RESULTS OF CIGUATERA ANALYSIS OF FISHES IN THE NORTHWESTERN HAWAIIAN ISLANDS

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ABSTRACT

Ciguatera is a form of fish poisoning caused by ingestion of certain species of fish. This study deals with the distribution of ciguatoxic fishes in waters of the Northwestern Hawaiian Islands (NWHI) and with a market sampling program to detect and remove ciguatoxic amberjack or kahala, *Seriola dumerili*, prior to its sale to the public. The results showed that ciguatoxic fish occurred throughout the entire NWHI from Nihoa to Kure Island. There were no detectable trends in rejection rate of the NWHI fishes with respect to area of capture; however, the analysis of data collected on kahala indicated a higher incidence of toxic fishes in the Nihoa-Raita Bank region. Of the 47 NWHI species tested between 1977 and 1979, the rate of rejection, based on a recently developed radioimmunoassay, was higher among fishes in the snapper-grouper complex. These included *Caranx ignobilis*, *C. cheilio*, *Seriola dumerili*, *Epinephelus quernus*, *Etelis carbunculus*, *Lutjanus kasmira*, and *Pristipomoides filamentosus*. Among inshore species sampled by the National Marine Fisheries Service and the Hawaii Division of Fish and Game, particularly high in rejection was *Kuhlia sandvicensis* and *Cheilinus rhodochrous*, followed by *Myripristis amaenus*, *M. murdjan*, *Mugil cephalus*, *Caranx melampygus*, *Polydactylus sexfilis*, and *C. ignobilis*. Of 926 kahala sampled between April and December 1979, 116 fish or 13% were rejected. The rate of rejection, high in April-May, declined steadily to December. The results also showed a low positive but significant correlation between fish size and toxicity.

Northwestern Hawaiian Islands
ciguatera
amberjack
snapper-grouper complex

INTRODUCTION

Ciguatera, a disease with certain characteristic neurotoxic and gastroenteritic symptoms produced by ingestion of a wide variety of fish belonging to groups such as the snappers, groupers, jacks, barracudas, surgeonfishes, and wrasses from toxic areas, appears to be widespread throughout the oceanic islands of the Pacific between the 30° parallels of latitude (Helfrich et al., 1968). The occurrence of ciguatera in some valuable food species found in the central Pacific affects not only the population by causing illness but also, as is often the case, deprives them of a major source of much needed protein. In areas that are seriously affected, it has restricted full development of fisheries and utilization of the available fish stocks.

At the outset of the Northwestern Hawaiian Islands (NWHI) survey and assessment investigation, provisions were made to conduct sampling for ciguatoxic fishes based on documented outbreaks of ciguatera occurring not only among the civilian population in the major Hawaiian Islands but also among U.S. naval and civilian personnel stationed at Midway Islands. It occurred to us that ciguatoxic fishes may also be found elsewhere in the archipelago and that the successful marketing of fishes caught in the NWHI would depend to a large extent on the wholesomeness of fishes from this area.

Together with the Hawaii Division of Fish and Game (HDFG), the Honolulu Laboratory of the Southwest Fisheries Center, National Marine Fisheries Service (NMFS), initiated an extensive sampling program to study the occurrence and distribution of ciguatoxic fishes in the nearshore and offshore waters of the NWHI.

In the spring of 1979, following a sudden outbreak of ciguatera among the local populace, the Honolulu laboratory, together with other state agencies and the fishing industry, also began a project of routinely sampling and pretesting amberjack or kahala, *Seriola dumerili*, which had been implicated in most of the outbreaks. By mid-April 1979, information supplied by various governmental agencies and fish retailers indicated that about 30 to 35 recent outbreaks could be documented and that perhaps another 50 to 100 individuals were affected but had not reported it to the health authorities.

Prior to 1977, ciguatera research throughout the Pacific relied on relatively crude bioassays to detect the presence of toxin in fish tissues (Banner et al., 1960, 1961). It was not until 1977, when two significant breakthroughs were announced, did researchers see any way to solve some of the pressing problems dealing with ciguatera. One was the discovery of a dinoflagellate, originally misidentified as *Diplopsalis* sp., but subsequently identified as a new species, *Gambierdiscus toxicus*, as the likely causative agent in ciguatera outbreaks (Yasumoto et al., 1977) and the other was the development of the radioimmunoassay (RIA) method of detecting ciguatoxin by scientists at the University of Hawaii John A. Burns School of Medicine, Department of Pathology (Hokama et al., 1977).

METHODS

Initially, our sampling effort was concentrated on commercially important species; however, if time and circumstances permitted, we also sampled other less valuable species. From each fish, we obtained tissue samples from the dorsal musculature (A), ventral abdominal musculature (B), gonads (C), and liver (D). Subsequently, liver collection was discontinued and additional muscle tissue were collected from the anal region (E).

At sea as well as at the United Fishing Agency auction market where only kahala are sampled, each tissue collected is placed in a plastic vial, labeled, and either frozen (while at sea) or kept in the fresh state until processed. Data collected on the fish include date, catch location, water depth, sex, length, weight, and vessel name. When it was not possible to obtain exact catch locality, particularly for many of the kahala sampled at the fish auction market, broad geographical areas were substituted. Obtaining data on catch locality for the kahala is very difficult or impossible in some cases because fishermen who land their catches to be auctioned may have fished several islands and banks during the course of a trip and cannot provide exact information on where a particular fish was caught.

The samples are processed by the University of Hawaii Department of Pathology to determine toxicity levels of the fish tissues from radioactive counts per minute per gram of tissue (c/m/g). Based on studies of fish involved in clinically evaluated cases of ciguatera, and on mouse and mongoose toxicity tests, levels of toxicity were established as follows (Y. Hokama, University of Hawaii, John A. Burns School of Medicine, Pathology Department, Honolulu, personal communication):

c/m/g tissue	Toxicity levels
<350,000	Negative
350,000 to 399,999	Borderline
>399,999	Positive

RESULTS

About 16% of all the fish sampled by NMFS and HDFG showed either a positive or borderline rejection level when tested by the RIA method. Of the 1,494 fish sampled, 1,250 were negative, 148 were borderline, and 96 were positive.

Table 1 shows the results of the RIA for fishes sampled by the HDFG in the nearshore areas of the NWHI. Because the list of species sampled is lengthy (624 fish; 60 species) and many of the species had few samples, the table includes only those species for which 10 or more samples were available for the 3 years--1977 through 1979--combined. Among the species tested, *Cheilinus rhodochrous* had a rejection rate of 71% which is exceptionally high. This was followed by *Myripristis amaenus* with 45%, *M. murdjan* and *Mugil cephalus*, both with 18%, *Caranx melampygus* with 14%, and *Polydactylus sexfilis* and *C. ignobilis*, both with 13%. All of these are commercially valuable on the Hawaiian fresh fish market.

TABLE 1. THE NUMBER OF NEGATIVE (n), BORDERLINE (b), AND POSITIVE (p) REACTIONS (n-b-p) OBTAINED WITH THE RADIOIMMUNOASSAY CONDUCTED ON FISHES CAUGHT DURING NEARSHORE SURVEYS CONDUCTED BY HAWAII DIVISION OF FISH AND GAME. ONLY SPECIES WHERE 10 OR MORE SAMPLES WERE COLLECTED IN 1977-79 ARE INCLUDED.[1]

Species	1977	1978	1979	Rejection (%)
Carcharhinus menisorrah	--	8-0-0	3-0-0	0
Myripristis murdjan	--	5-2-0	4-0-0	18
M. amaenus	--	4-3-1	2-1-0	45
Polydactylus sexfilis	--	7-0-1	27-3-1	13
Kuhlia sandvicensis	--	2-1-2	47-2-0	9
Caranx ignobilis	24-5-2	16-3-1	47-2-0	13
C. melampygus	--	3-0-2	9-0-0	14
Mulloidichthys flavolineatus	--	1-0-1	31-1-1	9
Kyphosus cinerescens	--	2-1-0	11-0-0	7
Mugil cephalus	--	4-3-1	19-1-0	18
Bodianus bilunulatus	--	3-0-5	53-1-0	10
Cheilinus rhodochrous	2-2-10	2-0-0	1-0-0	71
Thalassoma duperreyi	--	0-1-1	53-1-0	5
Acanthurus triostegus	--	0-2-0	52-0-0	4

[1]Data from Henry Okamoto, Aquatic Biologist, Hawaii Division of Fish and Game.

Whereas the HDFG concentrated their sampling effort on nearshore fishes, the NMFS sampling included many of the offshore species, although some inshore species were included as a result of recreational fishing conducted during the Cromwell's refueling and rest stops at Midway (870 fish; 47 species). From Table 2, it can be seen that among the members of the carangids where 10 or more samples were collected, Caranx ignobilis had the highest rate of rejection, reaching 32% from 19 fish sampled. This was followed by C. cheilio with 11% rejection and Seriola dumerili with 10%. The only species of serranid, Epinephelus quernus, sampled during our survey had a relatively high rejection rate of 18%. Among the snappers, 3 of the 10 Etelis carbunculus or 33% showed hazardous levels of ciguatoxin followed by a 23% rejection rate among Lutjanus kasmira. Pristipomoides filamentosus also showed a relatively high rate of rejection, reaching 15% in our samples. Table 2 shows that most of the rejected fish were sampled at Necker and French Frigate Shoals.

Among the inshore fishes caught and sampled, only Kuhlia sandvicensis showed alarmingly high levels of ciguatoxin. Of the 47 fish sampled, 27, or 57%, were positive or borderline according to the RIA. This rate of rejection is considerably higher than that obtained from the HDFG nearshore samples collected from 1977 through 1979.

Most of the other fishes sampled were few in total numbers; therefore, it is difficult to draw definite conclusions about whether ciguatoxin occurs in any significant amounts in their tissues. None showed

TABLE 2. THE NUMBER OF NEGATIVE (n), BORDERLINE (b), AND POSITIVE (p) REACTIONS (n-b-p) OBTAINED WITH THE RADIOIMMUNOASSAY CONDUCTED ON FISHES CAUGHT DURING THE NATIONAL MARINE FISHERIES SERVICE SURVEY CRUISES TO THE NORTHWESTERN HAWAIIAN ISLANDS IN 1977-79

	Nihoa	Necker	French Frigate Shoals	Brooks Banks	Gardner Pinnacles	Raita Bank	Maro Reef	Laysan	Northampton Seamount	Pioneer Bank	Neva Shoals	Lisianski	Unidentified Bank #8[1]	Pearl and Hermes	Midway	Kure	Unidentified Bank #10[2]	Total All Banks	Rejection (%)
Carangidae																			
<u>Carangoides ferdau</u>	--	--	--	--	1-1-0	--	--	--	--	--	--	--	--	1-0-0	--	--	--	2-1-0	33
<u>Caranx cheilio</u>	9-1-0	10-0-0	9-2-0	--	5-0-0	3-0-0	12-1-1	20-1-1	--	--	--	2-0-0	--	9-1-0	4-1-0	8-1-1	--	91-8-3	11
<u>C. ignobilis</u>	--	--	5-1-1	--	2-0-0	--	--	2-0-0	--	3-3-1	--	--	1-0-0	--	--	--	--	13-4-2	32
<u>C. lugubris</u>	--	--	--	--	1-1-3	--	--	--	--	--	--	--	--	--	--	--	--	1-1-3	30
<u>C. melampygus</u>	--	1-0-0	--	--	2-1-0	--	--	--	--	--	--	--	--	--	--	--	--	3-1-0	25
<u>C. speciosus</u>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	11-0-0	--	--	11-0-0	0
<u>Elagatis bipinnulatus</u>	--	2-0-0	--	--	1-0-0	--	--	--	0-1-0	--	--	--	--	--	--	--	--	3-1-0	25
<u>Seriola dumerili</u>	2-0-1	15-0-3	5-1-0	2-0-0	2-0-0	2-0-0	8-0-1	12-1-1	--	--	2-0-0	6-0-0	5-0-0	19-0-0	2-0-1	1-0-0	--	83-2-7	10
Serranidae																			
<u>Epinephelus quernus</u>	2-1-0	6-0-0	11-1-0	0-0-3	4-3-1	3-0-0	16-2-5	4-0-0	4-0-0	3-0-2	6-0-0	7-0-0	7-1-1	18-0-0	--	7-2-0	2-0-0	100-10-12	18
Lutjanidae																			
<u>Aprion virescens</u>	1-1-0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1-1-0	50
<u>Etelis carbunculus</u>	--	--	--	--	--	--	--	2-0-0	--	--	--	--	--	2-0-1	--	3-2-0	--	7-2-1	30
<u>E. marshi</u>	--	--	2-0-0	--	4-0-0	--	15-0-0	8-1-0	4-0-0	--	7-0-0	--	8-1-0	17-3-0	--	--	--	65-5-0	[illegible]
<u>Lutjanus kasmira</u>	11-1-3	1-0-0	11-2-2	4-0-0	--	--	--	--	--	--	--	--	--	--	--	--	--	27-3-5	23
<u>Pristipomoides filamentosus</u>	4-0-0	51-6-3	27-10-1	--	7-0-0	--	17-1-0	17-1-0	6-0-0	1-0-0	2-0-0	1-0-0	--	0-1-0	--	--	--	133-19-4	15
<u>P. sieboldii</u>	--	4-0-0	2-0-0	1-0-0	--	1-0-0	1-0-0	--	1-0-0	--	--	--	--	3-1-0	--	--	--	13-1-0	7
<u>P. zonatus</u>	--	--	5-0-0	1-0-0	--	--	6-0-0	0-0-1	2-0-0	--	1-0-0	--	7-0-0	1-0-0	--	--	--	23-0-1	[illegible]
Kuhliidae																			
<u>Kuhlia sandvicensis</u>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	20-12-15	--	--	20-12-15	57

[1]Unidentified bank #8 is located at lat. 26°17'N, long. 174°34'W.
[2]Unidentified bank #10 is located at lat. 28°58'N, long. 178°42'W.

exceptionally high rejection rates, although mention should be made of some members of the families Muraenidae and Acanthuridae that showed presence of ciguatoxin in varying degrees. These would include Gymnothorax flavimarginatus, G. hepaticus, Acanthurus triostegus, and A. nigroris.

In the kahala testing program, which began in April 1979, the number of fish tested until the end of December 1979 reached 926. Of these, 78 were borderline and 38 were positive in the RIA for a total rejection of 116 fish, or 13%. The rejection rate was relatively high in April 1979 when 63% of the kahala sampled were rejected but decreased in May to 24%, in June to 16%, then fluctuated between 3 and 6% until December when the rejection rate rose slightly to 10% (Table 3).

TABLE 3. THE NUMBER OF KAHALA SAMPLED WITH RIA, BY MONTH, THE NUMBER AND PERCENTAGE OF NEGATIVE, BORDERLINE, AND POSITIVE REACTIONS OBTAINED WITH THE RIA, AND THE TOTAL NUMBER AND PERCENTAGE OF FISH REJECTED PRIOR TO SALE

1979	Total Sampled	Negative	%	Borderline	%	Positive	%	Total Toxic	%
Apr.	52	19	37	10	19	23	44	33	63
May	122	93	76	21	17	8	7	29	24
June	174	147	84	24	14	3	2	27	16
July	153	149	97	4	3	0	0	4	3
Aug.	92	88	96	4	4	0	0	4	4
Sept.	73	71	97	2	3	0	0	2	3
Oct.	90	87	97	3	3	0	0	3	3
Nov.	65	61	94	1	1	3	5	4	6
Dec.	105	95	90	9	9	1	1	10	10
Total	926	810		78		38		116	

According to information provided by the fishing vessels, the area of capture of these kahala varied from as far south as the island of Hawaii northwestward to Raita Bank, a distance that spans 1,723 km. By geographical area, then, it appears that rejection was higher among kahala caught in the NWHI, principally from the area between Necker and Raita Bank, with the rejection level reaching 32% (Table 4). This was followed by a rejection rate of 29% among fish caught off the south coast of Oahu, and a rate of 24% among fish caught off Maui. Fishes caught at Penguin Bank also were rejected at a fairly high rate of 17%

Of particular interest is the relationship between fish size and toxicity. Prior to the start of the kahala sampling program, sale of fish weighing 9 kg (20 lb) or more was usually discouraged, because ciguatera outbreaks in the past allegedly implicated fish that were 9 kg or more in weight. Examination of our data showed that the size of kahala sampled between April and December 1979 varied from 0.57 to 45.4 kg (1.25 to 100.25 lb). Fish that were rejected fell in a range

TABLE 4. THE NUMBER OF KAHALA SAMPLED BY AREA AND THE NUMBER AND PERCENTAGE REJECTED BASED ON RESULTS OF RADIOIMMUNOASSAY

Area of Capture	Number Sampled	Number Rejected	% Rejected
Gardner Pinnacles	153	10	6
Necker-Raita Bank	132	42	32
Nihoa	1	0	0
Kauai	1	0	0
Oahu			
Southeast Coast	34	3	8
South Coast	77	22	29
North Coast	32	4	12
Molokai	5	0	0
Penguin Bank	151	26	17
Maui	17	4	24
Hawaii			
East Coast	192	12	6
South Coast	12	0	0
Unknown	119	9	7

from 0.79 to 26.88 kg (1.75 to 59.25 lb). Preliminary analysis showed that there is a low positive but significant correlation between toxicity level (tissue E) and fish size ($r = 0.101$; df = 1,202; $p < 0.01$).

DISCUSSION AND CONCLUSIONS

The preliminary results obtained from our NWHI samples show some agreement with those given by Sylvester et al. (1977). In the snapper-grouper complex of fishes in the Virgin Islands (includes snappers, groupers, grunts, jacks, porgies, triggerfishes, filefishes, and wrasses), the family Carangidae contains the most species prone to be ciguatoxic, followed by snappers and groupers. All are carnivores that attain relatively large sizes.

It is interesting to note that *Pristipomoides filamentosus*, considered to be a top-quality food fish and highly prized on the local fresh fish market, had a rejection rate of 15%. Yet, this species has never been implicated in any outbreaks among local citizens (Kubota, 1972).

According to Hokama et al. (1977), the RIA may produce "false positives." The binding of the anti-ciguatoxin-human serum albumin to non-toxic fish could be due to sensitivity of the test, to cross-reacting antigenic determinants, or to non-specific binding. There is also the possibility that most, if not all, marine fishes already contain low undetectable levels of ciguatera-like compounds. It should be emphasized, however, that although some "false positives" may occur as a result of the binding of anti-ciguatoxin-human serum albumin to non-toxic fish tissues, this should not detract from the value of the test. As demonstrated by Hokama et al. (1977), clinically documented toxic fishes

(fishes that have been actually implicated in ciguatera outbreaks) have given significantly higher c/m/g tissue than non-toxic fishes.

FUTURE RESEARCH NEEDS

Although NMFS field sampling included 47 species, most of which came from our offshore stations, it appears now that effort will need to be redirected to gather more data and samples from members of the families belonging to the snapper-grouper complex, all of which are commercially valuable in the Hawaiian Islands. With few exceptions, members of this complex showed varying rates of rejection with the RIA.

Processing of biological samples will continue so that the data base may be strengthened with respect to diet and sexual maturity and relationships of these variables to toxicity may be examined. Additional areal and seasonal coverage is also needed so that the distribution and seasonal occurrence of ciguatoxic fishes can be more clearly understood. Also planned are in-depth statistical analysis of the data collected from the NWHI fishes and of the data collected in the kahala testing program.

SUMMARY

From 1977 through 1979, NMFS and HDFG sampled 1,494 fish in the nearshore and offshore waters of the NWHI for ciguatera analysis. Of these, 244, or 16%, were rejected because the detected levels of ciguatoxin with RIA were considered hazardous to humans. Ciguatoxic fishes occurred throughout the NWHI and no apparent trend could be seen in their distribution. In the kahala sampling program, the data indicated a higher rate of rejection among fish caught between Nihoa and Raita Bank than among those caught in waters around the major islands. Of 926 kahala sampled between April and December 1979, 116 fish or 13% were rejected. Preliminary analysis suggests that there exists a low but statistically significant positive correlation between kahala size and toxicity.

Among the offshore species sampled by NMFS, those that belong to the snapper-grouper complex showed a relatively high degree of rejection. Nearshore species that showed high rejection rates included *Kuhlia sandvicensis* and *Cheilinus rhodochrous*.

REFERENCES

Banner, A.H., P.J. Scheuer, S. Sasaki, P. Helfrich, and C.B. Alender. 1960. Observations on ciguatera-type toxin in fish. *Annals of the New York Academy of Sciences* 90(3):770-787.

Banner, A.H., S. Sasaki, P. Helfrich, C.B. Alender, and P.J. Scheuer. 1961. Bioassay of ciguatera toxin. *Nature* 189(4760):229-230.

Helfrich, P., T. Piyakarnchana, and P.S. Miles. 1968. Ciguatera fish poisoning. I. The ecology of ciguateric reef fishes in the Line Islands. Occasional Papers of Bernice P. Bishop Museum 23(14): 305-369.

Hokama, Y., A.H. Banner, and D.B. Boylan. 1977. A radioimmunoassay for the detection of ciguatoxin. Toxicon 15:317-325.

Kubota, W. 1972. Ciguatera fish poisoning cases--a summary from year 1900 to May 1979. Special report prepared by State of Hawaii, Department of Health, Food and Drug Administration Branch, Honolulu, Hawaii. 10 pp.

Sylvester, J.R., A.E. Dammann, and R.A. Dewey. 1977. Ciguatera in the U.S. Virgin Islands. Marine Fisheries Review 39(8):14-16. (Also, MFR Paper 1260.)

Yasumoto, I., I. Nakajima, R. Bagnis, and R. Adachi. 1977. Finding of a dinoflagellate as a likely culprit of ciguatera. Bulletin of the Japanese Society of Scientific Fisheries 43(8):1021-1026.