GROUPER RESEARCH AT THE SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER AQUACULTURE DEPARTMENT

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INTRODUCTION

Research on breeding, seed production and culture of groupers at the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC AQD) was initiated following the recommendations of the 1987 Seminar-Workshop on Aquaculture Development in Southeast Asia (ADSEA) which prioritized the department's research and development activities. Initial activities focused on developing a captive broodstock, conducting a market survey of grouper species in the Philippines and in SE Asia (Kohno 1986; 1987; Kohno and Duray, 1989; Kohno et al. 1990), determining fry availability in reported fry grounds within Panay Island where the department is located, and conducting a survey of culture practices in the Philippines (Kohno et al., 1988). Since the most common grouper species cultured in the Philippines and fished from coastal waters is *Epinephelus coiodes (*syn. *E. suillus)*, esearch effort was focused on this species. This paper reviews the progress of research and development in breeding, seed production, nursery, and grow out culture of *E.coioides* at the SEAFDEC Aquaculture Department.

BROODSTOCK MANAGEMENT

Epinephelus coiodes like other Serranid species are protogynous hermaphrodites. They first mature as females at around 3-4 kg body weight. Some of the fastest growing females transform into males when they reach more than 6 kg body weight. Because of the difficulty in obtaining mature males, studies to develop methods to induce sex-inversion in juveniles and adults were undertaken (Tan-Fermin, 1992; Tan-Fermin et al., 1994). Female grouper juveniles (ave. body weight – 1.2 kg) given bi-monthly intramuscular injections of 17-alpha methyltestosterone (MT) at doses of 0.5 and 1.0 mg/kg BW developed mature testes within five months of hormone administration (Tan-Fermin, et al. 1994). A few of these sex-inversed males produced milt in very small quantities. However, 8-9 months after cessation of hormone treatment, the sex-inversed males reversed back to females (Tan-Fermin, 1992). To avoid frequent handling during hormone administration, MT in silastic capsules were implanted to adult females at a dose of 4 mg/kg BW. Functional males were obtained within 7-10 weeks of hormone implantation and milting was maintained by implanting MT capsules every three months. Spontaneous spawnings of females and these sex-inversed males were obtained producing viable eggs (Marte et al. 1994). Sex-inversion may also be enhanced by manipulating the social environment of groupers. A large female reared with several smaller females may spontaneously change to a male after 1-2 months of rearing in the same tank or cage (Quinitio et al.1997).

Epinephelus coiodes held in tanks or cages spawn monthly, usually within 4 days before or after the last quarter moon phase (Toledo et al., 1993). Spawning occurs for 5-17 successive days during each spawning run. However, egg numbers and quality vary considerably between each spawning run and for individual spawns in a series. Occurrence and frequency of spawnings also vary annually and may be related to environmental conditions. Attempts to improve egg quality by

enriching feeds with oils containing high levels of highly unsaturated fatty acids (HUFA) such as cod liver oil or by feeding broodstock with fish-by catch known to have high levels of HUFA have been unsuccessful to date. Present efforts are geared at improving the quality of spawned eggs through broodstock nutritional manipulation and determining morphological and biochemical parameters associated with good quality spawns.

SEED PRODUCTION

Intensive larval rearing techniques developed for other marine species such as milkfish and rabbitfish were modified to suit the requirements of grouper larvae. Early trials used oyster trochophore, artificial plankton and rotifers as feed for early larvae with little success. Based on information on mouth gape of early feeding grouper larvae (Duray and Kohno 1990), feeding behavior (Duray, et al., 1996) and known requirement of marine fish larvae for high levels of HUFA, the rearing protocol developed for grouper larvae involves feeding young larvae (Day 2-Day 15 larvae) with small rotifers obtained by passing a mixed rotifer culture through a fine mesh screen (Fig.1. Duray et al., 1997). Rotifers are previously enriched with high HUFA containing commercial larval food boosters. Older larvae (Day 20 to Day 50) are fed with enriched *Artemia* nauplii or increasing sizes of *Artemia* until larvae have metamorphosed to the juvenile phase when these are able to feed on minced trash fish. Survival rates for the first 21 days of culture improved considerably from less than 10% obtained during the early rearing trials to over 25% by using the improved feeding protocol.

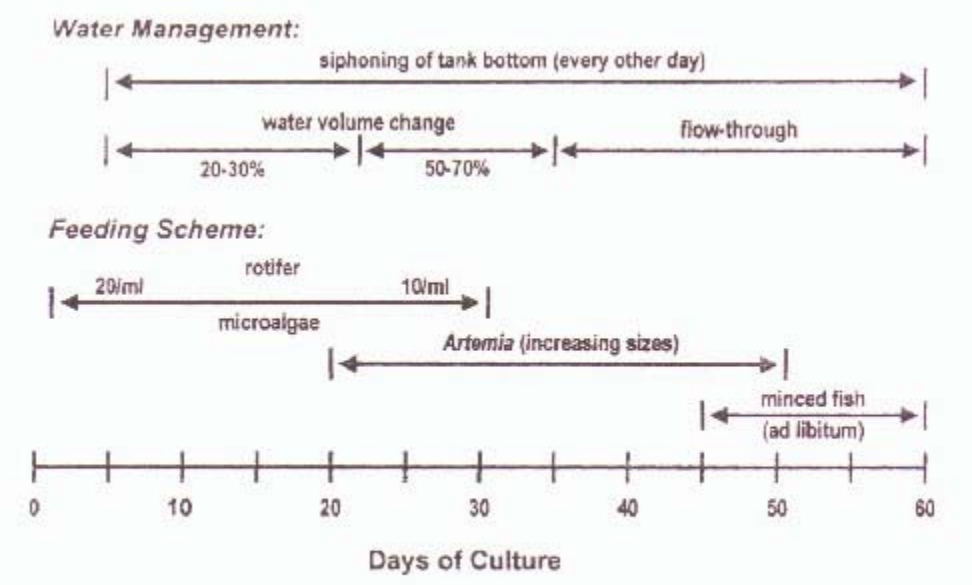


Figure 1. Generalized feeding and water management scheme for intensive rearing of grouper larvae (From: Duray et al., 1997)

Semi-intensive larval rearing using copepods collected from brackishwater ponds as initial food for first feeding grouper larvae has also been developed. The method differs from intensive rearing techniques in the low intial density of larvae used (10 larvae/l), minimal water change, and density of copepod nauplii fed to the larvae. Tests to determine food selectivity, and feeding behavior of grouper larvae indicated that the first feeding larvae preferred to feed on copepod nauplii (Fig 2., Toledo et al., 1997).

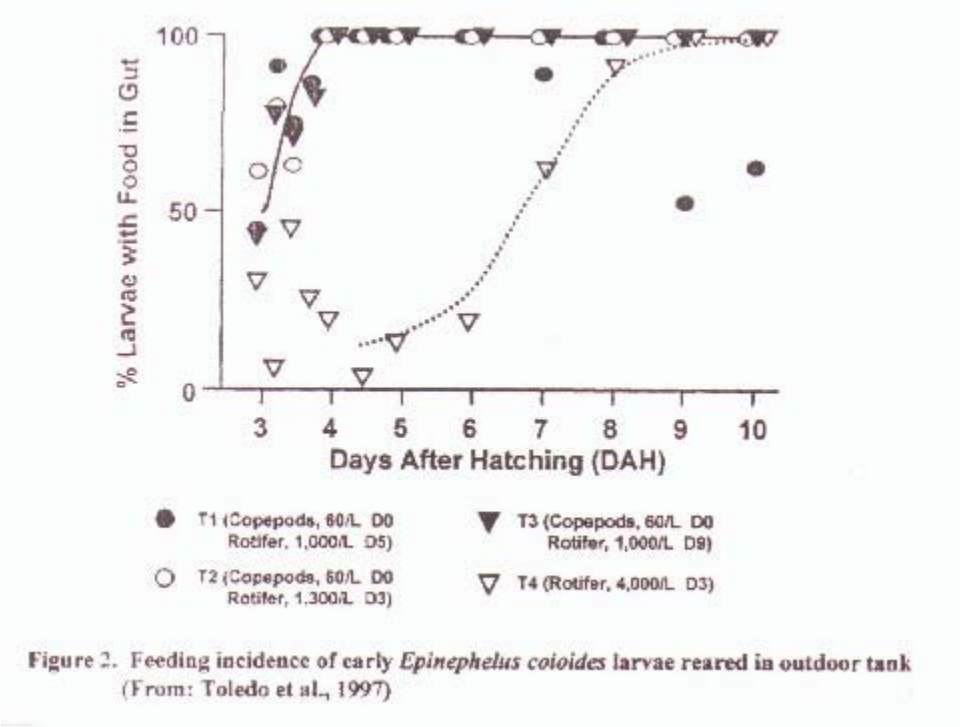
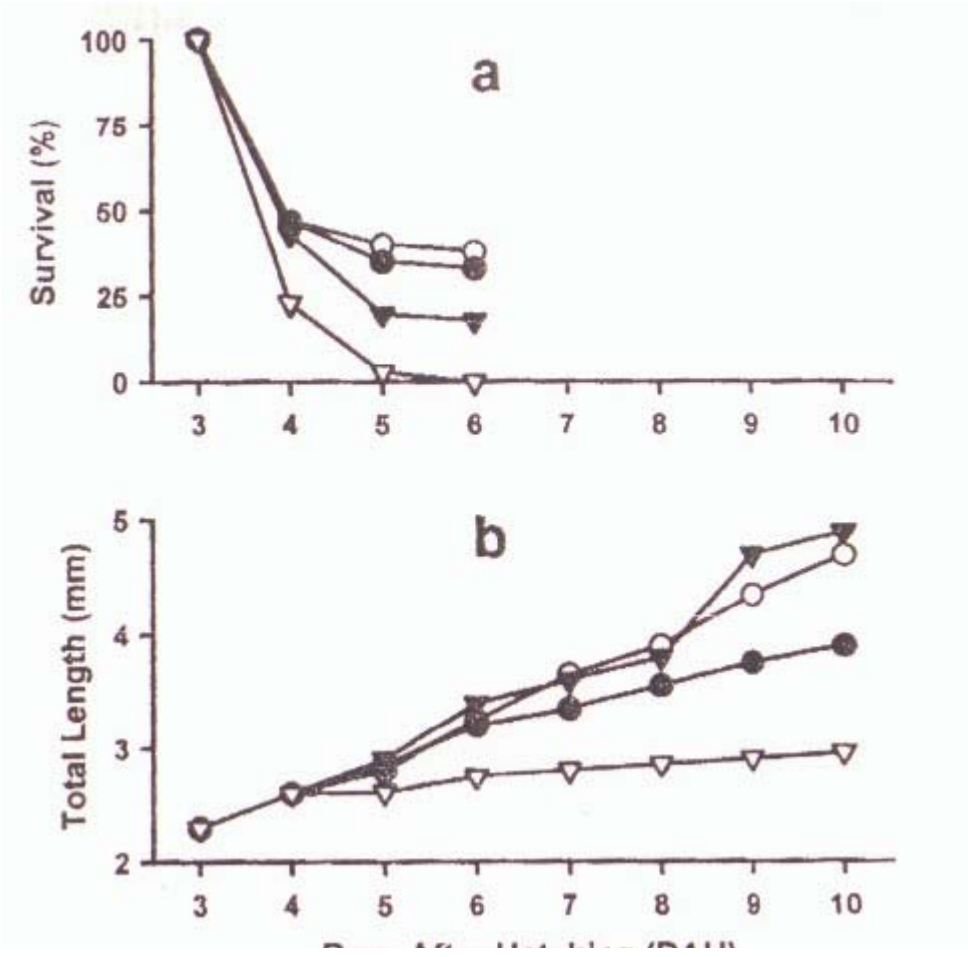


Figure 2. Feeding incidence of early *Epinephelus coioides* larvae reared in outdoor tank (From: Toledo et al., 1997)

Survival and growth of young larvae fed on copepod nauplii were also enhanced (Fig. 3).



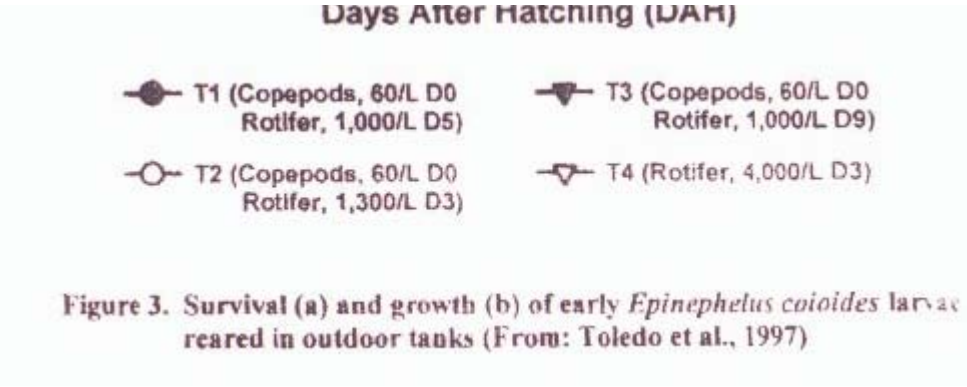


Figure 3. Survival (a) and growth (b) of early *Epinephelus coioides* larvae reared in outdoor tanks (From: Toledo et al., 1997)

Optimum densities of copepod nauplii and adults needed to support the food requirements of early feeding larvae were also determined (Toledo et al., 1996). These information together with modifications on water and tank management were the basis of the semi-intensive rearing scheme developed for grouper larvae. (Fig 4, Toledo et al., 1999).

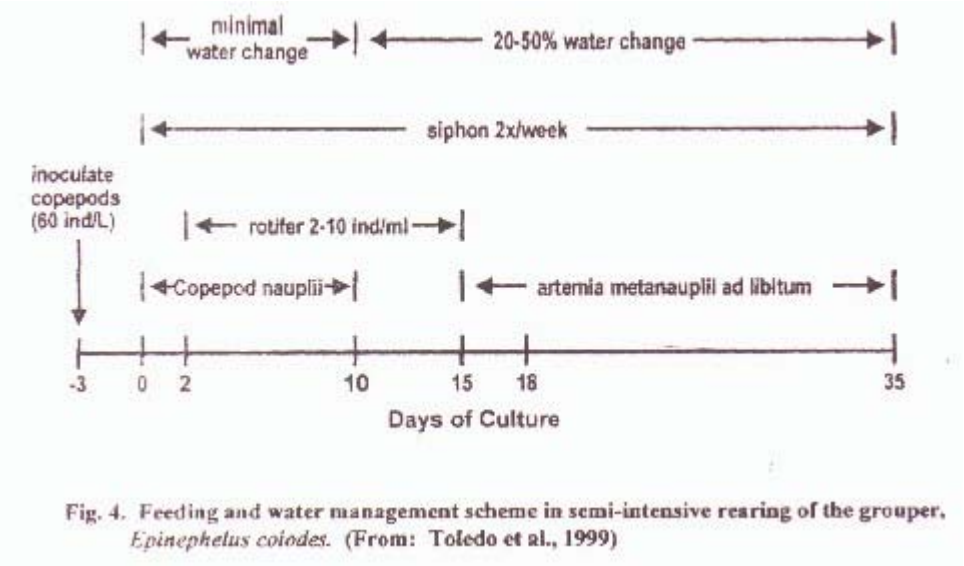


Fig. 4. Feeding and water management scheme in semi-intensive rearing of the grouper, *Epinephelus coioides*. (From: Toledo et al., 1999)

Grouper larvae reared at low densities and fed copepod nauplii during the early rearing phase grew faster and had higher survival rates at least up to the metamorphic stage. The enhanced nutritional quality of copepods particularly that of *Acartia tsuensis* can be attributed to the high levels of n3-HUFAs in these organisms (Table 1) particularly the fatty acid, docosahexaenoic acid (DHA) (Table 2) (Toledo et al., 1999). Continuing studies are being undertaken to refine this semi-intensive rearing scheme using copepod as live food. Studies to develop mass production techniques for *Acartia* and other copepods are also being undertaken to support the food requirement of grouper larvae.

Table 1. Fatty acid composition (% area) of food organisms used in the early feeding stages of grouper *E. coioides* larvae (From: Toledo et al., 1999)

Fatty Acid	*Pseudodiaptomus*	*Acartia*	*Oithona*	*Rotifer*
Σ Saturate	42.86	44.17	63.01	39.94
Σ Monoene	15.33	8.84	15.95	29.76
Σ n-6	7.72	8.14	1.60	10.50
Σ n-3	29.55	36.01	12.18	13.88
Σ n-3 HUFA	23.75	34.48	10.74	13.35

n-3/n-6	3.83	4.42	2.65	1.11
DHA/EPA	1.37	2.64	1.28	0.02

Table 2. Fatty acid composition (% area) of food organisms used in the early feeding stages of grouper *E. coiodes* larvae (From: Toledo et al., 1999)

Fatty Acid	*Pseudodiatomus*	*Acartia*	*Oithona*	*Rotifer*
14:0	8.97	4.54	10.88	4.34
16:0	24.33	26.68	32.49	28.38
16:1	8.22	2.85	6.43	13.28
18:0	4.94	8.26	12.45	5.10
18:1	5.15	4.53	7.30	12.22
18:2n-6	2.15	2.35	1.28	6.07
18:3n-6	0.46	0.32	0.37	0.50
18:3n-3	3.45	0.99	0.47	0.16
18:4n-3	1.97	0.43	0.67	0.17
20:1	0.46	0.26	1.50	2.61
20:2n-6	0.41	0.37	0.24	0.12
20:4n-6	1.71	2.33	1.35	3.64
20:4n-3	0.57	0.20	0.22	0.46
20:5n-3	9.24	9.25	4.22	8.26
22:0	0.33	0.43	0.48	0.10
22:1	0.23	0.19	0.13	0.59
22:5n-6	1.79	2.09	0.33	tr
22:5n-3	0.89	0.58	0.69	4.39
22:6n-3	12.70	24.41	5.42	0.17
24:0	0.36	0.26	0.75	0.23

tr - trace

While improvement in growth and survival of the early larval stages (Day 21 larvae) of *E. coiodes* has been achieved in both intensive and semi-intensive hatchery rearing schemes, final survival at harvest of grouper fry is still less than 5%. Grouper larvae undergo a long metamorphic phase before they assume the physical and behavioral characteristics of juvenile fish. Larval grouper undergoing metamorphosis are extremely sensitive to various stressors such as handling, water turbulence, and fluxes in environmental conditions. Metamorphosis involves resorption of the long dorsal and ventral fins, development of pigmentation patterns of adult fish, and assumption of benthic habits typical of the species. The process requires from 15 to 20 days and occurs when the larvae are 21days old until they are 35-40 days old (Doi et al.,1991).

The thyroid hormones, tri-iodothyronine (T3) and tetra-iodothyronine (T4) have long been known to enhance growth and accelerate metamorphosis in various vertebrates including fish such as flounder (Inui and Miwa, 1985). Following reports of Tay et al. (1994) on acceleration of metamorphosis in larval grouper, work to investigate the response of various ages of grouper larvae to T3 and T4 treatments was undertaken. Two, three and four-week old groupers immersed in rearing water containing 1 ppm of either T3 or T4 had resorbed dorsal and ventral fins (shorter than controls) and developed pigment patterns of juveniles within 3 days of hormone treatment (de Jesus et al., 1998). The response of grouper larvae to the hormones was directly related to the hormone dose. Larvae immersed in the higher T3 or T4 dose (1 ppm) completed metamorphosis within 2 days; larvae treated with 0.01 ppm of the hormones completed metamorphosis in 5-6 days; while untreated controls took 10-21 days to complete metamorphosis (de Jesus et al., 1998). The application of thyroid hormones in large scale hatchery runs is presently being verified.

NURSERY AND GROW-OUT CULTURE

Most of the culture practices for on-growing groupers in cages or ponds were developed by fishfarmer guided by their experiences on other species. Continuing research to improve these farming practices is being undertaken. Grouper farmers use trash fish to feed groupers. Trash fish feeding is not only inefficient as shown by its high feed conversion ratio (FCR), it also competes with the food requirements of human populations particularly in third world countries like the Philippines. A major research thrust to improve farming practices focus on the development of cost-effective diets to replace trash fish. Practical diets based on the requirements of other carnivorous marine fish species such as sea bass has been formulated. However, these feeds still contain considerable amounts of fishmeal and studies aimed at reducing the fish meal component of grouper feed is currently being pursued. The apparent digestibility of alternative protein sources from plant and animal sources, acceptability of compounded diets with these feed ingredients and growth of juvenile grouper fed these diets is being assessed. Among the locally available plant protein sources tested, preliminary results suggest that white cowpea meal can be used as a partial replacement for fishmeal in grouper diets (Eusebio, 1999, pers. com.). Other protein sources are being tested including commercially available meat solubles from slaughter house-by-products.

Increasing numbers of fishfarmers in the Philippines are now engaged in grouper culture and mortalities mostly from parasitic infestations is an emerging problem. Cage-reared groupers harbor more species of parasites with higher prevalence and intensity of infection than pond-cultured groupers (Lacierda, 1999 pers. com.). Parasites recovered from groupers cultured in ponds and cages include various species of protozoa, and parasitic flatworms and nematodes. Life cycles of the more common monogeneans are also being studied.

Grouper research at the Aquaculture Department will continue to focus on three major areas: broodstock development; seed production; and nursery and grow-out culture. The main objectives are: 1) to improve the quality of spawned eggs through improved broodstock nutrition; 2) to further improve seed production technologies that can be easily disseminated to private hatchery operators; 3) to develop cost-effective and environmentally-friendly feeds by searching for nutritionally adequate alternative protein sources to replace fish meal; and 4) to develop improved husbandry practices for cage and pond culture operations.

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