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**RADIOIMMUNOASSAY RESULTS OF CIGUATERA ANALYSIS OF FISHES  
IN THE NORTHWESTERN HAWAIIAN ISLANDS, 1980-81**

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**ABSTRACT**

As part of the Northwestern Hawaiian Islands (NWHI) survey and assessment investigation, tissues of various nearshore and offshore fish species were analyzed by radioimmunoassay (RIA) to evaluate the distribution of ciguatera fishes. From 1980 through 1981, the Honolulu Laboratory of the Southwest Fisheries Center, National Marine Fisheries Service (NMFS) sampled 43 different offshore species for a total of 1,831 tissue samples. A total of 962 samples representing 76 different nearshore species were collected by the Division of Aquatic Resources (DAR) of the Hawaii Department of Land and Natural Resources from 1980 through 1982. When analyzed by the RIA method, 2,292 (82 percent) of the samples from NMFS and DAR were negative, 278 (10 percent) were borderline, and 223 (8 percent) were positive. The results were similar to those of the 1977-79 surveys and show that commercially valuable species, especially those in the snapper-grouper complex from various NWHI locations, had moderate to high frequencies of borderline and positive RIA results. Of the nearshore species, Cheilinus unifasciatus (= C. rhodochrous) had the highest rejection rate (percentage of samples with RIA borderline and positive results). Although not previously implicated in ciguatera, Pontinus macrocephalus had the highest rejection rate among the offshore species, followed by Epinephelus quernus. Recent studies suggest that species having high rejection rates and not previously implicated in ciguatera may contain polyether compounds which are

similar in chemical reactivity to ciguatoxin but of lower toxicity.

ciguatera                      radioimmunoassay  
rejection rate                Northwestern Hawaiian Islands

## INTRODUCTION

Ciguatera is a disease caused by the ingestion of a variety of fish contaminated with ciguatoxin, a lipid originating in the dinoflagellate, Gambierdiscus toxicus (Yasumoto et al., 1977). Characterized by neurological and gastrointestinal symptoms, ciguatera has been a health problem as well as a deterrent to fisheries development and utilization in the oceanic islands of the Pacific.

As part of the Northwestern Hawaiian Islands survey and assessment investigation, various nearshore and offshore species of fish were sampled to determine the occurrence and distribution of ciguatoxic fishes. From 1977 until 1982, commercially important as well as other less valuable species were sampled through the cooperative efforts of the Division of Aquatic Resources (formerly the Division of Fish and Game) of the Hawaii Department of Land and Natural Resources and the Honolulu Laboratory of the Southwest Fisheries Center, National Marine Fisheries Service. Fish tissues were tested for the presence of ciguatoxin by a radioimmunoassay procedure developed at the University of Hawaii (Hokama et al., 1977).

Radioimmunoassay results of fish surveys conducted between 1977 and 1979 were reported previously (Ito and Uchida, 1980) and indicated that ciguatoxic fish occurred throughout the NWHI from Nihoa to Kure Atoll. Species among the snapper-grouper complex, including Caranx ignobilis, Pseudocaranx dentex (= C. cheilio), Seriola dumerili, Epinephelus quernus, Etelis carbunculus, Lutjanus kasmira, and Pristipomoides filamentosus, had high frequencies of borderline and positive RIA results for ciguatoxin. Among the nearshore species tested, Kuhlia sandvicensis and Cheilinus unifasciatus (= C. rhodochrous) had high frequencies of ciguatoxic fish.

The RIA results of nearshore and offshore surveys done from 1980 until 1982 are presented in this report. The data are similar to those of the 1977-79 surveys and show that some commercially valuable species from various NWHI locations had moderate to high frequencies of borderline and positive RIA results.

## METHODS

DAR sampled fish from the nearshore areas and NMFS concentrated on sampling offshore species although some nearshore species were sampled during refueling and rest stops. Shortly after capture, each fish was weighed and measured before tissue

samples were taken. Tissues collected by DAR were obtained from the anterior dorsal musculature (A); tissues collected by NMFS were taken from site A as well as from the anterior ventral abdominal musculature (B) and the posterior ventral musculature (E). Each tissue sample was placed in a plastic vial or bag and kept frozen until analyzed by the RIA method.

The RIA procedure was carried out according to the method of Hokama et al. (1977) with modifications described by Kimura et al. (1982). In the assay, precisely weighed tissue samples were incubated for 3 hours with <sup>125</sup>I - sheep anti-ciguatoxin antibody, washed, and then placed in a gamma counter to determine the radioactive counts per minute per gram of tissue. 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:

Counts per minute per gram	Toxicity level
<350,000	Negative
350,000 to 399,999	Borderline
>399,999	Positive

For all samples analyzed, tests were conducted "blind," i.e., samples were identified only by the original numbers assigned at the time of collection. Species identification and location of capture were revealed by NMFS and DAR only when all samples from a survey had been analyzed.

## RESULTS

Approximately 18 percent of all fish sampled by NMFS and DAR showed either a positive or borderline ciguatoxin level when tested by the RIA method. Of the fish tested, 2,292 were negative, 278 were borderline, and 223 were positive.

From DAR surveys of 1980 through 1982 a total of 76 different species accounting for 962 samples were tested. Of these samples, 873 (90 percent) were negative, 53 (6 percent) were borderline, and 36 (4 percent) were positive. Table 1 shows the RIA results for the nearshore fishes and includes only those species for which a total of 10 or more samples were tested over the 3-year period. The species having the highest rejection rate (50 percent) was C. unifasciatus followed by Polydactylus sexfilis (32 percent), Myripristis murdjan (27 percent), Mulloidichthys flavolineatus (24 percent), and Bodianus bilunulatus (13 percent). The rejection rates for NWHI samples in Table 1 varied from 5 percent in the summer of 1980, to 16 percent in the fall of 1980, and to 12 percent in the fall of 1981. In the summer of 1982, samples from Kure Atoll had a 2 percent rejection rate.

TABLE 1. NUMBER OF NEGATIVE (N), BORDERLINE (B), AND POSITIVE (P) REACTIONS OBTAINED WITH THE RADIOIMMUNOASSAY TEST ON FISHES CAUGHT DURING THE NORTHWESTERN HAWAIIAN ISLANDS NEARSHORE SURVEYS CONDUCTED BY THE HAWAII DIVISION OF AQUATIC RESOURCES IN 1980-82

Species*	1980		1981		1982		Rejection # (%)
	Summer N-B-P	Fall† N-B-P	Fall N-B-P	Spring-Summer‡ N-B-P			
<u>Acanthurus nigroris</u>	--	--	2-0-0	9-0-0	0		
<u>A. triostegus</u>	45-1-3	17-3-0	48-0-2	25-0-0	6		
<u>Bodianus bilunulatus</u>	12-1-2	14-5-1	34-0-0	2-0-0	13		
<u>Carangoides ferdau</u>	--	--	10-0-0	1-0-0	0		
<u>Caranx ignobilis</u>	10-0-0	1-0-0	4-0-0	1-0-0	0		
<u>C. melampygus</u>	5-0-0	4-0-0	5-1-1	--	12		
<u>Cheilinus unifasciatus</u>	--	2-4-1	4-0-1	--	50		
<u>Kuhlia sandvicensis</u>	51-1-0	19-0-0	38-5-0	37-2-0	5		
<u>Kyphosus bigibbus</u>	22-0-0	13-3-2	--	25-0-0	8		
<u>Mulloidichthys flaviolineatus</u>	1-0-0	10-6-1	11-0-0	--	24		
<u>Myripristis amaenus</u>	14-1-1	--	8-0-0	10-0-0	6		
<u>M. murdjan</u>	--	6-0-1	10-2-3	--	27		
<u>Parupeneus multifasciatus</u>	--	--	13-1-0	--	7		
<u>P. porphyreus</u>	5-0-0	27-1-1	3-1-2	1-0-0	12		
<u>Priacanthus sp.</u>	--	7-0-0	--	7-0-0	0		
<u>Polydactylus sexfilis</u>	--	--	19-1-8	--	32		
<u>Pseudocaranx dentex</u>	--	13-0-0	--	1-0-0	0		
<u>Thalassoma duperreyi</u>	--	19-1-0	--	--	5		
<u>Selax crumenophthalmus</u>	30-0-0	--	--	--	0		
TOTAL	195-4-6	153-23-7	209-11-17	119-2-0			

\*Only species of which 10 or more samples were collected are included.

†Fishes were collected only from Midway and Kure Atoll.

‡Fishes were collected only from Kure Atoll.

#Percent rejection = number borderline + positive fish/total number tested x 100.

From the NMFS surveys of 1980 through 1981, a total of 43 different species accounting for 1,831 samples were tested. Of these samples, 1,419 (78 percent) were negative, 225 (12 percent) were borderline, and 187 (10 percent) were positive. Table 2 shows the RIA results for species that had a sample size of at least 10 during this period. Although only 16 samples of Pontinus macrocephalus were analyzed from 6 locations, this species had a rejection rate of 50 percent, the highest among all species examined. Epinephelus quernus, the only serranid sampled, had the next highest rejection rate of 39 percent, based upon 308 fish from 19 locations. Among the carangids, Pseudocaranx dentex had the highest rejection rate (22 percent), followed by Caranx ignobilis (18 percent) and Seriola dumerili (10 percent). Among the snappers, Pristipomoides zonatus had a rejection rate of 36 percent, followed by P. filamentosus (26 percent), Etelis carbunculus (22 percent), and P. sieboldii (10 percent). Samples collected only from Midway gave moderate to high rejection rates: Acanthurus triostegus (25 percent), Selar crumenophthalmus (14 percent), and K. sandvicensis (10 percent).

For the NMFS surveys, comparison of rejection rates according to locations of capture indicates that Lisianski (44 percent) and Raita Bank (43 percent) had the highest rates of rejection. The following locations also showed high rates of rejection: Kure Atoll (33 percent), Laysan Island (28 percent), Pearl and Hermes Atoll (27 percent), French Frigate Shoals (26 percent), Maro Reef (23 percent), Necker Island (23 percent), Nihoa (21 percent), and Gardner Pinnacles (17 percent).

#### DISCUSSIONS AND CONCLUSIONS

The results of the NMFS and DAR surveys for ciguatoxic fish during 1980 through 1982 are similar to those obtained in the 1977-79 surveys (Ito and Uchida, 1980). Fish having borderline or positive RIA results were found among many of the species examined and appeared to be distributed throughout the NWHI. The results are in agreement with those of Sylvester et al. (1977) who reported that members of the family Carangidae, followed by snappers and groupers, were the species most likely to be ciguatoxic in the Virgin Islands.

A comparison of the data obtained by DAR and NMFS shows that higher rejection rates were found in the NMFS surveys: 22 percent vs 9 percent. A possible explanation for the difference is that the DAR rejection rates were based on only one tissue sampling site (A) for each fish whereas the NMFS rejection rates were based on results from three tissue sites (A, B, and E). No consistent relationship has been found between toxicity as determined by the RIA method and tissue sampling site. Therefore, the higher rejection rates for the NMFS samples may have resulted from the greater chance of detecting ciguatoxin since tissues from three sites rather than one were tested.

TABLE 2. NUMBER OF NEGATIVE (N), BORDERLINE (B), AND POSITIVE (P) REACTIONS OBTAINED WITH THE RADIOIMMUNOASSAY TEST ON FISHES CAUGHT DURING THE NATIONAL MARINE FISHERIES SERVICE SURVEY CRUISES TO THE NORTHWESTERN HAWAIIAN ISLANDS IN 1980-81

Species	Nihoa		Twin Banks		Necker		French Frigate Shoals		Brooks Banks		St. Rogatien		Gardner Pinnacles		Raita Bank		
	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	
Acanthuridae																	
Acanthurus triostegus																	
Carangidae																	
Caranx ignobilis	1-0-0				0-0-1		1-1-0							1-0-0			
Pseudocaranx dentex	3-0-0	4-0-0			8-3-2		9-3-3						12-0-0	22-2-2		11-4-0	
Seriola lalandi					15-1-0		12-0-0		1-0-0				3-0-0	21-5-1		12-1-0	
Seriola lalandi																	
Kuhliidae																	
Kuhlia sandvicensis																	
Kyphosidae																	
Kyphosus bigibbus																	
Lutjanidae																	
Etelis coruscans	2-0-0	1-0-0			13-3-4		6-2-2						1-0-0	4-0-0			
E. carbunculus	1-0-0	2-0-0			59-10-8		48-7-6					8-2-0	9-0-2				2-0-0
Pristigaster filamentosus	0-1-0	2-0-0			8-0-0		1-1-0		2-0-0			11-3-1	15-2-0				3-6-3
P. sieboldii					3-1-0		1-3-0					2-0-0	2-0-0				1-0-1
P. zonatus													4-0-0				1-0-0
Mullidae																	
Mulloidichthys flavolineatus																	
Priacanthidae																	
Priacanthus macki																	
Scombridae																	
Acanthocybium solandri	1-0-0				8-0-0				1-0-0					1-0-0			2-0-0
Euthynnus affinis	2-0-1				12-0-0												
Scorpaenidae																	
Pontinus macrocephalus																	
Serranidae																	
Epinephelus guerneus	1-1-0	3-0-0			9-6-2		25-5-3					13-1-0	11-1-1				9-8-8
TOTAL	11-2-1	12-0-0	135-24-17	103-22-14	4-0-0	51-6-1	90-10-6	41-19-12									

TABLE 2. NUMBER OF NEGATIVE (N), BORDERLINE (B), AND POSITIVE (P) REACTIONS OBTAINED WITH THE RADIOIMMUNOASSAY TEST ON FISHES CAUGHT DURING THE NATIONAL MARINE FISHERIES SERVICE SURVEY CRUISES TO THE NORTHWESTERN HAWAIIAN ISLANDS IN 1980-81 (continued)

Species	Maro Reef		Laysan		Northampton Seamount		Lisianski		Bank No. 9		Pearl and Hermes Reef		Salmon Bank	
	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P	N-B-P
Acanthuridae														
<i>Acanthurus triostegus</i>														
Carangidae														
<i>Caranx ignobilis</i>	2-0-0		13-2-2				2-1-0			1-0-1		25-4-0		
<i>Pseudocaranx dentex</i>	3-1-1						2-0-0			3-1-0		33-4-2		
<i>Seriola dumerili</i>	11-0-0		12-3-0				3-0-0					13-0-0		
<i>Selar crumenophthalmus</i>														
Kuhliidae														
<i>Kuhlia sandvicensis</i>														
Kyphosidae														
<i>Kyphosus bigibbus</i>														
Lutjanidae														
<i>Etella coruscans</i>	4-0-1		1-0-0							1-0-0		4-0-0		26-1-0
<i>E. carbunculus</i>	7-4-0		23-3-6		11-0-2					13-1-0		90-14-18		
<i>Fristipomoides filamentosus</i>			3-1-0		1-0-0					1-0-0		2-0-0		
<i>P. sieboldii</i>			8-0-0		1-0-0					2-0-0		6-0-0		6-0-0
<i>P. zonatus</i>	2-0-0		8-0-4		5-0-0					1-0-0		2-1-1		1-0-0
Mullidae														
<i>Mulloidichthys flavolineatus</i>														
Priacanthidae														
<i>Priacanthus meeki</i>														
Scombridae														
<i>Acanthocybium solandri</i>	3-0-0		1-0-0											
<i>Euthynnus affinis</i>			4-1-0							0-1-0				
Scorpaenidae														
<i>Pontinus macrocephalus</i>			0-0-1									2-2-2		1-0-0
Serranidae														
<i>Epinephelus quernus</i>	8-4-1		27-12-0		3-0-0		5-4-4			13-3-1		39-10-23		1-1-0
TOTAL	40-9-3		97-22-13		21-0-2		15-7-5			35-5-2		216-35-46		6-0-0
														35-2-0

TABLE 2. NUMBER OF NEGATIVE (N), BORDERLINE (B), AND POSITIVE (P) REACTIONS OBTAINED WITH THE RADIOIMMUNOASSAY TEST ON FISHES CAUGHT DURING THE NATIONAL MARINE FISHERIES SERVICE SURVEY CRUISES TO THE NORTHWESTERN HAWAIIAN ISLANDS IN 1980-81 (continued)

Species	Ladd Bank		Midway		Eastern		Nero and Pogy		Kure Atoll		Bank No. 11		Total All Banks		Rejection (%)
	N-B-P		N-B-P		N-B-P		N-B-P		N-B-P		N-B-P		N-B-P		
Acanthuridae															
<i>Acanthurus triostegus</i>			51-10-8		2-0-0								53-10-8		25
Carangidae															
<i>Caranx ignobilis</i>			1-0-0										32-6-1		18
<i>Pseudocaranx dentex</i>	2-1-0		1-0-0				1-0-0		2-1-0				121-21-13		22
<i>Seriola dumerili</i>			0-1-0		1-0-0		3-0-0						117-12-1		10
<i>Selar sirmenophthalmus</i>			24-3-1										24-3-1		14
Kuhliidae															
<i>Kuhlia sandvicensis</i>			223-14-11										223-14-11		10
Kyphosidae															
<i>Kyphosus bigibbus</i>			19-0-0										19-0-0		0
Lutjanidae															
<i>Etelis coruscans</i>													12-0-0		0
<i>E. carbunculus</i>	17-3-4		1-0-0				12-1-2		9-1-0				252-31-41		22
<i>Pristipomoides filamentosus</i>							0-1-1						154-35-19		26
<i>P. sieboldii</i>	2-2-0						1-0-0		1-0-0				45-4-1		10
<i>P. zonatus</i>													28-5-5		36
Mullidae															
<i>Mulloidichthys flaviolineatus</i>			13-0-0										13-0-0		0
Priacanthidae															
<i>Priacanthus meeki</i>			12-1-1										12-1-1		14
Scombridae															
<i>Acanthocybium solandri</i>													18-0-0		0
<i>Euthynnus affinis</i>													19-2-2		17
Scorpaenidae															
<i>Pontinus macrocephalus</i>							1-0-0		2-2-0				8-4-4		50
Serranidae															
<i>Epinephelus quernus</i>	7-0-6		1-0-0				5-1-4		7-5-1		1-2-2		188-64-56		39
TOTAL	28-6-10		346-29-21		3-0-0		23-3-7		22-9-2		1-2-2				



Similar to the results of earlier surveys, the nearshore species showing the highest rejection rate was Cheilinus unifasciatus which has been frequently implicated in ciguatera. Of the offshore species Pontinus macrocephalus had the highest rejection rate. However, this species has not been implicated in ciguatera in the past. Another offshore species having a high rejection rate was Epinephelus quernus, a member of the grouper family which has been implicated in ciguatera.

Results of surveys from 1977 through 1982 indicate that certain species (such as P. macrocephalus and Pristipomoides sp.), which have not been implicated in ciguatera, have high rejection rates based on the RIA. Recent studies describing the existence of compounds with structures similar to ciguatoxin suggested the possibility that the sheep anti-ciguatoxin antibody may also react with these compounds. Tachibana et al. (1981) reported that two marine sponges (genus Halichondria) contained okadaic acid, a cytotoxic polyether compound having close structural similarity to ciguatoxin. Murakami et al. (1982) identified okadaic acid as the toxic component in Prorocentrum lima, a benthic marine dinoflagellate, and found close similarity between okadaic acid and ciguatoxin in terms of chromatographic and ionophoric properties, and oxygenated polyether structure. Another marine toxin, brevetoxin B, from the red tide dinoflagellate Ptychodiscus brevis, also has a polyether structure similar to ciguatoxin (Lin et al., 1981).

Preliminary studies indicate that purified okadaic acid and brevetoxin partially inhibit the binding of sheep anti-ciguatoxin to toxic fish tissues, although at concentrations greater than those required for similar inhibition by purified ciguatoxin (unpublished observations). The presence of these ciguatoxinlike compounds in fish tissues may give positive RIA results and yet may not cause toxic symptoms because they are inherently less toxic than ciguatoxin. The LD<sub>99</sub> of highly purified ciguatoxin is 0.45 µg/kg when given intraperitoneally to mice (Tachibana, 1980) whereas the LD<sub>99</sub> of okadaic acid is 200 µg/kg (Murakami et al., 1982) and that of brevetoxin T34 is between 150 and 270 µg/kg (Baden et al., 1981).

In conclusion, results of the DAR and NMFS surveys of the nearshore and offshore waters of the NWHI indicate that approximately 18 percent of the fishes examined gave borderline or positive results by the RIA method. Offshore species that belong to the snapper-grouper complex showed a high degree of rejection. Among nearshore species, C. unifasciatus showed high rejection rates. More recent studies suggest that species with high rejection rates but not previously implicated in ciguatera may contain polyether compounds similar to ciguatoxin but of lower toxicity. Further studies are needed to examine this possibility, especially in view of the recent availability of techniques to develop monoclonal antibodies which could be used instead of the polyclonal sheep anti-ciguatoxin.

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