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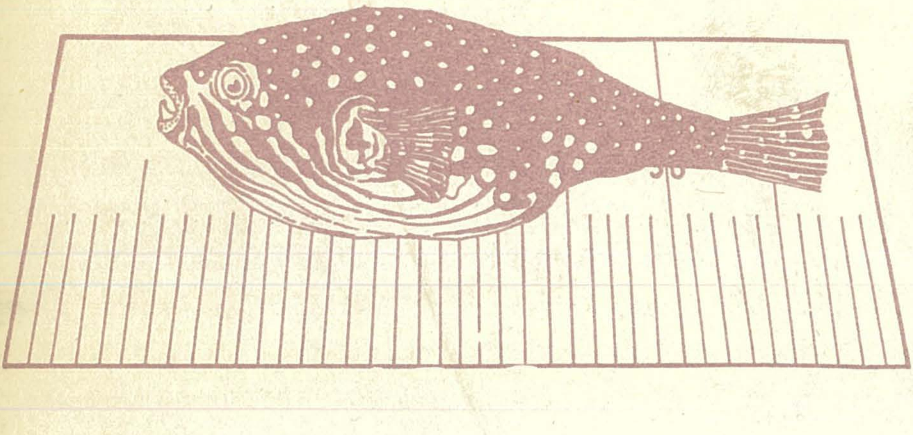
HANDBOOK ON SOME TOXIC MARINE ORGANISMS AND LOW RISK FISHERY PRODUCTS



by

Lourdes M. Arafiles

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FOREWORD

The urgent need for a local reference on toxic marine organisms including fish and shellfish products has long been felt by the industry and by the fish-consuming public. The efforts exerted by the author, Ms. Lourdes Martin Arafiles, to prepare a simplified handbook giving benchmark information on toxins from fish and aquatic invertebrates are highly commendable.

The manual includes basic and applied information on low risk fishery products such as "burong talangka" (salted small crab), oysters and smoked fish. Methods of reducing risks of toxicity are also discussed in detail including those involving puffer fish, jellyfish, tuna-like species, shellfish and other marine organisms.

It is noteworthy that the translation into Pilipino makes it highly relevant to the needs of the fishermen and fish processors in the coastal communities.

As Director of the Institute of Fisheries Development and Research, I am elated by the fact that the prospect of completing this handbook, which is very timely, has become a reality through the dedication, initiative and exceptional diligence of Ms. Lourdes Martin-Arafiles.

FLORIAN MAGNO-OREJANA
Director, Institute of Fisheries Development and Research
University of the Philippines in the Visayas

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INTRODUCTION

Very large amounts of aquatic resources are found in Philippine waters. Hence, basic information regarding the wholesomeness and safety of these resources is of primary importance. This is significant because many consumers and fishermen have been victims of "fish poisoning" owing to a number of factors, which include the following:

a) lack of knowledge and basic information regarding the constituents of marine organisms;

b) environmental variations;

c) lack of awareness of the importance of hygiene and sanitation in the processing plants.

Cases of puffer fish poisoning, scombroid poisoning and bacterial intoxication have been reported in the daily newspapers. However, there are no statistical records of the incidence rates.

As far as the fish processing industry is concerned, the problem requires stringent measures to help minimize or prevent incidences of poisoning.

There is also an immediate need to make known the experimental findings to the end-users or consumers. This manual, therefore, aims to disseminate information regarding fish poisoning, based on various experiments covering several poisonous species of fish and fishery products.

This manual has the following objectives:

1. To make consumers and fishermen aware of toxic marine organisms;

2. To give information on the types of toxins present in fish and fishery products;

3. To disseminate information and give recommendations on proper handling of toxic marine species;

4. To warn consumers and processors of the risk of post-process contamination.

I. TOXIC MARINE ORGANISMS

1. Definition of Toxin

Toxin is defined as a poison formed as a specific secretion in the metabolism of plant or animal organisms (Webster, 1960). On the other hand, toxin is any substance that may have a lethal effect on human beings, animals and plants. The substance may be of protein and of non-protein origin, and be produced from the metabolic processes of micro-organisms, plants, and animals or it may be a synthetic chemical substance (see Table 1).

2. Types of Toxin

Toxins can be classified into:

a) chemical toxins (e.g. mercury)

b) bacterial toxins (e.g. botulism)

c) animal toxins (e.g. crotalus)

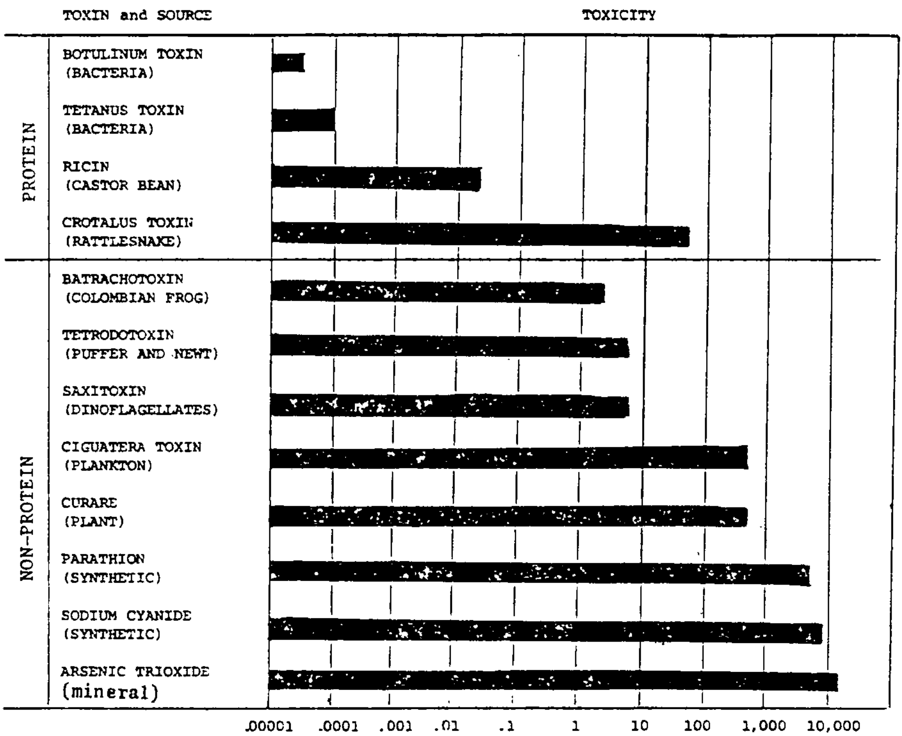
d) plant toxins (e.g. toxin from ricin)

e) marine toxins (e.g. puffer toxin, saxitoxin, ciguatera)

3. Differentiation between the Five Types of Toxin

(a) Chemical toxins can be extracted from plants and/or synthesized from chemical substances; or they may be industrial waste from factories, fertilizer and pesticide processing plants or nuclear fall-out, such as cyanide, parathion, mercury, asbestos, lead and strontium. These substances may be lethal to human beings.

Table 1. Lethal dose of toxin for mice (micrograms per kilogram of body weight). (After Fuhrman, 1967)



(b) Bacterial toxins are produced by the metabolism of microscopic organisms which can be present in food. Sumner (1980) distinguishes two forms of bacterial food poisoning, namely: (i) food infection, and (ii) food-borne intoxication.

(i) Food infections result from the growth of bacteria which have been ingested with the food, and which cause a pathogenic reaction in the host. Examples are *Salmonella, Vibrio* and *Escherichia coli* present in oyster, smoked fish, fish balls and "burong talangka" (fermented small crab). The infected host will experience diarrhea and vomiting. Toxins produced by such organisms as *Clostridium botulinum* and *Staphyloccocus aureus* in unhygienically handled and processed fishery products, namely, canned fish, frozen cooked shrimp, smoked fish, fermented products, etc., can be fatal to human beings (see Annex).

(ii) Food-borne intoxications occur when bacteria that grow in the food produce a toxin prior to consumption; the toxin then causes disease symptoms in the host.

(iii) Scombroid poisoning which is due to microbial enzyme decarboxylation of histidine to histamine is also an example of microbial toxin (see Fig. 1).

Scombroid toxin is common in frigate mackerel *(Auxis thazard)* (see Fig. 2) and other species of the Scombridae family such as tuna and tuna-like species and mackerel.

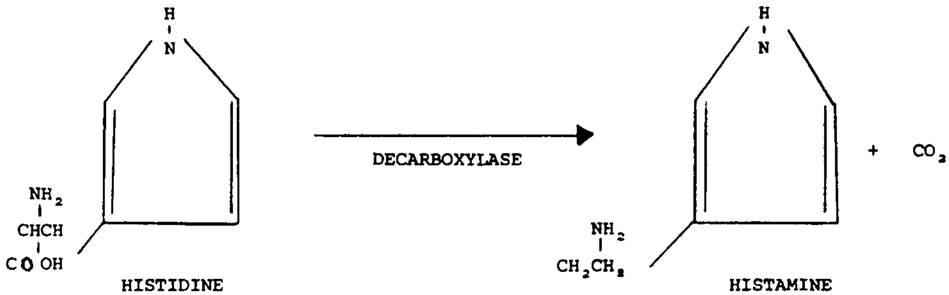
The amount of histamine formed in fish may vary according to species, composition of bacterial population, temperature, handling and storage of fish (Staruszkiewiez, 1967).

Histamine does not appear to be the sole causative agent in scombroid poisoning since histamine by itself is not toxic when taken orally (Bjeldanes and Chu, 1981). A possible interaction of histamine with an other substance or potentiator in food may be contributory to scombroid poisoning. The role of amines in scombroid poisoning in which the toxicity of histamine is induced has yet to be described clearly. However, some of these compounds, e.g., cadaverine, appear to occur at increased levels in toxic tuna. The average concentration of amines in wholesome and toxic tuna are as follows (Kim and Bjeldanes, 1979):

Wholesome	**Toxic**
Putrescine 0.35 mg%	1.53 mg%
Cadaverine 1.05 mg%	12.05 mg%
Histamine 2.74 mg%	116.00 mg%
Spermidine 3.26 mg%	2.37 mg%
Spermine 1.23 mg%	1.16 mg%

Low levels of putrescine, cadaverine and histamine were found to be typical in fresh tuna while in decomposed tuna, putrescine, cadaverine and histamine were significantly high.

Principle: Histamine will form from Microbial Decarboxylation of Histidine in Fish, Cheese, etc., as follows:



Among the suspect micro-organisms are *Proteus morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Citrobacter frerendii* and *Escherichia coli*.

Fig. 1. Decarboxylation of histidine to histamine.
(After Behling and Taylor, 1982)

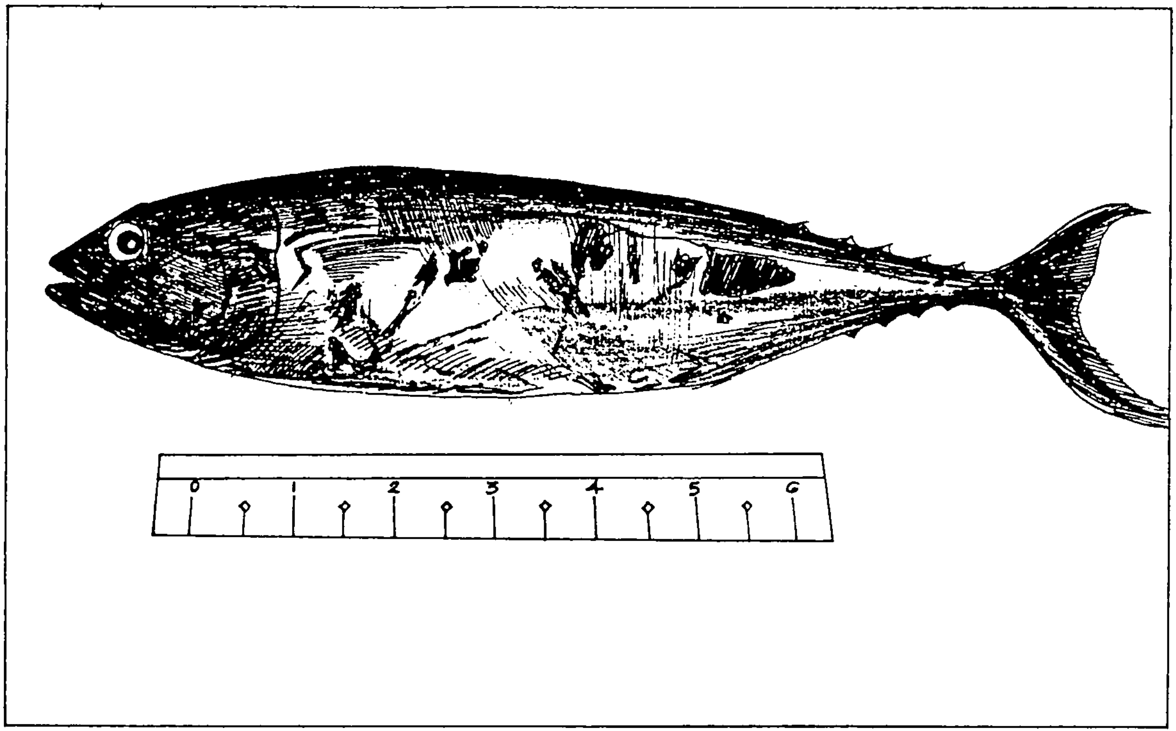


Fig. 2. Frigate Mackerel *(Auxis thazard)*

Dark meat fishes distinguish themselves from white meat fishes as regards the formation of histamine during bacterial spoilage (see Fig. 3). White meat has a higher histamine content than red meat owing to its high free histidine in the muscle tissues (see Table 2).

Several experiments have been conducted at the Department of Fish Processing Technology under the supervision of Dr. Florian M. Orejana and Ms. Lourdes M. Arafiles regarding histamine formation. Among these were the following:

(i) effect of delay in icing

(ii) effect of the ratio of ice to fish

(iii) effect of processing techniques

(iv) distribution of histamine in fish body parts

The results revealed that delay in icing and ratio of ice to fish are major factors in histamine formation (see Fig. 4, and Tables 3 and 4). From the tables and graph, it can be seen that the time of icing and the ratio of ice to fish had a significant effect on the formation of histamine.

Processing techniques were also considered in determining the histamine formation. The data gathered revealed that drying, smoking and fish ball making produce a higher level of

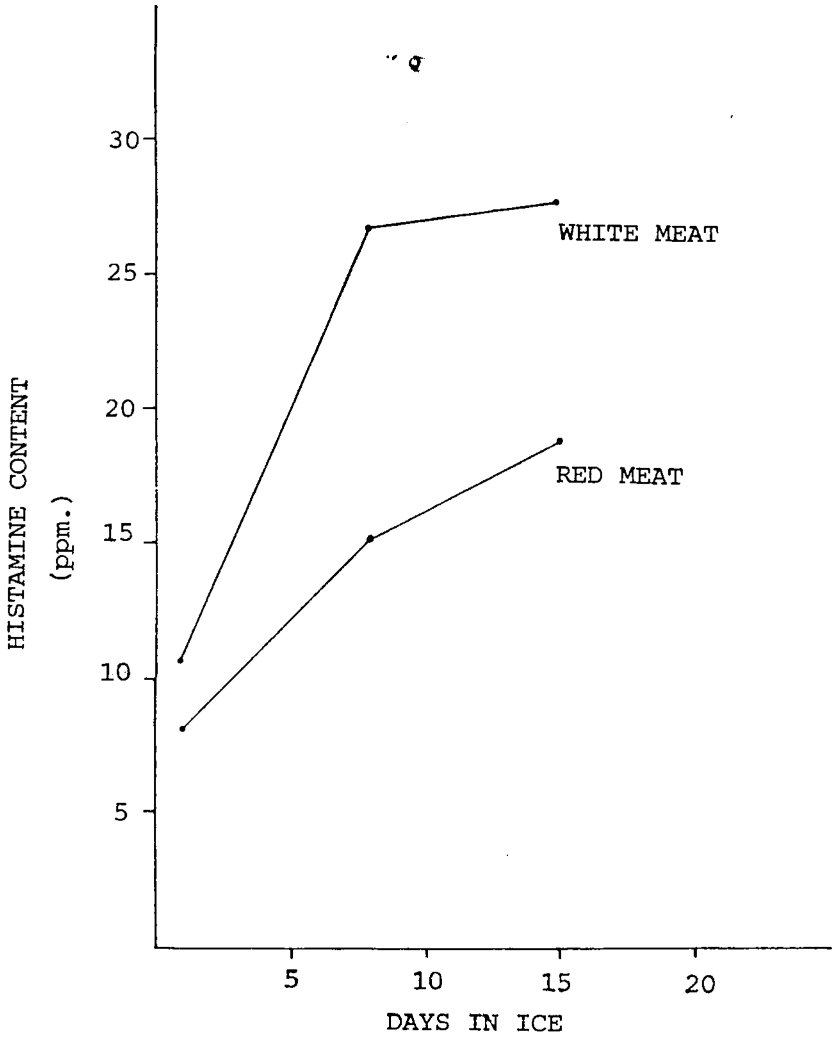


Fig. 3. Histamine levels in red and white meat of Skipjack Tuna (*Katsuwomus pelamis*) (After Kimata and Kawai, 1953).

Table 2. Amino acid composition in red and white meat of tuna (After Mukundan *et al.*, 1979).

AMINO ACID COMPOSITION g/100 g (DRY MUSCLE)		
	RED MEAT	WHITE MEAT
Isoleucine	5,00	5,53
Leucine	8,57	8,50
Lysine	4,17	9,48
Methionine + Cystine	3,88	3,80
Phenyl alanine	4,31	4,64
Tyrosine		
Threonine	4,99	5,38
Valine	4,24	5,36
Glutamic acid	2,38	5,36
Tryptophan	13,35	14,01
Arginine	0,45	1,70
Serine	3,83	4,59
Proline + Hydroxyproline	7,18	6,21
Aspartic acid	7,46	7,92
Glycine	3,93	2,86

histamine than canning (see table 5). This can be attributed to the time involved in processing such as smoking, drying, fish ball making. The above mentioned processes allow enough time for the micro-organisms to continue their activity, while canning inhibits microbial growth immediately owing to the high temperature of pre-cooking. However further studies need to be conducted to reach a conclusion on the effect of processing techniques on histamine formation. Furthermore, Sinaeng na Tulingan (boiled salted frigate mackerel) available in the local market has a histamine content ranging from 11.9 ppm to 17.9 ppm.

Apart from processing methods, storage temperature of processed products such as smoked skipjack tuna (*Katsuwomus pelamis*) also affects the histamine content significantly (see Fig. 5). Smoked skipjack kept at chilling temperature exhibited a slower rate of histamine formation than smoked skipjack kept at room temperature.

The body portions exhibited variations in histamine content (see Fig. 6 and Table 6). The head portion had the highest histamine content. This might be due to the gills' bacterial load. Further investigations on this aspect need to be undertaken.

Victims of scombroid poisoning may suffer flushing of the skin, throbbing or palpitations due to increased blood pressure, edema, and sometimes allergic reactions.

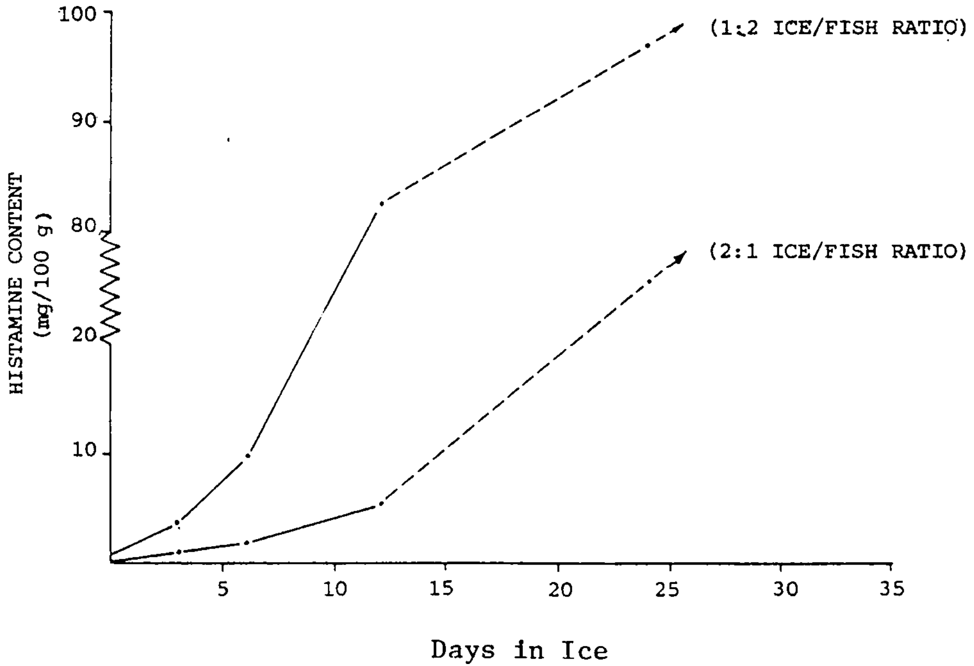


Fig. 4 Histamine content of Frigate Mackerel subjected to different ice ratios.

Table 3. Effect of delayed icing (ratio of ice/fish 2:1) on the histaminc level, TVB and TPC of Frigate Mackerel.

DELAY IN ICING (hr.)	HISTAMINE (ppm.)	HISTAMINE (mg/100 g)	TVB (mg/g)	TPC (log)
0	12.30	1.23	0.409	4.48
3	13.52	1.35	0.462	5.01
6	17.52	1.75	0.462	5.130
12	56.52	5.65	no data obtained	no data obtained
24	423.9	42.39	0.487	5.127
48	635.9	63.59	15.519	6.146

Ratio of ice/fish is 2:1

Table 4. Effect of delayed icing (ratio of ice/fish 1:2) on the histamine level, TVB and TPC of Frigate Mackerel.

DELAY IN ICING (hr.)	HISTAMINE (ppm.)	HISTAMINE (mg/100 g)	TVB (mg/g)	TPC (log)
0	14.6	1.46	1.04	4.18
3	37.2	3.72	2.37	4.66
6	93.1	9.31	2.08	5.66
12	826.4	82.64	3.41	6.30
24	954.0	95.4	5.94	7.30

Ratio of ice/fish is 1:2

Table 5. Effect of processing on histamine content of Frigate Mackerel.

PROCESS	MOISTURE CONTENT %	HISTAMINE (ppm.)	HISTAMINE (mg%)
A. DRYING			
Before	76.1	10.0	1.0
After	35.6	21.0	2.1
B. SMOKING			
Before	75.1	10.8	1.1
After	64.6	11.9	1.2
C. CANNING			
Before	75.54	18.62	1.8
After	70.95	12.95	1.2
D. FISH BALL MAKING			
Before (wet)	76.6	8.67	0.8
Minced	74.6	10.8	1.1
Formulated	59.2	12.4	1.2
Dried Fish Balls	28.3	11.9	1.2

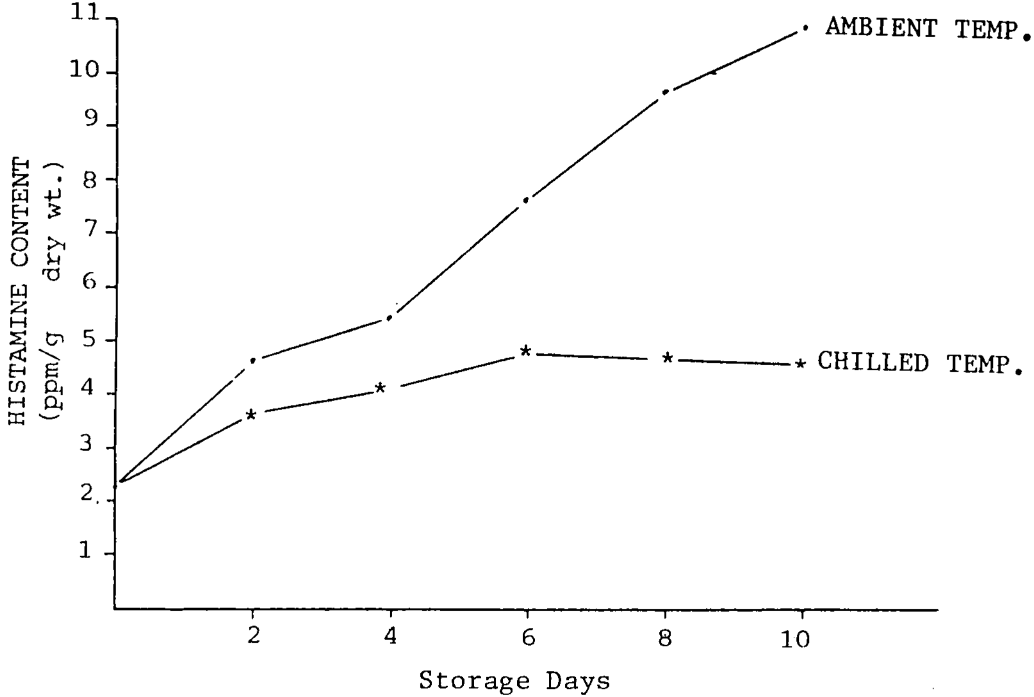


Fig. 5 Effect of storage temperature on histamine content of smoked skipjack tuna.

(c) Animal toxins are toxins produced within the body of the animal and generally secreted through a special organ. Examples are the venom of cobra which is secreted through a venom apparatus; the crotalus toxin of rattlesnakes; batrachotoxin present in the skin of a species of frog, etc.

(d) Plant toxins are produced by a plants metabolism. An example is ricin from the castor bean and curare, which is extracted from certain plants and used as arrow poisin by South American Indians.

(e) Marine toxins are toxic substances produced by marine plants and animals. The common ones can be further categorized into:

- Saxitoxin (paralytic shellfish poisoning),
- Ciguatera Poison
- Tetrodotoxin.

(i) Saxitoxin

The toxin originates in *Gonyaulax catenella, G. Tamarensis* and *Gymnodinium breve* and is accumulated by shellfish that feed on these dinoflagellates (Schantz *et. al.,* 1966).

Bates and Rapoport (as cited by Hashimoto, 1979) noted that human consumption of shellfish contaminated with saxitoxin causes poisoning and sometimes death; the human lethal dose is estimated to be approximately one mg when taken orally.

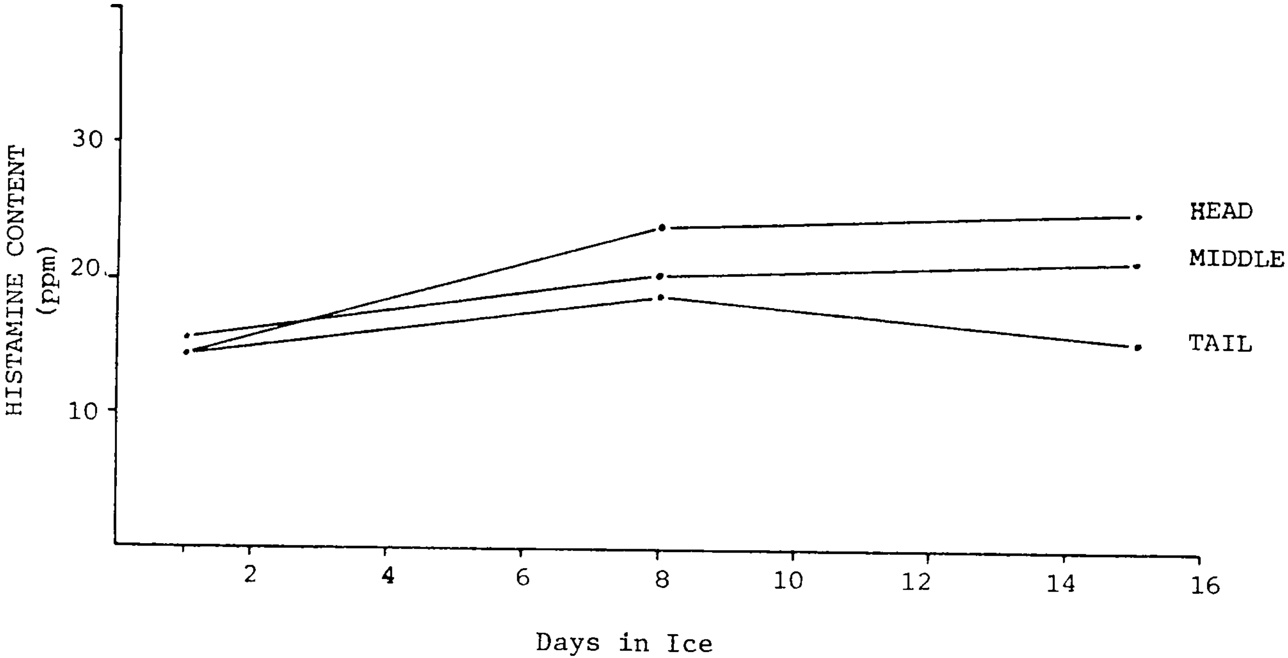
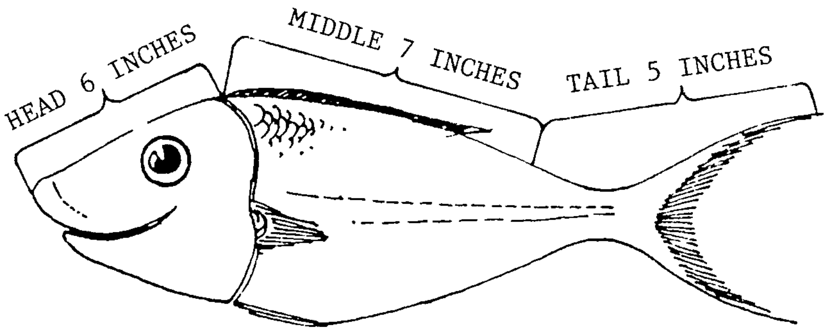


Fig. 6 Histamine levels of different portions of skipjack tuna.

Table 6. Histamine levels of the different portions of skipjack tuna stored in ice.

	1 DAY IN ICE		8 DAYS IN ICE		15 DAYS IN ICE	
	HISTA-MINE (ppm)	TPC (log)	HISTA-MINE (ppm)	TPC (log)	HISTAMINE (ppm)	TPC (log)
Head	14.85	3.52	24.2	3.81	25.3	4.04
Middle	15.8	2.55	20.5	3.69	21.85	3.98
Tail	14.5	2.31	18.4	3.9	15.7	4.15
Red meat	8.1	3.20	15.0	4.29	18.9	4.78
White meat	10.65	3.27	26.9	4.27	27.5	4.34



Average length of fish - 18 inches

Average weight/piece - 1.6 kg

Figure 7 shows some bivalves which can become toxic upon ingestion of *Gonyaulax* spp., *Gymnodinium breve* and *Pyrodinium bahamense* var. *compressa*.

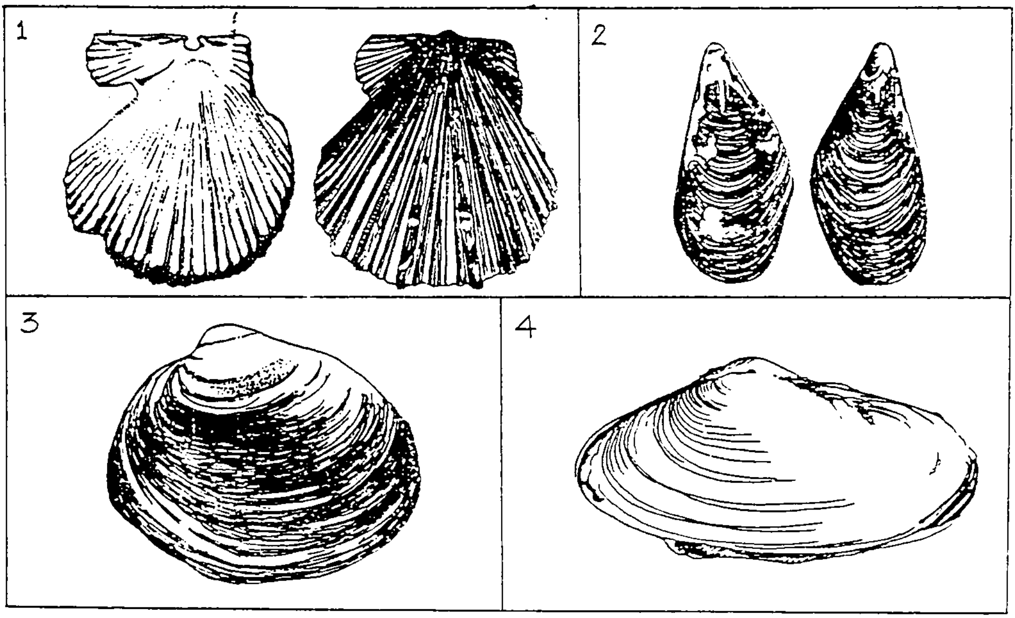


Fig. 7 Major bivalves that cause paralytic shellfish poisoning.

1. *Chlamys nipponensis akazara*
2. *Mytilus edulis*
3. *Saxidomus giganteus*
4. *Mya arenaria*.

(Adapted from Hashimoto, 1979).

Among the marine organisms that are able to accumulate saxitoxin are bivalves such as scallops and mussels. Paralytic shellfish poisoning according to Hashimoto (1979) has long been known along the Pacific and Atlantic coasts of the United States and Canada, and many fatal cases have been recorded. Outbreaks have also been reported in Britain, other European countries, Japan and Papua New Guinea, thereby suggesting that the problem is worldwide. Table 7 shows the mortality in Japan.

Schantz and Magnusson (1964) noted that there are marked differences among species in toxin distribution in the tissues and toxin retention. *Mytilus edulis* accumulates the toxin mainly in the digestive gland and retains the toxin for about two weeks. Prakash *et al.* (1971) observed that the soft shell clam *(Mya arenaria)* concentrates the toxin mainly in the digestive gland during summer and in the gills during autumn and winter. It takes up and releases the toxin slowly. Alaska butter clam *(Saxidomus giganteus)* stores two-thirds of the toxin in the siphon, where its concentration decreases slowly. Yasumoto and Kotaki (1977) isolated saxitoxin from the viscera of the green turban shell *(Turbo marmorata)*. Other parts of the body such as the liver may also contain the toxin and maintain a constant toxicity level for a long time.

Paralytic shellfish poison was detected by Sommer (1932) also in certain species of crabs such as the sand crab *(Emerita analoga)* during a period of red tides.

Table 7. Incidence of paralytic shellfish poisoning in Japan (After Hashimoto, 1979).

Date	Responsible Bivalve	Location	No. of Patients (deaths)
July, 1948	Short-necked clam, *Tapes japonica*	Toyohashi, Aichi Prefecture	12 (1)
May, 1961	Scallop, *Chlamys nipponensis akazara*	Ofunato Bay, Iwate Prefecture	12 (1)
Feb., 1962	Oyster, *Crassostrea gigas*	Miyazu, Kyoto	42 (0)

Arafiles *et al.* (1981) conducted, a survey of Philippine crabs which are seasonally toxic and found three species with traces of saxitoxin. They were *Cardisoma carnifex* (Herbst), a land crab with the vernacular names of "Kuray" in Llonggo, "Kural" in Aklanon and "Kamilong" in Ilocano; *Matuta Lunaris* (Forskal) or armed crab locally known as "Parag-parag" in Cebuano, "Alikamaw" in Aklanon and "Comaw" in Cebuano, the third species is still undentified but commonly called "Kaeas-kaeas" in Alkanon. The description of this species is as follows:

1) The carapace is about 4 cm in length, covered with a fine spine-like structure;

2) The color of the entire body is bluish-violet; the four pairs of walking legs are characterized by fine tendrils;

3) The species inhabits estuarine and mangrove areas and climbs on trees during high tide (see Figs 8-10)

Bioassays revealed that both the visceral argans and muscles contained traces of saxitxin.

Halstead (1965) describes the symptoms of patients who are victims of bivalve poisoning as follows:

1) Within 30 minutes after ingestion there is a tingling or burning sensation of the lips, tongue and face with gradual progression to the neck, arms, fingertips, legs, and toes.

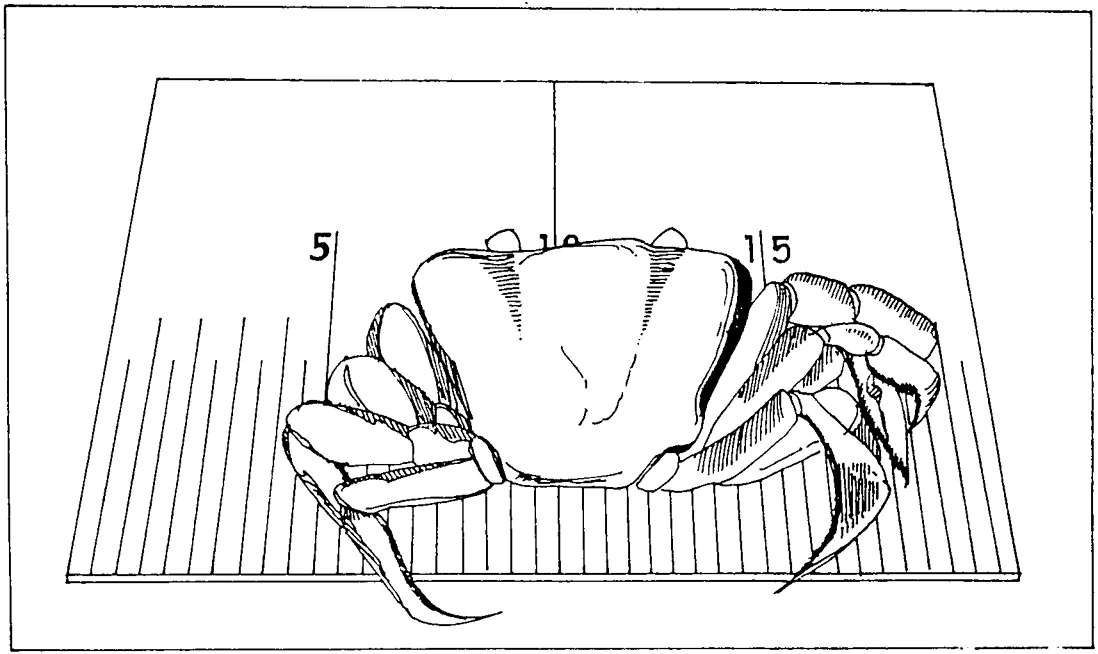


Fig. 8. *Cardisoma carnife*

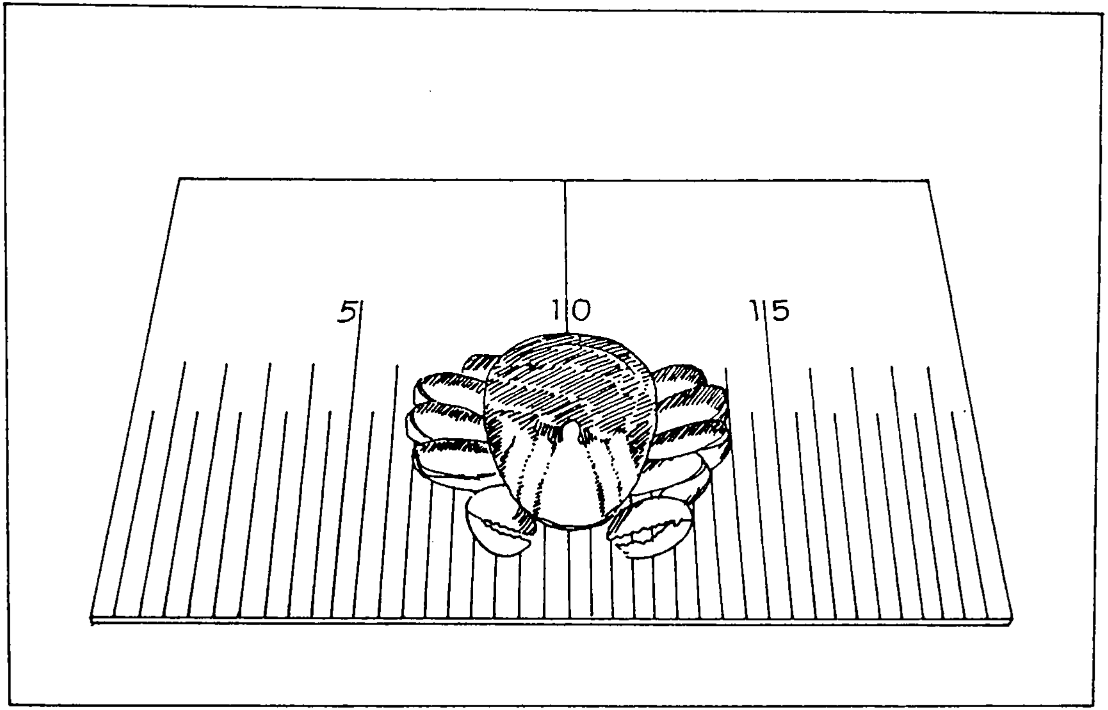


Fig. 9. *Matuta lunaris*

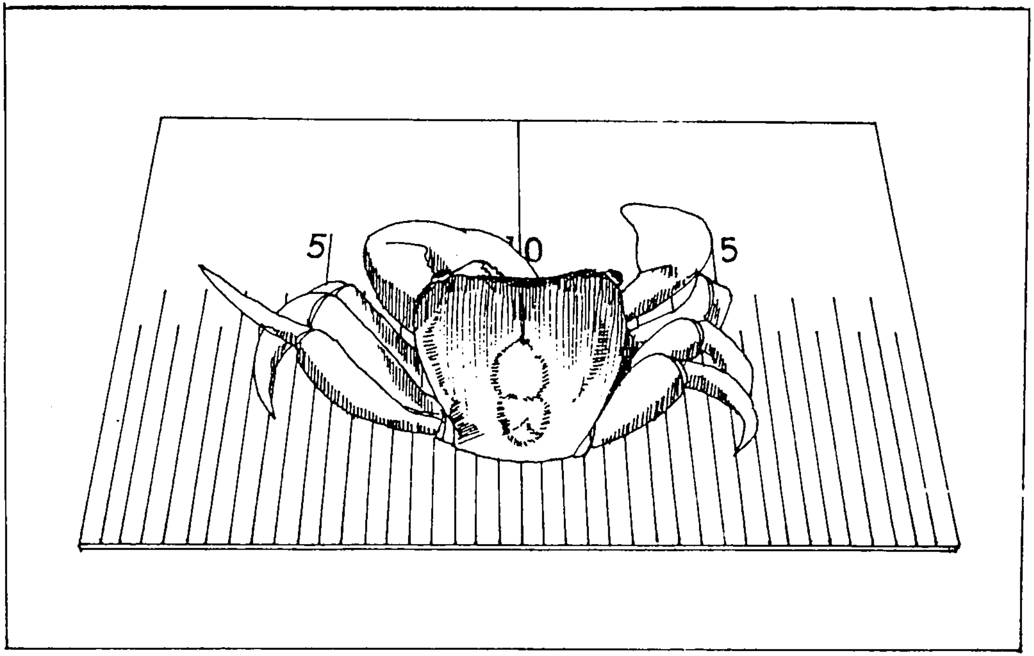


Fig. 10 "Kaeas - kaeas"

2) This sensation then changes to numbness, so that voluntary movements are made with difficulty.

3) In severe cases ataxia is accompanied by a peculiar feeling of lightness, as though floating on air, with aphasia, headache, thirst, nausea and vomiting.

4) In the terminal stages of the disease, paralysis becomes progressively more severe, and death occurs as a result of respiratory paralysis.

On the other hand, Hashimoto (1979) gives the following clinical picture of crab poisoning:

1) Within 15 minutes to a few hours after ingestion of crabs, numbness of the lips and limbs, and wobbling gait develop.

2) These symptoms are followed by severe vomiting, stupor, aphasia and respiratory difficulty.

3) Finally the victims fall unconscious and respiration fails. Death takes place within 4-6 hours after ingestion.

In the screening experiment on toxic crab, injected white mice exhibited hard breathing and partial paralysis of the hind legs. These symptoms are similar to those noted by Halstead (1965) and Hashimoto (1979).

(ii) Ciguatera Poison

The term ciguatera comes from a marine snail, *Cittarium* (Livona) *pica* called "cigua" in the Caribbean. It was first used by the Spanish conquistadores in Cuba to describe an illness including neurological and gastrointestinal disorders that was caused by eating the gastropod. Later, the term was extended to a similar disease induced by fish in the Caribbean.

Ciguatera poisoning is caused by eating toxic fish, mainly coral reef fishes. This type of poison is common in red snapper *(Lutjanus bohar)*, grouper and eel. According to Dr. John E. Randall as mentioned by Banner *et al.*, (1974), the toxin originates from a certain fine blue-green alga. As the alga is consumed by the herbivores, the toxin accumulates in the flesh and viscera. These herbivores are fed upon by the carnivores which are ingested by man (see Fig. 11).

The toxicity of ciguateric fish shows great individual, regional and seasonal variations. In general, toxicity of the viscera is greater than that of the flesh, with liver as the most toxic organ of the body, while ovaries and testes contain small amounts of toxic substances.

Halstead (1967) lists a wide spectrum of clinical symptoms of ciguatera poisoning as follows:

1) Prickling of the lips, tongue and throat;

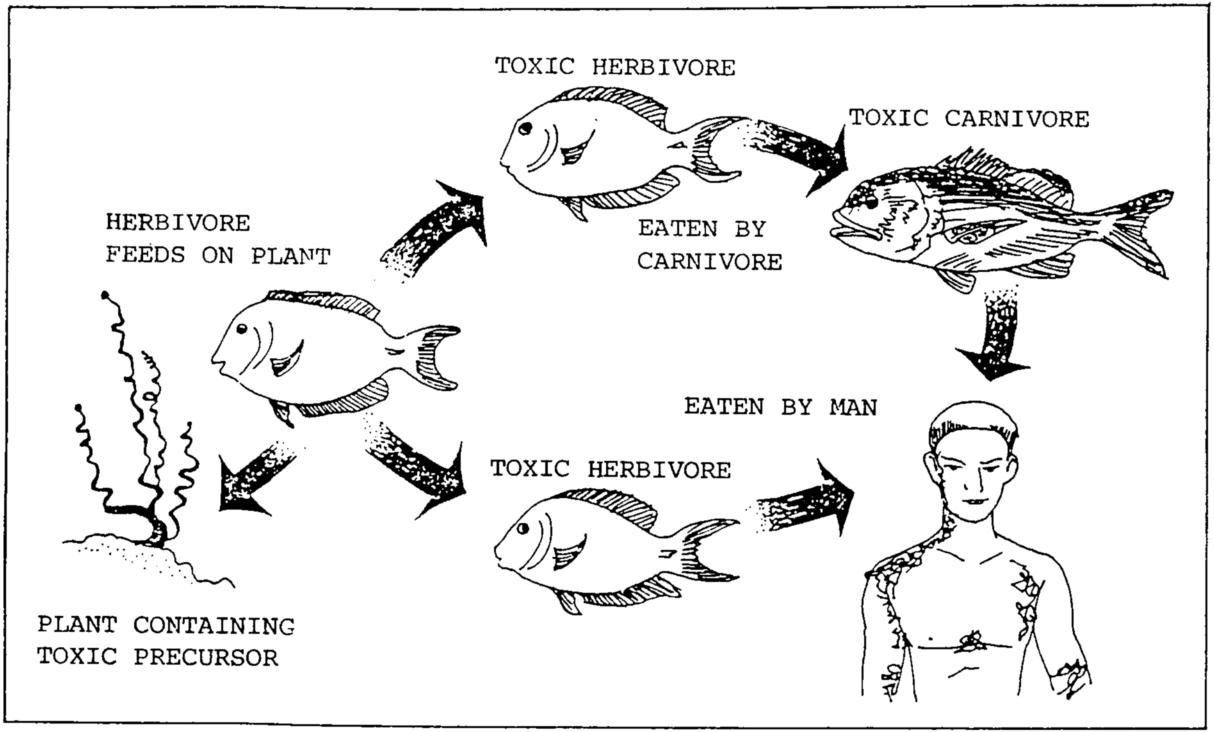


Fig. 11 Accumulation of toxin through the food chain (After Halstead, 1967)

2) numbness, nausea, vomiting, metallic taste, dryness of the mouth, abdominal cramps, insomia, prostration, ataxia and myalgia;

3) general symptoms such as numbness, headache, arthralgia, erethism, dizziness, cyanosis, insomia, prostration, ataxia and myalgia.

The victim complains of severe pain, especially in the arms and legs; his teeth feel loose; he has pain in the eyes, visual disturbances and skin disorders consisting of erythema, maculopapular eruption, blisters, a stinging sensation, and loss of hair and nails.

One of the most frequently noted symptoms is a reversal of temperature sensation, in which the victim interprets cold as a Dry Ice or electric shock sensation and hot objects as being cold.

General motor incoordination, paralysis and convulsions become progressively worse and are followed by coma and death.

Bagnis (1968) groups the symptoms into four:

1) gastrointestinal symptoms such as vomiting and diarrhea;

2) cardiovascular disorders such as decreased blood pressure and bradycardia;

3) neurologic disturbances such as sensory disturbances and myosis;

4) asthenia and arthralgia.

He also notes that gastrointestinal symptoms are more common in poisoning caused by herbivorous fish such as surgeonfish, while cardiovascular and other disorders are caused mainly by carnivores such as red snapper.

Halstead (1967) mentions that recovery from ciguatera poisoning is slow, in some cases requiring several months, because it is believed that more than two toxins are responsible for such poisoning. Examples of fishes which can cause ciguatera poisoning are given in Fig. 12 and Table 7.

(iii) Tetrodotoxin

Contrary to the two earlier mentioned types of marine toxin that are ingested, tetrodotoxin is inherent in the tissues of certain fish. This particular toxin is exhibited by species of the family Tetraodontidae, from which the word tetrodotoxin was coined (see Fig.13 for the chemical structure).

Several reports of puffer fish poisoning have been published in the daily newspapers. In the Philippines most deaths occurred in the province of Mindoro.

This type of poisoning is also rampant in other countries like Japan. Reports on poisoning incidences prompted the Department of Fish Processing Technology to conduct a

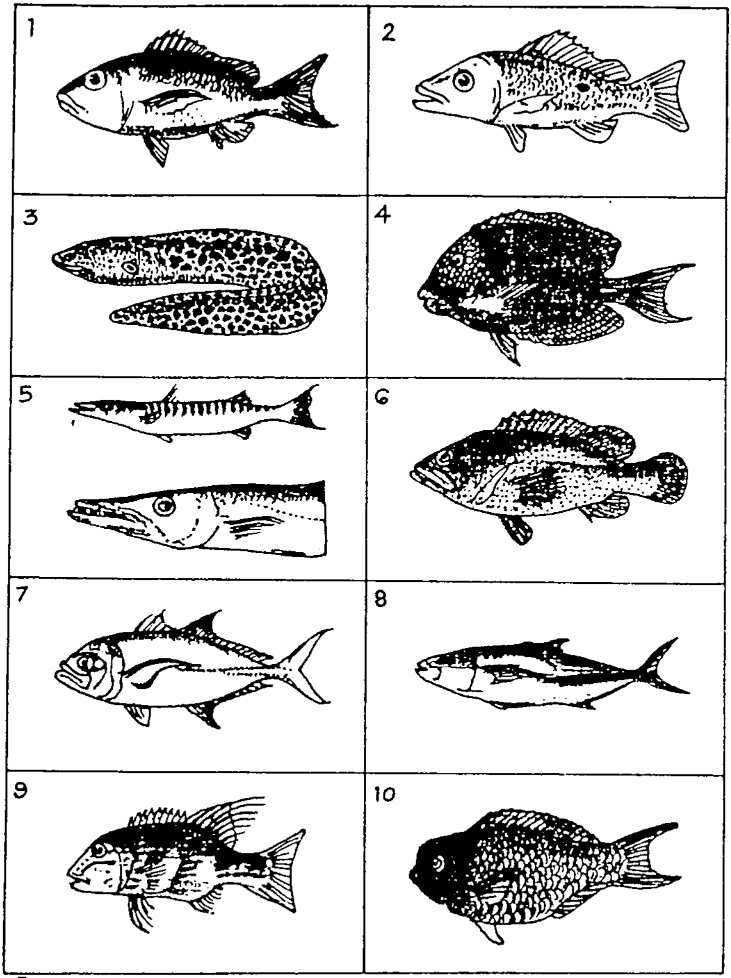


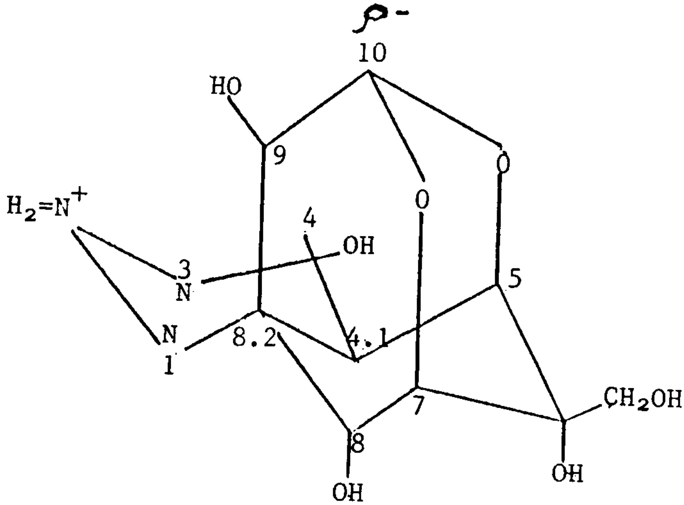
Fig. 12 Major ciguateric fish.

1. *Lutjanus bohar*
2. *Lutjanus monostigma*
3. *Gymnothorax javanicus*
4. *Ctenochaetus striatus*
5. *Sphyraena barracuda*
6. *Epinephelus fuscoguttatus*
7. *Caranx sexfasciatus*
8. *Seriola aureovittata*
9. *Glabrilutjanus nematophorus*
10. *Scarus gibbus*

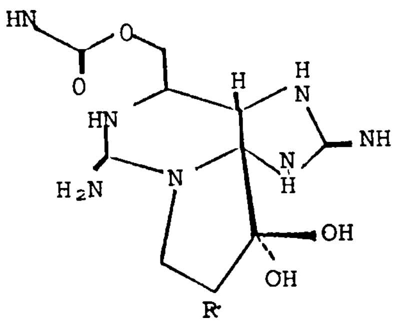
(Adapted from Kainuma and Halstead, 1967).

Table 7. Fish confirmed to contain ciguatoxin (After Rau and Rau, 1980).

ENGLISH NAME	SCIENTIFIC NAME	LOCAL NAME
Red snapper	*Lutjanus bohar*	Tingayg, Katambak Bambongon
Snapper	*Lutjanus monostigma*	Siksik
Moray eel	*Gymnothorax javanicus*	Tagbao, Hagmag Ubod
Surgeonfish	*Ctenochaetus striatus*	Mungit, Labahita
Barracuda	*Sphyraena barracuda*	Lingko, Lusod, Bikuda, Torsilyo, Tabangko
Grouper	*Epinephelus fuscoguttatus*	Garopa, Lapu-Lapu, Pugapalilug
Jack	*Caranx sexfasciatus*	Inggatan, Babakulan, Pinkit, Simbad, Muslo
Amberjack	*Seriola aureovittata*	(not available)
Chinamanfish	*Glabrilutjanus nematophorus*	Agba-on Langisi Bambongon
Parrotfish	*Scarus gibbus*	Molmol



AMINO PERHYDROXY QUINAZALINE COMPOUND
(TETRODOTOXIN) ($C_{11}H_{17}N_3O_8$)



GUANIDINE, 3-GUANIDINOPROPIONIC
PYRIMIDOPURINE (SAXITOXIN)
($C_{10}H_{17}N_7O_4$)

Fig. 13 Chemical structure of tetrodotoxin and saxitoxin.

study on the toxicity of puffer fish caught in Philippine waters, specifically in the Samar Sea.

Five species of puffer fish were identified according to their physical characteristics such as number of spines and body proportions (see Table 8). The species were found to be *Sphoeroides lunaris lunaris* (Bloch and Schneider). *S. lunaris spadiceus* (Richardson), *S. sceleratus* (Forster), *Tetraodon hispidus* (Linnaeus),(see Figs. 14-17) and *S. hyselogenion* (Bleeker).

A key to the Samar Sea species of puffer fish (dela Paz, Reynaldo, 1980 - personal communication) is given below to help consumers and fishermen in identifying the species.

A1 Body with white spots, black bands or irregular markings and prickles all over the body ----------- B

A2 Body without white spots, black bands or irregualar markings and prickles not all over the body ------------ C

B1 Black bands very prominent along the ventral region: *T. hispidus*

B2 Absence of black bands; color of the body is brownish: *S. hyselogenion*

C1 Dorsal side plain bluish-gray ----------- D

C2 Dorsal side brownish with irregular brown spots: *S. sceleratus*.

Table 8. Physical characteristics of the puffer fish species caught in Samar Sea and used as indices of identification

Physical Features	*Sphoeroides Hyselogenion*	*S. Scele-ratus*	*S. lunaris lunaris*	*S. lunaris spadiceus*	*Tetraodon hispidus*
NUMBER OF SPINES:					
Dorsal	8	10.5	12	12	10
Anal	7.5	10	11.5	11.5	9.5
Pectoral	14	15	16	16	17
Standard length (in cm)	2.6 - 5.2	14 - 15.0	12.3 - 24.0	12.3-24.0	11.0 - 76.2
BODY PROPORTIONS:					
HEAD/SL (a)	2.6	2.8	2.6	2.6	2.3
CAUDAL FIN/SL (a)	4.0	6.0	3.4	3.4	
SNOUT/HL (b)	2.5	1.4	2.2	2.2	2.2
EYE/HL (b)	3.2	3.0	2.8	2.8	3.2
Number of species observed	12	12	12	12	12

(a) size in relation to standard length of the body

(b) size in relation to the head length

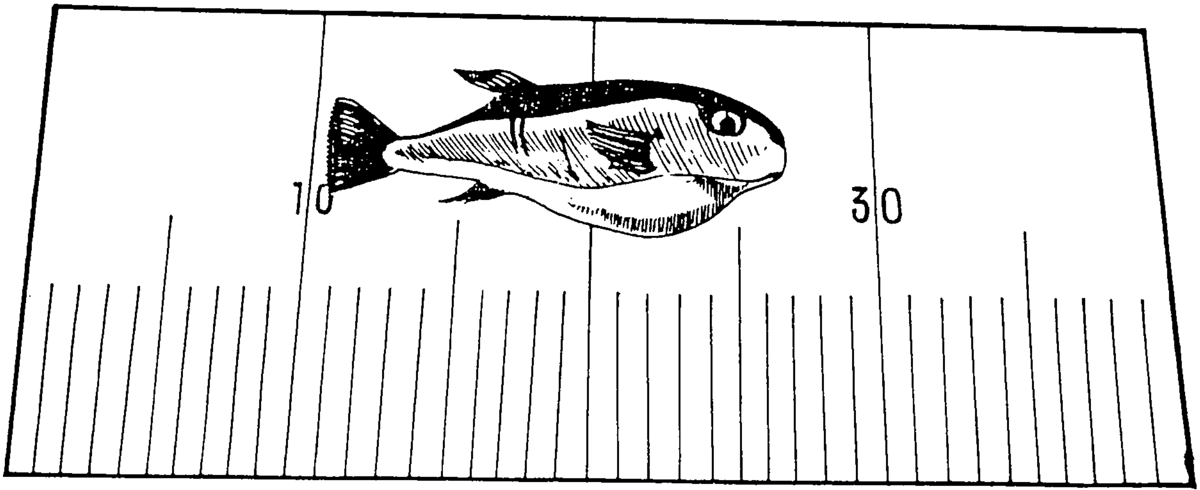


Fig. 14. *Sphoeroides lunaris lunaris*

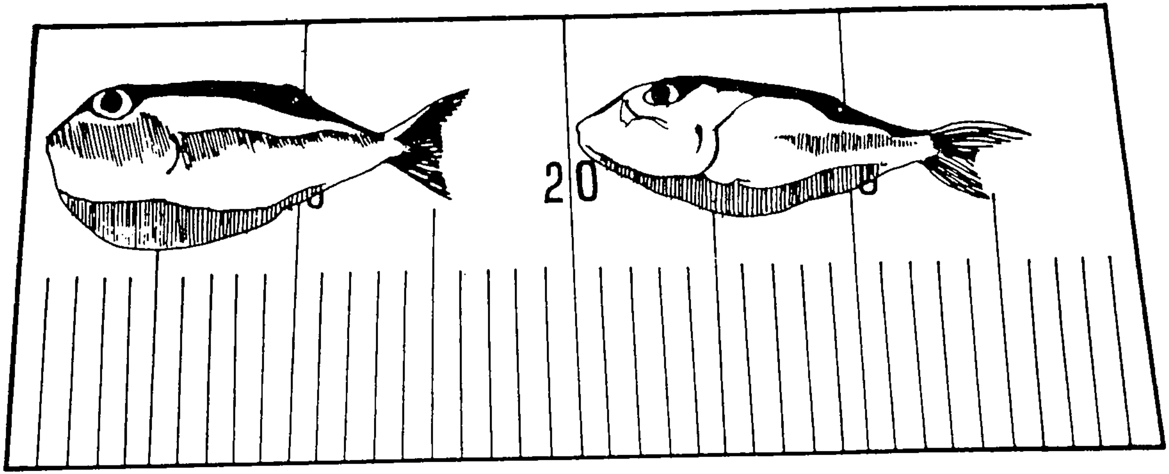


Fig. 15. *Sphoeroides lunaris spadiceus*

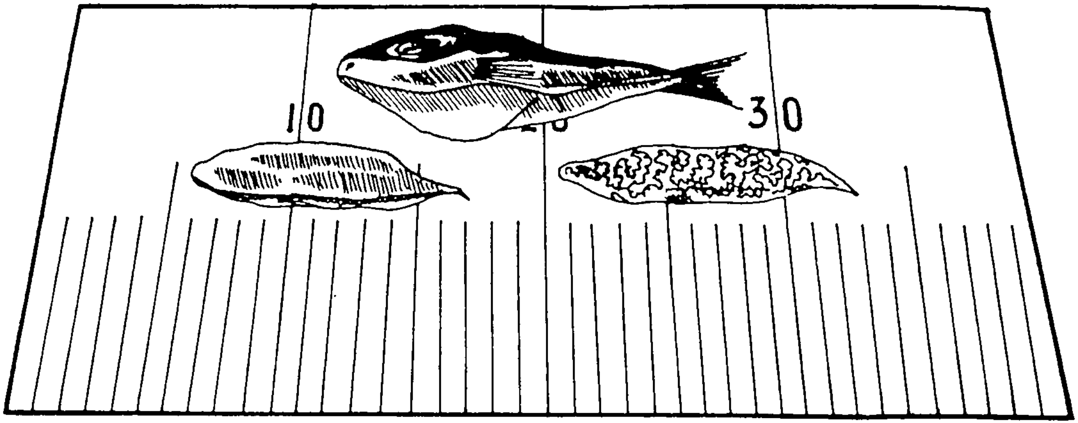


Fig. 16. *Sphoeroides sceleratus*

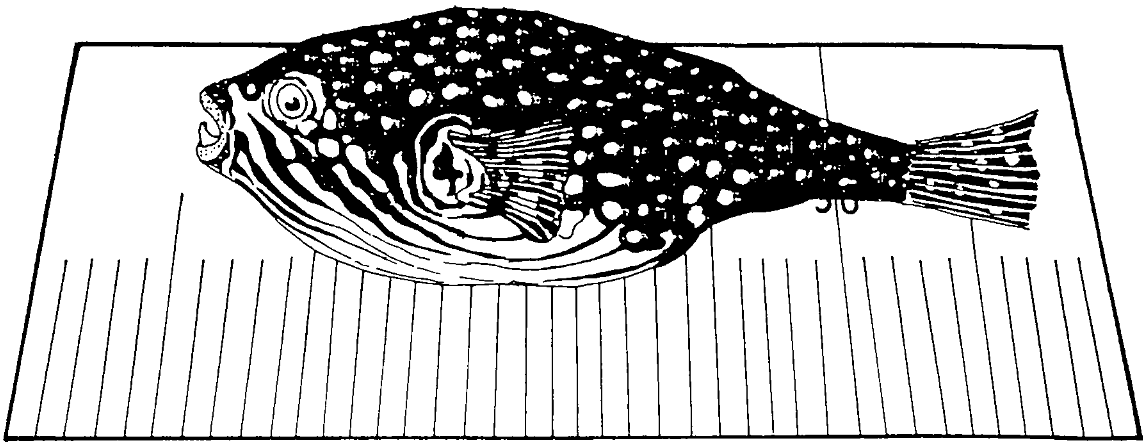


Fig. 17. *Tetraodon hispidus*

D1 Tail with black markings: *S. lunaris lunaris*

D2 Tail yellowish, without black markings: *S. lunaris spadiceus.*

S. lunaris and *S. lunaris spadiceus* are similar to *Lagocephalus lunaris lunaris* and *Logocephalus lunaris spadiceus* respectively found off Japan, (Masuda *et al.*, 1975). These two species of sphoeroides can be distinguished from each other by two prominent features: the presence or absence of black markings on the caudal fin and of small spines at the dorsal base. *S. lunaris lunaris* has black markings on the caudal fin and has small spines on the dorsal base while *S. lunaris spadiceus* has a yellowish caudal fin without black markings and the dorsal base has no spines.

Puffer toxin has been observed by Hashimoto (1979) to exhibit individual and seasonal variations (see Table 9) while Hoas and Randall (1969) noted the distribution of toxin in the body tissues (see Table 10). From their studies, they found that prior to and during the spawning season the fish become strongly toxic. Kawabata (1971) cited that the season for eating puffer is generally from October to March. Montalban *et al.*, (1964) and Russell (1968) added that toxicity of puffer varies considerably with species, sex, age, and season. However, these factors need further investigation.

Table 9. Individual and seasonal variations in toxicity of the ovary (O) and liver (L) of *S. rubripes rubripes* (as cited by *Hashimoto*, 1979).

	NOV		DEC		JAN		FEB		MAR		APR		MAY	
	O	L	O	L	O	L	O	L	O	L	O	L	O	L
Number of non-toxic specimens	9	9	3	8	1	7	1	5	5	7	1	5	4	7
Number of weakly toxic specimens	1	1	5	3	6	5	8	6	6	5	5	5	2	3
Number of strongly toxic specimens	0	0	3	0	8	3	3	1	3	1	5	1	1	0
Strongly toxic specimens	0	0	3	0	8	3	3	1	3	1	5	1	1	0
Total	10	10	11	11	15	15	12	12	14	13	11	11	7	10

Endean (1966), Ogura (1971) and Hashimoto and Fusetani (1978) reported that tetrodotoxin is found mainly in the ovary, liver, intestine and, to a lesser extent, in the skin and muscle as shown in Table 10. Yudkin (1944) believes that the toxin might cause poisoning only when the fish are improperly cleaned or when they remain ungutted for some time after their capture so that the poison can permeate into the muscle.

To test Yudkin's hypothesis, three species of puffer fish were immediately gutted after catching, and the tissues were separated. Comparative lethal effects of the tissue extracts were studied using white mice Figure 18 shows the distribution of toxin in the puffer fish tissues. Gonads contain the highest toxicity followed by the liver, intestine, skin and muscle. Among the three species subjected to bioassay, *S. sceleratus* is the most toxic, with all the body parts containing the toxin, while *S. lunaris lunaris* has non-toxic muscle. *S. lunaris spadiceus*, a species not frequently caught, is non-toxic.

Each body part of the toxic species was compared. Figure 19-21 show the comparative lethal effects of each body part. Skin of *T. hispidus* is more toxic than skin of *S. sceleratus;* the muscle of both *T. hispidus* and *S. sceleratus* are highly toxic; and the intestine of *S. sceleratus* is more lethal than that of *S. lunaris lunaris*. *S. hyselogenion* was not tested owing to lack of samples for crude toxic extract.

Table 10. Range of concentration of tetrodotoxin in ug/g of fresh tissues of 13 female species of sphoeroides (After KAO (1966), as cited in Hoas, 1969).

SPECIES	OVARY	LIVER	SKIN	INTES-TINE	MUSCLE	BLOOD
S. niphobles	400	1000	40	400	4	1
S.. alboplumbous	200	1000	20	40	4	-
S. pardalis	200	1000	100	40	1	1
S. vermicularis	400	200	100	40	4	-
S. porphyreus	400	200	20	40	1	-
S. oscellatus	1000	40	20	40	-0.2	-
S. basilewskianus	100	40	4	40	-0.2	-
S. chrysops	40	40	20	4	-0.2	-0.2
S. pseudommus	100	10	4	2	-0.2	-
S. rubripes	100	100	1	2	-0.2	0.2
S. xanthopterus	100	40	1	4	-0.2.	-
S. stictenetus	20	-0.2	2	1	-0.2	-
S. inermis	0.4	1	-0.2	0.4	0.4	-

-0.2 means less than 0.2 ug/g

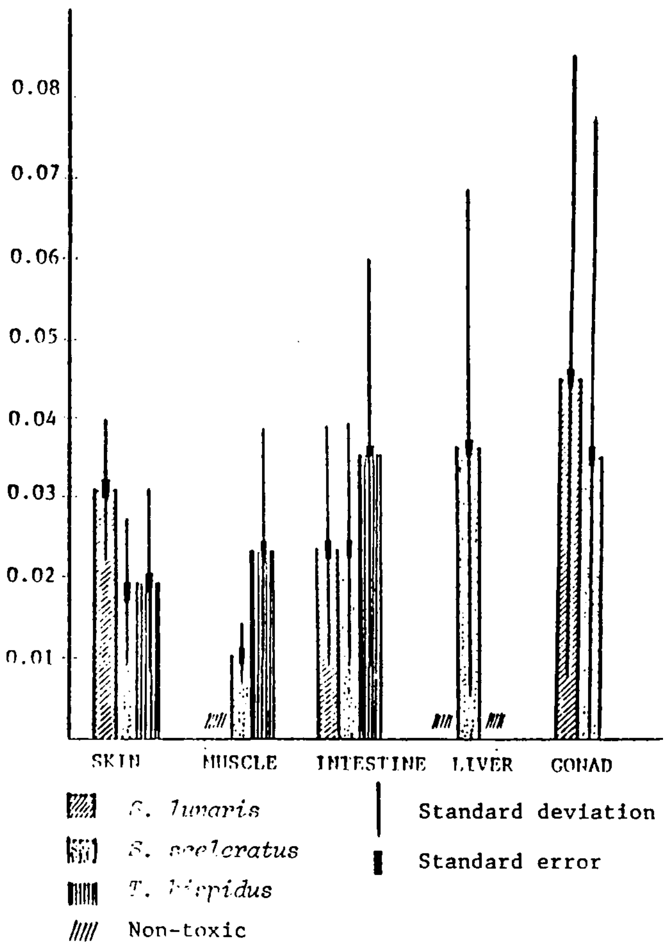


Fig. 18. Comparative lethal effects of the body tissues of *S. lunaris lunaris*, *S. sceleratus*, and *T. hispidus*

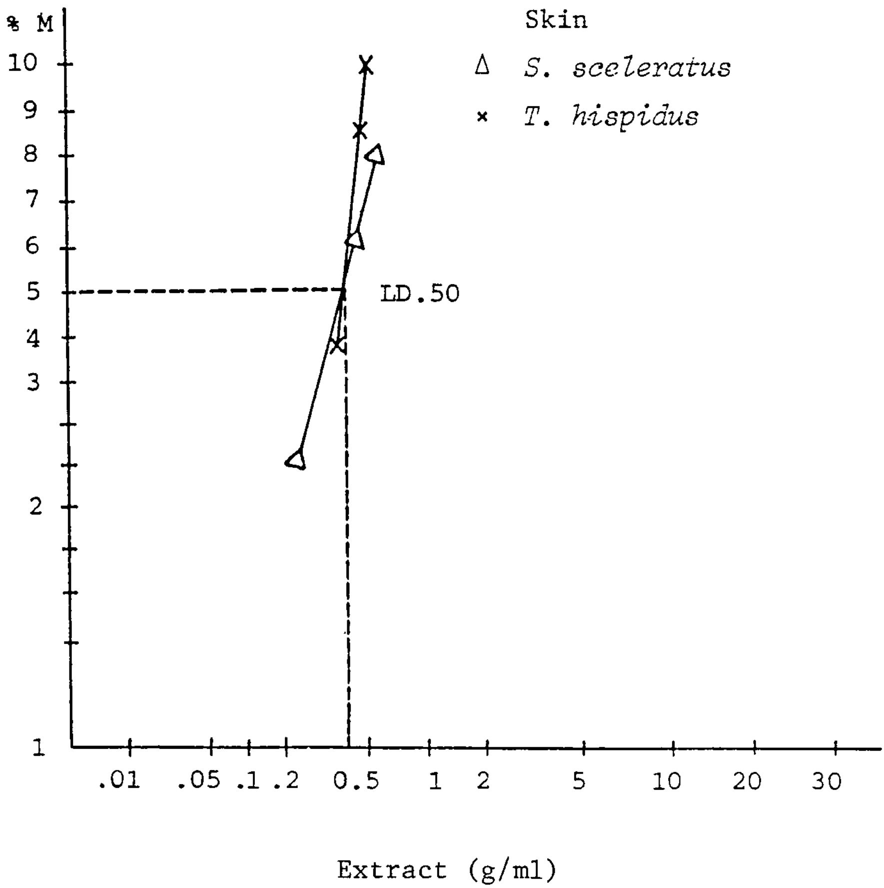


Fig. 19. Comparative lethal effects of *S. sceleratus* and *T. hispidus* skin on male and female mice

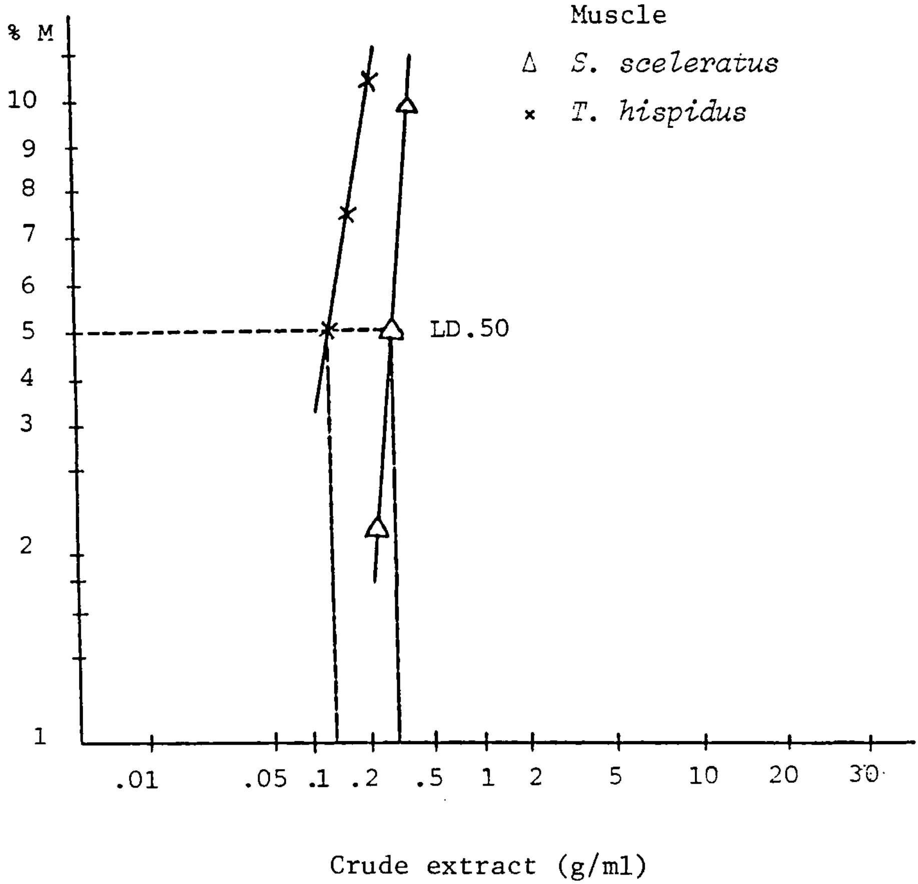


Fig. 20. Comparative lethal effects of *S. sceleratus* and *T. hispidus* muscle on male and female mice

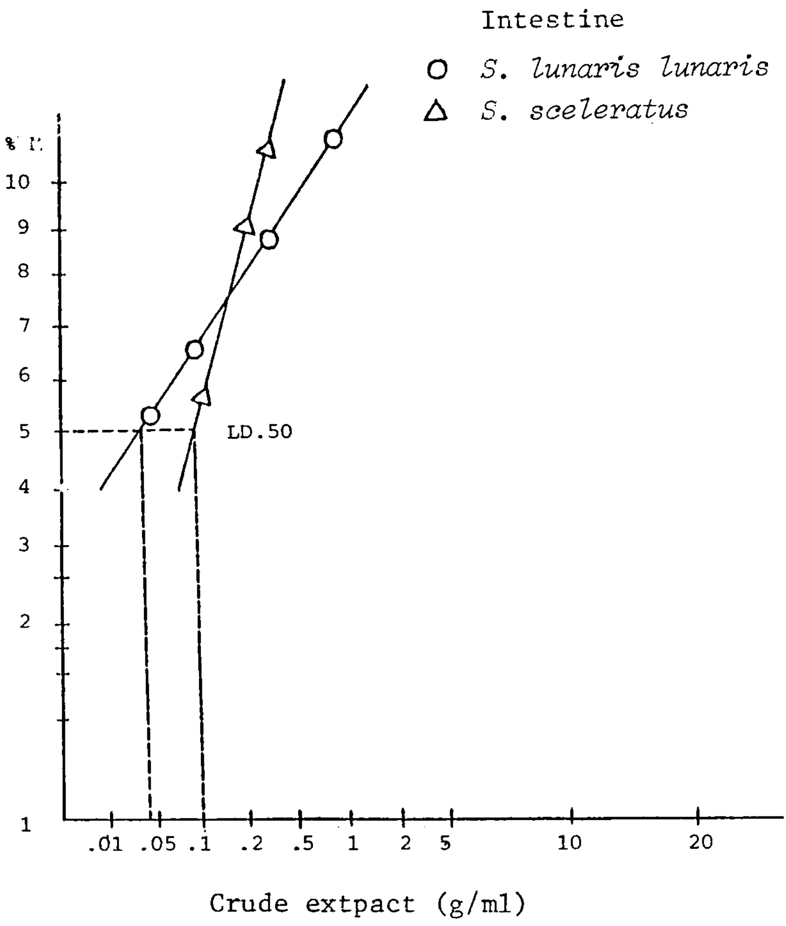


Fig. 21. Comparative lethal effects of *S. lunaris lunaris* and *S. sceleratus* intestine on male and female mice

In general, Hashimoto (1979), and Hoas and Randall (1969) described the symptoms of puffor fish poisoning as follows:

1) stupor - a condition in which the senses and faculties are suspended or greatly dulled;

2) aphasia - loss or failure of speech due to disorder in some of the cerebral centers;

3) ataxia - loss or failure of mascular coordination and loss of reflexes;

4) cyanosis - disordered condition of the circulation due to inadequat oxygenation of the blood causing the skin to appear blue;

5) dyspnea - labored or hard breathing;

6) dysphagia - difficulty in swallowing;

7) decreased blood pressure followed by a comatose condition or death due to respiratory arrest.

(iv) Other Toxins of Marine Organisms

Apart from the three major marine toxins, there are also toxins in the stings or bites of marine organisms such as species of coelenterates, echinoderms and molluscs, as well as a number of venomous fish.

a. Coelenterates

Representative examples are jelly fish, sea nettle and sea anemones (see Figs. 22-23). These organisms are notorious for their dangerous sting that contains a potent venom discharged from the nematocyst (Lane (1960) and Lane and Dogde (1958). See Fig. 24 showing a charged and discharged nematocyst.

Victims of the Portuguese Man-of-war experience severe pain with redness of the skin and erythematous wheals; in severe cases, the victims also have headaches, nausea, difficulty in respiration, abnormal pulse and convulsions, followed by death. Box jellyfish has been the cause of death of a considerable number of swimmers in waters off northern Australia.

Sea nettle is notorious for inflicting painful lesions upon bathers who come in contact with its tentacles. The nematocyst has been shown to contain proteinaceous toxins that affect not only the skin but also the circulatory system.

The sea anemone *Anemonia sulcata* is known to inflict lesions upon bathers. Skin contact with its tentacles produces cutaneous pain, scars, redness, edema, rashes and necrosis, and is usually accompanied by fever, drowsiness, dizziness and abdominal pain.

Fig. 22. Portuguese Man-of-war *(Physalia physalis)*

Box Jellyfish *(Chironex fleckeri)*

Fig. 23. Sea nettle (*Chrysaora quinuecirrha*)   Sea anemone (*Anemonia sulcata*)

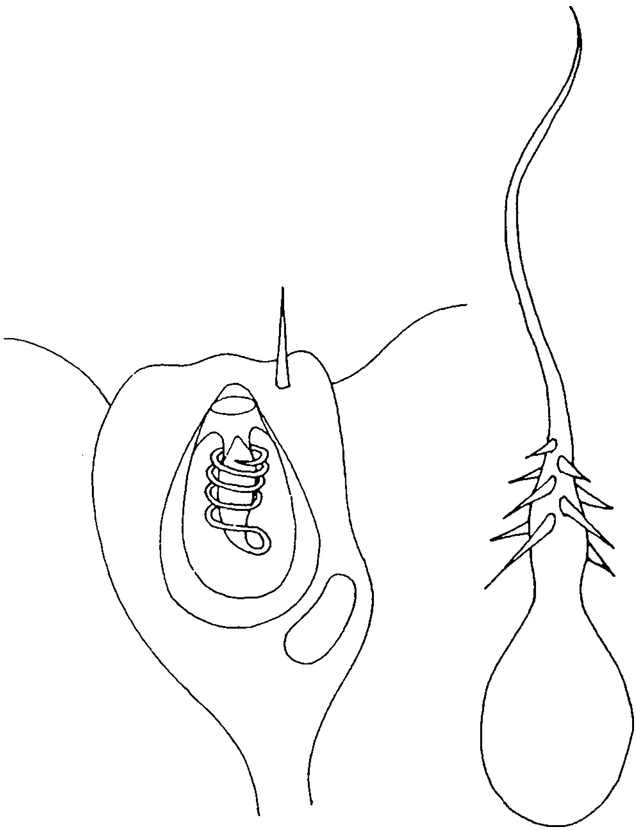


Fig. 24. Illustration of undischarged (left) and discharged (right) nematocysts (Adapted from Halstead, 1965)

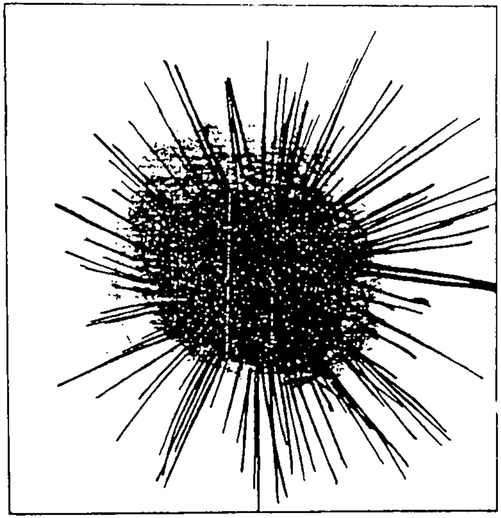
b. Echinoderms

Sea urchin, sea star, sea cucumber and starfish (see Figs. 25-26) are the only known echinoderms that are injurious to man.

Sea urchins possess a poison bag at the tip of sharp spines. When a person comes in contact with the spines, the tips break inside the body of the victim and the poison is released. The poison causes severe pain and swelling.

Apart from having these poisonous spines, sea urchins are also equipped with venomous organs, the pedicellariae (see Fig. 27), which are scattered among the spines. These function as a cleaning device of the sediments that accumulate on the body surface of sea urchins. The structure is species specific.

The head of a pedicellaria may reach a diameter of 2 to 3 mm and comprises three calcareous jaws or valves. The overall height of a pedicellaria is around 10 mm. Each of the three jaws opens like a flower. On stimulation of sensory hairs located on the inner side of each jaw, it closes like a bud, and a bite on the skin occurs. At the same time, venom is discharged from the venom gland in the head. Venom of pedicellariae is extremely toxic. It causes respiratory distress and a drop in body temperature, paralysis of the tongue, eyelids and facial muscles, relaxation of the lower limbs and difficulty of speech. The pain may disappear after about one hour but facial paralysis may last up to six hours.



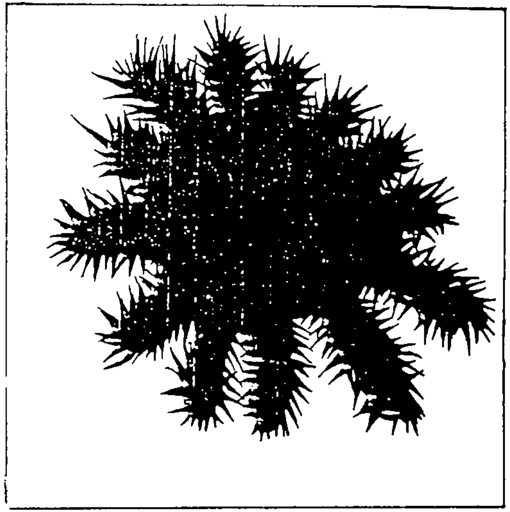


Fig. 25. Long-spined sea urchin *(Diadema setosum)*

Crown-of-Thorns sea star *(Acanthaster planci)*

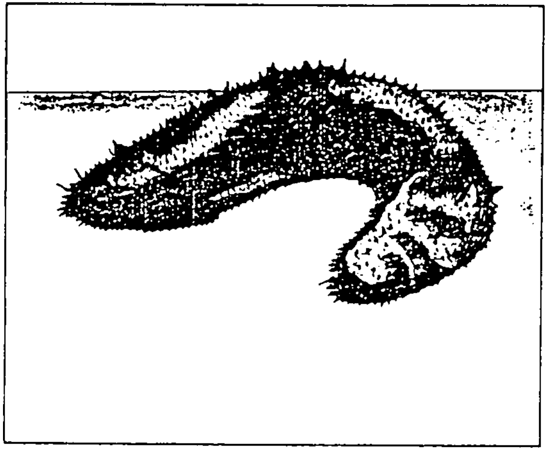
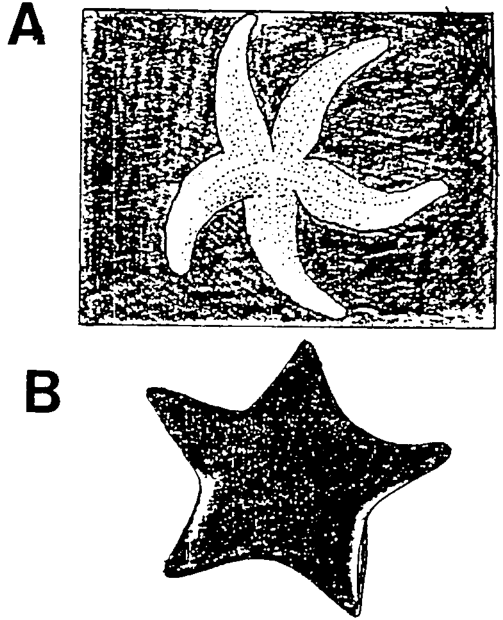


Fig. 26. Sea Cucumber
(Actinopyga agassizi)



Starfish (A) *Acterias amurensis*
and (B) *Asterina pectinifera*

Sea star has spines that are enveloped in a thin layer of integument that has glandular cells. When these spines accidentally become imbedded in a person's skin, they produce a painful wound and swelling.

In addition to spines and pedicellariae, some echinoderms, such as sea cucumber, starfish, brittle star, sea urchin and sand dollar, contains a toxic substance called saponin or holothurin.

Saponin contains sugar, several aglycones, sulfuric acid and steroid or triterpenoid moieties. Certain species of sea cucumbers (see Fig. 26) may discharge from their visceral cavities tenacious filaments which produce painful inflammation in contact with the human skin. It is also believed that if their body fluid comes in contact with the skin or the eye of a person it may cause dermatitis or blindness. The Bahamian sea cucumber *(Actinopga agassizi)* whose toxin is concentrated mainly in its cuvierian organ is highly toxic to fish.

Starfish such as *Asterina pectinifera* and *Asterias amurensis* also contain saponin.

c. Molluscs

The best known among molluscan toxins are (a) stinging toxins of cone shells of gastropods, and (b) a low molecular weight toxin (maculotoxin) and a peptide (eledoisin) from the salivary gland of cephalopods.

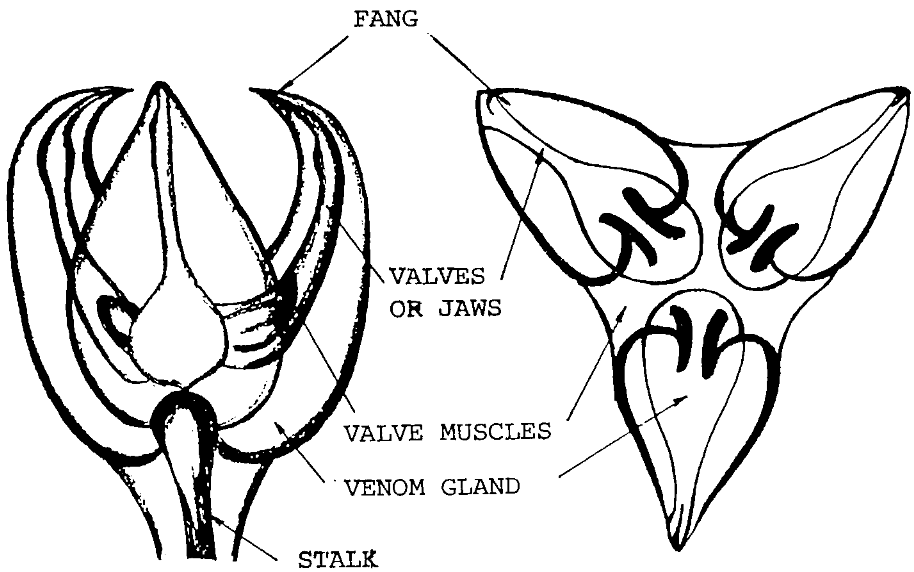


Fig. 27. A venomous globiferous pedicellaria of a sea urchin (Adapted from Halstead, 1965).

The cone shell toxin is used to paralyze prey. However, a sting from the *Conus* sp. is sometimes fatal to man. A person may be stung while swimming in waters where this species is present; or through carelessness while collecting specimens.

In the latter case, attempting to scrape the organic debris from the shell to see its pattern may stimulate the cone to extrude the proboscis far enough to reach the hand of the holder.

Figure 28 shows the structure of the venom apparatus which usually consists of venom bulb, venom duct, redular sheath, radular teeth and proboscis. The poison produced in the venom duct is transferred to the radular sheath where the radular teeth are charged with the venom. The venom bulb contains no poison and hence appears to serve as the pump that transfers the poison from the venom duct into the radular sheath which possesses a large number of hollow, chitinous radular teeth. The teeth are set one by one along the pharynx to the proboscis, where the foremost tooth points forward like a spear. Stinging is accomplished by extrusion of the proboscis, from which the redular tooth thrusts out. A tooth is used only once, and if it fails to reach the prey, a new one from the radular sheath is charged with poison.

Researchers are still actively studying the type of toxin present in cone shell and the specific symptoms as observed. However, Freeman, *et al.*, (1974), as cited by Hashimoto (1979), notes that the action of the venom on a muscular cell membrane is similar to that of tetrodotoxin.

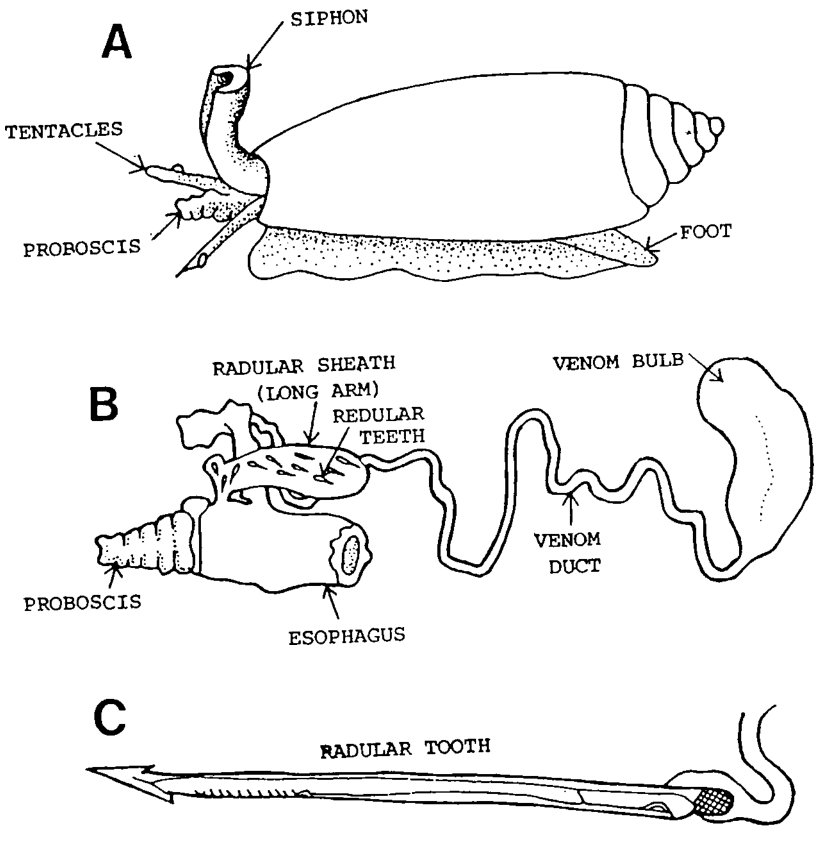


Fig. 28. Cone shell: External features (A); venom apparatus (B); and radular tooth (C). (Adapted from Halstead, 1965).

d. Venomous Fish

There are about 200 species of venomous fish with toxic spines and skins. Among them are stingray, scorpion fish and boxfish. Venomous fish are generally sedentary bottom dwellers, and their spines are considered defensive organs.

Stingrays are armed with a sharp single spine at the tail (see Fig. 29). The toxin contained in the spine is a mixture of unstable proteins. A sting generally occurs when the victim accidentally steps on the fish. A fierce pain, which lasts for a long period of time after the sting, can be felt. Death may occasionally follow. In many cases the symptoms are localized at the affected part, but sometimes nausea, weakness, unconsciousness, diarrhea, convulsions, and respiratory distress may occur.

Scorpionfish *(Inimucus japonicus)* and stonefish *(Synancya erosa)* possess stings and venom glands. Stonefish have caused a number of human deaths through stings by their extremely venomous spines (see Fig. 29). Boxfish on the other hand have a toxic substance in the mucous secretion of the skin.

To sum up, the most commonly encountered marine toxins or bio-toxins are shown in Table 11. The table gives the type of toxin, source, empirical formula, chemical classification, general toxicity, pharmacology and biological significance.

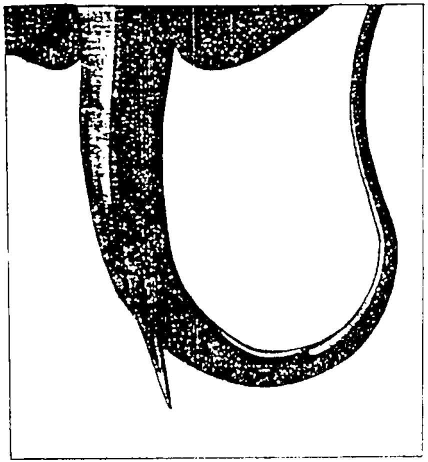
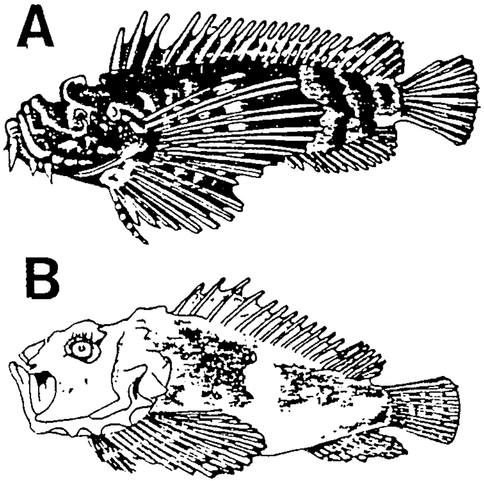


Fig. 29. The spine [illegible] a st[illegible]ngray



[illegible]corpionfish (A) *Inimucus* [illegible]*ponicus* and Stonefish (B) [illegible]*ja crosa*.

Table 11. Different toxins from species of marine organisms (After Angeles, 1981).

PROPERTY	OSTRACITOXIN-PAHUTOXIN	TETRADOTOXIN-TARICHATOXIN	CIGUATOXIN	HOLOTHURIN A
SOURCE ORGANISM	BOXFISH, OSTRACION LENTIGINOSUS	PUFFER, SPHOEROIDES RUBRIPES	MORAY EEL, GYMNOTHORAX JAVANICUS	SEA CUCUMBER ACTINOPYGA AGASSIZI
SOURCE ORGAN	SKIN MUCOUS SECRETIONS	VISCERA, GONADS AND SKIN	VISCERA AND MUSCLE	CUVIERIAN TUBULES
OCCURRENCE IN OTHER SPECIES	OTHER TRUNKFISHES (OSTRACIONTIDAE)	MOST PUFFERS (TETRAODONTIDAE. CANTHIGASTERIDAE AND DIODONTIDAE)	SEVERAL UNRELATED TROPICAL MARINE REEF FISHES	MOST SEA CUCUMBERS (HOLOTHUROIDAE)
EMPIRICAL FORMULA	$C_{23}H_{44}NO_5Cl$	$C_{22}H_{33}N_7O_{14}$	$C_{35}H_{65}NO_8$	$C_{50-52}H_{81-85}O_{5-6}SNa$
CHEMICAL CLASSIFICATION	CHOLINE CHLORIDE ESTER OF A FATTY ACID	(?)	LIPID CONTAINING QUATERNARY NITROGEN, HYDROXYL AND CARBONYL FUNCTIONS	STEROIDAL SAPONIN
GENERAL TOXICITY	ICHTHYOCIDAL WEAKLY TOXIC TO MAMMALS MLD TO MICE: 200,000 ug/k	NOT ICHTHYOCIDAL HIGHLY TOXIC TO ALL VERTEBRATES: MLD TO MICE 10 ug/k	NOT ICHTHYOCIDAL MODERATELY TOXIC TO WARM-BLOODED VERTEBRATES: MLD TO MICE 1,000 ug/k	ICHTHYOCIDAL; TOXIC TO A VARIETY OF MARINE ORGANISMS MLD TO MICE: 10,000 ug/k
PHARMACOLOGY	CAUSES HEMOLYSIS AND AGGLUTINATION OF RBC MUSCARINIC TYPE ACTIVITY ON NERVES	NOT HEMOLYTIC; RESEMBLES LOCAL ANESTHETIC, CAUSES RESPIRATORY FAILURE AND NEUROMUSCULAR BLOCK	NOT HEMOLYTIC; HAS CURARE-LIKE EFFECT ON NERVE-MUSCLE END PLATE	HEMOLYTIC; HAS IRREVERSIBLE EFFECT ON NERVES; DIRECT CONTRACTURAL EFFECT ON MUSCLE
BIOLOGICAL SIGNIFICANCE	ASSUMED TO BE REPELLENT	ASSUMED TO BE REPELLENT	UNKNOWN	ASSUMED TO BE REPELLENT

II. LOW RISK FISHERY PRODUCTS

1. Definition of Low Risk Fishery Products

Low risk fishery products are products that can cause sporadic cases of food infection or food-borne intoxication. The most common cause of this occurrence is post-process contamination due to poor hygiene and sanitation. Among the products examined are smoked fish, salted small crab, oysters and fish balls.

2. Some Low Risk Fishery Products

(a) Smoked Fish

Smoked fish or "tinapa" is a centuries-old product in the Philippines. This type of product is normally consumed as it is, without any cooking. In some parts of the Philippines such as the Tagalog region "tinapa" is incorporated in "pansit palabok" (a noodle recipe) or vegetable salads such as "kinilaw na labanos", while in other regions it is added in boiled or sauteed vegetables for flavoring.

A very serious problem in the fish smoking industry is the lack of attention given to hygiene. The unsanitary practices led researchers of the Department of Fish Processing Technology to conduct a survey on the microbial load of the locally processed and marketed smoked roundscad.

Based on some research findings, pre-cooked and cooked fishery products should have a minimum and maximum microbial load of

10^6 and 10^7 respectively (see Table 12). Furthermore, specifications for *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* have been established in Canada, UK, New Zealand, Australia and by FAO/WHO (see Table 13).

Survey results revealed that samples of smoked roundscad *Dicapterus macrosoma* processed in Tondo, Salinas and Navotas were far below the standard limits of microbial load, while those prepared by the Department of Fish Processing Technology met the standard (see Tables 14 and 15).

Market samples of smoked roundscad were found to contain pathogenic micro-organisms such as *E. coli*, *Staph. aureus*, *Vibrio* spp. and *Salmonella* spp. (see Table 16). Based on confirmatory tests (see Table 17), there were two species of *Escherichia* in the smoked roundscad, namely *E. aerogene* and *E. coli*.

Staph. aureus exhibited weak coagulation (see Table 18) but the possibility of toxin being produced in smoked roundscad remains high. *Vibrio* spp. and *Salmonella* were also confirmed to be present in smoked roundscad (see Tables 19 and 20). Determination of the product's water activity (Aw) showed that these pathogenic micro-organisms survived in the product because Aw was 0.92 to 9.8, a range very suitable for microbial growth and development (see Table 21).

It should also be noted that freshly smoked roundscad does not contain these pathogenic micro-organisms, if the processing plant observes good hygiene and sanitation (see Table 15). The products become contaminated only after a long

Table 12. Recommended microbial limits for pre-cooked and cooked fishery products. (After Cann and Taylor, 1981)

PRODUCT	ORGANISM	MINIMUM MICROBIAL LOAD	MAXIMUM MICROBIAL LOAD
PRE-COOKED FISH	Total count	10^6	10^7
	Faecal coliforms	4	400
	Staph. aureus	10^2	10^3
FROZEN, COOKED FISH	Total count	10^6	10^7
	Faecal coliforms	4	400
	Staph. aureus	10^3	2×10^7
	Vibrio parahaemolyticus	-	-

Table 13. Specifications for *Staph. aureus*, *E. coli* and *Salmonella* in cooked, frozen fishery products (recommended by ICMSF, UK, New Zealand, Australia and FAO/WHO).

COUNTRY/ AGENCY	*Staph. aureus*		*E. coli*		*Salmonella*	
	m*	M**	m	M	m	M
ICMSF	1×10^3	2×10^3	4	400	Not included in the specification	
UK	1×10^3	1×10^4	10	100	0	0
NEW ZEALAND	1×10^2	1×10^3	20	200	0	0
AUSTRALIA	1×10^2	1×10^3	9	70	0	0
FAO/WHO	5×10^2	5×10^3	Not included in the specification		0	0

* Minimum microbial load

** Maximum microbial load

Table 14. Comparative counts of presumptive pathogenic organisms of commercial and laboratory samples of smoked roundscad.

ORGANISMS	LABORATORY SAMPLE	SAMPLE FROM SALINAS	SAMPLE FROM NAVOTAS
* *E. coli*	2.7×10^3	2.65×10^5	6.3×10^5
* *S. aureus*	negative	2.85×10^5	2.0×10^4
* *V. parahaemolyticus*	negative	6.50×10^4	8.0×10^5
** *Salmonella* spp.	absent	present	present

* Count per gram sample

** Present in 25-gram samples

Table 15. Pathogenic micro-organisms of freshly smoked roundscad processed in Cavite, Tondo and the Department of Fish Processing Technology (DFPT).

PROCESSING PLANTS	*E. coli*	*Staph. aureus*	*Vibrio* spp.	*Salmonella* spp.
DFPT - University of the Philippines	-	-	-	-
CAVITE	-	-	-	-
TONDO	1.4×10^{6}	2.6×10^{7}	4.3×10^{6}	-

Table 16. Pathogenic micro-organisms of locally marketed smoked roundscad purchased in different markets

MARKET	*E. coli*	*Staph. aureus*	*Vibrio* spp.	*Salmonella* spp.
FARMER'S MARKET	5.95×10^5	5.0×10^4	5.0×10^4	+
NEPA-Q-MART	5.45×10^4	TNC $> 10^7$	TNC $> 10^7$	+
QUIAPO MARKET	5.50×10^4	TNC $> 10^7$	1.88×10^6	+
BLUMENTRITT MARKET	-	1.0×10^4	8.00×10^5	-

TNC - Too numerous to count

- Absent

+ Present

Table 17. Confirmatory test results of *E. coli* isolates from samples of smoked roundscad from different markets and Tondo processing plant

MARKET/PROCESSING PLANT	BIOCHEMICAL TESTS				ORGANISMS
	Indole	Methyl red	Voges-proskauer	Citrate	
FARMER'S	+	+	-	-	*E. coli*
QUIAPO	+	+	-	-	*E. coli*
NEPA-Q-MART	-	-	+	+	*E. aerogenes*
TONDO	+	+	-	-	*E. coli*

Table 18. Coagulase test results of presumptive *Staph. aureus* in samples of smoked roundscad from different markets and Tondo processing plant

MARKET/PROCESSING PLANT	COAGULASE TEST
FARMER'S	+
NEPA-Q-MART	+
QUIAPO	+
BLUMENTRITT	+
TONDO	+

Table 19. Confirmatory test results of *Vibrio* spp. isolates in samples of smoked roundscad from different markets and Tondo processing plant

BIOCHEMICAL TESTS	ORGANISMS		MARKET/PROCESSING PLANT				
	V.para	*V.cholerae*	FARMER'S	NEPA-Q MART	QUIAPO	BLUM.	TONDO
TRYPTONE 42°C	+	+	+	+	+	+	+
INDOLE	+	–	+	–	–	+	–
LYSINE DECARBOXYLASE	+	+	+	+	+	+	+
VOGES-PROSKAUER	–	+	–	+	+	–	+
TSI H2S	–	–	–	–	–	–	–
GLUCOSE FERMENTATION	Acid no gas	Acid no gas	Acid no gas	Acid no gas	Acid no gas	Acid no gas	Acid no gas
LACTOSE FERMENTATION	–	–	–	–	–	–	–
SUCROSE FERMENTATION	–	–	–	–	–	–	–

Table 20. Confirmatory test results of *Salmonella* spp. isolates in samples of smoked roundscad from different markets

MARKET	BIOCHEMICAL TESTS			
	TSL*	Lysine Decarboxylase	Lysine** Iron	Urea
FARMER'S	+	+	+	−
NEPA-Q-MART	+	+	−	−
QUIAPO	+	−	+	−

* H_2S Production, fermentation (acid + gas) of glucose

** H_2S Production, decarboxylation of Lysine, fermentation of dextrose.

Table 21. Moisture, salt content and Aw of market samples of traditionally smoked roundscad

MARKET	SOURCE	SPECIES	MOISTURE CONTENT%	NaCL %	Aw
QUIAPO	TONDO/NAVOTAS	ROUNDSCAD	55.3	5.36	0.93
NEPA-Q-MART	TONDO	"	57.6	6.37	0.92
	CAVITE	"	61.5	4.02	0.95
FARMER'S	CAVITE	"	66.05	1.58	0.98
	TONDO SMOKING PLANT	"	58.20	4.78	0.94
	CAVITE SMOKING PLANT	"	65.6	2.55	0.97

period of exposure to market conditions. Hence, post-process contamination plays a significant role in making the product risky to human health.

The presence of the pathogenic micro-organisms mentioned above can cause gastroentiritis.

(b) Salted Small Crab

Salted small crab is a delicacy in the northern part of the country, especially in the province of Pampanga. The product is prepared by simply sprinkling the crabs with a small amount of salt, and the product is kept at room temperature overnight. Sporadic cases of gastroenteritis after eating this type of product have been observed. However, no official report has been recorded.

Arafiles and Bulaong (1981) conducted a study on the appropriate ratio of salt to small crab, and the length of time the product should be stored to make it safe for human consumption. The study revealed that *Vibrio cholerae* might be responsible for sporadic gastroenteritis, especially if the small crab is salted with 1.20% salt (w/w) and kept overnight.

However, higher salt ratios of 25% and 30% with a longer storage time (48 hrs.) gave a different species of micro-organism (see Tables 22-23).

(c) Oysters

Oysters are commonly eaten raw with the addition of vinegar, onions, garlic and ginger. However, these shellfish become dangerous during the rainy season, especially if caught in polluted water or near a community. Since oysters are filter feeders most of the micro-organisms remain in their body. Upon microbial examination micro-organisms of public health interest were found to be present. Whereas smoked fish get pathogenic micro-organisms due to post-process contamination or dirty hands and equipment, oysters carry these micro-organisms because of their feeding habits.

Aside from pathogenic micro-organisms, viruses such as poliovirus have been isolated from oysters. In France, 10-20% of the shellfish examined were found to contain viruses. It was noted that shellfish are capable of bioaccumulating viruses from sea water, and retain the viruses for several days even when transferred to clean water. Shellfish become contaminated with viruses in areas where untreated human sewage is disposed of. Fig. 30 shows the typical shape of a virus called bacteriophage that attacks bacteria.

(d) Fish Balls

Most fish balls are made of flour and fish or shrimp extracts. The method of preparation is often so unhygienic that microbial examination has revealed the presence of all pathogenic micro-organisms. Fish balls were found to contain *Staph. aureus* beyond 10^7.

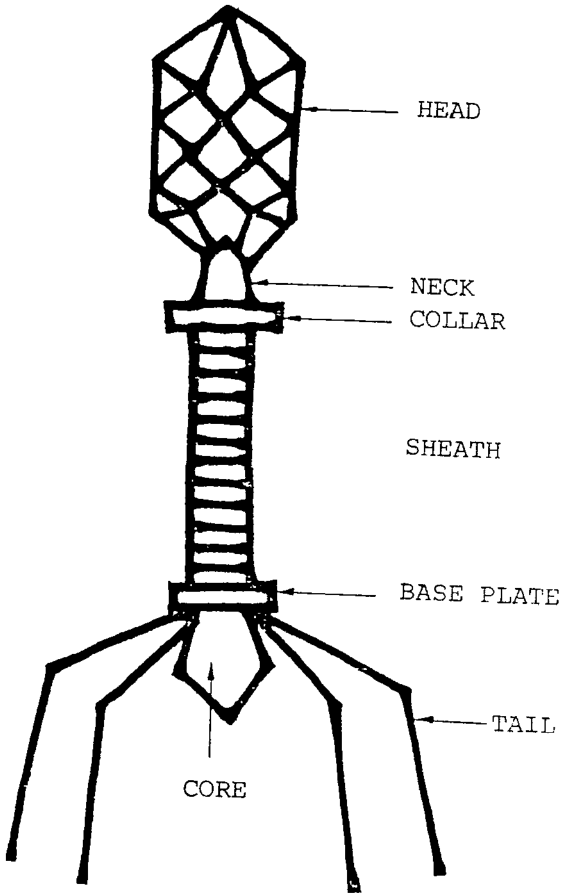


Fig. 30. Typical shape of a bacteriophage

Table 22. Confirmatory tests of salted small crab for *V. cholerae* at 24 hrs of storage.

BIOCHEMICAL TESTS	*V. cholerae*	ISOLATES FROM SALT %							
		1%	5%	7%	10%	15%	20%	25%	30%
GLUCOSE (ACID)	+	+	+	+	+	+	+	+	+
MANNITOL (ACID)	+	+	+	+	+	+	+	+	+
INOSITOL (ACID)	-	-	-	-	-	-	-	-	⊕
H_2S IN TSL	-	-	-	-	-	-	-	-	+
GAS FROM GLUCOSE	-	-	-	-	-	-	-	⊕	+
GROWTH AT 0% NaCL	+	+	+	+	+	+	+	+	+
GROWTH AT 3% NaCL	+	+	+	+	+	+	+	+	+
GROWTH AT 8% NaCL	-	-	-	-	-	-	-	-	-
GROWTH AT 11% NaCL	-	-	-	-	-	-	-	-	-
GROWTH AT 1% TRYPTONE	+	+	+	+	+	+	+	+	+
ARGININE DIHYDROLASE	-	-	-	-	-	-	-	⊕	-
LYSINE DECARBOXYLASE	+	+	+	+	+	-	+	+	+
ORNITHINE DECARBOXYLASE	+	+	+	+	+	+	+	+	+

NOTE: Encircled data are negative for *V. cholerae*.

Table 23. Confirmatory tests of salted small crab for *V. cholerae* with higher salt concentrations at different lengths of storage.

BIOCHEMICAL TESTS	*V. cholerae*	ISOLATES AT % SALT AT H.R. INDICATED							
		15%		20%		25%		30%	
		24	48	24	48	24	48	24	48
GLUCOSE (ACID)	+	+	+	+	+	+	+	+	+
MANNITOL (ACID)	+	+	+	+	+	+	(−)	+	+
INOSITOL (ACID)	−	−	−	−	−	−	(+)	(+)	(+)
H_2S IN TSL	−	−	−	−	−	−	(+)	+	+
GAS FROM GLUCOSE	−	−	−	−	−	+	+	+	+
GROWTH AT 0% NaCL	+	+	+	+	+	+	+	+	+
GROWTH AT 3% NaCL	+	+	+	+	+	+	+	+	+
GROWTH AT 8% NaCL	−	−	−	−	−	−	−	−	−
GROWTH AT 11% NaCL	−	−	−	−	−	−	−	−	−
GROWTH AT 1% TRYPTONE	+	+	+	+	+	+	+	+	+
LYSINE DECARBOXYLASE	+	(−)	(−)	+	+	+	+	+	(−)
ARGININE DIHYDROLASE	−	−	−	−	−	(+)	−	−	−
ORNITHINE DECARBOXYLASE	+	+	+	+	+	+	+	+	−

NOTE: Encircled data are negative for *V. cholerae*.

This organism is capable of producing enterotoxin which is heat resistant. Hence cooking will not completely eliminate the organism. Victims suffer from severe gastroenteritis and sometimes vomiting.

III. PROPER WAYS OF HANDLING AND PREPARING TOXIC MARINE ORGANISMS AND LOW RISK FISHERY PRODUCTS

There are several reasons why toxic marine organisms and low risk fishery products, may have a toxic effect on consumers. These include the following:

1. Lack of basic information on:

    a) toxic species of marine organisms;

    b) seasonal variation of the sea environment (e.g. effect of plankton bloom, pollution, etc.).

2. Absence of physical signs. For example, in scombroid poisoning, signs of toxic build-up are not observable; sensory evaluation of odor and other signs of toxic build-up is not possible; sensory evaluation of odor and appearance does not indicate the stage of spoilage of the fish and since the rate of spoilage is different in individual fish, it is very difficult to detect in the container of fish those that have a high histamine content.

3. Unhygienic practices and lack of proper handling and sanitation in fish processing plants.

4. Lack of quality control and standards for fish and fishery products.

It is therefore the aim of this manual to indicate easy methods for product preparation, as well as for proper handling and packaging of food so that it can be safely stored and will keep for a longer period of time. Some suggestions are given below.

(a) Puffer Fish

Following are suggestions for minimizing puffer fish poisoning:

1) Identify the fish first. The only species of puffer fish that can be eaten safely is *S. lunaris*.

2) Separate the muscle immediately from the rest of the body parts after catching the fish.

3) Liver and gonad or eggs or fish roe must not be included in the cooking or preparation of dishes.

4) If there is any uncertainty that the puffer fish can be utilized convert it to meal instead of consuming the flesh (see Fig. 31). Pressing the fish has been found to leach out toxins, thus, reducing the risk of poisoning.

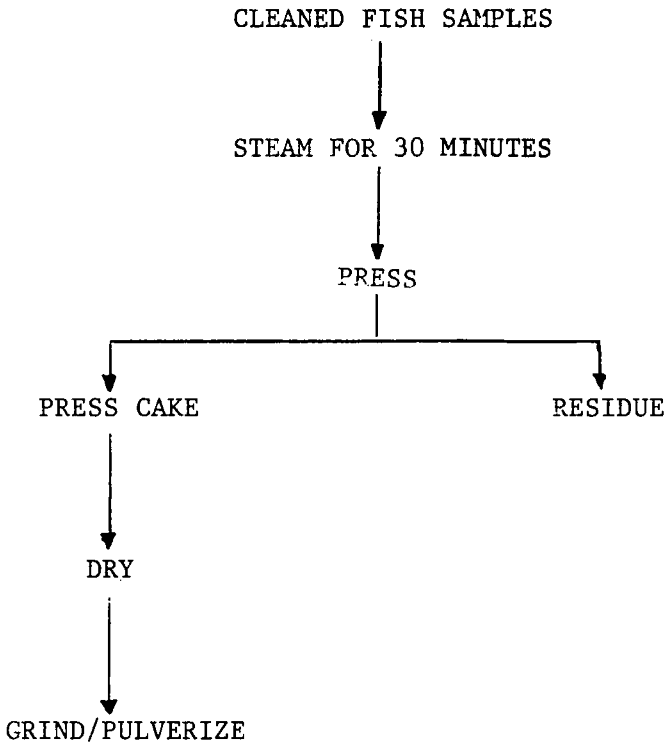


Fig. 31. Schematic diagram of puffer meal production.

(b) Species of Marine Organisms with Saxitoxin and Ciguatera Poison

These two types of toxins are very hard to detect and vary considerably depending upon the season of the year. For shellfish it is advisable to soak or rinse the shellfish in clean water for three or more days to allow the organisms to get rid of the content of the digestive system. In addition, it is suggested to keep a record of the months when fish and shellfish become toxic, and refrain from catching and consuming them during that season of the year.

(c) Tuna, Tuna-like species and Mackerel

The best way to prevent formation of histamine is to place the fish at once in a low temperature (from - 32°C to 0°C) environment. This condition will arrest some microbial activity. If freezer, refrigerator or cold storage facilities are not available, gutting and bleeding the fish at once and putting them in chilled water will also minimize histamine formation. It should be noted that when using ice the ratio of ice should be greater than the volume of fish for the treatment to be effective.

(d) Jellyfish

1) Identify the species first. The most common and widely distributed jellyfish is the *Aurelia* or moon jellyfish. It is umbrella-shaped, generally colorless but occasionally

tinted pink or violet. Sun jellyfish or sea blubber *(Cyanea)* is reddish in color and grows to a big size. *Pelagia* or pink jellyfish has the shape of a circular disk with scalloped margin. Much larger jellyfish than *Pelagia* are the purple-striped jellyfish, brown-striped jellyfish *(Chrysaora)* which is bluish in colour, streaked with brown, having 24 tentacles along its scalloped margin, the root-mouth jellyfish *(Stomolophus)* and others.

2) The method of processing jellyfish is as follows:

a. Soak in a saturated brine solution for one day to remove slime and materials causing itchiness, then take out and clean with a brush.

b. Place the cleansed jellyfish in another container and add salt, lime and sodium bicarbonate; leave there for three days to leach out the liquid; the amount of sodium bicarbonate should not be excessive to avoid spoiling the tissue of the jellyfish.

c. Remove from solution of salt, lime and sodium bicarbonate and soak again in a brine solution for 3-4 days stirring the solution occasionally.

d. Transfer to freshly prepared solution of salt, lime and sodium bicarbonate (at this stage the quantities of the ingredients are reduced).

e. Drain.

(e) Sea Cucumber

1) First identify the species.

2) Make a slit and remove the visceral organs and clean well; be sure to wear gloves to protect the hands.

3) Boil in a 15% brine solution for 10 to 20 minutes.

4) Drain off the liquid and dry in the sun.

5) Smoke to further reduce moisture.

(f) Low Risk Fishery Products

1) Always observe proper hygiene and sanitation in the processing plant, such as frequent washing of hands with soap and water or wear plastic gloves, to prevent post-process contaminations. Make sure that the equipment is clean and properly sanitized.

2) Always cook the products before eating. Avoid eating them raw.

3) Always re-heat refrigerated left-overs smoked fish, fish balls, etc.

4) Always pack the product carefully to prevent contamination from flies, rodents and other pests.

Annex

Food-caused Illnesses Their Sources and Symptoms.

Type of Microbial Poisoning/Source of Illness/Symptoms

Campylobacterosis (Food Infection)

Raw poultry, meat and unpasteurized milk. Other sources are not yet known.

Onset: 2-5 days after eating. Diarrhea, abdominal pain, fever and sometimes bloody stools. Lasts 2-7 days.

Guardiasis (Food Infection)

Unwashed raw fruits and vegetables that are contaminated by sewage or polluted water.

Diarrhea, abdominal pain and bloating; loss of appetite, and nausea.

Hepatitis A (Food Infection)

Sources are similar to the sources of viral gastroenteritis.

Onset: 2-6 weeks after ingestion. Fever weakness, loss of appetite, vomiting followed by yellowing of the skin and white of eyes.

Source: Good Housekeeping. 1983. vol. 196, No. 6, p. 219.

Salmonelliosis (Food Infection)

Raw meat, poultry, eggs, fish, milk or foods made from milk, egg, fish, meat and poultry. Bacteria multiply at room temperature.

Onset: 12-48 hrs. after eating. Nausea, fevèr, headache, abdominal cramps, diarrhea and sometimes vomiting. Can be fatal in infants, the elderly and the infirm.

Shigellosis (Food Infection)

Mixed salads which include fish, poultry, potato, macaroni, and egg salad. Bacteria introduced to food by infected persons handling them. Bacteria multiply at room temperature.

Onset: 1-4 days after eating. Abdominal pain, cramps, fever, diarrhea, sometimes vomiting, and blood, puss or mucus in stools. Can be serious in infants, the elderly and infirm.

Viral Gastroenteritis (Food Infection)

Chief sources: shell-fish from contaminated water and food that are handled a lot and eaten raw like vegetables.

Onset: 12-48 hrs. after eating. Severe diarrhea, nausea and vomiting. Mimics flu.

Botulism (Food-borne Intoxication)

Improperly canned or vacuum packed foods such as fish meat, poultry and vegetables.

Onset: 3 hrs. to 8 days after eating. Blurred or double vision, nausea, vomiting, cramps, diarrhea, inability to swallow, speak, breathing difficulty and uncoordinated movements. Get immediate medical help.

Staphylococcal Food Poisoning (Food-borne Intoxication)

Meat, poultry, egg products, tuna, potato-macaroni salads, cream-filled pastries. Bacteria multiply at room temperature.

Onset: 1-8 hrs. after eating. Vomiting, nausea, abdominal cramps. Lasts 24-48 hrs.

Clostridium perfringens Food Poisoning (Food-borne Intoxication)

Meat and poultry, and foods made with these. Bacteria multiply at room temperature.

Onset: 8-22 hrs. after eating. Abdominal pain and diarrhea. Sometimes nausea and vomiting. Lasts about 24 hrs.

Trichinosis

Raw or inadequately cooked or processed pork or pork products.

Onset: 12-24 hrs. after eating. Diarrhea, nausea, vomiting, abdominal cramps, fever, facial swelling (particularly of the eyelids), muscular pain.

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LIST OF SAFIS EXTENSION MANUALS

SEC/SM/1	Khumua liang pla namcheut (Freshwater Fish Farming: How to Begin), (original; English; translated: Thai)
SEC/SM/2	Oyster Culture (original: Bahasa Malaysia; translated: English)
SEC/SM/3	Mussel Culture (original: Bahasa Malaysia; translated: English)
SEC/SM/4	Ang pagpuna ug pagtapak sa pukot (Net Mending and Patching), (original: English; translated: Cebuano-Bisaya)
SEC/SM/5	Mussel Farming (original English)
SEC/SM/6	Menternak Ikan Airtawar (Freshwater Fish Farming: How to Begin), (original: English: translated: Bahasa Malaysia)
SEC/SM/7	Makanan dan Pemakanan Udang Harimau, *Penaeus monodon* (Nutrition and Feeding of Sugpo, *Penaeus monodon)*, (original: English; translated: Bahasa Malaysia)
SEC/SM/8	Macrobrachium Culture (original: Thai; translated: English)
SEC/SM/9	Selection of Marine Shrimp for Culture (original: Thai; translated: English)
SEC/SM/10	Induced Breeding of Thai Silver Carp (original: Thai; translated: English)
SEC/SM/11	Culture of Sea Bass (original: Thai; translated: English)

SEC/SM/12 Smoke-curing of Fish (original English)

SEC/SM/13 Cockle Culture (original English)

SEC/SM/14 Net Mending and Patching (original English)

SEC/SM/15 Kanliang hoy malangphu (Mussel Farming), (original: English; translated: Thai)

SEC/SM/16 Nursery Management of Prawns (original: Bahasa Indonesia; translated: English)

SEC/SM/17 Culture of Sultan Fish (*Leptobarbus hoevenii)*, (original: Bahasa Malaysia; translated: English)

SEC/SM/18 The Use of the Traditional Drying Method and Solar Drier for Croaker, Mullet and Herring (original English)

SEC/SM/19 Shrimp Culture (original: Thai; translated: English)

SEC/SM/20 Rok Plaa (Fish Diseases), (original: English; translated: Thai)

SEC/SM/21 Kanliang phomae pan *kung* kuladam (Broodstock of Sugpo, *Penaeus monodon*, Fabricius), (original: English; translated: Thai)

SEC/SM/22 Nakakaing Krustasyo ng Pilipinas (Field Guide to Edible Crustacea of the Philippines), (original: English; translated: Tagalog)

SEC/SM/23 Khumua kanliang plaa nai krasang thi Singapore lae Indonesia (Floating Net-cage Fish Farming in Singapore and Indonesia), (original: English; translated: Thai)

SEC/SM/24 Samll Ring Net Fishing (original English)

SEC/SM/25 Handbook on Some Toxic Marine Organisms (original: Tagalog; translated: English)

SAFIS

O What is SAFIS?

SAFIS is the Southeast Asian Fisheries Information Service. It is a project of the SEAFDEC Secretariat set up to provide extension materials for small-scale fishermen and fish farmers in the region.

O What are its objectives?

The immediate objectives are to collect and compile fisheries extension manuals, brochures, pamphlets and related aids for small-scale fisheries development, and to translate selected literature into local languages for distribution to fisheries extension workers in Southeast Asia.

O <u>What services will SAFIS provide?</u>

SAFIS will attempt to provide information and publications such as:

- lists of available texts in fisheries extension services,
- translation of suitable manuals,
- manuals on appropriate technologies,
- photocopies of appropriate fisheries extension literature,
- a current awareness service of regional fisheries.

O <u>How much will these services cost?</u>

A nominal cost of US $0.15 per page will be charged for photocopying, handling, and surface mail. Airmail costs will be extra. The cost of reproduction will vary according to number of page.

SAFIS is grateful for financial support received from the International Development Research Centre (IDRC) of Canada.

SAFIS is the Southeast Asian Fisheries Information Service. It is a project of the SEAFDEC Secretariat set up to provide extension materials for small - scale fishermen and fish farmers in the region. For additional information, contact the Project Leader of SAFIS

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