Improvement of larval rearing technique for Humpback grouper, *Cromileptes altivelis*

Ketut Sugama[1], Suko Ismi[2], Shogo. Kawahara[3] and Mike Rimmer[4]

1: Gondol Research Institute for Mariculture. Present Address: Central Research Institute for Aquaculture, Indonesia, Jl. KS Tubun Petamburan VI Jakarta, 3. Japan International Cooperation Agency, 4. Queensland Department of Primary Industries.

Introduction

Breeding of humpback grouper, *Cromileptes altivelis* has been underway in Gondol Research Institute for Mariculture Indonesia since 1995. Techniques for mass seed production of this fish were successful in late 1998 under research collaboration with JICA on the Multi-species Hatchery Project and the ACIAR Improved Hatchery Technology for Groupers Project and transferred to private hatcheries including backyard hatcheries in 1999. The techniques, which are based on natural spawning in captivity and the intensive rearing of larvae in tanks, are described in detail by Sugama et al. (2001). About one million juveniles (4-5 cm TL) were produced in 2000 and more than three million juveniles in 2001. Egg production is no longer a constraint since private hatcheries routinely produce billions of eggs from domesticated broodstock. In the research result carried in Research Institute, Sugama et.al. (2001) reported the highest known survival of 53.9 % in 10-ton tank on day 50 for this species. However, in recent times survival has been highly variable (low and irregular) due to various factors, chiefly due to infection with Viral Nervous Necrosis (VNN) infection (Koesharyani et.al.2001)

This paper summarize the larval rearing techniques that has been practicing in grouper hatcheries within Indonesia.

Broodstock management, maturation and spawning

Broodstock

At present, all of existing humpback grouper hatcheries are using wild caught fish as broodstock. This species is a protogynous hermaprodite, meaning that it is first sexually mature as a female and later on changes to be male. The smallest mature female is 1 kg body weight and only among broodstock more than 2.5 kg can males sometimes be found. In some cases, females do not change the sex even if their body weight is more than 3kg.

Broodstock Tank

The recommended volume of broodstock tanks can range from a minimum of 20 to a maximum of 200 tons. However, considering biological and management factors, the ideal volume of tank is 50-100 tons with 2.0-2.5 deep. The ideal shape of maturation tank is circular, with a flat bottom and 5-10 % gradient toward the central drain. The broodstock tank is equipped with a water inlet and outlet (over-flow) pipes and egg collection tank with a fine net (400mm) that is connected with the outlet pipe, and an aeration system.

Broodstock Care

The techniques for the capture, transportation, handling, sexing, sampling and acclimation of humpback grouper broodstock have been adequately developed. Prophylaxis using freshwater, antibiotics and quarantine is necessary before introducing broodstock into the maturation tank. A detailed description of prophylaxis and quarantine techniques is available in work by Sugama et.al.(2001). Recent advances have led to improved handling to reduce stress coupled with improved broodstock nutrition and good water quality and could increase overall egg quality. This leads to higher first feeding success rate and subsequent higher survival throughout the early developmental stages.

Maturation and spawning

Following quarantine and acclimation, broodstock fish are stocked in a maturation tank. The tank system is a flow-trough, achieving 200-300% water exchange daily. Generally, 30 fish are stocked in 100-ton tank with sex ratio of two females and one male. Fish are fed with mixed fresh or frozen trash fish (avoid using only *Sardinella* sp.) and squid mixed with 1% vitamin mix at 2-3 % of body weight per day.

Six to eight months after stocking in tank, the fish spawn naturally in captivity. The spawning usually occurs every month 7-10 days before and after the new moon phase and spawning takes place from midnight to early morning.

Early Cromileptes fry produced at Gondol

A closer view of the fry

Egg selection

Collected eggs are transferred into a transparent polycarbonate tank filled with filtered seawater, debris mixed with the eggs is removed using a 1.0 mm mesh net. In the tank, the eggs separate into three groups namely, floating, suspended and sinking eggs. Only floating eggs are recommended for further use in larval rearing. Floating eggs are soaked in 20 ppm iodine for 10 minutes or washed with UV treated sea water for 30 minutes to prevent a possible contamination of bacteria or other micro organism that may cause disease. In water temperatures of 28-29oC, the eggs hatch after 18-20 hours of incubation. Eggs from broodstock fed with fresh and mixed trash fish four times and squid three times a week with 1 % vitamin mix result in good quality larvae. The newly hatched larvae incubated without feeding can survive until seven days after hatching, while eggs from broodstock fed with sardine only mixed with 1% vitamin mix had completely died five days after hatching. I recommend using good quality eggs for seed production of this fish (Fig 1).

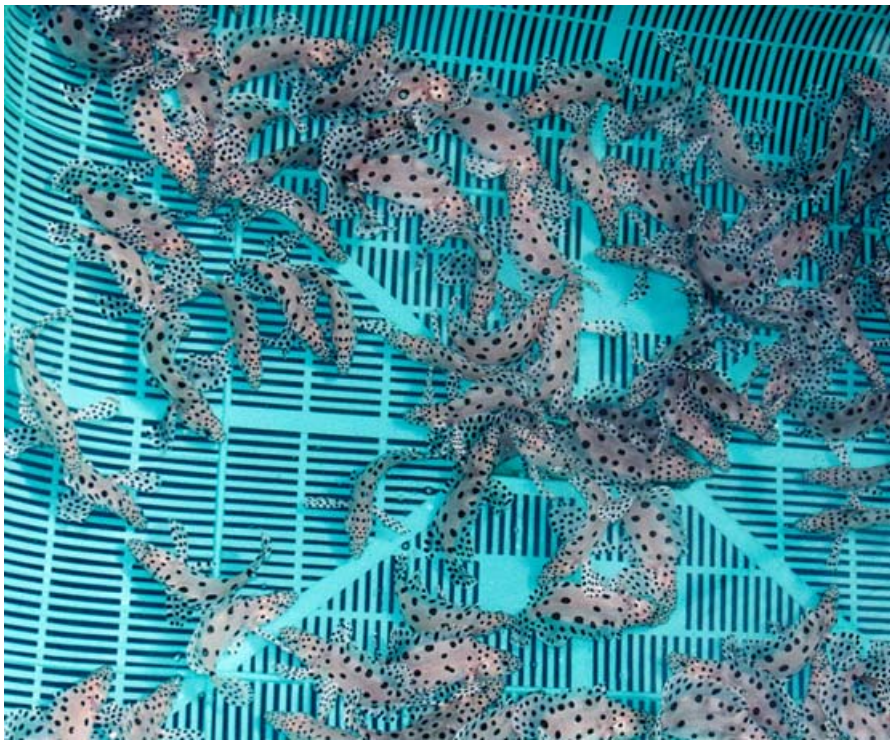
Larval rearing

Larval Rearing Tank

A particular feature of grouper hatcheries in Indonesia is use of the indoor method. The recommended size for larval rearing tanks is 10 ton with 1-1.2 metre depth. Both circular and rectangular tanks with flat bottoms can be used for larval raring. The tank should be painted with a light blue or yellow colour. For backyard hatcheries, the larval rearing tank should be roofed to avoid direct sunlight and rainwater. In order to minimize water temperature fluctuation, it is recommended to cover the tank with a transparent plastic sheet. The sheet is partially opened during the day and closed at night. The larval rearing tank should be filled with sand filtered sea water on the day of egg inoculation.

Feeding and Water Management

The eggs are generally added directly to the larval rearing tank with a density of 5-10 eggs/litre. Occasionally these are placed in hatching tanks and then the newly hatched larvae are transferred to the rearing tank, this process enables larval density to be estimated more accurately. The larval rearing is undertaken using green water techniques. The algal density (*Nannochlosopsis*) used for green water culture ranges from 300,000 to 500,000 cell/ml. Variables such as algal density are measured only in research hatcheries, commercial hatcheries and backyard hatcheries just add algal cells until the desired shade of green is reached. Two-day-old larvae are fed small rotifer (SS-strain, size 120-140m) usually for three days at density of 5-7



Fingerlings being graded for sale to local nurseries and farms

individuals/ml and followed by S-strain rotifer (180-200m) at density of 8-10 individuals/ml until day 20-24. Rotifers are enriched using a commercial fatty acid booster (DHA protein Selco) or by using concentrated *Nannochloropsis* before supply to the larval rearing tank. Commercial compound feed is used as an artificial diet introduced from day 17 onward and enriched *Artemia* is supplied from day 20 onward at density of 0.5 individuals/ml. A detailed feeding scheme and water management is presented in Table 1.

Survival rate

In 1999-2000 during five trials, the survival rate ranged from 2.65 to 5.13 % with total production of 22,000 juveniles (Sugama et.al 1999). At that time most mortality of the larvae occurred during the initial 2-5 days after hatching. Larval mortality was mainly due to poor quality of the newly hatched larvae. The newly hatched larvae were very weak, hence, easily trapped at the water surface by water tension. As well, the trapped larvae would be stressed and produce mucus that would accelerate the trapping of other larvae. In the 2000 trials, improvement of broodstock feed produced better quality of eggs and larvae. Furthermore, spreading squid oil on the surface of larval rearing water seemed to reduce such mortality.

Beginning on day 10-11, larvae have an elongated dorsal and pelvic fin spines, which often entangle larvae, especially when they swim to a common place in the tank wall near the water surface, probably in response to the light. Here they aggregated and clump together, which may be accelerated by the mucus and eventually die. Consequently, a high mortality (20-30 %) frequently occurred between day 10-25. To prevent total aggregation, a fluorescent tube lamp (40 watt) was hung above the larval rearing tank with minimum light intensity of 800 lux. The light intensity was adjusted as evenly as possible on the water surface. The colour of larval rearing water was maintained green through inoculation of *Naanochloropsis* at a density of 300-500 x 103 cell/ml. This might reduce larval aggregation.

Gradual larval mortality was usually observed after day 25, which was suspected to be due to nutritional deficiency. To prevent this problem, early weaning of larvae onto artificial diets that have sufficient nutritional value is recommended. In 2000-2001 trials, artificial diets were introduce at day 15 prior to feeding *Artemia*, and this minimized the demand of *Artemia* as food. The remaining Artemia in the larval rearing water should not be kept for more than one day. With this feeding management, mortality could be reduced and resulted in absence of lordosis. Based on our observation cannibalism was not the main factor of mortality in humpback grouper.

Figure 1. Survival of larvae without feeding from eggs that spawned by different broodstock

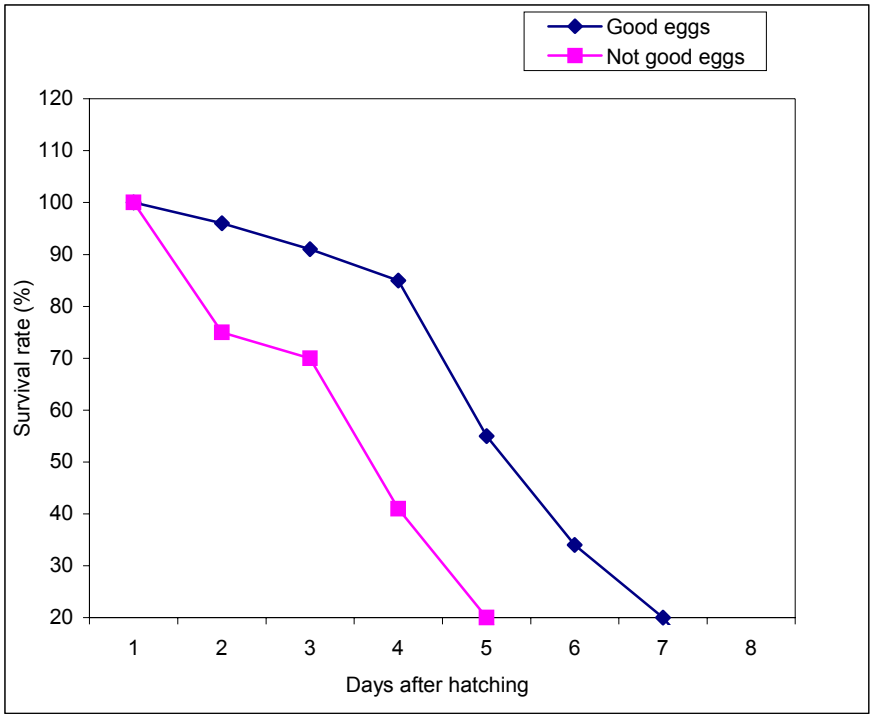


Table 1. Feeding scheme and water management in larval rearing of humpback grouper *Cromileptes altivelis*

Feeding scheme		
Day 2-25	*Nannochloropsis* (3-5 10^5 cell/ml	Control at 8:00 AM and 5 PM
Day 3-5	SS-strain rotifer (5-8 ind/ml)	Control at 8:00 AM and 5 PM
Day 5-25	S-strain rotifer (8-10 ind/ml)	Control at 8:00 AM and 5 PM
Day 15-31	Artificial diet (200-400µ)	1.5 g. each, four times daily
Day 20- 45	Artemia (0.5-1.0 ind./ml)	Supply at 5 PM
Day 28-39	Artificial diet (400-600µ)	2-5 g each, five times daily
Day 36 onward	Artificial diet (600-800µ)	5-10 g each, 7- 12 times daily
Water management		
Day 2-5	Spread squid oil in water surface	1 ml each, 10 AM and 15 PM
Day 11-17	10 % water exchange	Bottom siphoning at 9 AM
Day 18-30	20 % water exchange	Bottom siphoning at 9 AM
Day 30-35	50 % water exchange	Bottom siphoning at 9 AM
Day 35 onward	Running Water	Exchanged rate 100 %/day

The success of larval rearing depends on the control of Virus Nervous Necrosis (VNN). Once VNN broke out during larval rearing, most if not all larvae died within 2-3 days (Koesharyani et.al.2001). No treatment method is presently available. To avoid VNN infection, use only VNN-free broodstock by checking sperm and oocyte by PCR. In 2001-2002, an effort was made to reduce larval stress by decreasing the stocking density, improve nutritional quality of live feed (rotifer and *Artemia*) by enrichment and early weaning of artificial diet. In 2001-2002 data have shown that in Government hatcheries the survival at day 50 ranged from 23.4-53.9% in four commercial hatcheries ranged from 3.1-51.4 % and in 15 farmers backyard hatcheries ranged from 7.0-35.01 %.

Production

In 2001-2002, more than one million juveniles have been produced by hatcheries within Indonesia. At present, two Government, seven commercial and more than one hundred farmer backyard hatcheries are actively producing juveniles.

References

Sugama Ketut, Trijoko, Wardoyo, J. H. Hutapea and S. Kumagai, 1999. Natural spawning and larval rearing of Cromileptes altivelis. Report the APEC-NACA Cooperative Grouper Aquaculture Workshop, 7-9 April Hat-Yai, Thailand. 91-98 pp.

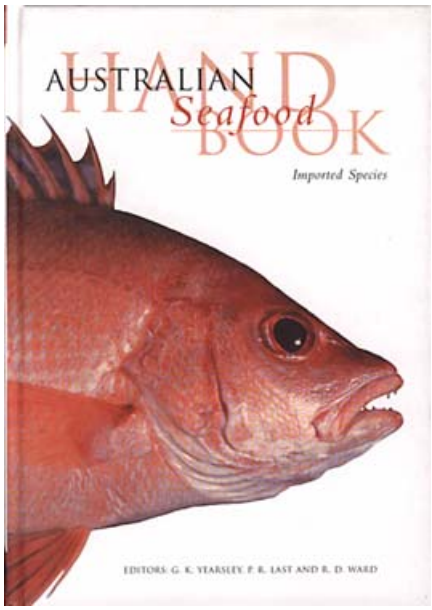
Sugama, Ketut, Trijoko, B. Slamet, S. Ismi, E. Setiadi and S. Kawahara. 2001. Manual for the seed production of Humback grouper Cromileptes altivelis, GRIM-JICA, Agency for Marine and Fisheries Research of Indonesia 37 p.

Koesharyani, I, D. Roza, K. Mahardika, F. Johnny, Zafran and K. Yuasa. 2001. Manual for fish diseases diagnosis II. 2001. GRIM-JICA, Agency for Marine and Fisheries Research of Indonesia 48 p.

Australian Seafood Handbook – an Identification Guide to Imported Species

This is the companion volume to a previously released (and also excellent) publication, the *Australian Seafood Handbook – an Identification Guide to Domestic Species*. The main purpose of the book is to help identify and standardize the naming of imported seafood products and link them with their official marketing name on the 'Australian Seafood Names List'.

Seafood marketing names have been an issue in Australia for some time. Consumers often pay a premium for certain species and there have been some high-profile product substitution rackets along with a lot of genuine confusion over interchangeable local names. Sometimes identical imports are sold side-by-side in the supermarket under different names. The Australian Seafood Names List was introduced standard marketing names to increase consumer confidence in the names used by vendors and to reduce mislabeling.



The guide is beautifully illustrated. Each of the 350 species of fish, crustaceans and shellfish covered is documented with color photographs of the whole animal and a representative fillet. Descriptions include identifying features, size, habitat, distribution and important marketing/trade notes.

A 'protein fingerprint' (electrophoresis gel) is also provided for each species to help confirm the identity of fillets since most imported product is imported in processed form. Regulatory and policing authorities can use these 'fingerprints' as a forensic test to detect product substitution or misrepresentation. The fingerprints depend on genetic variation between species and can be conducted outside the laboratory with only a small sample.

Price: AUD$ 49.95 + $35.00 for international freight. Available from CSIRO Publishing, PO Box 1139, collingwood, VIC 3066, Australia. Fax +61 (3) 9662 7555, email publishing.sales@csiro.au, www.publish.csiro.au.

Conclusion: Highly recommended for all involved in the Australian seafood trade. A very useful and beautifully presented book. Edited by G.K. Yearsley, P.R. Last and R.D. Ward. 480 pages.