviour seen in larvae fed traditional live feeds.

In 2005, the Rayong Coastal Fisheries Research and Development Center (Rayong CFRDC) made a breakthrough in mouse grouper (*Cromileptes altivelis*) larval rearing. The center uses the a simple recirculation system similar to Trad CAS for their broodstock holding facilities. The broodstock tanks are rather small at 3×5×1.2 m. Although mouse grouper broodstock successfully spawn in these tanks, egg production is low, which limits fingerling production.

Rayong CFRDC also operates a large broodstock holding cage facility at nearby Koh Samet. This facility holds broodstock of several grouper species including *P. maculatus*, *E. fuscoguttatus*, *E. lanceolatus*, *E. coioides*, mangrove snapper *Lutjanus argentimaculatus* and cobia *Rachycentron canadum*. Like Trad, Rayong have not been able to spawn their *P. maculatus* broodstock, despite attempts at hormonal induction of spawning.

There is considerable interest amongst the private sector in Thailand in developing marine finfish hatcheries. There is already considerable production of seabass (*Lates calcarifer*) in Thai hatcheries, and many are keen to diversify their production to higher-value species such as groupers. A major constraint to diversification amongst private hatcheries is access to eggs and larvae. Many are now working with the government centers and stations so that when fertilized eggs are available in government facilities, they can obtain them for grouper larviculture trials. The government also provides training and technical support on grouper hatchery technology to the private sector.

Mouse grouper fingerlings produced at Rayong Coastal Fisheries Research & Development Center.

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- Mr. Ekapol Wanagosoom, Partner and Manager of Aqua-Larval Farm, Thailand

E. coioides broodstock in the floating cage facilities, Krabi CFRDC.

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Marine finfish broodstock facilities of Rayong CFRDC near Koh Samet

Application of probiotics in rotifer production systems for marine fish hatcheries

Tawfiq Abu-Rezq and Charles M. James

Kuwait Institute for Scientific Research, Aquaculture Fisheries and Oceanography Dept., P.O. Box 1638, Salmiya 22017, Kuwait

There is an urgent need to develop microbial control strategies in marine fish hatcheries. Disease outbreaks are a major constraint to hatchery output, and use of antibiotics is generally considered undesirable within intensive grow-out systems. Recent advances in science have made it possible to use probiotics, so-called 'friendly' microbes, as an additional tool in health management. 'Probiotics' is a catch all term commonly used to describe microbes (or products containing microbes) that perform beneficial functions, and which are normally seeded within the production system in some manner. The seeding of biofilters with bacteria capable of breaking down nitrogenous wastes is a classic example of the use of 'beneficial' microbes within aquaculture. More recently, attention has focused on use of probiotics that can assist in disease control, for example by competing and interfering with other harmful microbes.

The 'probiotic approach' has some advantages over conventional use of antibiotics and other chemicals. The use of probiotics tends to focus on disease prevention rather than cure, and as a more 'natural' alternative, they are not currently subject to the same restrictions in international trade.

Probiotics are widely used in aquaculture applications, especially in commercial shrimp hatchery and farming systems. In recent times, use of probiotics has been also extended to marine finfish hatcheries, the shellfish industry and in live food production systems. Live food used in marine fish hatcheries, such as the rotifer *Brachionus plicatilis*, is an important carrier of bacterial pathogens that can result in larval fish mortalities. At the Aquaculture Fisheries and Oceanography Department (AFOD) of the Kuwait Institute for Scientific Research (KISR), the seawater used for live

Microalgal production facility.

food production is treated through use of protein skimmers, pressurized sand filters, cartridge filters and UV sterilizers before the water is stored in a reservoir for use. However, pathogenic bacteria still occur in the live food production system, especially in the intensive rotifer cultures using chemostats, and several incidences of larval fish mortality have been attributed to pathogenic bacteria from this source. To overcome this problem, the conventional rotifer batch culture method was adopted at AFOD instead of using intensive continuous chemostat cultures, since the continuous cultures build up undesirable bacterial loads over several months. Aiming to deliver desirable probiotics to marine fish larvae, recent studies at AFOD have focused on using commercially available probiotics in rotifer production systems. This article describes the efficiency of using commercially available probiotics for rotifer production and some of the possible advantages of using probiotics in the intensive rotifer production systems for commercial applications.

Source of probiotics and application method

We used a commercially available probiotic Alken Clear-Flo® 1006 (ACF-1006), procured from Alken Murray Corp., USA. ACF-1006 is a dry synergistic blend of bacteria specially designed to discourage disease proliferation in aquatic environments by enhancing the immune response of cultured species while eliminating specific pollutants that foster pathogenic *Vibrio* spp., and other disease causing species. This product uses a consortium of six gram-positive bacilli and ten gram-negative vegetative strains. It is a free flowing brown powder with a bacterial count of 3.5×10^{12} CFU/g. Before application, the dry product was mixed in the proportion of 10g of product to 150ml of seawater. The mixture was allowed to hydrate for two hours, stirring vigorously every 30 minutes. After final settling, the supernatant liquid layer was poured off for use. The first set of experiments evaluated the efficiency of ACF-1006 for feeding the rotifers using:

- ACF-1006 alone at a feeding rate of 1g/million rotifers/day;
- ACF-1006 at 1g/million rotifers/day plus *Chlorella* at a cell density of 25 million cells/ml;
- ACF-1006 at 0.5 g/million rotifers/day plus *Chlorella* and bakers' yeast at 0.5 g/million rotifers/day;
- *Chlorella* in combination with bakers' yeast at 1 g/million rotifers/day (control); and
- *Chlorella* alone at 25 million cells/ml.

The experiment was carried out in 5 litre beakers at a controlled temperature of 24.5-25ºC using three replicates for each treatment.

Based on the results obtained during the first experiment, the second experiment was carried out in 1m^3 capacity conical fiberglass tanks to understand the production dynamics of rotifers with and without probiotics. The water temperature was controlled at 25±1 ºC. In the first treatment with probiotics, seven replicated cultures were carried out using a feed combination of *Chlorella*, bakers' yeast and ACF-1006. Bakers' yeast and ACF-1006 were used at a feeding rate of 0.5g/million rotifers/day each. In the second treatment without probiotics, six replicated cultures were carried out using a feed combination of *Chlorella* and bakers' yeast at 1g/million rotifers/day. Rotifer harvests were made every alternative day as per requirement and the tank culture volume was adjusted by the addition of seawater and *Chlorella*.

Production dynamics of rotifers with and without probiotics

Among the different treatments used during the first set of experiments, a significantly higher (P<0.01) rotifer density and culture duration was observed when rotifers were fed with a combination of *Chlorella*, bakers' yeast and ACF-1006 compared to that of other treatments in the culture system. Maximum densities of 309.7±9.1 and 304.3±27.1 rotifers/ml were observed in this feed combination on days 8 and 9. The culture duration was also extended when using the probiotic combination in the feed compared to that of without probiotic. However, the rotifer growth was poor while using ACF-1006 alone and the culture declined on day 9 of the observation period showing that use of probiotic alone as a feed was not conducive for rotifer cultures.

In the second set of experiments significant increase (P<0.001) in the rotifer productivity was observed when using ACF-1006 along with *Chlorella* and bakers' yeast in the 1m^3 capacity rotifer production tanks compared to that of using *Chlorella* and bakers' yeast alone without probiotic. The rotifer productivity averaged 12.13±1.89 rotifers/ml/day when using ACF-1006 along with *Chlorella* and bakers' yeast. Without ACF-1006 the rotifer productivity averaged 6.64±3.60 rotifers/ml/day.

Extended rotifer cultures of more than 27 days were observed in the ACF-1006 feed combination compared to that of without ACF-1006, which declined on day 13 of the experiment. The rotifer growth rate and doubling time

Seawater treatment system for live food.

Rotifer chemostat production facility.

was also significantly higher (P<0.001) while using the probiotic in the culture system compared to that without it. The rotifer instantaneous growth rate (*K* value) averaged 1.96±0.45 when using ACF-1006 and averaged 0.78±0.17 without the probiotic. The rotifer doubling time averaged 0.37±0.09 days with probiotic and 0.92±0.20 days without, showing the efficiency of using the probiotic in the culture system. A significant increase (P<0.001) of the ciliate *Euplotes vannus* was also observed in cultures containing probiotic compared to those without.

Future prospects

The results of these investigations show that using of the ACF-1006 probiotic in the rotifer culture system can enhance rotifer growth rate and productivity, as well as extend the culture duration. In general, rotifer productivity under semi-continuous or batch culture systems at AFOD yields about 52 to 60 rotifers/ml/day. The intensive chemostat rotifer production system developed at AFOD using 1m^3 capacity tanks yields an average of about 255 to 261 rotifers/ml/day with a feed combination of *Nannochloropsis* and bakers' yeast. Such systems are desirable in commercial ventures due to their ability to produce a large rotifer biomass per unit space and time, compared to that of batch cultures. However, although the rotifer productivity is considerably higher in the chemostat culture system than conventional culture methods, we no longer use the chemostats due to the undesirable bacterial build up under long-term culture conditions. The results of our investigation suggests that application of commercial probiotics in the rotifer cultures can help to eliminate this problem and ensure the health of the marine fish larvae due to the encapsulation of probiotics in rotifers. However, further investigations are required to evaluate the beneficial effect of using probiotic fed rotifers to marine fish larvae. Further research is also required to assess the efficacy and use of other commercial probiotics and local isolates of beneficial bacteria towards bio-encapsulating rotifers as feed for marine fish larvae, as well as to enhance the rotifer productivity and culture tank conditions.

Collaborators

The following organizations and contacts are focal points for communication in the network:

Hong Kong
Dr Jim Chu, jim_cw_chu@afcd.gov.hk

India
Dr Mohan Joseph Modayil, mdcmfri@md2.vsnl.net.in

Indonesia
Dr Ketut Sugama, crifidir@indonet.id
Dr Muhammad Murdjani, lbapstbd@radnet.id

Iran
Dr Shapour Kakoolaki, bsh443@yahoo.com

Malaysia
Coastal marine fish culture
Mr Ali bin Awang, ppbuk@po.jaring.my

Fish quality
Mr Ismail Ishak & Mr Hamdan Jaafar
anasofiah@hotmail.com / hamdanj@yahoo.com

Low food chain species
Mr Hussin bin Mat Ali
pppil@po.jaring.my

Philippines
Ms Prescilla B. Regaspi, pregaspi@bfar.da.gov.ph
Ms Marygrace C. Quintero, mgquintero@bfar.da.gov.ph

Collaborating organisations