# Marine finfish section

*The Grouper Section has taken on a new and broader name beginning this issue: it has become the Marine finfish Section to take account of other species. This Section is almost wholly based on the Grouper Electronic Network 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).*

## Training Course on Grouper Hatchery Production

*Organized by the Gondol Research Institute for Mariculture, Bali, Indonesia in cooperation with NACA, JICA and the Asia-Pacific Grouper Network, Bali, Indonesia, 1–21 May 2002.*

A Grouper Hatchery Production course will be held in Bali, Indonesia, for hatchery operators, technicians and researchers involved in grouper aquaculture hatchery production, research, development and extension.

The training course is organized and supported by the Ministry of Marine Affairs and Fisheries, Indonesia, NACA, the Australian Centre for International Agricultural Research (ACIAR) and the Asia-Pacific Economic Cooperation (APEC). It is one of the activities of the Asia-Pacific Grouper Network (http://www.enaca.org/grouper/).

The Gondol Research Institute for Mariculture (GRIM) has extensive experience in short and long term training for Indonesian farmers and technical staff, in cooperation with Japan International Cooperation Agency (JICA). Such activities have contributed to the development of grouper hatchery in Indonesia. This is the first time that GRIM offers a grouper hatchery course for participants from the Asian region, in cooperation with NACA and the Asia-Pacific Grouper Network.

The objectives of this regional training course are to provide practical hands-on training on the following topics:

- Grouper broodstock management techniques, including handling, feeding, broodstock nutrition, control of the reproductive cycle, spawning techniques and egg collection and incubation.
- Larval rearing, including feeding and hatchery practices.
- Mass production of live food (phyto and zooplankton).
- Grouper diseases and health management, including viruses (VNN), and common diseases of marine fish.

The target grouper species for this training course will be mainly on *Cromileptes altivelis* (mouse grouper), but participants will gain experience with handling *Epinephelus fuscoguttatus* (tiger grouper) and other marine finfish species.

The training course will provide participants with a unique opportunity to visit private sector hatcheries and nurseries in the Gondol area, and some information on mariculture development in Indonesia.

The training course involves mainly practical hands-on teaching supported by short lectures and workshop discussion sessions. The course is intended for technicians and scientists from the private sector, NGO and government who are actively involved in grouper aquaculture development, research and extension. The course will be conducted in English.

The training course will involve: 40% lectures and small workshops, 50% practical work in the laboratory and on-station hatcheries and outdoor activities and 10% field trip.

The topics include management of broodstock, management of larval rearing, feed and feeding technique for broodstock, larvae and juveniles, fish diseases, prevention and control, mass production of live food for larvae, transportation of seed and broodstock, grow-out at floating net cages (brief introduction) and brief overview of mariculture development in Indonesia.

The fieldwork will be conducted around the island of Bali, at small-scale backyard hatcheries and private grouper hatcheries at Negara and grow-out and trading facilities at Denpasar.

All participants will be awarded a certificate of completion to certify that minimum performance requirements have been met, as evaluated by the Resource Persons, the Course Coordinator and the Board of Directors at GRIM. Performance will be based on the trainees' participation in class discussions and activities in the laboratory and outdoors.

All participants are required to complete the application form and send to the NACA Secretariat.

Selected participants are required to have a valid passport and an entry visa for Indonesia at least for the duration of the training course. Travel documents including passport, visa, fiscal and exit fee are to be arranged by the applicants at their own cost.

NACA will assist with visas, if required, in collaboration with GRIM and Indonesian authorities. Application for registration in the training workshop should be sent to the NACA Secretariat.

Qualified participants will be required to pay a course fee of US$1,500. This fee will cover the cost of training materials and supplies, administrative cost and local travel associated with the training.

Costs of accommodation and food at a nearby hotel will be the responsibility of the participant. Only one local hotel is available (see below), but participants will be advised of alternative options that may become available.

Accommodation will be at the Taman Sari Bali Cottages. This pleasant beachside resort hotel is 10 minutes drive from the Gondol station. Room rates for an air-conditioned room with hot water are around US$35 (to be confirmed) including breakfast. Details of the hotel can be found at www.balitamansari.com.

Payment of course fee can be made by either credit card or bank draft (details of payment are shown in the Registration Form). For further information, contact:

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### Mass Mortalities Associated with Viral Nervous Necrosis in Hatchery-reared Groupers in China

*L. Lin, J.G. He, K. Mori, T. Nishioka, J.L. Wu, S.P. Weng, K. Mushiake, M. Arimoto, T. Nakai-2001, Fish Pathology, 36(3): 186-188 (from Current Contents)*

The viral etiology of mass mortalities of groupers, *Epinephelus coioides* and *E. akaara,* cultured in the People's Republic of China was examined.

Disease outbreaks occurred in 7 to 45 day-old fish with erratic swimming motion and marked vacuolation was observed in the brain and retina of the affected fish. The piscine nodavirus (the Betanodavirus), the causative agent of viral nervous necrosis (VNN), was detected in the affected tissues by electron microscopy, indirect fluorescent antibody test and reverse transcription-polymerase chain reaction. This paper is the first record of the agent in China.

### The Potential for the Restoration of Marine Ornamental Fish Populations Through Hatchery Releases

*D.A. Ziemann-2001, Aquarium Sciences and Conservation, 3(1/3): 107-117*

Populations of tropical and subtropical marine fish are being depleted worldwide to supply increasing demands of the aquarium industry and fresh seafood market. Overfishing and destructive harvest techniques have left some marine fish populations virtually extirpated in a number of primarily underdeveloped countries.

In situations where only small remnant populations and significantly degraded habitat remain, population recovery even under the complete absence of collection will be slow, with the high potential for population loss due to natural environmental and recruitment variability. Stock enhancement, supplementing natural recruitment with hatchery produced fry, has the potential to significantly increase the rate of population recovery while maintaining population vigor.

Stock enhancement research on Pacific threadfin has demonstrated measurable positive impacts on recreational and commercial fisheries for this species in experimental scale releases; similar successes can be expected for enhancement efforts directed toward species of ornamental value.

The major technological barrier to ornamental fish enhancement, the development of appropriate culture capabilities, is being addressed in research directed to the commercial production of fish for the aquarium trade. (The Oceanic Institute 410202 Kalaniole Highway, Waimanalo, HI 96795, USA).

### Application of Marine Foodfish Techniques in Marine Ornamental Aquaculture: Reproduction and Larval First Feeding

*A.C. Ostrowski, Ch.W. Laidley-2001 Aquarium Sciences and Conservation, 3(1/3): 191-204*

The long-term sustainability of the marine ornamental industry is being threatened by environmental pressures that are severely degrading the health of coral reef ecosystems. There is now a compelling need to practice resource conservation through the development of 'reef friendly' aquaculture technologies as an alternative to wild collection practices and to restore degraded wild populations. The commercial culture of marine ornamental finfish is very much in its infancy, but advances can be made more rapidly using insights from years of research and development with marine foodfish species.

Many of the bottlenecks and constraints to developing marine ornamental fish culture are those now being addressed with the more challenging species of foodfish being attempted. The two key bottlenecks that currently limit expansion of the marine ornamental industry are the control of captive maturation and spawning and the identification of appropriate first-feed items for marine ornamental fish larvae.

This paper highlights basic principles and recent achievements in marine foodfish culture that might be applicable to rapid development of controlled reproduction and propagation techniques for marine ornamental finfish. (The Oceanic Institute Makapuu Point Waimanalo HI 96795, USA).

### Methods for Mass Rearing Stages I-IV Larvae of the American Lobster, Homarus americanus H. Milne Edwards, 1837, in Static Systems

*B.F. Beal, S.R. Chapman-2001 Journal of Shellfish Research, 20(1): 337-346 (from Current Contents)*

We conducted a series of five laboratory experiments (7-18 days in duration) to test the interactive effects of stocking density, aeration rates, and food types on survival of American lobster (*Homarus americanus*) larvae through their first three planktonic stages (I-III) to the postlarval stage (IV). Experimental units and culture protocols were designed to replicate a 1:100 scaled-down version of equipment used in association with a fishermen sponsored, stock enhancement lobster hatchery located in Cutler, Maine. The first four trials revealed that extremely high rates of aeration (ca. 240 mL air $sec^{-1}$ were necessary to distribute larvae and food sufficiently to reduce cannibalistic encounters; however, the best survival from stage I-IV (at stocking densities of 7-26 $L^{-1}$ fed ad libitum with enriched Artemia) was only 24%. The final experiment (stocking density = 20 $L^{-1}$) yielded a mean survival rate (+/- 95% CI) of 75.8 +/- 10.2% (range = 62.7% to 90.7%; n = 6). One important difference between the last and first four experiments was how stage I larvae were managed prior to their culture. In the first four trials, unfed larvae were collected from a relatively small (46 cm x 30 cm x 20 cm), screened capture basket located near the discharge pipe of a broodstock holding tank at the hatchery where they may have resided for > 12 hr. Larvae used in the final laboratory experiment were collected directly from the broodstock tank within 30 min after being liberated from the mother's swimmerets. Larvae, at relatively high densities within the screened box, likely had many more cannibalistic encounters prior to their culture than those collected directly from the broodstock tank and, therefore, suffered high rates of mortality during the first four laboratory trials. Mass rearing methods for larval American lobsters developed in conjunction with these laboratory experiments were used successfully by staff at the Cutler Marine Hatchery from

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1988 to 1992. During this period, survival from stages I-IV averaged 44%, and approximately 875,000 stage IV animals were released to the wild. These culture methods have withstood the test of time as a private lobster hatchery in Maine adopted our protocols in 1993, and they continue to be in use. Further, the general techniques described here have been used since 1994 to culture European lobsters (*Homarus gammarus*) at a commercial lobster hatchery in the southeast of Ireland.

### Vaccination of the Grouper, Epinephelus awoara, Against Vibriosis Using the Utrasonic Technique

*Yong-Can Zhou[a,b], Hui Huang[c], Jun Wang[b], Ben Zhang[a] and Yong-Quan Su[b]*
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A novel ultrasonic technique was used to facilitate the vaccination of fish against Vibrio alginolyticus. To establish the safety parameters, the effects of ultrasound treatment on juvenile groupers, *Epinephelus awoara* was first tested. Results showed that, at an intensity of 400 mW/cm$^2$, 10 minutes of cultrasound treatment were safe, whereas an ultrasound intensity of 600 mW/cm$^2$ produced a certain degree of damage to the experimental groupers. The ultrasound frequency had little effect on the survival of the treated fish. Next the protective effect of the ultrasound-facilitated vaccination was tested. A low frequency ultrasound (35kHz) with an intensity of 175 mW/cm$^2$ was used for vaccinating fish against vibriosis. Different ultrasonic vaccination methods were examined; each contains a total of 2-min continuous or pulsed ultrasound combined with or without 2-min imersion in the presence of vibriosis vaccine. Of all the eight ultrasonic inoculating methods tested, pulsed ultrasound followed by immersion and immersion, pulsed ultrasound and immersion again provided the best protection from bacterial challenge. Compared to other traditional methods, the protective effect provided by ultrasonic vaccination is comparable to that by the intraperitoneal injection method, and the operation convenience is comparable to that by the immersion method. Thus the ultrasound-facilitated vaccination provides an effective and practical approach for vaccinating fish on a large scale.

### Effects of Varying Dietary Fatty Acid Composition on Growth and Survival of Seahorse, Hippocampus Sp., Juveniles

*M. Chang, P.C. Southgate-2001*
*Aquarium Sciences and Conservation, 3(1/3): 205-214*

Three commercially available fatty acid enrichment emulsions (DC Selco, DC DHA Selco and DC Super Selco) were used to enrich *Artemia* nauplii fed to seahorse, *Hippocampus* sp. fry. The emulsions varied in their n-3 highly unsaturated fatty acid (HUFA) composition. Total n-3 HUFA content ranged from 200 to 450mg g$^{-1}$ between the three emulsions while levels of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) ranged between 47–220 and 80–190mg g$^{-1}$, respectively.

Survival and growth of seahorses at the end of the 30-day growth trial were greater in treatments receiving enriched Artemia. Seahorses receiving *Artemia* enriched with DC DHA Selco and DC Super Selco showed significantly ($p < 0.05$) greater mean survival (71.6 ± 6.0% and 78.3 ± 6.0%, respectively) than those receiving unenriched *Artemia* (48.3 ± 6.0%).

Mean standard length was also significantly greater ($p < 0.05$) in fry fed DC DHA Selco and DC Super Selco enriched *Artemia* (20.2 ± 0.3 and 19.7 ± 0.3mm, respectively) compared to those fed unenriched *Artemia* (18.1 ± 0.3mm). The results show that dietary n-3 HUFA are essential for optimal growth and survival of *Hippocampus* sp. and, based on the fatty acid compositions of the enriched *Artemia* used in this study, indicate that the level of dietary DHA supporting optimal growth and survival is greater than 9.3mg DHAg$^{-1}$ dry weight. (School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia).

### Clove oil used as an anaesthetic

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*Reprinted from the SPC Live Reef Fish Bull. #9.*

Clove oil has been used for a number of years to anaesthetise fish in seawater. In fish farming, this is essential for basic procedures such as weighing, tagging, experimental work and for transport. It considerably reduces pathology risks from stress, injury and accident during handling (Keene et al. 1998). It has also been recently proposed as a better alternative to cyanide for the capture of live reef food fish (Erdmann 1999). Clove oil is distilled from *Eugenia caryophyllata* stems, buds and leaves. In Indonesia, it has been used on humans for centuries as a local anaesthetic (Soto and Burhanuddin 1995). The active ingredients are phenol derivatives, essentially the $C_{10}H_{12}O_2$ eugenol compound (Taylor and Roberts 1999).

In a study conducted on coral fish farming on Reunion Island using wild-caught juveniles, a clove oil experiment protocol was required to find a means of handling fish regularly and efficiently. A series of experiments on two fish species was carried out so as initially to determine the optimum clove oil quantity for use on fish weighing less than 10 g and, subsequently, the effect of fish weight and the species under consideration.

#### Material and methods

The method used consisted of introducing the active ingredient of clove oil into the fishes' gills through the water, i.e. 'anaesthesia by immersion' (Brousse 1974). The substance is absorbed through the gills and travels through the bloodstream to the central nervous system. The fish then goes through several anaesthesia stages ranging from balance loss to total motionlessness and ventilatory arrest (McFarland 1960).

In the first part of the study, clove oil from an agricultural cooperative was mixed with seawater at rates of 0.025, 0.050, 0.1 and 0.2 ml · $l^{-1}$. Ethanol, which is normally used as a solvent, was not used in these experiments. The anaesthetic was simply prepared by vigorously shaking a small flask of clove oil and seawater to obtain a whitish emulsion.

A total of 100 fish were anaesthetised in four batches of 25 corresponding to the four clove oil doses: 0.025, 0.050, 0.1 and 0.2 ml $l^{-1}$. The average and standard deviation were calculated for each set. A Krusskal-Wallis non-parametric test conducted on all four batches demonstrated that induction times differed significantly ($H = 55.5$; $P < 0.01$). Mann-Whitney mean difference tests were then carried out on pairs of batches, revealing significantly different induction times for 0.025 ml. $l^{-1}$ doses as compared with the others. They fell by more than half from 0.025 ml $l^{-1}$ to 0.050 ml $l^{-1}$ but did not differ significantly thereafter as the dose increased. It should be pointed out that two specimens died at 0.2 ml $l^{-1}$, which may indicate the upper limit in this experiment. A 0.050 ml. $l^{-1}$ dose was subsequently selected for the remaining experiments. It had the advantage of anaesthetizing the fish quickly with a small dose.

### Induction times in terms of fish weight and species

The study on induction times in terms of fish weight was conducted using a 0.050 ml · $l^{-1}$ dose of clove oil on 100 specimens weighing from 0.05 g to 9.7 g. The induction times observed ranged from 13 to 56 seconds with an average of 30.4 ± 9.9 s. A Pearson correlation test indicated that there was no significant link between induction times and anaesthetized fish weights ($Cp = 0.13$; $P = 0.09$). The weight factor, therefore, had no effect on induction times when a 0.050 ml · $l^{-1}$ dose was administered to fish weighing less than 10 g.

Induction times were then compared for two species, *Valamugil cunnesius* and *Monodactylus argenteus* (Fig. 2). An average of 30.1 ± 10.8 s was obtained for 67 Valamugil cunnesius and 30.7 ± 7.9 s for 33 *Monodactylus argenteus*. A Mann and Whitney mean difference test revealed that the difference between samples was not significant and clove oil should, therefore, have the same effect on both species ($U = 1052$; $P = 0.23$).

### Conclusion

Clove oil proved to be highly effective and easy to use on juvenile tropical marine fish. The 0.05 ml · $l^{-1}$ dose selected in this experiment anaesthetised the fish in less than a minute and made it possible to handle them without any losses. Weight did not appear to have any effect on induction times in juvenile fish (< 10 g) and clove oil could even be used on small specimens weighing less than 1 g. No induction time difference was observed between the two species considered.

These observations may also apply to other juvenile fish. Methods that suit local conditions are becoming increasingly necessary for developing tropical marine fish breeding from spawners' eggs or wild-caught post-larval and juvenile fish. Clove oil, which is not well known or widely used, could become an alternative to the standard MS-222, Phenoxyethanol, Quinaldine or Benzocaine, which are hazardous, expensive, hard to come by in developing countries and sometimes less effective (Munday and Wilson 1997; Erdmann 1999). The results obtained may vary according to clove oil quality and active ingredient content, but this product has some potential in tropical aquaculture.

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## What is STREAM?

**STREAM** is the acronym for *Support to Regional Aquatic Resources Management*, a rural livelihoods oriented initiative that will support government's poverty alleviation programmes. The STREAM Initiative was founded by the Department for International Development (DFID) of the UK; NACA, FAO and the Voluntary Service Overseas (VSO), and with significant partners in AusAid and DFID NRSP.

STREAM became operational in November 2001 with the appointment of its Director, Graham Haylor (author of the article on the next page and former Programme Manager of DFID SEA's Aquatic Resources Management Programme which was hosted by NACA). During this period, start-up activities included the formal entry into partnership agreements with the Governments of Vietnam and Cambodia. Pilot activities were done, focusing on assessment and capacity building, before the planned expansion to other NACA member countries.

With the start of 2002, STREAM has moved on to a wider sphere. UK's DFID had provided the seed money for a Trust Fund for STREAM's initial period; and this was followed up by research funds from DFID NRSP to study their improved policy on aquaculture service provision for poor people in India. An AusAid grant in support of STREAM, to begin on 8 April 2002, will see the expansion of the Programme to include, aside from Cambodia, Vietnam and India, the Philippines, Nepal, Laos, Yunan in China, Myanmar and some others. A regional TCP project has been formulated and will be submitted to FAO in April 2002. It will provide support and catalyze wider regional activities to enable greater regional participation and sharing in STREAM.

While planning for the launch of the Philippine and Nepal STREAM Country Offices and implementing the planned activities for Cambodia and Vietnam, the author and Project Consultants S.D. Tripathi and W. Savage went on an inception mission to India in March as a preparatory activity for the DFID NRSP Project. This article, while including a pictorial on what they saw, and the rural folk and environment in which they will implement the Project, summarizes the other locations of STREAM.