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Author(s)	Duray, Marietta N.; Juario, Jesus V.
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BROODSTOCK MANAGEMENT AND SEED PRODUCTION OF THE RABBITFISH *SIGANUS GUTTATUS* (BLOCH) AND THE SEA BASS *LATES CALCARIFER* (BLOCH)

Marietta N. Duray and Jesus V. Juario
Aquaculture Department
Southeast Asian Fisheries Development Center
Tigbauan, Iloilo, Philippines

ABSTRACT

This paper reviews results of studies conducted on the rabbitfish, *Siganus guttatus* (Bloch) and the sea bass *Lates calcarifer* (Bloch) at the Aquaculture Department of the Southeast Asian Fisheries Development Center. Studies include broodstock development and management, induced breeding, effect of handling stress and diet on egg quality, early life history, food, feeding strategy, weaning to artificial diets, effect of stocking density and salinity on egg development, larval growth and survival, and advancement of metamorphosis in sea bass by using thyroxine.

A seed production technique had been developed for rabbitfish with survival rates ranging from 5-35% while the seed production technique for sea bass developed in Thailand had been modified to suit local conditions. Based on results from recent morphological and physiological studies, the stocking density, water management, and feeding scheme for the production of rabbitfish and sea bass fry had been modified to reduce cannibalism and improve survival.

INTRODUCTION

Rabbitfish and sea bass are two of the major marine and brackish-water species cultured in Southeast Asia (Rabanal, this volume). The major constraint to increase production of rabbitfish is seed supply (Juario et al 1985) while that of sea bass is availability of economically feasible weaning and grow-out diets and cannibalism in the hatchery production phase. The Department, therefore, started to conduct studies on the rabbitfish, *Siganus guttatus*, in 1980 to develop an economically feasible seed production technique and on the sea bass, *Lates calcarifer*, in 1982 to develop an appropriate and economically feasible weaning diet and to reduce cannibalism. This paper reviews the results of these studies.

BROODSTOCK MANAGEMENT

Rearing Facilities

Hatchery-bred or captive juveniles of *S. guttatus* and sea bass are reared to sexual maturity in floating net-cages at the Igang Substation, Guimaras Island. The cages are either rectangular (4 × 4 × 2 m) or circular (10 m dia × 3.0 m deep) and are made either of galvanized iron pipes or bamboo. Each cage is provided with cylindrical styrofoam floats. Fine-meshed net (0.5 cm) is used for fish weighing 50 g or less, 1.0 cm mesh net for 100-300 g, and about 3.0 cm for over a kg fish. Fish is stocked at a density of 2-3 kg/m³, thinned, and size-graded every 3-4 months. Nets are regularly cleaned of fouling organisms.

In Tigbauan, fish are reared to sexual maturity in 4-10 m dia × 1.0 m deep (15-40 t capacity) canvas tanks or in 6-50 t rectangular concrete tanks. The tanks are provided with aeration and flow-through system. A drain pipe is installed either at the center or periphery of the tanks. Bottom sediments are siphoned out weekly for *S. guttatus* (feeds are given on trays) and daily for sea bass. Tanks are thoroughly cleaned weekly for sea bass, monthly for siganids, or as the need arises. Broodstock tanks are either provided with green plastic roofing or covered with black sack cloth to prevent diatom or algal blooms.

Rearing Method

Juvenile *guttatus* are stocked at 20 fish/m³; sea bass juveniles at 10 fish/m³ in floating cages. The density is gradually thinned out as the fish grow. Rearing temperatures and salinity range from 26.1-30.9°C and 31.1-34.7 ppt, respectively. Sigamids are fed *ad libitum* twice daily with filamentous green algae or at 3-5% BW, with commercial fish pellets containing 35% protein or a combination of both. For spawning purposes, the breeders are fed with SEAFDEC lipid-enriched formulated diet at the same feeding level and frequency. Sea bass, on the other hand, are fed once daily with trash fish at 8-10% body weight (BW) if they are less than 100 g and 3-5% BW if bigger. Ration is reduced to 1-2% BW during the peak of spawning season.

Guttatus spawners are stocked at 1 female to 1 male ratio. Six pairs are stocked in each 6 m dia canvas tank provided with a flow-through system and aeration. For spawning, a pair is transferred to 500 l fiberglass tanks during the first lunar quarter. Sexes are determined by cannulation. Spawners are not fed during the spawning

period lasting 5-7 days. In sea bass, breeders with a sex ratio of 1:1 or 1:2 are transferred to spawning cages (1.5 × 3 × 2 m). As in *guttatus*, sexes are determined by cannulation in the absence of reliable external features that distinguish males from females.

Gonadal Maturation and Sexual Maturity

Captive wild *guttatus* matures at 200 g with a fork length (FL) of 34.0 cm (Soletchnik 1984). Hatchery-bred males mature in 10 months at 19.0 cm FL (Juario et al 1985) and females in 12 months at 21.5 cm FL (Soletchnik 1984, Juario et al 1985). One gonadal cycle is complete within 27-28 days (Soletchnik 1984, Hara et al 1986). Fish spawns every month throughout the whole year.

Gonadal development of sea bass is monitored every month by cannulation. Males mature ahead of females. Males mature when they are two years old, weighing about 1.5 kg with a TL of 45.0 cm, females at three years old, weighing 2.0 kg with a TL of 50.0 cm (Tan pers. comm.). Sexual maturation begins in January and peaks in February to August. The number of mature individuals decrease from October to November. Males appear to undergo gonadal regression earlier (October) than females (November), (Anon. 1985). Rematuration in the same season and multiple spawning were observed if gonadotropin-releasing hormone (GnRH-A) pellets are implanted in the breeders (Almendras pers. comm.).

Food abundance and diet quality proved to be important factors for *guttatus* maturation (Soletchnik 1984). Most of the females fed with commercial diet containing 43% protein spawned monthly for 11 consecutive months. However, Juario et al (1985) observed a decline in the fertilization and hatching rates and in larval quality with age of spawners fed with a commercial diet containing 43% protein.

Fecundity and Gonadosomatic Index (GSI)

A 400 g captive *guttatus* broodstock with GSI of 13.8% had 0.8 million eggs, while a 520 g with a GSI of 12.6 had 1.2 million eggs (Soletchnik 1984). About 400-500 g captive females spawned 0.45-1.3 million eggs. Hara et al (1986b) reported 0.2-1.2 million eggs from captive females averaging 410 g.

A 2.7 kg female sea bass had 3.6 million eggs; a 2.8 kg, 4.9 million eggs (Garcia pers. comm.).

Spawning

Natural or induced spawning for both species is not a problem. *Guttatus* spawns naturally the whole year 2-3 days after the first lunar quarter (Soletchnik 1984, Hara et al 1986b). Females mated for the first time spawned without failure. Weekly sampling of *Guttatus* collected from Cebu and Bohol, Philippines from April to June 1986 showed that a fish with a GSI value of 7.0 may spawn during the new to full moon period with a peak at the first quarter (Hara et al 1986d).

Guttatus having oocytes with an average dia of 0.46 cm spawned after one injection of human chorionic gonadotropin (HCG) at 2 IU/g body weight while those having oocytes with an average dia of 0.43 mm did not spawn or spawned only after several injections (Juario et al 1985). Harvey et al (1985) reported that LH-RH pellet implantation advanced spawning in both the first and second gonadal cycles after treatment. Stress due to routine hatchery operation also enhanced spawning but did not affect survival performance of resulting larvae (Ayson 1987).

Sea bass reared to sexual maturity in floating net-cages at the Igang Substation also spawn naturally (Anon. 1985). LH-RHa administered as saline injections or as cholesterol implants were comparably effective in inducing sea bass to spawn while osmotic pump triggered successive spawnings at 24 hr intervals (Harvey et al 1985). The same response was exhibited by females injected with LH-RH analogue D-ala⁶-D-Gly¹⁰-ethylamide at 100-400 µg/fish (Nacario and Sherwood 1986). An LH-RH dose of 150-300 µg/kg body weight induced a lower spawning frequency in sea bass, whereas lower dosages of 37.4-75.0 µg/kg induced higher spawning frequencies in mature females. At all doses tested, the total number of eggs collected per spawner decreased after four daily spawnings. Mean fertilization and hatching rates from four sequential spawnings of fish treated with 300 µg LH-RH were relatively lower compared to those implanted with lower doses. A dose with a range of 4.7-38.0 µg/kg increased spawning frequencies (Garcia pers. comm.).

The use of mammalian or salmon LH-RH during the new or first lunar phase was not clearly different. Although 1:1 sex ratio is effective, a ratio of 1 female to 2 males gave better fertilization and hatching rates. Pellets, pumps, and repeated injections induced multiple spawnings in sea bass, but the pellets proved to be more reliable, cheaper and less stressful to the fish (Almendras pers. comm.).

Spawning Behavior

Spawning behavior of *guttatus* is characterized by male chasing the female, nudging her abdomen, continuous swimming close to her nudging the operculum, anal region and caudal peduncle in sequence. After a minute of male display, female releases a small quantity of eggs and male releases milt. They stay quiescent for a time and another display is exhibited. Then more eggs and milt are released by both (Hara et al 1986c).

Active swimming of sea bass at night wherein male chases the female, characterizes their spawning behavior (Garcia pers. comm.).

Sperm Preservation

Significant results were obtained on the effect of pH, type of extender, dilution rate, and concentration of cryoprotectant on sperm viability of *guttatus* at 0.4-19.6°C (Anon. 1984).

After 24 hr in liquid nitrogen and cryogenic preservation in 150 mM KCl, 150 mM NaCl, and freshwater teleost Ringer's solution adjusted by tris-citric acid, good sperm motility was observed from pH 6.0-7.0. For 125 mM citrate, best sperm motility was at 6.5-1.0 pH, while glucose adjusted by HCl and NaOH at 4.0-10.0 pH.

Extenders like 100-200 mM KCl, 100-200 mM NaCl, 200-400 mM glucose, 75-175 mM Na citrate, freshwater Ringer's solution and Cortland medium yielded good results. Sera of tilapia, silver carp, milkfish, marine teleost Ringer's and Mounib medium yielded lower motility scores for cryopreserved sperm after thawing.

Cryoprotectant concentrations were best between 5-20% for dimethyl sulfoxide (DMSO) and 5-20% for glycerine. Ethyl alcohol yielded considerably lower scores than DMSO and glycerine.

Diseases

The ectoparasite, *Caligus epidemicus*, commonly infest captive *S. guttatus* spawners. This is effectively eradicated if salinity is kept at 0 ppt at least for 24 hr. Captive fish were also infected with nematodes leading to poor appetite.

No mortality due to infection or parasite have been reported for sea bass.

SEED PRODUCTION

Rearing Facilities

Larval rearing tanks vary in size and shape and are made of different materials. Size of experimental tanks ranges from 200-500 l. They are either circular or conico-circular and made of fiberglass. Pilot and large scale production tanks range from 3-10 t and are either circular, conico-circular or rectangular and are either ferroconcrete, concrete, or canvas. All tanks are housed in a roofed hatchery to protect larvae from direct sunlight or heavy rain. The roof is made of transparent corrugated plastic sheets.

Conico-circular concrete tanks are easier to clean than rectangular tanks; the former, however, are more expensive to construct and occupy more space. It is easier to maintain good water quality and to prevent harmful effects of aeration on young larvae in larger tanks. Thus, it is generally assumed that better survival rates are obtained in larger tanks. For rabbitfish results are contradictory (Juario et al 1985, Hara et al 1986b).

Egg Collection and Handling

Guttatus eggs are adhesive and demersal. Egg collectors consisting either of plankton nets or plastic sheets are placed at tank bottom prior to spawning. After spawning the collectors are removed and transferred to incubators.

Sea bass eggs are pelagic. Eggs from broodstock reared in floating net-cages are collected as follows: A day or two prior to quarter moons, cages are lined with hapa net (mesh size, 150 μm) to prevent loss of eggs. If spawning occurs, eggs are collected by using a net with a mesh size of 150 μm . Eggs spawned in tanks are collected in the same manner.

Sea bass eggs collected from floating net cages at Igang Substation are packed in doubled oxygenated plastic bags at a density of 100,000 eggs/10 l of sea water. Plastic bags with eggs are placed in *pandan* bags and transported to Tigbauan Research Station where they are reared to fry or until metamorphosis.

Incubation

Eggs are incubated in 500 l tanks or directly in 3 or 10 ton larval rearing tanks. A maximum stocking density of 400/l may be used for the former and 100 for the latter. Water is changed 1 or 3 times by allowing it to flow through for 30 min to 1 hr depending on water quality (Juario et al 1985). The total number of incubated sea bass eggs are estimated by collecting five samples from different parts of the tank by using a PVC pipe. Depending on temperature, incubation for *guttatus* ranges from 18-26 hr; and for sea bass, 15-18 hr, (Juario et al 1985, Duray et al 1986, Hara et al 1986b).

A comparative study on the effects of salinity on *guttatus* egg development and hatching showed that naturally spawned eggs are more tolerant to salinity changes than inductively spawned ones (Duray et al 1986). Higher hatching rates and greater number of viable larvae were obtained when eggs were transferred at the gastrula stage than at the blastomere stage. Highest total hatching and percentage of viable larvae were obtained at 24 ppt and lowest at 8 ppt. Larvae that hatched at lower salinities (8, 16 ppt) were relatively longer than those at 32 ppt and 40 ppt (Duray et al 1986).

Larval Rearing

Both *guttatus* and sea bass larvae are reared in semi-static system with aeration. Sediments and detritus that settle on tank bottom are siphoned out daily. Water management and feeding schemes in rearing *guttatus* and sea bass larvae to metamorphosis are presented in Fig. 1 and 2. The phytoplankton *Chlorella*, *Tetraselmis*, or *Isochrysis* are added to rearing tanks as water conditioners and as food for rotifers.

Newly hatched *guttatus* larvae were more resistant to low and high salinities (8-37 ppt) than 7-14 days old larvae while older larvae are more resistant to abrupt salinity (2-55 ppt) changes (Anon. 1984). Survival of larvae reared at 20-32 ppt salinity from 0-21 did not differ significantly (Hara et al 1986b). First-feeding larvae reared under continuous lighting grow and survive better than those reared under daylight (Duray and Kohno in press).

Survival of sea bass larvae reared at a density of 15, 30, and 45 ind/l did not differ significantly from each other. By day 21, however, the mean weight of larvae stocked at 5/l and 30/l were significantly larger than those stocked at 45/l. Based on size and weight of larvae,

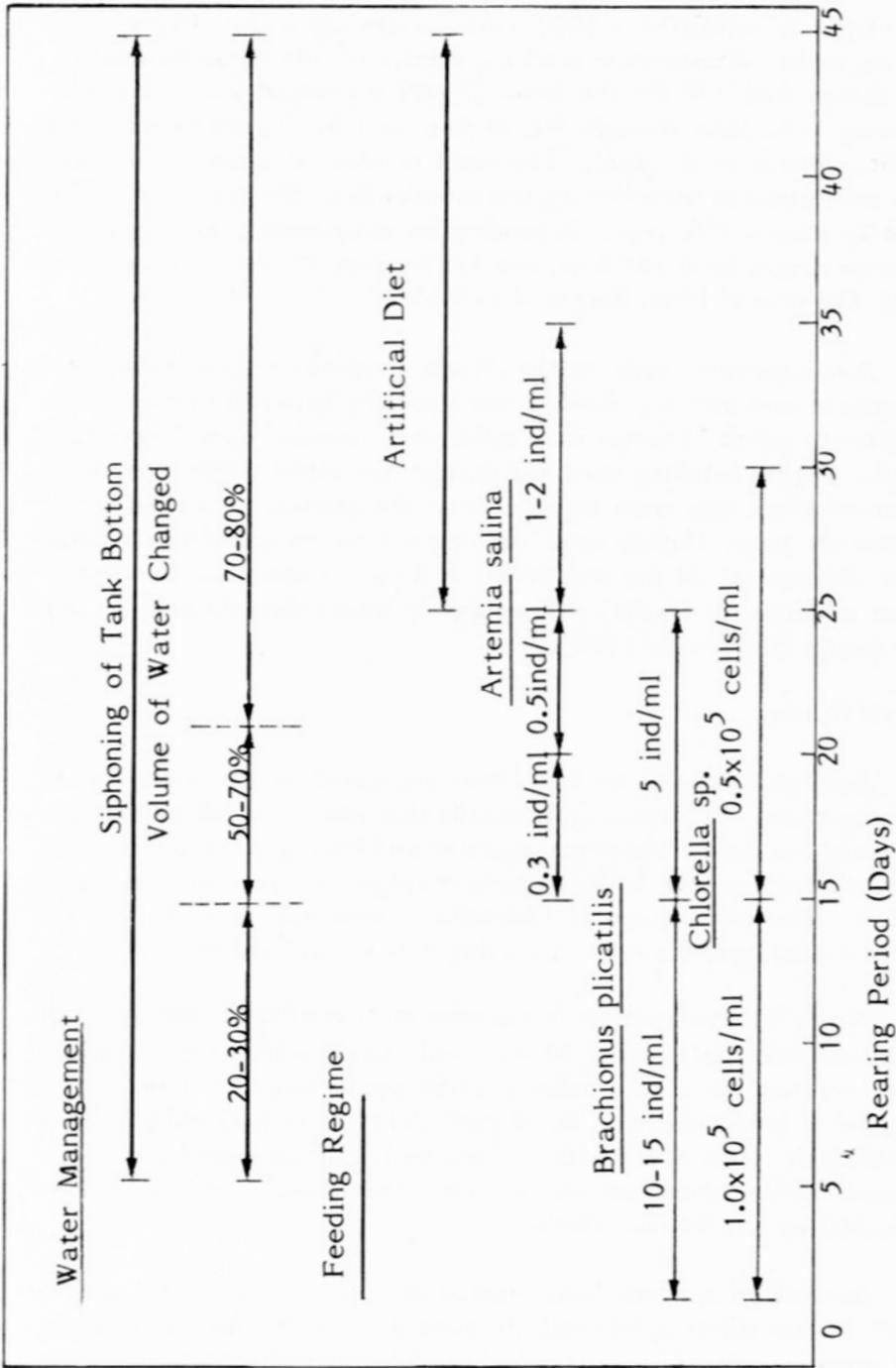


Fig. 1. Feeding scheme and water management for *Siganus guttatus*

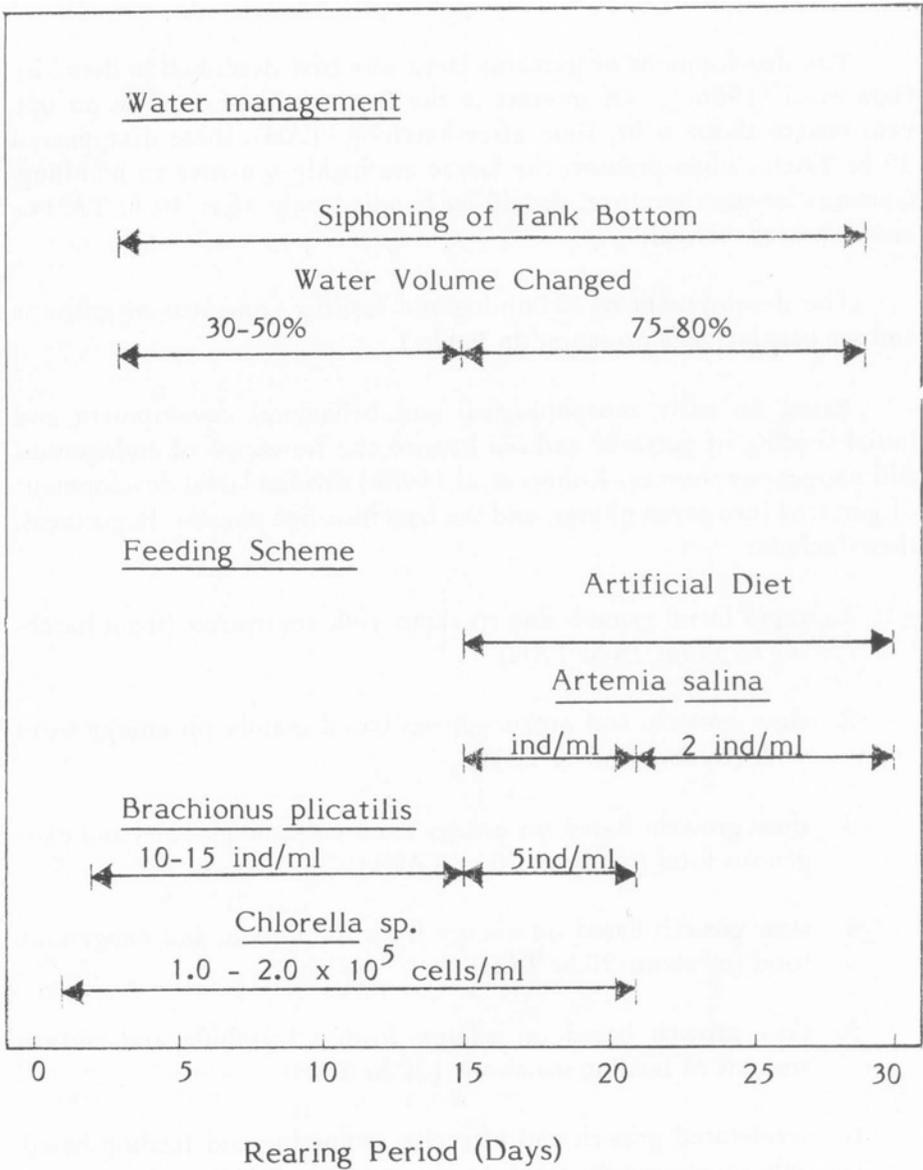


Fig. 2. Feeding scheme and water management for *Lates calcarifer*.

the stocking density of 30 ind/1 is the most appropriate in mass producing sea bass fry (Juario and Duray unpublished).

Larval Development

The development of guttatus larva was first described in detail by Hara et al (1986c). Of interest is the appearance of cupulae on free neuromasts about 6 hr, time after hatching (TAH); these disappeared 39 hr TAH. When present, the larvae are highly sensitive to handling. Guttatus larvae, therefore, should be handled only after 40 hr TAH to ensure better survival.

The development of swimming and feeding apparatus of guttatus and sea bass larvae is presented in Table 1.

Based on early morphological and behavioral development and initial feeding of guttatus and sea bass to the transition of endogenous and exogenous sources, Kohno et al (1986) divided larval development of guttatus into seven phases, and sea bass into five phases. In guttatus, these include:

1. rapid larval growth due to rapid yolk resorption (from hatching to about 15 hr TAH)
2. slow growth and organogenesis based mainly on energy from yolk (to about 50 hr TAH)
3. slow growth based on energy from yolk, oil globule, and exogenous food (to about 70 hr TAH)
4. slow growth based on energy from oil globule and exogenous food (to about 90 hr TAH)
5. slow growth based on energy from oil globule and certain amount of feeding (to about 120 hr TAH)
6. accelerated growth and effective swimming and feeding based only on exogenous food (to about 150 hr TAH); and
7. same mode as in the preceding but with increased feeding (beyond 120 hr TAH)

In sea bass, these include:

1. rapid early growth due to rapid yolk resorption (from hatching to about 15 hr TAH)

Table 1. Size of siganid and sea bass larvae at the first appearance and completion of morphological characters related to swimming and feeding (Kohno et al 1986, unpublished data)

Morphological Characters	Siganid		Sea Bass	
	Size at 1st Appearance	Size at Completion	Size at 1st Appearance	Size at Completion
A. Swimming-Related:				
Dorsal: Fin Supports	3.7	10.99	3.74	4.26
Fin Rays	3.93	8.04	4.23	5.30
Anal: Fin Supports	5.10	6.03	3.74	4.49
Fin Rays	5.94	8.54	4.45	5.85
Caudal: Fin Supports	4.14	8.94	2.95	8.40
Fin Rays	4.83	6.44	3.27	7.70
Pectoral: Fin Supports	2.52	10.80	2.06	2.52
Fin Rays	6.29	9.74	5.30	10.25
Ventral: Fin Supports	3.70	—	5.30	—
Fin Rays	3.93	7.44	5.30	7.09
Vertebrae	6.29	7.17	-	-
Notochord end flexion	4.97	6.50	3.27	6.5-7.5
B. Feeding-Related:				
Upper Jaw Teeth	4.49	7.5	2.57	5.85
Lower Jaw Teeth	4.12	5.94	3.20	—
Upper Pharyngeal Teeth	3.26	6.00	2.57	—
Lower Pharyngeal Teeth	7.08	—	3.84	—
Maxilla	2.52	—	1.92	—
Premaxilla	4.12	-	2.57	-

2. morphological differentiation and slow growth based on energy from yolk until yolk is exhausted (about 50 hr TAH)
3. slow growth with initiation of feeding and swimming activities, based on energy from oil globule and from exogenous food (to about 110 hr TAH)
4. accelerated growth and effective feeding and swimming based on the same two sources of energy as in the preceding stage until the oil globule is exhausted (to about 120-140 hr TAH); and
5. accelerated growth, effective feeding and swimming, and further development based solely on exogenous energy (beyond 140 hr TAH). The energy sources during the developmental phases are the yolk, oil globule, exogenous food, or any combination of these.

Sea bass eggs and larvae are bigger than *guttatus* (Bagarinao 1986, Avila and Juario 1987). At similar ambient temperatures (26-30°C), *guttatus* larvae grow much faster than sea bass in the first 24 hr TAH. This appears to be a compensatory mechanism for survival. Full eye pigmentation and opening of the mouth occur at 32-36 hr TAH for both species; sea bass larvae learn to feed earlier than *guttatus*.

Sea bass larvae of different ages were immersed in 0, 0.01, 0.10, and 1.0 ppm thyroxine to advance metamorphoses (Ordonio 1987). Growth, survival, and yolk resorption were not affected but fin differentiation and epidermal thickening in treated larvae were enhanced. Abnormalities in vertebral column were observed only among larvae exposed to a concentration of 1.0 ppm from day 7-14 and 0.1 and 1.0 ppm from day 15-21. Metamorphosis without deleterious effects was advanced in larvae exposed to T_4 from day 21-28. The absence of thyroid follicles from day 0-35, i.e., until metamorphosis, suggests that the thyroid gland may not be directly involved in the early development of sea bass larvae.

Food and Feeding

Guttatus larvae are reared on rotifers, newly hatched brine shrimp nauplii and artificial diet (Juario et al 1985, Hara et al 1986b). Initially they feed on rotifers at a total length (TL) of 2.6 mm (day 2) and on brine shrimp at 4.4 mm TL (day 12). A change in feeding habits occur at about 7.0-9.5 mm TL with rotifer as prey and at 7.2 mm TL with

brine shrimp as prey. Preference for brine shrimp over rotifers was observed in 8.0-9.0 mm TL or longer larvae. These changes in feeding habits coincide with the full osteological development of the feeding apparatus in 7.0-8.0 mm TL larvae.

In sea bass, initial feeding on rotifer was observed at 2.5 mm TL (day 2) and on brine shrimp at 4.0-4.5 mm TL (day 10). The amount of food consumed increased exponentially with larval growth. Food preference shifted from rotifers at 4.5 mm TL to brine shrimp at 6.0-7.0 mm TL.

The timing of events related to the onset of feeding in both species is presented in Table 2. Although sea bass larvae are about the same size as *guttatus* at the onset of feeding, mouth size of the former is twice larger than that of the latter. Thus, availability of food with appropriate size is critical to the survival of *guttatus* larvae during the first feeding period. Feeding *guttatus* larvae with rotifers less than 90 μm at a density of 10-20 ind/ml improved survival (Duray 1986). This was confirmed later by Hara et al (1986b).

The phytoplankton, *Chlorella*, *Tetraselmis*, or *Isochrysis* when given as the only food for *guttatus* larvae will not support life during the first-feeding days (Duray 1986). A feeding combination of the three phytoplankton and small-sized rotifers resulted in better survival although this was not significantly different from a feeding combination of *Isochrysis* and small-sized rotifers. A feeding combination of *Chlorella* and rotifers resulted in poor survival. Survival of larvae fed with rotifers at a density of 10-20 and 20-30 ind/ml did not differ significantly but differed significantly from those fed with rotifers at a density of less than 10/ml.

Guttatus larvae exhibit a diurnal feeding pattern. The percentage of larvae with food in the gut decreased in the evening and reached zero at 2200 hr. The time of active feeding shifted earlier in the day with larval growth. Satiation occurs at 0800-1000 hr (Hara et al 1986a).

Under natural illumination, the amount of rotifers in the gut of 10-day old sea bass larvae decreased from 1800 hr and started to increase at 1500 hr. No food was found in the gut at 0100 hr. The maximum food intake was 0800 hr at 20 rotifers/larva. Under artificial illumination, only 30-50% of the larvae had 0.5-11.0 rotifers in their guts from 2200-0500 hr. Maximum food intake was at 1300-1600 hr with 24-27 rotifers/larva (Anon. 1982).

Table 2. Some of the early life history characteristics of sea bass and siganid reared at 26-30°C (Bagarinao 1986)

Characteristics	Siganid	Sea Bass
Usual time of spawning	Midnight	Evening
Incubation period (hr)	20	14
Fertilized egg, type	Demersal	Pelagic
diameter (mm)	0.55	0.80
volume (ul)	0.871	0.2681
Larval length at hatching (mm)	1.50	1.72
Yolk volume at hatching (ul)	0.0251*	0.0859*
Maximal larval SL attained on yolk reserves (mm)	2.55	2.45
Time from hatching to attainment of maximal SL on yolk (hr)	24	24
Yolk volume remaining when maximal larval SL attained (ul)	0.0032*	0.0072*

*Total of yolk plus oil globule

Delayed feeding experiments showed that mortality occurred among unfed guttatus larvae in 88 hr while 7-12% of those fed within 32-56 hr survived beyond 88 hr. Unfed sea bass larvae died 144 hr TAH (Bagarinao 1986).

Guttatus larvae fed with newly hatched San Francisco Bay (SFB) brine shrimp nauplii for three days followed by SELCO-enriched Great Salt Lake (GSL) brine shrimp nauplii thereafter have significantly longer TL than those fed only with newly hatched GSL brine shrimp nauplii throughout the experimental period or newly hatched GSL brine shrimp followed by SELCO-enriched GSL brine shrimp nauplii (Duray unpublished). For sea bass larvae, better growth and survival are obtained if newly hatched GSL brine shrimp nauplii are fed on day 15 or 18 rather than on later stages (Kohno pers. comm.). A feeding level of 1.0 ind/ml is better than at lower (0.5/ml) or higher concentration (2.0/ml) (Gallego pers. comm.).

Diseases

Red spots sometimes appear on sides and bottom of rearing tanks. These red spots consist primarily of the bacteria, *Vibrio* sp. Continuous

direct application of fresh water for 2-3 days effectively controls the infection. Bacterial (*Vibrio* sp.) diseases observed in 22-25 days old sea bass larvae often result in total mortality of sea bass reared in outdoor tanks at high ambient temperatures (26-32°C), salinity (35-37 ppt), illumination, and dense diatom bloom (Bagarinao and Kungvankij 1986).

Transport and Handling of Fry

Fry are transported in doubled oxygenated plastic bags placed in *huri* or *pandan* bags for further protection. The density in each bag depends on fry size. Survival is better if sea bass fry are transported by day 21 when they are about 1.0 cm TL at a density of 3 000-5 000 fry/10 l water/bag (Juario pers. comm.).

Production Constraints

Although a technique to mass produce guttatus fry had already been developed, more work needs to be done before artificial propagation can be carried out on a routine basis. Survival rates are still very variable and there is no diet for guttatus broodstock that will lead to production of good quality eggs. In addition, practical diets for rearing guttatus larvae to metamorphosis and for its nursery phase still needs to be developed.

Although an appropriate diet had already been developed to successfully rear as early as 10 days old sea bass larvae to metamorphosis, its economic feasibility should be assessed and the most appropriate time and way of weaning the larvae to artificial diets should be determined. Cannibalism is still a serious constraint to sea bass fry and fingerling production. A technique to minimize it has to be developed.

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