AN ABSTRACT OF THE THESIS OF Steven Alan Lock for the Master of Science in Biology presented November 20, 1992.

Title: Factors influencing first feeding by larval rabbitfish, Siganus randalli.

Approved: _____
Stephen G. Nelson, Chairman, Thesis Committee

A set of experiments was designed to examine the effects of rotifer density, fish age and size, and preexposure to prey on the first feeding incidence and growth of larval rabbitfish, Siganus randalli. Rotifer density did not affect feeding incidence or larval growth, but the fish's age, time of day, and exposure of the prefeeding larvae to rotifers affected them significantly. The larvae began to feed approximately 60 hours after hatching, and feeding increased during the day and tapered off as sunset approached. Groups of larvae preexposed to rotifers had a higher feeding incidence than groups not preexposed.

TO THE OFFICE OF GRADUATE SCHOOL AND RESEARCH

The members of the Committee approve the thesis of Steven Alan Lock presented November 20, 1992.

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FACTORS INFLUENCING FIRST FEEDING BY LARVAL RABBITFISH, *SIGANUS RANDALLI*

BY
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INTRODUCTION

In the tropical Pacific, there is an increasing interest in the culture of siganids and other tropical marine fishes that are characterized by small larvae with rapid development. Several species of siganids have been examined as potential candidates for aquaculture development in this region, and recent work at Guam has primarily focused on a euryahaline, deep bodied species, Siganus randalli Woodland, 1990. However, the high larval mortality which occurs at first feeding and the transition from endogenous to exogenous nutrition presently restrict the successful large scale hatchery production of these fish.

Newly hatched siganid larvae have unpigmented eyes, no digestive tract, and no mouth (Duray, 1990) and the larvae therefore are not capable of feeding until they have developed sufficiently to be able to see and capture prey. The knowledge of when the larvae are capable of feeding is useful in developing feeding protocols for larval culture.

Prey density has been shown to be an important factor in larval survival in culture (Tamura et al., 1991). On one hand, high densities of rotifers may increase the chance of a larva encountering prey, and this in turn may increase the probability of prey capture. On the other hand, prey densities that are too high could be detrimental, either

directly, via confusion of the larvae, or indirectly through a decrease in water quality.

Prior experience with prey may also affect feeding success. In work done with the larvae of the cichlid Cichlasoma managuense a learning period was required for the fish to recognize differences between prey and non food items (Meyer, 1987). By being in physical proximity to prey, even before the eyes and mouth are fully developed, the larvae may learn to recognize prey. If learning is important in successful prey capture by siganid larvae, then larvae which are exposed to prey earlier have greater success at prey capture than larvae exposed to to prey later. I examined this by comparing the success at first feeding between larvae that had been exposed to rotifers before they were able to feed with that of larvae that were exposed to rotifers later in development.

Because rabbitfish larvae are among the smallest of cultured warmwater marine species, larger larvae might be expected to have the greater success at first feeding. Larger larvae may have a better swimming ability which would allow them to search a larger volume of water for prey items (Kiørboe et al., 1985). Larger larvae may also have an advantage in the ability to utilize a wider range of prey sizes (Meeren, 1991). To examine this I compared the sizes

of successful first feeding larvae with those of the same spawn that had not yet begun to feed.

As a first step in addressing this issue, I examined, through a series of replicate trials, factors affecting the first feeding of larval S. randalli. These included larval age and size, prey density, and exposure of prefeeding larvae to prey. Small (S-type) rotifers (110-230 μm), Brachionus plicatilis, were used as the first prey for larval S. randalli. This organism has been used in successful culture of other tropical marine fishes (Hara, 1986 a; Eda et al., 1990; Tamura et al., 1991).

MATERIALS AND METHODS

Study Site

The work described here was conducted at the University of Guam Marine Laboratory. Broodstock of Siganus randalli was originally collected from Apra Harbor, Guam. The fish were raised to maturity in a circular concrete pond 15 m in diameter and 1 m in depth at the Guam Aquaculture Development and Training Center and fed approximately 3% of their body weight per day with Kruz Catfish Pellets (El Monte, California).

Egg Collection

For spawning, broodstock were placed in 1500-l fiberglass tanks supplied with constantly flowing seawater and gentle aeration. Ripe males were detected by gently applying pressure to the abdomen and observing free flowing milt. Ripe females were chosen by applying pressure to the abdomen and observing the extrusion of the genital papillae. Spawning was conducted at periods of either the new moon or the full moon. The selected fish usually spawned within 2-3 days of being placed in the spawning tanks. Corrugated fiberglass plates had been placed on the bottom of the tanks in the evening as egg collectors, as the rabbitfish normally spawned in the early morning hours. Upon collecting sufficient spawn (≈70% coverage of the egg collection

plates) an estimated 5,000 eggs were removed from the plates and placed in each of ten 100-l cylindrical, fiberglass, rearing tanks. After hatching, the surfaces of the rearing tanks were skimmed with paper towels to remove floating debris, and the debris from the tank bottom was siphoned out. The night of hatching was referred to as day 0, the next as day 1, and so forth.

Water Quality

Salinity, pH, temperature, and dissolved oxygen were monitored twice daily throughout the trials. During the trials salinities ranged from 30 to 34 ppt, based on refractometer readings. Ph ranged from 7.6 to 8.1, the readings taken with a Nester Instrument (Millville, New Jersey) pH meter. Dissolved oxygen levels ranged from 2.8 to 6.6 mg/l. Water temperatures ranged from 24.3 to 27.5 °C; both parameters were recorded with a Horiba dissolved oxygen meter (Fritz Chemical, Dallas, Texas). Light measurements were taken just below the water surface of the rearing tanks with a Li-Cor underwater sensor and quantum meter. Light levels ranged from 3.2 to 104.6 $\mu Em^{-2}s^{-1}$ during the trials.

Larval Rearing

The rearing trials were conducted in static water and the only water exchange occurred during daily siphoning and

when plankton and rotifers were added. There was no additional mechanical aeration of the test tanks. Natural lighting was reduced by corrugated fiberglass roofing sheets.

To maintain the prey densities at targeted levels, the residual number of rotifers per test tank was determined twice daily, and rotifers were added as necessary. Samples of rotifers were drawn into a 1-ml pipet, and the rotifers were counted with the aid of a photographic slide viewing loop. Three samples per tank were taken and the average number of rotifers was calculated.

The rotifers were fed the phytoplankton Nanochloropsis oculata. Phytoplankton were added to a density of 250,000 cells/ml in the larval rearing tanks to provide adequate nutrition for the rotifers, as well as to help maintain the water quality by lowering the levels of metabolic wastes (Houde, 1975). The Nanochloropsis oculata were grown in 1500-l or 2000-l outdoor tanks in a medium of 100 mg ammonium sulfate, 30 mg monosodium phosphate, 5 mg urea, and 10 mg iron-ethylenediaminetetraacetic acid (EDTA) in 1.0 liter of seawater.

Those rotifers captured in a 45-μm mesh size bag were rinsed to remove ciliates and other contaminants and fed to the rabbitfish during the days of testing. Rotifers were added to the rearing tanks prior to sunrise.

The development of S. randalli has been previously described (Nelson et al., 1992). Hatching of S. randalli eggs takes place 17-20 h after spawning. The eyes of the larvae begin developing pigmentation approximately 36 h after hatching. Larval yolk is absorbed approximately 40 h after hatching. The mouths of the larvae become functional 50-60 h after hatching. The oil globule if fully absorbed 80-90 h after hatching.

Effect of Rotifer Density and Larval Age

The effects of rotifer density and larval fish age on feeding incidence four days after hatching were examined in two sets of experiments: one with rotifer densities of 10/ml and 30/ml; the other with rotifer densities of 1/ml and 20/ml. Sampling occurred every two hours beginning on day three from 0800 to 1800. A sample of twenty larvae was examined under a dissecting microscope at 50x magnification to determine the presence or absence of rotifers in the stomach. The sampling protocol was repeated on day four after hatching. The mean number of fish feeding on days three and four were compared seperately by a two-level, mixed-model, analysis of variance (BMDP 7D, BMDP Statistical Software, Inc; Los Angeles, California).

To test the effects of rotifer density on larval growth after four days, a sample of fifteen larvae was collected from each tank, and the notochord lengths were measured with

a dissecting microscope and ocular micrometer at 20x magnification. The mean notochord length was again calculated at the end of the fourth day after hatching. The initial and final lengths were compared with a mixed-model analysis of variance with equal cell sizes (BMDP 8V).

Exposure of Prefeeding Larvae to Rotifers

To test the effects of exposing prefeeding larvae to prey on feeding incidence, 20 rotifers/ml were added to five of the test tanks chosen at random at 0600 on day three after hatching. The remaining five tanks of fish were fed at 1300 on the same day. Samples were taken each hour between 1500 and 1800 on day three after hatching. The larvae began feeding in the afternoon of the third day after hatching. Samples were taken every two hours on the fourth day after hatching from 0800 to 1800. Twenty larvae from each tank were observed under a dissection microscope at 50x magnification, and the presence or absence of rotifers in the stomach was recorded. The mean number of fish feeding in the preexposed and non preexposed groups were compared by a two-level, mixed-model analysis of variance (BMDP 7D).

Larval Size and Feeding Success

To test the effects of larval size on feeding success, rotifers were added at a density of 1/ml on day three after hatching. On day four after hatching, in the mid morning

hours before a majority of the fish would actively begin to feed, a sample of ten fish per tank (total=100) was collected and examined under a dissecting microscope at 50x magnification. The numbers of those feeding and not feeding, as well as their final notochord lengths, were recorded. The final notochord lengths of fish feeding and not feeding were compared by a two-way, mixed-model analysis of variance (BMDP 7D).

Statistical Analysis

For the purpose of statistical analysis, the rotifer densities were considered to be fixed variables representing high and low densities; the times of day were also considered as fixed factors; and the rearing tanks were considered as random factors. Prior to the statistical comparisons with ANOVA, the data were analyzed to check the homogeneity of the variances with Levene's Test. The data were analyzed with the BMDP statistical programs 7D or 8V.

RESULTS

Effects of Rotifer Density and Larval Age

Prey density did not affect feeding incidence in either three or four day old larvae. There was no significant ($F_{1,5}$=0.01, P=0.9198) difference in feeding incidence between groups fed at rotifer densities of 10/ml and 30/ml three days after hatching (Table 1). There was also no significant ($F_{1,4}$=0.09, P=0.7633) difference in feeding incidence between those groups fed at rotifer densities of 1/ml and 20/ml (Table 2). For three day old larvae there was a general increase in feeding incidence after sunrise, peaking in the mid afternoon and levelling off, or decreasing, in the hours just prior to sunset (Figures 1 and 2). Four day old larvae fed at rotifer densities of 10/ml and 30/ml did not differ significantly ($F_{1,5}$=0.01, P=0.9198) in feeding success (Table 3). There were similar results at the rotifer densities of 1/ml and 20/ml ($F_{1,4}$=0.01, P=0.9293 Table 4). For four day old larvae, almost all larvae sampled had begun to feed by mid afternoon, with feeding incidence levelling, or dropping off slightly, as evening approached (Figures 3 and 4). Both time of day and the age of the larvae influenced feeding incidence, larvae did not feed during the night, and feeding success increased from the third to the fourth day after hatching.

Table 1. Effects of rotifer density on first feeding incidence of larval Siganus randalli three days after hatching. Rotifer densities tested were 10/ml and 30/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	0.0167	1	0.0167	0.01	0.9198
Time	87.5500	5	17.5100	10.78	0.0000
Interaction	6.0833	5	1.2167	0.75	0.5911
Error	78.0000	48	1.6250		

Table 2. Effects of rotifer density on larval Siganus randalli first feeding incidence three days after hatching. Rotifer densities tested were 1/ml and 20/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	0.2667	1	0.2667	0.09	0.7633
Time	169.3333	5	33.8667	11.64	0.0000
Interaction	19.7333	5	3.9467	1.36	0.2571
Error	139.6000	48	2.9083		

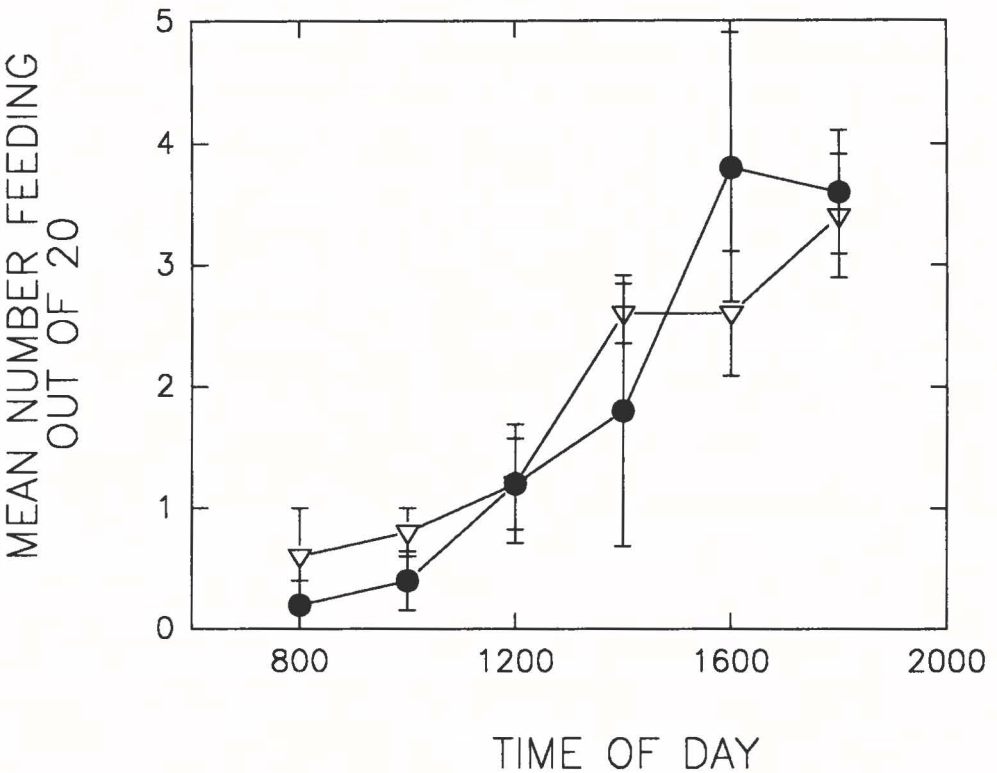

Figure 1. Effects of rotifer density on feeding incidence of Siganus randalli larvae three days after hatching. Triangles=High rotifer density 30/ml; Circles=Low rotifer density 10/ml; Bars are standard errors.

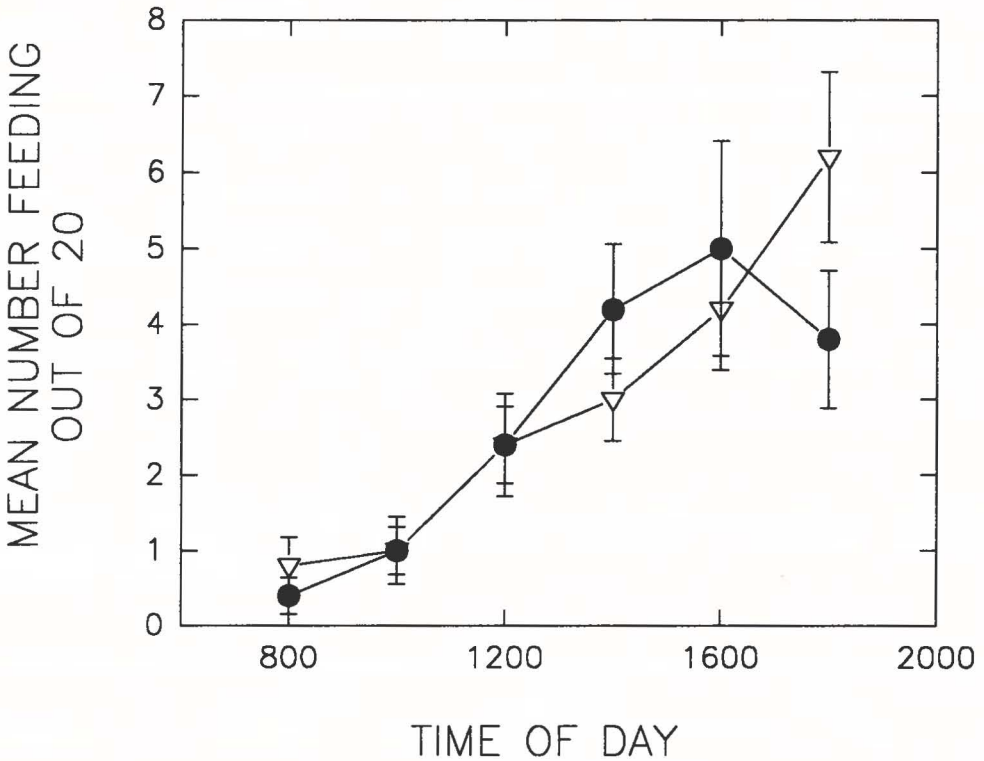

Figure 2. Effects of rotifer density on feeding incidence of Siganus randalli larvae three days after hatching. Triangles=High rotifer density 20/ml; Circles=Low rotifer density 1/ml; Bars are standard errors.

Table 3. Effects of rotifer density on feeding incidence of larval Siganus randalli four days after hatching. Rotifer densities tested were 10/ml and 30/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	2.0000	1	0.0167	0.01	0.9198
Time	87.5500	5	17.5100	10.78	0.0000
Interaction	6.0833	5	1.2167	0.75	0.5911
Error	78.0000	48	1.6250		

Table 4. Effects of rotifer density on feeding incidence of larval Siganus randalli four days after hatching. Rotifer densities tested were 1/ml and 20/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	0.0200	1	0.0200	0.01	0.9293
Time	1681.9200	4	420.4800	167.52	0.0000
Interaction	3.6800	4	0.9200	0.37	0.8310
Error	100.4000	40	2.5100		

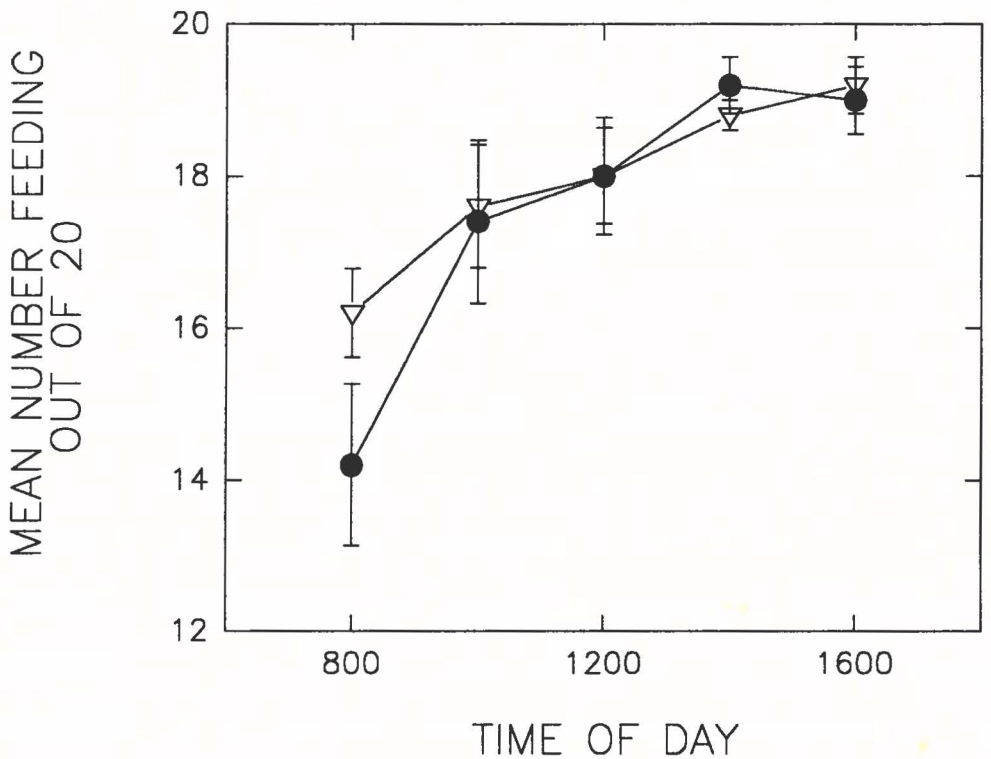

Figure 3. Effects of rotifer density on feeding incidence of Siganus randalli larvae four days after hatching. Triangles=High rotifer density 30/ml; Circles=Low rotifer density 10/ml; Bars are standard errors.

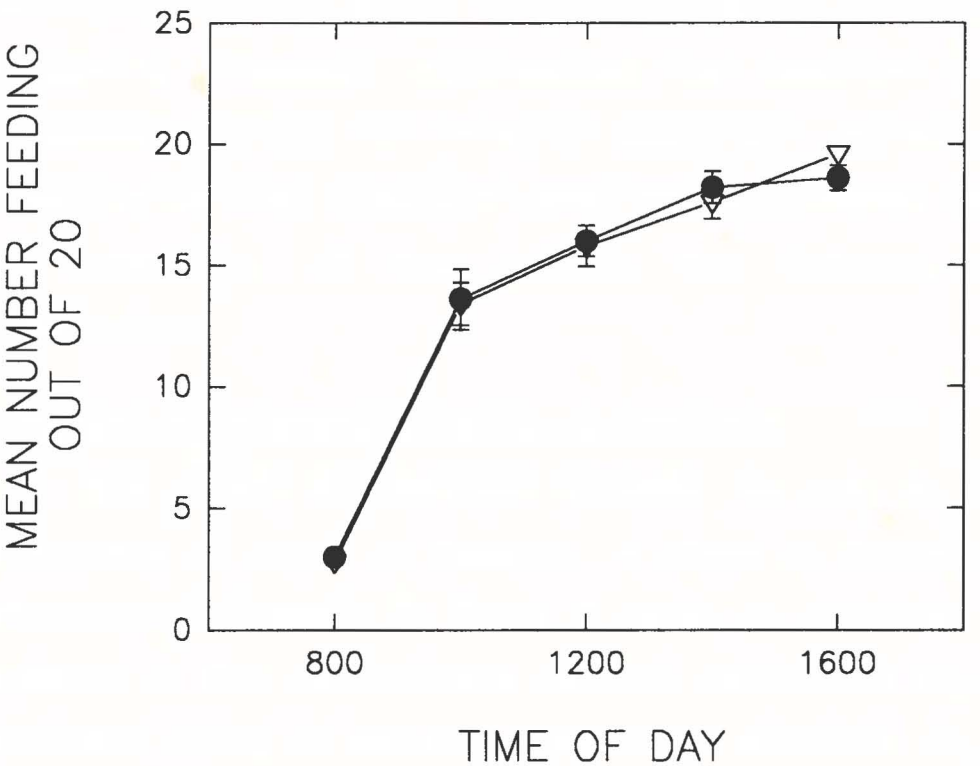

Figure 4. Effects of rotifer density on feeding incidence of Siganus randalli larvae four days after hatching. Triangles=High rotifer density 20/ml; Circles=Low rotifer density 1/ml; Bars are standard errors.

Effects of Rotifer Density on Larval Growth

Growth of the larvae in the four days after hatching was not related to prey density. Also there was no significant ($F_{1,8}$=0.03, P=0.08686) difference in mean length between those fish fed at 10 and those fed at 30 rotifers/ml (Table 5). Results were similar for fish fed 1 and 20 rotifers/ml ($F_{1,8}$=0.06, P=0.8066 Table 6). In both trials most larvae died five days after hatching, after the yolk was absorbed; and this limited the duration of the experiment.

Exposure of Prefeeding Larvae to Rotifers

The effect of previous exposure to prey on feeding incidence of larval S. randalli is shown in Figure 5. The statistical summary in Table 7 shows an increase in the numbers of fish feeding that had been previously exposed to rotifers early on the third day after hatching ($F_{1,3}$=5.20, P=0.0304). There was no significant interaction between treatment and time ($F_{1,3}$=2.06, P=0.1287), although the trend was for the differences between the groups to increase throughout the day. By day four there was no difference ($F_{1,4}$=0.39, P=0.5389) in feeding incidence between groups, as almost all of the fish had begun to feed by this time (Figure 6, Table 8).

Larval Size and Feeding Success

The comparison of the sizes of the larvae in the feeding and nonfeeding groups showed that there was no significant difference ($F_{1,98}$=0.33, P=0.5696), between the notochord lengths among those fish feeding and those not feeding. (Table 9, Figure 7) This suggests that the larger larvae did not have any particular advantage over smaller larvae in capturing rotifers as might be expected.

Table 5. Effects of rotifer density on growth of Siganus randalli larvae four days after hatching. Rotifer densities tested were 10/ml and 30/ml. T=tank, D=density, and F=number of fish feeding.

Source	Error Term	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	T(D)	0.2666703E-3	1	0.2666703E-3	0.03	0.8686
T(D)	F(TD)	1.7306668E-1	8	0.9133335E-2	0.58	0.7935
F(TD)		2.2066677E+0	140	0.1576191E-1		

Table 6. Effects of rotifer density on growth of Siganus randalli larvae four days after hatching. Rotifer densities tested were 1/ml and 20/ml. T=tank, D=density, and F=number of fish feeding.

Source	Error Term	Sum of Squares	DF	Mean Square	F Value	Prob.
Density	T(D)	0.0016667	1	0.0016667	0.06	0.8066
T(D)	F(TD)	0.2082667	8	0.0260333	0.84	0.5672
F(TD)		4.3280000	140	0.0309143		

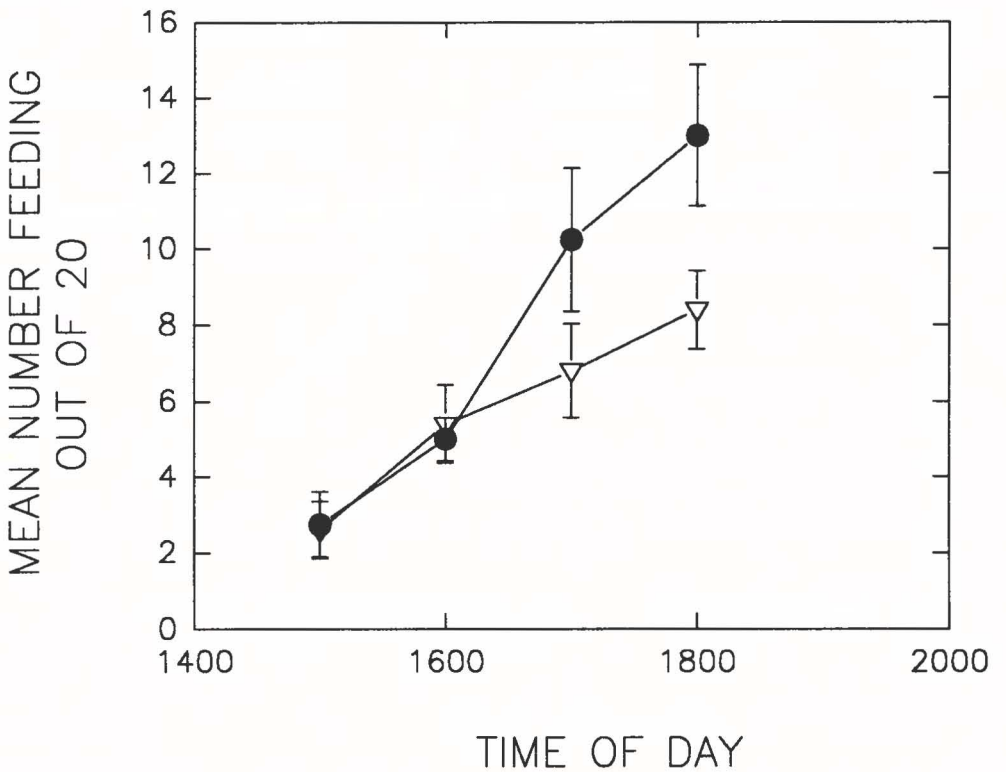

Figure 5. Effects of exposing prefeeding Siganus randalli larvae to rotifers on feeding incidence three days after hatching. Circles=Larvae preexposed to rotifers; Triangles=Larvae not preexposed to rotifers; Bars are standard errors.

Table 7. Effects of exposing prefeeding larvae to rotifers on feeding incidence three days after hatching. Rotifers were stocked at a density of 20/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Treatment	33.8000	1	33.8000	5.20	0.0304
Time	335.6333	3	111.8778	17.22	0.0000
Interaction	40.0778	3	13.3593	2.06	0.1287
Error	181.9000	28	6.4964		

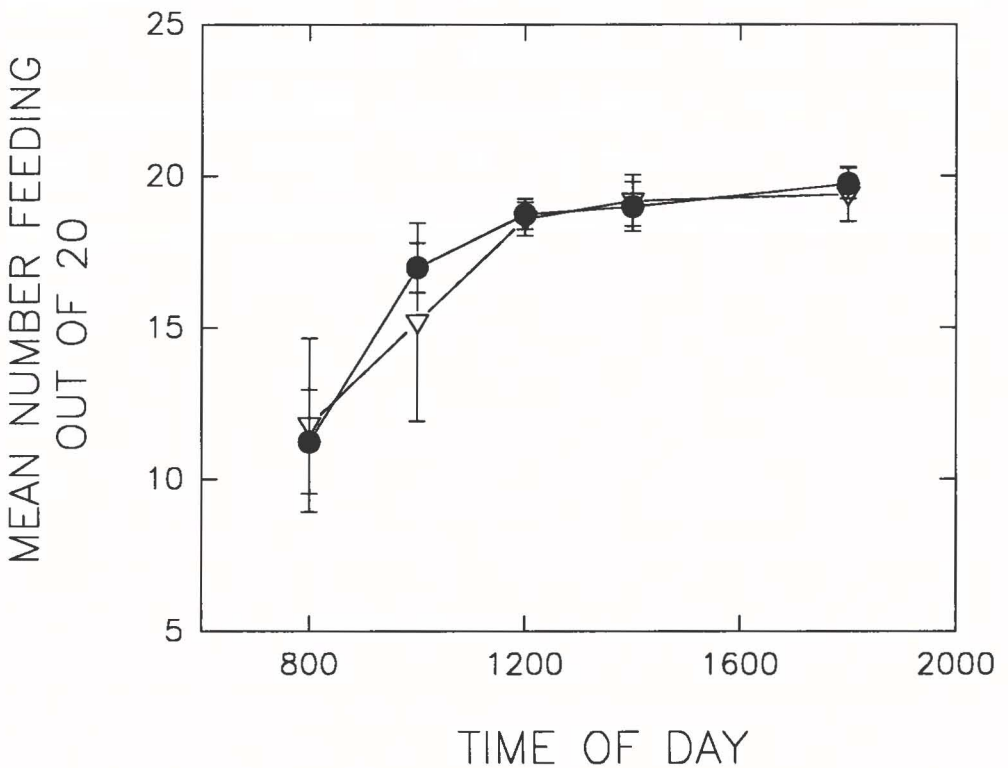

Figure 6. Effects of exposing prefeeding Siganus randalli larvae to rotifers on feeding incidence four days after hatching. Circles=Larvae preexposed to rotifers; Triangles=Larvae not preexposed to rotifers; Bars are standard errors.

Table 8. Effects of exposing prefeeding larvae to rotifers on feeding incidence four days after hatching. Rotifers were stocked at a density of 20/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Prob.
Treatment	1.0678	1	1.0678	0.39	0.5389
Time	396.7267	4	99.1817	35.77	0.0000
Interaction	7.2156	4	1.8039	0.65	0.6303
Error	97.0500	35	2.7729		

Table 9. Relationship between length and first feeding incidence of larval Siganus randalli four days after hatching. Rotifers were stocked at a density of 1/ml.

Source	Sum of Squares	DF	Mean Square	F Value	Probability
Feed	0.0111	1	0.0111	0.33	0.5696
Error	3.3460	98	0.0341		

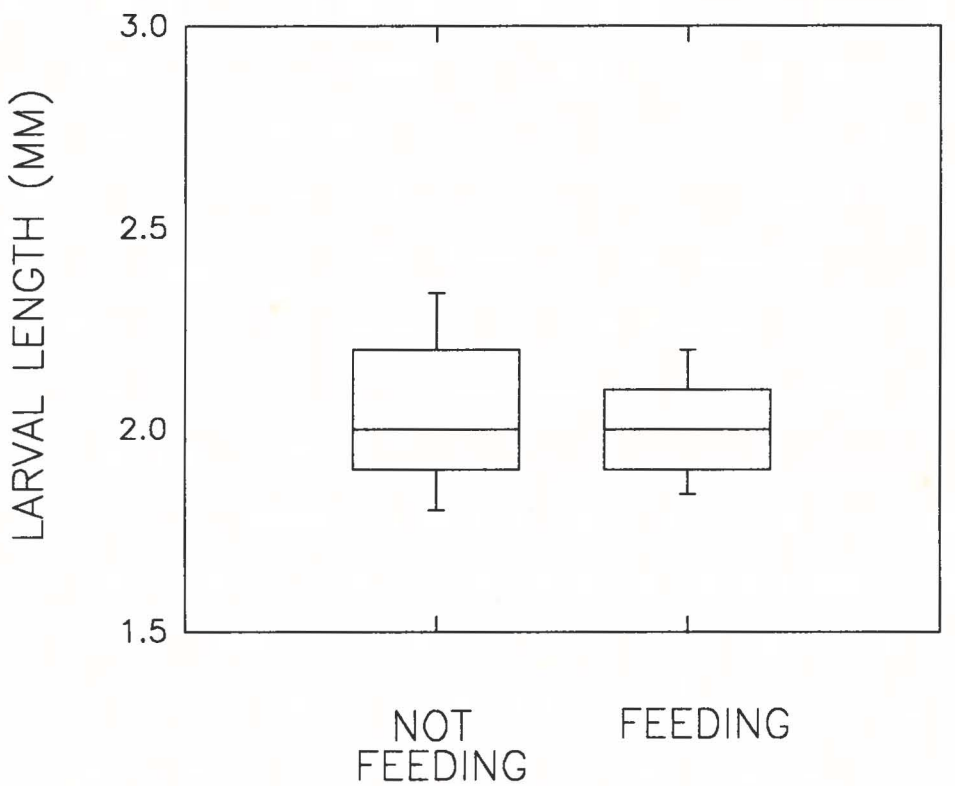

Figure 7. Comparison of the lengths of larval Siganus randalli with rotifers in their stomach to those without. Bars are standard errors.

DISCUSSION

Age of the larvae and time of day were the major factors influencing feeding incidence of S. randalli in this study. At water temperatures of 24.3 to 27.5 °C, larvae first began to feed approximately 60 h after hatching. In work with the rabbitfish S. guttatus, feeding was observed as soon as 2 d after hatching at temperatures between 25 to 30 °C (Hara et al., 1986 a). The milkfish Chanos chanos begins feeding somewhat later, at approximately 80 h after hatching at 25.3 to 26.5 °C (Eda, 1990). Almost all of the larval S. randalli were found to be feeding 84 h after hatching, which is similar to the mullet Mugil cephalus where the majority of larvae feed by 80 h after hatching (Tamura et al., 1991).

Larval S. randalli had a lower feeding incidence as sunset approached, and larvae sampled during the night had empty guts. Similar results have been obtained with S. guttatus larvae, where feeding incidence dropped as sunset approached, with few, if any, larvae feeding during the periods of darkness (Hara et al., 1986 b).

Effects of Rotifer Density and Larval Age

Over the 20-fold difference in prey densities tested, there was no difference in feeding incidence between groups. These results differ from those with the mullet Mugil

cephalus where first feeding was significantly higher with a rotifer density of 10/ml than of 1/ml (Tamura et al., 1991). In the herring Clupea harengus, feeding on copepod nauplii was initiated sooner at densities of 0.03/ml and 0.12/ml versus 0.0075/ml (Kiørboe et al., 1985). In some cases the C. harengus larvae feeding at the higher densities still had yolk reserves (Kiørboe et al., 1985). In studies of the bay anchovy Anchoa mitchilli, the sea bream Archosargus rhomboidalis, and the lined sole Achirus lineatus, the percentage of larvae feeding increased at prey densities above 0.1/ml (Houde and Schekter, 1980). None of the larvae held at a prey density of 0.01/ml survived long enough to achieve a dry weight of 200 μg, possibly because the availability of prey was not high enough to meet the nutritional needs of the larvae (Houde and Schekter, 1980).

The lower prey densities examined in my experiments (1 and 10/ml) with siganids were quite high compared to those used in similar studies of these other species. However, prey densities of 10/ml or higher are often used in the culture of larval fishes such as mullet Mugil cephalus, 10/ml, (Tamura et al., 1991), milkfish Chanos chanos, 10-20/ml, (Eda et al., 1990) and the rabbitfish Siganus guttatus, 5-20/ml, (Hara et al., 1986 a). I found that prey densities of 1/ml are adequate to ensure first feeding success of larval siganids.

The behavior of the larvae also affects the prey densities required for successful first feeding. Soon after hatching, S. randalli larvae were observed to stay in a large aggregation near the surface of the rearing tank. On the day after hatching, the larvae began to evenly disperse themselves around the edge of the rearing tank, 5 cm under the surface of the water. Feeding success of the larvae at low prey densities would be enhanced if the larvae were able to maintain their position in patches of rotifers, which may not have been uniformly distributed under the static water conditions of the rearing tank. Observations of larval anchovies Engraulis mordax showed that larvae were attracted to patches of the prey Gymnodinium (Hunter and Thomas, 1974). This behavior requires the larvae to search less water per unit time than if the larvae were outside a patch of prey (Hunter and Thomas, 1974).

Larval behavior apparently differs considerably between species. For example, larvae of the red grouper Epinephelus akaara are inactive upon hatching and float upside down (Tseng and Ho, 1988). Also on the second day after hatching, the larval E. akaara begin to rest on the bottom of the rearing tank at an angle of 30°-40° (Tseng and Ho, 1988). Neither of these behaviors were exhibited by larval S. randalli. Some fish larvae feed by assuming an S-shaped posture and lunging at the prey. These include such fishes

as the cichlid Cichlasoma managuense (Meyer, 1987) and the herring Clupea harengus (Rosenthal, 1969). However, preliminary observations indicate that first feeding siganid larvae capture prey by quick lateral motions of the head.

Although there was no effect of density on the growth of the larvae in the tests lasting four days, the fish had only been actively feeding for two days. If the trials had been carried out over a longer period, an effect may have been observed. However, in work with the scaled sardine Harengula pensacolae, no differences in growth were observed between those groups fed at low (0.5/ml) and high (1.5/ml) prey densities (Saksena and Houde, 1972). Results with the bay anchovy Anchoa mitchilli and the lined sole Achirus lineatus 16 d after hatching showed an increase in growth at higher (1/ml and 5/ml) than at lower (0.05/ml and 0.1/ml) prey densities (Houde, 1977). In studies of the herring Clupea harengus, an increase in larval growth was observed in the larvae as the prey density increased up to a threshold level of 0.0171 Artemia/ml (Werner and Blaxter, 1981).

Exposure of Prefeeding Larvae to Rotifers

The preexposure to prey before the larvae could feed influenced the feeding incidence later in the day. This effect may have been due to either physical or chemical stimulation of the larvae. The larvae may have gained

experience which helped them to identify or capture rotifers. Culturists often add rotifers to the larval rearing tanks prior to the time when the larvae are capable of feeding. For example, prey items are introduced to the mullet Mugil Cephalus 36 h post hatching at which time the mouth is open, although the larvae do not actively feed (Tamura et al., 1991). The timing of introduction of prey items is important in the culture of larval fishes, because if prey are added too soon the rotifers may reach densities that are detrimental to water quality and larval development. If prey items are added too late, larvae may starve (Noakes and Godin, 1988).

Larval Size and Feeding Success

The fish that successfully fed were no larger than those that had not. This suggests that feeding success of S. randalli may not be size dependent at this early stage of development. However when a more pronounced disparity in larval sizes develops, a wide range of prey size may need to be added to permit optimal feeding by the entire larval population (Meeren, 1991). In work with the milkfish Chanos chanos (Eda et al., 1990) and the rabbitfish Siganus guttatus (Juaria et al., 1985), no differences appeared in size between those larvae feeding and not feeding until five days after hatching. These results differ from those observed for mullet, Mugil cephalus, in which successfully

feeding larvae were larger 3.5 d after hatching (Tamura et al., 1991). Therefore, in comparison of these other larvae, the mode of prey capture by siganids may make them less dependent on size than other species.

This study is one of few of the feeding behavior of the larvae of a tropical marine fish. Several aspects of the work, particularly the relation between age and success at first feeding, and the influence of prior exposure to prey in subsequent feeding success, should be useful in developing techniques for the culture of siganids and other fishes. Also, from comparisons with other studies, these results indicate that larval behavior may differ considerably even between species with similar egg and larval sizes. It is hoped that this work will serve to stimulate interest in the behavior of larval fishes.

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