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Studies of the Use of Vertical Substrates for Improving Production of Pink Shrimp, *Penaeus duorarum* Burkenroad

William L. Rickards

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PREFACE

The Sea Grant Colleges Program was created in 1966 to stimulate research, instruction, and extension of knowledge of marine resources of the United States. In 1969 the Sea Grant Program was established at the University of Miami.

The outstanding success of the Land Grant Colleges Program, which in 100 years has brought the United States to its current superior position in agricultural production, was the basis for the Sea Grant concept. This concept has three objectives: to promote excellence in education and training, research, and information services in the University's disciplines that relate to the sea. The successful accomplishment of these objectives will result in material contributions to marine oriented industries and will, in addition, protect and preserve the environment for the enjoyment of all people.

With these objectives, this series of Sea Grant Technical Bulletins is intended to convey useful research information to the marine communities interested in resource development quickly, without the delay involved in formal publication.

While the responsibility for administration of the Sea Grant Program rests with the Department of Commerce, the responsibility for financing the program is shared equally by federal, industrial, and University of Miami contributions. This report, Studies of the Use of Vertical Substrates for Improving Production in the Culture of Pink Shrimp, *Penaeus duorarum* Burkenroad, is published as a part of the Sea Grant Program. Graduate research support was provided through a fellowship by the Shrimp Association of the Americas and a National Science Foundation contract.

TABLE OF CONTENTS

TABLE OF CONTENTS

(Continued)

LIST OF FIGURES

LIST OF FIGURES

(Continued)

LIST OF TABLES

LIST OF TABLES

(Continued)

LIST OF TABLES

(Continued)

TABLE PAGE

INTRODUCTION

In the past few years, efforts in the intensive farming of marine organisms have become popular in the United States and other countries, partly because of the realization that man is rapidly breeding himself into population and food crises and partly because of the profit incentive offered by the production of some seafoods. Of these factors, profit potential appears to be of greatest influence in determining the success or failure of the recent burst of enthusiasm for mariculture in the United States. For this reason, organisms with a high monetary value, such as shrimp, lobsters, and pompano, have been selected for use in large-scale sea-farming operations.

Penaeid shrimp have been farmed for many years in some parts of the world, largely in Asia and the Indo-Pacific region. Kow (1968) summarized earlier published accounts and described methods of prawn farming in Singapore. In that area, young prawns are not usually stocked by the farmer but are brought in by tidal flow which is regulated by sluice gates. Species of prawns present in quantity are *Penaeus indicus*, *P. merguiensis*, *Metapenaeus ensis*, *M. burkenroadi*, and *M. brevicornis*.

Bhimachar (1962), Gopinath (1956), and Menon (1955) described a similar method of prawn farming in the rice fields of India. Species involved were *Penaeus indicus*, *Metapenaeus dobsoni*, and *M. monoceros*.

Shrimp farming in the Philippines (Caces-Borja and Rasalan, 1968; Delmendo and Rabanal, 1956; Kesteven and Job, 1958; Villadolid and Villaluz, 1951) consists mainly of capturing young sugpo, Penaeus monodon, for stocking in rearing ponds. In some cases, young shrimp enter the pond via tidal flow. In the past, sugpo culture was incidental to the production of milkfish, but the recent development of pure sugpo culture has realized greater economic return than the mixed species culture technique previously employed.

Perhaps the most successful and highly developed shrimp culture operation is that of Dr. Motosaku Fujinaga (Hudinaga) in Japan. The Japanese work has been detailed in publications by Fujinaga (1963, 1968, and 1969), Fujinaga and Kittaka (1966), Hudinaga (1942), Hudinaga and Kittaka (1967), Hudinaga and Miyamura (1962), and Miyamura (1969). Fujinaga was the first to rear young shrimp, Penaeus japonicus, from eggs spawned in the laboratory, with subsequent stocking of the laboratory-reared postlarvae in ponds and tanks.

Summaries of the technology and biology of shrimp culture in Singapore, the Philippines, and Japan are presented by Ryther and Bardach (1968) from information gathered in interviews and from the published literature.

Rearing and culturing efforts in Korea are being directed along lines similar to those of Fujinaga. Kim (1967) reported the technology employed in farming Penaeus orientalis, while Lee and Lee (1968 and 1970) described the initial experiments on rearing Metapenaeus joyneri in anticipation of mass cultivation of "seed" (i.e. postlarvae) shrimp in Korea.

Shrimp and prawn farming projects are now underway or planned in Australia (Anon., 1969a) using Metapenaeus masterii, and in Taiwan (Anon., 1969b) using Penaeus japonicus.

Shrimp culture is a new field in the United States, starting with the work of Lunz (1958). Studies have been conducted on the Gulf and Southeastern Atlantic coasts, the regions in which penaeids are fished commercially.

Lunz's studies in South Carolina on pond culture of both white shrimp, Penaeus setiferus, and brown shrimp, P. aztecus, involved production from shrimp brought in by tidal flow as well as some which were placed in ponds treated to eliminate shrimp predators. Broom (1966 and 1969) used both brown and white shrimp in pond culture investigations in Louisiana, and Wheeler (1966, 1967a, 1967b, 1968, 1969, 1970) conducted similar studies on the same species in Texas.

Recent shrimp culture efforts have attempted to develop a system in which the entire life cycle of the shrimp is completed in the laboratory. Efforts in larval culture by Cook (1969), Cook and Murphy (1969), Dobkin (1961), Ewald (1965), Idyll, Tabb, and Yang (1969), and Tabb, Yang, Idyll, and Iversen (1969) have contributed to making possible such shrimp husbandry. However, spawning of laboratory-reared penaeid shrimp has yet to be accomplished in quantities sufficient for commercial propagation. Fujinaga (personal communication) has reported spawning of laboratory-reared shrimp but with no dependable regularity or quantity.

Despite this gap in the life cycle, several private organizations have undertaken the cultivation of shrimp in ponds or enclosed natural bays with the intention of commercial production in the near future (Anon., 1968 and 1970; Robinson, 1969).

Even though shrimp farming has been carried out for many years and controlled life-cycle culture may soon be a reality, little effort has been applied to developing techniques or mechanical innovations for improving the efficiency of shrimp production under controlled conditions. One possible innovation would involve the use of vertical surfaces or substrates in the culture enclosures. Such substrates would help make use of the entire water column in a farming technique which in the past has been inefficient with respect to utilization of the water volume employed. Because shrimp are interface feeders (i.e. they feed by browsing upon organisms growing in or on a surface or substrate) only the bottom few centimeters of water in a pond are used in feeding. The top part of the water column provides protection, cooling, and perhaps other environmental advantages but its use to increase the feeding area might be one step towards developing more efficient methods for culturing shrimp in any type of enclosure, whether pond, tank, or raceway.

Vertical substrates in culture enclosures might improve yields by increasing growth, survival, and total yield and reducing production costs. In shallow waters, the substrates could act as additional "bottom" by accumulating a community of fouling organisms composed of filamentous algae, benthic diatoms, and invertebrates upon which the shrimp could feed, possibly providing essential nutrients lacking in prepared diets. Also, the presence of additional food would decrease competition for food introduced into the system by the farmer.

Survival might be improved as a result of decreased contact between shrimp, particularly during molting when they are virtually helpless and subject to cannibalism. Under high density conditions, some organisms become extremely "nervous" and natural mortality may increase (Calhoun, 1962). Providing added surface area upon which the shrimp are able to move should reduce detrimental effects due to crowding by increasing the spatial distribution between individuals.

Production costs may be reduced by the additional surface area since the added source of food will decrease the amount of supplemental food which the farmer must provide. Also, increased survival and growth should increase the yield and produce greater profits.

Since a substrate fouling community may place an added demand for oxygen upon the system, flowing water must be used, at least to some degree. This requirement may eventually lead to an easy-to-handle enclosure such as a raceway system. Rate of water flow and placement of the substrates should be considered together, since the physical obstruction created by the vertical surfaces will influence the pattern and degree of water circulation within the enclosure.

Substrates must be constructed so that shrimp have free access to and from them, allowing for the maintenance of normal behavior and activity rhythms. Substrates must also be constructed of materials upon which the shrimp will move freely, and at the same time they must not interfere physically with diurnal activities such as burrowing into the bottom sediments and movement about the enclosures in search of food. Finally, the vertical surfaces must be easily moved to facilitate harvesting of the crop.

In only a few instances have published accounts of culture operations noted the use of any form of vertical configuration in enclosures for the purpose of making use of the entire water column for production. Encouragement of the "lab-lab" complex of organisms on the bottoms of fish and shrimp ponds in the Philippines, as recorded by Rabanal (1949) and Villadolid and Villaluz (1951), is well known. However, the "lab-lab" food source does not provide appreciably increased vertical surface area in the ponds. The same may be said of the scattering of rice husks over the bottoms of shrimp ponds documented by Wheeler (1968 and 1969).

The practice of encouraging algal growth in enclosures to provide shelter for the cultured organism has been cited for fresh-water prawns, Macrobrachium, (Bovbjerg, 1956) and stone crabs, Menippe, (Cook, 1969).

Rhyther and Bardach (1968) noted the use of stones, tiles, branches, and shells in tanks to provide shelter for newly molted Macrobrachium in Malaya and crabs, Portunus, in Japan.

Ingle and Witham (1969) and Sweat (1968) described the use of vertical plastic configurations as shelters to attract young spiny lobsters. The lobsters are removed from the artificial habitat "traps" and placed in culture enclosures where they are grown to marketable size.

Investigations are planned in Louisiana by de la Bretonne and Avault (1970) in which natural grasses will be tested for their suitability as cover for post-larval shrimp.

None of these innovations is intended to make use of the entire water column in culture enclosures by providing both shelter and food.

Ling (1962 and 1969) stated that in Malaysia production of the freshwater prawn, Macrobrachium rosenbergii, is enhanced by placing branches of bamboo or other woody plants in the ponds to provide shelter for the prawns. In addition, patches of the algae, Ipomoea, are grown on the pond surface to provide shelter, shade, and food for the prawns. However, the clumps of algae may become a hindrance during harvesting of the crop and if too abundant they may create an oxygen deficiency at night. This is perhaps the only published account of a practice in which an effort has been made to utilize the whole water column for production of the crop.

Present trends in the development of shrimp culture indicate that for the time being it will tend to concentrate upon high density, monospecific crops with supplemental feeding by the "farmer". These conditions require artificial manipulation of the ecology of the organism employed. Such manipulation must lead either to adaptation by the organism to the conditions imposed with subsequent successful production or to failure of the crop.

The three experiments described herein were designed to determine the manner in which pink shrimp, *Penaeus duorarum*, react to one artificial condition: the presence of man-made, vertical surfaces upon which the shirmp are intended to move and feed.

EXPERIMENT ONE

PROCEDURES

Substrate materials for this first experiment were two synthetic "grasses" selected with the hope that they would simulate the sea grass, Thalassia testudinum, which provides cover and a feeding surface for pink shrimp in the natural habitat (deBondy, 1969; Eldred et al., 1961; Hildebrand, 1955; Hoese and Jones, 1963; Hudson, Allen and Costello, 1970; Ingle, Eldred, Jones and Hutton, 1959; Tabb, Dubrow and Manning, 1962; Woodburn et al., 1957). It was anticipated that the shrimp would not avoid substrates made of these synthetic materials and that sufficient fouling would develop on them to provide food for the shrimp.

The materials to be used as substrates were Chemturf (Monsanto Chemical Company) and Olefern (Avisun Corporation). Chemturf is a carpet-like, polypropylene product designed to simulate short grass with a pile approximately 2.5 cm deep. Olefern, also made of polypropylene, is a ribbon 5mm wide and 0.01 mm thick.

Chemturf substrates were constructed of eight, 15 x 15 cm squares of the turf strung onto nylon line and separated by 5 cm lengths of vinyl plastic tubing. The top layer of Chemturf was at the water surface and the pile of each square was directed upwards. Each turf configuration was weighted down at one end by a 10 x 10 cm piece of concrete block.

Olefern substrates were constructed of 50 strands of the ribbon cut to a length of 110 centimeters. These strands were tied together at their mid-point, creating a bunch of 100 strands, 55 centimeters long. A 10 x 10 cm piece of concrete block provided weight for each configuration. The 55 centimeter length of the strands resulted in a slight amount of coverage of the water surface by the strands since the water depth was 50 centimeters. Both substrate materials were buoyant and required the concrete weights, and they remained buoyant throughout the study.

Enclosures for this initial experiment were six fiberglassed plywood tanks measuring one meter square and one meter deep. Tanks were supplied individually with continuously flowing water from the sea-water system of the laboratory; water was distributed to the tanks by PVC (polyvinylchloride) piping and plastic garden hose feed lines. Drainage from the tanks was maintained by both surface and bottom-level outlets to prevent stratification of the water. Because the drainage was a gravity-flow system, water inflow was adjusted to the rate which maintained a water depth of 50 centimeters without causing either an overflow or a drop in the water level below the surface outlets.

A plate glass window was built into the front side of each tank for the purpose of making day and night observations. The windows were provided with removable covers which prevented the entrance of light into the tanks during the night. Pink shrimp are known to react to both high and low light intensities (Mikulka, 1969), and passage of light from nearby laboratory buildings through the windows at night would have influenced the behavior patterns of the shrimp.

Each tank was provided with three to four centimeters of beach sand into which the shrimp could burrow in accordance with their natural diurnal rhythm; pink shrimp are generally found out of the bottom sediments during periods of low light intensity (Eldred *et al*., 1961; Fuss and Wathne, 1964; Hughes, 1968).

Lids for the tanks were constructed of green-tinted, corrugated fiberglass paneling mounted on wooden frames. The paneling is rated by the manufacturer* as transmitting 80 per cent of the incident light and 61-68 per cent of the heat. Corrugations in the paneling permitted some flow of air into the space between the water surface and tank lid while protecting the shrimp and excluding most of the rain which fell over the tank surface. The latter function prevented sudden decreases in the salinity of the tank water which could have had detrimental effects upon the shrimp.

Two tanks were supplied with substrates made of one type of synthetic grass, two had substrates of the other grass, and the remaining two tanks were without substrates and served as controls. Each of the substrate tanks contained six configurations of either Chemturf or Olefern which were arranged in a horse-shoe shaped pattern so that all of them could be seen through the observation windows. Figure 1.1 shows the arrangement of tanks and variables.

Shrimp used in this study, which lasted from 15 January through 17 April 1969, were obtained from the live-bait shrimp fishermen of Biscayne Bay, Florida. Although the shrimp population in Biscayne Bay is a mixture of *Penaeus duorarum* and *P. braziliensis*, the

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*Filon Corporation, Hawthorne, California

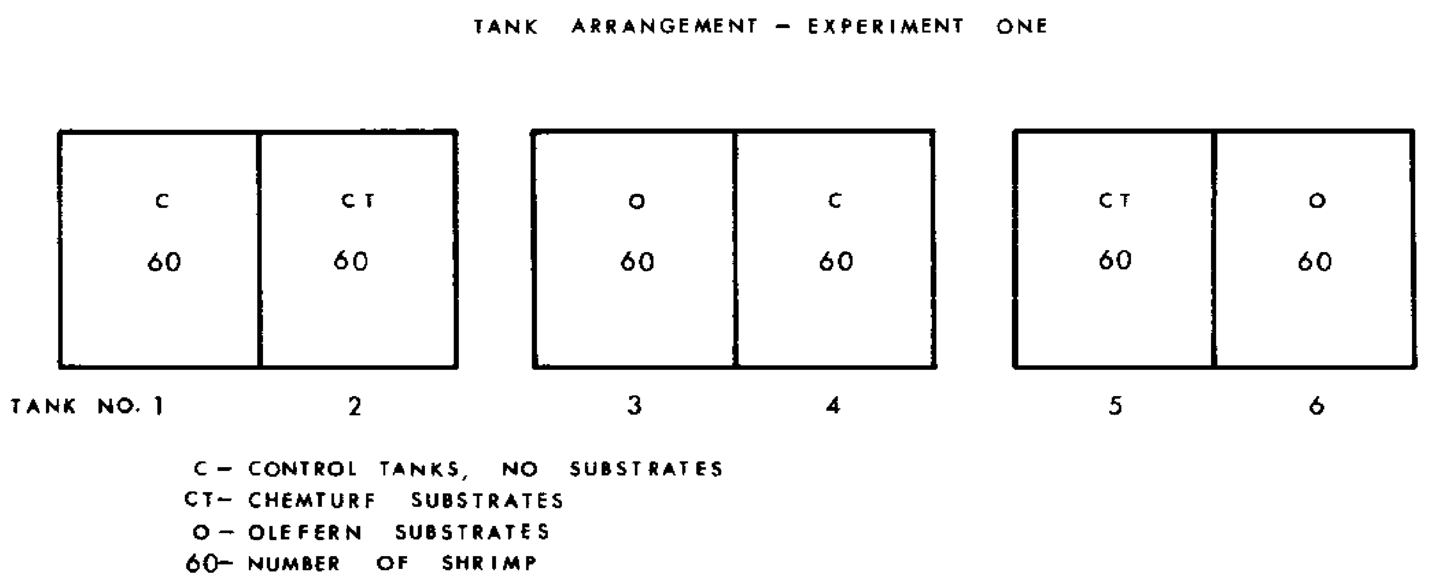

Figure 1.1. The arrangement of tanks and variables for Experiment One.

population at this time of the year is nearly 98 per cent P. duorarum (Saloman and Costello, 1968). Therefore, the possibility of obtaining a mixture of species from the fishermen was considered to be of little concern. The smallest juveniles were selected for use in the study so that sizes and weights were as uniform as possible.

The tanks were stocked with 60 shrimp apiece. Before being placed in the tanks, the shrimp were randomly selected from a holding tank in which they had been kept for two days. They were weighed as a group in water and placed in the tanks.

The amount of food to be given to the shrimp during the first two weeks of the experiment was determined on the basis of total shrimp weight at the time of stocking. Shrimp in all tanks were fed at the rate of ten per cent of the total wet weight per day. The ten per cent figure was chosen on the basis of preliminary studies (Tabb, personal communication) on the feeding of pink shrimp in captivity. This supplemental feeding was fixed at what was believed to be the approximate amount required for maintenance when no other food was available. The maintenance level is here defined as that amount of food which will permit an organism to remain at a given weight without gaining or losing.

Food was placed in the tanks late in the afternoon because the shrimp normally emerge from the sand late in the day to begin feeding (Eldred et al., 1961; Hughes, 1968). The food employed was ground, frozen squid. In preparation, the entire squid was ground into pieces about 6 mm square, washed to remove the ink and small squid fragments, drained, and re-frozen until needed.

Even though some food was left uneaten by the shrimp when temperatures dropped below 18°C, food was added every day so that it would be available if the shrimp wanted to eat. Some of the food was eaten every night regardless of water temperatures as low as 16°C, and uneaten food was left in the tanks.

Shrimp from each tank were weighed at night at approximately two-week intervals, corresponding to the new and full moon phases. The amounts of food provided were adjusted to the ten per cent level throughout the study.

The periods of new and full moon were selected as the times for weighing the shrimp since data of Tabb (unpublished) has demonstrated peaks in the molting frequency of pink shrimp on the quarter moon phases. Handling the shrimp at times of peak molting activity would risk injury and death to newly molted individuals.

Weighing was conducted at night because pink shrimp are normally active at this time, and a more representative sample of the population in a tank could be obtained when most of the shrimp were out of the sand and more susceptible to capture.

Estimates of the biomass of shrimp were obtained by weighing one-half of the population in each tank and multiplying the weight of this sample by two. The 30 shrimp sampled from each tank were located by using a red-filtered flashlight to which the shrimp did not react noticeably. Once located, the shrimp were dipnetted from the tank, placed in a container of water from that tank, and carried to the laboratory. There they were weighed as a group in water to the nearest 0.1 gram, and returned to the tank from which they had been taken.

Daily records were kept for each tank of the number of molts and the number of dead shrimp recovered. Whenever a dead shrimp was found it was removed from the tank and replaced with a live shrimp of similar size and weight. In this way the populations in all tanks were maintained as close as possible to the original level of 60 shrimp. Shrimp used as replacements were from among the supply originally obtained from the bait fishermen. These shrimp were kept in an aquarium in the laboratory and fed approximately ten per cent of their body weight per day so that their physiological condition was similar to that of shrimp already in the outdoor tanks.

In most cases the only evidence remaining from a molting was the carapace portion of the exoskeleton, this is the thickest section and is less likely to be consumed. Despite the fact that the entire exoskeleton was not left intact, the carapace was relatively easy to see against the background of sand in the tanks.

Daily records were kept of the salinity and temperature in the tanks. Salinities were determined to the nearest 0.1 °/₀₀ using a temperature-compensated refractometer. Temperatures were recorded to the nearest 0.1°C. These readings were taken in the morning, and temperature profiles reflect daily minimums.

Behavior observations were made intermittently for the purpose of determining the number of shrimp making use of the surface provided by the vertical substrates. General observations were made of the time of emergence from the sand and the time taken to locate and seize food after it was introduced.

Algal fouling was originally established from seedings of algae from other outdoor tanks which also used the laboratory sea-water system. Seedings were not put into control tanks. As fouling developed in the substrate tanks it soon became apparent that very little, if any, algae was actually growing attached to the substrates. The algae grew rapidly, but only across the water surface, resulting in extensive shading of the water column. Algae which grew below the water surface consisted of a few small patches on the sand and some extremely long filaments from algae on the surface which had become entangled in the substrates. Because of the limited growth of algae on the sides of the tanks, scrubbing or scraping was not necessary.

Tunicates were a very common fouling organism. However, because the tunicates did not provide food for the shrimp or compete with them for food or space, they were removed from the tanks only when they obstructed the flow of water or when they interferred with the floatation of a substrate.

At the conclusion of the experiment the substrate configurations were removed and the tanks were drained. In this way the entire population of shrimp in each tank was recovered, and final shrimp weights were taken. Six shrimp from each tank were preserved in ten per cent formalin for subsequent examination of the digestive tract contents. Identification of organisms in the gut were used to confirm that the shrimp were feeding upon the fouling organisms.

RESULTS AND DISCUSSION

Shrimp Growth

The average shrimp weights obtained by weighing one-half of each shrimp population at two-week intervals (Table 1.1) were used for computing growth regressions for each tank, with the formula Y = a + bX in which Y = shrimp weight, X = time, and a = starting shrimp weight. Regression slopes (b) are also shown in Table 1.1, and tank-type average regression lines are plotted in Figure 1.2 to show the differences in growth rates among the populations in the tanks.

Shrimp in tanks with Chemturf substrates grew at the fastest rate (b = .0373); those in Olefern substrate tanks grew slightly slower (b = .0322); and shrimp in control tanks with no substrates grew at the slowest rate (b = .0244).

A statistical comparison of the slopes of the growth regressions using an analysis of variance (Table 1.2), revealed that there was no difference between tanks with the two types of artificial grass. However, the comparison between control tanks and grass tanks showed faster growth in the grass tanks, at the 90 per cent level.

Intercept values were not different, indicating that selection of shrimp for stocking the tanks at the start of the study resulted in comparable sets of animals.

Table 1.1. Replicate average shrimp weights (in grams) determined at two-week intervals. Growth regression slopes were computed using the formula Y = a + bX in which Y = average shrimp weight, X = elapsed time, and a = starting weight.

Weighing period	Control tanks	Chemturf tanks	Olefern tanks
Start	1.90	1.93	1.83
	1.81	1.89	1.82
Ave.	1.86	1.91	1.83
I	2.40	2.25	2.34
	2.33	2.58	2.65
Ave.	2.37	2.42	2.50
II	2.65	2.70	2.89
	2.36	3.18	2.90
Ave.	2.51	2.94	2.90
III	2.78	3.10	3.43
	2.81	3.98	3.16
Ave.	2.80	3.54	3.30
IV	3.62	3.79	4.12
	3.42	4.00	3.62
Ave.	3.52	3.90	3.87
V	3.92	5.02	4.62
	3.69	5.16	4.28
Ave.	3.81	5.09	4.45
VI	4.25	4.81	5.08
	3.72	5.27	4.33
Ave.	3.99	5.04	4.71
Slope	.0262	.0361	.0368
	.0226	.0385	.0275
Ave.	.0244	.0373	.0322

Table 1.2. Analysis of variance with partitioned sum of squares of the growth slopes (Table 1.1).

Source	df	MS	F
Grasses vs. controls	1	.000142899008	8.10 ***
Between grasses	1	.000026132544	1.48 ns
Residual	3	.000017651074	
Total	5		

***Significant at the 90% level

ns = Non-significant F value

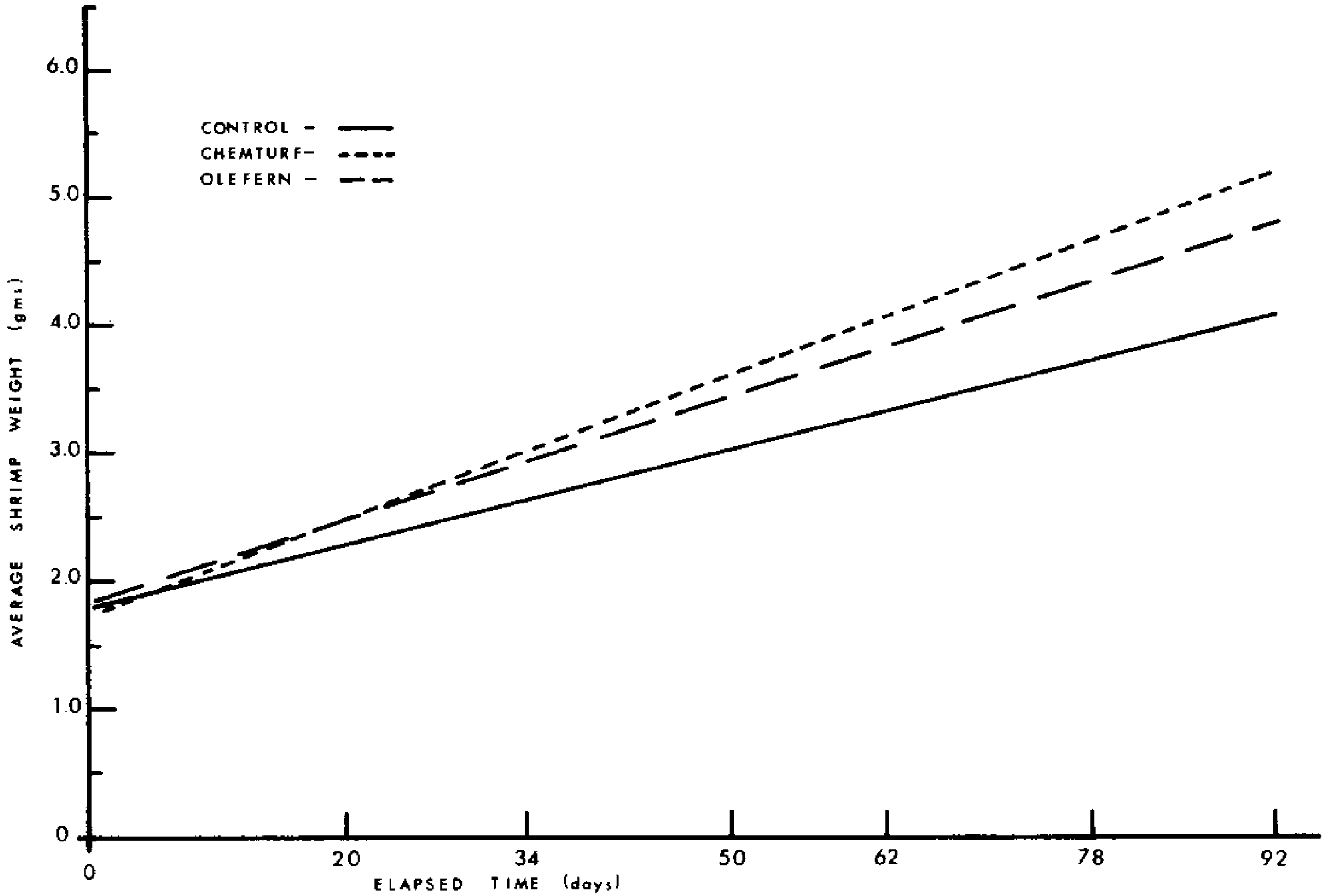

Figure 1.2. The average shrimp weight as a function of elapsed time for each of the three tank-types. Regression lines represent averages of replicated slope values (Table 1.1). Weighing intervals are shown on the X axis.

From an initial individual weight of approximately 1.8 grams for shrimp in each tank, Chemturf tanks produced shrimp averaging just over 5.0 grams, Olefern tank shrimp averaged 4.7 grams, and control tank shrimp averaged just under 4.0 grams after 92 days under the experimental conditions. Thus, shrimp in the artificial grass tanks grew at a rate approximating 1.0 gram per month compared to 0.7 gram per month for control tank shrimp.

Shrimp Mortality

Table 1.3 contains a summary of tank mortality totals and the results of a chi-square analysis of the total shrimp mortalities for each tank. The total chi-square value, 12.25, is significant at the 99 per cent level, indicating greater differences than expected in mortalities among the tanks. Examination of the individual tank contributions to the chi-square analysis shows that one control tank (Number 4) had greater mortality than expected at the 90 per cent level, and one Olefern tank (Number 6) had lower mortality than expected at the 95 per cent level. These two tanks accounted for most of the chi-square total; the remaining four tanks varied little from the expected values and differences were not significant.

A comparison of tank-type mortality totals shows that tanks with Olefern substrates had fewer deaths (9 and 1) than either Chemturf tanks (7 and 18) or control tanks (10 and 11).

As shown in Table 1.3, 28 dead shrimp were not located despite daily examinations of the tanks. These shrimp were presumably consumed by the remaining live shrimp in the tanks since no

Table 1.3. Tank-type mortality summary and results of a Chi-square analysis of the mortality data. Each tank was stocked with 60 shrimp at the start of the study.

Tank Type and Number	Control 1	4	Chemturf 2	5	Olefern 3	6	Totals
Dead shrimp replaced	9	4	4	10	1	0	28
Total shrimp used in tank	69	64	64	70	61	60	388
Shrimp recovered at harvest	59	53	57	52	52	59	332
Total mortality	10	11	7	18	9	1	56
Dead not accounted for in experiment	1	7	3	8	8	1	28
Mortality (%)	14	17	11	26	15	02	14
Contribution to Chi-square analysis	.0001	4.91	.15	.004	.49	6.7	12.25
Comparison with critical value	ns	***	ns	ns	ns	*	**

**Significant at the 99% level
* Significant at the 95% level
***Significant at the 90% level
ns = Non-significant value

parts of them were recovered at the end of the study. In addition, the number of dead shrimp observed equalled the number of dead shrimp not located. Thus, only half of the mortalities were detected by the investigator. If in future experiments feeding regimes are to be based upon the biomass of shrimp present, it may be desirable to obtain actual measures of that biomass when such undetected mortalities are considered.

Five of the deaths in one Chemturf tank (Number 5) occurred among freshly molted shrimp during the night of 22 March when water flow into the tank ceased and drainage from the tank continued and the water depth dropped to about ten centimeters. Water flow had also ceased in tank Number 6, but no dead shrimp or fresh molts were found in this tank. Therefore, it appears that the increased oxygen demand of molting shrimp (Costlow and Bookhout, 1958; Egusa, 1961; Egusa and Yamamoto, 1961; Passano, 1960; Skinner, 1962) and presumably reduced oxygen content of the water resulted in the deaths of these shrimp. Unfortunately, no readings of dissolved oxygen were taken. Resumption of water flow immediately increased the oxygen content of the water, making determination of dissolved oxygen of little value.

Shrimp Production

Replicate values of the total shrimp weight per tank-type for each weighing period are listed in Table 1.4. As was done for the average shrimp weight data, total weight regression lines were computed for each tank using the weighing period values and the formula total shrimp weight = initial total shrimp weight + b (time). Regression slopes (b) are also shown in Table 1.4, and the average tank-type regression slopes are plotted in Figure 1.3. Total production rate was greatest in Chemturf tanks (b = 2.057), slightly less in Olefern tanks (b = 1.769), and least in control tanks (b = 1.358).

The results of an analysis of variance of the production regression slope values (Table 1.5) disclosed no difference in production between the tanks with the two types of artificial grass. However, a comparison of grass tanks and control tanks showed greater production in the grass tanks at the 95 per cent level.

Total shrimp weight regression intercepts were not significantly different. From an initial total weight of approximately 110 grams in each tank, Chemturf tanks yielded an average of 274 grams, Olefern tanks 260 grams apiece, and control tanks 224 grams each after an elapsed time of 92 days.

Production (or yield) can be expressed as the result of the antagonistic processes of growth and mortality, and the relative effects of these two factors will determine the success or failure of any shrimp farming venture. In this study, the marginal differences detected in the growth and mortality rates of the grass and control tanks combined to give production rates in which the grass tanks were decidedly greater than those in control tanks. The differences in growth, mortality, and yield were presumably due to the availability of fouling organisms upon which the shrimp could graze in the grass tanks.

Table 1.4. Replicate total shrimp weights (in grams) determined at two-week intervals. Total weight slopes were computed using the formula Y = a + bX in which Y is the total shrimp weight, X is the elapsed time, and a = initial total shrimp weight.

Weighing period	Control tanks	Chemturf tanks	Olefern tanks
Start	113.7	115.9	110.0
	108.6	113.2	109.4
Ave.	111.2	114.6	109.7
I	143.8	135.0	140.6
	139.8	154.8	159.2
Ave.	141.8	144.9	149.9
II	159.0	161.8	173.6
	141.8	190.8	174.0
Ave.	150.4	175.9	173.8
III	166.8	186.0	202.4
	168.8	234.7	189.6
Ave.	167.8	210.4	196.0
IV	217.2	227.4	247.4
	205.4	256.0	217.0
Ave.	211.3	241.7	232.2
V	235.0	301.2	277.0
	221.4	309.8	256.6
Ave.	228.2	305.5	266.8
VI	250.8	274.0	264.1
	197.0	274.0	255.5
Ave.	223.9	274.0	259.8
Slope	1.543	2.063	1.923
	1.173	2.051	1.616
Ave.	1.358	2.057	1.769

Table 1.5. Analysis of variance with partitioned sum of squares of the total shrimp production rates (Table 1.4).

Source	df	MS	F
Grasses vs. controls	1	.41162552	10.68 *
Between grasses	1	.08291521	2.15 ns
Residual	3	.03854031	
Total	5		

*Significant at the 95% level
ns = Non-significant F value

Table 1.6. Stepwise multiple regression coefficients of the number of molts collected with the independent variables temperature, moon phase, and elapsed days in the experiment.

Analysis of Variance:

Source	df	MS	F	Multiple R^2
Regression	3	501.596	11.337**	0.2834
Residual	86	44.245		
Total	89			

Source	Coefficient	Standard error	F
Temperature	1.20434	0.40878	17.9862 **
Moon phase	1.04639	0.31577	10.6816 *
Elapsed days	0.05153	0.03109	2.7478 ns

**Significant at the 99% level
* Significant at the 95% level
ns = Non-significant F value

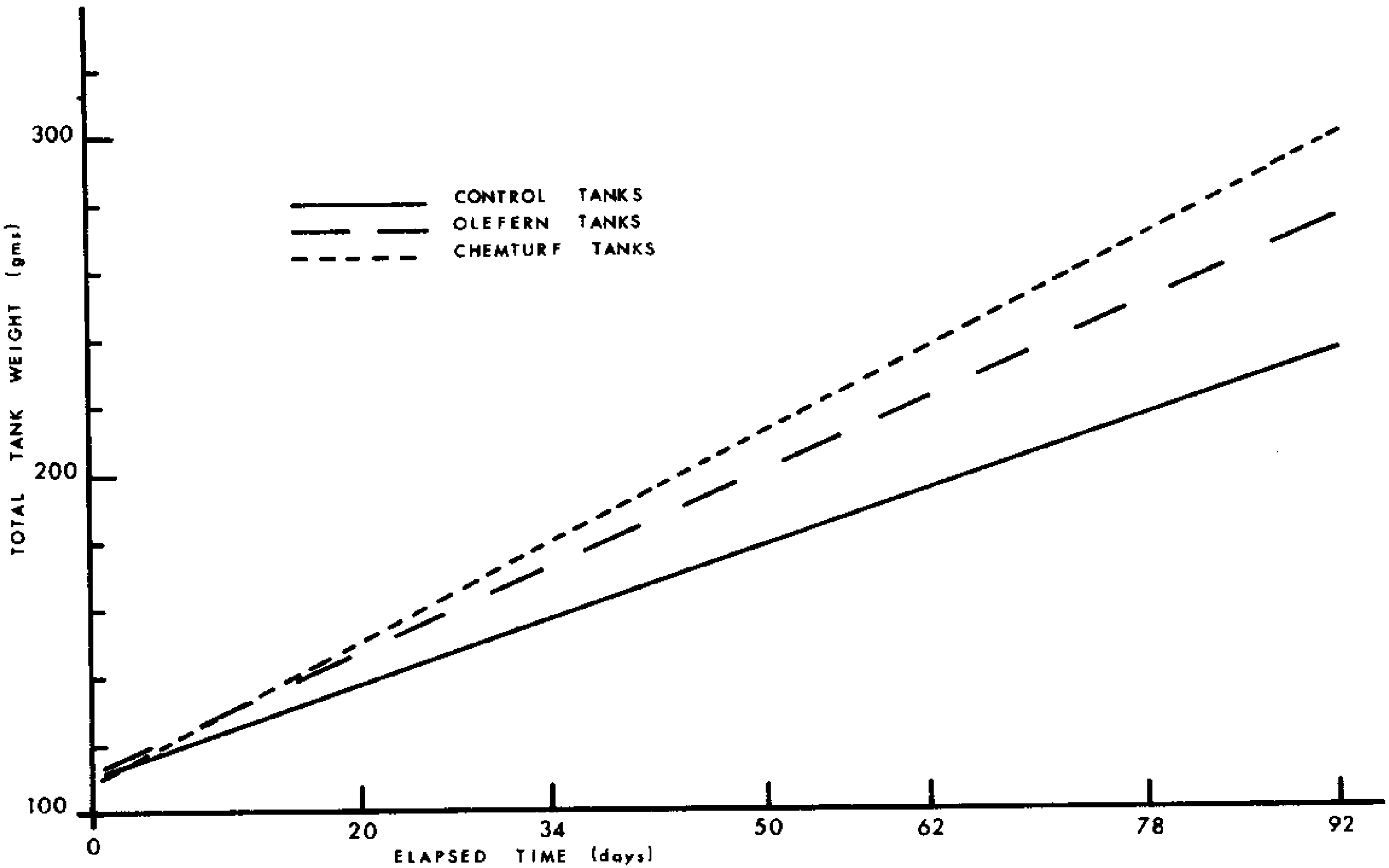

Figure 1.3. The total shrimp weight per tank-type as a function of elapsed time. Regression lines are averages of replicated slope values (Table 1.4). Weighing intervals are shown on the X axis.

Because of the manner in which algal fouling developed only across the water surface in the tanks, the artificial grasses played practically no part as feeding substrates. Little or no algae grew on these. It is not known whether this was due to a chemical in them, their smooth surfaces, or some other factor. It is likely that failure of the development of an abundant algal growth on the water surface of the four artificial grass tanks would have resulted in similar yields in all tanks since conditions were the same except for the presence of artificial substrates in the grass tanks.

Molting

Daily molt collection data are presented in Figure 1.4, with corresponding moon phases. Numbers of molts collected represent composite values for all six tanks.

On the basis of unpublished information from Dr. Durbin Tabb, it had been expected that peaks in molting frequency would occur on the quarter phases of the moon. Figure 1.4 shows definite peaks in molting, but they do not all occur on the quarter moons and they vary considerably in size. The first peak is believed to be the result of molting of shrimp whose time of molting had been determined by conditions in the environment prior to capture by the shrimp fishermen. The experimental conditions of reduced food and high population density had not yet begun to have any effect upon the physiology of the shrimp.

After the initial molting peak, the profile in Figure 1.4 becomes somewhat erratic. Comparison of the temperature and salinity data

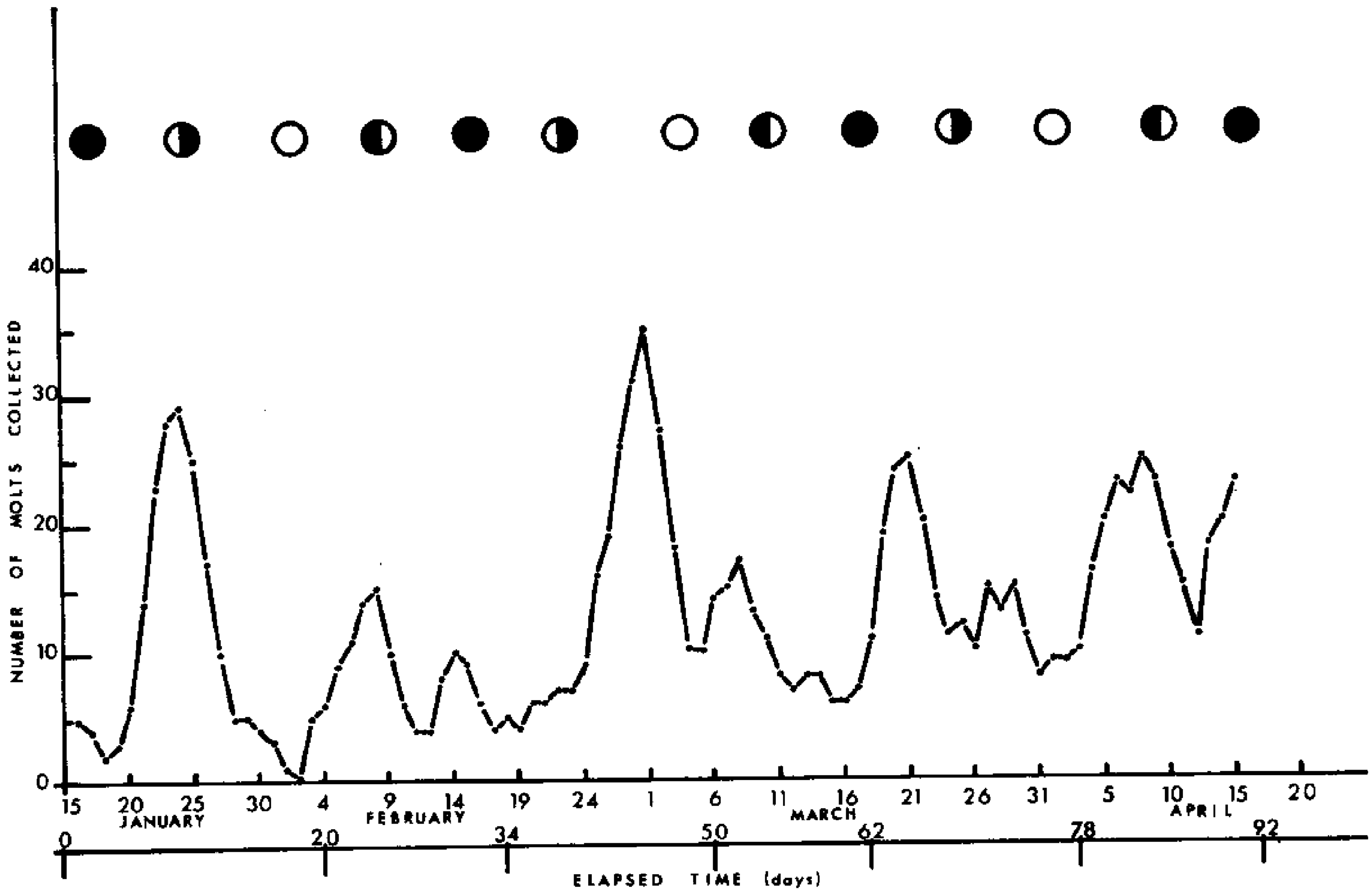

Figure 1.4. The total number of molts collected with corresponding moon phases. Open circles represent full moons, darkened circles are new moons, and half-darkened circles indicate quarter moons. Weighing intervals are shown on the X axis.

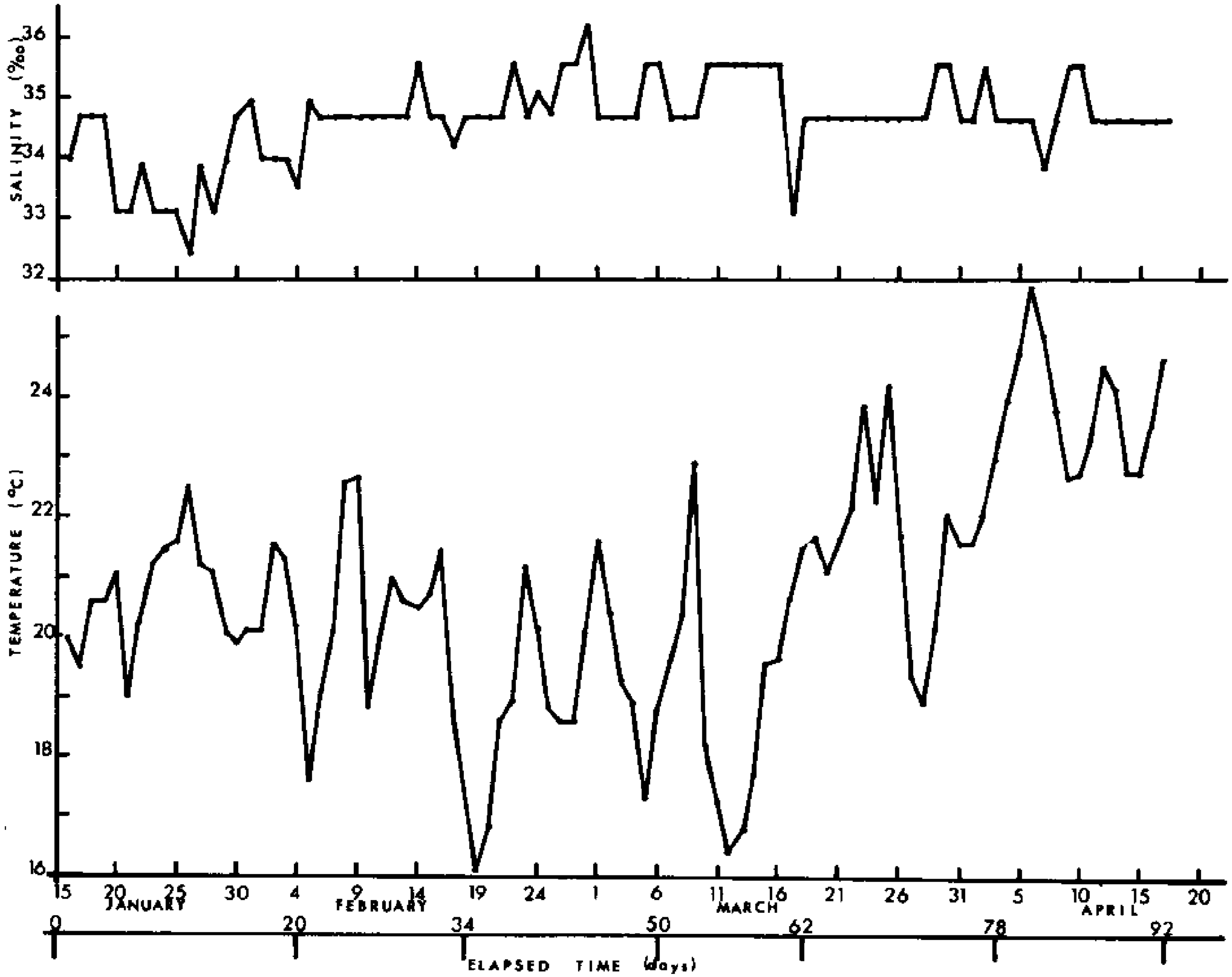

Figure 1.5. Daily temperature and salinity observations for the study period. Elapsed time values are placed at the dates of shrimp weighing.

(Figure 1.5) with the molting frequencies shows that while salinity remained relatively constant, i.e. within a range of about 3°/₀₀, water temperatures varied greatly (16.1 to 25.5°C). The cold spell beginning on 14 February, in which water temperatures dropped to almost 16°C, caused the first major departure from the natural molting rhythm. From Figure 1.4 it appears that temperatures below about 18 to 19°C depress molting in pink shrimp. As water temperatures rose above this level, after cold spells on 11 and 27 March, the molting frequency increased near the times of quarter moons, but not precisely at these times. Thus, the molting profile tends to mirror the temperature plot when the two are examined together.

Molting frequency data were correlated with temperature, moon phase, and the number of elapsed days, to determine the factor or factors having the greatest influence upon molting. Results of multiple regression correlations which employed the BMD02R stepwise regression computer program of the Health Sciences Computing Facility, University of California at Los Angeles, are presented in Table 1.6. The correlations revealed a significant analysis of variance F value (at the 99% level) for the regression equation with the Multiple R-squared value accounting for 28% of the variation in molting frequency. Analysis of the effects of individual factors indicated significant influence for both temperature (at the 99% level) and moon phase (at the 95% level); elapsed time was not significant.

Tabb's data mentioned above were obtained from shrimp studied at higher, more constant temperatures and fed at a greater rate than shrimp in this study. Thus, fluctuating low temperatures appear to disrupt the molting rhythm of the pink shrimp which tends to reach its greatest frequency at the times of quarter phases of the moon. Low temperatures may be of greater than normal consequence to molting when the shrimp are maintained under conditions of stress, i.e. reduced food and high population density, as in this experiment.

Growth Efficiencies

Further evidence of the differences in growth and yield among tank-types is presented in Table 1.7, which lists the growth efficiencies for each tank, and tank-type averages. The growth efficiency is here defined as the relationship of milligrams of wieght change per gram of initial weight per elapsed day in a weighing period to the milligrams of food consumed per gram of initial weight per elapsed day in the weighing period. Even though increases in shrimp size and weight occur in steps at molting, efficiencies are given on a daily basis so that data from periods of different length may be compared. Values listed are actually percentages of the amounts of food ingested which were used for growth. In other words, shrimp were fed at the rate of 100 milligrams of food per gram of body weight (10% of the wet weight) and weight changes are expressed as milligrams per gram of wet weight. Thus, assuming that each shrimp obtained 100 milligrams of food per gram of wet weight, the growth efficiencies are the percentages of that 100 milligrams of food which were used for growth.

In all cases except one (Period IV), average growth efficiencies for the artificial grass tanks were equal to or greater than those

Table 1.7. Growth efficiencies for each tank by weighing period. Values are presented as milligrams of weight change/gram of shrimp weight at the start of a period/day in that period. Since all shrimp were fed at the same rate (10% wet weight/day), feeding data are not listed.

Weighing period	Control tanks	Chemturf tanks	Olefern tanks
I	13.2	18.3	13.9
	14.4	9.3	22.8
Ave.	13.8	13.8	18.4
II	7.4	16.6	16.8
	0.9	10.0	6.7
Ave.	4.2	13.3	11.8
III	3.1	15.7	9.3
	11.9	9.3	5.6
Ave.	7.5	12.5	7.5
IV	23.2	0.4	10.1
	16.7	17.1	11.2
Ave.	20.0	8.8	10.7
V	5.2	6.6	7.6
	4.9	8.4	5.5
Ave.	5.1	7.5	6.6
VI	6.0	13.7	7.1
	0.6	8.5	7.1
Ave.	3.3	11.1	7.1

for the control tanks. Differences between efficiencies in artificial grass tanks and control tanks were presumably due to shrimp grazing upon fouling in the grass tanks. The differences vary in degree among the weighing periods, a result for which an explanation is not apparent.

The trend for each tank-type is for the growth efficiency to decline as the time in the tanks increases. Such a trend reflects the increasing cost of maintenance as the shrimp weight increased during the study leaving proportionally less food energy for growth.

Average growth efficiencies ranged from 7.5 to 13.8% in Chemturf tanks, 6.6 to 18.4% in Olefern tanks, and 3.3 to 20.0% in control tanks. Food conversion values (expressed as weight of food/change in shrimp weight) ranged from 13.3 to 7.2 for Chemturf tanks, 15.0 to 5.4 for Olefern tanks, and 30.0 to 5.0 for control tanks.

Utilization of the Artificial Grasses

Wilcoxon's signed rank test was used to compare the numbers of shrimp moving on the two artificial grasses. For both the day and night observations the test values were smaller than tabular critical values (Steel and Torrie, 1960). Thus, shrimp made significantly greater use of the Chemturf substrates than those of Olefern during the day (at the 99 per cent level) and during the night (at the 95 per cent level).

During the day, more shrimp remained out of the sand in Chemturf tanks than in Olefern tanks. A possible explanation for this is the relative shading produced by the two grasses. Pink shrimp are known to burrow into sand during daylight hours unless the light intensity is reduced by clouds or water turbidity (Eldred et al., 1961; Fuss and Ogren, 1966; Fuss and Wathne, 1964; Hughes, 1968 and 1969; Mikulka, 1969). The algal growth along the water surface, as well as shading by the artificial configurations, produced considerable reduction in incident light between the water surface and the bottoms of the tanks. Because of their physical structure, Chemturf substrates created more shade than did the Olefern substrates. Therefore, lower incident light levels in Chemturf tanks may have resulted in greater emergence from the sand by shrimp in these tanks.

In addition, Chemturf substrates were constructed of separated layers while Olefern substrates were of long, ribbon-like filaments which floated upwards together and presented essentially a solid, cylindrical structure to the shrimp. Shrimp were able to penetrate and sit on Chemturf substrates with ease. Increased shading with greater emergence of shrimp and easier access to the surfaces of the substrates in Chemturf tanks may have resulted in the observed preference shown by the shrimp for the Chemturf substrates.

Since light conditions were the same in all tanks at night, greater utilization of the Chemturf substrates may have been due solely to the ease of access to the surfaces of the substrates. The degree of difference in utilization of the two grasses at night is less than that detected for the daytime observations, possibly indicating more random movement of the shrimp at night. During the night shrimp were seen actively swimming in the tanks, but in the daytime most shrimp were not moving about. Clearly, more shrimp made use of the substrates at night, if only because more shrimp were out of the sand at this time than during the day.

Utilization of the Fouling Organisms as Food

Analysis of the stomach contents of shrimp preserved at the end of the study and relative abundances of food items are recorded in Table 1.8. Relative abundance values represent subjective evaluations of the organisms identified, and the values in Table 1.8 are averages of the appraisals made for shrimp from each tank.

The presence of food, other than squid, in control tank shrimp was not unexpected since small amounts of fouling grew on the sides of the tanks, accounting for the filamentous algae in the stomachs. However, these items were found only occasionally. Conversely, the common occurrence of the diatom, Pleurosigma, was due to grazing upon a well-developed diatom film on the sand in both control tanks. This film was evident by the end of January, and it caused a brownish coloration of the surface of the sand.

A diatom film did not develop in the artificial grass tanks, but filamentous algae were abundant in the fouling community on the water surface of the tanks. The shrimp demonstrated marked preference for the green algae, Cladophora and Enteromorpha, while evidently avoiding consumption of the blue-green, Oscillatoria, which was also abundant in the fouling community.

Table 1.8. Stomach content analysis, by tank-type, of shrimp preserved at the end of the experiment. Six shrimp from each tank were examined to obtain the relative abundance of food items listed.

Organism	Control tanks	Chemturf tanks	Olefern tanks
Copepods and copepod parts	*	****	****
Foraminifera	*	0	0
Algae			
Pleurosigma	***	0	0
Unident. diatom	*	0	0
Cladophora	**	***	***
Enteromorpha	**	***	***
Oscillatoria	*	0	0

0 = item not found
* = item found rarely
** = item found occasionally
*** = item found commonly
**** = item found abundantly

Copepods, both harpactacoid and cyclopoid, were abundant in the stomachs of shrimp from grass tanks. The copepods grew in the algal mats and were apparently actively selected by the shrimp since other equally abundant organisms, such as nematode worms, were not found in any of the stomachs examined. Nematodes have been listed among the stomach contents of pink shrimp by Eldred et al. (1961) and Idyll, Tabb and Yokel (1968), and the reason for their absence in shrimp stomachs from this study is not known.

Behavior Observations

The following observations were made intermittently as the study progressed, and they are, in most cases, simply qualitative interpretations of the behavior of pink shrimp in the culture tanks.

The circadian activity rhythm of the shrimp persisted throughout the 92 days of the study. Although it was not uncommon to find a few shrimp out of the sand early in the morning, all of them had usually burrowed into the sand by noon on sunny days. This included newly molted shrimp, an observation which is contrary to reports by Eldred (1958), who stated that pink shrimp do not burrow into the sand for about two days after molting. Confirmation that burrowing occurred only a few hours after molting was easy since shrimp molts and shrimp were readily seen out of the sand in the control tanks. On many occasions fresh molts were found on the bottom of a tank, but no shrimp were out of the sand. It was not believed that cannibalism resulted in the absence of newly molted shrimp out of the sand since the total number of molts found during the daily collections exceeded the total number of shrimp in the tanks. Also, the remnants of freshly molted and partially eaten shrimp were seldom found in the tanks. This would be expected if the newly molted shrimp were being cannibalized since it takes a few hours for a dead shrimp to be completely consumed.

A few of the shrimp in each tank remained buried in the sand during darkness. These were located with the aid of reflection of the red flashlight beam from their eyes just above the surface of the sand. It is not known why these shrimp remained in the sand at night.

When shrimp were out of the sand and moving about the tanks at night, presumably in search of food, few of them were in the center area of the tank bottom. Most of the shrimp were swimming up, down, and along the tank sides. A possible explanation for this is that because of the relative amounts of surface presented to the shrimp by the tank sides and the grass substrates, there was a much greater chance for a shrimp to come in contact with the sides of the tank rather than a substrate. If a shrimp did contact a substrate, it could go around it instead of onto it. However, contact with a tank side acted to concentrate the shrimp since they seldom swim backwards, unless alarmed, and they could not go around the barrier.

Some of the shrimp apparently molted during daylight hours. Occasionally, after checking for molts in the morning, fresh molts were found when examining the tanks prior to feeding in the afternoon.

The molts may have been overlooked in the morning but because of the fresh appearance of the molts it is not believed that this happened. Molts from the previous night were always at least partly eaten by shrimp in the tanks.

Two types of feeding behavior were observed. In the first, food was picked up with the chela or first pair of walking legs and passed to the mouth, and in the second the shrimp moved along the algal mat with the body inclined forward so that the mouth was in contact with the fouling organisms. It is assumed that the shrimp were actually feeding when the second feeding method was noted.

The shrimp soon became accustomed to the time of daily feeding. As Hughes (1969) stated, emergence from the sand may be influenced by previous feeding times. Thus, in all tanks, emergence of the shrimp increased markedly at about 4:00 p.m. each day, the time of feeding.

Shrimp did not consume the chitinous pen structure of squid. However, cartilaginous squid parts were readily seized and eaten.

When food was added as a single clump shrimp began searching movements within one or two minutes, and within about three minutes the first shrimp had made contact with the clump of food. This shrimp would pull the clump of food around the tank while attempting to remove some of the squid. In this way, food was distributed to the other shrimp in the tank within about 30 minutes.

Periods of low water temperature (below about 18°C) caused markedly decreased feeding, and food accumulated in the tanks. This food was left in the tanks to be eaten as the water warmed. Cold spells during the study were not prolonged, and accumulation of food did not often occur.

After approximately a month in the tanks, molts in the control tanks were being consumed to a greater degree than those in grass tanks. Whereas molts in the grass tanks were generally found to be lacking only the appendages, the only part remaining of control tank molts was the carapace.

Further indication of the difference in dietary conditions between grass and control tanks was the accumulation of fecal material in the grass tanks. No fecal pellets accumulated in the control tanks. Evidently the fecal material was consumed almost as soon as it was expelled by the control tank shrimp which did not have access to the added food source provided by the fouling organisms in the grass tanks.

Parasites

On 13 February, one shrimp in a control tank was noticed to have a whitish discoloration in its abdominal tissues. The shrimp was recovered at the end of the study and examined for parasites. It was found to be heavily infected by a microsporidian, *Thelohania* sp. (Iversen, personal communication). Iversen and Manning (1959) described the infection of pink shrimp by *Thelohania duorara* in Biscayne Bay, the location from which shrimp for this study were taken.

EXPERIMENT TWO

PROCEDURES

Substrates employed in the second study were panels of fiberglass window screen. The synthetic grasses used previously were discarded for the following reasons: 1) algae grew only on the water surface and did not penetrate the water column to an appreciable degree; 2) algae which did grow below the water surface was simply entangled in the artificial grasses and was easily dislodged if disturbed; 3) use of the synthetic grasses in pond culture would result in high costs because of both the increased labor necessary to manipulate the grass configurations during harvesting or pond preparation and the initial high cost of the materials themselves. A search for less expensive, easy to handle materials led to the selection of fiberglass window screen.

In addition to using a different substrate material, the number of tanks was increased to 24. The six tanks used in experiment one were each divided in half using plywood partitions, creating twelve tanks measuring one-half meter wide, one meter long, and one meter deep. Another set of twelve identical tanks was built, bringing the total to 24. The arrangement of the tanks for this experiment is shown in Figure 2.1.

The availability of 24 tanks meant that more than one variable could be incorporated into the study. Thus, stocking density and presence or absence of substrates were selected as the variables to be analyzed.

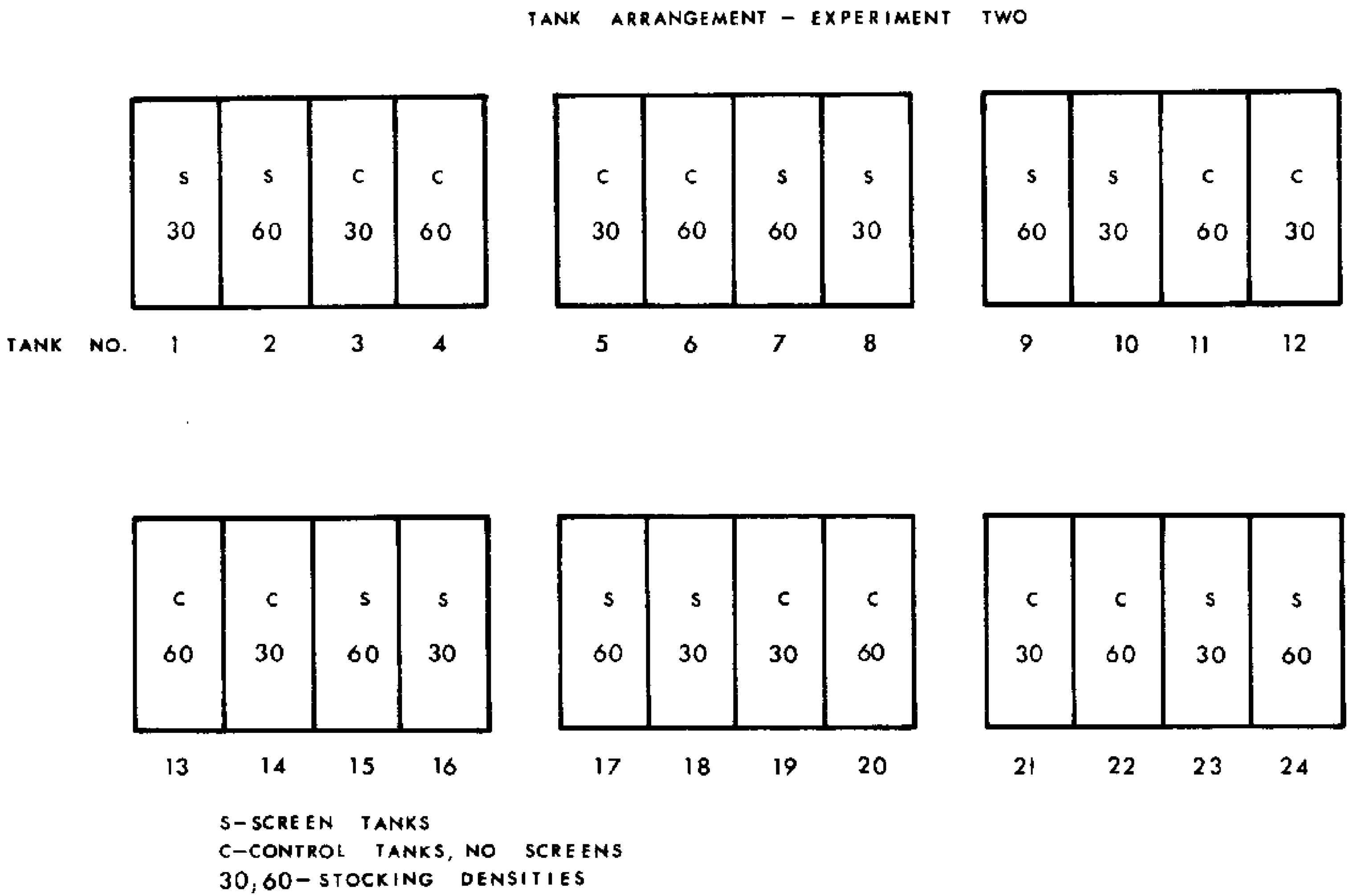

Figure 2.1. The arrangement of tanks and variables for Experiment Two.

Each experimental tank was equipped with two panels of screen; the control tanks had none. Half of the experimental tanks and half of the control tanks were stocked with 30 shrimp apiece. The remaining twelve tanks were stocked with 60 shrimp each. Thus, the following four combinations of the variables were employed, with six replicates of each: 1) 30 shrimp and 2 screen panels per tank (S-30); 2) 60 shrimp and 2 screen panels per tank (S-60); 3) 30 shrimp and no screens per tank (C-30); 4) 60 shrimp and no screens per tank (C-60).

The objectives of this study were essentially the same as those of the first experiment: to determine whether significantly better growth, survival, and total yield of shrimp could be obtained by using vertical substrates in the culture tanks. In addition, the effects of two stocking densities upon growth, survival, and yield were evaluated.

As in the previous study, tanks were supplied individually with continuous running sea water as well as surface and bottom drains. Water depth was maintained at 50 centimeters, and each tank had three to four centimeters of sand into which the shrimp could burrow.

Screen panels measured 80 centimeters wide (leaving approximately 10 cm at each end of the screen so that shrimp could move freely about the tanks after the screens were installed) and 80 cm long. Panels were constructed by folding 20 cm of the screen over a one-fourth inch wooden dowel and sewing along the dowel and lower edge of the overlapped portion of the panel with monofilament fishing line. Nylon cord was strung through holes in each end of the dowel, and the cords were tied to nails placed at appropriate intervals on the outside of the tanks. Thus, the dowels held the panels straight and the cords held the assemblage out of the water.

The nylon cords were adjusted in length so that the bottom edges of the screen panels rested on the sand and the dowels were 10 cm above the water surface. This created a double layer of screen from the water surface to a depth of 10 cm. The screening in the 10 cm portion of the overlap was used for periodic sampling to provide analysis of the composition of the fouling community and its standing crop.

The total underwater surface area per panel was 4,000 square centimeters (80 cm long, 50 cm deep) and this area remained constant since screen samples were taken only from the overlapped screen at the water surface. Panels were aligned parallel to each other at 16 to 17 centimeter intervals dividing the tanks into thirds. Panels were left as undisturbed as possible during the study so that fouling would develop on them.

Lead fishing net weights were tied to the bottom corners of each screen to hold it in a vertical position since algal fouling created sufficient buoyancy to life the screen off the sand if weights were not present.

Shrimp for this experiment, which lasted from 18 July through 13 October, 1969, were obtained from shrimp rearing ponds at the Turkey Point shrimp culture facility of the Rosenstiel School of Marine and Atmospheric Science. Juveniles were seined from the ponds and transported to the laboratory on Virginia Key where they were held for two days before stocking the tanks. All of the shrimp were Penaeus duorarum which had been raised from eggs spawned in the laboratory at Turkey Point. Before stocking the tanks, shrimp were randomly selected from the holding aquaria and weighed as in experiment one. The distribution of shrimp among the tanks is shown in Figure 2.1.

Supplemental food and feeding were the same as in the first study. The only change in the feeding procedure was in the preparation of the ground squid. Pens were removed from the squid before grinding for the following reasons: 1) pieces of the pens were not eaten by shrimp in experiment one; 2) pens clogged the grinder and added a great deal of time to processing the food. Amounts of food added were adjusted to the 10 per cent level after each weighing of the shrimp. At no time during the study was an appreciable amount of food left uneaten in the tanks, and food was added every day of the study.

Weighings were conducted during the day for this experiment so that the entire shrimp population from each tank could be removed and accurate measures of growth and mortality could be obtained. It was not possible to be certain that all shrimp were captured if weighing was conducted at night. A one-fourth inch mesh net, one-half meter wide and one meter long, was used for capturing the shrimp. The net was pulled through both the sand and water in a tank, and shrimp were removed to a container of water from that tank. All of the shrimp in a tank were not caught with one pass of the net, and sometimes five or six passes were required until two consecutive passes produced no shrimp. At this time, it was assumed that all of the shrimp in the tank had been removed. The shrimp were then taken into the laboratory and weighed by the same technique used in experiment one.

The first three weighings for this experiment were executed on the full or new moon phases for the same reasons given for experiment one. However, because of the number of shrimp which died during the first three weighings, the fourth weighing was conducted on the quarter moon in an attempt to reduce such handling mortality. The final weighing was also on the quarter moon because of the coincidental occurrence of the quarter moon and the total elapsed time of 90 days set aside for the study.

In this second experiment, dead shrimp were not replaced. Daily checks were made of each tank in the morning for shrimp mortalities, and any dead shrimp were removed from the tanks so that they did not serve as an added food source for the remaining shrimp. Because all of the shrimp were removed from each tank during weighing, accurate data on mortality were obtained.

During removal of the shrimp from the tanks for weighing it was not unusual for one or two shrimp from a tank to be captured in a condition in which the abdominal muscles were tightly contracted. Such abdominal flexure had not occurred in experiment one when weighing was conducted at night and the shrimp were normally active. It may be that since the pink shrimp is normally inactive during daylight a severe disturbance (such as that produced by capture in a net) is sufficient to cause extreme exertion which results in muscle tetanus and death. All shrimp which appeared with the flexed abdomen died soon after capture. However, because of the desire to weigh all shrimp in each tank and the type of net used to remove them, weighing during daylight was necessary even though some shrimp died in the process.

Water temperatures were monitored to the nearest 0.5°F at 24 hour intervals using two maximum-minimum thermometers. The thermometers were distributed among the tanks to detect an variations in water temperature from one end of the tank alignment to the other.

Salinity was monitored daily as in experiment one. Water flow rates were determined intermittently by measuring the amount of water inflow per minute and calculating the daily rate of tank volume overturn.

The diurnal dissolved oxygen cycle was monitored on 29-30 August for comparison of the dissolved oxygen among screen tanks and control tanks. Between about noon and 6:00 p.m., dissolved oxygen in both screen and control tanks was above 100 per cent saturation. After 6:00 p.m., the dissolved oxygen dropped below 100 per cent saturation and decreased to minimum values of 3.5 ppm in screen tanks and 4.0 ppm in control tanks. Egusa and Yamamoto (1961) noted that at a level of 1.0 ppm juvenile Penaeus japonicus showed signs of distress and died at a level of 0.5 ppm. The dissolved oxygen in all tanks was in excess of both of these levels, and it is assumed that oxygen was not dangerously low during the study as long as water flow was continuous. The water flow rate was equivalent to an average of 2.13 tank volumes per day.

Shrimp molts were collected from each tank at two day intervals in the morning. Collection of the molts was made easier than in the previous study by passing a small dipnet lightly over the sand to pick up carapaces and whole molts.

Behavior observations were made intermittently throughout the study to determine the degree to which shrimp at the two stocking densities utilized the screen surfaces at night. A red-filtered flashlight was used to locate and count shrimp on the screens. Observations were also made of the time taken for the shrimp to locate and seize food after its introduction into the tanks.

The twelve tanks with screen panels were seeded with algal fouling from other outdoor tanks using the laboratory sea water system. Consequently, fouling developed rapidly on the screens. Seedings and screens were placed in the tanks seven days prior to stocking with shrimp so that the system could stabilize, fouling could develop, and any toxic chemicals in the tanks or screens could leach out. As the experiment progressed and algal fouling developed in all 24 tanks, it became necessary to scrub the sides of all tanks every seven to ten days so that the only food sources were fouling on the screens and the daily ration of squid. Fragments of algae and other fouling organisms scrubbed from the tank sides were buoyant and were removed from the system by slightly increasing the inflow of water and letting the water spill freely out of the upper drain hole, carrying fouling material with it.

Screen samples taken before each weighing were preserved in five per cent formalin so that the fouling organisms could be identified. Algae were identified to genus and most other organisms were identified to class. Relative abundance estimates for the fouling organisms were established subjectively and were correlated with similar estimates for organisms found in the stomach contents of shrimp preserved at the end of the study.

The standing crop of the fouling community was determined by taking a 2.5 cm square piece of each preserved screen sample, drying the sub-sample, and weighing it to the nearest 0.0001 gram. The fouled sub-sample was then placed in 18N sulfuric acid in which all fouling was completely removed (the fiberglass screen was unaffected). The squares were then washed in tap water, dried, and weighed. Differences in weights for fouled and cleaned samples gave the biomass per 6.25 square centimeters (one square inch) of fouling on the screen. This assumed that fouling was uniform over the entire screen. In fact, it was not, but the samples are assumed to be representative of conditions as they existed.

Large fouling organisms, i.e. tunicates and barnacles, were generally left undistrubed unless they interferred with water flow into or out of the tanks. These organisms were a problem only in the drain lines since the tank sides were scrubbed regularly. Tunicate growth upon the screens was fairly heavy, but it did not appear to disrupt utilization of the screen surfaces by the shrimp.

Final shrimp counts and weights were obtained by draining the tanks and sifting through the sand to be sure that all shrimp were recovered. Six shrimp from each tank were preserved in 10 per cent formalin for future examination of the stomach contents and determination of whether or not the shrimp had been feeding upon fouling organisms on the screens.

RESULTS AND DISCUSSION

Shrimp Growth

The average shrimp weights obtained by weighing the entire population of shrimp in each tank at approximately two-week intervals (Table 2.1) were used for computing growth regressions for the tanks with the formula, average shrimp weight = initial shrimp weight + b (time). The regression slope values (b) are listed in Table 2.2 and the tank-type average slopes are plotted in Figure 2.2, demonstrating the differences in average growth rates among populations in the four tank-types.

Shrimp in the tanks with 30 shrimp and screen panels grew at the fastest rate (b = .02055), those in tanks with 60 shrimp and screens grew at the second fastest rate (b = .01391), shrimp in tanks with 30 shrimp and no screens grew slower (b = .00917), and growth in tanks with 60 shrimp and no screens was slowest (b = .00446).

A statistical comparison of the 24 growth slopes using an analysis of variance (Table 2.2) revealed that faster growth was obtained in tanks with 30 shrimp than those with 60 shrimp at the 99 per cent level. In addition, shrimp in tanks with screen panels grew faster than those in tanks with no panels at the 99 per cent level. Thus, from these results it would be expected that the combination of 30 shrimp and screen panels would produce the best growth under the

Table 2.1. Replicate average shrimp weights (in grams) determined at two-to-three week intervals for the four tank-types.

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
Start	1.72	1.88	1.71	1.73
	1.87	1.71	1.79	1.74
	1.82	1.81	1.73	2.00
	1.60	1.76	1.66	1.63
	1.64	1.87	1.80	1.97
	1.85	1.89	1.90	1.88
Ave.	1.75	1.82	1.77	1.83
I	2.17	2.12	1.97	1.83
	2.32	1.95	1.96	1.83
	2.39	2.11	1.95	2.11
	1.95	2.08	1.78	1.67
	2.22	2.23	2.09	2.13
	2.27	2.22	2.25	1.94
Ave.	2.22	2.12	2.00	1.92
II	2.44	2.27	1.97	1.88
	2.38	2.16	2.08	1.81
	2.60	2.14	2.15	2.18
	2.23	2.32	1.98	1.67
	2.40	2.43	2.06	2.14
	2.68	2.42	2.03	2.09
Ave.	2.46	2.29	2.05	1.96
III	2.98	2.55	2.21	2.03
	2.89	2.51	2.51	1.97
	3.04	2.67	2.46	2.19
	2.64	2.64	2.14	1.81
	3.04	2.73	2.25	2.26
	3.05	2.75	2.38	2.23
Ave.	2.94	2.64	2.33	2.08
IV	3.43	2.80	2.34	2.05
	3.24	2.72	2.53	2.02
	3.37	3.00	2.71	2.31
	3.23	3.17	2.46	1.91
	3.55	2.97	2.57	2.48
	3.50	3.05	2.68	2.47
Ave.	3.39	2.95	2.55	2.21

Table 2.1. Continued

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
V	3.81	2.96	2.33	2.03
	3.34	2.85	2.61	1.90
	3.50	3.12	2.79	2.47
	3.67	3.31	2.40	1.95
	3.80	3.15	2.61	2.45
	3.94	3.16	2.81	2.49
Ave.	3.68	3.09	2.59	2.22

Table 2.2. Replicate growth regression slopes for the four tank-types with an analysis of variance of the slope values. Regressions were computed with the formula, average shrimp weight = initial shrimp weight + b (time).

	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
Slope	.0223170	.0116600	.0066414	.0034014
	.0160230	.0125520	.0092890	.0022860
	.0175470	.0148520	.0118890	.0044778
	.0222750	.0172680	.0089902	.0038037
	.0232970	.0133290	.0086785	.0054623
	.0218510	.0137800	.0095399	.0073231
Ave.	.0205520	.0139070	.0091713	.0044591

Source	df	MS	F
Treatments			
Density (30 vs. 60)	1	.000650715666	88.41 **
Screens vs. controls	1	.000193476149	26.29 **
Among blocks	5	.000006233173	0.85 ns
Interactions			
Density X screens	1	.000005602124	0.76 ns
Density X blocks	5	.000000731568	0.10 ns
Screens X blocks	5	.000004467556	0.61 ns
Error	5	.000007360582	
Total	23		

**Significant at the 99% level
ns = Non-significant F value

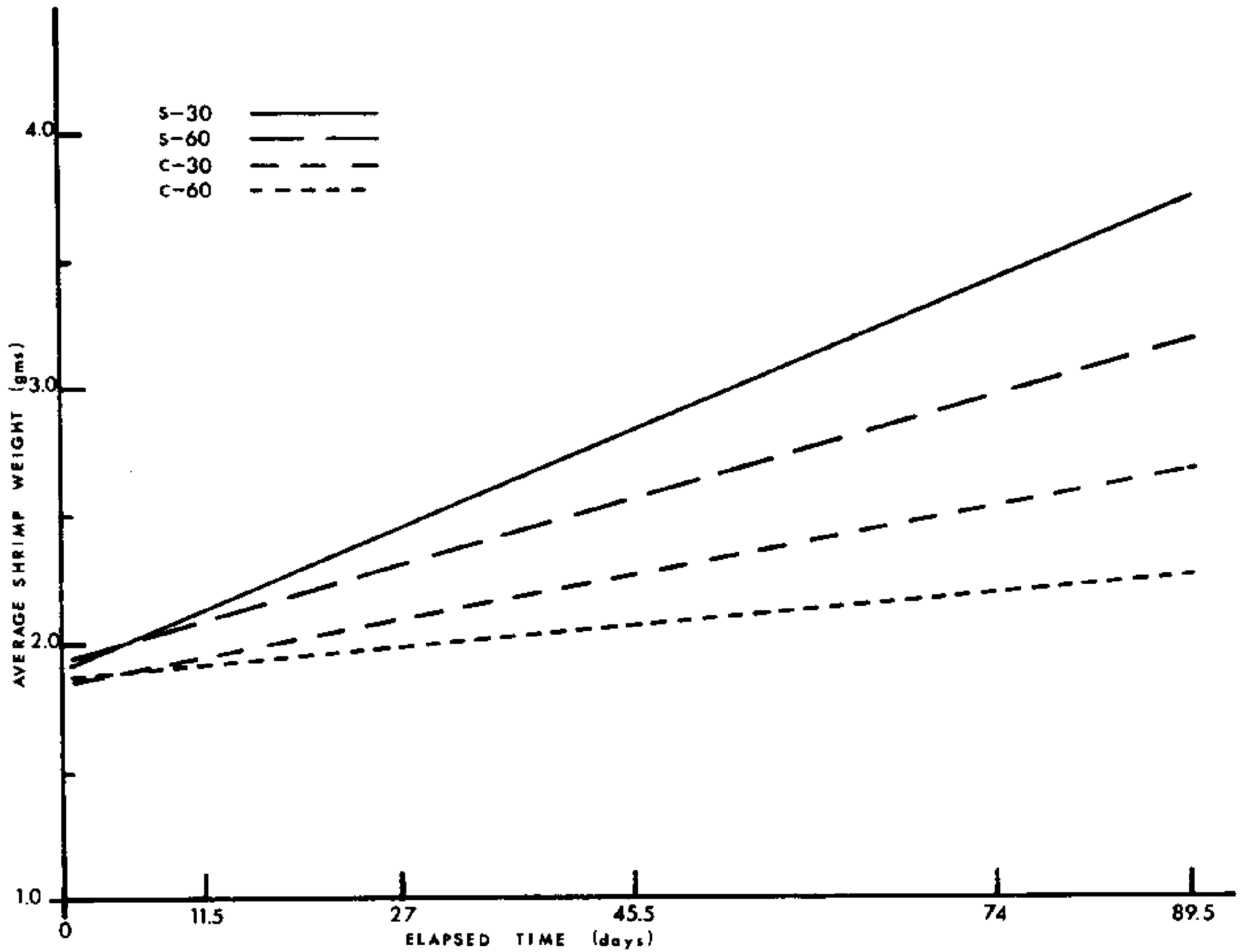

Figure 2.2. The average shrimp weight as a function of elapsed time for the four tank-types. Plotted lines represent the averages of six replicate slope values for each tank-type (Table 2.2). Weighing intervals are shown on the X axis.

experimental conditions. This was actually the case as shown in Figure 2.2.

No differences were detected for any of the interaction terms or among blocks (replicates). As in the first study, intercept values were not different, indicating that comparable sets of animals were stocked in the tanks at the start of the experiment.

From an average initial weight of approximately 1.8 grams for shrimp in each tank, S-30 tanks produced shrimp averaging 3.68 grams, S-60 tank shrimp averaged 3.09 grams, C-30 shrimp averaged 2.59 grams, and C-60 shrimp averaged 2.22 grams after 90 days under the experimental conditions. Shrimp in the S-30 tanks grew about 0.6 grams per month, and shrimp in the other tank-types grew proportionately less per month.

Shrimp Mortality

Since dead shrimp were not replaced in this experiment, mortality regressions could be computed for each tank and these were compared statistically as was done for the growth data. Because of the differences in stocking density, the ratio N_o/N_t, in which N_o is the number of shrimp stocked and N_t is the number remaining in the tank at the end of a weighing period, was employed as the Y value in computing the regressions so that all tanks would be on an equal basis for comparison of the mortality rates.

Mortality ratios for each tank at the times of weighings are listed in Table 2.3, and these ratios were used for computing the mortality regression slopes shown in Table 2.4, using the

Table 2.3. Replicate mortality ratios for the four tank-types. Ratios were calculated as the number of shrimp stocked initially/the number of shrimp remaining at the end of a weighing period. Figures in parentheses are the cumulative numbers of dead shrimp.

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
I	1.000 (0)	1.034 (2)	1.000 (0)	1.034 (2)
	1.034 (1)	1.091 (5)	1.000 (0)	1.000 (0)
	1.034 (1)	1.053 (3)	1.000 (0)	1.034 (2)
	1.000 (0)	1.071 (4)	1.034 (1)	1.053 (3)
	1.000 (0)	1.000 (0)	1.000 (0)	1.053 (3)
	1.000 (0)	1.071 (4)	1.034 (1)	1.034 (2)
Ave.	1.011	1.053	1.011	1.035
II	1.034 (1)	1.091 (5)	1.034 (1)	1.154 (8)
	1.071 (2)	1.154 (8)	1.111 (3)	1.154 (8)
	1.034 (1)	1.200 (10)	1.250 (6)	1.111 (6)
	1.000 (0)	1.091 (5)	1.071 (2)	1.091 (5)
	1.034 (1)	1.053 (3)	1.071 (2)	1.091 (5)
	1.034 (1)	1.071 (4)	1.071 (2)	1.053 (3)
Ave.	1.035	1.110	1.101	1.109
III	1.034 (1)	1.091 (5)	1.034 (1)	1.176 (9)
	1.071 (2)	1.176 (9)	1.111 (3)	1.154 (8)
	1.111 (3)	1.395 (17)	1.250 (6)	1.277 (13)
	1.111 (3)	1.154 (8)	1.071 (2)	1.111 (6)
	1.034 (1)	1.071 (4)	1.111 (3)	1.250 (12)
	1.071 (2)	1.091 (5)	1.250 (4)	1.111 (6)
Ave.	1.073	1.163	1.138	1.180
IV	1.071 (2)	1.091 (5)	1.034 (1)	1.200 (10)
	1.071 (2)	1.224 (11)	1.154 (4)	1.250 (12)
	1.154 (4)	1.579 (22)	1.304 (7)	1.500 (20)
	1.250 (6)	1.304 (14)	1.111 (3)	1.176 (9)
	1.034 (1)	1.091 (5)	1.111 (3)	1.538 (21)
	1.154 (4)	1.250 (12)	1.250 (4)	1.200 (10)
Ave.	1.122	1.257	1.161	1.311
V	1.071 (2)	1.200 (10)	1.071 (2)	1.224 (11)
	1.154 (4)	1.429 (18)	1.154 (4)	1.277 (13)
	1.429 (9)	1.765 (26)	1.500 (10)	1.622 (23)
	1.429 (9)	1.333 (15)	1.154 (4)	1.200 (10)
	1.304 (7)	1.224 (11)	1.364 (8)	1.538 (21)
	1.429 (9)	1.250 (12)	1.304 (7)	1.429 (18)
Ave.	1.303	1.367	1.258	1.382

Table 2.4. Replicate mortality slopes for the four tank-types with an analysis of variance of the slope values. Regressions were computed with the formula, $\log_e$ of the mortality ratio = b (time). All intercepts were at the origin of the graph.

	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
Slopes	.00159180	.00188260	.00053828	.00275770
	.00207300	.00386000	.00162510	.00236480
	.00155270	.00517560	.00354830	.00384960
	.00090459	.00319530	.00167370	.00237200
	.00144850	.00114250	.00162670	.00387030
	.00061731	.00266260	.00273190	.00226870
Ave.	.00136465	.00298643	.00195733	.00291385

Source	df	MS	F
Treatments			
Density (30 vs. 60)	1	.000009971472	12.31 *
Screens vs. controls	1	.000000405752	0.50 ns
Among blocks	5	.000002211134	2.73 ns
Interactions			
Density X screens	1	.000000663862	0.82 ns
Density X blocks	5	.000000254645	0.31 ns
Screens X blocks	5	.000000716374	0.88 ns
Error	5	.000000809765	
Total	23		

*Significant at the 95% level
ns = Non-significant F value

formula, $\log_e$ of the mortality ratio = b (time). The four tank-type mortality slopes in Table 2.4 were used for plotting the regression lines in Figure 2.3 in which lower slope values indicate better survival.

Examination of the slope values reveals that shrimp in the S-30 tanks had the lowest mortality (b = .00136), those in the C-30 tanks experienced slightly higher mortality (b = .00196), and shrimp in C-60 and S-60 tanks had nearly identical mortality rates (b = .00291 and b = .00299, respectively).

Statistical comparison employing an analysis of variance of the mortality slope values revealed no significant differences between tanks on the basis of the presence or absence of screen panels. However, tanks with 30 shrimp had significantly lower mortality rates than tanks with 60 shrimp ($Pr \leq .05$). Thus, survival of the shrimp during the study appears to have been inversely related to the stocking density.

As with the growth data, there were no differences among the six replicate tank blocks or among the interaction terms.

From an initial stocking density of 30 shrimp per tank the S-30 tanks lost an average of 6.7 shrimp or 22 per cent, and the C-30 tanks averaged 5.9 shrimp deaths or 19 per cent. From an initial stocking density of 60 shrimp per tank the S-60 tanks experienced an average loss of 15.3 shrimp or 26 per cent and the C-60 tanks incurred an average of 16.0 shrimp deaths or 27 per cent.

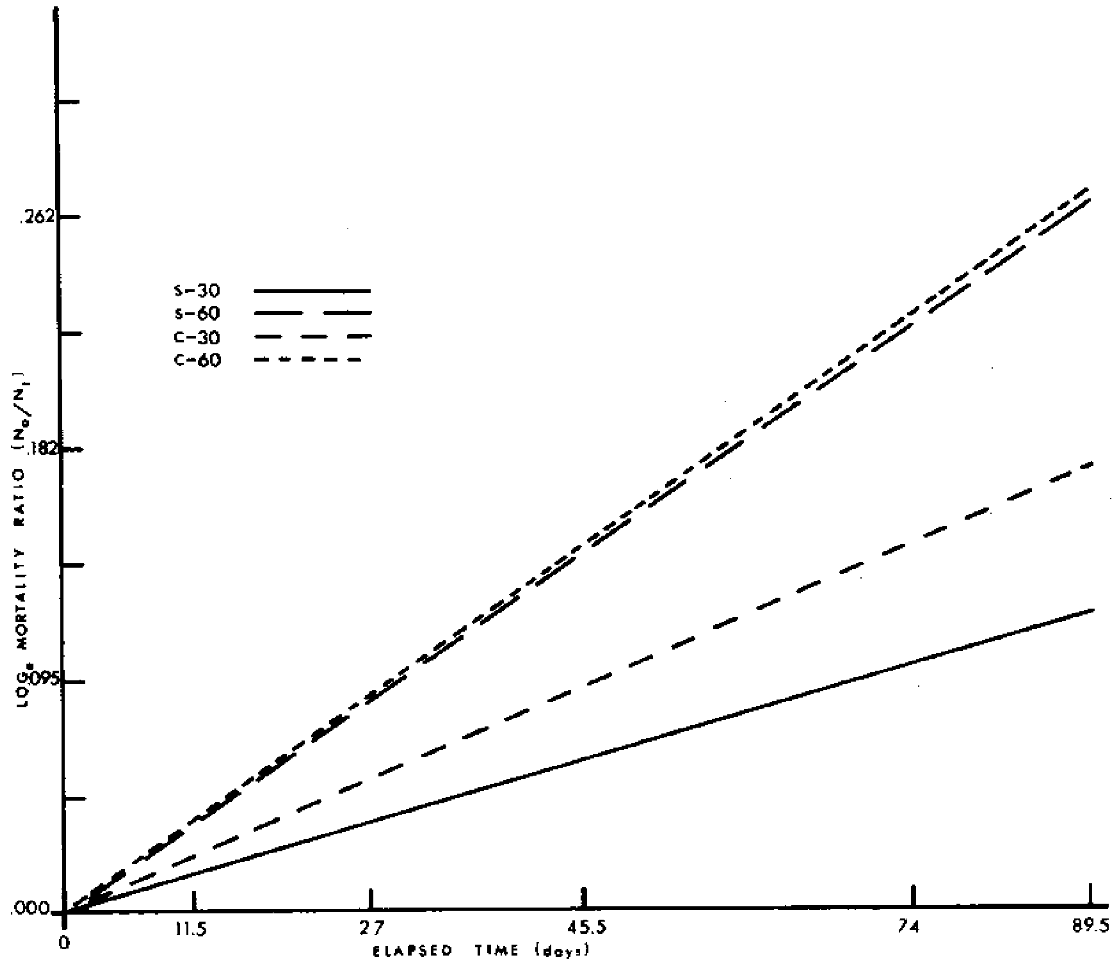

Figure 2.3. The tank-type mortality ratio as a function of elapsed time. N_o is the number of shrimp at the start of the study and N_t is the number remaining at the end of a weighing period. Regression lines are averages of six replicate slope values for each tank-type (Table 2.4). Weighing intervals are shown on the X axis.

It was stated previously that deaths apparently due to disturbing and handling the shrimp during daylight weighing were not uncommon in this experiment. Of a total of 45 such handling mortalities, only 4 were from the tanks stocked with 30 shrimp and the remaining 41 were from the tanks stocked with 60 shrimp. This difference was probably a result of stress conditions at the higher population densities rather than varying nutritional conditions among the shrimp, since all tanks received the same rate of supplemental feeding and the handling deaths were nearly evenly distributed among the screen and control tanks at their respective shrimp densities. Thus, handling and disturbances appear to have greater effects upon shrimp already stressed.

Shrimp Production

Replicate values of the total shrimp weights for the weighing periods are shown in Table 2.5, and these weights were used to compute the production regression slopes in Table 2.6 using the formula $\log_e$ total shrimp weight ratio = b (time). The average tank-type slope values were employed for plotting the production regression lines in Figure 2.4.

Total production was greatest in the S-30 tanks (b = .37846) and less in the S-60 tanks (b = .32489), but the rate was considerably lower in the C-30 tanks (b = .11050) while in the C-60 tanks (b = -.14123) the yield was actually less than the weight originally stocked.

Table 2.5. Replicate total shrimp weights (in grams) determined at two-to-three week intervals for the four tank-types.

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
Start	51.6	112.9	51.2	103.9
	56.1	102.7	53.6	104.1
	54.5	108.6	51.8	120.1
	47.9	105.5	49.8	97.9
	49.3	112.4	54.0	118.2
	55.5	113.3	56.9	112.9
Ave.	52.5	109.2	52.9	109.5
I	65.2	127.0	59.0	106.0
	67.2	116.7	58.7	109.5
	69.4	124.6	58.5	124.4
	58.6	118.7	51.5	98.4
	66.7	133.7	62.8	121.3
	68.1	128.9	67.4	116.4
Ave.	65.9	124.9	59.7	112.7
II	70.9	124.6	57.1	98.0
	66.6	112.3	58.1	94.1
	75.3	115.3	51.7	117.6
	66.9	127.5	55.3	92.1
	73.6	146.0	57.8	119.2
	80.3	135.4	56.7	118.9
Ave.	72.3	126.9	56.1	106.7
III	86.5	140.5	64.2	103.7
	80.8	130.6	67.7	102.4
	82.0	115.0	59.1	111.9
	71.2	137.5	59.8	99.5
	88.1	155.8	60.8	108.7
	85.3	153.8	57.0	124.6
Ave.	82.3	138.9	61.4	108.5
IV	96.1	154.2	68.0	102.5
	90.6	136.2	65.9	96.9
	87.6	126.0	65.0	99.4
	77.6	145.7	66.4	99.5
	103.0	163.4	69.3	101.7
	97.9	152.3	64.4	123.5
Ave.	92.1	146.3	66.5	103.9

Table 2.5. Continued

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
V	106.8	147.8	65.2	99.7
	86.9	119.9	67.8	89.4
	73.5	106.2	55.8	91.5
	77.0	148.8	62.5	97.4
	87.4	154.5	57.4	95.6
	82.8	151.7	64.7	104.4
Ave.	85.7	138.2	62.2	96.3

Table 2.6. Replicate total shrimp weight regression slopes for the four tank-types with an analysis of variance of the slope values. Regressions were computed with the formula, $\log_e$ total shrimp weight ratio = b (time). Ratios were expressed as the total shrimp weight at the end of a period/the total shrimp weight stocked initially. All intercepts were at the origin of the graph.

	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
Slopes	.57817	.41357	.15541	-.03668
	.36098	.24037	.15013	-.15631
	.22195	-.01453	.07384	-.35195
	.30390	.45674	.17234	.01657
	.46679	.44675	.06090	-.28633
	.33894	.40641	.05040	-.03268
Ave.	.37846	.32489	.11050	-.14123

Source	df	MS	F
Treatments			
Density (30 vs. 60)	1	.000094060219	259.02 **
Screens vs. controls	1	.000168223103	463.26 **
Among blocks	5	.000002265657	6.24 *
Interactions			
Density X screens	1	.000000707548	1.95 ns
Density X blocks	5	.000001095488	3.02 ns
Screens X blocks	5	.000001611361	4.44 ns
Error	5	.000000363132	
Total	23		

**Significant at the 99% level
* Significant at the 95% level
ns = Non-significant F value

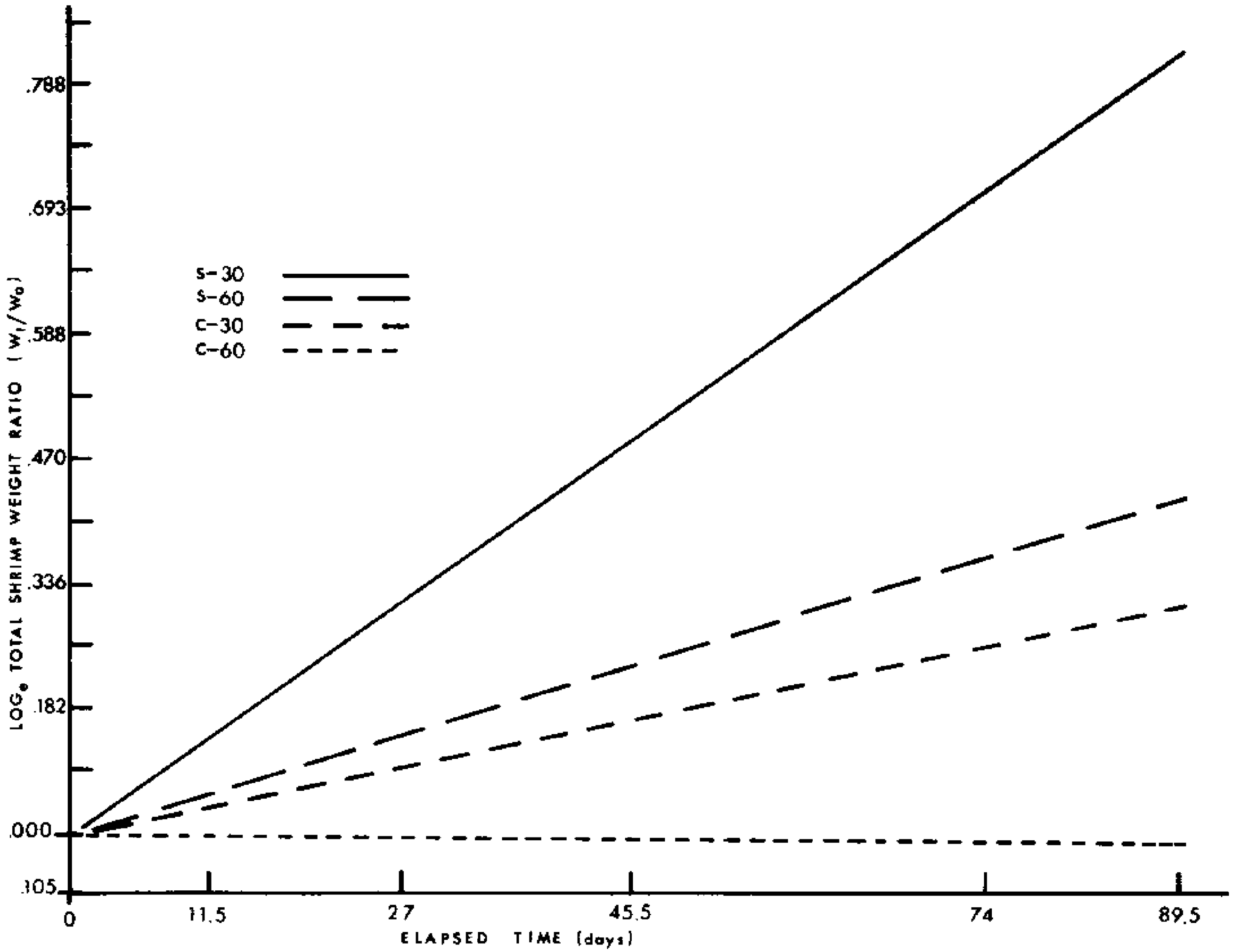

Figure 2.4. The total shrimp weight ratio as a function of elapsed time. W_t is the total shrimp weight at the end of a weighing period and W_o is the total weight stocked initially. Plotted lines represent averages of six replicate slope values for each tank-type (Table 2.6). Weighing intervals are shown on the X axis.

As with the growth data, an analysis of variance of the 24 tank slope values revealed that shrimp production in the screen tanks was greater than that in control tanks at the 99 per cent level. Also, production in tanks with 30 shrimp was greater than in tanks with 60 shrimp at the 99 per cent level.

None of the interaction terms were significant in the analysis, but the "among blocks" treatment was significant at the 95 per cent level. For some unexplained reason there was statistically detectable variation in production between the six tank groupings.

From an initial average stocked weight of 52.5 grams the S-30 tanks produced an average of 85.7 grams of shrimp while the C-30 tanks, which began with an average of 52.9 grams, yielded 62.2 grams of shrimp after 90 days. The S-60 tanks, which were stocked with approximately 109.2 grams of shrimp, each produced an average of 138.2 grams, and each of the C-60 tanks experienced a loss in total weight resulting in a decrease from an average starting point of 109.5 grams to an average final weight of 96.3 grams. Even though total shrimp weight was greater in tanks stocked with 60 shrimp at the end of the study, the production rate (slope) was greater in tanks stocked with only 30 shrimp, and it is likely that given a longer period of time the 30-shrimp tanks would have produced greater total yields than those which started with 60 shrimp.

It is evident that both stocking density and the availability of screen panels upon which the shrimp may move and feed have significant effects upon the total production of shrimp under the conditions imposed during this experiment. Furthermore, it appears that of the conditions examined the combination of 30 shrimp per square meter of bottom and the presence of screen panels (which had developed fouling communities) will produce the greatest weight of shrimp.

Molting

The total numbers of molts collected from all 24 tanks at two-day intervals are shown in Figure 2.5 with the corresponding moon phases for the study period.

As in the first experiment, peaks in molting frequency were expected on the quarter phases of the moon. For the first 60 days of the study, such peaks did occur on the quarter moons, but thereafter the molting frequency varied and the occurrence of peaks was erratic. Examination of the daily temperature and salinity data (Figure 2.8) shows that both parameters were fairly constant during the study. Temperatures varied between 26.0 and 34.5°C with diurnal variations usually limited to approximately 2.0°C, and salinity ranged from 30.7 to 34.5 °/oo. The trend toward lower temperatures during the last month of the study may have resulted in the variation in molting frequency peaks.

The multiple regression coefficients between molting frequencies and temperature, moon phase, and the number of elapsed days were examined to determine which factor or factors had the greatest influence upon molting. The BMDO2R computer program was used to provide multiple regression coefficients, and the results are contained in Table 2.7. The multiple F value proved non-significant,

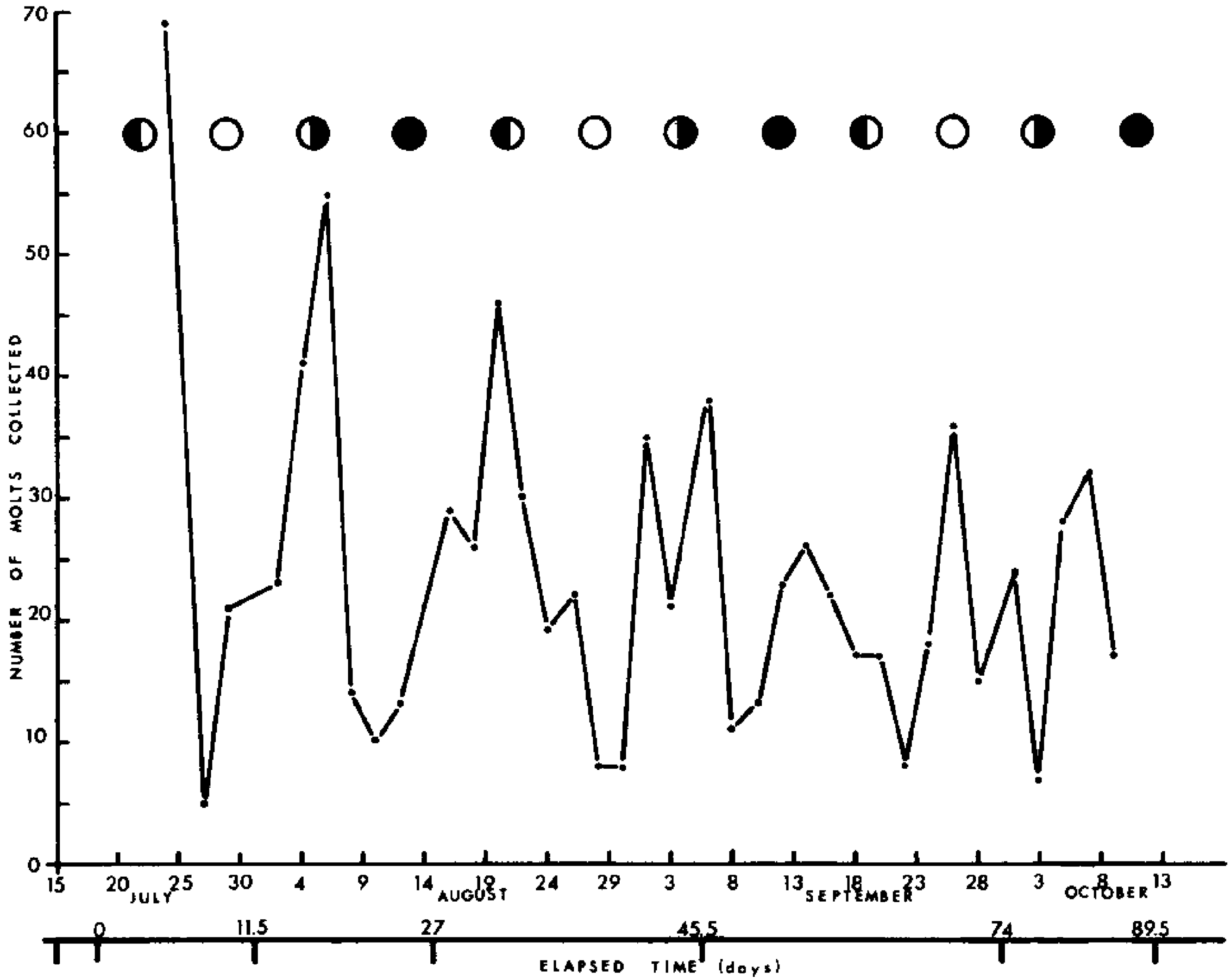

Figure 2.5. The total number of molts collected with the corresponding phases of the moon. Weighing intervals are shown on the X axis.

and the Multiple R^2 value accounted for only 18 per cent of the variation in molting frequency. Thus, under the conditions which existed during this experiment, some other factor or factors contributed considerably to determine the peaks in molting of the shrimp (Figures 2.6 and 2.7).

Further evidence of the differences in growth between shrimp in the screen tanks and control tanks is depicted by Figure 2.6 which is the total molt data separated into screen and control tank groups. After the first 30 days of the study, control tank molting had become very low while shrimp in the screen tanks continued molting at about the same rate. Thus, the progressive decline in size of the total molting peaks in Figure 2.5 was the result of decreased molting in the control tanks. Such a decrease was expected because of the absence of a fouling community upon which the shrimp could feed in the control tanks and the fact that the 10 per cent feeding rate approached the maintenance level in the control tanks, particularly in the C-60 tanks (Table 2.8). Wilcoxon's signed rank test revealed that molting frequencies were significantly higher in the screen tanks at the 99 per cent level.

Further evidence of the effects of stocking density are shown in Figure 2.7 which compares molting frequency in 30-shrimp and 60-shrimp tanks. The comparison is made on the basis of the percentage of the shrimp in each tank-type, and it shows that molting was clearly more frequent in the 30-shrimp tanks than in the 60-shrimp tanks. Wilcoxon's signed rank test revealed significantly higher molting frequencies in 30-shrimp tanks at the 99 per cent level.

Table 2.7. Stepwise multiple regression coefficients for the number of molts collected and the independent variables temperature, moon phase, and elapsed time in the experiment.

Analysis of Variance:

Source	df	MS	F	Multiple R^2
Regression	3	98.215	2.140 ns	0.1812
Residual	29	45.896		
Total	32			

Source	Coefficient	Standard error	F
Temperature	0.20011	1.89057	0.0112 ns
Moon phase	0.88313	0.53671	2.7075 ns
Elapsed days	-0.11298	0.09322	1.4688 ns

ns = Non-significant F value

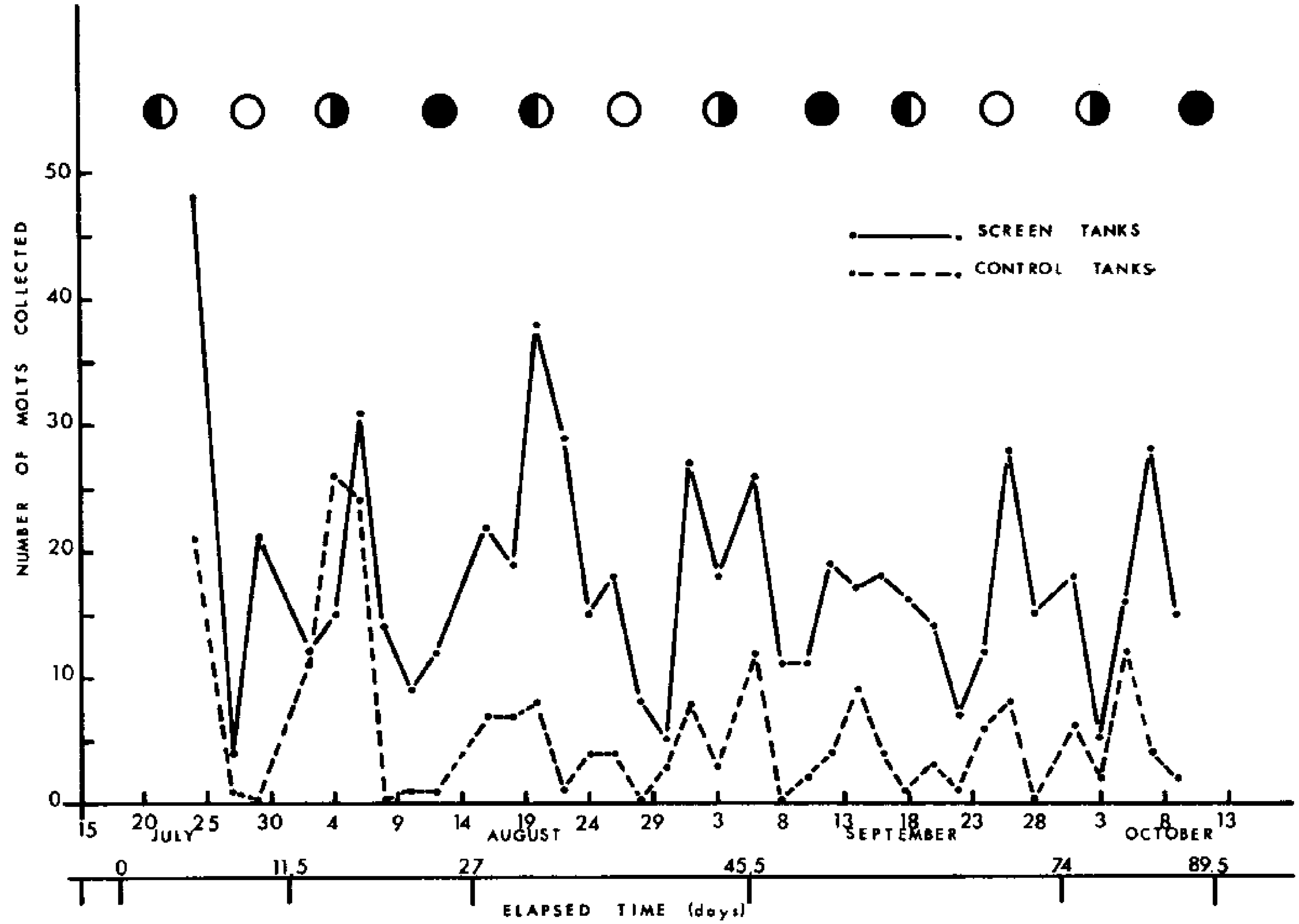

Figure 2.6. A comparison of the molting frequencies in screen tanks and control tanks, including phases of the moon. Weighing intervals are shown on the X axis.

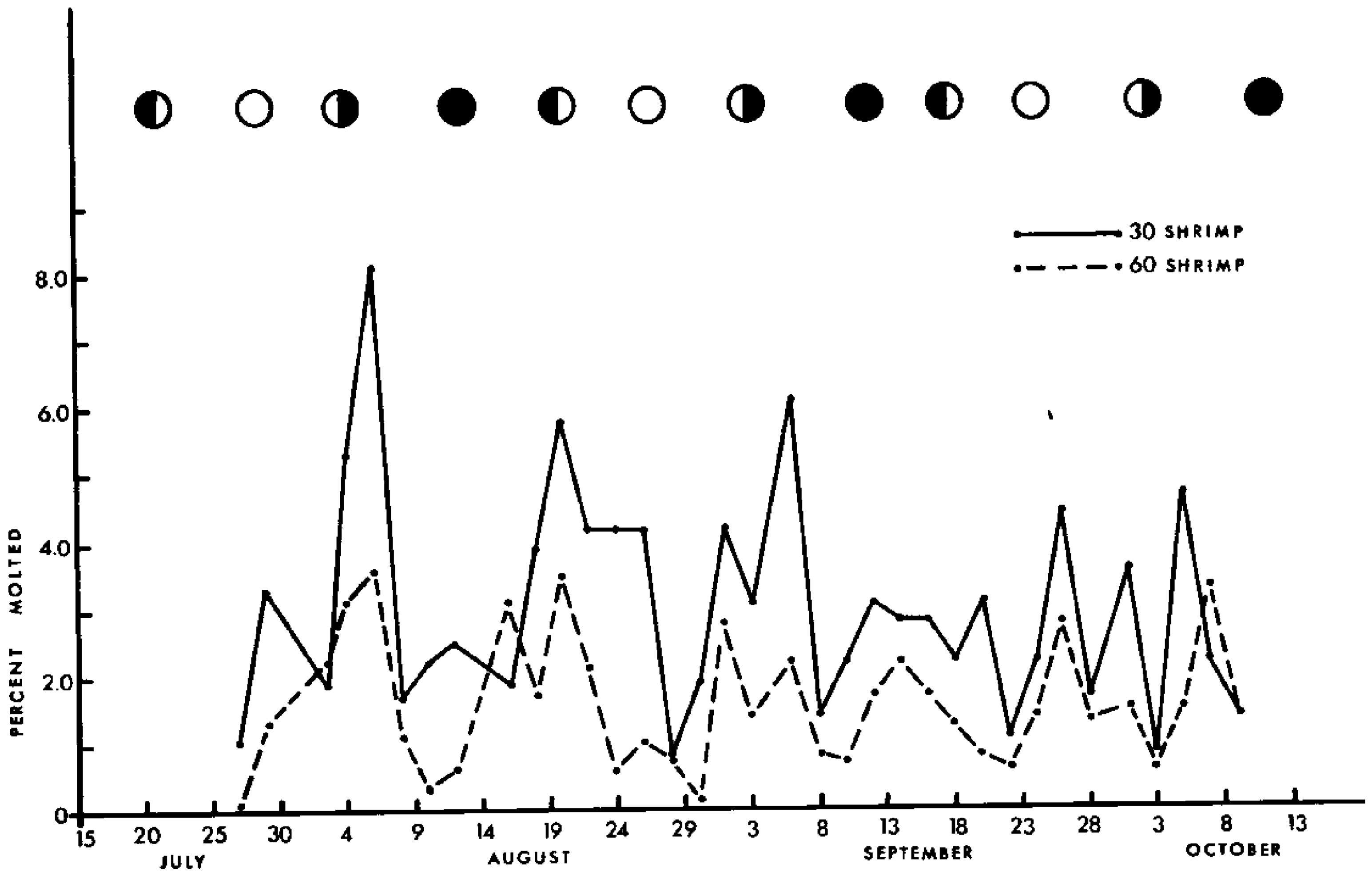

Figure 2.7. A comparison of the percent molting frequencies in tanks with 30 shrimp and tanks with 60 shrimp, including phases of the moon.

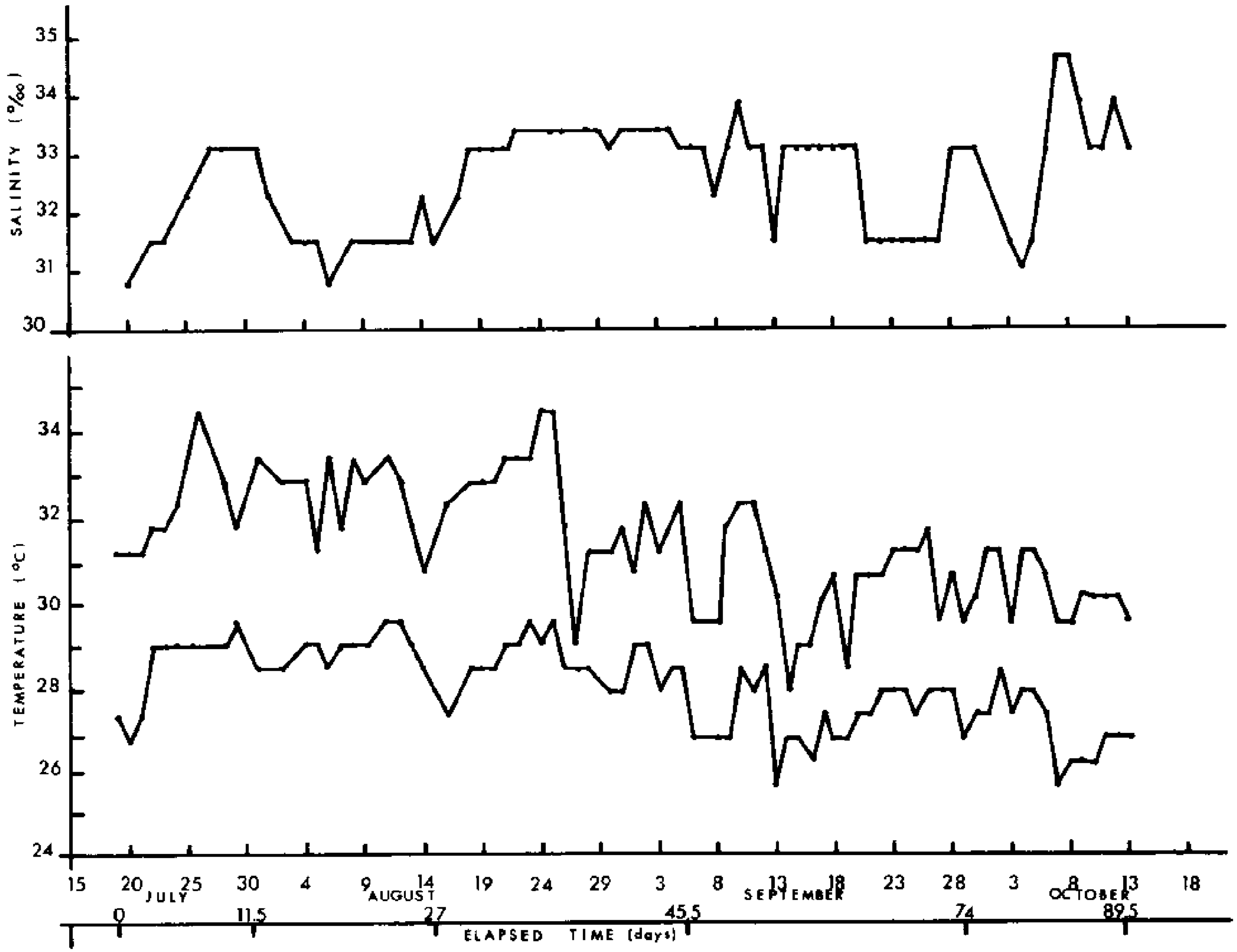

Figure 2.8. Daily salinity and maximum-minimum temperature observations for the study period. Weighing intervals are shown on the X axis.

Whereas differences in molting frequency between screen and control tanks did not develop for approximately 30 days, the effects of stocking density appear to have been felt almost immediately after the study began. During the entire experimental period, the percentage of molting shrimp in tanks with a starting density of 30 was higher than the percentage of molting shrimp in tanks originally stocked with 60 shrimp.

Growth Efficiencies

Additional evidence of the differences existing between the tank-types is shown in Table 2.8, which contains the weighing period growth efficiencies for each tank as well as the tank-type averages. The efficiencies were calculated using the relationship stated for experiment one.

In every weighing period shrimp in the S-30 tanks had the highest growth efficiencies and these were followed in order by the S-60, C-30, and C-60 tanks. This reflects the effects of density and fouled screens revealed in the analysis of the growth and yield slope values discussed previously.

In addition, the growth efficiencies generally decreased with each weighing period. This is as expected since as the shrimp grew the costs of metabolic maintenance increased per unit of food consumed and less food energy was available for growth. The variation in this trend between Periods II and III may have been the result of temperature fluctuations or changes in the physiological condition of the shrimp which resulted in slightly

Table 2.8. Growth efficiencies for shrimp in the four tank-types by weighing period. Since all shrimp were fed 10 per cent of their wet weight per day, feeding data are not included and efficiencies are presented as milligrams of weight change/gram of shrimp weight at the start of a period/day in the period.

Weighing period	Screens, 30 shrimp	Screens, 60 shrimp	Controls, 30 shrimp	Controls, 60 shrimp
I	21.8	10.6	12.7	4.8
	20.1	11.7	7.9	4.3
	26.1	13.8	10.6	4.6
	16.8	14.0	5.6	1.9
	27.2	14.8	12.4	6.2
	17.5	13.4	14.2	2.5
Ave.	21.6	13.1	10.6	4.1
II	8.3	4.7	0.0	1.8
	1.7	6.1	4.1	-0.7
	5.9	0.9	6.8	2.2
	9.6	7.3	7.5	-0.4
	5.4	6.0	-1.0	0.3
	12.0	6.0	-6.5	5.2
Ave.	7.2	5.2	1.8	1.4
III	10.5	5.9	5.8	3.8
	10.2	7.7	10.1	4.2
	8.1	11.8	6.9	0.2
	8.8	6.6	3.8	4.0
	12.7	5.7	4.4	2.7
	6.6	6.5	8.2	3.2
Ave.	9.5	7.4	6.5	3.0
IV	6.0	3.9	2.4	0.4
	4.8	3.0	0.3	1.0
	4.3	4.9	4.1	2.0
	8.9	8.0	6.0	2.2
	6.7	3.4	5.7	3.9
	5.9	4.5	5.0	4.1
Ave.	6.1	4.6	3.9	2.3
V	7.4	3.8	-0.3	-0.7
	2.1	3.2	2.1	-4.0
	2.6	2.4	3.3	4.6
	9.1	2.9	-1.6	2.5
	4.7	4.0	1.0	-0.5
	9.2	2.4	3.2	0.5
Ave.	5.9	3.1	1.3	0.4

higher efficiencies in Period III. As shown in Figure 2.8, the daily temperatures in Period III were slightly lower than during Period II, and such lower temperatures could have resulted in decreased metabolic rates in the shrimp which would leave proportionately greater amounts of food energy for growth processes.

The average growth efficiencies during the experiment ranged from 5.9 to 21.6 per cent for S-30 tanks, 3.1 to 13.1 per cent for S-60 tanks, 1.3 to 10.6 per cent for C-30 tanks, and 0.4 to 4.1 per cent for C-60 tanks. Food conversion values (expressed as weight of food/change in shrimp weight) ranged from 16.9 to 4.6 in S-30 tanks, 32.3 to 7.6 in S-60 tanks, 76.9 to 9.4 in C-30 tanks, and 250.0 to 24.4 in C-60 tanks. Clearly, the lower density of shrimp in the presence of fouled screen panels experienced the best food conversions with the S-60, C-30, and C-60 conditions producing successively poorer results.

Fouling Community Analysis and Its Utilization as Food

Table 2.9 is a list of the organisms identified on the screen samples and in the stomachs examined. In addition to these organisms, an occasional barnacle, *Balanus amphitrite*, jingle shell, *Anomia simplex*, bubble snail, *Haminoea* sp., and one nudibranch, *Phylaplesia* sp. were found in the tanks. These organisms are not included in Table 2.9 because they did not occur as fouling organisms on the screens or in the stomach contents examined at the end of the study.

The relative abundances of food and fouling organisms in Table 2.9 represent the averages of subjective evaluations for the

Table 2.9. Fouling community analysis by weighing period with stomach content analysis and electivity index values which were computed after the method of Cramer and Marzolf (1970).

Fouling Organisms	Weighing Periods I	II	III	IV	V	Contents	Electivity Index
Ceratium	*	*	*	0	0	0	
Foraminifera	*	*	**	****	****	*	- .600
Vorticella	*	0	0	0	0	0	
Hydroids	0	0	0	*	0	0	
Rotifera	***	**	***	*	*	0	-1.00
Nematodes	****	****	***	***	****	0	-1.00
Copepods	***	****	****	****	****	**	- .333
Nauplii	*	**	**	*	*	0	-1.00
Tardigrades	0	0	0	*	*	0	-1.00
Ascidians	*	**	***	****	****	0	-1.00
Diatoms							
Pleurosigma	*	***	***	***	***	**	- .200
Climacosphenia	***	*	*	0	0	0	
Licmophora	*	*	*	0	0	0	
Thalassionema	0	0	0	*	***	0	-1.00
Algae							
Enteromorpha	**	***	***	**	**	**	0.0
Chaetomorpha	*	0	*	0	*	0	-1.00
Cladophora	***	***	***	***	***	***	0.0
Lyngbya	***	***	**	**	**	*	- .333
Oscillatoria	****	***	****	****	****	**	- .333
Chroococcus	*	0	0	0	*	0	-1.00

0 = item not found
* = item found rarely
** = item found occasionally
*** = item found commonly
**** = item found abundantly

organisms identified. While the fouling organism abundances encompass values for all weighing periods, those for the stomach contents are for shrimp preserved only at the end of the study since food item analysis required sacrificing the shrimp.

For most organisms, relative abundances on the screens did not change appreciably between weighing periods. Exceptions to this included foraminiferans, ascidians, and Pleurosigma, which became more abundant as the study progressed. While the relative abundances remained fairly constant, the actual biomass of fouling organisms per square inch of screen surface increased progressively during the study period. The buildup of fouling biomass is shown by the dashed line in Figure 3.9.

Food selection for or against the various fouling organisms was analyzed by using a modification of the electivity index (EI) of Cramer and Marzolf (1970) in which the formula F-S/F+S expresses the relationship between the abundance of a fouling organism on the screens (F) and its occurrence in the stomachs of the shrimp (S).

Calculation of the EI values may produce ratios ranging from -1.0 to +1.0 in which -1.0 denotes selection against a food and +1.0 indicates selection for the food. Values between the extremes approach zero which indicates random feeding in which food items occur at approximately the same proportions in both the environment and in the gut contents.

Of the organisms listed in Table 2.9, several were considered to be of minor importance because of their rare occurrence in the fouling community; this group included the rotifers, copepod nauplii, tardigrades, Thalassionema, Chaetomorpha, and Chroococcus. All of these organisms had EI values of -1.0 largely because of their low abundance in the fouling community.

Two organisms, nematodes and ascidians, were found abundantly on the screens and had -1.0 EI values. Ascidians were too large to be consumed by the shrimp and were therefore selected against. Nematodes were not found in any shrimp stomach from experiment two, an identical situation to that noted for experiment one. Why the nematodes were not consumed is not known. Many of the nematodes were smaller than other organisms found in the stomachs, and nematode size did not appear to be an important factor. Perhaps the rapid body movements characteristic of the nematodes or an offensive taste or odor caused the shrimp to avoid eating them. The same may apply concerning taste or odor of the foraminiferans which were abundant on the screens but had an EI of -.6.

Copepods, Pleurosigma, Lyngbya, and Oscillatoria were selected against (EI = -.3 to -.2) to a lesser degree than the organisms noted above. The remaining two organisms, Enteromorpha and Cladophora, were both consumed in approximately the same proportions as they occurred in the tanks since the EI values for them equalled zero.

Of the organisms found in the shrimp stomachs, copepods were the most abundant animal while the filamentous green algae, Cladophora and Enteromorpha, and the diatom, Pleurosigma, were the most abundant plants.

An interesting difference between the filamentous green and blue-green algae was noted during examination of the stomach contents.

While the blue-green algae (Lyngbya and Oscillatoria) were consumed to almost the same extent as the green algae (Cladophora and Enteromorpha), it was noticed that 20 to 30 per cent of the cells in the green algae filaments were broken and emptied but less than 1 per cent of the blue-green cells were broken. A possible explanation for this is the relative width of the filaments of these algae and the structure of the gastric mill in the pink shrimp. The gastric mill is composed of a group of pads with recurved bristles, and the bristles are sufficiently close together to result in puncturing and breaking of cells in the wider green algal filaments. Filaments of the blue-green algae are much thinner and may slide into the spaces between bristles without being broken. Penaeid shrimp are not known to possess cellulase enzyme systems, and once past the gastric mill the algae cannot be broken down. Thus, even if the blue-green algae are ingested they are rarely broken during mastication of the food and they pass through the digestive tract relatively untouched. It appears that of the species of algae consumed by pink shrimp only the filamentous green algae may be of any appreciable value to the shrimp.

Behavior Observations

Observations of shrimp behavior during the study provided essentially the same information as obtained in the preceding experiment. Therefore, the following behavioral notes pertain to modifications of pink shrimp behavior stated previously and additional information relevant to the use of screen panels in the culture tanks.

Throughout the 90-day study period shrimp in tanks with screen panels adhered rigidly to the expected diurnal activity rhythm. Only a few shrimp were observed out of the sand during daylight hours, and all of these were in the S-60 tanks. This may have reflected the effects of high population density and the premium placed on burrowing space and food resources.

Shrimp in the control·tanks were frequently out of the sand during the day, and they appeared to be searching for food at this time. This points out a principal difference in physiological condition between shrimp in tanks with fouled screens and those in control tanks. Evidently control tank shrimp desired more food than was available and they were able to overcome their normal diurnal activity rhythm in order to remain out of the sand during the day in an attempt to find the food. Shrimp in tanks with fouled screens usually did not need to remain uncovered during the day because sufficient food was present as part of the fouling community on the screens and in the daily rations of ground squid.

Shrimp which were out of the sand during the day in control tanks were moving about the tanks and did not seem to be attempting to burrow, and shrimp were out of the sand in approximately even proportions in both C-30 and C-60 tanks. Thus, it is considered that the higher population densities had not resulted in a shortage of bottom space into which the shrimp could burrow but that a desire for additional food caused them to remain active during daylight hours.

Further indication of the insufficient food in control tanks was the complete lack of fecal material accumulation in these tanks and the partial consumption of all molts. In most cases, the only evidence remaining that molting had occurred in the control tanks was the carapace portion of the exoskeleton. In the screen tanks, fecal pellets began to accumulate almost immediately after the tanks were stocked with shrimp and molts were frequently found intact.

At night, most of the shrimp in every tank emerged from the sand to feed. As noted in experiment one, a large percentage of each population was observed moving along the sides of the tanks. In the S-30 tanks, the portion of the shrimp population observed on the screens at any one time ranged from 2.2 per cent to 23.3 per cent. In the S-60 tanks, these percentages ranged from 6.1 to 15.6. On only one occasion were more shrimp on the screens in the S-30 tanks than in the S-60 tanks, and a Wilcoxon's signed rank test showed that a significantly larger percentage of the shrimp in S-60 tanks made use of the screens than did shrimp in S-30 tanks. This is probably a result of the effects of higher population density imposed upon shrimp in the S-60 tanks.

Food seizure and feeding behavior were the same as in experiment one, but because of higher water temperature during this study food was seized and distributed more rapidly during experiment two.

Shrimp in tanks with screen panels were observed to pick up pieces of food on the sand bottom of the tanks and move up onto the screens in an effort to apparently "protect" their food from other shrimp which may not have yet found food. This may have been a density-dependent reaction to crowded conditions which helped to insure the successful completion of a meal by increasing the spatial distribution between individuals, thereby reducing the chance of conflict over food already in the possession of a shrimp.

Parasites

As in experiment one, parasites were apparently of little or no importance to the outcome of experiment two. Although a thorough search for parasites was not made, no obviously parasitized shrimp were found during the 90-day experimental period.

EXPERIMENT THREE

PROCEDURES

The screen panels used in the second experiment proved satisfactory for use as vertical substrates since an abundant fouling community developed on them and shrimp were able to move about freely in the tanks and on the screens. Screen panels were therefore also employed in the third study.

The question had arisen as to whether differences in production between screen and control tanks in the second experiment were the result of 1) grazing upon fouling organisms on the screens; 2) the added surface area upon which the shrimp could move and the resulting decreased contact between shrimp; or 3) a combination of these two factors. Therefore, the third experiment was designed to assess the relative effects of available surface area and the presence or absence of the fouling organism food source upon shrimp production.

In order to analyze the effects of surface area and fouling, both the number of screen panels and the presence or absence of fouling on the screens were selected as experimental variables. If fouling is necessary, the substrates must be favorable surfaces for the development of a fouling community as well as being a physical configuration which permits ready access of the shrimp to the food source. If surface area is important, fouling need not be considered, and any surface upon which the shrimp will move should suffice for improved production. If the two factors act together, the substrates must allow both growth of fouling organisms and access of the shrimp. The fiberglass screen panels allow both of these, and evaluation of the two factors was made possible through the arrangement of variables described below.

The same 24 tanks and water supply were used in this study as were employed in experiment two. Each tank had 3 to 4 centimeters of sand in which the shrimp could burrow, and water flow to each tank was continuous.

The tank and experimental variable arrangement is shown in Figure 3.1. The total screen surface area per tank was varied by using either 1, 2, 4, or 8 screen panels in a tank. Spacing of the panels in the tanks was as follows: 1 screen - in the middle of the tank; 2 screens - at 16 to 17 centimeter intervals; 4 screens - at 10 cm intervals; 8 screens - at 5 to 6 cm intervals.

Tanks designated as fouled were those in which the screen panels were not disturbed and upon which fouling was permitted to develop. Fouling in these tanks was initiated by means of algal seedings as in experiment two. Replaced tanks were those in which the screen panels were removed every three to four days and replaced by identical screens. Once removed from the tanks, the screens were washed and dried in the sun until they were used to replace the other set of screens in the tanks.

The tanks were arranged in three replicate blocks of eight tanks each. Tank types were assigned randomly within each block,

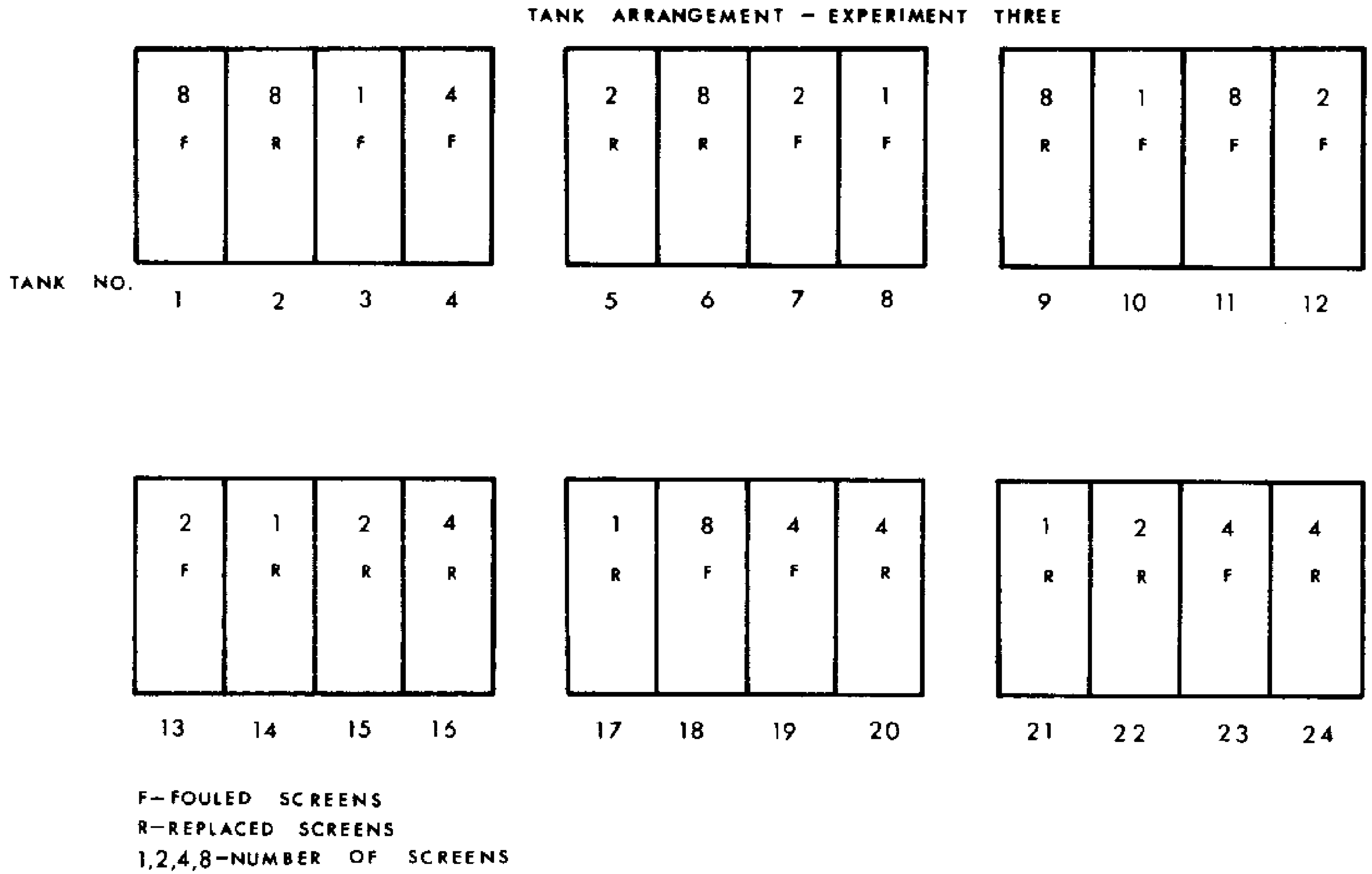

Figure 3.1. The arrangement of tanks and variables for Experiment Three. Each tank was stocked with 30 shrimp.

and each of the eight combinations of the experimental variables was represented once per block. Thus, Block One consisted of tanks 1, 2, 3, 4, 13, 14, 15, and 16; Block Two was composed of tanks 5, 6, 7, 8, 17, 18, 19, and 20; Block Three was the remaining eight tanks. Combinations of the variables were as follow: 1 screen and fouling (1-F); 1 screen replaced (1-R); 2 screens and fouling (2-F); 2 screens replaced (2-R); 4 screens and fouling (4-F); 4 screens replaced (4-R); 8 screens and fouling (8-F); 8 screens replaced (8-R).

Screen panels were of the same dimensions and construction as those used in experiment two. One screen in each of the fouled tanks was constructed so that screen samples, 2.5 cm by 10 cm, could be taken without altering the total screen surface area available to the shrimp. As in the preceding study, screen samples were taken from the upper 10 cm of the water column and they were used to provide an analysis of the composition and biomass of the fouling community.

Shrimp used in this experiment, which lasted from 3 January through 7 April, 1970, were obtained from the live-bait shrimp fishermen in Biscayne Bay. Mixing of the stock with species other than Penaeus duorarum was not considered a problem for the reasons stated in experiment one. Shrimp were held in aerated aquaria for two days before being taken randomly, weighed, and stocked in the experimental tanks at a rate of thirty shrimp per tank.

The food, food preparation, feeding rate, and time of daily feeding were the same as in experiment two. Food was withheld on one occasion. On 10 January, only a few days after the study had begun, a severe cold wave lowered the water temperatures in the tanks to between 10 and 11°C. Because of the low temperatures, feeding by the shrimp was minimal and food accumulated in all tanks. Therefore, no food was given to any tank on 10 January. On the following day, water temperatures began to rise and regular feeding was resumed.

Weighing of the shrimp was carried out at the same intervals and times of the month as in the previous experiment. Weighing was conducted at night in an attempt to avoid the handling mortality experienced formerly. As in the first study, only half of each shrimp population was weighed, and estimates of the total shrimp weight were calculated from the weights obtained. Locating the shrimp, sampling, and weighing procedures were the same as those used in experiment one.

Daily checks were made of each tank for dead shrimp, and any dead ones were removed so that they did not serve as an added food source for the remaining shrimp. Dead shrimp were not replaced and handling mortality was negligible. The actual number of shrimp surviving was determined only at the time of the final weighing. Numbers of shrimp in the tanks between the first and last weighings were based upon the initial number of stocked shrimp minus the cumulative number of dead found during the daily tank examinations.

Temperature, salinity, and water flow rates were monitored by the same methods used in experiment two. The diurnal dissolved oxygen cycle was recorded on 4-5 April. As before, the YSI oxygen meter was employed, and the data were used to compare oxygen cycles in fouled tanks with different numbers of screens, unfouled tanks, and the inflow water.

During the course of the study it became apparent that fouling was not growing evenly on all of the screen panels. Fouling developed over the entire screen surface in tanks with only one or two screens. However, in tanks with either four or eight screens, fouling grew only on the upper halves or upper quarters of the screens, respectively. It was also obvious that the degree of shading between screens increased in proportion to the number of screens in a tank.

In order to quantify the shading, incident light readings were taken in tanks of each type at the water surface, mid-depth, and at the bottom. Readings to the nearest 10 foot candles were obtained with a photometer (Model 200, Photovolt Corporation) which was wrapped in a single layer of transparent polyethylene as waterproofing. Two sets of readings were taken on the same day, one during full sunlight and one when the sky was heavily overcast, so that shading values for the two conditions could be compared.

Molts were collected in the morning at two-day intervals. Molt collections were made only in tanks with one or two screens since collection in the smaller between-screen spaces in the four and eight screen tanks required considerable disturbance of the screens.

Screen samples for fouling community analysis and biomass determinations were taken before each weighing as in experiment two. Organism identifications were also conducted in the manner used previously.

The sides of all tanks were scrubbed every 10 to 12 days so that fouling on these surfaces did not provide food for the shrimp. Thus, nearly the only food sources were fouling on the screens and the ground squid which was added to the tanks.

Large fouling organisms, such as tunicates, were generally left undisturbed unless they interfered with functioning of the tank system. On a few occasions, bubble snails, Haminoea sp., were found in the tanks. These gastropods had apparently come through the sea-water system as larvae, metamorphosed, grown, and matured in the tanks. The first evidence of their presence was the discovery of several egg masses on the screens and sides of the tanks. The egg masses were removed from the tanks, as were adults, whenever they were observed. The presence of these snails in the tanks was considered undesirable because they are grazing herbivores and competed for some food items with the shrimp.

Behavior observations were made intermittently during the study to determine whether or not more shrimp made use of the fouled screens than the replaced screens. Shrimp were located and counted by using a red-filtered flashlight.

At the conclusion of the study, all tanks were drained and the shrimp were removed so that final mortality and weight data could be obtained. Five shrimp from each tank were preserved in five per cent formalin for analysis of the stomach contents. Relative abundances of food items in the stomachs were compared with relative abundances of fouling organisms on the screens to determine whether or not any of the organisms were being selected for or selected against by the shrimp.

RESULTS AND DISCUSSION

Shrimp Growth

Table 3.1 contains the replicate average shrimp weights obtained at two-to-three week intervals and the weighing period averages for each of the eight tank-types. The individual tank weights were used for computing growth regressions for each tank with the formula, shrimp weight = initial shrimp weight + b (time), and the slopes (b) are listed in Table 3.2. The growth regression lines plotted in Figure 3.2 were calculated by averaging the growth slopes for the 12 fouled-screen tanks and the 12 replaced-screen tanks to show differences in growth among shrimp in tanks with fouled (average b = .025842) screens, versus unfouled screens (average b = .020757).

An examination of the average slope values for the eight tank-types (Table 3.2) reveals that, in every case, shrimp in tanks with 1, 2, 4, or 8 fouled screens grew at a greater rate than did their counterparts with replaced screens.

A statistical comparison of the 24 growth slope values (Table 3.2) revealed that there was no significant difference in growth rates among tanks with different numbers of screens. However, shrimp in tanks with fouled screens grew faster than those in tanks with replaced screens at the 95 per cent level. Because of these results, Figure 3.2 shows only the comparison of growth in fouled and replaced screen tanks.

Table 3.1. Replicate average shrimp weights (in grams) determined at two-to-three week intervals for the eight tank-types. Tank-types are designated by the symbols 1-F through 8-R in which F and R indicate tanks with fouled or replaced screens, respectively, and the numbers indicate the number of screens per tank.

Weighing Period	1-F	1-R	2-F	2-R	4-F	4-R	8-F	8-R
Start	3.03	2.83	3.05	2.97	2.48	2.83	2.79	2.76
	2.54	2.84	2.48	2.66	2.87	2.77	2.72	2.91
	3.08	2.95	2.99	2.81	2.67	2.77	2.74	2.76
Ave.	2.88	2.87	2.84	2.81	2.67	2.79	2.75	2.81
I	3.77	3.49	3.83	3.49	3.06	3.57	3.10	3.44
	3.14	3.59	3.13	3.22	3.17	3.35	3.21	3.25
	3.65	3.05	3.50	3.77	3.49	3.39	3.75	3.27
Ave.	3.52	3.38	3.49	3.49	3.24	3.44	3.35	3.32
II	4.27	4.01	4.39	4.16	3.73	4.17	3.85	3.72
	3.74	4.15	3.75	3.54	4.37	4.05	4.07	3.78
	3.73	3.94	4.33	3.83	3.92	4.25	4.01	3.78
Ave.	3.91	4.03	4.16	3.84	4.01	4.16	3.98	3.76
III	5.30	4.17	5.43	4.54	4.22	4.69	4.39	4.44
	4.21	4.60	4.36	4.08	4.78	4.62	4.15	3.95
	4.73	4.21	5.17	4.75	4.35	3.76	4.32	3.91
Ave.	4.75	4.33	4.99	4.46	4.45	4.36	4.29	4.10
IV	5.31	4.67	5.63	4.81	4.89	4.87	5.20	4.74
	4.42	4.93	4.91	5.23	5.31	5.05	4.82	4.27
	4.99	4.30	5.43	4.96	4.78	4.15	5.60	4.38
Ave.	4.91	4.63	5.32	5.00	4.99	4.69	5.21	4.46
V	5.29	4.90	5.61	4.89	5.12	5.19	5.24	4.75
	4.31	5.16	4.60	4.85	5.38	5.14	4.71	4.45
	4.68	4.31	5.47	4.86	5.34	4.36	5.60	4.41
Ave.	4.76	4.79	5.23	4.87	5.28	4.90	5.18	4.54

Table 3.2. Replicate growth regression slopes for the eight tank-types and analysis of variance of the slope values. Regressions were computed using the formula, average shrimp weight = initial shrimp weight + b (time).

	1-F	2-F	4-F	8-F
Slopes	.025233	.028668	.028500	.028464
	.019543	.024744	.028672	.022045
	.019651	.028262	.026105	.030214
Ave.	.021476	.027225	.027759	.026908
	1-R	**2-R**	**4-R**	**8-R**
	.020796	.020633	.023964	.021757
	.023799	.026428	.026019	.016150
	.015920	.022097	.014146	.017373
Ave.	.020172	.023053	.021376	.018427

Source	df	MS	F
Fouled vs. replaced	1	.000155138265	10.95 *
Numbers of screens	3	.000023040754	1.48 ns
Among blocks	2	.000001041260	0.07 ns
Fouled x No. of screens	3	.000014173141	0.91 ns
Error	14	.000015516296	
Total	23		

*Significant at the 95% level
ns = Non-significant F value

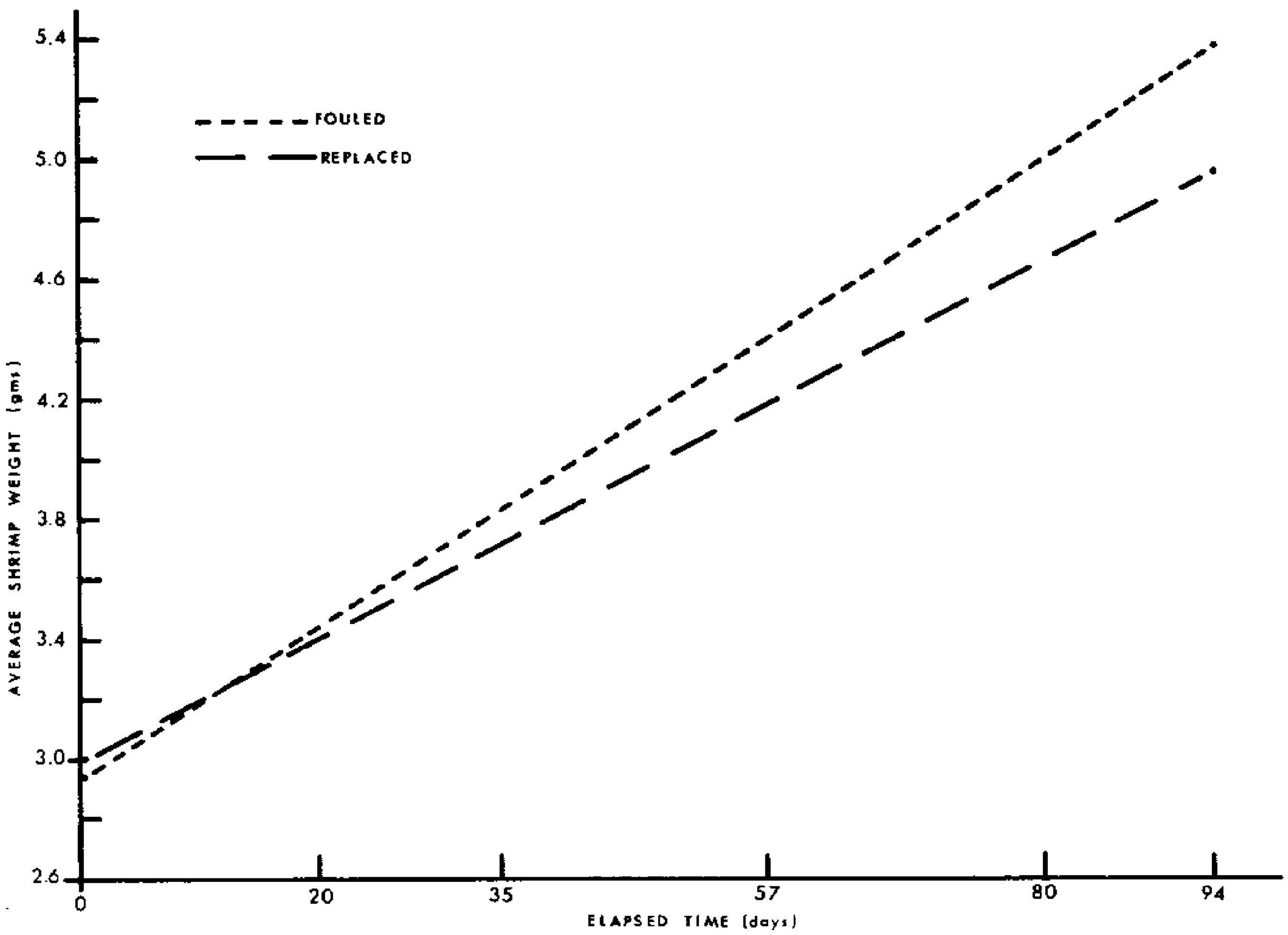

Figure 3.2. The average shrimp weight as a function of time for tanks with fouled screens and tanks with replaced screens. Plotted lines represent averages of growth regression slopes (Table 3.2). Weighing intervals are shown on the X axis.

There were no significant differences in growth among the three replicate blocks of eight tanks each, and the interaction term was not significant. Intercept values were not significantly different indicating, as in the two previous studies, that comparable sets of shrimp were selected for stocking the tanks at the beginning of the third experiment.

From an initial weight of approximately 2.8 grams per shrimp in all tanks, 1-F, 2-F, 4-F, and 8-F shrimp averaged 4.76, 5.23, 5.28, and 5.18 grams, respectively, after 94 days. After the same length of time and from the same starting weight, 1-R, 2-R, 4-R, and 8-R shrimp averaged 4.79, 4.87, 4.90, and 4.54 grams, respectively.

Of the conditions examined, the amount of surface area available to the shrimp appeared to have had little or no effect upon growth of the shrimp. On the other hand, permitting the screens to become fouled with filamentous algae and other organisms upon which the shrimp may feed has a definite beneficial effect upon shrimp growth at the stocking density employed.

Shrimp Mortality

Dead shrimp were not replaced with live ones during this experiment, and mortality regressions were computed for each tank for the purpose of statistical comparison as was done for the growth data. As in experiment two, the ratio N_o/N_t was employed as the Y value in the regression formula $\log_e Y = b$ (time).

Mortality ratios and cumulative numbers of dead shrimp for each tank at the times of weighing are listed in Table 3.3, and using these ratios in the regression formula yielded the mortality slope values (b) listed in Table 3.4. Lower slope values indicate better survival than higher slope values (mortality rates).

Figure 3.3 is a comparison of mortality rates in fouled and replaced tanks, and the regression lines shown are averages of the mortality rates for the 12 fouled screen tanks (average b = .00172730) and those for the 12 replaced screen tanks (average b = .00314849).

Examination of the eight tank-type average mortality rates (Table 3.4) reveals that in every case the tanks with fouled screens had lower mortality rates than their counterparts with replaced screens. Thus, the average slope for 1-F tanks was .000415 and for 1-R tanks it was .000813; 2-F slopes averaged .000768 while 2-R tanks averaged .001339; 4-F tanks averaged .000683 and 4-R tanks averaged .001129; 8-F tanks averaged .000436 and 8-R tanks averaged .000916.

Statistical comparison employing an analysis of variance of the 24 mortality slope values revealed no significant differences among tanks on the basis of the number of screens. However, fouled screen tanks experienced lower mortality rates (better survival) than the replaced screen tanks at the 99 per cent level. Therefore, only the comparison of fouled versus replaced tanks is depicted in Figure 3.3.

As with the growth data, there were no significant differences between replicate tank blocks, and the interaction term was not significant.

Table 3.3. Replicate mortality ratios for the eight tank-types. Ratios were computed as the number of shrimp at the start of the study/the number remaining at the end of a period. Values in parentheses are the cumulative numbers of dead shrimp found in the tanks.

Weighing Period	1-F	2-F	4-F	8-F
I	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
	1.034 (1)	1.000 (0)	1.000 (0)	1.000 (0)
	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
Ave.	1.011	1.000	1.000	1.000
II	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
	1.034 (1)	1.000 (0)	1.000 (0)	1.000 (0)
	1.000 (0)	1.034 (1)	1.000 (0)	1.000 (0)
Ave.	1.011	1.011	1.000	1.000
III	1.000 (0)	1.034 (1)	1.111 (3)	1.034 (1)
	1.034 (1)	1.111 (3)	1.000 (0)	1.000 (0)
	1.000 (0)	1.071 (2)	1.071 (2)	1.034 (1)
Ave.	1.011	1.072	1.061	1.026
IV	1.034 (1)	1.111 (3)	1.111 (3)	1.154 (4)
	1.111 (3)	1.154 (4)	1.034 (1)	1.000 (0)
	1.000 (0)	1.071 (2)	1.154 (4)	1.034 (1)
Ave.	1.048	1.112	1.100	1.069
V	1.071 (2)	1.200 (5)	1.250 (6)	1.364 (8)
	1.154 (4)	1.364 (8)	1.250 (6)	1.154 (4)
	1.071 (2)	1.154 (4)	1.364 (8)	1.154 (4)
Ave.	1.099	1.239	1.288	1.224

Table 3.3. Continued

Weighing Period	1-R	2-R	4-R	8-R
I	1.000 (0)	1.000 (0)	1.000 (0)	1.034 (1)
	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
Ave.	1.000	1.000	1.000	1.011
II	1.000 (0)	1.034 (1)	1.000 (0)	1.034 (1)
	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
	1.000 (0)	1.000 (0)	1.000 (0)	1.000 (0)
Ave.	1.000	1.011	1.000	1.011
III	1.111 (3)	1.200 (5)	1.111 (3)	1.111 (3)
	1.034 (1)	1.111 (3)	1.071 (2)	1.071 (2)
	1.111 (3)	1.111 (3)	1.071 (2)	1.000 (0)
Ave.	1.085	1.141	1.084	1.061
IV	1.154 (4)	1.250 (6)	1.200 (5)	1.200 (5)
	1.034 (1)	1.154 (4)	1.250 (6)	1.111 (3)
	1.200 (5)	1.250 (6)	1.154 (4)	1.000 (0)
Ave.	1.129	1.218	1.201	1.104
V	1.364 (8)	1.364 (8)	1.307 (7)	1.500 (10)
	1.154 (4)	1.250 (6)	1.429 (9)	1.250 (6)
	1.250 (6)	1.429 (9)	1.364 (8)	1.250 (6)
Ave.	1.256	1.348	1.366	1.333

Table 3.4. Replicate mortality regression slopes for the eight tank-types and analysis of variance of the slope values. Regressions were computed using the formula, $\log_e$ of the mortality ratio = b (time). All intercepts were at the origin of the graph.

	1-F	2-F	4-F	8-F
Slopes	.00019296	.00054338	.00084079	.00098668
	.00105280	.00104160	.00028746	.00006692
	.00000000	.00071854	.00092219	.00025589
Ave.	.00041525	.00076784	.00068348	.00043650
	1-R	2-R	4-R	8-R
Slopes	.00104160	.00167840	.00115900	.00176890
	.00031350	.00096404	.00126420	.00077198
	.00108360	.00137450	.00096543	.00020884
Ave.	.00081290	.00133898	.00112954	.00091657

Source	df	MS	F
Fouled vs. replaced	1	.000001346531	167.458 **
Number of screens	3	.000000249468	1.18 ns
Among blocks	2	.000000217144	1.03 ns
Fouled x No. of screens	3	.000000008041	0.04 ns
Error	14	.000000211319	
Total	23		

**Significant at the 99% level
ns = Non-significant F value

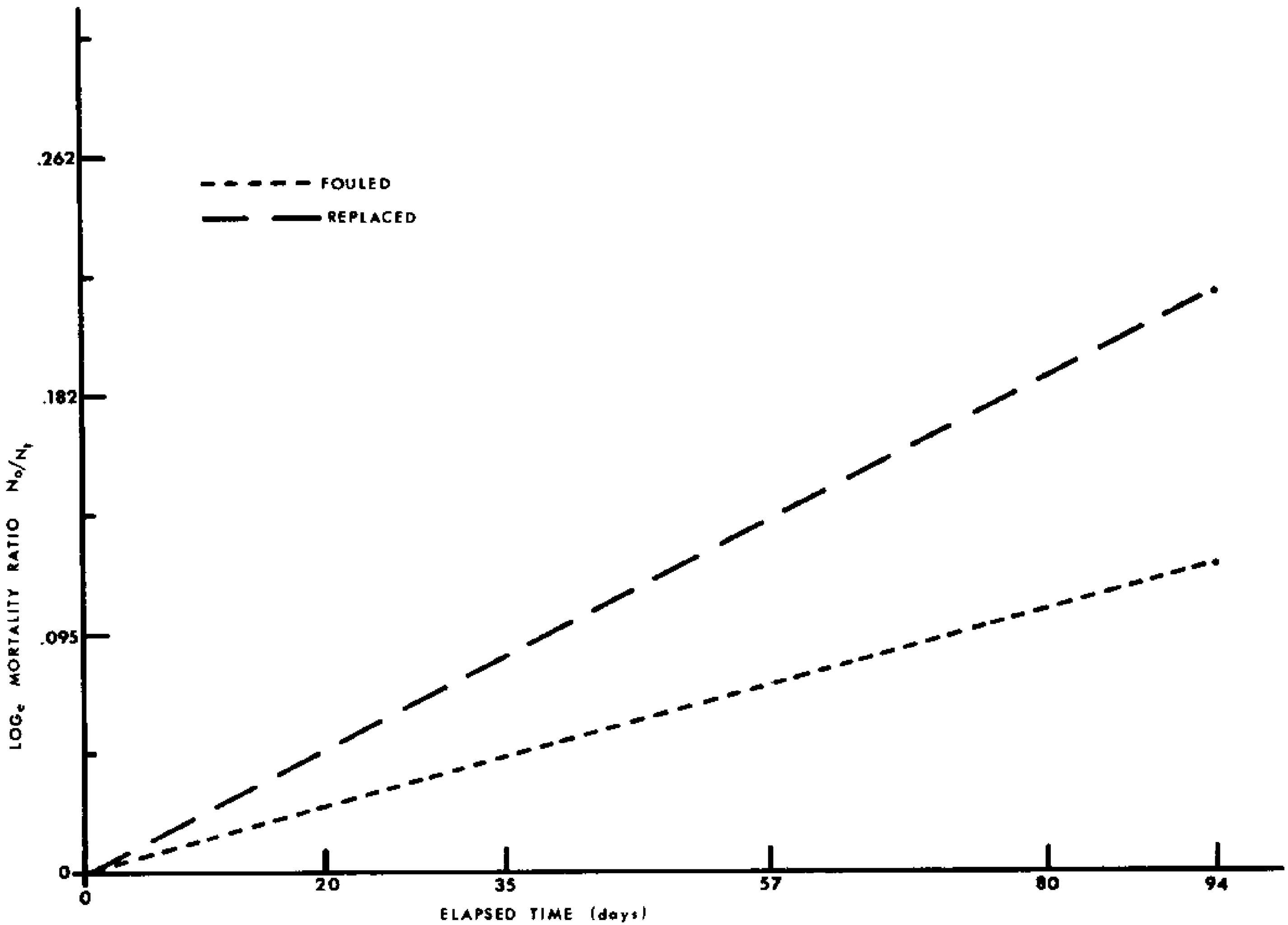

Figure 3.3. The tank-type mortality ratio as a function of time. Ratios were computed as in Experiment Two. Plotted lines represent averages of mortality regression slopes (Table 3.4). Weighing intervals are shown on the X axis.

From an initial stocking density of 30 shrimp per tank, average mortalities were as follow: 1-F tanks, 2.7 deaths or 9.0 per cent; 1-R tanks, 6.0 deaths or 20.0 per cent; 2-F tanks, 5.7 deaths or 19.0 per cent; 2-R tanks, 7.7 deaths or 25.7 per cent; 4-F tanks, 6.7 deaths or 22.3 per cent; 4-R tanks, 8.0 deaths or 26.7 per cent; 8-F tanks, 5.3 deaths or 17.7 per cent; 8-R tanks, 7.3 deaths or 24.3 per cent. Fouled tanks lost an average of 5.1 shrimp or 17.0 per cent while replaced tanks experienced an average mortality of 7.5 shrimp or 25.0 per cent.

Handling mortality was negligible during experiment three, evidently this was a result of weighing the shrimp at night when they are normally active rather than buried in the sand.

The amount of surface area available for shrimp to move about on appears to have had little effect upon survival at the stocking density employed. The presence of a fouling organism community on the screen panels produced significantly better survival than did an equivalent amount of surface area without such a fouling community.

Shrimp Production

Replicate values of the total shrimp weight for each tank and each weighing period are contained in Table 3.5. These weights were used to compute the yield regression slope values listed in Table 3.6 with the formula $\log_e Y = b$ (time). The ratio W_t/W_o, in which W_o is the total shrimp weight stocked in the tank and W_t is the total shrimp weight remaining at the end of a weighing period, was employed as the Y value in the regression formula. Average

Table 3.5. Replicate total shrimp weights (in grams) determined at two-to-three week intervals for the eight tank-types.

Weighing Period	1-F	1-R	2-F	2-R	4-F	4-R	8-F	8-R
Start	90.8	84.9	91.6	89.0	74.4	85.0	83.7	82.7
	76.1	85.3	74.4	79.9	86.1	83.1	81.5	87.4
	92.3	88.6	89.6	84.4	80.2	83.0	82.2	82.8
Ave.	86.4	86.3	85.2	84.4	80.2	83.7	82.5	84.3
I	113.2	104.6	114.8	104.6	91.8	107.2	93.0	99.8
	94.3	107.6	94.0	96.6	95.0	100.4	96.4	97.6
	109.6	91.4	105.0	113.0	104.6	101.6	112.6	98.2
Ave.	105.7	101.2	104.6	104.7	97.1	103.1	100.7	98.5
II	128.2	120.2	131.8	120.6	112.0	125.0	115.6	107.9
	108.5	124.6	112.6	106.2	126.8	121.4	122.2	113.4
	112.0	118.2	126.8	114.8	117.6	127.4	120.2	113.4
Ave.	116.2	121.0	123.7	113.9	118.8	124.6	119.3	111.6
III	159.0	112.6	157.6	113.5	113.9	126.5	127.4	119.9
	122.0	133.4	117.8	110.1	143.4	129.4	124.4	110.6
	142.0	113.6	144.8	128.3	121.8	109.2	125.3	117.4
Ave.	141.0	119.9	140.1	117.3	126.4	121.7	125.7	116.0
IV	153.9	121.4	152.0	115.4	131.9	121.7	130.0	118.7
	119.2	142.8	127.6	136.0	153.9	121.2	144.6	115.4
	149.8	107.5	152.0	119.0	124.4	108.0	162.4	131.4
Ave.	141.0	123.9	143.9	123.5	136.7	117.0	145.7	121.8
V	148.1	107.8	140.2	107.6	122.8	119.3	115.3	94.9
	107.7	134.1	101.2	116.3	129.1	108.0	122.5	106.7
	130.9	103.5	142.2	102.1	117.4	96.0	145.7	105.9
Ave.	128.9	115.1	127.9	108.7	123.1	107.8	127.8	102.5

Table 3.6. Replicate total shrimp weight regression slopes and analysis of variance of the slope values. Regressions were computed using the formula, $\log_e Y = bX$ in which Y is the ratio of the total weight at the end of a period/the total weight at the start of the experiment, and X is the elapsed time. All intercepts were at the origin of the graph.

	1-F	2-F	4-F	8-F
Slopes	.0070848	.0070104	.0070257	.0051291
	.0064067	.0069349	.0060829	.0064816
	.0052408	.0062988	.0068482	.0080980
Ave.	.0062441	.0067480	.0066523	.0065696
	1-R	2-R	4-R	8-R
	.0053886	.0043814	.0062815	.0049158
	.0069230	.0056478	.0059268	.0037811
	.0030371	.0061746	.0053341	.0053384
	.0051162	.0054013	.0058475	.0046784

Source	df	MS	F
Fouled vs. replaced	1	.000010025535	32.02 **
Number of screens	3	.000000554413	0.45 ns
Among blocks	2	.000000348984	0.28 ns
Fouled X Number of screens	3	.000000313131	0.25 ns
Error	14	.000001241707	
Total	23		

**Significant at the 99% level
ns = Non-significant F value

production slopes for the 12 fouled-screen tanks (average b = .0065535) and the 12 replaced-screen tanks (average b = .0052609) are presented in Figure 3.4 for comparison of the effects of the presence and absence of the fouling community food source upon the total shrimp yield.

An examination of the average production slopes (b) shows that in every case tanks with 1, 2, 4, or 8 fouled screens had higher production rates than their counterparts with 1, 2, 4, or 8 replaced screens (Table 3.6).

A statistical comparison of the 24 production slope values (Table 3.6) revealed that there were no significant differences in production rates among tanks with different numbers of screens. However, production rates in tanks with fouled screens were higher than those in tanks with replaced screens at the 99 per cent level. Thus, Figure 3.4 shows only the comparison of production between fouled and replaced screen tanks.

As with the growth and mortality data, neither the "among blocks" treatment or the interaction term were significant.

From an initial total shrimp weight of approximately 83.0 grams of shrimp per tank, 1-F, 2-F, 4-F, and 8-F tanks produced average final yields of 128.9, 127.9, 123.1, and 127.8 grams of shrimp, respectively, after 94 days. After the same length of time and from the same initial stocking weight per tank, 1-R, 2-R, 4-R, and 8-R tanks yielded an average of 115.1, 108.7, 107.8, and 102.5 grams of shrimp, respectively. Total yield was greater in fouled-screen tanks than replaced-screen tanks in all cases.

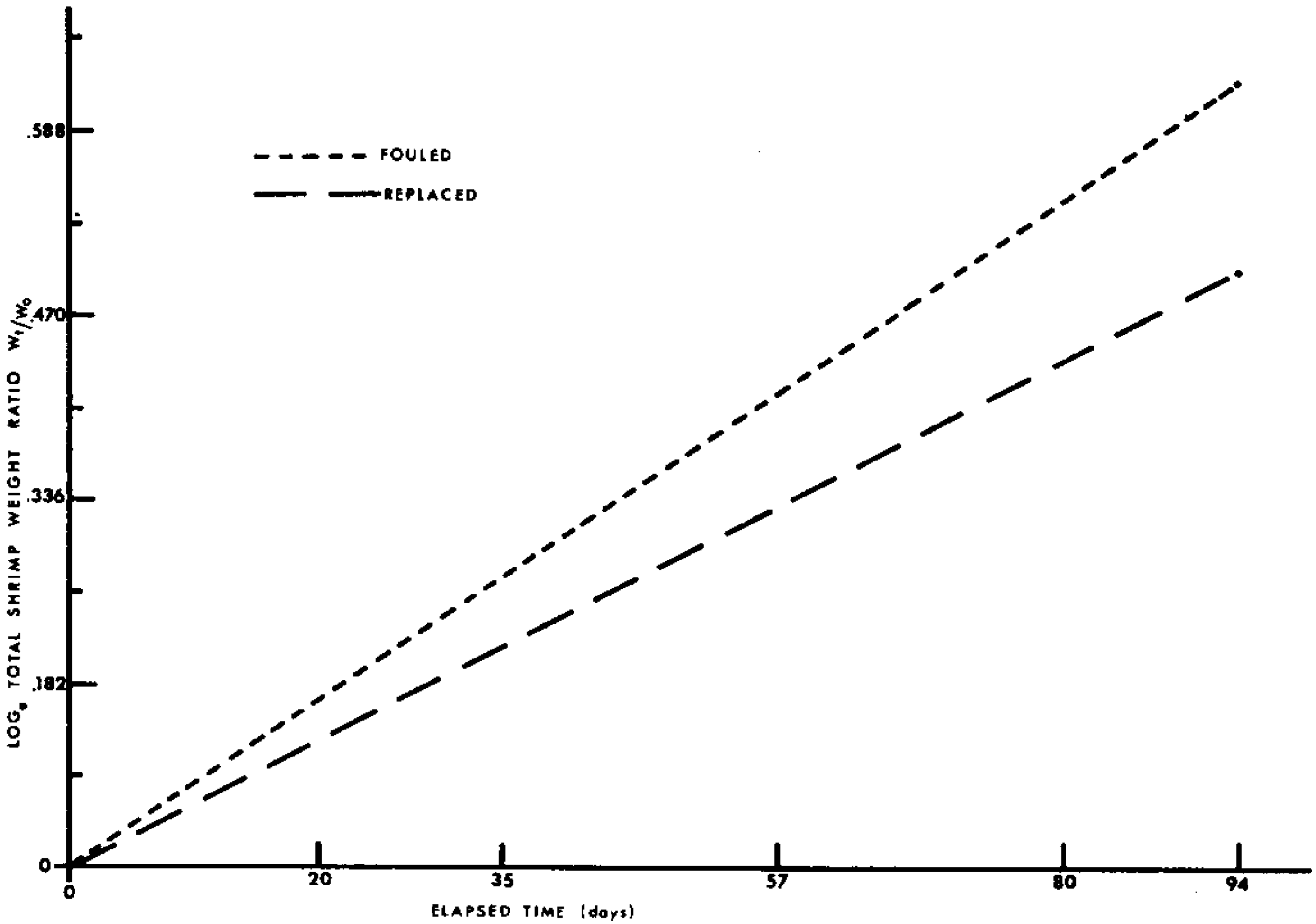

Figure 3.4. The total shrimp weight ratio as a function of time. Ratios were computed as in Experiment Two. Plotted lines represent averages of production regression slopes (Table 3.6). Weighing intervals are shown on the X axis.

As was revealed with the growth and mortality data, the amount of surface area available to the shrimp appears to have had little influence upon either total yield or production rate. However, the presence of a fouling community food source in addition to the daily ration of squid had a decided beneficial effect upon shrimp production at the stocking density employed.

Relative Effects of Varied Numbers of Screens

Figure 3.5 is a comparison of regression slope values as a function of the number of screens per tank for growth, mortality, and total production. Despite the fact that no significant differences were found among tanks with different numbers of screens, the figure provides an evaluation of the relative effects of the numbers of screens per square meter of bottom.

In all three categories, i.e., growth, mortality, and production, the 1-F, 2-F, 4-F, and 8-F slopes gave better shrimp yields than the 1-R, 2-R, 4-R, and 8-R slopes.

Among the fouled-screen tanks, the 2-F, 4-F, and 8-F tanks had nearly equal growth slopes which were higher than those for the 1-F tanks. Thus, the best growth with the lowest expense for equipment (screen paneling) could be obtained using two panels per square meter of tank bottom.

The 1-F and 8-F tanks had similar mortality rates which were lower than those of the 2-F and 4-F tanks. The best survival at the lowest cost for screening would be obtained with one panel per square meter of tank bottom.

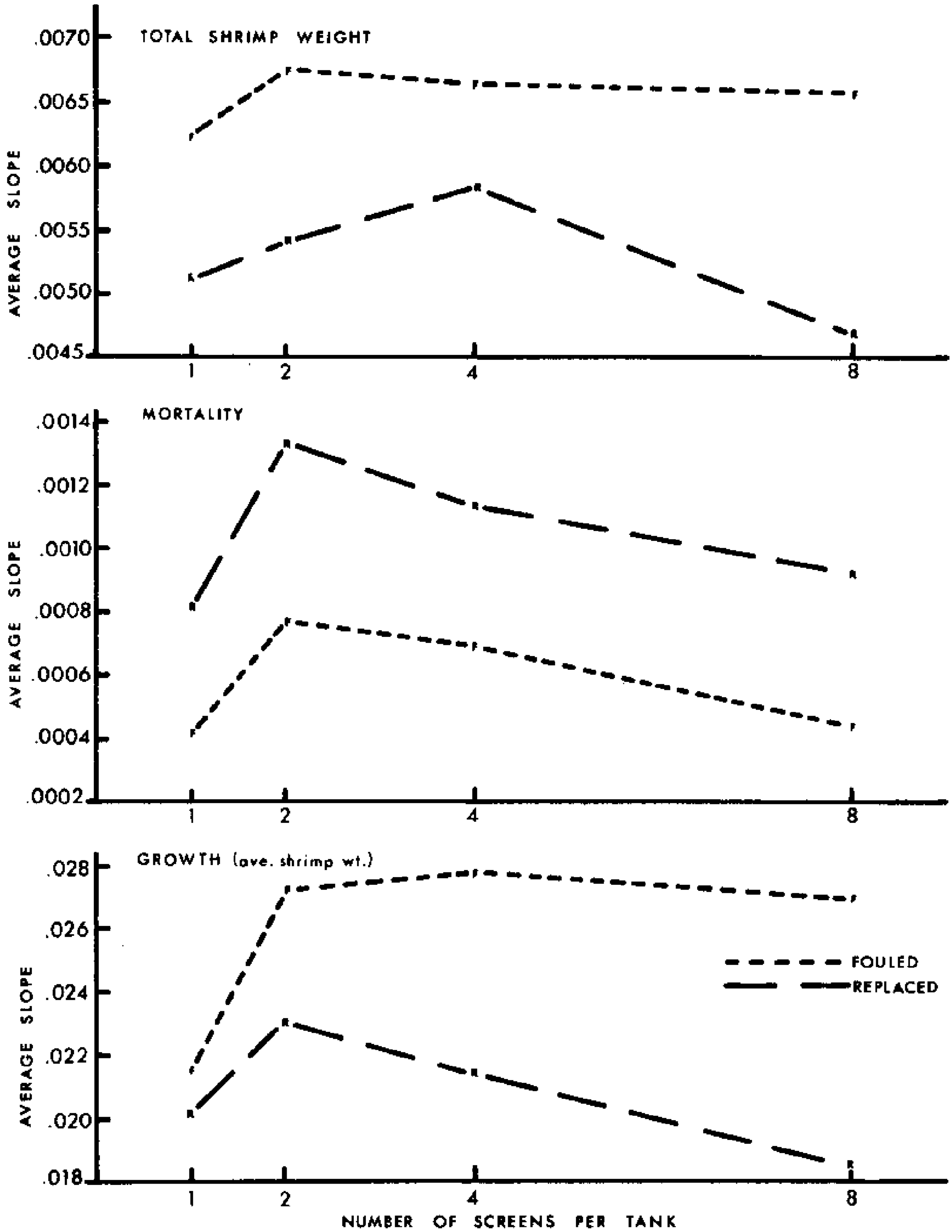

Figure 3.5. The average regression slope as a function of the number of screens present for growth (bottom), mortality (middle), and total shrimp weight (top). Tank-types are presented as fouled screens or replaced screens.

Production slopes, which reflect the combined effects of growth and mortality, were lowest in 1-F tanks and higher in the 2-F, 4-F, and 8-F tanks. Therefore, two screen panels per square meter of tank bottom produced the greatest yield at the lowest equipment cost.

Further examination should be made of the relative effects of different numbers of fouled screen panels per tank. Equipment costs may dictate that the somewhat lower production rates be accepted and one screen panel per tank may be used. However, if the additional yield to be realized by using two screen panels per tank compensates for the added cost of the screening, the 2-F combination may prove best.

Molting

The total number of molts collected from the 1-F, 2-F, 1-R, and 2-R tanks at two-day intervals are shown in Figure 3.6 with the corresponding moon phases for the 94-day study period.

Peaks in molting were expected on the quarter phases of the moon, but no definite peaks are discernible on the quarter moons in Figure 3.6. The reason for the lack of well-defined molting peaks throughout most of the study period may have been the low water temperatures experienced. During the first 60 days, daily temperatures averaged approximately 20°C with frequent dips below this average (Figure 3.7). Temperatures began to rise during the last month of the experiment, but the effects of previous low temperatures may have been felt even then as molting did not increase appreciably.

Daily minimum temperatures ranged from about 10.1 to 26.5°C and maximum temperatures ranged from 14.8 to 28.5°C. Diurnal

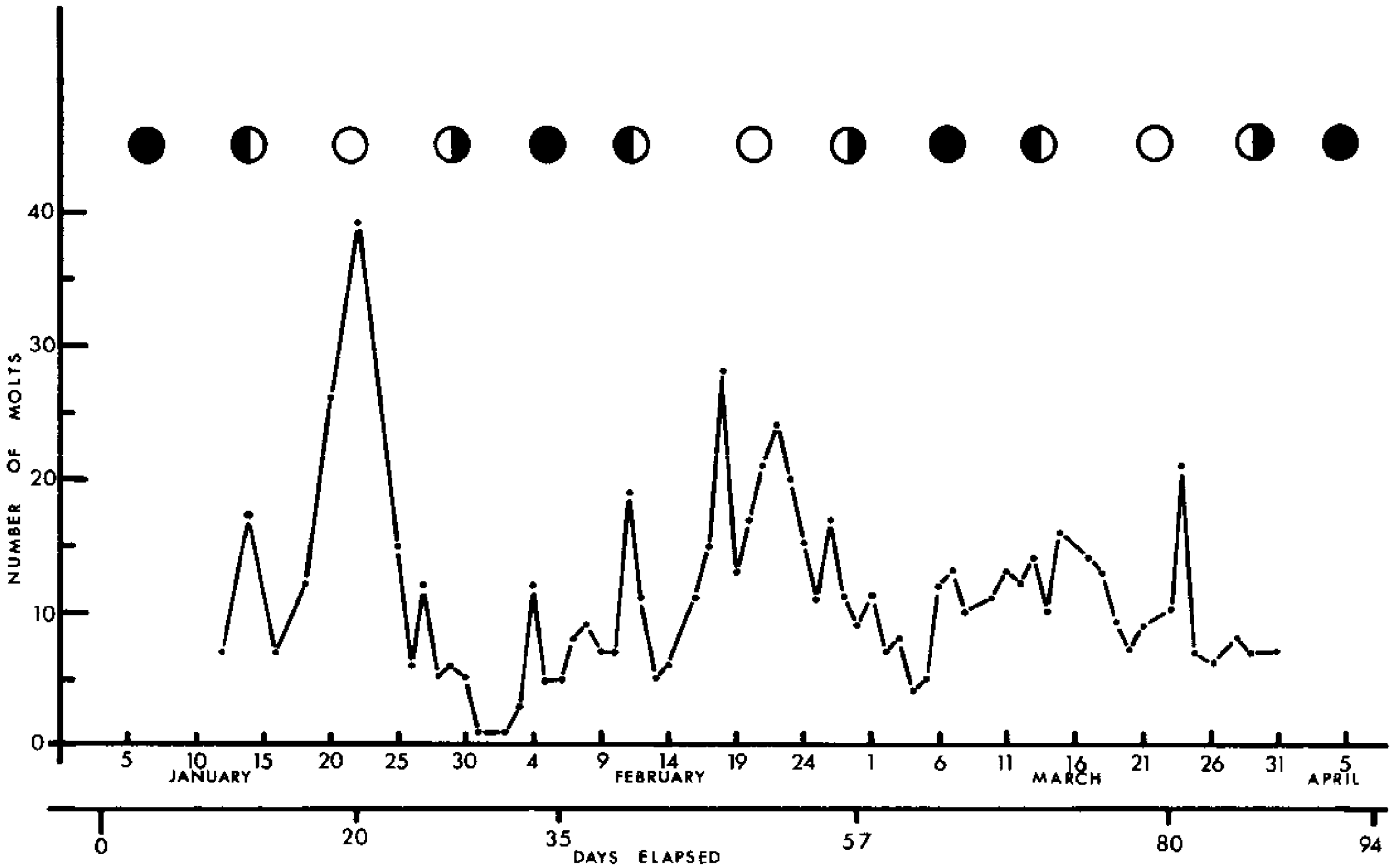

Figure 3.6. The total number of molts collected with corresponding phases of the moon. Weighing intervals are shown on the X axis.

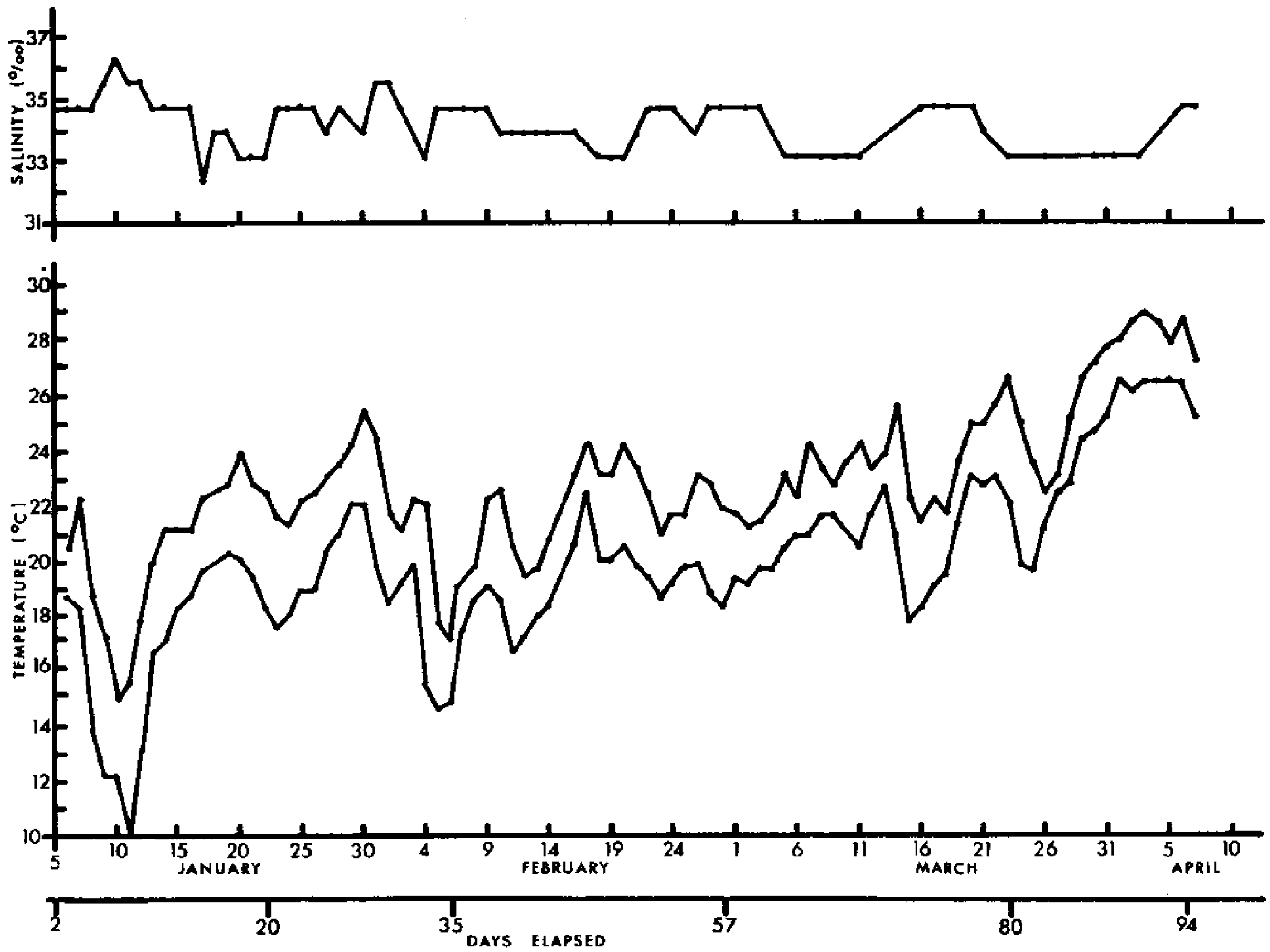

Figure 3.7. Daily salinity and maximum-minimum temperature observations for the study period. Weighing intervals are shown on the X axis.

temperature variations were usually 2 to 3°C. Salinity was relatively constant throughout the study and ranged from 32.3 to 36.3 ‰ (Figure 3.7).

Molting frequencies were analyzed with the independent variables temperature, moon phase, and number of elapsed days to determine which factor or factors had the greatest influence upon molting. Table 3.7 contains the results of computations employing the BMD02R step-wise multiple regression correlation computer program. Of the three variables, temperature and number of elapsed days were not significant, but moon phase was significant at the 95 per cent level. The Multiple R^2 value accounted for only about 12 per cent of the variation in molting frequency. Under the conditions which existed during the study, some other factor or factors contributed considerably in determining the molting frequency of the shrimp.

Further indication of the differences in shrimp growth between fouled-screen tanks and replaced-screen tanks is shown in Figure 3.8 which is the total molting data separated into fouled and replaced groupings.

Molting frequencies in the two groups followed nearly the same pattern throughout the study with shrimp in tanks with replaced screens sometimes molting more frequently than shrimp in tanks with fouled screens. However, analysis of the molting data by Wilcoxon's signed rank test revealed that over the entire 94-day period shrimp in tanks with fouled screens molted more frequently than shrimp in tanks with replaced screens at the 99 per cent level.

Table 3.7. Stepwise multiple regression coefficients for the number of molts collected and the independent variables temperature, moon phase, and elapsed time in the experiment.

Analysis of Variance:

Source	df	MS	F	Multiple R^2
Regression	3	69.002	2.645 ns	0.1185
Residual	59	26.091		
Total	62			

Source	Coefficient	Standard error	F
Temperature	0.61346	0.46593	1.7335 ns
Moon phase	-0.73473	0.29194	6.3340 *
Elapsed days	-0.06076	0.03835	2.5109 ns

*Significant at the 95% level

ns = Non-significant

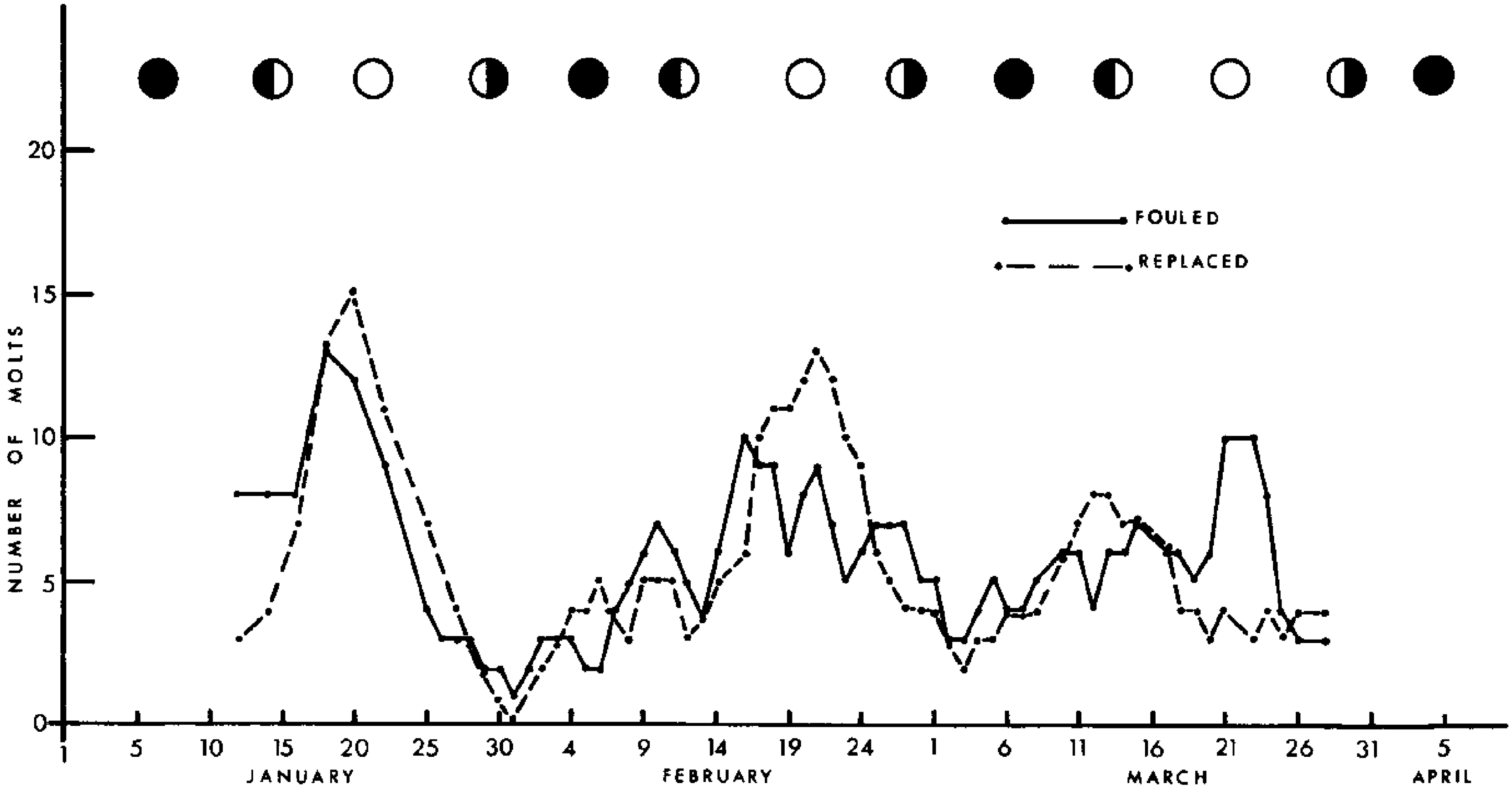

Figure 3.8. A comparison of the molting frequencies in tanks with fouled screens and tanks with replaced screens with corresponding moon phases.

The apparent closeness in molting pattern but clear differences in growth rates between shrimp in fouled versus replaced tanks may be accounted for by the possibility that shrimp in tanks with replaced screens may have molted with little or no growth. Molting without growth has been recorded for Penaeus duorarum by Eldred et al. (1961). Thus, shrimp in tanks with replaced screens in which food was limited may have had sufficient food for molting but not for the protein synthesis necessary for growth.

Growth Efficiencies

Further indication of the differences existing between the tank-types is shown in Table 3.8 which lists the growth efficiencies for each tank by weighing period as well as the tank-type averages. Growth efficiencies were calculated using the relationship stated for experiment one.

An examination of the average growth efficiencies for the eight tank-types shows that in nearly all instances values for fouled-screen tanks were higher than those for replaced-screen tanks with equal numbers of screens. As in experiment two, efficiencies followed the general trend of decreasing with time as the shrimp grew larger and the cost of maintenance per unit of food increased. Variations in the trend may be attributed to fluctuating water temperatures during the weighing periods (Figure 3.7).

Average growth efficiencies for the 12 fouled-screen tanks during the five weighing periods were 11.1, 12.4, 5.8, 4.0, and 2.9

Table 3.8. Growth efficiencies, for shrimp in each of the eight tank-types, expressed as milligrams of weight change/gram of body weight at the start of a period/day in the period. Since all shrimp were fed 10 per cent of their body weight per day, feeding data are not included.

Weighing Period	1-F	1-R	2-F	2-R	4-F	4-R	8-F	8-R
I	12.2	11.7	12.8	8.8	11.7	13.1	5.6	12.3
	11.8	13.2	13.1	10.5	5.2	10.5	9.0	5.8
	9.3	1.7	8.5	17.1	15.4	11.2	18.4	9.2
Ave.	11.1	8.9	11.5	12.1	10.8	11.6	11.0	9.1
II	8.8	9.9	9.7	12.8	14.6	11.2	16.1	5.4
	12.7	10.4	13.2	6.6	25.2	13.9	17.9	10.9
	1.5	19.5	15.8	1.1	8.2	2.9	4.6	10.4
Ave.	7.7	13.3	12.9	6.8	16.0	9.3	12.9	8.9
III	3.8	1.8	10.8	4.2	6.0	5.7	6.4	8.8
	5.7	4.9	7.4	6.9	4.3	6.4	0.9	2.0
	4.4	3.1	8.8	10.9	7.3	2.8	3.5	1.6
Ave.	4.6	3.3	9.0	7.3	5.9	5.0	3.6	4.1
IV	3.7	5.2	0.8	2.6	6.9	1.7	8.0	2.9
	0.6	3.1	1.5	4.8	4.8	4.0	3.5	3.5
	3.8	0.9	2.2	0.5	4.3	4.5	7.9	5.2
Ave.	2.7	3.1	1.5	2.6	5.3	3.4	6.5	3.9
V	3.8	3.5	1.0	1.2	3.4	4.7	0.6	0.2
	0.7	3.3	1.4	5.0	0.9	1.3	3.7	3.0
	3.7	0.2	0.5	0.7	8.4	3.6	7.0	0.5
Ave.	2.7	2.3	1.0	2.3	4.2	3.2	3.8	1.2

per cent. Average efficiencies for the 12 replaced-screen tanks for the same time intervals were 10.4, 9.6, 4.9, 3.3, and 2.3 per cent. Average food conversion values (expressed as weight of food/ change in shrimp weight) for the fouled-screen tanks were 9.0, 8.1, 17.2, 25.0, and 34.5 while replaced-screen tanks had average food conversions of 9.6, 10.4, 20.4, 30.3, and 43.5. In each weighing period average growth efficiencies were higher in fouled-screen tanks than in replaced screen tanks. These figures reflect the differences in growth, mortality, and production between fouled and replaced tanks because of the availability of the added food source in the fouling community on the fouled screens.

Fouling Community Analysis and Its Utilization as Food

Table 3.9 is a list of organisms identified on the screen samples and in the stomachs examined. In addition to these organisms, one jingle shell, _Anomia simplex_, several bubble snails, _Haminoea_ sp., and their egg masses, and the green algae, _Ulva_ sp., were found in the tanks. These organisms are not included in Table 3.9 because they did not occur in the shrimp stomachs or on the screens.

Relative abundances indicated for the food and fouling organisms in Table 3.9 were determined in the same manner used in experiment two. As the study progressed, the relative abundances of foraminiferans, rotifers, nematodes, copepods, and _Bacillaria_ sp. increased considerably. Relative abundances remained nearly unchanged for the remaining organisms.

Table 3.9. Fouling community analysis by weighing period with stomach content analysis and electivity index values computed after the method of Cramer and Marzolf (1970). Relative abundances* are averages for all screen tanks since there were no apparent differences in fouling community composition among the tanks.

Organism	Weighing Periods I	II	III	IV	V	Gut Contents	Electivity Index
Foraminifera	*	**	***	***	***	*	- .500
Vorticella	0	*	*	*	*	0	-1.00
Hydroids	*	**	*	*	*	0	-1.00
Turbellaria	**	**	**	**	**	**	0.00
Rotifers	**	*	**	***	***	**	- .200
Nematodes	**	***	***	***	***	0	-1.00
Copepods	**	**	***	***	****	***	- .143
Nauplii	*	*	**	**	**	0	-1.00
Ascidians	0	0	0	*	**	0	-1.00
Diatoms							
Pleurosigma	*	***	**	**	**	**	0.00
Thalassionema	*	*	*	*	**	0	-1.00
Licmophora	*	*	*	**	**	0	-1.00
Bacillaria	0	*	**	****	****	0	-1.00
Algae							
Enteromorpha	**	***	***	**	***	**	- .200
Chaetomorpha	0	0	0	0	*	0	-1.00
Cladophora	***	***	****	***	***	***	0.0
Lyngbya	**	**	**	**	**	*	- .333
Oscillatoria	***	***	****	****	****	**	- .333
Chroococcus	*	*	*	*	*	0	-1.00

*Relative abundance symbols represent the same values as in Table 2.10.

Food selection, for or against the fouling organisms, was analyzed as in experiment two using the electivity index.

Of the organisms in Table 3.9, Vorticella, hydroids, copepod nauplii, ascidians, Thallasionema, Licmophora, Chaetomorpha, and Chroococcus were considered to be of little importance because of their infrequent occurrence in the fouling community and absence in the stomach contents which resulted in electivity index (EI) values of -1.00 for these organisms.

Nematodes (EI = -1.00) were very abundant on the screens but were not found in any of the stomachs examined. Thus, nematodes were selected against by the shrimp. As stated in the previous studies, the reason for this is not known since Eldred et al. (1961) included nematodes among the food items of pink shrimp.

The diatom, Bacillaria sp. (EI = -1.00), was also very abundant on the screens but absent from the stomach contents. The reason for this is not known since another diatom of similar size but lower relative abundance, Pleurosigma sp., was found in approximately identical proportions in both the fouling community and the stomach contents. The electivity index for Pleurosigma was 0.00.

Other organisms which were fed upon randomly (EI = 0.00) were turbellarians (flatworms) and the filamentous green algae, Cladophora sp.

Foraminiferans were common on the screens but were selected against to the extent that the EI value was -.50. Rotifers, copepods, Enteromorpha sp., Lyngbya sp., and Oscillatoria sp. were selected against to a lesser degree (EI = -.33 to -.14).

Harpactocoid and cyclopoid copepods were the most abundant animals found in the shrimp stomachs, and Cladophora was the most abundant plant consumed by the shrimp.

As in experiment two, blue-green and green algae were consumed in nearly the same quantities. However, while approximately 30 per cent of the green algal cells in the stomachs were broken and empty, only about 2 per cent of the blue-green cells were damaged during ingestion. Similar observations were explained previously in experiment two.

While the relative abundances of most fouling organisms remained unchanged during the study, the biomass of fouling per square inch of screen surface increased progressively with time. Figure 3.9 shows the relative standing crops of fouling on the screens in 1-F, 2-F, 4-F, and 8-F tanks. After the third weighing period, the standing crop was greatest in 2-F tanks, approximately one-half as much in 1-F and 4-F tanks, and about one-fourth as heavy in 8-F tanks.

The standing crops of fouling on the screens were the result of the antagonistic processes of fouling production and consumption by the shrimp. Consumption by the shrimp was presumably nearly the same in all tanks since growth and mortality were not significantly different among tanks with different numbers of screens. Thus, differences in the standing crop may be attributed to differences in production of the fouling communities. A heavy

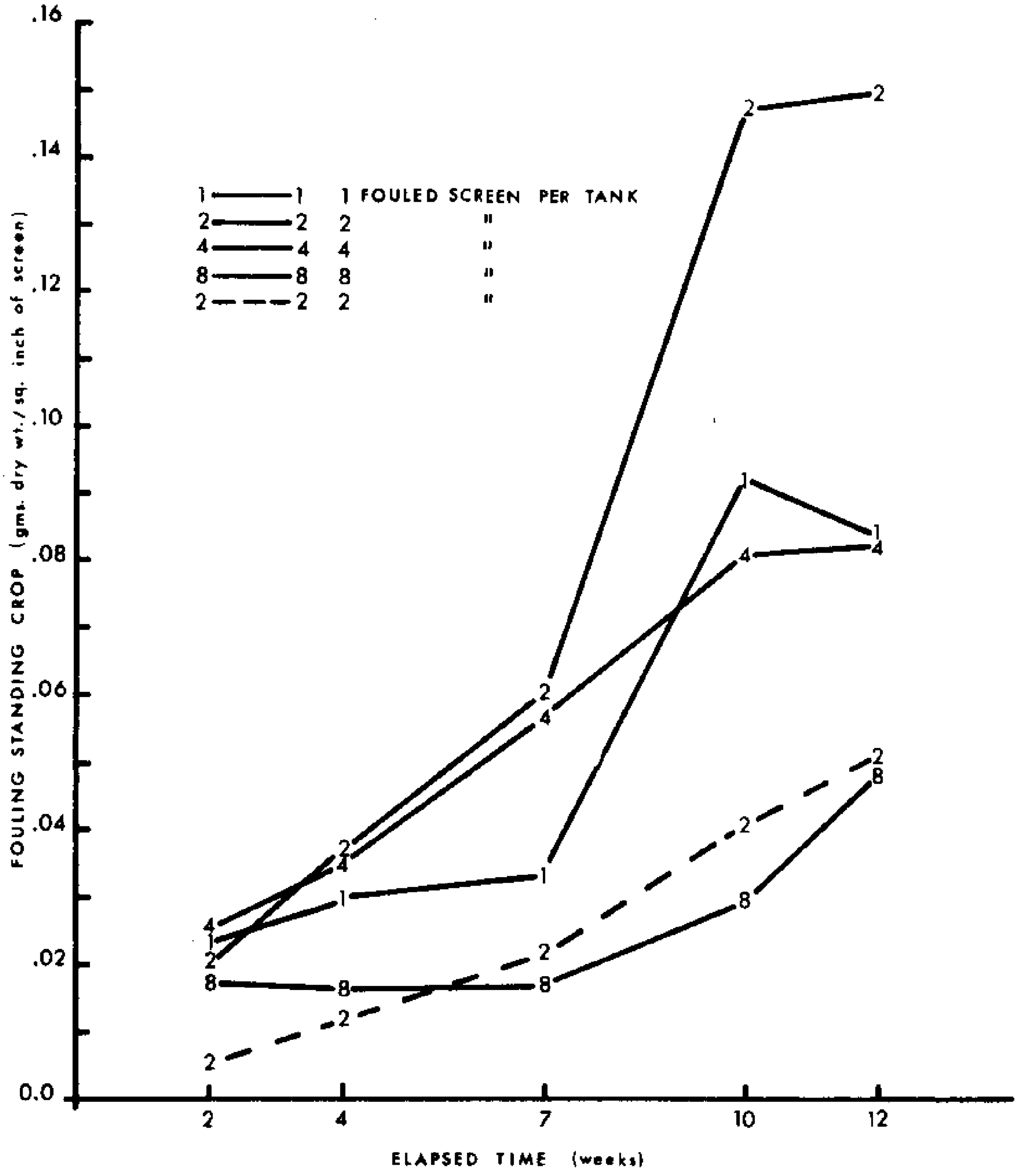

Figure 3.9. The average fouling standing crop as a function of elapsed time for tanks with different numbers of fouled screens. Solid lines represent tanks in the third experiment; the dashed line represents tanks in the second experiment.

growth of fouling organisms developed over the entire screen surface in 1-F and 2-F tanks, but it covered only the upper one-fourth of the screens in 4-F tanks and the upper one-tenth of some of the screens in 8-F tanks. Therefore, 2-F tanks had the greatest total of fouled screen surface (two full panels), 1-F and 4-F tanks had about one full panel of fouling, and in 8-F tanks only the equivalent of about 0.6 of a screen panel was fouled.

Differences in the extent of fouling production in the tanks may have been the result of shading between the screen panels. Therefore, incident light measurements were made to evaluate such shading in the tank-types. Figure 3.10 shows that shading is directly related to the number of screens, and as the screens are placed closer together the incident light decreases even in the upper half of the water column.

The growth of most filamentous algae falls off rapidly at light intensities below about 100 foot candles and algal production decreases proportionately as this value is approached (Dr. John Bunt, personal communication). Thus, in 1-F and 2-F tanks, in which the screens were completely covered by fouling, incident light exceeded 200 foot candles at the bottoms of the tanks. Light in the 4-F and 8-F tanks began to decrease before mid-depth, and little algae would be expected below this point since light was close to or less than 100 foot candles.

The light readings presented in Figure 3.10 were taken at noon on a sunny day and were near their maximum daily values due to the high angle of incidence and maximal penetration of the

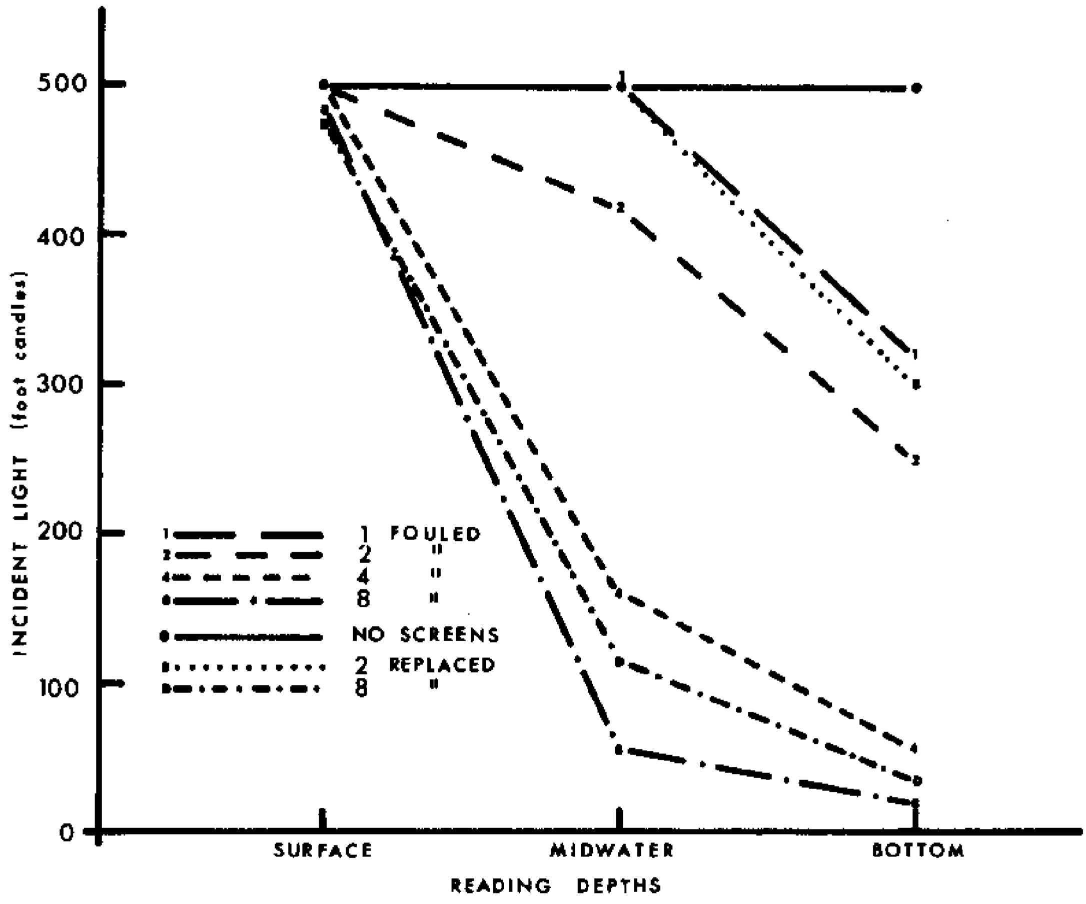

Figure 3.10. The average incident light at the surface, midwater, and bottom in tanks of each type, including readings for tanks with no screens.

water column. At other times of the day, light penetration would be less and the 100 foot candle level would be closer to the water surface, particularly in 4-F and 8-F tanks in which the screens were closer together. Therefore, the reduction of incident light as the number of screens per tank was increased apparently resulted in the limited fouling which developed in the tank-types.

The shading effects of unfouled screens are shown for tanks with 2 and 8 replaced screens. Clearly, much of the shading in all tanks was due to the screens themselves.

It would appear that 2-F tanks had the highest fouling standing crop per unit of screen area (Figure 3.9) because the entire surfaces of both screen panels became fouled, and consumption of fouling by the shrimp was exceeded by fouling production resulting in a progressive increase in the standing crop. The 1-F and 4-F tanks had only about one fully fouled panel per tank and the lower standing crop may have been due to proportionately greater shrimp grazing in these tanks. The 8-F tanks had less fouled screen surface and shrimp grazing kept the fouling standing crop at even lower levels than in the other fouled-screen tanks. Thus, the relative amounts of fouling production, as influenced by shading between the screens, and shrimp grazing combined to produce the fouling biomasses shown in Figure 3.9.

Behavior Observations

Modifications of behavioral patterns noted previously and observations relevant to the utilization of the screen surfaces by the shrimp are reviewed in the following discussion.

The severe cold wave mentioned previously resulted in minimal activity among the shrimp in all tanks. During the day of 10 January four shrimp from different tanks were out of the sand and were lying on their sides. These shrimp appeared nearly narcotized and moved their walking legs only slightly when disturbed. Further observations during the day and night found these shrimp in similar condition until the afternoon of 11 January when the water temperature began to rise. The shrimp then "recovered" and burrowed into the sand. It is possible that temperatures slightly lower than those experienced would have resulted in mass mortality of the shrimp.

Very few shrimp were observed out of the sand during the morning hours, and this may have been due to low water temperatures during the study period. Afternoon observations revealed more frequent occurrences of shrimp out of the sand, particularly in the tanks with replaced screens, and these shrimp appeared to be searching for food. Thus, in this experiment, the effects of less food in the replaced-screen tanks than in the fouled-screen tanks upon diurnal behavior of the shrimp were manifest largely in the afternoon when water temperatures had risen and the shrimp were active.

Further indication of differences in nutritional condition between tanks was the accumulation of fecal matter and the presence of entire molts in fouled-screen tanks. No fecal matter accumulated in replaced-screen tanks and molts in these tanks were always partially consumed.

Utilization of the fouled and replaced screens by the shrimp was analyzed with Wilcoxon's signed rank test which revealed that shrimp were observed more frequently on fouled screens than on replaced screens at the 99 per cent level. There were no detectable differences in the numbers of shrimp on screens between tanks with different numbers of screens. During any one observation, the portion of the shrimp population on the screens ranged from 2.2 to 10.0 per cent in tanks with replaced screens and from 3.6 to 22.5 per cent in tanks with fouled screens.

During the nights of 8 February, 10 March, and 5 April, counts of the number of shrimp on the screens were made before and after the daily ration of squid was added to the tanks. In each case, the number of shrimp on the fouled screens remained about the same after food was added. However, in the replaced-screen tanks, the number of shrimp on the screens increased by approximately 30 per cent after feeding and all of the shrimp held squid in their chela. This substantiates the comments made in experiment two in that the shrimp may have moved onto the added surface area provided by the screens to increase the distance between individuals and protect the food which they had seized. Shrimp in tanks with fouled screens did not appear to react in this manner since food was abundant on the screens and competition for food was less evident than in the replaced-screen tanks. Shrimp on the fouled screens before the squid was added were already feeding, possibly relieving crowding or competition for the squid among the entire population of shrimp in the tank. Such crowding and competition may have occurred in the replaced-screen tanks.

Parasites

As in the two previous studies, parasites were apparently of little consequence in experiment three. At the end of the 94-day study period, six shrimp were found with heavy parasitic infections, by the microsporidian Thelohania (Iversen, personal communication). Other shrimp in the tanks may have been lightly infected, but only these six showed the typical whitish discoloration of the abdominal tissues associated with the condition known as "cotton shrimp".

Shrimp used in the study were taken from Biscayne Bay, a location from which shrimp infected with Thelohania duorara have been taken previously (Iversen and Manning, 1959; Villela, Iversen and Sindermann, 1970).

Dissolved Oxygen and Water Flow

Measurements of the water flow into the tanks revealed that the rate was equivalent to an average of 2.3 tank volumes (1/4 cubic meter) per day. The water overturn ranged from 1.1 to 4.1 tank volumes per day depending on the water pressure in the laboratory seawater system.

Figure 3.11 shows a comparison of the diurnal cycles of dissolved oxygen monitored on 4-5 April in replaced-screen tanks,

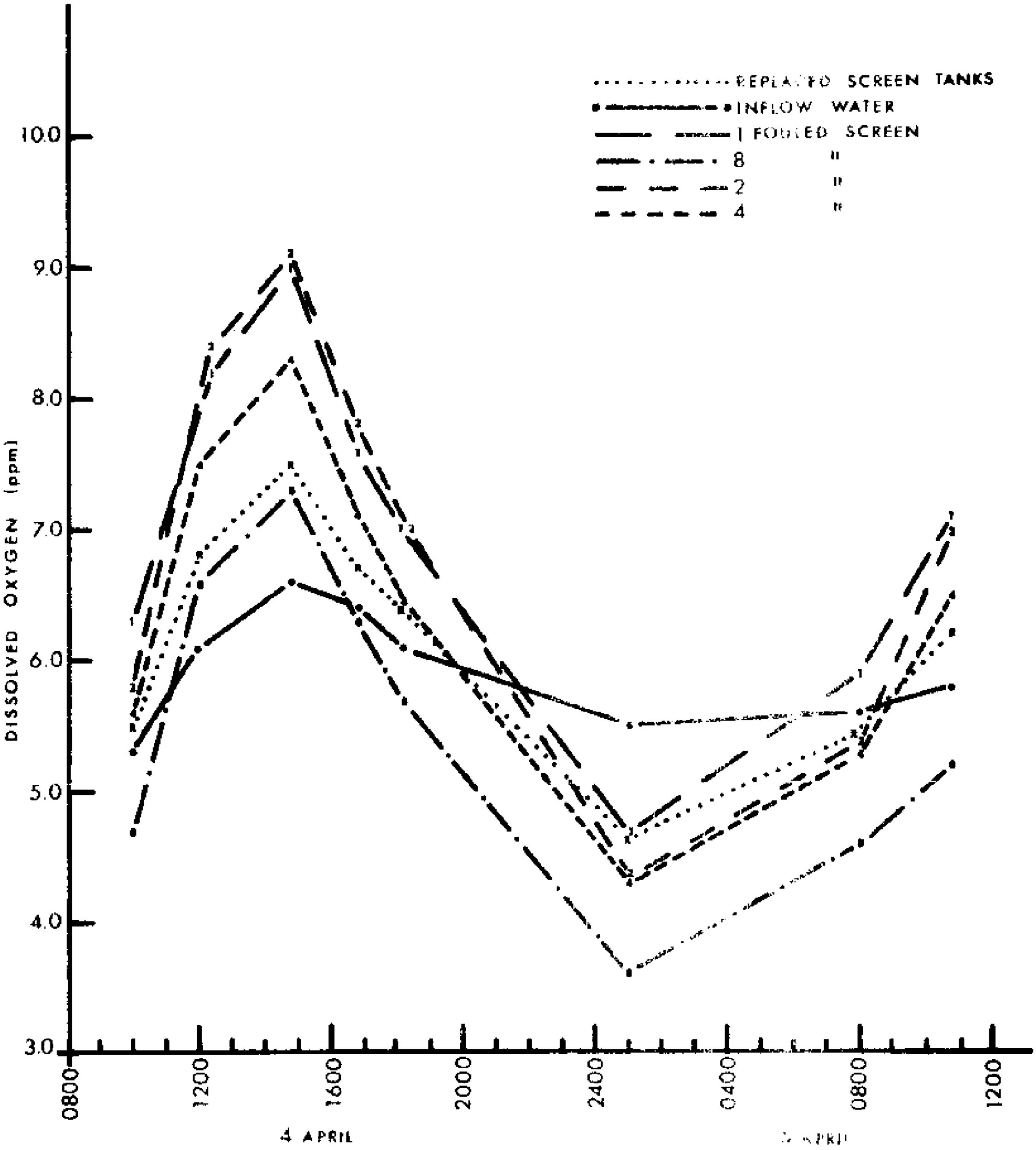

Figure 3.11. The average diurnal dissolved oxygen cycles in tanks with replaced screens, tanks with different numbers of fouled screens, and the inflow water.

1-F tanks, 2-F tanks, 4-F tanks, 8-F tanks, and the inflow water. Differences in the magnitude of oxygen values in the cycles were the result of relative production and consumption of oxygen in the tank-types. The cycle for the inflow water provides a baseline from which the fouled and replaced tanks varied in accordance with the animal and plant biomasses living in them.

Oxygen in replaced-screen tanks followed a typical cyclic pattern which was probably due to the presence of benthic diatoms and a limited amount of algal fouling on the bottoms and sides of the tanks.

The relative amounts of dissolved oxygen in the fouled-screen tanks reflect the fouling organism standing crops shown in Figure 3.9. The 2-F tanks supported the largest fouling biomass and had the highest dissolved oxygen during the day. These were followed in order by the 1-F, 4-F, and 8-F tanks which had proportionately lower fouling standing crops as well as lower dissolved oxygen values.

The oxygen values in 1-F and 2-F tanks were nearly equal during the day, but at night the values in 2-F tanks were lower than those for 1-F tanks. This indicated that the fouling communities in the two tank-types were composed of nearly equal amounts of algae producing similar quantities of dissolved oxygen during the day. Thus, differences in fouling biomass between 1-F and 2-F tanks (Figure 3.9) may have been due largely to animals which would result in lower oxygen in 2-F tanks than in 1-F tanks at night because of the greater demand for respiratory oxygen in 2-F tanks.

As in experiment two, dissolved oxygen values in fouled tanks were greater than 100 per cent saturation during the afternoon. The dissolved oxygen decreased to minimum values of 3.5 to 4.0 ppm at night. Thus, dissolved oxygen did not become dangerously low (approaching 1.0 ppm) while water flow into the tanks was uninterrupted.

Molting Analysis for the Three Experiments Combined

The molting frequency data for all three experiments were combined and analyzed with the corresponding temperatures, moon phases, and numbers of elapsed days to determine which factor or factors had the greatest influence upon molting over the entire range of conditions experienced. The BMD02R computer program was employed as before, and the results of the analysis are contained in Table 3.10.

Of the three independent variables, temperature was significant at the 99 per cent level, moon phase was significant at the 90 per cent level, and the number of elapsed days was not significant. The Multiple R^2 value accounted for approximately 34 per cent of the variation in molting frequency. Thus, of the factors analyzed, temperature had the greatest influence upon molting while moon phase had less influence. Elapsed time had no detectable effect upon the molting frequency in the shrimp used in the three studies.

Table 3.10. Stepwise multiple regression coefficients for the number of molts collected and the independent variables temperature, moon phase, and elapsed time for the combined data from the three experiments.

Analysis of Variance:

Source	df	MS	F	Multiple R^2
Regression	3	1340.410	31.237 **	0.3399
Residual	182	42.912		
Total	185			

Source	Coefficient	Standard error	F
Temperature	1.17598	0.12609	86.9787 **
Moon phase	0.40130	0.21533	3.4732 ***
Elapsed days	0.00237	0.02080	0.0130 ns

** Significant at the 99% level

***Significant at the 90% level

ns = Non-significant

CONCLUSION

Two basic considerations in shrimp culture are the total yield and the cost of production. Any technique which makes possible both improved shrimp production and reduced costs is of value to developing successful shrimp culture operations.

The results of the studies show that it is possible to realize significantly greater yields from shrimp grown in tanks in which an assemblage of fouling organisms is available as food than from shrimp with no fouling food source, even though all shrimp are given some supplemental protein. Although a certain amount of fouling will grow on the bottoms of shrimp ponds without encouragement by the culturist, in many cases the algae will consist mainly of blue-green species.

These studies have revealed that the filamentous green algae, Cladophora and Enteromorpha, are readily consumed by pink shrimp and are of greater nutritional value than the blue-green algae, perhaps because the latter are not broken in the gastric mill. Therefore, if conditions in the culture enclosures are controlled to encourage green algae rather than blue-greens, a greater percentage of the fouling community biomass will be composed of acceptable foods. Such conditions would include good light penetration of the water column, good water circulation, and the absence of uneaten food which could foul the water. These could be accomplished by employing relatively shallow culture enclosures with some form of water circulation, and carefully managed feeding regimes. In this situation, filamentous green algae could develop over the entire vertical substrate surface, creating a mechanism whereby the entire water column may be ultimately employed in shrimp production.

In pond culture, the fouling community on the vertical surfaces, e.g., screen panels, will create an added demand on the dissolved oxygen content of the water at night, and this will probably necessitate the use of flowing water or aeration. Also, because of the obstruction to water movement within the pond caused by screen panels, arrangement of the screen panels must be carefully designed so that water circulation is not greatly impeded. For example, screens may be aligned parallel to the direction of the prevailing winds to permit wind-generated water circulation, or the panels may be arranged in a staggered, alternating pattern to direct the circulation of pumped water to all parts of the pond.

It might be advisable to mechanize the culture operation so that the screens could be raised out of the water at night to avoid depletion of the dissolved oxygen by the fouling organisms. These organisms could be kept moist by periodic immersion in the pond so that they would not die. The periods of immersion at night would have to be long enough to permit feeding to allow the shrimp to feed, which in the case of *P. duorarum* is at night.

It is not known whether shrimp remaining out of the sand to feed during the day will grow at the same rate as shrimp receiving sufficient food at night and burrowing into the sand during the day.

The ability to raise the screens would also solve the problem of removing the substrates from the water during harvesting of the crop or preparation of the enclosure. But if the system were not mechanized, the substrates should be constructed so that manual handling is simple.

It may be desirable to employ a raceway culture system, in which water is circulated at a relatively rapid rate, permitting extremely high densities of shrimp to be grown in a small space. In such a situation, fouled screen panels could provide added food for the shrimp, but the fouling would be rapidly grazed off the screens and its standing crop would be limited or eliminated. This could be overcome by growing the fouling on replicate sets of screens in another body of water and placing them in the raceways when needed.

The number of vertical surfaces to be used in a pond or raceway would be an economic consideration. This study has shown that shrimp production rates in tanks with one screen panel per one-half square meter of bottom were nearly the same as production rates in tanks with greater numbers of screens. However, using one screen per meter of bottom in a pond would be unreasonably costly, and studies should be made to determine the optimum number of screens needed if ponds are to be used as the culture enclosures.

The number of screens to be used will be a function of (1) the shrimp stocking density (the higher the density the greater the amount of food needed); (2) the supplemental feeding rate (the greater the feeding rate the lower the amount of fouling needed as food); (3) the depth and clarity of the water (the greater the light penetration the lower the number of screens needed to produce equivalent amounts of fouling); and (4) the degree of water circulation (the greater the circulation the greater the fouling biomass which may be supported by the system).

Lunz (1966) stated that the maximum shrimp stocking density appears to be 10,000 to 12,000 per acre with heavy feeding and no water circulation. Broom (1968) stocked 12,000 to 18,000 shrimp per acre in ponds in Louisiana, while Wheeler (1968) reported stocking densities equivalent to 32,000 postlarvae per acre in Texas. Tabb (personal communication) has stated that a density of 20,000 juvenile shrimp per acre appears to be a reasonable estimate of that density which will produce the best yield under conditions of little water exchange and relatively heavy supplemental feeding. This is equivalent to a density of approximately four shrimp per square meter.

The stocking densities of 30 and 60 shrimp per one-half square meter of tank bottom employed in these experiments are equivalent to 15 and 30 times the estimate of optimal density cited above. The three experiments employed running water, supplemental feeding, and additional food from the fouling which developed on the screens. Therefore, it appears that the use of both water exchange and the fouling food source may permit stocking densities as high as 300,000 to 600,000 juvenile shrimp per acre. It must be realized that the shrimp density per unit of area must decrease if shrimp growth is to continue with time, and it is probable that such density figures apply only to shrimp in the weight range of 2.0 to 6.0 grams, as were used in the experiments. This leads to the consideration that it may be desirable and more profitable to employ an extremely high stocking density, i.e., 300,000 juvenile shrimp per acre, and harvest them after about 90 days for sale as bait shrimp, since their size (about 5 to 6 grams) would make them good bait but small for commercial processing. Partial harvesting of the crop at intervals could permit both the short-term production of bait shrimp and the longer-term production of market shrimp, since such periodic reductions in shrimp density would permit added growth by the shrimp remaining in the pond.

In the three experiments conducted in this study, tanks stocked with 30 shrimp per one-half square meter of bottom experienced mortalities which averaged approximately 20 per cent. Therefore, a stocking density of 300,000 shrimp (weighing 2 grams each) per acre may yield about 240,000 shrimp weighing 5 to 6 grams each. This is a total yield of about 1,200,000 grams, or 2,656 pounds per acre, if the entire crop were harvested after

90 days. The total yield could be greater if the proper balance of periodic harvesting and added shrimp production is maintained.

Anderson and Tabb (in press) have shown that the culture of pink shrimp in Florida does not appear profitable at any level of operation (up to 1,000 acres of ponds) at any land price for human food, and that bait shrimp culture appears profitable only at the levels of 500 or 1,000 acres of ponds at very low land prices. The bait shrimp estimates applied to an apparent limit of only one large farm when the total market for bait shrimp in Florida was considered.

However, if a stocking rate of 300,000 juveniles per acre may produce a yield of about 240,000 bait-sized shrimp (5 to 6 grams each) in 90 days, it may be that the yield could be 120,000 food shrimp (12 to 13 grams apiece) considering the added mortality during the time required for the added growth. This is equivalent to a total weight of about 3,333 pounds of food shrimp per acre. For a yield roughly equal to the 1,000 pounds for each of 1,000 acres cited by Anderson and Tabb, only about 333 acres of ponds would be required.

Thus, it appears that the use of vertical substrates in culture enclosures may reduce the land costs, the pond costs, and the labor costs for ponds, feeding, and other maintenance to about one-third of the quotations by Anderson and Tabb. This would change the entire economic picture expressed by them and would bring the possibility of successful culturing of pink shrimp onto more solid footing. Of course, the added cost of the substrates and related labor must be considered, but these will be offset to some extent by the reduced cost of feed for the shrimp.

The situation regarding bait shrimp would not appear to be improved through the use of vertical substrates to increase shrimp production per unit of area since the limitations cited by Anderson and Tabb related to the lack of a market with the ability to absorb massive additional production without radically reduced prices.

Research is needed to determine the optimum number of substrates to be used in any particular type of enclosure from both economic and practical standpoints. Studies should also be conducted to determine the optimum arrangement of the substrates, including the possibility of using fouled substrates as horizontal, layering surfaces in a tank or raceway system.

Additional knowledge is needed regarding stocking density and other factors affecting production, such as behavior. Perhaps a stocking density could be found at which some of the shrimp were feeding at all times and the system would be involved in shrimp production throughout the day and night. On the other hand, it may be possible to culture two species of shrimp together, i.e., *Penaeus duorarum* and *P. setiferus*, in which one would feed during the night and the other would feed during the day.

Since the total shrimp yield is the result of the effects of the initial stocking density, growth, and mortality, considerations for obtaining the greatest yield should include consideration of the stocking density. Growth and mortality are affected by the stocking density, but the detrimental effects of high densities may be moderated through the use of water exchange and the added food source provided by the fouling community which will develop on vertical surfaces in the culture enclosure. The beneficial effects of the fouling organism food source upon shrimp growth, mortality, and total production under tank culture conditions have been clearly demonstrated in the three experiments described above; it now remains for large-scale operations to apply the knowledge gained in a practical situation.

One of the areas in which little knowledge has been gathered is that of food energy utilization and optimum feeding rates of shrimp under culture conditions. Basic studies should involve determining essential nutritional requirements during the different stages in the life cycle of the shrimp; manipulations of the feeding regime could then be made regarding the amount and type of supplemental food and the extent to which fouling may be successfully utilized for optimum growth and production.

All aspects of research on shrimp culture may be interrelated with the use of fouled vertical substrates, and each of the aspects will affect the others as it is added to the culture program, resulting in complex multi-variate studies requiring intricate data treatment. However, the exact manner in which each part of the system meshes with the others will not be known until all of them are assembled as a unit. Such assemblages will represent the final stages in designing system models for successful shrimp culture operations.

SUMMARY

The three experiments were conducted with the principal objective of determining the effects of vertical substrates upon shrimp growth, mortality, and total yield. Secondary objectives were to study molting, behavior, and utilization of the substrates and the fouling organisms growing on them by the shrimp.

In experiment one, artificial grasses were used as the vertical substrates:

(1) Shrimp growth and total yield rates were significantly better in tanks with grass substrates than in tanks with no vertical surfaces.

(2) Mortality values were nearly the same in most tanks, but one control tank had more deaths than expected, and one grass tank had a lower number of deaths than expected.

(3) The grasses were judged unsatisfactory as substrates since fouling did not develop on them but only along the water surface.

(4) Peaks in the molting frequency correlated significantly with both fluctuations in water temperature and moon phase.

(5) Differences in growth efficiencies among shrimp in substrate and control tanks reflect the use of the fouling food source in the substrate tanks, with values for shrimp in these tanks being consistently higher than in control tanks. Growth

efficiencies declined progressively during the study as the shrimp grew larger and maintenance requirements increased.

(6) More shrimp made use of the Chemturf substrates than those of Olefern, probably because of the physical structure of the configurations.

(7) Shrimp showed a marked feeding preference for the filamentous green algae Cladophora and Enteromorpha while avoiding the blue-green Oscillatoria. Copepods were readily eaten but nematodes were avoided.

(8) The circadian activity rhythm of the shrimp persisted throughout the study. Two types of feeding behavior were observed, and feeding was radically depressed by water temperatures below 18°C. Neither fecal matter nor molts accumulated in the control tanks because of the lack of sufficient food.

In experiment two, panels of fiberglass window screen were used as vertical substrates, and stocking density was included as a variable:

(1) Shrimp growth and total yield were significantly higher in tanks with vertical screen surfaces and in tanks with 30 shrimp than in tanks with no substrates or tanks with 60 shrimp.

(2) Mortality was significantly lower in tanks with 30 shrimp than in tanks with 60 shrimp, but there were no differences in mortality due to the presence or absence of vertical surfaces.

(3) Peaks in molting frequency failed to correlate with any of the variables measured, apparently because of high water temperatures.

(4) Molting frequencies and growth efficiencies reflected the effects of stocking density and presence of the screens.

(5) The species represented in the fouling communities on the screen panels was correlated with the occurrence of items in the stomachs of preserved shrimp. Principal food items were copepods, the filamentous green algae, *Cladophora* and *Enteromorpha*, and the diatom, *Pleurosigma*. Nematodes were very abundant on the screens but were not found in the stomachs.

(6) The thicker green algae were apparently of greater nutritional use to the shrimp than the thinner blue-green filaments, since the green algae could be broken by the gastric mill.

(7) Shrimp in tanks with no substrates frequently remained out of the sand during the day, evidently to search for food, while shrimp in substrate tanks maintained the expected circadian activity rhythm noted for pink shrimp. Fecal material and molts did not accumulate in the control tanks as they did in the tanks with substrates.

In experiment three, screen panels were again used as substrates, while the amount of screen area was varied, and screens in some tanks were kept free of fouling:

(1) Shrimp growth, survival, and total yield were significantly greater in tanks with fouled screens than in those with screens with no fouling.

(2) There were no apparent effects on growth, mortality, or production due to the amount of screen surface available to the shrimp, and one screen per tank would be the least expensive for a culture operation.

(3) Peaks in molting frequency correlated significantly with moon phase.

(4) Differences in growth efficiency values between tank-types reflect the results noted for shrimp growth and production in the experiment.

(5) Fouling community analysis and food selection were essentially the same as in the preceding study.

(6) The standing crop of fouling organisms on the screens was studied, and its correlation with shading between the screens and dissolved oxygen in the water was discussed.

(7) Few shrimp were out of the sand during daylight hours, possibly because of low water temperatures.

(8) Fecal matter and molts accumulated in tanks with fouled screens but not in tanks with replaced screens.

(9) Shrimp were observed more frequently on fouled screens than on replaced screens, and the shrimp appeared to move up onto the screens after seizing pieces of squid to "protect" their food from other shrimp.

(10) Molting frequency data for the three experiments were combined, and these correlated significantly with water temperature and moon phase.

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