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Roy T. Tsuda

William J. Tobias, Patrick G. Bryan, William J. FitzGerald , Jr.

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Cover illustration: Siganus spinus, drawn by Terry Palumbo.

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Roy T. Tsuda
Principal Investigator
Marine Laboratory, University of Guam

William J. Tobias, Patrick G. Bryan, William J. FitzGerald, Jr.
Graduate Research Assistants
Marine Laboratory, University of Guam

Harry T. Kami and Isaac I. Ikehara
Consultants
Division of Fish and Wildlife, Department of Agriculture

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CONTENTS

SYNOPSIS

For the past four and a half years (1972-76), we have been investigating the feasibility of rearing the rabbitfish *Siganus* in Guam marine waters, with primary focus on the food requirements and general ecology of two local species (*S. spinus* and *S. argenteus*) in relation to juvenile-adult rearing. The six papers presented here by various authors represent our cooperative efforts toward this goal.

The interest in siganids for juvenile-adult rearing is the observed phenomenon, recorded by Harry T. Kami and Isaac I. Ikehara of the Division of Fish and Wildlife, that schools of juvenile *S. spinus* and *S. argenteus* (= *S. rostratus*) school onto the reef flats around Guam each year during the months of April and May, and occasionally during June and October. Even the time of schooling is predictable and occurs plus or minus two days of the last quarter moon during these months. Each run lasts for about one week. Likewise, runs of *S. canaliculatus* occur in Palau waters during these same months.

Since siganids are known herbivores, the obvious initial step in rearing the fish was to obtain information on their food habits. Observations on juvenile *S. spinus* and *S. argenteus* in the field and under laboratory conditions revealed that these fish feed only on the smaller filamentous-like algae which they can physically bite and ingest. The study was expanded in a M.S. thesis by Patrick G. Bryan who concluded that algal availability, and size and behavioral characteristics of *S. spinus* determine what kinds of algae are ingested. The studies showed that the green intertidal alga, *Enteromorpha*, was the most preferred food of juvenile and adult *S. spinus*. The assimilation values for the adult *S. spinus* when fed *Enteromorpha* ranged from 6 to 39 percent; those for the juveniles ranged from 9 to 60 percent.

Comparative growth rate studies were carried out on *S. spinus* and *S. argenteus*, as well as *S. canaliculatus* imported from Palau. Populations of each species were fed a diet of *Enteromorpha*, while other populations were fed this alga and trout chow. Trout chow is a commercial feed which contains about 40 percent protein; field collected *Enteromorpha* was found to contain 11-27 percent protein. The comparative studies on the three species of *Siganus* showed that *S. argenteus* had the fastest growth rate. In all cases, fishes fed a diet of both *Enteromorpha* and trout chow grew approximately twice as fast as those fishes fed only *Enteromorpha*. The legume *Leucaena leucocephala* was also tested as a possible feed for *Siganus*. Preliminary studies indicated that fish fed a constant diet of *Enteromorpha* and *Leucaena* became weak resulting in a high mortality rate.

Since our knowledge on the ecology of *S. argenteus* was almost nonexistent, a study was undertaken by William J. Tobias as a M.S. thesis topic to provide relevant information on the general biology of subadult and adult fish. The area of concentration was on habitat preference, feeding habits, behavior and diseases. In addition, the tolerance of *S. argenteus* to selected environmental parameters, such as, temperature, salinity and oxygen concentrations were investigated.

The other phase undertaken toward the overall goal of juvenile-adult rearing was the furthering of our knowledge on the alga *Enteromorpha*. Although a high source of protein, such as trout chow, is essential for faster growth in *Siganus*, the cost is prohibitive for commercial use. Thus, it seemed that *Enteromorpha* could be utilized as the primary diet of siganids until such time that a cheaper high protein source was found. Detailed laboratory and field studies were undertaken by William J. FitzGerald, Jr., as a M.S. thesis topic to characterize the various environmental parameters necessary for optimum growth in *Enteromorpha clathrata* found on Guam. Light intensity, salinity, temperature and nutrient levels for optimum growth were examined in the laboratory. In addition, field work concentrated on seasonality, zonation and substratum.

We are still far from rearing siganids economically, but feel that a foundation has been laid upon which a pilot mariculture project can be initiated on Guam. *Enteromorpha* can be used as the primary food source to rear *Siganus argenteus*. Although a diet of only *Enteromorpha* will maintain a slower growth rate in the fish, this trend will prove disadvantageous only in the initial years. However, the construction of a hatchery facility is essential to this mariculture endeavor since mere gathering of juveniles during the seasonal runs is not dependable.

Professional Placement

It is hearteningly to mention that the siganid study not only provided us a better insight into the feasibility of siganid mariculture on Guam, but has also proved to be an ideal training program for graduate students. Two of the three graduate students supported as research assistants on this project have already found professional employment in Guam and the Trust Territory. Patrick G. Bryan, originally employed as the siganid specialist at the Micronesian Mariculture Demonstration Center in Palau, is now the Fisheries Officer for the Marshall Island District. William J. FitzGerald, Jr., is employed as an aquaculturist within the Division of Fish and Wildlife, Department of Agriculture, on Guam. The third graduate student, William J. Tobias, has accepted a mariculture position with the University of Texas Artificial Upwelling-Mariculture Project on St. Croix, U. S. Virgin Islands.

FOOD PREFERENCE OF JUVENILE *SIGANUS ROSTRATUS* AND *S. SPINUS* IN GUAM[1].—Schools of juvenile *Siganus rostratus* and *S. spinus* (Teuthididae) swarm on the reef flats of Guam each year during the months of April and May, and occasionally during June and October (Ikehara, 1968). The time of schooling is predictable and occurs plus or minus two days of the third quarter moon during these months.

During the month of May 1972, the largest school thus far recorded over the past ten years invaded the reef flats of Guam and provided an opportunity to examine the food habits of the two species. The Fish and Wildlife Division of the Government of Guam estimated there were more than 13 million juvenile teuthidids on the reefs.

The first objective was to obtain information on the genera of algae eaten by the juveniles. Approximately 250 juveniles, consisting of an equally mixed population of the two species, were held in a 1000-liter circular plastic tank with running seawater. The mean standard lengths of *Siganus rostratus* and *S. spinus* were 80 mm and 45 mm, respectively. Forty-five of the common plant genera inhabiting the reefs during this time of year were collected and fed individually to the juveniles each morning (900 to 1000) and evening (1600 to 1700). The amount of plant material used in each trial varied slightly in quantity depending on the consistency of the thallus, but was enough to be consumed by the fishes in less than one minute.

The results (Table 1) of this test, based on five to 10 trials per algal genus, revealed that only 10 of the 45 plant genera tested were always eaten by the juveniles of both species. Eight of the genera were categorized as filamentous. The other two genera were noncalcareous fleshy algae, i.e., *Caulerpa racemosa*, a soft siphonaceous green alga, and *Hypnea pannosa*, a red alga less than 2 mm in diameter.

Eleven of the 18 noncalcareous fleshy plants, including two genera of seagrasses, and nine of the 11 calcareous algae tested were rejected by the juveniles. The hard consistency of these plants might be the reason for the rejection, i.e., the juveniles may not be physically adapted to feeding on these plants. Only two of the 16 filamentous algae were ignored by the juveniles. The high iodine content (Grimm, 1952) in *Asparagopsis taxiformis* most likely deterred the juveniles from feeding on that alga. The mucilaginous coating of the blue-green algae, *Schizothrix calcicola* and *S. mexicana*, possibly repelled the fishes from those algae.

There was also a third group of algae that was ingested during some trials but rejected at other times. The three blue-green algae, *Calothrix crustacea*, *Hormothamnion enteromorphoides* and *Microcoleus lyngbyaceus*, and the two green algae, *Bryopsis pennata* and *Chlorodesmis fastigiata*, could be eaten by the fishes but were avoided during most trials. The absence of blue-green algae in the diet of tropical reef fishes had previously been observed by Jones (1968a) and the senior author. The avoidance of juvenile fishes toward such algal genera as *Avrainvillea*, *Codium*, *Dictyota*, *Gelidium*, *Gracilaria*, *Padina* and *Jania* can probably be attributed to the large size of the individual thallus. Immature stages of these algae were eaten by the fishes, while larger thalli of the same genera were not. The juveniles attempted to feed on the larger thalli but soon swam away when they could neither bite nor swallow the algae. Thus, both species of *Siganus* feed preferentially on filamentous algae but do not eat blue-green and noncalcareous fleshy algae.

The second series of experiments was designed to determine differences in food preference between *Siganus rostratus* and *S. spinus*. Feeding tests, using the six more common algae ingested and one alga occasionally ingested by the mixed population, were conducted on separate populations of 100 *S. rostratus* and 100 *S. spinus*. The first difference observed was the avoidance of *Chlorodesmis fastigiata* by *S. spinus*, while *S. rostratus* quickly devoured the alga as soon as it was introduced into the tank. The second difference was the rejection of *Polysiphonia* sp. by *S. rostratus*, while *S. spinus* actively ingested this alga. Both species vigorously ate *Enteromorpha* spp., *Feldmannia indica*, *Derbesia* sp. and *Cladophoropsis membranacea*. In this experiment, neither species of *Siganus* ingested the blue-green alga, *Microcoleus lyngbyaceus*.

The goal of the third set of experiments was to determine which of the ingested algae were most preferred by the *Siganus* species. Different combinations of algae were inter-

[1] Contribution No. 33, The Marine Laboratory, University of Guam.

TABLE 1. RESULTS OF FEEDING EXPERIMENTS (0 REJECTED, – OCCASIONALLY INGESTED, + INGESTED) ON MIXED POPULATIONS OF *Siganus rostratus* AND *S. spinus* UTILIZING BENTHIC PLANTS FOUND ON THE REEFS OF GUAM DURING MAY TO JUNE, 1972. NUMBER OF ALGAL SPECIES TESTED ENCLOSED IN PARENTHESES.

Filamentous		Noncalcareous Fleshy		Calcareous	
Genera	Results	Genera	Results	Genera	Results
Cyanophyta		Chlorophyta		Chlorophyta	
Calothrix (1)	–	*Avrainvillea* (2)	–	*Halimeda* (2)	0
Hormothamnion (1)	–	*Boergesenia* (1)	0	*Neomeris* (1)	0
Microcoleus (1)	–	*Caulerpa* (2)	+	*Tydemannia* (1)	0
Schizothrix (2)	0	*Codium* (1)	–	*Udotea* (1)	0
Chlorophyta		*Dictyosphaeria* (1)	0	Phaeophyta	
Boodlea (1)	+	*Valonia* (1)	0	*Padina* (2)	–
Bryopsis (1)	–	Phaeophyta		Rhodophyta	
Chlorodesmis (1)	–	*Dictyota* (3)	–	*Actinotrichia* (1)	0
Cladophoropsis (1)	+	*Lobophora* (1)	0	*Amphiroa* (1)	0
Derbesia (1)	+	*Ralfsia* (1)	0	*Cheilosporum* (1)	0
Enteromorpha (2)	+	*Sargassum* (2)	0	*Galaxaura* (2)	0
Phaeophyta		*Turbinaria* (1)	0	*Jania* (2)	–
Feldmannia (1)	+	Rhodophyta		*Mastophora* (1)	0
Sphacelaria (1)	+	*Desmia* (1)	0		
Rhodophyta		*Gelidiella* (1)	0		
Asparagopsis (1)	0	*Gelidium* (1)	–		
Dasyphila (1)	+	*Gracilaria* (1)	–		
Leveillea (1)	+	*Hypnea* (1)	+		
Polysiphonia (1)	–	Seagrasses			
		Enhalus (1)	0		
		Halodule (1)	0		

mixed loosely into a single fist-size clump and introduced into both fish tanks. Both species of juveniles showed a rather surprising selectivity in that they would eat only one genus at a time, and would begin feeding on the next preferred alga only after the first alga was completely consumed. This experimental technique allowed us to rank the degree of preference of each fish species for a particular alga. Twenty such feeding tests revealed the order of preference which was similar for both species of *Siganus*—1. *Enteromorpha*, 2. *Feldmannia* and *Derbesia* and 3. *Cladophoropsis*.

Our data indicate that the juveniles of both *Siganus rostratus* and *S. spinus* are highly selective in the type of algae they eat. Results of gut content analyses of adult specimens of *Siganus spinus* from Guam indicate (Jones, 1968b) that the food preference of both juveniles and adults are similar. Jones' study of 11 specimens (92–169 mm SL) revealed that *Padina* (vaughaniella or immature stage), *Cladophoropsis*, *Gelidium* and *Hypnea* make up 80% of all food items ingested.

One of the more important findings of this study was to define the effect of selective grazing on the algae of the reef flats during those months when schooling occurred. A drastic change occurred in that certain algae were conspicuously absent. For example, the green alga *Enteromorpha* disappeared completely from the intertidal zone on several beaches around Guam. In fact, all of the *Enteromorpha* used in the feeding experiments had to be obtained from our outdoor holding tanks. There was also a conspicuous absence of all species of *Caulerpa* on the reef flats. Likewise, other green algae such as *Boodlea composita* and *Cladophoropsis membranacea*, which were abundant a month prior to the schooling, were absent from Pago Bay.

During the early part of June, staff members of both the Marine Laboratory and the Fish and Wildlife Division observed the juvenile *Siganus* in what seemed to be in a starved condition, i.e., large head and paper-thin body, swimming at the surface in an oblique position. Soon after, dead juveniles were reported from several localities around

TABLE 2. CONDITION OF GUTS IN JUVENILE *Siganus spinus* WHICH APPEARED STARVED DURING JUNE 5 TO 7, 1972 AT SIX SITES AROUND GUAM.

		Condition of Guts		
Location	N	Empty	Partially Full	Full
Agana	22	16	6	0
Asan	46	30	16	0
Neye	10	9	1	0
Tanguisson	29	14	12	3
Tumon	178	175	3	0
Ylig	46	0	46	0
Total	331	244	84	3

Guam. Starvation is a feasible explanation for the mass mortality, since the algal genera eaten by the juveniles were now absent from the reefs due to their prior feeding activity. No other reef fish were among the dead on the beaches.

Three hundred and thirty one fish specimens which appeared starved were collected from six localities and examined for food items present in their guts. The results (Table 2) show that 244 specimens (74%) had no food in their guts. Only three specimens had the guts filled with algal material. These three specimens, as well as the 84 specimens with partially filled guts, all possessed the same genera of filamentous and smaller noncalcareous fleshy algae reported earlier in the food preference experiments. Further, *Siganus spinus* was the only species that appeared starved and the only species collected at all six sites. This indicates that the larger and more aggressive *S. rostratus* was outcompeting *S. spinus* for food, since both species seemed to prefer the same algae.

We would like to thank I. I. Ikehara and H. T. Kami for fruitful discussions on this topic. We are indebted to R. S. Jones for constructive criticisms of the manuscript.

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ROY T. TSUDA *and* PATRICK G. BRYAN, *The Marine Laboratory, University of Guam, Agana, Guam 96910.*

Notes on the Annual Juvenile Siganid Harvest in Guam[1]

Harry T. Kami and Isaac I. Ikehara
Division of Fish & Wildlife, Department of Agriculture,
Agana, Guam 96910

Rabbitfish (Siganidae) are important food fishes throughout the Western Pacific. Eight species of siganids are recorded from Guam (Schultz et al., 1953; Kami et al., 1968; Kami, 1975), but only six of these have been observed near Guam within the last 20 years. Of these, *Siganus spinus* and *S. argenteus* are the predominant food species. Although the adults of these species are sought by the local residents, it is the juveniles that play an important part in the culture of the people of Guam. Traditionally, the annual harvesting of juvenile siganids when they first appear in the reef flats is a major village event. Based on records kept by the Division of Fish and Wildlife and according to local fishermen, the juveniles appear in the reef flats a few days before or after the last quarter of the moon (called Quarto Menguate locally) in April and May. Occasionally a third and fourth run may occur in June and October, again during the last phase of the moon.

When they first appear in the reef flats, these juveniles are called manahac hatang or manahac leso depending on their size by the local residents. The hatang is smaller than the leso and averages 43 mm in total length while the leso averages 60 mm in total length. The manahac hatang is the juvenile of *S. spinus* and manahac leso the juvenile of *S. argenteus*.

The approach of the run is usually signified when manahac are regurgitated by troll-caught tunas. On April 3, 1967 a "ball" of juvenile siganids was observed by crew members on the Division of Fish & Wildlife's exploratory fishing sampan PANGLAU ORO while anchored off Guam at a depth of 45 fathoms. A month later, the crew observed tunas chasing juvenile siganids on the lee coast of the island.

During the pelagic phase of their life cycle, these post-larval siganids are presumably plankton feeders and form large "balls"

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[1]This Paper has been accepted for publication in *Micronesica* 12(2) 1976.

appearing reddish-brown in color. Upon entering the shallow reefs, they become herbivorous and develop adult coloration. Tsuda and Bryan (1973) found that juvenile siganids are highly selective herbivores preferring benthic filamentous green algae over other algae. The local residents differentiate the plankton feeding stage (manahac) from the herbivorous stage called dage and prefer the manahac over the dage.

With the approach of the manahac run, the beaches are patrolled by the residents from early dawn to spot the reddish-brown "ball" of siganids as it enters the shallow reef flats. The "balls" are either a mixture of hatang and leso, or are schools of one or the other species. When "balls" are sighted, a long minnow net is used to encircle the school and pursed along the bottom lead line. Cast nets are also used to take smaller schools of siganids. The manahac is pickled in heavy brine and served either by itself or mixed with vegetables especially at the village fiestas.

Since 1963, the Division of Fish & Wildlife has been conducting creel censuses during the manahac runs. These creel censuses have several shortcomings. First, the catch data are based on sight estimate of the interviewing Conservation Officers. Actual weights were not taken because most of the people are anxious to take their catch home as soon as possible before it spoils and any delay to weigh their catch was not welcomed. In spite of this handicap, reasonable estimates can be made. Most of the manahac are put into cotton or gunny sacks. A sack full of manahac varied in weight from 45.4 to 49.9 kg (100 to 110 pounds). Hence, 45.4 kg was used as a standard equivalent weight for a full sack. Second, it is not possible to obtain a complete island wide coverage of the run. Therefore, the catch report is usually an underestimate. Because of high degree of variability associated with each run, the catch data were not subject to quantitative analysis. However, the total harvest is a fair estimate of the relative magnitude of the annual runs.

Figure 1 presents a thirteen year harvest record which shows wide variations in the annual total catch. A single-sample runs test for trends data (Sokal and Rohlf, 1969) showed that the sequence of the yearly variations did not differ significantly from randomness. There was no significant tendency toward regular cycling or continuously increasing or decreasing trends. In the 12 years following the initial year, there were eight sets of consecutive increases or decrease so,

$$t_s = \left(8 - \left[\frac{2.12 - 1}{3}\right]\right) \Big/ \sqrt{\frac{(16.12 - 29)}{90}} = .25$$

Five of the thirteen years (1964, 1968, 1969, 1973 and 1974) show exceptionally low harvests with 1973 amounting to less than 0.1 metric

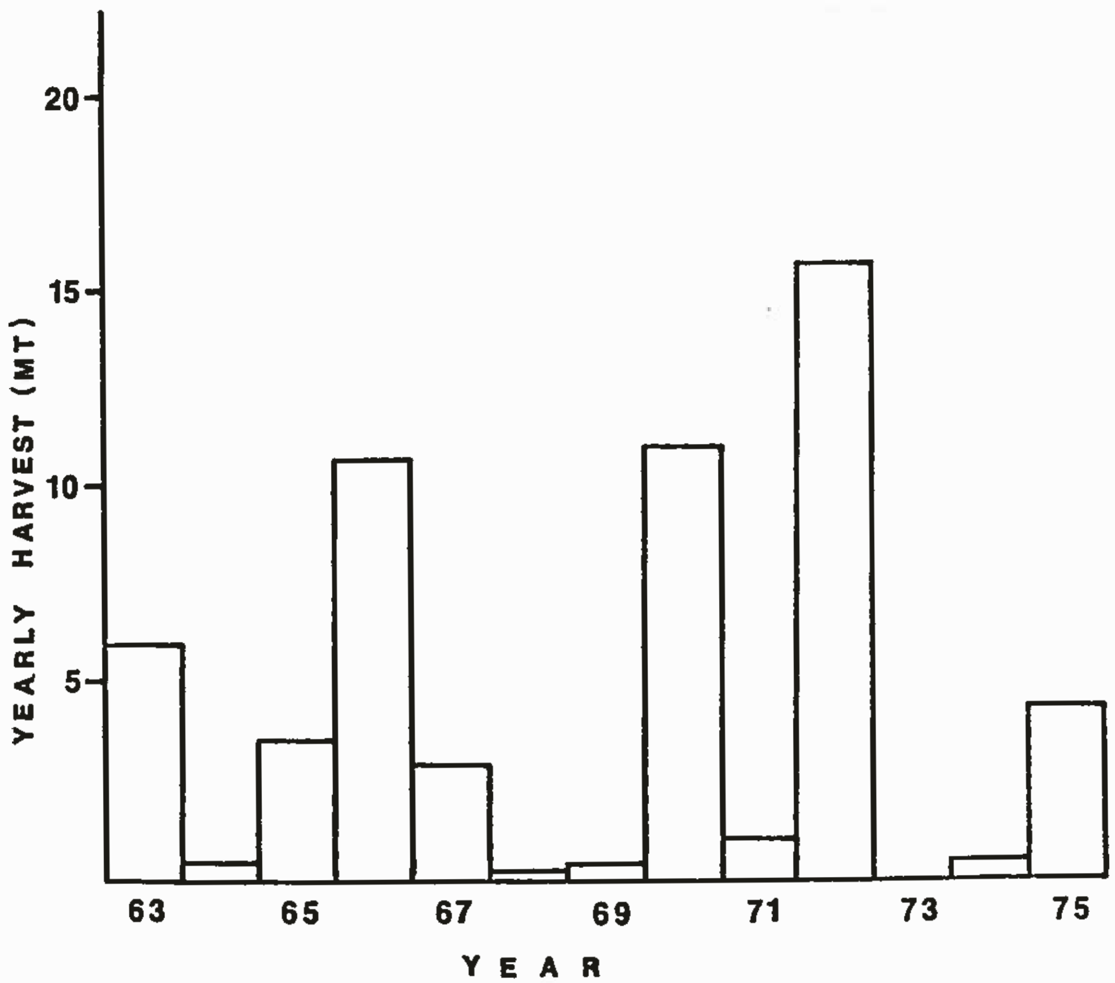

Figure 1. Yearly Harvest (MT) estimated from Creel Census.

ton. Curiously, the run preceding 1973 was exceptionally high. As a result of this unusually large run in June 1972, the schools of dage remaining on the reef flats depleted their food source and mass mortality caused by starvation became evident in several coastal areas (Tsuda and Bryan, 1973).

Based on data combined from 1963, 1965 and 1972, 454 gms of manahac hatang consisted of an average of 487 individuals while an equal weight of manahac leso comprised an average of 235 individuals.

Although the harvesting of juvenile siganids may appear to be an unwise conservation practice, if left unchecked, the millions of juveniles invading the reef flats will soon deplete their food source and succumb to starvation as they did in 1972. It is evident that the reef flats cannot sustain these masses of manahac for a prolonged period.

The annual harvesting of manahac, therefore, serves a double function of satisfying the culturally-bound gastronomical needs of the people as well as rendering biological control from over-grazing of algae. However, it would be prudent to investigate the optimum harvesting limits of this natural resource which varies greatly from year to year.

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Food Habits, Functional Digestive Morphology, and Assimilation Efficiency of the Rabbitfish *Siganus spinus* (Pisces, Siganidae) on Guam[1]

Patrick G. Bryan[2]

ABSTRACT: Analyses of stomach contents of *Siganus spinus* showed that algal availability and size and behavior characteristics of the fish determine what kinds of algae are ingested in the field. Sixty-two algal species were tested during multiple-choice food preference trials in the laboratory. Elimination trials and observation tests showed a ranked order of algal preference: (1) *Enteromorpha compressa*, (2) *Murrayella periclados*, (3) *Chondria repens*, (4) *Boodlea composita*, (5) *Cladophoropsis membranacea*, (6) *Acanthophora spicifera*, and (7) *Centroceras clavulatum*. An examination of the morphology of the digestive system showed that the fish are well-adapted herbivores, especially toward the filamentous algae. The assimilation values for the adults ranged from 6 to 39 percent; those for the juveniles ranged from 9 to 60 percent.

Among the fishes inhabiting Guam waters, members of the family Siganidae constitute one of the more important food resources for local consumption. In Guam, the family is represented by five species (Kami, Ikehara, and DeLeon 1968), of which *Siganus spinus* (Linnaeus) is the most abundant. Siganids are also the focus of considerable maricultural interest (unpublished data from the Siganid Mariculture Implementation Conference, Hawaii Institute of Marine Biology, 1-5 November 1972).

Each year during the months of April and May, and occasionally during June and October, schools of juvenile *S. spinus* swarm on the reef flats of Guam (Tsuda and Bryan 1973) where they spend several months foraging on benthic algae. On Guam adults are usually found in shallow water along the reef front and often venture onto the reef flats to browse on benthic plants. Both adults and juveniles are primarily diurnal feeders, feeding almost continuously during the daytime. These fish are often found in schools but may browse individually or in pairs, sometimes accompanying other siganids, scarids, and acanthurids.

This study deals with the herbivorous nature of *S. spinus*: types of benthic plants ingested and preferred, description of the morphological and functional aspects of the digestive mechanisms as related to ingestion and digestion, and the assimilation efficiency of the most preferred alga ingested.

A food preference study by Tsuda and Bryan (1973) on juvenile *S. spinus* and *S. argenteus* (= *S. rostratus*) indicated a selective feeding habit for filamentous algae and smaller fleshy algae. Jones (unpublished) reported on the stomach contents of 11 specimens of *S. spinus* and offered some insight into the food habits of this species. Descriptions of the digestive tract are available for *Siganus fuscescens* (Suyehiro 1942, Tominaga 1969) and for *S. argenteus* (Hiatt and Strasburg 1960). There are no values available on the assimilation efficiency for any member of this family.

Specimens used for all facets of this study were captured by spearing, throw net, or at night by hand with the aid of flashlights. Those fish captured alive at night were transferred to

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[2] University of Guam, The Marine Laboratory, P.O. Box EK, Agana, Guam 96910. Present address: Micronesian Mariculture Demonstration Center, P.O. Box 359, Koror, Palau, Caroline Islands 96940.

the laboratory. Benthic plants used in experiments were collected from various localities around Guam.

STOMACH CONTENTS

To estimate floral species common to each area, I made algal collections from those areas where the fish were collected. Stomach contents of *S. spinus* were preserved in individual vials with 10-percent formalin prior to examination. Food items found in the stomachs were treated as relative abundance (percentage composition by species) and relative frequency (percentage of occurance; that is, how often a species was found) with a modified version of the point method of Jones (1968*b*) being used. Whereas Jones used 17 points per grid, I used 81 points per grid in this study. The importance values (IV) were obtained by summing relative abundances (RA) and relative frequencies (RF).

Results of stomach content analyses are shown in Table 1. Everything found in the stomachs was identifiable. The four algae representing the highest importance values obtained in this study were *Gelidiopsis intricata* (27 IV), *Boodlea composita* (26 IV), *Sphacelaria tribuloides* (25 IV), and *Centroceras clavulatum* (24 IV). Crustaceans were few (2.1 IV). The pooled stomach contents of 53 young juveniles (30-50 mm fork length [FL]) also were examined. Only four food items were found: *Gelidium pusillum* (64 RA), *Centroceras clavulatum* (35 RA), *Ceramium* sp. (0.8 RA), and one annelid worm (0.2 RA).

TABLE 1

Food Items, Listed in Order of Importance Value, Present in Stomach Contents of *Siganus spinus*

CONTENTS	RA	RF	IV
Gelidiopsis intricata	15.0	12.0	27.0
Boodlea composita	18.0	8.0	26.0
Sphacelaria tribuloides	11.0	14.0	25.0
Centroceras clavulatum	15.0	9.0	24.0
Feldmannia indica	8.0	10.0	18.0
Gelidium pusillum	10.0	3.0	13.0
Sargassum polycystum	7.0	5.0	12.0
Calothrix pilosa	5.0	6.0	11.0
Enteromorpha compressa	2.0	5.0	7.0
Cladophoropsis membranacea	1.0	5.0	6.0
Hypnea pannosa	2.0	3.0	5.0
Jania capillacea	1.0	3.0	4.0
Ceramium sp.	0.7	3.0	3.7
Polysiphonia sp.	0.1	2.0	2.1
Crustaceans	0.1	2.0	2.1
Tolypiocladia glomerulata	1.0	1.0	2.0
Padina tenuis	0.5	1.0	1.5
Desmia bornemanni	1.0	0.3	1.3
Cladophora sp.	0.3	1.0	1.3
Dictyota bartayresii	0.2	1.0	1.2
Diatoms (epiphytes)	0.2	1.0	1.2
Champia parvula	0.1	1.0	1.1
Padina sp. (vaughaniella)	<0.1	1.0	1.0
Hormothamnion enteromorphoides	<0.1	1.0	1.0
Rhodymenia sp.	0.6	0.3	0.9
Caulerpa racemosa	0.3	0.3	0.6
Neomeris annulata	0.1	0.3	0.4
Microcoleus lyngbyaceus	<0.1	0.3	0.3

Note: *Siganus spinus* specimens measured from 65 to 200 mm fork length; N 70. They were taken from six localities on Guam. RA, relative abundance; RF, relative frequency; and IV, importance value.

FOOD PREFERENCE

Multiple-Choice Trials

Multiple-choice feeding trials were conducted to find out which species of benthic plants were preferred by the fish. Three groups of fish (nine fish in each group, 130-200 mm FL) were held in wooden holding tanks (145 × 85 × 28 cm) with running seawater. The fish were starved for 2 days before tests were begun. Equal quantities of 5 to 15 freshly collected algal species were fastened by clothespins and suspended for 30 to 60 minutes in each tank. The number of algal samples used per trial varied because it was not always possible to collect equal numbers of species and because many of the algae deteriorated quickly when held in holding tanks at the laboratory. Each species of alga was tested at least three times with each fish group (total of nine times); the positions of the algae were alternated for each new test. Sixty-two species (54 genera) of benthic plants were tested.

The results were similar to those found by Tsuda and Bryan (1973) for the juveniles. In that paper, 56 plant species were tested on the juveniles and only 12 were always completely consumed (by both *Siganus spinus* and *S. argenteus*). Of these, nine were filamentous and three were fleshy. The tests on adults revealed that, out of 62 species tested, only nine were always completely ingested; six of the algae were filamentous (*Boodlea composita*, *Cladophoropsis membranacea*, *Enteromorpha compressa*, *Centroceras clavulatum*, *Ceramium* sp., and *Murrayella periclados*) and three were fleshy (*Acanthophora spicifera*, *Chondria repens*, and *Gelidiopsis intricata*). Only 12 species were never consumed by the adults whereas 26 were rejected by the juveniles.

Elimination Trials

Two new tanks of fish (nine fish per tank, 150–200 mm FL) were used to run elimination trials among the seven preferred algal species, as determined from the above experiments, to obtain an indication of rank. (*Ceramium* sp. and *Gelidiopsis intricata* were not available at this time in quantities large enough for further tests.) Three 5-g blotted wet-weight samples of each of the seven preferred species were hung submerged by clothespins in alternating positions from boards placed across the tops of the two tanks. The algae were checked at intervals because the feeding rates of the fish varied. The number of samples of each species consumed was recorded. The algal species with all three samples consumed was eliminated from the next trial. For example, if all three samples of *Enteromorpha compressa* were consumed during the first trial, then that species was eliminated from the rest of the trials, and so on, until all the species were eliminated. If two or more species had all three of their samples consumed during a trial, then that trial was repeated with fresh algal samples. The test fishes were fed less-preferred algae between tests so that partiality among the fish toward those seven algae being tested would be avoided. The preferred algal species were then ranked in order of preference by the fishes. By order of consumption, they are *Enteromorpha compressa*, *Murrayella periclados*, *Chondria repens*, *Boodlea composita*, *Cladophoropsis membranacea*, *Acanthophora spicifera*, and *Centroceras clavulatum*.

Definitive Observation Trials

To test the hypothesis that a ranked order of algal preference existed, I ran six more tests, using new fish for each test. Tests were run in a 150-liter plastic tank containing two fish (140–170 mm FL) that had been starved for the preceding 24 hours. Two fish per experiment were used because solitary fish would not feed properly under experimental conditions. A solitary fish usually occupied its time swimming along the sides of the tank or hiding. However, two fish usually behaved more normally, one fish establishing dominance. Both fish spent most of their time feeding rather than seeking ways to escape from the container or hiding.

One 3-g blotted wet-weight sample of each of the ranked algal species was hung submerged by clothespins near the bottom of the tank. The placement order of the samples was alternated for each new test. A plywood blind was used to observe and record the order in which the algal samples were consumed. Those species that were consumed in the same order as that established by the previous elimination trials were rated 1. The species that were not consumed in the same order as in the elimination trials were rated zero. Even though the fish often fed alternately on several of the algae, the frequency of visits to a particular sample and the length of stay usually correlated nicely with the order of consumption of that alga. However, I strengthened the test statistically by recording two or more species as zero if they could not be distinguished as to their consumed order. The data were entered in a two-way table and the Cochran's test for related observations (Conover 1971) was used to test statistically the hypothesis that a ranked order of algal preference existed.

$$T = c(c-1)\left[\sum_{j=1}^{c}\left(C_j-\frac{N}{c}\right)^2\right]\Big/\left[\sum_{i=1}^{r}R_i(c-R_i)\right]$$

where R_i = row totals, C_j = column totals, r = number of blocks, c = number of treatments, and N = total number of 1's. T is expected to follow the χ^2 distribution with $c-1$ degrees of freedom.

Based on a 95-percent degree of confidence, the results of these trials led to the rejection of the null hypothesis that no definite order of preference exists ($P \leqslant 0.05$) according to Cochran's test as seen in Table 2. Therefore, the alternate hypothesis that a ranked order of

TABLE 2

RESULTS OF DEFINITIVE FEEDING TRIALS OF *Siganus spinus*

(FISH PAIRS)	*Enteromorpha compressa*	*Murrayella periclados*	*Chondria repens*	*Boodlea composita*	*Cladophoropsis membranacea*	*Acanthophora spicifera*	*Centroceras clavulatum*	R_i	$R_i(c-R_i)$
1	1	1	1	1	1	1	1	7	0
2	1	1	1	1	0	0	1	5	10
3	1	1	1	1	1	1	1	7	0
4	1	1	1	1	0	0	0	4	12
5	1	1	1	0	0	1	1	5	10
6	1	1	1	1	0	0	0	4	12
C_j	6	6	6	5	2	3	4	32	44

NOTE: Trials tested the order of preference among the seven most preferred algal species and the data used for computation of the Cochran's test for related observations (Conover 1971). c, number of treatments; r, number of blocks; R_i, row totals; C_j, column totals.

preference exists was accepted: (1) *Enteromorpha compressa*, (2) *Murrayella periclados*, (3) *Chondria repens*, (4) *Boodlea composita*, (5) *Cladophoropsis membranacea*, (6) *Acanthophora spicifera*, and (7) *Centroceras clavulatum*. Randall (1961) also found that *Enteromorpha* was one of the two most preferred algae of the convict tang *Acanthurus triostegus sandvicensis* in Hawaii.

DIGESTIVE MORPHOLOGY

Teeth, gill rakers, and pharyngeal structures were examined with the aid of a dissecting scope. Lengths of the gastrointestinal tracts were obtained by unwinding the gut and measuring from esophagus to anus.

Dentition

The upper jaw of *Siganus spinus* overlaps the lower jaw upon closing. The upper teeth are more pointed than the lowers, the lowers being somewhat rounded. Both the upper and the lower teeth are characterized by having a distinct notch and cusp protruding to one side (Figure 1*A*). The number of teeth present in the upper jaw (150–165 mm FL) is about 32; the number in the lower jaw, about 36. Feeding observations both in the laboratory and in the field revealed that the fish takes short quick bites, often biting, then backing away, pulling and cutting the algae as it does so. Sometimes it will make quick lateral jerks with its head as it bites.

Gill Arches and Pharyngeal Teeth

Siganus spinus has four distinct gill arches, the fifth being modified into pharyngeal tooth plates (Figure 1*C*). The gill rakers of the first and second arches are long and pointed, the central axis having lateral processes which may, in turn, branch in the second arch. The rakers of the third and fourth arches become progressively broader and have serrate upper margins. The lower pharyngeal elements of the fifth arch contain numerous fine spinelike projections oriented in a posterior manner on plates. The upper pharyngeals contain thicker thornlike spines (Figure 1*B*).

Gastrointestinal Tract

The stomach typically lies on the left side of the body and may be divided into the cardiac and pyloric regions. The wall of the cardiac stomach is about 0.5 mm thick in most adult specimens, thickening to about 1.0 mm as it constricts and merges into the pyloric region. Both regions are lined interiorly with thin longitudinal folds. The pyloric region constricts near the pyloric ceca, of which there are always five. The large lobe of the liver lies on the left side of the body, the smaller lobe on the right side. The gall bladder lies on the left side along the upper intestinal regions.

The intestinal wall is rather thick (about 0.8 mm), becoming thinner in the lower regions. The interior is lined with numerous tiny papillae. The complex winding nature of the

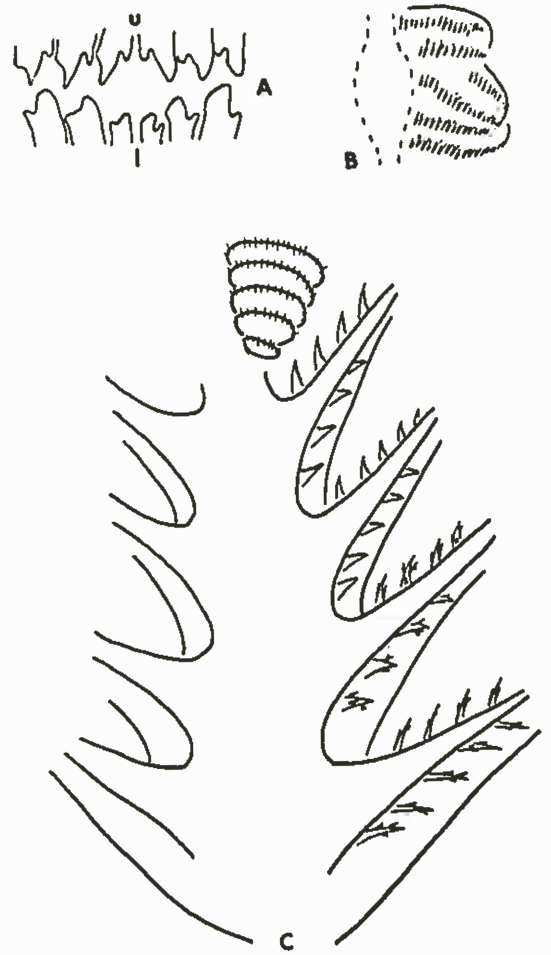

FIGURE 1. *Siganus spinus*. *A*, upper (u) and lower (l) median teeth; *B*, upper pharyngeals; *C*, pharyngeal apparatus showing gill arches 1–4 and the lower pharyngeal bones.

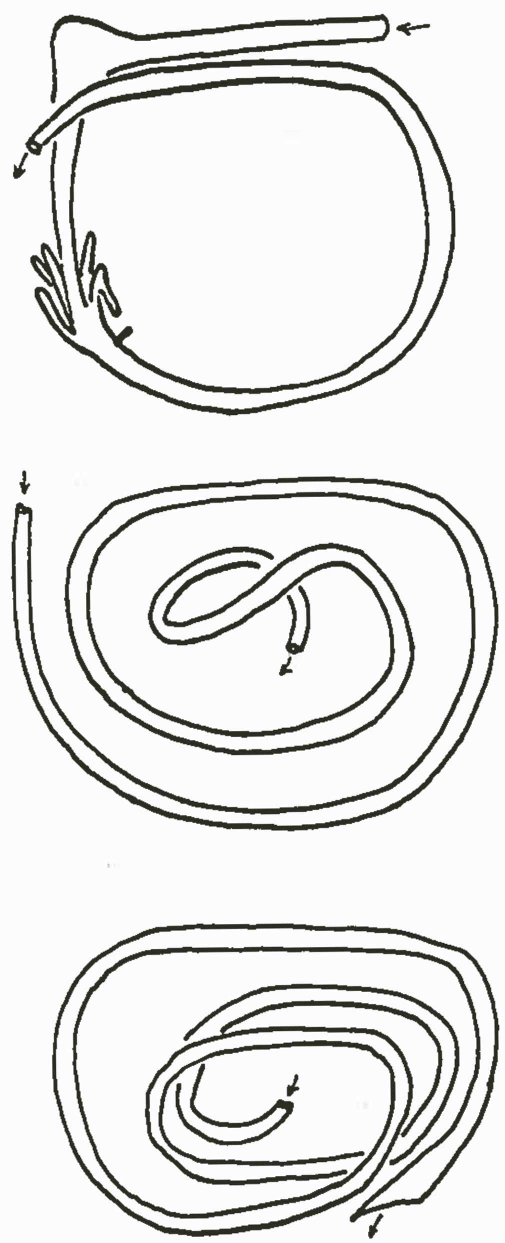

FIGURE 2. Diagrammatic drawing showing the complex coiling of the gastrointestinal tract of *Siganus spinus*.

gastrointestinal tract can be seen in Figure 2 and very nearly duplicates the intestinal winding of that described for *S. fuscescens* by Suyehiro (1942). The length of the gastrointestinal tract of *S. spinus* (102–155 mm FL) is 3.5 to 4.0 times the fork length of the body ($N = 5$).

The amount of time required for food to pass through the gut from the time of ingestion to the time of defecation was determined by feeding *Cladophoropsis membranacea* and *Enteromorpha compressa* to starved fish in a 150-liter tank and then by recording the time of appearance of the first feces. This time may be as short as 1.5 hours in the juveniles, although it usually takes 2 hours or as many as 3 hours in the adults. Because the fish were starved, these results may be biased.

TABLE 3

GROSS ^{14}C ASSIMILATED (%) BY ADULT AND JUVENILE *Siganus spinus* AFTER INGESTING LABELLED *Enteromorpha compressa*

STAGE OF DEVELOPMENT	TRIALS	$\bar{x}$ FORK LENGTH (mm)	GROSS ^{14}C ASSIMILATED (%)
Adults	1	107	20
(N = 2 per	2	114	11
trial)	3	119	7
	4	123	39
	5	131	6
	6	132	8
	7	140	24
Juveniles	1	51	47
(N = 5 per	2	52	60
trial)	3	54	27
	4	59	9

ASSIMILATION EFFICIENCY

A highly desirable method of determining assimilation rates in animals is that of using ^{14}C (Bakus 1969). For each experiment a 12-g blotted wet-weight sample of the most preferred alga, *Enteromorpha compressa*, was allowed to incorporate ^{14}C through photosynthesis. Incubation was made in 250 ml of seawater containing 2 μCi of [^{14}C]$NaHCO_3$ between the hours of 1000 and 1400 in a shaded outdoor area (500–1000 ft-c). The alga was then dipped in a saltwater solution of 0.001 N HCl and rinsed in running seawater for 3 minutes to remove any adsorptive ^{14}C. A subsample was retained and the remainder was fed to either one set of adults (two fish) or one set of juveniles (five fish). In all cases, the fish were previously starved and held in a 150-liter plastic tank with running seawater. Seven trials on adults and four trials on juveniles were run. As they appeared, feces were siphoned onto a paper towel for a period of 1 hour. Samples of the incubated alga at the time of feeding and feces were dried in an oven at 60° C. Equal subsamples of each were weighed to the nearest milligram, ground into a near-powder form, and placed in scintillation vials along with 15 ml of scintillation fluid. The samples were allowed to stabilize in darkness for at least 24 hours before being counted in a Beckman liquid scintillation counter. Percentage of carbon uptake was derived from the formula (fecal cpm/algal cpm) × 100 − 100% = gross (%) carbon assimilated.

The results (Table 3) showed that the amount of carbon assimilated by adults ranged from 6 to 39 percent ($\bar{x}$ = 16 percent). Results of tests on juveniles also varied considerably, ranging from 9 to 60 percent ($\bar{x}$ = 36 percent). These values represent gross assimilation as respiration was not accounted for in the experiments. The extreme nervousness of this fish made any accurate measurements of respiration impossible. In addition, Sorokin (1966), basing his conclusion on experiments by Monakov and Sorokin (1961) with *Daphnia*, decided that ^{14}C losses due to respiration are unimportant in short term experiments (3–6 hours).

DISCUSSION

A comparison of food habit vs. food preference data would seem to be pointless, as it would be expected that most of the preferred kinds of algae would make up a significant portion of the stomach contents. However, the most preferred alga, *Enteromorpha compressa*, grows only in abundance along the intertidal zone of two beach areas on the leeward side of Guam (Tumon and, to a lesser extent, Agana). Similarly, the second ranked alga, *Murrayella periclados*, is relatively rare on Guam, growing mostly intertidally on the basal portions of shaded limestone rocks. The third ranked alga, *Chondria repens*, is often found in inner reef flats. None of the above three algal species were found in the stomach contents of 11 specimens examined from Tumon Bay. Yet, all three algae were available to the fish in large quantities if the fish chose to move close to shore. The nervous behavior of *Siganus spinus* restricts the exploitation of these algae since the postjuvenile fish rarely venture close to the intertidal zones to feed.

Avoidance of blue-green algae by herbivorous acanthurids has been observed by Randall (1961) and by Jones (1968*a*), and for juvenile *S. spinus* and *S. argenteus* by Tsuda and Bryan (1973). Of the four blue-greens tested in food preference studies, only the genus *Schizothrix* was never ingested. This genus was not found in any of the stomach contents, even though it was available to the fish. However, *Calothrix pilosa* rated 5-percent abundance in those fish taken from one area in Pago Bay on the windward coast. These fish were collected in beds of *Sargassum polycystum* where *Sphacelaria tribuloides*, *Calothrix pilosa*, *Hormothamnion enteromorphoides*, *Schizothrix calcicola*, and *Jania capillacea* were growing luxuriantly as epiphytes on the *Sargassum*. *Sphacelaria tribuloides* was most abundant in the stomachs of these fishes. Of the 12 algal species which were never ingested in the food preference multiple-choice trials, only *Jania capillacea* (4 IV) was found in the stomach contents. *Jania capillacea* often grows intermixed with other epiphytic types such as *Sphacelaria tribuloides* and *Calothrix pilosa*.

From stomach samples taken from fish in another area of Pago Bay, *Gelidium pusillum* made up almost 90 percent of those algae found, and *Sphacelaria tribuloides* made up about 5 percent. *Gelidium pusillum*, *Sphacelaria tribuloides*, and *Padina tenuis* were the dominant species of algae found in this area at the time of collection. Similarly, *Boodlea composita*, representing the highest relative abundance (18 percent) and second highest importance value (26) was found almost exclusively in stomach samples taken from the Gun Beach reef flat on the northern end of Tumon Bay on the leeward side of Guam. *Boodlea composita*, *Gelidiopsis intricata*, and *Padina tenuis* were dominant in this area. *Gelidiopsis intricata* represents 15-percent relative abundance and the highest importance value (27) in the food habit data.

Only three species of algae and one annelid worm were found in the pooled stomach contents of 53 juveniles (30–50 mm FL) collected from Pago Bay in May, whereas eight species were found in 13 adults (150–200 mm FL) taken from the same area in March. The algal flora was essentially the same during these months. This suggests that the fish, as it matures, ingests a wider variety of algal types, including such species as *Sargassum polycystum* and *Mastophora macrocarpa*, a lightly calcified alga.

In the food preference tests, over twice as many species of algae were rejected by the juveniles (Tsuda and Bryan 1973) than by the adults. Adults are able physically to bite and ingest the larger, tougher algal species. This factor was probably significant for the high mortalities of starving juveniles recorded by Tsuda and Bryan (1973). They reported on the obvious absence of filamentous algae on the reefs of Guam shortly after the invasion by an extremely numerous run of juvenile *Siganus spinus* and *S. argenteus*. Although both juveniles and adult *S. spinus* prefer the filamentous algae, the young juveniles are probably dependent upon them for survival.

The occurrence of microcrustaceans (2.1 IV) and one annelid worm in the stomach contents is probably due to accidental consumption but may represent a significant supplementary dietary feature in the food of *S. spinus*. In a 42.8-g blotted wet-weight sample of *Centroceras clavulatum* and a 62.3-g sample of *Hypnea pannosa* taken from the outer reef flat of Pago Bay, Larson (1974) found 8,338 microcrustaceans and polychaetes. *Centroceras clavulatum* made up 71 percent of the food found in 13 stomach samples in fish taken from the outer reef flat of Pago Bay where *Centroceras clavulatum* was plentiful. Yet, microcrustaceans and polychaetes were almost absent in the stomach contents.

It is obvious that stomach contents of *Siganus spinus* are indicative only of the particular area where the fish has been feeding prior to capture. The algae must be available to the fish in order to be ingested. Algal availability is dependent on seasonality, zonation, and movement of the fish. Furthermore, preferred algal types will be selected for if they are available. Although a significant food preference exists among these fish, their size and behavior characteristics, combined with algal availability, determine the types of algae ingested by them in the field.

The incising nature of the teeth of *S. spinus* make them well adapted to browsing on benthic plants, particularly the filamentous types. The long coiling gastrointestinal tract increases time for digestion and allows for the almost constant feeding behavior of these fishes. Various similarities exist between the gastrointestinal tract of *S. spinus* and those of other herbivorous fishes, particularly the acanthurids

studied by Al-Hussaini (1947, 1949), Randall (1961), and Jones (1968*a*).

Nutritional studies of aquatic animals in which radioisotopic tracers are used are not new, although most of these studies have dealt with zooplankters (Marshall and Orr 1955, 1956; Lasker 1960; Monakov and Sorokin 1960; Berner 1962; Sorokin 1966). Others (Sorokin and Panov 1966) have investigated the nutritional factors of fish larvae by feeding them ^{14}C-labelled zooplankton. Reports of the use of ^{14}C as a tracer element to obtain an index of assimilation efficiency from the carbon uptake by marine herbivorous fishes are virtually nonexistent in the literature.

Although the assimilation efficiency of juveniles appears to be higher than that of the larger fishes, the wide ranges of assimilation values obtained were probably due to several factors such as individual variation among the fish, stress of the fish under experimental conditions, and variations in particle sizes of the ground up algal and fecal samples used in counting. The figures for average assimilation (16 percent) of *Enteromorpha compressa* by the adult specimens of *Siganus spinus* obtained in this study are not surprising. Johannes and Satomi (1966) determined that the average efficiency of converting food to feces by marine herbivores was about 20 percent. A compilation of literature by Welch (1968) showed that animals with low assimilation efficiencies tend to be plant or detritus feeders. Welch also hinted that animals with low assimilation efficiencies compensate by having high ingestion rates in order to supply enough energy for growth and maintenance. In the case of *Siganus spinus*, which feeds almost continuously, it seems reasonable that the constant ingestion rate would easily compensate for low assimilation.

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Juvenile-Adult Rearing of Siganus (Pisces: Siganidae) in Guam[1]

Roy. T. Tsuda, Patrick G. Bryan[2]

William J. FitzGerald, Jr.[3], and William J. Tobias

Marine Laboratory, University of Guam, P.O. Box EK
Agana, Guam 96910

Introduction

For the past two years, we have been investigating the feasibility of rearing Siganus in Guam waters. The technique of collecting, holding, and rearing these fishes from juveniles to marketable adults is similar to those currently used for the Japanese yellow-tail (Seriola quinqueradiata) in Japan. The information presented here summarizes the results obtained on the biology of this fish and the problems encountered as related to the goal of juvenile to adult rearing.

Species Survey

Only five species of Siganus (Kami et al., 1968) are present in Guam's waters. The five species are S. argenteus (Quoy and Gaimard), S. canaliculatus (Park), S. doliatus (Valenciennes), S. punctatus (Bloch and Schneider) and S. spinus (Linnaeus). Only two of these species, S. argenteus and S. spinus, are common on the reefs of Guam. Sub-adults and adults of S. argenteus are commonly seen beyond the reef margin in deeper water; S. spinus inhabit the reef flats and channels. The other three species are rare in Guam.

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[1]This paper was distributed at the South Pacific Commission, Seventh Technical Meeting on Fisheries, Nuku'Alofa, Tonga, July 15-19, 1974.

[2]Present Address: Fisheries Officer, Majuro, Marshall Islands 96960.

[3]Present Address: Division of Fish and Wildlife, Department of Agriculture, Agana, Guam 96910.

The interest in this fish for juvenile to adult rearing is the observed phenomenon that schools of juvenile Siganus argenteus and S. spinus swarm onto the reef flats around Guam each year during the months of April and May, and occasionally during June and October. Even the time of schooling is predictable and occurs plus or minus two days of the third quarter moon during these months. Each run lasts for about one week. Although the predictability of the occurrence of these juveniles is accurate, the quantity of juveniles swarming the reef flats varies each year to an extent that only a negligible amount was observed in 1973. Figure 1 shows the amount of juveniles estimated each year for a ten-year period. The data was taken by the Division of Fish and Wildlife through Creel Census[4].

To compound our problems in collecting the juveniles for rearing purposes, we found ourselves competing with the local residents in acquiring enough of the juveniles for our rearing experiments. The juveniles or "manahac" are prized by the Guamanians as a delicacy. The majority of the fishermen would not even consider selling the fish and, if they did, at a price of $10 for a 1.5 quart can.

Thus, it seemed more imperative that the production of fry under laboratory condition must be perfected before this maricultural venture is feasible. Recently larval spawning and rearing have been carried out successfully in Palau (May, Popper and McVey, In Press). If mass production of larvae can be perfected, there is a possibility of a market on Guam for juvenile Siganus.

Food Habits

Since Siganus are known herbivores, the obvious initial step in rearing the fish was to gather information on their food habits. Observations of juvenile Siganus spinus and S. argenteus in the field in 1972 revealed that these fish will feed on any algae which they could physically bite and ingest. This was confirmed by a more detailed stomach analyses (N=70) of adult S. spinus (Bryan, 1974) which showed that algal availability, and size and behavior characteristics of the fish determine what kinds of algae were ingested in the field. Westernhagen (1973) also obtained similar results in his report on the natural food of S. striolata (=S. spinus) in the Philippines. Stomach content analyses on 105 specimens of S. canaliculatus, another species which

[4]A corrected version of the yearly harvest is presented by Kami and Ikehara in this report.

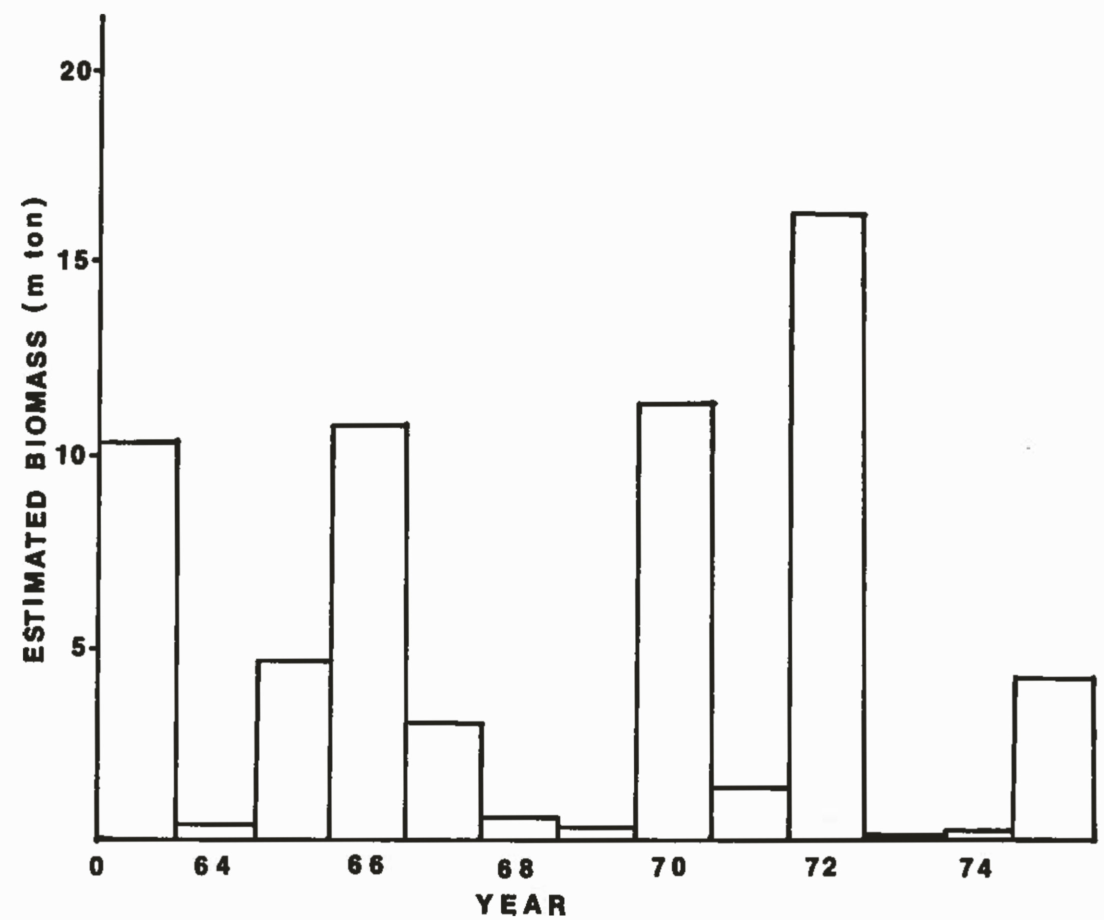

Figure 1. Yearly Biomass (m-tons) estimated from Creel Census taken by personnel of the Guam Division of Fish and Wildlife.

schools in Palau, reveal a different diet. The stomachs of this fish contained predominantly seagrass - Enhalus acoroides and Halophila ovalis. This was not too surprising since all of these specimens were caught in the seagrass beds.

The fact that these fishes are opportunist in their food habits prompted us to investigate which algae they preferred. Approximately 250 juveniles, consisting of an equally mixed population of S. argenteus and S. spinus, were held in a 1000-liter circular plastic tank with running seawater. Forty-five of the common plant genera inhabiting the reefs during the months of schooling were collected and fed individually to the juveniles each morning (0900 to 1000) and evening (1600 to 1700). The amount of plant material used in each trial varied slightly in quantity depending on the consistency of the thallus, but was enough to be consumed by the fishes in less than a minute. Only 10 of the 45 plant genera were always consumed by the juveniles of both species. When the species were separated into two holding tanks, the only difference observed between the food preference of the two species was the avoidance of the green siphonaceous alga Chlorodesmis fastigiata by S. spinus. On the other hand, S. argenteus devoured the alga as soon as it was introduced into the tank. The second difference was the rejection of a matted form of Polysiphonia, a red alga, by S. argenteus; S. spinus actively ingested this alga. The most preferred food of the juveniles (Tsuda and Bryan, 1973) and for adult S. spinus was Enteromorpha, a green alga which grows luxuriantly in the intertidal zone on certain beaches of Guam.

Growth Rates

Since we were not able to obtain any juveniles in 1973 on Guam, the growth rate studies focused on S. canaliculatus which was kindly sent to us by Dr. McVey of the Micronesian Mariculture Demonstration Center in Palau. Growth rate measurements (fork length and weight) were taken every two weeks for 42 weeks on two groups of fish (initially, 50 fish per 1000-liter tank). One group of fish was fed only Enteromorpha; the second group was fed Enteromorpha supplemented by a handful of trout chow (40% protein). The purpose of this experiment was to compare the growth of the fish on only an algal diet, which is more or less their natural diet in the wild, and a diet supplemented with a high protein source. The latter diet should provide some indication of their maximum growth under our holding conditions. After 42 weeks, the group fed Enteromorpha and trout chow increased in weight from 0.5 g to 65 g. This was nearly double the weight of those fishes whose diet consisted only of Enteromorpha (Fig. 2).

Preliminary growth studies on S. spinus and S. argenteus, which were placed in the same circular 7000-liter pool and fed on both Enteromorpha and trout chow, indicate that S. argenteus grows much faster

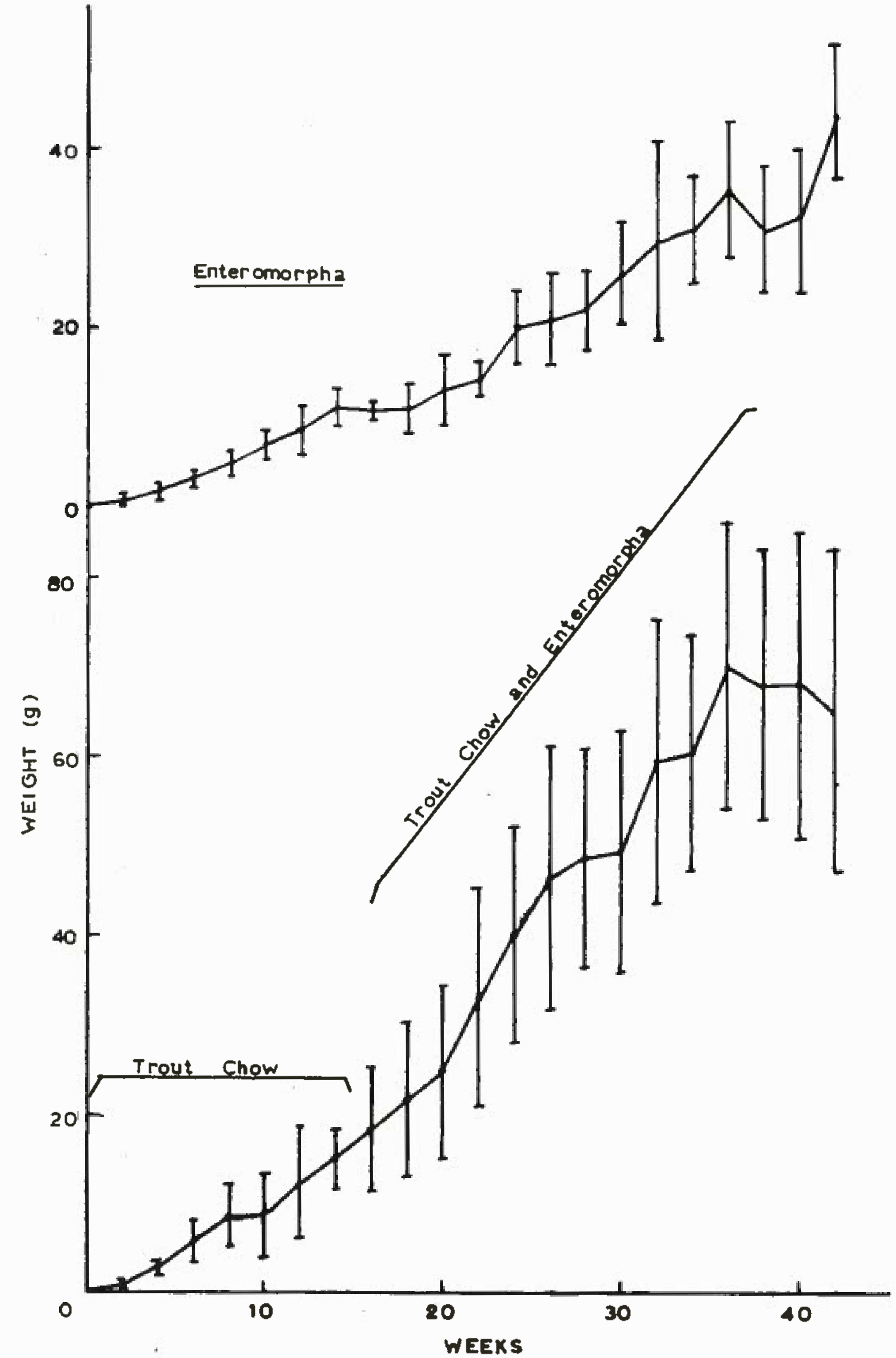

Figure 2. Growth rate of Siganus canaliculatus fed different diets. Each point represents mean of 10 fish; vertical line represents + or - 1 S.D.

than *S. spinus*. Over a 21 week period, *S. argenteus* increased in weight from 3 g to 88 g; *S. spinus* increased in weight from 1.5 g to 14.6 g. Crowding was definitely not a factor since only 30 fishes were present in this tank.

Weight-length data of *S. canaliculatus* held in tanks show a good log-log relationship.

$$W = 0.0163\ L^{2.96}$$

W = grams L = fork length (cm)

A diet based on a mixture of *Enteromorpha*, and *Leucaena leucocephala*, a high protein legume - 35% protein in leaves as denoted by Oakes (1968), in pellet form is presently being tested in a comparative growth experiment with duplicate populations (50 fishes each) of *S. argenteus* and *S. spinus* fed *Enteromorpha* and trout chow. Preliminary results after eight weeks indicate there is an adverse effect on the fishes fed on the legume.

	S. spinus		*S. argenteus*	
	Enteromorpha + Trout Chow	*Enteromorpha* + *Leucaena*	*Enteromorpha* + Trout Chow	*Enteromorpha* + *Leucaena*
Initial Measurements	63.8 mm 4.1 g	45.8 mm 1.55 g	8.56 mm 5.02 g	6.76 mm 4.97 g
Eight week Measurements	94.7 mm 12.08 g	56.37 mm 2.9 g	103.9 mm 17.45 g	71.7 mm 5.19 g

S. argenteus and *S. spinus* fed *Enteromorpha* and *Leucaena* grew 1/3 and 1/4 respectively slower than the *S. argenteus* and *S. spinus* fed *Enteromorpha* and trout chow. Mortality rate was high.

Discussion

Our studies have thus far shown that of the two species which show a schooling behavior on Guam, *S. argenteus* seems to have the faster growth rate than *S. spinus*. Although these fishes are basically herbivores and feed strictly on marine plants in the natural environment, an additional protein source must be included in their diet to promote a higher growth rate. Since trout chow is simply not economically feasible at 30 cents a pound as a high protein food supplement, other sources of local food must be tested. Until this is found, the mariculture of *Siganus* will not be economically feasible.

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Environmental Parameters Influencing the Growth

of Enteromorpha clathrata (Roth) J. Ag.

in the Intertidal Zone on Guam[1]

William J. FitzGerald, Jr.[2]
Marine Laboratory, University of Guam, P.O. Box EK
Agana, Guam 96910

Abstract

Laboratory studies were conducted to determine the light intensity, salinity, temperature, and nutrient levels that would provide optimum growth for Enteromorpha clathrata. The criteria for determining growth were the ratio of net productivity to respiration and mass volume change over a defined incubation period. Environmental conditions providing optimum growth were found to be a light intensity of 2600 ft-c or higher; 30°/₀₀; 25°C; and 150 µg-at/l of N, where the N:P ratio was maintained at 4:1.

Seasonality, zonation, and the influence of substratum were examined in the field. E. clathrata at Tumon Bay occurred year-round, and seasonal variations were correlated to wave height. Additional factors influencing the presence of this alga were wind-generated surge and grazing by herbivorous fish. The zone of E. clathrata growth in Tumon Bay occurred between mean tide level and mean lower low water. Adequate-sized substratum which varied with the degree of water movement, was necessary to maintain a population of Enteromorpha.

INTRODUCTION

Enteromorpha clathrata (Roth) J. Ag., a green alga in the family Ulvaceae, is common in the intertidal zone of certain bays and estuaries

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[1]This paper is based on a thesis submitted to the Graduate Division of the University of Guam in partial fulfillment of the requirements for the Master of Science degree, 1976.

[2]Present address: Division of Fish and Wildlife, Department of Agriculture, Agana, Guam 96910

on Guam. It is an important alga from several economic points of view. Previous studies on Guam (Tsuda and Bryan, 1973; Bryan, 1975) reveal that the genus Enteromorpha is the preferred food of the herbivorous rabbitfishes Siganus spinus and S. argenteus. It is also used as a food source by people in some Asian countries, such as the Philippines and Japan (Hoppe, 1966; Tamura, 1970; Velasquez, 1972). On the other hand, it is viewed as a nuisance by the hotel and tourist businesses on Guam since this alga accumulates on recreational beaches and must be raked and removed frequently. Enteromorpha has been reported as a source of pollution along beaches in Australia by Cribb (1953).

Despite the recognized importance of this alga, information on the major environmental factors which affect its growth, although extensive, is fragmentary. Most of the work concerning these parameters has been in determining the extreme tolerances of the alga. This information is important in ascertaining the possible range of growth in its natural habitat; however, the eury-tolerances have to be narrowed to a defined optimum point for maximum growth. The euryhaline character of Enteromorpha has been shown in various areas of the world by Nasr and Aleem (1949), Biebl (1956, 1962), Carpelan (1957), Taft (1964), Conover (1964), Umamaheswararao and Sreeramulu (1964), Salim (1965), Kapraun (1970), Nienhuis (1970), Woodson and Murley (1970), and Edwards (1972). Kjeldsen and Phinney (1972) found that the salinity tolerance of Enteromorpha exceeded its distribution in its natural habitat. Osterhous (1906) reported this alga's ability to tolerate drastic changes in salinity, i.e., 0 to 35°/₀₀, in cases of fouling on ships that regularly transit from freshwater rivers to salt water harbors.

The eurythermal properties of E. clathrata are evident by its wide distribution in both tropical and temperate waters (Setchell and Gardner, 1920; Taylor, 1960; Bliding, 1963; Kale, 1966; Kapraun, 1970, 1972; Krishnamurthy, 1972). Its tolerance to nutrient polluted waters has been noted by Grenager (1957), Munda (1967), Edwards (1972), and Tewari (1972).

This genus is extremely productive, as demonstrated by Partington and Jennings (1971), who found that thalli grew 91 cm in six weeks after germination from zygote or zoospore. Kanwisher (1966) found a P/R ratio in excess of 20:1 for Enteromorpha.

The purpose of this study is to ascertain optimal levels for those environmental parameters which are most important in influencing the growth of E. clathrata. The parameters discussed here are light, salinity, temperature, nutrients and substratum. In addition, standing-crop measurements were taken monthly to elucidate seasonal patterns over a 15-month period. The knowledge of how each of these parameters influences the optimum growth of Enteromorpha may make it possible to manipulate conditions for optimum algal growth in fish ponds, thus providing a constant natural food supply for fish mariculture. Environmental conditions might also be altered in other areas so as to decrease the growth rate or even eliminate the alga from beaches fronting hotels.

MATERIALS AND METHODS

Field Studies

The field studies were carried out on Guam at Tumon Bay, where lush stands of *Enteromorpha clathrata* inhabit the intertidal zone. This crescent-shaped bay (Fig. 1), stretching for three kilometers, is fringed by a shallow reef-flat platform and at most places bordered by sandy beaches along the shore. There are numerous natural (e.g., groundwater springs and intertidal seepages) and man-made (e.g., hotel storm drains) freshwater runoff areas along the beach.

Standing Crop and Zonation

A standing crop survey was carried out during the period of October 1973 through December 1974 to determine the seasonal availability of *E. clathrata* in Tumon Bay. A partial random sampling method (Kershaw, 1964) was employed at three sites selected along the intertidal zone. These sites encompassed the two extremities and the middle of the bay and were selected so that variation in one area would not dominate the cumulative results. A 50-m transect line was run parallel to the shore through the *Enteromorpha* stand. Ten random samples (0.25 m^2 quadrat) were taken monthly along the transect, and extending 0.5 m to either side. All *Enteromorpha* within each quadrat was collected, pooled with that from other quadrats within the site, dried at 65°C, and weighed. Necessity of drying large quantities of alga required the use of a plant dryer with a maximum temperature of 65°C.

Zonation was determined by measuring the water depth at the upper and lower range of the *Enteromorpha* stand. These depths were expressed in relation to datum (0.0 ft.) by recording the time of depth measurement and correcting it to the table of predicted tides.

The measured time and range of tidal fluctuation in Tumon Bay was found to be equivalent to that predicted. It was then assumed that the actual tide level was also the same as predicted.

Substratum and Water Movement

The relationship between the minimum suitable substratum size and the rate of water movement was determined at the sites selected for the standing crop studies. The degree of water movement served as a means of determining the minimum size of the substratum particles capable of providing sufficient anchorage to prevent the thalli from being swept away.

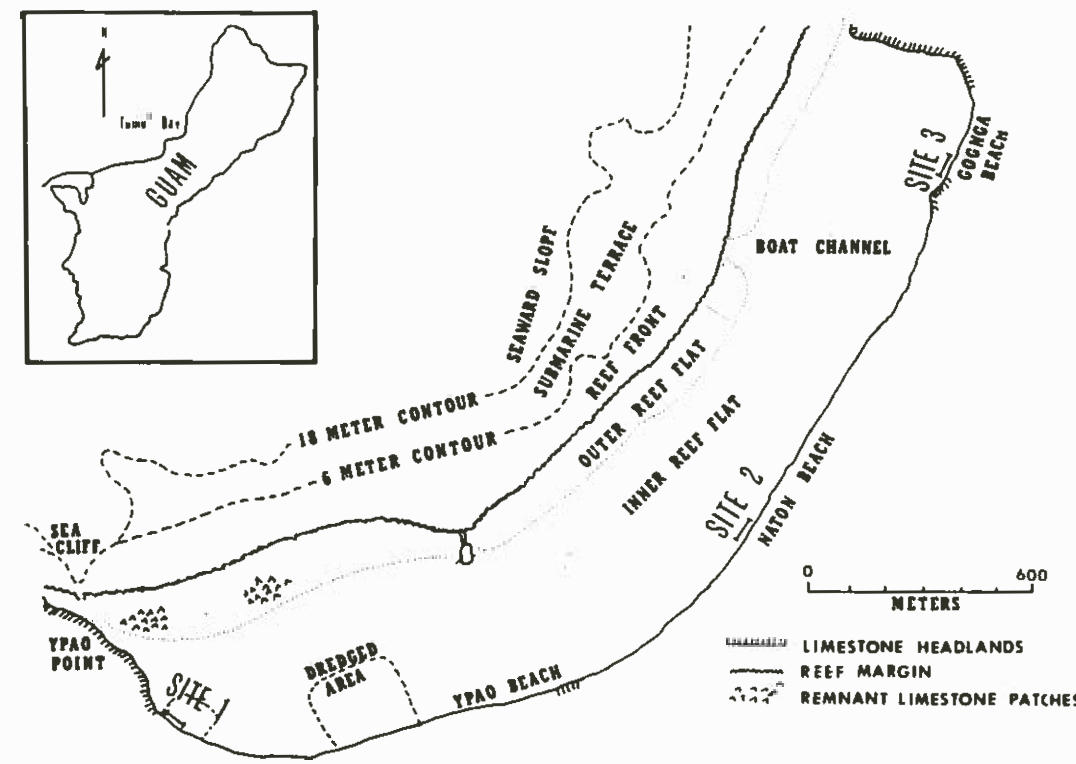

Fig. 1. Map of Tumon Bay, Guam showing standing crop collecting sites.

Water movement was quantified twice by the clod card method (Doty, 1971a). Five clod cards, tied to a cement block were submerged for 24 hours at each site within the Enteromorpha stand. During these two 24-hour periods, substratum particles were collected by removing them from Enteromorpha thalli which were 5 cm or greater in length. Thalli of 5 cm or greater in length, which were rarely supported by sand grains, required a stable substratum for anchorage. Three measurements of diameter with a micrometer were made on each substratum particle to estimate its volume.

Nutrients in Groundwater

Analysis of phosphorus was made by the ascorbic acid method (Strickland and Parsons, 1968) with the use of a spectrophotometer. Reactive nitrate reduction analysis (Strickland and Parsons, 1968) was used for determination of nitrate. The amounts of iron and manganese from groundwater percolating along the shoreline were analyzed by atomic absorption spectrophotometry.

Laboratory Studies

Laboratory studies were carried out to determine optimum conditions of growth under defined conditions.

Light Saturation Point

Light saturation level was determined and then used as a constant throughout the remainder of the laboratory experiments. The compensation intensity, defined by Jenkin (1937) as the light intensity at which photosynthesis and respiration balance over the period of an experiment, was also determined. Since optimum growth conditions were desired, the light intensity that produced the maximum photosynthetic rate was a desired factor.

The standard oxygen light-dark bottle method was used for measuring productivity. The experimental apparatus consisted of a rack holding 11 bottles (5 light, 5 dark, 1 control), each with a volume of 440 ml, submerged in a 15-gallon (57 liter) aquarium, which served as a constant temperature (ambient 28°C) water bath. The bottles were continuously agitated by a motor-driven system connected to the rack. Net productivity and respiration, over a 30-minute period, were measured with a YSI model 51A oxygen meter through a range of 15 different light intensities (50-5000 ft-c) to determine the light saturation point (Ryther, 1956; Kanwisher, 1966; Marsh, 1970). Light intensity was measured with a General Electric Type 213 light meter, which was placed in a water-tight housing and submersed to obtain light readings at the

point where the alga would be contained in the incubation trough. Two runs per day were made at each light intensity between 1130 and 1330, Guam Standard Time, to limit possible variance in the photosynthetic rate resulting from the diurnal rhythms found in some algae (Sweeney and Haxo, 1961).

A total of 10 light and 10 dark bottles constituted the sample size at each light intensity. Freshly collected Enteromorpha with a wet weight of 1-2 g was placed in the incubation bottles with water of known oxygen content. After the incubation period, oxygen measurements were made and the alga was removed, dried at 105°C for 16 hours, and weighed.

Salinity and Temperature

The alga was acclimatized for 24 hours prior to the experimental run since false results can be obtained with the sudden introduction of an alga into a different environmental setting. Often an initial shock response with a rise in photosynthetic rate is followed by a rapid decrease to a stable rate (Nellen, 1966). Acclimatization was conducted in a recirculating tank system (200-liter capacity, 8.6 liters/minute flow rate) under a light intensity of 1000 ft-c with a normal 12-hour light period. Temperature or salinity was varied to meet the experimental requirements. Holding the alga in the recirculating tank had no obvious detrimental effect after a 4-day period.

The incubation apparatus (Fig. 2) was a trough 150 x 17.5 x 30 cm I.D. Water was pumped from a 200-liter capacity tank into the incubation trough and then recirculated back to the tank. A motor-driven rack submerged in the trough provided constant agitation of the incubation bottles (5 light, 5 dark, 1 control; 440-ml volume) to prevent a gradient set up by gaseous diffusion, which might limit oxygen exchange between the algal material and the immediately surrounding water. The same procedures for incubation and measurement of net productivity and respiration (P/R) were used as in the light saturation experiment. The light source was a double layered bank of 12 cool white, 40 watt, fluorescent tubes. This was lowered into position 5 cm above the bottles.

Water samples with six salinity levels (10, 20, 25, 30, 35, and 40°/oo) were obtained by dilution of sea water with distilled water, and by concentration of sea water by freezing. Aquarium heaters were used for raising the water temperature and a Reisea Cooler unit was used for lowering the water temperatures (10, 20, 25, 30, and 40°C).

Nutrient Enrichment

Analyses of E. clathrata for nitrogen (N) and phosphorus (P) content were carried out to obtain a N:P ratio. This ratio was used in the enrichment studies.

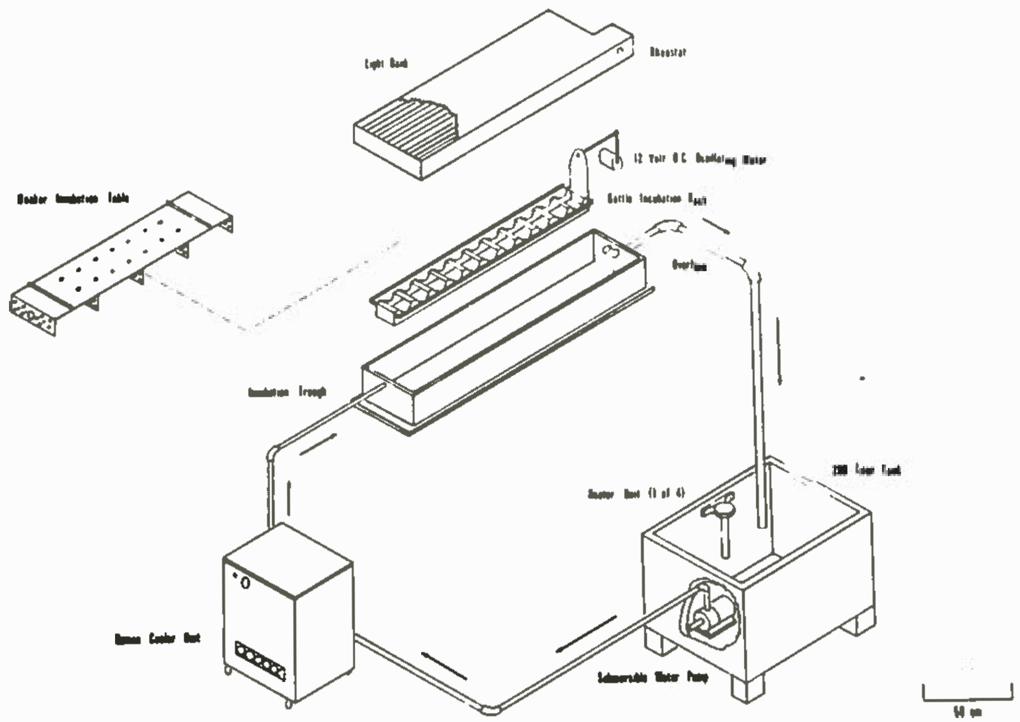

Fig. 2. Apparatus used for the incubation of <u>Enteromorpha clathrata</u> during salinity, temperature, nutrients, and mass growth experiments.

Nitrogen analysis was by the Kjeldahl method (Welcher, 1963). The method used for analyses of total phosphorus was a modification of the Fiske and SubbaRow (1925) method. Five milliliters of concentrated sulfuric acid was added to a sample (1-2 g dry wt.). The <u>Enteromorpha</u> was digested by heating the mixture until it turned brown. At this point the mixture was cooled and 2N nitric acid was added drop wise, followed by additional heating and nitric acid additions until the liquid became colorless and white fumes appeared. After cooling of the liquid, distilled water was added, bringing the total volume to 100 ml. One-milliliter samples were then placed in test tubes. One milliliter of molybate solution was added, followed by distilled water to a final volume of 7 ml. The reducing agent, stannous chloride, was added in powder form. After color development for 5 minutes, absorbency was measured in the colorimeter at 660 nm. Results were plotted against a standard curve obtained by using serial dilutions of a solution containing 1.361 g of KH_2PO_4 dissolved in 1000 ml of distilled water. Controls used were digestion with no sample, with a known amount of phosphate and alga sample, and with sucrose only.

Upon determination of the N:P ratio, nitrate/phosphate enrichment was carried out in the same incubation trough as that used for salinity and temperature. A table baffled to augment uniform water flow for heat exchange was used to support 25 beakers (350-ml volume). The trough was used as a water bath to maintain a constant temperature (25°C ± 1°C). The same light source was used as previously described (Fig. 2).

The nitrogen concentrations were 1, 10, 30, 70, and 130 times that normally found in the sea water system (5 µg-at/l). The phosphorus was added in amounts which matched the ratio of N to P measured in the alga (Table 1). To simplify the enrichment procedure the concentration of phosphate in normal sea water was assumed to be zero. To limit experimental bias due to possible variation in physical condition in the trough, a randomization scheme was used to arrange the samples in the trough. The medium in each of the enrichment beakers was changed once a day. The beakers were cleaned at the same time to limit growth of bacteria and diatoms.

Incubation of the samples was at the optimum salinity, temperature, and light saturation. Simulation of the day length on Guam of 12 hours was maintained during the incubation period. Glycylglycine (0.001 M) was used to maintain a pH between 8.2 and 8.6. The incubation period lasted for 5 days. The growth of the alga was measured by change in volume. To determine the volume a graduated cylinder (25-ml capacity) was filled to a given volume, then a blotted dry algal sample was placed in the cylinder and the change between initial and final volume was recorded. The results were reported as a mass growth factor which was obtained by dividing the final volume by the initial volume. This measurement was found by previous trials to be more reproducible than measurement of wet weight.

Table 1. Nutrient levels used in enrichment experiments.

Factor Increase	NO_3-N µg-at/l	PO_4-P µg-at/l	Fe µg-at/l	Mn µg-at/l
1x	5	0	0.358	0.592
10x	50	14	0.358	0.592
30x	150	38	0.358	0.592
70x	350	89	0.358	0.592
130x	750	189	0.358	0.592

Maximum P/R Quotient and Maximum Growth

Growth as measured by a P/R quotient would assume that the net photosynthesis in excess of respiration would result in the production of organic matter which would be incorporated into the alga, thus being a measure of growth. However, the use of a maximum P/R quotient as the criterion for optimum growth has been questioned as to whether it indicates maximum growth in terms of mass produced. An experiment was run to determine if the maximum P/R quotient and maximum growth are equivalent.

The experimental apparatus consisted of the same set-up used in the nutrient enrichment experiment. The variable was salinity (10, 20, 30, and 40°/₀₀). Measurement of growth was by volume displacement. The incubation period was for 7 days. These results were compared to those obtained by using the P/R quotient.

RESULTS AND DISCUSSION

Standing Crop

The standing crop study showed that *E. clathrata* is present throughout the year in Tumon Bay; however, there are large quantitative variations from month to month (Fig. 3). The mean standing crops at Sites 1, 2, and 3 were 7.5, 44.1, and 20.7 g/m^2 dry weight, respectively, during the 15-month sampling period.

The major physical factor influencing the presence of *Enteromorpha* was the surf condition (as recorded by Fleet Weather Central). Surf breaking 6 ft. (1.8 m) in height or greater on the reef margin at Tumon Bay is strong enough to generate a forceful surge and occasionally small breaking waves along the beach. During periods of high surf the standing crop of *Enteromorpha* is decreased, often to complete elimination. The degree of decrease in the standing crop is dependent on how long the high surf is sustained, and the distance from the reef margin to the beach. Areas with a wide reef flat have the effect of damping the surge generated by the surf, thus decreasing the impact of the surge on the beach. Areas close to the reef margin (Site 1) are very susceptible to surf conditions and are affected even by surf of less height. A highly significant negative correlation (-0.8160) occurred at Site 2 between standing crop and surf heights of 6 ft. or greater. Site 3 showed at all levels tested (4, 5, and 6 ft.) a highly significant negative correlation (-0.7254, -0.7611, and -0.7166) between standing crop and surf heights. This is indicative of Site 3's closer location to the reef margin. The distance from the reef margin to the beach is less here than at Site 2 (Fig. 1). Thus *Enteromorpha* at Site 3 becomes more susceptible to removal by a moderate surf height.

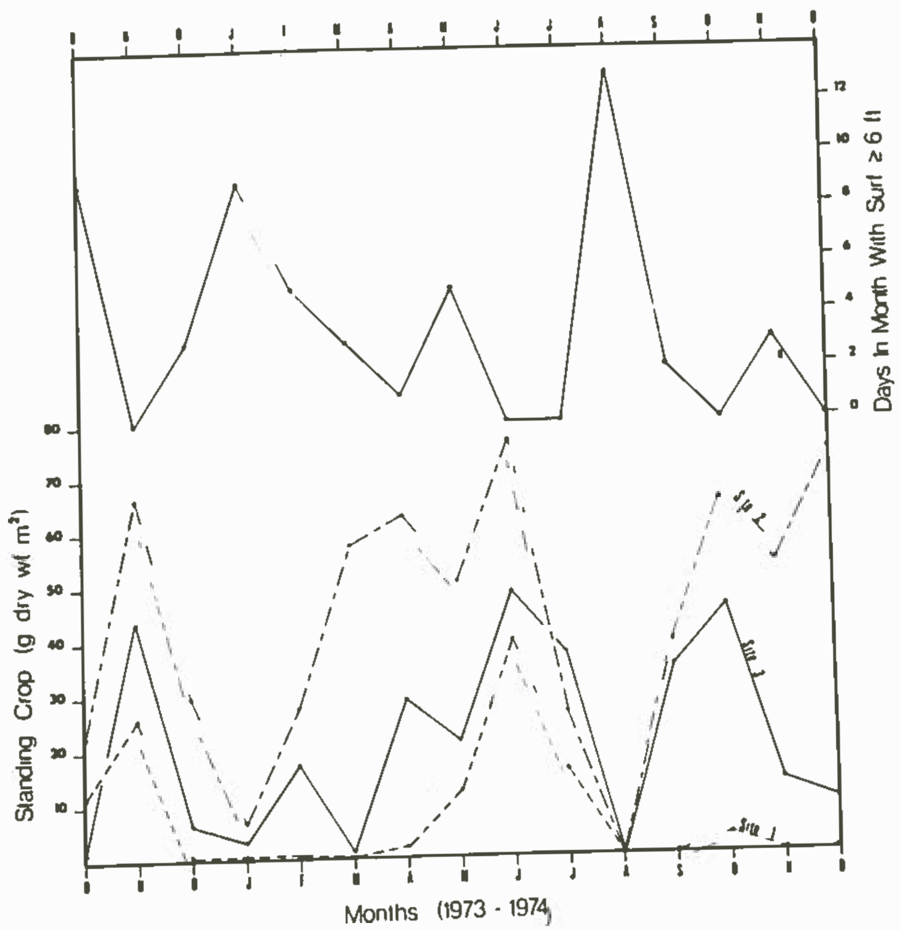

Fig. 3. Standing crop of Enteromorpha clathrata at Tumon Bay during the period of October 1973 through December 1974.

The importance of wave action has been noted by various authors (Stephenson, 1939; Southward and Orton, 1954; Southward, 1958; Kingsbury, 1962; Jones and Demetropoulos, 1968) to be a major factor influencing the presence, zonation, and structure of attached benthic algae. Doty (1971b) discusses the effects of "antecedent events" on modifying the standing crops of macro-algae. He cites the random occurrence of storm-generated waves as such an "antecedent event" in non-monsoonal tropical areas.

During the period of study the physical conditions of Site 1 were drastically altered. A jetty that ran parallel to the reef margin, which sheltered the beach at Site 1, was removed. This exposed the beach to a swift long-shore current. The area immediately in front of the beach was dredged, thus increasing the slope of the beach. Sand was trucked in and distributed over the beach area, further altering the contour and slope. This construction took place from December 1973 through April 1974.

Wind is a factor that also influences the variability in standing crops at Tumon Bay. The prevailing wind is from the NE, thus having the full length of the bay for its fetch, with the greatest resulting surge at the Hilton-Ipao Beach (Site 1). This heavy surge resulting from the wind keeps Site 1 a less suitable habitat for Enteromorpha. This is shown in the monthly standing crops (Fig. 3) and the clod card values (Table 2). The wind factor in the remaining two sites usually has less effect; however, with the occasional change in direction of the wind (i.e., from the southwest) Site 3 along with Site 2 to a lesser extent receives the wind blown surge. Nienhuis (1970) cites the tearing and washing away of algae during autumnal gales as the cause of Enteromorpha's disappearance during winter months in the Netherlands.

Grazing by herbivorous fish also affects the standing crop. Large runs of juvenile Siganus spinus and S. argenteus graze on the Enteromorpha resulting in its complete elimination. This was observed during May 1975 and due to continued grazing, Enteromorpha remained absent until Sept. 1975. This phenomenon was also observed in 1972 (P. G. Bryan, personal communication). Such extrinsic influence could lead to the the false conclusion that E. clathrata is seasonal. During the period of 1973 through 1974 an extremely small run of siganids occurred (Kami and Ikehara, In Press), thus having a minimal effect on the Enteromorpha standing crop.

Zonation

The range of growth of E. clathrata within the intertidal zone of Tumon Bay was found to be from 1.6 to -0.3 ft. (0.49 to -0.09 m). Guam has a semidiurnal tide with a mean tidal range of 1.6 ft. (0.49 m) and a diurnal range of 2.3 ft. (0.70 m). The extreme predicted tidal range is 3.5 ft. (1.07 m). Mean lower low water is datum (0.0 ft.) for Guam

Table 2. Water movement data obtained by the clod card method on August 7, 1974 and December 25, 1975.

	Seaward Side of Jetty 1974	Seaward Side of Jetty 1975	Site 1 1974	Site 1 1975	Site 2 1974	Site 2 1975	Site 3 1974	Site 3 1975
Clod-card Value (C.V.)	7.6 (n = 5, s = 0.46)	4.8 (n = 5, s = 0.2)	4.3 (n = 5, s = 0.31)	10.2 (n = 5, s = 0.15)	4.6 (n = 5, s = 0.93)	6.4 (n = 5, s = 0.29)	8.2 (n = 5, s = 0.39)	7.9 (n = 5, s = 0.32)
Diffusion Index Factor (D.F.)	13.6	26.4	7.7	18.2	8.1	11.4	14.6	14.1
Wind (Knots)*	4-, SW	8-13 NE	4-7 SW	8-13 NE	6-9 SW	2-4 NE	8-12 SW	2-4 NE
Surf Height (ft.)	1-2	2-4	1-2	2-4	1-2	2-4	1-2	2-4

(Randall and Holloman, 1974). Based on this information, the approximate zone of E. clathrata growth is from mean tide level to mean lower low water. During spring mean lower low tides, the upper zone of E. clathrata in Tumon Bay is exposed for approximately 10 hours. The period of exposure is critical in defining the site a species inhabits in the intertidal zone (Doty, 1946).

Townsend and Lawson (1972), using a tidal simulation apparatus, found that the maximum period of emersion tolerated by E. flexuosa under a semidiurnal tidal cycle was 4 hours (25°C and 65-75% relative humidity). Biebl (1938) found that E. linza tolerated emersion for 14 hours at a relative humidity of 83-86%. In another study, Biebl (1956) found E. clathrata to survive for 14 hours at 83.9% relative humidity, but not at 60.6%.

Relative humidity is thus an important factor in determining the tolerated period of emersion. Humidity levels recorded at the center of the island (by the Aerological Branch, Naval Operations) show a mean maximum of 89% and a mean minimum of 66% throughout the year. Relative humidity at the intertidal zone, by nature of its location, would show an even higher level, often saturation.

Substratum and Water Movement

A direct relationship between water movement and minimum size of substratum required to provide anchorage for thalli greater than 5 cm in length was found. A mean minimum substratum size was 0.70 cm^3 (n = 143, s = 0.28) during surf conditions of 2-4 ft. (0.61-1.22 m) with a clod card value of 6.4 (DF = 11.4) at Site 2. Site 1, which is usually exposed to a greater water movement than the remaining sites (Table 2), had a mean minimum substratum size of 2.70 cm^3 (n = 110, s = 1.2) with a clod card value of 10.2 (DF = 18.2) under the same surf conditions and a NE wind of 8-13 knots.

A transplantation experiment was carried out at Site 1 prior to the removal of the rock jetty. The phenomenon of the absence of Enteromorpha on the seaward side of the jetty while it occurred immediately leeward, was examined. Large rocks of 6500 cm^3 or greater with a profuse growth of Enteromorpha were placed at various heights within the intertidal zone on the seaward side of the jetty. After 3-4 days the rocks had been completely denuded of all Enteromorpha, due to abrasion and almost complete sand burial. The factor preventing Enteromorpha's growth on the seaward side of the jetty was the instability of the substratum caused by strong water movement (CCV = 14.8, DF = 26.4).

It becomes evident that the presence of Enteromorpha is dependent upon adequate substratum size to maintain a stable support. The most common substrata supporting Enteromorpha in Tumon Bay were coral rubble, fragments of mollusk shells, and rocks; however, any material that supplied a stable substratum was utilized. Nienhuis (1970) cited the importance of a stable substratum in sandy areas and found a positive correlation between the abundance of Enteromorpha and the presence of suitable substratum. He found an increase of up to 90% in the standing crop of Enteromorpha in areas of adequate substratum. Scoffin (1970) found in his work on the role of marine algae in trapping and stabilizing substratum that the pioneer population of Enteromorpha first became established on large stable objects (e.g., large gastropod shells) followed by the utilization of smaller substratum particles including sand grains as the substratum became stabilized by dense mats of Enteromorpha growth. He found a sand substratum bound by dense Enteromorpha mats to withstand dislodgement by water currents up to five times the velocity required to dislodge sand particles alone.

Site 2, having a low water movement, has a smaller substratum size requirement than Site 1 which has a greater water movement. This is further exemplified by the lack of Enteromorpha growth in the areas adjacent (windward of the jetty) to Site 1, where variables other than water movement - substratum stability are minimal.

Nutrients In Groundwater

The northern half of Guam is formed of limestone which is moderately to highly permeable to water. The water table in this area extends from the shoreline to the interior where it reaches a height of several feet above sea level. Outflow of the groundwater occurs mainly along the shoreline, and is continuous (Randall and Holloman, 1974).

Groundwater introduced along the shoreline by springs and outflows contains high concentrations of nutrients (Table 3). Nitrate showed up to an 8.5 times increase while phosphate did not show a significant increase over reef flat water. Marsh (unpublished data) recorded for groundwater at Tumon up to an 87-times increase of nitrate over reef flat water (due mainly to lower readings on the reef flat). It is evident that the numerous areas of groundwater percolation along Tumon Bay add to the enrichment of the bay, especially the shoreline, and stimulates a rich growth of Enteromorpha (Fig. 4). Boalch (1957) found a positive correlation of the distribution of Enteromorpha with groundwater with high nitrogen levels.

Drainage water from the hotels also adds high levels of nitrate and occasionally high levels of phosphate (12.5 µg-at/l) (Marsh, unpublished data). However, drainage water is of a variable nature, and it is not a reliable source of nutrients for Enteromorpha. On the other hand, a lack of Enteromorpha growth was sometimes observed in the immediate area of drainage plumes, with rich growth to either side of the plumes. This could indicate that some drainage waters contain a noxious element that deters Enteromorpha growth.

Table 3. Water analysis results. The samples were collected on a -0.4 tide at 0100. Surf conditions were 1-2 ft., with a light NE breeze. These conditions resulted in very little water movement on the reef flat.

Source	Distance From Shore m	NO_3 µg-at/l	NO_2 µg-at/l	PO_4 µg-at/l	Fe µg-at/l	Mn µg-at/l	Salinity ‰
Runoff Site							
Groundwater	0	116.79	0.056	0.225	0	0.182	0.5
Groundwater	0	106.03	0.078	0.225	0	0.364	0
Drain Water	0	98.70	1.178	0.238	0	0.182	0
Transect (Perpendicular to the Shoreline)							
Groundwater	0	57.52	0.056	0.325	0	0.182	5.5
Reef Flat	5	18.72	0.199	0.825	0	0.729	14.4
Reef Flat	10	14.24	0.099	0.913	0.537	0.546	22.2
Reef Flat	50	13.97	0.033	0.663	0.716	0.911	30.5
Reef Flat	100	13.58	0.155	0.288	0.716	0.546	30.5

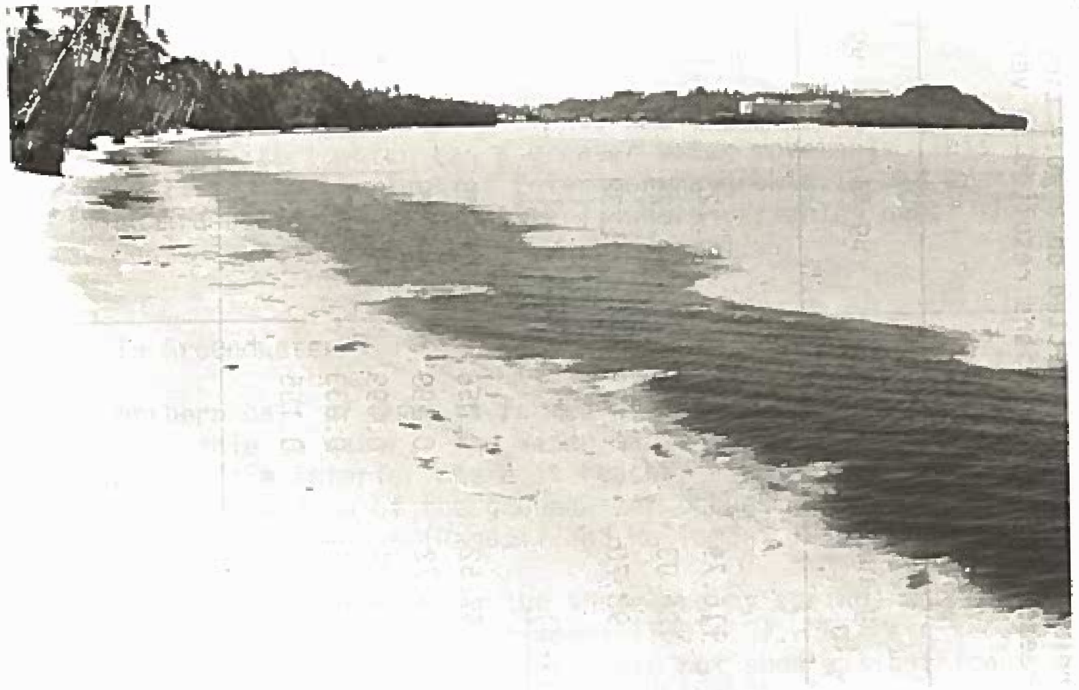

Fig. 4. Growth of *Enteromorpha clathrata* at Site 2, Tumon Bay.

Light Saturation

Light saturation occurs at 2600 ft-c (Fig. 5). Further increase in the light intensity to 5000 ft-c showed no statistically significant effect. Below the intensity of 2600 ft-c the photosynthetic rate was presumably limited by the photochemical stage and above this intensity the enzymatic stage became the limiting factor. No detrimental effect was observed at 5000 ft-c for the duration of the experiment. *Enteromorpha*'s occurrence in the high intertidal zone means that it is commonly subjected to intensities of this level and higher. Nasr and Aleem (1949) point out *Enteromorpha*'s tolerance to high intensity of light in the natural habitat. The compensation intensity was found to be 150 ft-c. Thus *Enteromorpha* has a wide range of light intensities under which it is productive.

Salinity

The optimum P/R quotient for salinity was found at 30°/oo. Both the net photosynthesis and dark respiration showed significant differences (Table 4) among the salinity levels ($p<0.05$). The findings are similar to those of Conover (1964), who found an optimum growth between 25 and 35°/oo for *E.* ~~clathrata~~. Kapraun (1970) found the development of *E. clathrata* to be best in a salinity of 27 ± 2.5 °/oo, and sporulation at 15-30°/oo. These findings indicate that *E. clathrata* grows best in near-to-normal salinity. Significantly lowered salinity by itself has a detrimental effect on the growth of *E. clathrata*. The euryhaline character of this alga can still be recognized in the P/R quotient (Fig. 6) where within the range of 10-40°/oo it is still above the compensation point. Biebl (1956) found *E. clathrata* to tolerate 0.5-2.0 times the concentration of normal sea water for up to seven days. It was also able to tolerate distilled water for three days and recover if transferred back into normal sea water. He attributes the ability to resist plasmolysis in *E. clathrata* to its osmoregulatory mechanism, which is capable of accumulating salts against a diffusion gradient.

The frequent introduction of nutrients along with fresh water may lessen the detrimental effect of lowered salinity. This synergism might be responsible for the frequent occurrence of luxuriant growths of *E. clathrata* in areas of large fresh water effluents. Kier and Todd (1967) found that blooms of *E. prolifera* occurred in a lagoon with consistently high phosphate conditions (13 µg-at/l) under conditions of high (45°/oo) or low (12°/oo) salinity.

Temperature

The optimum P/R quotient for temperature (at 30°/oo) occurred at 25°C. Temperature had a significant effect on the respiration (Table 4) with all levels being significantly different ($p<0.05$). Lampe (1935)

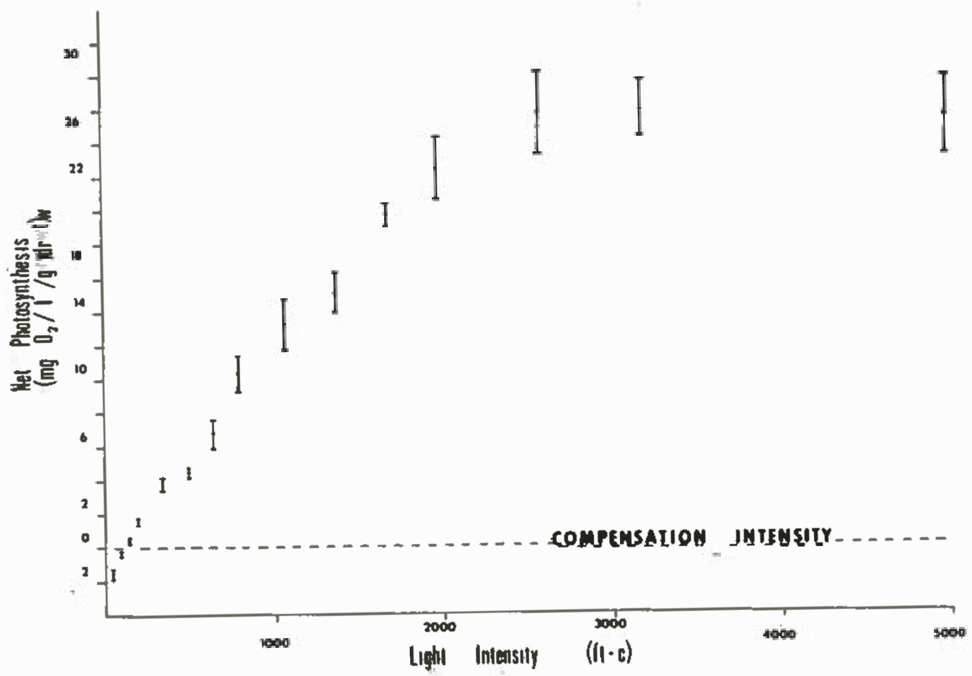

Fig. 5. Light saturation curve for Enteromorpha clathrata (28°C ± 0.5 C, 34°/oo ± 1°/oo).

Table 4. Student-Newman-Kuels Procedure, Multiple Comparisons Test. The means are arrayed in assending order of magnitude. Those sets of means that are underlined are not significantly heterogeneous. Those means that are not connected by a line are considered significantly different (p<0.05).

SALINITY

Photosynthesis n = 10

10°/oo	40°/oo	20°/oo	35°/oo	25°/oo	30°/oo

Respiration n = 10

30°/oo	25°/oo	20°/oo	35°/oo	10°/oo	40°/oo

TEMPERATURE

Photosynthesis n = 10

10°C	40°C	20°C	30°C	25°C

Respiration n = 10

10°C	25°C	20°C	30°C	40°C

NUTRIENTS n = 15

	130x	10x	70x	30x
1x	16x	2x	8x	4x

SALINITY (Mass Growth Indicator) n = 18

40°/oo	10°/oo	20°/oo	30°/oo

showed that eurythermal marine algae are capable of adapting to different temperature regimes; this occurs most rapidly under high light intensities. Associated with a sudden change in temperature there is generally an increase in respiration within 24 hours. E. clathrata shows its eurythermal characteristics by its relatively high P/R quotient through the 10-40°C range (Fig. 5).

Nutrient Enrichment

Nitrogen content of E. clathrata was 4.5% of the dry weight (n = 10, s = 0.526) and the phosphorous content was 1.1% of the dry weight (n = 4, s = 0.096). The resulting N:P ratio is 4:1. This ratio was used as a constant in the enrichment experiment. Imbamba (1972) found a N:P ratio of 1.92:1 in his analysis of the green alga Ulva lactuca. This tissue ratio is very close to the optimum growth enrichment ratio for NH_3:PO_4 (2.26:1) as found by Waite and Mitchell (1972) for U. lactuca. The relative percentages of nitrogen and phosphorous incorporated into the alga's tissue should reflect the ratio required for these nutrients when they are not stored in excess. With this balance one nutrient will not become the limiting factor influencing the utilization of the other nutrient. The N:P ratio in Enteromorpha is probably variable depending on the concentration in usable form of the elements in the medium, as found for phytoplankton (Ketchum, 1939; Ketchum and Redfield, 1949; Ryther and Dunstan, 1971). However, a ratio found in plants growing profusely in a natural environment should reflect a ratio of these elements that is condusive and not limiting to growth.

Data from the nutrient enrichment experiment (Fig. 6, Table 4) showed an optimum at 30x (150 µg-at/l) the nitrate level found in 1x sea water with a N:P ratio of 4:1. This value indicates Enteromorpha's tolerance to high nutrient levels, which makes it a suitable species to inhabit and predominate in polluted eutrophic areas (Grenager, 1957; Munda, 1967; Tewari, 1972).

During the preliminary trials of the enrichment experiment, growth was poor at all levels of enrichment. A factor limiting the growth or limiting the utilization of the enriched medium was suspected. Addition of various combinations and amounts of earth extract, Enteromorpha extract (1000 g Enteromorpha boiled in 1 liter of distilled water for 1 hour), $NaHCO_3$ (possible carbon source), and ammonium chloride (alternate nitrogen source) proved to be of no aid in the further stimulation of growth. However, the separate addition of the micronutrients iron (iron citrate) and manganese (manganese sulfate) both gave positive stimulus to the growth (up to 3-fold increase) of Enteromorpha when added to the enriched medium (NO_3/PO_4). No stimulus to the growth occurred in the 1x medium. Thereafter, iron at a concentration of 0.358 µg-at/l (0.020 PPM) and manganese at 0.592 µg-at/l (0.0325 PPM) were added to all incubation beakers (Table 1) including the controls of unenriched sea water (1x).

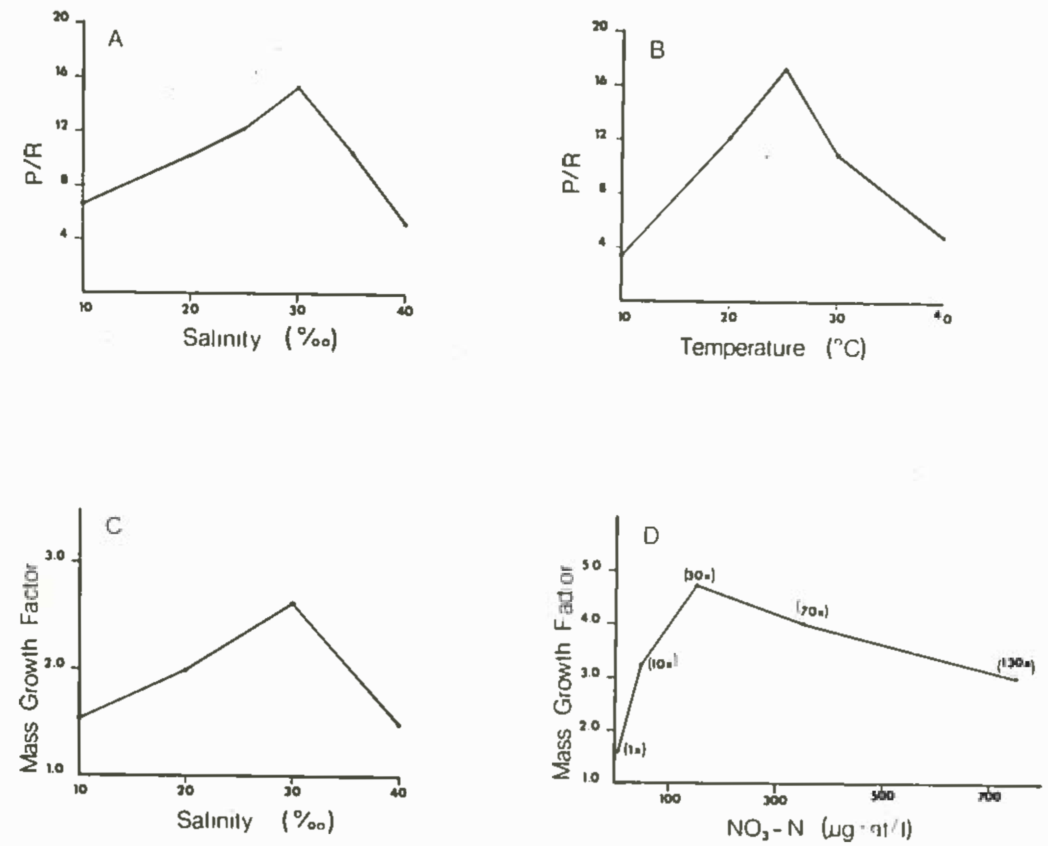

Fig. 6. Optimum growth conditions for Enteromorpha clathrata. A. Optimum salinity at ambient temperature 28°C ± 1°C, B. Optimum temperature at 30°/oo, C. Optimum salinity at 25°C using the mass growth factor, D. Optimum nutrient enrichment at N/ = 4:1, 30°/oo, and 25°C. All experiments were carried out at light saturation (2600 ft-c).

Kylin (1945) showed that manganese (4.5 µg-at/l) exerted a highly significant influence on the assimilation of nitrogen supplied as sodium nitrate in Ulva lactuca. Assimilation of other nitrogen sources, e.g., amides, amino acids, and ammonium, are not influenced by the addition of manganese. Sodium nitrate (72 µg-at/l, N) gave a higher growth rate than ammonium salts when manganese was added. It was suggested (Kylin, 1945) that iron may compensate for the lack of manganese in the medium. Results from this study indicate that iron does in fact stimulate growth when added to a medium containing sodium nitrate as the nitrogen source. Evidence of iron's involvement in nitrate reduction has been found in Chlorella (Trubachev, 1968) and Anabaena cylindrica (Hattori and Uesugi, 1968). Iron was found to be a constituent of the enzyme that catalyzes the stage in nitrate reduction of nitrite to ammonium in Chlorella (Aparicio et al., 1971). The presence of iron in the growth medium of Ankistrodesmus braunii, and Chlorella fusca was found to increase the capacity for nitrite reduction and the level of nitrite reductase (Kessler and Czygan, 1968; Cardenas et al., 1972). Iron's effect on growth is probably primarily through its incorporation in enzymes and porphyrins and its importance in the energy transport system (Walker, 1954; Hewitt, 1958; Epel and Butler, 1970). Its effect on nitrate reduction is possibly mainly involved with an interaction with manganese (Hopkins, 1930; Noack and Pirson, 1939; Alberts-Dietert, 1941; Treharne and Eyster, 1962). Harvey (1966) and Velichko (1968) review the effects of iron and manganese on algal growth, photosynthesis, and respiration.

Foyn (1934a, 1934b) found poor growth of Ulva lactuca and Cladophora subriana in sea water enriched with nitrate and phosphate until the addition of soil extract, which then produced normal growth. De Valera's (1940) work on culturing Enteromorpha intestinalis and E. linza with nitrate as the nitrogen source showed that the addition of earth extract further promoted growth in addition to the iron citrate. The addition of earth extract may have supplied a sufficient quantity of manganese to further promote the utilization of the available nitrate. The ill-defined and varying constituents of earth extract leaves one with no concrete information on what the specific stimulating compound or compounds may have been.

A difference in color of alga samples appeared as incubation progressed. A resulting light green coloration of alga in the 1x medium, a darker green in the 10x, and a very dark green in 30x, 70x, and 130x media were observed. The same result occurred whether the initial alga specimen was of light or dark pigmentation at the beginning of the experiment. De Valera (1940) also noted a color change in cultured E. intestinalis and E. linza, with both species having a pale green color in unenriched sea water and a good green color in enriched medium. This variance in pigmentation intensity occurs naturally in the field. The darkest pigmented specimens were found in areas of drainage or groundwater percolation. Since it was found that groundwater (mainly a nitrate source) and drainage systems are also carriers of enriched nutrients, this pigmentation variance can possibly be used as an indicator of a nutrient-enrichment area. Butcher et al. (1937) found a similar situation

for Cladophora glomerata in its natural habitat, with growth in nutrient rich waters a dark green, richly branched specimen occurred, while in unenriched waters a pale green, densely tufted specimen occurred.

Chlorophyll content can vary due to a number of causes (Emerson, 1929; Mandels, 1943; Granick, 1951; Neeb, 1952; Ichioka and Aron, 1955; Yentsch, 1962); however, it appears in this study to be directly related to the nitrate concentration in the media. Yentsch and Scagel (1958) concluded that under culture conditions of high light intensity, a decrease in chlorophyll was due to the exhaustion of nutrients by active photosynthesis and growth. Nitrogen deficient cells show an increase in chlorophyll content as they recover (Harvey, 1953; Bongers, 1956).

Calculation of the dry weight to volume ratio (0.11:1) of alga specimens grown at the different enrichment levels gave no significant difference. This shows that the growth indicated by the volume change is actually of the increase in tissue matter not interstitial volume by the addition of gaseous spaces of water.

Salinity Mass Growth Factor Indicator

Cultivation of E. clathrata at 10, 20, 30, and 40°/∘∘ showed a significant difference between consecutive salinities in volume change (Table 4). The maximum volume increase occurred at 30°/∘∘ (Fig. 6). This concurs with the results obtained by the P/R quotient method thus supporting the validity of the net excess of photosynthesis above respiration as an indicator of growth (Kanwisher, 1966). However, a direct convergence from a P/R quotient into a corresponding volume of alga is not possible since they are not proportionately equivalent. A ratio for 30°/∘∘/10°/∘∘ resulting from the P/R method gives 2.27:1, and for the growth factor method gives 1.66:1. This would represent a loss of 27% of the organic matter photosynthesized. The P/R quotient seems to be more sensitive near the optimum range. This discrepancy could possibly be due to an increase in extracellular products (Bergland, 1969), with a higher proportion of energy going towards this production, and less towards growth at 30°/∘∘ as compared to that at 10°/∘∘. There has been found to be an excretion of 20 to 40% of the total organic matter photosynthesized in some algae (Lewin, 1956; Allen, 1956; Fogg, 1962; Stewart, 1963; Silburth, 1969; Aaronson, 1971; Guillard and Helleburst, 1971).

CONCLUSIONS

Enteromorpha clathrata shows a wide tolerance to salinity, temperature, and nutrient levels. The optimum conditions for growth are at a light saturation of 2600 ft-c or higher, 25°C, 30°/∘∘, 150 µg-at/l nitrogen, where the N:P ratio was maintained at 4:1. The high P/R values (e.g., in excess of 17 at 25°C, 30°/₀₀, and 2600 ft-c) demonstrate this alga's production capabilities.

Surf conditions, water flow, and grazing by herbivorous fish are the main causes of fluctuations in standing crop.

Adequate substratum is necessary to maintain anchorage and was shown to be a function of the intensity of water movement (e.g., surf, surge, and currents).

Nutrient enrichment of the shoreline by percolating groundwater stimulates a luxuriant growth of E. clathrata. The slight reduction in salinity is secondary to its introduction of high nitrate levels. The optimum salinity (30°/₀₀), being only slightly less than normal sea water, indicates that influx of fresh water alone would not be of a growth stimulating nature, other than to reduce possible competition.

The presence of E. clathrata within the intertidal zone on Guam is dependent on a number of variables, the major ones being substratum stability (as influenced by wave height and currents) and nutrient enrichment. Salinity, temperature, and light intensity would not be limiting factors in most cases.

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Ecology of Siganus argenteus (Pisces: Siganidae) in Relation to its Mariculture Potential on Guam[1]

William J. Tobias
Marine Laboratory, University of Guam, P.O. Box EK
Agana, Guam 96910

Abstract

To assess the mariculture potential of Siganus argenteus on Guam, a study was conducted to integrate the known information on S. argenteus, to provide relevant information of the general biology (i.e., habitat preference, feeding habits, behavior, growth rate, and diseases) of sub-adult and adult fish and to determine their tolerance to environmental parameters (i.e., temperature, salinity, and oxygen), as encountered on Guam's fringing reefs.

Field observations have shown that S. argenteus juveniles migrate from the reef flat to spend their sub-adult and adult lives feeding diurnally on the algal turf of the submarine terrace and seaward slope (3-40 m in depth).

Analysis of stomach contents from 20 sub-adult/adult specimens has shown Tolypiocladia glomerulata (Importance Value=47.8), Halimeda discoidea (IV=24.1), Dictyota sp. (IV=19.3), and Galaxaura marginata (IV=10.4) to be the most important algae consumed in the field. Comparison of stomach content analysis with quantitative field analysis of the dominant macro-algae present indicates that the algal species ingested directly reflect the algal availability of that specific area. S. argenteus exhibits no active food selection, with the exception of possible avoidance of the blue-green alga Schizothrix calcicola.

The growth rate of S. argenteus is faster than S. canaliculatus and S. spinus. Fork length and weight measurements of similar initial size and weight S. argenteus, S. spinus, and S. canaliculatus grown under similar laboratory conditions after seven months were 187 mm/114 g, 124 mm/29 g, and 158 mm/59 g, respectively. Length-weight regression line slopes for S. argenteus, S. canaliculatus, and S. spinus were not significantly different. S. argenteus was sexually mature in 11 months at 201 mm fork length and approximately 150 g.

Some laboratory reared S. argenteus developed symptoms similar to those caused by deficiencies of B-complex vitamins. S. argenteus juveniles develop exopthalmia in water with a mean temperature of 33.2°C ± 1.3°C and 6.68 ppm dissolved oxygen concentration.

The survival rates of fish subjected to water temperatures of 28, 30, and 32°C were 100, 94, and 79%, respectively, over 14 days. Fish in 34°C water had 50% mortality in 2.7 days, 96% mortality in 5 days, and 100% mortality in 8 days. S. argenteus juveniles are tolerant to reduced salinities and oxygen concentrations. The lower lethal salinity limit range was 4-7°/∘∘. The survival of S. argenteus is reduced to 50% after two days at 4°/∘∘ salinity. The growth rate of S. argenteus was not significantly different at salinities of 10, 20, and 30°/∘∘ over a 1-month period.

The 24-hour lower dissolved oxygen concentration limit for S. argenteus held in 48-liter aquaria was 1.0-2.0 ppm. The average fish survival time was 1.5 and 4.6 hours, respectively, for the .5 and 1.0 ppm oxygen experiments; 100% survival was recorded for fish maintained in 2.0 and 3.0 ppm dissolved oxygen concentrations. An oxygen consumption rate of .1 mg l^{-1} hr^{-1} was recorded for fish maintained in 2.0 and 3.0 ppm dissolved oxygen concentrations.

INTRODUCTION

Eight species of siganids (rabbitfish) are reported from Guam; however, only six have been observed recently (Kami and Ikehara, In Press). Of these, only Siganus spinus and S. argenteus support heavy fishing demand. Although both species have high local demand (Bryan, 1975; Kami and Ikehara, In Press) and have juveniles equally available during certain times of the year (Tsuda and Bryan, 1973), the faster growth rate (based on preliminary observations in holding tanks) and the larger attainable size of adult S. argenteus favor this species as the more desirable siganid for mariculture on Guam.

Siganus argenteus (Quoy and Gaimard) is the most widely distributed of the siganid species, occurring from the Tuamotu Islands to the Red Sea (Herre and Montalban, 1928; Fowler and Bean, 1929; Schultz et al., 1953; Hiatt and Strasburg, 1960; Smith and Smith, 1963; Ben-Tuvia et al., 1973; Popper and Gundermann, 1975; and D. J. Woodland, personal communication).

[1]This paper is based on a thesis submitted to the Graduate Division of the University of Guam in partial fulfillment of the requirements for the Master of Science degree, 1976.

S. argenteus juveniles (mean fork length range: 54-64 mm) school onto the reef flats of Guam at the same time as *S. spinus* juveniles (two days plus or minus the last quarter moon during the months of April, May, and occasionally in June and October) and begin to feed on filamentous and smaller fleshy algae (Tsuda and Bryan, 1973). The number of juveniles entering the reef flat areas varies yearly as indicated by creel census figures collected over a 13-year period by the Guam Division of Fish and Wildlife (Kami and Ikehara, In Press). Statistical analysis of creel census information shows no pattern or cycle to these fluctuations.

Little information is available on the general biology of sub-adult and adult fish of this species on the reefs. Some data are available on its habitat preference (Schultz et al., 1953), food habits (Hiatt and Strasburg, 1960), and behavior (Popper and Gundermann, 1975); however, this information is at best fragmentary.

Growth rate experiments have been conducted on a variety of siganids to determine their mariculture potential - *S. rivulatus* (Ben-Tuvia et al., 1973; Popper and Gundermann, 1975), *S. canaliculatus* (Lavina and Alcala, 1973; May et al., 1974; Westernhagen and Rosenthal, 1975), *S. spinus* (Westernhagen, 1974), *S. guttatus* (Westernhagen and Rosenthal, 1975), and *S. luridus* (Popper and Gundermann, 1975). However, there is no published literature on the growth rate of *S. argenteus*. No information is available on the tolerance and growth of *S. argenteus* at elevated temperatures or varying salinities. Oxygen data relevant to siganid culture, particularly tolerance to low dissolved oxygen concentrations often encountered in reef flat environments, are nonexistent.

The purpose of this study is to integrate the known information on *S. argenteus*, to provide relevant information on the general biology (i.e., habitat preference, feeding habits, behavior, growth rate, and diseases) of sub-adult and adult fish, and to determine their range of tolerance to environmental parameters (i.e., temperature, salinity, and oxygen), as encountered on Guam's fringing reefs. The results of the data obtained are of significance in assessing the mariculture potential of *Siganus argenteus* on Guam.

MATERIALS AND METHODS

Field Observations

Field observations of the behavior and feeding associations of sub-adult and adult *Siganus argenteus* were made with the aid of scuba gear on the submarine terrace and seaward slope reef zones off the Tanguisson Power Plant, which is located between Tanguisson Point and Amantes Point on the northwest coast of Guam. *Siganus argenteus* is abundant in this area and provides adequate opportunity for observation.

A description of the submarine terrace and seaward slope reef zones off the Tanguisson Power Plant has been provided by Jones et al. (1976). The first submarine terrace is approximately 100 m in width and slopes gradually from 3-15 m in depth to the seaward slope. The seaward slope, approximately 70 m in width, slopes sharply from 15-40 m in depth to the second submarine terrace.

Stomach Content Analysis

A quantitative analysis of stomach contents was conducted on 20 specimens of *S. argenteus* speared on the submarine terrace and seaward slope reef zones off the Tanguisson Power Plant in water 6-40 m deep. The stomach contents were preserved in 70% alcohol for later identification under the microscope. A modified point method was used to quantify the food items (Jones, 1968a; Bryan, 1975). Relative abundance (percent composition by species), relative frequency (percentage of occurrence), and importance values (relative abundance + relative frequency) were calculated for the items ingested.

The electivity index, $E = \frac{r_i - p_i}{r_i + p_i}$, formulated by Ivlev (1961)

to determine the degree of choice a predator shows in its feeding behavior, was used to determine the electivity for certain algae exhibited by *S. argenteus* in its feeding behavior (E = electivity, r_i = percent composition of an alga in the stomach contents, and p_i = percent composition of that alga in the field). Values of -1, 0, and +1, respectively, indicate complete avoidance, no active selection, and complete selection.

Growth Rates

Holding Tanks

Juvenile *S. argenteus* and *S. ~~Pinus~~ spinus*, netted from the reef flats of Guam in May 1974, were transported to the Marine Laboratory and reared in 1000-liter circular polyethylene tanks, with 50 fish per tank. Each tank was equipped with a recirculating seawater system. Seawater was continuously pumped from the rearing tank by a submersible magnetic pump (Iwaki Pump Co., Model MDS-15) up through an external gravel filter box (64 cm^2; 200 liters), containing crushed coral separated by plastic-coated fiber window screening. Aeration was supplied via a 1/3-hp dri-air pump (Conde Pumps, Model No. 2MMRB).

A diet consisting of the green alga Enteromorpha clathrata and Purina Trout Chow was fed twice daily (0800 and 1700 hours) to groups of S. argenteus and S. spinus (50 fish each) from May to December 1974. Enteromorpha has been shown to be the most preferred food of juvenile siganids (Tsuda and Bryan, 1973). Previous growth experiments with S. canaliculatus (July 1973-February 1974), reared under identical laboratory conditions, revealed that the addition of trout chow with Enteromorpha yielded a faster growth rate in fish (Tsuda et al., unpublished report).

Enteromorpha and trout chow were fed in excess but replenished with a fresh supply twice daily. Excess dry food and fecal material were removed with a siphon hose prior to each feeding. Ten fish were selected at random from each tank and weighed in a preweighed container of seawater (wet weight) on a torsion balance (Torbal, Model PL-12) and measured (fork length) every two weeks during the 7-month period.

Floating Pens

Juvenile S. argenteus were also raised in three floating fish pens from May to December 1974. The fish pens, measuring 1.5 x 1.5 x 1.2 m, were constructed of a synthetic mesh net (.7 cm mesh) held rigid by a 2.5-cm PVC frame and supported by pre-cast foam floats on each corner and in the center. The nets were tied across an intake channel leading to the Piti Power Plant and stocked with approximately 500 fish per net. This group of fish simulated a "wild" population, existing under conditions similar to those in a natural environment, but without predators. This site was chosen because of its good water flow qualities and because it provided protection from possible outside interference.

The fish in each pen were fed approximately 9 kg (20 lbs) of Enteromorpha twice a week; however, due to the occasional unavailability of this alga, it was necessary to alter the feeding pattern to one heavy feeding per week. A natural algal growth, predominantly of the blue-greens Schizothrix calcicola and Microcoleus lyngbyaceus, and the brown algae Dictyota bartayresii and Sphacelaria tribuloides, covered the nets, thus providing an additional food source.

Ten fish selected at random from each of the three nets were weighed (wet weight) on a triple beam balance (O'Haus, Model 700) and measured (fork length) monthly over a 7-month period.

Diseases

An important consideration in evaluating the mariculture potential of an organism is its susceptibility to diseases under controlled rearing conditions. The diseases encountered during the comparative

growth experiments of S. argenteus, S. spinus, and S. canaliculatus are described and causes discussed.

S. argenteus and S. canaliculatus, fed a diet of trout chow and Enteromorpha clathrata, developed symptoms similar to those caused by deficiencies of B-complex vitamins in salmon, trout, carp, and catfish (Lagler et al., 1962; Halver, 1972). To determine if this condition was caused by a vitamin B-complex deficiency, a preliminary vitamin enrichment experiment was conducted for one week with S. argenteus juveniles having various degrees of the symptoms. Nine groups of fish, four fish per group, were given intramuscular injections (.01-.03 mg) of combinations of thiamine (vitamin B_1), riboflavin (vitamin B_2), and pyridoxine (vitamin B_6) at ten times the minimum daily requirement (.15-.68 mg/kg body wt/day) for salmonid fish (Phillip and Brockway, 1957). Information on the vitamin requirements of herbivorous fish is fragmentary. The dosage of ten times that required for salmonids was chosen because the vitamin requirements of herbivores are believed to be greater than those of carnivores (Halver, 1972). Two control groups (four fish per group), one group injected with 1 cc of sterile water and one group not injected, were also monitored.

Environmental Parameters

Thermal Tolerance

Mass mortality of S. argenteus juveniles maintained in laboratory holding tanks has been observed during extreme low spring tides where tidal range is about 1 m and mid-day air temperatures are 29.5-30.4°C on the reef flat. Reef flat water, ranging in temperature from 26.2-32.0°C, exits from the reef flat through a shallow intake channel from which the laboratory draws its seawater supply.

In an effort to determine the cause of the fish kills, the effects of thermal stress (tolerance and growth) on S. argenteus were studied in a thermal simulator apparatus (Jones et al., 1976) consisting of four series of 79 x 59 x 50 cm polyethylene tanks (Series A, B, C, and D), three tanks per series, equipped with standpipes to hold the water level at 39 cm (182 liters). All tanks were set up with an open seawater system and flow rates were regulated at 7.5 liters/min for a tank turnover time of 2.5/hr. Series A tanks received ambient temperature seawater (≈28°C). The water, before entering Series B, C, and D tanks, was regulated at temperatures of +2 (≈30°C), +4 (≈32°C), and +6°C (≈34°C), respectively, in 200-liter holding tanks with a Pac-Tronics temperature controller (Model 1442) and Clepco quartz immersion heaters (Model No. 6-2215-V). The controllers automatically adjust the experimental temperatures to 2-degree increments above the existing ambient temperature of about 28°C. Stratification of heated water in the holding tanks was prevented by vigorous aeration through air stones in the corners. The water temperature in Series A, B, C, and D tanks was continuously monitored via temperature probes with a scanning telethermometer (Yellow Springs Instrument Co., Model 47) and recorder (Yellow Springs Instrument Co., Model 80A). Dissolved oxygen (ppm) was measured at about 0900 hours daily with an oxygen meter (Yellow Springs Instrument Co., Model 51A).

Four sub-adult S. argenteus per tank were measured (fork length) and weighed (wet weight) before and after each temperature experiment, the duration of which was two weeks. The mean range of fish size and weight for the four thermal experiments was 114-119 mm and 24-28 g, respectively. Growth was recorded for fish in each temperature series after the 2-week period.

The fish to be placed in the +2, +4, and +6°C temperature series tanks were acclimated in 16-liter plastic containers, half filled with ambient temperature seawater. An individual container was then placed into the 182-liter tank containing the heated water. When the water temperature inside the smaller container warmed up to within .5°C of the temperature of the larger tank, the fish were released into the larger tank. Acclimation times were less than two hours.

All fish were fed Enteromorpha clathrata and trout chow, a high protein supplement, twice daily (0800 and 1700 hours) during the experiments.

Salinity Tolerance

Salinity tolerance studies were conducted to determine the lower lethal salinity limit and the effects of reduced salinity on the survival and growth of S. argenteus.

The first salinity study was conducted to determine the range of the lower salinity limit. Three resin-coated wood tanks (143 x 84 x 21 cm; 252 liters), two experimental and one control, were filled with seawater of 31.6°/oo salinity. Ten fish were selected at random and placed in each tank. Aeration was added via a diaphram pump (Cole-Palmer, Model P-200) and the tanks were half covered to prevent outside stimuli from exciting the fish.

The salinity was reduced in the two experimental tanks by 10°/oo daily to about 12°/oo and reduced again the following day to 8°/oo by the addition of tapwater that was vigorously aerated for 24 hours to drive off residual chlorine (American Public Health Association, 1971). Prior salinity experiments had shown that S. argenteus will tolerate daily salinity reductions of about 10°/oo from 30 to 8°/oo; behavior and feeding habits remained normal. The salinity was further reduced from 8°/oo in the experimental tanks by 1°/oo daily and percent mortality was recorded.

Daily temperature and oxygen measurements were made at about 0900 hours with a calibrated mercury thermometer (Yoshino, No. 467361) and oxygen meter. Salinity was determined with a refractometer (American Optical Corporation, Model 10402). All fish were fed Enteromorpha clathrata and trout chow daily for the duration of the experiment (10-12 days). Four replicates of the experiment were conducted.

A second salinity tolerance study was conducted to narrow the boundaries of the lower lethal salinity limit and to determine the short term effects of reduced salinity on S. argenteus. The salinity of seawater in a circular 1000-liter polyethylene tank containing 60 S. argenteus (mean fork length: 85 mm; mean weight: 10 g) was reduced from 31.6 to 10°/oo by the method described above. A series of 200-liter tanks (Series A, B, C, and D), three tanks per series, were set up in which the salinity of water in each series had been reduced to 10, 8, 6, and 4°/oo, respectively. Aeration was supplied to each tank.

Twelve fish were selected at random from the 1000-liter tank and four fish were placed in each of the three tanks of each series to test for survival rates. Temperature and dissolved oxygen were recorded daily and pH samples were taken before and after the 96-hour experiment. All fish were fed Enteromorpha daily.

Quantitative observations were made on the fish's response to tactile and light stimuli, equilibrium, coloration, and feeding ability in the reduced salinity water. Normal responses to tactile and light stimuli are to avoid touch contact and to back out of the path of light when disturbed at night by a flashlight beam. Normal equilibrium and coloration responses are exhibited by the fish's ability to maintain a horizontal swimming position and to adapt to background shading. Feeding observations are reported for tank group response.

A third study was conducted to determine the effects of reduced salinity on the growth rate and survival of S. argenteus over a 1-month period. The salinity of seawater in three 1000-liter circular polyethylene tanks was reduced to 10, 20, and 30°/oo, respectively, as described above. Each tank was equipped with a submersible magnetic pump which recirculated the water through an external gravel filter box (64 cm^2; 200 liters). Aeration was supplied via a 1/3-hp dri-air pump.

The fish used in the experiment had been previously held in a tank identical to the experimental tanks. The salinity was reduced to the experimental level by 10°/oo daily (from 30 to 20 to 10°/oo) and the fish selected at random and placed into the corresponding experimental salinity tank. The mean length and weight of fish in the 10, 20, and 30°/oo salinity tanks were 88 mm/10 g (n=26), 90 mm/12 g (n=16), and 90 mm/12 g (n=16), respectively. A greater sample number of fish was inadvertently used in the 10°/oo salinity group; however, crowding was definitely not a problem.

The fish were fed Enteromorpha and a combination of chicken starter crumbles and rabbit feed twice daily, due to the unavailability of trout chow.

Oxygen Tolerance

A series of four oxygen experiments with fish held in water of dissolved oxygen concentrations of .5, 1.0, 2.0, and 3.0 ppm were conducted to determine the lower lethal oxygen concentration limit of S. argenteus. Oxygen consumption rates for S. argenteus were calculated at each level of treatment.

The respirometry tanks consisted of six covered 40-liter aquaria, four experimental and two control tanks. A total of four oxygen experiments were run; one each at dissolved oxygen concentrations of .5, 1.0, 2.0, and 3.0 ppm. These dissolved oxygen levels were selected from a series of pre-trial runs which indicated that the fish's lower lethal oxygen concentration was within these limits.

The dissolved oxygen concentration in the experimental tanks was reduced to the desired level by the addition of nitrogen gas bubbled through air stones placed at opposite ends of the tank. Graham (1949) and Carpenter and Cargo (1957) employed a similar technique to gradually reduce the oxygen content of seawater. Shelford and Allee (1913) studied the behavior of 16 species of fish to gradients of oxygen, carbon dioxide, nitrogen, and ammonia. They concluded that there was no significant response to the nitrogen gradient and the concentration of that gas is of no importance as long as it is not present in such excess as to cause "gas bubble" disease (exopthalmia).

To assure even dispersion of the nitrogen gas and subsequent reduction of oxygen in the seawater, a plastic impeller connected by a glass rod to a small electric motor was used to gently stir the water without disturbing the air-seawater interface. The motor, located above the tank, rotated the impeller at a rate of 110 rpm. The dissolved oxygen concentration was read from a continuously reading oxygen meter (Yellow Springs Instrument Co., Model 54). Czaplewski and Parker (1973) reported the accuracy of the oxygen probe equivalent to or better than the Winkler method. The oxygen probe was placed close to the impeller blades to allow a continuous flow of water past the probe membrane. The uneven rotation of the impeller also imparted a gentle back and forth movement to the oxygen probe. Two small holes were drilled in each of the aquaria lids to allow access for the oxygen probe and impeller-motor apparatus.

Opaque partitions were placed between and at the ends of all tanks containing fish. This eliminated inter-tank stimuli of fish and subsequent hyperactive states which may result in abnormal metabolic increases.

The rabbitfish were randomly selected and transferred from 1000-liter holding tanks (temperature - 28°C; dissolved oxygen - 6.4 ppm; salinity - 33.3°/oo) to a 200-liter tank at 2100 hours while the fish were in a semi-quiescent state. Fish of the same cohort (mean fork length: 116 mm; mean weight: 29 g) were removed and placed directly into the deoxygenated water in the experimental tanks, one fish per tank including the first control tank. Upon addition of the fish into the control tank, the aeration was removed and the fish allowed to slowly deplete the oxygen supply for the duration of the experiment. A second control tank series with no fish, but with the dissolved oxygen reduced to that of the experimental tanks, was monitored separately. The change in oxygen concentration of the water due to diffusion of oxygen from the tank atmosphere across the water interface was recorded at hourly intervals.

If the dissolved oxygen concentration varied more than ± .2 ppm from the previously set concentration, nitrogen or oxygen was added to adjust the oxygen concentration back to the desired level.

The duration of each oxygen experiment was 24 hours or until death of all the fish. Dissolved oxygen readings were taken at hourly intervals for the .5 and 1.0 ppm experiment, 2-hour intervals for the 2.0 ppm experiment, and at approximately 4-hour intervals for the 3.0 ppm experiment. The fish were not fed during the experiment.

Salinity was measured with a hand-held refractometer. Samples for pH determination were taken at the beginning and end of each experiment and measured with a Beckman Expandomatic SS-2 pH meter (Beckman Instrument Co., Model 76). Temperatures were recorded with a calibrated mercury thermometer.

RESULTS AND DISCUSSION

Feeding Habits

Foraging Behavior

Field observations show that after approximately 4-8 weeks of feeding on the reef flat, S. argenteus juveniles (<105 mm fork length) migrate from the reef flat community to spend their sub-adult (105 to <170 mm fork length) and adult (sexual maturity, >170 mm fork length) lives in deeper water of the submarine terrace and seaward slope.

Surge channels and reef flat areas are less frequently used as feeding grounds.

Tsuda and Bryan (1973) concluded after examining 331 dead juvenile *S. spinus* from seven locations around Guam, 74% of which had empty guts, that *S. argenteus* outcompeted *S. spinus* for food. My observations indicate that *S. argenteus* did not outcompete *S. spinus* for food, but simply had migrated off the reef flat. Attempts to net juvenile *S. argenteus* were fruitless and none were observed in reef flat areas where they were caught in abundance several weeks before. Groups of juvenile and sub-adult *S. spinus* (10-200 individuals) were observed in the same area feeding on benthic algae.

Observations made after a fish kill, believed caused by aerial application of the organophosphate pesticide Malathion on a portion of the reef flat on the northwest coast of Guam (May 23, 1975), further support this hypothesis. An estimated 65,000-85,000 fish were reportedly killed in an area of 360 m^2 (Kami, Division of Fish and Wildlife); approximately 40% of the fish were *S. spinus* juveniles and sub-adults and 60% were species other than siganids. No juvenile or sub-adult *S. argenteus* were found. Juveniles of both species first appeared in this area during the first week of April 1975. By the date of the spraying, it is believed that *S. argenteus* juveniles had migrated from the reef flat to the submarine terrace and seaward slope and therefore were not affected by the pesticide in the shallow reef flat waters.

S. argenteus is predominantly a schooling rabbitfish, occasionally occurring in pairs but most often in aggregates of less than 100 individuals, feeding diurnally on the algal turf of the submarine terrace and seaward slope (3-40 m in depth). These results are derived from 50 hours of underwater observation at the Tanguisson station location. Hobson (1972) found that during daylight hours, the behavior of Hawaiian reef fish is dominated by feeding, and twilight and dark periods are dominated by measures to enhance security. As observed by Popper and Gunderman (1975) for *S. rivulatus*, the schools tend to move randomly over larger portions of the submarine terrace and seaward slope.

External body morphology (i.e., fusiform shape, pointed snout, low fins, narrow caudal peduncle, and deeply incised caudal fin) and countershading coloration (blue dorsally overlain with yellow dots and commas with lighter sides) indicate that *S. argenteus* is a free-swimming, open water fish (Herre and Montalban, 1928; Schultz et al., 1953; D. J. Woodland, personal communication). Jones and Randall (1971) commonly found *S. argenteus* on the seaward slope in the vicinity of the Agana outfall. Schultz et al. (1953) stated that this species enters shallow reef flat areas and frequently becomes trapped in pools during low tide; 103 of 106 specimens collected were from Marshall Islands atolls and only three were from Guam. It is believed that his observations reflect those areas where specimens available for study were most abundant. As mentioned by Bryan (1975) for *S. spinus*, it is possible that due to the

flighty or nervous behavior of *S. argenteus* and increased recreational use of the reef flat areas of Guam, sub-adult and adult *S. argenteus* less frequently enter shallow reef flat waters to feed.

Two types of feeding associations commonly occur, conspecific feeding and mixed aggregate feeding. The latter feeding association consists of occasional congeners and fish from the families Scaridae, Labridae, Acanthuridae, Mullidae, and Chaetodontidae. *S. argenteus* occupies a relatively higher position in the water column than would normally be expected for a herbivore.

S. argenteus is the last species of fish to enter the mixed feeding aggregates and the first to leave when approached by a diver. *S. argenteus* in the field approaches the turf substratum at an angle of 45-90° and with one or more quick bites crops the algal turf close to the bottom substratum, occasionally removing calcareous sediments with the turf. When disturbed, the siganids reschool and head for deeper, more open waters. Similar observations have been made by Woodland (personal communication) for this species.

S. argenteus reared in 1000 and 7000-liter tanks under laboratory conditions (both open and closed seawater systems) and fed a diet of *Enteromorpha* and trout chow exhibit schooling behavior similar to wild populations (swimming and feeding together). Occasionally, one or two fish per tank exhibited aggressive displays (i.e., raised dorsal, pelvic and anal fins; altered color pattern different from that exhibited for fright; and lowered head swimming position) and became territorial, defending the area where food was placed in the tank. Similar conditions believed caused by improper diets, were observed by Lam (1974) and Westernhagen and Rosenthal (1975) for *S. canaliculatus* reared under laboratory conditions. I found that *S. argenteus* fed eagerly on pieces of shrimp and fish when dried food or *Enteromorpha* was not available; however, aggressive behavior became more prominent.

Stomach Content Analysis

Stomach content analysis (Table 1) of 20 sub-adult and adult specimens of *S. argenteus* collected during 1974-1976, ranging from 133-256 mm fork length and 38-284 g in weight, indicate a more diverse diet than the juveniles and reflect the ability of larger fish to bite and ingest a greater variety of fleshy and calcareous algae. *Dictyota*, *Gelidium*, *Bryopsis*, *Jania*, and *Codium*, avoided by the juveniles, were consumed by the larger fish (respective importance values of 19.3, 6.1, 5.8, 4.5, and 1.6). The red alga *Tolypiocladia glomerulata* had the highest importance value of 47.8; the highly calcified green alga *Halimeda discoidea*, rejected by juvenile *S. argenteus*, was the second most important food item in the stomach analysis (IV=24.1). *Galaxaura marginata*, a calcareous red alga, had an importance value of 10.4. The highly calcified green alga *Neomeris annulata* (IV=5.0) was also common.

Table 1. Relative abundance (RA), relative frequency (RF), and importance value (IV) of stomach contents from Siganus argenteus (n=20), speared from the submarine terrace and seaward slope reef zones off the Tanguisson Power Plant, 1974-1976, compared to the RA, RF, and IV of algae quantified in the same area by R. T. Tsuda, July 13, 1970. Food items are arranged in the order of importance value. A modified point method (Tsuda, 1972) was employed to quantify the benthic algae by placing a 25 cm^2 quadrat frame, subdivided into 5 cm^2, five times around designated transect stations so that overlapping occurred.

SPECIES	STOMACH CONTENTS RA	STOMACH CONTENTS RF	STOMACH CONTENTS IV	FIELD OCCURRENCE (61% algae, 31% rock, 7% live coral, 1% invertebrates) RA	FIELD OCCURRENCE RF	FIELD OCCURRENCE IV
Tolypiocladia glomerulata	34.8	13.0	47.8	36.3	18.9	55.2
Halimeda discoidea	17.9	6.2	24.1	13.0	20.8	33.8
Dictyota sp.	9.7	9.6	19.3	8.3	7.5	15.8
Galaxaura marginata	5.6	4.8	10.4	4.4	7.5	11.9
Microcoleus lyngbyaceus	3.0	6.8	9.8	2.0	1.9	3.9
Antithamnion sp.	4.5	4.1	8.6	-	-	-
Ceramium sp.	1.6	6.8	8.4	3.9	1.9	5.8
Leveillea jugermannioides	2.6	5.5	8.1	-	-	-
Gelidium sp.	2.0	4.1	6.1	-	-	-
Bryopsis pennata	2.4	3.4	5.8	-	-	-
Neomeris annulata	.9	4.1	5.0	-	-	-
Sphacelaria furcigera	1.0	4.0	5.0	-	-	-
Jania sp.	1.8	2.7	4.5	9.3	5.6	14.9
Feldmannia indica	2.1	2.0	4.1	-	-	-
Cladophoropsis sp.	1.6	2.1	3.7	-	-	-
Calothrix pilosa	1.7	1.4	3.1	-	-	-
Rhodymenia sp.	.7	2.0	2.7	-	-	-
Sargassum sp.	1.2	1.4	2.6	-	-	-
Sponge/spicules	.6	2.0	2.6	-	-	-
Polysiphonia sp.	1.0	1.4	2.4	-	-	-

Table 1. Continued

SPECIES	STOMACH CONTENTS RA	STOMACH CONTENTS RF	STOMACH CONTENTS IV	FIELD OCCURRENCE (61% algae, 31% rock, 7% live coral, 1% invertebrates) RA	FIELD OCCURRENCE RF	FIELD OCCURRENCE IV
Chondria repens	.3	1.4	1.7	-	-	-
Schizothrix calcicola	.2	1.4	1.6	1.5	3.8	5.3
Codium edule	.9	.7	1.6	-	-	-
Laurencia sp.	.8	.7	1.5	-	-	-
Enteromorpha tubulosa	.1	1.4	1.5	-	-	-
Ectocarpus sp.	.4	.7	1.1	-	-	-
Benthic diatoms	.1	.7	.8	-	-	-
Fish scales	.1	.7	.8	-	-	-
Acrochaetium sp.	<.1	.7	.7	-	-	-
Anadyomene wrightii	<.1	.7	.7	-	-	-
Padina (vaughaniella)	<.1	.7	.7	-	-	-
Asterocystis ornata	<.1	.7	.7	-	-	-
Annelid worm	<.1	.7	.7	-	-	-
Copepod	<.1	.7	.7	-	-	-
Foraminifera	<.1	.7	.7	-	-	-
Porolithon onkodes	-	-	-	11.1	7.5	18.6
coralline sp.	-	-	-	4.4	5.6	10.0
Halimeda opuntia	-	-	-	1.5	5.6	7.1
Lobophora variegata	-	-	-	2.0	3.8	5.8
Amphiroa sp.	-	-	-	1.0	1.9	2.9
Lithophyllum kotschyanum	-	-	-	.5	1.9	2.4
Chlorodesmis fastigiata	-	-	-	.5	1.9	2.4
Hemitrema sp.	-	-	-	.5	1.9	2.4

The rank order of representation by algal divisions of the stomach contents is 42% reds, 27% greens, 21% browns, and 10% blue-greens.

Of particular interest is the relatively high frequency (RF=6.8) and importance value (IV=9.8) for the blue-green alga Microcoleus lyngbyaceus. The absence of blue-green algae in the diets of herbivorous fish have been reported by Randall (1961) and Jones (1968b) for acanthurids and by Tsuda and Bryan (1973) and Bryan (1975) for juvenile S. argenteus and S. spinus and adult S. spinus.

It would appear from the present analysis that micro-invertebrates are not significant in the diet of S. argenteus; however, it has been suggested that micro-invertebrates may represent a substantial dietary supplement for S. spinus (Bryan, 1975).

Tsuda and Bryan (1973) determined that juvenile Siganus argenteus and S. spinus were highly selective in the type of algae ingested and exhibited some food preferences. Chlorodesmis fastigiata was avoided by S. spinus and devoured by S. argenteus, and Polysiphonia sp. was rejected by S. argenteus but devoured by S. spinus. As juveniles, both siganids prefer filamentous algae and avoid blue-green and calcareous algae.

Hiatt and Strasburg (1960) provide additional data on the food habits of sub-adult and adult S. argenteus, based on 15 specimens collected from the Marshall Islands. They report 60% calcareous material ingested with scraped algal filaments in the stomach contents. Although this species is described to be a typical browsing herbivore, they have observed adult S. argenteus in the vicinity of garbage dumps where they reportedly consume waste meat scraps. This indicates that it is not an obligatory herbivore and that its feeding and digestive apparatus can convert to a carnivorous diet on occasion.

A comparison of the most important algal species identified from the stomach content analysis agrees quite well with the important algal species found by R. T. Tsuda in July 1970 on the submarine terrace and seaward slope (Table 1) off the Tanguisson Power Plant. Tolypiocladia glomerulata, Halimeda discoidea, Dictyota sp., and Galaxaura marginata had importance values of 47.8, 24.1, 19.3, and 10.4, respectively, from stomach content analysis and 55.2, 33.8, 15.8, and 11.9, respectively, from field analysis.

The electivity index of Ivlev indicates no active selection for Tolypiocladia glomerulata (-.02), Halimeda discoidea (+.16), Dictyota sp. (+.08), Galaxaura marginata (+.12), or Microcoleus lyngbyaceus (+.20). An electivity index of -.76 for the blue-green alga Schizothrix calcicola indicates possible avoidance. It would appear that the algal species ingested directly reflect the algal availability of that specific area.

Morphological Adaption for Feeding

Table 2 presents proportional measurements and angles of possible significance in feeding of juvenile, sub-adult, and adult S. argenteus and S. spinus. The measurements used to determine morphological adaptations for feeding were those used by Jones (1968b) in comparing Hawaiian and Johnston Island Acanthuridae. S. argenteus is larger than its congener in all respective age classes as indicated by standard length, standard depth, and head length measurements. The increasing head length sets the eye back on the head allowing for a more horizontal line of sight. The ability of S. argenteus to see in a horizontal plane would also aid in predator recognition in the open waters of the submarine terrace and seaward slope. The smaller eye/mouth angle of S. argenteus (as with N. hexacanthus and N. brevirostris) indicates that the mouth is located higher on the head more in line with the eye so the fish must look ahead for its food. Small eye/mouth angles may be advantageous for certain herbivores in selecting individual algal thalli (Jones, 1968b).

Preliminary observations of S. argenteus for the presence of a notch and cusp on both upper and lower teeth, premaxillary overlap of dentary, and feeding behavior on filamentous algae in the laboratory are similar to those described by Bryan (1975) for S. spinus.

Growth Rates

Figure 1 presents the growth rates of Siganus argenteus in comparison to S. spinus and S. canaliculatus fed diets of Enteromorpha and trout chow and held in similar holding tanks. S. canaliculatus, reared from July 1973-February 1974 were fed only trout chow for 15 weeks before the addition of Enteromorpha. Temperature, salinity, and oxygen varied from 26.7-29.8°C, 31-33°/oo, and 4.8-5.8 mg/l, respectively, during the growth rate experiments. Nephelometric Turbidity Units and pH ranged from .27-.42 and 8.1-8.6, respectively. In seven months, the mean fork length and weight of S. argenteus was 187 mm and 114 g, compared to 124 mm and 29 g for S. spinus, and 158 mm and 59 g for S. canaliculatus. S. argenteus, raised in a 7000-liter circular tank with an open seawater system, and fed the same Enteromorpha and trout chow diet, measured 270 mm in fork length and weighed 390 g in 11 months. Similar growth rates were obtained for S. argenteus reared in floating cages with carangids in Tahiti (A. Michel, personal communication).

S. argenteus and S. canaliculatus remained parasite-free for the 7-month comparative growth-rate study. However, after three months, the S. spinus group suffered heavy losses due to infestation of the fish's gills by a parasitic monogenic trematode and its growth rate was significantly reduced.

Table 2. Comparative measurements and angles of possible significance of *Siganus argenteus* and *S. spinus* which may indicate morphological adaptations for feeding. j=juvenile, s-a=sub-adult, a=adult; FL=fork length, SL=standard length, SD=standard depth, SNT=snout length, EYE=eye diameter, HL=head length, EYE/MOUTH=angle between eye and mouth. Each measurement represents the mean of 10 fish per age class. All measurements are in millimeters except the EYE/MOUTH angle which is measured in degrees.

SPECIES	FL			SL			SD			SNT			EYE			HL			EYE/MOUTH		
	j	s-a	a	j	s-a	a	j	s-a	a	j	s-a	a	j	s-a	a	j	s-a	a	j	s-a	a
Siganus argenteus	53.8	105.0	222.6	49.6	95.1	203.3	15.4	32.0	74.2	4.9	9.3	19.1	3.8	7.4	14.9	12.8	24.9	48.7	22.6	31.8	31.7
Siganus spinus	41.5	89.0	190.0	37.3	76.0	164.3	12.2	27.6	54.3	3.5	7.2	16.1	3.5	7.4	14.5	10.4	20.4	39.7	32.8	38.0	41.2

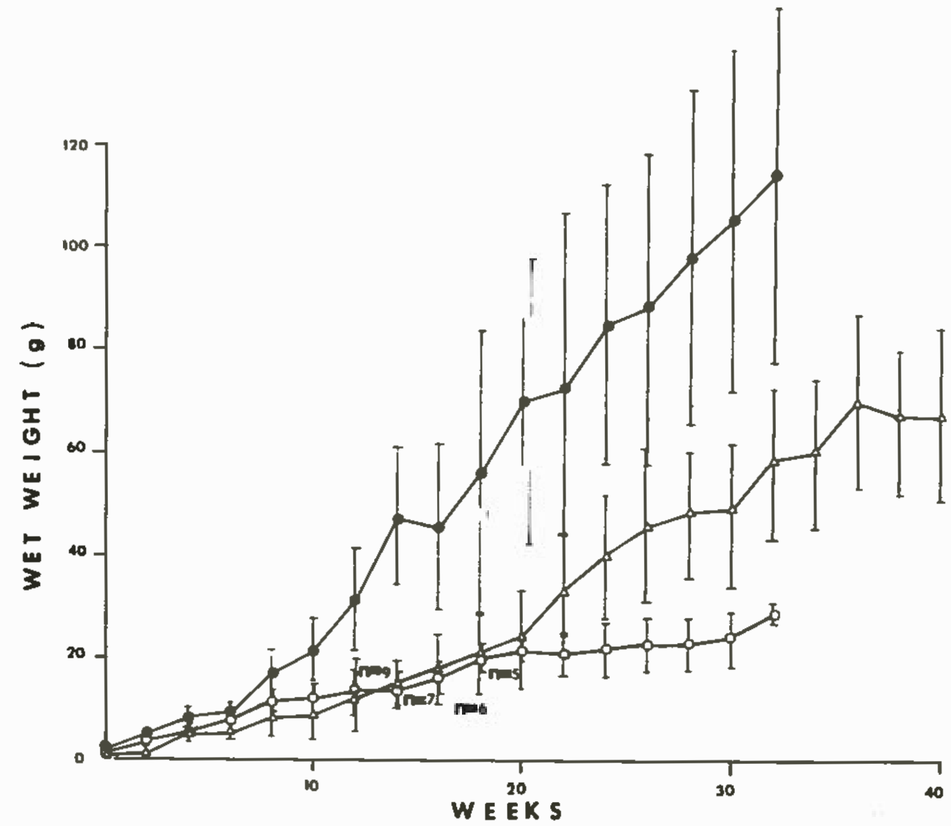

Figure 1. Growth rates of *Siganus argenteus* (●), *S. canaliculatus* (Δ), and *S. spinus* (o) raised in a closed system and fed *Enteromorpha clathrata* and trout chow (*S. canaliculatus* was fed only trout chow for 15 weeks before addition of *Enteromorpha*). Each data point represents the mean of 10 fish except as indicated for *S. spinus* (after 18 weeks, n=5).

Figure 2 shows the length-weight relationship of S. argenteus, S. canaliculatus, and S. spinus reared under similar laboratory conditions in a closed seawater system. Regression line slopes for S. argenteus, S. canaliculatus, and S. spinus were not significantly different.

The growth rate of S. argenteus held in the three floating pens for the first four months was slow (72 mm/5 g final length and weight) compared to the laboratory group (141 mm/46 g). This is attributed to insufficient food supply, competition for food between siganids, competition for food by other herbivorous fish, and/or crowding.

When Enteromorpha was placed in the holding pens, a natural competition for food between siganids resulted, with the larger, more aggressive fish eating more algae than the smaller, slower growing fish. This was evident by the high standard deviation of the fish group in the floating pens ($\bar{x}$=47.3 g, S.D.=22.4 g, n=10) at the end of the 7-month period compared to similar size fish reared in the laboratory ($\bar{x}$=47.3 g, S.D.=13.9 g, n=10) for three months.

In constructing the holding pens, a net mesh size was chosen which would retain the juvenile fish and algae food and allow for good water flow through the net. The initial stocking density of 500 fish/net (2.7 m^3/fish) provided ample room for growth. However, algal thalli extending outside the net provided food for the resident herbivorous fish population in the intake channel, thus decreasing the food source to the confined siganids. Fish of the families Scaridae, Pomacentridae, and Acanthuridae were observed feeding on Enteromorpha hanging through the net.

No sexually mature S. argenteus were observed at the end of the 7-month growth experiments. A second group of S. argenteus (n=41) reared in a 7000-liter tank from June 1975- May 1976 with an open seawater system and fed Enteromorpha and trout chow were sexually mature in 11 months. The mean fork length of these fish was 201 mm (approximately 150 g). Popper and Gundermann (1975) have reported that S. argenteus may reproduce at a larger size than other siganids, based on ripe field specimens collected in the Red Sea (450-550 g). However, S. argenteus in Tahiti showed maturation signs at 200 g and natural spawnings were observed during the months of September, October, November, and December. The eggs were pelagic, 700 microns in size. An induced spawning attempt with human chorionic gonadotropin hormone has also been successful.

Diseases

Behavioral observations made of fish suffering from possible vitamin B-complex deficiencies (approximately 25% of laboratory stock

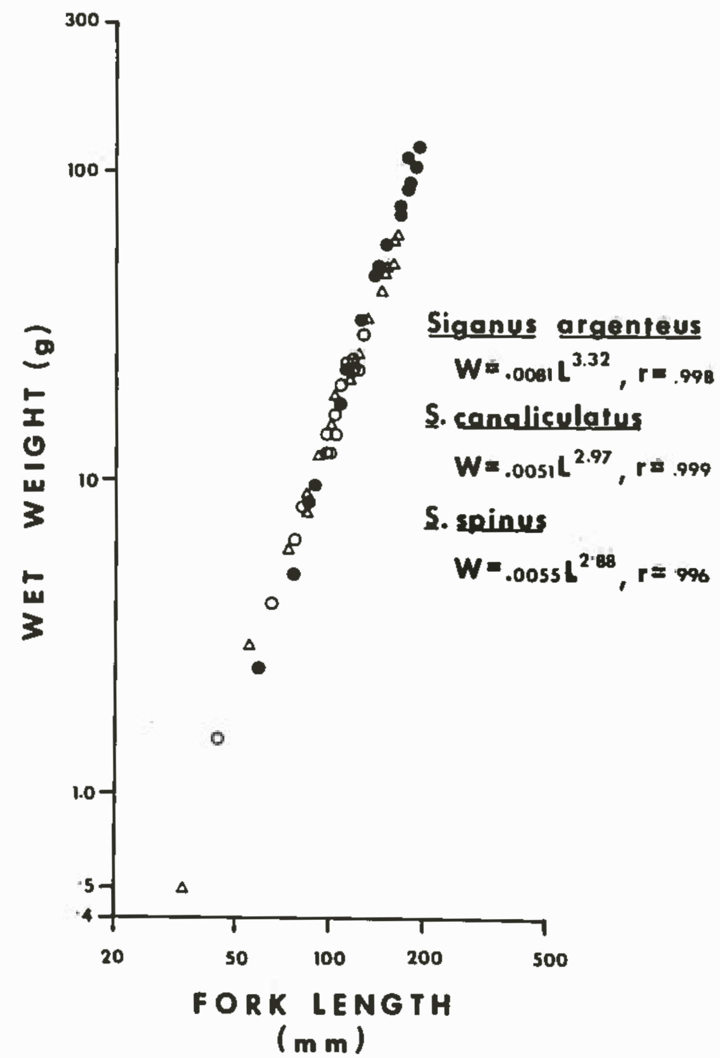

Figure 2. Logarithmic length-weight relationship of Siganus argenteus (●), S. canaliculatus (Δ), and S. spinus (o) raised under similar conditions in a closed seawater system. Each point represents a mean of 10 fish (except S. spinus over 101 mm, n=5). Regression line equations are given (r=coefficient of correlation).

at times) showed a decrease in feeding efficiency, resulting in poor growth. In addition, the fish became dark in color and were unable to change their coloration pattern to blend in with background surroundings. Swimming activity decreased and the fish remained in one area of the tank. Eventually, all fish in this condition regressed to a continual swimming stage, in which the lens of the eye became cloudy, and mono- or bilateral cataracts developed. The lens of the eye later became transparent and feeding ceased altogether. Some fish survived in this condition for several weeks until death resulted from apparent starvation.

During the growth experiments, it was noticed that the trout chow had become damp and partially moldy. Commercial feeds, such as trout chow, will retain thiamine during dry storage; however, upon contact with moisture, thiaminase hydrolysis occurs and the thiamine moiety is destroyed (Halver, 1972). The coloration and activity of the fish did improve slowly over three months when they were fed fresh trout chow,and Enteromorpha; however, no improvement was observed in fish with eye cataracts.

After seven days of vitamin injections, results from the preliminary vitamin enrichment experiment have shown that the activity and coloration of 8% of the vitamin-injected fish improved, but no other changes were observed. The incomplete recovery may indicate that a lack of B-complex vitamins was not the cause of the fish's condition. It is also possible that the vitamin dosages were too weak, the injections should have been continued for longer than seven days, or the fish may have been beyond recovery.

Halver (1972) reported that riboflavin deficiencies result in high salmonid mortality with eye opaqueness of most obvious external sign. The addition of riboflavin to the diet of salmonids will reduce the deficiency symptoms except when cataracts have developed and the protein crystal structure of the eye is lost (Halver, 1957). Westernhagen (personal communication) reported a similar condition affecting S. canaliculatus and S. guttatus when they were fed a diet of commercial chicken and rabbit feeds. He attributed this condition to a deficiency in folic acid in the feed.

During preliminary experiments in 200-liter holding tanks, S. argenteus juveniles developed exopthalmia in supersaturated water conditions (mean water temperature, 33.2°C ± 1.3°C; dissolved oxygen, 6.68 ppm, 111% saturation). This disease, resulting from gas embolism, is caused by the inability of the fish to maintain the internal partial pressure of oxygen in the blood and external partial pressure of oxygen in the water near equilibrium by normal gas diffusion across the gill filament surfaces (Duijn, 1971). As a result, nitrogen becomes supersaturated in the blood and gas bubbles accumulate between the cornea and lens of the eye.

The affected siganids recovered when aeration was added to increase gas diffusion in the water of the influent troughs. Exopthalmia was reported by Lam (1974) in culturing S. canaliculatus; however, the cause of the condition was not stated.

Parasitic trematodes have previously been described from siganids by Goto and Ozaki (1929), Yamaguti (1934, 1939), Young (1967), and Manter (1969). Several trematode species may be specific to siganids (Paperna, 1972). Although I have not found any trematodes on S. argenteus, a parasitic trematode tentatively identified by D. I. Gibson as Microcotyle mouwoi has been found on the gills of S. spinus from Guam and caused heavy losses to this species during growth studies. This microcotylid monogenean was originally described by Ishii and Sawada (1938) from the gills of S. fuscescens from Japan.

No symptoms of the disorder prior to the fish's death were noticed. Upon examination, the fish's gills appeared pale and were covered with a heavy mucous secretion. This secretion, consisting of slime, presumable produced by the fish to rid the gill of parasites and destroyed epithelial cells, decreased the respiratory surface area on the gill filaments, causing the fish to suffocate (Davis, 1953). Lam (1974) described a similar infestation of monogenean trematodes in attempts to culture S. canaliculatus. Three field specimens of S. argenteus examined by Paperna (1972) were free from monogenea.

Environmental Parameters

Temperature Tolerances

The mean survival rates based on four runs of Series A (ambient), B (+2°C), and C (+4°C) fish were 100, 94, and 79%, respectively, for the 14-day experiments (Figure 3). Series D (+6°C) fish had 50% mortality in 2.7 days, 96% mortality in 5 days, and 100% mortality in 8 days. The upper tolerance limit, defined by Hoff and Westman (1966) as the temperature by and at which 50% of the experimental animals can no longer live for a designated exposure period, for S. argenteus is 33.7°C ± .3°C in 2.7 days. Earlier trial experiments with S. argenteus juveniles in Series D tanks resulted in 100% mortality in 20 hours when temperatures rose to 36.3 C.

To maintain the desired temperatures (30, 32, and 34°C), adjustments with the thermal controller were made at 0800, 1200, 1700, and 2100 hours when necessary. Regulation of thermal Series C and D temperatures was most difficult during two of the runs because of heavy rains and frequent power outages. As a result, the lower mean temperatures may have increased the survival time of these fish. The dissolved oxygen concentrations in the thermal series tanks ranged from 6.01 to 6.68 ppm (94-113% saturation).

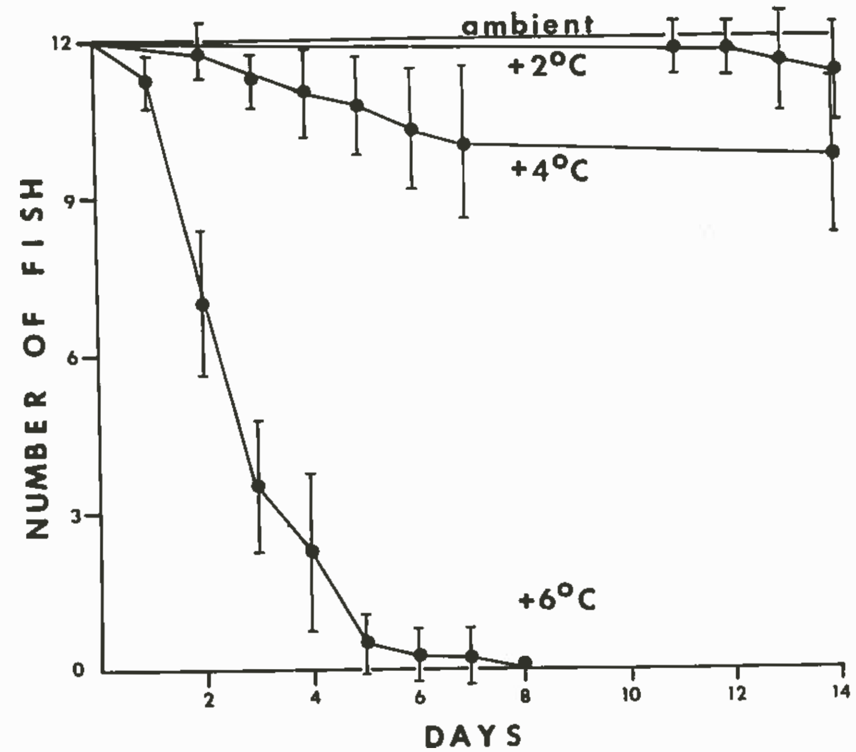

Figure 3. Mean survival rate of *Siganus argenteus* in four replicate thermal experiments at ambient (28°C ± .4°C), +2 (29.7°C ± .4°C), +4 (31.8°C ± 1.4°C), and +6°C (33.7°C ± .3°C) temperatures. Vertical lines indicate standard deviations.

The fish in Series A, B, and C thermal tanks acted normally, either schooling or hiding along the tank bottom. The fish in Series B tanks consumed more food than those in Series C. Normal consumption occurred in Series A; no food was consumed by Series D fish. The behavior of Series D fish was noticeably different from that of the other fish. After schooling for two hours, the rabbitfish separated and remained in the more shaded corners of the tank. In 24 hours, the bodies of the fish appeared swollen. The coloration of the frontal head area of the fish became dark and the body turned pale gray with yellow outlines around the base of the dorsal and pelvic fin and caudal peduncle. Activity of the fish progressed from quiescent and sluggish swimming periods to erratic, spasmotic movements near death.

Previous growth measurements made on *S. argenteus* indicated substantial increments of growth in 14 days (see Figure 1). Analysis of covariance revealed no significant weight increase of or between Series A, B, or C fish (Sokal and Rohlf, 1969). Regressions of weight gain for each temperature series were not significantly different.

Drew (unpublished report) stated from field experiments that *S. canaliculatus* can tolerate temperature fluctuations of 23-36°C recorded *in situ* in the *Enhalus acoroides* beds in Palau. Lam (1974) also reported the ability of *S. canaliculatus* to tolerate temperature fluctuations of 25-34°C while maintained in laboratory holding tanks. *S. canaliculatus* and *S. guttatus* have also been reared by Westernhagen and Rosenthal (1975) in 26-30°C water. These reports indicate tolerance to brief temperature fluctuations but provide no information on the effects of sustained elevated temperatures in siganid culture.

Salinity Tolerances

While present on the reef flat areas around Guam, *S. argenteus* juveniles feed on the filamentous alga *Enteromorpha clathrata*, which grows luxuriantly in the intertidal zone (FitzGerald, 1976). Because of the structure of Guam's limestone aquifer and ground-water reservoir (Randall and Holloman, 1974), the salinity in this area is reduced, with exact levels depending on the amount of ground-water runoff. It is evident from the duration of time the fish spends feeding in this reef flat area that *S. argenteus* can tolerate some degree of salinity reduction.

The mean size and weight of fish used to determine the lower salinity limit range was 74 mm fork length and 6 g, respectively. Oxygen concentration and temperature in the experimental and control tanks varied from 6.1 to 7.0 ppm ($\bar{x}$=6.4 ppm) and 24.7-30.3°C ($\bar{x}$=26.1°C), respectively. A mean temperature change of .3°C occurred in the experimental tanks after the addition of dechlorinated tap-water.

Figure 4 shows the survival of S. argenteus (n=80) after daily salinity reductions of 1°/oo from 8 to 2°/oo. Survivorship ranged from 100% at 7°/oo salinity to zero at 2°/oo salinity. At salinities of 6, 5, 4, and 3°/oo, survivorship was 99, 91, 36, and 6%, respectively. The lower salinity limit, defined as the interval from which survivorship is reduced from 100% to 50% as determined by this experiment, is between 4°/oo and 7°/oo salinity.

As the lower salinity limit was approached, the fish appeared light in color, unable to match their coloration with background surroundings. Their bodies were noticeably swollen with spines erect, and they were unable to maintain a horizontal, upright swimming position. Short erratic periods of activity continued until death. Apparently, the fish were unable to remain hypo-osmotic to the seawater environment, due to the increased ion and water absorption, and equilibrium was impaired.

During the second salinity study conducted for 96 hours, the mean water temperature was 30.6°C and the dissolved oxygen concentration and pH varied from 6.2 to 6.6 ppm and 8.11 (initial) to 8.78 (final), respectively. Although 100% survival was recorded for fish held in Series A (10°/oo), B (8°/oo), and C (6°/oo), the quantitative observations made of fish response to tactile and light stimuli, and effect of reduced salinity on coloration, equilibrium, and feeding indicate various degrees of metabolic inhibition (Table 3).

The first noticeable negative response to reduced salinity is a change in color from a light to dark phase. This is followed by a negative light response and reduced tactile (flight) and equilibrium response. In 72 hours, 25% of the fish in Series A (10°/oo) and B (8°/oo) tanks were dark in color and exhibited a negative response to light. In Series C (6°/oo), the same conditions occurred in 48 hours to 50% of the fish. Equilibrium was also impaired and tactile response reduced. Feeding ability was positive throughout the study for Series A and B fish and negative for Series D fish. Feeding ability, initially negative for Series C fish, became positive after 24 hours for the remainder of the study. This may indicate partial acclimation at the 6°/oo salinity level.

The survival of S. argenteus at 4°/oo salinity is reduced to 50% after 48 hours (Figure 5). At 96 hours, survivorship was reduced to 25%.

Results from the third study to determine the effects of reduced salinity on the growth rate and survival of S. argenteus over a 1-month period have shown an increase in growth (weight) of 77, 59, and 51%, respectively, for fish held in 20, 10, and 30°/oo salinity water. Analysis of covariance (Sokal and Rohlf, 1969) has shown no significant difference in growth rate for S. argenteus grown in the reduced salinity

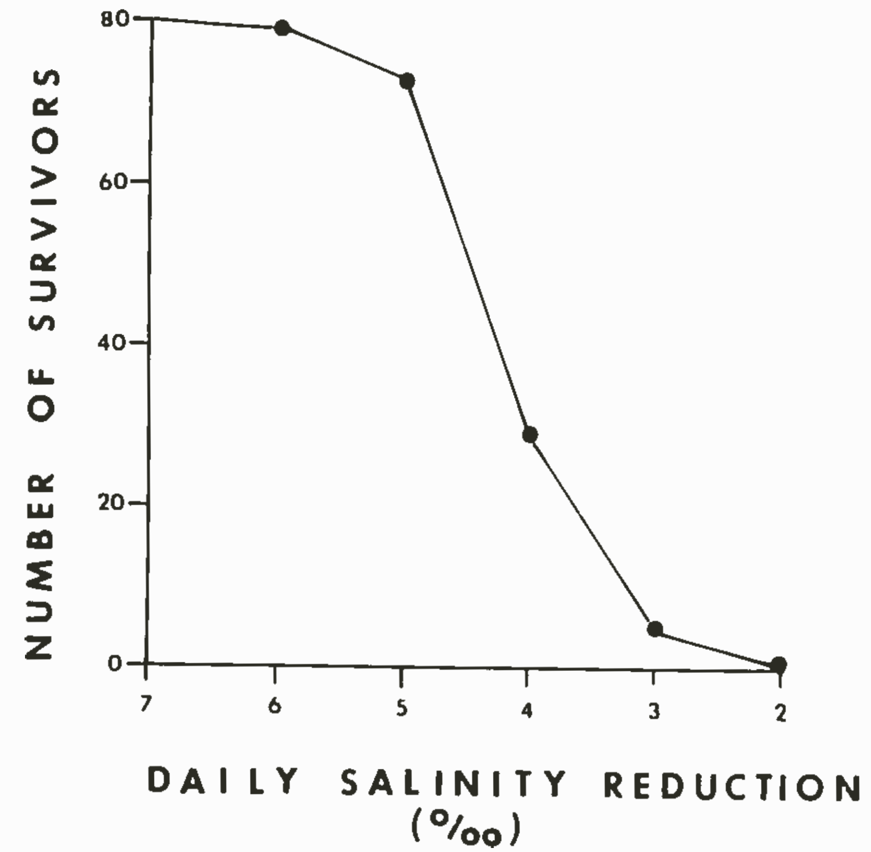

Figure 4. Survival of Siganus argenteus after daily salinity reductions of 1°/oo from 8 to 2°/oo.

Table 3. Response of Siganus argenteus to salinities of 10 (Series A), 8 (Series B), 6 (Series C), and 4°/oo (Series D) for 96 hours. A (+) indicates a normal response for feeding.

			NUMBER OF FISH SHOWING NORMAL RESPONSES				
SERIES	HOURS	NUMBER OF FISH	TACTILE	LIGHT	EQUILIBRIUM	COLORATION	FEEDING
	24	12	12	12	12	11	+
A	48	12	12	12	12	10	+
	72	12	12	9	12	9	+
	96	12	12	8	12	8	+
	24	12	12	10	12	10	+
B	48	12	12	10	12	9	+
	72	12	12	9	12	9	+
	96	12	12	8	12	8	+
	24	12	12	5	10	7	-
C	48	12	12	5	10	7	+
	72	12	11	5	10	5	+
	96	12	9	6	9	6	+
	24	8	2	2	2	2	-
D	48	6	2	1	2	1	-[a]
	72	5	2	1	2	2	-[a]
	96	3	2	1	2	2	-

[a] Indicates a positive response by one fish in one of the three series tanks.

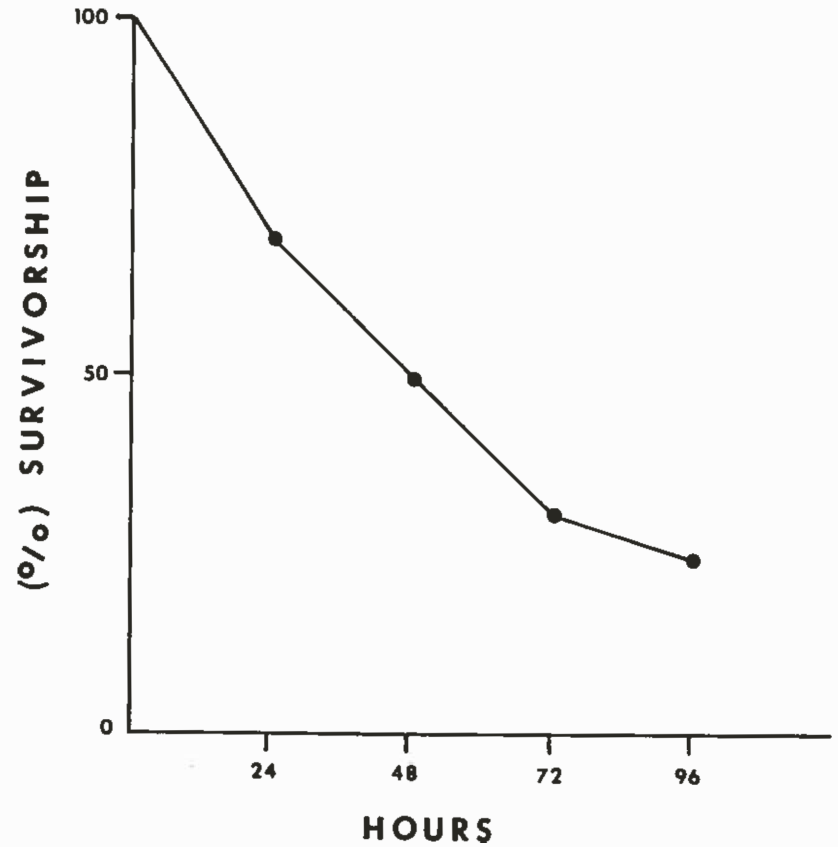

Figure 5. Survival of Siganus argenteus in 4°/oo salinity water.

water. The survival rate was 94% for fish held in 20 and 30°/oo salinity water and 81% for the 10°/oo salinity fish.

Habitat observations of siganids indicate that they are subjected to wide fluctuations in salinity. Drew (unpublished report) stated that S. canaliculatus in Palau tolerates salinity fluctuations of 17-37°/oo. Lam (1975) reported that S. canaliculatus can be gradually acclimated to 5°/oo salinity and thrives in 33-50% seawater. Similar acclimation studies were conducted by Lavina and Alcala (1973), but they found S. canaliculatus unable to tolerate a medium of 25% seawater (8.75°/oo salinity). S. rivulatus and S. luridus have established populations in the Mediterranean Sea by passing through the Suez Canal from the Red Sea (Ben-Tuvia, 1966). Popper and Gundermann (1975) concluded that S. rivulatus and S. luridus tolerate 20-50°/oo salinity water; however, mortality increases and growth decreases at the extremes. They also found no significant difference in growth rate for juvenile S. rivulatus and S. luridus reared in 20, 30, 40, and 50°/oo salinity water for six months.

Oxygen Tolerances

Temperatures in the experimental and control tanks ranged from 26.0-29.4°C. A maximum variation of 1.5°C occurred over 24 hours from insolation in the aquaria with 3.0 ppm dissolved oxygen. A general trend of decreasing pH, ranging from 8.39 to 8.04, was observed during each experiment with a maximum decrease of .30 in the 2.0 ppm dissolved oxygen aquaria. Salinity remained constant at 33°/oo throughout the experiments.

Table 4 shows the mean change in dissolved oxygen concentration in the experimental and diffusion control tanks. In the .5 and 1.0 ppm dissolved oxygen experiments, the diffusion of oxygen from the tank atmosphere into the water in the experimental tanks (+.1 mg l^{-1} hr^{-1}) was approximately the same as the control tank #2 diffusion rates (+.07 and +.06 mg l^{-1} hr^{-1}). At dissolved oxygen concentrations of 2.0 and 3.0 ppm, the change in oxygen concentration from fish respiration (-.19 and -.13 mg l^{-1} hr^{-1}, respectively) was greater than the diffusion rate of oxygen into the water (+.09 and +.04 mg l^{-1} hr^{-1}, respectively).

Oxygen consumption rates, calculated from the mean change in oxygen concentration per hour in the experimental tanks minus the diffusion rate, were approximately .1 mg l^{-1} hr^{-1} for fish maintained in dissolved oxygen concentrations of 2.0 and 3.0 ppm. Fish in control #1 tanks at an initial oxygen concentration of 5.4 ppm showed an average oxygen uptake of .15 mg l^{-1} hr^{-1}.

Stress signs developed immediately after the fish were placed in the .5 and 1.0 ppm experimental tanks. All fish remained in the lower

Table 4. Mean change in dissolved oxygen concentration in the .5, 1.0, 2.0, and 3.0 ppm experimental tanks (n=4) and control tanks. A (+) indicates diffusion of atmospheric oxygen into the water and a (-) indicates removal of the dissolved oxygen in the water by fish respiration.

INITIAL $[O_2]$ ppm Experimental tanks	Δ $[O_2]$ mg l^{-1} hr^{-1} Experimental Tanks (1 fish/tank)	Control #2 Tanks[a/] (no fish)	EXPERIMENT DURATION (hr)
.5	+.10	+.07	2.6
1.0	+.10	+.06	6.5
2.0	-.19[b/]	+.09	24
3.0	-.13[c/]	+.04	24

[a/] Diffusion rate across the tank atmosphere-seawater interface.

[b/] Δ $[O_2]$ mg l^{-1} 2 hr^{-1} divided by 2.

[c/] Δ $[O_2]$ mg l^{-1} 4 hr^{-1} divided by 4.

half of the tank, resting against the side of the tank or tank bottom, except for occasional erratic movements to the surface. A general lack of coordination and equilibrium was observed. The coloration of the fish became pale with dark areas around the head and sides, unlike the mottled fright pattern coloration. Opercular movements became more shallow and ventilation rates increased from 139/min to 300+/min for fish in the 1.0 and .5 ppm dissolved oxygen concentrations. The average survival time for S. argenteus in water of .5 and 1.0 ppm dissolved oxygen concentration was 1.5 and 4.6 hours, respectively.

The activity of S. argenteus held in water of 2.0 and 3.0 ppm dissolved oxygen concentration was characterized by periods of inactivity on the tank bottom, occasionally disrupted by periods of short swimming activity. Opercular movements appeared slower and deeper than in fish held in .5 or 1.0 ppm dissolved oxygen concentration water. Ventilation rate, monitored by opercular movements, averaged 270/min and 192/min for fish in the 2.0 and 3.0 ppm water, respectively. The experiments were terminated after 24 hours with 100% survival of fish.

Westernhagen and Rosenthal (1975) provide the only account of dissolved oxygen concentrations continuously monitored during growth experiments with S. canaliculatus and S. guttatus (3.81 ppm ± .97 ppm). Lavina and Alcala (1973) and Lam (1974) both report that S. canaliculatus is sensitive to dissolved oxygen concentrations below 2.0 ppm.

CONCLUSIONS

The mariculture potential of Siganus argenteus on Guam is encouraging. Juvenile stocks may be acquired locally during the months of April, May, June, and October. Despite being an open water, schooling herbivore, the fish adapts well to confinement in 1000 and 7000-liter holding tanks and accepts pelletized commercial food, which indicates its suitability for pond culture. The conditions which provide optimum growth of Enteromorpha clathrata, the preferred food of the juveniles, are known (FitzGerald, 1976) and may be utilized to provide an unlimited, inexpensive food source. S. argenteus has spawned naturally in captivity in Tahiti and initial induced spawning attempts have been successful (A. Michel, personal communication). Of the two most abundant siganids on Guam, S. argenteus has a faster growth rate and larger attainable size in seven months than S. spinus grown under similar laboratory conditions and fed a diet of Enteromorpha and trout chow (terminal lengths and weights of 187 mm/114 g and 124 mm/29 g, respectively).

S. argenteus juveniles are tolerant to increased water temperatures and reduced salinity and oxygen concentrations. Survival rates of fish subjected to water temperatures of 28, 30, and 32°C were 100, 94, and

79%, respectively, over 14 days. Fish held in water of 34.0°C had 50% mortality in 2.7 days, 96% mortality in 5 days, and 100% mortality in 8 days. The lower lethal salinity limit of S. argenteus was 4-7°/oo with survival reduced to 50% after two days at 4°/oo. Survival of S. argenteus for 24 hours at 2.0 and 3.0 ppm dissolved oxygen concentrations was 100%; however, at .5 and 1.0 ppm, survival times were reduced to 1.5 and 4.6 hours, respectively. Environmental extremes such as those tested on S. argenteus infrequently occur in mariculture operations; however, knowledge of adaptation to these extremes is essential to prevent complete loss of a mariculture stock during operation failures.

Major disadvantages must be minimized before the mariculture of S. argenteus on Guam will become a reality. Because of the yearly fluctuation in the number of juveniles appearing on the reef flat (Kami and Ikehara, In Press), induced spawning and larval rearing techniques must become routine to provide a continuous supply of juveniles. An inexpensive, high-protein dietary supplement must be found to replace expensive trout chow ($.60 lb including shipping). Areas needing additional research, as implied above, include induced spawning and larval rearing, larvae-adult nutrition and food conversion, and diseases.

It is interesting to note that the local demand and market value of siganid juveniles (manahac) are higher than for the adults. Serious consideration should be given to rearing fish only to the juveniles stage for market in lieu of strict juvenile-to-adult culture. Advantages of the former would be decreased management costs, faster growth of fish to marketable stage, and several marketable crops per year. The juveniles may also represent an important source of baitfish which would be essential in developing Micronesia's tuna fishing industry. Juvenile-to-adult culture would be continued to provide a dependable source of breeding stock.

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