PROGRESS REPO

U. S. PUBLIC HEALTH SERVICE
NATIONAL COMMUNICABLE DISEASE CENTER
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EFFECTS OF PESTICIDES ON ESTUARINE ORGANISMS

DEPARTMENT OF FISHERIES AND WILDLIFE
OREGON STATE UNIVERSITY
CORVALLIS, OREGON

JULY 31, 1969

Progress Report

EFFECTS OF PESTICIDES ON ESTUARINE ORGANISMS

June 1, 1966 through July 31, 1969

U. S. Public Health Service
National Communicable Disease Center
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SUMMARY

Studies relevant to five specific aims (listed on pp. 7, 8) have been completed and the results either published (aim numbers 1, 2a, 2b in part, 3c in part, and 5) or a manuscript summarizing the results has been submitted for publication (aim no. 2b in part). The findings of these studies are the following.

1. The acute toxicity of Sevin and its hydrolytic product 1-naphthol to 10 species of marine animals was determined. Sevin was more toxic to larval and adult crustaceans than to larval and adult mollusks and juvenile fishes. Sevin was 30 to 300 times more toxic than 1-naphthol to crustaceans but less toxic than 1-naphthol to mollusks and fishes.

2. A Sevin concentration of 0.8 mg/liter killed all larvae of cockle clams by day 7 of the test, and the growth of those exposed to 0.4 mg/liter was reduced by 15%. Sevin was less toxic than 1-naphthol to juvenile clams. The food consumption of juvenile clams exposed to 1.6 mg/liter of Sevin was markedly reduced and their food conversion efficiency was impaired. Adult clams exposed to Sevin concentrated the pesticide in their tissues, but tissue concentrations decreased sharply after the clams had been in clean sea water for 12 hours.

3. We have shown that a free prezoeal stage of short duration is normal in the life history of the Dungeness crab. With increasing salinity, the duration of the prezoeal stage decreased, and the numbers of prezoeae that molted to first-stage zoeae increased.

4. The short-term and long-term effects of Sevin on various life history stages of the Dungeness crab were determined. The early larval stages are the most sensitive to Sevin. A concentration of 1.0 mg/liter prevented molting of all prezoeae to zoeae. Survival of zoeae after 25 days exposure to concentrations ranging from 0.0001 to 0.01 mg/liter decreased from 83 to 0%. Young juvenile crabs are more sensitive to Sevin than are older juveniles or adults. The behavior, growth, and survival of juvenile crabs are not affected when the animals are exposed to 0.032 mg/liter of Sevin for 24 hours and then held in clean sea water for 44 days. Adult crabs were irreversibly paralyzed within 6 hour after eating cockle clams that had just been exposed to Sevin.

5. Ages, lengths, and weights were determined for 124 female shiner perch collected in Oregon, and lengths, weights, and numbers per female parent were determined for 1,005 embryos. Equations were computed for the relationships of: embryo number to parent female fork length, weight, and age; total length of embryo at birth to parent female fork length and weight; and embryo weight to embryo length at birth.

6. In laboratory aquaria without mud, the concentration of Sevin decreased approximately 50% in 38 days at 8° C. Most of this decrease was accounted for by the production of 1-naphthol. After 17 days at 20° C, the Sevin had almost completely disappeared with 43% converting to 1-naphthol. When mud was present, both Sevin and 1-naphthol declined to less than 10% in

the sea water in 10 days. Radioactive carbon dioxide was produced in the aquaria containing ^{14}C carbonyl-labeled and ^{14}C ring-labeled Sevin, indicating decomposition by hydrolysis of the carbamate and oxidation of the naphthyl ring. The total recovery of the ^{14}C activity was only 40%. It is postulated that much of the remainder was evolved as methane. In a field experiment, Sevin could be detected in the mud for 42 days, but 1-naphthol persisted for only one day.

Studies relevant to the other aims of the project are in progress. The following are the results thus far.

1. Sevin is less toxic to juvenile chum salmon in freshwater than in sea water. At 15° C, the respective 96-hour TL_m's (median tolerance limits) were 3.9 and 2.5 mg/liter of Sevin.

2. Three continuous flow dilution systems of different design have been built for use in studies on the effects of the pesticide Dursban on survival, growth, and reproduction of shiner perch. Dursban is more toxic to shiner perch than it is to several species of freshwater fish. In a preliminary experiment, concentrations of 0.16 µg/liter and less did not affect growth of perch, but higher concentrations appeared to have affected growth and food consumption.

3. Laboratory models of an intertidal mud flat have been constructed. Tidal cycles are simulated in them with a system of time switches and pumps. We have successfully maintained communities of intertidal animals in the models for periods up to 75 days. Different environmental conditions such as types of tidal cycles, amount of substrate, and rates of water exchange were studied for their effects on establishment of the animal communities. We are now studying effects of light intensity and animal numbers on the establishment of the animal community. In early 1970, we will begin studies on the effects of Sevin on an animal community in the laboratory models.

4. Studies on acetylcholinesterase activities in tissues of the Dungeness crab were continued. Attempts to purify the enzyme were unsuccessful, however, the crude homogenate showed a high degree of homogeneity. Studies were continued on characterization of the enzyme and its inhibition by Sevin.

STAFFING

Time on 12 month basis

Personnel	Percent time	Period of appointment
Millemann, Raymond E. Professor of Fisheries	30 to 35	June 1966 to present
Doudoroff, Peter Professor of Fisheries	5	June 1966 to present
Terriere, Leon C. Professor of Biochemistry	5	June 1966 - Sept. 1968
Breese, Wilbur P. Assoc. Prof. of Fisheries	5	June 1966 to present
Stewart, Nelson E. Assistant In Fisheries	100	June 1966 to present
Karinen, John F. Assistant In Agric. Chemistry	100	June 1966 - Sept. 1968
Butler, Jerry A. Research Assistant in Fisheries	50	June 1 - June 30, 1966
Buchanan, David V. Research Assistant in Fisheries	50	July 1967 to present
Wilson, Dennis C. Research Assistant in Fisheries	50	July 1967 to present

PUBLICATIONS

1. Stewart, N. E., R. E. Millemann, and W. P. Breese. 1967. Acute toxicity of the insecticide Sevin and its hydrolytic product to some marine organisms. Trans. Amer. Fish. Soc. 96: 25-30.

2. Karinen, J. F., J. G. Lamberton, N. E. Stewart, and L. C. Terriere. 1967. Persistence of carbaryl in the marine estuarine environment. Chemical and biological stability in aquarium systems. J. Agric. Food Chem. 15: 148-156.

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4. Buchanan, D. V. and R. E. Millemann. 1969. The prezoeal stage of the Dungeness crab, Cancer magister Dana. Biological Bulletin. Oct. (In press).

5. Wilson, D. C. and R. E. Millemann. 1969. Relationships of female age and size to embryo number and size in the shiner perch, Cymatogaster aggregata Gibbons. J. Fish. Res. Bd. Canada. Sept. (In press).

6. Buchanan, D. V., R. E. Millemann, and N. E. Stewart. Effects of the insecticide Sevin on survival and growth of the Dungeness crab, Cancer magister. Submitted to: J. Fish. Res. Bd. Canada.

In preparation

1. Stewart, N. E. and R. E. Millemann. Simulation in the laboratory of an intertidal mud flat. To be submitted to: J. Exptl. Marine Biol. and Ecology.

THESES

1. Butler, J. A. 1968. Effects of the insecticide Sevin on the cockle clam, Clinocardium nuttalli (Conrad). M. S. Thesis. Oregon State University, Corvallis, Oregon.

2. Buchanan, D. V. 1969. Effects of the insecticide Sevin on the Dungeness crab, Cancer magister Dana. M. S. Thesis. Oregon State University, Corvallis, Oregon.

INTRODUCTION

Estuaries are the temporary or permanent habitats for many valuable species of shellfish and fish. Pesticides may be carried into estuaries by streams emptying into them or by rain runoff from adjacent ground, or they may be applied directly to estuarine waters either through accidental or careless use of the chemicals during spray operations or purposely in efforts to control shellfish pests or predators. Thus, these chemicals may seriously affect coastal life.

We must know the effects of a pesticide on as many life history stages of an animal as possible before we can critically evaluate the hazards involved in its use. For this reason, our research has included not only acute toxicity studies, but also studies involving short-term and long-term exposures of animals to sublethal concentrations of pesticides.

Initially, our work has been with the insecticide Sevin or carbaryl (1-naphthyl methyl carbamate) because this chemical alone or with others has been used successfully in the field on oyster grounds to control oyster pests such as the burrowing shrimps Callianassa californiensis and Upogebia pugettensis. These shrimp turn over estuarine mud much as earthworms do to the soil. The burrowing activities of the shrimp can render the substrate unsuitable for oyster production. The ground is softened by their continuous digging, and the oysters as they mature and increase in weight, sink into the mud, are buried, and die. Field tests have shown that Sevin can be used to control the shrimp, but it is also toxic to other valuable animals. Therefore, we need more information on the effects of Sevin and its degradation products on estuarine organisms before its widespread use can be recommended.

The organophosphorous insecticide Dursban is registered for use in mosquito control programs, and it is possible that it may eventually enter estuaries. Therefore, we began studies recently of the effects of this pesticide on an estuarine fish, the viviparous shiner perch, and we will shortly begin studies of the effects of this chemical on oysters and the Dungeness crab.

This progress report summarizes the results of our investigations on the effects of pesticides on estuarine organisms for the period from June 1, 1966 through July 31, 1969. It has been prepared for submission with an application for research grant support that would enable us to continue and expand the studies made possible by the current research grant.

Objectives of this Research

The general objective of this research is to investigate the effects of various pesticides, which might be expected or are known to occur in estuaries, on the larval and adult stages of estuarine organisms, and to study the fate of these compounds in the organisms and the environment. This information is necessary for the development of safe and rational control methods. The following are the specific aims of the study.

1. To determine the acute toxicity of Sevin to some estuarine organisms through short-term bioassays.

2. To determine the short-term and long-term effects of Sevin on a:
   a. representative estuarine mollusk;
   b. representative estuarine crustacean;
   c. representative estuarine fish.

3. To determine the short-term and long-term effects of Dursban on a:
   a. representative estuarine mollusk;
   b. representative estuarine crustacean;
   c. representative estuarine fish.

4. To develop a laboratory model of an estuary containing a functioning community of animals, and then to determine the effects of Sevin and other pesticides on such a community.

5. To follow the fate of Sevin in laboratory aquaria under simulated natural conditions.

6. To determine the mode of action of Sevin and its metabolites on estuarine organisms.

Studies relevant to specific aims 1, 2a, and 5 have been completed and the results published. Reprints of these publications are included in this progress report and will serve as a summary of accomplishments to date for that phase of the project. The results of studies relating to aims 1 and 5 were given in our first progress report of May 31, 1966, but at that time they had not been published. Therefore, reprints of these publications are included here for background information and for completeness.

Studies relating to specific aim 2b have been completed. One manuscript reporting the results of a background study has been accepted by Biological Bulletin and will appear in the October 1969 issue. A second manuscript reporting the principal results has been submitted to the Journal of the Fisheries Research Board of Canada for publication.

Studies pertaining to aims 2c, 3c, 4, and 6 are in progress. One manuscript reporting results of a background study relevant to aim 3c has been accepted by the Journal of the Fisheries Research Board of Canada and will appear in the September 1969 issue. Studies relating to the first phase of aim 4 are almost completed. A manuscript reporting the successful development of a laboratory model of an estuary containing a community of animals is being submitted to the Journal of Experimental Marine Biology and Ecology.

Studies relevant to aims 3a and 3b will begin this year.

The remainder of this progress report is divided into sections, each section relating to one of the above specific aims.

ACUTE TOXICITY OF SEVIN TO MARINE ORGANISMS

Specific aim no. 1. To determine the acute toxicity of Sevin to some estuarine organisms through short-term bioassays.

This phase of the project is completed. See the following reprint for summary of the results.

EFFECTS OF SEVIN ON COCKLE CLAMS

Specific aim no. 2a. To determine the short-term and long-term effects of Sevin on a representative estuarine mollusk.

This phase of the project is completed. See the following reprint for summary of the results.

EFFECTS OF SEVIN ON DUNGENESS CRABS

Specific aim no. 2b. To determine the short-term and long-term effects of Sevin on a representative estuarine crustacean.

This phase of the project is completed. See the following two manuscripts for summary of the results. The first manuscript will appear in the October 1969 issue of Biological Bulletin, and the second has been submitted to the Journal of the Fisheries Research Board of Canada for publication.

SHEET I. Send proof to: Dr. Raymond E. Millemann
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THE PREZOEAL STAGE OF THE DUNGENESS CRAB, *CANCER MAGISTER* DANA[1]

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Running head:

PREZOEAE OF CANCER MAGISTER

[1] Technical Paper No. 2645, Oregon Agricultural Experiment Station. This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia.

There is controversy in the literature as to the normalcy of the occurrence of a free prezoeal stage in the life history of the Dungeness crab, Cancer magister. MacKay (1942) stated that the eggs hatched into protozoeae (= prezoeae), but others reported that zoeae normally emerged from eggs and that free prezoeae were abnormal and died (Mir, 1961) or resulted from premature hatching of eggs (Poole, 1966). Our preliminary observations on egg hatching and on subsequent molting of prezoeae supported MacKay's belief. Before we could begin studies on the effects of the insecticide Sevin on survival and growth of C. magister larvae we had to resolve the controversy. This paper reports the results of a study undertaken to determine if salinity (1) affects egg hatching, (2) determines the type of larva that emerges from the egg, and (3) affects development of these larvae.

MATERIALS AND METHODS

Ovigerous crabs caught in the ocean off the Oregon coast were held in the laboratory in flowing sea water for 3 days. One crab was then transferred to a 31-gallon tank containing filtered, sterilized, standing sea water of 30‰ at 11° C and it was held for an additional 3 days. Eggs began to hatch at the end of this time. We could predict from previous experience that eggs would hatch within a few days when egg coloration changed from a light to a dark brown. When hatching began, about 2000 eggs of normal appearance with all cuticular layers and ovigerous setae intact were gently removed in small bunches from the crab and held for 3 hours in filtered, sterilized sea water of 32 ‰ salinity at 10.5° C. Only a few eggs hatched during this time and the larvae, some of which may have hatched prematurely because of the recent egg handling, were discarded. At the end of the 3 hours, unhatched eggs still attached to ovigerous setae and with their cuticular layers intact were then selected at random and separated into 14 groups of 20 and each group was placed into a 250 ml beaker containing sea water at 10.5° C and of one of the following salinities: 10, 15, 20, 25, 30, and 32 ‰. A temperature-salinity combination of 17.5° C and 32 ‰ was also tested. Duplicate vessels were used for each test. The larvae, upon hatching, were immediately transferred to another beaker containing sea water of the salinity and temperature at which they had hatched. All vessels were held under constant light. Eggs were examined for hatching at intervals of about 5 minutes for 36 hours, and the hatched larvae were observed for molting at the same intervals for the first hour after they had hatched and then again at the end of the 36-hour experiment.

RESULTS

Some eggs hatched at all the test salinities, and 94% of these are known to have hatched into prezoeae (Table I). Of the remainder (6%), all of the eggs but one were in the two highest salinities at which the prezoeae may molt to zoeae as early as 2 minutes after hatching. Therefore, we believe that all of the hatching eggs hatched into prezoeae, but because of the short duration of this larval stage, especially at the higher salinities, some prezoeae could have hatched and molted between observations and thus they would not have been seen. The percentage of eggs hatching at 10.5° C increased as salinity decreased to an optimum of 15 ‰, but at 10 ‰ was lowest (Fig. 1). At a salinity of 32 ‰, the mean percentages of eggs that hatched at 10.5 and 17.5° C were 30 and 73%, respectively, indicating a marked temperature effect (Table I).

TABLE I

Effects of salinity and temperature on hatching of Cancer magister eggs and on molting of the prezoeae to zoeae*

Salinity	Hatched eggs		Free prezoeal stage confirmed		Duration of prezoeal stage (min.)**		Prezoeae molted to zoeae	
(‰)	No.	%	No.	%	Mean	Range	No.	%
10	4	20	4	100	-	-	0	0
10	3	15	3	100	-	-	0	0
15	12	60	12	100	>60	-	1	8
15	11	55	11	100	>60	-	2	18
20	9	45	8	89	>44	13->60	3	36
20	11	55	11	100	>22	9->60	6	54
25	8	40	8	100	14	7-31	8	100
25	7	35	7	100	14	7-27	6	86
30	6	30	4	67	10	7-14	4	100
30	4	20	3	75	4	2-7	3	100
32	4	20	4	100	12	9-18	4	100
32	8	40	7	88	10	6-16	7	100
32†	17	85	15	88	11	3-35	15	100
32†	12	60	12	100	11	4-30	12	100

* Twenty eggs were used in each test and they were observed for hatching approximately every 5 minutes for 36 hours. All tests were done at 10.5° C unless noted otherwise.

** Hatched prezoeae were observed for molting approximately every 5 minutes for the first hour after they had hatched and again at the end of the 36-hour experiment.

†Test temperature was 17.5° C.

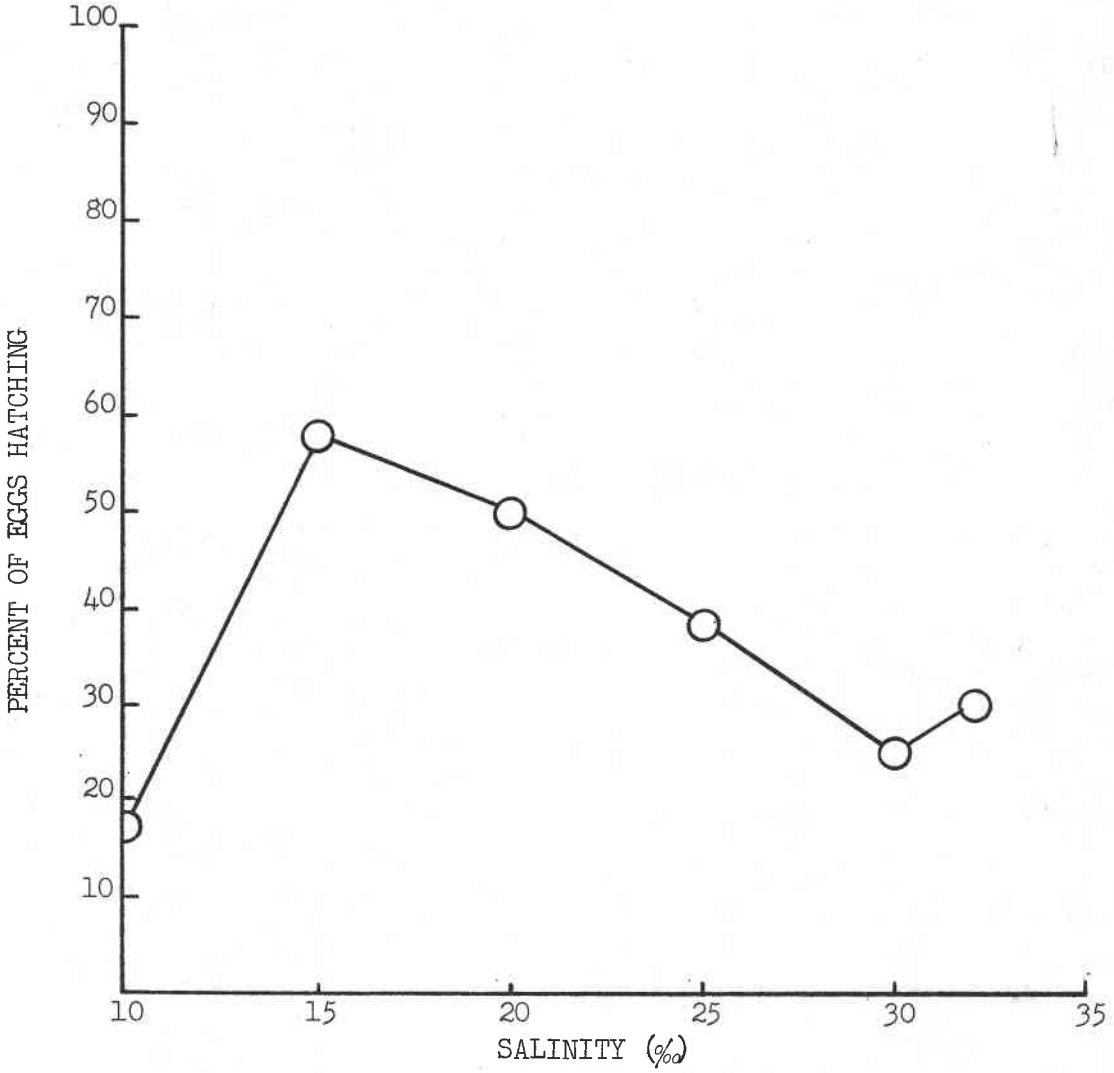


Figure 1. Effect of salinity on hatching of Cancer magister eggs at 10.5° C.

The mean percentages of prezoeae that molted to zoeae increased with increasing salinity from 0% at 10 ‰ to 100% at 30 and 32 ‰ (Table I and Fig. 2). With increase of salinity from 15 to 32 ‰, the mean duration of the prezoeal stage decreased from more than 60 minutes to 11 minutes (Table I). At 32 ‰ salinity, all prezoeae molted to zoeae at the two test temperatures, and there was no effect of temperature on duration of the prezoeal stage.

Our morphological observations of the prezoeae agree with those of MacKay (1934) except that we did not see the lateral spines on the cephalothorax. The prezoeae have large eyes, a five-segmented abdomen, and a forked telson with spines (Fig. 3). They lack the rostral and dorsal spines of the zoeae. A further distinguishing characteristic, not mentioned by MacKay (1934), is the shortness of the natatory hairs on the maxillipeds. These hairs on the maxillipeds of the zoeae are much longer.

Prezoeae swam erratically, using their abdomen and telson for propulsion. As first noted by MacKay (1942) their movement resembled that of a mosquito larva. It was weak at the intermediate salinities and at 10 and 15 ‰ salinity the prezoeae did not swim.

Molting of prezoeae to zoeae required only a few seconds. The prezoeae first settled to the bottom of the container; then they extended the maxillipeds, the cuticle split, and the dorsal and rostral spines emerged. Our first-stage zoeae conformed morphologically with those described by Mir (1961) and Poole (1966).

Five zoeae, which had developed from prezoeae, were randomly selected at the end of the experiment and held in sea water of 32 ‰ at 10° C for 3 days. All of them survived and appeared normal during this time.

DISCUSSION

Our observations support the contention of MacKay (1934) that the free prezoea is a normal stage in the life history of C. magister and not the abnormality that it was believed to be by Mir (1961) and Poole (1966). The results of our study support the statement of MacKay (1942) that the prezoeal stage is of short duration. This short duration may explain Poole's (1966) failure to see prezoeae, his observations having been made apparently no more frequently than once a day. Mir (1961), who also apparently observed his eggs no more frequently than once daily, stated that prezoeae, when present, were "imperfect" and died. He saw prezoeae apparently only when eggs were refrigerated or contaminated with protozoa and undoubtedly for these reasons his prezoeae were indeed abnormal and died without molting.

Churchill (1942), on the basis of laboratory studies, stated that a free prezoeal stage, which lasted from 30 to 60 minutes, was normal in the life history of the blue crab, Callinectes sapidus. However, Sandoz and Rogers (1944) believed that their experimental data did not support Churchill's statement. They concluded that unfavorable environmental conditions, such as suboptimal salinities, were responsible for the occurrence of free prezoeae, because in their experiments the numbers of free prezoeae increased as salinities decreased. They found no prezoeae when eggs were

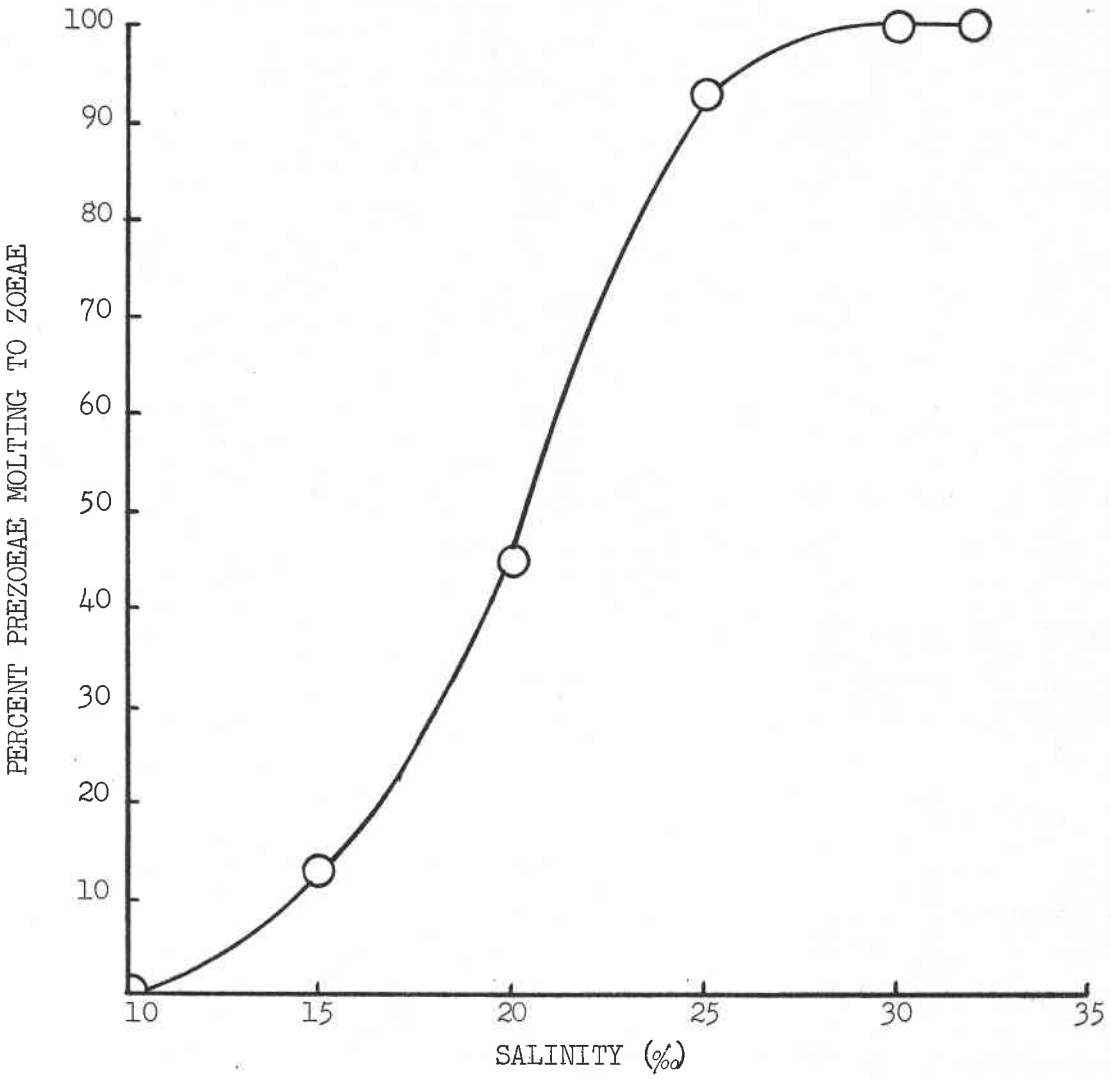
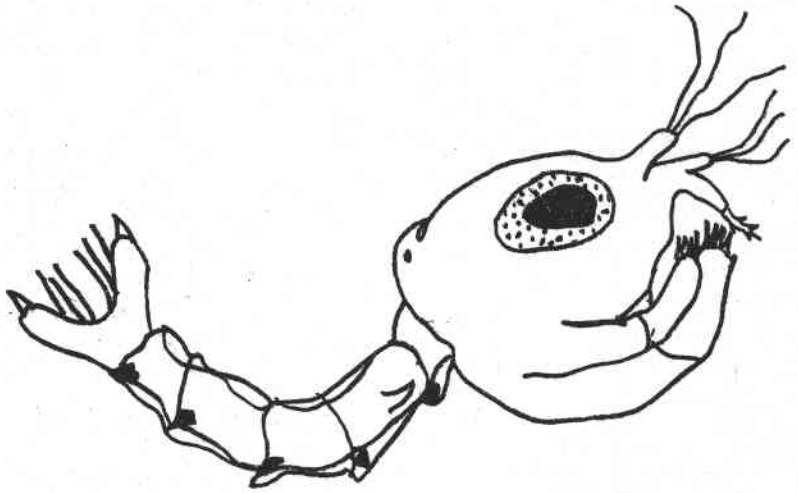


Figure 2. Effect of salinity on molting of Cancer magister prezoeae to zoeae.



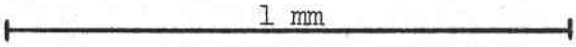


Figure 3. Prezoeae of Cancer magister after hatching from the egg.

hatched at salinities ranging from 23.4 to 32 ‰ but at a salinity of 10 ‰ 90 to 100% of the hatched larvae remained as prezoeae. We found the same for C. magister prezoeae at low salinities, but at high salinities all of our prezoeae molted to zoeae. Sandoz and Rogers (1944) observed their eggs only twice daily, and therefore they could have failed to see the short-lived prezoeae at the higher salinities. The data of Sandoz and Rogers (1944), therefore, do not refute the belief of Churchill (1942), and for the same reason neither do the data of Costlow and Bookhout (1959), who also reported that eggs of C. sapidus at salinities ranging from 20.1 to 32 ‰ always hatched as first-stage zoeae. Costlow and Bookhout (1960) observed the eggs of six species of Brachyura, including C. sapidus, once daily and reported that most of the eggs of all species hatched as zoeae. Knudsen (1959) and Williams (1968) found that eggs of the crabs Paraxanthias taylori and Carcinus maenas, respectively, normally hatched as prezoeae, and the latter author reported that the average duration of the prezoeal stage of C. maenas was 4 to 5 minutes. It is clear, therefore, that the early life history of the blue crab and of other crabs in which the normal occurrence of a free prezoeal stage is doubted must be restudied.

The range of salinity that proved optimal for molting of our prezoeae was between 25.0 and 32 ‰ at 10.5° C; at 32 ‰ salinity, prezoeae molted equally well at 10.5 and 17.5° C. Reed (1969) found that the optimal salinities and temperatures for development in the laboratory of C. magister first-stage zoeae to the megalops stage were between 25 and 30 ‰ and between 10.0 and 13.9° C.

We wish to thank Mr. Paul H. Reed, Fish Commission of Oregon, for suggesting to us the possibility that free prezoeae are normal in the life history of C. magister, and Mr. Dennis E. Anderson and Mr. Nelson E. Stewart, Oregon State University, for making some of the observations.

SUMMARY

1. Ninety-four per cent of eggs of the Dungeness crab, Cancer magister, held in sea water at 10.5 or 17.5° C and at salinities of 10 to 32 ‰ hatched into prezoeae during a 36-hour observation period. The highest and lowest numbers of eggs hatched at salinities of 15 and 10 ‰, respectively. At a salinity of 32 ‰ the mean percentages of eggs that hatched at 10.5 and 17.5° C were 30 and 73%, respectively.

2. With increase of salinity, the percentages of prezoeae that molted to first-stage zoeae increased from 0% at 10 ‰ to 100% at 30 and 32 ‰.

3. With increase of salinity from 15 to 32 ‰, the mean duration of the prezoeal stage decreased from more than 60 minutes to 11 minutes.

4. The experimental results show that the occurrence of a free prezoeal stage of short duration is normal in the life history of C. magister. The possibility that this is true for other Brachyura is discussed.

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EFFECTS OF THE INSECTICIDE SEVIN ON SURVIVAL AND GROWTH OF THE DUNGENESS CRAB, *CANCER MAGISTER*[1,2]

By David V. Buchanan, Raymond E. Millemann, and Nelson E. Stewart

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Running head:

BUCHANAN ET AL.: EFFECTS OF SEVIN ON CRABS

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[1] Technical Paper No. 2727, Oregon Agricultural Experiment Station. A contribution from the Pacific Cooperative Water Pollution Laboratories, Oregon State University.

[2] This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia.

Buchanan, David V., Raymond E. Millemann, and Nelson E. Stewart. 1969. Effects of the insecticide Sevin on survival and growth of the Dungeness crab Cancer magister. J. Fish. Res. Bd. Canada 26: -- .

The short-term and long-term effects of the insecticide Sevin on various life history stages of the Dungeness crab, Cancer magister, were determined. Early larval stages were the most sensitive to Sevin. A concentration of 1.0 mg/liter did not affect egg hatching but prevented molting of all prezoeae to zoeae. The 96-hr EC_{50} (effective concentration that killed 50% of the animals) for first-stage zoeae and adults were estimated to be 0.01 and 0.26 mg/liter, respectively.

Few zoeae were killed in 24 hr by 82.0 mg/liter, but the 24-hr EC_{50} based on death within 15 days after the exposure as the criterion of effect was estimated to be 0.015 mg/liter. The 24-hr EC_{50} based on cessation of swimming, which was not always permanent, as the criterion of effect was 0.0065 mg/liter.

Survival of zoeae after 25 days exposure to concentrations of 0.0001, 0.00032, 0.001, 0.0032, and 0.01 mg/liter were 83, 60, 69, 21, and 0%, respectively, and control survival was 79%. Molting was delayed at a concentration as low as 0.0001 mg/liter.

Young juvenile crabs are more sensitive to Sevin than are older juveniles or adults. The 24-hr EC_{50}'s (concentration that irreversibly paralyzed 50% of the animals) for second stage juveniles, ninth stage juveniles, and adults were estimated to be 0.076, 0.35 to 0.62, and 0.49 mg/liter, respectively.

The behavior, growth, and survival of juvenile crabs were not affected when the animals were exposed to 0.032 mg/liter of Sevin for 24 hr and then held in clean sea water for 44 days.

After eating cockle clams that had just been exposed for 24 hr to 1.0, 3.2, and 10.0 mg/liter of Sevin 22, 77, and 100% of adult crabs, respectively, were irreversibly paralyzed within 6 hr.

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INTRODUCTION

Pesticides may be carried into estuaries by streams emptying into them or by rain runoff from adjacent ground, or they may be applied directly to estuarine waters either through accidental or careless use of the chemicals during spray operations, or purposely in efforts to control shellfish pests or predators. Thus, these chemicals may seriously affect marine life; this subject has been recently discussed by Butler (1966).

The insecticide Sevin, 1-naphthyl methylcarbamate, has been used in the field to control oyster pests and predators but may adversely affect nontarget organisms also (Butler et al., 1968). The Dungeness crab, Cancer magister, is abundant in estuaries and could be exposed to chemicals used in oyster pest control programs. The only reported laboratory study of the effects of pesticides on this crab is that of Stewart et al. (1967), who studied the acute toxicity of Sevin and its first hydrolytic product, 1-naphthol, to juvenile crabs.

The purpose of our study was to determine the short-term and long-term effects of Sevin on survival and growth of C. magister at various stages of its life history. The Dungeness crab was chosen as the test animal because of its abundance in estuaries, its widespread distribution, its economic importance, and its ready availability. The experiments were carried out from September 1967 to April 1969 in the Pacific Fisheries Laboratory of Oregon State University's Marine Science Center at Newport, Oregon.

MATERIALS AND METHODS

The Sevin, obtained from Miller Products Company, Portland, Oregon, was a microfine wettable powder that contained 80% active ingredient and 20% inert materials. Stock solutions were prepared by dissolving 100 mg of active Sevin in 1 liter of filtered sea water. Each stock solution was stirred for 2 hr to dissolve the toxicant. Test solutions were prepared from the stock solutions by serial dilution, using filtered, sterilized sea water adjusted to 25‰ salinity with distilled water. A logarithmic series of concentrations was used. Stock and test solutions were used within 5 hr after preparation. In every experiment, each concentration was tested in duplicate or in triplicate. With a few noted exceptions, the tests were done under continuous light with standing sea water at 10 ± 1 C. Each EC_{50} value (the effective concentration of Sevin that produced a designated effect on 50% of the test animals) was determined by straight-line graphical interpolation commonly used for the estimation of median tolerance limits (American Public Health Association et al., 1965). The EC_{50} is equivalent to the TL_m when death at the end of the stated exposure period is the criterion of toxic effect. The numbers and kinds of experiments done are shown in Table 1.

EGG AND PREZOEAL TESTS

Ovigerous crabs caught in the ocean off the Oregon coast were held in the laboratory in 31-gal (117.8-liter) tanks containing flowing sea water at approximately 10 C. When hatching began, usually several days

Table 1. Kinds of experiments done to evaluate the toxicity of Sevin to Dungeness crabs.

Life history stage	Expt. no.	Sevin concentrations (mg/liter)	Exposure time	Postexposure observation time	Criteria of toxic effect
Fertilized eggs and prezoeae[a]	1-3	0.0001-1.0	24 hr	8 days	Prevention of hatching and molting
Zoeae	4-7	0.0032-82.0	24, 48, or 96 hr	0	Death
Zoeae	8, 9	0.0003-10.0	1, 5, 24 hr	8 and 25 days	Cessation of swimming, prevention of molting and death
Zoeae	10	0.0001-0.01	25 days	0 days	Prevention of molting and death
Juveniles					
2nd stage	11	0.0032-10.0	1, 24, 48 hr	3 days	Death or paralysis[b]
3rd stage	12	0.01 and 0.032	24 hr	44 days	Prevention of molting, and death or paralysis
9th stage	13, 14	0.18-1.0	96 hr	0	Death or paralysis
Adults					
	15	0.18-1.0	96 hr	0	Paralysis
	16	1.0-10.0[c]	24 hr	0	Death or paralysis

[a] Prezoeae are those that hatched from eggs during exposure to Sevin.

[b] Paralysis was irreversible and was considered equivalent to death.

[c] These crabs were exposed to Sevin by feeding them cockle clams that had just been exposed for 24 hr to the indicated concentrations of Sevin.

later, eggs of normal appearance with all cuticular layers intact were gently removed from the crabs and placed into 250 ml flasks. Each flask contained approximately 20 eggs and 200 ml of test solution. Eggs from one female only were used in each experiment, but different crabs were used in the three experiments. Nine concentrations of Sevin ranging from 0.0001 to 1.0 mg/liter were tested. The number of eggs that hatched after a 24-hr exposure to each concentration was recorded.

A free prezoeal stage of 5 to 15 min in duration is normal in the life history of C. magister (Buchanan nd Millemann, 1969). In these three experiments the effect of Sevin on molting of prezoeae to zoeae was also determined. The numbers of prezoeae and zoeae in the test vessels were recorded at the end of the 24-hr exposure period. At this time, 117 exposed prezoeae that failed to molt to zoeae in two of the three experiments were transferred to flasks containing clean, filtered, sterilized sea water to determine if the effect of Sevin on molting of prezoeae was irreversible. The prezoeae were fed either brine shrimp larvae (Artemia salina) or barnacle larvae (Balanus glandula) on alternate days. The water in the flasks was changed at the time of feeding. The tests were terminated at 8 days and the numbers and kinds of surviving larvae recorded.

ZOEAL TESTS

Seven experiments were done to determine the short-term and long-term effects of Sevin on survival and molting of first-stage zoeae. The larvae were progeny of crabs held in the laboratory. Those used in any one experiment all came from one female. Larvae used in experiments 4, 5, and 7 all came from the same female, and those used in the remaining experiments came from different females. At the beginning of each experiment, swimming larvae were selected randomly from the holding vessels and usually 10 were placed into each test vessel (250-ml beaker) containing 200 ml of the test solution.

In the first four experiments, the zoeae were exposed for either 24, 48, or 96 hr to eight or more Sevin concentrations ranging from 0.003 to 82.0 mg/liter. In experiments 4, 5, and 6, unfed larvae 1 to 2 days old were tested at 10 $\pm$ 1 C, and also at 17 $\pm$ 1 C in experiment 6. In preliminary tests Buchanan (MS, 1969) found that feeding is not necessary during tests of such brief duration; 90% or more of unfed larvae survived in sea water for at least 7 day. In experiment 7, 10-day-old larvae were tested at 10 $\pm$ 1 C. These larvae, in groups of 50 held in 1-gal (3.8-liter) glass jars, had been fed barnacle larvae on alternate days for 10 days before they were used. The sea water was changed at the time of feeding. In these experiments, death (cessation of larval heart beat) was used as the criterion of effect in estimating EC_{50}'s.

In experiments 8 and 9, the first-stage zoeae were 1 and 8 days old, respectively. The 8-day-old larvae were held in groups of 50 in 1-gal (3.8-liter) glass jars and were fed brine shrimp larvae on alternate days before use. The larvae were exposed to eight or more Sevin concentrations ranging from 0.0003 to 10.0 mg/liter for 1, 5, or 24 hr at 10 $\pm$ 1 C. They were then transferred to beakers containing 200 ml of clean, sterilized sea water and held for a total of 25 days in experiment 8, and 8 days in

experiment 9. The zoeae in each beaker were fed about 300 to 400 brine shrimp larvae three times a week. The water was renewed in each beaker at the time of feeding, and at this time the larvae were observed for survival and molting.

The 2-day-old crab larvae used in experiment 10 were exposed continuously for 25 days to five Sevin concentrations ranging from 0.0001 to 0.01 mg/liter at 10 ± 1 C. The larvae were exposed alternately to light for 16 hr and to darkness for 8 hr. Each concentration was tested in triplicate. The zoeae in each beaker were fed about 1,000 brine shrimp larvae daily for the first 15 days of the experiment and three times a week thereafter. In a preliminary experiment, we found that brine shrimp larvae are not affected in 24 hr even by 1.0 mg/liter of Sevin. The test solutions were changed at the time of feeding. The crab larvae were observed daily for survival and molting.

JUVENILE TESTS

In experiment 11, second stage juvenile crabs, averaging 9.5 mm in carapace width, were held for 2 days in sea water at the test temperature of 14 ± 1 C and at the test salinity before transfer to 1-gal (3.8-liter) glass jars containing 2 liters of test solution. They were exposed in groups of six either to seven concentrations of Sevin ranging from 0.01 to 10.0 mg/liter for 1 hr, or to six concentrations ranging from 0.0032 to 1.0 mg/liter for 24 or 48 hr. After exposure, they were transferred to clean, standing sea water and observed for 3 days for delayed toxic effects, using death or irreversible paralysis as the criterion of effect. The animals were not fed during the experiment.

Experiment 12 was done to determine if 24-hr exposure to sublethal concentrations of Sevin affects survival, molting, and the behavioral interactions between exposed and unexposed crabs held together. The crabs were third stage juveniles with an average carapace width of 14.5 mm. Sex of the crabs was used as a marker to distinguish between exposed and unexposed animals because there is no other known marker for crabs of this size that will persist through molting. The sex of third stage juvenile crabs can be determined by raising the abdomen and looking for oviduct openings. However, this procedure can damage the brittle abdomen. We have found that the sex of third stage juveniles can be quickly and easily determined by the external shape of the second abdominal segment. The edges of this segment are straight in males and curved in females.

In experiment 12, the crabs were held for 5 days in sea water at 12 ± 1 C before transfer to 1-gal (3.8-liter) glass jars containing 2 liters of test solution. They were exposed in groups of six to Sevin concentrations of 0.01 or 0.032 mg/liter for 24 hr. Then a group of 10 crabs of each sex was removed from each exposure concentration and subdivided into two equal subgroups. Each subgroup was placed with five unexposed crabs of the opposite sex and approximately the same size into a 13.3-liter plastic container. Thus, there were eight containers each containing five exposed crabs of one sex and five unexposed crabs of the opposite sex. Two additional containers each had five male and five female crabs from one exposure concentration and two other containers each had

five male and five female crabs from the other exposure concentration. There were also two control containers each with five unexposed female and five unexposed male crabs. The 14 containers were supplied with flowing, filtered, aerated, sea water at 12 ± 1 C at a rate of 400 ml/min. The bottom of each container was covered with sand and broken clam shells 4 cm deep. The crabs were exposed alternately to light for 16 hr and darkness for 8 hr. They were fed chopped cockle clams daily, and at that time deaths and molts were recorded. Carapace widths of the crabs were measured at the end of the experiment and compared with initial widths.

The crabs used in experiments 13 and 14 were probably ninth stage juveniles. Their carapace widths ranged from 58 to 80 mm and averaged 67 mm. They were acclimated to the test temperature of 18 ± 1 C and salinity of 25‰ for 4 days, and then placed in groups of five into 5-gal (19.0-liter) glass jars containing 15 liters of aerated test solution. The test solutions were changed daily. The four Sevin concentrations used ranged from 0.18 to 1.0 mg/liter. The experiment was ended after 96 hr and the EC_{50}'s estimated using death or irreversible paralysis as the criterion of toxic effect.

ADULT TESTS

Two experiments using adult crabs were done. The crabs used in experiment 15 were females and averaged 110 mm in carapace width. They were acclimated to the test temperatures of 11 or 18 C and salinity of 25‰ for 4 days, and then placed in groups of three into 5-gal (19.0-liter) glass jars containing 15 liters of aerated test solution. The test solutions were changed daily. The four Sevin concentrations used ranged from 0.18 to 1.0 mg/liter. The experiment was ended after 96 hr and the EC_{50}'s estimated using death or irreversible paralysis as the criterion of toxic effect.

Experiment 16 was a secondary poisoning test. Adult male and female crabs with an average carapace width of 100 mm were fed cockle clams that had just been exposed to different concentrations of Sevin. Clams with a mean shell length of 60 mm were exposed to Sevin in groups of 11 for 24 hr in 5-gal (19.0-liter) glass jars containing 15 liters of test solution at 13 ± 1 C. The concentrations used were 1.0, 3.2, and 10.0 mg/liter. Clams in the last concentration became paralyzed, i.e., they were unable to close the shell or retract the extended foot. After exposure, the clams were removed from their shells, rinsed in clean sea water, and weighed. Some of these clams were fed to the crabs and the others were saved for tissue analyses. A group of three crabs was placed into each of twelve 13.3-liter plastic containers provided with flowing sea water of 25 to 32‰ salinity at 11.0 ± 1 C. The 12 containers were arranged into four equal subgroups. Each container in each subgroup received either three unexposed clams or three clams from one of the above exposure levels. The numbers of paralyzed or dead crabs were recorded at 6 hr and again at the end of the 24-hr experiment. The clam tissue in each container was weighed at the beginning and end of the experiment, and the amount eaten by the crabs was computed.

TISSUE ANALYSES

Tissues from animals used in the above experiment were analyzed for

Sevin and free 1-naphthol, the first hydrolytic product of Sevin. Twenty-seven clams, nine from each of the three exposure groups, and nine crabs, one from each of the nine groups that had received exposed clams, were analyzed. Nine additional crabs that had been exposed for 24 hr to 0.32, 1.0, and 3.2 mg/liter solutions of Sevin were analyzed to compare their uptake of Sevin with that of crabs fed treated clams. Control animals for the analyses were nine unexposed clams and six unexposed crabs. Clams that had been removed from their shells and whole crabs were treated and analyzed as follows: (1) animals from each group were pooled and thoroughly homogenized in a blender; (2) 20 g and 30 g of clam and crab homogenate, respectively, were mixed with 90 g of anhydrous sodium sulfate; (3) the mixtures were placed in a paper extraction thimble in a glass jar, and about 100 ml each of pentane and ether were added; (4) the samples were stored at 3 C until analyzed; (5) the samples were extracted and the extracts purified using a modification of the procedure of Johnson et al. (1963); and (6) the colorimetric procedure described by Karinen et al. (1967) was used for determination of Sevin and free 1-naphthol.

RESULTS

EGG AND PREZOEAL TESTS

Sevin did not affect hatching of eggs. The percentages of control eggs that hatched within the 24-hr test period ranged from 53 to 76%, and for eggs at the highest concentration of Sevin (1.0 mg/liter) the hatching percentages were 65 and 77%.

Of the prezoeae that hatched in the control vessels, 85 to 95% molted to zoeae within the 24-hr test period. With Sevin concentrations increasing from 0.0001 to 1.0 mg/liter the numbers of prezoeae that molted decreased from about 85 to 0%, respectively (Fig. 1). The 24-hr EC_{50}'s for the three experiments are 0.006, 0.02, and 0.03 mg/liter.

The 117 prezoeae that did not molt during the test were transferred to clean water and held for 8 days. None of the larvae that had been exposed to Sevin concentrations greater than 0.032 mg/liter were alive at the end of the 8-day observation period. Only 3% of those exposed to the lower concentrations molted and survived.

ZOEAL TESTS

Experiments 4 through 7 were acute toxicity tests. At 10 $\pm$ 1 C, the 24- and 48-hr EC_{50}'s could not be estimated because few larvae were killed even at concentrations as high as 82.0 mg/liter. However, the majority of larvae were not swimming at concentrations as low as 0.005 mg/liter. In the one 96-hr experiment at 10 $\pm$ 1 C, the 96-hr EC_{50} was found to be 0.01 mg/liter. In the one experiment at 17 $\pm$ 1 C, the 24- and 48-hr EC_{50}'s were 0.08 and 0.005 mg/liter indicating a marked temperature effect.

In experiments 8 and 9, we tested the delayed effects of Sevin on swimming, molting, and survival of zoeae 1 and 8 days old, respectively. In experiment 8, all larvae were alive after the 1, 5, and 24 hr exposures after which the larvae were returned to clean sea water, but on

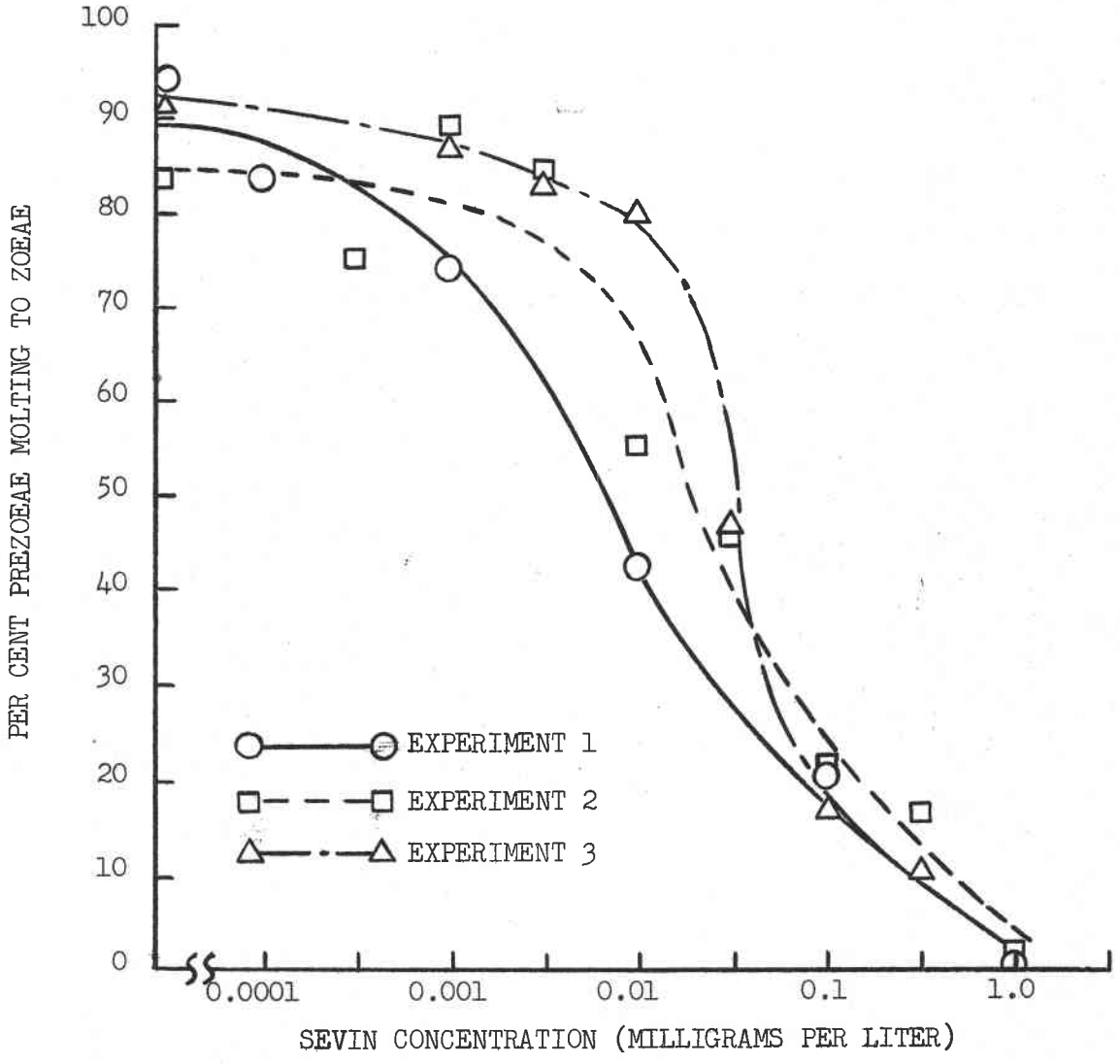

Fig. 1. Effect of a 24-hr exposure to Sevin on the molting of Cancer magister prezoeae to zoeae.

day 3 dead larvae were observed. Many larvae ceased swimming during the periods of exposure to Sevin but resumed swimming after return to clean water. Thereafter a number again stopped swimming, and therefore the EC_{50} values based on cessation of swimming as the criterion of effect decreased with increasing observation time after the third day (Table 2). These EC_{50} values for any observation period decrease progressively with increase of exposure time. EC_{50} values based on death as the criterion of effect did not show the same consistent relation to exposure time during the first 6 days of observation. However, by day 15 the EC_{50} values based on death as the criterion of effect approximated or equalled those based on cessation of swimming as the criterion (Table 2). Therefore, by day 15, the consistent inverse relation between exposure time and EC_{50}'s that was noted for all observation times when cessation of swimming was the criterion of effect can be seen also when death is the criterion. Thus, on day 15, the percentages of survival of zoeae exposed to Sevin concentrations of 0.03 to 1.0 mg/liter decreased markedly as the duration of exposure to these concentrations increased. (Fig. 2). In this experiment, Sevin did not affect molting of the larvae. Approximately equal numbers of control and experimental larvae molted to second stage zoeae during days 13 to 23 of the experiment.

In experiment 9, 8-day-old larvae were exposed to Sevin for 1 and 24 hr and then held in clean sea water for 8 days. The results were generally the same as those obtained with larvae 1 day old in experiment 8. All larvae were alive at the end of the exposure periods, but on day 3 some were dead. The EC_{50}'s at this time for the 1- and 24-hr exposures were 0.57 and 0.024 mg/liter, respectively, on day 6 they were 0.24 and 0.019 mg/liter, and on day 8 they were 0.19 and 0.017 mg/liter. As in the previous experiment, there was an immediate effect on swimming. Again, many larvae recovered from this paralysis but some of these died later. On day 8, the EC_{50}'s, based on cessation of swimming as the criterion of effect were found to be identical with those based on death as the criterion. Survivals of control larvae on days 6 and 8 were 93 and 77%, respectively.

The larvae in experiment 10 were exposed continuously to Sevin for 25 days. During this time they were observed for effects on molting and survival. All larvae were alive at the end of day 1, but on day 2 some of those exposed to the two highest Sevin concentrations, 0.0032 and 0.01 mg/liter, were dead (Fig. 3A). The 96-hr EC_{50} was estimated to be 0.009 mg/liter. This value nearly agrees with the 96-hr EC_{50} of 0.01 mg/liter based on the results of the acute toxicity test mentioned previously. Survival of larvae at the highest Sevin concentration of 0.01 mg/liter decreased from 97% on day 3 to 10% on day 5, and on day 20 it was 3%. Survival of larvae at the other concentrations tested (0.0001 to 0.0032) ranged from 73 to 88% on day 20, and at this time 83% of the controls were alive. On day 25, the percentages of larvae surviving at Sevin concentrations of 0.0001, 0.00032, 0.001, 0.0032, and 0.01 mg/liter were 83, 60, 69, 21, and 0%, respectively, and control survival was 79%. The 20- and 25-day EC_{50}'s based on death as the criterion of effect were estimated to be 0.005 and 0.002 mg/liter of Sevin, respectively.

There was a direct relationship between Sevin concentration and delay or prevention of molting of larvae in experiment 10 (Fig. 3). The effect on

Table 2. Effects of previous exposures to Sevin on swimming and survival of Dungeness crab zoeae held in clean sea water for periods up to 25 days.[a]

Exposure time (hours)	Criterion of toxic effect	EC_{50}'s (mg/liter) for observation days indicated below:										
		0[b]	3	6	8	11	13	15	18	20	22	25
1	Cessation of swimming	0.056	1.7	1.7	1.6	1.6	1.4	1.2	0.56	0.26	0.21	0.21
	Death	>10.0	2.5	1.9	1.8	1.7	1.6	1.3	0.56	0.26	0.21	0.21
5	Cessation of swimming	0.018	0.78	0.73	0.55	0.52	0.22	0.13	0.12	0.11	0.032	0.024
	Death	>10.0	>10.0	2.1	1.4	0.62	0.45	0.18	0.12	0.11	0.032	0.024
24	Cessation of swimming	0.0065	0.017	0.016	0.016	0.016	0.016	0.015	0.013	0.012	0.0032	0.0032
	Death	>1.0	0.13	0.017	0.016	0.016	0.016	0.015	0.014	0.012	0.0032	0.0032

[a] The larvae were exposed to Sevin in sea water of 25‰ salinity at 10 ± 1 C for the indicated exposure times. Then they were transferred to clean sea water of the same salinity and temperature and fed brine shrimp larvae three times a week. Survival of control larvae on days 15 and 25 was 90 and 79%, respectively.

[b] At the end of the exposure period.

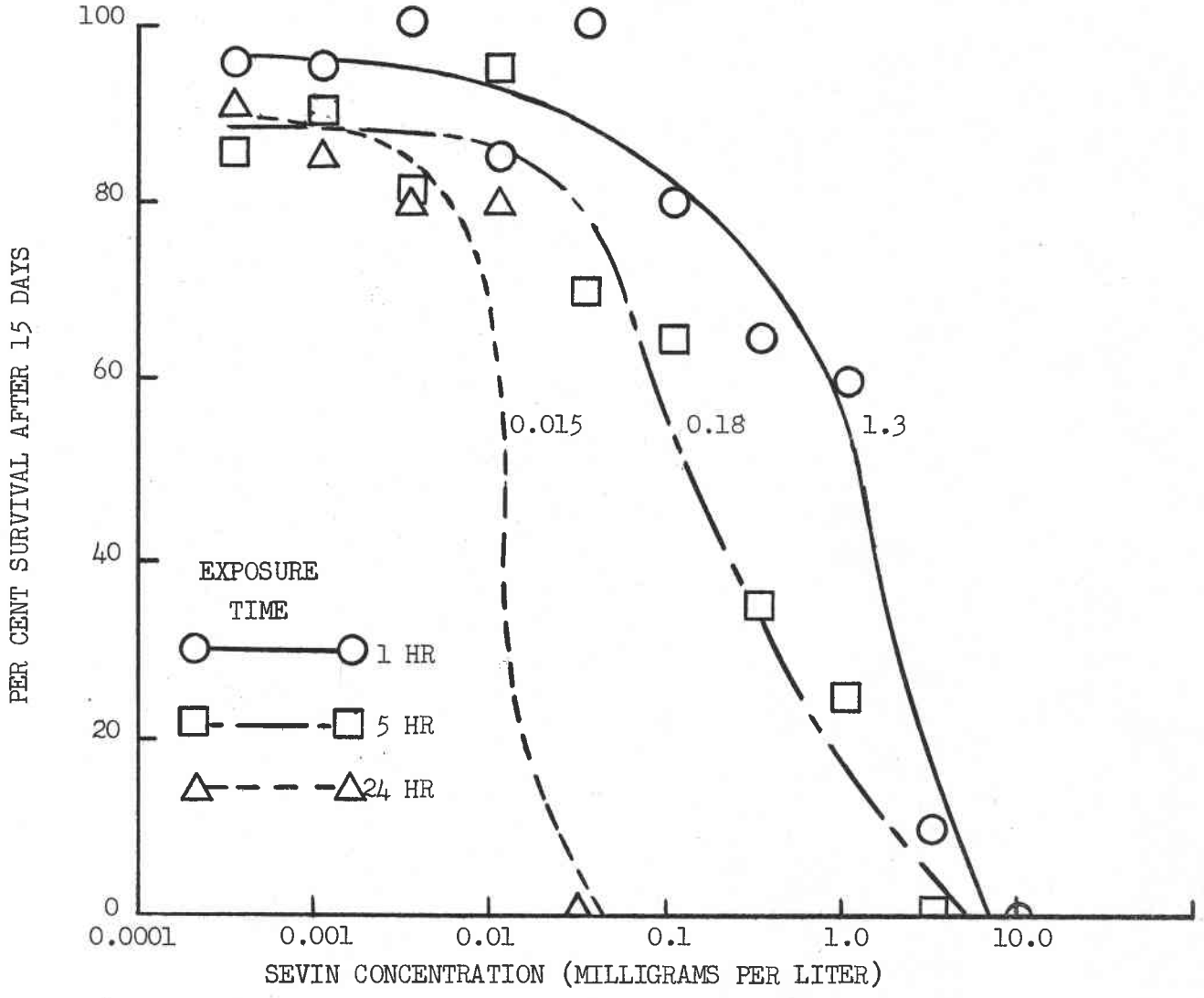

Fig. 2. Survival of Dungeness crab zoeae on day 15 in clean sea water after exposure to Sevin for different time periods. The number beside each curve is the EC_{50} value. Survival of control larvae was 90%.

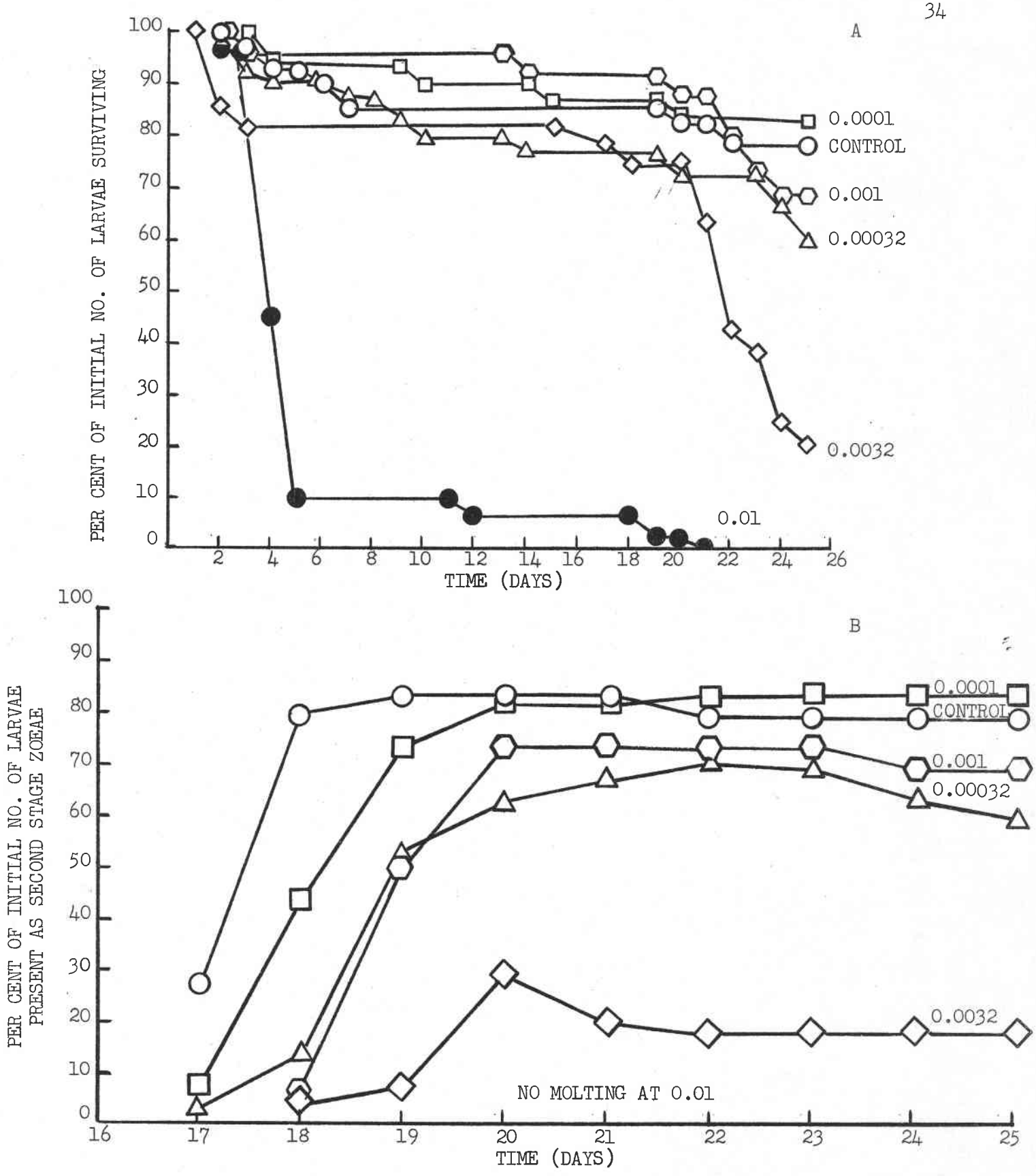

Fig. 3. Effect of different Sevin concentrations on survival (A) and molting (B) of first stage Dungeness crab zoeae. The larvae were exposed continuously to Sevin for 25 days. The number beside each curve is the toxicant concentration used.

molting of larvae to second stage zoeae could first be observed on day 17. At that time, 28% of the controls had molted and were living, whereas only 7% of the larvae at the lowest Sevin concentration of 0.0001 mg/liter and none at the three highest concentrations had molted and survived (Fig. 3B). On day 18, 79% of the controls and 43% of the larvae exposed to 0.0001 mg/liter of Sevin and less than 15% of those exposed to higher concentrations had molted and were still living. At the end of the experiment, however, all surviving larvae except one exposed to 0.0032 mg/liter were second stage zoeae.

On day 3 of experiment 10, we found all of the larvae infested with a microorganism, which was either an alga or a fungus. The infestation was lost when the larvae molted. We do not know if the infestation affected survival and molting of the larvae, but at the end of the experiment 79% of the controls, all of which had been infested, had molted and were alive. This percentage was the same as that of control larvae in experiment 8, which also lasted 25 days and in which no infestation of the larvae was seen.

JUVENILE TESTS

In experiment 11, crabs were exposed to different Sevin concentrations for 1, 24, or 48 hr and then held in clean sea water for 3 days. The 1-hr EC_{50} was estimated to be 4.3 mg/liter when death or paralysis by the end of the exposure period was the criterion of effect; it decreased to 1.5 mg/liter when death or paralysis within 3 days after exposure was the criterion of effect. The 24-hr and 48-hr EC_{50}'s were found to be 0.076 and 0.057 mg/liter, respectively, when death or paralysis by the end of the exposure period was the criterion of effect; they were found to be the same when death or paralysis within 3 days after exposure was the criterion of effect.

In experiment 12, crabs were exposed to sublethal concentrations of Sevin for 24 hr and then held in clean sea water for 44 days, during which they were observed for delayed effects of Sevin on molting and survival, and on the interaction between exposed and nonexposed crabs held together. Sevin did not affect molting or growth of crabs. The mean per cent increases in carapace width for controls and for crabs exposed to 0.01 mg/liter of Sevin when the unexposed and exposed crabs were held together were 92 and 91%, respectively. They were 94 and 92% for controls and crabs exposed to 0.032 mg/liter and held together with the controls. The mean per cent increases for control crabs and for those exposed to 0.01 and 0.032 mg/liter of Sevin when these groups were held separately were 93, 93, and 96%, respectively. Sevin did not affect survival of the crabs. Survival percentages for all controls and for all crabs exposed to 0.01 and 0.032 mg/liter of Sevin were 88, 88, and 85%, respectively. Those deaths that did occur were due to cannibalism, newly-molted crabs having been eaten by others. Sevin apparently did not affect the normal behavioral interaction of crabs. For example, we did not observe differences in aggressiveness or in burrowing ability between control and exposed crabs.

Experiment 13 and 14 were acute toxicity tests with older, probably ninth stage, juvenile crabs. For the two experiments, the 24-, 48-, and

96-hr EC_{50}'s were 0.35 and 0.62, 0.30 and 0.32, and 0.22 and 0.28 mg/liter, respectively.

ADULT TESTS

Experiment 15 was a 96-hr acute toxicity test done at two temperatures, 11 and 18 C. We found that Sevin was more toxic to the crabs at the higher temperature (Fig. 4). The 24-hr EC_{50}'s at 11 and 18 C were 0.49 and 0.32 mg/liter, and the 96-hr EC_{50}'s at 11 and 18 C were 0.26 and 0.18 mg/liter, respectively.

Experiment 16 was a secondary poisoning test. Crabs were fed cockle clams that had been exposed to Sevin. They were observed for paralysis at 6 hr and again at the end of the 24-hr experiment. At 6 hr, 22, 77, and 100% of the crabs were paralyzed after eating clams that had been exposed to 1.0, 3.2, and 10.0 mg/liter of Sevin (Table 3). One additional crab, which had eaten clams exposed to the intermediate Sevin concentration, became paralyzed after more than 6 hr. Control crabs, which had eaten unexposed clams, remained normal during the experiment. Crabs fed clams exposed to 0.0, 1.0, 3.2, and 10.0 mg/liter of Sevin ate 14.4, 13.8, 10.8 and 9.0 g of clam tissue, respectively (Table 3).

Clams exposed to Sevin in the water had higher tissue concentrations of Sevin and 1-naphthol, measured together, and also of Sevin alone, than did crabs exposed to the same Sevin concentrations (Table 4). For example, clams and crabs exposed to 3.2 mg/liter of Sevin had respective tissue concentrations of 8.60 and 0.91 ppm of Sevin. Crabs exposed to Sevin in the water had higher tissue concentrations of Sevin and 1-naphthol, and of Sevin alone, than did crabs that ate clams exposed to the same Sevin concentrations (Table 4).

DISCUSSION

We must know the effects of a pesticide on as many life history stages of a species as possible before we can critically evaluate the hazards involved in its use. For this reason, acute toxicity studies per se are not adequate. We need studies involving short-term and long-term exposures of animals to sublethal concentrations of pesticides, and this study was planned accordingly.

Of the life history stages of C. magister that we tested, the early larvae were the most sensitive to Sevin. A Sevin concentration of 1.0 mg/liter did not affect egg hatching, but it prevented molting of all prezoeae to zoeae, and a concentration as low as 0.006 mg/liter prevented molting of 50% of the larvae. Exactly 50% of first stage zoeae were killed in 96 hr when exposed to 0.01 mg/liter of Sevin at 10 C, whereas at nearly the same temperature of 11 C the 96-hr EC_{50} for adult crabs was 0.26 mg/liter.

Few zoeae were killed in 24 hr after exposure to a Sevin concentration as high as 82.0 mg/liter. The 24-hr EC_{50} based on death within 15 days after the exposure as the criterion of effect was estimated to be 0.015 mg/liter. These results clearly show delayed toxic effects resulting from a short-term exposure. However, the 24-hr EC_{50} based on cessation of

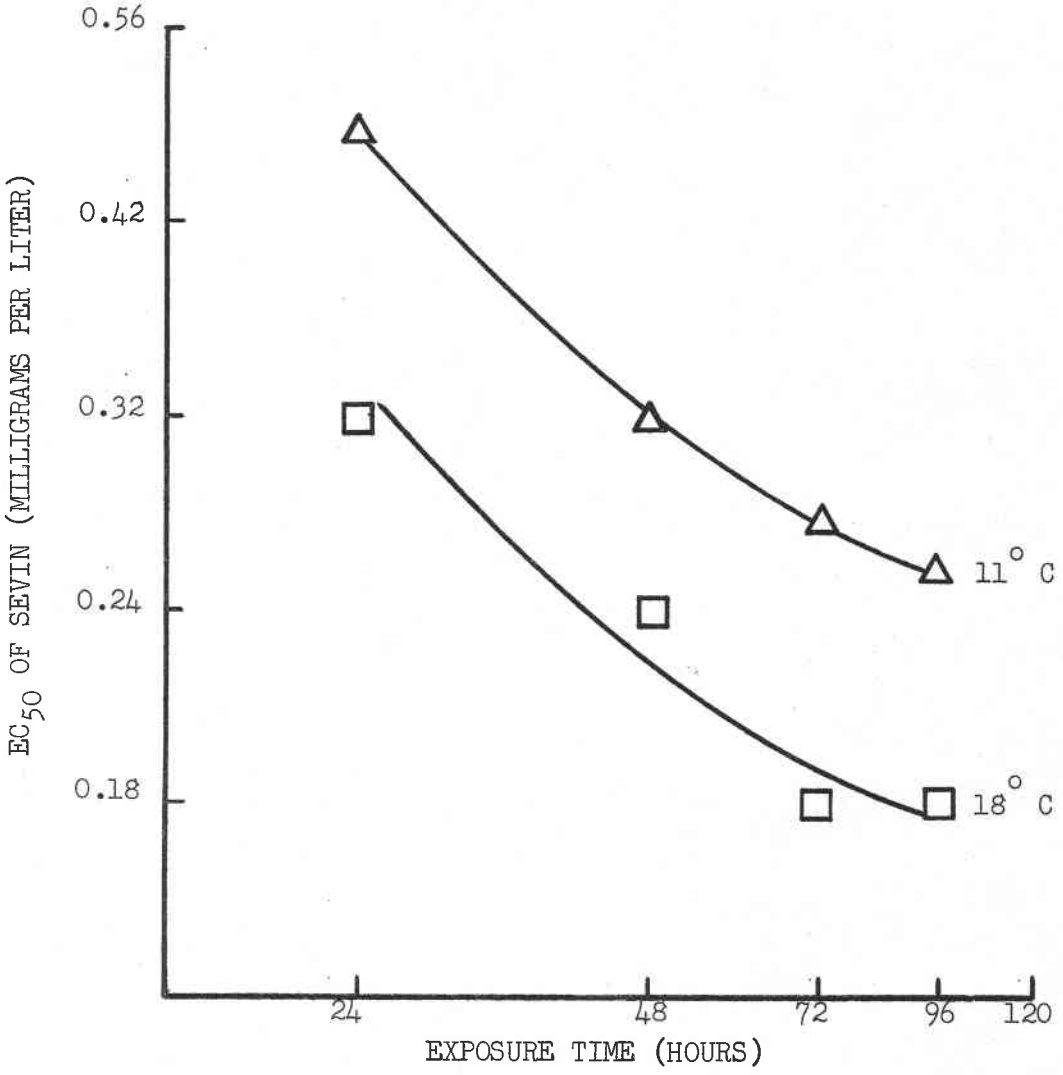

Fig. 4. Effect of Sevin on survival of adult female Dungeness crabs at 11 and 18 C.

Table 3. Effect on adult Dungeness crabs of feeding on cockle clams previously exposed to Sevin.[a]

No. clams exposed	Sevin concentration (mg/liter)	% survival of clams[b]	No. crabs	Mean wt (g) of clam tissue		% crabs paralyzed after:	
				Fed	eaten	6 hr	24 hr
9	0.0	100	9	14.4	14.4	0	0
9	1.0	100	9	15.0	13.8	22	22
9	3.2	100	9	15.4	10.8	77	89
9	10.0	0	9	15.4	9.0	100	100

[a] Crabs in groups of three were fed exposed clams and were observed for irreversible paralysis at 6 and 24 hr.

[b] At the end of the 24-hr exposure period.

Table 4. Concentrations of Sevin and 1-naphthol found in tissues of cockle clams and Dungeness crabs exposed for 24 hr to different concentrations of Sevin in water, and concentrations found in Dungeness crabs exposed to Sevin by feeding them clams previously exposed to Sevin in water.

Animal	Kind of exposure	Sevin concentration (mg/liter)	Tissue concentration (ppm)[a]	
			Sevin plus free 1-naphthol	Sevin alone
Clams	In water	1.0	3.45	----[b]
		3.2	13.38	8.60
		10.0	39.45	31.30
Crabs	In water	0.32	<0.10	0.10
		1.0	----[b]	0.10 - 0.30
		3.2	0.94	0.91
Crabs	In food	1.0[c]	<0.10	<0.10
		3.2[c]	<0.10	<0.10
		10.0[c]	0.95	0.88

[a] On a wet-weight basis. The crab sample included the exoskeleton.

[b] Sample was lost during analysis.

[c] Concentration in water to which clams used as food were exposed.

swimming, which was not always permanent, as the criterion of effect was 0.0065 mg/liter. Therefore, this criterion is more sensitive than death. Also, it may be more significant ecologically because in nature nonswimming larvae perhaps would not often survive. Thus, the 24-hr EC_{50} based on cessation of swimming as the criterion of effect is probably more meaningful than the EC_{50} values determined after observation periods of 15 days using either death or cessation of swimming as the criterion.

Long-term effects of Sevin on zoeae exposed continuously to sublethal concentrations of the pesticide were shown. Sixty per cent or more of larvae exposed for 25 days to Sevin concentrations ranging from 0.0001 to 0.001 mg/liter survived and molted. However, molting was delayed at a concentration as low as 0.0001 mg/liter. None of the larvae exposed to the highest concentration of 0.01 mg/liter molted. Most of those exposed to the next highest concentration of 0.0032 mg/liter should have molted soon after day 20 (Fig. 3B) but at this time there was a marked decrease in survival (Fig. 3A). This high mortality may have been due to an increase in larval sensitivity to Sevin associated with the molting process. However, increased mortalities of larvae that were exposed to lower concentrations, and that had molted earlier was observed between days 19 and 25. Therefore, molting cannot be said definitely to have been a cause of the greater mortality at the 0.0032 mg/liter concentration.

Young juvenile crabs are more sensitive to Sevin than are older juveniles or adults. Sevin concentrations of 0.076, 0.35 to 0.62, and 0.49 mg/liter irreversibly paralyzed within 24 hr 50% of 2nd stage juveniles, 9th stage juveniles, and adults, respectively. Our 24-hr EC_{50}'s for 9th stage juveniles agree with those of Stewart et al. (1967), who reported 24-hr EC_{50}'s ranging from 0.55 to 0.70 mg/liter for late juvenile Dungeness crabs. Crabs of other species have about the same sensitivity to Sevin as Dungeness crabs. The 24-hr EC_{50}'s for small stone crabs (scientific name not given) was 1.0 ppm (Butler, 1962), for juvenile blue crabs, Callinectes sapidus, it was 0.55 ppm, (Butler, 1963) and for adult shore crabs, Hemigrapsus oregonensis, it was 0.06 to 1.05 mg/liter (Stewart et al., 1967). The only other published study of the effects of Sevin on crabs known to us is that of Andrews et al. (1968), who reported that a Sevin concentration of 10.0 mg/liter eliminated parasitic pea crabs, Pinnotheres ostreum, from live oysters in 12 hr.

We found that Sevin did not affect behavior, growth, or survival of juvenile C. magister when the crabs were exposed to Sevin concentrations as high as 0.032 mg/liter for 24 hr and then held in clean sea water for 44 days. In contrast with these results are those we obtained in experiments using zoeae, where we did find delayed toxicity to the larvae after their short-term exposure to Sevin.

We have shown that adult crabs can be killed within 6 hr after feeding on cockle clams previously exposed to Sevin. These results may explain in part the findings of Snow and Stewart (1963), who observed many paralyzed cockle clams on an oyster ground in Oregon that had been treated 30 min previously with Sevin to control burrowing ghost and mud shrimps. A day later they found a large number of dead Dungeness crabs on the grounds.

They suggested that the crabs may have moved on to the treated area during flood tide and been killed either by direct contact with the chemical or by feeding on paralyzed clams.

Butler et al. (1968), suggested, on the basis of their results and those of Karinen et al. (1967), that the best time for treatment of estuaries with Sevin to control oyster pests would be the period when maximum water temperatures occur. The insecticide at this time would be more rapidly degraded and perhaps be accumulated by clams in minimal amounts. Our results suggest that minimal damage to the Dungeness crab population from field application of Sevin would occur after the larvae had molted into juveniles. This larval molt in nature occurs predominantly in June in central California (Poole, 1965), from April to July in Oregon (Waldron, 1958), and in September in British Columbia (Butler, 1961).

ACKNOWLEDGMENTS

Grateful acknowledgment is expressed to the following individuals of Oregon State University: Mr Donald Bennett, Mr Jerry Butler, and Mr Dennis Wilson for technical assistance; Mr John Lamberton for the tissue analyses; Mr Dean Satterlee for making the graphs; and Dr Peter Doudoroff for advice given during this study.

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EFFECTS OF SEVIN ON CHUM SALMON

Specific aim no. 2c. To determine the short-term and long-term effects of Sevin on a representative estuarine fish.

INTRODUCTION

Lowe's (1967) study is the only published one known to us on the effects of Sevin on growth of an estuarine fish. He reported that growth of spot, Leiostomus xanthurus, was not affected after their exposure to 0.1 mg/liter of Sevin in flowing sea water for 5 months.

We have started studies on the short-term and long-term effects of Sevin on juvenile chum salmon, Oncorhynchus keta. This fish was chosen as a test animal because of: (1) its widespread distribution on the Pacific coast from northern California to the Bering Sea; (2) its economic importance; and (3) because it passes through estuaries during its migrations to and from sea. Also, the chum salmon spawns in the lower reaches of streams, which sometimes are under tidal influence. Thus, young fish could be exposed to pesticides in both freshwater and salt water. Therefore, their use in the laboratory to study the effects of pesticides in waters of different salinity would not be unnatural.

MATERIALS AND METHODS

PRELIMINARY STUDIES

Several preliminary experiments were done to determine if juvenile chum salmon could be used as an experimental animal in the laboratory. We found that the fish could be transferred successfully from fresh water directly to normal sea water. They showed signs of distress immediately after transfer, but they recovered within a few hours. Ten fish were held in 10 liters of freshwater at 15° C and 10 others in 10 liters of sea water at 15° C. They were fed daily an unrestricted diet of processed fish food. The mean initial weights of the two groups were 0.56 and 0.48 grams, and at the end of the seven day experiment, they had increased by 41% and 45%, respectively. These results suggest that juvenile chum salmon can be adapted readily to sea water in the laboratory.

ACUTE TOXICITY STUDIES

Toxicants

The Sevin was a microfine wettable powder, which contained 80% active ingredient and 20% inert materials. The 1-naphthol, the first hydrolytic product of Sevin, was a recrystallized reagent.

Experimental Fish

The juvenile chum salmon were obtained from the Oregon Fish Commission's Big Creek Salmon Hatchery on the Columbia River. Their mean total lengths and weights ranged from 41.2 to 54.0 mm and from 0.47 to 1.08 grams.

Bioassay Procedures

The fish were acclimated to the test conditions of temperature and salinity for 4 to 8 days before use in an experiment. Tests were done in 5-gal (19-liter) glass jars each containing 15 liters of test solution and 10 fish. The test

solutions were prepared from 1-liter stock solutions containing 100 mg of active ingredient dissolved in either freshwater or in sea water of 25 ‰ salinity. The fish were not fed during the experiment. The test solutions were replaced at the end of each 24 hour period and at that time the numbers of dead fish were recorded. The appropriate median tolerance limits (TL_m's), that is, concentrations killing 50% of the fish in the specified time periods, were estimated by straight-line graphical interpolation as suggested by the American Public Health Association et al. (1965).

RESULTS

The toxicity of Sevin to fish, which had been acclimated to the test conditions for 4 to 8 days, was greater at 20° C than at 11 or 15° C (Table 1). The respective mean 96-hr TL_m's are 1.5, 2.4, and 2.5 mg/liter. However, in one experiment the 96-hr TL_m for fish, which had been in sea water for 34 days before use, was 1.6 mg/liter at 15° C (Table 1). Sevin was less toxic to chum salmon in freshwater than in sea water (Table 1). The 96-hr TL_m was 3.9 mg/liter for fish in freshwater at 15° C. Sevin is less toxic than 1-naphthol to fish. The mean 96-hr TL_m for 1-naphthol is 0.39 mg/liter, whereas the lowest mean TL_m value for Sevin is 1.5 mg/liter.

These acute toxicity experiments were done in 1967. We had planned then to study the effect of sublethal concentrations of Sevin on growth of juvenile chum salmon; however, most of our fish at that time developed furunculosis and died. In 1968, we obtained another stock of fish but we lost most of them because of a Vibrio infection. We will try again in early 1970 to begin this growth study, and we hope to maintain disease-free fish in the laboratory using appropriate prophylactic procedures.

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Table 1. Acute toxicity of Sevin and 1-naphthol to juvenile chum salmon.

Toxicant and no. of Expts.	Temperature (C°)	Salinity (‰)	Acclimation time in days	TL_m (mg/liter)			
				24-hr	48-hr	72-hr	96-hr
				Mean(Range)	Mean(Range)	Mean(Range)	Mean(Range)
Sevin							
2	11	25	4-8	3.0(2.4-3.5)	2.7(2.4-2.9)	2.5(2.4-2.6)	2.4(2.2-2.6)
3	15	25	4	3.1(2.4-4.0)	3.1(2.4-4.0)	2.6(2.3-3.8)	2.5(2.2-2.7)
1	15	25	30(4)[a]	3.2(---)	2.2(---)	2.0(---)	1.6(---)
2	20	25	4-8	2.3(2.0-2.6)	2.2(2.0-2.3)	1.7(1.4-2.0)	1.5(1.3-1.6)
1	15	0	4[b]	>4.5(---)	>4.5(---)	>4.5(---)	3.9(---)
1-naphthol							
2	15	25	4	1.3(1.3 only)	0.52(0.42-0.62)	0.42(0.42 only)	0.39(0.35-0.42)

[a] Fish were held 30 days in flowing sea water and then acclimated to the experimental conditions for 4 days.

[b] Acclimated to temperature only.

EFFECTS OF DURSBAN ON SHINER PERCH

Specific aim no. 3c. To determine the short-term and long-term effects of Dursban on a representative estuarine fish.

A background study necessary for the main study on the effects of Dursban on reproduction of shiner perch has been completed. The following manuscript, which will appear in the September 1969 issue of the Journal of the Fisheries Research Board of Canada, summarizes the results. Following this manuscript is a summary of the results to date of the study of the effects of Dursban on shiner perch.

SHEET I. Send proof to Dr. Raymond E. Millemann, Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97331

Relationships of female age and size to embryo number and size in the shiner perch, *Cymatogaster aggregata* Gibbons[1,2]

By Dennis C. Wilson and Raymond E. Millemann

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WILSON AND MILLEMANN: REPRODUCTION OF SHINER PERCH

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[1] Technical Paper No. 2649, Oregon Agricultural Experiment Station.

[2] This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia, and consititutes part of thesis research conducted by the senior author at Oregon State University.

Ages, lengths, and weights were determined for 124 female shiner perch, Cymatogaster aggregata, collected in Yaquina Bay, Oregon, from May 17 through June 29, 1968. Embryos, numbering 1,005, were obtained from 111 gravid females either by dissection (737) or at parturition in the laboratory (268). Embryo lengths, weights, and numbers per female parent were determined.

With increase in age of females from 1 to 6 years, their mean fork length increased from 9.32 to 13.65 cm, their weight from 13.65 to 47.78 g, and the mean number of embryos per parent female increased from 5.83 to 20.00. The equations and their correlation coefficients (R) computed for the relationships of embryo number (Y) to parent female fork length, weight, and age are respectively: $\log Y = -1.892 + 2.735 \log X$ ($R = 0.89$); $Y = 1.131 + 0.313 X$ ($R = 0.70$); and $Y = 4.23 + 1.73 X$ ($R = 0.51$). Embryo size at birth was also directly related to female parent size. The equations computed for the relationships of total length of embryo at birth (Y) to parent female fork length and parent female weight are respectively: $Y = 24.211 + 1.620 X$ ($R = 0.79$); and $Y = 0.384 + 0.015 X$ ($R = 0.72$). The equation computed for the relationship of embryo weight (Y) to embryo length at birth is: $Y = -2.266 + 0.712 X$ ($R = 0.95$).

Received April , 1969

INTRODUCTION

The viviparous shiner perch, Cymatogaster aggregata Gibbons, is distributed along the Pacific coast from southern California to Alaska. These fish inhabit in large numbers the bays and estuaries during the summer months, and at this time breeding and parturition occur. For these reasons, we chose the shiner perch as a test animal for use in studies on the effects of pesticides, which may enter estuaries, on growth and reproduction of representative estuarine organisms. However, before beginning these studies we needed to assess the natural variation of the relationships between female size and embryo size and number. This information which is the subject of the present paper, is not available in sufficient detail from the studies of Suomela (MS, 1931) and Gordon (MS, 1965) on age and growth of C. aggregata or from the reports of Wiebe (1968 a,b), who studied the reproductive cycle of shiner perch and the effects of temperature and daylight on the reproductive physiology. Data similar to ours have been reported for three other embiotocid species, namely, the barred surfperch (Hubbs, 1921; Carlisle et al., 1960), the striped perch (Swedberg, MS, 1965), and the pile perch (Wares, MS, 1968).

MATERIALS AND METHODS

Four samples consisting of 100 adult female shiner perch were collected in Yaquina Bay, Oregon, at 2-week intervals from May 17, 1968 through June 29, 1968. The weight of each female less that of their embryos was recorded to the nearest 0.05 g and the fork length to the nearest 0.1 cm. Ages of the fish were determined by examination of scales. Scales from a key area under the left lateral line of each fish were removed, pressed on acetate cards, and read separately by three persons.

Embryos were removed individually from the female uterus and counted. Their weights to the nearest 0.001 g and total lengths to the nearest 0.01 cm were recorded. Total length was used because the caudal fin fork does not develop until just before birth of the embryo.

The relationship between female size and size of embryos at birth was determined from an additional sample of 24 fish collected in Yaquina Bay, Oregon, on July 1, 1968. These fish were held in the laboratory in 12-liter glass jars with continuously flowing sea water of about 30 ‰ salinity and at about 12.5 C. The fish were fed minced cockle clams, Clinocardium nuttalli, daily. At the time of parturition, which was 3 to 14 days after the females were collected, the young fish were removed from the jars and their weights and total lengths recorded, as well as those of a few removed from the females by dissection. Weights and fork lengths of the parent females also were recorded.

Standard length (SL) and total length (TL) were recorded for each of 71 females, which were 5.4 to 13.4 cm in fork length (FL). We computed equations from these measurements for conversion of FL to SL or TL, so that we could compare our results with those of others. These equations are:

$$TL = 0.54 + 1.05\ FL$$

and

$$SL = -0.34 + 0.92\ FL.$$

The respective correlation coefficients (R) are 0.96 and 0.97.

The age, length, and weight data were compiled on computer cards using a separate card for each embryo. A linear regression analysis program was used to obtain the equations for the various relationships. Logarithmic transformation was used to compute the equation for one relationship which did not prove linear when arithmetic coordinates were used.

RESULTS

Age Group Relationships

The 121 female fish, of which 109 were gravid, were 1 to 6 years old; 108 of the 121 females were 1 to 3 years old (Table I). With increase in age of females from 1 to 6 years, their mean fork length increased from 9.32 to 13.65 cm, their mean weight increased from 13.65 to 47.78 g, and the mean number of embryos per female increased from 5.83 to 20.00. The equations computed for the relationships of embryo number (Y) to parent female fork length (Fig. 1), weight (Fig. 2), and age are respectively:

$$\log Y = -1.892 + 2.735 \log X \quad (R = 0.89);$$

$$Y = 1.131 + 0.313 X \quad (R = 0.70);$$

$$\text{and } Y = 4.23 + 1.73 X \quad (R = 0.51).$$

The number of embryos per female ranged from 3 to 25. Yearling and 2-year-old females can be recognized by their size and number of embryos per female, but with fish in age groups II through VI there is some overlap of the relationships between these characteristics and age (Table I).

Sampling Time Relationships

The number of embryos measured was 1,005; these were obtained from 111 gravid females. The mean total length and mean weight of embryos collected on May 17 and June 29 were 2.23 cm and 0.102 g and 3.62 cm and 0.484 g, respectively; and the mean total length and mean weight of embryos at parturition were 4.37 cm and 0.842 g (Table II). The sample of newborn young was a highly selective one and this explains the large difference in mean size between these fish and the embryos obtained in the field only 1 to 2 weeks earlier.

Size of embryos at birth was directly related to female parent size (Figs. 3 and 4). The equations computed for the relationships of total length of embryo at birth (Y) to parent female fork length and parent female weight are respectively:

$$Y = 24.211 + 1.620 X \quad (R = 0.79);$$

$$Y = 0.384 + 0.015 X \quad (R = 0.72).$$

Size of embryos collected in the field was also directly related to female parent size for each sample date. The smallest embryo, which came from a parent 9.7 cm long and 14.4 g in weight, was 3.90 cm long and 0.454 g in weight at birth; the largest embryo, which came from a parent 14.0 cm long and 40.0 g in weight, was 4.90 cm long and 1.256 g in weight at birth. The equation computed for the relationship of embryo weight (Y) to embryo length at birth (Fig. 5) is:

$$Y = -2.266 + 0.712 X \quad (R = 0.95).$$

DISCUSSION

Our data show that shiner perch females born during the summer reach maturity and give birth to young during their second summer. This finding

Table I. Mean lengths and weights of female shiner perch, Cymatogaster aggregata, and the mean number of embryos per female fish for each age class.

	Female fish[a]			
Age group	No. examined/ No. gravid	Mean fork length (cm)	Mean weight (g)	Mean no. embryos per female
I	51/46	9.32 ± 0.59[b]	13.65 ± 2.64[b]	5.83 ± 0.86[b]
II	32/28	11.11 ± 0.54	24.12 ± 3.55	9.30 ± 1.69
III	25/24	11.78 ± 0.61	28.60 ± 4.50	11.10 ± 1.89
IV	9/8	12.96 ± 0.84	37.09 ± 6.05	15.40 ± 4.33
V	2/1	13.35	44.95	15.00
VI	2/2	13.65	47.78	20.00
Totals	121/109			

[a] Scales of three of the 124 fish collected were not readable.

[b] Standard deviation of mean.

Table II. Mean lengths and weights of female and embryo shiner perch, Cymatogaster aggregata, for each sampling period.

Sample date (1968)	Adults			Embryos		
	No. examined/ No. gravid	Mean fork length (cm)	Mean weight (g)	Sample size	Mean total length (cm)	Mean weight (g)
5/17	28/25	11.33 ± 1.55[a]	26.95 ± 11.45[a]	255	2.23 ± 0.61[a]	0.102 ± 0.085[a]
6/2	27/23	10.62 ± 1.50	21.36 ± 9.38	174	2.73 ± 0.59	0.173 ± 0.077
6/16	25/25	11.09 ± 1.14	24.66 ± 7.39	216	3.77 ± 0.37	0.474 ± 0.145
6/29	20/14	10.08 ± 0.96	18.04 ± 6.11	92	3.62 ± 1.02	0.484 ± 0.222
7/4-7/15[b]	24/24	12.01 ± 1.11	30.39 ± 8.16	268	4.37 ± 0.23	0.842 ± 0.171
Totals	124/111			1005		

[a] Standard deviation of the mean.

[b] These fish were collected July 1, 1968 and held in the laboratory to the time of parturition.

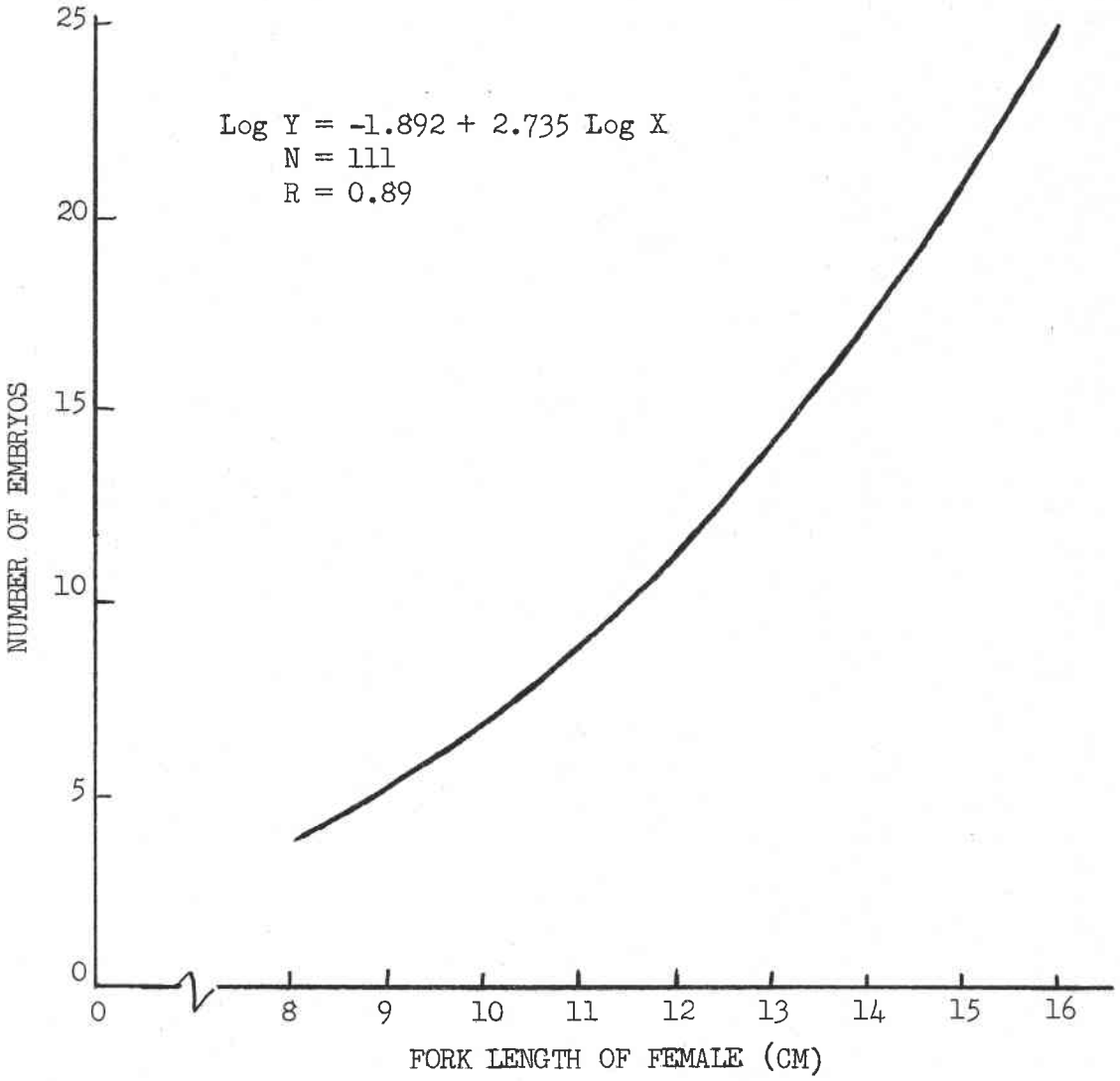

Fig. 1. Regression of the number of Cymatogaster aggregata embryos compared with fork length of parent females. (Points in this and succeeding figures not shown for reason of clarity.)

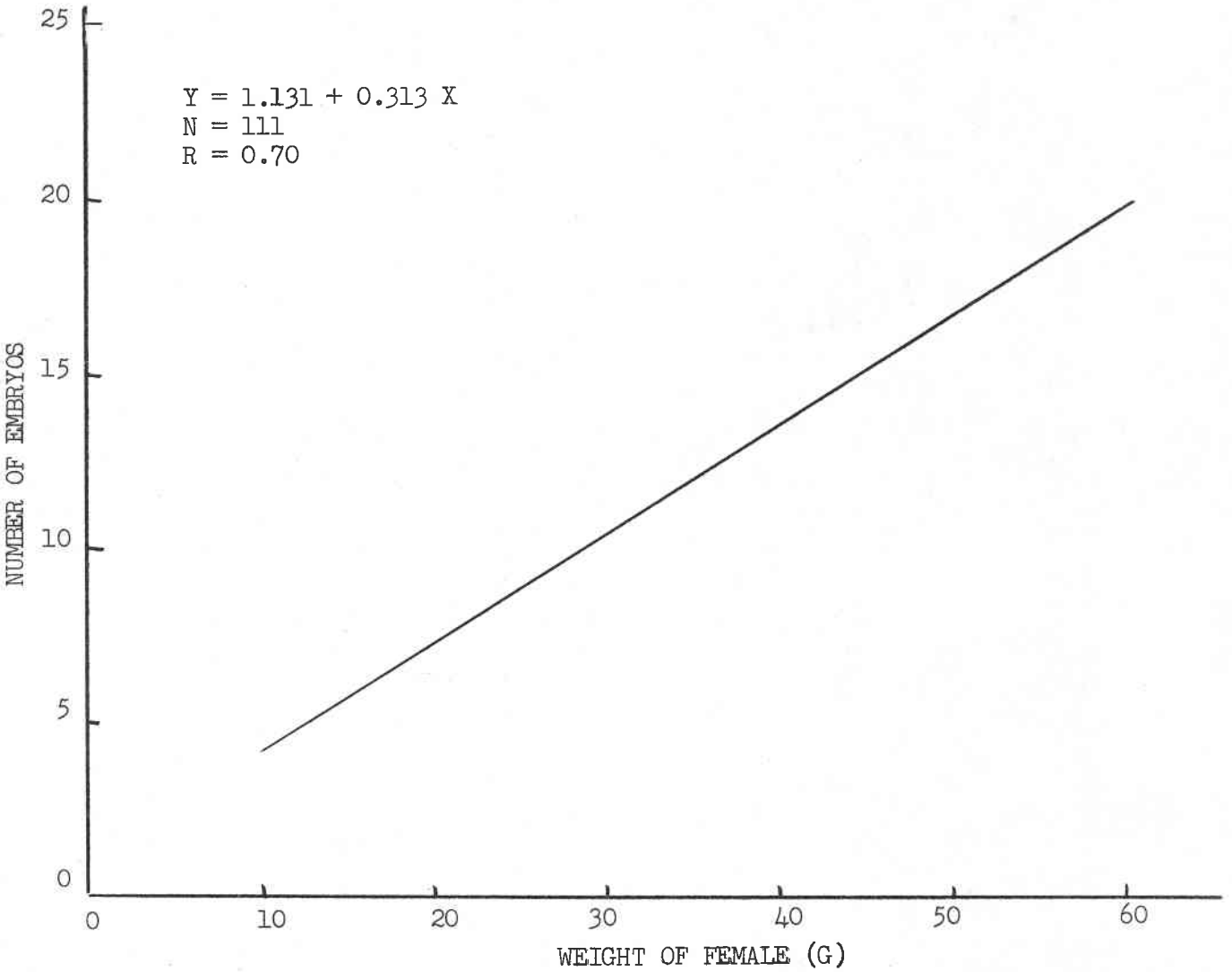

Fig. 2. Regression of the number of Cymatogaster aggregata embryos compared with weight of parent females.

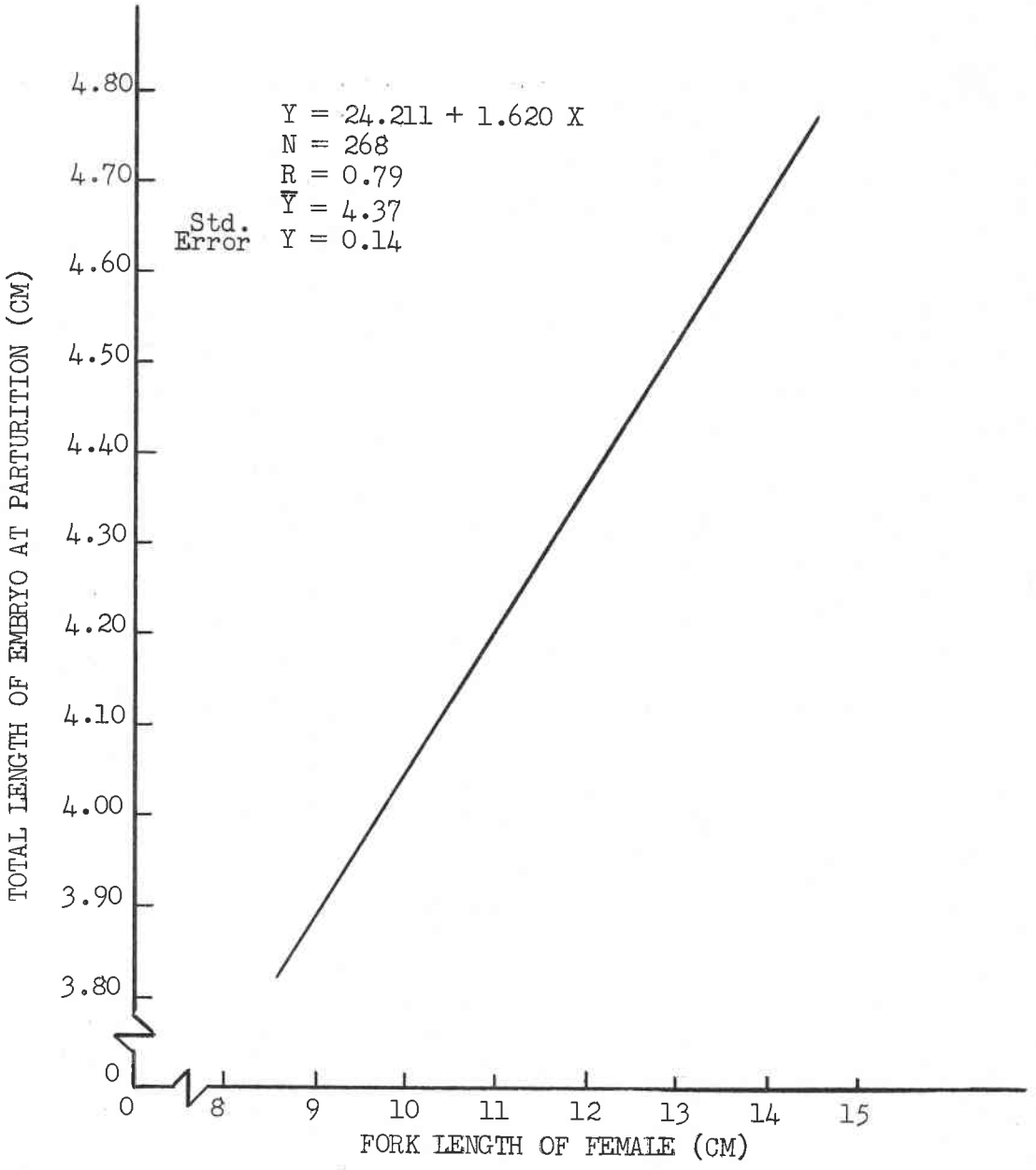

Fig. 3. Regression of the total length of Cymatogaster aggregata embryos at parturition compared with the fork length of 24 parent females

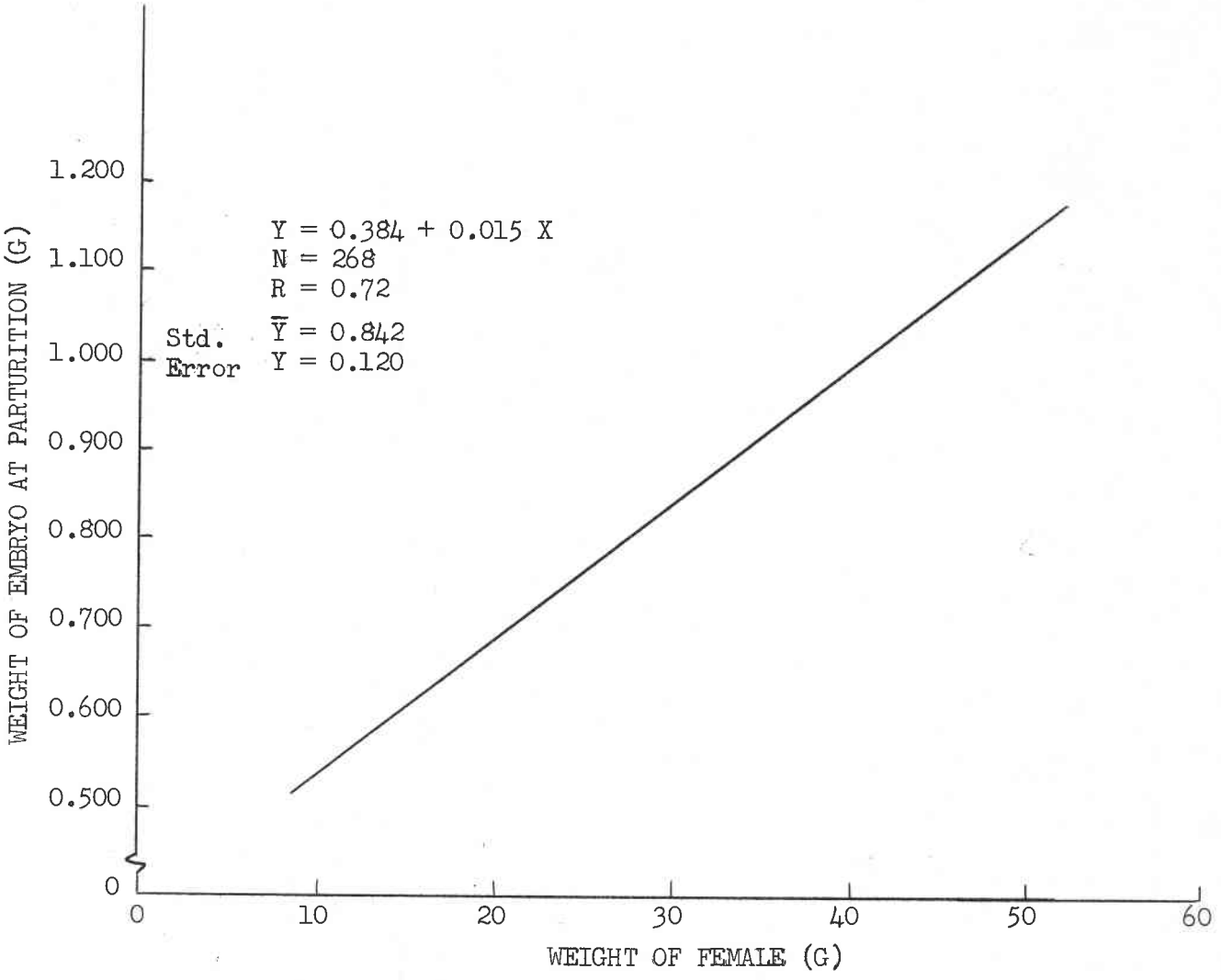

Fig. 4. Regression of the weight of Cymatogaster aggregata embryos at parturition compared with weight of 24 parent females.

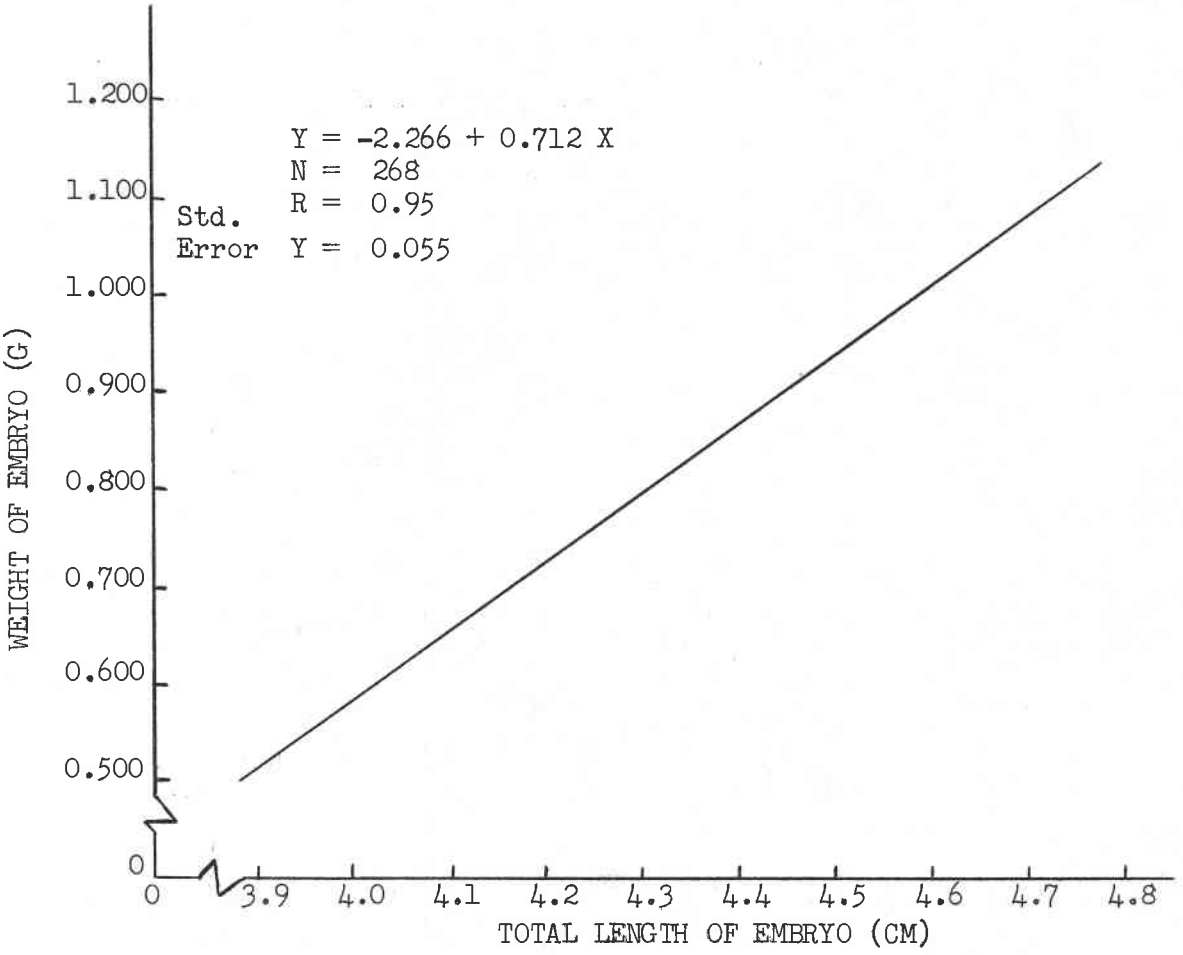

Fig. 5. Regression of the weight of Cymatogaster aggregata embryos compared with their total length at birth.

agrees with that of Suomela (MS, 1931) but not with that of Gordon (MS, 1965). The latter author reported that yearling females did not contain embryos, but more than 99% of his fish 2 years of age and older had embryos. Unfortunately, he did not state the type of length measurement he used; however, we assume it was fork length because the relationship of female length to embryo number that he found was similar to the relationship that we obtained using fork length. If this assumption is correct, then our 1-year-old fish are about 2 cm longer than Gordon's fish of the same age. It appears, therefore, that maturity of shiner perch may be dependent more on size than on age. The difference in size between our 1-year-old fish and those of Gordon's from British Columbia could be due to some environmental factor, perhaps temperature, that affects growth.

The relationship of female parent size to embryo size at parturition has not been studied previously in detail. Suomela (MS, 1931) reported that the standard length of embryos at birth was 2.7 to 3.8 cm, but he estimated these lengths from the lengths of unborn embryos. Our study complements that of Wiebe (1968b), who made a general statement that the largest females gave birth to the largest offspring and the greatest number of offspring. Neither Suomela nor Wiebe related embryo length at birth to female parent length.

We found that fecundity is directly related to female parent size. The same relationship was reported for shiner perch from California (Eigenmann, 1892), Washington (Suomela, MS, 1931), and British Columbia (Gordon, MS, 1965). However, our equations for fecundity are not necessarily applicable throughout the geographic range of the species.

ACKNOWLEDGMENTS

We wish to thank Mr Dennis E. Anderson and Mr Nelson E. Stewart, Oregon State University, for help in reading the fish scales, and Dr Roger G. Petersen, Oregon State University, for help with the statistical analyses.

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EFFECTS OF DURSBAN ON SHINER PERCH

ABSTRACT

Three continuous flow dilution systems of different design have been built for use in studies on the effects of Dursban on survival, growth, and reproduction of shiner perch.

The estimated concentrations of Dursban that killed 50% of shiner perch in 96 hours were 3.5 to 4.1 µg/liter under standing water conditions and 3.7 µg/liter in flowing sea water. Dursban is more toxic to this marine fish than it is to several freshwater fishes.

On the basis of a preliminary experiment, Dursban concentrations of 0.16 µg/liter and less do not affect growth of perch, but higher concentrations appear to affect growth and food consumption.

INTRODUCTION

Dursban, O, O-diethyl 0,3,5,6,-trichloro-2-pyridyl phosphorothioate, is one of a new group of organophosphorous insecticides, the "pyridal phosphates" (Gray, 1965). Its solubility in fresh water is between 0.4 and 2.0 ppm, and the half life for hydrolysis is 80 to 100 days (Smith, 1966). Lewis, Christenson, and Eddy (1966) reported that Dursban had a longer residual effectiveness than did other organophosphorous or carbamate insecticides that they tested.

Dursban is a broad spectrum insecticide that has been registered for use in mosquito control programs on noncrop areas only. Linn (1968) stated that the amount of Dursban required to control mosquitos may be fatal for fishes. The only published study known to us on the acute toxicity of this pesticide to fishes is that of Ferguson, Gardner, and Lindley (1966). They reported 36-hr TL_m's (median tolerance limits) of 35, 215, 22.5 µg/liter for the golden shiner (Notemigonus crysoleucas), mosquitofish (Gambusia affinis), and green sunfish (Lepomis cyanellus), respectively. Ferguson et al. (1966) stated that Dursban is less toxic to fish than most chlorinated hydrocarbon insecticides, but more toxic than other organophosphorous insecticides.

Boyd (1964) reported that several chlorinated hydrocarbon insecticides caused abortion of gravid mosquitofish. Johnson (1967) found that concentrations of endrin that were not lethal for adult Japanese rice fish (Oryzias latipes) affected their reproduction. There is little information on the effects of organophosphorous insecticides on reproduction of fishes and no information on the effects of Dursban on fish reproduction.

Organophosphorous insecticides inhibit acetycholinesterases in animals. The action of these insecticides on the enzyme levels in the brains of fishes has been studied by Holland and Lowe (1966) and Holland, Coppage, and Butler (1967). These insecticides also reduce the hematocrit and hemoglobin levels in the blood of fishes (Eisler, 1967).

Because of the predicted widespread use of Dursban in the aquatic environment and its residual life, studies are needed on the effects of this

pesticide on nontarget organisms. There is no information on the effects of Dursban on fishes of the Pacific coast of North America.

The specific subaims of this phase of the project are to study: (1) the acute toxicity of Dursban to shiner perch (Cymatogaster aggregata) using both standing and continuous flow seawater systems; (2) the effects of Dursban on growth of juvenile shiner perch; (3) the effects of Dursban on reproduction of shiner perch; and (4) the effects of Dursban on the acetycholinesterase level in the brain of perch, and on the hematocrit level in the blood of shiner perch.

We chose the shiner perch, Cymatogaster aggregata, as our test animal because of: (1) its occurrence in large numbers in estuaries from southern California to Alaska; (2) its ready availability; (3) its small size and short life cycle, which makes it a convenient laboratory animal; (4) its close biological relationship with larger economically important species of perch; and (5) its viviparity. The last characteristic allows us to study the effects of pesticides on development of embryos exposed indirectly to the chemical. With oviparous fishes, the developing embryos in the water would be exposed directly to the pesticide.

MATERIALS AND METHODS

ACUTE TOXICITY TESTS

Standing water bioassays were done in 5-gallon glass jars each containing 15 liters of filtered, aerated sea water of 25 ‰ salinity and at 15° C. A logarithmic series of test concentrations was used. Each concentration was prepared using technical grade Dursban mixed in acetone. The final acetone concentration in each test jar was adjusted to 480 ppm. Duplicate vessels were used for each test concentration.

A continuous flow sea water dilution system was constructed for use in both the short-term and long-term toxicity studies (Figs. 1, 2). The system automatically delivers a series of pesticide concentrations to individual test chambers. Filtered sea water in a head tank is heated to 15° C by a glass immersion heater. The pesticide stock solution is mixed with the sea water in a primary dilution chamber. The other dilutions are obtained by mixing proportionate amounts of pesticide from the various chambers with sea water in additional dilution chambers. Water and pesticide flows are maintained by glass siphons, which are held by eye bolts to a pegboard backing. Large adjustments in flow rates are made by regulating the height of the siphon with respect to the head level in the chamber, or by changing the diameter of the glass tubing. Final adjustment in flow rate is made by turning the eye bolts to change the siphon height. The variation in the flow rate is about 3%. The dilution ratios in the test vessels can be varied within the concentration range used in a particular test.

Juvenile shiner perch averaging 55 mm in length and 1.8 gm in weight were used in the acute toxicity tests. Mortalities at 24, 48 and 96 hours were recorded; the respective median tolerance limits (TL_m's), that is, concentrations that kill 50% of the test animals in the specified time periods were estimated by straight-line graphical interpolation as suggested by the American Public Health Association et al. (1965).

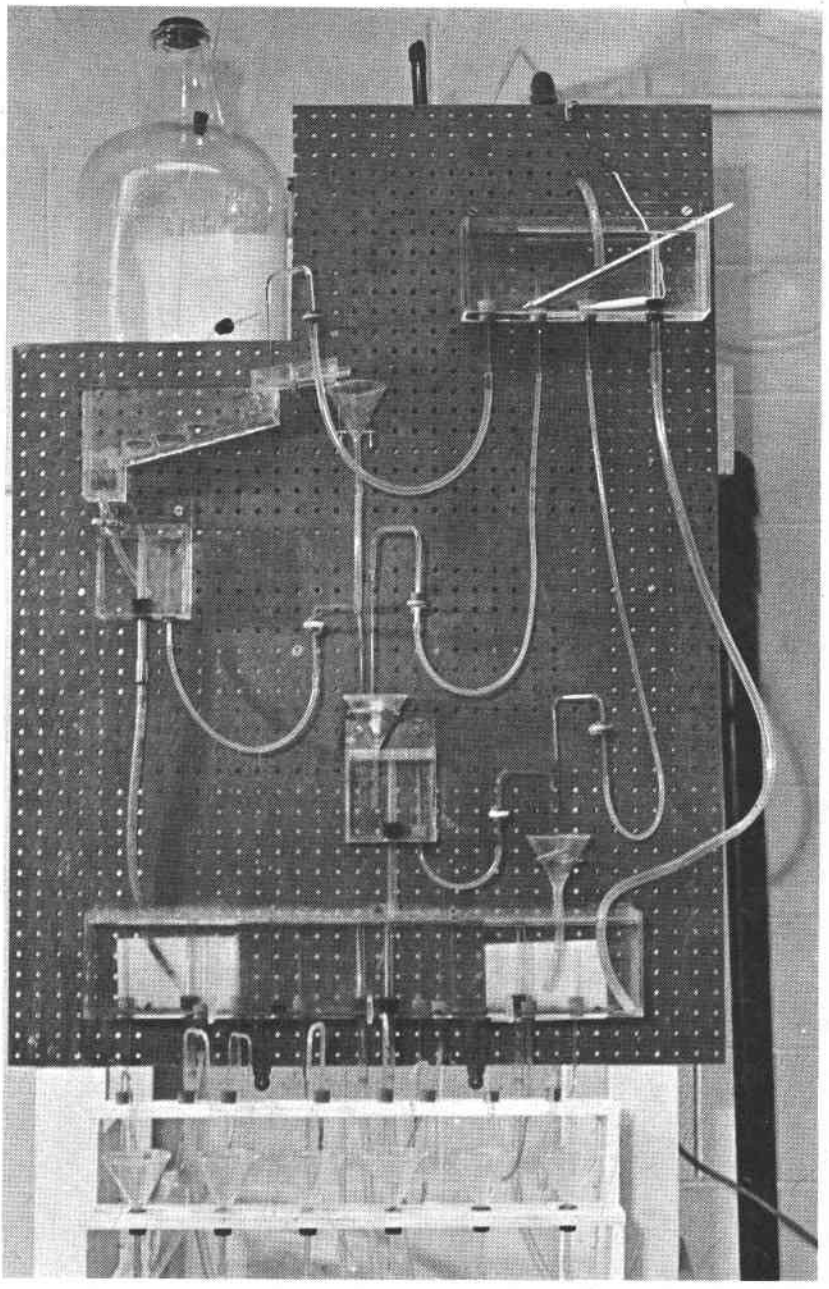

Fig. 1. Continuous flow sea water dilution system for exposure of fish to different concentrations of Dursban.

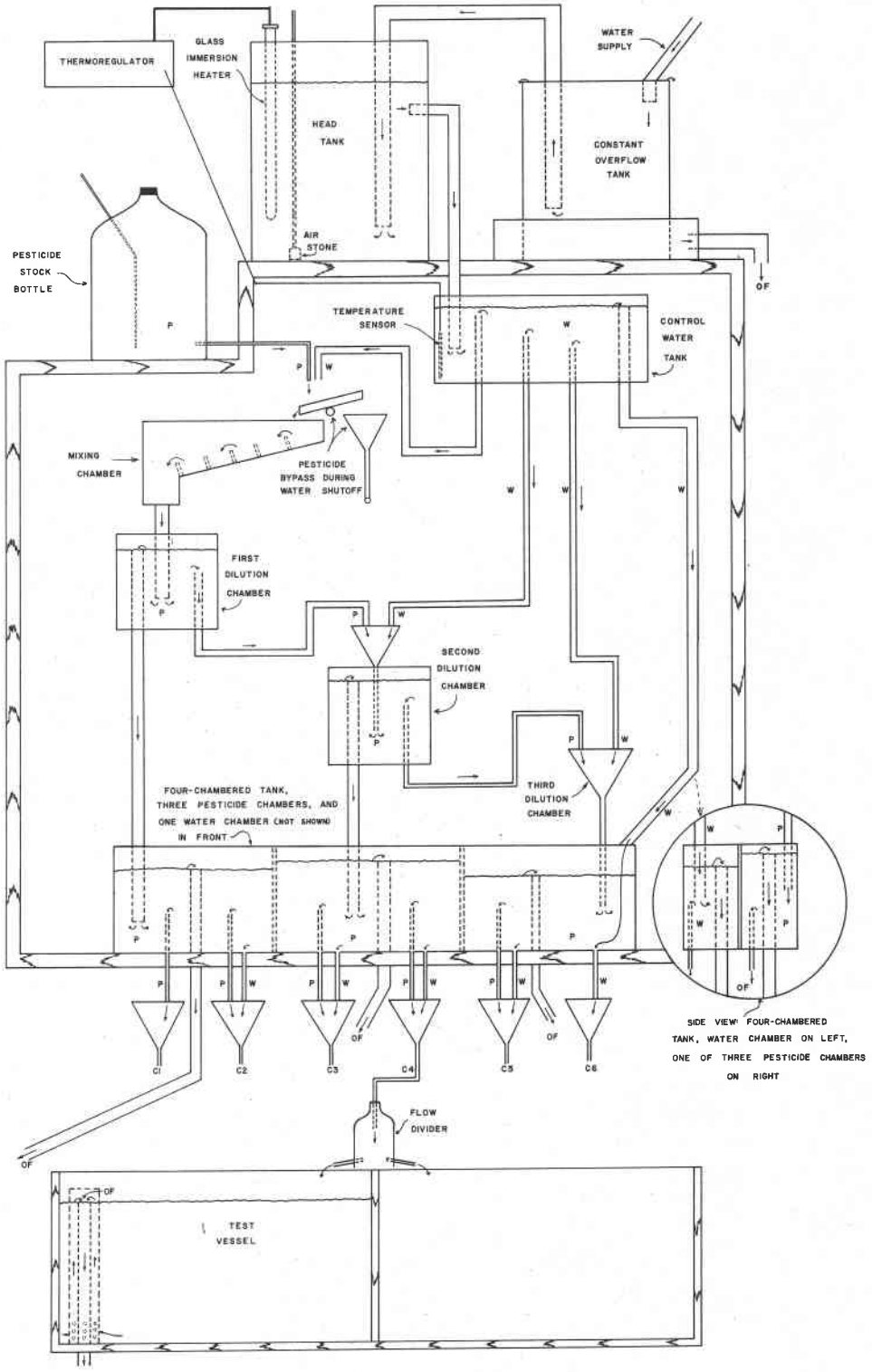

Fig. 2. Schema of continuous flow dilution system shown in Figure 1. P = pesticide, W = sea water, C = pesticide concentrations, OF = overflow. Arrows indicate sea water and pesticide flows.

A second dilution system has been constructed (Fig. 3). This system, which is a modification of the proportional diluter designed by Mount and Brungs (1967), has a maximum dilution ratio of 50%. It will be used for additional acute toxicity experiments.

GROWTH STUDIES

The continuous flow dilution system described above was used in a preliminary experiment on the effects of Dursban on growth of juvenile shiner perch. Sixty fish were weighed to the nearest 0.01 gm and measured to the nearest 0.1 cm and then divided into 12 equal groups. Each group was placed into a 15-liter test chamber and exposed to flowing solutions of Dursban in sea water at 15° C for 22 days. Duplicate vessels were used for the control and for each of the pesticide concentrations of 2.50, 1.00, 0.40, 0.16, and 0.064 µg/liter. The test was done under continuous light. The fish were fed an unrestricted diet of minced squid twice daily. Uneaten food was removed from the chambers before each feeding. Survival, growth, food consumption, and food conversion efficiencies were determined at the end of the experiment.

REPRODUCTION STUDIES

A third continuous flow dilution system has been constructed for use in this phase of the study (Figs. 4, 5). It is similar in design to the first one described above except that the volume of flow has been increased ten-fold to 2 liters/minute, and the number of test chambers has been reduced to four. Each chamber is a 200-gallon plastic tank, which will hold up to 100 adult shiner perch.

RESULTS

ACUTE TOXICITY TESTS

The toxicity of Dursban to perch was about the same in standing and in flowing sea water (Table 1). The 24-hour and 96-hour TL_m's in standing water were 6.0 to 7.8 µg/liter and 3.5 to 4.1 µg/liter, respectively; the 24-hour and 96-hour TL_m's in flowing sea water were 8.0 and 3.7 µg/liter, respectively. Dursban is considerably more toxic to shiner perch than it is to the freshwater fishes tested by Ferguson et al. (1966). The last authors reported a 36-hour TL_m of 22.5 ug/liter for the green sunfish, which was their most sensitive species.

These studies will be continued during the coming year.

Table 1. Acute toxicity of Dursban to shiner perch under conditions of standing and flowing sea water.

Test condition	No. fish/ test concentration	TL_m (µg/liter)		
		24-hour	48-hour	96-hour
Standing sea water	16	7.8	6.9	3.5
Standing sea water	14	6.0	4.2	4.1
Flowing sea water	10	8.0	6.2	3.7

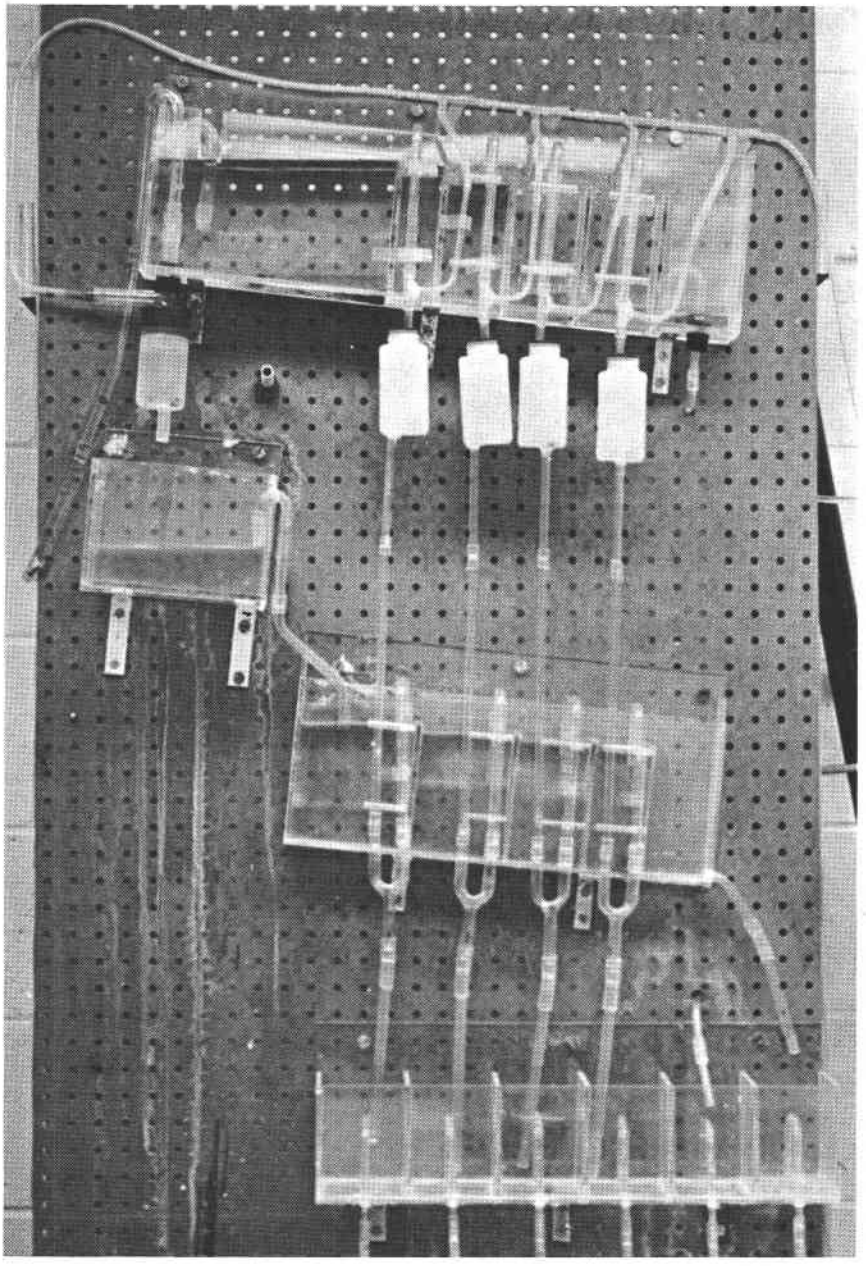

Fig. 3. Proportional dilution continuous flow sea water system for exposure of fish to different concentrations of Dursban. The design is a modification of the one described by Mount and Brungs (1967).

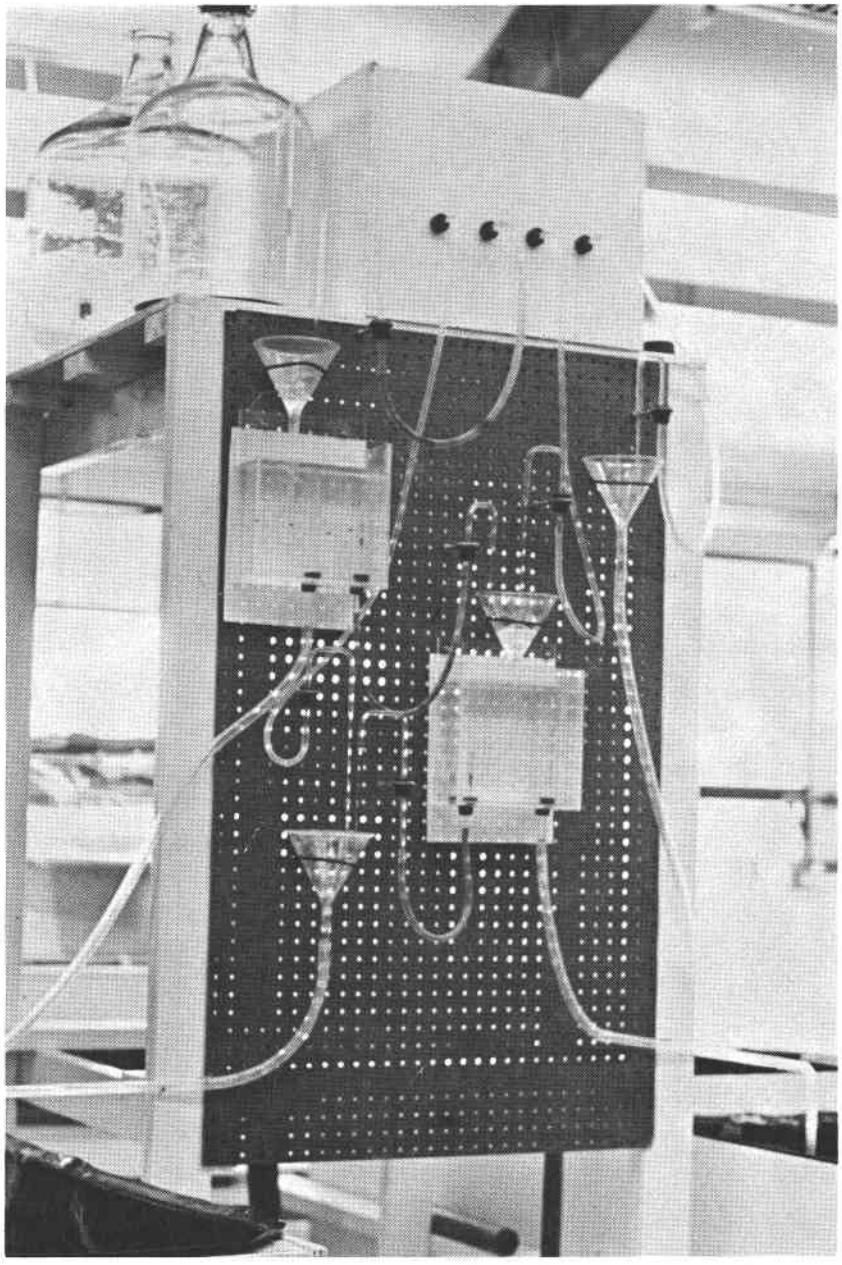

Fig. 4. Continuous flow sea water dilution system for use in studies on the effects of Dursban on reproduction of shiner perch.

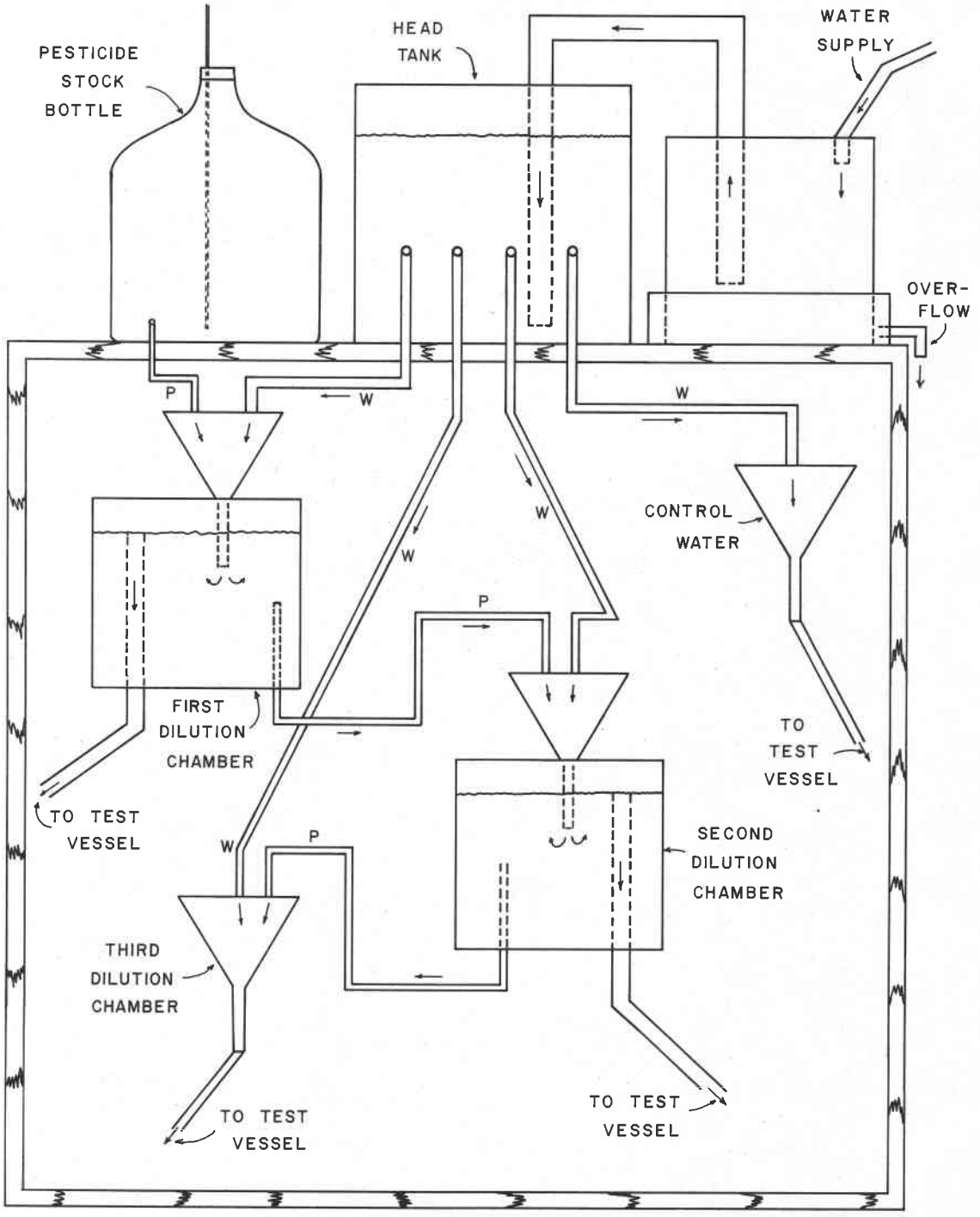

Fig. 5. Schema of continuous flow dilution system shown in Figure 4. P = pesticide, W = sea water. Arrows indicate sea water and pesticide flows.

GROWTH STUDIES

The fish exposed to the highest concentration of Dursban, 2.50 µg/liter, stopped feeding on day three of the experiment. On day nine, 50% were dead and all were dead by day 17 (Fig. 6). Those exposed to 1.00 µg/liter of Dursban stopped feeding by day 7, and 50% had died between days 12 and 15. The mean weight loss of five fish that survived exposure to 1.00 µg/liter was 11.0%, whereas all other fish gained weight (Table 2). The growth rates of control and experimental fish were proportional to their food consumption (Figs. 7, 8). The fish exposed to 0.064 and 0.16 µg/liter consumed more food and grew better than either the controls or those exposed to 0.40 µg/liter of Dursban (Table 2; Figs. 7, 8). The food conversion ratios decreased slightly with increasing concentrations of Dursban (Table 2; Fig. 9); however, additional experiments are necessary to establish this relationship.

On the basis of these preliminary results, it appears that Dursban concentrations of 0.16 µg/liter and less do not affect growth of shiner perch, but higher concentrations may affect growth and food consumption. In an experiment now in progress, we are testing the effects of concentrations of 0.125, 0.25, 0.50, 0.75, and 1.00 µg/liter on growth of perch to define better the relationship between pesticide concentration and growth.

These studies will be continued and expanded during the coming year.

REPRODUCTION STUDIES

Before we could begin experiments on the effects of Dursban on reproduction of perch, we needed information on the natural variation of the relationships between female size and embryo size and number. As noted previously, this study has been completed. We will now be able to study the effects of Dursban on reproduction of perch. These studies will be initiated during the coming year.

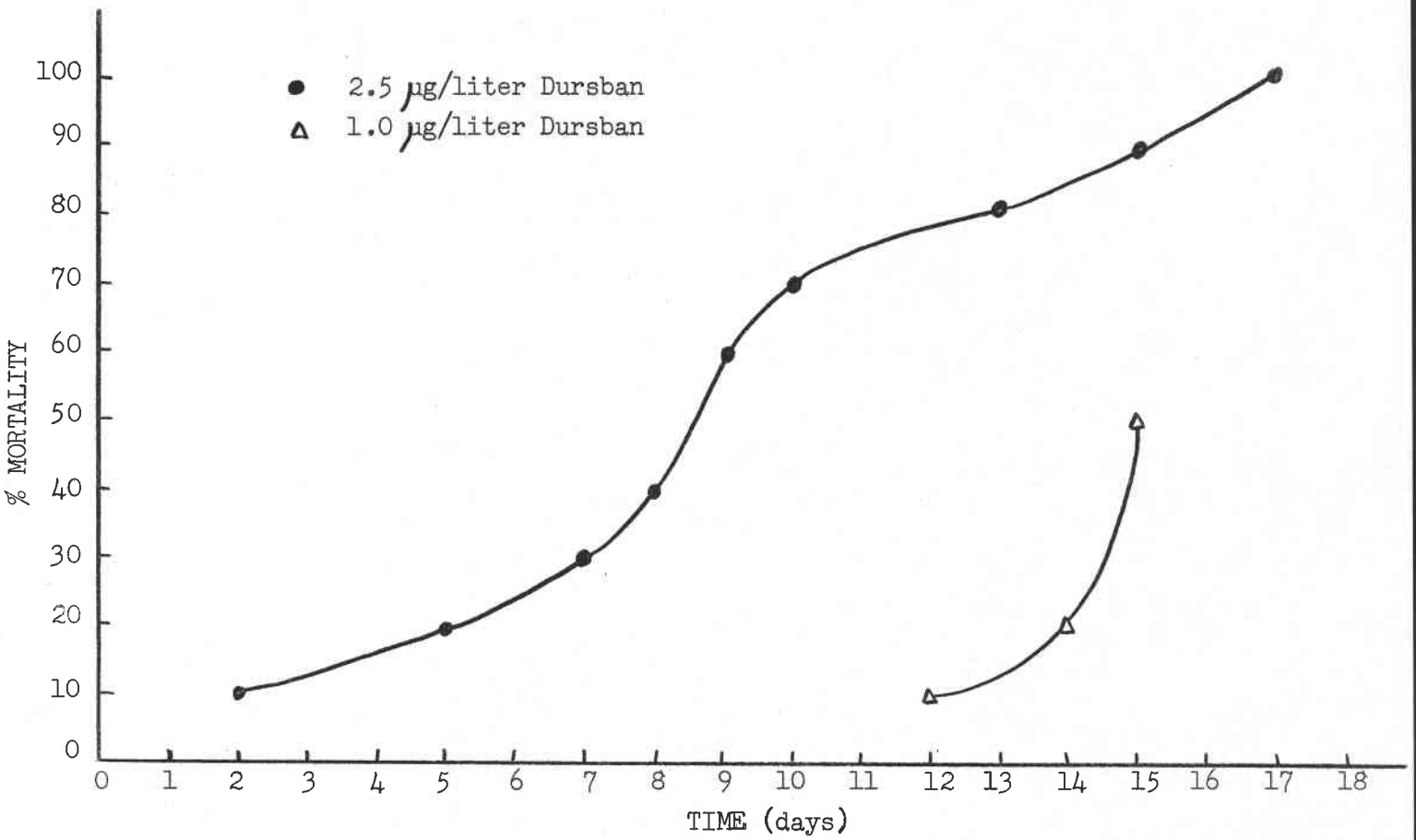

Fig. 6. Effect of continuous exposure to Dursban on survival of shiner perch.

Table 2. Growth, food consumption, and food conversion ratios of juvenile shiner perch exposed continuously to different concentrations of Dursban for 22 days.

Dursban concentration (ug/liter)	Mean wt. (grams)		Mean wt. gain/fish	Mean % wt. gain	Food consumed/fish (grams)	Grams food consumed/day gram initial fish wt.	Food conversion ratio[a]
	Initial	Final					
0.0	3.60	4.63[b]	1.03	28.6	8.80	0.111	0.129
0.0	3.95	5.07	1.12	28.4	10.09	0.116	0.111
Mean				28.5		0.114	0.120
0.064	3.57	4.88	1.31	36.8	10.60	0.135	0.124
0.064	3.77	4.92[b]	1.15	30.5	10.90	0.132	0.106
Mean				33.6		0.1335	0.115
0.16	4.03	5.57	1.54	38.2	13.75	0.155	0.112
0.16	3.45	4.72	1.27	36.8	12.42	0.154	0.102
Mean				37.5		0.1545	0.107
0.40	3.68	4.39	0.71	19.3	8.96	0.111	0.079
0.40	3.81	5.04	1.23	32.3	10.48	0.125	0.117
Mean				25.8		0.118	0.098
1.00	4.60	3.98	-0.62[c]	---	---	---	---
1.00	4.32	3.92	-0.40[d]	---	---	---	---
2.50	3.00	No	---	---	---	---	---
2.50	3.73	survivors	---	---	---	---	---

[a] Weight gain/fish in grams divided by grams of food consumed/fish weight in grams.

[b] One fish died, it was identified and its initial weight subtracted from the initial group weight.

[c] Three fish died, data was corrected as in footnote b.

[d] Two fish died, data was corrected as in footnote b.

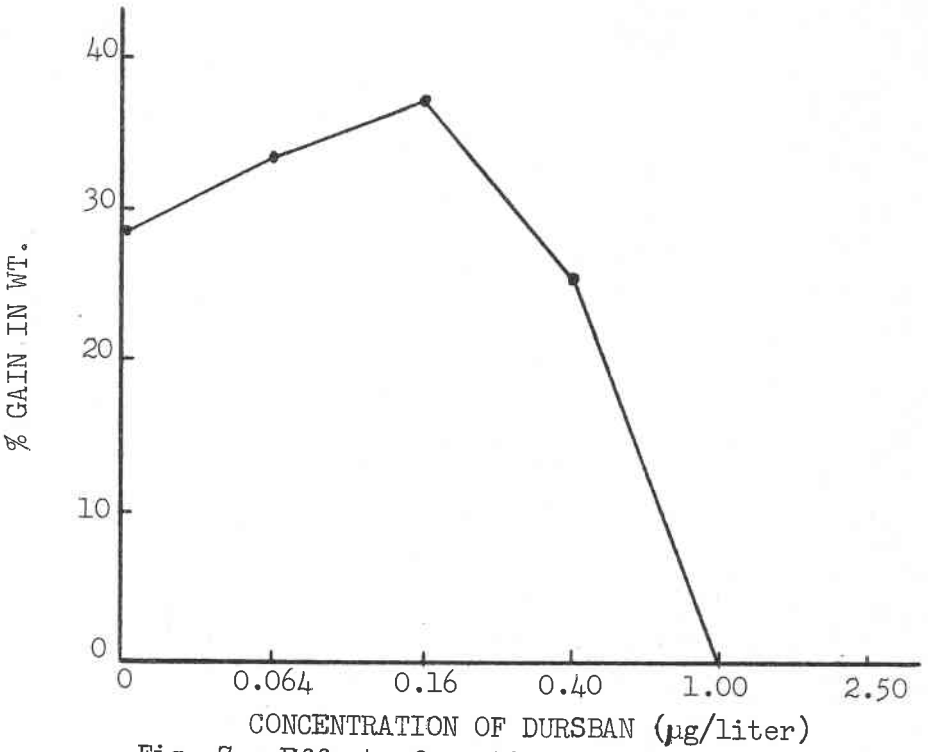

Fig. 7. Effect of continuous exposure to Dursban on growth of shiner perch. Fish surviving 1.00 µg/liter had a mean weight loss of 11%.

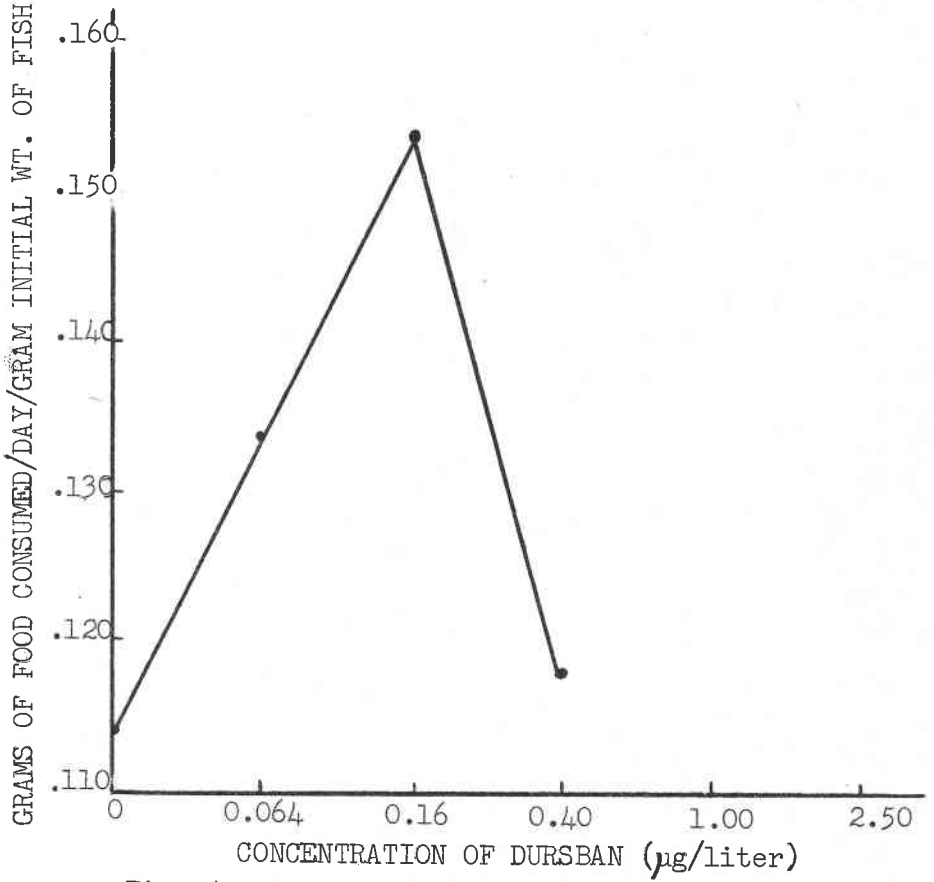

Fig. 8. Effect of continuous exposure to Dursban on food consumption of shiner perch.

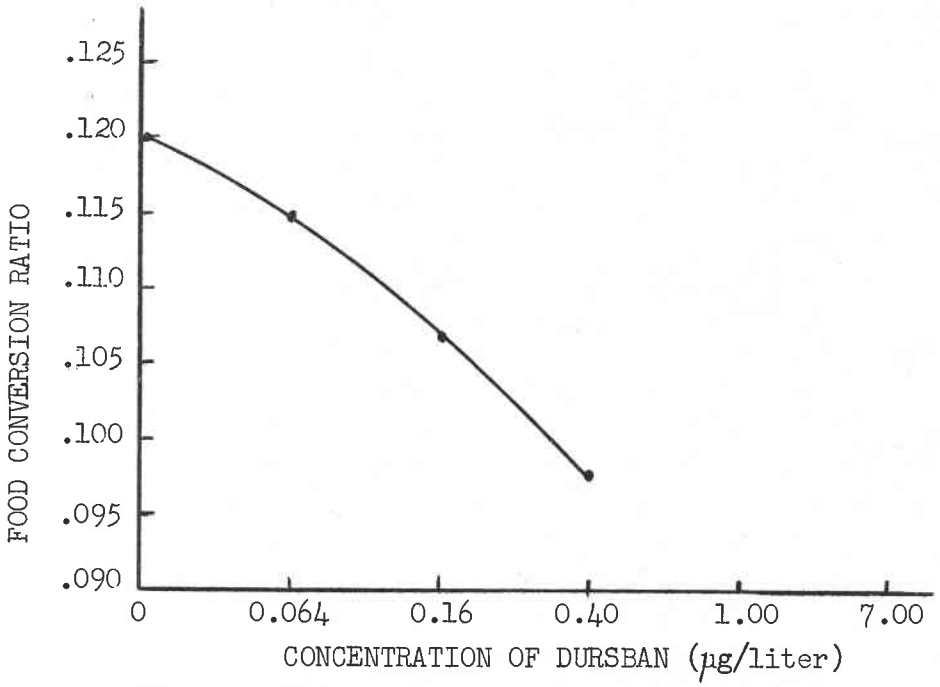

Fig. 9. Effect of continuous exposure to Dursban on food conversion ratios of shiner perch. The ratios are computed from the gain in weight of fish in grams divided by the grams of food consumed per weight of fish in grams

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LABORATORY MODEL STUDIES

Specific aim no. 4. To develop a laboratory model of an intertidal mud flat containing a functioning community of animals, and then to determine the effects of Sevin and other pesticides on such a community.

An experiment now in progress will evaluate the effects of light intensity and differences in numbers of animals of a given species on the establishment of the animal communities in the models. This experiment will be terminated in October 1969 and the first part of the above aim will be completed. The results of this last experiment will be incorporated into the following manuscript, which will then be submitted to the Journal of Experimental Marine Biology and Ecology for publication.

In early 1970, we will begin the studies on the effects of Sevin on a community of animals in the laboratory models.

SIMULATION IN THE LABORATORY OF AN INTERTIDAL MUD FLAT[1]

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Running head: LABORATORY MODEL OF AN INTERTIDAL MUD FLAT

[1] Technical Paper No. , Oregon Agricultural Experiment Station. This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia , and constitutes part of thesis research conducted by the senior author at Oregon State University.

ABSTRACT

Laboratory models of intertidal mud flats are being used to study the influence of different environmental conditions on the establishment of animal communities. The wooden tanks housing the animals are 2.1 m long, 1.1 m wide, and 0.8 m deep. Tidal cycles are simulated in them with a system of time switches and pumps.

Communities of intertidal animals have been successfully maintained in the laboratory models, which had different types of tidal cycles, for periods up to 75 days. Generally, the different types of tidal cycles tested did not affect survival and growth of shore crabs (Hemigrapsus oregonensis), Dungeness crabs (Cancer magister), and bay mussels (Mytilus edulis). Survival and growth of Pacific oysters (Crassostrea gigas) were not affected by tidal cycles when their exposure to air did not exceed about 4 hours each day; however, when the oysters were exposed to air daily for 12 hours or more, their survival and growth were reduced considerably. The results of one experiment indicated that cockle clams (Clinocardium nuttalli) may, under certain conditions, grow better if exposed to air for short periods of time each day, but additional experiments are needed to establish this point. English sole (Parophrys vetulus) did not survive in models with tidal cycles, whereas some sculpins (Leptocottus armatus) did. Generally, differences in mud depth, which ranged from 9 to 27 cm, did not directly affect survival and growth of the animals. However, in one experiment, Dungeness crabs grew best in those models having the larger volume of substrate probably because of the greater number of food organisms present in the mud.

INTRODUCTION

Laboratory models of flowing streams have been used successfully for studying plant and animal communities (Davis & Warren, 1965; Brocksen et al. 1968; McIntire, 1968). However, few studies have been done with marine animal communities in laboratory models having simulated tidal cycles. Vader (1964) studied the reactions of some infaunal animals from tidal flats to tidal cycles in laboratory aquaria, which had a maximum capacity of about 24 liters. The tidal apparatus that he used was described by De Blok (1964). This system is operated by a siphon device that is controlled by an electric motor. A similar system, but having a 45-liter experimental tank, was described by Evans (1964). De Santo (1967) has described a timer system that will operate an aquarium pump to simulate natural marine tidal intervals.

The purpose of our study was to develop a laboratory model of an intertidal mud flat, which would be suitable for studying the influence of pesticides on a community of animals under conditions similar to those found in their natural environment. However, before we could begin this study, we first had to know the influence of such variables as type and amount of substrate, rate of water exchange, different tidal cycles, salinity, temperature, and light on the establishment of an animal community. This study, therefore, was planned accordingly.

MATERIALS AND METHODS

THE LABORATORY MODELS

Six wooden experimental tanks were housed in a large laboratory room. Each tank was 2.1 m long, 1.1 m wide, and 0.8 m deep and was lined with vinyl plastic, which was removed and replaced at the end of each experiment. The liners protected the wood from marine fouling organisms, and they will also prevent absorption by the wood of pesticides used in future experiments. Figure 1 shows one of the six experimental systems.

Sea water was pumped from Yaquina Bay, Oregon, into the laboratory through nontoxic plastic pipes and then into three plastic constant head tanks. Each head tank served two models. Simulation of daily tidal cycles in the models was achieved with a system of pumps and time switches (Figs. 1, 2, 3). Each of the experimental tanks was equipped with a centrifugal pump that was operated by a series of timers mounted on a central control panel. During the filling period (flood tide), sea water was pumped from one of the constant head reservoirs into a perforated plastic standpipe located at one end of the experimental tank. During this time, some of the water left through a similar standpipe located at the opposite end of the tank. The system was adjusted so that the flood tide period was followed by a slack high tide during which the water left the experimental tank through the standpipe and an overflow pipe (Fig. 1). Each experimental tank was emptied (ebb tide) by shutting off the incoming water and allowing the water in the tank to leave by gravity flow through the standpipe. A period of slack low tide was simulated by delaying the starting of the flood tide. No attempt was made to control water temperatures or salinities. Lighting was controlled to simulate day and night.

The shortest on-off cycle obtainable with the time switches is 90 min. To obtain cycles of shorter duration, two control timers were wired in series. The "on" and "off" trippers on these timers were set so that the electrical current passing through the timers was stopped for a period of time each day. The current from the two control timers operated the pump timers; therefore, all input current to the pump timers was shut off for predetermined periods of time each day (Fig. 3A). This arrangement delayed the pumping cycle and so permitted daily advancement of tidal cycles in the models. This advancement was about the same length of advancement of tides in nature. This timer system is similar in design to the one described by De Santo (1967).

Titanium electrodes in the constant head tanks were connected to a 10 milliampere relay through which the output power from the control timers passed. Thus, if the water being pumped from the bay was interrupted, the pumps for the models were shut off automatically.

After the second experiment, a set of titanium electrodes was installed in the outlet standpipe of each experimental tank (Fig. 1). These electrodes were connected to a relay that turned an electric clock off when the tank emptied and on again when it began to fill with water (Fig. 3B). Thus, the slack low tide period (exposure period) could be determined by comparing the time on this clock with that on a continuously running control clock.

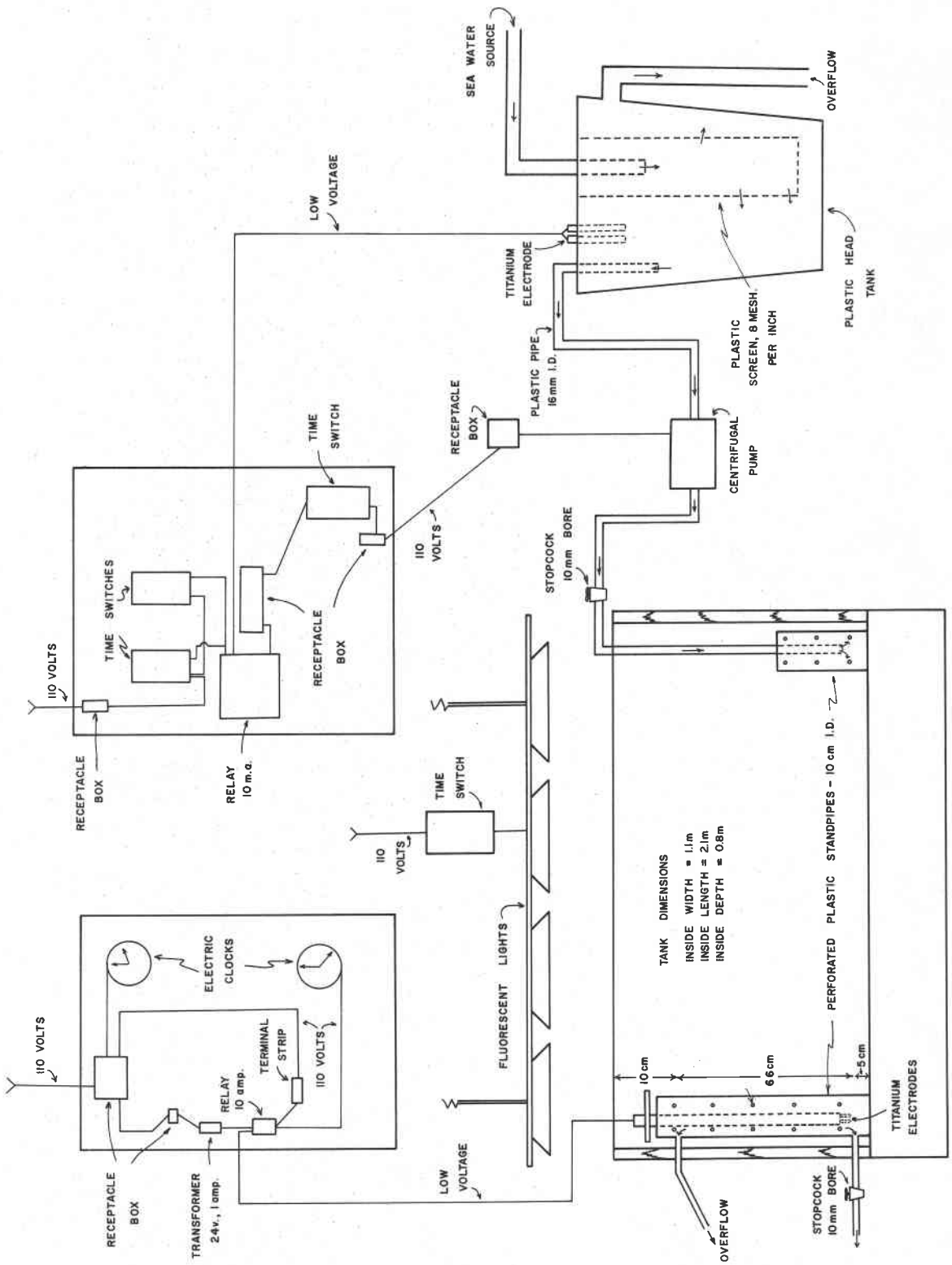

Fig. 1. Schema of one of the six laboratory models of an intertidal mud flat.

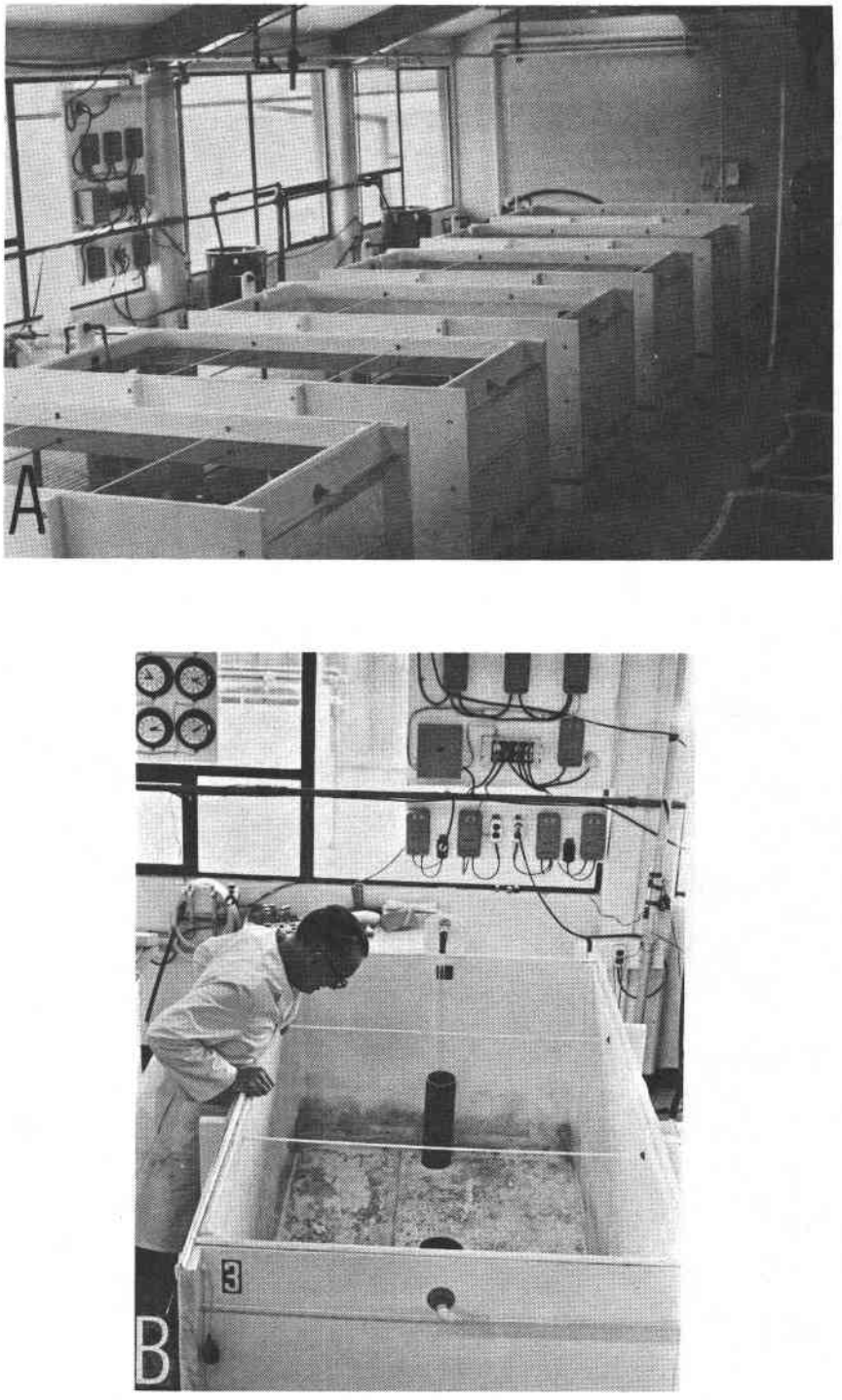

Fig. 2. Laboratory models of an intertidal mud flat. A. The six experimental tanks each with a community of animals and a mud substrate. B. Empty tank showing standpipes and control panels in the background.

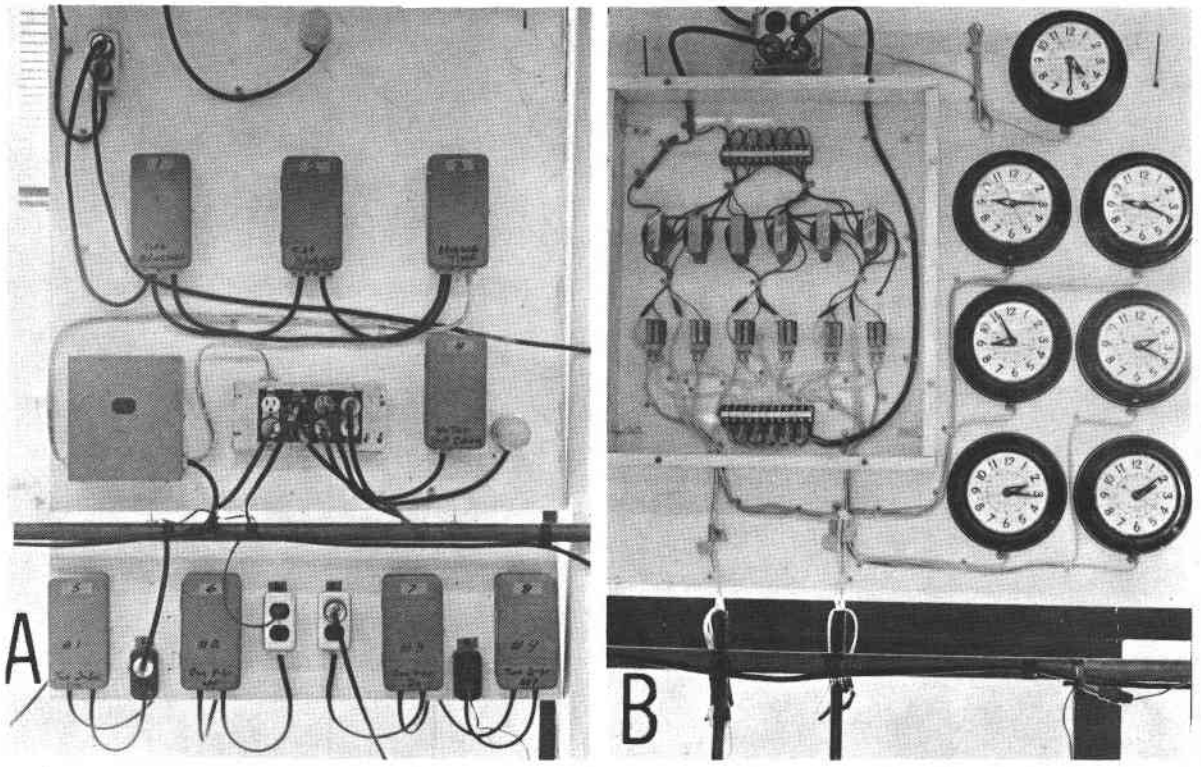

Fig. 3. Control panels for the laboratory models. A. Timers that operate the pumps in each tank to produce tidal cycles. B. Relay switches and clocks connected to electrodes in each tank that record the length of the exposure period during low tide. The top clock is the continuously running control clock; one of each of the others serves a tank.

During the first two experiments, the fluorescent ceiling lights in the room were controlled by a time switch to give alternate periods of 16 hours of light and 8 hours of darkness each day. Each experimental tank is now provided with a bank of 16 cool white fluorescent tubes, 122 cm long, which can be raised or lowered to control light intensity. These lights are controlled by a time clock. The experimental tanks are next to windows and so they receive some natural sun light.

TEST ANIMALS

The test animals included crustaceans, mollusks, and fishes (Table I). The juvenile Pacific oysters were reared in our laboratory; the other animals were collected from Yaquina Bay, Oregon.

EXPERIMENTAL PROCEDURES

In each experiment, the tanks were provided with a substrate of bay mud collected from an intertidal area in Yaquina Bay. Only material from about the top 20 cm of the mud flat was used. The mud was collected in 10-liter buckets and then was distributed randomly among seven 76-liter plastic containers. The latter were used to transport the mud to the laboratory. Mud from six of these containers was used for the experimental tanks. The mud from the remaining container was placed in a "sample box" to a uniform depth. The box was 109 cm square and 20 cm deep.

Three 1 square foot (30 cm^2) samples of mud and three core samples (2.1 x 17 cm) of mud were taken from the sample box on the day of collection in experiment 1 and 2 days later in experiment 2. These samples were used for estimating the numbers and species of animals originally present in the mud. The core samples were used for estimating the numbers of nematodes and small annelids, and the square foot samples were used for estimating numbers of all other animals. The square foot samples were washed through a screen having 14 or 32 meshes per inch and core samples through a 32-mesh screen. The animals retained by the screen were preserved in alcohol or formalin for later study. Three additional core samples of mud taken from the box were used for particle size analysis. These samples were dried at 70° C, weighed, and then resuspended in water. The suspensions were passed through a series of Tyler screens. The material retained by each screen was washed into a beaker and then filtered using preweighed No. 1 filter paper. The samples were then dried at 70° C, weighed, and the particle size composition based on weight was computed (Table II). This procedure for analysis of particle size was adapted from the one described by Krumbein and Pettijohn (1938).

After the mud was placed into the experimental tanks, sea water was passed through each tank at a rate of 15 liters/min for 3 days in experiment 1 and at 8 liters/min for 8 days in experiment 2 to aid in settling the substrate. Two rocks and two oyster shells were placed on the mud in each tank to provide cover for the crabs.

The animals used in experiment 1 were measured and added to the tanks during a 3-day period. The oysters, which had previously set on "old" oyster shells, were suspended in the tanks at a distance approximately 15 cm below the surface of the water when the tanks were full. Therefore, the length of time

TABLE I

Species, life history stages, numbers, and mean sizes of animals used in the laboratory model experiments.

Species	Life history stage	No. animals per model		Initial mean size			
				Expt. 1		Expt. 2	
		Expt. 1	Expt. 2	Length[1] (mm)	Wt. (grams)	Length[1] (mm)	Wt. (grams)
Crustaceans							
Ghost shrimp (Callianassa californiensis)	Adult	10	20	66.0	---	47.6	---
Shore crab (Hemigrapsus oregonensis)	Adult & juvenile	10	10	12.0	---	12.9	---
Dungeness crab (Cancer magister)	Juvenile	10	10	25.8	---	20.5	---
Mollusks							
Pacific oyster (Crassostrea gigas)	Juvenile	20	20	3.5	---	8.3	---
Cockle clam (Clinocardium nuttalli)	Juvenile	24	44	21.6	---	13.1	---
Bay mussel (Mytilus edulis)	Juvenile	20	20	13.4	---	15.5	---
Fishes							
Pacific staghorn sculpin (Leptocottus armatus)	Juvenile	5	10	66.1	2.9	70.0	3.6
English sole (Parophrys vetulus)	Juvenile	10	5	112.9	12.2	89.1	6.3

[1] The mean "length" for shore crabs and Dungeness crabs was the width of the carapace, for oysters the length of the shell perpendicular to the hinge, and for cockles the length of the longest rib. Total length is given for all other species.

TABLE II

Summary of particle size of substrate material used in the laboratory models.

Screen size meshes/ inch	Particle grade size (mm)	Mean % of sample retained[1]		Cumulative %	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
5	4.0 & above	0.15	0.01	0.15	0.01
16	1.0 - 4.0	0.04	0.06	0.18	0.07
32	0.5 - 1.0	0.31	0.34	0.50	0.41
60	0.25 - 0.5	18.82	11.03	19.32	11.44
115	0.125 - 0.25	40.83	34.35	60.15	45.79
250	0.063 - 0.125	15.99	16.86	76.14	62.65
<250	Below 0.063	21.83	29.45	97.97	92.10
		Mean sieve loss		2.03	7.90
			Total	100.00	100.00

[1] The initial dry weight of the core samples ranged from 53.62 to 80.26 grams.

that oysters were exposed to the air each day was not the same in each tank because of differences in the type of tidal cycle being tested. Bay mussels were placed on empty oyster shells, to which they subsequently attached, located on the surface of the mud. Cockle clams were placed just below the mud surface. Sea water was started into the tanks and then the other animals were added. The rates of flow of sea water were adjusted and the tidal cycles were started on the third day after all the animals had been placed in the models.

In experiment 2, all the animals were measured and added to the experimental tanks on the same day. Individual unattached oysters and bay mussels were placed into 4.8-liter perforated plastic buckets and suspended 2-5 cm above the mud surface. The other animals were introduced into the tanks as described for experiment 1. Flow rates were adjusted and tidal cycles started on the same day the animals were added to the tanks. Exogenous food was not added to the tanks in either experiment 1 or 2.

In experiment 1, which lasted 75 days, we studied the effects of mud depth and duration of exposure period (slack low tide) on the animal community. The timer system was set to turn the pumps on at 2:00 a.m. and 2:00 p.m. and off at 6:00 a.m. and 6:00 p.m. each day for four of the models. Thus, a complete tidal cycle occurred each 12 hours. The different exposure periods were attained by adjusting the emptying rates (water exchange rates) of the models accordingly. In the remaining two models, there was a continuous flow of water.

In experiment 2, which lasted 37 days, we compared the effects of tidal cycles synchronized with those occurring naturally with unsynchronized cycles on the animal community. For four of the models, the two control timers were set to turn off the electricity to the pump timers, for 24 min at 12:00 midnight and again at 12:00 noon each day. This procedure advanced the tidal cycle 48 min each day. Two of these models had cycles synchronized with those occurring in Yaquina Bay, Oregon. The remaining two had unsynchronized cycles so arranged that during a low tide in nature a high tide occurred in the models. The different exposure periods were attained as described for experiment 1. The two remaining models had continuous flows of water but one of them received twice the volume of water as the other.

The experimental conditions for both experiments were maintained by making necessary measurements and adjustments daily. The detailed experimental conditions of these experiments are summarized in Tables III and IV.

At the end of each experiment, the experimental tanks were drained and the surviving animals recovered. Three 1 square foot (30 cm^2) mud samples and three core samples (2.1 x 17 cm) were taken from each tank and washed through screens having 14 and 32 meshes per inch, and the animals excluding those that we had added,were preserved for later analysis. The square foot samples taken at the end of the experiment were the only means used to recover the ghost shrimp. Survival estimates of the other experimental animals were based on the actual numbers collected at the end of the experiments.

TABLE III

Test conditions of the laboratory experiments

Expt. no. and test period	Purpose of experiment	Model no.	Mean mud depth (cm)	Number of daily exposures[1]	Length of each exposure period (hrs)[2]		Total mean daily exposure (hrs)
					Mean	Range	
Expt. 1 Aug. 26- Nov. 9, 1967	To evaluate effect of mud depth and exposure period on the animal community	1	9	0	0	0	0
		2	27	0	0	0	0
		3	9	2	1.4[3]	1.1-1.8	2.8
		4	25	2	3.8	1.8-4.3	7.6
		5	27	2	1.6	1.0-2.3	3.2
		6	9	2	3.8[3]	3.3-4.2	7.6
Expt. 2 July 23- Aug. 29, 1968	To evaluate effects of tidal cycles synchronized and unsynchronized with those in nature on the animal community	1	18	2[4]	2.3	2.0-2.5	4.6
		2	18	1[5]	4.1	3.3-4.3	4.1
		3	18	1[4]	4.5	4.0-4.9	4.5
		4	18	2[5]	2.1[3]	1.5-2.5	4.2
		5	18	0	0	0	0
		6	18	0	0	0	0

[1] The exposure periods were at the same time each day, unless noted otherwise.

[2] The exact length of at least one exposure period was determined daily for each model except in experiment 2, when the daily check for models 1, 2, and 4 was missed on one occasion and for model 3 on two occasions. However, because the water flows on these days approximated the flows on the immediately preceding days, we assumed that the length of the exposure periods were the same as those for the preceding days.

[3] On one occasion a pump failed. The exact time of the malfunction could not be determined, and therefore, the exposure time for that day is not known.

[4] Tidal cycle synchronized with natural cycle.

[5] Tidal cycle unsynchronized with natural cycle.

TABLE IV

The mean temperatures and water flows in the laboratory model experiments.[1]

Expt. and model no.	Temperature (°C)[2]				Water flow (liters/min)				Approx. hrs. pump on each day	Mean vol. of water passing through model each day (liters)
	Water[3]		Mud[4]		Incoming		Outgoing[5]			
	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
Expt. 1										
1	14.0	11.0-16.5	----	----	4.1	2.0- 4.9	2.4	1.4-2.6	24	5,904
2	13.9	12.0-16.0	----	----	4.3	2.2- 5.2	2.4	1.2-2.9	24	6,192
3	13.6	10.5-16.0	15.7	13.0-18.0	13.0	9.6-14.8	4.7[6]	3.4-4.9	8	6,240
4	13.6	10.5-16.0	16.1	14.0-18.0	12.5	6.0-14.0	5.0	4.5-5.4	8	6,000
5	13.6	10.5-16.0	15.6	13.0-18.0	12.3	6.2-14.0	3.5[6]	2.0-3.8	8	5,904
6	13.6	10.5-16.0	15.9	14.0-18.0	13.2	12.0-14.0	7.5[6]	6.0-7.8	8	6,336
Expt. 2										
1	13.3	10.0-16.0	13.8	12.0-15.0	12.1	12.0-12.4	4.3	3.0-4.8	8	5,808
2	13.8	11.0-16.0	16.5	15.0-17.5	6.8	4.2- 7.4	4.3	3.7-4.8	14	5,712
3	13.8	10.0-16.0	15.4	13.0-18.0	6.8	6.0- 7.6	4.4[6]	4.1-5.0	14	5,712
4	13.8	12.0-16.0	16.3	15.0-17.5	12.1	11.8-12.6	4.4[6]	3.6-5.0	8	5,808
5	13.7	11.5-16.0	----	----	4.0	2.5- 4.4	1.8	1.8-2.0	24	5,760
6	14.7	12.5-16.5	----	----	1.9	0.7- 2.2	1.1	1.0-1.2	24	2,736

[1] The salinities ranged from 27 to 34 ‰ and averaged 33 ‰ in experiment 1, and ranged from 31 to 34 ‰ and averaged 33 ‰ in experiment 2.

[2] The air temperatures ranged from 21 to 28° C and averaged 21° C in experiment 1, and ranged from 21 to 22° C and averaged 22° C in experiment 2.

[3] Temperature of surface water in the experimental tank.

[4] Temperature of the mud surface was taken near the end of an exposure period periodically during the experiment.

[5] Measured at bottom of the stand pipe.

[6] See footnote 3 in Table III.

RESULTS AND INTERPRETATION

THE MODEL COMMUNITIES

In experiment 1, the most numerous animals initially present in the mud were small polychaetes, small clams (Cryptomya californica), tanaidaceans, and amphipods (Corophium sp.). The average numbers of these species in the mud samples were 17, 68, 54, and 13, respectively. By the end of the experiment in all models, the numbers of clams, tanaidaceans, and amphipods were reduced by an average of 95, 98, and 100%, respectively. This decline may have been due either to normal cyclical fluctuations or failure of the animals to re-establish in the models. However, the decline in numbers of C. californica may have been due to predation by the crabs because we did not find dead clams in the models of either experiment during the 3 and 8 days that the models were operated before they received the experimental animals. We found live clams just below the mud during the above mentioned periods, but on day 2 of each experiment we observed empty clam shells on the surface of the mud. The decline in numbers of tanaidaceans and amphipods may also have been caused by crab or fish predation, but stomach analyses of crabs and fishes were not done to verify this. Numbers of polychaetes did not change significantly during experiment 1. At the end of the experiment, we found small ectoprocts, barnacles, gastropods, and mussels (Mytilus sp.) on the rocks and oyster shells in about equal numbers in all models. They apparently were introduced as larvae with the incoming water.

In experiment 2, C. californica was the only abundant organism present in the initial mud samples. There was an average of 85 per sample. We found only a few other invertebrates and, except for one tanaidacean, no crustaceans were present. However, in this experiment we took the mud samples from the sampling box 48 hours after the mud was brought into the laboratory. During this time, the mud was exposed to the air and some organisms may have died before the samples were taken. The final mud samples from experiment 2 have not yet been analyzed. At the end of this experiment, the only animals we found on the rocks and oyster shells were a few barnacles and they occurred only in the two models that had continuous water flows.

We did not observe any difference in behavioral patterns of experimental animals in those models with tidal cycles. On the basis of the number of openings on the surface of the mud, the degree of burrowing was apparently the same in all models, including those without tidal cycles. Usually, Dungeness crabs were buried in the mud around the two standpipes during the exposure periods. Shore crabs were either buried or under the rocks and oyster shells. The crabs appeared to be more active at night than during daylight hours. Occasionally, we saw a cockle clam on the surface of the mud but it would soon bury itself when the water flow resumed.

Fish were at a disadvantage in the models with tidal cycles, because we did not have tide pools that could have been place for retreat during low tides. Rarely, were fish present in the water that was usually retained in a depression in the mud around the standpipe at the end of the models where the water was introduced. This depression was formed by the incoming water flowing out through the perforated standpipe. More frequently, we observed fish partially buried in small depressions in the mud at the out-

let end of the models. There were also a few small depressions scattered throughout the models that retained a few ml of water during the exposure periods. These "pools" sometimes contained fish. The inlet standpipe retained a volume of water equal to the mud depth, but the standpipe extended above the mud surface and therefore, the animals could not reach this water as the model emptied. However, some crabs and fish did enter the standpipe during high tides.

At times, parts of thalli entered the experimental tanks through the water supply. However, visible macroalgal communities did not develop. Possibly because the light intensity at the surface of the mud during the exposure periods was only about 50 foot-candles, and the experiments were too short in duration.

SURVIVAL AND GROWTH OF EXPERIMENTAL ANIMALS

We do not know how many ghost shrimp survived. They burrowed throughout the substrate so it was not practical to examine all the mud to recover them. However, the numbers of burrow openings on the mud surface indicated that survival of ghost shrimp was approximately equal in all models in an experiment.

Survival of shore crabs ranged from 20 to 90% in the two experiments (Table V). At the end of an experiment, only about the top 5 cm of the mud was examined and it is possible that some crabs were lost. This large range in survival suggests that a larger number of crabs should be used in future experiments. Because of these large differences in survival, we can make only a few general statements about the effects of the different environmental conditions on the shore crabs. In experiment 1, there was no apparent relationship between survival and type of tidal cycle, and survival was best in those models with the least substrate (Table V). In experiment 2, survival was greater in those models with two daily low tides. The mean percentage increases in carapace width for shore crabs ranged from 2 to 23% and from 2 to 13% in experiments 1 and 2, respectively (Table V).

Of the Dungeness crabs, 70% or more survived in both experiments (Table V). In experiment 1, the mean percentage increases in carapace width of Dungeness crabs in the three models that had mud depths of 25 or 27 cm ranged from 74 to 85% and averaged 80%; whereas in the three models that had a mud depth of 9 cm, it ranged from 45 to 51% and averaged 47% (Table V). The greater growth of crabs in models with the larger volumes of mud was probably due to the greater quantity of food that was introduced with the mud. The type of tidal cycle did not appear to affect growth of the Dungeness crabs in experiment 1; however, in experiment 2 the crabs grew the most in those models with a continuous flow of sea water (Table V). In experiment 2, the mean percentage increases in carapace width of crabs in models with tidal cycles ranged from 53 to 74%, whereas for crabs in models having a continuous flow of sea water, they were 86%.

Survival and growth of juvenile oysters in experiment 1 were best in those models having a continuous flow of sea water (Table VI). They averaged 100% and nearly 600%, respectively, whereas the best survival and increase in shell length of oysters exposed to tidal cycles were 68 and 82%, respectively. In this experiment, the oysters were suspended several cm above the mud, and

TABLE V

Survival and growth of crabs used in the laboratory model experiments.

Species	Expt. no. and model no.	Mud depth (cm)	Exposure period: No./day[1]	Exposure period: Mean duration (hrs)	% survival	Carapace width (mm): Initial Mean	Carapace width (mm): Initial Range	Carapace width (mm): Final Mean	Carapace width (mm): Final Range	% increase in carapace width
Shore crab	Expt. 1 1	9	0	0	60	12.1	11-14	14.5	13-16	20
	2	27	0	0	20	12.5	11-15	15.0	15 only	20
	3	9	2	1.4	50	12.1	9-17	13.8	10-19	14
	4	25	2	3.8	30	12.0	9-15	14.7	14-15	23
	5	27	2	1.6	20	12.3	10-14	12.5	12-13	2
	6	9	2	3.8	80	10.8	9-13	12.6	10-16	17
	Expt. 2 1	18	2[2]	2.3	90	13.4	12-15	14.0	13-16	4
	2	18	1[3]	4.1	60	13.6	12-15	15.3	13-17	13
	3	18	1[2]	4.5	60	13.2	12-15	14.3	13-16	8
	4	18	2[3]	2.1	80	12.6	12-14	13.0	11-14	3
	5	18	0	0	30	12.6	12-14	13.3	13-14	6
	6	18	0	0	60	12.0	11-14	12.2	10-14	2
Dungeness crab	Expt. 1 1	9	0	0	80	26.5	17-36	38.8	32-52	46
	2	27	0	0	90	24.8	17-34	43.1	36-51	74
	3	9	2	1.4	90	26.0	17-35	39.2	29-49	51
	4	25	2	3.8	70	26.0	17-35	46.1	40-54	80
	5	27	2	1.6	70	24.8	18-33	45.9	35-57	85
	6	9	2	3.8	80	27.3	22-33	39.5	29-46	45
	Expt. 2 1	18	2[2]	2.3	90	20.9	19-23	31.9	28-38	53
	2	18	1[3]	4.1	80	20.8	19-25	36.1	33-38	74
	3	18	1[2]	4.5	100	20.4	18-22	34.3	28-39	68
	4	18	2[3]	2.1	80	20.7	20-23	35.8	27-39	73
	5	18	0	0	80	20.6	20-23	38.3	36-44	86
	6	18	0	0	100	19.5	18-22	36.2	33-41	86

[1] The exposure periods were at the same time each day, unless noted otherwise.

[2] Tidal cycle synchronized with natural cycle.

[3] Tidal cycle unsynchronized with natural cycle.

TABLE VI

Survival and growth of mollusks used in the laboratory model experiments.

Species	Expt. no. and model no.	Mud depth (cm)	Exposure period: No./day[1]	Exposure period: Mean duration (hrs)	% survival	Shell length (mm): Initial Mean	Initial Range	Final Mean	Final Range	% increase in shell length
Pacific oysters										
Expt. 1	1	9	0	0	100	3.2	3 -4	21.1	9-29	563
	2	27	0	0	100	3.2	3 -4	23.5	13-30	634
	3	9	2	1.4	45	3.6	3 -5	5.0	4- 7	39
	4	25	2	3.8	30	3.7	3 -5	6.2	5- 8	68
	5	27	2	1.6	68	3.8	3 -5	6.9	4-11	82
	6	9	2	3.8	0	3.6	3 -4	---	----	--
Expt. 2	1	18	2[2]	2.3	95	8.2	6 -12	14.0	8-20	71
	2	18	1[3]	4.1	100	8.3	6 -12	14.3	7-21	72
	3	18	1[2]	4.5	100	8.2	6 -12	14.3	9-19	74
	4	18	2[3]	2.1	100	8.2	6 -12	15.2	10-20	85
	5	18	0	0	90	8.2	6 -11	13.5	10-18	65
	6	18	0	0	100	8.4	6 -12	11.0	8-15	31
Cockle clams										
Expt. 1	1	9	0	0	96	22.0	14-30	25.9	17-35	18
	2	27	0	0	88	22.5	14-32	26.3	18-37	17
	3	9	2	1.4	100	21.4	13-28	26.4	18-32	23
	4	25	2	3.8	96	21.7	13-28	23.6	14-30	9
	5	27	2	1.6	100	20.3	13-29	25.2	17-33	24
	6	9	2	3.8	92	21.5	14-29	24.1	18-31	12
Expt. 2	1	18	2[2]	2.3	95	13.1	12-15	16.2	14-19	24
	2	18	1[3]	4.1	95	13.1	12-15	16.3	14-19	24
	3	18	1[2]	4.5	89	13.1	12-15	16.3	14-19	24
	4	18	2[3]	2.1	95	13.1	12-15	16.3	14-19	24
	5	18	0	0	91	13.1	12-15	17.3	14-19	32
	6	18	0	0	93	13.1	12-15	16.0	14-19	22

1, 2, and 3, see corresponding numbered footnotes in Table V.

Table VI (continued) Survival and growth of mollusks used in the laboratory model experiments.

Species	Expt. no. and model no.	Mud depth (cm)	Exposure period: No./day[1]	Exposure period: Mean duration (hrs)	% survival	Shell length (mm): Initial Mean	Shell length (mm): Initial Range	Shell length (mm): Final Mean	Shell length (mm): Final Range	% increase in shell length
Bay mussels	Expt. 1									
	1	9	0	0	0	13.4	12-15	----	-----	--
	2	27	0	0	70	13.4	12-15	23.9	15-31	78
	3	9	2	1.4	30	13.4	12-15	20.7	16-24	54
	4	25	2	3.8	0	13.4	12-15	----	-----	--
	5	27	2	1.6	45	13.4	12-15	23.6	21-27	76
	6	9	2	3.8	35	13.4	12-15	20.4	14-24	52
	Expt. 2									
	1	18	2^{2}_{3}	2.3	85	15.4	14-17	20.2	16-23	31
	2	18	1^{3}_{2}	4.1	75	15.4	14-17	19.9	16-23	29
	3	18	1^{2}_{3}	4.5	95	15.8	14-17	19.9	16-24	26
	4	18	2	2.1	95	15.6	14-17	18.5	15-25	19
	5	18	0	0	80	15.3	14-17	19.8	15-26	30
	6	18	0	0	85	15.3	14-17	17.4	14-21	14

1, 2, and 3, see corresponding numbered footnotes in Table V.

therefore they were exposed to the air for much longer periods of time than the exposure period given in Table VI. The number of hours the oysters were covered with water each day was determined for each model twice during the experiment. The relationships of survival and growth of oysters to the average number of hours they were in sea water each day in experiment 1 are shown in Figs. 4 and 5. In experiment 2, juvenile oysters survived equally well in all models (Table VI). In this experiment, the oysters were close to the mud surface, and thus they were exposed to the air for approximately the same period of time as the rest of the animals. The type of tidal cycle did not affect growth of the oysters in experiment 2 (Table VI). The mean percentage increases in shell length of oysters in models with tidal cycles and in model no. 5, which had a continuous flow of sea water at a rate of 5,800 liters/day, ranged from 65 to 85% (Table VI). However, the mean increase in shell length of oysters in model 6, which had a continuous flow of sea water but at a rate of 2,700 liters/day, was only 31%.

Survival of cockle clams was not affected by the different experimental conditions. In the two experiments, survival of the clams ranged from 88 to 100% (Table VI). Differences in mud depth did not affect growth of cockle clams in experiment 1; however, the clams grew the most under conditions of two 1.5 hour daily exposure periods and the least in models that had two 3.8-hour exposure periods (Table VI). The mean percentage increases in shell length for clams exposed for 1.5 hours twice daily was 24% and for those exposed for 3.8 hours twice daily, it was 11%. The mean percentage increases in shell length of clams in models with a continuous flow of sea water was 18%. In experiment 2, cockle clams in all models with tidal cycles increased in shell length by 24%, but in model no. 5, which had a continuous flow of 5,800 liters of sea water per day, they increased in shell length by 32% (Table VI). We do not know why clams in experiment 1 grew more in models having short exposure periods than in models having continuous flows of sea water.

Survival of bay mussels in experiment 1 ranged from 0 to 70%, but the average survival was only about 30% (Table VI). Most of the mortality probably was due to predation by Dungeness crabs. The mussels were on the surface of the mud and so were available to the crabs. Nearly all of the empty mussel shells that we observed were broken along the edges, and on one occasion, we saw a Dungeness crab trying to open a live mussel. Increase in shell length of the surviving mussels ranged from 52 to 78%; however, because of the low survival in most models, we can not make any inferences on the effects of the experimental conditions on growth of mussels. In experiment 2, 75% or more of the mussels survived (Table VI) probably because they were suspended in plastic containers above the surface of the mud and so were not readily available to the crabs. In experiment 2, the type of tidal cycle tested did not influence the growth of mussels (Table VI). The mean percentage increases in shell length for mussels in models with tidal cycles ranged from 19 to 31%. The increase in mean shell length of mussels in model no. 5, which had a continuous flow of 5,800 liters of sea water per day, was 30%, and in model 6, which had a continuous flow of 2,700 liters of sea water per day, it was 14%.

In experiment 1, some sculpins survived in models with tidal cycles as well as in one of the two models that had a continuous flow of sea water, but no more than three fish survived in any one model (Table VII). We do not know

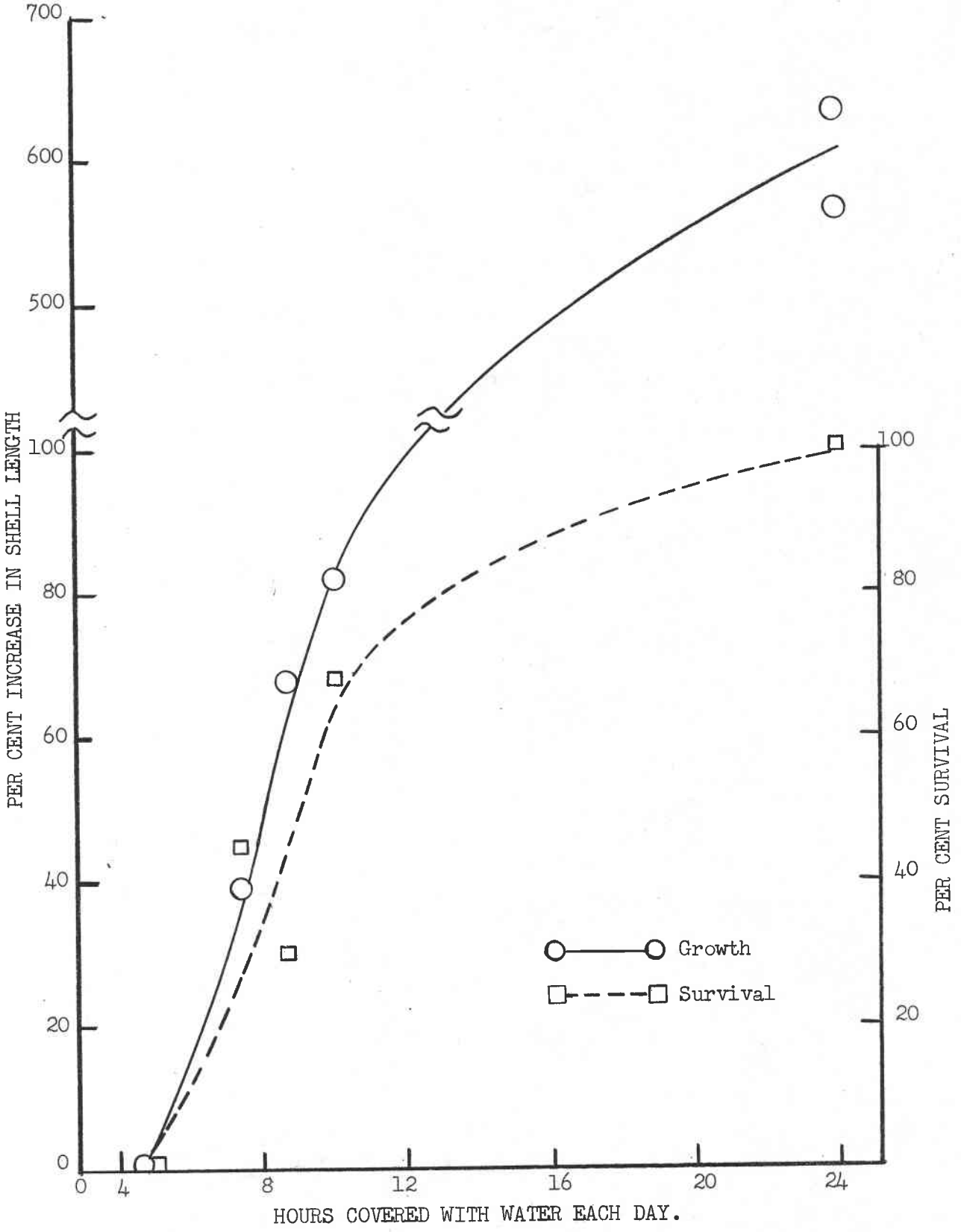

Fig. 4. Survival and growth of Pacific oysters in the laboratory models in relation to the number of hours they were covered with sea water each day.

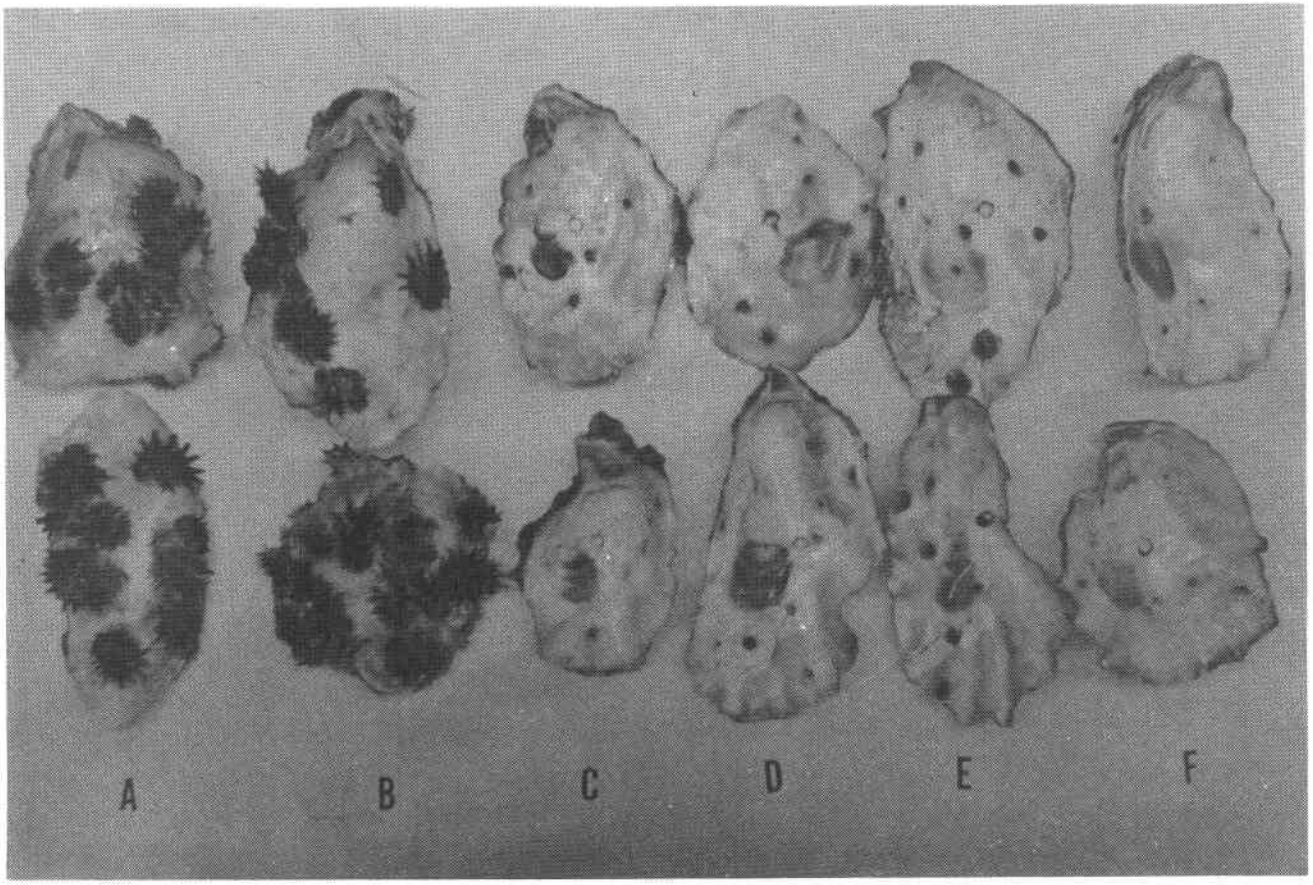

Fig. 5. Photographs of juvenile Pacific oysters showing relative effects of exposure to air on growth after 75 days in the laboratory models. The mean number of hours the oysters were covered with sea water each day were: A and B 24, C 7.5, D 8.5, E 10.0, and F 4.5.

TABLE VII

Survival and growth of staghorn sculpins used in the laboratory model experiments.

Expt. no. and model no.	Mud depth (cm)	Exposure period		No. surviving/ no. used	Weight in (grams)				% change in weight
		No./ day[1]	Mean duration (hrs)		Initial		Final		
					Mean	Range	Mean	Range	
Expt. 1 1	9	0	0	0/5	2.89	2.40-3.35	----	-----	--
2	27	0	0	3/5	2.93	2.15-3.50	2.97	2.50-3.25	+1
3	9	2	1.4	2/5	2.97	2.55-3.35	3.15	2.85-3.45	+6
4	25	2	3.8	2/5	2.93	2.70-3.25	2.88	2.35-3.40	-2
5	27	2	1.6	1/5	2.83	2.35-3.00	4.05	4.05 only	+43
6	9	2	3.8	0/5	2.99	2.65-3.20	----	-----	--
Expt. 2 1	18	2[2]	2.3	8/10	3.56	2.45-4.95	2.63	2.20-3.70	-26
2	18	1[3]	4.1	7/10	3.59	2.25-5.40	3.45	2.75-4.60	-4
3	18	1[2]	4.5	2/10	3.55	2.70-5.25	3.05	2.90-3.20	-14
4	18	2[3]	2.1	7/10	3.64	2.30-5.30	3.01	2.50-3.80	-17
5	18	0	0	9/10	3.67	2.45-4.70	3.21	2.05-3.95	-12
6	18	0	0	8/10	3.56	2.45-4.90	3.08	2.30-3.95	-14

1, 2, and 3, see corresponding numbered footnotes in Table V.

why sculpins failed to survive in the other model that had a continuous flow of sea water. The survival of fish in models with tidal cycles was unexpected because there were no pools in the mud that fish could retreat to during low tides. Most of the surviving sculpins increased in weight, but too few were used to determine the effects of the experimental conditons on their growth and survival. Some sculpins survived in all models in experiment 2 but they lost weight (Table VII).

In experiments 1 and 2, the English sole survived only in the models that had continuous flows of sea water. The respective survival percentages were 90 and 100%. In the first experiment, the mean weight of the fish decreased by 22 and 30%, and in the second experiment, it increased by 34 and 46%. We have no satisfactory explanation for this discrepancy.

DISCUSSION

The purpose of this study was to develop a laboratory model of an intertidal mud flat that would be suitable for studying the effects of pesticides on a community of marine animals. The models described in this paper are satisfactory for this type of study. We have shown that communities of intertidal animals can be successfully maintained in laboratory models, which have simulated tidal cycles, for periods up to 75 days.

Generally, the different types of tidal cycles that we tested did not affect survival and growth of shore crabs, Dungeness crabs, and bay mussels. Survival and growth of Pacific oysters were not affected by tidal cycles when their exposure to air did not exceed about 4 hours each day; however, when the oysters were exposed to air daily for 12 hours or more, their survival and growth were reduced considerably. The results of one experiment indicated that cockle clams may, under certain conditions, grow better if exposed to air for short periods of time each day, but additional experiments are needed to establish this point. English sole did not survive in models with tidal cycles, whereas some sculpins did. Generally, differences in mud depth, which ranged from 9 to 27 cm, did not directly affect survival and growth of the animals. However, in one experiment, Dungeness crabs grew the most in those models having the larger volume of substrate, probably because of the greater number of food organisms present in the mud.

Our experimental tanks can easily be modified so that other variables in addition to the ones we have tested can be studied. For example, the tanks are large enough to accommodate paddle wheels or similar devices for moving water to study the effects of water currents on the animals, or an electrical fan system as described by Evans (1964) for simulating wind action. Also, pools that would retain water during low tides could be incorporated into the experimental tanks so that fishes, in addition to bottom fish, could be used as test animals.

Studies using laboratory models of a marine environment will complement highly controlled laboratory studies and field studies, which are difficult to control. With such laboratory models, the effects of pesticides, as well as other pollutants, on marine animal communities can be studied under controlled simulated natural conditions.

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CHEMICAL STUDIES

1. Fate of Sevin in laboratory aquaria.

Specific aim no. 5. To follow the fate of Sevin in laboratory aquaria under simulated natural conditions.

This phase of the project is completed. See the following reprint for summary of the results.

CHEMICAL STUDIES

2. Mode of action of Sevin.

Specific aim no. 6. To determine the mode of action of Sevin and its metabolites on estuarine organisms.

Studies on acetylcholinesterase activities in tissues of the crab, Cancer magister, were continued using the pH-stat method. Attempts to purify the acetylcholinesterase from crab ventral nerve tissue were unsuccessful; therefore, it was decided to test the crude homogenate for substrate specificity to determine its homogeneity with respect to types of cholinesterases. Benzoyl choline is not hydrolyzed at 0.65×10^{-3}, 1.95×10^{-3} or 13.0×10^{-3} moles/liter. Benzoyl choline inhibits the hydrolysis of acetylcholine bromide by the enzyme. A concentration of 1.95×10^{-3} M benzoyl choline gives 86.3% inhibition when acetylcholine bromide is present at 0.65×10^{-3} moles/liter. Butyrl choline at a concentration of 0.65×10^{-3} moles/liter shows insignificant hydrolysis (less than 5%). Acetyl B methyl choline showed a significant rate of hydrolysis by the enzyme at a concentration of 0.65×10^{-3} moles/liter (25% of the rate for acetycholine bromide at the same concentration). The data suggests that acetycholine bromide competes with acetyl B methyl choline for the same site on the enzyme and is preferentially hydolyzed. Benzoyl choline (0.65×10^{-3} M) inhibits acid production from both acetyl B-methylcholine and acetylcholine bromide when they are present at a concentration of 0.65×10^{-3} mole/liter.

The major cholinesterase enzyme in the crude crab homogenate is apparently a true cholinesterase. Because the crude homogenate showed a considerable degree of homogeneity with regard to substrate specificity, we decided to proceed with the characterization of the enzyme and to study its inhibition by Sevin without attempting further purification of the enzyme.

A study of enzyme activity versus temperature in which the enzyme was preincubated at temperatures from 5 to 37°C for 8 minutes before assay gave an asymptotic curve with activity increasing with temperature up to 37°C. There was no inhibition over the entire temperature range but the standard deviation between duplicate assays increased with increasing temperature. Addition of K^+, Na^+, Mg^{++}, Ca^{++} and Cl^- ions increased the activity of the enzyme by 30%, stabilizing and lengthening its period of activity. Further studies on the inhibition of crab acetylcholinesterase by Sevin were done. The reversibility of the inhibition was studied by incubating a mixture of the enzyme and Sevin for 32 minutes, and then diluting the mixture 200 times with slightly buffered isotonic salt solution. The recovery of the enzyme was followed by assaying at approximately 30 minute intervals. Temperature and pH were controlled during the incubation and assay periods. A control enzyme preparation without Sevin was diluted and assayed in a similar manner.

Recovery of the inhibited enzyme was slow and incomplete. Duplicate studies on different enzyme preparations gave similar results. A semilog plot with percent of inhibition on the ordinate and time on the abscissa showed a linear recovery of the enzyme from 100% to 63% inhibition during the first 100 minutes after dilution. The recovery half-life from this slope was 156 and 150 minutes for the two experiments. The decarbamylation constants (K_3) calculated from these slopes were 0.00444 min^{-1} and 0.000462 min^{-1}, respectively.