THE EFFECTS OF PARALYTIC SHELLFISH POISON ON FISHES, PARTICULARLY THE STAGHORN SCULPIN, LEPTOCOTTUS ARMATUS

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THE EFFECTS OF PARALYTIC SHELLFISH POISON

ON FISHES, PARTICULARLY

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ABSTRACT

Four Euryhaline fishes, Leptocottus armatus, Pholis ornatus, Gasterosteus aculeatus and Platichthys stellatus exhibited symptoms of Paralytic Shellfish Poison intoxication and died when doses were administered by intraperitoneal injection. The LD_{50} of PSP as determined by intraperitoneal injection for L. armatus was 0.014 mouse/units/gram. The regression equation of the relationship between ln dosage and ln "death $time_{1/5}$" for intraperitoneal doses of PSP was obtained for L. armatus. L. armatus can tolerate oral doses of PSP at least 200 times greater than the intraperitoneal LD_{50}. Only three of forty L. armatus given oral doses died. Sensitivities of L. armatus and mice to PSP are approximately the same. L. armatus is possibly capable of retaining orally administered PSP in the body and toxic fishes were found to occur naturally. Toxic L. armatus produced experimentally contained almost 20 times the intraperitoneal LD_{50}. The levels of toxicity demonstrated in fish would probably not be harmful to fish predators.

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INTRODUCTION

The toxin which causes Paralytic Shellfish Poisoning on the American west coast is produced by dinoflagellates of the genus Gonyaulax, most commonly the species Gonyaulax catenella. The toxin may then be stored in the tissues of organisms that ingest the toxic dinoflagellates (Sommer et al., 1937). Mussels, clams, snails, scallops, cockles, oysters, chitons, starfish and sand crabs are some of the organisms known to transvect the toxin (Sommer and Meyer, 1937).

The effects of Gonyaulax toxin are well documented for mice and men as well as rats, monkeys, cats, rabbits, dogs, guinea pigs, and pigeons (McFarren et al., 1956). Symptoms include nervousness, ataxia, respiratory distress and paralysis which may lead to death. These animals, all terrestrial vertebrates, rarely contact the toxin in nature. There are, however, vertebrates both terrestrial and marine which may have occasion to ingest either the dinoflagellates themselves or other toxic organisms. Flatfishes often feed on clam siphons; herring and other clupeid fishes feed on plankton throughout their lives (Clemens and Wilby, 1949); diving ducks (subfamily Aythyinae), gulls and crows feed on mussels (Bent, 1921, 1925, 1946); otters, mink and bears living along the coast may also eat shellfish. The effects of the toxin on these and other species which may frequently ingest

the toxin have not been studied.

The term Paralytic Shellfish Poison (PSP) is also used to include toxins produced by other dinoflagellate types. Evans (1970) reported the death of many sea birds due to a toxin produced by Gonyaulax tamarensis, similar to that of Gonyaulax catenella. In Florida, Gymnodinium breve produces mass mortalities among fish and a bioassay using Fundulus heteroclitus has been perfected (Galstoff, 1948). Shilo and Aschner (1953) developed a bioassay for Prymnesium parvum toxin using Gambusia. However, Burke et al. (1960) state that, "Blooms of G. catenella, although fatal to man through the shellfish chain, have never been reported as the direct cause of fish kills."

The purpose of this study was to determine the effects of Paralytic Shellfish Poison on a fish which may contact it in nature. Behavioral responses to oral, intramuscular and intraperitoneal dosages were observed for four species of fish. The LD_{50} and dosage vs. death time relationships of PSP when administered to the sculpin Leptocottus armatus were studied. Finally, an attempt was made to determine what percentage of an oral dose of the toxin would be retained in the fish after digestion. The probability of accumulation of the toxin with repeated oral dosages was investigated.

The sculpin, L. armatus (family Cottidae), was the primary fish used in this study. It was chosen because

large numbers of fish weighing 3 to 20 grams were readily obtainable. Furthermore, they are easy to keep alive, and are bottom feeders which have been known to feed on clams and clam siphons (R. L. Smith, unpublished). Other fishes studied were saddled blennies (Pholis ornatus), sticklebacks (Gasterosteus aculeatus) and starry flounders (Platichthys stellatus).

METHODS AND MATERIALS

Collection and Maintenance of Fishes

Fishes were obtained by seining in a large tidepool at the mouth of Fish Creek on Douglas Island near Juneau, Alaska. This pool is situated relatively high up the beach at the upper border of *Fucus* growth. The bottom of the pool is muddy and houses large numbers of small clams, some mussels and the isopods which were found to be the predominant food item in the stomachs of those sculpins examined. Water in the collecting area was brackish, though the pool was in contact with salt water only at high tide. Although the fish used in this study rarely exceeded 15 grams, specimens of *L. armatus* measuring up to 40 cm and weighing more than 500 g are often caught by fishermen in deeper, more saline water. The collecting area seemed to be a nursery area for these fish, as fish weighing 3 to 4 grams which were common in May were uncommon by late August; the average size then being 8 to 12 grams. This is in agreement with Jones (1962).

Fishes were maintained in seawater aquaria at the Douglas Marine Station at room temperature (17° C). Air was bubbled continuously into the three 20 gallon aquaria used. When given oral doses of the toxin and in determining a dose-response curve for these fish, individuals were kept in disposable plastic mouse cages in about 1 liter of

unaerated seawater for periods up to 90 hours. Fish placed in plastic mouse cages as above were never observed to be in distress due to anything other than the toxin administered.

Toxin Samples and Extraction

The method used for assay of Paralytic Shellfish Poison is a bioassay in which the amount of toxin present is determined from the length of time required for an intraperitoneal injection of an acid extract of shellfish tissue to kill a mouse of a standard weight. The amount which kills a 20 g mouse in 15 minutes is defined as one "mouse unit". 0.2 ug of purified PSP equals approximately one mouse unit (Halstead, 1965).

PSP was obtained by extraction of butter clam (Saxidomus giganteus) siphons from North Porpoise Island in Icy Straits in Southeast Alaska (58° 20' N, 135° 28' W). Of 123 siphons examined, 8 showed signs of predation; their tips had been removed. One neck showed severe lacerations. Clam siphons were homogenized with 0.1N HCl in a blender with heating element (Norelco brand). One ml of acid was used per gram of clam meat. Once ground, the mixture was boiled and blended for five minutes. It was then allowed to cool to room temperature and the pH adjusted to 3.5 using 5N HCl and pH paper (pHydrion papers, Micro Essential Laboratory, Brooklyn, N. Y.) as an indicator. The mixture was centrifuged until a clear supernatant was obtained. The supernatant was decanted off and the mouse bioassay as described in Halstead (1965) was used in evaluating

the toxicity of these and similar extracts. PSP toxin extracts of this variety are reported to be stable for up to one year (Schantz et al., 1958).

In extracting toxin from whole fish the volume of acid used was determined primarily by the amount necessary to cover the blender blades and insure uniform blending. Therefore, 30 g of whole fish might require the addition of 60 ml of 0.1N HCl. Since at this dilution the small amount of toxin present could not be detected by the mouse bioassay, the supernatant obtained after centrifugation was concentrated to 0.05 - 0.10 of its original volume by boiling and evaporation. This concentrate was then injected into mice as above.

Injection Technique and Behavioral Observations

Intraperitoneal injection was chosen as preferable to intramuscular injection because large volumes (up to 1 ml in a 3 g fish) could be administered without causing severe tissue damage. Volumes of 0.1 ml could not be readily injected into the muscle of a 5 g fish without causing blisters under the skin. In addition, both intraperitoneal and intramuscular methods seemed to produce toxic effects in equal times. Injections were administered with a 27 gauge x 1/2 inch needle on a 1 cc disposable tuberculin syringe.

In the preliminary stages of the study and at various times subsequently, observations were made of the behavior

of fishes injected or force-fed with toxin. Any change in behavior was then noted as was the time of the occurrence of the new behavior. In this way, the sequence of symptoms of PSP intoxication was obtained for four species. Observations of this type were made singly, fish being placed in a 15 gallon aquarium 1/3 full of aerated seawater.

LD_{50} - Leptocottus armatus

The LD_{50}, or dosage level which kills 50% of all fish injected with an equal dosage of PSP, was determined for Leptocottus armatus.

Fish were injected in groups of 9 to 36 at a time with 0.1 ml intraperitoneal injections of 0.09 mouse units of paralytic shellfish poison each. Fish varied in size from 3 to 12 grams. Thus dosage and volume injected/gram varied according to the size of the fish tested. After two hours time, weights of live and dead fish were taken. Dosage vs. per cent survival was plotted by dividing the dosage range into small segments and plotting per cent survival for each segment. The LD_{50} was determined from the equation for the regression line of the natural logarithm of dosage vs. per cent survival. As a control, 0.2 ml of 0.1N HCl was injected into each of ten fish. Volumes of 0.2 ml were chosen in order to indicate what effect fluid volume played in the results obtained. Weights of the controls were taken two hours after injection.

Dose-Response Curve - *Leptocottus armatus*

Results of work on LD_{50} determination for *L. armatus* indicated that sublethal doses of PSP would be less than 0.02 mouse units/gram. An accurate mouse assay requires at least 3 ml (enough for three mice) of an extract with a toxicity greater than 1.0 mouse units/ml (Halstead, 1965). Therefore, any attempt to measure the amount of toxin recovered from fish given sublethal doses (after the fish have destroyed, assimilated or eliminated the toxin) could end in failure unless a more sensitive assay than the mouse bioassay were used. With this in mind, the relationship of dosage versus death time was investigated for *L. armatus* to determine if it would provide that sensitivity. In addition, a volume of 0.1 ml would be sufficient for each fish injected whereas a 1.0 ml sample is necessary for each mouse injected.

A dose-response curve was established by administering a series of dilutions of a clam siphon extract (0.1 ml volumes) to fish of varying weights (2.0 to 9.2 g). Dosages ranged from 0.01 to 0.22 mouse units/gram. Distribution of fish weights used in bioassay determination is given in Table 1. The end point used in the mouse assay of paralytic shellfish poison is death time. However, death times determined by observation of the external appearance of the fish gave end points which varied by more than 5 minutes at the same dosage. More consistent results were

TABLE 1. Weight Distribution of Fish Used to Determine Dosage vs. Death Time$_{1/5}$ Relationship for *L. armatus*

Fish Weight (Grams)	Number
2.5 - 3.4	3
3.5 - 4.4	11
4.5 - 5.4	12
5.5 - 6.4	22
6.5 - 7.4	22
7.5 - 8.4	13
8.5 - 9.4	8

Total Number of Fish = 91

Mean Weight of Fish = 6.3 grams

Standard Deviation (wt.) = .67 grams

obtained by using the time when the rate of opercular ventilation movements had dropped to one in five seconds as the end point. Death followed soon after this decrease in opercular contraction rate. The time when the rate of opercular ventilation movements dropped below one per five seconds was called "death $time_{1/5}$." Controls were the same as those used in LD_{50} determination.

Force Feeding PSP and Extraction of L. armatus

In order to give the fish measured oral dosages of the toxin, they were force-fed a known volume of a toxin extract and flour paste. A shellfish extract of known toxicity was mixed with flour in a tared beaker. The amount of toxin extract in the mixture was known and the weights of beaker plus mixture and average weight of 0.1 ml of the mixture were found. It was then possible to calculate how many mouse units of toxin were contained in the mixture per volume. The mixture was then force fed with a 1 cc syringe via a polyethylene tube. The tube was passed through the mouth down the throat and into the stomach of the fish. Volumes force-fed in this manner ranged from 0.1 to 0.5 ml depending upon the size of the fish. In order to determine what effects flour had on the paralytic shellfish poison, a toxin extract plus flour mixture and a quantity of the same extract used to make the mixture were stored in a refrigerator for 72 hours. The mixture was then centrifuged and the supernatant assayed.

The results of this assay were then compared to the results of an assay of the portion of the toxin extract (used to make the mixture) that was set aside at the beginning of the experiment.

In order to determine what percentage of the toxin force-fed to fish could be recovered by extraction from whole fish, groups of fish were force-fed and the extraction procedure carried out immediately thereafter.

To find what percentage of the oral dosage was present in the fish after the toxin-flour mixture had been eliminated, groups of 3 or 4 fish were placed in individual containers after force feeding and extracted after the flour became evident in their feces.

An attempt was also made to demonstrate the possibility of accumulation of toxin residues in fish that were force-fed the toxin-flour mixture repeatedly. In this case, groups of 3 or 4 fish were force-fed and placed in individual containers as above. When the flour became visible in their excreta, the fish were force fed a second time. One of the two groups given repeated oral doses was force fed a third time. Extracts of the fish in each group were made after flour from the last portion fed had been eliminated.

Fish extracts were assayed by routine mouse assay except that in some instances only 2 rather than the 3

mice normally required were used due to the small volume of the extract sample.

As a control, fish that had received no toxin were extracted in the same manner as those used in the feeding experiments. The extract was evaporated by boiling to one-tenth its original volume and a mouse assay was performed.

RESULTS

Behavioral Observations

Leptocottus armatus

When given a lethal intraperitoneal dosage of Paralytic Shellfish Poison, the symptoms of intoxication follow the general sequence below. Times given are approximate and are included only to give some idea of the chronology of symptoms. Since dosage determines how rapidly death occurs, this sequence represents what would happen if a 4 to 5 gram fish were given a dosage of about 0.02 mouse units/gram.

1 Minute: Opercular ventilation movements are deep and regular, fish lies on the bottom of the aquarium.

2 Minutes: Ventilation movements ("breathing") become more shallow and irregular.

2.5 Minutes: Ventilation movements the same with a few gasping "breaths."

3.5 Minutes: Gasping with lifting of head or with lifting of head and tail in some cases.

4 - 6 Minutes: Rapid uncoordinated swimming; weak opercular movements, irregular and decreasing in frequency.

7 Minutes: Violent writhing and swimming movements. Fish often spiralling through the water.

8 Minutes: Fish may come to lie on its back, opercular movements decrease ("death time$_{1/5}$") and finally cease altogether.

9 - 10 Minutes: Fish dies, opercular spines may be elevated in death.

Gasterosteus aculeatus

Four sticklebacks were observed for their reactions to intraperitoneal doses of Paralytic Shellfish Poison. The general sequence of symptoms is as follows:

1. Pectoral fins cease fanning.
2. Erratic movement.
3. Fish lie on side, either floating or on the bottom.
4. Infrequent spasmodic body movement.
5. Spasmodic twitching of jaw, cheek, and opercular muscles; erection or dorsal spines.
6. Reddening of throat, cheek and jaw area, in males only.
7. Three of the four died floating with head downward; the fourth came to lie on the bottom.

Two starry flounders (Platichthys stellatus) and one blenny (Pholis ornatus, family Pholidae) showed roughly the same symptoms as Leptocottus when given intraperitoneal doses of PSP.

Two starry flounders that were given oral doses of PSP showed no symptoms of PSP poisoning. The same is true of

all sculpins tested except three which died overnight while not under observation.

In general, the symptoms of Paralytic Shellfish Poisoning in fish are equivalent to their mammalian counterparts in mice; i.e., fish die in convulsions from respiratory failure.

LD_{50} - Leptocottus armatus

In order to determine the LD_{50} for L. armatus, 133 fish were injected with 0.1 ml volumes of toxin extract each containing 0.093 mouse units of toxin. Fish weights ranged from 2.9 to 11.4 grams. Dosages ranged from 0.0079 mouse units/gram to 0.0310 mouse units/gram.

The regression equation of $\log_e$ (ln) dosage versus per cent survival was obtained by dividing the dosage range into 0.0029 mouse unit segments. Twenty-three of these dosage intervals were used. They overlapped so that the midpoint of any one interval was the same as the end points of the intervals above and below it. The equation for the $\log_e$ dosage versus per cent survival regression line was then determined by the method of least squares from the natural logarithm of the average dosage for each interval and the per cent survival for that interval. The equation is:

$$Y = -230.86 - 65.78 \ln X$$

where Y = % survival

X = dosage (mouse units/gram)

The correlation coefficient for per cent survival and lnX (Pearson r) is 0.95. This is highly significant at $P < .001$ with 21 degrees of freedom.

The LD_{50} calculated from the above equation is 0.014 mouse units/gram. Figure 1 shows the graph of the regression line and its relation to the data points.

As a control, ten fish ranging in size from 6.5 to 7.7 grams were injected with 0.2 ml of 0.1N HCl. All survived with no toxic symptoms for 2 hours at which time the control experiment was terminated.

Dose-Response Curve - L. armatus

Of the 100 fish injected to obtain the data which generated the curve shown in Figure 2, only 12 survived. A ln dosage versus ln death $time_{1/5}$ regression was computed by the method of least squares. The equation of the regression line is:

$$\ln Y = 4.30 - .575 \ln X$$

where Y = Death $Time_{1/5}$

X = Dosage (mouse units/gram)

Death time is expressed in seconds and dosage in mouse units/gram. The correlation coefficient (Pearson r) for ln death $time_{1/5}$ and ln dosage is 0.77, which is highly significant, $P < .001$. Controls for this determination are the same as those for the LD_{50}.

Force Feeding and Extraction of L. armatus

In force feeding toxin to fish, a flour and toxin

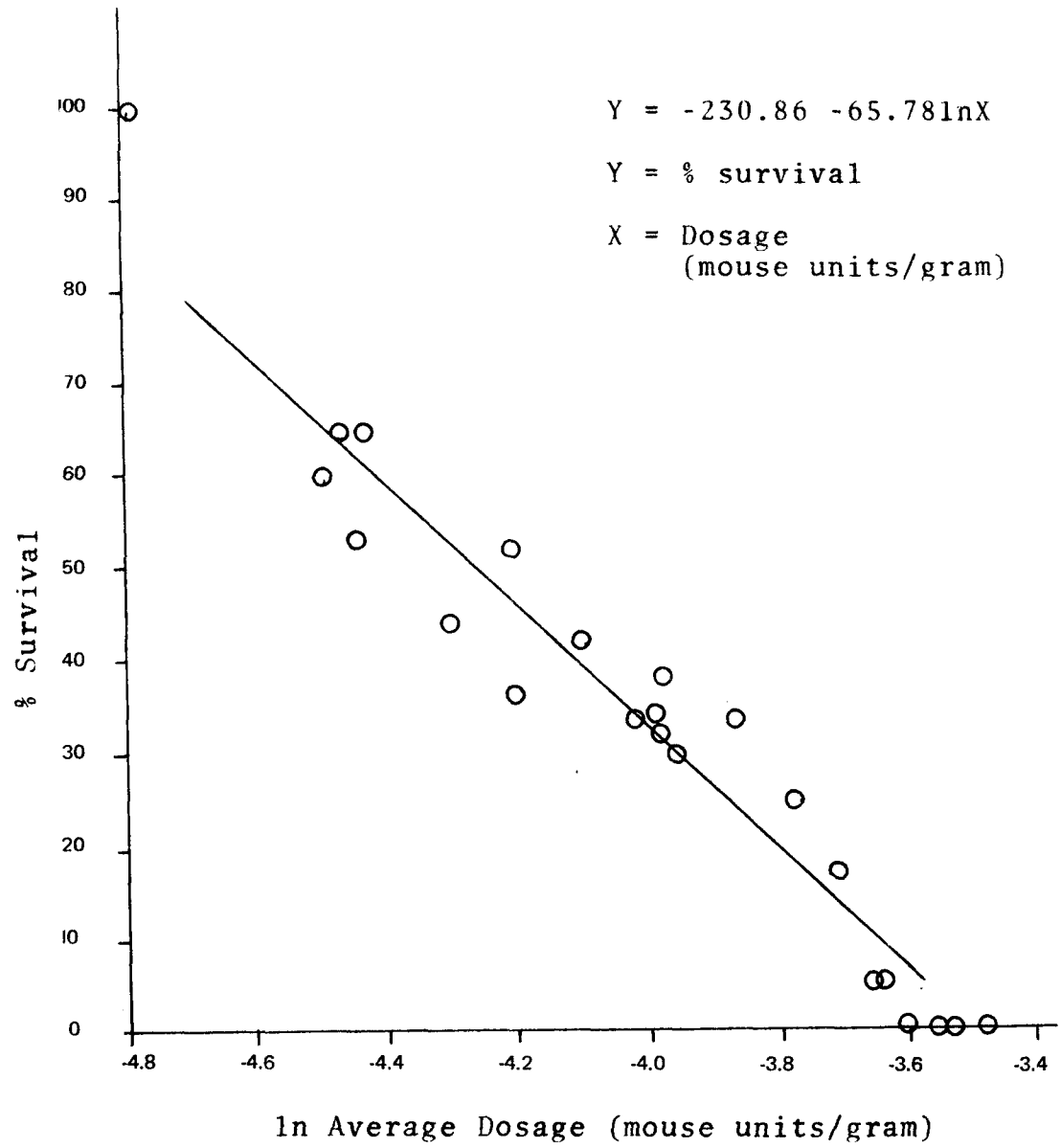

FIGURE 1. Relationship between per cent survival and ln average dosage for groups of Leptocottus armatus. $r = .881$; $p < .001$

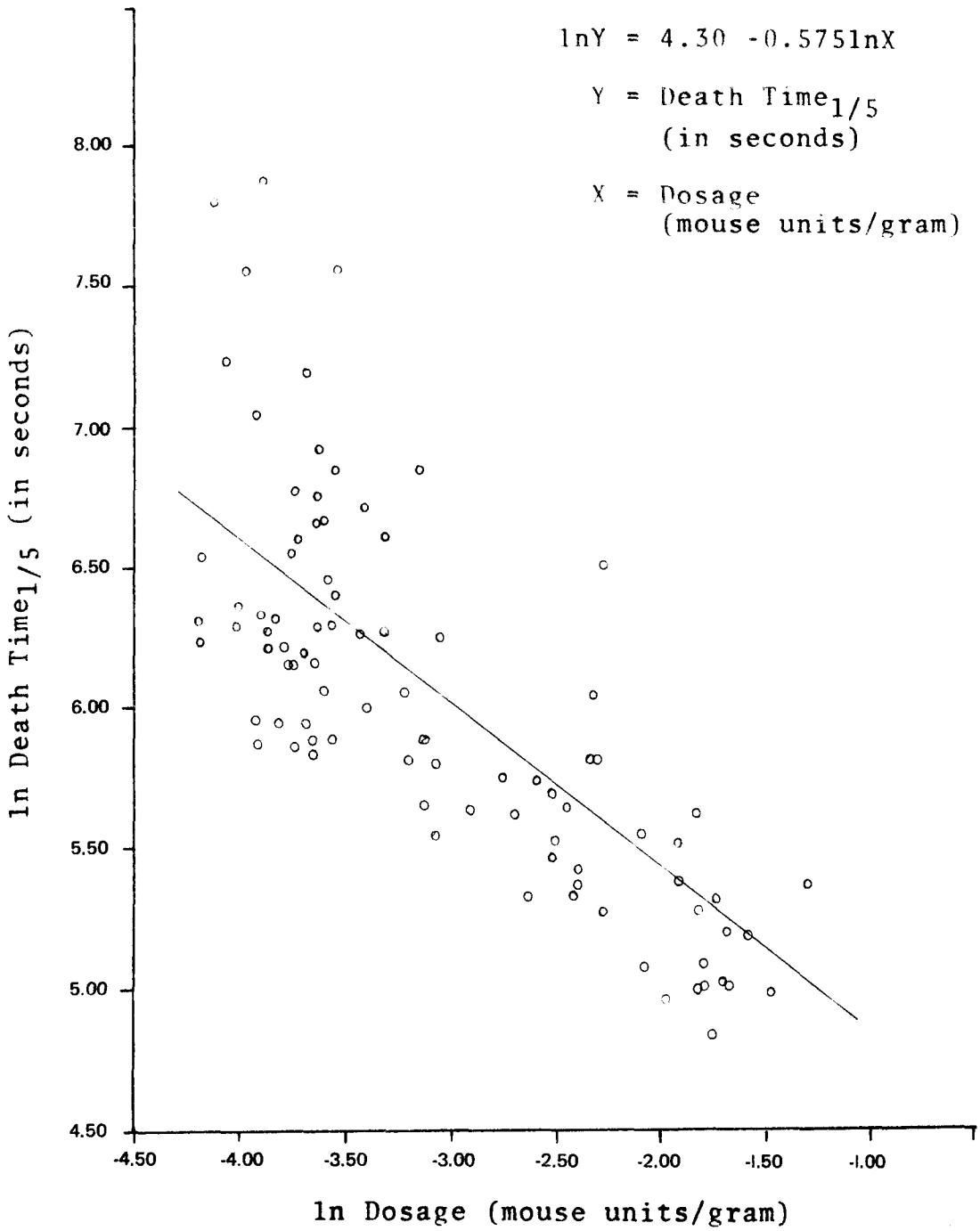

FIGURE 2. Relationship between ln Dosage and ln Death $Time_{1/5}$ for Leptocottus armatus. $r = .770; p<0.001$.

mixture was used. The results of an assay of a 3-day-old toxin-flour mixture were the same as those of an assay of the toxin extract used to make the mixture which was aged for the same period. It was therefore concluded that flour has no effect on the toxicity of such a mixture.

Since whole fish extracts were used to recover the toxin from fish that had been given oral dosages, an extract of fish which had been given no toxin was prepared, concentrated to 1/10 volume, and assayed using mice. This control extract proved to be lethal to mice and showed that the sculpins used had a toxicity comparable to 0.24 mouse units/gram.

Feeding and Immediate Extraction:

Three groups of fish were force fed a toxin-flour mixture, their weights were taken and an extract of each group was made immediately (see Table 2, extracts 3, 4, and 5). In extracts 3 and 4 the percentage of the original dosage recovered by extraction was greater than 100% (approx. 120%). This "excess toxicity" can be attributed to toxin present in the fish themselves before force feeding as shown by the control group. If we assume that the fish in groups 3 and 4 (extract number and group number are the same) showed the same basal level of toxicity as the controls (0.24 mouse units/gram), we would expect the toxicity of the extracts to be 160% to 200% of the amount administered. However, the average excess toxicity of groups 3

TABLE 2. Results of Force Feeding and Subsequent Recovery of PSP from L. armatus

Group or Extract Number	Mouse Units Toxin Fed	Mouse Units Toxin Recovered	Per Cent Recovery of Toxin Fed	Mouse Units PSP Expected from Toxicity of Control Extract	Total Wt. of Fish in Group	Mouse Units Recovered in Excess of Amt. Fed/Gram Fish
Control	0.0	22.8	---	---	142.8	.24
3 [a]	8.3	9.6	116	6.2	26.0	.05
4 [a]	19.6	24.1	123	11.5	48.0	.09
5 [a]	37.8	26.5	70	7.4	30.5	---
6 [b]	37.8	16.7	44	6.2	26.4	---
7 [b]	99.0	10.7	11	7.9	33.0	---
8 [c]	108.0	21.9	20	11.3	46.5	---
9 [d]	35.2*	14.3	40	7.2	30.3	---
10 [d]	23.2*	7.3	31	6.5	27.0	---

a) Force Fed and Immediately Extracted

b) Force Fed Once

c) Force Fed Once, Fish Dead

d) Multiple Feedings

* Average

and 4 is only 0.07 mouse units/gram rather than the 0.24 mouse units/gram indicated by the control.

The toxicity expected of each group based on the weight of fish used and the assay of the control group was calculated and is given in Table 2. Immediate extracts 3 and 4 had toxicities in excess of the amount administered. The "excess toxicity" per gram of fish as well as total weight of fish in each group extracted is also given in Table 2.

Extraction After One Feeding:

Two groups of fish were weighed and force fed measured amounts of toxin. One group (No. 6, Table 2) was placed in individual containers as mentioned earlier; the other was placed in a refrigerated (10°C) aerated seawater system (Instant Ocean). When flour became evident in the feces, extracts were made of the fish in both groups. Three of the five fish kept in the refrigerated seawater system died before extraction. This group was divided by extracting dead (No. 8, Table 2) and survivors (No. 7, Table 2) separately. Upon dissection of the dead fish, flour was seen in their upper intestines, stomach, and caeca. When survivors were examined, none of the flour was evident in their digestive tracts. The three fish from which extract No. 8 was made were the only fish that died during the force feeding experiments. They also received the highest dosages except for those in group 7. The dosages of groups 7 and 8

ranged from 2.5 mouse units/gram to 3.1 mouse units/gram; fish weighed from 13 to 18 g. Fish in group 7 weighed 17 and 15 grams and received 3.2 and 2.8 mouse units/gram respectively. Fish in group 8 died before extraction and the extract showed 20% toxin recovery as compared to 11% for group 7 which lived. This was expected, since some of the toxin-flour mixture remained in the guts of the dead fish. However, of the 108.0 mouse units of toxin administered to group 8, only 21.95 remained. This indicates that even though the mixture fed these fish had not passed through the gut, at least 80% of the toxin was lost in the 12 hours of the experimental period. Extracts of the two groups of live fish extracted after the mixture had been eliminated, Numbers 6 and 7, showed 44% and 11% recovery respectively. Again, if we assume the toxicity of the fish themselves to be no more than demonstrated by the control, only 6.24 mouse units of the 16.65 mouse units present in Extract 6 can be accounted for by this factor (Table 2). In extract 7 which showed 11% recovery, there are only 2.8 mouse units of toxin which cannot be attributed to toxicity of fish tissue used to make the extract. The difference is probably not significant.

Extraction After Repeated Feedings:

Repeated oral dosages of toxin were given to groups 9 and 10 (Table 2). The percentage recovery of the average dose administered was 40% for extract 9 and 31% for extract

10. Of the two extracts of groups that were repeatedly force fed, only extract 9 showed a greater amount of toxin recovered than would be expected from the weight of the fish and the toxicity demonstrated by the control group. In this instance the amount recovered is twice that expected (Table 2).

CONCLUSIONS

Sensitivity of L. armatus to PSP

Only three of the forty fish used died during the course of force feeding experiments (Group 8, Table 2) and in these cases the dosage averaged 2.8 mouse units/gram. Two other fish (Group 7, Table 2), given dosages of 3.0 mouse units/gram, lived. Since the intraperitoneal LD_{50} is 0.014 mouse units/gram, it appears that sculpins can tolerate 200 times this dosage orally, and that this is approximately the oral LD_{50}. If this is the case, the tolerances of sculpins and mice for PSP are very similar. Mice have an oral LD_{50} of 2.1 mouse units/gram (McFarren et al., 1956).

Dose-Response Curve

The dose-response curve (Figure 2) obtained for Leptocottus armatus was not utilized as a bioassay mechanism to indicate dosage from time of death. Fish death times of 3 to 8 minutes probably would be the most accurate indicators of dosage, since the dosage versus death $time_{1/5}$ relationship is most linear in that range (Figure 3). Since this represents a dosage range of about 0.02 to 0.07 mouse units/gram, if 6 gram fish were used for assay, toxicities of from 0.12 to 0.42 mouse units could be determined. This is approximately the same useful range as that of the mouse assay (Halstead, 1965) though, as mentioned previously, smaller volumes are involved.

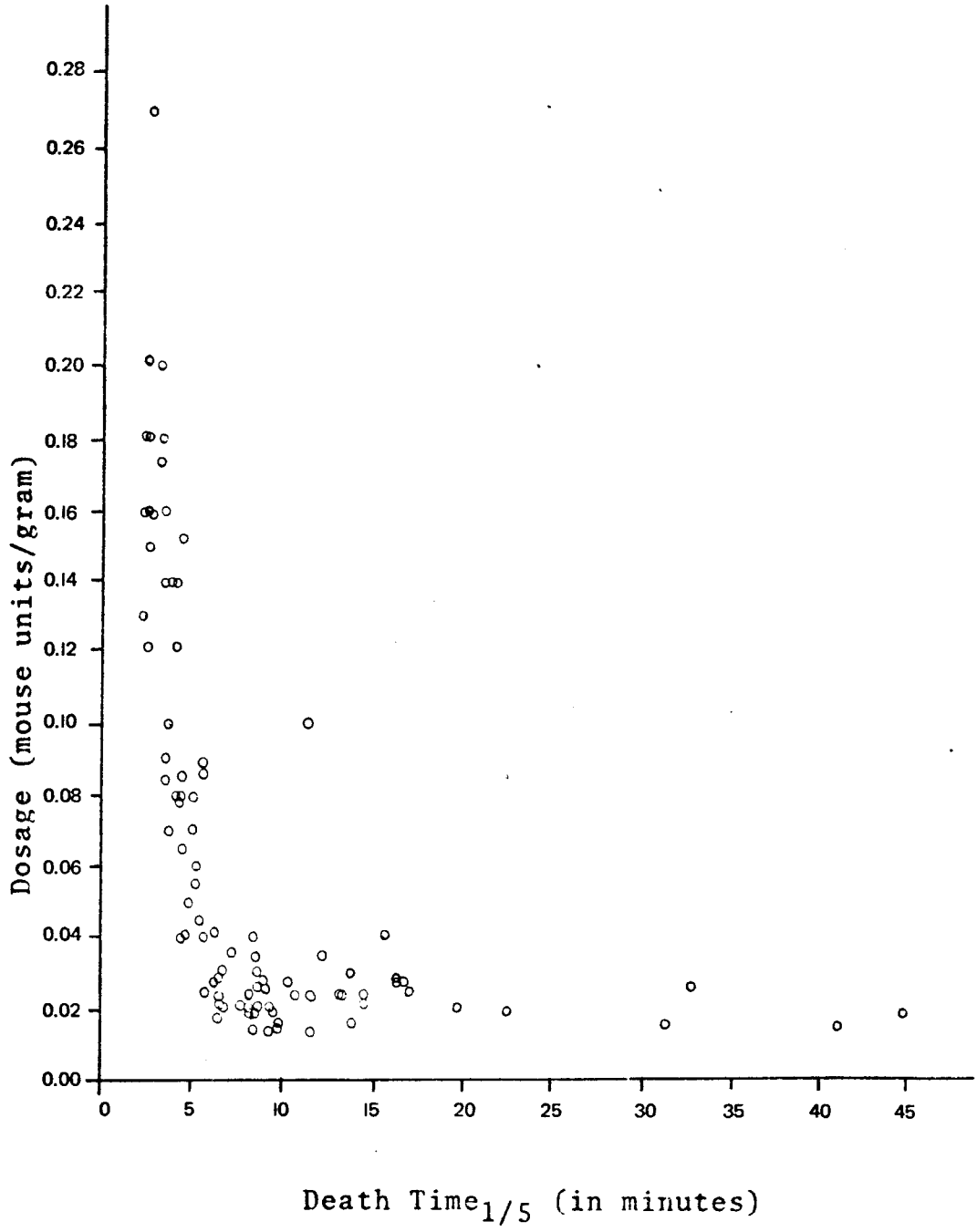

FIGURE 3. Relationship between Dosage and Death $Time_{1/5}$ for Leptocottus armatus

Force Feeding and Extraction - *Leptocottus armatus*

The control extract for the force feeding experiments (Table 2) had a toxicity of 0.24 mouse units/gram. If we assume that all fish used in the study had the same toxicity prior to force feeding, then we would expect the toxicity of fish extracted immediately after force feeding of toxin to be higher than it actually was (Table 2 and p.26). The results therefore indicate that toxicity per gram of fish tissue for each group extracted was not the same.

Of the four groups (6, 7, 8 and 9, Table 2) that were force fed either once or repeatedly and allowed to eliminate the mixture before extraction, all four showed toxicities higher than would be expected due to the weight of fish tissue used to make the extracts and the toxicity of the control group. This indicates that some of the toxin is retained in the fish tissue. Another possibility is that the fish used in these experiments were twice as toxic as the control group to begin with.

The unexpected toxicity of the fish themselves may be due to a variety of factors acting either singly or in combination. Obviously, the fish may truly contain PSP. This substance is one of the most potent of the non-protein toxins (Shantz, p. 18, Alaska Dept. of Health and Welfare, 1965) and produces definite symptoms of convulsions and death in mice. In the mouse assay of the fish extracts in question, the mice died with definite symptoms of PSP

intoxication in 3 to 5 minutes. It is possible that either PSP or a similar substance is formed from fish tissue during the extraction procedure (Halstead, 1965). The gut contents of the fish used may have been toxic. The best explanation at this time, and the most exciting, is that the fish actually do contain Paralytic Shellfish Poison which they either manufacture themselves or acquire through the food chain. Suggestions for further study are:

1. Extracts of fish that could not have come in contact with PSP (perhaps freshwater species) should be made and assayed to determine if the toxicity shown by the fish used in this study is merely an artifact of the extraction procedure.

2. Additional force feeding experiments should be conducted in order to further substantiate these preliminary findings.

3. Various parts of toxic fish should be assayed to determine where the toxin is located in the fish.

4. Collection and assay of L. armatus as well as other demersal fishes (particularly those from areas where toxic shellfish are found) could better indicate the range of toxicities found in fishes in nature.

PSP and Fishes in Nature

Any conclusions based on the force feeding experiments are necessarily tenuous due to the toxicity of fish which received no toxin (Table 2, control group) and the small

number of samples. However, even though Leptocottus armatus, Pholis ornatus, Gasterosteus aculeatus and Platichthys stellatus showed signs of PSP intoxication and died when toxin was administered intraperitoneally, L. armatus was shown to be capable to retaining at least part of an oral dose. In fact, toxicity of the control group for the force feeding experiments (0.24 mouse units/gram) was twenty times the LD_{50} as determined by intraperitoneal injection (0.014 mouse units/gram). Toxicity shown by the extract of the control group also indicates that L. armatus becomes toxic in nature.

Results of the force feeding experiments therefore indicate that PSP is retained at the third trophic level. The effects of toxic fishes upon the fourth trophic level is of course dependent upon the size of the predator, number of fish eaten, tolerance of the predator to PSP, and whether or not toxic portions of the fish are eaten.

The most toxic fish produced experimentally contained approximately 0.62 mouse units/gram (Extract 6, Table 2). It is doubtful that such levels of toxicity would be hazardous to fish predators. However, since the intraperitoneal LD_{50} is 0.014 mouse units/gram, it might well be asked why this amount of toxin is not hazardous to the fish. The toxin is probably not circulating freely in the blood but is bound or stored somewhere in the fish; possibly in a slightly altered, nontoxic form. Passage of

toxin from the digestive tract into the body may occur slowly enough (or alteration of the toxin quickly enough) so that lethal concentrations are not present in the blood unless large amounts of toxin are ingested.

SUMMARY

1. Leptocottus armatus, Pholis ornatus, Platichthys stellatus and Gasterosteus aculeatus showed symptoms of convulsion and paralysis of the respiratory musculature when PSP was administered by intraperitoneal injection.
2. The LD_{50} of PSP for L. armatus was 0.014 mouse units/gram when toxin was administered intraperitoneally.
3. The regression equation of the relationship between ln dosage and ln "death $time_{1/5}$" for intraperitoneal doses of PSP was obtained for L. armatus.
4. L. armatus can tolerate oral doses of PSP at least 200 times greater than the intraperitoneal LD_{50}.
5. Three L. armatus died from oral dosages of PSP that averaged 2.8 mouse units/gram. The other 37 fish that were force fed the toxin showed no ill effects.
6. Sculpins and mice exhibit approximately the same sensitivity to PSP.
7. It appears that L. armatus can retain orally administered PSP after digestion and that toxic L. armatus may occur naturally.
8. The most toxic fish produced experimentally contained approximately 0.62 mouse units/gram. Since the intraperitoneal LD_{50} is 0.014 mouse units/gram, PSP in fish with toxicities of 0.62 mouse units/gram is probably stored or bound possibly in a slightly altered and

nontoxic form.

9. Though toxic fish might be eaten, such levels of toxicity probably would not prove harmful; it would depend upon the size of the predator and its feeding habits.

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