AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF THE INSECTICIDE SEVIN ON THE DUNGENESS CRAB, CANCER MAGISTER DANA

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Dr. Raymond E. Millemann

This study was undertaken primarily to determine the short-term and long-term effects of the insecticide Sevin on various life history stages of the Dungeness crab (Cancer magister). Also determined was the normalcy of the occurrence of a free prozoeal stage in the life history of this crab. Also, some morphological observations on the sex of third stage juvenile crabs were made.

When eggs of the crab were held in sea water at 10.5 or 17.5°C and at salinities of 10 to 32‰, 94% hatched into prozoeae during a 36-hour observation period. 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‰. With increase of salinity from 15 to 32‰, the mean duration of the prezoeal stage decreased from more than 60 minutes to 11 minutes. 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.

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-hour EC_{50}'s (effective concentration that killed 50% of the animals) for first stage zoeae and adults were 0.01 and 0.26 mg/liter, respectively. Few zoeae were killed in 24 hours by 82.0 mg/liter, but the 24-hour EC_{50} for death within 15 days after the exposure was 0.015 mg/liter. The 24-hour EC_{50} for cessation of swimming, which was not always permanent, 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-hour EC_{50}'s (concentration that irreversibly paralyzed 50% of the animals) for second stage juveniles, ninth stage juveniles, and adults were 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 hours and then held in clean sea water for 44 days.

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

Effects of the Insecticide Sevin on the Dungeness Crab, Cancer magister Dana

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF THE INSECTICIDE SEVIN ON THE DUNGENESS CRAB, *CANCER MAGISTER* DANA

INTRODUCTION

The primary purpose of my study was to determine the short-term and long-term effects of the insecticide Sevin on survival and growth of the Dungeness crab (*Cancer magister*) at various stages of its life history. Minor objectives also investigated included the following: (a) to resolve the controversy about the normalcy of the occurrence of a free prozoeal stage in the life history of the crab; and, (b) to find a marker or tag that would identify crabs and persist through several molts.

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.

The insecticide Sevin, 1-naphthyl methylcarbamate, has been used in the field to control oyster pests and predators such as mud shrimp (*Upogebia* sp.), ghost shrimp (*Callianassa* sp.), aquatic gastropods, and starfish, but it may also adversely affect nontarget organisms such as blue crab (*Callinectes sapidus*), Dungeness crab

(Cancer magister), cockle clams (Clinocardium nuttalli), and fish (Haven et al., 1966; Lindsey, 1961; Loosanoff, 1962; Loosanoff, MacKenzie and Shearer, 1960; Snow and Stewart; 1963).

The acute toxicity of Sevin to five species of marine fish has been studied in the laboratory by Butler (1963) and Stewart, Millemann and Breese (1967). For expressing toxic levels, Butler used TL_m values (the median tolerance limit, that concentration lethal to 50% of the test animals within a certain time period); whereas, Stewart et al. used EC_{50} values (the effective concentration which produced a designated effect on 50% of the test animals). When death is used as the criterion of toxic effect TL_m and EC_{50} values are equivalent. The 24-hour and 48-hour EC_{50} or TL_m values for the five species of fish studied by the above authors ranged from 1.75 to 6.7 mg/liter or ppm (parts per million).

Davis (1961) studied the effect of Sevin and other pesticides on the development of eggs and growth of larvae of oysters (Crassostrea virginica) and clams (Venus mercenaria). He found that 2.5 ppm of Sevin inhibited the development and growth of the clams, whereas only 1.0 ppm inhibited the development of the oysters. Stewart et al. (1967) also studied the effect of Sevin on the cockle clam and reported that the 24-hour EC_{50} value was 7.3 mg/liter when death was the criterion of toxic effect. Butler, Millemann and Stewart (1968) reported on the effect of Sevin on the growth of the cockle clam. They found that clams

exposed to 1.8 mg/liter of Sevin had increased in growth by 13% at the end of 15 days compared with a 64% increase in shell length for control clams.

There are only a few reported studies on the toxicity of Sevin to crustaceans. Butler (1962) reported that the 48-hour TL_m's of Sevin for brown shrimp (Penaeus aztecus) and white shrimp (Penaeus setiferus) adults were 0.027 and 0.013 ppm, respectively. Muncy and Oliver (1963) reported 24-, 48-, and 72-hour TL_m's for red crayfish (Procambarus clarki) were 5.0, 3.0, and 2.0 ppm, respectively. Stewart et al. (1967) studied the toxicity of Sevin and its first hydrolytic product 1-naphthol to various species of marine crustaceans. They used loss of equilibrium or paralysis as the criterion of toxic effect, and reported mean 48-hour EC_{50} values ranging from 0.03 to 0.09 mg/liter for mud shrimp and ghost shrimp larvae.

Butler (1962, 1963) reported a 24-hour EC_{50} value of 0.55 ppm of Sevin for juvenile blue crabs, and Stewart et al. (1967) reported 24-hour EC_{50} values ranging from 0.06 to 1.05 mg/liter for adult shore crabs (Hemigrapsus oregonensis) and values ranging from 0.55 to 0.70 mg/liter for juvenile Dungeness crabs. Male and female Dungeness crabs were equally sensitive to Sevin.

There have been no reported studies on the long-term effects of pesticides on Dungeness crabs. Lowe (1965) reported on the chronic effects of DDT on growth of blue crabs. He exposed juvenile crabs to

DDT in flowing sea water for nine months and found no significant differences between control crabs and those exposed to 0.25 ppb (parts per billion) DDT, but crabs exposed to 0.5 ppb solution exhibited typical signs of insecticide poisoning.

MacKay (1942) reported that female Dungeness crabs attain sexual maturity at a carapace length of approximately four inches. According to Cleaver (1949), a large female can lay in excess of 2.5 million eggs. Several months after copulation the eggs are fertilized and immediately extruded. They become attached to the abdominal appendages and are carried by the female until hatching several months later. The eggs are a bright orange when first extruded but become a dark brown or black as they mature.

Poole (1966) studied larval development of the crab under laboratory conditions. He believed that eggs hatched into first stage zoeae. He observed larval development through five zoeal and one meglopa stage. MacKay (1942) stated that the eggs of the crab hatched into protozoeae (= prezoeae) before molting into first stage zoeae, but Poole (1966) believed that prezoeae resulted from premature hatching of eggs. Mir (1961) also reported that zoeae normally emerged from eggs and that free prezoeae were abnormal and died. My preliminary observations on egg hatching and on subsequent molting of prezoeae supported MacKay's belief. Before I could begin studies on the effects of Sevin on survival and growth of C. magister larvae I had to resolve

the controversy.

Poole (1966) reported that complete larval development required four months, and that the meglopa then metamorphosed directly into the first juvenile stage or instar. Growth of juvenile crabs was studied first by MacKay and Weymouth (1935) and later by Butler (1961).

The Dungeness crab was chosen as the test animal for my study because of its abundance in estuaries where it could be exposed to pesticides used in oyster pest control programs, 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.

METHODS

Life History Studies

To determine whether or not a prezoea was normally the stage of hatching, ovigerous crabs caught in the ocean off the Oregon coast were held in the laboratory in flowing sea water for three days. One crab was then transferred to a 31-gallon tank containing filtered, sterilized, standing sea water of 30‰ at 11° and held for an additional three days. Eggs began to hatch at the end of this time. I 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 three 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 three 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 250 ml beakers containing sea water at 10.5°C and 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 five 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.

Sevin Studies

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 one liter of filtered sea water. Each stock solution was stirred for two hours 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. Logarithmic series of concentrations were used. Stock and test solutions were used within five hours after preparation. In every experiment, each concentration was tested in duplicate or in triplicate. With a few noted exceptions, the experiments were done under continuous light with standing sea water at $10 \pm 1^{\circ}C$. Each EC_{50} value (the 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). In the 17 different experiments, the criterion of toxic effect varied with the life history stage tested (Table 1).

Eggs and Prezoeae

Ovigerous crabs caught in the ocean off the Oregon coast were held in the laboratory in 31-gallon tanks containing flowing sea water at approximately 10°C. When hatching began, eggs of normal appearance with all cuticular layers intact were gently removed from the crabs and placed into 250 ml flasks. Each flask contained an average of 20 eggs and 200 ml of test solution. Eggs from one female only were used in each experiment but eggs from different crabs were used in the three experiments. Nine concentrations of Sevin ranging from 0.0001 to 1.0 mg/liter were tested.

The numbers of unhatched eggs, prezoeae, and zoeae in the test vessels were recorded at the end of the 24-hour exposure periods. 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

Table 1. Conditions of experiments and toxicity criteria for each life-history stage of the Dungeness crab exposed to Sevin.

Life-history stage	Experiment number	Range of Sevin concentrations (mg/liter)	Exposure time (hours)	Postexposure observation time (days)	Criteria of toxic effect
Fertilized eggs and prezoeae[1]	1-3	0.0001-1.0	24	8	Prevention of hatching and molting
Zoeae	4-7	0.0032-82.0	24, 48, or 96	0	Death[2]
	8	0.0003-10.0	1, 5, 24	25	Cessation of swimming, prevention of molting and death[2]
	9	0.0003-10.0	1, 24	8	"
	10	0.0001-0.01	25 days	0	Prevention of molting and death[2]
Juveniles					
2nd stage	11	0.0032-10.0	1, 24, 48	3	Death or paralysis[3]
3rd stage	12	0.01 and 0.032	24	44	Prevention of molting and death or paralysis[3]

(Continued on next page)

Table 1. (Continued)

Life-history stage	Experiment number	Range of Sevin concentrations (mg/liter)	Exposure time (hours)	Postexposure observation time (days)	Criteria of toxic effect
5th stage[4]	13	0.032-1.0	24	0	Death or paralysis[3]
9th stage	14, 15	0.18-1.0	96	0	"
Adults					
	16	0.18-1.0	96	0	"
	17	1.0-10.0[5]	24	0	"

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

[2] Cessation of heart beat.

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

[4] Survivors from preceding experiment.

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

time of feeding. The tests were terminated at eight days and the number and kinds of surviving larvae recorded.

Zoeae

Seven experiments (Table 1) were done to determine the effects of Sevin on survival, molting, and swimming of first stage zoeae. Experiments 4-9 involved short-term exposures (96 hours or less) to the chemical, whereas in experiment 10 the larvae were exposed for 25 days.

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 ten were placed into a test vessel (250-ml beaker) containing 200 ml of the test solution.

A preliminary experiment was done to determine the length of survival of unfed larvae. Three groups of ten larvae and three groups of five larvae were held in beakers for 11 days in 200 ml of sea water of 25‰ salinity at 10°C. The larvae were observed for mortality at least every two days and at those times the sea water in the beakers was renewed.

In the first four experiments, the zoeae were exposed for either

24, 48, or 96 hours to eight or more Sevin concentrations ranging from 0.003 to 82.0 mg/liter. In experiments 4, 5, and 6, unfed larvae one to two days old were tested at 10 ± 1°C, and also at 17 ± 1°C in experiment 6. In experiment 7, ten-day-old larvae were tested at 10 ± 1°C. These larvae, in groups of 50 held in one-gallon glass jars, had been fed barnacle larvae on alternate days for ten 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 one and eight days old, respectively. The eight-day-old larvae were held in groups of 50 in one-gallon 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 hours at 10 ± 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 eight 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, molting, and cessation of swimming.

The two-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 hours and to darkness for eight hours. Each concentration was tested in triplicate. The zoeae in each beaker were fed about 1,000 brine shrimp daily for the first 15 days of the experiment and three times a week thereafter. In a preliminary experiment, I found that brine shrimp larvae are not affected in 24 hours 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.

Juveniles

In experiment 11 (Table 1), second stage juvenile crabs, averaging 9.5 mm in carapace width, were held for two days in sea water at the test temperature of 14 ± 1°C and at the test salinity before transfer in groups of six to one-gallon glass jars containing two liters of test solution. They were exposed either to seven concentrations of Sevin ranging from 0.01 to 10.0 mg/liter for one hour, or to six concentrations ranging from 0.0032 to 1.0 mg/liter for 24 or 48 hours. After exposure, they were transferred to clean, standing sea water and observed for three 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 (Table 1) was done to determine if 24 hour 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. Thus, exposed males were held with unexposed females in some containers while in other containers unexposed males were held with exposed females.

In experiment 12, the crabs were held for five days in sea water at 12 ± 1°C before transfer to one-gallon glass jars containing two liters of test solution. They were exposed in groups of six to Sevin concentrations of 0.01 or 0.032 mg/liter for 24 hours. Then a group of ten 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 3.5 gallon 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 (Figure 1). The bottom of each container was covered with sand and broken clam shells four

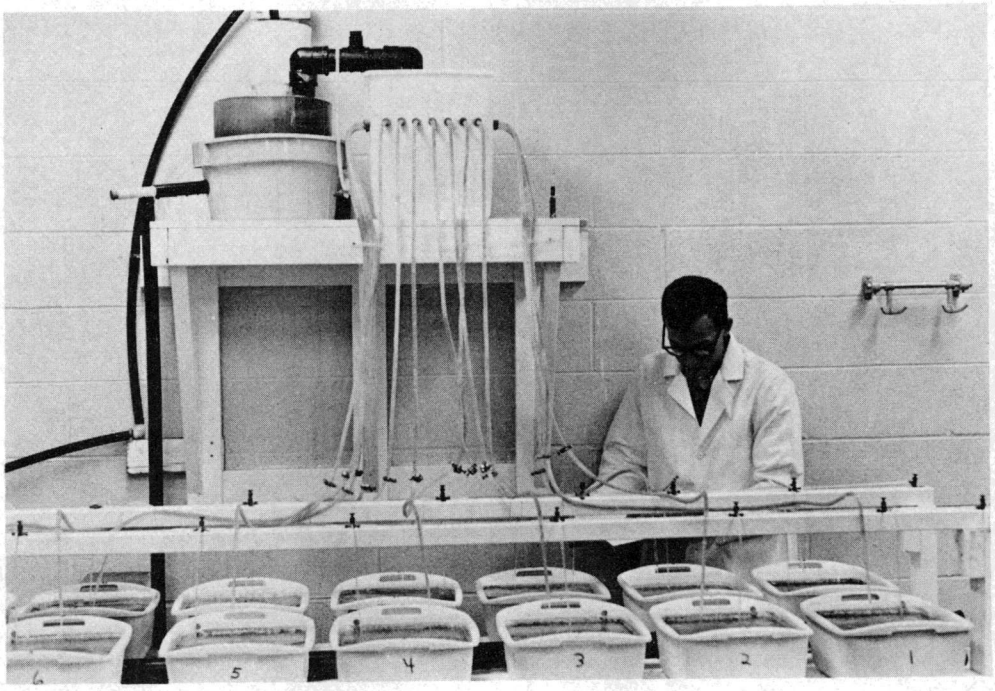

Figure 1. Apparatus used in experiment 12.

cm deep. The crabs were exposed alternately to light for 16 hours and darkness for eight hours. They were fed chopped cockle clams (Clinocardium nuttalli) daily, at which time deaths and molts were recorded. Carapace widths of the crabs were measured at the end of the experiment and compared with initial widths.

Animals used in experiment 13 were 30 control crabs from experiment 12 and 30 crabs from each of the two exposure groups in experiment 12. These crabs were now fifth stage juveniles. Experiment 13 was done to determine if resistance to Sevin developed in crabs that had survived previous exposure to Sevin. Of the 60 crabs previously exposed to Sevin, 48 were re-exposed in groups of six to four Sevin concentrations ranging from 0.032 to 1.0 mg/liter for 24

hours at 12 ± 2°C. The remaining 12 crabs were not re-exposed and served as controls. Of the 30 control crabs from experiment 12, 24 in groups of six were exposed to the above Sevin concentrations for 24 hours, and the remaining six again served as unexposed controls. The test was terminated at the end of the exposure period and the 24-hour EC_{50}'s determined using irreversible paralysis or death as the criteria of toxicity.

The crabs used in experiments 14 and 15 were probably ninth stage juveniles. Their carapace widths ranged from 58 to 80 and averaged 67 mm. They were held at the test temperature of 18 ± 1°C and salinity of 25‰ for four days, and then placed in groups of five into five-gallon 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 hours and the EC_{50}'s estimated using death or irreversible paralysis as the criterion of toxic effect.

Adults

Two experiments using adult crabs were done (Table 1). The crabs used in experiment 16 were females and averaged 110 mm in carapace width. They were held at the test temperature of 11 or 18°C and salinity of 25‰ for four days, and then placed in groups of three into five-gallon glass jars containing 15 liters of aerated test solution.

The solutions were changed daily. The four Sevin concentrations used ranged from 0.18 to 1.0 mg/liter. The experiment was ended after 96 hours and the EC_{50}'s estimated using death or irreversible paralysis as the criterion of toxic effect.

Experiment 17 was a secondary poisoning test (Table 1). 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 hours in five-gallon glass jars containing 15 liters of test solution at 13 $\pm$ 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 others were saved for tissue analysis. A group of three crabs was placed into each of 12 3.5-gallon plastic containers provided with flowing sea water of 25 to 32‰ salinity at 11.0 $\pm$ 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 six hours and again at the end of the 24-hour 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 experiment 17 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 hours 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 colormetric procedure described by Karinen et al. (1967) was used for determination of Sevin and free 1-naphthol.

RESULTS

Life History Studies

Prezoeae

Some eggs hatched at all the test salinities, and 94% of these are known to have hatched into prezoeae (Table 2). 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 two minutes after hatching. Therefore, I 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 precentage of eggs hatching at 10.5°C increased as salinity decreased to an optimum of 15‰, but at 10‰ the fewest hatched (Figure 2). 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 2).

The mean percentages of prezoeae that molted to zoeae increased with increasing salinity from 0% at 10‰ to 100% at 30 and 32‰ (Table 2 and Figure 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 (Table 2). At 32‰ salinity, all prezoeae molted to zoeae at the two test temperatures, and there was no effect of temperature on

Table 2. Effects of salinity and temperature on hatching of Cancer magister eggs and on molting of the prezoeae to zoeae.[1]

Salinity ‰	Hatched eggs		Free prezoeal stage confirmed		Duration of prezoeal stage (min.)[2]		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	>25	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[3]	17	85	15	88	11	3-35	15	100
32[3]	12	60	12	100	11	4-30	12	100

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

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

[3] Test temperature was 17.5°C.

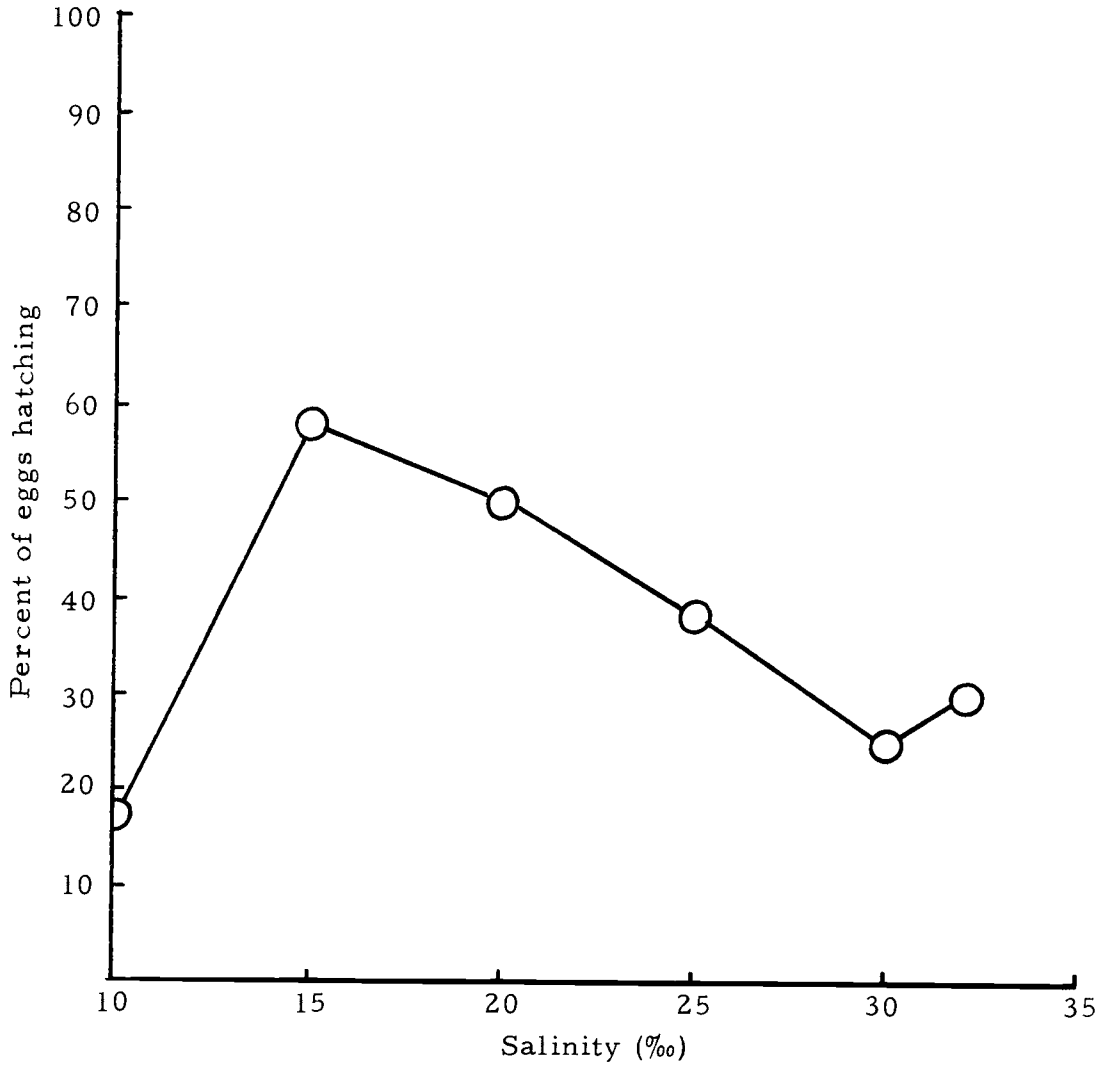

Figure 2. Effect of salinity on hatching of Cancer magister eggs at 10.5°C.

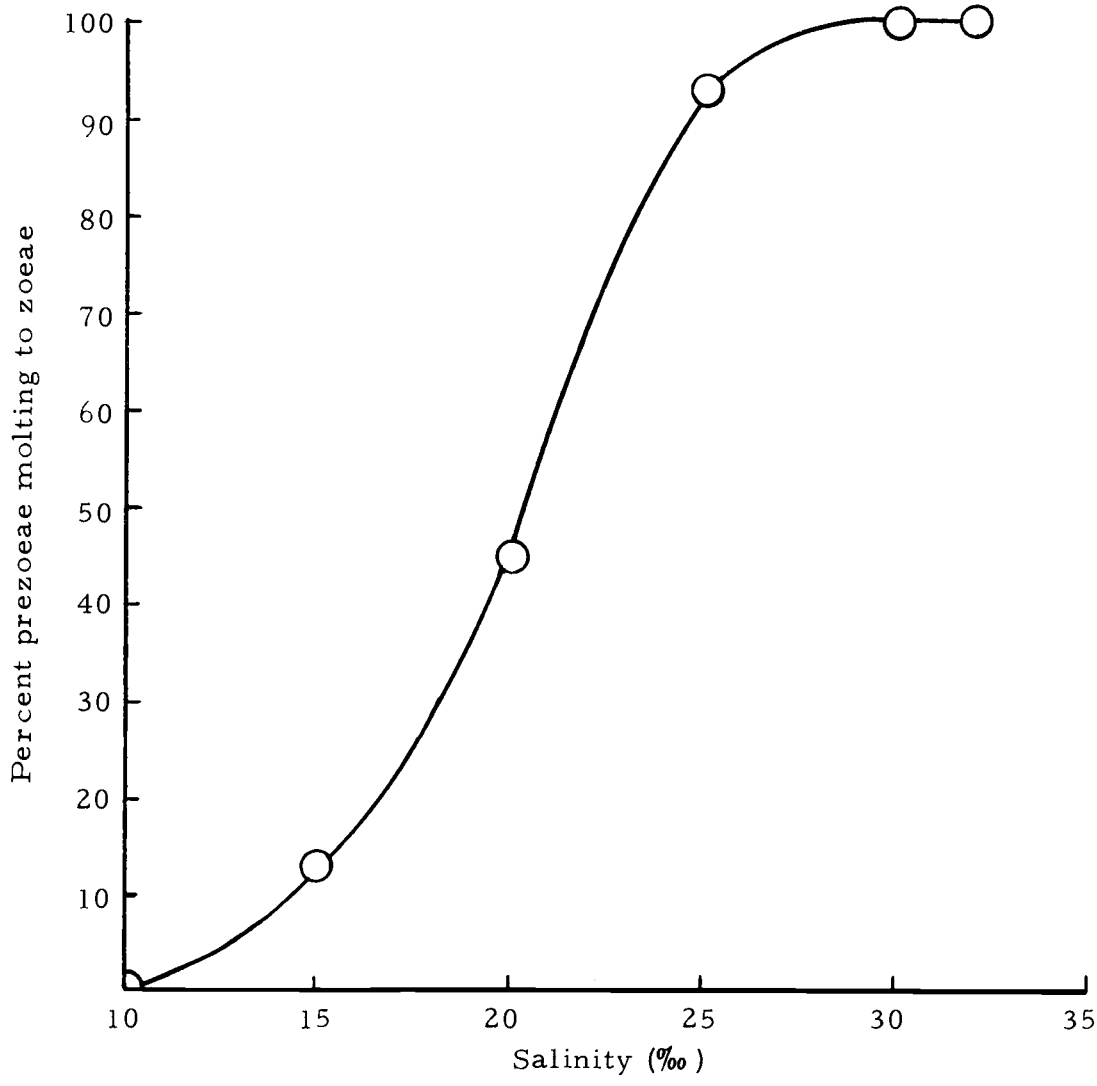

Figure 3. Effect of salinity on molting of Cancer magister prezoeae to zoeae.

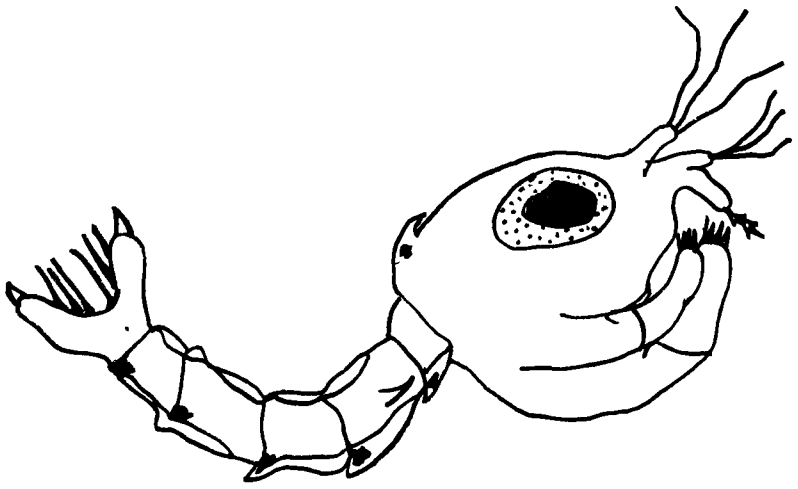

Figure 4. Prezoea of Cancer magister after hatching from the egg.

duration of the prezoeae stage.

My morphological observations of the prezoeae agree with those of MacKay (1934) except that I did not see the lateral spines on the cephalothorax. The prezoeae have large eyes, a five-segmented abdomen, and a forked telson with spines (Figure 4). 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 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. My first stage zoeae were morphologically identical 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‰ salinity at 10°C for three days. All of them survived and appeared normal during this time.

Sex Identification

The sex of third stage juvenile crabs can be determined externally by raising the abdomen and looking for oviduct openings. However, this procedure can damage the brittle abdomen. I 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 in the 100 crabs that I examined by raising the abdomen were always straight in males and curved in females (Figure 5). Thus, I was able to use the sex of the crabs as a marker to distinguish exposed

and unexposed crabs in experiment 12.

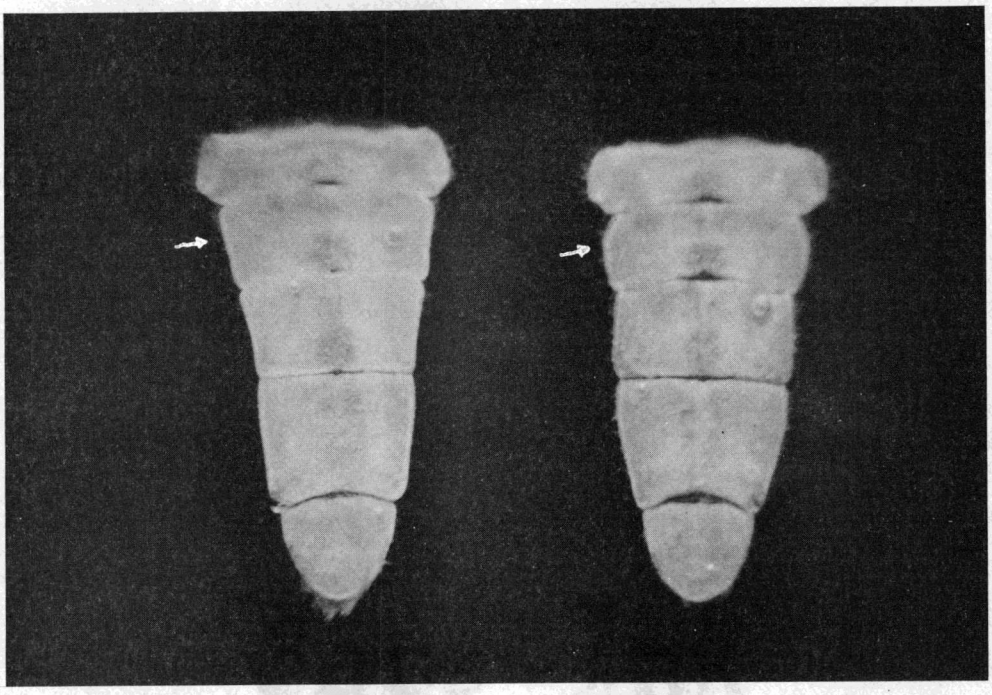

Figure 5. Male (left) and female (right) abdomens from third stage juvenile Dungeness crabs (second abdominal segment indicated by arrow).

Sevin Studies

Eggs and Prezoeae

Sevin did not affect hatching of eggs. The percentages of untreated, control eggs that hatched within the 24-hour 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 to 77%.

I have found that a free prezoeal stage of five to fifteen minutes in duration is normal in the life history of C. magister. Of the prezoeae

that hatched in the control vessels, 85 to 95% molted to zoeae within the 24-hour exposure 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 (Figure 6). The 24-hour EC_{50}'s for the three experiments were 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 eight days. None of the larvae that had been exposed to Sevin concentrations greater than 0.032 mg/liter was alive at the end of the eight-day observation period. Only 8% of those exposed to the lower concentrations molted and survived.

Zoeae

In the preliminary experiment, unfed first stage zoeae in concentrations of five per 200 ml and ten per 200 ml of sea water survived at the same rate throughout the 11 day experiment (Table 3). Of all larvae, 93% were alive at the end of seven days. Therefore, I did not feed the larvae used in subsequent experiments of 96 hours or less in duration.

In experiments 4 through 7 at 10 $\pm$ 1°C, the 24- and 48-hour 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-hour experiment at 10 $\pm$ 1°C, the 96-hour EC_{50} was found

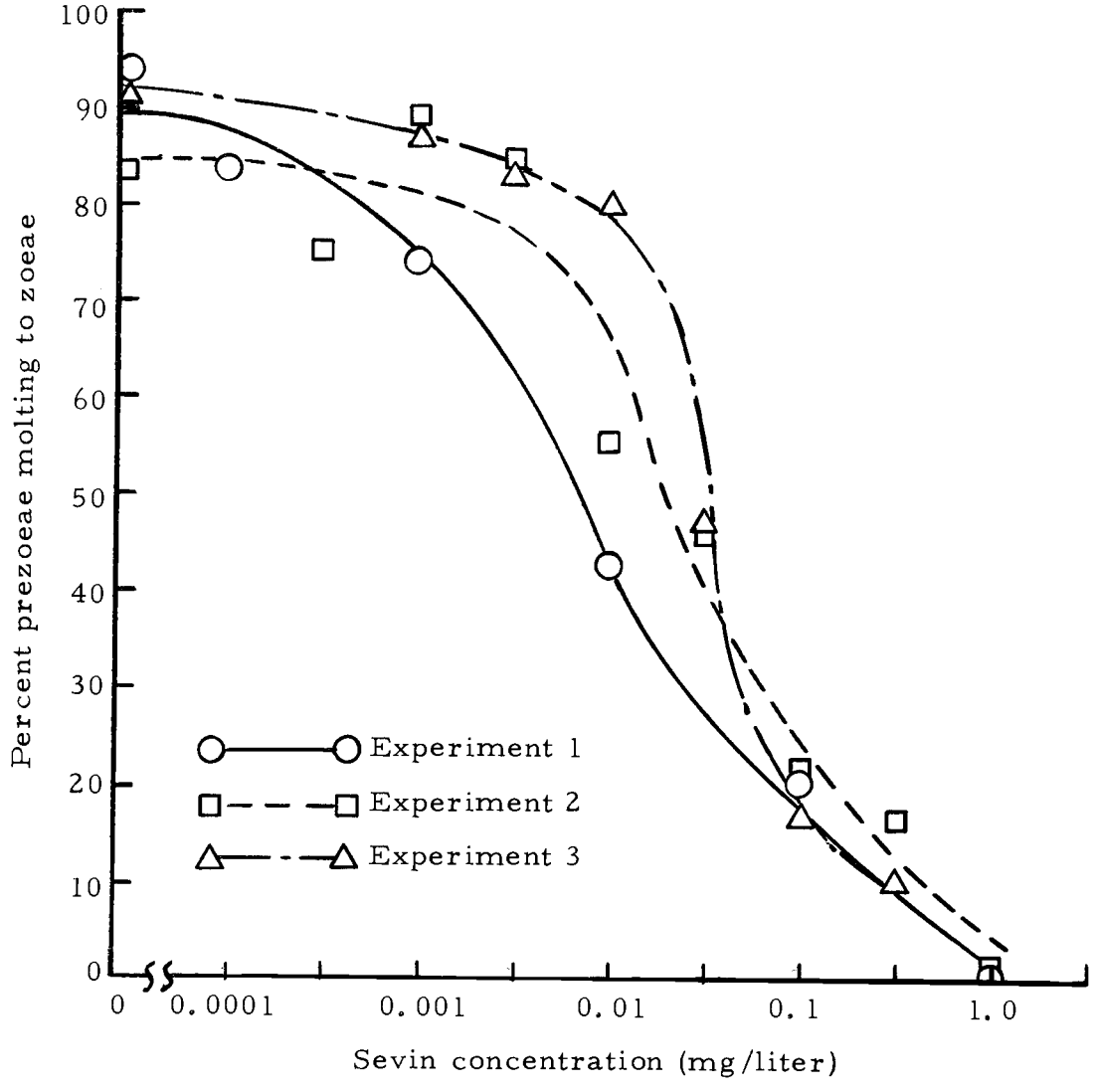

Figure 6. Percentages of Dungeness crab prezoeae that molted during a 24-hour exposure to various concentrations of Sevin. The prezoeae hatched from eggs that were obtained from three female crabs, one for each experiment.

Table 3. Survival of unfed Cancer magister first stage zoeae at 10°C and 25‰ salinity.[1]

No. larvae	Percent survival after: 2 day	4 day	6 day	7 day	9 day	11 day
5	100	80	80	80	40	0
5	100	100	100	100	80	0
5	100	100	100	100	60	40
10	100	100	100	80	60	30
10	100	100	100	100	90	0
10	100	100	100	100	90	0

1. Larvae were held in 200 ml of filtered, sterilized sea water.

to be 0.01 mg/liter. In the one experiment at 17 ± 1°C, the 24- and 48-hour EC_{50}'s were 0.08 and 0.005 mg/liter indicating a marked temperature effect.

In experiments 8 and 9, I tested the delayed effects of Sevin on swimming, molting, and survival of zoeae one and eight days old, respectively. In experiment 8, all larvae survived after 1-, 5-, and 24-hour exposures after which the larvae were returned to clean sea water. 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 for cessation of swimming decreased with increasing observation time after the third day (Table 4). These EC_{50} values for any observation period decrease progressively with increase of exposure time. EC_{50} values for death did not show the same consistent relation

Table 4. EC_{50}'s (mg/liter) for cessation of swimming and for death for 1-day-old zoeae exposed for different periods to Sevin and then held in clean sea water for periods up to 25 days.[1]

Exposure time (hours)	Criterion of toxic effect	Days after beginning of exposure										
		≤1[2]	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

[1] For both exposure and observation periods the salinity of the sea water was 25‰ and the temperature 10 ± 1°C. After exposure the larvae were transferred to clean sea water and fed brine shrimp larvae three times a week. Survival of control larvae on days 15 and 25 was 90 and 79% respectively.

[2] Calculated at the ends of the exposure periods; no larvae died during these times.

to exposure time during the first six days of observation. However, by day 15 the EC_{50} values for death approximated or equalled those for cessation of swimming (Table 4). 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 (Figure 7). In this experiment, Sevin did not affect molting of the surviving larvae. All surviving control and exposed larvae molted to second stage zoeae during days 13 to 23 of the experiment.

In experiment 9, eight-day-old larvae were exposed to Sevin for one and 24 hours and then held in clean sea water for eight days. The results were generally the same as those obtained with larvae one day old in experiment 8. All larvae were alive at the end of the exposure periods, but on day three some were dead. The EC_{50}'s for death at this time for the one- and 24-hour exposures were 0.57 and 0.024 mg/liter, respectively, on day six they were 0.24 and 0.019 mg/liter, and on day eight 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 eight, the EC_{50}'s for cessation of swimming were

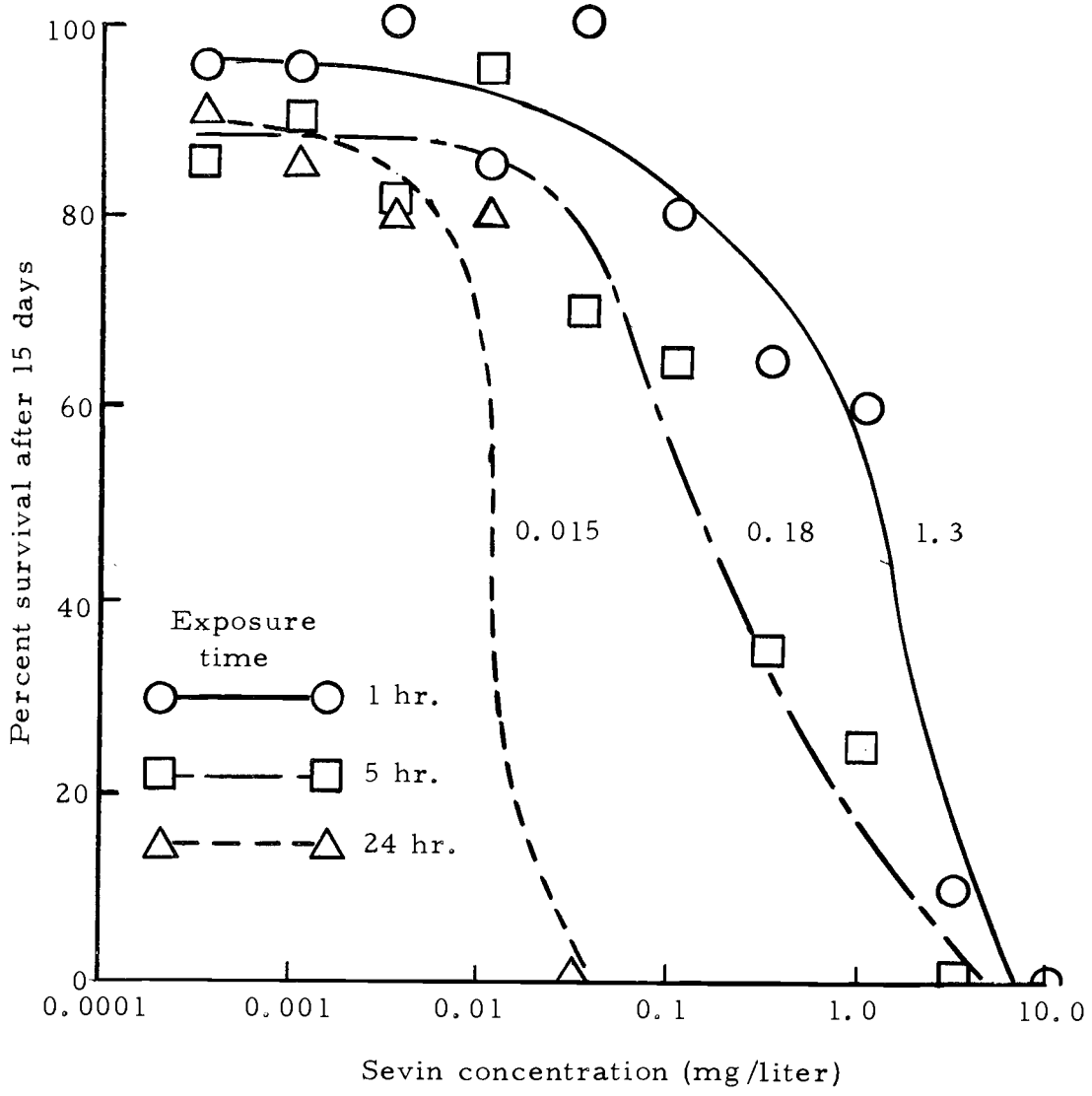

Figure 7. Percentages of survival of zoeae on day 15 in clean sea water at 10 ± 1°C after exposure at one day of age to various concentrations of Sevin for three time periods (experiment 8). The number beside each curve is the EC_{50} value. Survival of control larvae was 90%.

identical with those for death. Survivals of control larvae on days six and eight 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 one, but on day two some of those exposed to the two highest Sevin concentrations, 0.0032 and 0.01 mg/liter, were dead (Figure 8). The 96-hour EC_{50} for death was 0.009 mg/liter, which nearly agrees with the 96-hour EC_{50} of 0.01 mg/liter based on the results of acute toxicity test mentioned previously. Survival of larvae at the highest Sevin concentration of 0.01 mg/liter decreased from 97% on day three to 10% on day five, and on day 20 it was 3%. On day 20, survival of larvae at the other concentrations ranged from 73 to 88%, in comparison with 83% for the controls. 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 for death were 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 (Figure 9). The effect on molting of larvae to second stage zoeae was first observed on day 17. At that time, 28% of the controls had molted and were living in comparison with 7% at the lowest Sevin concentration of

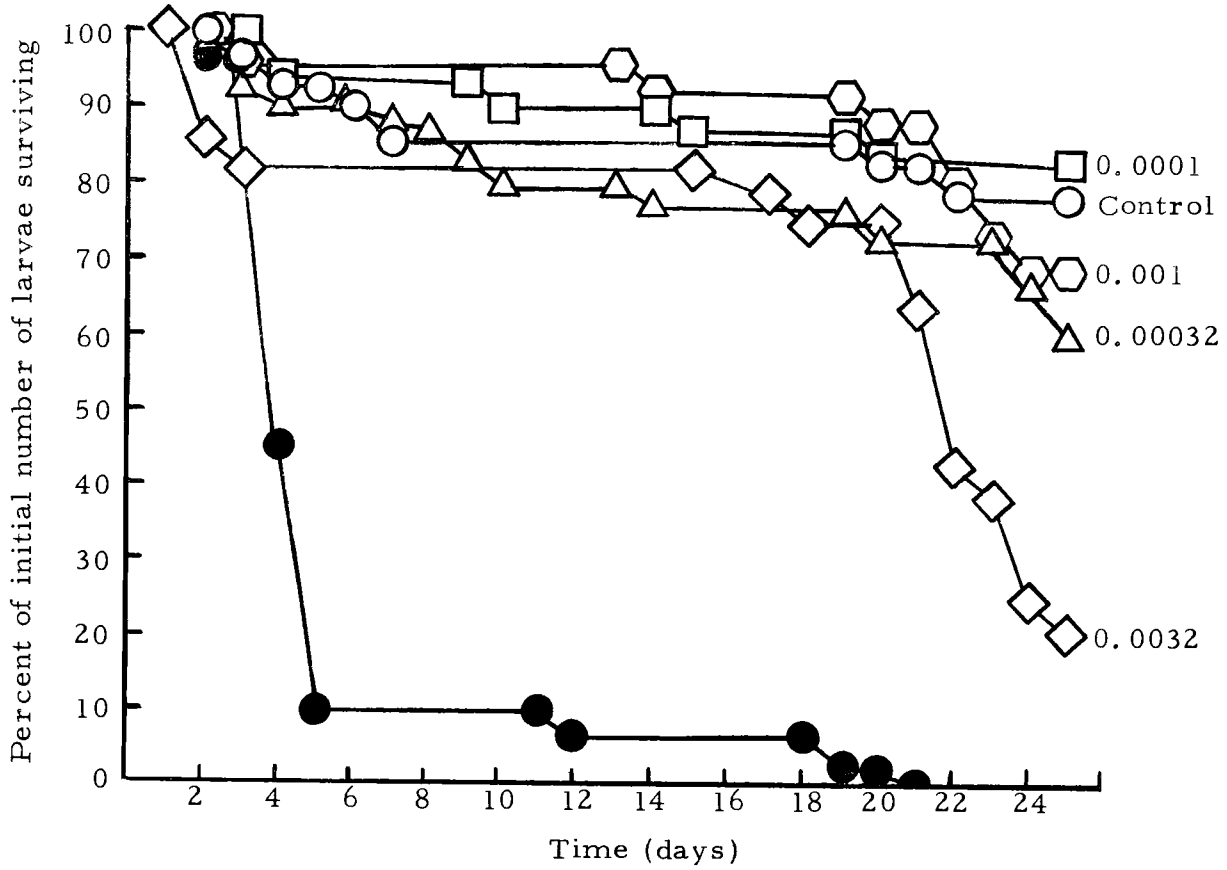

Figure 8. Effects of different Sevin concentrations on survival of first stage Dungeness crab zoeae. The larvae were exposed continuously to Sevin for 25 days. The number beside each curve is the toxicant concentration used.

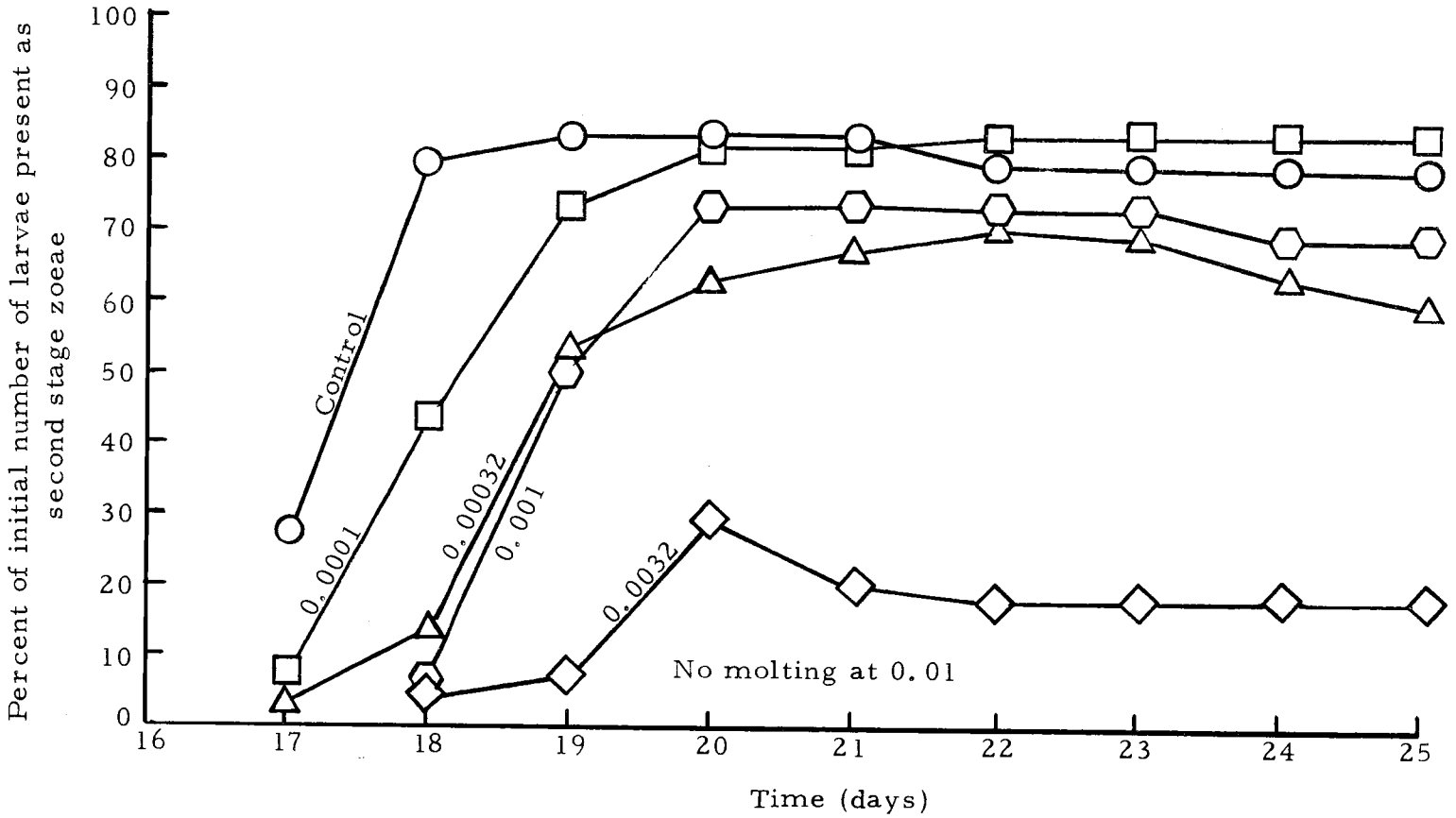

Figure 9. Effects of different Sevin concentrations on molting of first stage Dungeness crab zoeae. The larvae were exposed continuously to Sevin for 25 days. The number beside each curve is the toxicant concentration used.

0.0001 mg/liter and none at the three highest concentrations had molted and survived (Figure 9). 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 three of experiment 10, I 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. I 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 the control larvae in experiment 8, which also lasted 25 days and in which no infestation of the larvae was seen.

Juveniles

In experiment 11, crabs were exposed to different Sevin concentrations for 1, 24, or 48 hours and then held in clean sea water for three days. The one-hour EC_{50} for death or paralysis was 4.3 mg/liter at the end of the exposure period; but it decreased to 1.5 mg/liter three days after exposure. The 24-hour and 48-hour EC_{50}'s for death or paralysis were 0.076 and 0.057 mg/liter, respectively, at the end of the exposure period; they were the same three days after

exposure.

In experiment 12, crabs were exposed to sublethal concentrations of Sevin for 24 hours 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 unexposed crabs held together. Sevin did not affect molting or growth of crabs (Table 5). The mean percent 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. The mean percent 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 (Table 5). 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, I did not observe differences in aggressiveness or in burrowing ability between control and exposed crabs.

Experiment 13 was done to determine if exposed crabs from the preceding experiment on re-exposure to Sevin were more resistant

Table 5. Effect on the growth of juvenile Dungeness crabs exposed for 24 hours to sublethal concentrations of 0.01 and 0.032 mg/liter of Sevin.[1]

Vessel no.	Crabs per vessel		Concentration of Sevin (mg/liter)	Percent[2] survival	Mean carapace width (mm)		Mean percent increase in carapace width
	Sex	No.			Initial	Final	
1	male	5	0.0	100	15.4	29.0	88
	female	5	0.01	100	14.6	27.4	88
2	male	5	0.0	80	14.8	29.5	99
	female	5	0.01	80	14.2	27.1	91
3	male	5	0.01	100	14.0	27.2	94
	female	5	0.0	100	14.2	26.7	88
4	male	5	0.01	80	14.8	28.5	93
	female	5	0.0	60	14.5	27.3	88
5	male	5	0.0	80	14.1	29.2	107
	female	5	0.032	80	14.0	26.7	91
6	male	5	0.0	100	13.8	26.0	88
	female	5	0.032	100	14.1	26.3	86
7	male	5	0.032	80	14.5	27.6	90
	female	5	0.0	60	14.5	27.9	92
8	male	5	0.032	100	15.3	30.8	101
	female	5	0.0	100	14.5	27.7	91
9	male	5	0.0	100	14.9	29.6	99
	female	5	0.0	100	14.4	29.0	101
10	male	5	0.0	100	14.4	25.8	79
	female	5	0.0	80	14.3	27.6	93

(continued on next page)

Table 5. (Continued)

Vessel no.	Crabs per vessel		Concentration of Sevin (mg/liter)	Percent[2] survival	Mean carapace width (mm)		Mean percent increase in carapace width
	Sex	No.			Initial	Final	
11	male	5	0.01	60	14.7	27.7	88
	female	5	0.01	100	14.5	28.8	99
12	male	5	0.01	100	14.8	27.8	88
	female	5	0.01	80	15.0	29.6	97
13	male	5	0.032	40	14.3	30.4	113
	female	5	0.032	100	14.9	26.0	74
14	male	5	0.032	100	14.5	29.4	103
	female	5	0.032	80	15.0	28.9	93

[1] Crabs were exposed to Sevin in standing water and then transferred to clean flowing sea water and held for an additional 44 days.

[2] Survival of crabs after 44 days. There were no mortalities of crabs during the exposure period.

than crabs exposed only once to the chemical. I found no indication of resistance. The three 24-hour EC_{50}'s for crabs exposed once, and for those previously exposed to 0.01 and 0.032 mg/liter of Sevin and then re-exposed were 0.057, 0.15, and 0.057 mg/liter, respectively.

Experiments 14 and 15 were acute toxicity tests with older, probably ninth stage, juvenile crabs. For the two experiments, the 24-, 48-, and 96-hour EC_{50}'s were 0.35 and 0.62, 0.30 and 0.32, and 0.22 and 0.28 mg/liter, respectively.

Adults

Experiment 16 was a 96-hour acute toxicity test done at two temperatures, 11 and 18°C. I found that Sevin was more toxic to the crabs at the higher temperature (Figure 10). The 24-hour EC_{50}'s at 11 and 18°C were 0.49 and 0.32 mg/liter, and the 96-hour EC_{50}'s were 0.26 and 0.18 mg/liter, respectively.

Experiment 17 was a secondary poisoning test. Crabs were fed cockle clams that had been exposed to Sevin. They were observed for paralysis at six hours and again at the end of the 24-hour experiment. At six hours, 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 6). One additional crab, which had eaten clams exposed to the intermediate Sevin concentration, became paralyzed after more than six hours. Control crabs, which had eaten unexposed clams,

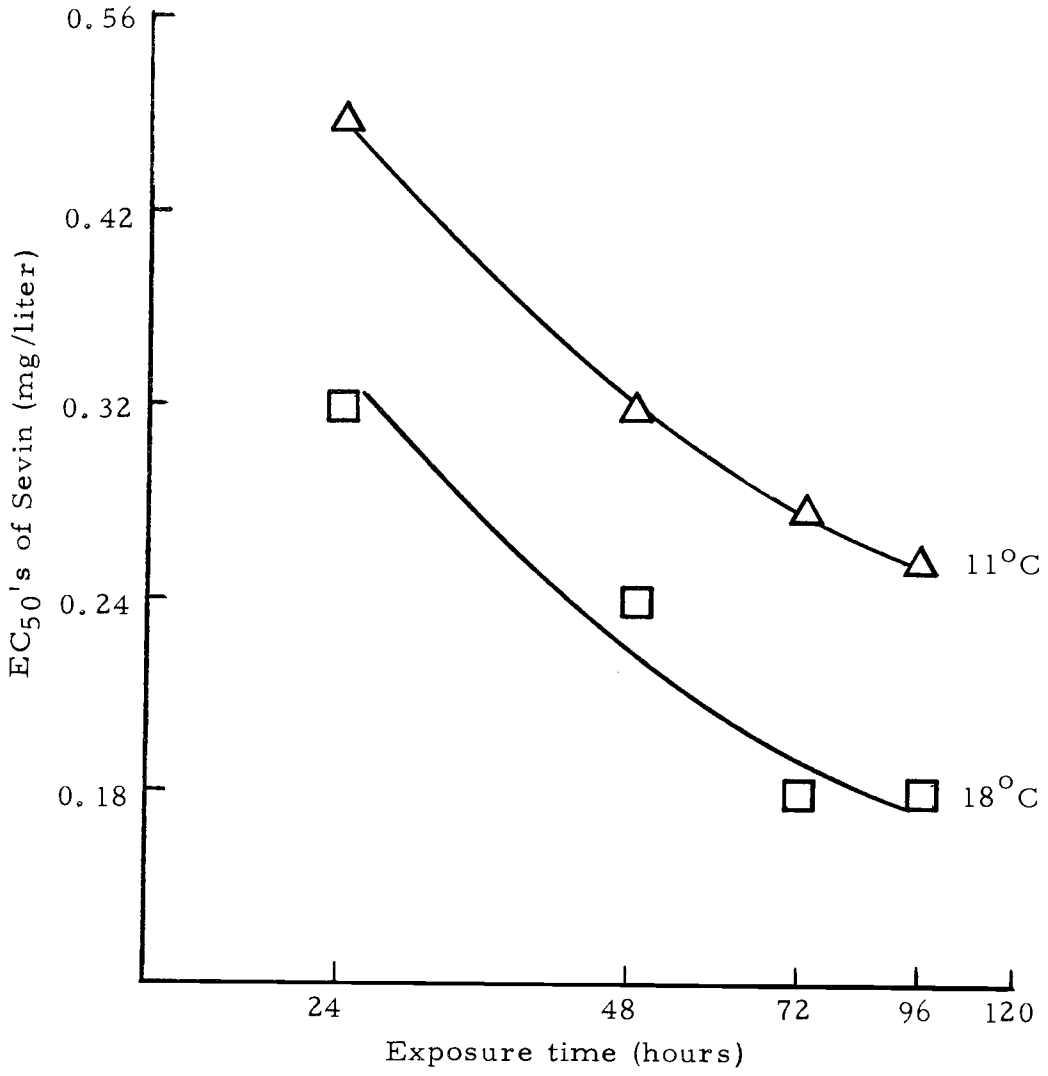

Figure 10. EC_{50}'s of Sevin for death or paralysis of adult female crabs at 11 and 18°C.

Table 6. Effects on adult Dungeness crabs of feeding on cockle clams previously exposed to Sevin.[1]

No. clams exposed	Sevin concentration (mg/liter) in water	% survival of clams[2]	No. crabs	Mean weight (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

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

[2] At the end of the 24-hour exposure period.

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 6).

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 7). 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 7).

Table 7. Concentrations of Sevin and 1-naphthol found in tissues of cockle clams and Dungeness crabs exposed for 24 hours 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)[1]	
			Sevin plus free 1-naphthol	Sevin alone
Clams	In water	1.0	3.45	--[2]
		3.2	13.38	8.60
		10.0	39.45	31.30
Crabs	In water	0.32	< 0.10	0.10
		1.0	--[2]	0.10 - 0.30
		3.2	0.94	0.91
Crabs	In food	1.0[3]	< 0.10	< 0.10
		3.2[3]	< 0.10	< 0.10
		10.0[3]	0.95	0.88

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

[2] Sample was lost during analysis.

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

DISCUSSION

Life History Studies

My 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 my 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 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. I found the same for C. magister prezoeae at low salinities, but at high salinities all of my 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 Bookout (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 Bookout (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 four to five 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 my 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.

Sevin Studies

We must know the effects of a pesticide on as many life stages of a species as possible before one 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 I 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 the first stage zoeae were killed in 96 hours when exposed to 0.01 mg/liter of Sevin at 10°C, whereas at nearly the same temperature of 11°C the 96-hour EC_{50} for adult crabs was 0.26 mg/liter.

Few zoeae were killed in 24 hours after exposure to a Sevin concentration as high as 82.0 mg/liter. The 24-hour EC_{50} for death within 15 days after the exposure was 0.015 mg/liter. These results clearly show delayed toxic effects resulting from a short-term exposure.

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

Long-term effects of Sevin on zoeae exposed continuously to sublethal concentrations of the pesticide were shown. Sixty percent 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 normally would have molted soon after day 20 (Figure 9), but at this time there was a marked decrease in survival (Figure 8). 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 hours 50% of second stage juveniles, ninth stage juveniles, and adults respectively. My 24-hour EC_{50}'s for ninth stage juveniles agree with those of Stewart et al. (1967), who reported 24-hour 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-hour 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 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 me 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 hours.

I 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 hours and then held in clean sea water for 44 days. In contrast with these results I found delayed toxicity to zoeae after their short-term exposure to Sevin.

I have shown that adult crabs can be killed within six hours after feeding on cockle clams previously exposed to Sevin. These results

may explain in part the findings of Snow and Stewart (1963). They observed many paralyzed cockle clams on an oyster ground in Oregon that had been treated 30 minutes 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. My 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).

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