Poison Fish Projects Report*

SINCE 1958, the problem of ciguatera has been investigated at the Hawaii Marine Laboratory. Fish poisoning in the tropical Pacific has become increasingly common and, according to doctors in New Caledonia, cases of fish poisoning occur almost daily. Unfortunately, research is often hampered because patients rarely know the proper name of the fish they have eaten or recall where it was caught. This report covers the calendar year 1963, and describes the progress made in pursuing research into the problem.

The investigation has concerned itself with many phases of the problem; the biology of the fish that carry the toxin causing ciguatera and the possible origin of the toxin; the chemical isolation and identification of the toxin; the pharmacological action of the toxin; the epidemiology and symptomatology of the disease; the preliminary investigation of other toxic forms to see if they carry a toxin similar to that of the ciguateric fish; preliminary studies of native remedies for ciguatera; and even a compilation of names of plants and animals in the Pacific languages to facilitate the gathering of information. These various phases of the projects are coordinated and interdigitated, sharing personnel, laboratory space and facilities, specimens, costs of field work and collecting, and office and laboratory help.

Of major importance in 1963, and affecting all phases of the project, was the loss of a suitable location for the procurement of specimens and for field studies. Throughout the summer of 1962, fish were obtained and studied from Palmyra Atoll in the Line Islands, with supplemental catches and information being obtained from Christmas Island in the same archipelago. However, Palmyra no longer can be reached either by military or civilian transport, and the fish at Christmas Island, once moderately toxic, have so declined in toxicity as to render the island unsatisfactory for all phases of the study. Efforts to find a new site are discussed below.

It should be noted that the programme has almost entirely recovered from the losses of the Hawaii Marine Laboratory fire of 1961, as it was possible to move personnel into a new, but small, laboratory in the late spring of 1963.

BIOLOGY

Field Investigations

In the early summer a field party was sent to Christmas Island, under the direction of Dr. Helfrich, with the objectives of studying the habits of the toxic red snapper, *Lutjanus bohar*, to contrast the fish, and the reef and food chain in toxic and non-toxic sections of the reef, to collect a large amount of the toxic fish for laboratory study, and finally to collect potentially toxic fish in the food chain of the snapper. While all of these aims were accomplished in the field, the decline in toxicity, discovered upon the return of the specimens to the laboratory, rendered most of the studies meaningless.

During the autumn of 1963, Dr. Helfrich visited Fiji to make field observations and to investigate the possibilities of procuring a steady supply of toxic fish. While there, he was able to visit several islands in addition to the main island of Viti Levu. He arranged his trip to coincide with the rise of the balolo and to obtain an extensive sample of the sardines which are reportedly toxic in the season of the balolo rise. His trip continued to the Society Islands, where he spent one week in the Windward Group, again making field observations and arranging for possible future procurement.

Dr. Helfrich shown recording details of fish caught during a visit to New Caledonia.

The possibility of Samoa for field studies was investigated by the principal investigator while he was there for other purposes during the summer. Evidently the problem of toxic fish in both American and Western Samoa is not acute, and the archipelago would not be suitable either for regular procurement or for field studies. Arrangements were made to obtain a sample of the reputedly toxic fish from Tutuila.

For procurement of highly toxic fish, one of the most promising sites is Johnston Island. On this island *Lutjanus bohar* does not occur and most of the reef fish are non-toxic (and are eaten by the civilian workers), but the large and abundant Gymnothorax are highly toxic. Plans have been made to work jointly with the Atomic Energy Commission investigation on the biological

Under the heading "Assistance to Research," the Commission, for some years, has given financial assistance to research on fish toxicity. This report indicates that one of the problems causing delay in this field is the poor supply of poisonous fish to the Laboratory. As the dispatch of whole fish is costly, the best method is to crush the flesh, and, after placing it in a drier at 167°F, reduce it to flakes. This is possible in some territories. To our readers in New Caledonia, where fishing is such a popular sport, we would suggest that they would be well advised to test a small quantity of any suspect fish on an animal, and keep the rest in a refrigerator pending the outcome of the experiment. If the fish proves to be toxic it could be sent to the South Pacific Commission in Noumea, who will ensure its subsequent dispatch to the Laboratory in Hawaii.

* From the Hawaii Marine Laboratory, University of Hawaii, 1963.

effects of dredging of the lagoon. There it is hoped to conduct a study on settlement of "new surfaces" that are postulated as the ultimate source of the toxin by Randall (1958), using, both test panels and the surfaces exposed by the dredging operations.

Food Chain Transmission of the Toxin

During 1963, the five-year study on the comparative biology and food habits of *Lutjanus bohar,* the red snapper, and other similar groupers and snappers was completed. The study embraced a total of about 1,800 specimens, and included both the food habits and field observations on their biology.

As the food habit study of *Lutjanus bohar* indicated that the single most common food are the acanthurids, and as it is known that acanthurids will cause ciguatera, a study is being initiated on the food habits of *Acanthurus triostegous* and *Ctenochaetus striatus.* These fish appear to be algal feeders specializing in the finer algae, as indicated by Randall (1958). However, as they appear to eat any of the finer algae available, it is not known whether any decisive leads to the possible source of the toxin will be found.

From the epidemiological study by Mrs. M. J. Cooper, an officially appointed associate, there came an indication of a possible algal source of the toxin. She reported that the Gilbertese on the island of Marakei stated that, coincidental with the development of a highly toxic condition in the reef fishes, there appeared conspicuous patches of a previously uncommon algae. They attribute the toxicity to this algae. Mrs. Cooper, when on the island in 1962, observed that the acanthurids appeared to feed upon this algae, and she was able to collect and preserve a small sample of this. It has been identified as *Plectonema terebrans,* one of the cyanophytes. It was planned to make a special trip to the isolated island during the summer of 1963, but arrangements could not be made before the start of the autumn school-term. Through the cooperation of the Honolulu Biological Laboratory, the U.S. Fish and Wildlife research ship was able to make a one-day stop at Marakei during the autumn and to collect some potentially toxic algae and fish (received at the laboratory in late December). It is now planned to visit the atoll during the early summer of 1964.

Laboratory Studies on Food Chain Hypothesis

As a necessary corollary to the food-chain hypothesis of the origin and transmission of the toxin, the fish, when feeding on toxic food, must become toxic themselves without harm to their own physiology and they must store, rather than metabolize or excrete, the toxin. The induction of toxicity through diet in normally non-toxic fish was studied previous to the Hawaii Marine Laboratory fire, and fortunately enough specimens, although without adequate data, were salvaged from the fire to publish a short paper on this in 1963. In this study it was found possible to induce toxicity in a normally non-toxic Hawaiian species by feeding it the flesh of toxic *L. bohar.* Unfortunately, the data were not quantitative, and a new experiment has been started to show the quantitative transmission of the toxin.

In December, 1961, a number of toxic *Lutjanus bohar* were introduced from Christmas Island to the laboratory ponds. Since that date they have been fed on a non-toxic diet. A small number of these fish are sampled for toxicity periodically. Because the samples have been small and the original level of toxicity not high, no statistical studies on the possible decline of toxicity can be made until the experiment is concluded some time in 1964. However, in the two-year period there has been no obvious loss of toxicity.

Toxins of Other Species of Fish

Preliminary extractions of the grouper, *Epinephelus fuscoguttatus,* and the morays, *Gymnothorax flavomarginatus* and *javanicus,* have shown that toxins may be extracted in a fashion exactly parallel to that method used for *Lutjanus bohar.* Also, no difference was observed in the symptomatology of the toxin carried by these fish in either of the test animals, the mongoose or the mouse. Pending the actual chemical identification of the toxin of *L. bohar,* it is presumed on the basis of this evidence that the toxin in these fish is similar to or identical with that found in *L. bohar.* Similarly, it has been impossible to detect chemical or pharmacological differences between the toxins from Gymnothorax from either the Line Islands or from Johnston Island.

There are now available toxic *L. bohar* or other species of fish from New Caledonia, Fiji, the Society Islands, Johnston, and Wake Islands. During 1964 it is hoped to make similar comparative studies on their toxins.

However, parallel chemical extracts on the acanthurid, *Ctenochaetus striatus,* have shown a difference in their toxin from that found in the large carnivores. In the extraction method the toxin is separated from ethanol-water mixture by the use of diethyl ether, with virtually all of the toxin of the carnivores going into the diethyl ether layer. However, on the few acanthurids tested, the toxin remains in the aqueous layer. This might be the result of one of three things—

(1) That the acanthurids bear a precursor of different chemical structure and solubility which is chemically changed by the metabolism of the carnivore;

(2) That the acanthurids bear an entirely different toxin, and that the food chain hypothesis is incorrect;

(3) That because of different water content and a different series of fat-soluble compounds—therefore a different extraction system—the toxin, while the same, will appear in the alcohol-water fraction. This will be investigated again as soon as an adequate supply of toxic acanthurids can be obtained.

Bioassay

Dissatisfaction continues with the present methods of bioassay, which use the reactions of the mongoose to test feedings for original screening of the potentially toxic fish, and the reactions of mice to intraperitoneal injections of potentially purified extracts. At present a new system for simple and rapid extraction of the toxin from the fish is being explored. The Bligh and Dyer tri-solvent method of lipoid extraction was tested and found to be rather unreliable. The use of acetone is now being investigated to extract small samples of fish. The extract is to be injected intraperitoneally into mice. It is hoped that this method will lead to a more rapid and more quantitative method of screening of fish in the laboratory and may also lead to a more simple field test.

To substitute for the mouse test in the chemical extract of the toxin, the Laboratory again explored the impedence of the nerve impulse at the neuromuscular junction in pharmacological preparations. The results were unsatisfactory, and the project has been deferred until a qualified pharmacologist can be obtained.

CHEMISTRY

Procurement

As indicated above, the supply of highly toxic fish obtained at Palmyra was exhausted during the year. With the fish from the Line Islands no longer available, the major effort during the autumn was to obtain a new source of fish for the chemical studies. Unfortunately, the chemical studies demanded a very large and steady supply of highly toxic fish, and the lack of this supply impeded the investigations. It is hoped that the possible procurement systems in Fiji, the Societies, and Johnston Island will soon be producing an adequate supply.

Isolation and Identification

During the year, new steps were added to the procedure for chemical isolation of the toxin. These include precipitation of impurities in low temperatures using different solvent systems in silicic acid columns. This improved procedure has led to the final isolation of the toxin.

The toxin, as obtained, has been subjected to a series of tests including thin-layer chromatography, paper electrophoresis, and counter-current distribution, and gives every indication of a pure compound. However, the compound has not been crystallized. The amounts obtained to date have been too small for chemical tests for structural analysis (the present yield is about two parts in a million of raw fish flesh). As soon as an adequate supply of toxic fish is obtained, the studies will continue on the elemental and structural analysis of the toxin.

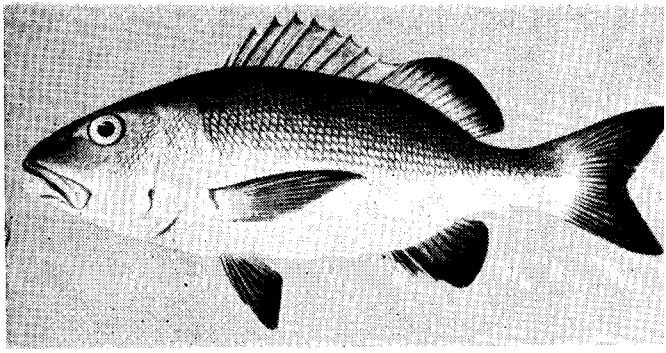
Colorimetric Chemical Tests for the Toxin

During the year 1962-1963, Dr. Motokazu Asano, a biochemist from Japan, was associated with the programme. With the aid of funds from the University of Hawaii East-West Center, he explored the reaction of the flesh of toxic fish to over forty known chemical tests for steroids and related compounds. He found some correlation between toxicity and colour reaction in the Lieberman-Burchard and Salkowski tests, but evaluation of the data revealed that the results did not have sufficient statistical reliability for use as a bioassay. It is now presumed that the colour resulted not from the toxin but from some steroid coincidental in its distribution with the toxin.

Other Toxins

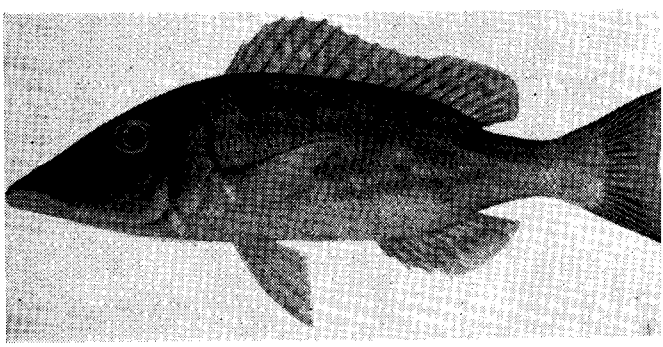
In 1962 preliminary investigations of a poisonous coelenterate, *Palythoa vestitus*, indicated that this coelenterate was indeed toxic, but the toxin was not closely related to that causing ciguatera. With the partial support of this investigation, but with major supports from other grants, Dr. Richard Moore, working with Dr. Scheuer, was able to isolate the toxin from this zoanthid. They anticipate publishing upon their methods and the empirical formula of the toxin within a few months.

Two students, one for his master's degree and the other for his doctorate, completed theses during the year on marine toxins; both had the partial support of the combined investigations. Mr. William Eger, for his master's thesis, was able to show that the puffer fish, *Arothron hispidus*, produced in its skin a toxin similar to, if not identical with, the toxin found in the liver and gonads. Mr. Eger also reported upon a survey of similar toxins in other plectognath fishes. Mr. Donald A. Thompson established that the boxfish, *Ostracion lentiginosus*, when subjected to stress, produced a toxin from skin glands and that this water-soluble toxin had a lethal effect upon fish in the vicinity.



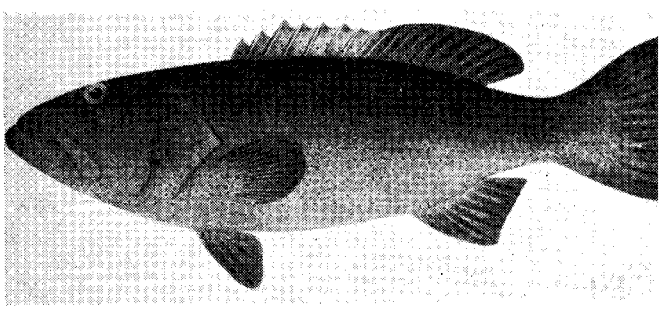
LUTJANUS BOHAR Forksal.

The red snapper, red emperor, l'anglais. This fish is probably the most consistently toxic fish in the tropical Pacific. Alive, it is a bluish hue on top, and pink to red along the belly; however, soon after dying the fish turns a brilliant red.



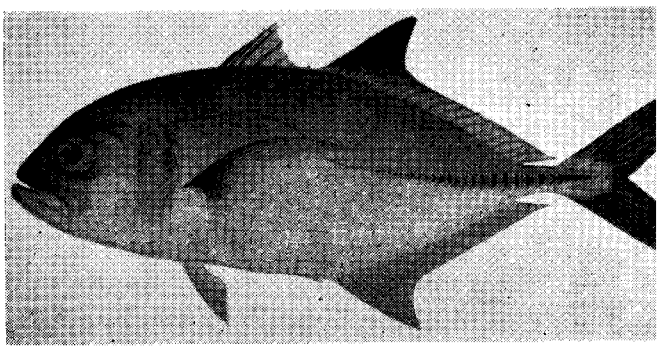
LUTJANUS MINIATUS (Schneider).

Scavenger fish, sweetlips. This is a silvery grey fish, and is often quite toxic in poisonous areas.



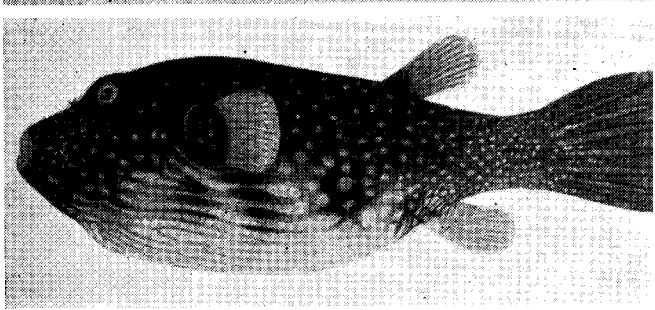
PLECTROPOMUS TRUNCATUS Fowler.

One of the groupers; light-brownish with numerous bright blue spots. It is frequently violently toxic.



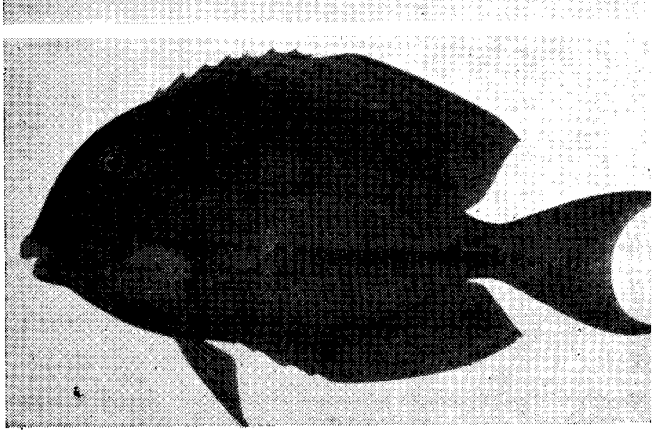
CARANX LESSONII Cuvier & Valenciennes

One of the jacks, trevally, or crevally. This fish, usually highly esteemed as food, becomes toxic in areas such as the Palmyra Atoll. In Fijian waters it is reported to be toxic only during the balolo season.



AROTHRON HISPIDUS (Linnaeus); also known as **TETRAODON HISPIDUS.**

One of the numerous puffers or toadfish, which are characterized by their ability to inflate themselves into a balloon. These fish, in all parts of their range, are reputed to be highly toxic unless correctly prepared for cooking. Also related is the toxic balloon fish, with sharp spines, often known as the porcupine fish (Diodon).



CTENOCHAETUS STRIGOSUS (Bennett).

A black surgeon fish with stripes —known in Tahiti as the Maito. This is an herbivorous fish that becomes highly toxic in certain areas, and is fed upon by the larger snappers and groupers; it may be important in the transmission of the toxin along the food chain.

PHARMACOLOGY

Because of the inability to find a qualified pharmacologist to work full-time with the Laboratory, only slight progress was made in pharmacological studies. On the advice of the consultant, Dr. Hamilton Anderson, the graduate assistant explored the "beer-can test" developed by Dr. Dermott B. Taylor, of U.C.L.A., for drugs. While the mice responded to ciguatera toxin in this test, it was found that the test did not give more accurate results than the previously used test and took considerably longer. As noted above, no further advances were made on the test using neuro-muscular preparations. At the end of 1963 we were hopefully awaiting permission to be granted by the U.S. Consulate, for Mr. Kwan-ming Li, from Hong Kong, a man experienced in pharmacology, to join the staff for one to two years, and we were also arranging for Dr. Yasumi Ogura, a leading Japanese pharmacologist working on the fish toxin, to visit the Laboratory for three months during the summer of 1964.

NATIVE REMEDIES

Through the help of Dr. Jacques Barrau of the South Pacific Commission, samples of *Duboisia myoporides* from New Caledonia were obtained during the year. This species of *Duboisia* has been reported to be of specific use by the New Caledonians for the treatment of ciguatera, and has been shown by chemical studies to contain a number of alkaloids. Preliminary studies of the extract gave ambivalent results when injected into mice in which controlled ciguatera had been produced. More precise experiments on this reaction are continuing.

During his visit to Fiji, Dr. Philip Helfrich was able to obtain samples of some *Ficus obliqua* which is used for the treatment of ciguatera in those islands. It is planned to investigate this remedy as soon as studies on *Duboisia* are completed.

Through epidemiological questionnaires (see below), information is being obtained on other native remedies.

EPIDEMIOLOGY

Dr. Philip Helfrich published a compilation of all cases of fish poisoning reported in the Hawaiian Islands in this century. Most cases were the result of eating fish brought from known toxic areas, such as Palmyra, Johnston, or Midway Islands. No ciguatera from fish of Hawaiian origin was reported previous to 1956, but from that date to 1963, five outbreaks, afflicting 38 persons, were reported (since publication, the first death from ciguatera caused by a local fish has occurred). Outbreaks of ciguatera from fish caught in Hawaii appear to be increasing.

The study of Mrs. M. J. Cooper on the epidemiology of ciguatera in the Gilbert Islands has been completed and submitted for publication. In this island-to-island survey of the Gilbert Archipelago, Mrs. Cooper, speaking in Gilbertese, interviewed village elders and fishermen on the present extent and past history of toxic conditions, together with the species involved. Her study produced the most accurate information yet obtained on the geographical limitations of toxic areas.

In 1962, booklets prepared by Dr. Helfrich explaining fish toxicity in the Pacific were distributed together with questionnaires to be answered on the general picture of ciguatera in Pacific islands and on specific cases of poisoning. To date 53 questionnaires have been returned. Dr. Guy Loison, of the South Pacific Commission, reported that the replies to the French questionnaires sent to the French-administered islands were much more complete, and that they are now being translated by the South Pacific Commission. It is expected to receive these early in 1964.

From the questionnaires and from personal interviews, it is now established that ciguatera exists in every major archipelago in the South Pacific, but that its seriousness varies. The Laboratory has also been informed of a recent marked increase in toxicity of fishes about the Marquesas and American-held Wake Island, and is now planning to extend its studies to include the latter.

In epidemiological studies it is often found impossible to identify fish referred to, as only their native names were used. As an aid in this study, glossaries of plant and animal names for the major languages of Polynesia and Micronesia have been prepared. (Because of the extremely large number of languages involved—over 200 reported from New Guinea alone—Melanesia was not included in the study.) In the summer of 1963, nine different glossaries were issued in mimeographed form. These glossaries, based solely upon library studies, are as yet incomplete. They are now being sent to people in the field who have a knowledge of the local language and of the scientific identification of the local flora or fauna. It is hoped that in the course of the year, sufficient additions and corrections will be obtained to warrant the publication of interlingual glossaries of biological names of the Pacific. To date, 170 copies of the mimeographed glossaries have been distributed.

In-service Training at SPC Headquarters

Internships

In order to develop further the direct association of islanders with the work of the Commission, the Commission at its Twenty-fifth Session agreed to a proposal that a small number of internships within the three work-programme sections of the Secretariat and in its general administrative-financial organization might be arranged in 1964 on an experimental basis. In particular, the Commission decided that—

(a) One intern might be attached to each section during 1964.

(b) Interns should possess qualifications or experience in the relevant field necessary to their useful training and to their making a useful contribution while in the service of the Commission.

(c) Salaries and travel to and from Commission headquarters should not be a charge on Commission funds.

The Commission has made a small budgetary provision for a modest allowance to meet some local expenses of up to four interns in 1964. Accommodation can be provided at Commission headquarters. It would be necessary that the salary (or stipend) and travel to and from Nouméa be taken care of by the territory concerned in each case, or by the territory with the assistance of a fellowship from some other source. As a result of an approach to the United Nations Technical Assistance Board, we are in a position to count on three fellowships of a reasonable amount for 1964, with an excellent prospect of the same for 1965. It should thus be possible to make arrangements for the travel and salary expenses to be met in the case of up to three territories desiring to place an intern with the Commission for six months of in-service training this year, and also next year if the Commission considers the 1964 experiment successful and approves its continuance in 1965.

It is intended to place one of these interns in each of three out of four sections of the Secretariat Organization, i.e., health, education and social development, economic development, and administrative-financial; but this depends upon the needs of the Secretariat, the wishes of the territory, and the qualifications of the nominees.

Territorial administrations have been invited to consider sponsoring interns during this year. Among the requirements for the fellowships are an official request from the government concerned and a medical certificate of physical fitness.