# Aspects of Nutrition and Reproduction in *Siganus guttatus* with Emphasis on Applications to Aquaculture

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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# INTRODUCTION

The rabbitfish Siganus guttatus, one of the common species in Philippine waters is being studied at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) in Iloilo, Philippines for eventual mass seed production. Several problems were encountered in the rearing of this species in the hatchery. One of those suspected was egg quality. This study was conducted to investigate the problem. At the start of the study, the orientation was to improve the feeding management of the broodstock, and to monitor variables strictly dependent on the parents: maturation, spawning frequency, fertilization rates, egg diameter, hatching rates, normality of larvae. Subsequently, the quality of the larvae was investigated using variables such as size at hatching, speed of yolk resorption and early growth. The later part of the study included feeding of broodstocks with formulated experimental diets and rearing the larvae produced.

On the whole, this investigation included several aspects of the biology of S. guttatus and provides information on nutrition, growth and reproduction, with emphasis on their applications to aquaculture.

# MATERIALS AND METHODS

## Experimental Fish and Holding Systems

*Siganus guttatus* that had been reared in the hatchery of the SEAFDEC Aquaculture Department, as well as some that were captured from the wild were used in the study. Four broodstocks were designated (Table 1), together with a batch of wild juveniles. Broodstocks A and B both consisted of 1.5 yr old hatchery-bred adults; A was fed a pelletized commercial crustacean diet, while B was fed fresh food. Broodstock C was the fish of B turned over to experimental diets. Broodstock D consisted of fish captured in Guimaras Island. The juveniles were captured in Pandan, Panay Island. The fish were kept in 4 m and 6 m-diameter cylindrical canvas tanks with a flow-through system. Water depth was adjusted to obtain 200% turnover daily. Stocking rate varied between 0.5 and 1.5 $kg/m^3$.

For spawning, groups of broodstock fish, 1-2 male-female pairs were transferred to cylindrical fiberglass tanks of 200-600 l water volume with aeration. Spawned eggs or the hatched larvae were then transferred to a series of 15 250-l cylindroconic fiberglass tanks for larval rearing. The tanks were aerated and the water partially changed at intervals.

Table 1. The different broodstocks of <u>Siganus guttatus</u> used in this study in March 1983 - January 1984, fed various diets.

| Broodstock | Number of fish | Source | Feeding Treatment | Availability of broodstock | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | M | A | M | J | J | A | S | O | N | D | J |
| A | 22 | Hatchery | Commercial diet | X | X | X | X | X | X | X | X | X | X | X |
| B | 22 | Hatchery | Fresh Food | | X | X | X | X | | | | | | |
| C | 16 | Hatchery | Experimental diets | | | | | | X | X | X | X | | |
| D | 22 | Guimaras | Fresh food | | | | X | X | | | | | | |
| Juveniles | 100 | Pandan | Experimental diets | | | | | | X | X | X | X | | |

Broodstock Diet and Feeding Experiments

The commercial crustacean diet given to broodstock A was used as the control. Fresh food given to broodstock B consisted of Enteromorpha sp., squid, mussel, shrimp and trash fish, chopped to suitable size. In one experiment of 15 days duration, fish were given all 5 kinds of fresh food; in a subsequent experiment of 35 days duration, only Enteromorpha and squid were used. The fish were fed ad libitum from feeding trays. Weighted aliquots (30-40 g each, depending on kind) were added to the feeding trays as soon as they were lacking. The exact food consumption by the fish in the canvass tanks was determined daily from the amount of aliquots removed by the fish from the feeding trays, corrected for the amount leached out as the chopped food got into the water.

Samples of all diets were dried in an oven at 60°C, dry weights were measured and samples sent for proximate analysis. Analysis showed that the commercial diet had 43% protein; Enteromorpha, only 11%; and the other kinds of fresh food, 62-74% (Table 2). Enteromorpha, which turned out to be a preferred food of the broodstock, became scarce in August-November, so formulated artificial diets were resorted to. These diets were processed at the Department's Feed Laboratory, with the advice of Dr. F. Pascual. Three

Table 2. The proximate analysis of the various diets used for the broodstocks of *S. guttatus*.

| DIETS | Protein (%) | Fat (%) | Fiber (%) | Ash (%) | Nitrogen free Extract (%) | Moisture (%) | Energy Protein + Fat (Kcal/kg) | Total Energy of diet (Kcal/kg) |
|---|---|---|---|---|---|---|---|---|
| *Enteromorpha* | 11.4 | 1.0 | 4.0 | 49.8 | 33.8 | 88.5 | 546 | 1898 |
| Squid | 73.9 | 7.5 | 0.5 | 6.7 | 11.4 | 83.8 | 3631 | 4067 |
| Mussel | 61.9 | 8.4 | 0.3 | 13.2 | 16.2 | 86.8 | 3232 | 3880 |
| Shrimp | 73.9 | 4.0 | 1.2 | 11.7 | 9.3 | 82.0 | 3316 | 3688 |
| Fish | 72.7 | 10.0 | 4.0 | 11.4 | 5.6 | 80.4 | 3808 | 4032 |
| Commercial crustacean diet | 43.0 | 5.2 | 1.7 | 8.3 | 41.8 | 11.7 | 2188 | 3860 |
| Experimental diet 1 | 18.6 | 3.2 | 3.4 | 10.6 | 64.3 | 11.9 | 1032 | 3604 |
| Experimental diet 2 | 21.9 | 9.3 | 4.3 | 12.9 | 51.7 | 10.8 | 1713 | 3781 |
| Experimental diet 3 | 25.8 | 13.8 | 4.2 | 13.7 | 42.5 | 11.0 | 2274 | 3974 |

experimental diets with similar ingredients (Table 3) were prepared. Their protein contents were relatively low (18-26%), and fat levels varied (3, 9 and 14%) (Table 2). The diets were steamed to assure stability in seawater for several hours. These experimental diets 1, 2 and 3 were used in feeding experiments run in parallel in August-November on broodstock C and on the juveniles.

Observations on growth, maturation and spawning

The fish were regularly sampled for body lengths and weights to determine the effects of the various diets on growth. After sexual differentiation of the adults, males were marked from the females by a cut in the dorsal fin. This permitted subsequent identification of the sexes without the stress of anaesthesia and cannulation. The mark was recognizable several months after the operation. To determine sexual maturation, the fish were anaesthesized with 2-phenoxyethanol and cannulated. A polyethylene tubing 0.86 mm in diameter was inserted in the urogenital opening of the fish to sample the gonadal material. The maturation of oocytes in 5 females in July was monitored through cannulation.

Table 3. Composition of the experimental diets fed to *S. guttatus*.

| Ingredients | Percent weight | | |
|---|---|---|---|
| | Diet 1 | Diet 2 | Diet 3 |
| Fish meal | 10.0 | 10.0 | 10.0 |
| Soybean meal | 15.0 | 26.5 | 28.0 |
| Defatted soybean meal | 8.5 | 0.0 | 0.0 |
| Rice bran | 4.0 | 15.0 | 25.0 |
| Ipil ipil leaves | 5.0 | 5.0 | 5.0 |
| Corn starch | 44.5 | 25.0 | 3.5 |
| Rice hulls | 8.0 | 12.8 | 20.0 |
| Soybean oil | 0.0 | 0.7 | 3.5 |
| Mineral mixture + Vic C + BHT | 5.0 | 5.0 | 5.0 |

Spawning was very easy to monitor among the siganid broodstock since the eggs are adhesive and demersal. The fish spawned without hormonal treatment. The dates on which eggs appeared in the tanks were recorded to determine the spawning periodicity. The frequency of spawners every month was determined from the degree of maturation of the particular broodstock, from the physical appearance of the females before and after the spawning date, and from direct observation of isolated pairs of fish in spawning tanks. Fecundity measurements were made by the volumetric method using ovaries in the pre-spawning stage of maturation, or by direct counting of the adhesive eggs in small collectors set in the spawning tanks. The diameters of eggs spawned by the different broodstocks each month were measured to determine effects of the different diets.

## Larval Rearing

In June and July, it became clear that it was necessary to include larval survival as a criterion in the evaluation of the quality of broodstock. Siganid eggs were collected from the spawning tanks and transferred usually at the gastrula stage to rearing tanks. Rotifers (*Brachionus plicatilis*) and algae (*Chlorella*, *Tetraselmis* and *Isochrysis*) were given to the larvae once a day, and oyster trochophores and artificial feeds 2-5 times daily. Nitrate and ammonia concentrations were monitored to control water quality. Salinity was 29-32 ppt; temperatures ranged 27-31°C.

There is as yet no standardized technique to rear *S. guttatus* larvae although the species has been reared at the Aquaculture Department since 1981. My attempts at rearing are efforts in that direction.

## RESULTS

### Consumption of Fresh Food and Preference for *Enteromorpha* and Squid

Table 4 shows the amounts in dry weight of *Enteromorpha*, squid, mussel, shrimp and trash fish taken by adult *S. guttatus* (broodstock B) during a day's feeding from trays averaged for a number of fish over periods of 15 days and 35 days. The feeding rates, determined from the dry weight of the fresh food consumed and the average weight of the fish in the treatment, were about 2.7% and 3.6% during the 15-day and 35-day periods, respectively (Table 4).

Table 4. Daily consumption of fresh food by adult *S. guttatus*.

| Type of fresh food | Daily Food Consumption (g dry wt/kg fish) | | | | Daily Food Consumption (% dry weight) | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 day expt. | | 35 day expt. | | 15 day expt. | | 35 day expt. | |
| | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 |
| *Enteromorpha* | 7.6 | 7.2 | 15.0 | 14.4 | 28 | 26 | 41 | 44 |
| Squid | 9.8 | 9.2 | 21.3 | 18.7 | 36 | 33 | 58 | 56 |
| Mussel | 3.0 | 4.9 | | | 11 | 18 | | |
| Shrimp | 4.2 | 3.6 | | | 16 | 13 | | |
| Fish | 2.3 | 2.8 | | | 9 | 10 | | |
| Feeding rate (%) (dry weight food)/(wet weight fish) | 2.69 | 2.77 | 3.63 | 3.31 | | | | |

Each tank had 11 fish.

In terms of wet weight, this feeding rate is about 20% of fish body weight.

It appears that the fish preferred *Enteromorpha* and squid over the 3 other animal food sources. During the 15-day experiment, chopped squid and *Enteromorpha* respectively accounted for 34.5% and 27% in dry weight, and 26.5% and 38.5% in wet weight of the total daily food consumption of *S. guttatus*.

## Biochemical Quality of the Food Actually Consumed

The quality of the fresh food actually consumed was determined for broodstock B for the periods April-May when they were fed all the 5 kinds, and June-July when they were fed only *Enteromorpha* and squid. The biochemical quality was estimated from the daily average consumption of each kind (Table 4) and the proximate analysis of these (Table 2). Results indicate that the actual fresh food intake (Table 5) has 31-51% protein, 3-5% fat, a higher ash content (20-27%) and a lower nitrogen-free extract (22-36%) than the commercial diet and the experimental diets (Table 2). The total energy of the fresh food diets were 3000-3400 Kcal/kg (Table 5). In comparison, the total energy from *Enteromorpha* alone was low (1900 Kcal/kg), and those from

Table 5. Biochemical quality of the fresh food diets actually consumed by *S. guttatus* broodstock.

| DIETS | Protein (%) | Fat (%) | Fiber (%) | Ash (%) | Nitrogen free Extract (%) | Moisture (%) | Energy Protein + Fat (Kcal/kg) | Total Energy of diet (Kcal/kg |
|---|---|---|---|---|---|---|---|---|
| Fresh Food Diet (April- May) | 51.1 | 5.2 | 1.8 | 19.5 | 22.4 | 80.90 | 2512 | 3408 |
| Fresh Food Diet (June-July) | 31.4 | 3.1 | 2.9 | 26.5 | 36.1 | 80.90 | 1535 | 2979 |

the experimental diets were higher (3600-4000 Kcal/kg) (Table 2).

Table 6 shows the total energy and ash contents of the different diets and percentages of total energy in the diets that come from protein, carbohydrate and fat. It is to be noted that the total energy in the diet decreases when the ash content increases.

Feeding Rate and Growth of Adults and Juveniles

Table 7 shows the growth, feeding rate and food efficiency of hatchery-bred adults during the periods April-May, June-July and August-November when fed the commercial diet (broodstock A), fresh food (broodstock B) and experimental diets (broodstock C). Feeding rate on the commercial diet was 1.7% of body weight for which broodstock A realized a 0.07-0.1 food efficiency (wet weight gain over the dry weight of food consumed). Broodstock B that fed *ad libitum* at 3% body weight

gained weight (food efficiency = 0.17), while those that fed at 1.6% lost weight. Broodstock C did not take well to the experimental diets 2 and 3, feeding only at 2% and losing weight; diet 1 was only slightly better (food efficiency = 0.01). Food efficiency is quite low for adult *S. guttatus*, positive for broodstock fed

Table 6. Distribution of energy in the diets.

| DIET | Total energy (Kcal/kg) | Energy from protein (% of total energy) | Energy from carbohydrate (% of total energy) | Energy fat(% of total | Ash content (%) | Total energy relative to diet 3 (%) | Ash content relative to *Enteromorph* (%) |
|---|---|---|---|---|---|---|---|
| *Enteromorpha* | 1898 | 24 | 71 | 5 | 50 | 47 | 100 |
| Fresh food (April-May) | 3408 | 60 | 26 | 14 | 20 | 86 | 40 |
| Fresh food (June-July) | 2979 | 42 | 48 | 10 | 27 | 75 | 54 |
| Experimental diet 1 | 3604 | 21 | 71 | 8 | 11 | 91 | 22 |
| Experimental diet 2 | 3781 | 23 | 55 | 22 | 13 | 95 | 26 |
| Experimental diet 3 | 3974 | 26 | 43 | 31 | 14 | 100 | 28 |
| Commercial diet | 3860 | 45 | 43 | 12 | 8 | 97 | 16 |

Table 7. Growth, feeding rate and food efficiency of hatchery-bred adult *S. guttatus* fed various diets during various periods.

| Broodstock | Number of fishes | Feeding treatment | Period covered (1984) | Initial mean weight (g) | Final mean weight (g) | Feeding rate[1] (%) | Food efficiency[2] |
|---|---|---|---|---|---|---|---|
| A | 22 | Commercial diet | March-November | 288.0 | 429.5 | 1.7 L | 0.10 |
| | 4 | | August-November | 364.3 | 419.8 | 1.7 L | 0.07 |
| B | 22 | Fresh food | March-April | 303.5 | 415.0 | 3.0 L | 0.17 |
| | 12 | | June-July | 415.0 | 381.0 | 1.5 | -0.09 |
| C | 4 | Experimental diet 1 | August-November | 345.5 | 351.8 | 2.5 L | 0.01 |
| | 4 | Experimental diet 2 | | 424.0 | 413.3 | 2.1 L | -0.01 |
| | 4 | Experimental diet 3 | | 403.3 | 394.0 | 1.9 L | -0.01 |
| | 10 | Enteromorpha | June-July | 400.0 | 358.0 | 1.6 | -0.10 |

[1] Feeding rate $= \frac{\text{dry weight of food consumed}}{\text{wet weight of fish}}$ ; L denotes fish fed *ad libitum*.

[2] Feed efficiency $= \frac{\text{gain wet weight of fish}}{\text{dry weight of food consumed}}$

*ad libitum* on fresh food and commercial diet, about zero for fish fed the experimental diets and negative for fish fed restricted amounts of fresh food.

Fig. 1 shows the growth of male and female hatchery-bred broodstocks during almost a year's observation. The best growth was realized by broodstock B fed *ad libitum* with fresh food. Growth was subsequently depressed in these fish when the diet was restricted to 1.6% body weight, and levelled off when the experimental diets were given for 4 months. About 50% of the females of broodstock C spawned monthly and lost 5-10% of their body weight in broadcast eggs.

Table 8 shows the growth of wild juveniles reared in captivity on the commercial diet and the experimental diets. During a 4-week observation period, the juveniles fed at low rates of 2-2.5% body weight. They gained weight on the commercial diet and on the experimental diet 3, but not on diets 1 and 2. During a 12-week observation period, feeding rates were higher and growth was realized in all feeding treatments. It is interesting that for diet 3 and the commercial diet where feeding rate was low (2-2.9%), food efficiency was high (0.35-0.42), while in diets 1 and 2 where feeding rates were higher (3.5-3.9%), food efficiency was much lower (0-0.23).

On the commercial diet and the experimental diets, the feeding rate of juveniles is higher than of the adults. The food efficiency is about 4-5x greater for juveniles

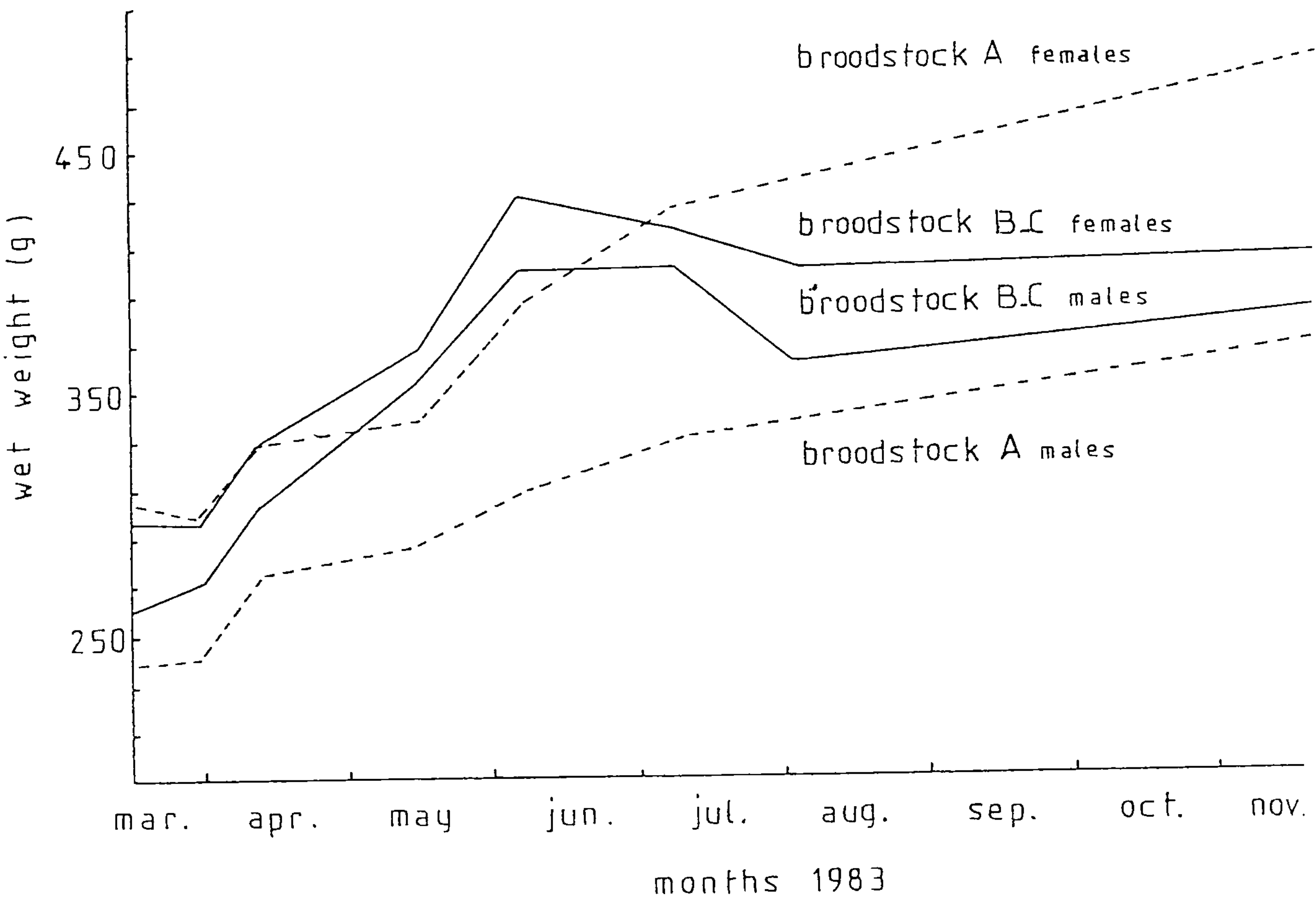


Fig. 1. Growth of S. guttatus adults, males and females, fed on commercial diet - broodstock A, on fresh food and experimental diets, broodstock B and C.

Table 3. Growth, feeding rate and food efficiency of juvenile *S. guttatus* from the wild reared in canvas tanks in August-November 1983.

| Feeding treatment | Number of fishes | Period covered (Weeks) | Initial mean weight (g) | Final mean weight (g) | Feeding rate (%)[1] | Food efficiency[2] |
|---|---|---|---|---|---|---|
| Commercial | 25 | 4 | 47.2 | 64.8 | 2.5 | 0.42 |
| Diet | 25 | 12 | 64.8 | 170.4 | 2.9 | 0.38 |
| Experimental | 25 | 4 | 51.2 | 50.0 | 2.4 | -0.03 |
| Diet 1 | 25 | 12 | 50.0 | 105.8 | 3.3 | 0.23 |
| Experimental | 25 | 4 | 51.6 | 51.8 | 2.1 | 0.00 |
| Diet 2 | 25 | 12 | 51.8 | 92.6 | 3.5 | 0.20 |
| Experimental | 25 | 4 | 50.9 | 60.9 | 2.0 | 0.30 |
| Diet 3 | 25 | 12 | 60.9 | 132.9 | 2.6 | 0.35 |

1. Feeding rate $= \frac{\text{dry weight of food consumed}}{\text{wet weight of fish}}$

2 Food efficiency $= \frac{\text{gain wet weight of fish}}{\text{dry weight of food consumed}}$

than for adults fed the commercial diet. Among the experimental diets, the feeding rate was highest and food efficiency tended to be lowest in diet 1 which had the least % protein and least % fat; the converse was true for diet 3.

The feeding rate is higher in juveniles than in adults on any one diet. The feeding rate in both groups seems to be associated with the total energy content of the diet (Fig. 2). The regressions can be expressed by the equations:

Adults: $R = -0.00233\ E + 10.89$ $\quad r = -0.94,\ P < 0.01$

Juveniles: $R = -0.00366\ E + 17.16$ $\quad r = -0.97,\ P < 0.05$

where R is feeding rate in % body weight (dry) and E the total energy in the diet in Kcal/kg.

The extrapolation of these regressions to a theoretical feeding rate of zero gives respectively for juveniles and adults 4690 and 4680 Kcal/kg as the limiting values of total energy in the diet for S. guttatus.

From the feeding rates (Tables 7 and 8) and the biochemical composition of the diets (Tables 2-5), the daily energy intake from the diet as a whole, and those from proteins, from fats and from carbohydrates were estimated. I looked for correlations between food efficiency and the energy values. Significant correlations were seen between the food efficiency and the energy from protein + fat in the adults and juveniles (Fig.3).

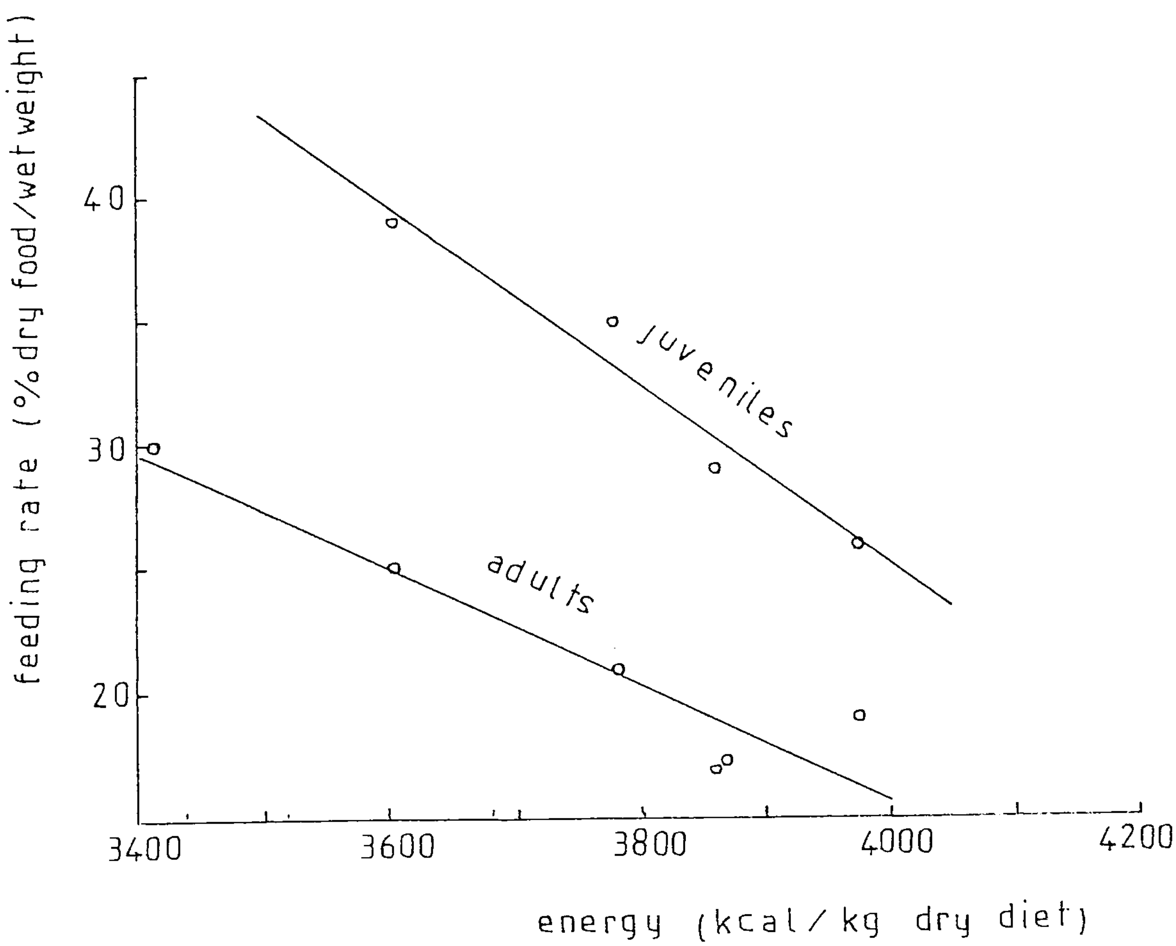


Fig. 2. Relationships: feeding rate - energetic value of the diet for adults and juveniles fed ad libitum on various diets.

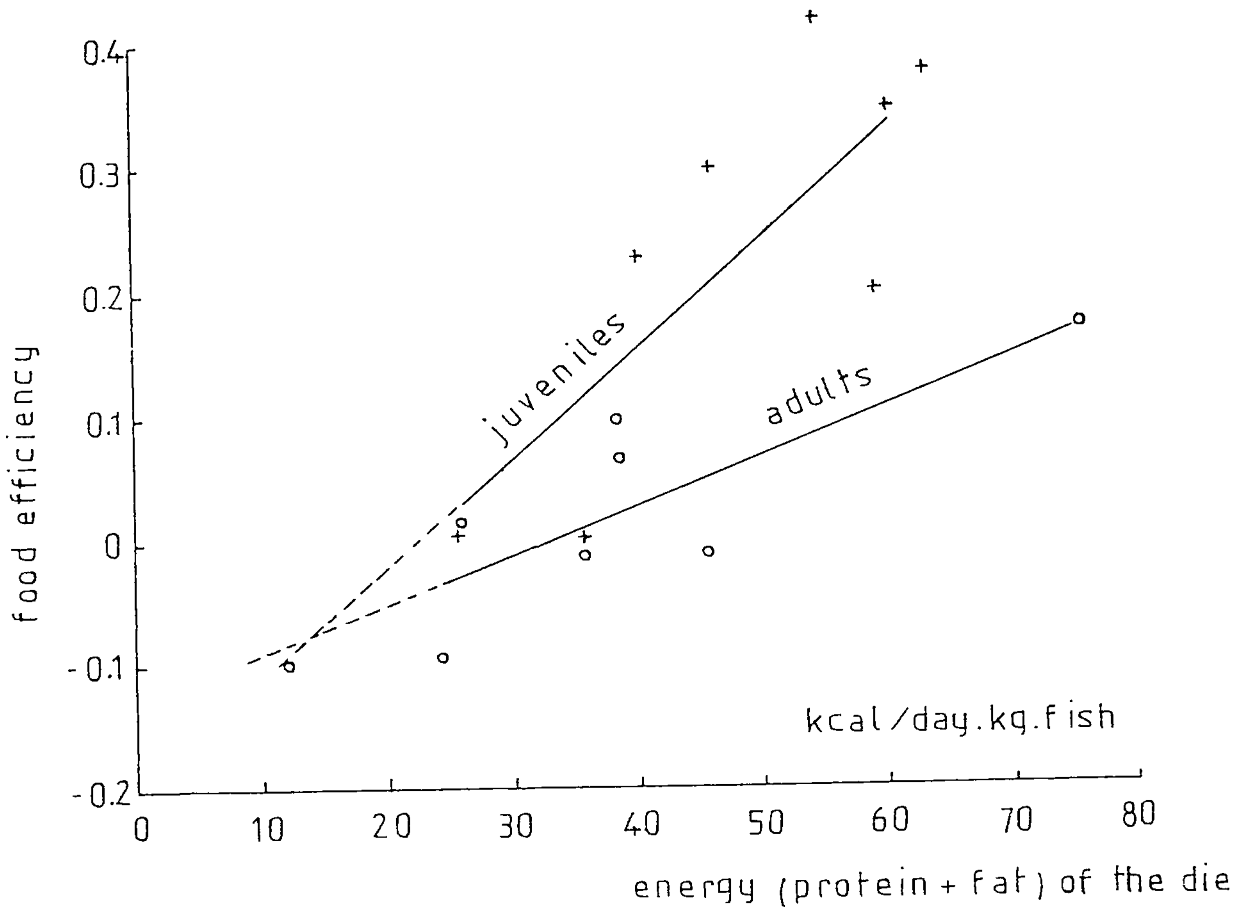


Fig. 3. Relationships: food efficiency and daily (protein + fat) energy intake for juveniles and adults, fed on various diets.

The regressions can be represented by the equations:

$$\text{Adults: } F = 0.0040\, e - 0.125 \qquad r = 0.79,\ P < 0.05$$

$$\text{Juveniles: } F = 0.0092\, e - 0.203 \qquad r = 0.80,\ P < 0.05$$

where F is the food efficiency and e is the daily energy intake from protein in adults and protein plus fat in juveniles. These relationships were obtained where the energy from protein and fat constitute respectively 80-20% of this energy for the adults and 60-40% of this energy for the juveniles.

At any defined level of energy intake, the food efficiency of juveniles is higher than that of adults (Fig. 3).

## Length-Weight Relationship

The length-weight relationship in various groups of fish was determined to estimate general bodily condition (Table 9). Fig. 4 shows the regression for two batches of newly-caught wild fish: the broodstock from Guimaras and the juveniles from Pandan. The regression line for the 2 batches combined is given by the equation:

$$W = 0.0105\, L^{3.24}$$

where W is body weight in grams and L is fork length in cm.

Table 9. Length-weight relationships in various groups of _S. guttatus_.

| Fish, diet, period of sampling | Sampling date[1] | Length-weight relationship[2] | | | Sample size | Condition factor |
|---|---|---|---|---|---|---|
| | | Regression | b | ln a | | |
| Juveniles from Pandan | July (13) | 6 | 2.64 | -3.07 | 41 | 0.046 |
| D, newly caught adults | JuneD19 | 3 | 3.19 | -4.37 | 22 | 0.013 |
| Adults D + juveniles | | 7 | 3.24 | -4.56 | 63 | 0.010 |
| A, commercial diet | June D 19 | 1 | 2.38 | -1.73 | 22 | 0.177 |
| A, commercial diet | July D 17 | 2 | 2.79 | -2.97 | 22 | 0.051 |
| B, _Enteromorpha_, 1 month | July D 19 | 4 | 3.38 | -4.92 | 10 | 0.007 |
| B, _Enteromorpha_, 1 month | Aug D 6 | 5 | 2.62 | -2.55 | 10 | 0.078 |
| A + B, beginning experiment, females | March (14) | 8 | 2.38 | -1.78 | 22 | 0.168 |
| D, newly caught adult, females | June D 19 | 9 | 3.35 | -4.90 | 11 | 0.007 |
| A + B, beginning experiment, males | March (14) | 10 | 2.69 | -2.82 | 16 | 0.060 |
| D, newly caught adults, males | June D 19 | 11 | 3.11 | -4.12 | 10 | 0.016 |

[1] Sampling date is the stage of maturation $D_1$ - $D_{30}$ or date of month (in parenthesis).

[2] Regressions are significant ($P < 0.001$).

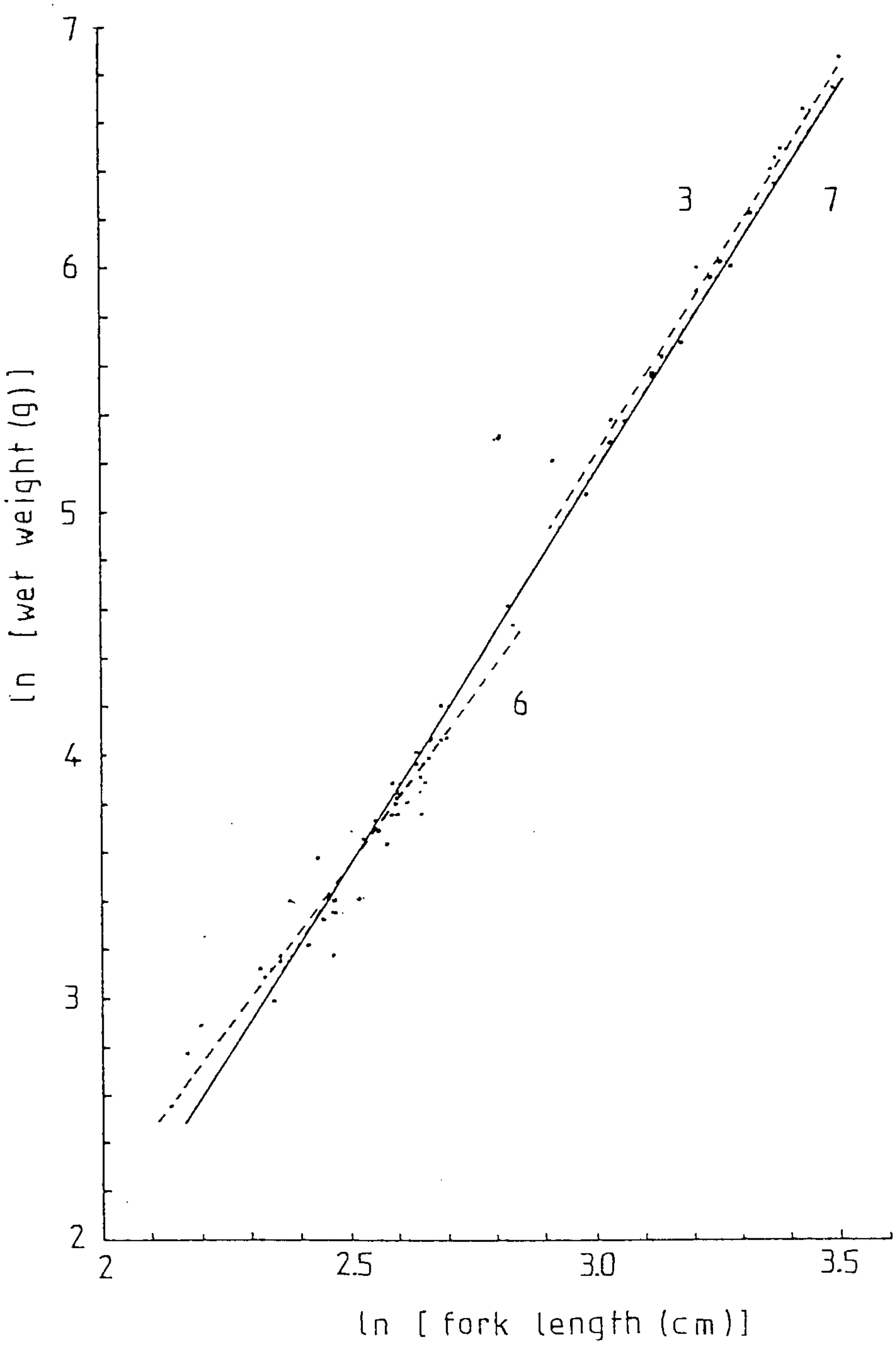


Fig. 4. Length weight relationship of newly-captured wild S. guttatus.
3 - broodstock D, Guimaras
6 - juveniles E, Pandan

Fig. 5 compares this regression for wild fish with that of broodstocks A and B. It shows that only the fish that had been fed Enteromorpha for one month and sampled in the post-spawning period had a relationship comparable to that of the newly-caught adults from Guimaras. At the same time in the maturation cycle in June and July, the hatchery-bred adults had higher weights at any given length than the newly-caught wild adults. Fed *ad libitum* with the same commercial diet, the hatchery-bred broodstock had higher weights in July than in June.

Fig. 6 resolves the length-weight relationship for male and female fish from both the hatchery and the wild. It appears that the regression for hatchery-bred females is different, i.e. they have higher weights at given lengths than the others. No difference is seen between males and females among the newly-captured wild fish.

The general average condition factor for wild fish (0.023) is lower than that of hatchery-bred fish (0.090). The lowest value is seen in fish fed Enteromorpha for one month and in females from the wild (0.007).

## Growth Curve of *Siganus guttatus*

Fig. 7 shows a plot of the weights against age of specimens of hatchery-bred adults, hatchery-bred juveniles

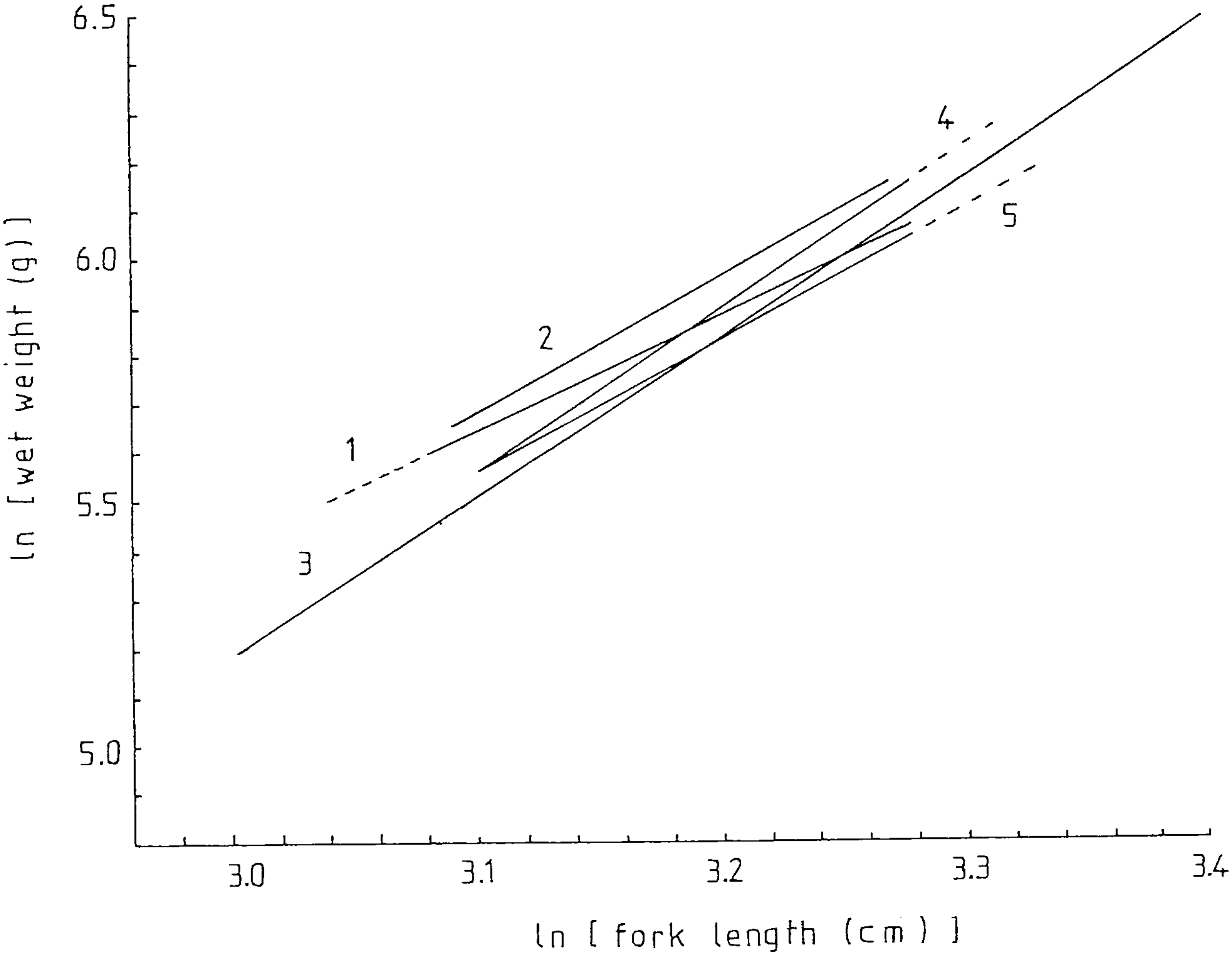


Fig. 5. Length-weight relationship of bred broodstock and newly-captured broodstock, fed on various diets.

1 Broodstock A fed on commercial diet (June D 19)
2 Broodstock A fed on commercial diet (July D 17)
3 Adults newly caught (June D 19)
4 One month fed enteromorpha (July D 19)
5 One month fed enteromorpha (August D 6)

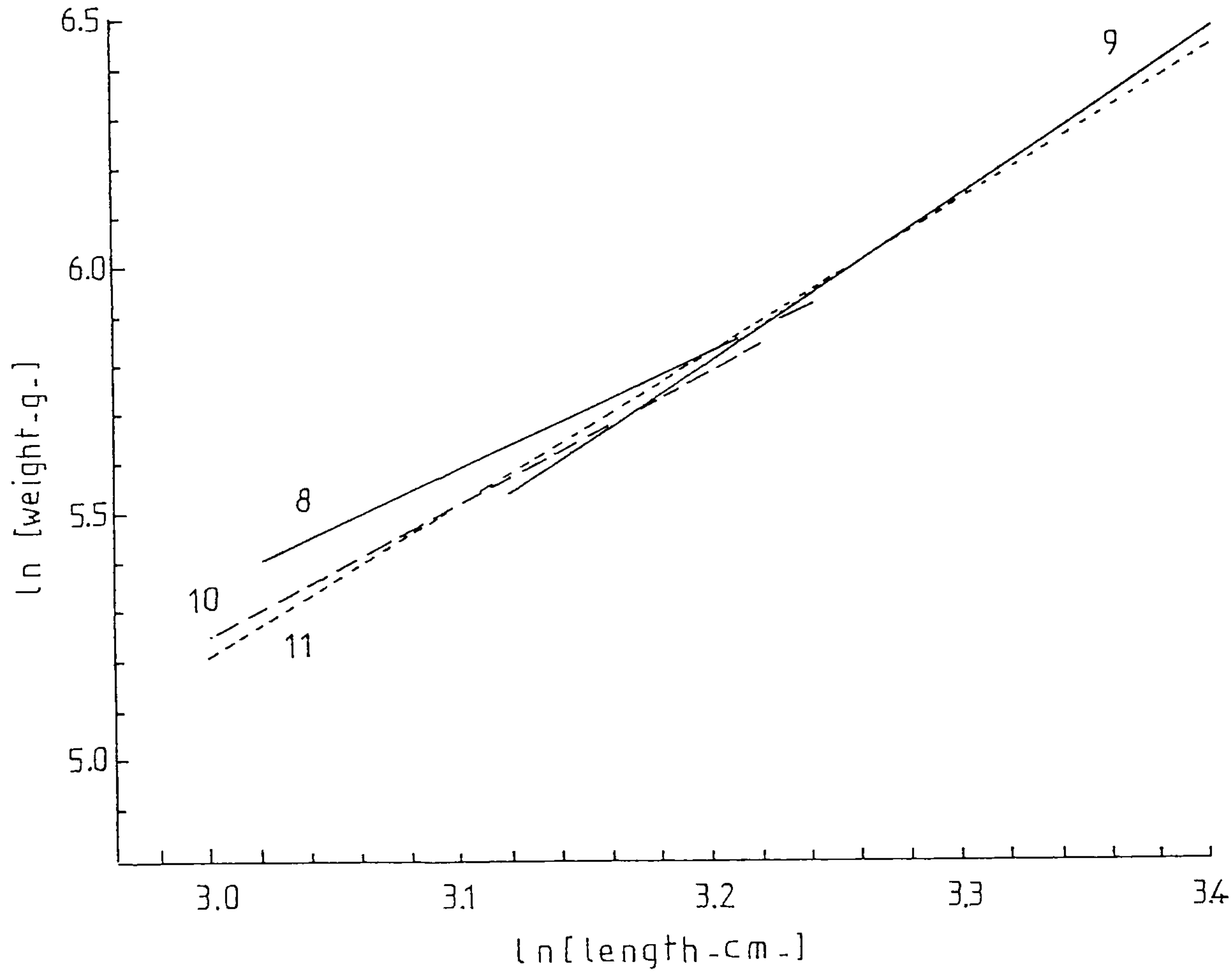


Fig. 6. Length-weight relationships of hatchery-bred and newly captured S. guttatus broodstocks.

8 females A+B hatchery-bred (March 1983)
10 males A+B hatchery-bred (March 1983)
9 females D newly-captured (June 1983)
11 males D newly-captured (June 1983)

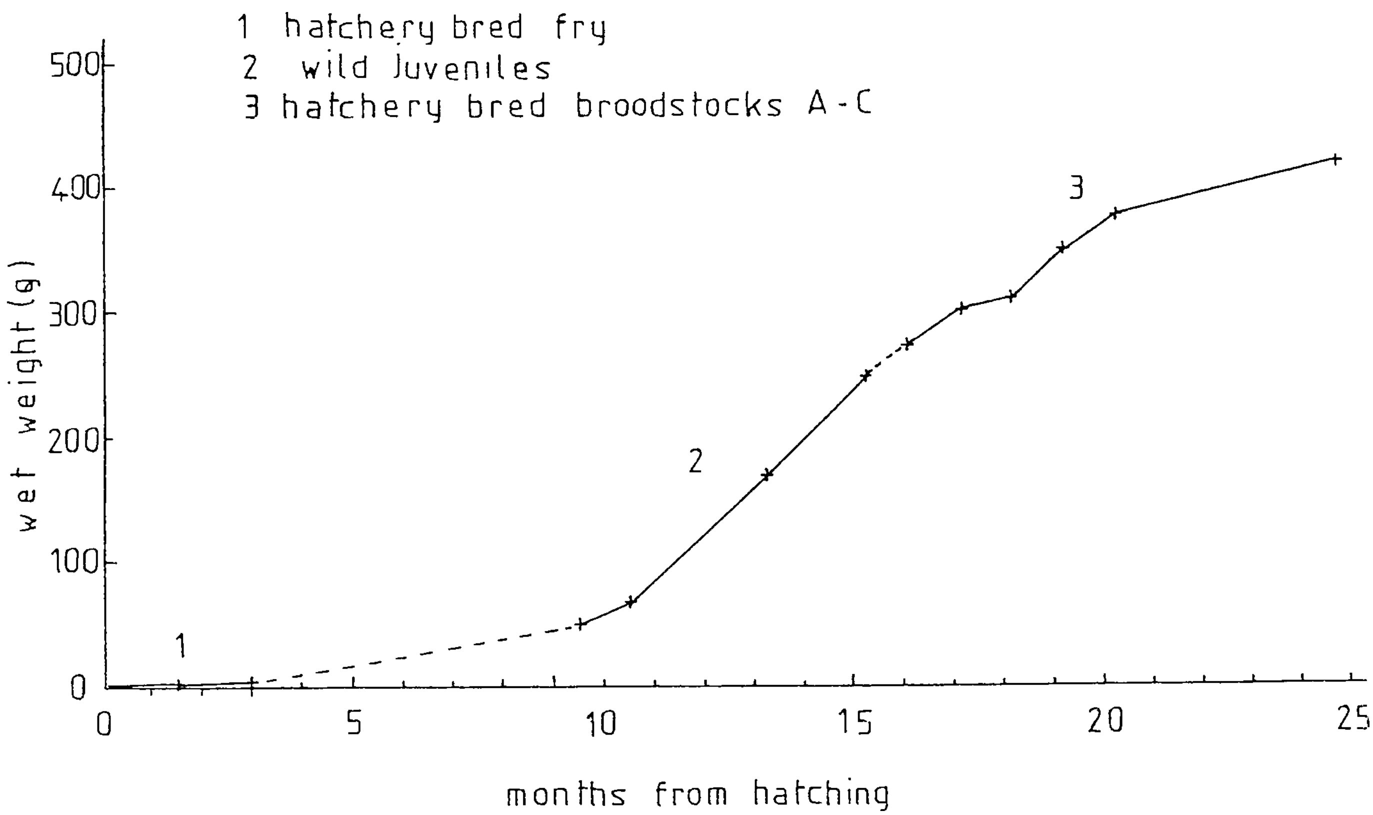


Fig. 7. Growth curve of S. guttatus.

and wild juveniles. The curve shows slow growth (4 g/month weight gain) up to 9 months, fast growth (33 g/month) up to age of 15 months and again slower growth (23 g/month) up to age 22 months.

## Occurrence of Spawning

The dates of spawning of the different broodstocks are shown in Table 10. From March 1983 to January 1984, the fish spawned every month on 2 consecutive nights (sometimes 3 nights) 2-5 days before the full moon, i.e. during the first quarter period. The spawning could be predicted with reasonable accuracy and it became routine to transfer male-female pairs into spawning tanks 2-3 days before the expected spawning dates.

Some fish kept in dark conditions during the two spawning nights in December spawned as expected that month, together with fish kept in the open moon-lighted tanks. It was observed, however, that in January their spawning was shifted out of the regular period; they spawned during the full moon instead of 3 days before (Table 10).

Table 10. The dates of recorded natural spawning of *S. guttatus* in canvas tanks in 1983-84.

| Month | Dates of natural spawning | Dates of first quarter of moon | Date of full moon | Inter-spawning period (days) |
|---|---|---|---|---|
| March | 23-24 | 22 | 29 | - |
| April | 22-23 | 20 | 27 | 30 |
| May | 20-22 | 20 | 27 | 28.5 |
| June | 19-20 | 18 | 25 | 29.5 |
| July | 18-20 | 17 | 24 | 29.5 |
| August | 18-19 | 16 | 23 | 30.5 |
| September | 18-19 | 14 | 21 | 31.0 |
| October | 16-18 | 13 | 20 | 28.5 |
| November | 15-16 | 12 | 19 | 29.5 |
| December | 12-13 | 11 | 18 | 27.0 |
| January | 14-15 | 10 | 17 | 32.5 |
| January [1] | 17-18 | 10 | 17 | 35.5 |

[1] Fish were in covered tanks in December and spawned when expected in that month; in January, spawning was shifted later than predicted, and coincided with the full moon.

## Spawner Frequency

The eleven females (together with 11 males) fed *ad libitum* with the commercial diet were maintained under the same conditions for nearly one year. Table 11 shows the number of females that spawned of the number observed each month. About 75-100% of mature females spawn each month under captive tank conditions.

## Maturation of Oocytes

Since *S. guttatus* spawns monthly, one maturation cycle covers the day after spawning (D 1) to the day of spawning (D 28 - D 32). Fig. 8 shows the maturation process in June-July as determined by cannulation and shown by the increase in the frequency of oocytes with the largest diameter. The vitellogenesis was seen to take 28 days for one batch of oocytes, without other batches maturing in the ovary.

In October and January, the ovaries of fish in the prespawning stage (D 27) were dissected. The oocyte diameter distribution is shown in Fig. 8. A difference is seen in the size-frequency distribution of oocytes between the July fish and the October and January fish probably due at least in part to the difference in the techniques employed.

Table 11. The frequency of female *S. guttatus* that spawned every month in 1983-1984.

| Month | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Spawner frequency | 10/11 | 10/11 | 11/11 | /9 | 10/10 | 10/11 | 6/6 | 6/6 | 2/2 | 6/6 | 6/8 |

Table 12. Mean diameter of oocytes of broodstock *S. guttatus* at the same maturations stages.

| Stage of maturation | Broodstocks | Mean oocyte diameter (um) | Number oocytes examined |
|---|---|---|---|
| $D_{20}$ (June) | A | 400 | 182 |
| | B | 405 | 292 |
| | C | 398 | 233 |
| $D_{17}$ (July) | A | 390 | 281 |
| | B | 395 | 166 |
| | C | 398 | 308 |

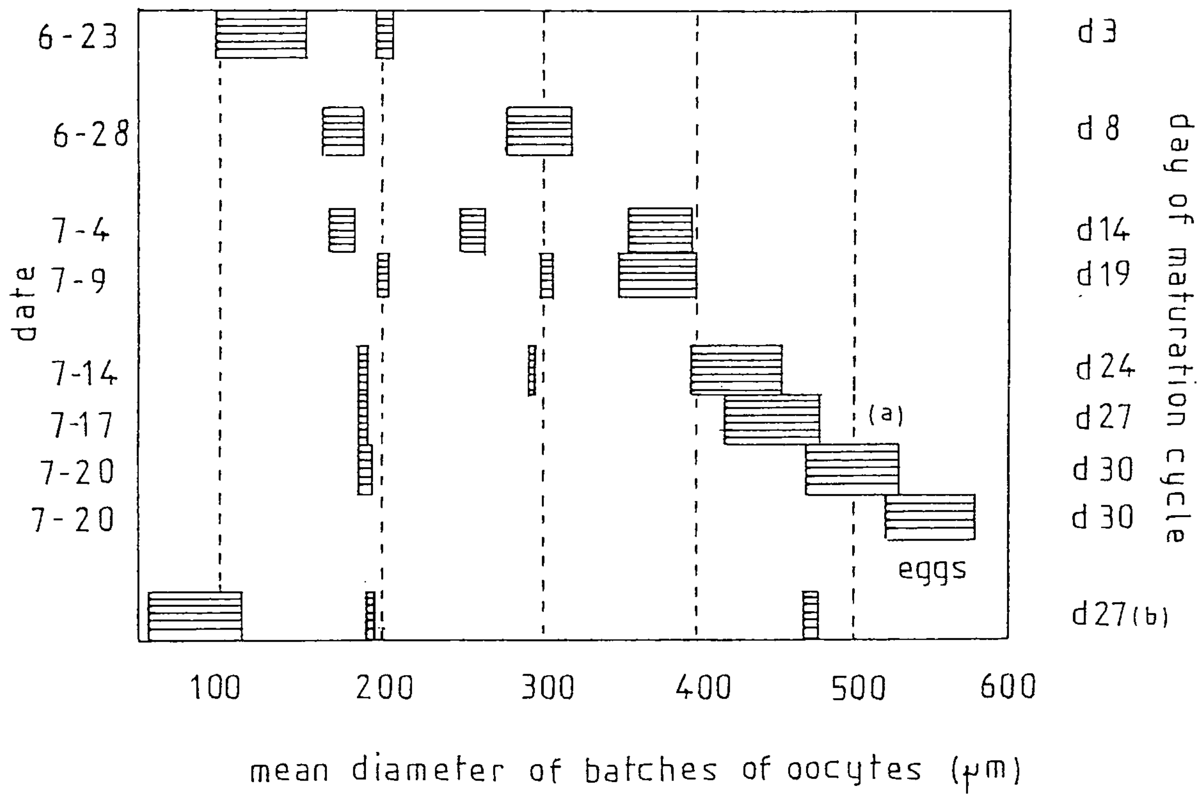


Fig. 8. Development of the oocytes in the gonads of S. guttatus during one month maturation. [Technique used: canula].

a) Ova broadcasted and not fertilized
b) After dissection of ovaries in October and January

Fig. 2 approximate the volume increment associated with the increase in mean oocyte diameter. About 2/3 of the gonadal material seems to be synthesized during the last 10 days of the maturation cycle.

The degree of maturation of oocytes was determined for broodstocks A, B and C at D 20 in June and D 17 in July. Table 12 shows that the mean diameter of the oocytes was similar among the 3 broodstocks, 401 µm in June and 394 µm in July.

## Some Fecundity Measurements

Measurements made in October and January using dissected ovaries showed that a 400 g fish of GSI (gonadosomatic index) 13.8 had 0.8 million eggs, while a 520 g fish of GSI 12.6 had 1.2 million eggs. Estimates from direct counts of eggs in spawning tanks indicate that 450-500 g females spawned 0.45-1.3 million eggs in January.

It was estimated that the eggs broadcasted during one spawning constitute about 12% of the total number of ovarian oocytes (Fig. 8) and account for some 5-10% body weight loss of the females every month.

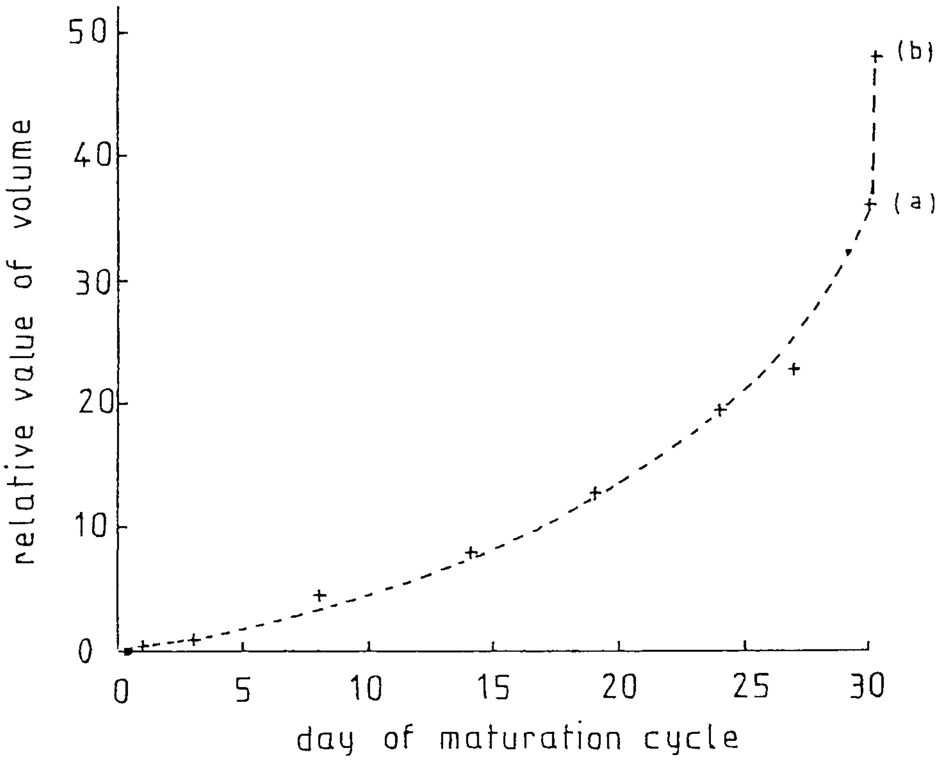


Fig. 9. Increment of the volume of the oocytes in the gonad of S. guttatus.

a) After broadcasting without fertilization
b) Eggs

Fertilization and Hatching Rates

The fertilization rates ranged 94-98% and hatching rates ranged 96-98% in both hatchery-bred broodstocks and newly-captured wild broodstocks in April-July. Subsequently, however, the quality of spawn did not stay as good. Monitoring did not remain quite systematic but it was noted that in October-December, hatching rates were not as high. In fish fed the experimental diet 1, the hatching rate was 90-100%; in diet 2, 40-60%; in diet 3, 5-10%. In January, 2-18% of the eggs from fish fed the commercial diet for 10 months and the diet 1 for one month, died in early embryonic development.

It seems that broodstock diet affects the quality of the spawn, but information from fertilization and hatching rates is not conclusive.

Diameter of Eggs from Different Broodstocks

Siganus guttatus eggs range 520-590 μm in diameter (mean 555 μm ± 7%). Broodstock A under constant feeding and water management showed fluctuations in the egg diameter, with low values in March-May, September and November, and high values in July–August, October and January (Fig. 10). A similar pattern of low and high values was seen in broodstock B and C, although the diameters (540–560 μm) were significantly greater than in broodstock A. The wild fish from Guimaras produced eggs of bigger diameter (580 μm) in June and of equivalent diameter (570 μm) in July as broodstock B.

Three sources of variability of the egg diameter can be pointed out. The interfish variability can modify the egg diameter by ± 1%. Seasonal fluctuations account for ± 3–4% of the variability, while interbroodstock differences make for ±4%. Table 13 and Fig. 11 show the relationship between the condition factor of the spawners and the diameter of the eggs they produce. An inverse correlation exists of the equation:

$$D = 566 - 76.9\ C \qquad r = 0.78,\ P < 0.01$$

where D is the egg diameter (μm) and C is the condition factor of the spawner. A condition factor of 0.6 is associated with an egg diameter of 520 μm, and 0.01 with 560 μm.

## Larval Rearing

Little success was realized in the rearing attempts (Table 14). In the June to September trials, total mortality of larvae occurred within 5 days, except in July when

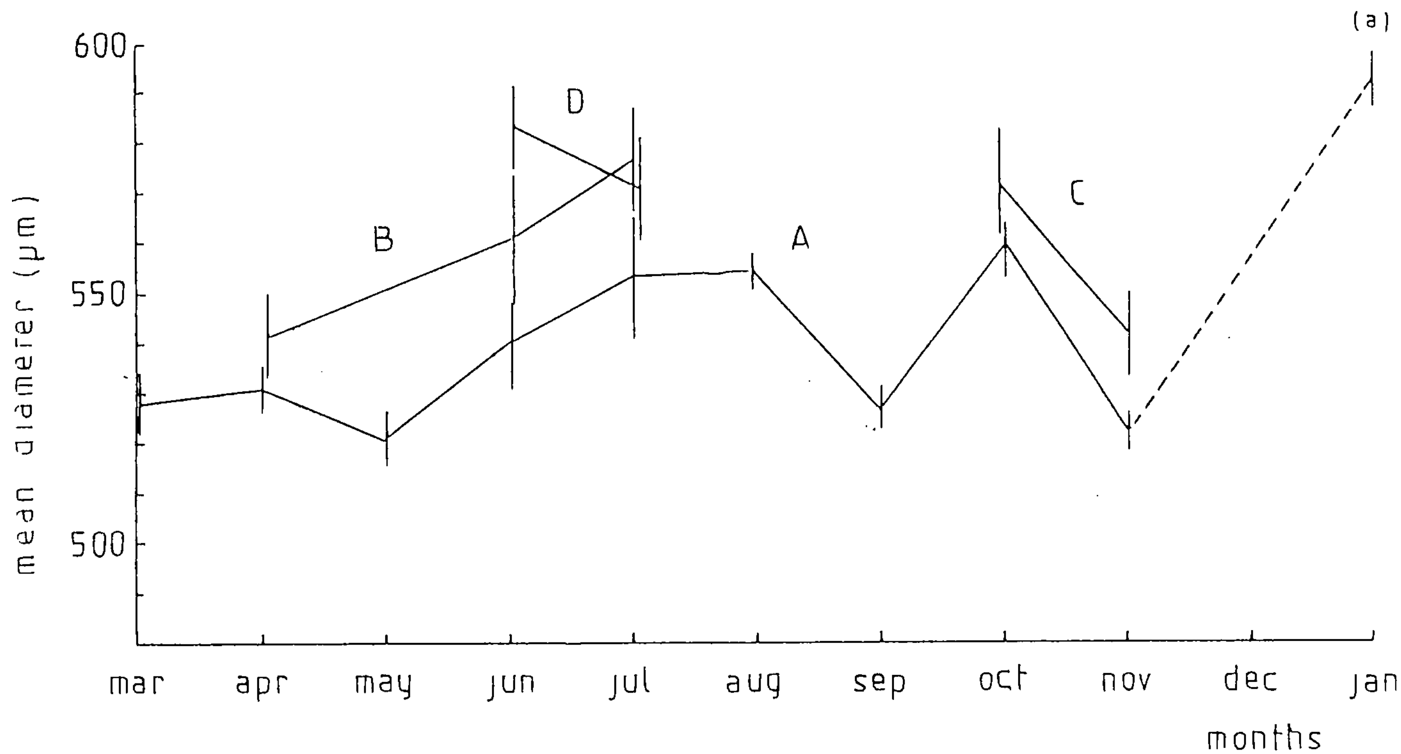


Fig. 10. Egg diameter of S. guttatus broodstock.

A Broodstock A (March 1983-January 1984)
B Broodstock B (April - July)
C Broodstock C (October - November)
D Broodstock D (June - July)

(a) Broodstock A fed the last month on Experimental diet 1

Table 13. Relationship between the condition factor of spawners and egg diameter.

| Broodstock | Month | Condition factor of spawner | Egg diameter (um) |
|---|---|---|---|
| A | March | 0.353 | 527 |
| | April | 0.657 | 530 |
| | May | 0.440 | 521 |
| | June | 0.177 | 540 |
| | July | 0.051 | 543 |
| B | April | 0.364 | 541 |
| | June | 0.023 | 561 |
| | July | 0.109 | 576 |
| C | June | 0.013 | 583 |
| | July | 0.029 | 571 |

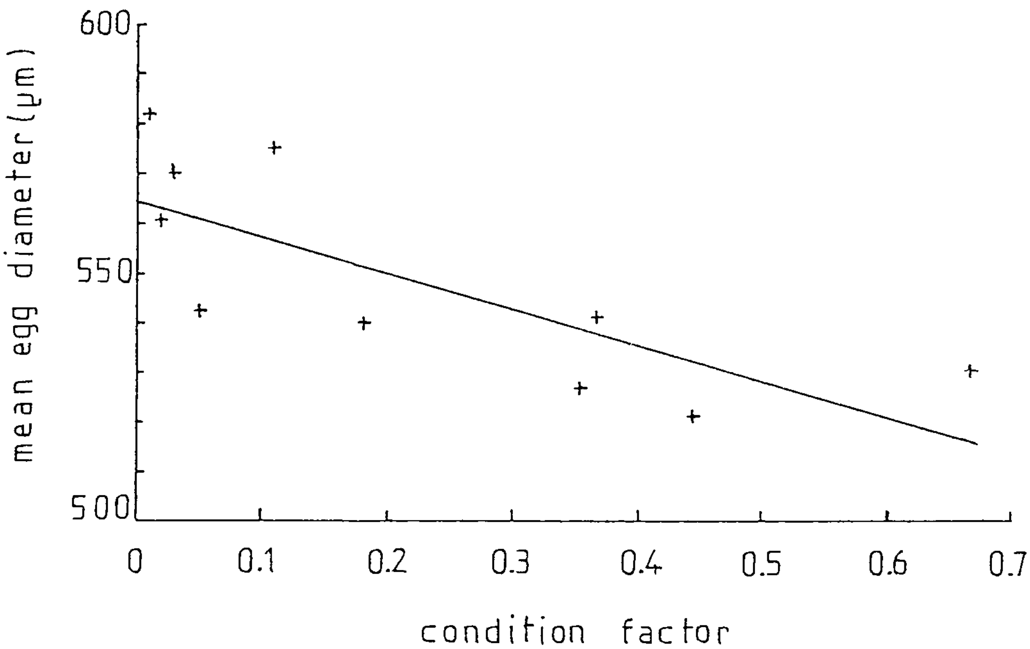


Fig. 11. Relationship egg diameter - condition factor of broodstocks. A-B-C during March-June period.

Table 14. Results of larval rearing of *S. guttatus* in 1983-84.

| Month | Broodstock | Feeding Scheme | Results |
|---|---|---|---|
| June | A and B | Isochrysis, Brachionus (1a) and chlorella used together | Total mortality within 5 days. |
| July | A-B-D | Isochrysis, Chlorella and Tetraselmis used singly, with Brachionus (1a) | No differences among algae. Mortality in 5 days. |
| | | + Brachionus (1b) | Survival to 9 days |
| August and September | A | Microencapsulated diets-dried powdered Brachionus eggs and larvae of oysters | Mortality in 5 days. |
| October | C | Oyster trochophores and Brachionus (2) alone | Survival to metamorphosis (<1 %) |
| November | C | Oyster trochophores and Brachionus (2) alone<br>Isochrysis + Brachionus (2) | Survival to metamorphosis in the two cases (<< 1 %) |
| December | A | Isochrysis + Brachionus (2) | Survival to metamorphosis (<1%) |
| January | A | Isochrysis + Brachionus (2) | Survival to meta morphosis (> 5%) |

Brachionus (1a) "big" strain reared at 20-24°/oo.
Brachionus (1b) "big" strain reared at 30°/oo.
Brachionus (2) "small" strain reared 20°-24°/oo.

larvae survived 9 days. These July larvae were fed *Brachionus* strain 1 reared in 30 ppt rather than 20-24 ppt. Less than 1% of larvae survived past metamorphosis in October, very few in November-December and more than 5% in January, all fed with *Brachionus* strain 2.

Finding the right-sized food particle was obviously part of the problem in larval rearing. Finely powdered (40-100 μm) artificial diets were used, likewise oyster eggs and trochophores (60 μm). Results were not clear-out, probably complicated by the water management. In July, the ammonia concentration in the rearing tanks with live algae reached 1.1 ppm within 4 days. In all other runs, the ammonia concentration stayed less than 0.6 ppm in all tanks. Under static water conditions and temperatures about 30°C, bacteria developed on the artificial diets within 24 hr. In 200 l rearing tanks with a water turnover of 250 ml/min, total mortality occurred significantly earlier than in tanks under static conditions, although the ammonia level did not exceed 0.2 ppm.

In October, when survival of larvae past metamorphosis was first obtained, the rearing scheme was as follows: No algae were added at all. Oyster eggs and larvae were given on day 2 from hatching, 3-5 times a day, every day until day 4. A strain of small *Brachionus* (strain 2) was first given on day 2 and maintained at concentrations of 10-20/ml. Water was not changed during the first 5 days of rearing; subsequently, only 30% of the volume was replaced every day until metamorphosis. The ammonia concentration was less than 0.5 ppm even in tanks with high (40-50/ml) rotifer densities.

## Size and Survival of Larvae from Different Broodstocks

In June and July, the newly-hatched larvae of broodstock A measured 1.3-1.55 mm; of broodstock B, 1.40-1.65 mm; of broodstock C, 1.5-1.7 mm. However, all larvae grew to 2.5 mm within 24 hr. This positive exponential growth is related to the negative exponential depletion of the yolk (more than 90% resorbed in 48 hr).

Table 14 shows that broodstock C fed diet 1 produced larvae which survived past metamorphosis in October and November. Broodstock A fed the commercial diet produced larvae which survived past metamorphosis in December. From December to January, broodstock A was fed diet 1, and in January, produced larvae that also survived past metamorphosis. Larvae from broodstock C fed diets 2 and 3, and from broodstock A reared under the same conditions in October and November did not survive past metamorphosis.

# DISCUSSION

## Growth and First Sexual Maturity

Ben-Tuvia *et al*. (1973) and von Westernhagen and Rosenthal (1976) compared the growth rates of different species of rabbitfish. It seems that growth-wise, *S. guttatus* is not suited for aquaculture. Horstmann (1975) and Tahil (1978) both worked on juvenile fish in sea cages; the former found that 15 g fry reached 35 g in 5 weeks; the latter, in 5-6 months. In the present study in flow-through canvas tanks, the 35 g size would be obtained in 2 months. Von Westernhagen and Rosenthal (1976) found that fish in closed water systems need 10 months to grow from 40 g to 150 g; in this study, only 3 months. Fig. 7 shows that it takes *S. guttatus* 16 months to reach 280 g weight.

Fed on commercial diets, the males of the batch of juveniles from Pandan reached first sexual maturity at about 200 g. Females of the hatchery-bred broodstock A fed commercial diet were first sexually mature at 260 g. This 200-260 g weight at first sexual maturity is marked by the second inflection of the curve in Fig. 7. Adult fish do not grow as fast as juveniles.

The length-weight relationships (Figs. 4, 5, 6 and Table 9 indicate that hatchery-bred fish are heavier at a given weight than newly-captured wild fish. This difference is mostly due to the hatchery-bred females which shift the range of weights for fork length 20-22 cm from 180-240 g to 210-27 g. This indicates that first sexual maturity is reached at a smaller size in hatchery-bred fish than in wild fish. This may be related to the high energy content and high protein content of the formulated diets given to the captive broodstock.

The aquaculture of S. guttatus, if pursued, will have to depend on the natural fry sources until technical problems in hatchery production of fry are solved. As the growth performance of the species is specially slow between the fry and fingerling stage, it is better to collect the fingerlings which can soon achieve fast growth. In intensive culture systems, two crops a year may be possible. The fish in grow-out ponds should be harvested when they reach 200-250 g size because thereafter the growth slows down.

## Effect of Diet Quality on Growth

The commercial crustacean diet with 43% protein taken to a total of 67 Kcal/day. kg fish resulted in good growth performance of adult S. guttatus. Fresh food with 51% protein and 102 Kcal/day. kg fish as total energy intake apparently overfed the fish and went to waste. Fresh food with 31% protein content and only 48 Kcal/day. kg fish

underfed the fish and inhibited growth.
The experimental diet with lower energy content than diets 2 and 3 was consumed at a higher rate, to a total of 140 Kcal for juveniles and 88 Kcal for adults. Despite this high energy intake, growth was not any better on diet 1.

Marais-Kissil (1979) likewise found that sea bream juveniles and adults consume more of those diets that have low energy content. However, they found that faster growth always accompanied high energy intake. This may have been due to the high protein content of the diets they used, 43% in contrast to 18% in this study's diet 1. This suggests that energy from a high protein diet and energy from a low protein diet are not equivalent in terms of the resultant growth. Moreover, the protein quality differs according to its source. Diet 1 had 6% protein from animal sources and 12% from vegetable sources. Vegetableproteins usually lack cystein and tryptophan. When these amino acids are not added to the diets, vegetable proteins do not perform as well as animal proteins (Dabrowska et al. 1977).

## Consumption of Fresh Food

Rabbitfishes are mostly recognized as herbivorous species. Tsuda and Bryan (1973) and Westernhagen (1974) noted the preference of S. argenteus, S. spinus and

S. striolata for Enteromorpha among many other species of algae. Nevertheless, through the studies of various investigators, Lam (1974) summarized the food tendency of siganids as "potentially omnivorous".

S. guttatus broodstock is able to consume fresh food with high protein content, as much as 130 g food per kg fish daily. They select their food; they preferred Enteromorpha and squid over shrimp, mussel and trash fish. They can easily overfeed themselves with fresh food, particulary squid.

With these high protein fresh food diets, there was no evident improvement in the egg quality. The little increase in egg diameter may, however, be useful in larval rearing, as longer, stronger larvae hatch from larger eggs (Hunter, 1981).

## Food Efficiency and Energy Requirements

Fig. 3 shows the relation between the food efficiency (gain wet weight of fish/dry weight of food consumed) and the daily energy intake of the fish. The food efficiency is poorly correlated with the total energy but is significantly correlated with the energy from protein in adults and with the energy from protein + fat in juveniles. Thus weight gain is mostly due to these two energy sources, with the protein source of energy apparently required first. Under sufficient protein supply, fat improves the food efficiency in juveniles. The importance of a fat energy source for the adult fish is debatable.

However, the correlation between the food efficiency and the energy content of the food may actually be better than it appears, considering the fact that adults lose 5-10% of their weight monthly after spawning (and there were 8 spawnings during the period under study). The high protein content of the diets may also have contributed to the occurrence of spontaneous spawning every month.

Table 15 shows the inverse relation between the food conversion factor and the protein content of the diet. The food conversion factor was computed from the formula by Metailler _et al._ (1981):

$$\text{feed conversion factor} = \frac{\text{feeding rate}}{\alpha}$$

where $\alpha$ is from the formula:

$$W_T = W_o\,(1 + \alpha/100)^T$$

where $W_T$ is final weight of fish

$W_o$ is the initial weight of the fish

T is the number of days of feeding

Smaller amounts of food are required for a unit weight gain of fish when the food has a high protein content. Protein may be the main factor in growth and reproduction. It was observed that fish fed fresh food (47-55% protein) and the commercial diet (43% protein) had hyperdistended abdomens during the prespawning stage whereas the fish fed on the 3

Table 15. Feed conversion factor in *S. guttatus*

| Diet | Protein content (%) | Feed conversion factor | Author |
|---|---|---|---|
| Commercial trout pellet | 40 | 3.2 | Westernhagen *et al*. (1976) |
| Rabbit pellet | 12.5 | 4.0-6.5 | Westernhagen *et al*. (1976) |
| Commercial crustacean pellet | 43 | 2.5 | This study |
| Experimental diets | 18 - 25 | 2.8-3.0 | This study |

The feed conversion factor in this study was computed from the formula of Metailler *et al*. (1981): feed conversion factor = $\frac{\text{feeding rate}}{\alpha}$

where $w_T = w_0 \left(1 + \frac{\alpha}{100}\right)^T$, where

$w_0$ = initial weight of fish

$w_T$ = final weight of fish

T = number of days feeding

experimental diets (19-26% protein) and *Enteromorpha* (11% protein) had little or no swelling.

## Model for the Formulation of Diets for *S. guttatus*

From the relationships between feeding rate and energy in the diet (Fig. 2) and between the food efficiency and the energy from protein plus fat (Fig. 3), a model is here presented that considers the biological and economic aspects of nutrition and growth of *S. guttatus*.

Definition of variables:

$R$ = feeding rate (%)

$F$ = food efficiency

$e_{p+f}$ = daily energy intake of fish in terms protein plus fat (Kcal/day. kg fish)

$e_p$ = daily energy intake of fish in terms of protein (Kcal/day. kg fish)

$E$ = total energy content of the diet (Kcal/kg)

$E_{p+f}$ = energy from protein plus fat in the diet (Kcal/Kg)

$E_p$ = energy from protein in the diet (Kcal/Kg)

$K_1 = E_p/E$

$K_2 = E_{p+f}/E$

Relationships:

R is a function of E

F is a function of $e_{p+f}$ in juveniles, r=0.80

F is a function of $e_p$ in adults, r=0.85

$e_p \quad = R \times E \times K_1$ for adults

$e_{p+f} = R \times E \times K_2$ for juveniles

From these relationships, another emerges of the form:

$F \quad = aKE^2 + bKE - c$

The food efficiency is thus correlated with the total energy in the diet E and the ratio ($0<K_1<1$ in the adult fish) of the energy from protein, or the ratio ($0<K_2<1$ in the juveniles) of the energy from protein plus fat relative to the total energy in the diet.

The derived equations are:

For the adults:

$$F = -1.07 \times 10^{-7}K_1E^2 + 5 \times 10^{-4}K_1E - 0.095$$

For juveniles:

$$F = -3.37 \times 10^{-7}K_2E^2 + 1.58 \times 10^{-3}K_2E - 0.203$$

A series of curves are drawn in Fig. 12 for various $K_1$ and $K_2$ values. A parabolic relationship appears between the food efficiency and the energy in the diet. All the curves are asymptotic at the same energy value: 2300-2400 Kcal. The theoretical extremes of diet energy would be 0 Kcal and 4700 Kcal. Could the value 2350 Kcal be translated as the ideal caloric content of the optimal diet for S. guttatus? This value is greater than the energy content of Enteromorpha (1900 Kcal). On this low energy diet, the adults would have to feed at 5.5% and the juveniles at 8.6% of the body weight to obtain optimal food efficiency.

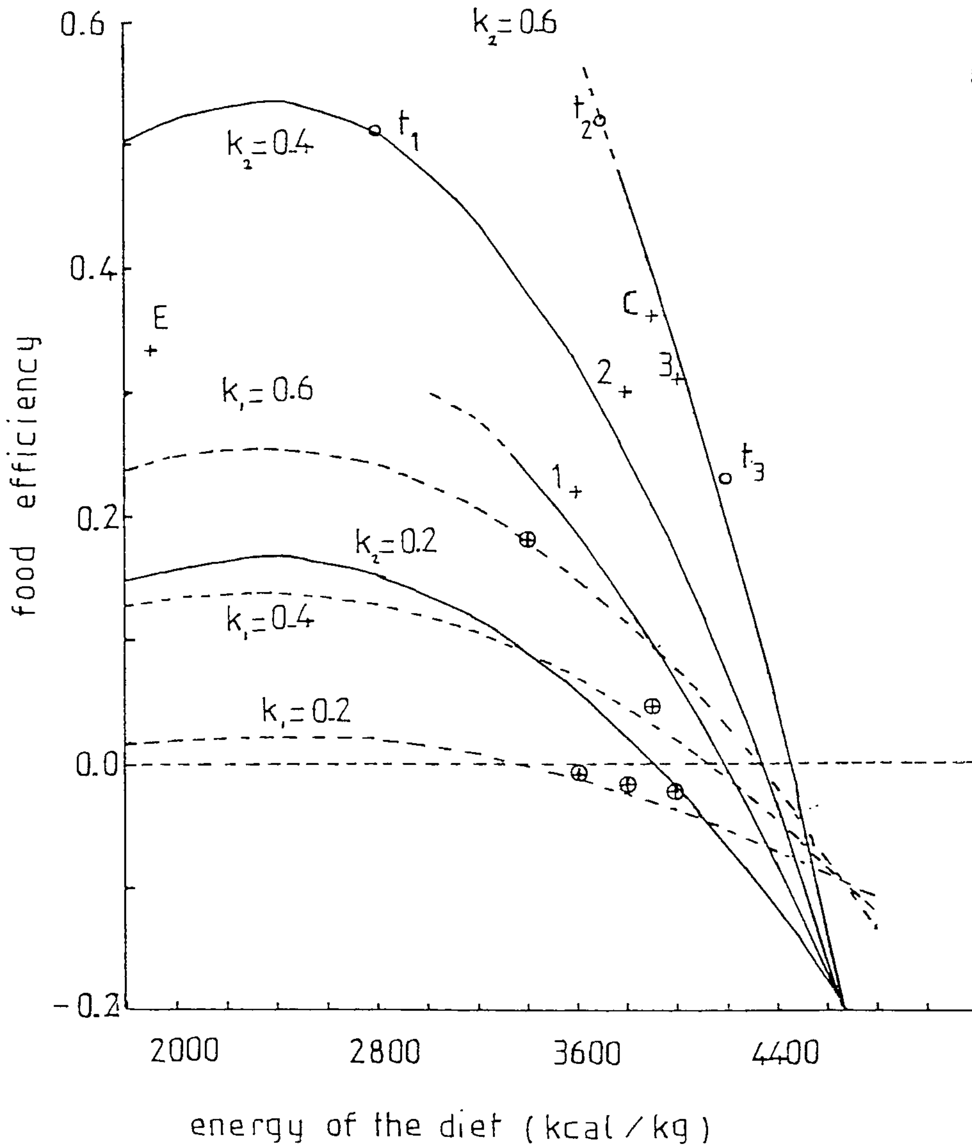


Fig. 12. Relationships: food efficiency - energy content in the diet for juveniles (——) and adults ( - - -).

$K_1$ : % energy from protein in the diet

$K_2$ : % energy from (protein + fat) in the diet

1.2.3. c - Experimental values of food efficiency from the juveniles fed respectively on diet 1.2.3 and commercial diet.

$t_1 t_2 t_3$ - 3 theoretical diets - E - theoritical value of food efficiency for enteromorpha.

⊕ - formulated diet and fresh food - results from adults.

On *Enteromorpha* alone, adults have to feed at a rate of 6.5% and the juveniles at 10.2%. Will the fish feed at these high rates? It is obviously economically disadvantageous to have to provide great amounts of food to cultured fish.

Table 16 considers different possible feeding schemes with theoretical diets 1, 2 and 3 for juvenile *S. guttatus*. A diet with only 2800 Kcal and 17% protein will permit a food efficiency of 0.5, but can the fish feed at 7% of its body weight? A diet of 3700 Kcal and about 33% protein will permit the same food efficiency at a feeding rate of 3.5%. A high energy diet with 4200 Kcal/kg will be consumed at the least amount but will not permit high food efficiency (Fig. 12). Economically then the second diet is best. A diet with 3700 Kcal and 33% protein will be a little more expensive but the amounts required will be half that in a diet with 2800 Kcal.

The too low protein + fat content of the experimental diet 1 did not permit it to be as efficienct as theoretical diet 2 inspite of the equivalence of their total energy (3600-3700 Kcal) (Table 16). At similar $K_2$, the commercial diet ($K_2$=0.56) and the theoretical diet 2 ($K_2$=0.60) result in different food efficiencies. The higher % ash in the theoretical diet 2 reduces the total energy content of the diet at the same $K_2$, the consequent increase in feeding rate increases the food efficiency.

Table 16. Comparison between theoretical diets and diets used in experiment on <u>S. guttatus</u>

| Diet | Total energy | Feeding rate (%) | $K_2$ | Food efficiency | % Protein | % Fat | % NFE | % Ash + Fiber |
|---|---|---|---|---|---|---|---|---|
| Theoretical diet 1 | 2800 | 7 | 0.4 | 0.51 | 17 | 5 | 42 | 36 |
| Theoretical diet 2 | 3700 | 3.5 | 0.6 | 0.52 | 33 | 10 | 37 | 20 |
| Theoretical diet 3 | 4200 | 1.75 | 0.7 | 0.22 | 44 | 13 | 32 | 11 |
| Experimental diet 1 | 3600 | 4.0 | 0.29 | 0.23 | 19 | 3 | 64 | 14 |
| Commercial diet | 3900 | 2.9 | 0.56 | 0.38 | 43 | 5 | 42 | 10 |
| Enteromorpha | 1900 | 10.0 | 0.28 | 0.33 | 11 | 1 | 34 | 54 |

Table 17. The size of spawned eggs and newly hatched larvae of siganids.

| Siganus sp. | Egg diameter (mm) | Length newly hatched larvae (mm) | Author |
|---|---|---|---|
| <u>argenteus</u> | 0.65 | 2.5 | Burgan, 1979 |
| <u>fuscescens</u> | 0.62-0.66 | 2.6 | Fujita and Ueno, 1954 |
| <u>lineatus</u> | - | 2.5 | Bryan and Madraisau, 1977 |
| <u>vermiculatus</u> | 0.56 | 1.8 | Popper <u>et al</u>., 1976 |
| <u>rivulatus</u> | - | 1.8-2.3 | Popper <u>et al</u>., 1973 |
| <u>canaliculatus</u> | 0.55 | 2.1 | May <u>et al</u>., 1974 |
| <u>canaliculatus</u> | 0.42-0.46 | 1.55 | Soh and Lam, 1973 |

The adult fish have even lower tolerance for high energy diets than the juveniles. The feed ng rates are lower. Only fish fed *ad libitum* on fresh food with 3400 Kcal/kg realize a food efficiency of 0.17. About 70-100% of the females fed fresh food and the commercial diet spawned monthly while only 50% of those fed the experimental diets did so. The 19-26% protein content associated with the high total energy (3600-4000 Kcal/kg) in the experimental diets apparently do not permit high enough food intake, nor enough protein intake to support greater growth and reproductive activity.

Caution is necessary in the consideration and application of the above results and extrapolations. The relationships in Figs. 2 and 3 are significant inspite of the varied quality of the diets used. The energy values were calculated from the proximate analysis of the diets and the caloric equivalents of the different classes of energy sources, without due consideration of the origin of the feedstuffs (Hastings, 1976; Smith, 1978). Another point is that the $K_2$ values are defined at 60% of the energy from protein and 40% of the energy from fat in juveniles. The model can only work within food efficiencies less than 0.5, which is the maximum that can be expected (Hastings, 1976). At too high protein content in the diet, the fish will waste energy and the increment of food efficiency will be reduced. Finally, the model considers the total diet energy associated with maximal feeding rates. For example, juveniles could obtain a food efficiency of 0.33 only if the feeding rate is 10% (i.e. 100% feeding rate in terms of fresh Enteromorpha). Is this physiologically possible?

In summary, the model considers the biological and economic aspects of S. guttatus nutrition and growth. It implies that production of juvenile fish (200-250 g) would require a formulated diet with about 3500-3700 Kcal/kg total energy, 33% protein, 10% fat, 20% ash. For seed production purposes, the feeding management of the broodstock should take into account the herbivorous habit of the fish. The model suggests that a diet of about 2400-2800 Kcal/kg total energy be given to adults at 4-5% feeding rate. An increment in ash content will permit reduction of the total energy in the diet. A protein content of 17-20% will maintain a low food efficiency. Low food efficiency is associated with low condition factor, which in turn is correlated with bigger egg diameters. If the food efficiency is too low and the fish do not spawn, a diet with higher protein content would induce maturation.

## Spawning Season of S. guttatus

It has been shown that in captivity under conditions of high protein diet, S. guttatus spawned every month. The general condition of the fish appears to get progressively better towards July, and is particularly greater in June-July and November than in March-May. It was also shown that broodstock A fed the same diet the whole year produced significantly bigger eggs in July-August, October and January, than in March-May, September and November. Within the same month, the difference in mean egg diameter from the same broodstock does not exceed 10-15 μm, whereas between May and October, the difference is 40 μm. This suggests a genetic tendency to produce bigger eggs at certain months of the year, probably coincident with the natural spawning season.

The spawning season of *S. guttatus* in the wild may not extend the whole year, but will probably cover June-July. Fishermen in Guimaras note that adults with well-developed gonads occur in shallow-water fish traps starting June each year. In nearby Cebu Island, *S. guttatus* fry could be collected the whole year from different localities, and for periods of 3-12 months in any one locality (Tahil, 1978). If fry are considered to be one month old, the highest frequency of *S. guttatus* spawning around Cebu then appears to be from February to May, in 70-90% of the places surveyed.

The spawning season differs among species of *Siganus*. The spawning season of *S. canaliculatus* in Negros Island is February-September (Alcala and Alcazar, 1979), whereas for *S. vermiculatus* in Fiji, it is almost the complementary period, November-February (Popper et al., 1976). Information from various authors (Lam, 1974; Popper and Gundermann, 1975; Johannes, 1978) indicate that the spawning seasons of siganids extend 2-8 months, usually 4-5 months, in one or two periods of the year. The main period in most species is February-June; the second when it occurs is usually around November.

In tropical seas where the annual environmental fluctuations are minimal, time to maturation tends to be considerably reduced and the frequency of spawning increased (Hempel, 1979). *S. guttatus* has the potential to spawn every month, but in the wild, it probably has "rest" periods, imposed by fluctuations in food availability and abundance. Siganid species that have omnivorous tendencies will tend to have longer spawning seasons (Lam, 1974). *S. guttatus* has omnivorous food habits, can spawn every month in captivity, and thus can very possibly spawn most months of the year in the wild.

## Lunar Periodicity in Spawning of *S. guttatus*

In the one-year study conducted, *S. guttatus* spawned during the first quarter period, 5-2 days before each full moon. This periodicity is so ingrained that the 2-3 spawning nights could be predicted plus or minus one. A lunar periodicity in spawning is also shown by various species of *Siganus* and many other reef fishes (Johannes, 1978).

This lunar periodicity in spawning has been explained various ways. McVey (1972) is of the opinion that the most important factor influencing the spawning of *S. fuscescens* (should be *S. canaliculatus*) is the change in tidal levels. In the present study, *S. guttatus* experienced little change in water level and water quality in the canvass tanks, yet the fish spawned as predicted every month. On the other hand, Schwassman (1971) suggested that in the grunion, "the time of spawning seems to be determined by the interaction of a physiological rhythm of gonadal maturation and some factor related to the second preceding full moon or new moon". Results of my preliminary experiment suggest that the environmental factor releasing maturation is strongly associated with the light of the moon, since no other factor fluctuated regularly under the conditions I studied. It is of interest to mention that the guppy reacts directly to different intensities and spectral ranges of the moon (Lang, 1967).

Johannes (1978) argues that spawning during the new moon and full moon periods, as observed in many coral reef species would enhance the offshore flushing of eggs and larvae away from benthic reef predators. The siganids appear to be one of the few groups that spawn, not during the full moon nor new moon period but during the first quarter period (Johannes, 1978). Of the species of *Siganus* common in Philippine waters, *S. vermiculatus* and *S. guttatus* spawn after the first quarter, while *S. canaliculatus* spawns before the first quarter (Manacop, 1937; MacVey, 1972; Popper and Gundermann, 1976; Johannes, 1981; this study).

## Maturation Process

While it is common for cold-water fish species to alternate during the year periods of intensive feeding, spawning and starvation, tropical species, particularly reef fishes, have a more stable environment, short maturation cycles and increased frequency of spawning. Vitellogenesis takes 5 months in the black sea bream (Soletchnik, 1982) but only 20 days in the loach (Suzuki, 1983). Vitellogenesis is completed within 27-28 days in *S. guttatus* (Fig. 8). In some months, the cycle is longer probably because a short resting period follows the spawning. Feeding rates are reduced during these rest periods of 2-3 days.

The rate of maturation is influenced by environmental conditions (Lam, 1974). For *S. guttatus*, the important factor appears to be food abundance and diet quality. Most of the female *S. guttatus* fed the crustacean diet with 43% protein spawned monthly for 11 consecutive months. In contrast, *S. vermiculatus* under the same conditions of captivity and fed the same diet from June to October did not spawn (unpublished data). Suzuki (1983) induced captive loach to spawn 22 times in 22 consecutive months.

In January, a female of stage D 29-D 30 showed an increment of 2% body weight after 9 days starvation during which the males and juveniles lost 5-10% of their body weight, and the females that spawned, 11-14%. In other words, this female was 7-12% heavier than the others after 9 days starvation. This weight increment is not due to food intake and synthesis of materials, but due to an increase in gonad weight which is probably due to water intake. On the same score, Taylor (1980) suggested that the ovarian weight increase in the killifish is due to the accumulation of water in the maturing oocytes. This uptake of water in S. guttatus is also suggested by the fact that the diameters of spawned eggs of broodstock A, B and D are significantly different in June (Fig. 10), although there were no significant differences in oocyte diameters at D 20 June and D 17 July among the 3 broodstocks. Indeed, when the egg diameter increases as the fecundity decreases, one physical explanation may be proposed: that crowded eggs present less accessibility to water.

If uptake of water by maturing oocytes does take place, then seawater quality becomes an important factor that should be controlled in S. guttatus breeding systems.

The amount of oocytes in the ovary of S. guttatus is 7x the quantity of eggs that are spawned. About 6/7 of the total number of oocytes is thus constituted by primary oocytes. The maturation cycle I have considered (Fig. 8) is the maturation of that batch of oocytes that occurs with the highest frequency in the ovary. In fact, the gametogenic cycle includes the period of accumulation of nutrients by the fish, the period of high metabolic activity from the oogonium stage to the primary oocyte (Giese and Pearse, 1974). How long is the gametogenic cycle compared with the follicular maturation cycle (Taylor, 1980)? How important is the food intake many months before (back 6-7 months), compared with the food intake during vitellogenesis? These questions need to be answered so that the broodstock could be appropriately managed.

## Size and Quality of Eggs and Larvae

S. guttatus eggs measure 555 µm on the average, and the newly hatched larvae about 1.5 mm, although Avila (1980) recorded 660 µm for the eggs in 1977 and Duray (pers. comm.) recorded 2.1 mm for the larvae in 1981. The Siganidae tend to have small eggs and larvae (Table 17). The egg diameter is positively correlated with the size of larvae at hatching. S. guttatus larvae are significantly smaller than S. argenteus,

S. fuscescens and S. lineatus.

The highest values of egg diameter (580-590 μm) were recorded in June with the newly-captured fish from Guimaras and in January with the hatchery-bred fish fed commercial diet and then fed experimental diet 1 for one month before the spawning. The spawners of these large-sized eggs had low condition coefficients (0.012-0.013). In S. guttatus, a negative correlation is seen between the condition of the spawners and the diameter of spawned eggs (Table 11) that is highly significant ($P < 0.01$). Hempel (1979) has shown for the cod and brook trout a similar correlation.

A negative correlation between fecundity (number of eggs/gram ovary) and egg diameter was seen in females of broodstock A in January. A relationship that is significant ($P < 0.05$) can be given by the equation:

$$F = 599.6 - 7.29\ D$$

where F is number of eggs/gram and D is egg diameter. This relationship, however, is restricted to the higher range of egg diameters (580-595 μm); if extrapolated to 525 μm, an unlikely fecundity of 5 million eggs would be attributed to a 500 g female. The inverse relation between fecundity and egg size is usually demonstrated interspecifically (Blaxter, 1969), but apparently can be resolved also intraspecifically in S. guttatus.

The biochemical composition of S. guttatus eggs is not known, but from studies of other species, it is shown that protein is the major constituent (Blaxter, 1969). Protein in egg yolk is what mostly goes into larval tissue, and protein is what the spawners should have in quantity to synthesize the eggs. Thus the quality of eggs depends on the diet of the broodstock.

## Rearing and Feeding of S. guttatus Larvae

Rearing siganid larvae is generally difficult (Lam, 1974; May et al., 1973) because of the need for small-sized prey organisms. In the 6 monthly rearing trials of S. guttatus with about 30 spawns and feeding schemes, the 0.1-5% survival was achieved with Brachionus of the small strain (< 150 μm) plus oyster larvae, and with Brachionus plus Isochrysis. Oyster larvae and Isochrysis may not really be necessary if small Brachionus (less than 100 μm) could be provided in adequate amounts. S. guttatus larvae of 1.5 mm length have mouth width of about 125 μm (Bagarinao, pers. comm.); the optimal size of first prey is 70-100 μm. Yufera (1982) has shown that depending on the strain, 1-2% or 80% of Brachionus plicatilis can be smaller than 150 μm. The culture method is also important. Brachionus cultured at 20 ppt weakened and perished in larval rearing tanks at 30 ppt sooner than those cultured at 28-30 ppt (unpublished observations). On the other hand, unicellular algae such as *Tetraselmis* and *Isochrysis* are too small to be of direct utility to *S. guttatus* larvae.

Bigger larvae tend to have greater chances for survival (Hunter, 1981). In 1981, Duray (pers. comm.) recorded 15% survival of *S. guttatus* larvae; these were 2.1 mm long at hatching. The 5% survival of larvae in January 1984 (this study) may be associated with the larger egg diameter (590 μm); larger eggs give rise to larger larvae.

It would seem from the size-related rearing problem that *S. guttatus* is not so suitable for aquaculture. Other tropical species used for aquaculture have bigger eggs: milkfish, 1.2 mm; tilapia, 2-3 mm; sea bass, 0.8 mm; grey mullet, 0.93 mm (Liao *et al.*, 1979; Maneewongsa and Tattanon, 1982; Nash *et al.*, 1974); rearing of these species is less problematic.

When the newly hatched larvae are bigger than 2.5-3.0 mm, there is less of a feeding problem. The sole can be fed low concentrations of *Brachionus* and *Artemia* nauplii without any kind of algae and survive at high rates of 70-80% during the first month (Fuchs, 1981). Ciliates and tintinnids were found to be important for first feeding of the marbre (Divanoch Kentouri, 1983). Although oyster trochophores are of the right size (60 μm) for most newly hatched larvae, they do not consistently produce good larval survival (Minton *et al.*, 1983; Lasker *et al.*, 1970).

The naked dinoflagellate Gymnodinium splendens has been used successfully in rearing various subtropical species at the Southwest Fisheries Center in La Jolla, California. It has to be noted, however that different species of larvae may react differently to the same prey. Gymnodinium is not considered appropriate for grey mullet larvae (Nash et al., 1974).

Various species of unicellular algae are used in larval rearing, of which "green" water Chlorella is most popular. Survival of many species of larvae is enhanced by phytoplankton blooms in rearing tanks (Houde, 1973), but not of all. S. guttatus larvae in my study, provided with 3 kinds of algae did not survive past day 5 (Table 14). Popper et al. (1973) noted total mortality of S. rivulatus 2-12 days after they fed them unicellular algae. Popper et al (1979) obtained similar results for S. luridus and S. argenteus. Bryan and Madraisau (1977) obtained respectively for S. vermiculatus and S. lineatus, 9% survival out of 200 larvae and 16% survival out of 5000 larvae in big volumes of 30 $m^3$ and 8 $m^3$ with high concentrations of multi-species algae under field conditions.

The effect of phytoplankton on larval rearing is not clear. It apparently acts as a water conditioner that removes metabolites produced by fish larvae and their zooplankton prey, through photosynthetic activity. It also serves as food for the zooplankton. For the rotifer

<u>Brachionus</u>, it may not be necessary to add algae to the rearing tanks, if freshly fed rotifers are given to larvae every day, rotifers survive 2-3 days without algae at 28°C. On the other hand, algal blooms in rearing tanks may reduce the light intensity to levels favorable to the larvae. However, in static systems, these blooms may induce wide fluctuations in environmental parameters within 24 hr. The oxygen requirement increases considerably at night. Dead algae are an important substrate for bacterial infection that very likely contaminates the larvae. The presence of high concentrations of algal cells in rearing tanks may stress the larvae that continually ingest them without gaining any energy.

# CONCLUSIONS AND RECOMMENDATIONS

1. In captivity, *S. guttatus* broodstocks under conditions of high (43%) protein diet spawn every month during the first quarter period, without hormonal treatment.
2. Broodstocks fed commercial diet (43%) protein and the experimental diet 1 (18% protein) produced larvae that survived to metamorphosis, with results being more consistent with diet 1.
3. The length of newly-hatched larvae is correlated with the egg diameter. The egg diameter varies with the broodstock source (wild or hatchery-bred) and diet, the season, and perhaps salinity. Thus, broodstock diet influences the quality of the larvae through the egg size.
4. The major problem in siganid larval rearing is the availability of the right-sized prey for first-feeding larvae. Larvae are 2.5 mm at first feeding, with mouth width of 125 μm. The optimal first prey required is 70-80 μm in size. Brachionus smaller than 100 μm constitute only a small percentage of the population. The quality of *Brachionus* (culture salinity, diet) is also an important factor in successful larval rearing. Oyster eggs and trochophores do not seem so appropriate for *S. guttatus* larvae. Other small living prey like tintinnids and ciliates could be tried. Other siganid species such as *S. argenteus*, *S. fuscescens* and *S. lineatus* produce bigger eggs than *S. guttatus*. They

should be tried for aquaculture where they occur. Rearing of the larvae of these species would be less problematic, assuming that the nutritional requirements would be satisfied by readily available rotifers.

5. For *S. guttatus* fed *ad libitum*, the food intake is inversely correlated with the total energy in the diet. This increase in consumption is associated with the energy requirements. The tolerance of juveniles for high energy diets is higher tha n that of adults. The optimum diet for juveniles would be different than for adults. Food efficiency is correlated with the daily protein intake of the adults and the protein plus fat intake of the juveniles. At the same daily protein intake, the food efficiency is higher for juveniles than for adults. The protein content of the diet seems to induce the maturation process, and is associated with high fecundity and high spawning frequency in females.

6. For optimum growth of juveniles, a diet with about 3700 Kcal/kg total energy, 33% protein, 10 % fat and 20% ash is advisable.

7. The biggest eggs (530 μm). produced in January by fish fed the commercial diet (43%protein, 3900 Kcal/kg) for 9 months, then the experimental diet 1 (18% protein, 3600 Kcal/kg) for 1 month resulted in 5% larval survival past metamorphosis. Assuming that this result is associated with the low food efficiency and low

condition factor of fish on diet 1, it is suggested that S. guttatus broodstocks be maintained on a diet with low energy content, low % protein, high % ash (cheap, similar to the natural herbivorous diet of the fish). For maturation and spawning purposes, the fish may subsequently be fed a diet with high energy content, high % protein and lower % ash. For example, for maintenance:

Feeding rate: 4.0% body weight

Total energy of the diet: 2500 Kcal/kg

Protein: 17%

Fat: 5%

NFE: 42%

Ash + fiber: 36%

Food efficiency expected: 0.35

For maturation and spawning:

Feeding rate: 2.5% body weight

Total energy: 3500 Kcal/kg

Protein: 40%

Fat: 5%

NFE: 36%

Ash + fiber: 19%

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# Errata

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|---|---|---|
| Page 22 | read: | These relationships were obtained where the energy from protein and energy from fat constitute respectively 80% and 20% in the adults, and 60% and 40% in the juveniles. |
| Page 24 | read: | 7 combined regression |
| Page 46 Line 19 | read: | (Dabrowska and Wojno, 1977) |
| Page 49 Fig. 15 | read: | Westernhagen and Rosenthal (1976) |
| Page 54 Table 17 | read: | Burgan and Zeeleczky (1979) |