# THE EFFECT OF PLANT STEROLS ON THE GROWTH, SURVIVAL, AND STEROL LEVELS IN *MACROBRACHIUM ROSENBERGII*

by

Cynthia Soule

Biology Department

Submitted in Partial Fulfillment of the Requirements of
the University Undergraduate Fellows Program

1976-1977

Approved by:

Robert W. Brick

Dr. Robert W. Brick

May 1977

# ABSTRACT

Growth in Macrobrachium rosenbergii was studied using diets in which plant sterol was substituted for cholesterol. Weight changes, mortality, molt cycle, and sterol levels were observed. Results of a study of the effect of increasing the percentage of plant sterol in the diets were inconclusive due to cannibalism among experimental animals. In a subsequent study, all animals were held in separate containers. In that study, a level of 0.5% plant sterol showed no signigicant difference in weight change or molting cycle from the same amount of cholesterol in a prepared diet fed the shrimp. Plant sterols seemed to successfully substitute for cholesterol in this study.

# ACKNOWLEDGEMENTS

The author wishes to express her most sincere gratitude to Dr. Robert W. Brick whose assistance, encouragement, and very capable supervision were invaluable in the completion of the work.

Sincere thanks are expressed to Dr. Jerry Darsie for his guidance, support, and supervision which were gratefully received.

Thankful acknowledgement is also made of Dr. Raymond Reiser, whose suggestions have been extremely valuable.

This paper is dedicated to the late Dr. Bryant F. Cobb, who first suggested the research program, and whose encouragement and inspiration will always remain in loving memory.

TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# INTRODUCTION

Recent studies have shown that crustaceans are unable to synthesize sterols and must receive a dietary sterol for growth (1-4). Previous work with dietary cholesterol in crustaceans has shown it to be a precursor of vitamin D (5), sexual hormones (6) and the molting hormone, ecdysone (7). Cholesterol is a structural component of the hypodermis and has been shown to vary during the molting cycle (8-9). Numerous insects have been shown to be able to convert plant sterols, or phytosterols into cholesterol. The Virginia pine sawfly, Neodiprion pratti (10), the tobacco-tomato hornworm, Protoparca sexta (11), and the German cockroach Blatella germanica (1) all have shown the ability to convert various phytosterols such as ergosterol, stigmasterol, B-sitosterol, or campsterol into cholesterol or 7-dehydrocholesterol. Studies on Crustacea include work with the brine shrimp, Artemia salina (12), the crab, Portunus trituberculatus (4), and the shrimp, Penaeus japonicus (4). In the latter study, the phytosterols, ergosterol, stigmasterol and B-sitosterol, were fed to the shrimp to compare their use with that of dietary cholesterol on growth and survival. Survival rates were similiar though growth rates on the phytosterol diet were inferior (4). The amount of phytosterols fed was 0.5 grams per 100 grams diet, which Kanazawa found to be the

---

the citations on the following pages follow the style of the Journal of Nutrition.

dietary requirement of cholesterol in Crustacea. The effect of increasing levels of phytosterols has not been thoroughly investigated.

In this study, phytosterols were substituted for cholesterol in the diet of the freshwater shrimp, Macrobrachium rosenbergii. Growth rate, mortality, length of molt cycle and total sterol level of shrimp tail muscle were parameters compared in shrimp fed phytosterol diets, a diet containing cholesterol and a commercial diet (Ralston-Purina, M-25). These parameters determined whether or not plant sterols could be successfully substituted for cholesterol in a shrimp diet. Increasing levels of plant sterol were used in a preliminary experiment to determine a level of plant sterols needed to maintain maximum growth and/or survival.

The freshwater shrimp, M. rosenbergii, is an omnivorous shrimp commercially farmed for food in Southeast Asia and in Hawaii (13). Its culture potential in the United States is currently being evaluated. It lives in brackish to fresh water, and has a rapid growth rate, often attaining 22 centimeters in length, and 120 grams in weight. Studies were carried out on this shrimp to determine if this species can convert phytosterols to cholesterol, and to observe the effects on its growth, molting rate, and sterol level. This information would be relevant to the commercial manufacturer of feed for this potentially important species of shrimp.

# MATERIALS AND METHODS

Juvenile Macrobrachium rosenbergii, ranging in size from 3.0 to 10.0 grams, were used as experimental animals, obtained from the Texas Agricultural Experimental Station, Aquaculture Research Center. The animals were held in 30-gallon aquaria under controlled conditions of temperature (26°C), lighting (12/12), and salinity (0.5-1.0ppt). The substrate was oyster-shell gravel.

EXPERIMENT I. M. rosenbergii were fed a diet which contained increasing levels of plant sterols in place of cholesterol. These levels were 0.0, 0.1, 0.3, and 0.5% plant sterol, with 0.5% cholesterol as a control. (See Table 1). In the diet preparation, plant sterols were added at the expense of the corn starch, and they were dissolved in the lard. The mixture was cooked at 100°C for 30 minutes. The shrimp were held for one month in five 30-gallon aquaria, each divided into three compartments. Initial weight was recorded, at two weeks, and at one month. Feeding rate was 8.0% biomass daily, and excess food was removed as far as possible. Mean weight change, molting cycle interval, and percent mortality were determined at the end of the one-month period. Mean weight change was found by subtracting initial weight from final weight. Percent mortality was found by the formula: $\frac{\#\text{ of deaths}}{\#\text{ of initial animals}} \times 100$. Molting cycle was the number of days between molts.

EXPERIMENT 2. In this study, the diet from the previous experiment was reduced and the 0.1 and 0.3% plant sterol diets were eliminated due to lack of experimental animals. An additional diet was introduced which was a commercial food diet later analyzed to have 0.8% sterol. Due to aggressiveness and cannibalism in the previous experiment and the resulting high mortality rates, the shrimp were placed in small cylindrical nettings which kept them isolated. The feeding rate was reduced to 6.0% body-weight daily due to a build-up of excess food at higher feeding levels. The observation time was extended to two months to observe greater growth rates. Lengths were observed as well as weights during this period, and taken initially, at one month, and at two months. Mean weight change was found as in Experiment 1, and percent growth rates were found by the formula:

$$\frac{\text{Final Weight - Initial Weight}}{\text{Initial Weight}} \times 100.$$

Sterol analysis was done using a saponification and colorimetric technique. (14, 15)

# RESULTS

From Experiment 1, the mean weight changes for shrimp fed the diets containing plant sterols were not significantly different from each other. In fact, the growth increases followed mortality rates closely. (Fig, 1,2). The highest weight increase, from the 0.1% plant sterol diet, also exhibited the highest mortality. The next highest weight increase was the diet containing no sterol which is illogical since the shrimp need a dietary level of sterol to maintain growth. In addition, the 0.5% cholesterol diet showed the least amount of growth, but had no mortality. Results therefore indicated that considerable aggressiveness and cannibalism was leading to a high number of deaths and more growth from the nutritive value of the dead shrimp.

In experiment 2, no mortality was seen, and better overall growth resulted from the separation of shrimp as well as reduced the feeding rate. There were significant differences in mean weight change between the various diets, as shown in Figure 3, with the commercial-food diet showing a great increase, and the sterol-free diet showing a significant decrease in mean weight. The plant sterol and cholesterol diets showed no significant differences in mean weight change, nor percent weight change from each other. Molting cycle showed no significant differences in the three experimental diets. The commercial food diet was introduced after one month, and thus a cycle was not established for these shrimp. (See Table 5)

Shrimp fed the sterol-free diet had the least sterol per gram of tissue after the two-month study, while those fed the commercial diet had the most sterol although fed for one month only. Those fed the plant sterol diet had slightly more sterol than those fed cholesterol. Circumstances surrounding the sterol analysis of muscle tissue permitted only one analysis for each experimental and control diet. As a result, these data lack replication and statistical analysis and must be considered no more than preliminary indicators of possible trends. (See Table 4)

# DISCUSSION

Overall results from Experiment I indicated the need to separate the animals and extend the observation period. These modifications of technique greatly improved the results of Experiment 2, which had no mortality and exhibited greater growth rates as a whole. Results from the outstanding growth rate on the commercial diet versus the experimental diet prepared in Experiment 2 indicated that the latter was not as complete a diet as the commercial one. Semi-purified diets generally seem to produce reduced growth rates. The semi-purified diets used in this study resulted in poor growth, overall, and reduced sterol levels in tail muscle. Natural ingredients, a better balance of nutrients, and attractants in the commercial food may have caused better growth in shrimp. In addition, the extra level of sterol in the commercial diet at 0.8%, allowed increased growth also. In this study, plant sterol successfully substituted for cholesterol when growth rates and molting cycle were compared. The mean per-cent weight change among the two experimental diets, 0.5% plant sterol and 0.5% cholesterol, were not significantly different, with standard deviations overlapping (Fig. 3). Molting cycle between these two diets also showed no significant difference, although there was also no difference in the sterol-free diet. This indicates that probably the shrimp may have a reserve of sterol when they began feeding on the sterol-free diet, and this reserve was enough to allow them to molt, but not to grow. In fact, these shrimp showed a decrease in growth, yet had no change in molting cycle.

Results from the comparison in sterol levels in muscle tissue were inconclusive. Plant sterol showed an increase in sterol in the tissue over cholesterol-fed shrimp. More experimentation is needed in this area.

In future studies, various levels of plant sterol could again be used to determine an exect level needed to maintain maximum growth in shrimp. This level could be tried at 0.8%, the level found in the commercial food used in this study. A diet could be prepared that is similiar in content to the commercial food with its natural ingredients, except that plant sterol be substituted for animal sterols, and the same parameters measured including growth rates, molting cycle, and sterol levels. Incorporation of plant sterols into shrimp feeds has been shown here to be feasible. It remains to be shown if this substitution for animal sterol would result in any particular benefit, i.e., product quality or economic.

Table 1. Composition of the diets used to test the effect of dietary sterols on shrimp growth and survival.

| SUBSTANCE | PERCENTAGE OF DRY DIET |
|---|---|
| Corn starch | 59.0 |
| Egg albumin (wet weight) | 25.0 |
| Stripped lard | 10.0 |
| Mineral mix[1] | 4.0 |
| Fortification mix[2] | 2.0 |
| Sterols[3] | 0.0-0.5 |
| | 100.0 |
| Agar | 5.0 |
| Distilled water | 100.0 |

[1]Mineral mix (16)

[2]Vitamin mix - Teklad Test Diet No. 40040060

[3]Sterols in experiment I were as follows: 0.0%, 0.1%, 0.3% and 0.5% plant sterols and 0.5% cholesterol. Sterols in experiment II were as follows: 0.0%, 0.5% plant sterol and 0.5% cholesterol.

The plant sterol mixture had the following composition: B-sitosterol, 63.5%, campesterol, 32.2%, and stigmasterol 4.2%.

Table 2. Results of feeding Stage I experimental diet during the period which extended from 11/17 to 12/18.

| | DIET | # OF MOLTS | # OF ANIMALS | # OF DEATHS | % MORTALITY | MEAN WEIGHT CHANGE/TWO WEEKS/SURVIVING ANIMALS |
|---|---|---|---|---|---|---|
| I. | No Sterol | 4 | 11 | 5 | 45.4% ±14.3 | 0.30 ± 0.20 |
| II. | 0.1% plant sterol | 2 | 11 | 7 | 63.6% ±14.3 | 0.33 ± 0.22 |
| III. | 0.3% plant sterol | 4 | 10 | 3 | 33.3% ±14.3 | 0.13 ± 0.18 |
| IV. | 0.5% plant sterol | 2 | 12 | 4 | 33.3% ±14.3 | 0.12 ± 0.17 |
| V. | 0.5% cholesterol | 1 | 6 | 0 | 0.0% ±14.3 | 0.5 ± 0.02 |

Table 3. Results of Stage II feeding study which extended from 2/10 to 4/10. (Data represents measurements ± standard deviation.)

| DIET | # SHRIMP | MEAN LIVE WEIGHT | | | PERCENT CHANGE WEIGHT | | MEAN TOTAL LENGTH | | | PERCENT CHANGE LENGTH | | MEAN NO. MOLTS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2/10 | 3/10 | 4/10 | 3/10 | 4/10 | 2/10 | 3/10 | 4/10 | 3/10 | 4/10 | |
| No sterol | 4 | 4.95 ±1.62 | 4.69 ±1.40 | 4.55 ±1.31 | -5.5 ±4.9 | -8.79 ±5.40 | 6.35 ±1.7 | 6.33 ±0.69 | 6.4 ±0.49 | -0.3 ±0.12 | -0.7 ±0.5 | 2 |
| 0.5% plant sterol | 5 | 4.55 ±1.64 | 4.54 ±1.63 | 4.62 ±1.65 | 1.5 ±1.1 | 4.62 ±1.65 | 6.32 ±0.77 | 6.34 ±0.61 | 6.42 ±0.82 | 0.3 ±0.2 | 1.5 ±1.7 | 2 |
| 0.5% chole-sterol | 4 | 6.38 ±2.79 | 6.40 ±3.01 | 6.57 ±3.07 | 0.31 ±0.32 | 2.89 ±1.40 | 6.98 ±0.99 | 7.05 ±1.06 | 7.06 ±1.10 | 1.0 ±0.5 | 0.1 ±0.15 | 2 |
| 0.8% sterol* | 4 | 8.12 ±3.99 | 10.26 ±5.05 | ---- ---- | 20.9 ±5.60 | ---- ---- | 7.13 ±1.30 | 7.78 ±1.30 | ---- ---- | 9.1 ±2.3 | ---- ---- | -- |

*Shrimp fed a commercial diet (Ralston Purina, M-25) were held for study from 3/10 to 4/10.

Table 4. Sterol contents of shrimp tissue* from Stage II experiment.

| DIET | STEROL (mg/gm tissue) |
|---|---|
| No sterol | 0.0** |
| 0.5% plant sterol | 0.59 |
| 0.5% cholesterol | 0.36 |
| commercial food | 0.95 |

*TAIL MUSCLE TISSUE

**Not enough to be measured

Table 5. Molting cycle of the shrimp from Stage II experiment.

| DIET | MOLTING CYCLE (days) |
|---|---|
| No sterol | 40.0 + 4.8 |
| 0.5% plant sterol | 41.2 + 3.3 |
| 0.5% cholesterol | 40.0 + 9.3 |

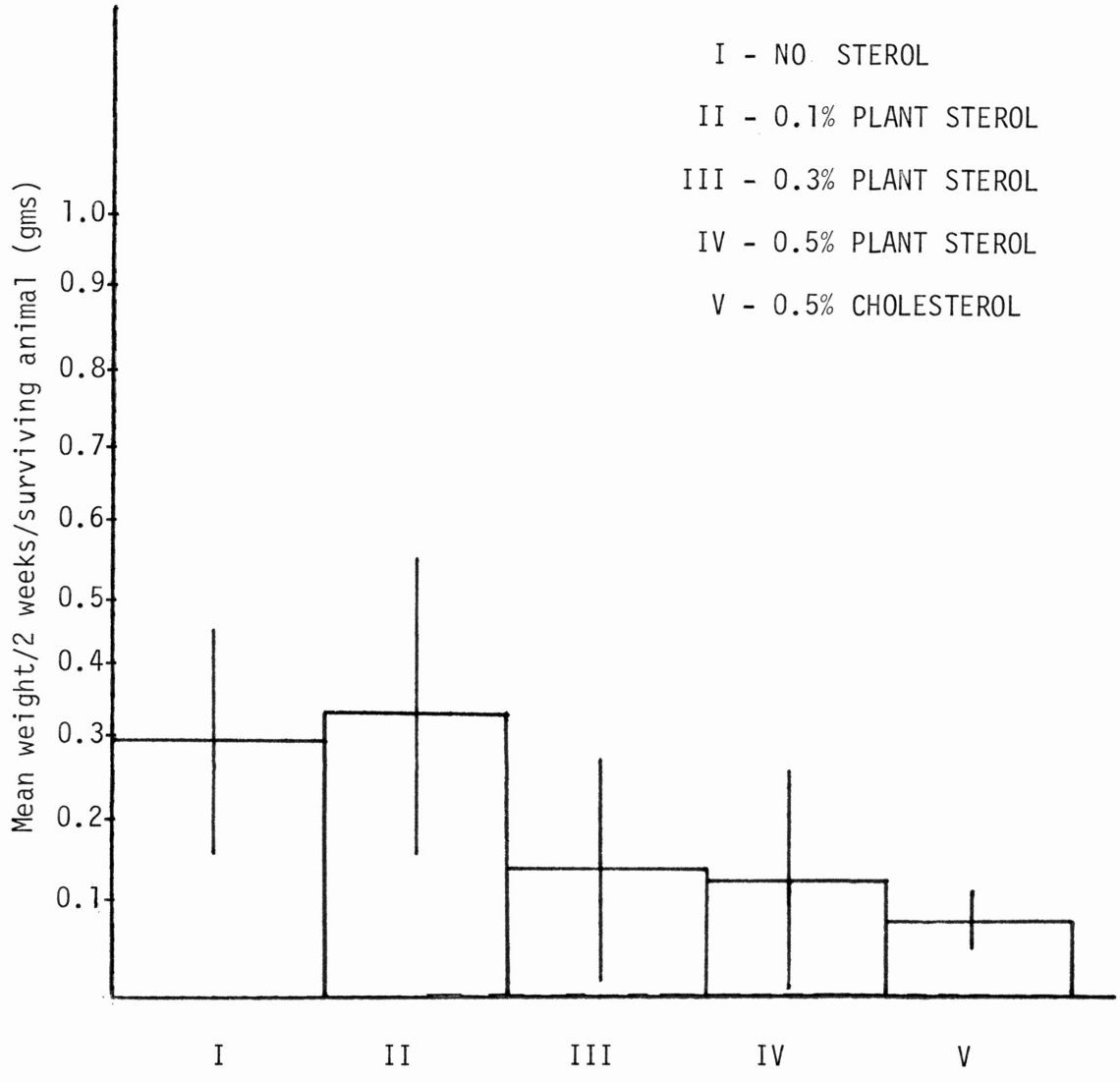


Figure 1. Mean weight change of shrimp fed Experiment I diet. Vertical bars indicate ± one standard deviation.

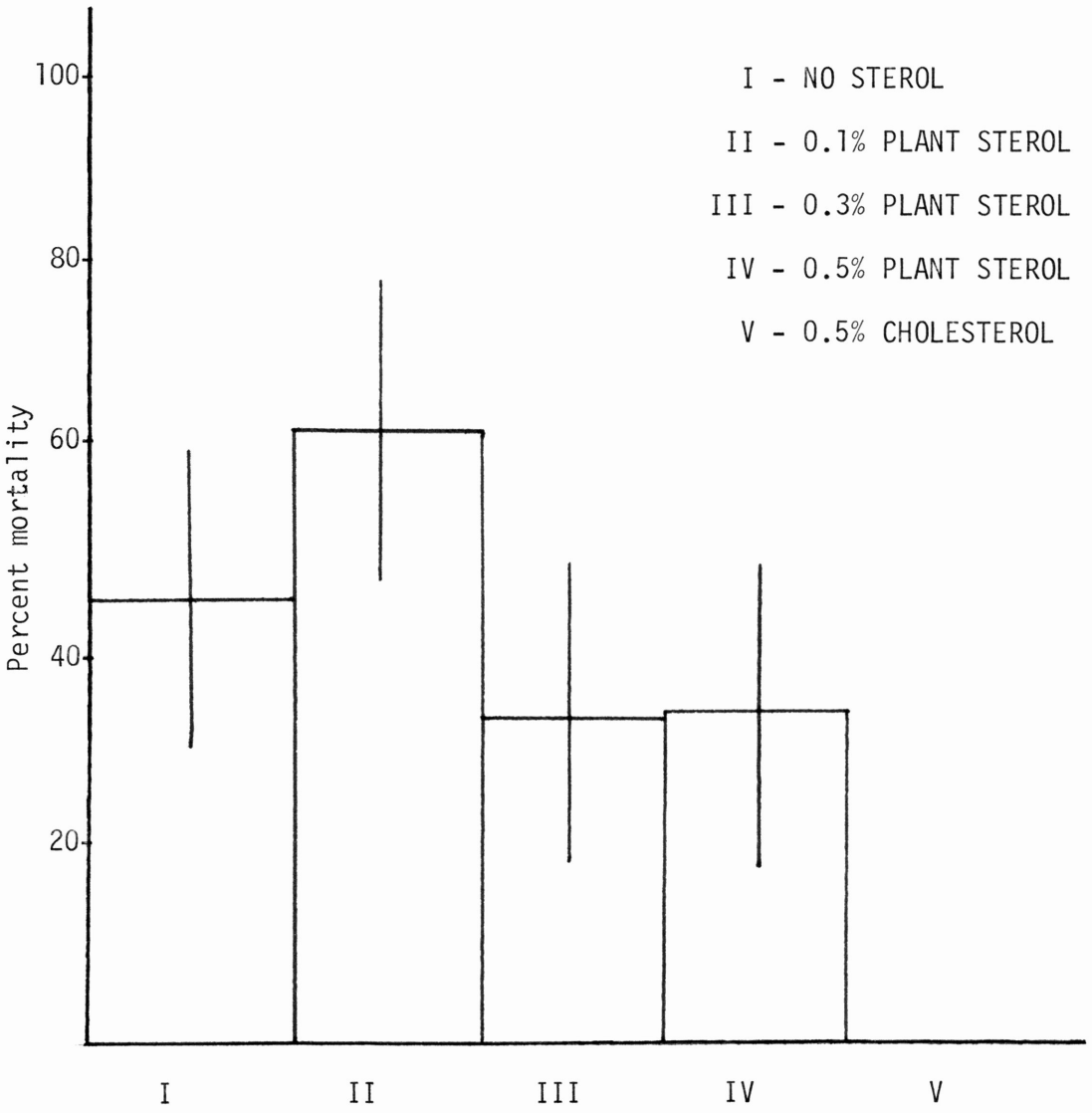


Figure 2. Mortality rates of shrimp fed Experiment I diet. Vertical bars indicate ± one standard deviation.

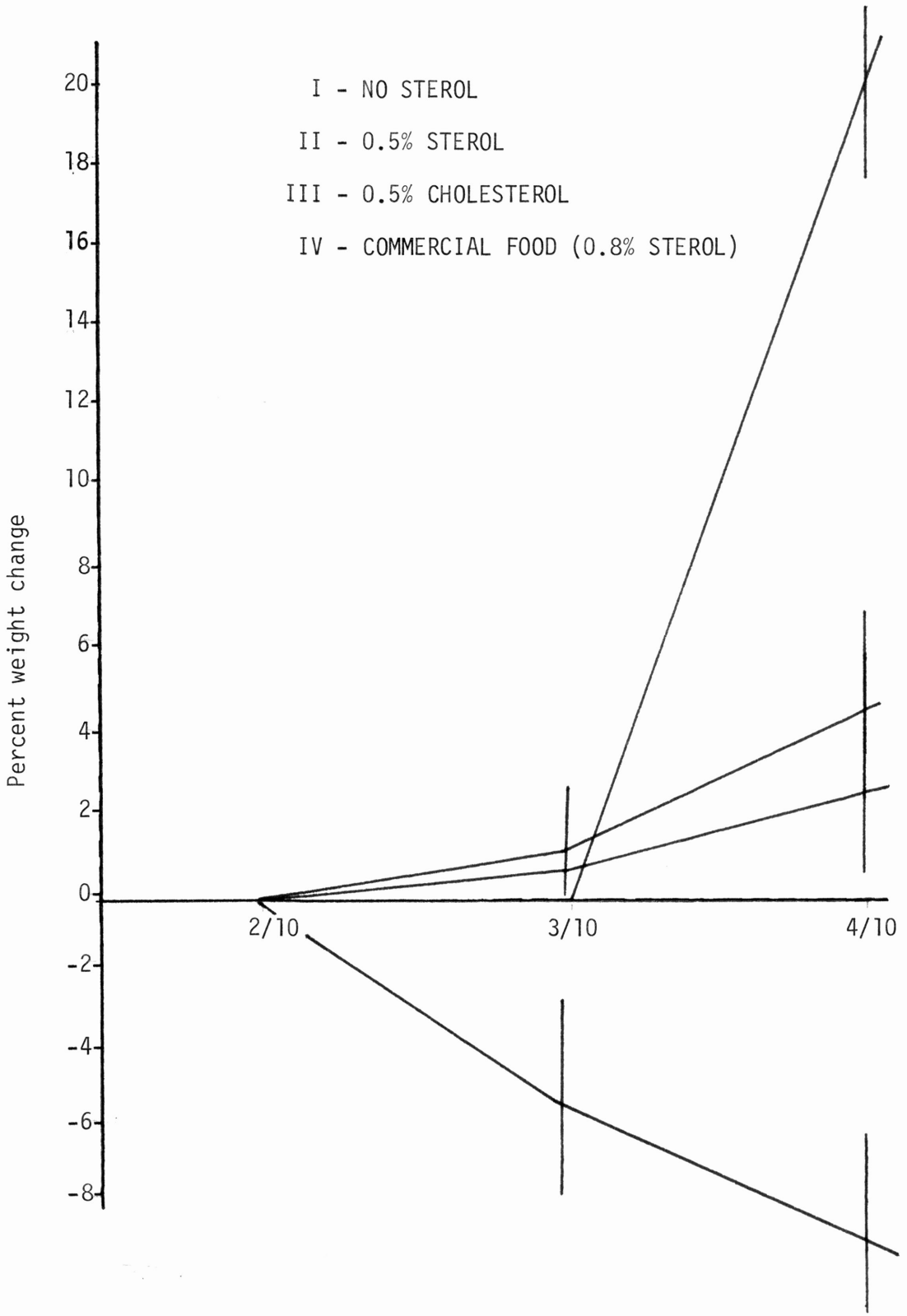


Figure 3. Percent weight changes of shrimp fed Experiment II diet. Vertical bars indicate ± one standard deviation.

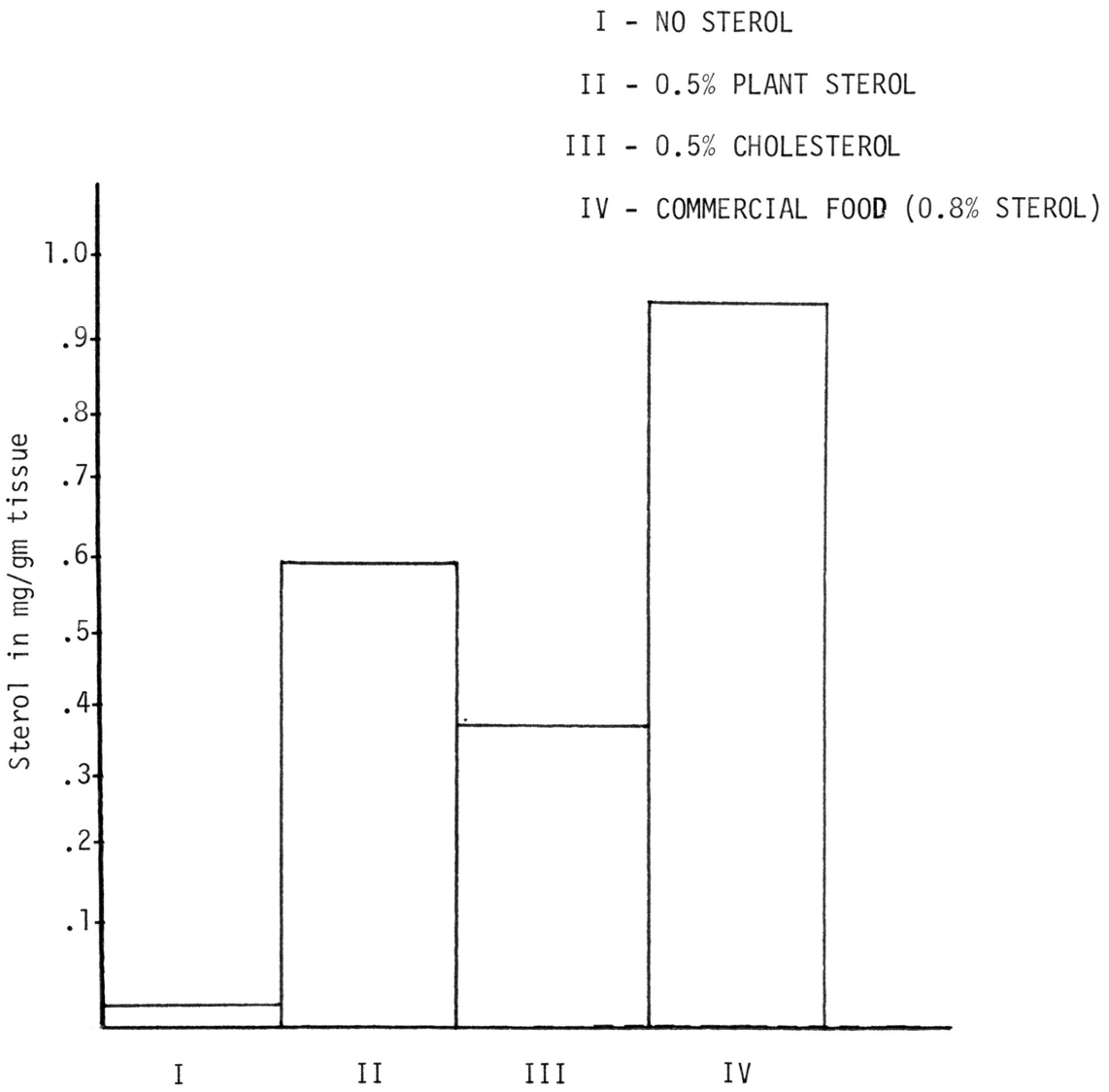


Figure 4. Sterol content of shrimp tissue fed Experiment II diet.

## LITERATURE CITED


1. Clark, A.J.and Block, K. (1959) J. Biol. Chem. 234:2578-2583, 2583-2588.

2. Levinson, Z.H. (1962) J. Ins. Physiol. 8: 191-198.

3. Whitney, J.O. (1969) Mar. Biol. 3: 134-135.

4. Teshima, S. (1971) Bull. Jap. Soc. Sci. Fish. 37 (7): 671-673.

5. Robbins, W.E., Thompson, M.J., Kaplanis, J.N., and Shortino, T.J. (1964) Steroids 4: 635.

6. Smissman, E.E., Jenny, N.A., and Beck, S.D. (1964) J. Pharm. Sci. 53: 1515.

7. Karlson, P. and Hoffmeister, H. (1963) Z. Physiol. Chem. 331: 298.

8. Guary, J.B., and Kanazawa, A. (1973) Comp. Biochem. Physiol. 46A: 5-10.

9. Kanazawa, A., Teshima, S., and Sakamoto, Y. (1975) Bull. Jap. Soc. Sci. Fish. 41 (11) : 1185-1189.

10. Schaeffer, C.H., Kaplanis, J.N., and Robbins, W.E. (1965), J. Ins. Physiol. 11: 1013-1021.

11. Svoboda, J.A., Thompson, M.J., and Robbins, W.E., (1967) Life Sci. 6: 395.

12. Kanazawa, A., Tanaka, N., Teshima, S. and Kashiwada, K. (1971) Bull. Jap. Soc. Sci. Fish. 37 (3): 211-215.

13. Goodwin, H. and Hanson, J. (1974) The Farming of Freshwater Shrimp The Oceanic Institute, Waimanalo, Hawaii.

14. Abell, L.L., Levee, B.B. Brodie, B.B., and Kendle, F.E. (1952) Journ. Biol. Chem. 195: 357.

15. Searcy, R., Berquist, L. (1960) Clin. Chim. Acta 5: 192.

16. Jones, H. and C. Foster. 1942. Journal of Nutrition 24:245-256.