Ciguatera On the Island of Hawai`i: Windward vs. Leeward Shores

A Look at Two Species of Fish:

Cephalopholis argus (Roi)
&
Ctenochaetus strigosus (Kole)

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Abstract

Approximately 60% of all reported cases of Ciguatera Fish Poisoning (CFP) in the state of Hawaii were due to toxin-contaminated fish caught in the coastal waters of the Big Island. The vast majority of these cases (87%) were reported from the West, or leeward side, of the island. However, studies have shown that toxin-producing dinoflagellates, the causative agents for ciguatera, are present on both sides of the island. To date, there have been no studies focusing on fish in East Hawaii to make a preliminary determination whether the difference in reported cases of ciguatera is based on fish toxicity or other factors (e.g., shoreline accessibility, fishing pressures, environmental conditions, etc.). This study focused on two species, *Ctenochaetus strigosus* (Kole) and *Cephalopholis argus* (Roi), both of which are listed in the top five fish species implicated in ciguatera outbreaks on the Big Island since 1980. Samples obtained were cut from the epaxial musculature posterior to the head. The samples were tested for presence of ciguatoxins using a monoclonal immunoassay (MIA) stick test. The occurrence of toxicity in the fish was compared in leeward (West) vs. windward (East) samples using Chi Square analyses. Of 117 fish tested (51 windward & 66 leeward), there was no significant difference found in east vs. west samples: i.e. fish on both sides of the island appear to be equally prone to ciguatera.

Introduction

Ciguatera fish poisoning (CFP) is today a well-known malady of social and economic concern, endemic to tropical and subtropical regions (Anderson *et al.*, 1982; Hokama *et al.*, 1987; Epidemiology, State of Hawaii, 1988; Chinain *et al.*, 1999). CFP is conservatively estimated to affect from 25,000 (Lewis *et al.*, 1999) to possibly greater than 60,000 people annually (Hokama & Yoshikawa-Ebesu, 2001). The toxins responsible for CFP, known as ciguatoxins, are made up of lipid soluble, heat-stable polyether compounds that promote movement of sodium ions through cell walls of nerves and muscles (Shirai *et al.*, 1991; Ebesu & Hokama, 1995; Manger *et al.*, 1995; Lewis *et al.*, 1999, Hawaii Dept. of Health, 2001). The primary source of the ciguatoxins is benthic dinoflagellates, the best-known being *Gambierdiscus toxicus,* Adachi *et* Fukuyo (1979) named after its origin of discovery in the Gambier Islands, French Polynesia. Since then, several species of *Gambierdiscus* have been described. *Gambierdiscus* spp. are known to grow on a variety of macrophytes that are ingested by herbivorous fishes (e.g. filamentous algae, Abbott & Wilder, 1995). In the next higher trophic level, carnivorous fish that consume the contaminated fish can become contaminated themselves. Human ingestion of fish bearing the ciguatoxins results in CFP of varying degrees and symptoms, and in rare cases, death (Anderson *et al.*, 1982; Shirai *et al.*, 1991; Hokama & Yoshikawa-Ebesu, 2001).

It is believed that CFP is grossly underreported as well as misdiagnosed due to the flu-like symptoms (e.g. weakness, nausea, diarrhea, and aches) often associated with the disease (Anderson *et al.*, 1982; Hokama *et al.*, 1988, Lewis, personal comm.). To date; over 175 symptoms have been associated with ciguatera (Becker & Sanders, 1991). Ciguatera affects the gastrointestinal, neurological, and cardiovascular systems in humans and other mammals. Temperature reversal (hot feels cold and cold feels hot) and circumoral paresthesias (tingling) are both characteristic of reported cases (Anderson *et al*, 1982). Symptoms typically onset within one to three hours after ingestion of the fish, although they sometimes can be delayed as much as 24-48 hours, and can last hours to days, or weeks to months (Katz *et al.,* 1993; Legrand, 1998; Lewis, 2001; Hawaii Dept. of Health, 2001). Symptoms are also known to be recurrent, showing up after exercise, alcohol consumption, or ingestion of more contaminated fish (Higerd, 1982). Additionally, peanuts, chicken, and pork have been implicated as prompting recurrent symptoms as well (Lewis, 2001).

Ciguatera is the major cause of fish poisoning in Hawaii, where fishing is a cultural mainstay (Shirai *et al.*, 1991). Of the four most populated Hawaiian Islands, approximately 60% of all reported cases since 1980 occurred on the island of Hawaii, also known as the Big Island (Hawaii Dept. of Health, 1988). The vast majority of reported CFP cases (87%) and related ciguatera research on Hawaii have been on the west or leeward side of the island (Anderson *et al.*, 1982; Ichinotsubo *et al.*, 1994; State Department of Health, 2000). This difference in reported CFP cases for East vs. West Hawaii is known to the general public: i.e. fish known to occasionally be "hot", or ciguatoxic, on the west or leeward side are considered to be safe to consume on the east or windward side of the island (personal obs.). However, this belief may be unfounded, as *Gambierdiscus* spp. and other toxin-producing dinoflagellates have been found on macrophytes in windward waters, indicating a potential for toxicity in fish as well as increasing the validity of the previously reported cases (Robbins, 1999; Page, 2000; Parsons *et al.,* 2002). Additionally, the Hawaii State Department of Health (1992) reports "fish have tested positive even where no human illnesses have been reported", although it is unknown if East Hawaii fish were tested.

In addition to an apparent geographic distribution of ciguatera on the Big Island, several observations point to a possibility of seasonal occurrences of ciguatera. The reported cases of ciguatera on the Big Island when compiled for a 20-year period (1980-2001) show peaks in number in May and September. Puako on the leeward shore of the Big Island is an area known for persistent outbreaks where, in a study by Ichinotsubo *et al.* (1994), high cell counts of *G. toxicus* were found in April, and the highest percentage of fish showing presence of ciguatoxin was in April and August. At Mauna Lani, an area just a few miles south of Puako, *Gambierdiscus* spp. was found to be abundant in summer and fall months (Abbott & Wilder, 1995). Seasonal variation in cell abundances has been observed in other regions as well (Bomber *et al.*, 1988; Chinain *et al.*, 1999). However, there are studies that have found no evidence of seasonal fluctuations of *Gambierdiscus* spp. abundances (Ballantine *et al.*, 1985; McCaffrey *et al.*, 1992).

Seasonal fluctuations in toxicity in fish are apparently less studied and less understood. Tosteson *et al.* (1992) observed seasonal fluctuations in the toxicity of barracuda,; however, Lewis *et al.* (1992) did not detect any seasonal variability in their eel samples. This may be a reflection of several factors including stability of physical environmental conditions, decay of the toxins in the fish over time, fluctuations in toxicity of the prey, and/or seasonal variability of toxigenic benthic dinoflagellates and bacterial flora (Lewis, 2001).

While limited studies on ciguatera have been done in East Hawaii, none to date are known to focus on fish; the only data come from the CFP cases reported to the State Department of Health (DOH). The DOH data infer that ciguatoxic fish are more prevalent on the leeward shore, possibly resulting in a misconception; ciguatera may be deemed nearly absent in East Hawaii because it hasn't been properly studied.

One could address several variables that could contribute to the higher number of reported cases of CFP in West Hawaii:

- Fish toxicity: Are ciguatoxic reef fish more prevalent on the leeward coast?
- Shoreline accessibility: The windward coast is considerably more rugged in its topographical features and less accessible than the leeward coast.

- Fishing pressures: Has overexploitation of nearshore resources caused a shift in the diet of some fish?
- Environmental conditions: Is the windward shore environment less hospitable for *Gambierdiscus* spp., inhibiting cell proliferation or toxin production?
- Ratio of fish being caught (east vs. west): Are there 8-9x more fish being caught on the leeward coast to substantiate the 87% of reported ciguatera cases on the leeward coast?

For my project, I proposed to answer the first of these questions:

Are ciguatoxic reef fish more prevalent on the leeward shore than the windward shore of the Big Island?

My hypotheses are:

H_0: There is no difference in the prevalence of ciguatoxic reef fish for leeward (west) vs. windward (east) coasts of the Big Island.

H_1: Ciguatoxic reef fish are more prevalent on the leeward shore of the Big Island.

Reef associated fishes are predominantly responsible for ciguatera outbreaks in Hawaii (Hawaii Dept. of Health, 2001). For this study two nearshore reef fish: the Peacock Grouper, *Cephalopholis argus* (Tahitian and local name: roi), and the Gold Ring Surgeonfish, *Ctenochaetus strigosus* (Hawaiian name: kole), were chosen as target species (recommended by Dr. William J. Walsh, Division of Aquatic Resources, DLNR) due to the high incidence of CFP cases related to these fish

(Shirai *et al.*, 1991, Ichinotsubo *et al.*, 1994, Hawaii Dept. of Health, 2001). Both have been listed in the top five fish implicated in ciguatera outbreaks since 1980, and roi has been listed as the number one fish responsible for reported cases of ciguatera in the past five years as published by the Hawaii Department of Health (2001).

Materials and Methods

Fish samples were obtained from many people including personal, solicited, and unsolicited contacts. Samples were solicited using flyers (Appendix A) advertising specifics posted at local fishing supply stores, and by speaking at both the East and West Hawaii YMCA Freediving Club meetings. Additionally, students from the West Hawaii Explorations Academy (WHEA) contributed to our collection effort as part of a school assignment. Samples were collected by myself, friends, WHEA students, local fishermen, and with the help of the Division of Aquatic Resources (DAR) West Hawaii Aquarium Project (WHAP) divers, laboratory and boat resources. Personal collection of samples was conducted via freediving (snorkeling) and/or SCUBA diving, using a three-prong pole spear.

For standardization, samples of the fish were cut from the epaxial musculature posterior to the head (Okomoto, 1981, Ito *et al.*, 1984, Ichinotsubo *et al.*, 1994). The size of the sample varied due to the variety of sources (solicited and donated) and relative sizes of the fishes. Care was taken to extract a clean core subsample from the collected samples (i.e. from the interior) for the analysis using a biopsy punch or a scalpel and tweezers.

The Membrane Immunobead Assay (MIA) procedure was used to analyze for the presence/absence of ciguatoxins in the fish tissue samples (Hokama & Yoshikawa-Ebesu, 2001). The Membrane Immunobead Assay (MIA) and related materials used to test for ciguatoxins were graciously donated by Dr. Yoshitsugi Hokama, Department of Pathology, John A. Burns Medical School, University of Hawaii. A muscle sample approximately the size of a grain of rice was placed in a vial containing 12 drops methanol. A test stick was placed membrane-end down into the vial and left to soak for 15 minutes. The test stick was then removed and allowed to air dry for at least 15-20 minutes. The dry test stick is then set into a test tube containing 12 drops of the blue latex bead solution to soak membrane-side down for 10 minutes. The membrane stick was then rinsed using distilled water and patted dry on a paper towel.

Dr. Hokama supplied control sticks illustrating a weakly positive (2.5 ppb) result and strongly positive (5.0 ppb) result, and a negative control stick. Additionally, negative controls were run with each test session. Any color present on a stick indicates some ciguatoxin present and is considered to be unfit for consumption (Hokama, personal comm.). An interpretation of positive (+), borderline positive (+/-), or negative (-) was assigned to the test results. Positive sticks illustrate a blue coloration present with a clearly defined meniscus, the borderline positive sticks show a blue coloration without a clearly defined meniscus, and negative sticks demonstrated negligible coloration present on the test stick.

The occurrence of toxicity in the fish was compared in leeward (west) vs. windward (east) samples using Chi-square analysis. Seasonality was analyzed using ranked test results in a One-way ANOVA. Fisher's Exact test was used for all other analyses.

Results

A total of 117 samples were collected (Table 1). Windward samples totaled 51 fish, comprised of 11 *Cephalopholis argus* (roi), 19 *Ctenochaetus strigosus* (kole), and 21 additional samples including one *Aphareus furca* (wahanui), four *Caranx melampygus* (`omilu), nine *Chlorurus sordidus* (uhu), one *Monotaxis grandoculis* (mu), one *Naso lituratus* (umaumalei), two *Sphyraena helleri* (kawele`ā), one *S. barracuda* (kākū), and two *Scarus rubroviolaceus* (pālukaluka). Leeward samples totaled 66 fish, comprised of 30 *C. argus*, 21 *C. strigosus*, and 15 supplemental samples including one *Ctenochaetus hawaiiensis* (Hawaiian kole), one *C. melampygus* (`omilu), one *Lutjanus kasmira* (ta`ape), one *Scomberoides lysan* (lai), two *Oxycheilinus unifasciatus* (po`ou), one *Parupeneus multifasciatus* (moano), three *Acanthurus* spp. (palani), and five uhu (*Scarus* spp. and *Chlorurus* spp.) (identifications from Shore Fishes of Hawaii, by J.E. Randall, 1998). A complete summary of test results for species sampled is listed in Table 2.

Table 1: Summary of total samples of collected

SPECIES	*WINDWARD*	*LEEWARD*	*Total*
ROI	11	30	41
KOLE	19	21	40
OTHER	21	15	36
Total	51	66	117

Table 2. Summary of species sampled and Membrane Immunoassay (MIA) test results. Test results were placed into three categories: positive (+), borderline-positive (+/-), and negative (-). Positive sticks illustrated a blue coloration present with a clearly defined meniscus, the borderline-positive sticks showed a blue coloration without a clearly defined meniscus, and negative sticks demonstrated negligible coloration present on the test stick.

SPECIES	*Positive* (+)	*Borderline Positive* (+/-)	*Negative* (-)
Aphareus furca (wahanui)	1	0	0
Acanthurus spp. (palani)	3	0	0
Caranx melampygus (` omilu)	4	1	0
Cephalopholis argus (roi)	28	9	4
Chlorurus sordidus (uhu)	9	1	0
Ctenochaetus hawaiiensis (H. kole)	1	0	0
Ctenochaetus strigosus (kole)	21	10	9
Lutjanus kasmira (ta` ape)	1	0	0
Monotaxis grandoculis (mu)	1	0	0
Naso lituratus (umaumalei)	1	0	0
Oxycheilinus unifasciatus (po` ou)	2	0	0
Parupeneus multifasciatus (moano)	1	0	0
Scarus rubroviolaceus (pālukaluka)	3	0	0
Scarus sp. & Chlorurus sp. (uhu)	3	0	0
Scomberoides lysan (lai)	1	0	0
Sphyraena barracuda (kākū)	1	0	0
Sphyraena helleri (kawele` ā)	1	1	0

The majority of the MIA test results fell between the negative and weakly positive controls; i.e., the coloration left on the test stick membrane ranged from some blue with no clear meniscus to more blue with a clearly defined meniscus.

The resultant color variations on the test stick were subtle and somewhat ambiguous; therefore, in order to attain an accurate interpretation, 15 of the test sticks were sent to Dr. Hokama on Oahu for his interpretation. Those sticks were then used as guidelines by Dr. Mike Parsons, Dr. Randy Kosaki and myself in evaluating the remaining test sticks.

Table 3. Summary of test results for windward vs. leeward samples. Membrane Immunoassay (MIA) test results were placed into three categories: positive (+), borderline-positive (+/-), and negative (-). Positive sticks illustrated a blue coloration present with a clearly defined meniscus, the borderline-positive sticks showed a blue coloration without a clearly defined meniscus, and negative sticks demonstrated negligible coloration present on the test stick.

Shoreline/Fish	*Positive* (+)	*Borderline Positive* (+/-)	*Negative* (-)
Windward	40 (78%)	8 (16%)	3 (6%)
Leeward	42 (64%)	14 (21%)	10 (15%)
Kole - Windward	13 (68%)	3 (16%)	3 (16%)
Kole - Leeward	8 (38%)	7 (33%)	6 (39%)
Roi - Windward	9 (82%)	2 (18%)	0 (0%)
Roi - Leeward	19 (63%)	7 (23%)	4 (13%)
Other-Windward	18 (86%)	3 (14%)	0 (0%)
Other-Leeward	15 (100%)	0 (0%)	0 (0%)
Carnivores	41 (73%)	11 (20%)	4 (7%)
Herbivores	41 (67%)	11 (18%)	9 (15%)
Carnivores - Windward	16 (80%)	4 (20%)	0 (0%)
Carnivores - Leeward	25 (69%)	7 (19%)	4 (11%)
Herbivores - Windward	24 (77%)	4 (13%)	3 (10%)
Herbivores - Leeward	17 (57%)	7 (23%)	6 (20%)

Chi-square analysis for windward vs. leeward samples pooled (all species) revealed no significant difference in prevalence of ciguatoxins in the fishes tested regardless of shoreline (P-value 0.166). I therefore accepted my null hypothesis, indicating no difference found between the prevalence of ciguatoxic fish for windward vs. leeward facing shores.

Additionally, Fisher's Exact statistical test was used to compare toxicity frequency between species on the same shore (roi vs. kole for both windward and leeward shores), within species but between shores (windward kole vs. leeward kole & windward roi vs. leeward roi), and carnivore vs. herbivore (uhu were classified as herbivores and po`ou as carnivores) for same and opposing shores. A summary of MIA test results for grouped by category is found in Table 3. Positive and borderline-positive results were pooled to accommodate test parameters. All comparisons generated a P-value = >0.05, thereby indicating no significant differences (Table 4).

Table 4. Summary of statistical analyses using Fisher's Exact test. Positive and borderline-positive results were lumped to accommodate test parameters. All comparisons generated a P-value = >0.05, thereby indicating no significant differences.

Variables	***Fisher's Exact Test P-value***
Windward vs. Leeward samples lumped	0.097486
Kole: windward vs. leeward shores	0.280231
Roi: windward vs. leeward shores	0.27061
Carnivore vs. Herbivore for both shores	0.155360
Windward: carnivore vs. herbivore	0.21585
Leeward: carnivore vs. herbivore	0.140954
Carnivore: windward vs. leeward shores	0.22642
Herbivore: windward vs. leeward shores	0.219782

A one-way ANOVA was applied to samples when ranked by test result as follows: Positive (+) = 2; Borderline-positive (+/-) = 1; Negative (-) = 0. Ranked test results were analyzed statistically for seasonality, where seasons were grouped as follows: Fall = September, October, & November; Winter = December, January, & February; Spring = March, April, & May; Summer = June, July, & August. Since only two samples were collected from the summer season and both tested positive, they were excluded from the ANOVA in order to obtain a clearer analysis. Analysis yielded a P-value of 0.005 (n = 115), indicating significantly higher frequency of toxicity in samples procured in the spring months vs. both fall and winter months (Figure 1).

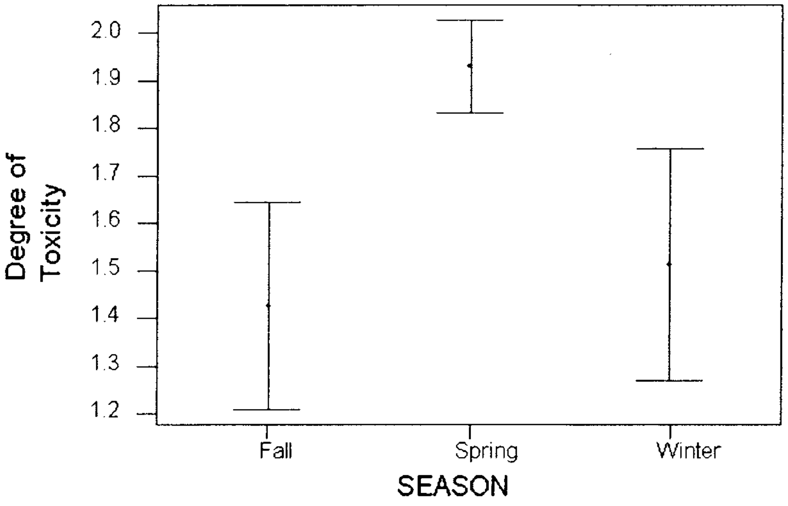

Figure 1. Interval plot of ranked mean degree of toxicity for each season sampled. A one-way ANOVA was applied to samples when ranked by test result as follows: Positive (+) = 2; Borderline-positive (+/-) = 1; Negative (-) = 0. Ranked test results were analyzed statistically for seasonality, where seasons were grouped as follows: Fall = September, October, & November; Winter = December, January, & February; Spring = March, April, & May; Summer = June, July, & August. Analysis yielded a P-value of 0.005, indicating significantly higher frequency of toxicity in samples procured in the spring months vs. both fall and winter months.

Discussion

According to this study, reef associated fishes are just as likely to be ciguatoxic on the east or windward shore as they are on the west or leeward shore of the island of Hawaii. If there is a difference in the presence of toxicity in reef-associated fishes for windward vs. leeward shores, this study did not find one. The belief that fishes caught in windward waters are safe to eat may simply be a misconception.

A remarkably high number of samples tested positive for ciguatoxins. This may be due to this study's focus on two of the most implicated fish in ciguatera outbreaks, kole and roi. However, the supplemental species turned out to have a higher incidence of positive results, possibly due to less frequent targeting of more difficult to catch and/or less frequently seen species (except with respect to uhu) or toxin sensitivity to the MIA test method (as expounded on below).

If ciguatoxins are so prevalent in fishes here, why are there not more frequent cases of ciguatera? The effectiveness of the test was questioned by many of the fishermen I obtained samples from since they did not feel symptoms after ingestion of a fish that tested positive. It is pertinent to reiterate that the majority of my test results corresponded to the range of color between the negative and weakly positive control sticks, which signifies that maybe the levels or amounts of toxins present are not sufficient to produce recognizable symptoms in those consuming the fish. The epidemiology of ciguatoxin is very complex, with variables such as "individual susceptibility, dosage, and toxin profile" (Lewis, 2001).

Recently, twenty structures of ciguatoxins have been resolved (Yasumoto *et al.*, 2000). Ciguatoxins biotransform as they move through the marine food chain (Legrand *et al.*, 1992; Lewis, 2001), increasing in toxicity by up to ten fold in carnivores (known as P-CTX-1) from the origination of the toxins produced by *Gambierdiscus* spp. (known as P-CTX-3C; Yasumoto *et al.*, 1992; Lewis, 2001). The MIA test stick method is reported to have a "sensitivity of 92% and a specificity of 86%" (i.e., sensitivity = the number of positive DOH fish detected by MIA x 100/ total number of positive fish implicated in ciguatera, & specificity = the number of negative results in DOH fish using MIA x 100/ total number of fish tested, presumed to be negative for ciguatera; Hokama & Yoshikawa-Ebesu, 2001). However, it is still unconfirmed which of the many structures of ciguatoxin the MIA stick test is reacting to (Hokama, personal comm.; Yoshikawa-Ebesu, personal comm.).

The Oceanit Cigua-check® test kit (MIA procedure) is a simple test to use; however, it is expensive for everyday use given the large amount of fishing typical to Hawaiian culture, and the results are ambiguous without some training as to how to read them. As mentioned before, any blue coloration of the membrane on the test stick indicates that ciguatoxin is present in the fish. The degree of toxicity (i.e., amount of toxin present) is gauged by the intensity of the color and the strength of definition of the meniscus, the latter being the key signifier. Negligible coloration on the test stick indicates a negative result. It is noteworthy to mention that Dr. Joanne Yoshikawa-Ebesu at Oceanit Test Systems re-tested my first 46 samples for confirmation of my results, using non-ciguatoxic fish as negative controls, and found all 46 samples to be positive to some degree. My results for the same fish agreed for 36 of the samples, however 10 disagreed, as they were interpreted as negative results.

Gambierdiscus toxicus has been confirmed as the origin of Pacific ciguatoxins (Yasumoto *et al.*, 2000). However, several other benthic dinoflagellates produce toxins, including *Gymnodinium* spp., *Prorocentrum* spp., *Ostreopsis* spp., and *Coolia* spp. (Anderson & Lobel, 1987; Faust, 1995; Landsberg *et al.* 1999; Parsons, personal comm). Although further study is needed, these dinoflagellates have been proposed as contributing to the many symptoms observed in ciguatera cases (Ballantine et al., 1985; Tosteson et al., 1992; Hokama et al., 1995; Ebesu & Hokama, 1995). Structural similarity of polyether toxins, such as okadaic acid produced by *Prorocentrum* spp., has been shown to cross react with monoclonal immunoassay test procedures and is therefore speculated to influence the results of the MIA test methods (Lewis, 1995).

Given the low levels yet high frequency of ciguatoxins present in the samples tested leads to the question of seasonality. As stated earlier, the peaks in numbers of reported ciguatera cases for the last twenty years have occurred in

May and September, inferring seasonal fluctuations in the disease on the Big Island. Additionally, Bomber (1987) noted similar fluctuations in ciguatera cases in Florida, hypothesizing the phenomena to be a reflection of seasonally fluctuating environmental conditions. On the Big Island as well as in other regions *Gambierdiscus* spp. has been shown to exhibit seasonal fluctuations in cell abundances, with marked peaks in cell abundance in the beginning and/or end of the hot (summer) season (Bomber *et al.*, 1988; Ichinotsubo *et al.*, 1994; Abbott & Wilder, 1995; Chinain *et al.*, 1999). Increases in *Gambierdiscus* spp. coincide with increases in host macrophytes during summer and fall months, when algae are most abundant (Grzebyk et al., 1994; Abbott & Wilder, 1995). Tosteson, et al. (1995) found "apparent seasonal variability" in ciguatoxic barracuda. Furthermore, analysis of ranked samples showed significantly higher frequency of toxicity in fish samples caught during spring months as opposed to both fall and winter months (Figure 1). In two of my latest samples acquired in mid-April, the coloration of the meniscus on the test stick was a strikingly darker blue than any previously tested samples. This leads me to anticipate higher toxicity levels in samples procured in the upcoming summer months.

Summary

On the island of Hawaii where 87% of reported cases of ciguatera occur on the west or leeward side of the island, tests for ciguatoxins in reef-associated fishes indicated no difference in the prevalence of ciguatoxic reef fish for windward vs. leeward shores. The local idea that fishes from windward waters are safe may simply be a misconception. The elucidation of twenty different structures of ciguatoxins with different levels of toxicity and the presence of structurally reminiscent toxins produced by other benthic dinoflagellates indicates that more studies are needed with regard to detection methods. The high frequency yet low toxicity of ciguatoxins in fishes tested may indicate a seasonal trend, with expected increases in toxicity appearing throughout the upcoming summer months, though further study is needed. The higher incidence of reported CFP cases on the leeward shore of the Big Island is most likely due to ease of accessibility and preferable environmental conditions, both of which influences the amount of fishing taking place on the leeward shoreline vs. the windward shoreline.

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References

Abbott, I. A. and G. P. Wilder. 1995. Hawaiian herbivorous fish; what algae are they eating, or what's left? p. 11-18. In Y. Hokama, P. J. Scheuer, and T. Yasumoto, (eds.), Proceedings of the international symposium on ciguatera and marine natural products, Asian Pacific Research Foundation, Honolulu, HI. 96814.

- Adachi, R., and Y. Fukuyo. 1979. The thecal structure of a marine toxic dinoflagellate *Gambierdiscus toxicus* gen. et sp. nov. collected in a ciguatera-endemic area. Bull. Japan. Soc. Sci. Fish. 45:67-71.

Anderson, B. S., J. K. Sims, N. H. Wiebenga, and M. Sugi. 1982. The Epidemiology of ciguatera fish poisoning in Hawaii, 1975-1981. Hawaii Medical Journal 42:326.

Anderson, B. S. 2001. Ciguatera fish poisoning: a five-year review. Communicable Disease Report, September/October p. 4-6. Hawaii Deptartment of Health Communicable Disease Division, Honolulu, Hawaii.

- Anderson, D. M., and P. S. Lobel. 1987. The continuing enigma of ciguatera.

- Ballantine, D. L., A. T. Bardales, and T. R. Tosteson. 1985. Seaonal abundance of *Gambierdiscus toxicus* and *Ostreopsis* sp. in coastal waters of southwest Puerto Rico, 4:417-422. In Proceedings of the Fifth International Coral Reef Congress, Tahiti.

- Becker, S. A. and W. E. Sanders, Jr. 1991. Ciguatoxins. FJPH 3:38-41.

- Bomber, J. W. 1987. Ecology, genetic variability and physiology of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus* Adachi and Fukuyo. Doctoral dissertation, Florida Institute of Technology, Melbourne, Florida, 32901.

Bomber, J. W., R. R. L. Guillard, and W. G. Nelson. 1988. Roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera-causing *Gambierdiscus toxicus* Adachi & Fukuyo (Dinophyceae). Journal of Experimental Marine Biology and Ecology 11: 53-65.

Chinain, M., M. Germain, X. Deparis, S. Pauillac, and A-M. Legrand. 1999. Seasonal abundance and toxicity of the dinoflagellate *Gambierdiscus* spp. (Dinophyceae), the causative agent of ciguatera in Tahiti, French Polynesia. Marine Biology 135: 259-267.

Ebesu, J. S. M., and Y. Hikama. 1995. Novel toxins in the gut of *Acanthurus triostegus*, Manini, p. 37-50. In Y. Hakama, P.J. Scheuer, and T. Yasumoto, (eds.), Proceedings of the international symposium on ciguatera and marine natural products. Asian Pacific Research Foundation, Honolulu, HI. 96814.

Faust, M. 1995. Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. Journal of Phycology 31: 996-1003.

Grzebyk, D., B. Berland, B. A. Thomassin, C. Bosi, and A. Arnoux. 1994. Ecology of ciguateric dinoflagellates in the coral reef complex of Mayotte Island (S. W. Indian Ocean). Journal of Experimental Marine Biology and Ecology 178: 51-66.

- Higerd, T. 1982. Ciguatera seafood poisoning: a circumtropical fisheries problem. Natural Toxins and Human Pathogens in the Marine Environment, A Maryland Sea Grant Publication p. 1-7.

Hokama, Y., L. K. Shirai, L. M. Iwamoto, M. N. Kobayashi, C. S. Goto, and L. K. Nakagawa. 1987. Assessment of a rapid enzyme immunoassay stick test for the detection of ciguatoxin and related polyether toxins in fish tissues. Biology Bulletin 172: 144-153.

Hokama, Y., J. S. M. Ebesu, J. L. R. Y. Shirai, H. Nagai, and J. T. Miyahara. 1995. Sodium channel inhibitor (SCI) toxin(s) and maitotoxin or palytoxin-like compounds in extracts of herbivore gut and algae as assessed by mouse and guinea pig atrial assays, p. 51-72. In Y. Hakama, P. J. Scheuer, and T. Yasumoto, (eds.), Proceedings of the international symposium on ciguatera and marine natural products. Asian Pacific Research Foundation, Honolulu, HI. 96814.

- Hokama, Y., W. E. Takenaka, K. L. Nishimura, J. S. Ebesu, R. Bourke, and P. K. Sullivan. 1998. A simple membrane Immunobead assay for detecting ciguatoxin and related polyethers from Human Ciguatera Intoxication and Natural Reef Fishes. Journal of the Association of Official Analytical Chemists International 81(4): 727-735.

Hokama, Y., and J. S. M. Yoshikawa-Ebesu. 2001. Ciguatera fish poisoning: a foodborne disease. Journal of Toxicology - Toxin reviews 20(2), 85-139.

- Ichinotsubo, D., A. Y. Asahina, E. Titius, S. Chun, T. W. P. Hong, J. L. Shirai, and Y. Hokama. 1994. Survey for ciguatera fish poisoning in West Hawaii. Memoirs of the Queensland Museum 34(3): 513-522. Brisbane.

- Ito, B. M., R. N. Uchida, L. K. Shirai, M. A. Abad, L. H. Kimura, and Y. Hokama. 1984. Radioimmunoassay results of ciguatera analysis of fishes in the Northwestern Hawaiian islands, 1980-81. In Proceedings of the Second Symposium on Resource Investigations in the Northwestern Hawaiian Islands Volume 2, May 25-27, 1983.

Katz, A. R., S. Terrell-Perica, and D. M. Sasaki. 1993. Ciguatera on Kauai: investigation of factors associated with severity of illness. American Journal of Tropical Medicine and Hygene 49: 448-454.

Landsberg, J. H., G. H. Balazs, K. A. Steidinger, D. G. Baden, T. M. Work, and D. J. Russell. 1999. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. Journal of Aquatic Animal Health 11:199-210.

Legrand, A-M., M. Fukui, P. Cruchet, Y. Ishibashi, and T. Yasumoto. 1992. Characterization of ciguatoxins from different fish species and wild *Gambierdiscus toxicus*, p. 25-32. In T. R. Tosteson (ed.), Proceedings of the Third International Conference on Ciguatera Fish Poisoning, Puerto Rico 1990. Polyscience Publications, Quebec.

Legrand, A-M. 1998. Ciguatera toxins: origin, transfer through the food chain and toxicity to humans, p. 39-43. In B. Reguera, J. Blanco, L. Fernandez, and T. Wyatt (eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela, Spain.

Lewis, R. J., M. Selllin, R. Street, M. J. Holmes, and N. C. Gillespie. 1992. Excretion of ciguatoxin from moray eels (Muraenidae) of the central Pacific, p. 131-143. In T. R. Tosteson (ed.), Proceedings of the Third International Conference on Ciguatera Fish Poisoning, Puerto Rico 1990. Polyscience Publications, Quebec.

Lewis, R. J. 1995. Detection of ciguatoxins and related benthic dinoflagellate toxins: *in vivo* and *in vitro* methods, p. 135-161. In G. M. Hallegraeff, D. M. Anderson, A. D. Cembella (eds.), Manual on Harmful Marine Microalgae. IOC Manuals and Guides No. 33. UNESCO.

Lewis, R. J., A. Jones, and J. P. Vernoux. 1999. HPLC/Tandem electrospray mass spectrometry for the determination of sub-ppb levels of Pacific and Caribbean ciguatoxins in crude extracts of fish. *Analytical Chemistry*, 71: 247-250

Lewis, R. J. 2001. The changing face of ciguatera. Toxicon 39: 97-106

Manger, R. L., S. Y. Lee, L. S. Leja, J. M. Hungerford, Y. Hokama, R. W. Dickey, H. R. Granade, R. Lewis, T. Yasumoto, and M. M. Wekell. 1995. *In vitro* analysis of ciguatoxins, brevitoxins, and saxitoxins, p. 123-136. In Y. Hakama, P. J. Scheuer, and T. Yasumoto, (eds.), Proceedings of the international symposium on ciguatera and marine natural products. Asian Pacific Research Foundation, Honolulu, HI. 96814.

- McCaffrey, E. J., M. M. K. Shimizu, P. J. Scheuer, and J. T. Miyahara. 1992. p. 145-153 In T. R. Tosteson (ed.), Proceedings of the Third International Conference on Ciguatera Fish Poisoning, Puerto Rico 1990. Polyscience Publications, Quebec.

Morton, S. L., J. W. Bomber, and P. M. Tindall. 1992. Effect of temperature, salinity, and light intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera. Journal of Experimental Marine Biology and Ecology 157: 79-90

- Okamoto, H. 1981. Status report of ciguatera research in the Northwestern Hawaiian Islands by the Department of Land and Natural Resources, Division of Fish and Game (Division of Aquatic Resources), *Ciguatera Workshop* March 18-20, 1981, Honolulu, Hawaii

- Shirai, J. L., L. K. Shirai, and Y. Hokama. 1991. Seafood poisoning: Ciguatera. Hokama Family Trust, Gardena, CA. University of Hawaii.

Tosteson, T. R., R. A. Edward, and D. G. Baden. 1992. Caribbean ciguatera and polyether dinoflagellate toxins: correlation of barracuda ciguatoxins with standard toxins, p. 89-102. In T. R. Tosteson (ed.), Proceedings of the Third International Conference on Ciguatera Fish Poisoning, Puerto Rico 1990. Polyscience Publications, Quebec.

Tosteson, T. R., R. F. Bard, I. M. Gonzalez, D. L. Ballantine, and G. S. Bignami. 1995. Toxin diversity in the ciguatera food chain, p. 73-87. In Y. Hakama, P. J. Scheuer, and T. Yasumoto, (eds.), Proceedings of the international symposium on ciguatera and marine natural products. Asian Pacific Research Foundation, Honolulu, HI. 96814.

Yasumoto, T., M. Murata, Y. Ishibashi, M. Fukui, and A-M. Legrand. 1992. Structure determination of ciguatoxin of moray eels and *Gambierdiscus toxicus*, p. 3-11. In T. R. Tosteson (ed.), Proceedings of the Third International Conference on Ciguatera Fish Poisoning, Puerto Rico 1990. Polyscience Publications, Quebec.

- Yasumoto, T., T. Igarashi, A-M. Legrand, P. Cruchet, T. Bracchi, M. Chinain, T. Fujita, and H. Naoki. 2000. Structural elucidation of twenty ciguatoxin congeners. In: 9th Harmful Algal Blooms Conference, Hobart, Australia, 68.

Sources of Unpublished Materials

Page, K. N. 2000. Distribution and abundance of the epiphytic dinoflagellate, Prorocentrum on the Big Island of Hawaii. Marine Science Thesis Project, University of Hawaii at Hilo, Marine Options Program.

Parsons, M. L. 2001. Nutrient loading, turtle tumors, and ciguatera: Is there a connection? Grant proposal, University of Hawaii at Hilo, Marine Science Department.

Robbins, I. C. 1999. Survey of toxic dinoflagellate populations on the Big Island of Hawaii. Marine Science Thesis Project. University of Hawaii at Hilo, Marine Options Program.