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The Effect of Temperature on the Growth of the Zebra Mussel, Dreissena Polymorpha (Pallas)

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THE EFFECT OF TEMPERATURE ON THE GROWTH OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA* (PALLAS)

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ABSTRACT

Dreissena polymorpha (Pallas), zebra mussels, are recent invaders of North American freshwater systems. They have a high reproductive rate and settle in high densities which can clog water intake valves and pipes. Many studies investigating the use of heat as a control measure have examined the effects of high temperatures on zebra mussel mortality. Much less is known about the effect of temperature on the actual growth rate and development of zebra mussels. This study examined the growth rates of zebra mussels at 10°C, 20°C, and 25°C over two four-week periods in the laboratory. Mussels were placed in culture dishes (five similarly sized mussels per dish) and fed 100 ml of the algae *Chlorella pyrenoidosa* (Chick) daily at a concentration of $4.13 * 10^5$ cells/ml. Shell length and shell height measurements were taken three times during the experiment. Although shell length is the measurement typically used in growth studies, it has not been documented whether increases in shell length are accurate indicators of increases in tissue weight. This study compared both shell length and shell height with tissue weight. Since the correlation between shell length and tissue weight ($r^2 = 0.811$) was slightly higher than that between shell height and tissue weight ($r^2 = 0.723$), shell length was used as the growth indicator in this study. Growth was significantly greater at 25°C than at either 10°C or 20°C during one two-week period (Scheffe's, $p < 0.05$). Mortality was also significantly greater at 25°C than at 10°C. The results from this study are important because factors which reduce growth should decrease the long-term success of the zebra mussels and therefore should be useful as possible control methods. Also, scientists working with zebra mussels in the laboratory will find the results of this study useful in determining the best conditions in which to raise zebra mussels.

ACKNOWLEDGEMENTS

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INTRODUCTION

Since its first sighting in North America in 1988 (Hebert *et al.*, 1989), *Dreissena polymorpha* (Pallas), the zebra mussel, has rapidly invaded much of North America's freshwater systems, including all five Great Lakes and the Mississippi, Illinois, Ohio, and Arkansas Rivers (Boydstun and Benson, 1993). The zebra mussel is believed to be a native of the Caspian or Black Sea region and was first introduced to the United States from Europe when ballast water was emptied from ships (Wiktor, 1963; as cited by Haag and Garton, 1992). Populations of zebra mussels typically occur in such high densities (100,000 mussels per square meter) that they can cause serious problems, including clogging or reducing flow through industrial water pipes, competing with native species for survival, and affecting the taste of drinking water (Lewandowski, 1976; O'Neill and MacNeil, 1991; Auger, 1993; LePage, 1993; Ohnesorg *et al.*, 1993).

The rapidity of the zebra mussel invasion has been attributed to its life history. Mature females, one year old and older, produce, on average, 30,000 to 40,000 eggs per year (O'Neill and MacNeill, 1991). On average, zebra mussels live 3.5 years, but can live up to ten years (O'Neill and MacNeill, 1991). Fertilization occurs externally in the water (McMahon, 1991). Then the fertilized eggs develop into veliger larvae, which feed on phytoplankton (O'Neill and MacNeill, 1991). The planktonic larvae float along with currents and are believed to aid in the dispersal of the zebra mussel population (Griffiths, 1990). After about three weeks, the veligers undergo metamorphosis and settle to the

bottom where they move around by propelling themselves with their foot (O'Neill and MacNeill, 1991). The filter-feeding adults eventually attach via byssal threads to any convenient solid substrate including metal, rocks, and the shells of other mussels (O'Neill and MacNeill, 1991).

Zebra mussels seem to be able to adapt and survive in a wide variety of conditions (high and low temperature, high and low salinity, high and low turbidity, fast and slow moving water, and at various depths), which makes them especially difficult to control (Kachanova, 1962 as cited by Morton, 1969; Stanczykowska, 1977; Mackie *et al.*, 1989; Dobson and Mackie, 1993; Kilgour and Kepple, 1993). One of the most common methods of controlling zebra mussels involves the use of heat (Harrington *et al.*, 1993; Neuhauser *et al.*, 1993). Kornobis (1977) found that lakes in Poland treated with heated water contained zebra mussel populations of smaller densities that were composed of smaller sized mussels than the mussel populations found in untreated lakes. He found the most dense populations in waters ranging from 20-25°C. Several studies have investigated mortality rates of zebra mussels at various temperatures in the laboratory. Nichols (1993) found increased mortality in adult zebra mussels at temperatures above 24°C. Other studies have demonstrated that adult zebra mussels can withstand heat up to 40°C (Mikheev, 1961; as cited by Morton, 1969). In *Mytilus edulis* L., the blue mussel, a marine bivalve with a similar life history to the zebra mussel, the upper lethal limit of temperature tolerance is 27°C to 30°C and the lower limit is -10°C to -15°C (Newell, 1989).

In most invertebrates, the temperature range at which growth occurs is much narrower than the temperature range which can be tolerated (Kinne, 1970). In the blue mussel, temperatures above 5°C are necessary for growth to occur (Newell, 1989). For invertebrate larvae, as temperature increases, growth rate increases up to a maximum point, above which the growth rate slows considerably (Pechenik, 1987). Several species of marine bivalve larvae have been reported to have maximum growth rates at 25°C to 26°C (Culliney *et al.*, 1975) whereas *D. polymorpha* larvae have been shown to have a maximum growth rate at 30°C (Robert *et al.*, 1988). In a study by Walz (1978), growth rates (measured in terms of dry shell weight, dry tissue weight of the tissue separated from the shell, and shell length) in zebra mussels at warmer, shallower depths were greater than those of mussels at colder, deeper depths. O'Neill and MacNeill (1991) reported that zebra mussels grow at temperatures between 7°C and 32°C in the field.

The optimum temperature range for adult zebra mussel growth has been demonstrated to be between 10° - 15°C (Mackie *et al.*, 1989). Growth rates have also been shown to be greater for smaller zebra mussels than for larger ones (Smit *et al.*, 1992; Nichols *et al.*, 1990). For this reason, small zebra mussels (< 6 mm in length) should be expected to show more growth during short-term projects than larger mussels.

The standard measure of growth used in zebra mussel studies has been shell length. Increased shell length of mollusks does not always reflect an equal increase in biomass (Pechenik, 1980, 1987; Bayne, 1983). In the blue mussel, initially, more resources are

allocated to shell growth than tissue growth (Newell, 1989). Later in development, resources shift toward tissue growth, and later still, resources are allocated for gamete production (Newell, 1989). Mussels at different stages of gonadal development also have different weights (Sprung and Borcherding, 1991). Therefore, the shell length of an individual zebra mussel would not always be the best measure of growth. Previous studies of the zebra mussel have only examined the relationship between shell length and total dry weight (shell and tissue weight) without measuring tissue weight (Haag and Garton, 1992). The total dry weight includes inorganic mass, and therefore does not provide a complete description of growth (Paine, 1964). The relationship between tissue weight (biomass) and shell length must be determined to accurately estimate the growth of zebra mussels.

Although shell length is an important indicator of growth, few studies have examined the use of shell height as a measure of growth in the zebra mussel (Fig. 1). Shell height has been utilized as an indicator of growth in other freshwater mussels (Bailey and Green, 1988). Moreover, growth studies with marine mollusks have also described the relationship between shell length, shell height, and biomass to estimate growth (Epifano *et al.*, 1976; Sprung, 1984; Lima and Pechenik, 1985; Dobberteen and Pechenik, 1987). Preliminary results in this laboratory have shown a possible relationship between shell height and dry tissue weight (Jill Brown, unpublished data).

In this study, shell length and shell height of zebra mussels were measured to determine their relationship with tissue weight.

If a linear relationship exists, shell length or shell height could then be used as a measure of growth in determining the effect of temperature on growth. For growth to occur, the proper amount of food was needed. Since zebra mussels feed by filtering particles from the water (O'Neill and MacNeill, 1991), measuring filtration rates would provide a guideline for how much food should be fed to the mussels each day. It was anticipated that growth would be more evident at higher temperatures up to an optimal point, and that these temperatures would provide a range at which zebra mussels could be raised successfully in the laboratory, as well as provide information for possibly controlling the spread of zebra mussels by limiting their growth.

Zebra Mussel Shell Length and Shell Height Dimensions

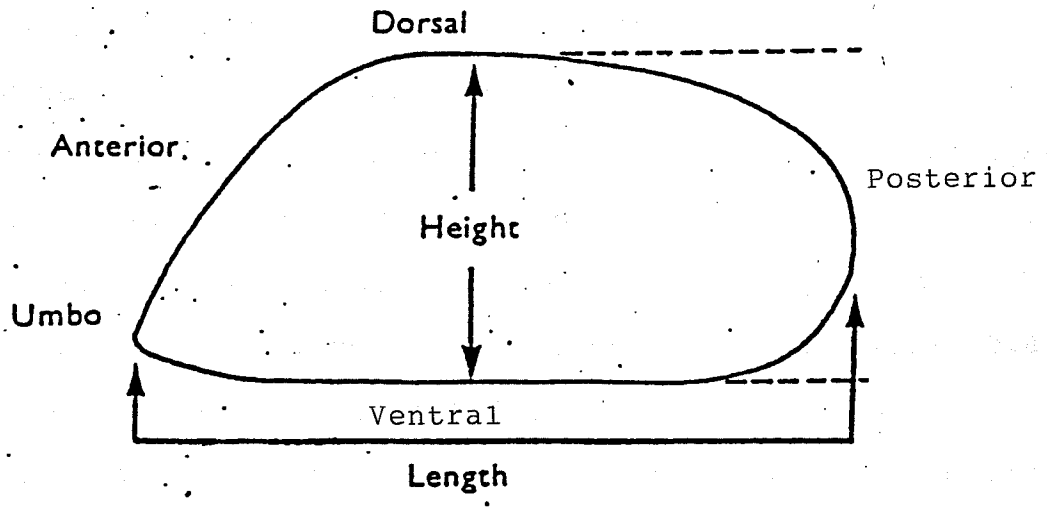

FIGURE 1. Shell length was measured as the maximum dimension between the umbo and the posterior end. Shell height was measured as the maximum dimension between the dorsal and ventral sides perpendicular to the shell length. Shell height and shell length were highly correlated (r^2= 0.974, $p < 0.05$). (Fig. from Seed 1968)

METHODS

Animals

Adult Dreissena polymorpha were collected in August 1992 from Lake Erie at Sterling State Park in Monroe, Michigan and in October 1992 from Lake Michigan at Diversey Harbor in Chicago, Illinois. They were packed moist in newspapers and transported on ice to the laboratory. The mussels were maintained in Percival incubators at 10°C with cycles of 12 hours of light and 12 hours of dark in 2.5-gallon aquaria containing water collected from Lake Evergreen in Bloomington, IL and filtered to 0.45 micrometers. Nitrites and ammonia were at acceptable levels (N < .1 mg/L, ammonia < 1ppm) as measured by water test kits. Mussels were fed daily (or as the water cleared) a diet of dried algae, *Chlorella* sp. (Nature's Herbs Better *Chlorella*), at a concentration of one capsule (0.410 g) diluted with 2500 ml of 0.45 µm filtered lake water. Dried *Chlorella* sp. had been previously demonstrated to support the survival and growth of zebra mussels (Nichols, 1993).

Filtration Rate

Filtration rate was determined by placing five mussels, ranging in length from four to six millimeters, in five separate 5 ml test tubes with 2 ml of live *Chlorella pyrenoidosa* (Chick) cultured in the lab (Culture Collection of Algae at the University of Texas at Austin). A previous study by de Kock and Bowner (1993) also used *C. pyrenoidosa* as food for zebra mussels. A test tube containing the same concentration of *C. pyrenoidosa* as in the feeding solution, but without a mussel was used as a control. The initial algal cell density was measured with a hemacytometer. The

tubes were then placed at 10°C. After thirty minutes, the final cell density was determined for each tube using a hemacytometer. The average filtration rate was calculated using the equation given by Pechenik and Fisher (1979).

$$Y\ \frac{(ml)}{(animal)} = \frac{(C_o - C_f) * (\text{ml of suspension in tube})}{(\#\text{ of animals}) * (\text{duration of experiment})} * 24\ \text{hour}$$

In this equation, Y is the average filtration rate, C_o is the initial concentration of algae, and C_f is the final concentration of algae. The filtration rate calculated is the maximum amount of feeding solution that the mussels can filter in one day.

Influence of temperature on growth

One hundred five small mussels (< 11 mm in length) collected from Lake Erie and Lake Michigan were removed from the aquaria at 10°C in November and placed in fresh 0.45 µm filtered lake water in 250 ml culture dishes overnight also at 10°C. The wet weights of individual mussels were determined with a Mettler M-3 pan micro-balance. Using a Fowler Dial caliper, shell length and shell height were measured as described by Seed (1968) (Fig. 1). Mussels of similar size were then placed five to a dish in 250-ml culture dishes with 100 ml of feeding solution. Seven dishes were stacked and placed in Percival incubators with 12-hour light and dark cycles at each temperature (10°C, 20°C, and 25°C).

The mussels were fed *C. pyrenoidosa* that had been cultured in the lab. Algal cell densities were determined using a hemacytometer to calculate the amount of algae needed to obtain the desired concentration for feeding ($4.13 * 10^5$ cells/ml of lake water). This amount is less than the maximum amount of algae that

the *D. polymorpha* can filter from the water as determined through the filtration experiments described above. However, studies have shown that a lower concentration of food might be more beneficial to the mussels' long-term survival. Reid (1983) found that many bivalves do not feed constantly. Hawkins and Bayne (1992) determined that excess food filtered, but not digested, by mussels leads to the production of pseudofeces which can reduce the amount of oxygen in the water. Therefore a feeding solution with a lower concentration of algae than the maximum amount that can be filtered in twenty-four hours should improve overall survival.

The amount of algae needed in the feeding solution was determined using the following calculation:

$$\frac{100 \text{ ml} * 5 \text{ animals} * 4.13 * 10^5 \text{ cells/100 ml water}}{\text{concentration of algae (in cells/ml)}} = \frac{\text{ml algae}}{\text{100 ml water}}$$

Each day, 100 ml of feeding solution were added to each dish. Every two days, the water was changed and the dishes replaced to eliminate waste buildup. Dishes were checked daily for dead mussels which were removed as noticed.

After two weeks, the mussels were weighed and measured again and returned to the incubators. After four weeks, the mussels were fixed in 95% ethanol to preserve them for later measurement. Wet weights, shell lengths, and shell heights were measured and then the mussels were placed in a drying oven at 65°C. After 24 hours, the total dry weights were determined.

The experiment was repeated a second time (Experiment II), beginning in February, using the same procedure outlined above for Experiment I except for the following changes. Only mussels

collected from Lake Michigan were used because of an incubator malfunction in August (before the Lake Michigan mussels had been collected) which resulted in high temperatures that may have stressed the Lake Erie mussels. Many mussels collected from Lake Erie died in the aquaria maintained at 10°C after the incubator malfunction. The mussels were maintained in Springdale Spring Water during Experiment II instead of lake water due to the high levels of ammonia and nitrites in Lake Evergreen at that time of year (ammonia > 2 ppm, nitrites > 0.25 mg N/liter water; water test kits). At the termination of the experiment, mussels were fixed in 10% buffered formalin for future use instead of ethanol because in long-term storage, ethanol may break down lipids and proteins in tissue (Humason, 1972). Twenty-two of these mussels were then dried and combusted to determine ash-free dry weights (see below).

Estimate of growth

Random samples of mussels were taken from the 2.5 gallon aquaria maintained at 10°C and weighed and measured as previously described. The sampled mussels were fixed and preserved in 10% buffered formalin for later use in ash weight determinations. These mussels and twenty-two from the second temperature experiment (see above) were then dried for 48 hours at 65°C in a drying oven and reweighed. They were then ashed for 12 hours at 500°C in a muffle furnace and weighed again. The tissue weight was calculated by the following equation:

total dry weight - ash weight = tissue weight

using the weights obtained in the drying and ashing procedures.

Growth rates for the temperature experiments were defined as

shown below.

$$\text{Growth rate} = \frac{\text{shell length}_2 - \text{shell length}_1}{\text{time in days}}$$

Mean shell lengths from each dish were used to determine the growth rate at each temperature. Separate growth rates were determined for the first two weeks of the experiment, the second two weeks of the experiment, and the entire four-week experiment.

Statistical analyses

The relationships between tissue weight and shell length, and tissue weight and shell height were determined using regression analysis. Regression analyses of the logarithmic transformation of these data were conducted to confirm these relationships. One-way analysis of variance was performed ($\alpha = 0.05$) to compare growth rates and mortality at 10°C, 20°C, and 25°C. Pairwise comparisons between means were made using Scheffe's multiple comparison test ($\alpha = 0.05$) as appropriate. All statistical tests were conducted using the Statistical Package for the Social Sciences - PC version (Norusis, 1990).

RESULTS

Shell length vs. shell height as an indicator of growth

An increase in tissue weight was reflected by both an increase in shell length and shell height. Both shell length and shell height showed strong relationships with tissue weight (Fig. 2 and 3). More of the variation in tissue weight was explained by the relationship with shell length ($r^2 = 0.811$, $N = 35$) than with shell height ($r^2 = 0.723$, $N = 35$). Regression analyses of log transformations of the data confirmed these relationships (for length vs. tissue weight: $r^2 = 0.982$, $N = 35$; for height vs. tissue weight: $r^2 = 0.966$, $N = 35$). Length and height also showed a strong relationship with each other ($r^2 = 0.974$, $N = 35$).

Relationship between temperature and growth

The combined data for both Experiment I and Experiment II demonstrated that mean shell lengths and mean shell heights did not increase at either 10°C or 25°C (Table I). Moreover, at 20°C, mean shell length increased, though not significantly ($p > 0.05$), but mean shell height decreased. Since the incubator malfunction could have affected the results of Experiment I, Experiment II was analyzed separately. Data from Experiment II alone indicated, in general, slight increases in mean shell lengths at all three temperatures and small increases in mean shell heights at both 20°C and 25°C (Table II, Fig. 4 and 5). No significant differences in shell length were evident over any time period at any temperature for Experiment II (ANOVA, $p > 0.05$).

No significant differences were found between growth rates from each dish from Experiment I at any temperature for any time

period -- the first two weeks, second two weeks, or the entire four-week experiment -- (Table III, ANOVA, $p > 0.05$). Growth rates from Experiment II were found to be significantly higher at 25°C than at either 10°C or 20°C for the first two weeks of the experiment (Table IV, Scheffe's, $p < 0.05$). No significant differences occurred between any temperatures during the other time periods in Experiment II (Table IV, ANOVA, $p > 0.05$).

Relationship between mortality and temperature

Mortality was much higher overall in Experiment I than Experiment II. Thirty-nine mussels (38%) were found dead during Experiment I while eleven mussels (10%) died during Experiment II. In both experiments, mortality increased at higher temperatures (Fig. 6). In Experiment II, mortality at 25°C was significantly greater than at 10°C (Scheffe's, $p < 0.05$). No significant differences were observed at the other temperatures in Experiment II or between any temperatures for the combined data from both experiments (ANOVA, $p > 0.05$).

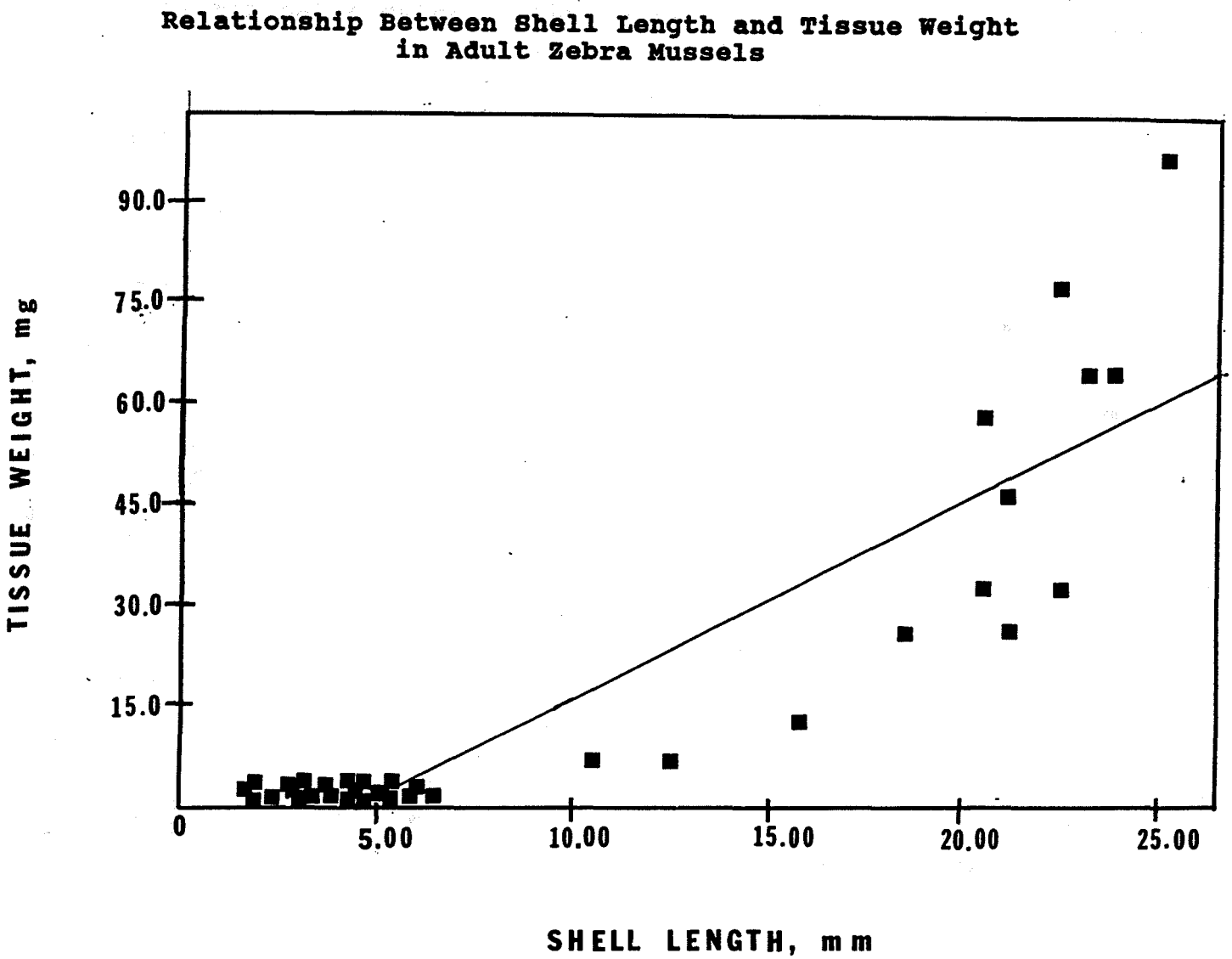

FIGURE 2. Shell length showed a strong relationship with tissue weight (r^2=0.81065, N=35, y = 3.01401x - 13.0826). Tissue weight was determined by subtracting ash weight from total dry weight. The linear relationship was confirmed by regression analysis on the logarithmically transformed data.

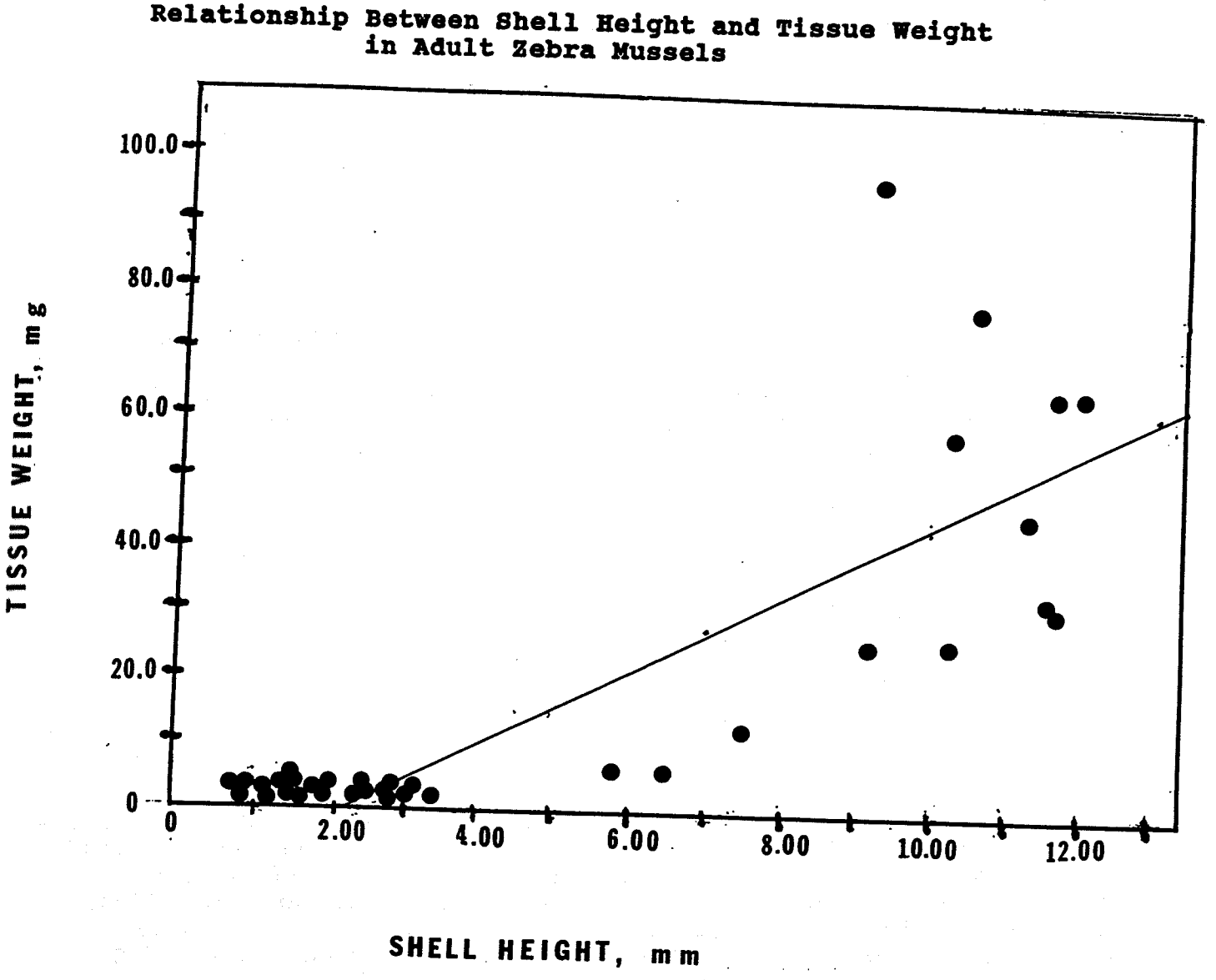

FIGURE 3. Shell height showed a strong relationship with tissue weight (r^2=0.72296, N=35, y=0.12983x + 2.93757). Tissue weight was determined by subtracting ash weight from total dry weight. The linear relationship was confirmed by regression analysis on the logarithmically transformed data.

Mean Shell Length and Height at 10°C, 20°C, and 25°C
at Two-Week Intervals for Experiment I and II Combined

SHELL LENGTH

Temp., °C	Week	Mean Shell Length (+/- S.D.), mm	N
10	0	4.60 (2.09)	70
	2	4.52 (2.07)	66
	4	4.38 (2.06)	62
20	0	3.75 (1.57)	67
	2	3.76 (1.63)	53
	4	3.79 (1.61)	46
25	0	4.06 (2.03)	70
	2	3.91 (1.51)	49
	4	4.01 (1.34)	45

SHELL HEIGHT

Temp., °C	Week	Mean Shell Height (+/- S.D.), mm	N
10	0	2.44 (1.00)	70
	2	2.40 (0.99)	66
	4	2.31 (1.00)	62
20	0	2.04 (0.75)	67
	2	2.00 (0.77)	53
	4	2.02 (0.74)	46
25	0	2.14 (0.96)	70
	2	2.08 (0.75)	49
	4	2.12 (0.65)	45

TABLE I. Mean shell length and shell height at Week 0 (initial), Week 2, and Week 4 (final) for zebra mussels in Experiment I and II combined. Slight decreases in mean shell height and mean shell length were obtained at some temperatures because of the high mortality rates in Experiment I. Note that the initial N value at 20°C is less than at the other temperatures because 3 mussels were crushed during handling when measuring the mussels at the beginning of Experiment I.

Mean Shell Length and Height at 10°C, 20°C, and 25°C at Two-Week Intervals for Experiment II

SHELL LENGTH

Temp.,°C	Week	Mean Shell Length (+/- S.D.), mm	N
10	0	4.05 (1.68)	35
	2	4.06 (1.66)	35
	4	4.06 (1.68)	35
20	0	3.61 (1.74)	35
	2	3.64 (1.72)	34
	4	3.79 (1.68)	32
25	0	3.69 (1.78)	35
	2	4.16 (1.63)	26
	4	4.32 (1.63)	24

SHELL HEIGHT

Temp.,°C	Week	Mean Shell Height (+/- S.D.), mm	N
10	0	2.15 (0.78)	35
	2	2.15 (0.79)	35
	4	2.11 (0.79)	35
20	0	1.94 (0.81)	35
	2	1.91 (0.77)	34
	4	1.99 (0.74)	32
25	0	1.95 (0.83)	35
	2	2.18 (0.79)	26
	4	2.26 (0.78)	24

TABLE II. Mean shell length and shell height at Week 0 (initial), Week 2, and Week 4 (final) for zebra mussels in Experiment II. A slight increase in mean shell length was demonstrated at all three temperatures, and a small increase in mean shell height occurred at 20°C and 25°C. No significant difference was demonstrated between mean shell lengths at any time period for any temperature (ANOVA, $p > 0.05$).

Mean Shell Length of Adult Zebra Mussels at Two-Week Intervals at 10°C, 20°C, and 25°C

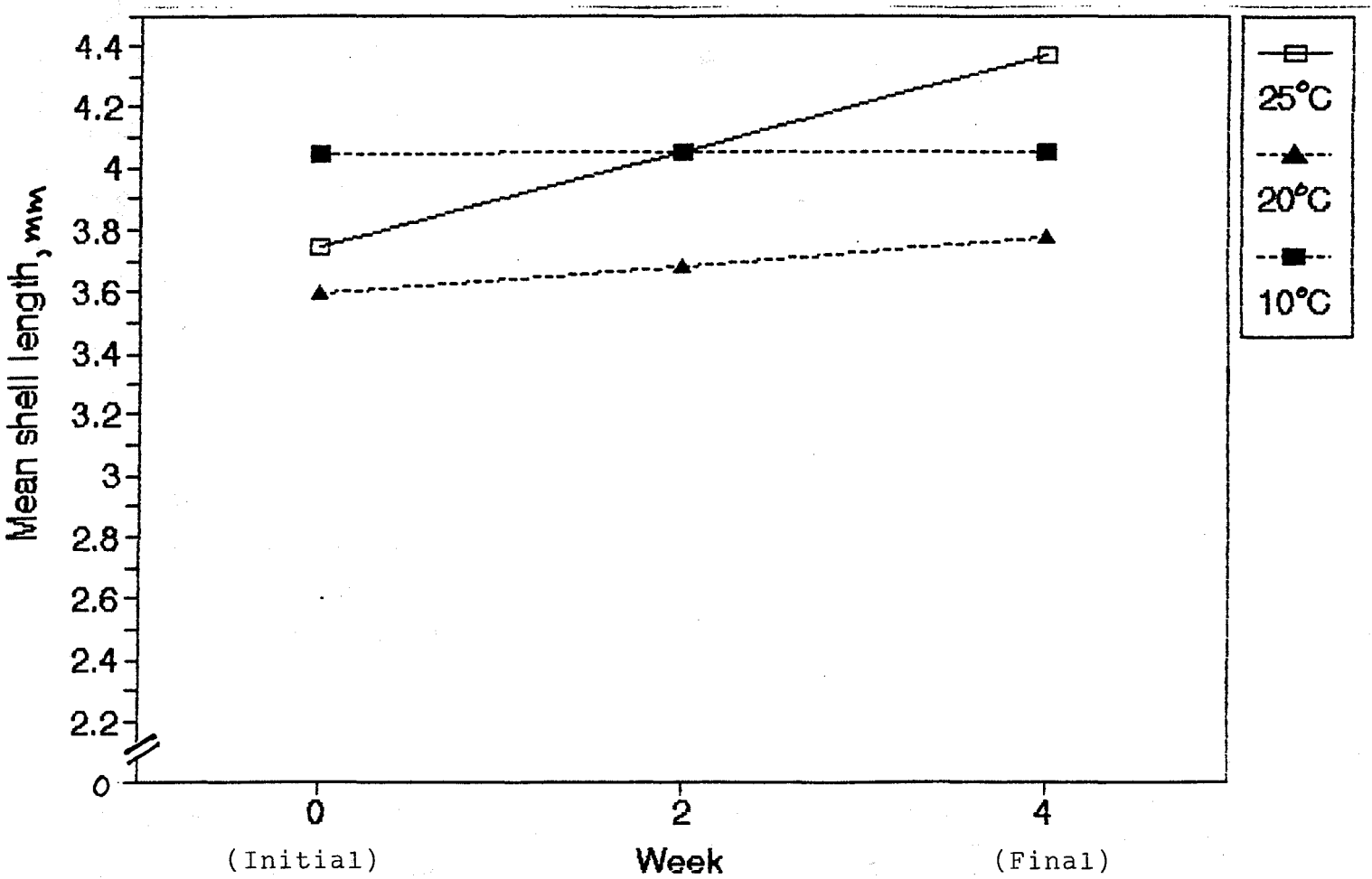

FIGURE 4. The mean shell length of zebra mussels in Experiment II showed the largest increase at 25°C. At 10°, no increase was found, and at 20°C, only a slight increase occurred. The standard deviation of all points is less than +/- 1.8 mm.

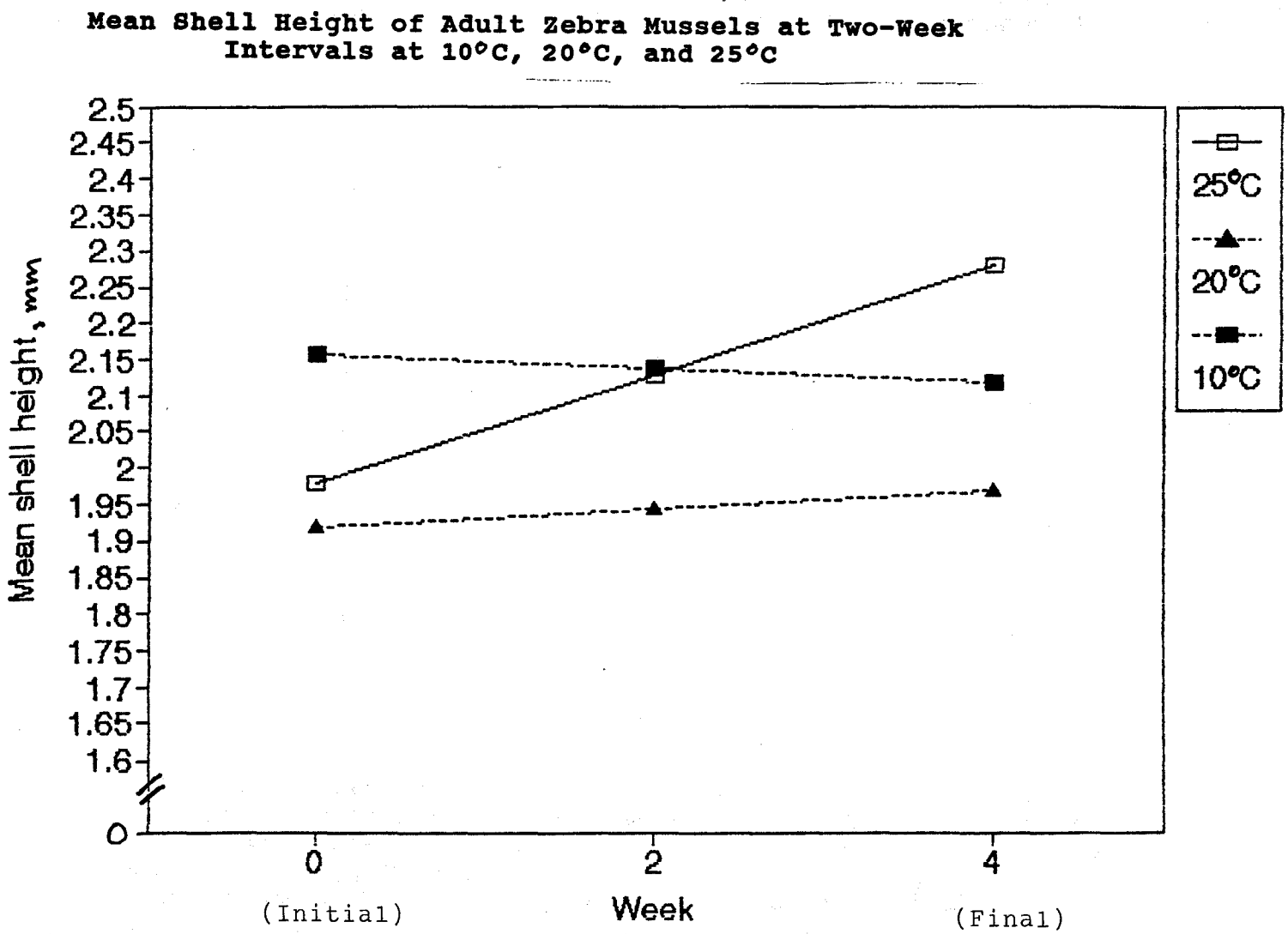

FIGURE 5. Mean shell height of zebra mussels at 10°C, 20°C, and 25°C from Experiment II was measured at two-week intervals. Mean shell height showed the greatest increase at 25°C. For each point (N = 35) the standard deviation is less than +/- 0.8.

Zebra Mussel Growth Rates from Experiment I at 10°C, 20°C, and 25°C over a Four-Week Period

		Growth in mm/day		
Temp., °C	Dish	Growth Rt1	Growth Rt2	Overall Growth Rt
10	1	-.02	.00	-.01
	2	.02	.00	.01
	3	.00	.05	.03
	4	-.01	.00	.00
	5	.00	.00	.00
	6	.00	.00	.00
	7	.00	.00	.00
20	1	.00	-.01	.00
	2	.06	-.38	-.16
	3	.01	.00	.00
	4	.00	.02	.01
	5	.02	-.08	-.03
	6	.02	.05	.03
	7	.02	.01	.02
25	1	-.01	.02	.01
	2	.00	.01	.01
	3	.00	.01	.00
	4	-.47	.00	-.23
	5	.02	.02	.02
	6	-.01	-.59	-.30
	7	.03	.04	.04

TABLE III. Growth rates were calculated by subtracting mean shell length per dish at time 1 from time 2 and dividing by the number of days. Growth Rt1=(mean shell length at week 2 - initial mean shell length)/14 days), Growth rt2=(final mean shell length - mean shell length at week 2)/14 days, Overall growth rt=(final mean shell length - initial mean shell length)/28 days. No significant differences in growth rates occurred between any of the three temperatures (ANOVA, $p > 0.05$).

Zebra Mussel Growth Rates from Experiment II at 10°C, 20°C, and 25°C over a Four-Week Period

Temp.,°C	Dish	Growth in mm/day Growth Rt1	Growth Rt2	Overall Growth Rt
10	1	.00	-.35	-.18
	2	.01	.00	.00
	3	.00	.00	.00
	4	.00	-.01	-.01
	5	.00	.00	.00
	6	.00	.01	.01
	7	.00	.00	.00
20	1	.01	.00	.00
	2	.00	.00	.00
	3	-.01	.00	.00
	4	.00	.00	.00
	5	.00	.00	.00
	6	.00	.01	.01
	7	.00	.01	.00
25	1	.00	.00	.00
	2	.00	.00	.00
	3	.00	.00	.00
	4	.01	.01	.01
	5	.01	.01	.01
	6	.03	.01	.02
	7	.02	.00	.01

TABLE IV. Growth rates were calculated by subtracting mean shell length per dish at time 1 from time 2 and dividing by the number of days. Growth Rt1=(mean shell length at week 2 - initial mean shell length)/14 days), Growth rt2=(final mean shell length - mean shell length at week 2)/14 days, Overall growth rt=(final mean shell length - initial mean shell length)/28 days. Significantly higher growth rates occurred at 25°C than at 10°C and 20°C in the first two weeks of the experiment (Scheffe's test, $p < 0.05$, $F = 9.76$).

Zebra Mussel Mortality Levels at 10°C, 20°C, and 25°C

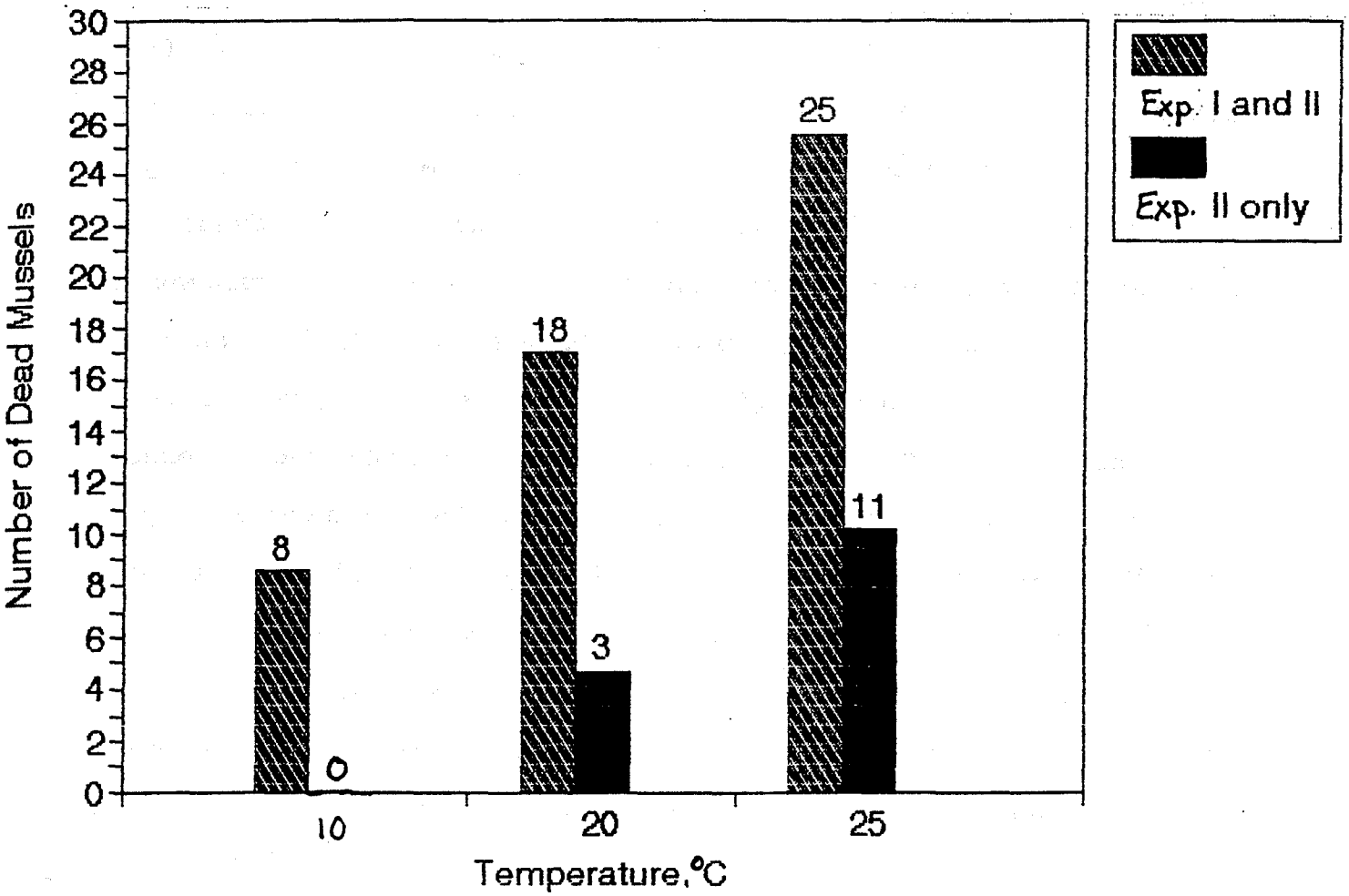

FIGURE 6. The number of dead mussels was greater at higher temperatures. Experiment I had higher levels of mortality overall (38% to 10% for Experiment II). In Experiment II, significantly more mussels died at 25°C than at 10°C (Scheffe's, $p < 0.05$).

DISCUSSION

A linear relationship between shell length and tissue weight was determined from the results of this study. These results will increase the validity of growth estimates in zebra mussel growth studies. Methods used to measure growth have included wet weight, shell length, and dry tissue weight (Kornobis, 1977; Walz, 1978; Smit *et al.*, 1992). Studies that use measurements such as shell length are not the most accurate investigations of growth unless a relationship between these measures and biomass (tissue weight) can be demonstrated. Both shell length and shell height were related to tissue weight in this study. However, shell length ($r^2 = 0.811$) accounted for more of the variability in the relationship with tissue weight than did shell height ($r^2 = 0.723$). Since shell length accurately reflects tissue weight, it can confidently be used as an indicator of growth in zebra mussels. Other studies of mollusks have found a similar linear relationship between shell length and tissue weight (Sprung, 1984; Lima and Pechenik, 1985). These data validate the results of field studies which only measured shell length when determining zebra mussel growth (Nichols *et al.*, 1990; Smit *et al.*, 1992; Sprung, 1992). We are currently expanding our study to include mussels from a wider range of size.

The strong relationship between shell length and shell height ($r^2 = 0.974$) found in this study is not surprising since bivalves have been shown to have growth bands around the shell (Farrow, 1971). Such growth bands indicate that the shells of mussels do not grow in length only. Although molluscan growth bands do not always occur in equal numbers around the entire shell, band width

compensates to keep the shell somewhat uniform (Gruffydd, 1981). In other words, shell height usually increases when shell length increases. Seed (1968) reported that in *Mytilus edulis*, increases in height began to level off as a mussel aged, but increases in length continued. This suggests that shell length and shell height measurements from larger zebra mussels may not show as strong of a relationship with each other as found in this study.

Shell height was also demonstrated to be a valuable estimate of growth in our study. However, since shell length was more strongly related to total biomass, the dimension of shell length was used as an indicator of growth instead of shell height. In this study, two experiments were conducted comparing the growth rates of adult zebra mussels maintained at 10°C, 20°C, and 25°C.

Temperature is an important part of any living organism's environment. Different temperatures result in different feeding, assimilation, and respiration rates due to the influence of temperature on the chemical reactions underlying these activities (Pechenik, 1987). An optimal temperature exists for each organism at which the chemical reactions necessary to sustain life occur most efficiently (Hainsworth, 1981). The maximum growth rate, which would be ideal for successful competition and reproduction, occurs at the optimal temperature. This study was designed to identify the optimal temperature for growth in the zebra mussel.

Some problems occurred in Experiment I which could have affected the data. Mean shell length did not increase significantly when data from both experiments were combined (ANOVA,

$p > 0.05$). Experiment I did not show significant differences in growth rate at any temperature (Table III, ANOVA, $p > 0.05$). In fact, actual shell dimensions decreased slightly at 10°C and 25°C due to the high level of mortality in Experiment I that reduced the total sample size. Mortality was much greater in Experiment I than in Experiment II (39 dead versus 11 dead). This represents a 38% mortality rate in Experiment I and a 10% mortality rate in Experiment II. An incubator malfunction in August resulted in an elevation of the temperature that may have stressed the Lake Erie mussels while they were being maintained at 10°C. The mussels from Lake Michigan were collected after this incident and, thus, were not affected. Higher mortality was evident in the aquaria at 10°C containing mussels collected from Lake Erie than in the aquaria with mussels from Lake Michigan. Also, for the first few days of Experiment I, water in the dishes was emptied, but the dishes were not replaced with clean dishes every other day. This could have allowed nitrogenous wastes to accumulate and may have also decreased the oxygen content of the water, resulting in reduced growth and higher levels of mortality. For these reasons, data from Experiment I were excluded from the growth analysis.

Mean shell length increased slightly at all three temperatures in Experiment II, while a small increase in mean shell height was observed at 20°C and 25°C. The growth rate for the first two weeks was significantly greater at 25°C than at either 10°C or 20°C (Scheffe's, $p < 0.05$). These data do not support the report that the optimum growth range of the zebra mussel in the field is from 10°C to 15°C (Mackie *et al.*, 1989). However, the growth in shell

length at all three temperatures tested in our experiment supports the observations of the temperature range for growth in European mussels of 7°C to 32°C (O'Neill and MacNeill, 1991). The growth rates observed in our study were much less than the 53% average weekly growth observed in the ongoing field study by Nichols *et al.* (1990). The growth rates obtained for Experiment II (-.01 to .03 mm per day) are similar to those obtained in a field study by Mackie (1993). In his study, zebra mussels born in the spring exhibited a growth rate of .126 mm per day over a 194-day period and zebra mussels born in the fall showed a growth rate of .100 mm per day over the same length of time.

Studies conducted in the field have shown that increased temperature during the summer resulted in decreased food availability which led to a decrease in zebra mussel growth (Walz, 1978). This phenomenon did not occur in our study because food concentration was kept constant at all three temperatures. Consequently our study showed an increase in growth at higher temperatures.

In our study, significant differences in growth rate were only exhibited in Experiment II during the first two-week period ($p < 0.05$). The growth rates were very low at all temperatures (Table IV) although similar to those determined from other studies (Mackie, 1993). The length of time over which growth is measured can have an effect on the growth rate (Yamaguchi, 1975). Therefore, lengthening the time period over which our study was conducted could result in greater changes in size at all temperatures. Previous studies of zebra mussel have generally

examined growth over longer time periods and have been able to measure larger increments of growth (Stanczykowska, 1977; Walz, 1978; Sprung, 1992). An optimum temperature for growth cannot be predicted from our data. In order for an optimum temperature for growth to be determined, this study would have to be extended over a longer period of time in order to examine how long-term growth of zebra mussels is affected by temperature.

Temperatures above or below the optimal temperature approach the limits to the range which can be tolerated by an organism. High temperatures can cause death in animals by denaturing essential proteins, inactivating enzymes, increasing the demand for oxygen, affecting metabolic rates, and changing membrane structure (Schmidt-Nielsen, 1990). In this study, mortality was much higher in Experiment I than Experiment II which could have been the result of stress from high temperatures when an incubator malfunctioned during maintenance at 10°C. In Experiment II, mortality at 25°C was significantly higher (ANOVA, Scheffe's test, $p < 0.05$) than at 10°C. These results are consistent with the study by Nichols (1993) which reported increased mortality in zebra mussels in the lab at temperatures above 24°C.

Other studies suggest that temperature tolerance in bivalves is dependent on how they were acclimated to the temperatures being tested (Bayne et al., 1977). This could explain the difference observed in mortality between the mussels at 25°C and those at 10°C since the mussels were stored at 10°C until the study began. Mortality could also have been the result of stress due to handling when the dishes were changed or when water was added each day.

However, Nichols (1993) reported that zebra mussels were not affected by repeated handling in the lab. Also, since all of the dishes were treated similarly, any handling effects should be the same at all temperatures.

The results of this study are unusual in that the maximum growth rate and the highest mortality level occurred at the same temperature. The reasons for this are unknown, but could be explained if 25°C was close to the upper level of temperature tolerance. A wider range of temperatures need to be studied in order to have a better indication of the optimum temperature for growth.

The results from this study are important in the continuing effort to understand the biology of zebra mussels. The correlation between shell length and tissue weight provides an easy way to measure growth in the field without sacrificing the subjects. Moreover, if an optimum temperature could be determined, new thermal control measures would be possible. Heat treatments above the optimal temperature should reduce growth rates and the long-term success of zebra mussel populations. The extremely high temperatures needed to kill large zebra mussel populations cannot be used in all circumstances since some industrial plants do not have the ability to flush water of that temperature through their pipes, or have sensitive equipment which might be damaged during the process (Harrington *et al.*, 1993). Therefore, a control method based on optimal temperature would provide an alternative. In addition, knowing the optimum temperatures for growth would assist scientists studying zebra mussels in the laboratory and the field.

Information on the range of temperatures at which growth can occur will help in predicting where zebra mussels can potentially colonize so that these areas can prepare for the upcoming invasion.

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