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Full Length Research Paper

Variability of Ciguatoxicity in *Cephalopholis Argus* Across Spatial and Temporal Scales in the Hawaiian Islands

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Decades following its introduction, the peacock grouper *(Cephalopholis argus)* developed a reputation for causing ciguatera fish poisonings in Hawaii. This study examined the frequency of ciguatoxicity in this carnivorous reef species, and the nature of variability in ciguatoxicity associated with season, location (i.e., island : island, leeward : windward), temperature, and specimen size. Overall, 36.4% of the 1,447 specimens were positively ciguatoxic. The frequency of ciguatoxic fish showed only modest seasonal differences. No sampled portion of any coastline was free of ciguatoxic fish. The frequency of ciguatoxic specimens from leeward areas (38%) was roughly twice that for windward areas (18%). Roughly 50% more samples were collected from leeward areas; such disproportional fishing effort in leeward areas contributes to an exaggerated public perception of ciguatera differences between leeward and windward areas. Though there was a higher frequency of ciguatoxic fish in larger specimens, there was no correlation between the calculated ciguatoxin concentrations and fish body weight. These ciguatoxicity-size data refute the popular expectation that the smaller specimens should be safe to eat. The average thermal regime showed negligible (<0.2°C) differences between windward and leeward areas, and only modest (~2.1°C) seasonal variation. We conclude that seasonal and/or spatial differences in temperature are of insufficient magnitude to elicit a perceptible change in the propensity for ciguatoxic fish in the Main Hawaiian Islands such as has been observed in Caribbean and South Pacific localities.

Keywords: Ciguatera, Marine toxin, *Cephalopholis argus*, Fish, Hawaii.

INTRODUCTION

Ciguatera fish poisoning (CFP) incidence occurs pantropically throughout the world, affecting coastal populations and travelers within the 35 °N – 35 °S latitudinal band; see recent reviews (Lehane and Lewis, 2000; Bienfang et al., 2008; Dickey, 2008; Dickey and Plakas, 2010). CFP is caused by the ingestion of coral reef fishes that have accumulated naturally-occurring marine ciguatoxins that are produced by dinoflagellates of the genus *Gambierdiscus spp*. *Gambierdiscus* is a

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predominantly epiphytic genus that grows on marine macroalgae; the ciguatoxins it produces are a powerful suite of polyether compounds (Yasumoto et al., 1977; Holmes, 1998; Chinain et al., 2010; Roeder et al., 2010). The ciguatoxin congeners are polar, lipid-soluble, heat stable, tasteless, and odorless. Ciguatoxins enter the coral reef food web when herbivorous fishes graze on macroalgae in the littoral zone, and inadvertently ingest the *Gambierdiscus spp*. and the ciguatoxins contained within. The grazing of these dinoflagellates by herbivorous fishes begins the processes of bioaccumulation and biomodification through the reef food web and ultimately to humans.

Public health institutions throughout the world rank CFP as the most common food-borne disease related to the consumption of marine finfishes (De Fouw et al., 1999; Lehane, 2000; Dickey and Plakas, 2010). The frequency of CFP cases is thought to approach 500,000 per year (Quod and Turquet, 1996; Lewis, 2001; Pearn, 2001; Arena et al., 2004), and it has been estimated that in some Caribbean and South Pacific Islands, 25-50% of the inhabitants have suffered from CFP (Lewis, 1986; Lange, 1994; Fleming et al., 1998; Fleming et al., 2001; Llewellyn, 2010; Tester et al., 2010). Ciguatoxin produces gastrointestinal, neurological, and/or cardiovascular symptoms that may persist in some form for weeks or longer (Cameron et al., 1991; Benoit et al., 2000; Arena et al., 2004; Friedman et al., 2008). Ciguatoxic fishes are not distinguishable by any visual or organolyptic signal, and clinical symptomologies in humans are elicited at extremely low (i.e. sub-ppb) ciguatoxin concentrations in fish.

From 16th century ship logs (Fraga et al., 2010), through WWII chronicles when CFP was a serious problem for military troops in Pacific Island locals (Hokama and Yoshikawa-Ebesu, 2001), this marine toxin has affected humans. In part, it was those experiences in the Pacific that initially focused scientific attention on CFP by researchers from Hawaii (Randall, 1958; Banner, 1974, 1976; Banner et al., 1960; Banner and Helfrich, 1964, Helfrich and Banner, 1963). These early contributions, indicating that ciguatoxin was derived from food consumed by the fishes, showed that toxicity could be transferred to non-toxic fishes via consumption of toxic fishes, and evolved into the food web concept that is held to this day.

Cephalopholis Argus, known commonly as roi, blue-spotted grouper and/or peacock grouper, is a demersal carnivore of the family Epinephelinae (Smith and Craig, 2007). This species is found in reef habitats between 2 – 40m depth throughout the tropical Indo-Pacific (Heemstra and Randall, 1993). As per other members of the genus, *C. argus* displays complex social behaviors such as sequential hermaphrodism, a haremic social system, and vigorous territoriality, particularly in areas of high rugosity (Shpigel and Fishelson, 1999; Donaldson, 1995). As protogynous hermaphrodites, *C. argus* change from females to males at approximately 0.5-1.0kg depending upon social structure; thus males tend to be larger than females. *C. argus* tend to be highly site attached; harems are composed of one male and several females, and in Hawaii males and females have similar home size ranges (Meyer, 2008). *C. argus* prey on the early juveniles of several endemic reef fish species (Dierking et al., 2009).

C. argus was introduced to Hawaii from stocks taken from Moorea, French Polynesia. Between 1956 and 1961, the Hawaii Division of Fish and Game made two introductions off Oahu (~2,000 juveniles), and one off Hawaii Island (~400 juveniles) with the intent of enhancing local fisheries by establishing a game fish that was highly-valued elsewhere in Polynesia (Randall, 1987). Sightings of *C. argus* were rare till the 1980's, and then were seen to increase to the point where its biomass exceeds that of all other reef fish predators combined in some areas (Dierking, 2007). This progression was supported in large part by both the remoteness of Hawaii, and a curtailment of fishing pressure on this carnivore. The reef systems of Hawaii support some of the highest endemism in the world (Demartini and Friedlander, 2004), and there is a limited presence of several fish families that are found in much of the Indo-Pacific (Randall, 1995, 1998) and the southern Pacific Ocean.

Decades following its introduction, *C. argus* gained its reputation for often being associated with ciguatera fish poisonings in Hawaii; as a consequence spear fishermen eschewed it as a desirable target, and inadvertently removed a key force of biomass control from its populations (Dierking and Campora, 2009). In the early 2000's, Hawaii's spear fishing community became increasingly aware of the predominance of this predator in the reef ecosystem, associated it with the concomitant demise of numerous native species, and began to aggressively target *C. argus* as an unwanted invasive species whose predation on native fishes needed to be reduced to allow these species to recover. Following establishment of communication and coordination, these ongoing efforts to harvest *C. argus* created samples of opportunity that supplied the fish for this research effort. This enabled direct sampling of a top reef carnivore prominently associated with ciguatera over broad spatial and temporal dimensions; this information may be accessed at www.fish4science.com. The objectives of this study were to describe the frequency of ciguatoxicity in this carnivorous reef species throughout the Main Hawaiian Islands (MHI), and to describe the nature of variability in ciguatoxicity that is associated with season, spatial (i.e., island: island, leeward: windward), temperature, and specimen size.

METHODS

Collection of Fish

Local spear fishermen from coastal localities throughout the MHI collected 1,447 specimens of the obligate carnivore, *C. argus* between February, 2008 and June, 2010. Specimens were identified according to the collector, the date and location of collection, placed in Ziploc bags, and transported to the laboratory. Collection locations were specified using numbered grid maps for all MHI. These grids of 2 minutes latitude by 2 minutes longitude were distributed around the coastlines of each island. Upon arrival in the lab, specimens were logged into the system, weighed, subsampled for ciguatera, and frozen prior to analyses for ciguatera. In the laboratory, weights of *C. argus* samples were measured to the nearest 0.1g using a Mettler PC 4000 Analytical Balance. Based on 687 C. argus samples, the weight-total length relationship is $W_{(g)} = 0.0261 \times (TL_{(cm)})^{2.917}$ (M. Donovan, University of Hawaii, USA, personal communication); this differs only slightly from the relationship of $W_{(g)} = 0.0125 \times (TL_{(cm)})^{3.122}$ that was calculated from 110 *C. argus* samples by Dierking et al. (2009).

Sample Preparation

To prepare the fish extracts for bioassay testing, approximately 10-20 g of muscle from each fish was minced, placed in falcon tubes, frozen at -80 °C and then lyophilized for 48-72 hours. Lyophilized samples were ground into powder, extracted [2:1 v/v of methylene chloride (CH_2Cl_2)] for 5 minutes under sonication with a solid state ultrasonic FS-9 bath (Fisher, Houston, TX), and filtered for separation. The filtered CH_2Cl_2 was then transferred to glass, round bottom flasks. The sonication/filtration extraction process was repeated twice with fresh CH_2Cl_2, and subsequent CH_2Cl_2 volumes were added to the round bottom flask. Extracts were dried on an R-114 rotary evaporator (Büchi, New Castle, DE), and then reconstituted with approximately 2 mL of CH_2Cl_2. The extract, along with two subsequent rinses, were transferred to a glass 20 mL vial, where it was then dried and stored at -20 °C until use in the mouse neuroblastoma (N2a) assay.

Sample Testing

The Na^+-channel specific N2A assay was developed by Manger et al. (1993) and Dickey et al. (1999) and used with some modifications to assess the presence of ciguatoxin in fish extracts. The assay is based upon the combined cytotoxic activity of ouabain, veratridine, and ciguatoxin, a sodium channel activator toxin. A cell suspension 200,000 cells/mL was made using mouse neuroblastoma cells in RPMI-1640 cell media supplemented with 5% fetal bovine serum. 96-well plates were plated with the cell density 20,000 cells/well, and placed in an incubator at 37˚C with 5% CO_2 enriched and humidified air, and allowed to acclimate overnight. The 96-well plates were dosed the following day with various concentrations of crude fish extract in triplicates. Each dose titration set occurred in replicates. One set additionally contained ouabain (0.5 mM) and veratridine (0.05mM), and the other set did not. Control wells, those with no added fish extract, with and without ouabain and veratridine, were used to assess baseline cell growth and baseline chemical cytotoxicity. All well volumes were brought to 200 µL using the RPMI media, and plates were placed in the incubator overnight. The following day, CellTiter 96 Aqueous One Solution was added to each well. This solution contains a tetrazolium compound that is bioreduced by metabolically active cells to produce a colorimetric response. Plates were incubated for 1 hour to allow for color development (used to measure cell viability) and then read on a Multiskan MCC/340 Eliza plate reader at 492 nM. Wells containing only cells and fish extracts (i.e., no ouabain and veratridine) were used to first assess the cytotoxicity of the extracts to neuroblastoma cells alone. Only when cytotoxicity was not evident did the interpretive analyses proceed to assessment for aberrant sodium channel activity. Non-cytotoxic samples were analyzed for sodium channel disruption by comparison of readings from wells containing cells plus fish extract, and ouabain and veratridine, versus the control wells plus ouabain and veratridine to determine if significant decreases in cell metabolic activity were evident. Sodium channel disruption is demonstrated if the viability of the cells exposed to the fish extract and ouabain and veratridine is significantly reduced from that of the control cells exposed to ouabain and veratridine alone. Results were computed and statistically analyzed using Students t-test (Snedecor and Cochran, 1980) to identify significant differences between various controls (n=10) and fish sample means (n=5). The standard dose – response curve for the N2a bioassay is shown in Figure 1. The P-CTX concentration in fish tissue that has been reported to cause intoxication in humans has ranged from 0.1 -1.0 ppb (= 0.1-1.0 ng P-CTX / g wet weight fish). The practical limit of detection for the N2a bioassay as applied here is approximately 50% below the lower (0.10 ppb) concentration that is understood to elicit clinical symptomology in humans.

Sea Surface Temperature (SST) data came from several sources. The National Oceanographic Data Center (www.nodc.noaa.gov), and the World Ocean Atlas (WOA) 2009 (Locarnini et al., 2010) were used for a spatial climatological perspective (Figure 4). The SST climatologies are the average of five “decadal”

Dose-Response Curve for the N2a Neuroblastoma Bioassay to Pacific Ciguatoxin

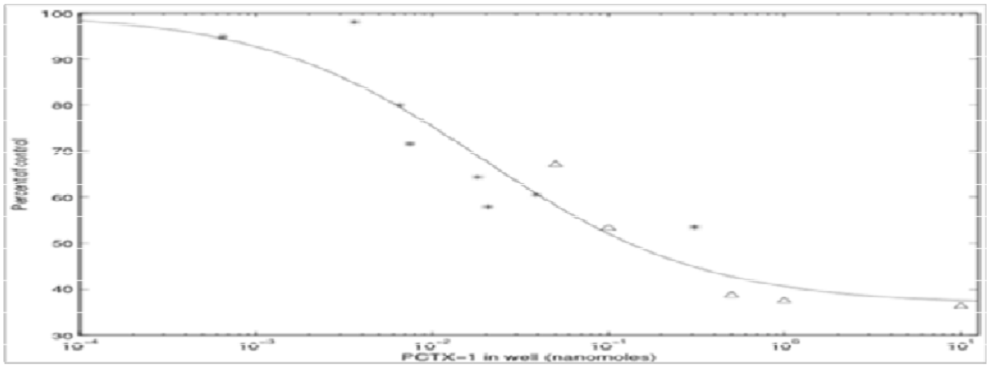

Figure 1. Signal strengths in response to a range of PCTX-1 concentrations are given as % of control wells (n=20) following addition of ouabain and veratridine. Relative standard deviations about the means for standards and controls averaged 11% and 14%, respectively.

Cephalopholis argus Metadata

	No. (#)	**W_{mean} (gm)**	**W_{sd} (gm)**	**W_{rsd} (%)**	**W_{min} (gm)**	**W_{max} (gm)**	**W_{med} (gm)**
All Samples	1436	855.1	433.3	50.7	126.1	3500.2	773.4
Quartile 1 (Q1)	359	386.5	96.4	24.9	126.1	529.9	395
Quartile 2 (Q2)	718	788.7	164.4	20.8	530.5	1123.9	773.4
Quartile 3 (Q3)	359	1456.2	312.8	21.5	1125	3500.2	1377.7
Maui	714	983	395.1	40.2	250	3500.2	908.2
Oahu	580	701.2	421.5	60.1	126.1	2580	579.8
Hawaii	67	928	433.4	46.7	127.5	2004.2	849.3
Kauai	75	761.1	471.1	61.9	137	2376.7	608

Table 1. Metadata for *C. argus* samples give average weight (W_{mean}), median weight (W_{med}), standard deviation (W_{sd}), relative standard deviation (W_{rsd}) for all quartiles as well as the upper (W_{max}) and lower (W_{min}) boundaries for the inter-quartile ranges observed.

Climatologies for the following time periods: 1955-1964, 1965-1974, 1975-1984, 1985-1994, and 1995-2006. The Internet-based WOA Select Tool was used to define 0.25 by 0.25 degree areas just offshore to the leeward and windward sides of Oahu and Maui for finer spatial resolution in deriving annual and seasonal means. The mean is an average of the minimum and maximum range provided by WOA Select tool. *In situ* SST data also came from point locations. SST data collected at 30 minute intervals from the Coastal Data Information Program's Mokapu buoy http://cdip.ucsd.edu), located 4 km windward of Oahu, were available starting in 2000 (Figure 5). The Hawaii Ocean Time Series (HOTS) program (http://hahana.soest.hawaii.edu/hot/hot_jgofs. Html) provided temperature data from a station 10 km off leeward Oahu; data from the 0-10m depth were used to acquire the seasonal and annual means (Figure 6). For seasonal averages from the WOA, Mokapu buoy, and HOTS CTD sensor, summer is defined as the mean monthly averages for July-September and winter is defined as the mean monthly averages for January-March. An additional source for spatial SST came from the Advanced Very High Resolution Radiometer (AVHRR) Pathfinder v4.1 (http://oceanwatch.pifsc.noaa.gov), which provides monthly means. In this study, months during 2010 were used to derive summer and winter means, defined as above. For each island and side, the same offshore location was used in selecting the monthly values. Since this source was only derived for one year, it contains an annual bias that was smoothed out of the other sources given in this study.

RESULTS

The metadata for *C. argus* collected from the MHI are summarized in Table 1. Overall, specimens ranged in size from 126.1 – 3500.2 g, and > 90% of the specimens came from Maui and Oahu. The number of specimens

Ciguatoxic *Cephalopholis argus* Metadata

	No. (#)	Ciguatoxic (%)	W_{mean} (gm)	W_{rsd} (%)	W_{min} (gm)	W_{max} (gm)
All Samples	526	36.4	985.5	41.9	127.5	2580
Quartile 1 (Q1)	131	36.5	513.6	24.0	127.5	675.9
Quartile 2 (Q2)	264	36.8	940.7	18.7	677.9	1240.5
Quartile 3 (Q3)	131	36.5	1547.9	16.5	1241	2580
Maui	371	52.0	1040.8	35.1	339.5	2107.3
Oahu	98	16.9	791.4	64.2	194.6	2580
Hawaii	51	23.1	957.3	44.2	127.5	1994.4
Kauai	6	8.0	971.4	57.7	380	1859.5

Table 2. Metadata for positively ciguatoxic *C. argus* within various size groupings. Data give average weight (W_{mean}), relative standard deviation (W_{rsd}), and the upper (W_{max}) and lower (W_{min}) boundaries for the interquartile ranges for all positively ciguatoxic *C. argus* for all samples and for Maui, Oahu, Hawaii, and Kauai individually.

Spatial distribution of all specimen collections of *Cephalopholis argus* within the MHI

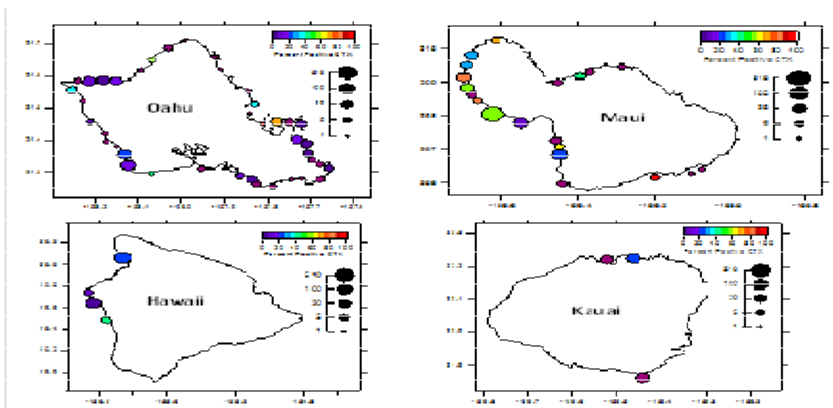

Figure 2. Dot locations are associated with equivalently-sized grids specifying locations where collections were made; dot diameters reflect the number of specimens from each location; dot colors give the percentage of positively ciguatoxic fish within the group from that location.

from the individual MHI were distributed as follows: Maui (50.4%) > Oahu (40.8%) > Kauai (5.3%) > Hawaii (3.5%). The average weight of specimens among islands was not significantly different ($p>0.05$). The mean body weight was 529.4 g. Body weight data were separated into quartiles (i.e., Q_1 , Q_3 and Q_2) representing the smallest 25%, the largest 25%, and the middle 50% of the samples; the Q_1-Q_2 and Q_2-Q_3 boundaries fell at 529.4 g and 1127.7 g, respectively (Table 1). Body weight data were not available for all 1447 samples; only 1436 samples were analyzed for body weight relationships.

Of the 1,447 specimens evaluated 526 (36.4%) were positively ciguatoxic (Table 2). By island, the frequency of positively ciguatoxic specimens were as follows: Maui (52.0%) > Hawaii (23.1%) > Oahu (16.9%) > Kauai (8.0%). The mean weights were not significantly different ($p>0.05$) among the positively ciguatoxic samples from the four islands.

Figure 2 shows the collection locations within the MHI and the distribution of positively ciguatoxic fish from various locations. The absence of uniform fishing effort over the coastlines within the MHI is evident by the range of dot diameters (showing numbers of specimens) from various locations. The figures show an absence of large-scale spatial differences in the frequency of ciguatoxicity on and/or among any of the islands. The indication of a higher frequency of ciguateric specimens on the leeward coast of Maui relative to its windward area may simply reflect higher fishing effort along the leeward coast. These figures indicate that no portion of any sampled coastline was free of ciguatoxic *C. argus* on any of the islands.

Spatial distribution of specimen collections of *Cephalopholis argus* from 2008-2010 for the islands of Maui and Oahu within the MHI

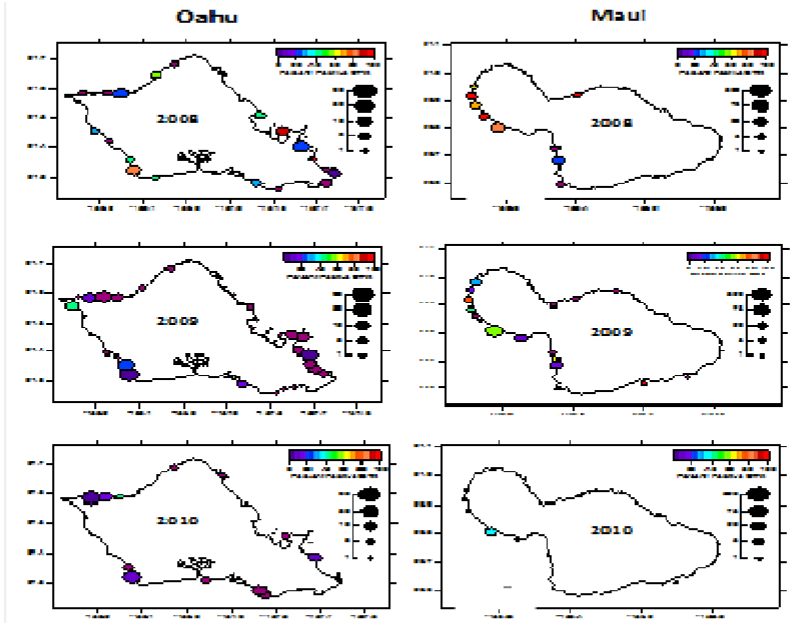

Figure 3. Dot locations are associated with equivalently-sized grids specifying locations where collections were made. Dot diameters reflect the number of specimens from each location, and dot colors give the percentage of positively ciguatoxic fish within the group from that location.

Sea Surface Temperature (SST) average within the geographic range of the Main Hawaiian Islands

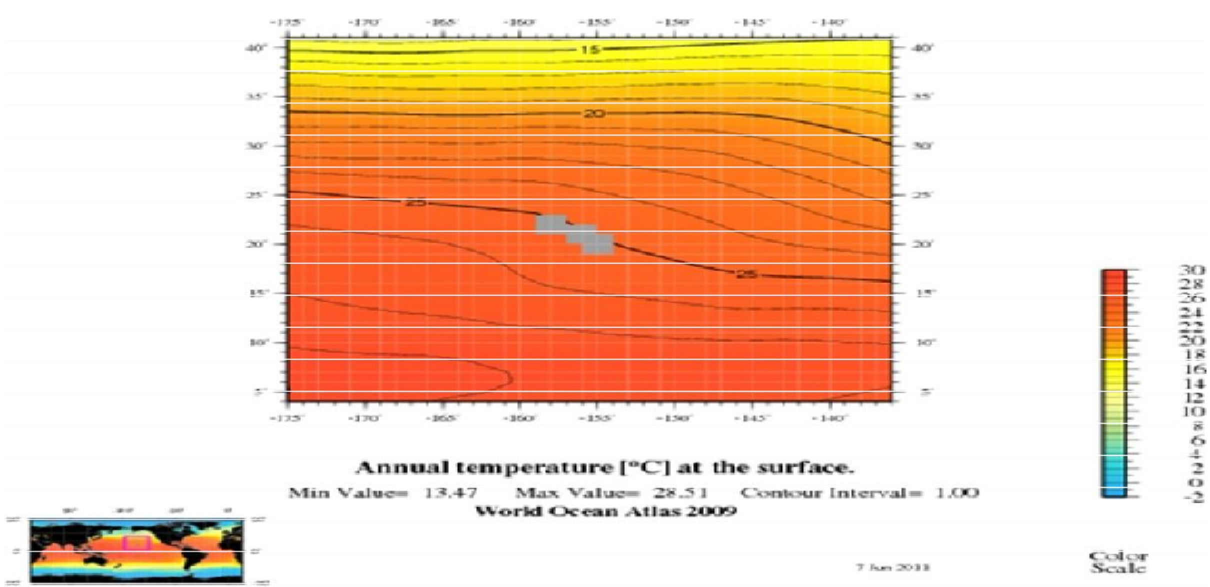

Figure 4. The Main Hawaiian Islands located along the 25ºC isotherm.

For all islands, the frequency of positively ciguatoxic *C. argus* specimens from the leeward areas were approximately twice that from the windward areas (i. e., 38% and 18 %, respectively). The sampling efforts in the three-year period of 2008-2010 collectively contributed approximately 2.9-times more fish from leeward areas relative to windward areas (Figure 3). Due to this increased sample size from the leeward coasts, this study observed roughly a six-fold greater likelihood of encountering a positively ciguatoxic fish originating from the leeward areas than from windward areas.

Only modest temporal (i.e., seasonal) differences were observed in the frequency of positively ciguatoxic fish that were collected at various times of the year. Each year was separated into quarters (i.e., January-March, April-June, July-September and October-December). The frequencies of ciguatoxic *C. argus* collected during each of these periods were 41.3%, 31.5%, 33.5%, and 24.5%, respectively. Comparing the warmest (i.e., July-September) and coolest (i.e., January-March) periods, the frequency of ciguatoxic fish collected differed by less than 8%, and the number of fish that were collected during the warmest period was more than twice that collected during the coolest period. We conclude that the seasonal temperature expression in the MHI is of insufficient magnitude to elicit a perceptible change in the propensity for ciguatoxic fish.

Water temperature is generally regarded as a prominent parameter underlying variability that may be associated with season, island-island, and/or leeward-windward locations. Water temperature was examined to evaluate the thermal context for these ciguatera results. The 25 ^{o}C isotherm of annual mean sea surface temperature (SST) approximately overlays the geographical axis through the MHI chain, as shown in Figure 4. This pattern places cooler water to the northeast or windward side in reference to the trade winds of each island and warmer surface waters to the southwest or leeward sides. The seasonal patterns have a similar orientation of SST isothermal alignment. Temporally, variations in solar signals drive the diurnal and annual temperature cycles (Figure 5). Significant temporal variation may also be seen on the order of days associated with weather and order of weeks associated with small scale oceanographic features such as mesoscale eddies (Firing and Merrifield, 2004), or interannual to interdecadal variations (Firing et al., 2004). The Mokapu buoy SST time series was used to investigate the magnitude and duration of temperature extremes. Figure 5 shows that for the decade 2000-2010, there were only 29 days when the temperature exceeded 28°C for at least one 30 minute sample, and 20 of those days were in September-October 2004 which lies outside the temporal window of this study. These results indicate that in this mid-Pacific locality, SST greater than 28°C occur in a minute fraction (i.e., < 0.03-0.8%) of days, and excluding 2004 were near nil. Moreover, there were no >28°C periods of sufficient duration to influence *Gambierdiscus* physiological activity. Temperature records also indicated that there were no days having SST readings greater than 29°C.

Examination of the SST data shows negligible (i.e., <0.2°C) leeward-windward differences for or between any of the islands. Inspections of annual means and seasonal variations of the leeward versus windward SST of Oahu and Maui were made from various data sources (Figure 6). These data show negligible leeward-windward differences, ~2.1°C summer-winter variance for both sides, and negligible SST differences between Oahu to Maui (Figure 6).

Toxicity results were examined for a relationship between *C. argus* size and ciguatoxicity in several ways. There was no difference (p>0.05) between the mean weight of all samples, and the 36.4% of all samples that were positively ciguatoxic. For the results from all MHI, the frequencies of positively ciguatoxic samples were similar among the three weight quartiles. Relative ciguatoxicity was determined by calculating percent difference between baseline O/V (control) and the O/V value of the smallest fish extract concentration that elicited a significant response in assay, and normalized to the equivalent wet weight of fish tissue represented by each extract. There was no significant difference (p>0.05) in the relative toxicity values for the positive fish within the three size quartiles (Table 2). The mean relative toxicity of fish showed relative standard deviation values of 75.0-88.5% within quartiles Q1, Q2, and Q3, and varied by only 2.1 % between quartiles. There were also no significant differences (p>0.05) between the mean weights of the positively ciguatoxic samples from either the smallest and largest specimens from each of the individual islands (Table 2). Ciguatoxin concentrations (ng/g) in the positively ciguatoxin fish were calculated using the dose-response relationship given in Figure 1. The distribution of ciguatoxin concentration as a function of body size (Figure 7) illustrates the general lack of strong correlation between fish size and the degree of ciguatoxicity. With the exception of a single sample with a 71.3 ng/g concentration, ciguatoxin concentrations in these positive *C. argus* ranged from 0.08 ng/g to 24.4 ng/g, and averaged 2.61 ng/g. Fish were subsequently analyzed for the percentage of ciguatoxic specimens that were grouped into a number of bins based on $\log_{10}$ of body weight (Figure 8). This analysis, showing higher frequencies of positively ciguatoxic fish in the larger fish groupings gave the sole indication of a relationship between size and ciguatoxicity in this top carnivore.

DISCUSSION

As a high trophic level reef carnivore, *C. argus* is thought to represent a sentinel species presenting an above average likelihood for ciguatoxicity (Lehane and Lewis, 2000). These results from a relatively large sample set of this single species may therefore provide insights regarding some of the beliefs based on considerably smaller samplings and/or anecdotal inference concerning ciguatera. Note also that rather small temperature variations, either seasonally (~2.1°C) or spatially (both

Sea Surface Temperature (SST) variation off Oahu, Hawaii for the 2001-2010 period

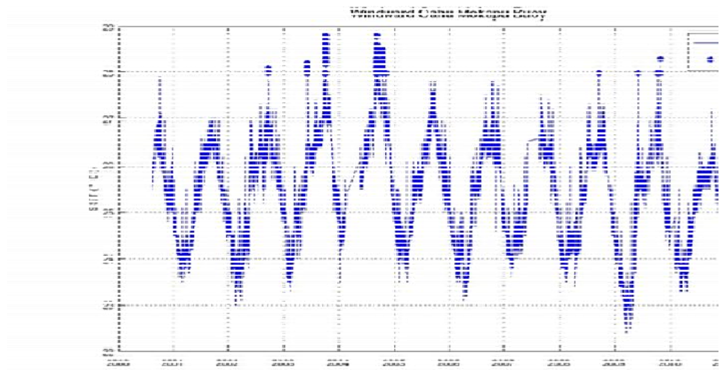

Figure 5A. Dots indicate the 29 days during this 10 year period when SST > 28°C.

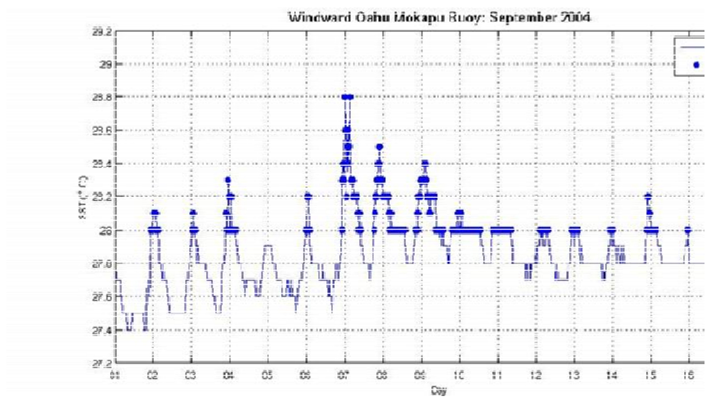

Figure 5B. SST variation for the September 2004 period when 20d of 29d of SST > 28°C occurred.

island-island and/or windward-leeward differences were ~0.2°C) are characteristic for this mid-Pacific local. This presents opportunity for evaluation of several types of variability in the absence of large concomitant variations in the temperature parameter per se.

It is part of the ciguatera lore that fish on the leeward side of islands are more likely to be ciguatoxic than fish in windward areas. It is sometimes implied that this is presumably due to the preference of *Gambierdiscus spp*. for quiescent rather than turbulent growth environments. To a degree, these data support the expectation for higher ciguatera rates in fish from leeward areas. However, in this study, the frequency of ciguateric fish collected from leeward areas was about twice that taken from windward areas. Given the absence of large temperature differences, this represents a first-order approximation for any leeward-windward influence factor due to parameters (e.g., turbulence, access, fishing effort, etc.) other than temperature differences coincident with other windward-leeward differences. These results also illustrate the relative effect of ancillary factors that exaggerate the windward-leeward perception. The number of fish collected from leeward areas was about three times the number from windward areas. The leeward/windward bias for positively ciguatoxicity was influenced roughly 50% more by number of samples collected (i.e., fishing effort) than by the actual frequency of ciguatoxic fish in the two areas. Also, during the three years of this study, the ratios of ciguatoxic *C. argus* individuals that came from the leeward and windward areas in a given year ranged over a factor of more than ten, and was due primarily to differences in the number of samples (i.e., fishing effort) that took place between these two areas. It is suggested that experiences associated with actual numbers of samples encountered within individual periods/years contributes to the perception of differences in ciguatoxicity between leeward and windward areas that is disproportionate to actual frequency of ciguatoxic fish within leeward and windward areas.

This study showed evidence for negligible seasonality in the frequency of positively ciguateric *C. argus* in the

Average Sea Surface Temperature (SST) variations for the windward and leeward coasts of Oahu and Maui

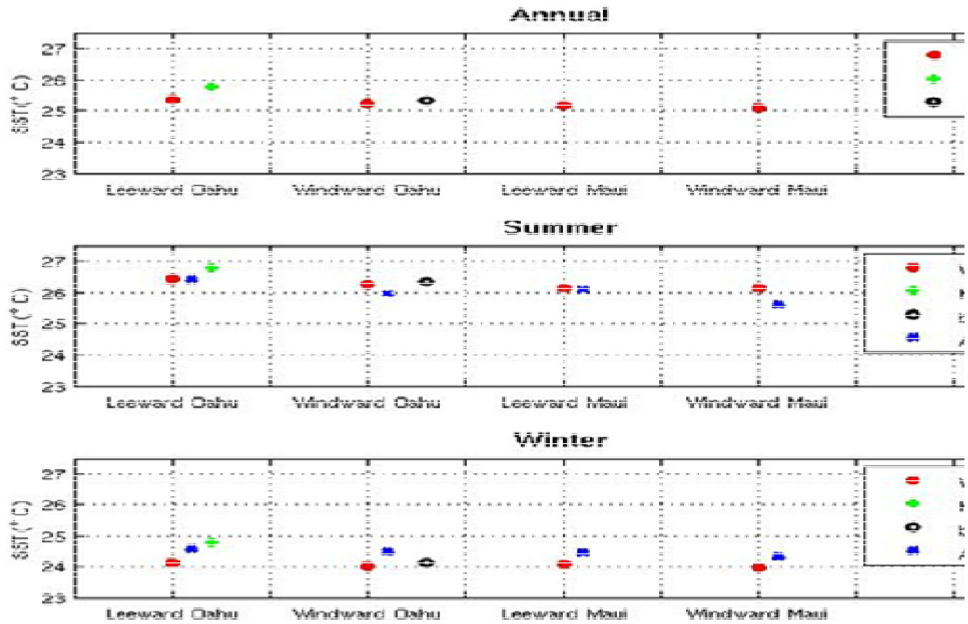

Figure 6. Annual and seasonal SST variations are shown. Summer months include July through September and winter months include January through March.

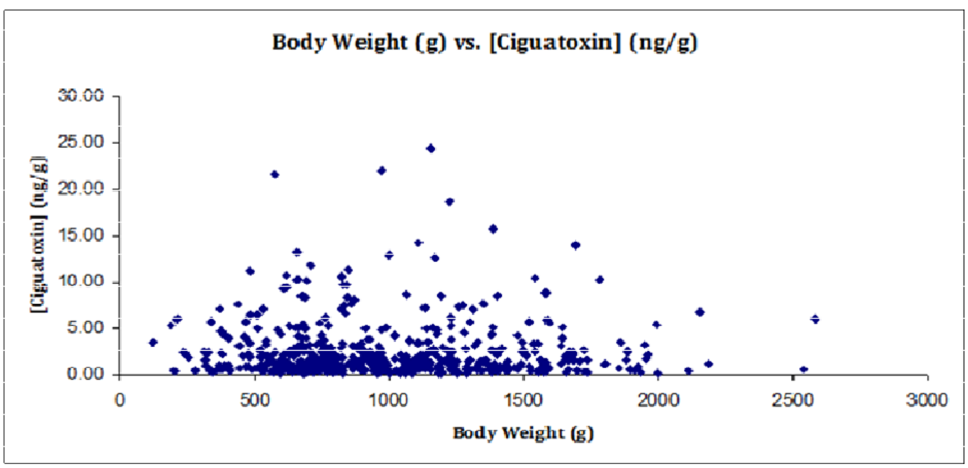

Figure 7. Calculated ciguatoxin concentrations as a function of *C. argus* body weight

Hawaiian Archipelago. This is somewhat in contrast to expectations for higher frequencies of ciguateric fish in the warmer months of late summer. Two reasons for this lack of seasonality likely lie in (a) the modest seasonal variation in SST within the MHI (Figures 4, 5, and 6), and (b) the fact that the normal temperature regime within the MHI is considerably lower that the upper thermal limit for *Gambierdiscus spp*. Annual temperature variations within the 24-27°C range (Figure 6) fall in the middle of the thermal preference for *Gambierdiscus spp*. Perhaps more importantly, the temperature variation almost always fails to embrace the 28-30°C range that is the thermal optimum for *Gambierdiscus spp*. and the thermal range that has been shown to coincide with physiological changes in *Gambierdiscus spp.* that are associated with toxin production, and concomitant increases in ciguatoxicity in resident fish populations. Exhaustive analyses (Tester et al., 2010; Llewellyn, 2010) have shown that SST exceeding 28°C are common seasonally in some Caribbean and Pacific locations, and attribute changes in ciguatoxicity to such temperature regimes. These results for the MHI, showing negligible ciguatera variations temporally or spatially, lend support that the reported correlations between ciguatera and temperature

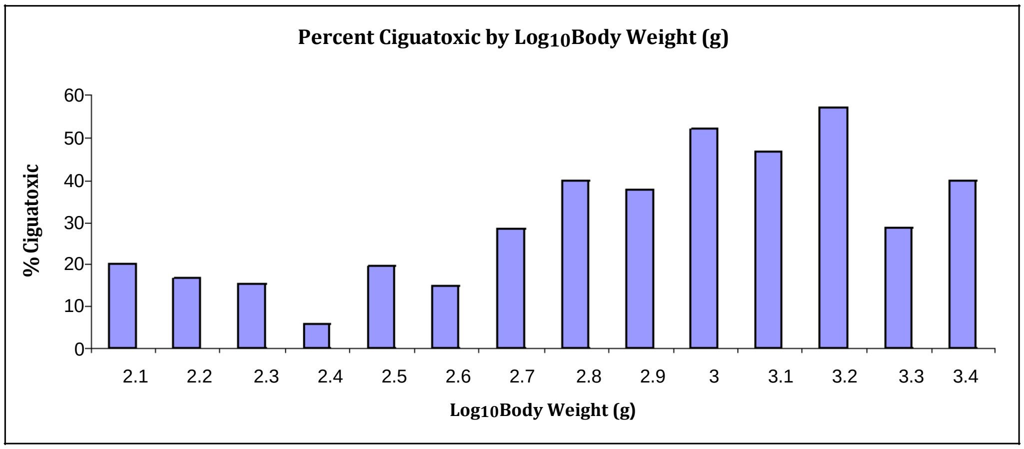

Figure 8. Frequency of positively ciguatoxic *C. argus* individuals within groups of increasing size, indexed by Log_{10} of total body weight, indicates an increased frequency of ciguatoxicity with increased size

reported in other environments may be heavily influenced by the >28°C thermal experience *per se* in those regions. Only modest (seasonal) differences were observed in the frequency of positively ciguatoxic *C. argus* that were collected at various times of the year. It is concluded that the seasonal temperature expression in the MHI is of insufficient magnitude and infrequently has periods >28°C that might elicit perceptible changes in the propensity for ciguatoxic fish.

Fishing communities frequently suggest that smaller fish should be safer to eat. This belief is rooted in a deduction that the larger (older) specimens must have fed more and bioconcentrated the ciguatoxin for longer periods than smaller (younger) fish. The results for this top reef carnivore indicate a lack support for this belief. Most of these analyses that were based on fairly large sample size showed negligible relationship between ciguatoxin concentration and fish size; it was only when fish were grouped by size (Figure 8) was correlation of the frequency of positively ciguatoxic fish with size evident. The examination of ciguatoxicity in this local provides insights on the relative roles of individual factors (e.g., temperature, leeward/windward location, fish size, season) on the prevalence of ciguatera in the carnivorous fish populations.

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REFERENCES

Arena P, Levin B, Fleming LE, Friedman MA, Blythe DG (2004). A pilot study of the cognitive and psychological correlates of chronic ciguatera poisoning. Harmful Algae 3: 51-60.

Banner AH (1974). The biological origin and transmission of ciguatoxin. In: Hun HJ, Lance CE (eds) Bioactive products from the sea, Marcel Dekker, New York, pp 15-36.

Banner AH (1976). Ciguatera: a disease from coral reef fish. In: Hun HJ, Lance CE (eds) Bioactive products from the sea, Marcel Dekker, New York, pp 177-215.

Banner AH, Helfrich P (1964). The distribution of ciguatera in the tropical Pacific. Tech. Rep. Hawaii Mar. Lab. 3: 1-48.

Banner AH, Scheuer P, Sasaki S, Helfrich P, Alender C (1960). Observations

Benoit E, Juzans P, Legrand AM, Molgo J (2000). Reversal of Pacific ciguatoxin-1B effects on myelinated axons by agents used in ciguatera treatment. Cybium. 24: 33-40.

Bienfang PK, Parsons ML, Bidigare RR, Laws EA, Moeller PDR (2008). Ciguatera fish poisoning: a synopsis from ecology to toxicity. In: Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele H, Gerwick WH (eds) Oceans and human health: risks and remedies from the sea. Elsevier Science Publishers, New York, pp 257-270.

Cameron J, Flowers AE, Capra MF (1991). Electrophysiological studies on ciguatera fish poisoning in men (Part II). J. Neurol. Sci. 101: 93-97.

Chinain M, Darius HT, Ung A, Cruchet P, Wang Z, Ponton D, Laurent D, Pauillac S (2010). Growth and toxin production in the ciguatera-causing inoflagellates *Gambierdiscus polynesiensis* (Dinophyceae) in culture. Toxicon 56: 739-750.

De Fouw JC, Van Egmond HP, Speijers GJA (1999). Ciguatera fish poisoning: a review. RIVM Report No. 388802021. Available from: www.rivm.nl/bibliotheek/rapporten/388802021.html.

DeMartini EE, Firedlander AM (2004). Spatial pattern of endemism in shallow water reef fish populations of the Northwestern Hawaiian Islands. Mar. Ecol. Prog. Ser. 271: 281-296.

Dickey RW (2008). Ciguatera toxins: chemistry, toxicology and detection. In: Botana LM (ed) seafood and freshwater toxins: pharmacology, physiology, and detection, 2nd edition. CRC Press, Taylor and Francis Group, Florida, pp 479-500.

Dickey RW, Jester E, Granade R, Mowdy D, Moncrief C, Rebarchik D, Robl M, Musser S, Poli M (1999). Monitoring brevetoxins during a Gymnodinium breve red tide: comparison of sodium channel specific cytotoxicity assay and mouse bioassay for determination of neurotoxic shellfish toxins in shellfish extracts. Natural Toxins 7: 157-165.

Dickey RW, Plakas SM (2010). Ciguatera: A public health perspective. Toxicon 56(2): 123-136.

Dierking J (2007). Effects of introduced predatory fish *Cephalopholis argus*, on native reef fish populations in Hawaii. Dissertation, University of Hawai'i.

Dierking J, Campora C (2009). Ciguatera in the introduced fish *Cephalopholis argus* (Serranidae) in Hawai'I and implications for fisheries management. Pacific Science 63(2): 193-204. Doi: 10.2984/049.063.0203

Dierking J, Williams ID, Walsh WJ (2009). Diet composition and prey selection of the introduced grouper species peacock hind (*Cephalopholis argus*) in Hawaii. Fish. Bull. 107: 464-476.

Donaldson TJ (1995). Courtship and spawning behavior of the pygmy grouper (*Cephalopholis spiloparaea* (Serranidae Epinephelinae), with notes on *C. argus* and *C. urodeta*. Environmental Biology of Fishes 43(4): 363-370. Doi 10.1007/BF00001171

Firing YL, Merrifield MA (2004). Extreme sea level events at Hawaii: influence of mesoscale eddies. Geophysical Research Letters 31: L24306. Doi 10.1029/2004GLO21539

Firing YL, Merrifield MA, Schroeder TA, Qiu B (2004). Interdecadal sea level fluctuations at Hawaii, Journal of Physical Oceanography 34: 2514–2524.

Fleming LE, Baden DG, Bean JA, Weisman R, Blythe DG (1998). Seafood toxin diseases: issues in epidemiology and community outreach. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful Algae. Zunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, pp 245-248.

Fleming LE, Katz D, Bean JA, Hammond R (2001). Epidemiology of seafood poisoning. In: Hui YH, Kitts D, Standfield P (eds) Foodborne disease handbook. Marcell Dekker, New York, pp. 287-310.

Fraga S, Rodriguez F, Caillaud A, Diogene J, Raho N, Marin I, Zapata M (2010). Ciguatera in Macaronesia: an emerging problem. Contribution to identification of Gambierdiscus species. Presentation to the 14th Conference of the International Society for the Study of Harmful Algae. Crete, Greece.

Friedman MA, Fleming LE, Fernandez M, Bienfang P, Schrank K, Dickey R, Bottein MY, Backer L, Ayyar R, Weisman R, Watkins S, Grande R, Reich A (2008). Ciguatera fish poisoning: treatment, prevention and management. Marine Drugs 6: 456-479.

Heemstra PC, Randall JE (1993). FAO Species Catalogue Vol. 16. Groupers of the world (family Serranidae, subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail Species known to date. FAO Fish. Synop. 125(16): 1-382.

Helfrich P, Banner AH (1963). Experimental induction of ciguatera toxicity in fish through diet. Nature 197: 1025–1026.

Hokama Y, Yoshikawa-Ebesu JSM (2001). Ciguatera fish poisoning: a foodborne disease J. Toxicol. Toxin Rev. 20(2): 85-139.

Holmes MJ (1998). *Gambierdiscus yasumotoi* sp. nov. (Dinophyceae), a toxic benthic dinoflagellate from Southeastern Asia. J. Phycol. 34: 661-668.

Lange WR (1994). Ciguatera fish poisoning. Am. Ram. Physician 50: 579-586.

Lehane L, Lewis RJ (2000). Ciguatera: recent advances but the risk remains. International Journal of Food Microbiology 61: 91–125.

Lewis RJ (1986). Disease and development: ciguatera fish poisoning. Soc. Sci. Med. 23: 983-993.

Lewis RJ (1993). The changing face of ciguatera. Toxicon 39: 97-106.

Llewellyn LE (2010). Revisiting the association between sea surface temperature and the epidemiology of fish poisoning in the South Pacific: Reassessing the link between ciguatera and climate change. Toxicon 56: 691-697.

Locarnini RA, Mishonov AV, Antonov JI, Boyer TP, Garcia HE, Baranova OK, Zweng MM, Johnson DR (2010). World Ocean Atlas 2009, Volume 1: Temperature. In: Levitus S (ed) NOAA Atlas NESDIS 68, U.S. Government Printing Office, Washington, D.C., pp 184.

Manger RL, Leja LS, Lee SY, Hungerford JM, Wekell MM (1993). Tetrazolium-based cell bioassay for neurotoxins active on voltage-sensitive: semi-automated assay for saxitoxins, brevetoxins, and ciguatoxins. Analytical Biochemistry 214 (1): 190-194.

Meyer AL (2008). An Ecological Comparison of *Cephalopholis argus* between native and introduced populations. Dissertation, University of Hawai'i. on ciguatera-type toxin in fish. Ann. NY Acad. Sci. 90: 770–787.

Pearn J (2001). Neurology of ciguatera. J. Neurol. 70(1): 4-8.

Quod JP, Turquet J (1996). Ciguatera in Reunion Island (SW Indian Ocean): epidemiology and clinical patterns. Toxicon 34(7): 779-785.

Randall J E (1995). Zoogeographic analysis of the inshore Hawaiian fish fauna. In: Maragos JE, Peterson MNA, Eldredge,LG, Bardach JE, Takeuchi HF (eds) Marine and coastal biodiversity in the tropical island Pacific region: Vol. 1, Species systematic and information management priorities. East-West Center, Honolulu, pp 193-203.

Randall J E (1998). Zoogeography of shore fishes of the Indo-Pacific region. Zool. Stud. Taiwan 37: 227-268.

Randall JE (1958). A review of ciguatera tropical fish poisoning with a tentative explanation of its cause. Bull. Mar. Sci. Gulf Carib. 8: 236-267.

Randall JE **(**1987). Introduction of marine fishes to the Hawaiian Islands. Bull. Mar. Sci. (41): 490-502.

Roeder K, Erler K, Kibler S, Tester P, Van The H, Nguyen-Ngoc L, Gerdts G, Luckas B (2010). Characteristic profiles of Ciguatera toxins in different strains of Gambierdiscus spp. Toxicon 56: 731-738.

Shpigel M, Fishelson L (1999). Territoriality and associated behavior in three species of the genus *Cephalopholis* (Pisces: Serranidae) in the Gulf of Aqaba, Red Sea**.** J. Fish. Biol. 38: 887-896.

Smith WL, Craig MT (2007). Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of Serranid and Pereid fishes. Copeia 1: 35-55.

Snedecor GW, Cochran WG (1980). Statistical Methods, 7th edn. The University Press, Ames, Iowa, pp 1-507.

Tester PA, Feldman RL, Nau AW, Kibler SR, Litaker RW (2010). Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and West Indies. Toxicon 56: 698-710.

Yasumoto T, Bagnis R, Thevenin S, Garçon M (1977). A survey on comparative toxicity in the foodchain of ciguatera. Bull. Jap. Soc. Sci. Fish. 43: 1015-1019.