ABSTRACT

Mitchell E. Landen. EFFECTS OF FOOD AND TEMPERATURE ON SIPHON REGENERATION AND GROWTH IN THE ESTUARINE CLAM RANGIA CUNEATA (Under the direction of Dr. Carlton Heckrotte). Department of Biology, Autumn 1984.

Rates of siphon regeneration in the mactrid clam Rangia cuneata(1831) were measured under varied temperature and food levels. The rates of siphon regrowth were found to be dependent on temperatures, but were food-independent. The temperatures involved were 11o C, 21^{o} C, and 28o C. The food source was Pseudomonas aeruginosa, a motile bacterium. The concentrations of bacteria maintained were a) 0/ml (starvation) b) 2.5 X 10^{5} /ml, and c) 1.0 X 10^{6} /ml.

Each of the nine tanks used in the research contained a group of experimental clams (those having their siphons snipped) and a similar group of control clams. Total weights and lengths were measured for each subject at the beginning and the end of testing, as well as the siphon weights of the experimental clams. The data was compared and contrasted using an analysis of variance.

Time necessary for clams to regenerate their snipped siphons increased at lower temperatures. Food levels had no effect on the time for regeneration. Total clam weight and length increases were attributed to the higher temperature levels, whereas the higher food levels showed no major effect, possibly because of the clam's ability to regulate food intake by regulating water intake.

Mortalities occurred during the course of the research, but neither

temperature level, food level, nor snipping appeared to have caused a notable rise in deaths among the subjects. Mortality in the clams remained below 14%, generally, with no statistically important differences between the experimental and control clams.

EFFECTS OF FOOD AND TEMPERATURE ON SIPHON REGENERATION AND GROWTH IN THE ESTUARINE CLAM *RANGIA CUNEATA*

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Mitchell E. Landen

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EFFECTS OF FOOD AND TEMPERATURE ON SIPHON REGENERATION AND GROWTH IN THE ESTUARINE CLAM RANGIA CUNEATA

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Introduction

Regeneration, the redevelopment of normal functional tissue, is a phenomenon not seen on a major scale in humans or most vertebrates. With many invertebrates, however, it is commonplace. Regeneration of siphon tissue in the estuarine clam Rangia cuneata is the subject of this study. The chief goal of the study was to determine how siphon regeneration in the clam was affected by temperature and food levels. A secondary goal was to ascertain food and temperature effects on clam growth (weight and shell-length). Also investigated was the impact of siphon removal by snipping on clam growth and mortality.

Natural History

Rangia cuneata, of the class Pelecypoda and family Mactridae, is found from the mid-Atlantic states to the Gulf coast (Fairbanks, 1963; Grzimek, 1974). This clam is readily adaptable to temperature and salinity variations. Attesting to this is the record of its rapid increase in distribution. The first report of living, non-Pleistocene Rangia found on the eastern United States coast was in 1955 in the Newport River of North Carolina. By 1960, the clam was reported in Virginia and Maryland (Hopkins and Andrews, 1970). Rangia inhabit low-salinity embayments. Though many can survive in fresh water, they cannot reproduce in it. In tests done with Rangia to determine tolerance to salinity, populations of the clam survived in salinities from 0 to 39 o/oo (Bedford and Anderson, 1972). In North Carolina's Pamlico River,

Rangia are most common in areas with salinities between 0.5 and 10.0 o/oo (Crump, 1971). Their ability to acclimate to temperature extremes has also been examined. Rangia have shown 100% mortality below 0.5° C and above 35° C, but can survive between those ranges (Naylor, 1965; Cain, 1973; Sample and Landy, 1978; and Landy, 1979). Another indicator of Rangia's hardiness is its constant densities in spite of substantial salinity and temperature changes in the Pamlico River (Tenore, 1972; Sutherland, 1982). The clam itself is an ecological asset to the Pamlico, since it converts detritus to meat, which is then used as food by birds, fish, and crustaceans.

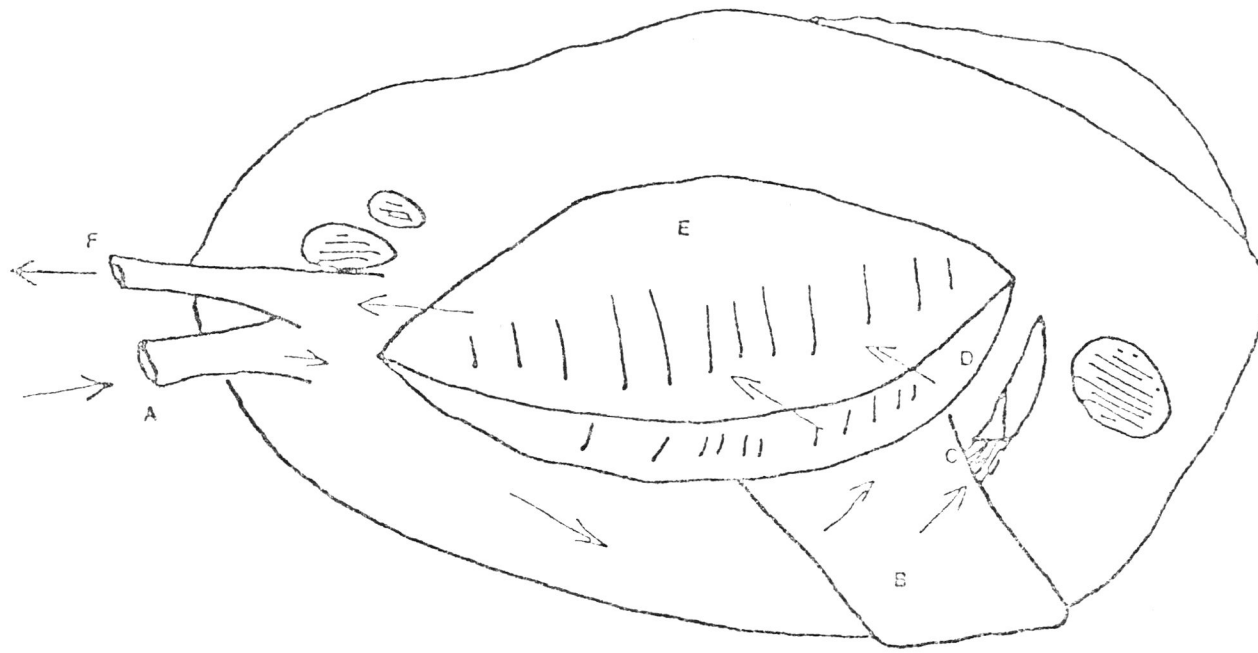
Molluscans form 45% of the benthic invertebrate species list of the Pamlico estuary (Tenore, 1972). In the food web of Lake Pontchartrain, Louisiana, Rangia densities place it among the three most important invertebrates, the others being the mud crab, Rithropanopeus harrisii, and the blue crab, Callinectes sapidus (Green, 1968). This implies Rangia's ecological significance in some aquatic systems. The filtering activity of the clam is as high as 40 l/h according to some estimates (Potts, 1967; Duffy, 1980). Therefore, major decreases in the clam population could result in an acute ecological imbalance.

Rangia Siphons: Functions and Ecological Value

Rangia are filter-feeders, subsisting on bacteria, dinoflagellates, diatoms, dead organic matter, and other microscopic organisms (Hyman, 1967; Vernberg, 1977; Peterson and Quammen, 1982). Two types of bacteria, *Serratia marcescens* and *Serratia liquefaciens*, have been shown in experiments to be adequate food sources for *Rangia* (Brooks, 1978; Jeffreys, et al, 1980). Ingestion and egestion in *Rangia* are performed by two siphons, the dorsal excurrent (exhalant) and the ventral incurrent (inhalant) seen in Figure 1. These are apertures controlling water intake and elimination (Jorgenson, 1966). The siphons are modifications of the posterior edges of the mantle. They are commonly retractable within the shell. This is performed by the siphonal retractor muscle, which is a local exaggeration of the pallial retractor muscle, located along the entire mantle edge (Pratt and Campbell, 1956). Some clams, such as the *Mya arenaria*, lack a totally retractable siphon and tend to remain so deeply buried that even a slight retraction brings the siphon safely under the sediment surface (Feder, 1972; Bernard, 1975). The *Rangia* siphon, however, is totally retractable. Sensory tentacles line the opening of the incurrent siphon, allowing exclusion of undesirable particles without forcing the retraction of the entire siphon (Reid and Crosby, 1980).

Where large populations of *Rangia* exist, their siphons may be of some importance to small and juvenile fish as a food source in eastern North Carolina estuaries. Small benthic-feeding fish, among them

Figure 1. Water circulation in the clam (Ellis, 1978)
A) incurrent siphon
B) foot
C) mouth
D) interior gill
E) exterior gill
F) excurrent siphon



juvenile flatfishes, feed heavily upon bivalve siphons and other soft parts of benthic fauna , as determined by examination of gut contents (Edwards and Steele, 1968). In studies by Peterson and Quammen (1982) off the southern California coast on the clam Prototheca staminea, it was noted that in addition to fish such as the California halibut (Paralichthys californicus), other predators feeding on the siphons were small cancer crabs, Cancer anthonyi, moon snails, Polinices reclusianus, staghorn sculpins, Leptocottus armatus, and diamond turbots, Hypsopsetta guttulata. In research by Currin (1984) at Rose Bay, North Carolina, gut contents of spot and croaker were scrutinized. Among the contents were dipterans, small fish, amphipods, calanoids, whole bivalves, and bivalve siphons. The high number of siphons present suggests a significant role for them in the diet of the fish species under investigation. From work done at Loch Ewe, Scotland, Edwards and Steele (1968) found that in juvenile plaice gut contents examined, bivalve siphons constituted the highest percentages overall of individual structures present. Amphipods, cumoceans, and calanoid copepods were also found. Among common dabs, the numbers of siphons found followed in abundance that of amphipods, cumoceans, and harpacticoid copepods. The smaller fish (plaice and common dabs) tended to feed on siphons of the bivalve Tellina tenuis and tentacles of the polychaete Nerine cirratulus. In a Dutch study (Kuipers, 1977), Macoma siphons were found in abundance in the gut contents of juvenile plaice.

The nutritional value of the bivalve siphon has not been positively established, but the siphon is believed to impart a notable

measure of calories to the predator. Edwards, et al (1969), discovered that bivalve siphons were a major component of the natural food of plaice in Loch Ewe during the first two months after metamorphosis. In their research, high growth rates for plaice were detected when the fish were allowed to feed on Tellina siphons exclusively.

Morowitz (1968) performed analyses of Macoma soft tissues to determine caloric values for them. The predominant components of the tissues were proteins, carbohydrates, and lipids, percentages of which varied according to the season (Beukema and de Bruin, 1977). The average caloric value for all seasons was 4.1 $kcal \cdot g^{-1}$ for the carbohydrate, 5.5 $kcal \cdot g^{-1}$ for the protein, and 9.3 $kcal \cdot g^{-1}$ for the lipid. Percentages of carbohydrate in the soft tissues were somewhat higher than those for protein and much greater than those of lipids. The average caloric content for soft tissues was put between 5.5 and 6.0 $kcal \cdot g^{-1}$. In 1979, Beukema and de Bruin used Macoma balthica for an energy study involving a biochemical method (designated the "indirect method") and a method using a micro-bomb calorimeter (the "direct method"). From the indirect method, using conversion factors for various components to find calorific values, a figure of 5.47 $kcal \cdot g^{-1}$ was derived for the tissue caloric content. Using the direct method, they got 5.59 $kcal \cdot g^{-1}$.

Whether siphons may contribute significantly to energy needs of fish can be determined by investigation of fish requirements in general. For pike, 14 to 33 % of its caloric consumption was found to have been converted into new tissue, while between 50 to 70 % was metabolized in

such processes as respiration, digestion, excretion, and maintenance of existing tissue (Lagler, et al, 1977). Although energy efficiencies for younger fish are greater than those of older fish (45 % as opposed to 15%, generally), metabolic rates of younger fish are also higher. Therefore, food demand is higher. The daily maintenance ration of red hind (Epinephelus guttatus), for a 250 g specimen between 19 and 28°C, ranges between 1.7 and 5.8 percent of body weight. Carp yearlings (Cyprinus carpio) in ponds may have a daily maintenance ration of 16 % of their body weight at the beginning of the growing season. These rations naturally vary due to food availability, reproductive stage, and season. Blue gill (Lepomis macrochirus), for example, may consume up to 5 % of its body weight daily in summer when the mean water temperature is 20°C, but the food intake in winter, when the mean water temperature is 3°C, may only be 0.14 % per day. According to a report edited by Neuhaus and Halver (1969), 1000 ml of oxygen consumed by a fish (in this case, salmon) corresponded to 4.69 kcal expended. A 100 g fish requires 16.48 ml of oxygen per hour, equalling 395.5 ml per day, therefore the daily maintenance requirement for a 100 g fish would be 1.8 kcal. From this study, using adult Rangia averaging 40 mm in length and 39 g in weight, a mean dry siphon weight of 0.0020 g was calculated. Approximately 500 siphons, then, would be needed to constitute 1 g (dry weight) and 5.59 kcal. The 1.8 kcal required by a 100 g fish is 31 % of the number of calories found in 1 g of siphon tissue. Therefore, around 155 siphons would be needed to fill the daily maintenance requirement for a fish of this size. Flounder, croaker, and spot, known for their

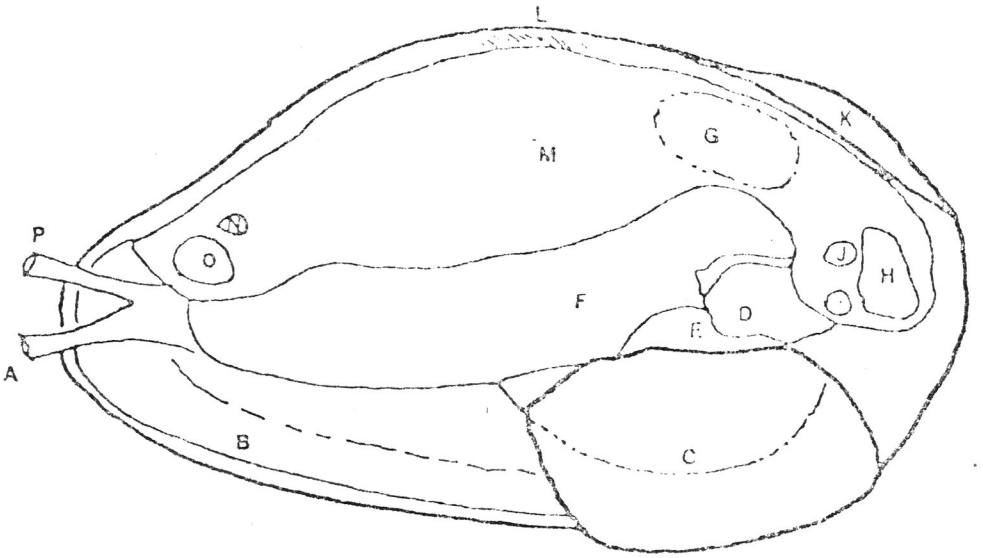
predation of bivalve siphons, abound in the Pamlico estuary and probably rely to some extent on the sizeable Rangia and Macoma populations for sustenance. Other species of fish, such as striped bass, have not been found to feed heavily on bivalve siphons, although examination of their guts has revealed the presence of some siphons (Dr. T.J. Lawson, ECU, personal communication 1984). Gut contents of yellow perch, white perch, channel catfish and white catfish from the western Albemarle Sound yielded few siphons when examined. Whether the siphons found were from Macoma , Rangia, or some other bivalve species was not determined, since the purpose of that study was identification of predators of juvenile yellow perch. If regeneration of snipped siphons is the norm, an almost perpetual crop of siphon tissue would be available for the fish to feed upon.

Digestive Process

The food particles entering by the incurrent siphon are sorted out according to size by the labial palps, with the over-sized ones rejected (Bernard, 1975). Smaller particles are passed via cilia action (because the clam's alimentary canal is devoid of muscle) to the esophagus and thence to the stomach. The crystalline style, a gelatinous rod-like structure, is then rotated against the gastric shield, aiding gastric enzymes in gradually digesting the stomach contents (Barnes, 1968). The food is sent to ducts of the digestive gland (Figure 2), where cells ingest it. Following this, the nutrients are digested intracellularly (Fretter and Graham, 1976).

Figure 2. Anatomy of Rangia cuneata (Fretter and Graham, 1976)

A) incurrent siphon
B) mantle
C) foot (containing loops of intestine and gonads)
D) palps
E) interior gill
F) exterior gill
G) digestive gland
H) adductor muscle
I) protractor muscle
J) retractor muscle
K) umbo
L) ligament
M) visceral mass
N) retractor muscle
O) adductor muscle
P) excurrent siphon



Solid matter not absorbed by these cells in the diverticula enters the intestinal groove and passes into the intestine. More digestion occurs there, because the lumen of the intestine possesses amoeboid cells capable of phagocytosis (Morton,1960). Wastes are removed from the blood by the nephridial tubules, U-shaped kidneys below the heart (Hickman, 1967). The wastes are then excreted by the excurrent siphon. The siphons, therefore, serve necessary functions in the clam, with the sensory tentacles also having a role.

The Pamlico River

The Pamlico River has salinities ranging from 1 to 20 o/oo, with temperature variations between 0 and 31° C (Jarrett, 1966; Tenore, 1972). The river is divided into three zones (using the Venice classification of estuarine waters):

a) The oligohaline region, dominated by Rangia and Nereis succinea, with salinities from 0.5 to 5.0 o/oo, and having the highest concentration of Rangia,

b) The mesohaline region, dominated by N. succinea, Macoma balthica, and Heteromastus filiformis, with salinities from 5.0 to 18.0 o/oo, and

c) The polyhaline region, dominated by Macoma Phenax, Mulinia lateralis, and Glycera dibranchiata (Tenore, 1972).

The Pamlico River, a wide and shallow estuary, extends from Washington, N.C., for 65 km to the Pamlico Sound. It has an average

depth in its central muddy areas of 2 to 3 m, and an average depth in the near-shore zones of 1 m. Rangia is most abundant in the latter zone. In 1967, the density of Rangia in some places was as high as 275 clams/m (Tenore, 1972).

Regeneration

Regeneration occurs in many organisms, but the physiological prompting of regeneration is unknown. The ability of the planarian to regenerate is well-documented, the first known experiments having been done in 1774 by Pallas (Brønsted, 1969). In salamanders, legs and tails are regenerated fully, with normal motor function resulting (Brookbank, 1978). Other amphibians, like the newt, are also capable of limb regeneration (Ede, 1978). After amputation, the wound is closed by epithelium. This is accompanied by proliferation of subepithelial growth. The stump blastema formed is undifferentiated at first, but eventually becomes adult tissue (Fulton and Klein, 1976). According to one theory, nerve supply to the amputation site in the newt may be involved in tissue regeneration, because nerve supply is much greater relative to the total cross-sectional area of the limb itself as compared to that of an animal incapable of regeneration (McKenzie, 1976). In studies involving the cockroach Leucophaea, legs were regenerated but only if amputation were conducted, and not a mere wounding (Grant, 1978). Success has been noted in regeneration of severed or crushed optic nerves in adult frogs (Graham and Wareing,

1978). Although tests involving weak pulses of electricity through damaged tissue in humans have had some positive results, regeneration in post-embryonic humans occurs only in the liver, by normal replacement phenomena (e.g., uterine endometrium), and through wound-healing (Clark et al, 1980; Borgens, 1981).

Previous Bivalve Siphon Studies

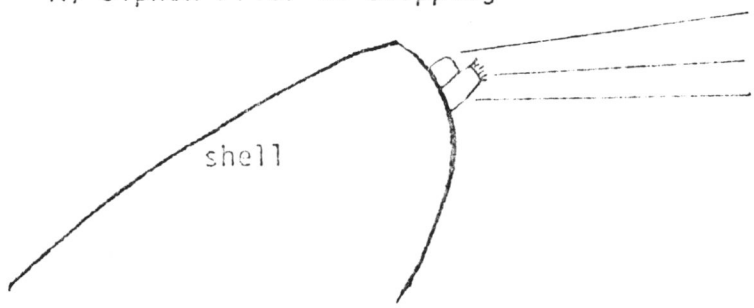
Sutherland (1981) placed cages with two densities of bivalves (Macoma balthica and Macoma phenax) and three densities of fish (juvenile spot, Leiostomus undulatus, and juvenile croaker, Micropogon xanthurus) in Rose Bay, N.C., to find effects of siphon snipping by fish on growth of the clams. The cages were made of plastic restaurant bussing trays with a 1.8 cm pipe frame, covered by a 0.63 cm^2 CONWED plastic mesh. The volume of each cage was 0.046 m^3 , with a surface area of 0.177 m^2 and a height of 0.26 m. Biweekly, fish were taken from the cages and placed in 10% formalin, and their stomach contents examined for siphons. The Macoma showed no difference in growth when subjected to siphon snipping by juvenile spot than did those serving as controls. Only those Macoma being snipped by juvenile croaker showed a decline in growth rates. Control clams initially 10 mm long grew 3.3 mm during 15 weeks in cages, whereas those in cages with croaker showed only 1.5 mm growth -- a reduction of 45%. In a similar experiment done in California, a two-fold drop in growth of Prototheca staminea was attributed to siphon-snipping (Peterson and Quammen, 1982). In a siphon-

snipping experiment done in the Netherlands with M. balthica, little growth reduction and little increase in mortality were seen after the bivalves were subjected to siphon snipping by juvenile plaice and flounder (de Vlas, 1981).

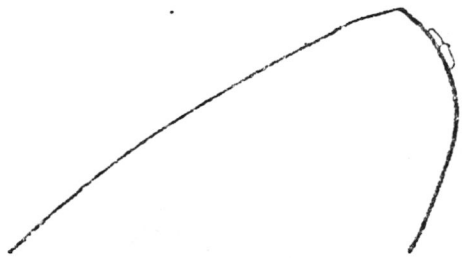
In research done to establish general characteristics and results of siphon-snipping in Rangia, Landen (1983) used 30 Rangia in one tank. Salinity was maintained at 1 o/oo and temperature at 21° C (room temperature). The clams ranged in length from 50 to 55 mm and in weight from 70 to 77 g. Feeding was done once a week by addition of water (approximately 1 l) from Euglena and Paramecium cultures. Fifteen clams were snipped by hand and fifteen were used as controls. On the average, 7 days were required for new siphon tissue to be detected. After this period, the siphon length increased by increments of about 0.5 mm/day. Minute siphon sensory tentacles (~0.3 mm) were observed by day 15, with siphon length at this point averaging 4 mm. By day 19, sensory tentacle length had increased to 1.3 mm, and by day 21, the maximum average of 1.5 mm was attained (Figure 3). By this time, siphon length was between 5 to 6 mm and remained at this length. After 21 days, no increase in siphon or tentacle length was noted. Mortality of 20% was seen among the snipped clams, but none occurred among the controls.

Figure 3. Observations on stages of siphon regrowth in Rangia

A) Siphon Prior to Snipping

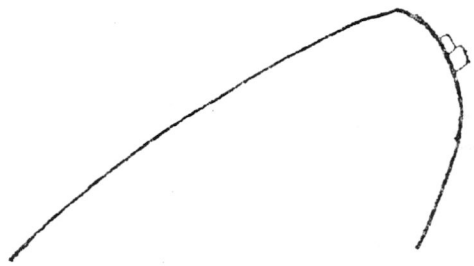


B) Siphon 10 Days After Snipping



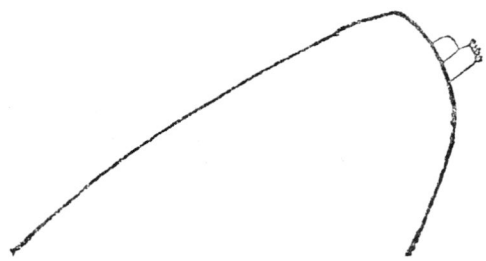
sensory tentacles unseen
incurrent siphon ~1.5 mm

C) Siphon 15 Days After Snipping



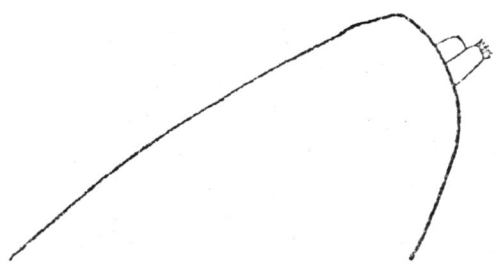
sensory tentacles ~0.3 mm
incurrent siphon ~4.0 mm

D) Siphon 19 Days After Snipping



sensory tentacles ~1.3 mm
incurrent siphon ~5.0 mm

E) Siphon 21 Days After Snipping



sensory tentacles ~1.5 mm
incurrent siphon ~5.0 mm

Materials

Nine plastic containers ("rat cages") which measured 0.17 m (width) X 0.29 m (length) X 0.13 m (height), providing a bottom area of 0.49 m^2 and a total volume of 0.064 m^3, were used to contain the clams. Into each container were placed four liters of water with a salinity of 1 o/oo . This water was made from distilled water to which "7 Seas" marine salts was added to get the necessary salinity (Table 1). This salinity level was chosen because the average salinity of Chocowinity Bay, the site of the clam collection, is between 0.5 o/oo and 1.0 o/oo (Figure 4). The salinities were checked at 3-day intervals with a YSI Model 33 salinity meter. Oxygen concentrations were kept high by bubbling air through the water, and were checked twice weekly with a YSI Model 57 oxygen meter. For those tanks requiring a heat supply, Appco and Wil-Nes 77 thermoregulators were used.

The food source used was the bacterium Pseudomonas aeruginosa (ATCC 10145), a motile bacteria commonly found in most natural waters. This bacterium was selected for several reasons:

a) The relative ease and time-effectiveness in handling the cultures and growing the bacteria,

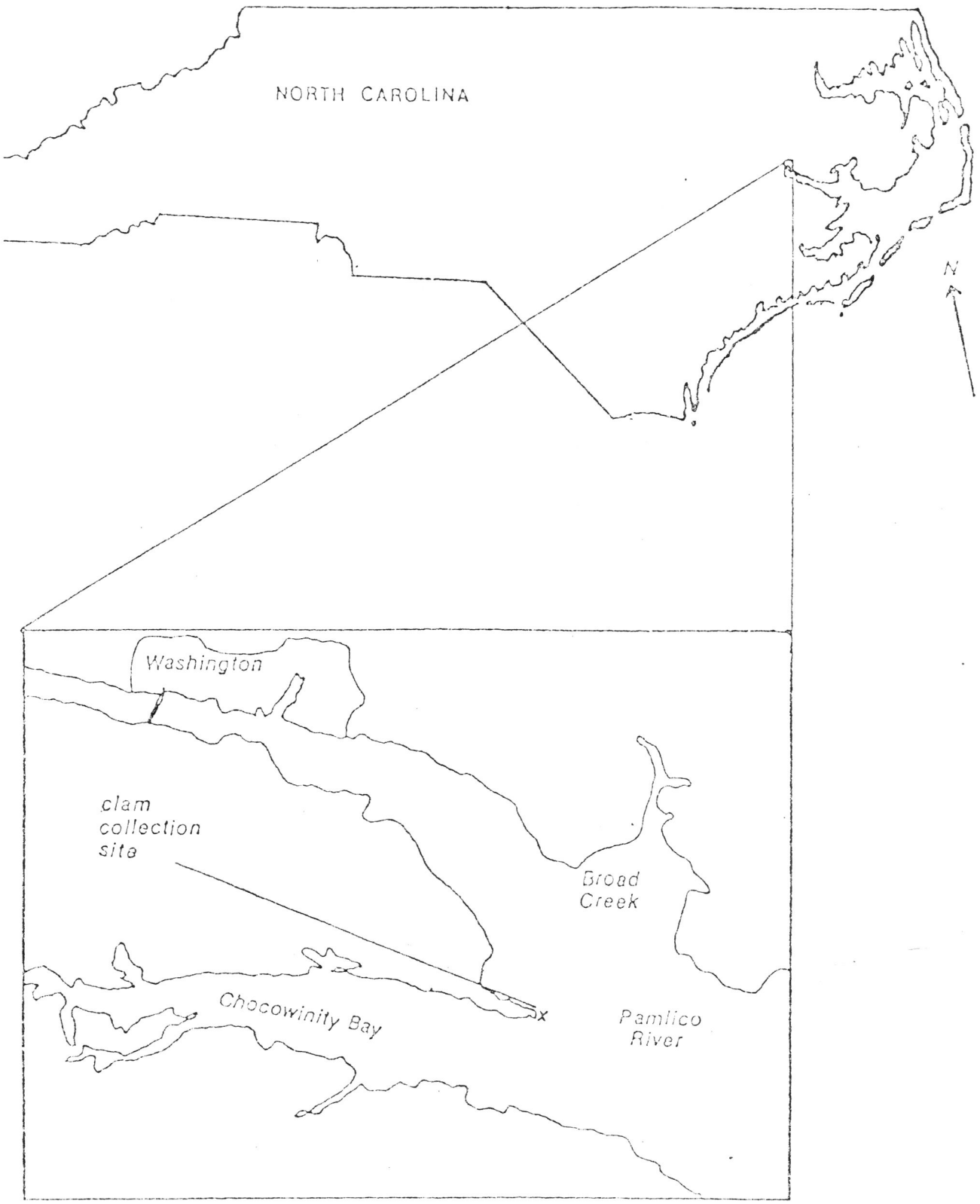
b) The ubiquitous distribution of P. aeruginosa, leading one to assume that wherever Rangia are found, so are these bacteria, and

c) the ability of P. aeruginosa to produce and release the antibiotic-like bacteriocins called pyocins (Pelczar and Reid, 1972). These substances are lethal to some forms of bacteria (via adsorption on specific receptors of host-cell walls) and should

Table 1. Composition of Utility Marine Mix (Utility Marine Mix Bulletin #127 -- "7 Seas" sea salts)

Compound	Chemical Formula	%
Sodium Chloride	($NaCl$)	37.0
Magnesium Chloride	($MgCl_2 \cdot 6H_2O$)	7.0
Magnesium Sulfate	($MgSO_4 \cdot 7H_2O$)	9.3
Potassium Chloride	(KCl)	0.9
Sodium Bicarbonate	($NaHCO_3$)	0.2
Strontium Chloride	($SrCl_2 \cdot 6H_2O$)	27.0
Manganese Sulfate	($MnSO_4 \cdot H_2O$)	5.0
Disodium Phosphate	($Na_2HPO_4 \cdot 7H_2O$)	4.0
Lithium Chloride	($LiCl$)	1.3
Sodium Molybdate	($Na_2MoO_4 \cdot 2H_2O$)	1.3
Calcium Chloride	($CaCl_2$)	1.7
Calcium Gluconate	($Ca(C_6H_{11}O_7)_2$)	0.8
Potassium Iodide	(KI)	0.1
Potassium Bromide	(KBr)	Trace
Aluminum Sulfate	($Al_2(SO_4)_3$)	0.6
Cobalt Sulfate	($CoSO_4$)	Trace
Rubidium Chloride	($RbCl$)	0.2
Copper Sulfate	($CuSO_4 \cdot 5H_2O$)	0.6
Zinc Sulfate	($ZnSO_4 \cdot 7H_2O$)	0.1

Figure 4. Site of the collection of Rangia cuneata at Chocowinity Bay, North Carolina (Radford et al, 1968)



keep non-pseudomonad bacterial levels low or eliminate them altogether. Pseudomonads were cultured using tripticase soy agar (TSA) and were transferred to sterile 10-ml Pyrex test-tubes, which contained 8 ml each of trypticase soy broth (TSB). These are conventional media used for many bacterial experiments (Table 2). The inoculated tubes were incubated in an oven at 30° C for 24 hours to allow the bacterial populations to reach 1.0 X 10^9 cells/ml. The 24-hour period is the standard time requirement for such a population of Pseudomonas to be reached. Confirmation was attained by plate count.

The clams, ranging from 37 to 45 mm in length, were numbered 1 to 90 with red Maybelline fingernail polish. This polish proved the best of several tried, since it dried quickly (which reduced the time the clams were kept out of the water), peeled seldom, and was non-toxic.

Table 2. Componants of the media used (trypticase soy broth and trypticase soy agar)

TSA typical formula (for 1 l of distilled water):

15.0 g Peptone 140 (Pancreatic Digest of Casein)

5.0 g Peptone 110 (Papaic Digest of Soy Protein)

5.0 g Sodium Chloride

15.0 g Agar

(pH 7.3 +/- 0.2 at 25° C)

TSB typical formula (for 1 l of distilled water):

17.0 g Peptone 140 (Pancreatic Digest of Casein)

3.0 g Peptone 110 (Papaic Digest of Soy Protein)

2.5 g Dextrose

5.0 g Sodium Chloride

2.5 g Potassium Phosphate Dibasic

Methods

Ten clams were placed in each container, and randomly divided into two groups: 5 experimentals were placed on one side and 5 controls on the other side of the container. The weight and length of the clams was measured (length defined as distance from umbo to base of siphon). They were then paired, experimental to control, according to length (intrapair comparisons, however, were not made). The experimental design in this research called for three levels of temperature and three levels of food to be used (Figure 5). The temperature levels chosen were 11° C, 21° C, and 28° C, which are the approximate average temperatures found in Chocowinity Bay in March, May, and July, respectively. The three tanks designated 11° C were placed in cold rooms, which are maintained at 4° C. Heaters were attached to each tank and adjusted, over a period of two days, to raise the water temperature to 11° C. The three containers used for 28° C water were placed in a room maintained at 21° C, and heaters were used to raise the water temperature to 28° C. The three tanks at 21° C were kept at the room temperature of 21° C. These three were placed alongside the tanks holding the 28° C water.

The three food levels chosen were:

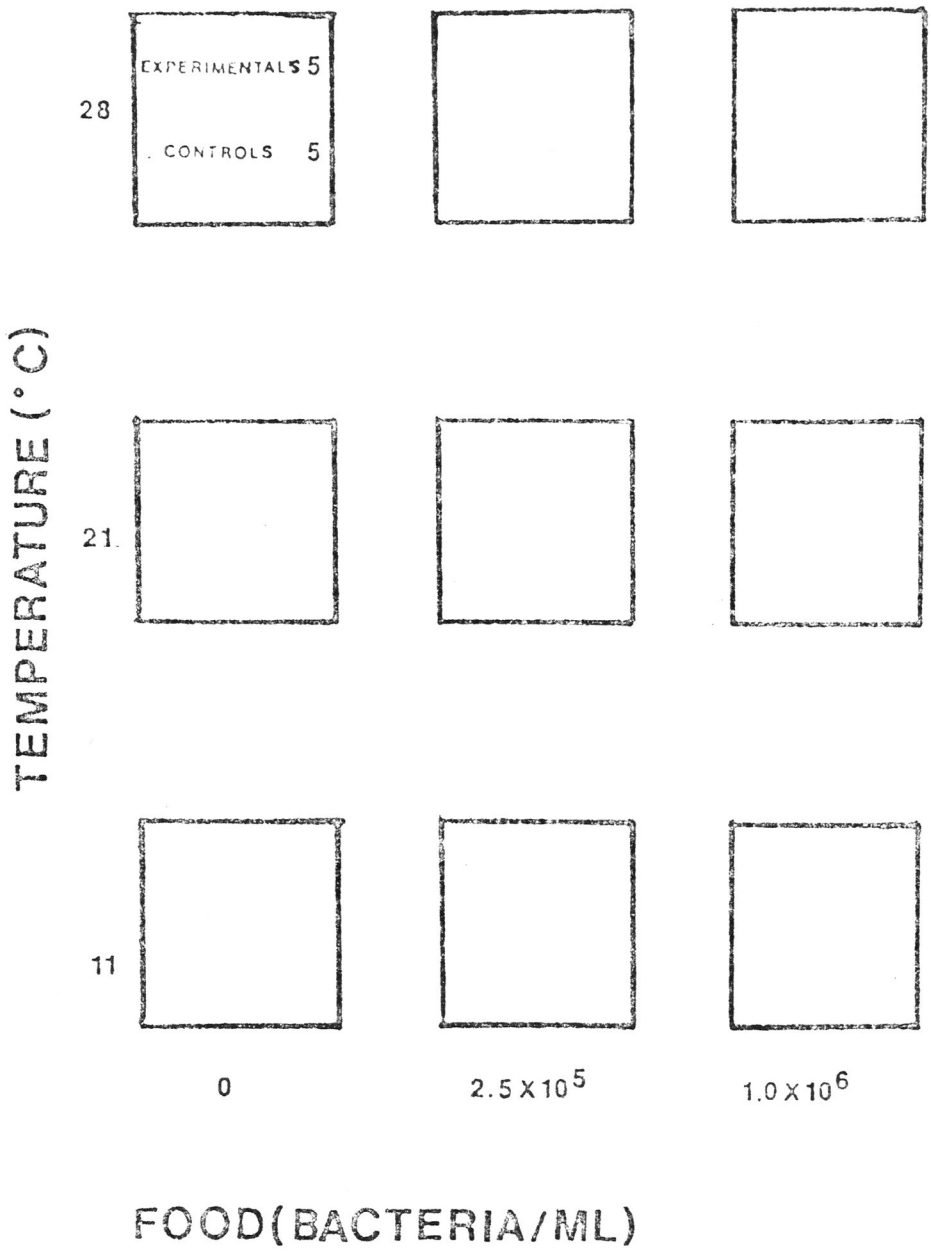
0) 0 bacteria/ml (starvation level),

1) 2.5×10^5 bacteria/ml, and

2) 1×10^6 bacteria/ml

The level of bacteria in natural waters, including the Pamlico River, is generally around 1×10^6/ml (Dr. R. Christian, ECU, personal communication 1982), hence the "base" amount used. To achieve this level

Figure 5. Experimental design used in research



of pseudomanal bacteria in the three high-food-group containers, four ml of the inoculate (containing 1 X 10^9 Pseudomonas/ml) were applied. This resulted in a nearly-immediate Pseudomonas population of 1 X 10^6/ml. For the second food-level tanks, one ml of the inoculate was added to the water, giving a Pseudomonas population of about 2.5 X 10^5/ml. No Pseudomonas were added to the tanks containing clams designated for starvation.

After the clams were placed in the containers, the water temperature was slowly changed , over a 72 hour period, to allow time for the clams to acclimate to the temperature. Twenty four hours had been used initially, only to result in high and sometimes total mortality within 36 hours, particularly among those clams in 28° C water. For the clams designated for 28°C, the water was slowly heated. For those assigned the 11°C temperature, the water was slowly cooled.

A total water change was done every 48 hours. Care was taken to adjust the temperature of the new water to the experimental temperature to prevent possible shock to the clams by a quick change in temperature. Appropriate food levels were administered immediately following the water replacements.

Snipping of the experimental subject's siphons was conducted at the beginning of the experiment, prior to the first feeding. This cutting was performed manually with scissors. The clams were observed individually on a daily basis to determine mortality and to ascertain when full siphon regeneration had occurred (i.e., when the sensory tentacles had reached pre-snipped size). The time needed for this

regeneration was recorded . At this time, the siphon was again snipped. After this second snipping, total clam weight and length measurements were recorded. An analysis of variance was done using the Statistical Analysis System (SAS) computer program (SAS Institute, Cary, North Carolina, 1982). The level of significance used in all comparisons of the analysis was the 0.05 level. Mortalities were noted as they occurred.

Results and Discussion

Results from this experiment indicate variation in siphon regeneration rates (Figure 6, Table 7), variation in total weight gain (Figure 7, Table 8), and variation in total length increases (Figure 8, Table 8) as being dependent on varying temperature levels. The food levels used were found to have no notable impact on these variables (Figures 9, 10, and 11; Tables 7 and 8). It must be noted that with respect to the mean length and weight differences, statistical significance did not necessarily indicate biological significance, primarily because of the small differences in means.

The degree of independence of the variables used (food and temperature) from each other is seen in Tables 3, 4, and 5. The correlation coefficients and p values of food level with the clam length and weight illustrate food's overall impact in the course of the research to be insignificant. The correlation values for temperature indicate a very significant effect on the two variables length and weight. The lack of significant interaction allowed the effects of temperature and food to be assessed seperately.

Figure 6. Relationship of temperature level to mean number of days required for full siphon regeneration

(S.E. = standard error)

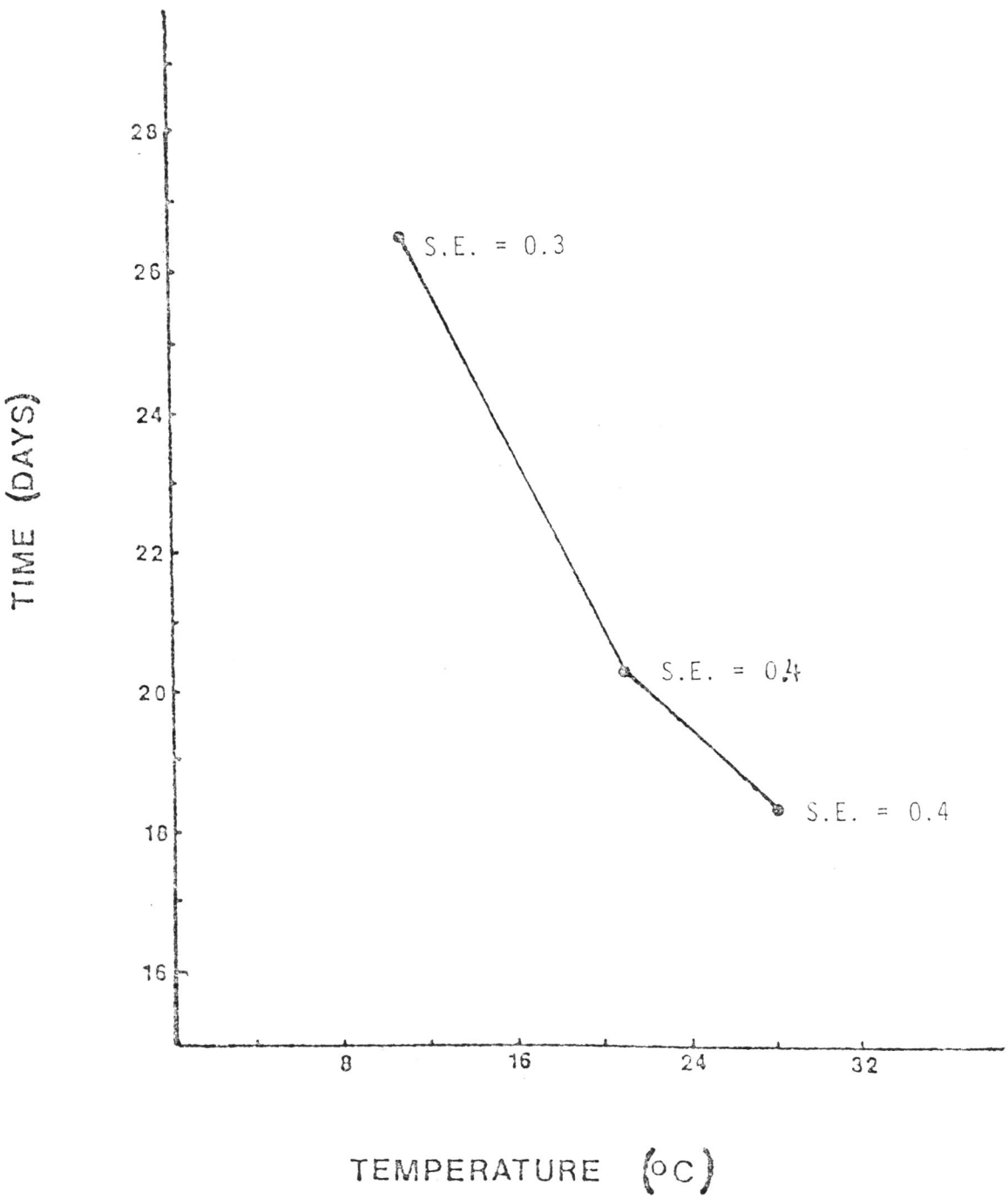


Figure 7. Relationship of clam weight gain to experimental temperature level
(S.E. = standard error)

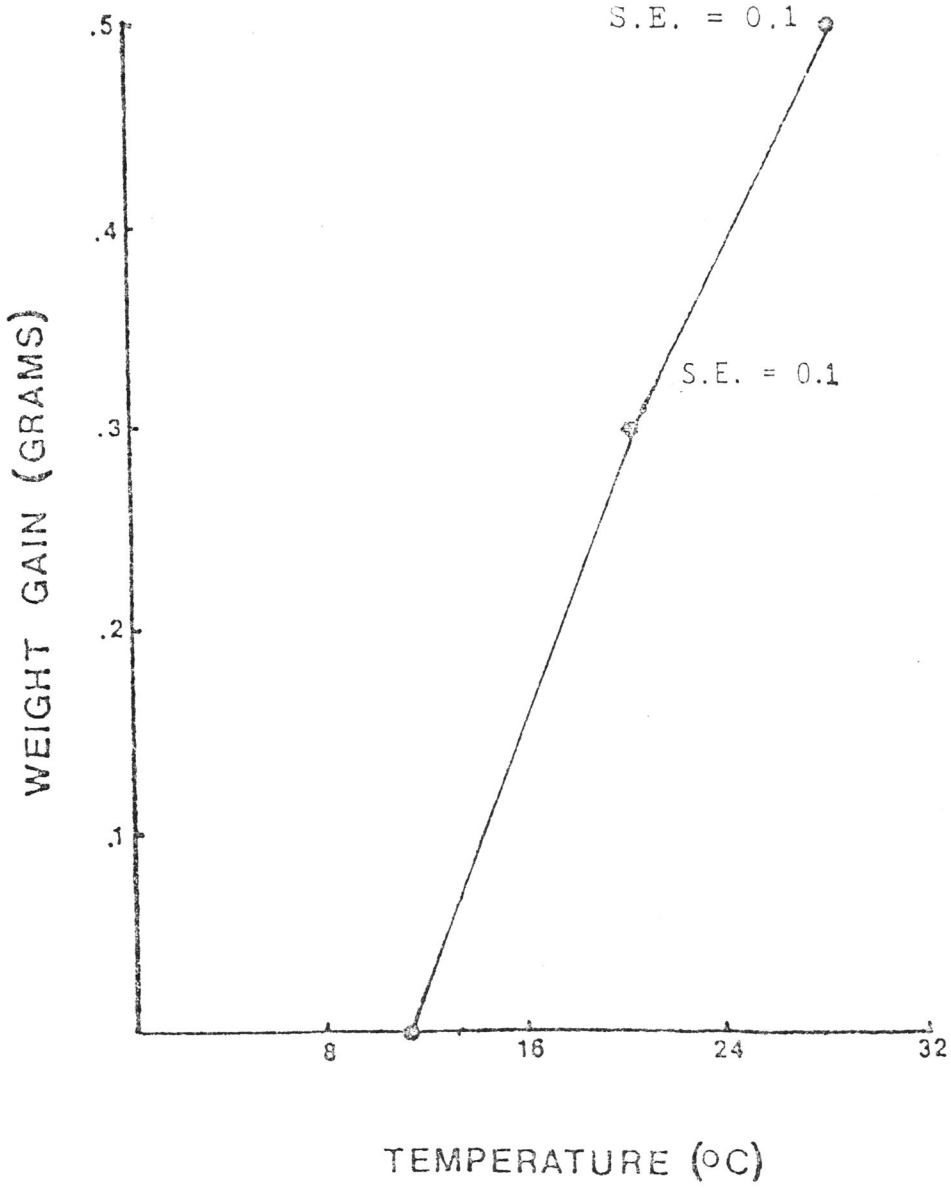


Figure 8. Relationship of clam length gain to temperature levels used
(S.E. = standard error)

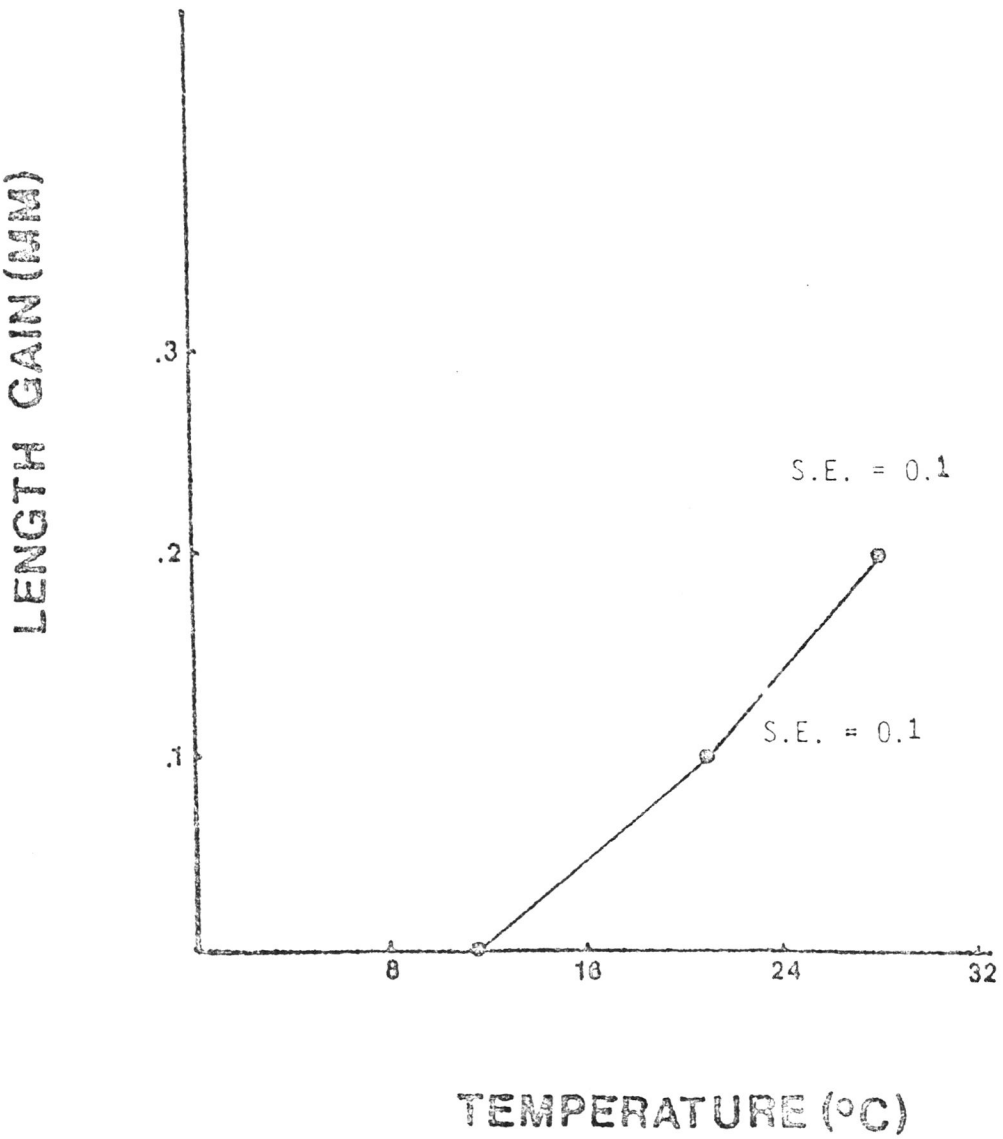


Figure 9. Relationship of food levels to days required for full siphon regeneration
(S.E. = standard error)

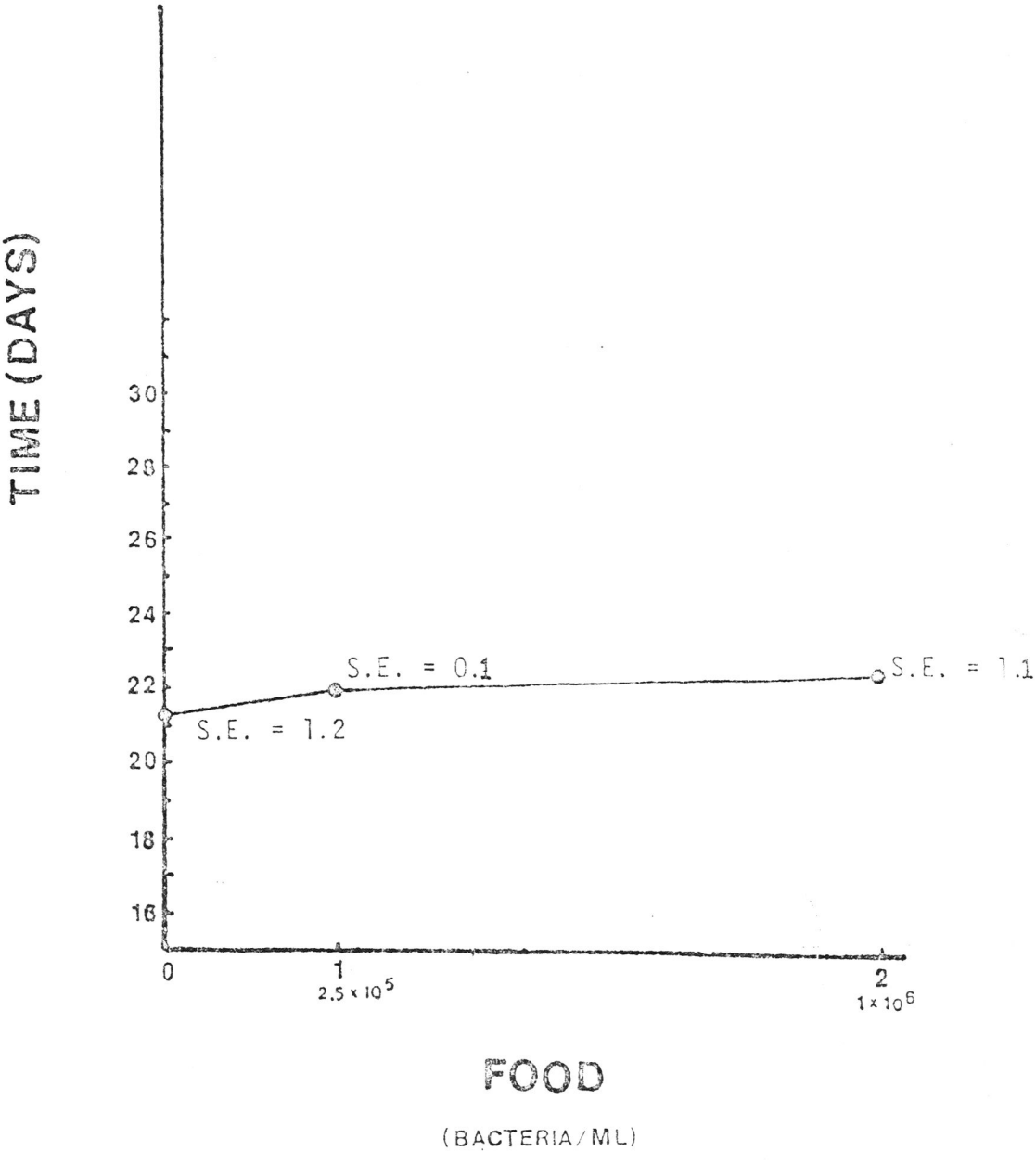


Figure 10. Relationship of clam weight gain to varying food levels
(S.E. = standard error)

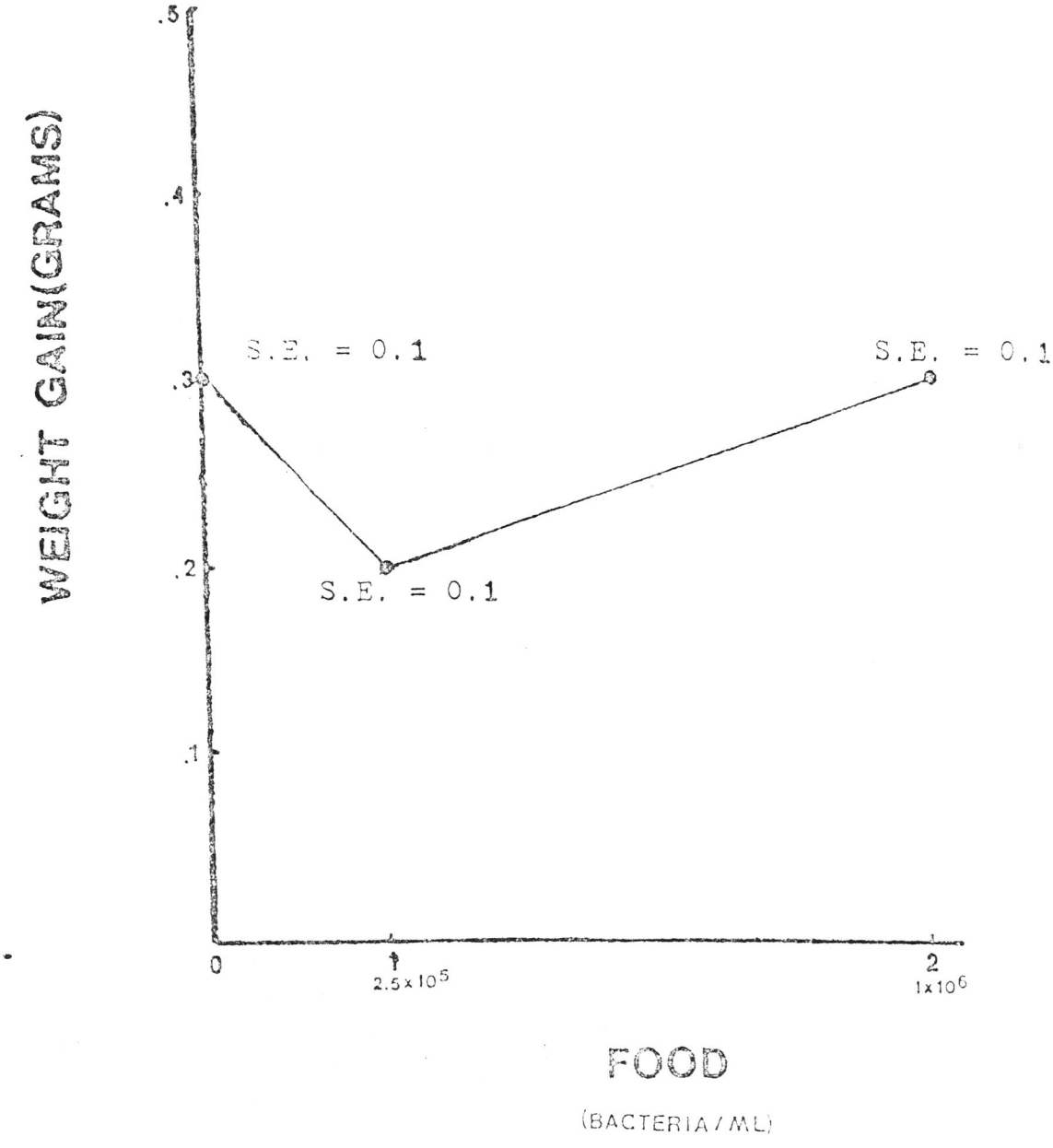


Figure 11. Relationship of clam length gain to varying food levels
(S.E. = standard error)

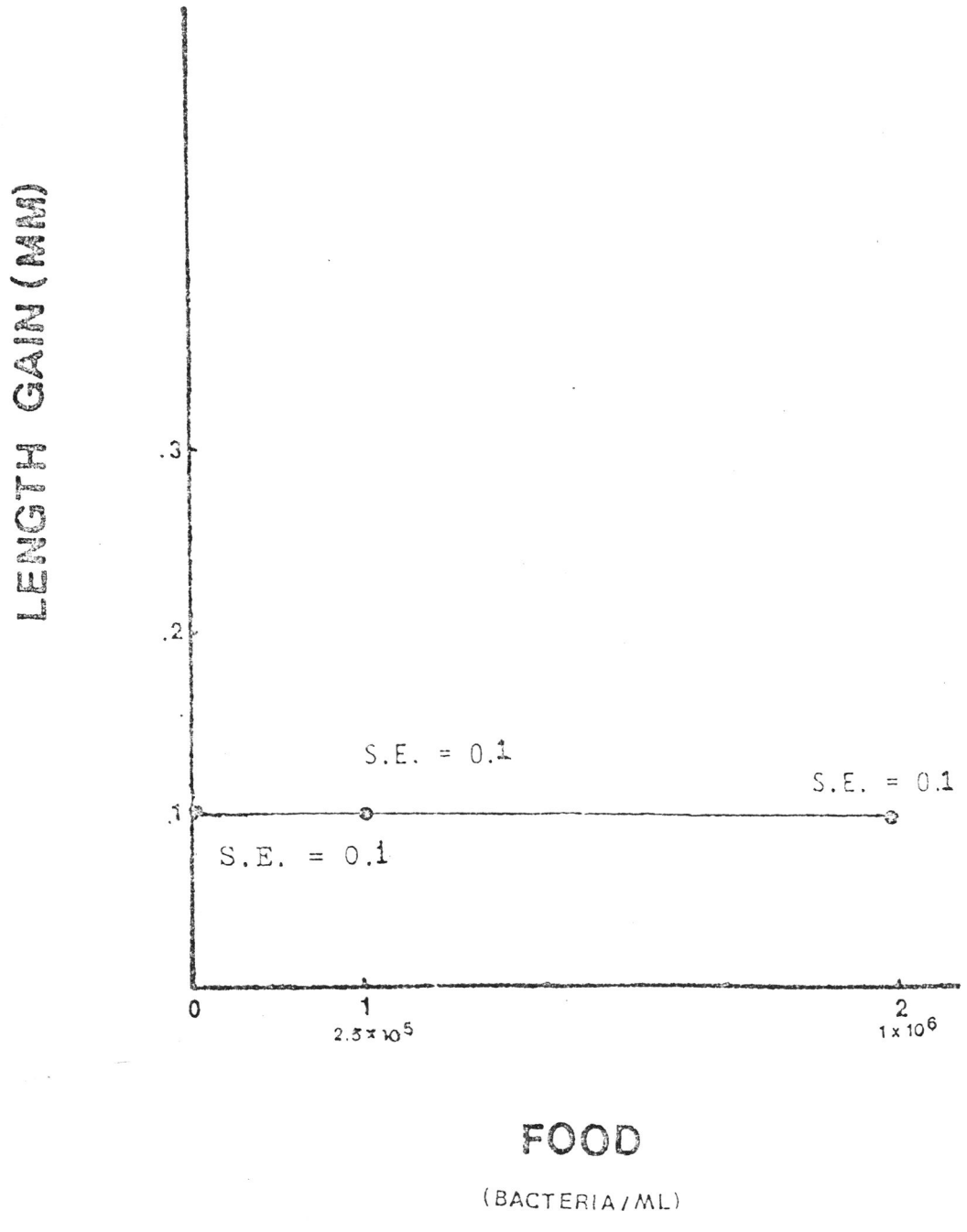


Table 3. Statistics on independent variable relationship to each other with regard to time for regeneration

Source	Degrees of Freedom	F Value	P Value
Food	2	1.76 NS	0.4772
Temp	2	129.87 **	0.0001
Temp*Food	4	1.32 NS	0.2840

(Mean square for error was 1.865, with 30 degrees of freedom)
NS = not significant
** = significant
(snipping relationships not applicable with regard to time)

Table 4. Statistics on independent variable relationship to each other with regard to weight gain

Source	Degrees of Freedom	F Value	P Value
Food	2	0.18 NS	0.8394
Temp	2	8.50 **	0.0005
Temp*Food	4	0.34 NS	0.8500
Food*Snip	2	0.42	0.6605
Temp*Snip	2	0.48	0.6227
Temp*Food*Snip	4	0.35	0.8461
Snip	1	1.06	0.3060

(Mean square for error was 0.1796, with 64 degrees of freedom)
NS = not significant
** = significant

Table 5. Statistics on independent variable relationship to each other with regard to length gain

Source	Degrees of Freedom	F Value	P Value
Food	2	0.00 NS	0.9983
Temp	2	3.28 **	0.0489
Temp*Food	4	0.01 NS	0.9997
Food*Snip	2	0.44	0.6476
Temp*Snip	2	0.19	0.8448
Temp*Food*Snip	4	0.35	0.8442
Snip	1	0.03	0.8573

(Mean square for error was 0.1093, with 64 degrees of freedom)
NS = not significant
** = significant

Table 6. p values and correlation coefficients among variables in the experiment

p values

	temp	food	time	weight gain	length gain
temp					
food	0.2840				
time	0.0001	0.5112			
weight gain	0.0001	0.8209	0.0032		
length gain	0.0069	0.9628	0.1533	0.0001	
snipping	0.0000	0.0000	0.0000	0.3140	0.8451

correlation coefficients

	temp	food	time	weight gain	length gain
temp					
food	0.0000				
time	0.9186	0.1084			
weight gain	0.7401	0.0253	0.4596		
length gain	0.2962	0.0052	0.2331	0.9809	
snipping	0.0000	0.0000	0.0000	0.1125	0.0219

Table 7. Effects of food and temperature levels on days required for full siphon regeneration

Food Level	Number of Subjects	Days Needed For Total Siphon Regeneration(1)
0	13	21.3 ±1.2
1	13	21.9 ±0.9
2	13	22.3 ±1.1

Temperature Used (°C)	Number of Subjects	Days Needed For Total Siphon Regeneration(1)
11	13	26.6 ±0.3
21	14	20.3 ±0.4
28	12	18.3 ±0.4

Temperature Used (° C)	Food Level	Number of Subjects	Mean Number of Days Needed For Total Siphon Regeneration(1)
11	0	4	27.2 ±0.6
11	1	4	26.2 ±0.5
11	2	5	26.6 ±0.7
21	0	5	19.8 ±0.9
21	1	5	20.6 ±0.5
21	2	4	20.7 ±0.5
28	0	4	17.2 ±0.5
28	1	4	19.2 ±0.9
28	2	4	18.5 ±0.6

(1)values reported are means and the standard error of the mean

Table 8. Effects of food and temperature levels on weight and length increases for both experimentals and controls

Food Level	Number of Subjects	Mean Weight Gain Among Subjects(1) (g)	Mean Length Gain Among Subjects (1) (mm)
0	27	0.3 ±0.1	0.1 ±0.1
1	27	0.2 ±0.1	0.1 ±0.1
2	26	0.3 ±0.1	0.1 ±0.1

Temperature Used (°C)	Number of Subjects	Mean Weight Gain Among Subjects(1) (g)	Mean Length Gain Among Subjects(1) (mm)
11	28	0.0 ±(2)	0.0 ±(2)
21	28	0.3 ±0.1	0.1 ±0.1
28	26	0.5 ±0.1	0.2 ±0.1

Temperature Used (° C)	Food Level	Number of Subjects	Mean Weight Gain Among Subjects(1) (g)	Mean Length Gain Among Subjects(1) (mm)
11	0	9	0.0 ±(2)	0.0 ±(2)
11	1	9	0.0 ±(2)	0.0 ±(2)
11	2	10	0.0 ±(2)	0.0 ±(2)
21	0	9	0.3 ±0.2	0.1 ±0.1
21	1	10	0.2 ±0.1	0.1 ±0.1
21	2	9	0.4 ±0.2	0.1 ±0.1
28	0	9	0.4 ±0.2	0.2 ±0.1
28	1	8	0.5 ±0.2	0.3 ±0.2
28	2	9	0.4 ±0.2	0.2 ±0.1

(1)values reported of means and the standard error of the mean
(2)No Observed Change; Standard Error Not Defined

Effects of Temperature

Analysis of the siphon regeneration (Table 7 and Figure 6) showed significant differences in required time for regeneration with respect to the three temperatures the clams were subjected to. The mean number of days taken for total siphon regeneration was 18.3 at 28oC, 20.3 at 21o C, and 26.6 at 11o C. The relationship of time and temperature was linear. The correlation coefficient was 0.9186 ($p = 0.0001$), showing that as temperature increased, the number of days needed for siphon regeneration decreased significantly (Table 7). This fits well with the fact that biological rates increase with increasing temperature up to a point. The increased growth could result from increased enzymatic activity as a result of changes in types of enzymes and modulation of pre-existing enzymes (Hochachka and Somero, 1973).

Increases in total clam weight and shell-length were also related to temperature (Table 8). With respect to weight gain, the correlation coefficient with temperature was 0.7401 with a p value of 0.0001, indicating a significant positive linear relationship between weight gain and temperature (Figure 7). By the end of experimentation, the mean weight increases found (including both experimental and control subjects) at the temperatures of 28o C and 21o C were 0.5 g and 0.3 g, respectively. No measurable weight gain was found in clams kept at 11o C (Figure 7 and Table 8). The overall mean weight increase at all temperatures in the experimental clams was 0.3 g, with a standard error of 0.1 (Table 9). For the controls, the increase was 0.2 g, with a standard error of 0.1 (The

Table 9. Weight, length, and mortality between snipped and control clams

	Controls	Snipped
Number of Clams	45	45
Proportion Died and Standard Error*	0.04 ±0.031	0.13 ±0.051
Mean and Standard Error of Weight Increase	0.2 g ±0.1	0.3 g ±0.1
Mean and Standard Error of Length Increase	0.1 mm ±0.0	0.1 mm ± 0.0

*proportion does not differ significantly for controls and experimentals using z-tests for proportions (See Dixon and Massey, 1983)

relationship between temperature and weight was linear; See Figure 7). Formation of new body tissue, combined with deposition of calcareous material to the shell, would account for the elevation in weight. Whether the flesh weight of the clam increased is unknown. However, flesh weight is roughly ten percent of total weight (shell plus soft tissues). Since the correlation coefficient of shell-length to total body weight was 0.5984 with p value of 0.0001, one might assume that increased shell-length was indicative of increased flesh weight in the clams (Tenore, 1968).

Using Sidak's T tests (Kirk, 1982), significant differences between the means of weight increases with regard to temperature were assessed. The mean weights of clams at 28°C and 21°C differed by only 0.1 g, which was insignificant (Table 8). However, the difference between means at 28°C and 11°C was 0.5 g, a significant difference. The difference in means of the clams at 21°C and 11°C was 0.3 g, also significant.

Shell-length growth was affected by temperature differences (Figure 8 and Table 8). At 28° C , a mean growth of 0.2 mm was recorded among all (experimental and control) clams. At 21° C, the mean was 0.1 mm. However, at 11° C, no measurable increase in shell-length was found. The correlation coefficient for the shell-length to temperature was 0.2962, with a p value of 0.0069 , indicating a significant linear relationship. For the experimental clams, the overall mean length increase in all temperatures was 0.1 mm, with a standard error of 0.0. The mean for the controls was found to be 0.1 mm, with a

standard error of 0.0 (Table 9). The difference between means for clams at 28°C and 11°C was 0.2 mm, which was significant using Sidak's multiple comparisons test. The difference between means from clams at temperatures 28°C and 21°C was 0.1 mm -- not significant. An insignificant difference in means was also found from clams in the 21°C and 11°C groups.

Effects of Food Levels

The food levels used [(0) 0 bacteria per ml{starvation}; (1) 2.5 x 10^5 bacteria per ml; (2) 1.0 x 10^6 bacteria per ml] had no significant effect on the time required for siphon regeneration in Rangia (Figure 9 and Table 7). The mean number of days required for the clams to completely regenerate their siphons at the three food levels were :21.3 days for the starved group, 21.9 days for the 1 ml of (1 X 10^9) Pseudomonas group, and 22.3 days for the 4 ml of (1 X 10^9) Pseudomonas group. Although fluctuations in weight between groups occurred (Figure 10), it was of such small magnitude as to be inconsequential. The correlation coefficient for the food-to-time relationship was 0.1084, with a p value of 0.5112, denoting a lack of significance in food amount's impact on the regeneration time requirement.

Food availability did not substantially affect overall weight gain in the clams (Table 6). Recorded weight gains are found in Figure 10. For the food effect on weight increase, a correlation coefficient of

0.0253 and p value of 0.8209 were found, indicating no significant impact by food. Surprisingly, starved clams showed a mean weight increase greater than that found among clams administered the 1 ml of bacteria, but lower than the mean from clams at the 4-ml-of-bacteria level. One explanation for this uneven increase could be measurement errors due to procedures in the data-gathering process, or some aspect of Pseudomonas. Another possibility for the similarity in results regarding food level may have been that the amounts of bacteria administered were not sufficient for optimal clam growth. One ml of 1 x 10^9 Pseudomonas aeruginosa equals approximately 1 mg (dry weight), and there are 5 cal/ml of the bacteria at that level (Christian, 1982). With this amount diluted to 1 x 10^6/ml and 2.5 x 10^5, the number of calories available to the clams would have been small. Whether this amount would have been sufficient would depend on the metabolic rates of the clams. At the higher temperature and food levels, 6 doublings of the bacterial populations over a period of 48 h could be expected, but this amount would provide a limited energy supply, i.e., about 160 cal/ml. Hughes (1970) found that the bivalve Scrobicularia plana had a caloric expenditure of 4.83 calories per each ml of oxygen consumed. Bayne et al (1976) discovered that the amount of oxygen utilized by the bivalve Mytilus californianus was 0.232 ml/hr/1 g dry flesh weight. If these readings are applied to the Rangia used in this study, one finds a daily caloric maintenance requirement of 26.4 calories. For 10 clams, this amount would total 264 calories per day. An additional possible explanation for the inconsistancies in the varied-temperature means in

weight gain is that the clams digest only the amount of food they require at the time, passing unnecessary particles through the digestive tract without digestion taking place, or merely reducing the water-intake rate. This selectivity is known to occur in many bivalves, so Rangia - in this respect - probably conforms. By use of the Sidak multiple comparisons method (Kirk, 1982), it was found that differences in means for weight increase were not significant between one food level to that of another food level. Between levels 2 and 0, the means did not measurably vary. When levels 2 and 1 were contrasted, the difference was 0.1 g. For the means from levels 1 and 0, the difference was 0.0 g.

Effects of a possible temperature, food, and snipping interaction on weight increases in the clam were found to be negligable in this research (Table 4). A p value of 0.8461 was calculated for F = 0.35 with 4 degrees of freedom .

The food levels used also did not have any significant impact on shell-length increases. The means of growth in length varied only minimally among the three levels (Figure 11 and Table 8). For food levels 0 and 1, a mean of 0.1 mm growth was noticed, whereas for level 2, at which the most food was provided, a mean of 0.1 mm was also calculated. No significant statistical difference among these means was found, using Sidak's multiple comparisons method. The correlation coefficient for the relationship of food level to length was 0.0052, and the p value was 0.9628. For experimental snipped clams, the mean length increase was 0.1 mm, with a standard error of 0.0. For the controls,

the mean was 0.1 mm, with a standard error of 0.0. No difference is seen. From this evidence, one can theorize that the clam is able to procede unabated with food and water intake regardless of a severely damaged siphon, since the unharmed clams showed little more increase in length despite being given the same amounts of food as the experimental clams.

As in the case of the weight increases, the length increase relationship to a possible temperature, food, and snipping interaction was found to be insignificant, with F = 0.35, 4 degrees of freedom, and a p value of 0.8442 (Table 5).

Snipping Effects

Snipping had no significant effect on the mortality of the clams, as determined by comparisions of the experimental and control groups (See Table 9). Among the fifteen clams snipped in 11° C water, there were two deaths (13.3% of all those snipped in those three tanks), whereas there were no deaths among the controls. In the 21° C water, there was one death each for the experimental and control groups (6.6%). In the 28° C tanks, there were three deaths among the snipped groups (20.0%) and one death among the control clams (6.6%). The differences in percentages may seem striking. The sample sizes, however, were small and lacking power. The differences, then, are not so meaningful. Had a larger sample size been used, the differences may have been significant. For experimental clams, the total proportion dead was 0.13, with a

standard error of 0.051, while for the controls, the total proportion was 0.04 with a standard error of 0.031. From these findings, one is led to believe that stress from the loss of the siphons is tolerated well by the clams, with shock of the initial wounding not sufficient to bring on death. However, possibility of infection of injured tissue is present. In nature, bacteria and viruses not commonly seen in the lab setting could make the siphon-snipped clam more vulnerable to infection, with an accompanying increase in mortality. Also, stress from continual siphon cropping performed by fish or other predators may affect the mortality rate. Further work needs to be done on this aspect of clam life. Since only two snippings were performed in this study, concrete conclusions about Rangia's tolerance of siphon loss are difficult to make.

In another study currently underway involving bacterial effects on Rangia (Dr. B. Kane, Dept. Environmental Health, ECU, personal communication, 1984), a lack of substrate is believed to have had an effect on Rangia mortality. Among clams in tanks without a substrate, high mortality was noted. Among those in tanks with a layer of silt present, mortality was lower. In this study, no substrate was used, but clam mortality was not high in any tank, regardless of temperature, food level, or performance of snipping. The mortality-substrate relationship may be a problem requiring resolution for future testing with Rangia.

Snipping was found to have little effect on the outcome of experimental clam weight gain and length increase (Tables 9 and 10). The mean weight among control and experimental clams differed by only

Table 10. Impact of snipping, alone and in conjunction with food and temperature levels

Clam Snipping (0=done; 1=omitted)	Number of Subjects	Mean Weight Gain Between Snippings (g) (1)	Mean Length Gain Between Snippings (mm) (1)
0	39	0.2 ±0.1	0.1 ±0.1
1	43	0.3 ±0.1	0.1 ±0.1

Food Level	Clam Snipping	Number of Subjects	Mean Weight Gain Between Snippings (g) (1)	Mean Length Gain Between Snippings (mm) (1)
0	0	14	0.2 ±0.1	0.0 ±0.0
0	1	13	0.3 ±0.1	0.1 ±0.1
1	0	14	0.2 ±0.1	0.1 ±0.1
1	1	13	0.2 ±0.1	0.1 ±0.1
2	0	15	0.2 ±0.1	0.1 ±0.1
2	1	13	0.4 ±0.1	0.1 ±0.1

Temperature used (o C)	Clam Snipping	Number of Subjects	Mean Weight Gain Between Snippings (g) (1)	Mean Length Gain Between Snippings (g) (1)
11	0	15	0.0 ±(2)	0.0 ±(2)
11	1	13	0.0 ±(2)	0.0 ±(2)
21	0	14	0.3 ±0.1	0.1 ±0.1
21	1	14	0.4 ±0.1	0.1 ±0.1
28	0	14	0.4 ±0.1	0.2 ±0.1
28	1	12	0.6 ±0.1	0.3 ±0.1

(1) values reported of means and the standard error of the mean
(2) No Observed Change; Standard Error Not Defined

0.1 g. The mean shell length growth between the two groups were not seen to vary. Also, Sidak's test showed no statistical significance in the differences between the means. No weight gain was measured at 11°C, thus the difference between means of experimental and control clams was 0 g. The difference calculated for the experimentals and controls at 21°C was 0.1 g, while the difference found at 28°C was 0.2 g. The test for the snipping-to-temperature interaction with weight was $F = 0.48$, with 2 degrees of freedom and p value 0.6227 (Table 4). The difference in mean lengths of growth between experimental and control clams at 11°C was 0 mm, since no increases were recorded for either group (Table 8). The difference between experimentals and controls at 21°C was 0.1 mm, and that for the clams at 28°C was 0.1 mm (Table 10). The reactions of the clams at the temperatures used were similar then, regardless of snipping. These results indicate that siphon-snipping in Rangia , done on the scale used in this research, did not have a major detrimental impact on the clam's abilities to get nourishment and grow. These results parallel Hodgson's study (1982) of the bivalve Scrobicularia plana to siphon-snipping, in which he found no appreciable effect on water pumping, valve movements, and heart rate during and after siphonal wounding.

Further siphon studies may be conducted to determine periods of siphon regrowth in Rangia over a time period more extensive than that used in this research. Variations of temperature and/or food levels could be utilized to ascertain more comprehensively the reaction of the clam regarding its siphon regeneration. This could involve snipping

of the structure at 10 to 15 day intervals under one set of conditions, then another snipping done after another time interval under other conditions. Perhaps this would allow detection of "spurts" of regrowth activity at differing times and states, as well as provide information on the clam's capacity to tolerate repeated siphon damage. Also, a bioenergetics study could be pursued to determine the caloric contribution of Rangia's siphon to a fish diet and to indicate the degree of importance its siphon may have as a food source for various fish species in the Pamlico estuary.

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