INFLUENCE OF DIET ON THE FEEDING BEHAVIOR, GROWTH, AND THERMAL RESISTANCE OF POSTLARVAL *Penaeus aztecus* AND *P. setiferus*

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Results indicated that initial diet preference, survival, growth, and resistance to high temperature are independent qualities of foods as indicated by these two species of shrimps.

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INFLUENCE OF DIET ON THE FEEDING BEHAVIOR, GROWTH AND THERMAL RESISTANCE OF POSTLARVAL *Penaeus aztecus* and *P. setiferus*

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ABSTRACT

Laboratory studies were conducted on the effects of diet on the food preference, survival, growth and temperature tolerance of two species of penaeid shrimps, *Penaeus setiferus* and *P. aztecus*. *Artemia* and five artifically compounded foods were tested. Both *P. setiferus* and *P. aztecus* usually showed food preferences when given options. *P. setiferus* demonstrated stronger food preferences than the other species.

Diets influenced survival, growth and temperature resistance of the shrimps. The effects of some diets differed markedly between species.

Far better growth was produced in the shrimps fed *Artemia* than any of the artificial diets used in the experiment.

Results indicated that initial diet preference, survival, growth and resistance to high temperature are independent qualities of foods as indicated by these two species of shrimps.

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In the memory of my father,

KASEM ALI

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INTRODUCTION

There is perhaps more enthusiasm for the possibilities of commercial culture of crustaceans than of any other kind of seafood. This is a consequence of high demand for shrimp and other crustaceans in many parts of the world. For its rapid growth, and high nutritional value, shrimp has been of continued interest to the mariculturist.

Shrimp in the U.S.A. is a food delicacy. U. S. shrimp landings in 1969 were 195.5 million pounds, heads-off weight and accounted for nearly a fourth of the $518 million dollar value of landings for all U. S. fisheries (Whitaker and Surdi, 1970). Total imports were 220.1 million pounds, heads-off weight. Domestic landings accounted for 47 percent of the combined total of landings and imports. The nation had to depend on imports to meet more than 50 percent of its demand. In terms of dollar value, shrimp constitutes the most important fishery in the U.S.A. Shrimp contributes significantly to the protein supply and economy of many other countries. Pakistan's foreign exchange, for example, appreciably depends on shrimp export. In many of the oriental countries shrimp support important fisheries.

The citations on the following pages follow the style of the journal "Transactions of the American Fisheries Society".

For its high demand for human consumption, the necessity for its increased production by artificial propagation has been appreciated by the aquaculturists. The culture of *Penaeus japonicus* has become a many-years-research-project in Japan (Hudinaga, 1942; Fujinaga, 1963), its culture now being a commercial enterprise (Hudinaga and Kittaka, 1966). Similar attempts have also been made in the U.S.A. with limited success so far (Cook and Murphy, 1966; Cook, 1969). The brown shrimp, *Penaeus aztecus* and the white shrimp, *P. setiferus* are two of the most important penaeid shrimps in the U.S.A. The success in hatching the eggs of these and some other species enhances the possibility of farming shrimp in American coastal ponds or other suitable waters in an economically practical way.

The success of shrimp farming depends, among other factors, on a constant supply of foods to the animals. This fact leads us to investigate the possibility of using an artificial diet which is readily acceptable to the shrimp, has good growth stimulating qualities, yet is cheap and easily available. Such food also should not reduce the animal's natural tolerance to environmental stresses such as extremes of temperatures (Hoar and Dorchester, 1949 and others). In the present study some potential diets were tested to study their influence on the feeding behavior, survival, growth and temperature tolerance of *P. setiferus* and *P. aztecus*.

REVIEW OF THE LITERATURE

Opinions vary about the food habits of penaeids in general. They have been designated as omnivorous, carnivorus, plant or detritus feeders by different investigators.

Gopalakrishnan (1952) analyzed the stomach contents of 380 specimens of Penaeus indicus from the coastal waters of India. He found that plant matter and crustacean debris formed the bulk of the stomach contents, but as the remains of other animals were also found, he concluded that the species must be omnivorous.

Panikkar and Menon (1956) maintain that the food of penaeid shrimp consists of detritus, both animal and plant, that accumulate in their benthic habitat. These authors suggest that shrimp also naturally take in large quantities of sand and mud along with the detritus. In their study, the analysis of stomach contents of numerous species of the Family Penaeidae, including Penaeus carcinatus, P. indicus, Metapenaeus monoceros, M. dobsoni, M. brevicornis, and others, revealed the presence of large numbers of diatoms, particularly when these algae were abundant in the plankton. Both M. dobsoni and P. indicus seem to consume large quantities of algal matter when available. But in Parapeneopsis stylifera, plant matter, excepting some diatoms, was rarely noticed. Small molluscs and worms were also found in the animals examined by Panikkar and Menon (1956).

Hall (1962) made a general analysis of stomach contents of 765 specimens of 31 species of Indo-West-Pacific penaeid shrimp collected from Malayasian waters. His findings are different from those of Panikkar and Menon (1956) in that the sand grains and diatoms were not present among stomach contents in any significant numbers. Further, he found detritus as a very minor item of diet. In his work he found both plant and animal matter in varying proportions. The animal portion of the food included polychaetes, echiurids, crustaceans including copepods, ostracods, mysids, isopods, amphipods, decapod larvae and crustacean eggs, arachnids, molluscs, fish larvae and fish eggs. Plant matter included diatoms and some other algae.

Hall, however, maintains that although many penaeids appear to feed on what is most readily available, some species certainly select their food. On the basis of his analysis, he classified the penaeid family into six groups: Group A - species feeding mainly on polychaetes; Group B - species feeding mainly on small crustaceans; Group C - species feeding mainly on large crustaceans; Group D -

species feeding mainly on plant matter; Group E - species with a general carnivorous diet; Group F - species which are quite omnivorous.

Hall includes Penaeus indicus with Group C, but Gopalakrishnan (1952) designates it an omnivorous animal. Panikkar and Menon (1956), on the other hand, found vegetable matter as the predominant portion of the stomach contents of this species. Again, the great bulk of the stomach content of Metapenaeus brevicornis of Indian waters was found to be diatoms and other algal matter (Panikkar and Menon, 1956), while the main bulk of the stomach contents of the same species from Malaysian waters was found to be angiosperm tissue (Hall, 1962).

The studies of Ikematsu (1955), Kubo (1956) and Yasuda (1956) on Japanese species of Penaeidae indicated a general carnivorous diet for the family. Kubo concluded that Penaeus japonicus has a preference for small animals such as fishes, molluscs and crustaceans. Hall (1962) also described this species collected from Malacca Strait to be carnivorous, feeding on polychaetes, crustacea, arachnids, molluscs and fishes.

Food of the Australian commercial penaeid shrimps, Penaeus esculentus, P. merguiensis, P. plebejus, Metapenaeus bennettae, and M. macleayi, was found to consist of the "remains of small animals and a large amount of unrecognizable material" (Dall, 1968). He suggested that the latter item forms the main component of the diet, and that shrimps derive this by browsing on the microorganisms (bacteria, algae and microfauna) which grow on the surface of the substrata.

Williams (1955) analyzed the stomach contents of 184 young and adult penaeid shrimp (*Penaeus aztecus*, *P. duorarum*, and *P. setiferus*) from the estuaries of North Carolina and came to the conclusion that the main bulk of the food consisted of unrecognizable debris. Darnell (1958), based on the stomach analysis of 10 white shrimp in Lake Pontchartrain, Louisiana, concluded that whereas both young and adult white shrimp were omnivorous, they fed largely upon organic detritus of the bottom. Darnell did not, however, explain how he identified a bulk of unrecognizable food in the stomach as bottom detritus and not triturated and partially digested food which had been ingested alive. Flint (1956) studied the food of "shrimp" from Grand Isle, Louisiana, and Biloxi, Mississippi. He found that small shrimp (about 1 cm) contained chiefly cropped filaments of blue-green algae and such diatoms as ordinarily would have been found adherent to them in the natural habitat. Adult shrimp were found to have a preference for animal food. Flint, however, concluded that in the turbid coastal waters of Louisiana and Mississippi, blue-green algae serve as a major food for both young and adult shrimp, although the latter consume a diversity of materials. Weymouth *et al.* (1933) noting that *Penaeus setiferus* in the Gulf of Mexico and along the coast of southeastern United States may feed on its own species, and on other crustacea, worms, small molluscs and plant debris, concluded the species was omnivorous.

The above review suggests that penaeid shrimp have a wide range of diets. It is also clear that on the basis of stomach analysis, workers vary in their conclusions regarding food habits of single penaeid species. This problem may arise from the basic limitations of the stomach analysis method. The presence in a stomach of a high proportion of a particular food does not indicate whether the food material was in fact selected, or whether the stomach contents were just a reflection of what was available to the organisms. It is not sufficient merely to record the constituents of the stomachs; one must determine whether the elements had been ingested selectively as pieces of food material or accidentally along with other food, or as an aid to trituration.

A search of the literature indicates that no direct observations have been made on the food preference behavior of postlarval shrimp. By direct observation, I mean visual observation of the process of ingesting or selecting a food by a shrimp provided with a variety of potential diets. Since it is logical that an animal's food preference may influence its growth produced on various diets, the study on the food preference of a commercially important penaeid shrimp can have practical application. The data may be useful to future shrimp farmers, as well as to laboratory investigators who need to hold shrimp for scientific studies.

For farming shrimp, or other animals, a constant supply of appropriate food to the animal is of primary consideration. In certain laboratories, scientists have successfully raised penaeid shrimp on cultures of *Skeletonema*, *Thalassiosira*, *Dunaliella*, *Exuviella*, and *Gymnodinium splendens* during larval stages and *Artemia* during postlarval to juvenile stages (Dobkin, 1961; Ewald, 1965; Cook and Murphy, 1966; Cook, 1969). Laboratory growth of postlarval *Penaeus aztecus* fed brine shrimp larvae required 2 to 3 grams of food for each gram of weight increment at salinity and temperature levels of 15‰-35‰ and 18-25 C (Zein-Eldin and Aldrich, 1965). This food is expensive and a less costly diet is needed for commercial production of postlarvae. This suggests that a shrimp farmer will have to feed his captive shrimp with a prepared food, or else provide a rather large area of pond bottom per shrimp in order to maintain an adequate supply of natural food. Farming shrimp using *Artemia* as food is not practicable and natural food production in ponds limits shrimp production. As a result, an artificial diet is needed which has good growth stimulating qualities, yet is cheap and easily available.

There are many cases in which the natural foods of an animal have been partially or completely substituted for prepared foods. Ling (1962) found cooked fish and cooked chicken egg to be good material for the various larval stages of a caridean shrimp, *Macrobrachium rosenbergi*. Subrahmanyam and Oppenheimer (1970) used squid and three combinations of prepared food, namely (1) fishmeal (77.5%), stick water (20%), and vitamins (2.5%); (2) fishmeal (50%), stick water (18%), vitamins (2%), and Soya (30%); and (3) fishmeal (49%), algae (10%), banana starch (28.5%), cod liver oil (10%), vitamins (2.5%) in a growth study of *P. duorarum* 37.6 - 38 mm in length. The prepared food was mixed and compressed into pellets 1 mm round and 2 mm long. They found good acceptance of these foods by the shrimp. Best growth (0.5 mm length increase per day) was observed with the first diet, as against 1.7 mm per day in the natural habitat of the estuary (Williams, 1955; St. Amant *et al.*, 1963). Lewis *et al.* (1969) have experimentally shown that when large-mouth bass (*Micropterus salmoides*) are crowded in ponds, some of the bass can be conditioned to accept non-living food. Under these conditions the fish accept prepared foods and may also grow well. In nature, salmon eat live food, but Locke and Linscott

(1969) fed captive Atlantic salmon (*Salmo salar*) with a prepared dry diet, Ewos brand salmon feed, and obtained very satisfactory results. During the second year of feeding tests, salmon reared on the Ewos diet were twice as large as those on 100 percent beef liver (control group). Bryan and Allen (1969) used floating type commercial trout feed for feeding channel catfish fingerlings in the hatchery pond. The S-value ($S = \frac{\text{Pounds feed added}}{\text{Total pounds fish produced by added plus natural foods}}$ Swingle, 1959) ranged from 0.73 to 1.24 in individual ponds, and averaged 0.9. Periodic checks of the stomach contents indicated that the amount of natural food used by the fish was small. Snow (1968) tested the Oregon Moist Pellet (OMP) as a growing ration for fingerlings of large-mouth bass (*Micropterus salmoides*). During four trials involving 5,600 fish, from 75 to 90 percent of the fish readily accepted the diet. A weight increase of 568 percent and a food conversion of 1.65 were obtained. Several species of esocids which generally eat live food have been successfully raised on trout starter, trout crumble and sucker fry (Delano, 1968).

All these instances lead us to speculate that some of the prepared foods may also be acceptable and may cause good growth to the white and brown shrimp, particularly since adult white shrimp seem to be more or less omnivorous (Weymouth *et al.*, 1933). But even if these foods are accepted by the shrimp and proved to have growthpromoting qualities, certain problems still remain. Food has a profound influence on the general physiology of the animal. It may modify the resistance of the animal to the fluctuations of the environmental factors, such as temperature (Hoar and Dorchester, 1949; Hoar and Cottle, 1952; Irvine et al., 1957; House et al., 1958), resistance to disease (Allison, 1950; Wolf, 1951; Rucker et al., 1952; Zobairi, 1956), or molting and larval development (Broad, 1957).

The proposed research will, however, study only the influence of diet on heat tolerance. The lethal temperature for an animal is, of course, not only influenced by diet, but may also be affected by many other factors. Past thermal history including acclimatization or acclimation of animals and the importance of its variance to survival times has been discussed by Loeb and Wasteneys (1912), Hathaway (1927), Doudoroff (1942), Brett (1956), Fry (1957), Morris (1960), Bowler (1963), Naylor (1965) and Vernberg and Vernberg (1966). Hoar and Robertson (1959) provided direct evidence of a photoperiod effect through tests on goldfish exposed to different light cycles. Fish exposed to longer periods of illumination and shorter periods of darkness showed greater resistance to high temperatures and lesser resistance to low ones than did fish that were exposed to the reverse cycle of illumination. Hoar (1955) had previously demonstrated a seasonal variation in the thermal resistance of goldfish fed on a standard diet and acclimated to a constant temperature. Hart (1952) made similar observations on wild fish. Hutchinson and Kosh (1965) reported a clear-cut example of the effect of photoperiod on the CTM (critical thermal maximum) value in the painted turtle, *Chrysemys picta,* in which the difference between acclimation to a 16-hour versus an 8-hour light period changed the CTM value by about the equivalent of a 4° increase in acclimation temperature. The lethal temperature is reported to be influenced also by oxygen saturation in the environment (Thomas, 1954; Prosser, 1961; Sprague, 1963); salinity (Bert, 1871; McLeese, 1956; Zein-Eldin and Aldrich, 1965; Lewis and Hettler, 1968); age and size (Belehradek, 1935; Huntsman and Sparks, 1924; Hart, 1952); molting stage in crustacea (McLeese, 1956); genetic background (Prosser, 1961); and by non-genetic influence at developmental stage (Gibson, 1954).

In the present work, attempts were made to maintain all conditions constant except food, so that effects of diet could be most readily observed.

It will be relevant in this connection to cite some examples of the influence of food on the animal's resistance to temperature.

Mason *et al*. (1965) found that domesticated brook (*Salvelinus fontinalis*), brown (*Salmo trutta*) and rainbow (*Salmo gairdneri*) trout reared on dry diets survived and grew well. Later in 1966, the same group of workers made a study on the survival and growth of these trout after release into the wild environments of lakes and streams. In some of the cases the survival of the trout fed only on dry food was significantly lower than that of the control groups. The low survival of the fish was suspected to be their susceptibility to the fluctuating environmental temperatures.

A variety of effects of dietary lipids on temperature tolerance in poikilotherms has been reported. Heat tolerance of goldfish is said to be increased by high dietary cholesterol; and goldfish had greater heat tolerance when fed lard than when fed fish oil; fats of warm-acclimated fish have lower iodine numbers, hence fewer unsaturated bonds (Irvine *et al*., 1957). Blowfly larvae reared at high temperatures were more heat resistant and their fats had lower iodine numbers (Fraenkel and Hopf, 1940); a diet of highly saturated fat increased their heat tolerance (House *et al*., 1958). Earlier, Hoar and Dorchester (1949) and Hoar and Cottle (1952) demonstrated the same general relationship between degree of unsaturation of the body fats and heat tolerance, but they did not find a strict correlation between these two factors. Heat tolerance was not always dependent upon the melting point of the body fats, suggesting that some other properties of the fats are also involved. Different fats may bring about changes in the water content of the body tissues (Mayer and Schaeffer, 1914, cited by Hoar and Dorchester, 1949) and may also change the permeability of the cell membrane (Brooks and Brooks, 1941); thus affecting the heat tolerance of the animal.

GENERAL PROCEDURE

Collection of Postlarvae

Large numbers of postlarval white shrimp were collected from the Gulf of Mexico entrance to the Galveston Bay in waters 2-4 feet deep just north of the South Jetty and about 1/2 mile southeast of the Galveston seawall. Postlarval brown shrimp were seined from the same general area but along the beach near the South Jetty (Figure 1). All the collections of white shrimp were made during June and early July 1969 and those of the brown shrimp during February and early March 1970. Attempts were first made to collect brown shrimp postlarvae at the identical area from which postlarval white shrimp were collected, but that area was then inaccessible due to excessive siltation.

A beam net (Renfro, 1963) was used to collect all postlarvae. Each collection was immediately washed with clear sea water contained in a plastic bucket to remove any mud and trash. As far as possible, fishes, big shrimps, crabs and other unsought animals were removed from the catch in the field. This was found useful in decreasing mortality and deterioration of the condition of the postlarvae. In the field, shrimp were stored in an 8-liter plastic bucket or a 10-gallon Styrofoam box. A battery operated aerator was used

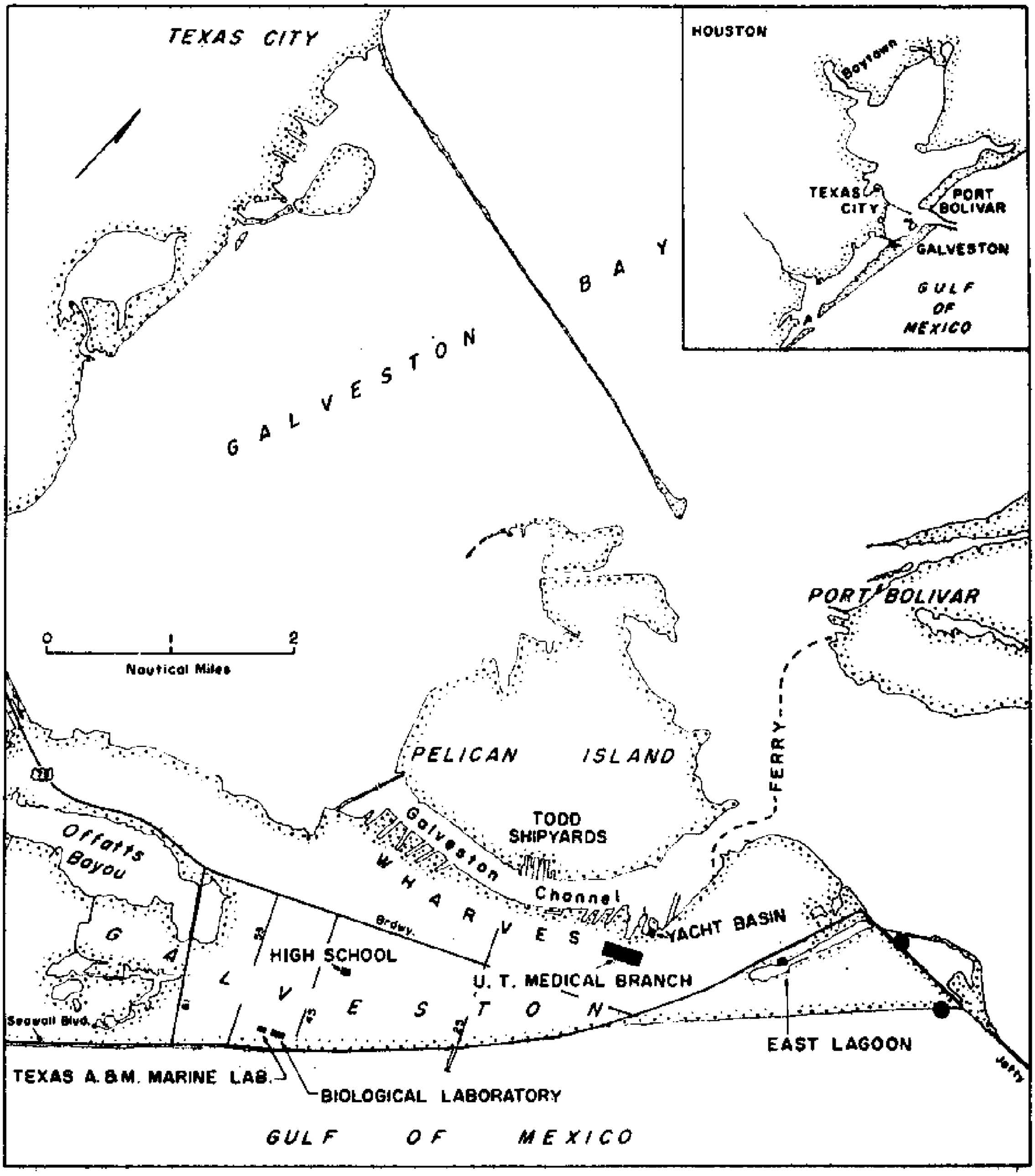


Figure 1. --Map of Galveston Bay area. Black solid circles indicate sites at which postlarval shrimp were collected.

to ensure oxygen supply to the crowded animals. The collections were always taken to the laboratory within 1-2 hours for final separation, identification and further care.

Field temperatures and salinities for white shrimp varied from 25 to 27 C, and 23‰ to 26‰, respectively. Salinity was determined to the nearest 0.5‰ (± 0.25‰) by means of a refractometer (American Optical Co., Rochester, N.Y.). For brown shrimp, temperature ranged from 15 to 18 C and salinity varied from 24‰ to 29‰ during collection periods.

Laboratory Method

After being brought to the laboratory, the collections were dispersed into several containers of aerated sea water. Drastic changes in temperature and salinity were avoided. No special arrangements were made to acclimate the postlarvae to the room temperature and the holding salinity. During 3-4 hours of sorting, the water (and shrimp) temperature gradually approached the room temperature (22 - 26 C). The field salinities at collection for both the species were within 4‰ of the holding salinity.

For separation of postlarvae, small aliquots of the collections were placed in a small finger-bowl over a fluorescent bulb. Under these conditions the postlarvae were clearly visible. The shrimp were transferred to aquaria containing clean seawater, with a small piece of polyethylene mesh. The aquarium water was covered by a thin polyethylene film to prevent the postlarvae from sticking to the tank walls above water level or jumping completely out of the tank. The water of each aquarium was kept aerated through an air stone. The stored animals were fed *Artemia* nauplii.

FOOD PREFERENCE STUDIES

Procedure

Before conducting food preference studies, the shrimps were held without any food for a sufficiently long time until they readily accepted food when it was provided. In most cases 3-5 days were sufficient. To starve the shrimps, they were held in 1500 ml fingerbowls with seawater filtered through fine glass wool. Filtration through glass wool was a rapid process and it was also effective to eliminate most microorganisms. The bowls were placed under bright light supplied from a pair of 40 W fluorescent bulbs.

Five artificial foods and laboratory-hatched brine shrimp, *Artemia* nauplii, were tested. The constituents of the five prepared foods are provided in Table 1.

The artificial foods were stored in the refrigerator to help preserve their qualities.

Pellets of the prepared foods were broken into small pieces with a mortar and pestle. Food particles were uniformly sized to eliminate any possibility of the influence of particle size on the animals' food selection. Particles of sizes between .0059 and .007 inch were

TABLE 1.--Approximate percentage of constituents of the prepared foods used in the food preference studies.

Constituents	Silver Cup Fish Feed[1]	Purina Trout Chow[2]	Purina Commercial Catfish Chow[2]	Purina Fish Chow[2]	Vio-Bin Fish Flour[2]
Crude protein	48*	40*	30*	35*	70
Crude fat	8*	2.5*	2.5*	2.5*	2.5*
Crude fiber	5**	5.5**	10	8**	0.5***
Ash	17**	13**	not known	not known	16***
Water	not known	not known	not known	not known	8

*Minimum %
**Maximum %
***Based on the analysis of deodorized fish flour from the same manufacturer.

[1] Murray Elevators
118 West 4800 South
Murray, Utah, U.S.A.

[2] Ralston Purina Co.
St. Louis, Missouri, U.S.A.

[3] Vio Bin Corporation
Monticello, Illinois, U.S.A.

separated out by using metallic sieves of U. S. Bureau of Standards Series. Particles of this size were chosen for the following characteristics:

1. suitability for easy manipulation by postlarval shrimps (8-12 mm);
2. visibility at magnifications (7.5 and 10 x) which permit visual observation of postlarval shrimp feeding behavior;
3. small enough for the animals to eat a number of them in a reasonably short time.

Sized food particles were kept in labeled vials in a refrigerator when not in use.

Only two types of food were tested at a time. To permit visual identification of food particles when mixed together, the two types of foods were stained with different U. S. certified food colors (The Kroger Co., Cincinnati, Ohio, U.S.A.).

The stained particles of each type of food were clearly distinguishable under a microscope. Two types of observation showed no influence of food colors on the animals' food selection. Particles of one type of food stained with different colors were selected with equal frequency. Moreover, the shrimp retained its preference for one type of food over the other even though the colors of the foods were mutually exchanged.

For staining, food particles were taken in 1 ml depressions of two labelled depression slides. Two or three drops of food coloring fluid were enough to stain a large number of particles. An hour or so was sufficient to stain the foods. Red and green colors were found most satisfactory because of their sharply contrasting hues. Immediately before using, the particles were washed in saline water to remove any excessive coloring fluid. Seawater of the same salinity and temperature as that in which the shrimp were held was kept at hand in the laboratory.

Artemia, which is too active to follow visually when alive, were killed by holding them in a freezer until the water was completely frozen. They were left in that condition until used. *Artemia*, so killed, could be stained only faintly and this faint color was immediately removed when the animal was washed in clear water. *Artemia*, was, therefore, used unstained but it could be easily distinguished from any other stained foods under the microscope.

For visual observations of their actual feeding behavior, the animals were confined in a sufficiently small area to be viewed in the field of a stereoscopic dissecting microscope at 7.5 magnifications. Specially designed transparent plastic cages were used for holding the animals (Figure 2). Each cage, about 20 mm long, 4 mm and 13 mm wide at the bottom and top, respectively, and 13 mm high

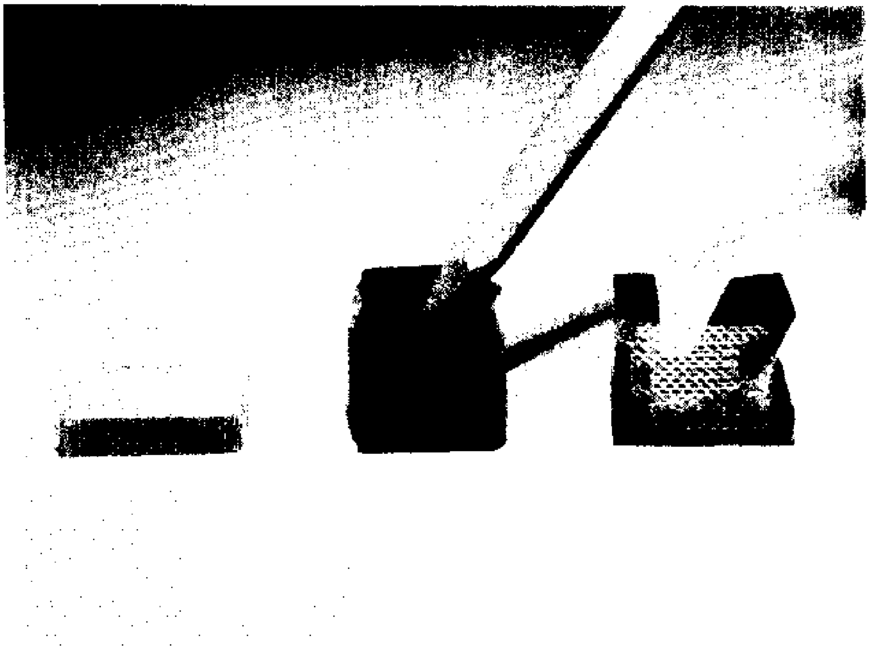


Figure 2. -- Holding cage top with front and side views of holding cage (left to right).

(inside measurements), was made of polyethylene mesh and 6 mm thick Plexiglas. The two side walls were made of polyethylene mesh which allowed free circulation of water but prevented the escape of small shrimp. The back wall was slightly slanting with the base closer to and the upper side away from the front wall. Thus, the floor of the cage was reduced to a narrow longitudinal strip which forced the shrimp to rest close and parallel to the front wall and the plane of focus. The side profile of the shrimp was thus clear and the feeding behavior could be clearly observed. The broader upper part of the cage facilitated the introduction of postlarvae. The outside of the back wall and the floor was painted in black so that the almost transparent whitish shrimp could be better seen against the dark background. In addition, the colors of food particles were also distinct against the black background when light was provided on the top and sides. Focusing microscope illuminators were used as a source of concentrated light. Each cage was provided with a transparent top.

The cages were placed in a Plexiglas tray 25 cm square and 5 cm high to provide the enclosed animals a large volume of water (Figure 3). Enough filtered seawater was put in the tray to nearly cover the cages. The water was gently aerated by an air stone. The water of the small cages was confluent with the constantly

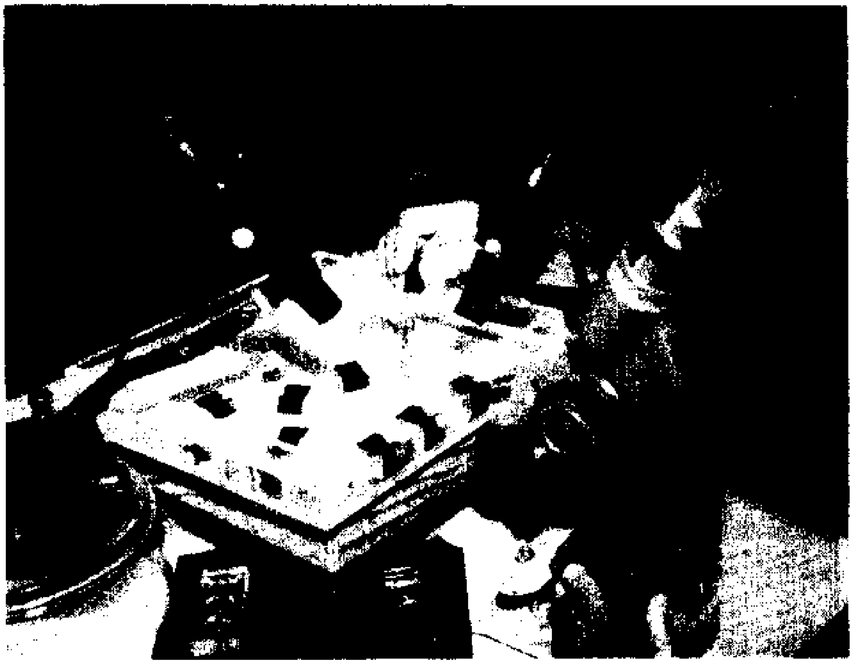
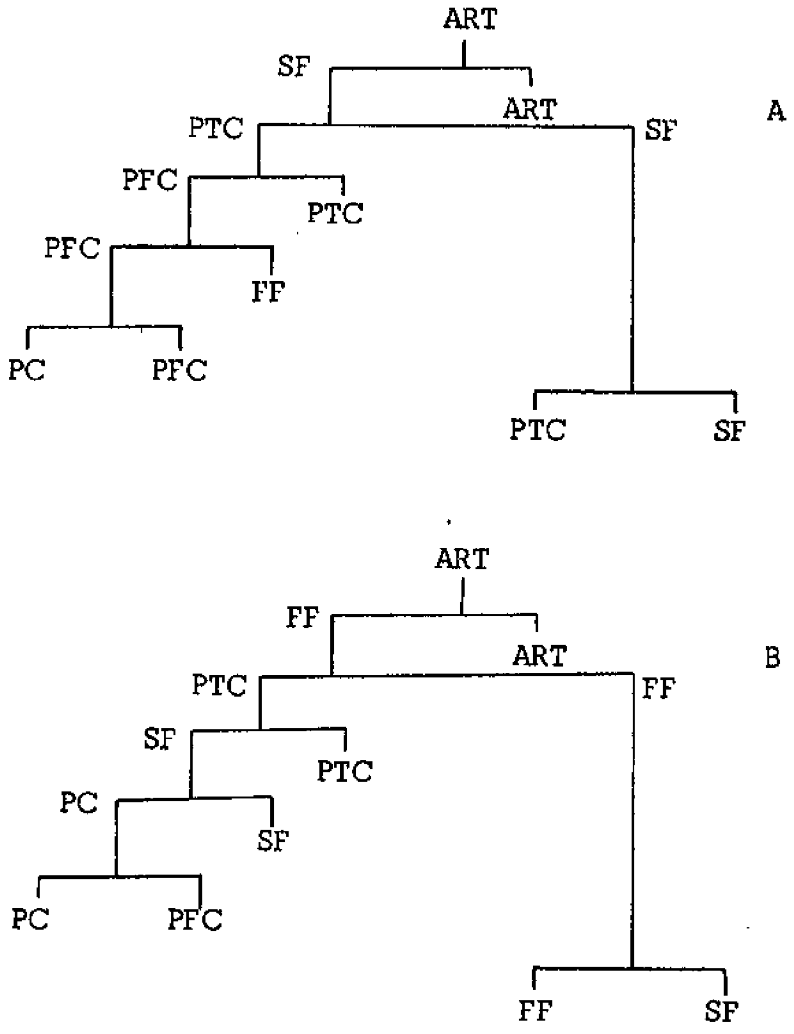


Figure 3. --Experimental set up for food preference studies.

aerated water of the tray so that oxygen and temperature would not become limiting to the experimental shrimp.

At the beginning of a food selection experiment, the starved test animals were transferred to these cages. The animals, one in each cage, were kept in the experimental conditions of light and limited space until no behavioral response to these conditions could be observed. This period of adjustment usually occupied 10 to 30 minutes. Sufficient numbers of stained food particles were then randomly distributed on the floor of the cage with an eye dropper, giving the animal an equal opportunity to reach both types of food at the same time. In the present work, only the initial preference of the animal was considered. For this purpose I noted the order of consumption of the first three particles by a shrimp. One pair of foods was tested at one time. Thirty shrimp were tested for each pair of foods. For evaluating the data, 1, 2 or 3 points were scored for third, second and first choices, respectively. The preferred member of the first pair of food was tested with the third food and so on, so that at the end of a series of tests the six types of foods could be arranged in order of preference shown by the shrimp (Figure 4).

Figure 4. --Sequence and qualitative results of food preference studies of postlarval *P. setiferus* (A) and *P. aztecus* (B).



ART = *Artemia*
FF = *Vio Bin* Fish Flour
PC = Purina Commercial Catfish Chow
PFC = Purina Fish Chow
PTC = Purina Trout Chow
SF = Silver Cup Fish Feed

Results

In the food preference studies of *P. setiferus*, *Artemia* and Purina Commercial Catfish Chow were the most and least preferred diets, respectively (Figure 4). Among the prepared foods, Silver Cup Fish Feed was found the most preferred.

In the case of *P. aztecus*, the most and least preferred diets were *Artemia* and Purina Fish Chow, respectively, and Vio Bin Fish Flour ranked first among the prepared foods.

The overall ratings of the six types of diets on the basis of the preference of the two species of shrimps are presented in Table 2.

Three pairings of foods (Purina Commercial Catfish Chow vs. Purina Fish Chow; Purina Trout Chow vs. Silver Cup Fish Feed, and the most preferred artificial food vs. *Artemia*) were common in the feeding tests between the two species of shrimp. Comparison of data on these three pairs of diets indicates stronger food preference by *P. setiferus* than *P. aztecus* (Table 2).

TABLE 2.--Qualitative and quantitative food preference of P. setiferus and P. aztecus

Species	Pairing of food	Total first particles		Total second particles		Total third particles		Total of all particles		Score of all particles	
P. setiferus	PC vs PFC	23	7	20	10	20	10	63	27	129	51
	PFC vs FF	21	9	19	11	22	8	62	28	123	57
	PTC vs PFC	22	8	21	9	22	8	65	25	130	50
	PTC vs SF	20	10	17	13	17	13	54	36	111	69
	ART vs SF	24	6	17	13	24	6	65	25	130	50
P. aztecus	PC vs PFC	20	10	12	18	9	21	41	49	93	87
	SF vs PC	17	13	20	10	18	12	55	35	109	71
	PTC vs SF	18	12	9	21	19	11	46	44	92	88
	FF vs PTC	15	15	20	10	18	12	53	37	103	77
	ART vs FF	19	11	17	13	19	11	55	35	110	70

ART = *Artemia*; FF = Vio Bin Fish Flour; PC = Purina Commercial Catfish Chow; PFC = Purina Fish Chow; PTC = Purina Trout Chow; SF = Silver Cup Fish Feed

SURVIVAL, GROWTH AND TEMPERATURE RESISTANCE STUDIES

Procedure

Survival and Growth Studies

In these experiments I attempted to use animals similar in size to those used in the food preference studies (*P. setiferus*, 8.1 to 11.5 mm; *P. aztecus*, 11.4 to 12.6 mm). The animals were held in the laboratory in aerated water of salinity approximately 25‰ and 24 C for about 26 hours prior to use to allow the animals to overcome any shock of handling. All sick or weak postlarvae were removed.

From each laboratory population of about 300-400 postlarval shrimp, an initial random sample of 30 animals was taken to obtain a record of the initial length and weight. For determining length (distance from tip of rostrum to end of telson), the postlarvae were measured under a stereoscopic microscope at 7.5 x with a metric ruler graduated in millimeters. Length was estimated to the nearest 0.1 mm and weight was determined to the nearest 0.1 mg. For weighing the postlarvae, an analytical balance (Sartorius Selectra Rapid, Germany) with 100 gm capacity and 0.1 mg sensitivity was used. Similar random samples of 30 shrimp each were placed in a series of 9-liter fish bowls under identical conditions (excepting diet) for 4-week survival and growth studies. Through a mistake, the bowls of P. setiferus receiving one diet (Artemia), contained 35 shrimp each. Each experiment was replicated once.

The substrate in the bowl was a 1-inch-thick layer of thoroughly washed fine beach sand. Filtered seawater was used for initial filling and for all subsequent replacements. A large polyethylene barrel of filtered seawater of the desired salinity and temperature (25‰ and 24 C, respectively) was maintained in the laboratory to provide a common water supply for growth experiments. In each aquarium, the surface of the water was covered with a thin polyethylene film as a preventive measure against any loss of shrimp through escape. Gentle aeration was provided through an air stone.

For feeding the shrimps in the growth studies, big pellets of the various artificial foods were crushed and particles of sizes between 0.0117 and 0.0234 inch were sieved out. Particles of these sizes were handled by the animals easily. Smaller particles than these had been found to bring about fouling of bowl water.

A constant supply of Artemia nauplii was maintained for shrimp receiving this diet. Freshly hatched nauplii were filtered from the hatching container with a piece of fine nylon net. The nauplii were then thoroughly washed with clean water to remove any poisonous metabolites produced during hatching of the eggs.

At feeding, each washed artificial food was carefully pipetted onto a plastic petri dish on the floor of the appropriate bowl. Some food particles were also scattered outside the petri dish to attract the animals to the main source of food.

In the growth studies of the shrimps, foods were supplied three times a day, once between 8:00 and 10:00 AM, once at about 5:00 PM and the last meal was given between 10:00 and 11:00 PM. Any left-over food was pipetted out before morning and night feedings to avoid bacterial fouling of the water. The water removed in this process was carefully checked for any shrimp that might have been removed with the food particles. Cleaning of the left-over food from each bowl was always accompanied with the removal of at least 500 ml of water each time. This was immediately replaced with an equal volume of clean water.

Light was provided with a pair of overhead 40 W fluorescent tubes about 10 feet away from the bowls. Lights were continuously on from 8:00 AM to 5:00 PM and again at the evening feeding time. At other times, the lights were off.

The experimental shrimps were kept under frequent observation. Any death or cannibalism in the postlarvae was noted.

The growth and survival experiments with *P. setiferus* were started on August 1, 1969. Diets tested included the most preferred diet (*Artemia*) and the least and most preferred artificial diets, as determined in the food preference experiment (Figure 4). The latter two diets were Purina Commercial Catfish Chow and Silver Cup Fish Feed, respectively.

In addition to the regular partial renewal of water, three almost complete changes of water were provided in the case of white shrimp. Water of each bowl was changed on the 16th, 19th and 23rd day of the experiment. These changes were made in an attempt to alleviate the cause of mortality in tanks containing shrimp fed Silver Cup Fish Feed and to standardize the water condition in all tanks.

The growth and survival experiments of *P. aztecus* were started on March 11, 1970. As with *P. setiferus*, diets tested for *P. aztecus* included the most and least preferred foods. These were *Artemia* and Purina Fish Chow (Figure 4). To permit further comparison of the two shrimps' responses to diets, the least preferred and most preferred artificial diets for *P. setiferus* (Purina Commercial Catfish Chow and Silver Cup Fish Feed) were also included.

Temperature Resistance Studies

To test for possible effects of diet on temperature resistance of shrimp, animals were sampled from each tank and subjected to a lethally high temperature on the 15th and 25th day of the experiments. On the 15th day, 10 animals and on the 25th day all survivors from each tank were exposed to lethal temperature. The lethal temperature used in the case of *P. setiferus* was 38.6 C and that for *P. aztecus*, 37 C. A preliminary study suggested that these temperatures would yield reasonably comparable ranges of survival times for the two species, and thus facilitate interspecies comparisons of dietary influence on temperature resistance.

During exposure to lethal temperature, the tiny transparent shrimps were individually contained in small specially designed cages to permit systematic observation of all members of each test group. The cages, made of Plexiglas and polyethylene mesh, had a detachable top. The two sides of each cage were made of polyethylene mesh which allowed free circulation of water through the cage. The internal dimensions of the cages were 2.5 to 6.0 cm long, 1.3 to 3.5 cm wide and 2.5 to 4.0 cm tall.

The lethal temperature baths consisted of a series of plastic-painted wooden tanks, each 42 cm long, 25 cm wide and 30 cm tall and with a capacity of 30 liters. The front wall and the removable top were made of transparent plexiglas. The transparent front wall allowed clear observation of the shrimp in the cages arranged near the front of the tank (Figure 5). The water (salinity = 25‰) in each tank was preheated to the selected test temperatures and maintained at that temperature by a thermoregulator and a 50 W heater. A thermometer (directly readable to 0.1 C) was used to check the tank temperatures. Vigorous aeration through two air stones kept the water circulating to provide a uniform temperature throughout the tank.

Heat resistance of the shrimps fed different diets was determined by placing the cages with the animals in the lethal temperature tanks and measuring the death time of each shrimp to the nearest 0.1 minute. Complete cessation of the movements of all body parts including the movements of scaphognathites, stomach and intestine were taken as the criteria for death. Length and weight of each shrimp were determined soon after death.

The survival and growth studies were originally intended for a 4-week period, but due to rapid mortality, the numbers of white shrimp in two of the replicate bowls (Silver Cup Fish Feed) were reduced to 9 and 12 on the 25th day of the experiment. Since at least 20 animals on each diet were desired for the temperature

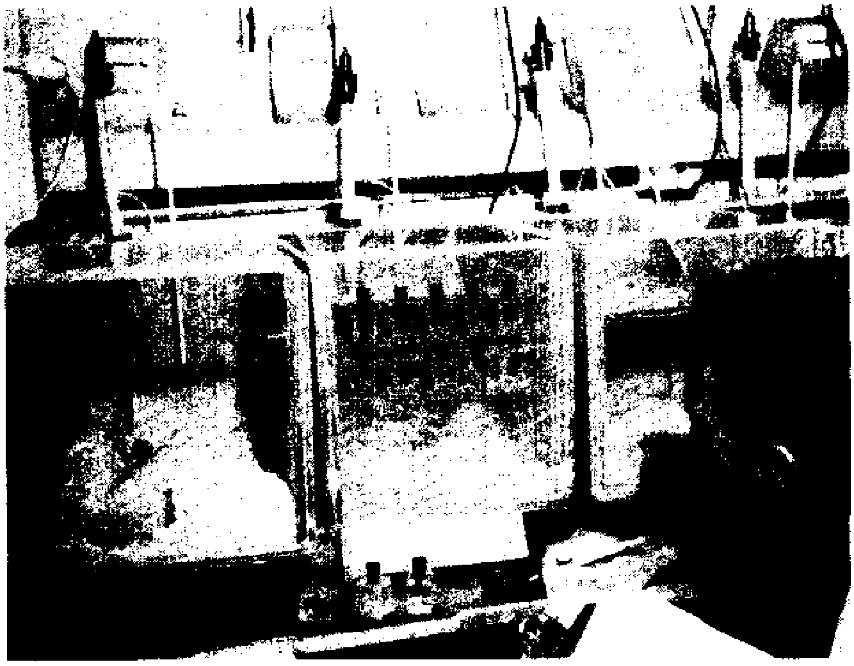


Figure 5. --Experimental set up for temperature resistance studies of postlarval P. setiferus and P. aztecus fed various diets.

resistance test, the growth experiment was terminated on the 25th day.

For comparison of data, the growth study of brown shrimp was also discontinued after 25 days.

The survivors of all the groups of _P. setiferus_ were subjected to the same lethal temperature (38.6 C) as used in the 15th day experiment. But _P. aztecus_ were tested with a slightly elevated temperature. The lethal temperature (37 C) selected through a preliminary study was found unsuitable in the 15th day temperature experiment. For the second exposure, the lethal temperature used was 37.3 C to further reduce the range of death times.

Results

Survival

Excellent survival for _P. setiferus_ occurred in all the experimental groups except in those receiving Silver Cup Fish Feed (Table 3). In the latter case, regular mortalities were noted from the 15th day onward during the experiment. Water changes on the 16th, 18th and 23rd day did not bring about any change in the mortality rate.

TABLE 3.--Survival and mortality of postlarval P. setiferus fed different diets

Elapsed time (days)	Silver Cup Fish Feed		Purina Commercial Catfish Chow		Artemia	
	Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2
6			1[1]			
12		1				
15	1	2				
16[2]		1				
17		1[1]				
18[2]		1[1]				
19	1					
20		1				
22		1				
23[2]		1				
24	2	1				
25	1					

TABLE 3.--Survival and mortality of postlarval P. setiferus fed different diets (continued)

Elapsed time (days)	Silver Cup Fish Feed		Purina Commercial Catfish Chow		Artemia	
	Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2
Observed mortalities	5	10	1	0	0	0
Unaccounted for mortalities	3	1	1	0	2	0
Total mortality	8	11	2	0	2	0
Total survival (%)	53		95		96	

[1] cannibalism noticed

[2] water changed

Increased population pressure, at least to the given extent, 35 vs. 30 animals, in the bowls receiving Artemia as food seemed to have no adverse effects on survival of white shrimp postlarvae.

In P. aztecus, survival in all tanks was excellent (Table 4). No comparable mortality, as in P. setiferus which were fed Silver Cup Fish Feed, was noticed in P. aztecus. The survival percentage (90%) in this species fed Silver Cup Fish Feed perhaps could have been still better if two shrimp were not lost through accident.

Overall survival in the bowls receiving Artemia as diet was 88%, slightly less than that for other groups. But the case of at least three out of five mortalities in this group of shrimp was escape from the tank. Had there been no such escape mortality, the possibility of better survival with this food would have been increased.

Growth

The effect of diet on growth of postlarval P. setiferus and P. aztecus was quite marked. Significantly higher growth was noted with Artemia than with any other foods used in the experiments (Figures 6, 7, 8; Tables 5, 6, 7).

By the 25th day of the experiment, the mean length and weight of P. setiferus fed Artemia respectively increased 160% and 1957% over the initial length (9.2 mm) and weight (4.2 mg). The final length and

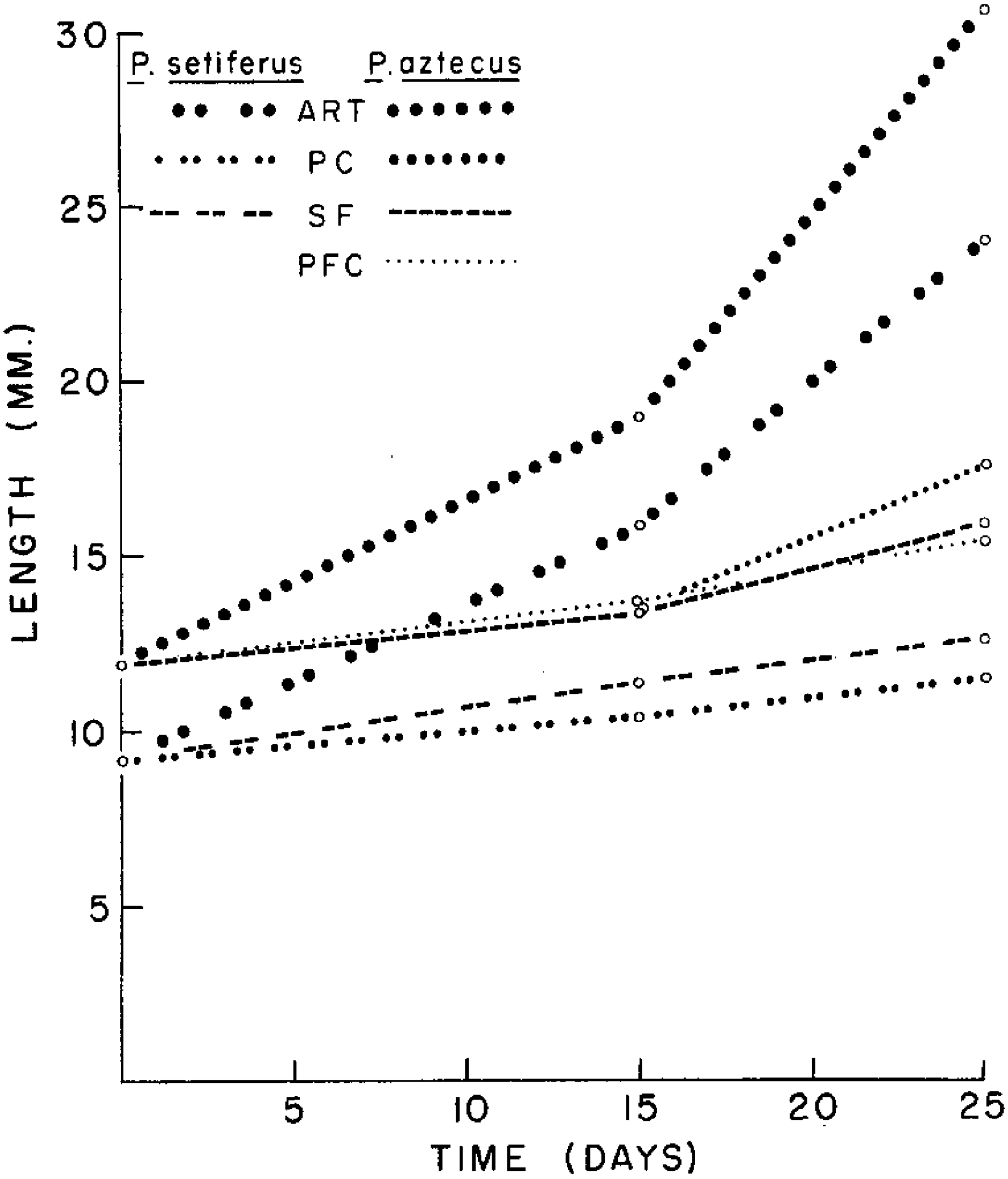


Figure 6. -- Length increase of postlarval *P. setiferus* and *P. aztecus* fed various diets.

ART = *Artemia*
PC = Purina Commercial Catfish Chow
PFC = Purina Fish Chow
SF = Silver Cup Fish Feed

TABLE 4.--Survival and mortality of postlarval P. aztecus fed different diets

Elapsed time (days)	Silver Cup Fish Feed		Purina Commercial Catfish Chow		Purina Fish Chow		Artemia	
	Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2
5								1[1]
8				1[1]				1[1]
9								1
16[2]		2[3]						
17								1[1]
Observed mortalities	0	2	0	1	0	0	0	4
Unaccounted for mortalities	2	-	3	0	2	0	1	0
Total mortalities	2	2	3	1	2	0	1	4
Total survival (%)	90(95)[4]		90(93)[4]		95		88(95)[4]	

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[1] escaped through the polyethylene film

[2] water changed

[3] accidentally killed during water change

[4] survival figures in parentheses indicate survival as calculated excluding mortality due to accident

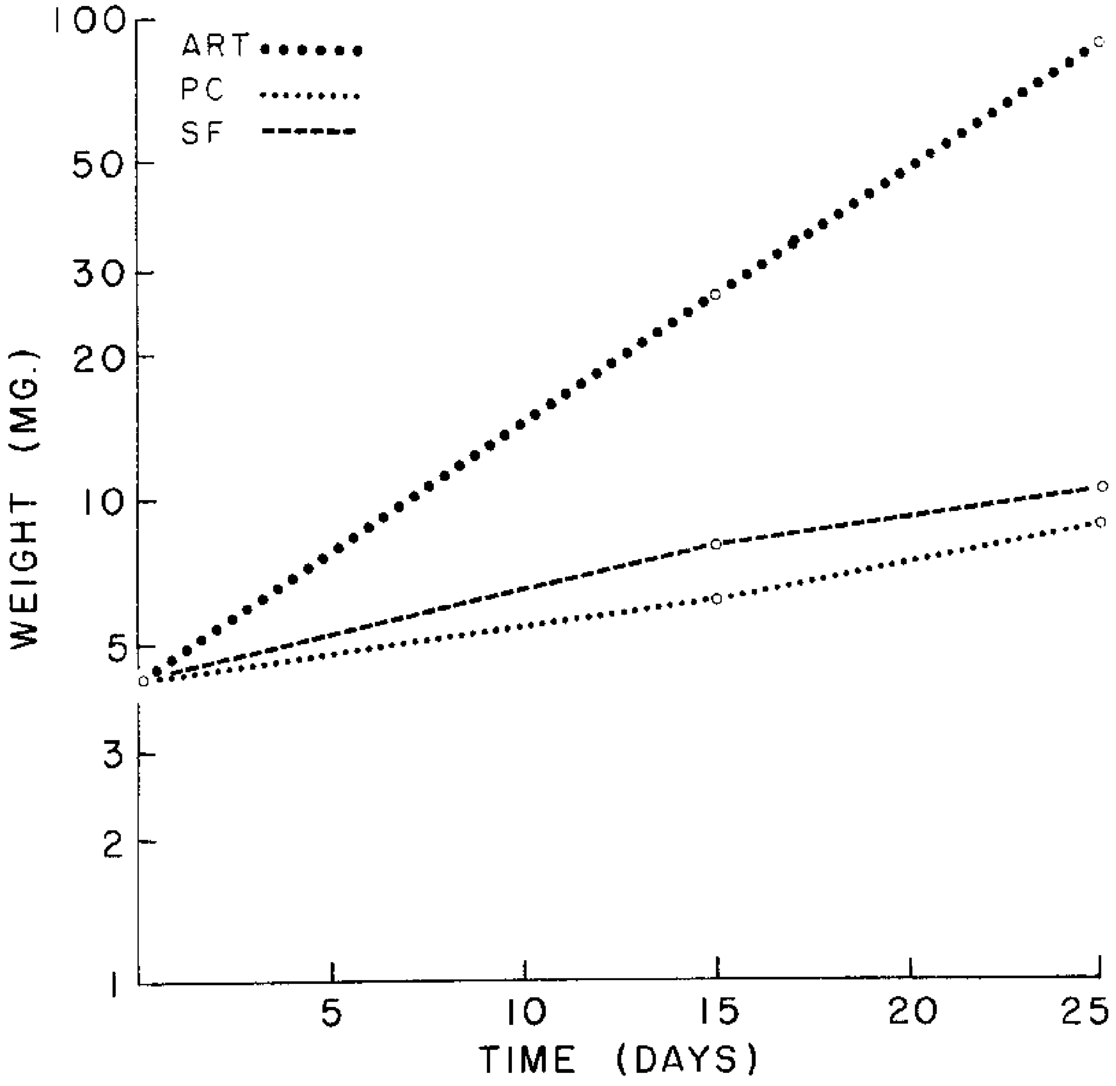


Figure 7. -- Weight increase of *P. setiferus* fed various diets.

ART = *Artemia*
PC = Purina Commercial Catfish Chow
SF = Silver Cup Fish Feed

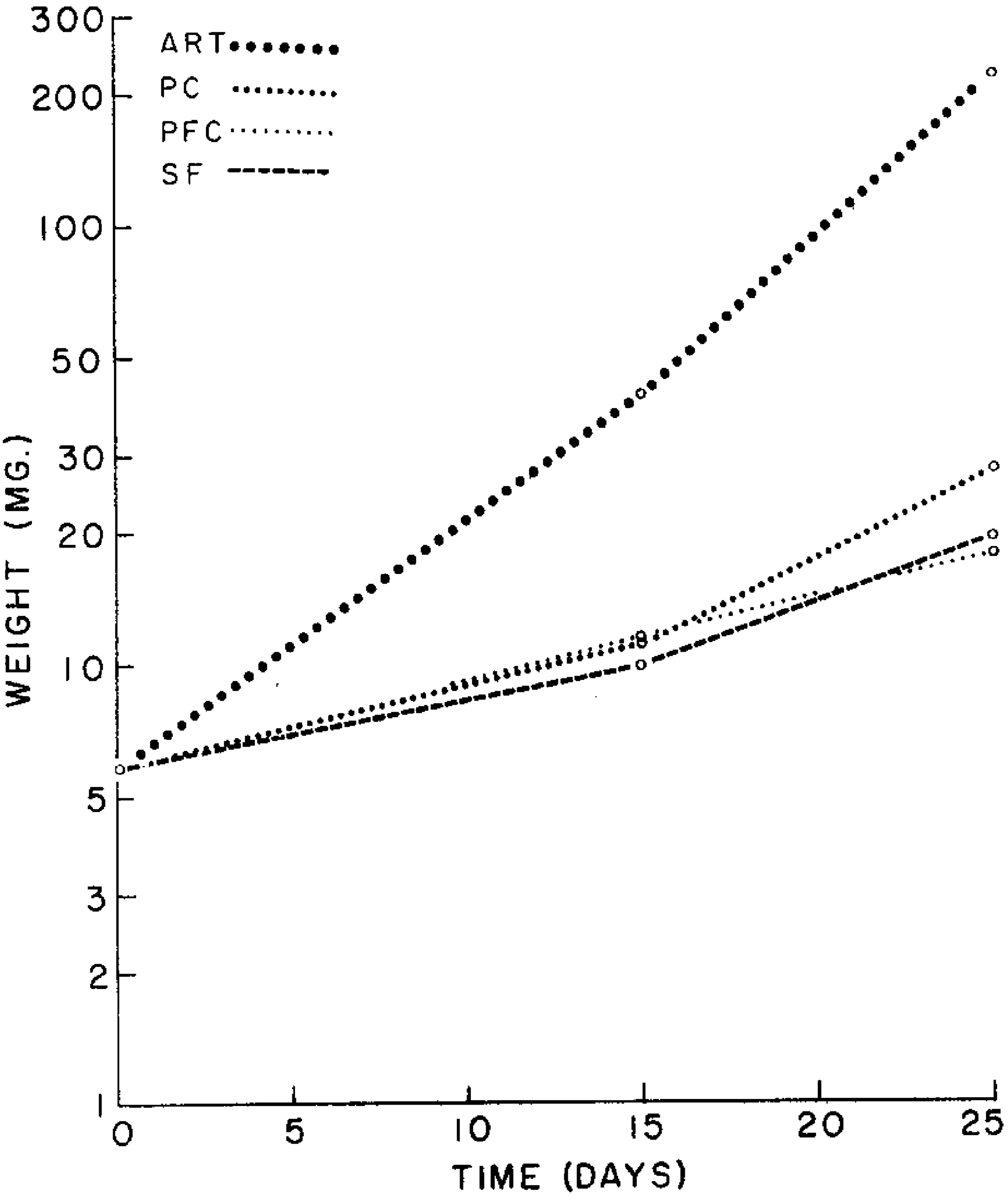


Figure 8. -- Weight increase of P. aztecus fed various diets

ART = Artemia
PC = Purina Commercial catfish chow
PFC = Purina Fish Chow
SF = Silver Cup Fish Feed

TABLE 5.--Mean lengths and weights of postlarval P. setiferus and P. aztecus fed various diets

Species	Elapsed time (days)	Silver Cup Fish Feed		Purina Commercial Catfish Chow		*Artemia*		Purina Fish Chow	
		length (mm)	weight (mg)	length (mm)	weight (mg)	length (mm)	weight (mg)	length (mm)	weight (mg)
P. setiferus	0	9.2	4.2	9.2	4.2	9.2	4.2	-	-
	15	11.4	8.0	10.4	6.1	15.9	26.3	-	-
	25	12.7	10.5	11.6	8.9	24.0	86.4	-	-
P. aztecus	0	12.0	5.7	12.0	5.7	12.0	5.7	12.0	5.7
	15	13.4	9.9	13.6	11.0	19.0	41.0	13.5	11.4
	25	15.9	19.4	17.5	27.5	30.6	222.4	15.4	17.7

TABLE 6.--Mean lengths (mm) and weights (mg) of replicate groups of postlarval P. setiferus fed various diets

Elapsed time (days)		Silver Cup Fish Feed		Purina Commercial Catfish Chow		*Artemia*	
		Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2
0	Mean length	9.2	9.2	9.2	9.2	9.2	9.2
	Overall mean length	9.2		9.2		9.2	
	Mean weight	4.2	4.2	4.2	4.2	4.2	4.2
	Overall mean weight	4.2		4.2		4.2	
15	Mean length	11.1	11.6	10.6	10.2	15.4	16.3
	Overall mean length	11.4		10.4		15.9	
	Mean weight	7.6	8.3	6.3	5.8	23.8	28.8
	Overall mean weight	8.0		6.1		26.3	
25	Mean length	13.3	11.9	11.9	11.3	22.9	25.2
	Overall mean length	12.6		11.6		24.1	
	Mean weight	11.3	9.7	9.8	8.6	76.0	106.3
	Overall mean weight	10.5		8.9		86.4	

Table 7.--Mean lengths (mm) and weights (mg) of replicate groups of postlarval P. aztecus fed various diets

Elapsed time (days)		Silver Cup Fish Feed		Purina Commercial Catfish Chow		Purina Fish Chow		Artemia	
		Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2	Tank 1	Tank 2
0	Mean length	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
	Overall mean length	12.0		12.0		12.0		12.0	
	Mean weight	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
	Overall mean weight	5.7		5.7		5.7		5.7	
15	Mean length	13.4	13.5	13.4	13.9	13.5	13.5	18.8	19.2
	Overall mean length	13.5		13.6		13.5		19.0	
	Mean weight	9.8	10.0	10.0	11.9	11.8	11.0	40.0	42.0
	Overall mean weight	9.9		11.0		11.4		41.0	
25	Mean length	15.8	16.0	17.5	17.5	15.5	15.3	29.7	31.5
	Overall mean length	15.9		17.5		15.4		30.6	
	Mean weight	18.9	19.9	26.6	28.4	18.1	17.2	206.5	238.3
	Overall mean weight	19.4		27.5		17.7		222.4	

weight in this group of shrimp were at least 85% and 723%, respectively, more than with either Silver Cup Fish Feed or Purina Catfish Chow. Growth of Artemia-fed shrimp was very significantly different (t-test) from that of animals receiving the other diets ($P < 1\%$). This result is even more striking in view of the fact that population pressure was higher among the Artemia-fed groups than among those shrimp fed other diets (35 vs. 30 per group). Growth in the two groups of shrimp fed artificial diets was similar.

Already by the 15th day, the growth of Artemia-fed shrimp was markedly different from that of the other groups. The fast growth rate of this batch of animals continued throughout the experiment, accounting for a daily mean increase of 0.6 mm in length and 3.3 mg in weight.

As for P. setiferus, Artemia also yielded the maximum growth in P. aztecus. In the 25 days, the average shrimp gained 19.0 mm in length, nearly 155% increase over the initial mean length (11.99 mm). This growth rate is only half that observed for P. aztecus in nature (Williams, 1955; St. Amant et al., 1963). The final mean length (30.6 mm) for Artemia-fed shrimp, was 76% higher than that (17.5) for the next most effective food (Purina Commercial Catfish Chow). In keeping with this result, weight gain in P. aztecus with Artemia was also very pronounced. The mean weight (222.4 mg) of this group of shrimp was 722% higher than that (27.5 mg) obtained with Purina Commercial Catfish Chow and 3800% higher than the initial weight of the group (5.7 mg).

The growth on all the three artificial diets was quite similar in the first 2 weeks. On the 25th day, both length and weight of shrimp fed Purina Commercial Catfish Chow were significantly ($P < 1\%$) higher than those fed any other artificial diet.

Temperature Resistance

P. setiferus postlarvae held under identical experimental conditions, but receiving different diets had somewhat different survival times when subjected to lethally high temperature (Figures 9, 10; Table 8). Survival times of postlarval *P. aztecus*, on the other hand, were similar in all the four diet groups (Figures 11, 12; Table 8).

Results of the 15th day and 25th day test of *P. setiferus* were similar: postlarvae supplied with Silver Cup Fish Feed had the least resistance to the experimental temperature (38.6 C). In the first 10 minutes of the heat exposure 80%-90% of these shrimp died (Figures 9, 10), and the mean survival time was less than 20 minutes. The survival times of the other two groups of shrimp were much longer (means > 50 minutes). In the latter two groups, 80%-95% of the shrimp survived the lethal temperature for more than 20 minutes. The

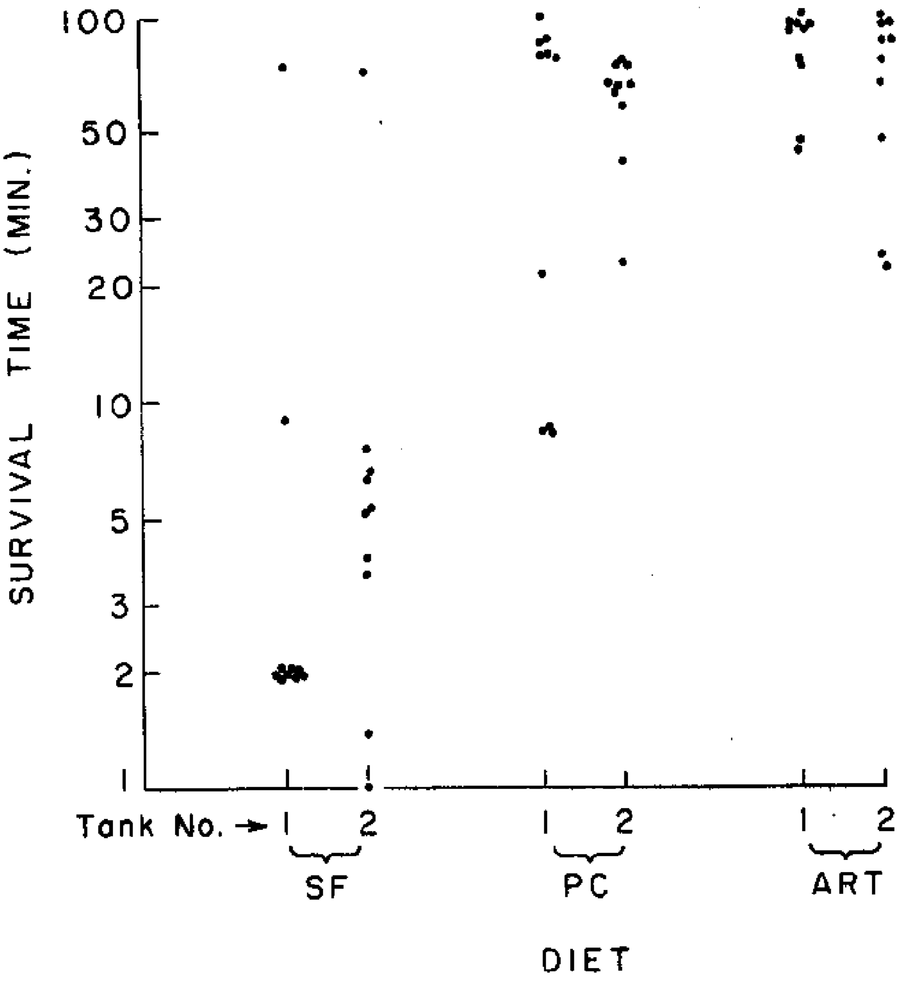


Figure 9. -- Thermal resistance of postlarval *P. setiferus* fed various diets for 15 days.

SF = Silver Cup Fish Feed
PC = Purina Commercial Catfish Chow
ART = *Artemia*

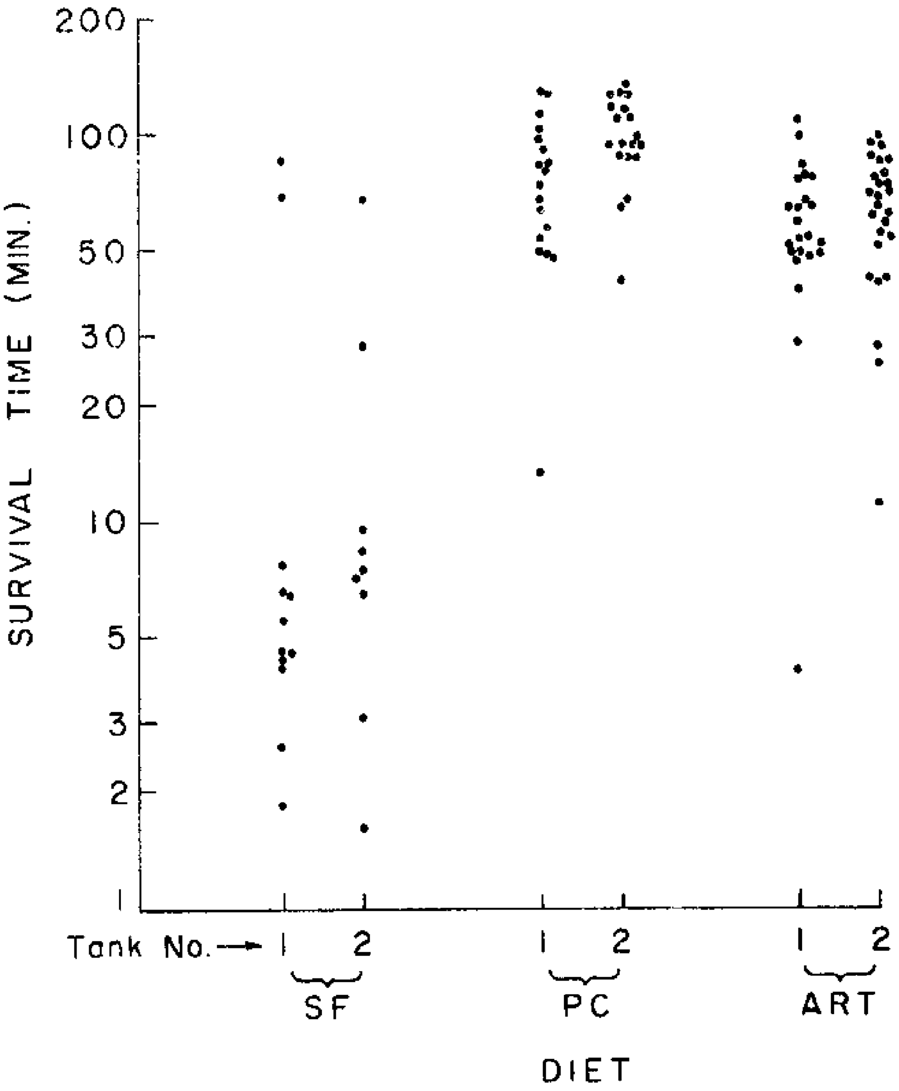
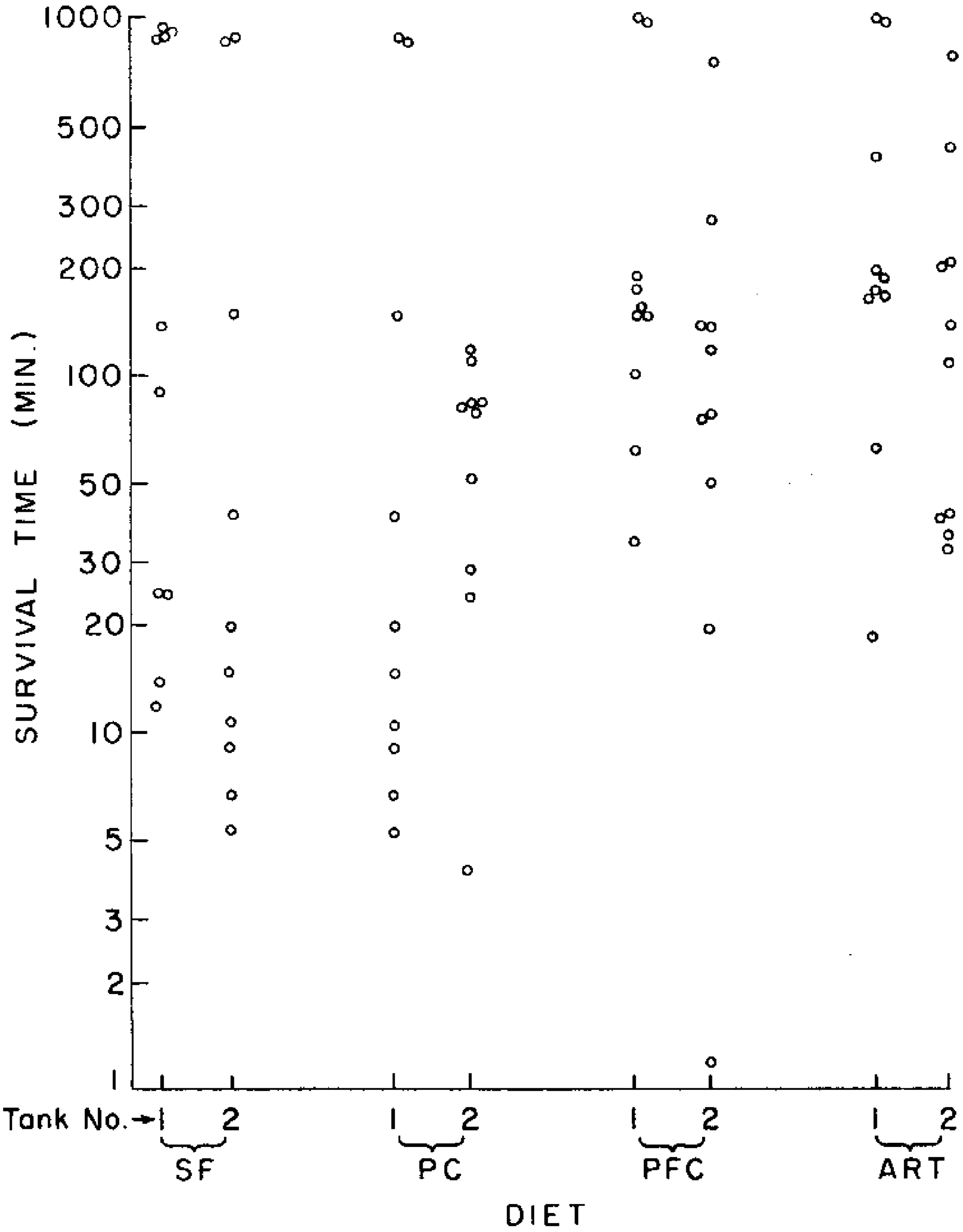


Figure 10. -- Thermal resistance of postlarval *P. setiferus* fed various diets for 25 days.
SF = Silver Cup Fish Feed
PC = Purina Commercial Catfish Chow
ART = *Artemia*

Figure 11. --Thermal resistance of postlarval P. aztecus fed various diets for 15 days.



SF = Silver Cup Fish Feed
PC = Purina Commercial Catfish Chow
PFC = Purina Fish Chow
ART = *Artemia*

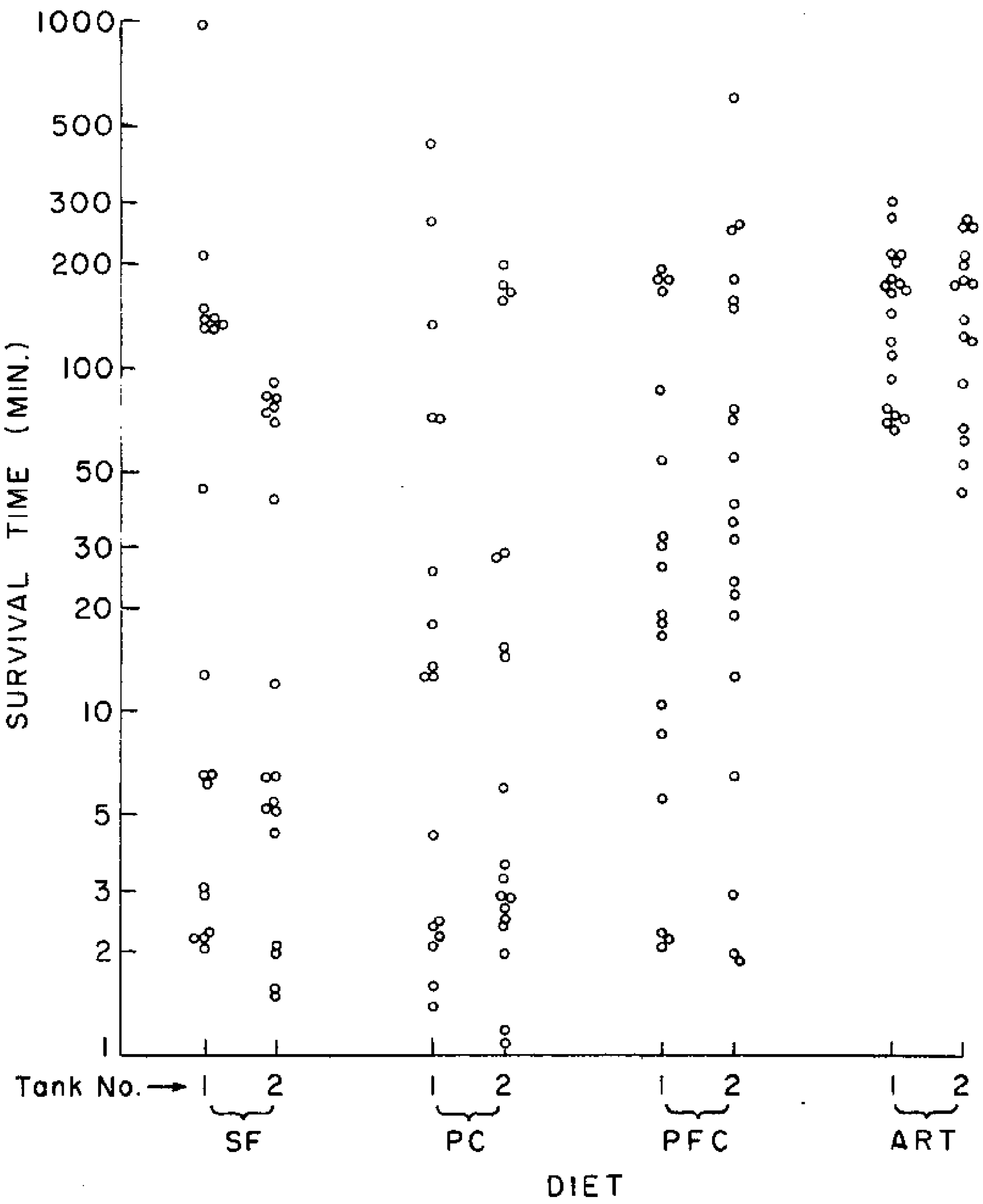


Figure 12. -- Thermal resistance of postlarval <u>P. setiferus</u> fed various diets for 25 days.

SF = Silver Cup Fish Feed
PC = Purina Commercial Catfish Chow
ART = <u>Artemia</u>
PFC = Purina Fish Chow

TABLE 8.--Mean and range of thermal resistance times (minutes) of postlarval P. setiferus and P. aztecus fed various diets

Diets	15th day				25th day			
	P. setiferus 38.6°C[1]		P. aztecus 37.0°C[1]		P. setiferus 38.6°C[1]		P. aztecus 37.3°C[1]	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
ART	76.7	(24.4-100.2)	228.4	(18.7-993.2)	61.3	(4.2-105.5)	141.9	(44.3-310.4)
SF	10.9	(2.0-82.6)	265.3	(0.4-921.5)	16.6	(1.6-82.6)	77.9	(1.5-998.2)
PC	54.3	(2.5-89.8)	130.4	(4.5-858.4)	84.4	(13.3-132.2)	54.8	(1.1-452.3)
PFC	-	-	227.6	(1.8-988.2)	-	-	77.8	(1.9-610.6)

[1] lethal temperature

ART = Artemia; SF = Silver Cup Fish Feed; PC = Purina Commercial Catfish Chow; PFC = Purina Fish Chow survival times of each of these groups were significantly higher ($P<1\%$) than those of Silver Cup Fish Feed group shrimp (Table 9).

Significant difference ($P<1\%$) was also noted between the survival times of the two groups of shrimp fed Purina Commercial Catfish Chow and *Artemia* with a higher mean value (84 minutes vs. 61 minutes) for the former on the final experiment day. But on the 15th day, the difference between these groups was smaller and less significant ($1\%<P<5\%$) (Table 9) and the longer survival time was observed in the *Artemia* group (76 minutes vs. 54 minutes) of shrimp. Analysis of variance between replicates of each group yielded no significant difference ($P>5\%$).

A preliminary experiment suggested that a lethal temperature of 37 C for *P. aztecus* would yield ranges of survival times reasonably comparable to those of *P. setiferus* at 38.6 C, but the 15th day temperature experiment with *P. aztecus* revealed that many of the brown shrimp survived much longer than the white shrimp at the respective lethal temperatures. While all of the white shrimp died within 150 minutes (except one individual that lived slightly longer) at 38.6 C, many of the brown shrimp survived beyond 150 minutes at 37 C. To reduce further the survival range of this species, a higher temperature of 37.3 C was used for the final experiment. This elevated temperature reduced the survival times of the brown shrimp, but not

TABLE 9.--Results of statistical analyses of thermal resistance times for postlarval P. setiferus fed different diets. Significance of differences within and between experimental diet groups

Diet	15th day		25th day	
	Difference between replicates within diets	Difference between diets	Difference between replicates within diets	Difference between diets
SF	NS		NS	
		$P < 1\%$		$P < 1\%$
PC	NS	$P < 1\%$	NS	$P < 1\%$
		$1\% < P < 5\%$		$P < 1\%$
ART	NS		NS	

ART = *Artemia*; NS = difference not significant at 5% level;
PC = Purina Commercial Catfish Chow; SF = Silver Cup Fish Feed

sufficiently to make mean death times comparable with those of the white shrimp.

Interspecies comparisons of dietary influence on temperature resistance was not possible. However, the range of survival times of P. aztecus was greater than that of white shrimp (Table 8; Figures 9, 10, 11, 12). The early and late mortalities of Silver Cup Fish Feed and Purina Commercial Catfish Chow fed P. aztecus were earlier and later, respectively, than those of P. setiferus fed the same diets.

When exposed to a lethal temperature, the response of postlarval P. aztecus differed from that of P. setiferus in that no significant difference was noticed between the survival times of shrimp from any two of the experimental diet groups or between any replicate groups of a given diet, except for two instances. Statistical comparisons (Table 10) of survival yielded significant difference ($1\% < P < 5\%$) between shrimp of two diet groups, Artemia and Purina Fish Chow on the 15th day and between the replicates of Artemia-fed shrimp on the 25th day experiments.

Close examination of P. aztecus resistance times suggests clustering of survival times at different intervals. Such grouping of survival times was not noted for P. setiferus.

TABLE 10.--Results of statistical analyses of thermal resistance times for postlarval *P. aztecus* fed different diets. Significance of differences within and between experimental diet groups

Diet	15th day: Difference between replicates within diets	15th day: Difference between diets				25th day: Difference between replicates within diets	25th day: Difference between diets			
SF	NS					NS				
		NS					NS			
PC	NS		NS			NS		NS		
		NS					NS			
ART	NS			NS		$1\% < P < 5\%$			NS	
		$1\% < P < 5\%$			NS		NS			NS
PFC	NS					NS				

ART = *Artemia*; NS = difference not significant at 5% level;
PC = Purina Commercial Catfish Chow; PFC = Purina Fish Chow;
SF = Silver Cup Fish Feed

DISCUSSION

Food Preference

Food supply information is very important to a fisheries biologist, for it may explain such characteristics of a species as differential distribution in different geographical areas, seasonal or diurnal variations in their abundance in a particular area; their growth and resistance to environmental factors. If any of the environmental factors is not limiting, the animals tend to concentrate in an area where they find abundance of preferred foods.

The literature review (p. 3-14) suggests that shrimp can utilize various sources of food so that fluctuations in any one or the other kind of food may not have any severe effect on the animal. Sanders (1963) comments that a species should evolve to feed on as wide a scope of foods as possible. If it is tied too rigidly to a single prey or a single source of food, and its food supply fluctuates, this animal's population level also must fluctuate. Ray (1963) also maintains that animals which are highly selective of their food and consistently eat one kind of thing only have a lesser survival chance and a narrower range of distribution in nature than animals having a great diversity in the ability to eat and utilize different kinds of food. It is of interest to consider the possible significance of the present findings in relation to these principles.

My laboratory studies under controlled conditions revealed three important points: first, shrimp select food when given options (Table 2); second, P. setiferus is far more selective of food than P. aztecus; third, food preference of these two species of shrimp differ somewhat. These findings suggest an explanation for differences in the distribution and abundance of these two closely related shrimps.

Catch statistics (1959-1963) compiled and analyzed by Osborn et al. (1969) of the U. S. Bureau of Commercial Fisheries (USBCF) suggest that in the Gulf of Mexico the area of moderate or greater abundance for P. aztecus is more than twice that for P. setiferus. (Moderate or greater abundance is arbitrarily defined here as indicated by annual catches of at least 240,000 lbs of P. setiferus or 291,000 lbs of P. aztecus per USBCF statistical area depth zone unit. These values were chosen in order to compare the most nearly equal catch categories supplied by the USBCF for the two species.) During the statistical period mentioned, the average annual landings of brown and white shrimp were 87,000,000 and 43,000,000 lbs, respectively, accounting for 52% and 26% of the total catch of shrimp from the Gulf of Mexico.

Osborn, Maghan and Drummond illustrated that the moderate and higher abundance zone for P. aztecus was continuous from the continental shelf of Alabama around the western Gulf of Mexico to northern Mexico. A smaller zone exists near the Yucatan Peninsula. On the other hand, P. setiferus in moderate and higher abundance levels was found in three separate areas: one off the northwest coast of Florida; one adjoining Alabama and Mississippi; and the other extended from the Mississippi River delta to Padre Island of Texas.

The greater abundance of P. aztecus in wider geographical range in the Gulf of Mexico may be due to the fact that this species of shrimp is not as selective of foods as is P. setiferus. Although no natural diets were tested in the food preference studies, the results clearly suggest a stronger taste sense in P. setiferus than in P. aztecus. Since P. aztecus is not particularly selective of the diets tested, food may be less limiting to their distribution, survival and multiplication than it is for P. setiferus. Thus the weaker taste sense of P. aztecus may have adaptive value for this species, enhancing its chance of survival in diversified conditions of food. In contrast, the stronger taste sense of P. setiferus may delimit the abundance level of this species to areas only where food of its preference is available.

The results (Figure 4) indicate that the two species of shrimp, P. setiferus and P. aztecus, differed somewhat from each other in their food preference. Although Artemia was most preferred by both the species, the order of their preference for other diets did not coincide. The observed differential preference of these shrimps for the available diets under the laboratory conditions may also be true when these shrimps are exposed to natural foods in the marine environment. The differential preference for a food among different species of an area has great ecological significance. Through much of their ranges, P. setiferus and P. aztecus live in the same ecological environment. If laboratory studies are indicative of shrimp's natural feeding behavior, then their differential food preference would favor their coexistance in the same habitat by reducing competition for food.

Survival and Growth

The survival of both P. setiferus and P. aztecus was excellent with all but one of the tested foods for a period of 25 days. The exception was P. setiferus fed Silver Cup Fish Feed, the most preferred artificial food of the experimental diets for this species. The shrimp fed this food suffered high mortality. Animals' initial preference for a food was thus not always indicative of long-range good effect.

The reason for mortality in P. setiferus fed Silver Cup Fish Feed was first suspected to be fouling of tank water due to rapid bacterial decomposition of this food. The water was always reasonably clear, however, and did not produce any objectionable smell. In addition, three changes of water did not remove the cause of mortality. Moreover, excellent survival of P. aztecus with the same food under the same experimental conditions suggest that the mortality of P. setiferus with this food was not due to fouled water. What specific deleterious effect this food had on P. setiferus cannot be ascertained without further studies.

Growth of both the species of experimental shrimp was maximum with Artemia which was also the most preferred food used in these experiments. But in both species, initial food preference was undependable as an indicator of an effective food in respect to sustaining shrimp survival and growth.

In P. setiferus, the final mean weight (10.5 mg) was slightly higher in the animals fed Silver Cup Fish Feed (most preferred artificial food) than that (8.9 mg) of shrimp fed Purina Commercial Catfish Chow (least preferred artificial diet). But due to poor survival, the total weight gain (222.3 mg) of the former group of shrimp was less than that (337.4 mg) of the latter.

The three artificial diets, Silver Cup Fish Feed, Purina Fish Chow, and Purina Commercial Catfish Chow, which were included in the growth study of *P. aztecus* represent the 4th, 5th and 6th foods in order of the animals' preference. By the 15th day, the growth of shrimp in all the three diet groups was similar. But, by the 25th day, the Purina Commercial Catfish Chow-fed shrimp were significantly larger than animals fed the other two diets.

Temperature Tolerance

Significantly different survival time at lethally high temperature among the three diet groups of white shrimp appears to be the influence of food. Silver Cup Fish Food had apparently adversely modified the shrimp's heat tolerance. As noted above, the same food seemed to have a deleterious effect on the shrimp's survival in the growth study.

In *P. aztecus*, the influence of diets on the shrimp's temperature tolerance was not perceptible.

The temperature experiment exposed another point -- two of the contributions of food, growth and resistance to temperature, are independent qualities. *Artemia* was far superior to Purina Commercial Catfish Chow and Purina Fish Chow in terms of its effect on the animal's temperature resistance.

Deaths occurring in a logarithm-normal distribution in time may be ascribed to one lethal effect, whereas deaths occurring in more than one logarithm-normal distribution are believed to represent more than one lethal effect (Fry, 1957). Both in the 15th and 25th day experiments, the survival times of the *P. setiferus* groups were more or less clustered together for each diet, whereas in *P. aztecus* the scattering of survival times into several groups is noticeable. This difference suggests that more lethal effects were expressed in *P. aztecus* than for *P. setiferus*.

The basic physiological mechanisms of heat death in shrimp are not known. However, the results of Bowler's (1963) experiments with crayfish, *Astacus pallipes*, suggest that heat-induced death in this species results from the failure of the nervous system and/or hepatopancreas. The causes of heat death in fishes have been reviewed by Brett (1956). Heat death in fish has been attributed by Fisher (1958) to synapse failure occurring in the pace maker, myoneural junctions, and during smooth muscle peristalsis. Kusakina (1963) ascribes it to be thermal denaturation of body cells and inactivation of cholinesterase. Pegel and Remorv (1961) maintain that at high temperatures enzyme structure changes. The cause of heat death in killifishes (*Fundulus heteroclitus* and *F. majalis*) is believed to be failure of some coordinating mechanism of the central nervous system

(Orr, 1955). Battle (1929) believed that heat death in skates (*Raja erinacea*) was caused by failure of the automatic mechanism of the heart.

GENERAL CONCLUSION

A short-term effect of a diet may not be very revealing of other qualities of that food. A food initially preferred may prove unsuitable for sustaining survival and growth to the shrimp, and may also reduce the animals' resistance to a sudden temperature rise. Such a food cannot be used for a purpose where shrimp's survival and growth and resistance to a sudden high temperature are desirable.

If P. setiferus and P. aztecus are raised in the same pond or are held in the same tank for scientific studies, the food selection must be made carefully. A food may be good for one species of shrimp, but may adversely affect the other.

Consideration of the total influence of foods on P. setiferus and P. aztecus in relation to their preference, survival, growth and temperature tolerance is important from the practical standpoint. Since the qualities of food may be independent of each other, the overall effects of a diet must be known before it is selected and used by a shrimp farmer or a laboratory scientist.

Apart from good survival and growth, resistance to sudden fluctuations of temperatures must be a quality for a diet which is intended for shrimp of farm ponds along American coasts. This is suggested in view of the fact that considerable fluctuations of temperatures are often encountered in the shallow waters of such areas (Duff and Teal, 1965; Aldrich, 1966; Phleger and Bradshaw, 1966).

The circumstances thus call for further research into the influence of food on temperature resistance of shrimps. Responses to low as well as high temperatures should be studied as potentially susceptable to dietary influence. Normal seasonal declines in temperature of estuarine waters of the Gulf of Mexico are strongly suspected of bringing about the seasonal emigration of P. setiferus from the bays (Lindner and Anderson, 1956). Furthermore, unusually rapid decreases in bay water temperatures are recognized as causes of occasional natural mortalities among estuarine and coastal organisms (Brongersma-Sanders, 1957; Collier and Hedgpeth, 1950). The present research dealt only with upper temperature extremes because careful study of low temperature induced lethal effects is logistically quite difficult. The existence of good methodology, as developed by Fry and co-workers, presently permits more meaningful study of upper temperature extremes.

With the exception of Silver Cup Fish Feed, which was found to have deleterious effects on P. setiferus, other artificial foods used in the experiment had excellent survival and temperature resistance qualities. But none of the artificial diets supported growth approaching natural levels, or even that observed in Artemia-fed shrimps.

Since a major objective of farming is to maximize growth of farm animals, the tested artificial foods seem inadequate for use in pond culture of shrimp. This, however, does not exclude the possibility of using artificially compounded diets for shrimp farming. The intensive nature of the present work designed to test for a variety of possible dietary effects, precluded the testing of many diets. So, an endless variety of other diets remain to be considered. In any case, the variety of independent effects which a diet may have should be considered and tested before it is recommended for general use.

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