Marine Finfish Section

The Grouper Section has taken on a new and broader name: It has become the Marine Finfish Section to take account of other species. This section is almost wholly based on the Marine Finfish Aquaculture Newsletter which is prepared by Sih Yang Sim (Editor), Michael Phillips (NACA Environment Specialist) and Mike Rimmer (Principal Fisheries Biologist of the Queensland Department of Primary Industries). Visit www.enaca.org/grouper for more information on the network or email sim@enaca.org.

Coral trout: World First Breeding Success in Indonesia

The National Seafarming Development Centre (NSDC) in Lampung, Indonesia, has succeeded in breeding the bar-cheeked coral trout *Plectropomus maculatus*. Around 100 healthy and active 10cm fingerlings have been produced from 50,000 fertilized eggs. Although at an early stage of development, the result is very promising and further refinement of the hatchery techniques should enable improved survival rates of this new species for hatchery rearing.

In Bahasa Indonesia language NSDC is also known as Balai Budidaya Laut (BBL). BBL has been working on grouper hatchery mass production technique since 1999. It has had success in production of several grouper species, including Tiger grouper *Epinephelus fuscoguttatus*, Green grouper *E. coioides*, Malabar grouper *E. malabaricus* and Hump-back grouper *Cromileptes altivelis*. The hatchery now produced large quantities of all four species, and the evidence of this success can be seen in the increasing number of commercial grouper grow-out farms in the Lampung area.

NSDC was established in 1982 and its development supported through a FAO/UNDP Technical Assistance Project. It is a NACA Seafarming Centre.

For further information, contact Mr Sudjiharno (Director of BBL) at asts@indo.net.id. The Asia-Pacific Marine Finfish Aquaculture Network also welcomes further information and experiences on the aquaculture of this and other marine finfish species. Please send contributions to grouper@enaca.org.

Report of the Grouper Hatchery Production Training Course, May 2003

The second regional grouper hatchery production training course was held in May 2003 with 14 participants from Australia, Brunei Darussalam, India, Malaysia, Singapore and Vietnam.

The course covered twelve topics: Broodstock management; egg collection, quality checking and treatment; larviculture and hatchery management; cleaning of culture tanks; harvestinglive feed and feeding larvae; harvesting lavae, grading and sorting sizes; packaging and transportation; disease laboratories; PCR testing; and artificial feed production. The full report of the training course, which is illustrated with many photographs, is available for free download from: the MFAN website, http://www.enaca.org/Grouper/.

NACA has been following the 2002 participants with interest. Three of last year's participants have since reported successful larval rearing trials of species. In Thailand, green grouper (*Epinephelus coioides*) was the only grouper species that has been regularly produced in government hatcheries. However, in August 2002, Krabi Coastal Aquaculture Development Station of Department of Fisheries Thailand produced tiger grouper (*E. fuscoguttatus*) seed for the first time. In Vietnam, green grouper fingerlings were successfully produced in June 2002. Around 100,000 fingerlings were reared and sold to fish farmers by the Cat Ba Research Centre for Mariculture of Research Institute for Aquaculture No 1 (Ministry of Fisheries Vietnam). In Malaysia, Mr Lu Kien Chee and Mr Yazid Bin Sahjinan of the Department of Fisheries Sabah reported that they were able to produce tiger grouper fingerlings (6 cm TL and above) in January 2003, and 1,200 fish were sent for grow-out trials. These success stories, following the training course, are a welcome development, and a credit to the staff at the Research Institute for Mariculture - Gondol in Bali, Indonesia, who put significant effort into delivering this well received course.

The training course was organised by the Asia-Pacific Marine Finfish Aquaculture Network under the coordination of Network of Aquaculture Centres in Asia-Pacific (NACA) in cooperation with Northern Fisheries Centre, Queensland, Australia (QDPI) and Research Institute for Mariculture - Gondol. Support for the training course came from the Ministry of Marine Affairs and Fisheries, Indonesia, NACA, the Australian Centre for International Agricultural Research (ACIAR), the Asia-Pacific Economic Cooperation (APEC) and the Japan International Cooperation Agency (JICA). The training course

was conducted in the Research Institute for Mariculture at Gondol, northern Bali, Indonesia.

Joint DPI / JCU Project Targets Captive Spawning of Barramundi Cod / Humpback Grouper (Cromileptes altivelis)

The Department of Primary Industries' (DPI) Northern Fisheries Centre (NFC) in Cairns, Queensland, Australia, is conducting research into the captive breeding of barramundi cod / humpback grouper (*Cromileptes altivelis*). The research focuses on resolving the issues of poor performance and spontaneous sex reversal of male broodstock in captive culture systems. Newly acquired broodstock are being held at the new Aquaculture and Stock Enhancement Facility at NFC, Cairns in 30 and 60 m^3 fibreglass tanks fitted with recirculating biofiltration systems. Current research is investigating the differential response between male and female sex steroids levels (testosterone, 11a-ketotestosterone and 17a-estradiol) to various hormonal treatments. To date, DPI has successfully achieved the sex inversion of females to males (masculinisation) using methyltestosterone implants. Implanted females responded to treatment within 6 days by significantly reducing 17a-estradiol production, with significant proliferation of testicular tissue and viable milt production observed after 35 days. Exogenous hormone implants are being used to artificially induce sex change from female to male (inversion) and male to female (reversal) in order to identify the mechanisms involved in sex change. Future research will focus on prolonged hormonal manipulation to control the sex ratio of captive spawning populations. In conjunction with this work, a quantitative real-time PCR assay to study the gene expression of the enzyme aromatase is being developed, using the facilities at DPI's Bribie Island Aquaculture Research Centre and James Cook University. Aromatase converts the precursory steroid testosterone to feminising oestrogen steroids and is the primary enzyme involved in the sex change process. The down or up regulation of aromatase gene expression in protogynous fishes signals the onset of sex inversion or reversal respectively. In in vivo studies expression of aromatase gene will be assessed using gonad biopsies during induced sex change. In addition, specific *C. altivelis* brain and gonadal cell lines have been developed to study the regulation of aromatase gene expression. This in vitro research will study the response in gene expression to specific stimulatory and inhibitory compounds in isolation. Subsequent research (in 2004) will focus on developing a successful delivery system to induce spawning behaviour in broodstock, utilising existing procedures and prostaglandins. The projected outcomes of this research are to increase our current knowledge of the mechanisms controlling sex change in *C. altivelis* and development of techniques to ensure functional reproductive males in captive populations. This research is funded by the Queensland Government's Aquaculture Industry Development Initiative. For further details contact: Adam Reynolds (adam.reynolds@dpi.qld.gov.au); Elizabeth Cox (elizabeth.cox@dpi.qld.gov.au) or Abigail Elizur (abigail.elizur@dpi.qld.gov.au)

Philippines Working on Becoming Self-Sufficient in Milkfish Fry

A program to make the Philippines self-sufficient in bangus (milkfish) fry in a few years is on target, the Bureau of Fisheries and Aquatic Resources (BFAR) recently said. Westly Rosario, Chief of the Dagupan-based National Integrated Fisheries Technology Development Center (NIFTDC) of the BFAR, said since the program was launched in October 2002, some 16 million good milkfish eggs had already been produced. P10 million (US$188,000) has been made available from Countrywide Development Fund for the establishment of the central bangus hatchery. The target of the program is the production of 200 million milkfish eggs for distribution to 18 satellite breeding centers and private hatcheries across the nation. Rosario said that Dagupan is known for its tasty bangus. Through the program, the proper technology in raising bangus, the way they do it in the aquaculture farms of Dagupan, is being disseminated all over the country. At least 6.5 million eggs have already been delivered to private satellite hatcheries in Labrador, Pangasinan; and Damortis, La Union. These private hatcheries will raise the eggs until they grow into fry, for sale to fish-farmers who are in short supply of this commodity in their respective provinces. Rosario said in later months, the central hatchery here will also supply fish eggs for the other satellite hatcheries in Cabangan, Zambales; Bais, Negros Occidental; and the BFAR satellite hatcheries in Tiwi, Albay; Naujan, Occidental Mindoro; Guian, Eastern Samar; Dumangas, Illoio; and Calape, Bahol. To date, bangus fry being used by the country's fish-farmers are coming from Taiwan and Indonesia, draining the country of its much-needed dollar reserves, Rosario said. Source: Asia Pulse, April 8, 2003.

Enhancing Reef Recovery in Komodo National Park, Indonesia: Coral Reef Rehabilitation at Ecologically Significant Scales

Fox H.E, Mous P.J. Muljadi A., Purwanto & Pet J.S.*

Illegal fishing with homemade bombs or dynamite is rampant throughout Southeast Asia and has devastated many coral reefs in the region. In addition to fish and other organisms being indiscriminately killed, coral skeletons are shattered by the blasts, leaving fields of broken rubble. This rubble shifts in the current, abrading or burying any new coral recruits, thereby slowing or preventing reef recovery. Due to effective management, blast fishing has decreased in Komodo National Park (KNP), Indonesia, making restoration efforts worth investigating. Based on 4 years of pilot data testing three different methods (rock piles, cement slabs, and netting pinned to the rubble) rocks were selected for large-scale rehabilitation. Many more corals per square meter grew on the rock piles compared to untreated rubble. Rocks also provided the most natural, complex substrate, were easiest to scale up, and are relatively inexpensive compared to reef rehabilitation methods being investigated elsewhere. Mid-scale rock piles were installed in 2000; cover by hard corals on the rocks continued to

increase as of this most recent visit (March 2003). In 2002, rehabilitation efforts in KNP were further scaled up, testing four rock pile designs at each of four different rubble field sites, covering more than 6,000 m^2 total. If the rubble fields have adequate source coral larval supply from nearby live coral, using rocks for simple, low-cost, large-scale rehabilitation could be a viable option to restore the structural foundation of the reefs, thereby facilitating the return of coral, fish, and other reef-associated life. For further information contact: Dr. Peter J. Mous Science, Training and Partnerships Manager The Nature Conservancy Coastal and Marine Program Indonesia E-mail: pmous@TNC.ORG The complete report in pdf format (1.3 Mb) is available for download from: http://www.komodonationalpark.org/downloads/foxetal2003.pdf

SPC Live Reef Fish Information Bulletin, Number 11 – April 2003

Articles that are of interest in this issue:

- Aquaculture suitability of post-larval coral reef fish
- Market and industry demand issues in the live reef food fish trade
- Live reef food fish trade – Pacific awareness materials project
- Developing industry standards for the live reef food fish trade
- Protecting and managing reef fish spawning aggregations in the Pacific

There are many more interesting articles listed on this issue, for direct connection to these articles visit SPC website at www.spc.int/coastfish/.

LiveFish HK

A new venture, LiveFish HK, has been established in Hong Kong, the biggest demand center in the world for live reef food fish. The company provides services to sectors interested in the trade in live reef fish, including the fishing industry, mariculutrists, governments, multi-governmental agencies and non-governmental and environmental organizations. For more information go to the website www.livefishhk.com.

Fish Health Abstracts

Intervet Aquatic Animal Health Newsletter, March 2003

Characterisation of a Pathogenic Virus Isolated from Marine Threadfin Fish (*Eleutheronema tetradactulus*) during a Disease Outbreak

An unknown virus was isolated from massive mortality of cultured threadfin (*Eleutheronema tetradactulus*) fingerlings. The virus replicated in BF-2 fish cell line and produced a plaque-like cytopathic effect. Electron micrographs revealed non-enveloped, icosahedral particles approximately 70-80 nm in diameter composed of a double capsid layer. Viroplasms and subviral particles approximately 30 nm in diameter and complete particles of 70 nm in diameter were also observed in the infected BF-2 tissue culture cells. The virus was resistant upon pH 3 to 11 and ether treatment. It is also stable to heat treatment (3 h at 56C). Replication was not inhibited by 5-iododeoxyuridine (5-IudR). Acridine orange stain revealed typical reovirus-like cytoplasmicinclusion bodies. Electrophoresis of purified virus revealed 11 segments of doublestranded RNA and five major structural polypeptides of approximately 136, 132, 71, 41 and 33 kDa. Based on these findings, the virus isolated was identified to belong to the genus Aquareovirus and was designated as threadfin reovirus. This virus differed from a majority of other aquareovirus by its increase in virus infectivity upon exposure to various treatments such as high and low pH, heat (56C), ether and 5-IudR. The RNA and virion protein banding pattern of the threadfin reovirus was shown to differ from another Asian isolate, the grass carp hemorrhage reovirus (GCV). Artificial injection of the threadfin fingerlings resulted in complete mortality, whereas seabass (*Lates calcarifer*) fingerlings infected via bath route showed severe mortality within a week after exposure. These results indicate that the threadfin virus is another pathogenic Asian aquareovirus isolate that could cross-infect into another marine fish, the seabass. Original published in Aquaculture 214: 1-18, 2002. Seng K, Fang Q, Chang SF, Ngoh GH, Qin QW, Lam TJ, Sin YM (Singapore, China).

Nodavirus Infection in Hatchery-reared Orange-spotted Grouper Epinephelus coioides: First Record of Viral Nervous Necrosis in the Philippines

Mass mortality occurred in 34-day old larval orange-spotted grouper *Epinephelus coioides* reared at a hatchery in the Philippines with clinical signs such as anorexia and abnormal swimming behavior. Histopathology of moribund fish demonstrated marked vacuolation of the brain, spinal cord and retina. Cytopathic effects were observed in SSN-1 cells inoculated with the tissue filtrate of affected grouper. Electron microscopy revealed non-enveloped virus particles measuring 20 to 25 nm in diameter in the cytoplasm of degenerated SSN-1 cells. Piscine nodavirus (betanodavirus), the causative agent of viral nervous necrosis (VNN), was detected in the affected tissues and SSN-1 cells inoculated with the tissue filtrate of affected fish by RT-PCR. This is the first record of VNN in the Philippines. Original published in Fish Pathology 37: 87-89, 2002. Maeno Y, de la Pena LD, Cruz-Lacierda ER (Japan)

Tuna News and Abstracts

Tuna – The New Goldrush

The development of Bluefin tuna (*Thunnus thynnus*) in aquaculture has been given a boost by the recent success in two crucial areas of tuna aquaculture. The first is the success in closing the lifecycle of bluefin tuna by Japanese researchers in 2002, which enable fingerlings to be produced in hatchery rather than rely on wild caught juveniles. The second being able to produce commercial pelleted diet for tuna grow-out is also on the way and the result has been encouraging based on grow-out trial with southern bluefin. The full article is available from *Fish Farming International, May 2003, Volume 30, No. 5.*

Multi-national Bluefin Study

An EU project on "Reproduction of the Bluefin Tuna in Captivity, A Feasibility Study for the Domestication of *Thunnus thynnus*" (REPO-DOTT) started in 2003. This is a three years project brings researchers from seven countries to work on bluefin reproduction in captivity. The aims of the project are:

- Improving knowledge of the reproductive biology of bluefin tuna in the wild as well as in captivity;
- Assessing the capability of broodstock to mature and spawn in captivity; and
- Determining the feasibility of obtaining and hatching viable eggs from breeders.

Tuna farming is still relatively new so researchers need to develop skills such as handling techniques, transport system, eggs collection methods, etc. The full article is available from *Fish Farming International, May 2003, Volume 30, No. 5*.

Genetic Monitoring for Spawning Ecology of Captive Yellowfin Tuna (*Thunnus albacares*) using Mitochondrial DNA Variation

Y. Niwa, A. Nakazawa, D. Margulies, V.P. Scholey, J.B. Wexler, S. Chow-2003 Aquaculture, 218(1-4): 387-395
Mitochondrial DNA genotypes of captive broodstock of yellowfin tuna (*Thunnus albacares*) were compared with those of their offspring in order to monitor spawning frequency and periodicity. Among 38 broodstock individuals, 27 genotypes were observed, 18 of which established a single individual's identity. Spawned eggs and hatched larvae were collected on 48 sampling days over a period of 1 year. Among 538 eggs and larvae analyzed, 10 genotypes were observed; eight of them established a single female's identity, and two females shared two types. The spawning profiles of these females were determined by observing the occurrence of these genotypes in the offspring. Based on the dates when genotypes first occurred and on growth trajectories estimated for individual fish, the size of a female at first spawning was estimated to be 12–28 kg and 75–112 cm. Usually, multiple females spawned on a given date. The same genotypes were observed on almost any sampling day throughout the year. The results indicated that some individual females were capable of spawning almost daily for extended periods of time as long as they remained in the appropriate range of water temperatures and had sufficient food. (INTEM Consulting, Inc., 7-22-18-K201 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan, email of S. Chow: chow@affrc.go.jp)

Grouper Research Abstracts
Transport of hatchery-reared and wild grouper larvae, *Epinephelus* sp

Ch.B. Estudillo, M.N. Duray-2003 Aquaculture, 219(4): 279-290
Optimum packing conditions for the transport of hatchery-reared and wild grouper larvae were investigated under simulated condition or actual air transport. Simulation of transport motion was done through the use of an electric orbit shaker to identify the best packing conditions for the transport of grouper larvae at various ages. Simulated transport was conducted in hatcheryreared grouper larvae at day 35 (mean TL=14.73 mm), 45 (mean TL=15.23 mm) and 60 (mean TL=28.16 mm) at packing densities of 50, 100 and 200 larvae l-1 and at high (28 °C) or low (23 °C) temperatures. Packing density of 50 larvae l-1 was best for 45- and 60-day-old larvae 8 h transport at low temperature. However, packing density could be increased to a maximum of 100 larvae l-1 8 h transport at 23 °C with mortality rates ranging from 2.3% to 5.3%. The increase in total NH_3 level was dependent on temperature, packing density and size of larvae. High packing density (100–200 larvae l-1) and temperature (28 °C) resulted in increased NH_3 level and mortality rates during transport. In addition, regardless of the temperature, NH_3 levels were consistently higher for 60-day-old larvae. Day-60 grouper larvae displayed strong resistance to handling/mechanical stress compared to 35-day-old larvae probably because most are already fully metamorphosed at this stage. Based on these results, a packing density of 50 larvae l-1, a temperature of 23 °C and larval age of 60 days were considered as the best transport conditions for hatchery-reared grouper larvae. When these transport conditions were used in experiment 2, for 26-day-old hormone-metamorphosed, 60-day-old naturally metamorphosed or 60-day-old pre-metamorphosing hatchery-reared grouper larvae, a 100% survival rate was attained in all treatments. Seven days of hormone (T3) treatment did not accelerate metamorphosis of wild-caught transparent grouper larvae (tinies) significantly. Survival rates of hormone-treated transparent tinies (H-tinies), untreated black tinies (B-tinies) and untreated transparent tinies (T-tinies) were also similar after 8–9 h air transport (experiment 3). The results of the current study suggest that T3 treatment did not affect the performance of hatchery-reared and wild-caught transparent tinies/larvae during transport. In addition, mass mortalities of these transported tinies during the nursery phase were associated with nutritional aspect and the sudden confinement of these undomesticated wild-caught grouper to small space rather than transport or hormone treatment effects. Source: *Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD), 5021, Tigbauan, Iloilo, Philippines, e-mail: chonae@aqd.seafdec.org.ph.*

Induction of Ovulation in Captive-reared Dusky Grouper, Epinephelus marginatus (Lowe, 1834), with a Sustained-release GnRHa Implant

G. Marino, E. Panini, A. Longobardi, A. Mandich, M.G. Finoia, Y. Zohar, C.C.
Mylonas-2003 Aquaculture, 219(4): 841-858 Captive-reared dusky grouper were induced to ovulate using a sustained-release delivery system (implant) loaded with gonadotropin-releasing hormone agonist [D-Ala6, Pro9, NEt]-GnRH (GnRHa). Thirteen females were implanted at doses ranging from 30.5 to 68.3 µg kg-1 during three experiments between late June and early September. Of the injected females, 85% responded positively to the GnRHa implant and ovulated between 60 and 238 h after treatment, whereas none of control fish showed any sign of maturation. No spontaneous spawning was observed, and the eggs were manually removed

from the females using gentle abdominal pressure. The mean number of ovulations per fish was 3.8, with a maximum of nine for one female. Overall, a total of 42 ovulations were obtained, resulting in the production of more than 5 million eggs. The average relative fecundity was 118.3±16.0×103 eggs kg-1 BW, with a maximum of 202.2×103 eggs kg-1 BW. Mean percentage fertilisation and hatching were 48.2% and 52.2%, respectively. The results demonstrate that GnRHa administration via controlled delivery systems is an effective method for producing good quality eggs in captive dusky grouper. (ICRAM Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare, 300 Via Casalotti, 00166, Rome, Italy, e-mail: g.marino@icram.org)

Morphometric Prediction of Cannibalism in Larviculture of Orange-spotted Grouper, *Epinephelus coioides*

J.-R. Hseu, H.-F. Chang, Y.-Y. Ting-2003 Aquaculture, 218(1-4): 203-207.
This study developed a linear regression model to predict the occurrence of cannibalism in larviculture of orange-spotted grouper, *Epinephelus coioides*. Based on measurements of mouth width (MW), body depth (BD), and total length (TL), a model of prey length (mm) to cannibal length was constructed: TLprey=0.80 TLcannibal-1.50. According to the equation, we suggest that 30% is a threshold for TL differences to use in grading grouper fry, and that beyond the threshold, potential cannibals should be removed. (Institute of Fisheries Science, National Taiwan University, 1 Roosevelt Road, Section 4, Taipei 106, Taiwan, e-mail; jrhseutfri@pchome.com.tw)

The Effects of Exogenous Androgens on Ovarian Development and Sex Change in Female Orange-spotted Protogynous Grouper, Epinephelus coioides

S.-L. Yeh, Ch.-M. Kuo, Y.-Y. Ting, Ch.-F. Chang-2003 Aquaculture, 218(1-4): 729-739
The efficacy of various doses of an androgen mixture, containing testosterone (T), 17-methyltestosterone (MT), and testosterone propionate (TP) in equal ratios, for induction of sex change in protogynous orange-spotted grouper, *Epinephelus coioides*, was examined. The androgen mixture, with doses from 1 to 20,000 µg/kg BW, was implanted into each fish (body weight 1.7 kg) in July (post-spawning season), and gonadal stage and plasma T were monitored at various time intervals for a period of 90 days. Gonadosomatic (GSI) and hepatosomatic indices (HSI), gonadal histology, sex steroids (T, 11-ketotestosterone=11-KT, and estradiol=E2) in plasma were determined after 90 days of implantation. The implanted T was released to plasma for 60 days. All androgen mixtures at doses higher than 1000 µg/kg BW were capable of inducing a sex transition and completion of spermatogenesis up to the functional male phase. Low doses of androgens induced ovarian development and higher GSI and HSI indices than in the control and other groups. Significantly higher plasma T levels were found in the developing and spermiating males as compared to the females and intersextransitional fish. No significantly difference of plasma levels of E2 and 11-KT was found in the control and all the androgen-treated groups (during the nonreproductive season). Therefore, it is concluded that the stimulation of sex change or ovarian development is dependent on the dose and time course of implanted androgens. Plasma T levels were correlated with the development of controlled male phase in protogynous grouper, *E. coioides*. (Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan, ROC, email of CH.-F. Chang: b0044@mail.ntou.edu.tw).

Induced Sex Change, Spawning and Larviculture of Potato Grouper, Epinephelus tukula

Shinn-Lih Yeh, Quen-Chai Dai, Yeong-Torng Chu, Ching-Ming Kuo, Yun-Yuan Ting and Ching- Fong Chang Aquaculture, In Press*
The potato grouper (*Epinephelus tukula*) is a new aquaculture species with high economic potential. This is the first report of induced sex change, reproduction and larval rearing in this species. The smallest body size at which mature females were observed was 90 cm in total length and 16 kg in body weight. The diameter of tertiary yolk globule stage of oocytes was 505±10 mm for 16-24 kg individuals. Larger oocytes (552±13mm) were obtained from individuals. (Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan, ROC, email of CH.-F. Chang: b0044@mail.ntou.edu.tw)

Grouper Nutrition Abstracts

All the grouper nutrition abstracts in this section are obtained from Department of Fisheries, Thailand and most of these articles were published in Thai language with English abstracts.

Effect of Ash and Inoganic Phosphorus in Diets on Growth and Feed Performance of Orange-spotted Grouper, *Epinephelus coioides*

Mali Boonyaratpalin and Atra Chaimongkol
Juvenile grouper (*Epinephelus coioides*) initial weight 5.62 g were fed with low-ash diets (diets 1-4) and high-ash diets (diet 5-8) supplemented with mono-sodiumphosphate containing phosphorus at 0, 0.25, 0.50 and 1.0 %, respectively. Fish were fed to satiation twice daily for 12 weeks in a flow-through system; 40 liter aquarium. The effect of dietary treatments on growth, survival, feed intake, feed efficiency ratio, protein efficiency ratio, protein retention, phosphorus retention, Ca, P, and Zn in vertebrae, protein digestibility coefficient, energy digestibility coefficient, dry matter digestibility coefficient and phosphorus absorption were evaluated. The result showed that no significant difference on growth and survival among dietary treatments. While feed efficiency ratio was highest in diet 2 for low-ash diets and diet 6 for high-ash diets. Phosphorus retention and phosphorus absorption in low-ash diets range from 26.81-93.78% and 87.16-91.94%, respectively. In contrast, phosphorus retention and phosphorus absorption in high-ash diets slightly low, 14.78-24.25% and 44.88-65.73%, respectively. There were interactions between ash content and supplemented P level for phosphorus

retention and phosphorus absorption. From this experiment can be concluded that low-ash and high-ash diets without supplemented phosphorus satisfy the needs of the fish for growth. Unfortunately, the utilization of low-ash diets is less polluting than high-ash diets.

Effect of Fishmeal Source in Diets on Growth. Feed Efficiency and Body Composition for Orange-spotted Grouper, *Epinephelus coioides*

Atra Chaimongkol, Mali Boonyaratpalin, Chusak Borisut and Sujin Boonchuy

Fishmeal produced from pelagic fish (catch by purse seine: diet 1), ground fish (catch by trawl net: diet 2), by-product of surimi fishery processing (diet 30, and by-product of tuna fishery processing (diet 4) were used as single protein source in grouper diet. Experiment diets were fed to satiation twice a day to juvenile grouper (initial body weight 1.33 g) in 30 liter aquaria for 10 weeks. From the experiment, fish fed diet 1 showed significantly highest growth among dietary treatments. Feed conversion, feed intake and survival rate were not significantly different between fish fed diet 1 and diet 2, but significantly higher than fish fed diet 3 and 1. Protein and ash content in whole body of fish fed diet 1 and 2 slightly lower than fish fed diet 3 and 4. Based on these results, fishmeal that is produced from pelagic fish was suitable for use as protein source in grouper diet. Further investigations should e conducted to determine effect of fishmeal source for large grouper.

Effect of Dietary Protein to Lipid Ratio on Growth and Body Composition for Orangespotted Grouper, *Epinephelus coioides*

Atra Chaimongkol, Mali Boonyaratpalin, Chusak Borisut and Sujin Boonchuy

The suitable protein to lipid ratio for juvenile orange-spotted grouper was determined using practical diets in a factorial experiment. Fish (initial weight 6.2 g) were fed with nine formulated diets that contain protein : lipid (%) ratio as 43:13, 43:17, 48:13, 48:17, 48:21, 53:13, 53:17 and 53:21, respectively. Fish were fed to satiation for 10 weeks in a 40 liter aquarium. The effect of dietary treatments on survival, growth, feed conversion, feed intake, protein efficiency ratio and whole body composition of the fish were evaluated. Best growth was observed in fish fed diets contain protein : lipid ratio of43:17 (diet 2), 48:17 (diet 5), 48:21 (diet 6), 53.13 (diet 7), 53.17 (diet 8) and 53.21 (diet 9). Feed conversion ratio ranged from 0.98 to 1.32, the highest value was obtained in fish fed diet 1 (43% protein and 13% lipid). Protein efficiency ratio ranged from 1.76-2.34 and was highest in fish fed diet 2 (43% protein and 17% lipid). Whole body lipid content was correlated positively to dietary lipid. Dietary treatments did not effect on survival and feed intake. There was no interaction between dietary protein and lipid except for growth. From this experiment can be concluded that dietary protein : lipid ratio of 43:17 appeared to be suitable fro juvenile orange-spotted grouper.

Effect of feeding 3-17 Day Grouper Larvae, Epinephelus coioides with omenga-3 HUFA Enriched Rotifer, *Brachionus rotundiformis*

Mavit Assavaaree, Tida Pechmanee and Paiboon Boonliptanon

Grouper larvae age 1-day-old was used in this study. The larvae were put in six 500 Liter plastic tanks (culture volume was 450 L) at 25 ind./L. The larvae were fed with rotifer until 17-days old. We started feeding rotifer to larvae when they were 3-days old. After the first three days of experiment (larvae 3-5 days old) small sized rotifer filtered through 120 micron net were fed to larvae at density of 5-10 ind./ml. Larvae 6-17 days old were fed with all sized rotifers at 10-15ù he result showed that the larvae fed enriched rotifer had significantly higher survival (4.77%) than larvae fed rotifer without enrichment (2.59%). However, total length and body weight did not show significant differences between with and without enrichment. The culture conditions during experiment were, temperature 27-30 C, salinity 30-31 ppt, pH 7.6-8.1 and dissolve oxygen 5.6-6.6 ppm. It could be concluded that feeding enriched rotifer to grouper larvae will increase essential fatty acid at early stage and can improve their survival and health.

Fish Identification Cards

The Secretariat of the Pacific Community (SPC) has published a set of 16 waterproof identification cards for fish commercially taken for the live reef fish trade, mostly covering groupers but also several species of wrasse. Each card provides the English and Hong Kong names, a description and a clear photograph. The reverse of the card has notes on the biology, reproduction maximum size, distribution, commercial importance and IUCN conservation status.

Available from: SPC Information Section, BP D5, 98848 Noumea, New Caledonia, Email cfpinfo@spc.int.

Weekly Live Marine Fish Prices – Hong Kong

A new service – average wholesale prices in the Hong Kong market are published for a range of live reef fish species. Updated weekly. http://www.enaca.org/Grouper/FishPrices/FishPricesIndex.htm

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).

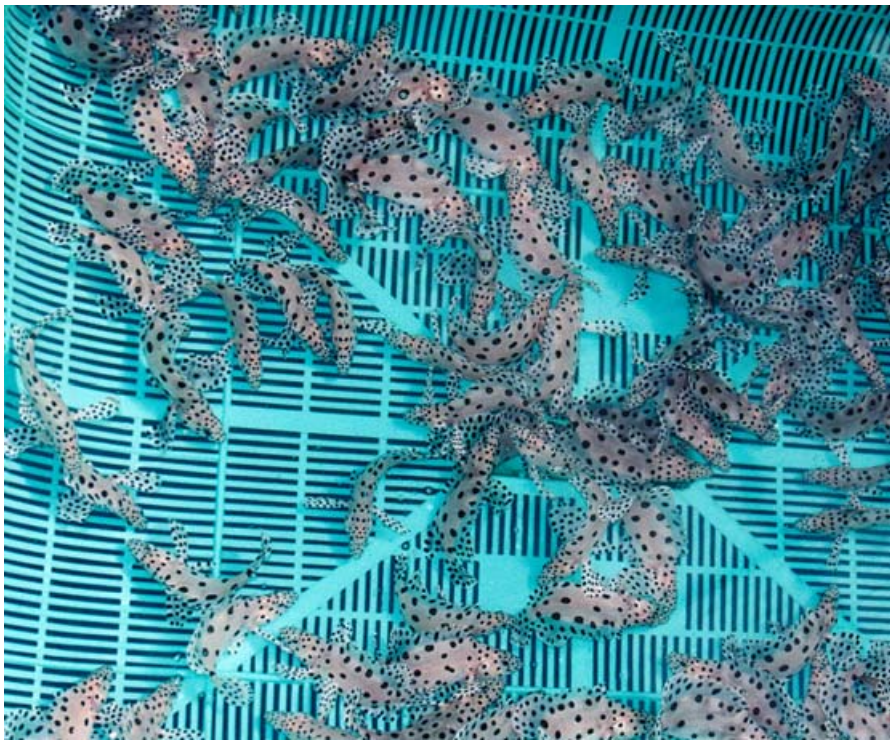
Larval rearing

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

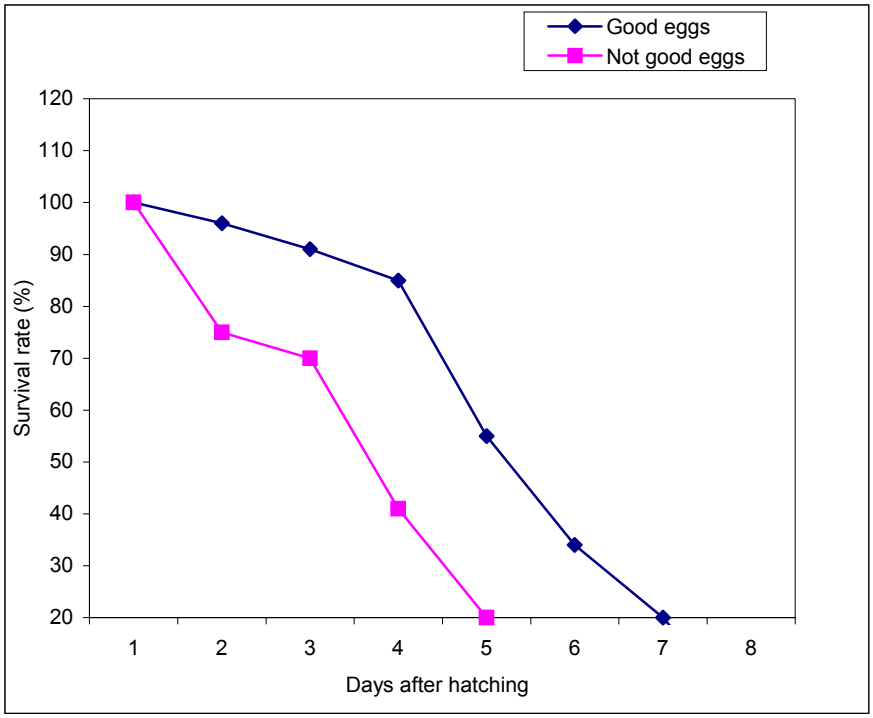


Table 1. Feeding scheme and water management in larval rearing of humpback grouper *Cromileptes altivelis*

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.

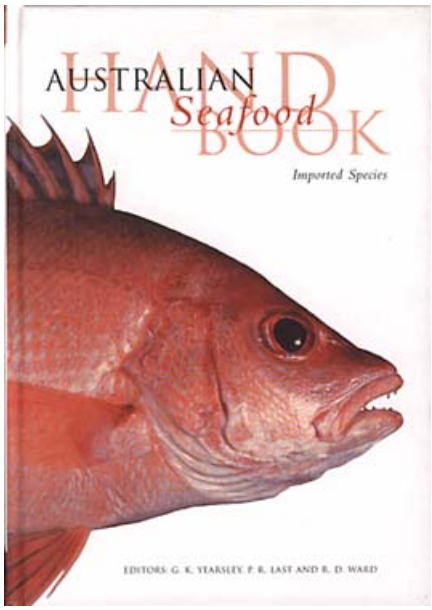
Sugama, Ketut, Trijoko, B. Slamet, S. Ismi, E. Setiadi and S. Kawahara. 2001. Manual for the seed production of Humback grouper Cromileptes altivelis, GRIM-JICA, Agency for Marine and Fisheries Research of Indonesia 37 p.

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.