BIOLOGICAL MAGNIFICATION OF CIGUATOXIN: A QUANTITATIVE APPROACH

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By Natalie J. Wallsgrove

Thesis Committee:

Brian N. Popp, Chairperson
Paul N. Bienfang
Jeffrey C. Drazen

DEDICATION

This work is dedicated to my brothers for the inspiration and encouragement that they have always provided.

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ABSTRACT

Ciguatoxins, the causative agent of ciguatera fish poisoning, are a group of potent neurotoxins produced worldwide in tropical and subtropical marine coastal ecosystems by several dinoflagellate species within the genus *Gambierdiscus.* It has been hypothesized that the multiple chemical congeners of ciguatoxin are lipid-soluble molecules that are biologically magnified through coral reef food webs. This study attempts for the first time to quantify this biological magnification via correlation between estimations of fractional trophic position and estimations of ciguatoxin concentrations for individual wild-caught fish within a known feeding relationship. This study focuses on the carnivorous grouper, *Cephalopholis argus*, and 22 potentially ciguatoxic prey fish species.

Prevalence and concentration of ciguatoxin within this predator/prey relationship were analyzed using a mouse neuroblastoma bioassay on 924 *C. argus* samples and 156 prey samples all collected from the reefs along the south and/or west shores of the Hawaiian Islands of Maui and Oahu. To determine the biological magnification of ciguatoxin, the bioassay results were combined with the trophic position estimates obtained via compound-specific nitrogen isotope analysis of amino acids (AA-CSIA). AA-CSIA is a novel technique which allows for the estimation of fractional trophic position for organisms without the necessity of separate analysis to determine the $\delta^{15}N$ values of photoautotrophs within the food web of interest. This technique therefore circumvents many of the short-comings of bulk stable isotope analyses that have traditionally been applied

for trophic position determination. AA-CSIA was performed on 56 fish samples consisting of both *C. argus* and prey and trophic position was estimated using three separate calculation methods for comparison. These results were also compared to trophic position estimations based on stomach content analysis as reported on the website www.fishbase.org, which is a global database on the ecology of fish. The results of these comparisons indicate that an empirically derived trophic enrichment between glutamic acid and phenylalanine (3.9 ± 1.3 ‰) applied for trophic positions greater than 2 (previously published trophic enrichment of 7.6 ± 1.3 ‰ is applied for the step between trophic positions 1 and 2) yields the most accurate and precise estimation of trophic position.

Of the 924, 41.3% *C. argus* and 35% of the 156 prey samples contained ciguatoxin above the detection limit of the mouse neuroblastoma bioassay. An increased frequency of ciguatoxicity with total body weight was found for *C. argus*, however, no such relationship was observed for prey species. A significant positive relationship was found between trophic position and total body weight for *C. argus* that was not found for prey species. No significant positive relationship was found between ciguatoxin concentration and trophic position for individual samples. The results of this study indicate that trophic position alone is not sufficient to explain variation in ciguatoxin concentrations observed in individual members of the studied fish population. However, the results of this study provide evidence to support the hypothesis of biological magnification of ciguatoxin within the studied populations.

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CHAPTER 1

Introduction

Ciguatoxins (CTX) are a class of lipid-soluble neurotoxins that are concentrated, amplified and modified through tropical and subtropical coral reef food webs worldwide and are considered to be among the most potent toxins known (Pearn 2001, Lewis and Holmes 1993). Ciguatera fish poisoning (CFP) in humans results from the consumption of reef fish with accumulated CTX (Randall 1958). About 50,000 cases of CFP are reported globally each year. It is estimated that this number could be as high as 500,000 when under-reporting and mis-diagnosis are considered, making CFP the most common fish poisoning globally (Lewis 2001). Symptoms of CFP include gastrointestinal, neurological and cardiovascular disorders, and, although rare, cases of death have been reported (Pearn 2001). Carnivorous predatory reef fish, such as *Cephalopholis argus* are commonly implicated in cases of CFP due to assumed biological magnification of CTX through the food web.

While the effects of biological magnification of CTX seem relatively easy to quantify in simple linear food chain models, the feeding relationships of coral reef ecosystems are much more complicated and are best represented with complex food webs that are characterized using fractional trophic levels (Pilos and Strong 1996).

Bulk tissue nitrogen isotope ($\delta^{15}N$) values have been widely used in food web studies to establish trophic position of marine organisms (Fry 2006). However, interpreting the $\delta^{15}N$ values of animals is complicated by the fact that

these values are a consequence of two variables, i.e., variation in the isotopic composition of nitrogen available to primary producers and the mean number of steps the consumer is removed from feeding directly on phytoplankton (Martinez del Rio et al. 2009 and references within). Characterizing $\delta^{15}N$ values at the base of the food web is challenging because phytoplankton, bacteria and heterotrophic protists respond quickly to changes in biogeochemistry and are difficult to isolate and analyze (Hannides et al. 2009, Rolff 2000, O'Reilly et al. 2002) .

Compound-specific nitrogen isotope analysis of amino acids (AA-CSIA) is a promising new technique that avoids many of the short-comings of traditional bulk stable isotope analyses (McClelland and Montoya 2002, Chikaraishi et al. 2007). In samples of consumer muscle tissue, "source" amino acids (e.g. phenylalanine, glycine and serine) appear to retain the isotopic composition of the nitrogen (N) sources at the base of the food web, whereas "trophic" amino acids (e.g. glutamic acid, alanine, valine, leucine, isoleucine, and proline) become ^{15}N enriched at each step up in trophic level (Chikaraishi et al. 2009, Popp et al. 2007). One key advantage of this technique is that predator tissue alone is sufficient for quantitative determination of trophic position, making separate analysis of the isotopic composition at the base of the food web and the potential dietary constituents unnecessary.

The mouse neuroblastoma (N2a) bioassay that is used for the detection of ciguatoxin in animal tissue is a sodium-channel-specific bioassay (Manger et al. 1993 and 1995, Dickey et al. 1999 and Bienfang et al. 2008). This method

measures sodium channel disruption as a proxy for ciguatoxin concentration (Bienfang et al. 2008).

Extensive analyses of ciguatoxic *C. argus* around the main Hawaii Islands indicate substantial spatial and temporal variability in ciguatoxicity of fish (Figure 1). This study utilizes AA-CSIA of *C. argus* and selected prey species in an attempt to place CTX biomagnification into a food web context. Prey species targeted are a subset of prey species determined from the ecological study of *C. argus* undertaken by Dierking (2007) who identified the prey items in 156 full *C. argus* stomachs (Table 1). The families and species listed in Table 1 are restricted to *C. argus* prey fish that are non-zooplanktivorous.

The controls on the distribution of ciguatoxic fish, and therefore the potential for human illness, have remained elusive (Lewis and Holmes 1993, Dickey and Plakas 2010 and references within). Ciguatoxins originate as gambiertoxins in the benthic dinoflagellate genus *Gambierdiscus* and are later transformed into CTX after entering the food web through herbivorous reef fish (Yasumoto et al. 1977, Bomber and Aikman 1989). Factors controlling both the abundance and toxin production of *Gambierdiscus* are yet to be fully understood (Chinain et al. 1999), although sea surface temperature (Chateau-Degat et al. 2005), nutrient load (Lartigue et al. 2009), physical disturbance of coral reefs and algal cover as the preferred substratum for *Gambierdiscus spp.* (Kaly and Jones 1994) have all been linked to changes in abundance of this dinoflagellate.

The utilization of the N2a bioassay for the detection of CTX in fish tissue, combined with AA-CSIA for the determination of trophic position, allows for the

quantification of the biological magnification of CTX. My primary research objective is thus to place CTX in a food web context using these techniques in an effort to gain better understanding of the biomagnification of CTX in the coral reef ecosystems of the Hawaiian Islands of Maui and Oahu.

My overarching hypothesis is that CTX is concentrated via food web magnification and the degree of magnification can be quantified with the determination of trophic position by AA-CSIA. Biomagnification of CTX through a food web has not been quantified in the published literature to date, and this work provides a unique opportunity to attempt this task. Any further insight into the occurrence of ciguatoxic fish in this ecosystem may help to prevent future human illness due to CFP.

CHAPTER 2

Methods

Collection

Samples of *C. argus* were obtained through collaboration with local sports fishermen who provided the date, approximate depth and location of the catch. Total body weight was recorded after the fish were received from the fishermen, and total length and standard length measurements were recorded after muscle samples were taken from the fish. Samples of *C. argus* prey species were collected by scientific divers via spear using either SCUBA or closed circuit re-breather from near-shore reefs around Oahu and Maui, and detailed descriptions of date, depth and location of the catch were provided. Total body weight, total length and standard length were recorded prior to muscle tissue sampling. Fish length is reported here as total length to avoid uncertainty that may result from error in standard length measurements of the rounded tail which is characteristic of *C. argus.*

For both AA-CSIA and N2a bioassay analysis lateral muscle tissue was sampled from each fish, lyophilized and ground into a homogenous powder.

N2a Bioassay

This method follows that outlined in Bienfang et al. 2008. CTX was extracted from fish tissue powder (~5-20 g wet weight) using 2:1 v/v methylene chloride (CH_2Cl_2):fish powder. The 2:1 mixture was allowed to sit at room temperature for at least 1 hour with gentile agitation prior to overnight storage at 4 °C. After at least 16 hours, samples were sonicated for 5 min and vacuum filtered

(Whatman GF/F, nominal prosity 0.7 μm). The powdered fish was scraped from the filter and the sonication/filtration process was repeated twice. The CH_2Cl_2 containing CTX extract was dried using a rotary evaporator, reconstituted in ~ 5 ml CH_2Cl_2, sonicated for 30 sec and transferred to a clean 20 ml scintillation vial. The addition of ~5ml CH_2Cl_2 and sonication process was repeated twice, and each rinse was transferred to the scintillation vial. The ~15 ml of CH_2Cl_2 was allowed to evaporate overnight in a ventilation hood. An additional ~2 – 3 ml CH_2Cl_2 was added to concentrate the dry CTX extract in the bottom of the scintillation vial, then allowed to dry overnight in the hood. The extracts were capped and stored at -20°C before being submitted to the laboratory of Dr. Paul Bienfang at the Center for Oceans and Human Health at UH Mānoa for analysis of sodium channel activity via mouse neuroblastoma (N2a) bioassay.

The N2a used to assess changes in sodium channel activity follows the procedures as outlined by Manger et al. (1993 and 1995), Dickey et al. (1999) and Bienfang et al. (2008). One day prior to analysis of CTX extracts, 96-well plates were prepared with 100μl of mouse neuroblastoma cell suspension (200,000 cells ml^{-1}) added to the 60 inner wells and a phosphate-buffered saline added to the outer perimeter wells. The plates were allowed to acclimate overnight in an incubator at 37 °C with 5% CO_2-enriched and humidified air. CTX extracts were re-dissolved in 2 mL methanol and sonicated for 3-5 min and added to the prepared plates. Plates were dosed with extract in 1μl, 2μl 3μl and 4μl per well concentrations, replicating each concentration in 6 wells. Ouabain (0.3 mM) and veratridine (5uM) (O/V) were added to 3 of the 6 wells per concentration to

depolarize the cellular membranes and elucidate a sodium-channel disruption as caused by the presence of ciguatoxin in the extracts. Each plate also contained 10 cells-only control wells (i.e., no sample or O/V) representing uninhibited growth and 10 baseline wells (i.e., cells plus O/V) representing the baseline decrease in cell viability as a result of the addition of these chemicals. Well volumes were brought to 200µl using RPMI-1640 cell media and plates were allowed to incubate overnight.

Following the incubation, 10µl of CellTiter 96 Aqueous One Solution (Promega, Inc., Madison, WI) was added to each well and allowed to incubate for 1 hour. The tetrazolium compound in this solution is bioreduced by metabolically active cells to produce a colorietric response that was measured via a Multiskan MCC/340 Eliza plate reader (Thermo Labsystems, Cincinnati, OH) at 492nM. Results were analyzed using a Student's *t*-test to identify significant differences between control and sample means. Wells containing only cells and extract were used to assess the cytotoxcity of the extract to the cells prior to interpretive analysis of sodium channel activity. Wells were considered non-cytotoxic when sample means between extract-containing wells and control wells were not significantly different ($p > 0.05$). Sodium channel disruption analysis was conducted on non-cytotoxic samples by comparing the response from wells with extract plus O/V and control wells plus O/V to determine if significant decreases in cell metabolic activities occur. Differences in the mean of control wells, both with and without O/V (n=10 each), and the mean of extract-plus O/V wells (n=3) were used to determine statistical differences.

The concentration of CTX muscle tissue of fish (pg/mg wet weight basis) was determined using an N2a bioassay dose-response curve for PCTX-1 generated by two separate sets of experiments. The equation describing the data is as follows:

$$y = y_{low} + \frac{y_{high} - y_{low}}{1 + \left(\frac{x}{EC50} \right)^{n}} \qquad (1)$$

where y is the percent control, x is the amount of PCTX-1 (nmol/L), y_{high} = 100, y_{low} = 36.7, EC50 = 19.2 nmol, and n = 0.69. To calculate the amount of CTX in muscle tissue the inverse of equation 1 was used:

$$x = EC50 \times \left(\frac{y_{high} - y_{low}}{y - y_{low}} - 1 \right)^{1/n} \qquad (2)$$

The equivalent wet weight of fish added to each well of the bioassay was used to express the CTX concentration in units of pg CTX/mg fish. The limit of detection of this method is estimated to be ~0.07 pg/mg.

The larger sample size for *C. argus* (n=924) and the individual prey species *C. strigosus* (n=74) allowed for the utilization of more elaborate methods for the determination of median CTX concentrations. The median concentration for these groupings was inferred from plots of x vs. z-statistic, where x is the individual value for $\log_{10}$[CTX] measured in a particular fish, and z-statistic is defined as:

$$z = \frac{(x - \mu)}{\sigma} \quad (3)$$

where μ is the theoretical mean and σ is the theoretical standard deviation. The z-statistic is back-calculated from the cumulative probability (p) associated with each $\log_{10}$[CTX] value (x) using the computing software Matlab. Cumulative probability of x refers to the probability that a randomly selected value from the distribution of x will be less than or equal to x. Assuming a normal distribution for x, and using p, the inverse of the normal cumulative distribution yields the z-statistic for x. If the data are normally distributed, the plot of x vs. z-statistic is linear and has an intercept of μ, and a slope of σ. The x value at z-statistic = 0 can therefore be inferred as the median value of x. The inverse $\log_{10}$ of x is reported as the median CTX concentration in fish.

Bulk Isotope Analysis

Bulk tissue nitrogen (N) and carbon (C) concentrations and isotopic compositions were determined from powdered and homogenized muscle samples (300-500 μg) using either a Thermo Finnigan ConfloII/Delta S mass spectrometer coupled to a Carlo Erba NC2500 Elemental Analyzer or a Thermo Finnigan Delta^{Plus} XP mass spectrometer coupled to a Costech Instruments Model 4010 Elemental Combustion System. The University of Hawaii Stable Isotope Biogeochemistry Laboratory underwent an equipment upgrade during the time frame of this study, and repeated isotopic analyses of internal reference materials of known isotopic composition (glycine and yellowfin tuna muscle) and NIST

certified reference materials were used to ensure consistency in results. Isotopic values are reported in δ-notation relative to atmospheric N_2 and V-PDB, for N and C respectively. Average accuracy and precision of all stable isotopic analyses determined by replicate analysis of glycine and samples was less than ±0.1‰ (1 S.D.).

Amino Acid Compound Specific Isotope Analysis

Prior to amino acid compound specific isotope analysis (AA-CSIA), dried and homogenized fish muscle tissue was subjected to acid hydrolysis, esterification of the carboxyl terminus and trifluoroacetylation of the amine group (Macko et al. 1997; Popp et al. 2007).

Amino Acid Hydrolysis and Derivitization

Muscle tissue (~5 mg) of fish was hydrolyzed at 150 °C for 70 minutes using 6 N hydrochloric acid (HCl) in a culture tube that was flushed with dinitrogen gas (N_2) and fitted with a Teflon-lined cap. The HCl was either evaporated to dryness at 55°C under a stream of N_2 or using a Thermo Savant Speed Vac concentrator coupled with a UVS400 at 55°C for 1.5 hr. The residue was re-dissolved in 1 ml 0.01 N HCl and purified by filtration (0.45 μm hydrophilic filter), and the filter washed with 1 ml 0.01 N HCl. Amino acids were separated from sugars and organic acids using a cation exchange column (~ 5 cm Dowex 50WX8-400 in a Pastuer pipette). The filtered hydrolysate was added to the ion exchange column in 0.01 N HCl and amino acids eluted with 4 ml ammonium hydroxide and evaporated to dryness under a stream of N_2 at 80 °C.

The samples were re-acidified by adding 0.5 ml of 0.2 N HCl, the vials were flushed with N_2, heated to 110 °C for 5 minutes and then dried either at 55 °C under a stream of N_2 or using the Speed Vac concentrator for 1.5 hr at 55 °C. The hydrolyzed muscle samples were esterified using 2-3 ml of 1:4 acetyl chloride:isopropanol in N_2-flushed vials heated to 110 °C for 60 minutes. Excess solvents were then dried under a stream of N_2 at 60 °C. Trifluoroacetylation of the amine group was accomplished by adding 3:1 methylene chloride:trifluoroacetic anhydride (TFAA) to each vial and heating to 100 °C for 15 minutes. The samples were further purified by solvent extraction following Ueda et al. (1989) using 2 ml of P-buffer (KH_2PO_4 + Na_2HPO_4 in distilled water, pH 7). The acylated amino acids were partitioned into chloroform, the chloroform evaporated to dryness and the trifluoroacetylation step repeated to ensure full derivitization. Samples were stored at -20 °C in 3:1 methylene chloride:TFAA for up to one month until isotope analysis.

Compound Specific Isotope Analysis

Just prior to isotope analysis of samples the 3:1 methylene chloride:TFAA was evaporated under a stream of N_2 at room temperature and samples were re-dissolved in 100 µl of ethyl acetate. The stable N isotope composition of the amino acids were determined using either a Delta^{Plus} XP or Delta V plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980 °C), reduction furnace (650 °C), and liquid nitrogen cold trap. The samples (1-2 µl) were injected (split/splitless injector in split mode with

a 10:1 split ratio) onto a BPx5 capillary column (30m x 0.32mm x 1.0 μm film thickness) at an injector temperature of 180 °C with a constant helium flow rate of 1.4 ml min^{-1}. The column was initially held at 50 °C for 2 minutes and then increased to 190 °C at a rate of 8 °C per minute. Once at 190 °C, the temperature was increase at a rate of 10 °C per minute to 300 °C where it was held for 7.5 minutes. Internal reference compounds, aminoadipic acid and norleucine of known nitrogen isotopic composition, were co-injected with samples and used to normalize the measured $\delta^{15}N$ values of unknown amino acids. All samples were analyzed in triplicate and isotopic values are reported in δ-notation relative to atmospheric N_2. Reproducibility associated with isotopic analysis of glutamic acid and phenylalanine averaged 0.40‰ and ranged from 0.04‰ to 1.37‰. The accuracy of the measurements was determined by using the known $\delta^{15}N$ value for norleucine to determine the measured $\delta^{15}N$ value of aminoadipic acid as an unknown. The accuracy averaged 0.67‰ and ranged from 0.01‰ to 1.97‰.

Calculation of trophic position from AA-CSIA

The fractional trophic positions of fish samples were calculated in three ways. The first (Method 1) utilizes the measured $\delta^{15}N$ values of glutamic acid and phenylalanine as described by Chikaraishi et al. (2009).

$$TP_{TEF=7.6} = \frac{(\delta^{15}N_{glu} - \delta^{15}N_{glu}) - \beta}{TEF_1} + 1 \qquad (4)$$

In eqn. 4, β is the difference between the $\delta^{15}N$ values of glutamic acid and phenylalanine in marine photoautotrophs (assumed to be 3.4 ± 1.0‰) and TEF is

the trophic enrichment factor (assumed to be 7.6 ± 1.2‰ (TEF_1)) (Chikaraishi et al. 2009).

The second method (Method 2) was adopted from Sherwood et al. (2010) where the mean values for "source" (Sr-AA) and "trophic" (Tr-AA) amino acids are used. In this study, Sr-AA include glycine, serine and phenylalanine, and Tr-AA include alanine, valine, leucine, isoleucine, proline and glutamic acid. Using a weighted average based on the uncertainty associated with these amino acids in photoautotrophs and in feeding experiments as described in Chikaraishi et al. (2010), a β value of 3.4 ± 0.6‰ and a TEF_2 value of 5.6 ± 0.7‰ were used for equation 5:

$$TP_{Tr-Sr} = \frac{(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Sr-AA}) - \beta}{TEF_2} + 1 \qquad (5)$$

To address concerns of underestimation of TP for fish with an expected TP>2, an empirical derivation of TEF (TEF_3) was utilized (Method 3). Using AA-CSIA data for 123 marine fish across 29 species with an expected TP>2 derived from FishBase (see below), TEF_3 = 3.9 ± 1.3‰ was calculated using equation 6:

$$TEF_3 = \frac{(\delta^{15}N_{glu} - \delta^{15}N_{phe}) - \beta - TEF_1}{TP_{FB} - 2} \qquad (6)$$

This new TEF was then used to calculate TP using equation 7:

$$TP_{TEF=3.9} = \frac{(\delta^{15}N_{glu} - \delta^{15}N_{phe}) - \beta - TEF_1}{TEF_3} + 2 \qquad (7)$$

so that TEF_1 is applied between TP=1 and TP=2, and TEF_3 is applied for all TP>2. This method was chosen to explore variation in CTX concentrations in the fish samples included in this study.

Expected TP (TP_{FB}) was determined from www.fishbase.org. FishBase uses the modeling software Ecopath (Polovina 1984, Christensen and Pauly 1992, 1993; Pauly and Christensen 1993; Christensen and Pauly 1995) to estimate TP from diet composition as determined by published stomach content studies or by individual food items when complete diet composition data is not available.

Propagation of Error

The uncertainty associated with the trophic position calculation was determined by propagation of error using the uncertainly in β and the TEF established by Chikaraishi et al. (2009, 2010) and the measured analytical reproducibility for the $\delta^{15}N$ values of amino acids for each sample.

For Method 1:

$$\sigma_{TP}^2 = \left(\frac{\partial TP}{\partial \delta^{15}N_{glu}}\right)^2 \sigma_{\delta^{15}N_{glu}}^2 + \left(\frac{\partial TP}{\partial \delta^{15}N_{phe}}\right)^2 \sigma_{\delta^{15}N_{phe}}^2 + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma_{\beta}^2 + \left(\frac{\partial TP}{\partial TEF_1}\right)^2 \sigma_{TEF_1}^2$$

For Method 2:

$$\sigma_{TP}^2 = \left(\frac{\partial TP}{\partial \delta^{15}N_{Tr-AA}}\right)^2 \sigma_{\delta^{15}N_{Tr-AA}}^2 + \left(\frac{\partial TP}{\partial \delta^{15}N_{Sr-AA}}\right)^2 \sigma_{\delta^{15}N_{Sr-AA}}^2 + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma_{\beta}^2 + \left(\frac{\partial TP}{\partial TEF_2}\right)^2 \sigma_{TEF_2}^2$$

$$\sigma_{\delta^{15}N_{Tr-AA}} = \sqrt{\sigma_{\delta^{15}N_{Ala}}^2 + \sigma_{\delta^{15}N_{Val}}^2 + \sigma_{\delta^{15}N_{Leu}}^2 + \sigma_{\delta^{15}N_{Iso}}^2 + \sigma_{\delta^{15}N_{Pro}}^2 + \sigma_{\delta^{15}N_{Glu}}^2}$$

$$\sigma_{\delta^{15}N_{Sr-AA}} = \sqrt{\sigma_{\delta^{15}N_{Gly}}^2 + \sigma_{\delta^{15}N_{Ser}}^2 + \sigma_{\delta^{15}N_{Phe}}^2}$$

For Method 3:

$$\sigma_{TP}^2 = \left(\frac{\partial TP}{\partial \delta^{15} N_{glu}}\right)^2 \sigma_{\delta^{15}N_{glu}}^2 + \left(\frac{\partial TP}{\partial \delta^{15} N_{phe}}\right)^2 \sigma_{\delta^{15}N_{phe}}^2 + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma_{\beta}^2 + \left(\frac{\partial TP}{\partial TEF_1}\right)^2 \sigma_{TEF_1}^2 + \left(\frac{\partial TP}{\partial TEF_3}\right)^2 \sigma_{TEF_3}^2$$

Statistical Analysis

Normality and homogeneity of variance were examined using Kolmogorov-Smirnov (K-S) and Levene's test, respectively. CTX concentrations were $\log_{10}$-transformed to improve normality and homogeneity of variance. The α value for all statistical tests was set at 0.05 and analyses were performed using Minitab (version 16) software.

CHAPTER 3

Results

Ciguatoxin concentration

The muscle tissue from 924 *C. argus* and 157 prey specimens from 20 species were collected from near-shore reef locations on the islands of Oahu and Maui and analyzed using the N2a bioassay for the detection of ciguatoxin (Table 2). Of the 924 *C. argus* tested, 382 tested positive (i.e., having a concentration exceeding the limit of detection, estimated to be below ~0.07 pg/mg) for CTX (CTX^+) (41.3%). 17.5% of the *C. argus* collected off the island of Oahu tested CTX^+, and 55.3% of the specimens collected off the island of Maui tested CTX^+ (Table 2, Figure 2). Of the 157 prey specimens tested, 55 tested CTX^+ (35%). 31.9% of the prey specimens collected from Oahu were CTX^+, and 37.5% of those collected from Maui were CTX^+ (Table 2, Figure 2). Prey species *C. strigosus* and *A. nigrofuscus* had large enough sample sizes (n = 74 and n = 22, respectively) to assess in this manner. *C. strigosus* had CTX^+ results in 47.3% of samples, with 41.5% of samples from Oahu CTX^+, and 56.3% of samples from Maui CTX^+. 45.5% of *A. nigrofuscus* samples were CTX^+, 11.1% of samples from Oahu were CTX^+, and 69.2% of samples from Maui were CTX^+ (Table 2, Figure 2).

Due to the high frequency of fish with non-detectable levels of CTX and occasional high CTX concentrations, median CTX concentrations in positive *C. argus* and prey were used to evaluate concentration across these groups. Median CTX for CTX positive *C. argus* was 1.50 pg/mg and ranged from 0.08 pg/mg to

71.78 pg/mg for individual fish. Median CTX concentration in CTX positive prey was 2.93 pg/mg (Table 2) and ranged from 0.35 pg/mg to 514.13 pg/mg for individual fish.

Histograms of % CTX positive (i.e., above limit of detection) by $\log_{10}$ of total body weight for *C. argus* (Figure 3a) indicates an increased frequency of ciguatoxicity with increased body weight. No such relationship was found for all prey or *C. strigosus* (Figure 3b and 3c, respectively).

Bulk Isotopic Analysis

Bulk tissue isotope analysis for both carbon and nitrogen was performed prior to selection of samples for AA-CSIA. 108 *C. argus* samples and 126 prey samples (including 70 *C. strigosus,* and 34 *A. nigrofuscus*) from both Maui and Oahu were analyzed for bulk C and N isotope values. A plot of the $\delta^{15}N$ (‰) vs. $\delta^{13}C$ (‰) show groupings of *C. argus*, *C. strigosus* and *A. nigrofuscus* (Figure 4). Sample catch location (i.e., Maui or Oahu) had no effect on the observed grouping.

CTX is a lipid soluble compound; therefore, variable lipid concentrations in muscle tissue samples could bias results. To address this concern, C:N molar ratios were used as a proxy for variable lipid content (Post et al. 2007). Lipid synthesis favors the incorporation of ^{12}C, resulting in low $\delta^{13}C$ values for lipid-rich tissues (Post et al 2007). To verify that there is no systematic bias in $\delta^{13}C$ values due to variation in C:N molar ratios, potential correlation between molar C:N ratios and $\delta^{13}C$ values was examined. No correlation was found (Figure 5a). The C:N ratios of

samples averaged 3.8 ± 0.5 mol/mol for *C. argus* and 3.8 ± 0.1 mol/mol for prey, and there is no significant correlation between C:N molar ratios and CTX concentration. A significant ($p<0.05$) positive relationship was found between molar C:N ratios and total body weight for *C. argus* (Figure 5b), but not for *C. stigosus* or all prey.

Amino Acid Compound Specific Isotope Analysis

A subset of samples was selected for AA-CISA analysis (n=56); 8 of which were not analyzed for CTX concentration. Samples of both CTX^+ (n=16) and CTX^- (n=6) *C. argus* and CTX^+ (n=20) and CTX^- (n=6) prey species were also analyzed. Prey species include *C. strigosus* (n=11), *A. nigrofuscus* (n=3), *H. cruentatus* (n=3), *S. xantherythrum* (n=2), *A. triostegus* (n=1), *A. thompsoni* (n=1), *S. dubius* (n=1), *S. bursa* (n=1) and *S. psittacus* (n=3).

A linear regression model of the $TP_{TEF=3.9}$ vs. total body weight (g) of *C. argus* shows a significant positive relationship between the two variables ($p<0.05$) (Figure 6a). No such relationship is found for CTX^+ and CTX^- *C. strigosus* or all prey (Figure 6b and 6c, respectively).

Quantification of Biological Magnification of Ciguatoxin

A linear regression model of the concentration of $TP_{TEF=3.9}$ vs. $\log_{10}$CTX (pg/mg) for all CTX^+ species analyzed shows no significant relationship between the two variables (Figure 7).

Assuming that the samples chosen were not a statistically random sampling of fish, median trophic position was compared with median [CTX]. Comparison of median CTX concentrations and median TP estimations shows *C.*

argus (TP of 3.9) has a median CTX concentration of 1.52 pg/mg and *C. strigosus* (TP of 1.81) has a median CTX concentration of 0.39 pg/mg.

CHAPTER 4

Discussion

Ciguatoxin

Frequency of detection

Patterns in the frequency of CTX^+ *C. argus* and prey species vary between Oahu and Maui (Figure 2). *C. argus* and the prey species *C. strigosus* and *A. nigrofuscus* all showed an increased frequency in ciguatoxicity on the island of Maui compared to the island of Oahu, while all prey as a combined group shows no such pattern. This increase in frequency could potentially be the result of a limited sample collection area on the island of Maui that was targeted for prey collection upon reviewing the results of Dr. Paul Bienfang's study of *C. argus* (Figure 1). It is possible that this area may have been experiencing a relative 'bloom' of *Gambierdiscus spp.* during the course of these studies.

An increased frequency of CTX^+ *C. argus* was found in fish with larger total body weight (Figure 3a). However, while there is a greater percentage of CTX^+ in larger fish, there is no significant relationship between total body weight and CTX concentration. The large number of no-detects across all sizes of fish could possibly be a contributing factor for this lack of relationship. From a human health standpoint, these findings are significant as they indicate a higher likelihood of encountering a ciguatoxic *C. argus* when consuming larger fish.

Similar relationships are not found when all prey items were combined or when *C. strigosus* was examined (Figure 3 b, c). These results indicate that for herbivorous fish such as C. *strigosus,* there is a similar probability of

ciguatoxicity regardless of fish size. The overall frequency of ciguatoxicity in prey (35%) may have implications for the quantification of biological magnification of CTX.

It is important to note that most prey specimens targeted for this study are smaller than the maximum length of adults for each species. For example, max standard length for *C. strigosus* is 14.6 cm (Randall and Clements 2001), but the largest *C. stigosus* used in this study is 13.6 cm (mean of 9.1 cm). Analysis of 156 full *C. argus* stomachs revealed that 83.5% of fish prey were less than 10 cm in total length (mean of 7.2 cm) and 76.2% weighed less than 15 g (mean undigested weight of 11.4 g) (Dierking 2007). In light of those findings, smaller *C. strigosus* and other prey specimens were targeted when possible in an attempt to constrain samples to those that are likely to be preyed upon by *C. argus*. However, the prey specimens included in this study are slightly larger with a mean weight of 48.5 g and a mean total length of 12.1 cm. The lack of correlation between the frequency of detection of CTX vs. total body weight (Figure 3b, 3c) and between estimated TP vs. total body weight (Figure 6b, 6c) in prey species indicates that this discrepancy is likely negligible. Additionally, many of the prey species included in this study are herbivorous, and, therefore, diet is not likely to change with increasing size.

Median Concentration

The median concentration of CTX for *C. argus* and *C. strigosus* was inferred from plots of x vs. z-statistic (Table 3). A z-statistic is a measure of the divergence of an individual result from the most probable result, the median. A z-statistic is expressed in terms of the number of standard deviations from the mean where x is the experimental value, μ is the mean and σ is the standard deviation (Equation 3).

One key advantage to this method is that it allows for the determination of a median concentration even if that concentration falls below the limit of detection for the N2a bioassay. This is accomplished through the inclusion of the CTX^- samples (i.e., below the limit of detection) in cumulative probability calculations required for the determination of the associated z-statistic.

A second advantage of this approach is the assumption of a normal distribution of x can be confirmed via the linearity of the plot x vs. z-statistic. Because the z-statistic represents the distance between the raw score and the population mean in units of standard deviation, a plot of x vs. z-statistic should be a straight line with the intercept equal to the median. It should be noted that because x is normally distributed, the mean and the median of x are equal, but the mean and median of the inverse log of x are not necessarily equal. For this reason, the inverse log of x at z-statistic = 0 is reported as the median [CTX].

The distribution of $\log_{10}$[CTX] in *C. strigosus* resembles the right side of a normal distribution (Figure 8a), suggesting that the $\log_{10}$[CTX] is normally distributed when CTX^- values are included and that CTX^+ and CTX^- sample

constitute a single distribution or population of *C. strigosus*. Confirmation of single population with a log-normal distribution of CTX was attained via linearity in x vs. z when CTX^- samples are included in the calculation of the z-statistic for *C. strigosus* (Figure 8b). The y-intercept of x vs. z for *C. strigosus* is -0.41, and the inverse $\log_{10}$ of the y-intercept is 0.39.

Contrary to the distribution of CTX in *C. strigosus*, the distribution of CTX^+ in *C. argus* is log-normal (K-S, $p>0.15$) without the inclusion of the CTX^- samples (Figure 9a). This indicates two distinct populations of *C. argus* exist, those that are CTX^+, and those that are not. Non-linearity in x vs. z when CTX^- *C. argus* are included in the calculation of the z-statistic (Figure 9b) further supports the suggestion that two types of *C. argus* are found in the study area. For the calculation of median CTX concentrations, CTX^- *C. argus* were not included in the calculation of the z-statistic (Figure 9c). The y-intercept of x vs. z for *C. agus* is 0.18, and the inverse $\log_{10}$ of the y-intercept is 1.52.

One possible ecological explanation for the difference in distributions of CTX may be found in behavioral differences between *C. argus* and its prey. *C. argus* is known to be a territorial species with a single male occupying a large territory with up to 12 females occupying sub-territories within that region (Shpigel and Fishelson 1989, 1991). Relative 'blooms' of *Gambierdiscus spp*. are patchy in distribution (Lehane and Lewis 2000), so a particular *C. argus* occupying its territory may have a low likelihood of encountering CTX^+ prey if there is no *Gambierdiscus* blooms nearby. Prey species that may be less

sedentary than *C. argus* over their lifespan have an increased likelihood of eventually encountering an area where a *Gambierdiscus* boom is occurring, thereby being exposed to CTX. For example, *A. nigrofuscus* has been observed in the Red Sea to undergo daily migrations of up to 1.5 km to feeding sites (Mazeroll and Montgomery 1998). Additionally, an acoustic telemetry study of a territorial species within the family Acanthuridae found two different behavior patterns within the species; one making daily migrations of several hundred meters between daytime foraging areas and nighttime refuge holes (Meyer and Holland 2005).

Trophic Position Determination

Quantitative determination of TP was evaluated in this study by comparing stomach content analysis and various combinations of isotopic analysis of individual amino acids. One key advantage to using stable isotopic compositions for TP estimation is the relative ease (when compared to stomach content analysis) with which one can quantitatively determine fractional trophic level, and, therefore, have an ability to express the complexities of the food web associated with coral reef ecosystems (Post 2002).

Stomach Content Analysis from FishBase

Trophic position using results in FishBase (TP_{FB}) is determined from stomach content analysis (SCA) in combination with EcoPath software, and is therefore susceptible to the shortcomings of SCA (i.e., assumptions of TP of prey, susceptibility to variation in digestions rates of prey, failure to integrate long-term

foraging habits, etc. (Hyslop 1980)). In cases where complete diet composition data from published stomach content studies are available, TP_{FB} is calculated as the weighted mean TP of food items (weighted by contribution of food items) plus 1 (Christensen and Pauly 1992, 1993; Pauly and Christensen 1993; Christensen and Pauly 1995), which implicitly assumes that the TP of prey are known. In addition, information from all diet studies available for a particular species is used to calculate trophic position. Consequently, if geographic variation in the TP of a species exists, the calculated TP will be biased towards the location where the most complete diet data exist and that may not be the location of interest.

In the second approach utilized by FishBase, TP_{FB} is calculated using TP for a number of individual food items by a random resampling routine (Sachs 1984). The individual food items approach requires certain assumptions about the relative importance of food items and their TP based on an empirical model derived from examination of data entered into FishBase until mid-1999 (www.fishbase.org). These assumptions make the individual food item estimate of TP the weaker of the two approaches, and were, therefore, only used in this study with species for which no complete diet composition data was available (*C. strigosus, A. triostegus, A. nigroris* and *S. psittacus*). For some prey species, the only TP estimations available were either based upon a single food item or upon size and TP of the closest relatives; no TP_{FB} values were used for these species (*S. dubius*).

Bulk Isotope Analysis

A plot of the $\delta^{15}N$ (‰) vs. $\delta^{13}C$ (‰) show groupings of *C. argus, C. strigosus* and *A. nigrofuscus* (Figure 4). Sample catch location (i.e., Maui or Oahu) had no effect on the observed groupings. The groupings by species are not entirely unexpected as species-specific feeding habits will affect isotope values. Given that both *A. nirgofuscus* and *C. strigosus* are herbivorous, the distinction observed in the two groups is likely due to specific feeding behavior associate with each species; *A. nigrofuscus* is known to feed on filamentous and turf algae (Sano et al. 1984) while *C. strigosus* feeds on plants and detritus by whisking its comb-like teeth over substrate as it closes its mouth (Honebrink 1990).

Amino Acid Compound Specific Isotope Analysis

Method 1

TP calculated using method 1 ($TP_{TEF=7.6}$) resulted in TP estimates that were consistent with SCA for known herbivorous fish (i.e., $TP_{FB}=2$), however, for all omnivorous and carnivorous fishes (i.e., $TP_{FB} > 2$), $TP_{TEF=7.6}$ systematically underestimated trophic position compared to TP_{FB} (Figure 10). Two critical assumptions exist when estimating TP from AA-CSIA: 1) a constant difference between the $\delta^{15}N$ values of source and trophic amino acids in primary producers (β value), and 2) a constant ^{15}N trophic enrichment factor (i.e., the extent of ^{15}N enrichment between source and trophic amino acids in consumers).

The first critical assumption has likely been sufficiently addressed for most marine environments. Chikaraishi et al. (2009) examined the constancy between the $\delta^{15}N$ values of source and trophic amino acids in primary producers by analyzing AA $\delta^{15}N$ values in 25 photoautotrophs from various locations and growth experiments, including cyanobacteria, green algae, red algae, brown macroalgae as well as mixed ice algae. Results of analyses of these organisms were combined with published data for cyanobacteria (McClelland et al. 2003), green algae (McClelland and Montoya 2002), red and brown macroalgae (Chikaraishi et al. 2007) and a diatom (McCarthy et al. 2007). The patterns of $\delta^{15}N$ values for amino acids were amazingly similar for all samples regardless of whether they were natural or cultured samples. Chikaraishi et al. (2009) found a constant difference (3.4 ±1.0‰) between the $\delta^{15}N$ values of phenylalanine and glutamic acid, which is very close to the 4‰ difference originally found by McClelland and Montoya (2002). However, most natural samples used to calculate β = 3.4‰ were photoautotrophs sampled from Japanese waters with the exception of the sample of ice algae from Antarctica (Chikaraishi et al. 2009) and the equatorial Pacific diatom (McCarthey et al. 2007), therefore lacking a truly extensive evaluation of the spatial variability in β. In particular, zooxanthelle, which are important components of the photosynthesis associated with coral reef ecosystems (Falkowski et al. 1984), have not been evaluated with respect to β values in the published literature to date.

The second assumption concerning a constant trophic enrichment factor has been examined in only a limited number of organisms, tissue types and

physiological conditions. Chikaraishi et al. (2009) examined the trophic enrichment factor in four controlled feeding experiments using green algae, zooplankton and newly hatched fish. They found the $\delta^{15}N$ value of phenylalanine changed slightly (0.4 ± 0.5‰, 1stdev) and the $\delta^{15}N$ value of glutamic acid changed markedly (8.0 ± 1.2‰) with each trophic position, resulting in an enrichment factor of 7.6‰. The uncertainty in this value can be calculated from the standard deviations of ^{15}N trophic transfer for phenylalanine and glutamic acid and is 1.3‰. Chikaraishi et al. (2009) conclude that the most appropriate pair for precise estimates of TP is glu/phe, with a $\beta = 3.4 \pm 1.0$‰ and TEF = 7.6 ± 1.3‰. However, TEF=7.6‰ is based upon very few direct and previously published feeding studies for organisms with a TP≤3, with no evaluation of TEF for fish with TP>3 (Chikaraishi et al. 2009).

To address some of these concerns, samples of pen-raised *Pristipomoides filamentosus* and feed items were provided by Dr. Clyde Tamura and Dr. Chris Kelley with the Hawaiian Institute of Marine Biology (HIMB). These carnivorous fish (TP_{FB} =3.64 ± 0.49) were reared in pens at HIMB for between 4 and 15 years and fed a regular diet of squid (41.1%), krill (16.7%), and either anchovies or sardines (41.1%) for about 1 year prior to our analysis. The fish component of diet was composed of only anchovies for ~6 months prior to this study, and, therefore, anchovies were the only fish evaluated here. The feed in this time period was provided by a single distributor located in Monterey, Ca. who verified that all squid and fish (~84% of diet) were caught in the Monterey Bay area. This factor is important because samples of feed over time were not

available for analysis, so consistency in the isotopic composition of the feed must be assumed for these purposes. It should be noted that inter-annual variability of a few permil in $\delta^{15}N$ values of zooplankton has been observed off California's central coast (Rau et al. 2003), thus the results of this mini-study should be interpreted with some caution.

The source of the krill was less consistent, but could be narrowed down to the N. Pacific. However, the krill only compose ~16% of the regular diet, implying less concern for variability associated with changes in catch location and thus $\delta^{15}N$ value. The consistency in diet and long time frame make these samples somewhat analogous to a controlled feeding experiment, and provide the opportunity to evaluate TEF for higher level carnivores.

Recent AA-CSIA results from the muscle tissues of the brown stingray *Dasyatis lata* and the hammerhead shark *Sphyrna lewini* from Kaneohe Bay, Oahu, Hawaii suggest that the TEF =7.6 may be too large for elasmobranchs (Dale et al. 2011). These authors suggested that the lower TEF could be related to the use of urea for osmoregulation and specifically to increased importance of the glutamate-glutamine-urea pathway in sharks and rays, which could result in lower glutamate catabolism (see Speers-Roesch et al. 2006) and reduced ^{15}N enrichment in glutamic acid in muscle tissue. Elasmobranchs use a unique carbamoyl phosphate synthetase (CPSase III) that utilizes glutamine in the pool of free amino acids in liver mitochondrial cells as the nitrogen-donating substrate rather than ammonia for urea formation (Julsrud et al. 1998). Dale et al. (2011) thus speculated that reduced hepatic glutamate catabolism resulted in lower ^{15}N

enrichment of glutamic acid in muscle tissue of *D. lata* and *S. lewini*. The findings of Dale et al. (2011) highlight the need for consideration of biochemical controls on ^{15}N enrichment of amino acids and while a TEF of 7.6‰ may be appropriate for herbivorous organisms, the Dale et al. (2011) results cast doubt that a TEF of 7.6‰ for glu and phe is can be used to calculate TP in all marine organisms.

Because all of the fish evaluated in this study produce ammonium as a waste product as opposed to urea formation in elasmobranchs, TEF was evaluated using AA-CSIA and bulk isotope analysis of both *P. filamentosus* and feed. Bulk isotope analysis was performed for 5 *P. filamentosus*, 4 anchovy, 3 squid and 3 krill samples. The results of the bulk isotope analysis indicate a high level of consistency in $\delta^{15}N$ values within each feed type (Figure 11), so a subset (5 *P. filamentosus*, 2 anchovies, 1 squid and 1 sample of krill) of samples was analyzed using AA-CSIA. These results were utilized for the evaluation of the TEF for a nitrogen isotope shift of glu and phe across one trophic level (TEF = $\delta^{15}N$-(glu-phe)$_{consumer}$ - $\delta^{15}N$-(glu-phe)$_{feed}$). The average $\delta^{15}N$ values for glu$_{feed}$ and phe$_{feed}$ were weighted by the relative contribution of each feed type to total diet. The results are summarized in Table 4 and yield a TEF for glu and phe of only 1.7‰. In contrast, the TEF associated with bulk tissue $\delta^{15}N$ values for these samples is 3.0 ± 0.91‰; a value that is in agreement with estimations of bulk $\delta^{15}N$ trophic enrichment observed for carnivorous fish in previous studies (Vanderkilt and Posnard 2003, McCutchan et al. 2003, Post 2002).

These results indicated a substantial decrease in TEF across glu and phe for carnivorous fish and highlight a need for further investigation into the biochemical controls on this value. The value of 1.7‰ is suspect in light of the uncertainty associated with the $\delta^{15}N$ values for feed over time. It is also important to note that these fish may not be representative of wild populations due to the length of time they were reared in captivity. For this reason, further study of both natural systems and controlled feeding experiments are merited to fully constrain TEFs for marine organisms. However, the results taken together suggest that a TEF of <7.6‰ may be required to calculate TP for marine fish with TP>2 using the $\delta^{15}N$ values of glutamic acid and phenylalanine.

Method 2

Determination of TP via AA-CSIA relying on values for only two AAs (i.e., glu and phe) can be susceptible to error associated with any single value. McCarthy et al. (2007) and Sherwood et al. (2010) suggested that a potentially more robust approach utilizes the difference in averages of broadly grouped "source" amino acids (Sr-AAs) (i.e., gly, ser and phe) and "trophic" amino acids (Tr-AAs) (i.e., ala, val, leu, iso, pro and glu). Following this approach, TP was estimated for a subset of samples of *C. argus* and prey. For this subset, a weighted average based on the uncertainty associated with these amino acids in photoautotrophs and feeding experiments described by Chikaraishi et al. (2010) was used to estimate a β and TEF. The key advantage to using the weighted average to calculate these values is that the resulting TP estimation is more

strongly based upon the AA in which the isotopic relationship are best constrained by the available data. This approached yielded estimates closer to those reported in FishBase; however, the propagation of analytical error for Sr-AAs and Tr-AAs in error calculations for TP leads to large associated variability in TP (Figure 12).

Method 3

To address concerns with methods 1 and 2, an empirically derived TEF (TEF_3) for fish with TP>2 was used to calculate TP for this study. While several studies (Chikaraishi et al. 2009, McClelland and Montoya 2002) support a large TEF for glu and phe between photoautotrophs and herbivorous organisms, evidence from the evaluation of TEF for *P. filamentosus* (described above) indicates a substantially lower TEF between herbivorous and omnivorous and carnivorous fishes. This change in TEFs from herbivores to higher TP's is potentially the result of differences in assimilation efficiencies. Assimilation efficiencies for herbivores are notably lower (~10-20%) than for carnivores (~95-98%) due to a high amount indigestible plant material in herbivorous diets (Horn et al. 1989). Studies investigating the assimilation efficiency of protein and/or nitrogen in herbivorous fish have shown that, while these efficiencies can be much higher than 10-20%, they still fall below the 95-98% efficiencies observed in carnivorous fish (Edwards and Horn 982, Lassuy 1984). Because the processes of ingestion, assimilation and excretion occur in an open system, the larger fraction of material going to excretion in herbivores may result in a relatively large associated isotopic fractionation (Fry, 2006). However, a meta-analysis

performed by Vanderkilt and Posnard (2003) evaluating variation in trophic enrichment in bulk $\delta^{15}N$ values (evaluation of 134 estimates from controlled studies of consumer-diet $\delta^{15}N$ values) found that carnivores and herbivores are not significantly different in this respect. Discrepancies between our findings and those for bulk tissue ^{15}N trophic enrichment in several studies (Vanderkilt and Posnard 2003, McCutchan et al. 2003, Post 2002) speak again to the need for further investigation in this regard.

In an initial attempt to address this issue, $\delta^{15}N$ values of glu and phe for 123 marine fishes, combined with expected TP from FishBase, were used to calculate TEF for fish with TP>2 using equation 6. This approached yielded a TEF = 3.9 ± 1.3 for TP>2. TEF=7.6 was applied for the step between TP=1 and TP=2, and the new TEF was applied for all steps above TP=2 using equation 7. This method of TP estimation most closely agreed with TP estimations based on SCA from FishBase and had an acceptable level of associated variability (Figure 13). For these reasons, this method was chosen for use in this study. A concern to note about this method is the use of TP_{FB} for the derivation of TEF, and, thus it is susceptible to the same shortcomings as was discussed for SCA.

Ciguatoxin and Amino Acid Compound Specific Isotope Analysis

There is a significant positive relationship between $TP_{TEF=3.9}$ and total body weight for *C. argus* (Figure 5a). Combining this relationship and the relationship between frequency of toxicity and total body weight as described

above, there is evidence to support a higher frequency of toxicity with increased TP for this species.

Due to a high degree of variability associated with the acquisition and concentration of CTX in fish, reliable data on CTX concentration is only available for *C. argus* and *C. strigosus.* An attempt to quantify the biological magnification of CTX therefore relies on the examination of these two species alone. For this predator/prey relationship we find an indication of biological magnification of CTX via multiple approaches to TP estimation.

FishBase estimates the TP of *C. strigosus* to be 2 ± 0 based upon individual food items (i.e., the weaker of the two approaches utilized by FishBase). TP estimation for *C. strigosus* based upon AA-CSIA is in fairly close agreement to TP_{FB} across multiple approaches. $TP_{TEF=7.6}$ (n = 11) ranges from 1.66 to 2.09 with a mean of 1.90. TP_{Sr-Tr} (n = 5) ranges from 1.79 to 2.29 with a mean of 2.05. $TP_{TEF=3.9}$ (n = 11) ranges from 1.33 to 2.18 with a mean of 1.81.

FishBase estimates the TP of *C. argus* to be 4.48 ± 0.79 based upon diet composition data. Average TP estimation based upon AA-CSIA falls within this range for both methods. $TP_{TEF=7.6}$ (n = 26) ranges from 2.41 to 3.41 with a mean of 2.95. TP_{Sr-Tr} (n = 4) ranges from 3.43 to 4.14 with a mean of 3.69. $TP_{TEF=3.9}$ (n = 26) ranges from 3.10 to 4.75 with a mean of 3.90.

It is well established that CTX are initially produced by the dinoflagellate genus *Gambierdiscus* and propagated up the food web via consumption of prey by predators (Randall 1958, Banner et al. 1960, 1966, Banner and Helfrich 1964, Yasumoto et al. 1977, Bomber and Aikman 1989). Biological magnification is

defined as increased concentration of a substance obtained from lower trophic positions by organisms at higher trophic positions (Bienfang et al. 2011). With this definition in mind, there is clear evidence in support of the biological magnification of CTX. The inclusion of only two species in the attempt to quantify this biomagnification is clearly a shortcoming of this study; however, using the change in TP between *C. strigosus* and *C. argus* via $TP_{TEF=3.9}$ (2.09 TPs) and the median [CTX] estimates found in Table 3, it can be concluded that there is a 0.54 pg/mg increase in CTX concentration per trophic level found for this predator/prey relationship.

This study fails to constrain for several factors associated with *Gambierdiscus* abundance and ciguatoxin production. For example, spatial and temporal variability in abundance and toxicity of *Gambierdiscus spp*. is well documented in the literature; however, the environmental controls on this variability are still poorly understood (Lewis 2000 and references within). The sampling efforts for this study were somewhat opportunistic, and therefore did not constrain spatial and temporal variability; an aspect that may be necessary to fully understand the biological magnification of ciguatoxin in a natural system. Another important factor that was not addressed in this study is the retention time of ciguatoxin in fish tissue. This factor is also poorly understood, though one study found that after 30 months captive feeding of a non-toxic diet, red snapper showed no significant decline in toxicity (Banner et al. 1966).

Conclusions

This study has provided insight into the yet to be resolved concerns for the quantitative estimation of trophic position using AA-CSIA for carnivorous marine fish. Additionally, it has evaluated ciguatoxin concentrations for the predator *C. argus* and its prey species *C. strigosus* with an initial evaluation of 21 other prey species.

The quantitative determination of trophic position via AA-CSIA requires further investigation into the biochemical controls of $\delta^{15}N$ enrichment between consumers and diet for specific amino acids. Results from this investigation are incomplete in this regard, but provide evidence of a decreased ^{15}N trophic enrichment of glutamic acid for carnivorous fish.

The increase in frequency of toxic fish that is observed in larger *C. argus* has implications for human health. It has been observed that repeated exposures to ciguatoxin may be associated with more severe symptoms of ciguatera fish poisoning (Bagnis et al. 1979), indicating that frequency of exposure is an important factor when evaluating risk of ciguatera fish poisoning. An increased frequency of toxicity in carnivorous fish with size implies an increase in frequency of exposure to ciguatoxin when large carnivores are targeted for catch and consumption, as they often are.

My overarching hypothesis at the initiation of this study was that ciguatoxin is concentrated via food web magnification and that the degree of the magnification could be quantified with the determination of trophic position by AA-CSIA. Trophic position estimated via AA-CSIA alone does not explain

variations in concentrations of ciguatoxin for individual fish. However, evidence from this study supports the hypothesis of biological magnification of ciguatoxin across trophic positions in the near-shore reef ecosystem in the main Hawaiian Islands. This initial effort to quantify biological magnification is unique in its attempt and provides the foundation for further work towards this goal.

Table 1. Select *C. argus* prey by family and their index of relative importance (Pinkas et al. 1971) and percent weight as determined by J. Dierking, unpublished PhD dissertation. The families and species listed in Table 1 are restricted to *C. argus* prey fish that are non-zooplanktivorous.

***C. argus* prey taxon**	**% IRI**	**% W**
Fish	**97.7**	**94.5**
Scaridae	25.2	27.8
Scarus psittacus		
Acanthuridae	17.3	12
Acanthurus nigrofuscus		
Acanthurus nigrosis		
Acanthurus triostegus		
Zebrasoma flavescens		
Ctenochaetus strigosus		
Ctenochaetus striatus		
Priacanthidae	8.6	10.9
Heteropriacanthus cruentatus		
Balistidae	1.3	5.5
Xanthichthys auromarginatus		
Monacanthidae	12.7	4.6
Pervagor aspricaudus		
Cantherhines verecundus		
Holocentridae	16.4	4.6
Sargocentron diadema		
Sargocentron xantherythrum		
Pomacentridae	0.9	3.1
Labridae	0.5	2.6
Total	**82.9**	**71.1**

Table 2. Median and mean ciguatoxin concentrations [CTX] (pg/mg) of CTX positive *C. argus* and prey species. Also shown are sample size (n) for total sample and CTX positive fish, mean body mass and standard deviations for both positive and negative samples.

	n	n ctx $^{+}$	% CTX^{+}	Median [CTX] (pg/mg)	Mean [CTX] (pg/mg)	Stdev [CTX]	Mean Mass $^{+}$ (g)	Stdev Mass^{+}	Mean Mass $^{-}$ (g)	Stdev Mass^{-}
***C. argus* (roi)**	924	382	41.34	1.50	2.73	4.70	1005.99	405.74	779.97	401.74
Oahu	342	60	17.54	1.93	2.91	3.44	1068.67	368.05	775.45	413.71
Maui	582	322	55.33	1.41	2.70	4.91	994.31	411.86	784.87	389.08
Prey	157	55	35.03	2.93	16.87	69.56	52.52	37.91	49.04	56.11
Oahu	69	22	31.88	4.50	35.13	108.53	50.45	20.93	46.52	29.42
Maui	88	33	37.50	2.58	4.69	7.34	53.89	46.18	64.11	59.87
***C. strigosus* (kole tang)**	74	35	47.30	2.81	22.10	86.84	43.53	20.01	57.25	53.07
Oahu	40	16	40.00	5.07	13.61	20.02	42.73	22.62	66.17	67.08
Maui	34	19	55.88	1.60	29.26	117.43	44.19	18.14	44.76	18.04
***A. nigrofuscus* (brown surgeonfish)**	22	10	45.45	3.86	3.93	2.01	30.32	8.69	31.58	16.06
Oahu	9	1	11.11	5.02	5.02	-	26.10	-	25.45	9.61
Maui	13	9	69.23	2.96	3.81	2.09	30.79	9.08	43.85	20.72
***Z. flavescens* (yellow tang)**	13	-		-		-	-	-	21.83	10.98
Oahu	4	-		-		-	-	-	12.23	5.36
Maui	9	-		-		-	-	-	26.10	10.17
***S. xantherythrum* (Hawaiian squirrelfish)**	6	1		34.01	34.01	-	35.00	-	24.78	5.39
Oahu	-	-		-		-	-	-	-	-
Maui	6	1		34.01	34.01	-	35.00	-	24.78	5.39
H. cruentatus	6	3		3.55	9.68	11.73	149.70	10.01	157.23	19.91

(‘aweoweo)									
Oahu	6	3	3.55	9.68	11.73	149.70	10.01	157.23	19.91
Maui	-	-	-		-	-	-	-	-
***A. thompsoni* (Thompson’s surgeonfish)**	5	1	0.83	0.83	-	79.40	-	73.30	19.03
Oahu	1	0	-		-	-	-	46.50	-
Maui	4	1	0.83	0.83	-	79.40	-	82.23	8.02
***A. olivaceous* (orangebar surgeonfish**	5	-	-		-	-	-	57.34	16.13
Oahu	-	-	-		-	-	-	-	-
Maui	5	-	-		-	-	-	57.34	16.13
***C. sordidus* (bullethead parrotfish)**	3	2	1.03	1.03	0.95	125.10	92.07	109.10	-
Oahu	-	-	-		-	-	-	-	-
Maui	3	2	1.03	1.03	0.95	125.10	92.07	109.10	-
***S. dubius* (regal parrotfish)**	3	1	0.97	0.97	-	101.70	-	106.65	35.85
Oahu	-	-	-		-	-	-	-	-
Maui	3	1	0.97	0.97	-	101.70	-	106.65	35.85
***S. psittacus* (palenose parrotfish)**	3	-	-		-	-	-	68.10	33.36
Oahu	-	-	-		-	-	-	-	-
Maui	3	-	-		-	-	-	68.10	33.36
***S. diadema* (crown squirrelfish)**	3	-	-		-	-	-	57.33	17.78
Oahu	-	-	-		-	-	-	-	-
Maui	3	-	-		-	-	-	57.33	17.78

***S. bursa* (lei triggerfish)**	2	1	9.70	9.70	-	116.30	-	72.00	-
Oahu	2	1	9.70	9.70	-	116.30	-	72.00	-
Maui	-	-	-		-	-	-	-	-
***P. meeki* (Hawaiian bigeye)**	2	-	-		-	-	-	187.60	71.56
Oahu	-	-	-		-	-	-	-	-
Maui	2	-	-		-	-	-	187.60	71.56
***T. dupery* (saddle wrass)**	2	-	-		-	-	-	56.10	36.63
Oahu	-	-	-		-	-	-	-	-
Maui	2	-	-		-	-	-	56.10	36.63
***P. aspricaudus* (yellowtail filefish)**	2	-	-		-	-	-	14.65	11.53
Oahu	1	-	-		-	-	-	6.50	-
Maui	1	-	-		-	-	-	22.80	-
***G. varius* (bird wrass)**	1	1	38.30	38.30	-	30.20	-	-	-
Oahu	-	-	-	-	-	-	-	-	-
Maui	1	1	38.30	38.30	-	30.20	-	-	-
***C. verecundus* (shy filefish)**	1	-	-		-	-	-	44.00	-
Oahu	1	-	-		-	-	-	44.00	-
Maui	-	-	-		-	-	-	-	-
***A. nigroris* (bluelined surgeonfish)**	1	-	-		-	-	-	115.80	-
Oahu	-	-	-		-	-	-	-	-
Maui	1	-	-		-	-	-	115.80	-

***N. hexacanthus* (sleek unicornfish)**	1	-	-	-	-	-	124.10	-
Oahu	-	-	-	-	-	-	-	-
Maui	1	-	-	-	-	-	124.10	-
***X. auromarginatus* (gilded triggerfish)**	1	-	-	-	-	-	69.80	-
Oahu	1	-	-	-	-	-	69.80	-
Maui	-	-	-	-	-	-	-	-
***C. agilis* (agile chromis)**	1	-	-	-	-	-	14.90	-
Oahu	-	-	-	-	-	-	-	-
Maui	1	-	-	-	-	-	14.90	-

Table 3. Median ciguatoxin (CTX) concentration inferred from plots of x vs. z-statistic (see text). Also showing total sample size, CTX positive sample size, mean $TP_{TEF=3.9}$ with StDev and median $TP_{TEF=3.9}$.

	n	n CTX $^{+}$	Median [CTX]	Median $TP_{TEF=3.9}$	Mean $TP_{TEF=3.9}$	StDev $TP_{TEF=3.9}$
C. argus	924	382	1.52	3.90	3.85	0.44
C. strigosus	74	35	0.39	1.81	1.81	0.24

Table 4. Nitrogen isotope values ($\delta^{15}N$) for glutamic acid and phenylalanine in *P. filamentosus* and feed used to calculate trophic enrichment from diet to consumer in carnivorous fish (TEF). The TEF for this experiment was calculated using TEF = $\delta^{15}N$-(glu-phe) $_{P.\ filamentosus}$ - $\delta^{15}N$-(glu-phe) $_{\text{weighted total feed}}$ and is 1.7‰.

	% Diet Contribution	$\delta^{15}N$ glu	$\sigma\ \delta^{15}N$ glu	$\delta^{15}N$ phe	$\sigma\ \delta^{15}N$ phe
P. filamentosus		25.95	0.38	8.75	1.26
Ancovy	41.17	23.66	0.35	9.12	0.18
Squid	41.17	25.25	0.03	7.23	0.66
Krill	16.67	16.33	0.30	4.80	0.26
Weighted $Total_{feed}$	100	23.10	0.47	7.61	0.73

Figure 1. Maps of the distribution of *C. argus* that have tested positive or negative for ciguatoxin on the Hawaiian islands of Maui and Oahu. Map created by Dr. Alexandria Boehm using data generated by Dr. Paul Bienfang and Sue DeFelice. www.fish4science.com.

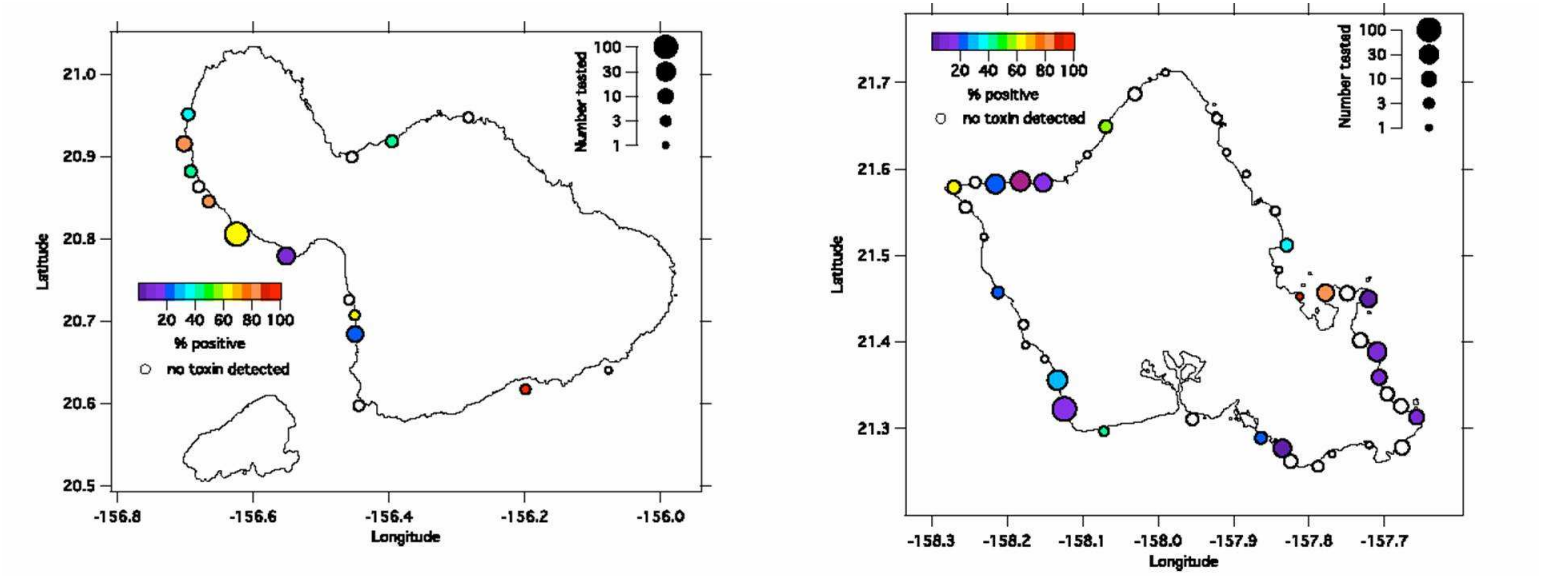


Figure 2. Percent fish tested positive (CTX^+) for ciguatoxin by location. Comparison of the percent of fish that tested positive for CTX for *C. argus*, all prey, *C. strigosus,* and A. *nigrofuscus* evaluated by catch location (island-scale).

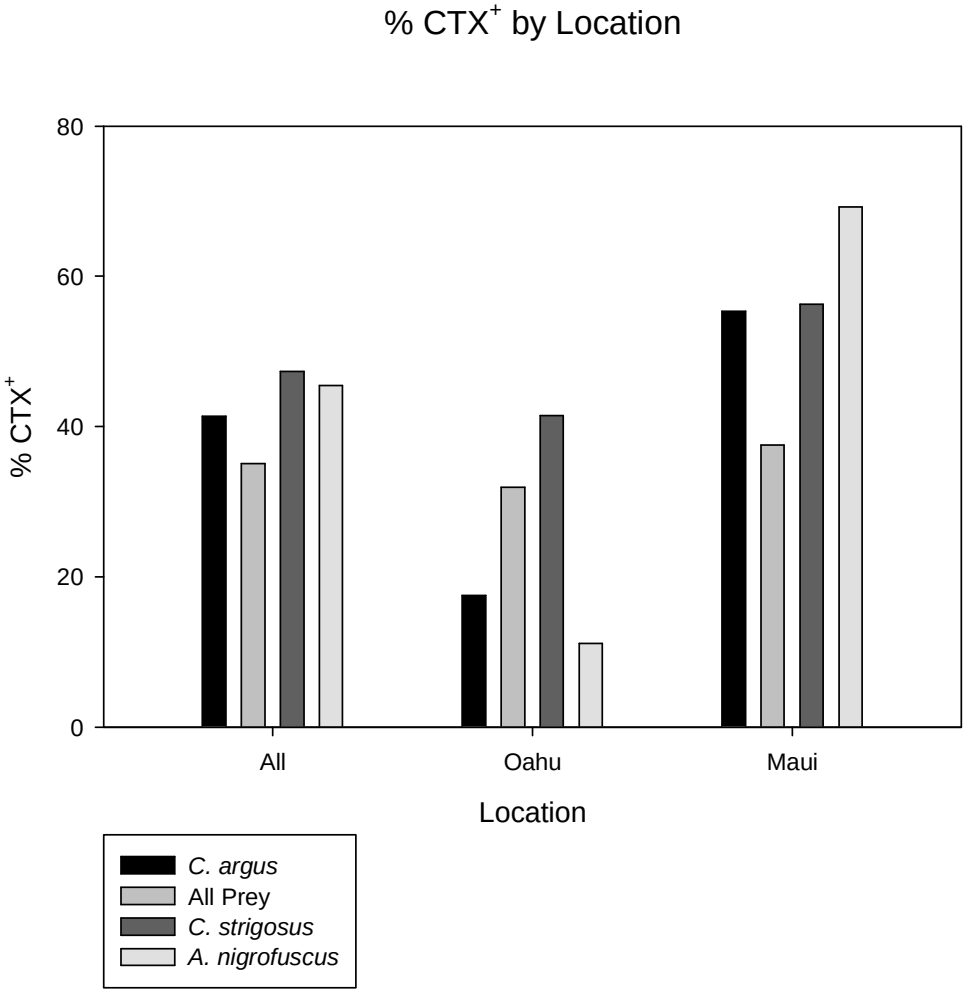
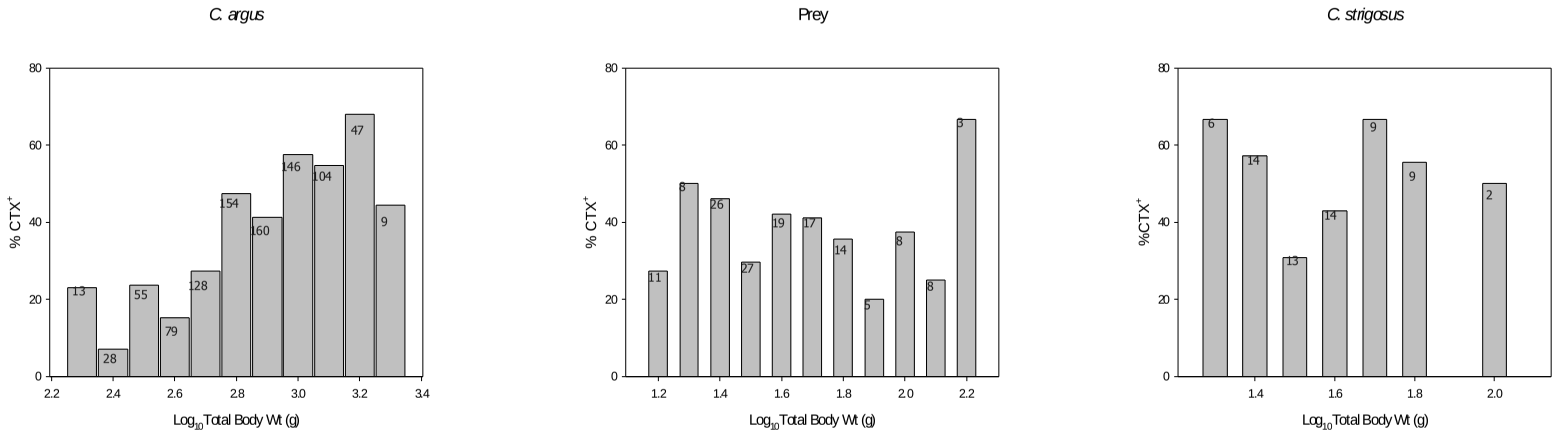


Figure 3. Histogram of percent of fish tested positive for ciguatoxin (CTX) by Log_{10} Total Body Weight (g). a) % CTX positive *C. argus* by Log_{10} Total Body Weight (g). b) %CTX positive prey by Log_{10} Total Body Weight (g). c) %CTX positive *C. strigosus* by Log_{10} Total Body Weight (g).



a) b) c)

Figure 4. Bulk isotope results for *C. argus* and prey. $\delta^{15}N$ (‰) vs. $\delta^{13}C$ (‰) for 108 *C. argus* samples and 126 prey samples (including 70 *C. strigosus* and 34 *A. nigrofuscus*).

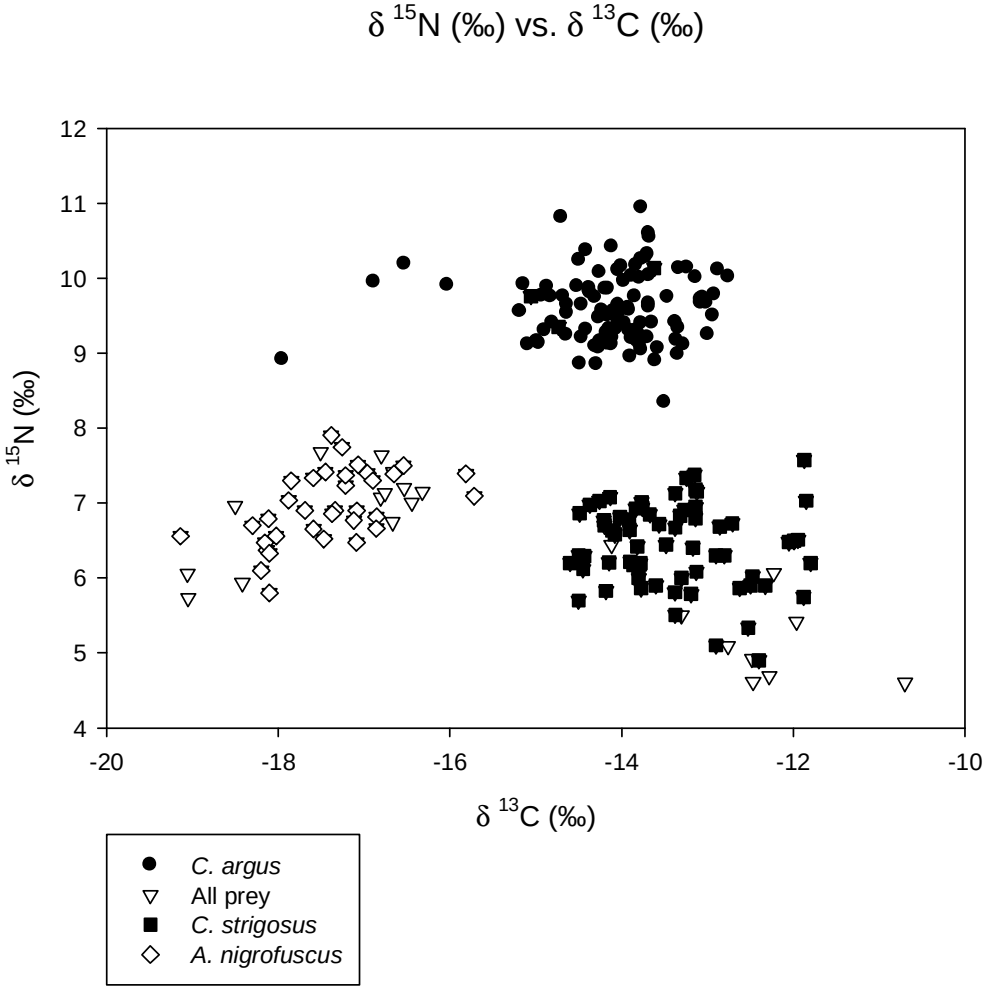
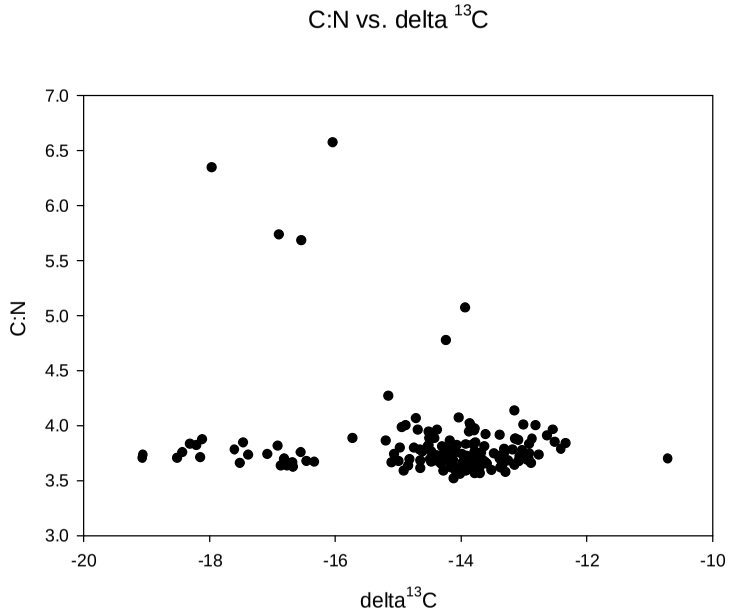
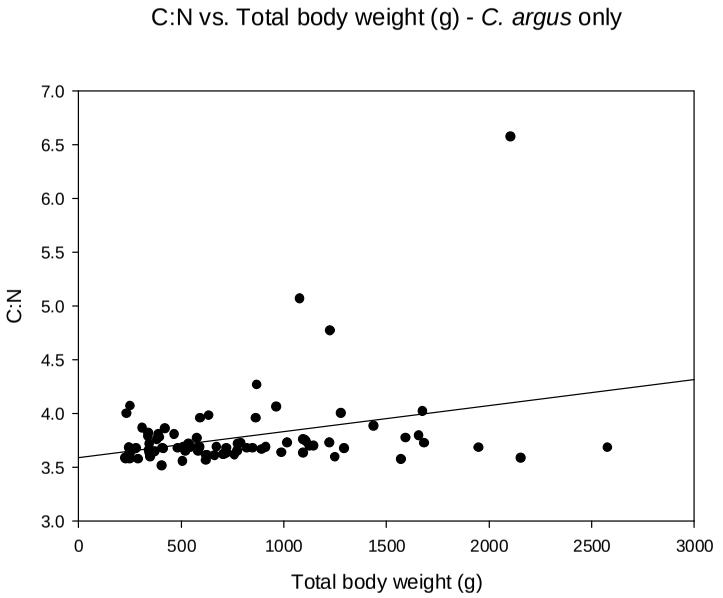


Figure 5. Molar C:N ratios as a proxy for lipid concentration**. a)** Molar C:N ratios versus bulk $\delta^{13}C$ measurements for samples analyzed for CTX concentration. b) Molar C:N ratios versus total body weight for *C. argus* only (r^2=0.103).

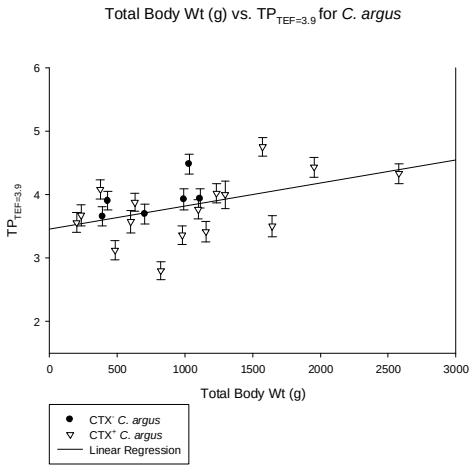


a)

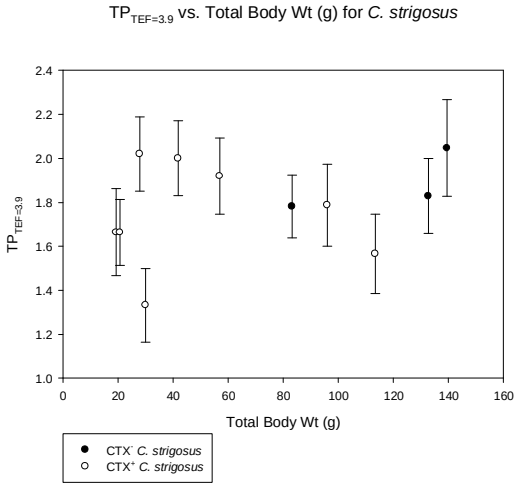


b)

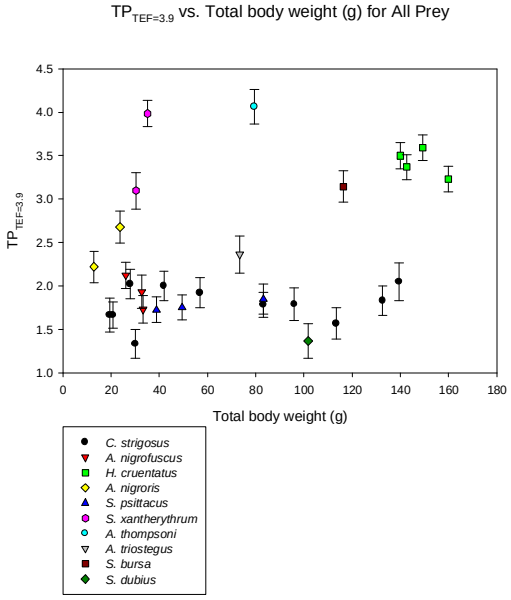
Figure 6. Trophic position (TP) estimated using a trophic enrichment factor of 3.9 for TP>2 and a trophic enrichment factor of 7.6 for TP 1 to TP 2 ($TP_{TEF=3.9}$) versus total body weight (g). a) $TP_{TEF=3.9}$ vs. Total Body Weight (g) for *C. argus*. b). $TP_{TEF=3.9}$ vs. Total Body Weight (g) for *C. strigosus* c) $TP_{TEF=3.9}$ vs. Total Body Weight (g) for all prey.



a)



b)



c)

Figure 7. Ciguatoxin concentration ([CTX]) as a function of trophic position (TP) calculated using a trophic enrichment factor of 3.9 for TP>2 and 7.6 for TP 1 to TP 2 ($TP_{TEF=3.9}$) (see text) for individual fish within sampled community. Linear regression analysis show no significant relationship between the two variables ($p>0.05$).

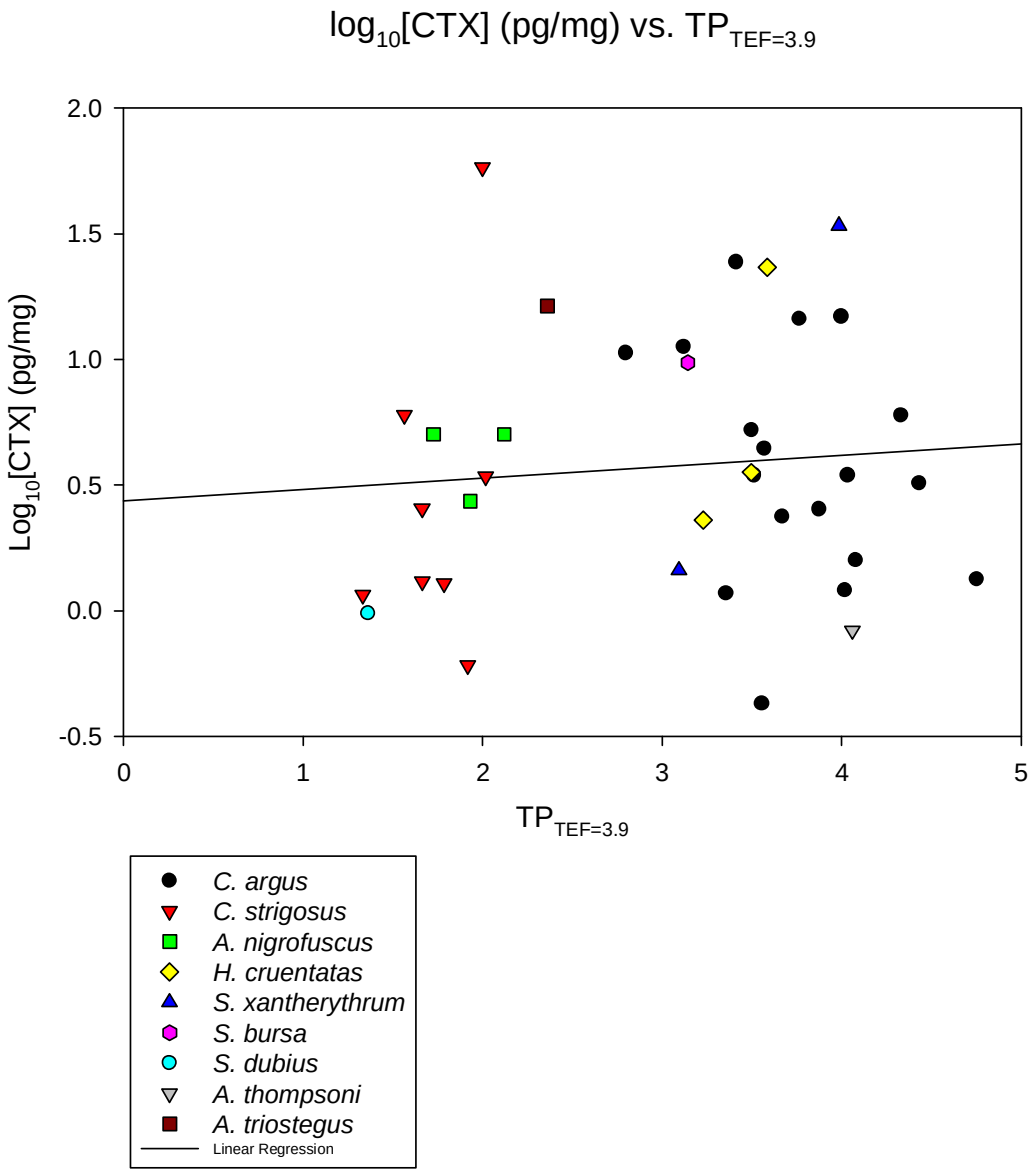
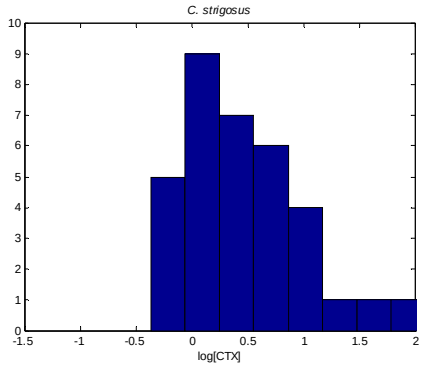
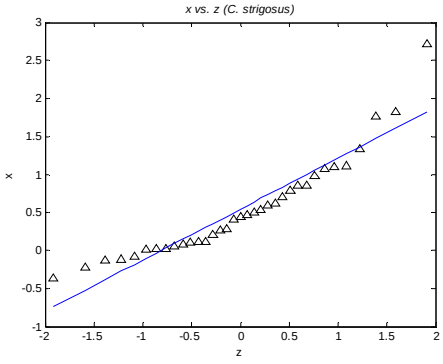


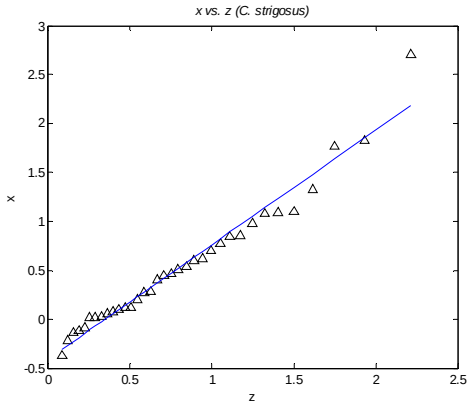
Figure 8. Median ciguatoxin (CTX) concentration determined by x vs. z-statistic for *C. strigosus*. a) Histogram of Log_{10} of CTX concentrations (pg/mg). b) Plot of x vs. z-statistic when CTX negative *C. strigosus* are not included in the calculation of the cumulative probability for the determination of the z-statistic (see text). c) Plot of x vs. z-statistic when CTX negative *C. strigosus* are included in the calculation of the cumulative probability for the determination of the z-statistic (see text), r^2=0.966.



a)

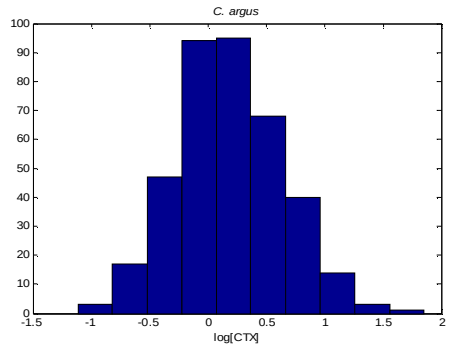


b)

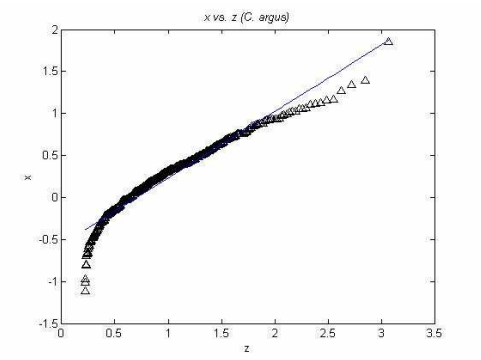


c)

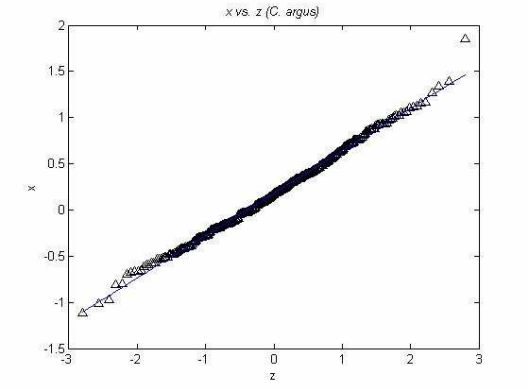
Figure 9. Median CTX concentration determined by x vs. z-statistic for *C. argus.* 9a) Histogram of Log_{10} of CTX concentrations (pg/mg). 9b) Plot of x vs. z-statistic when CTX negative *C. argus* are included in the calculation of the cumulative probability for the determination of the z-statistic (see text). 9c) Plot of x vs. z-statistic for CTX positive *C. argus* only are used in calculation of the cumulative probability for the determination of the z-statistic (see text), r^2=0.995.



a)



b)



c)

Figure 10. Trophic position determined using a trophic enrichment factor of 7.6 versus trophic position as determined by FishBase. Also shown is the 1:1 line. A linear regression analysis yields r^2=0.89 for the equation y = 0.504x + 0.914.

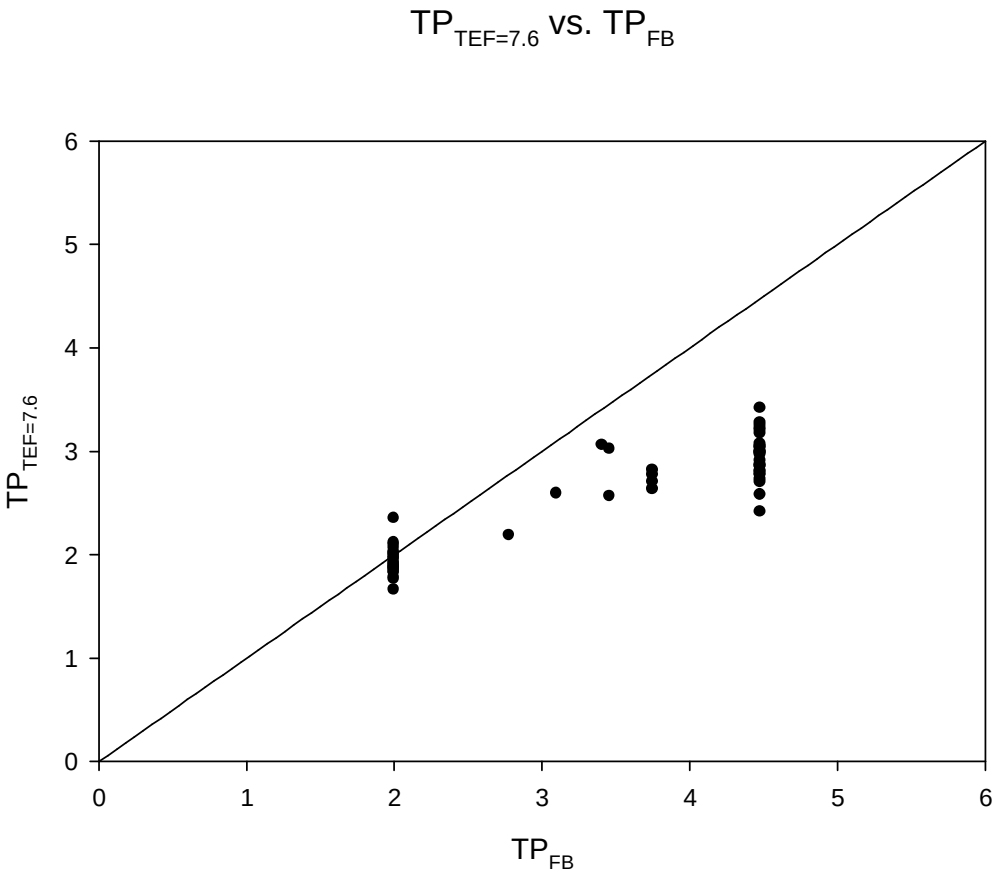


Figure 11. Bulk nitrogen and carbon isotope values for *P . filamentosus* and feed.

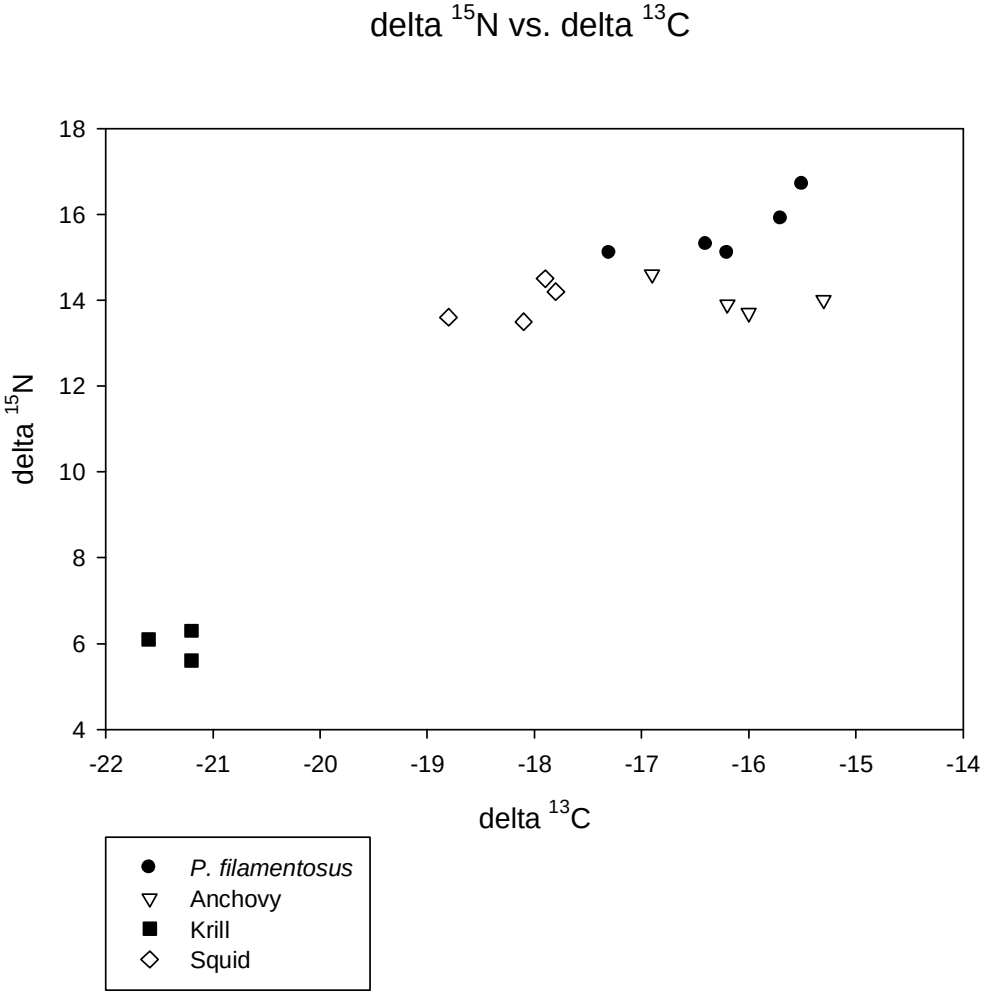


Figure 12. Plot of trophic position estimates from FishBase versus trophic position estimates using "trophic" and "source" amino acid groupings. Also shown is the 1:1 line. A regression analysis yields r^2=0.89 for the equation y = 0.651x + 0.838.

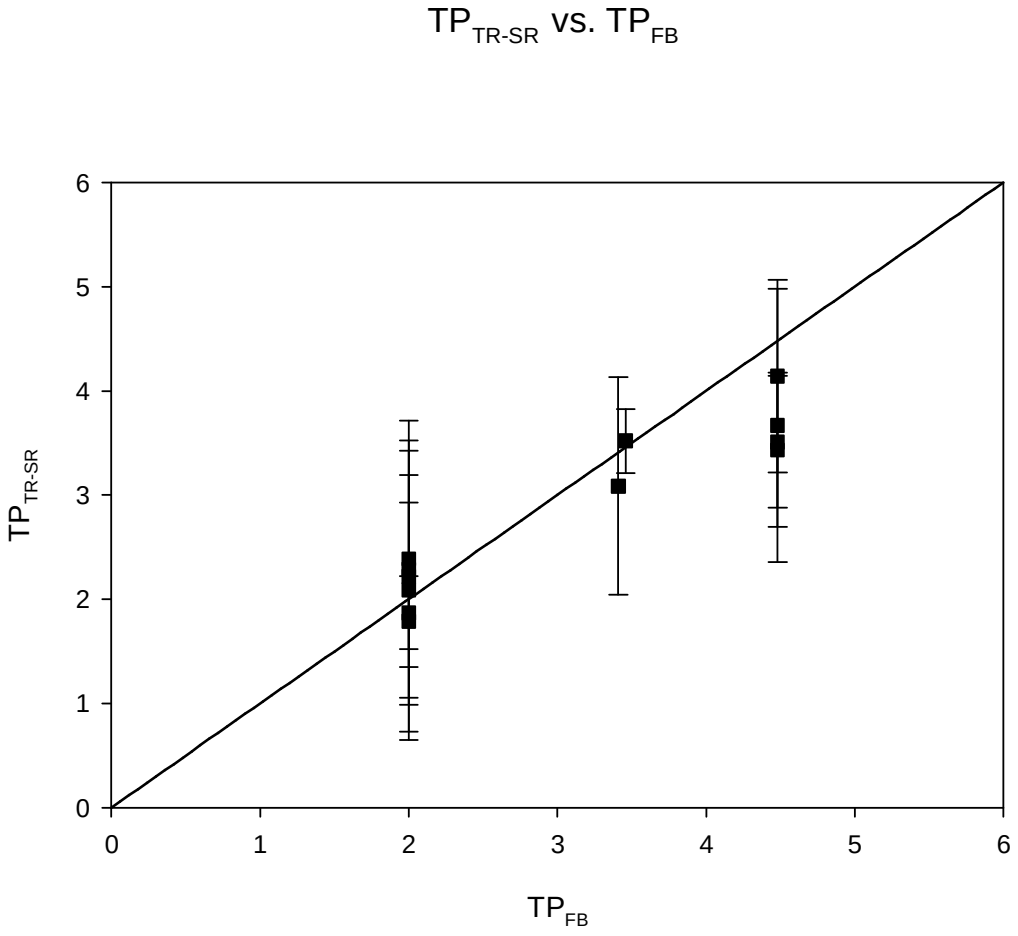
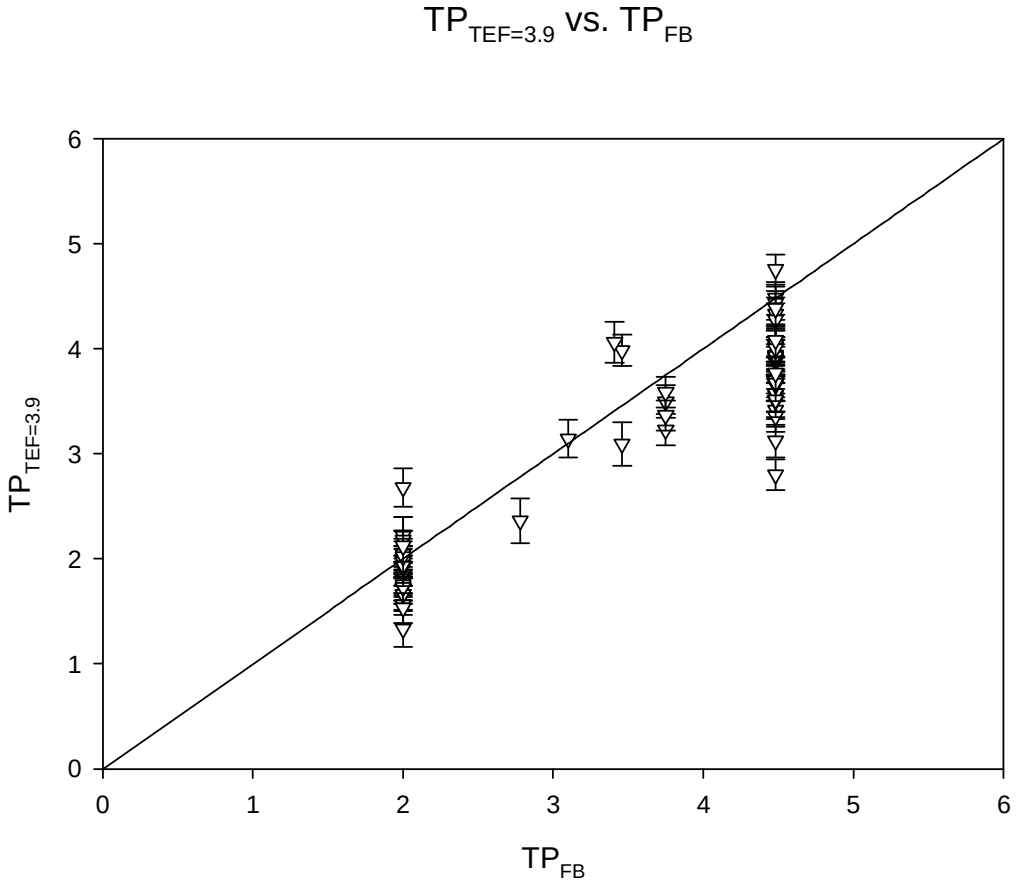


Figure 13. Plot of trophic position determined from FishBase versus trophic position calculated using a trophic enrichment factor of 7.6 between photoautotrophs and a trophic enrichment factor of 3.9 for all trophic positions greater than 2. Also shown is the 1:1 line. A regression analysis yields r^2=0.878 for the equation y = 0.981x – 0.116.



APPENDIX A

Metadata tables

Table 1. Fish samples collected for this study; information including family, species, catch location, total body weight in grams, total length in millimeters and standard length in millimeters.

Genus	Species	Catch Location	Total Body Weight (g)	Total Length (mm)	Standard Length (mm)
Acanthurus	nigrofuscus	Ewa, Oahu	21.1	100	83
Acanthurus	nigrofuscus	Ewa, Oahu	10.3	79	67
Acanthurus	nigrofuscus	Ewa, Oahu	12.1	82	70
Acanthurus	nigrofuscus	Ewa, Oahu	13.2	82	67
Acanthurus	nigrofuscus	Ewa, Oahu	21.2	97	89
Acanthurus	nigrofuscus	Ewa, Oahu	19.8	90	75
Acanthurus	nigrofuscus	Ewa, Oahu	13.2	82	68
Acanthurus	nigrofuscus	Ewa, Oahu	17.2	93	81
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	12.4	77	64
Acanthurus	nigrofuscus	Ewa, Oahu	14.1	83	69
Acanthurus	nigrofuscus	Ewa, Oahu	7.9	72	59
Acanthurus	nigrofuscus	Ewa, Oahu	12.9	79	66
Acanthurus	nigrofuscus	Ewa, Oahu	12.1	82	60
Acanthurus	nigrofuscus	Ewa, Oahu	20.2	100	83
Acanthurus	nigrofuscus	Ewa, Oahu	15.6	90	73
Acanthurus	nigrofuscus	Ewa, Oahu	7	66	55
Acanthurus	nigrofuscus	Ewa, Oahu	7.1	69	57
Acanthurus	nigrofuscus	Ewa, Oahu	11.8	81	61
Acanthurus	nigrofuscus	Ewa, Oahu	12.5	83	69
Acanthurus	nigrofuscus	Ewa, Oahu	13.2	85	72

Acanthurus	nigrofuscus	Ewa, Oahu	12.2	78	66
Acanthurus	nigrofuscus	Ewa, Oahu	23.9	110	90
Acanthurus	nigrofuscus	Ewa, Oahu	30.6	107	87
Acanthurus	nigrofuscus	Ewa, Oahu	32.1	113	94
Acanthurus	nigrofuscus	Ewa, Oahu	14.52	88	73
Acanthurus	nigrofuscus	Ewa, Oahu	18.9	97	81
Acanthurus	nigrofuscus	Ewa, Oahu	17.5	93	76
Acanthurus	nigrofuscus	Ewa, Oahu	17.8	95	78
Acanthurus	nigrofuscus	Ewa, Oahu	31.8	107	88
Acanthurus	nigrofuscus	Ewa, Oahu	26.1	103	86
Acanthurus	nigrofuscus	Ewa, Oahu	24.8	107	88
Acanthurus	nigrofuscus	Ewa, Oahu	20.8	99	81
Acanthurus	nigrofuscus	Ewa, Oahu	18.2	92	76
Acanthurus	nigrofuscus	Ewa, Oahu	16.4	90	75
Acanthurus	nigrofuscus	Ewa, Oahu	15.6	93	79
Acanthurus	nigrofuscus	Ewa, Oahu	14.7	89	73
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Ewa, Oahu	-	-	-
Acanthurus	nigrofuscus	Marnies Rock, Oahu	16.2	93	74
Acanthurus	nigrofuscus	Marnies Rock, Oahu	17	88	72
Acanthurus	nigrofuscus	Marnies Rock, Oahu	-	-	-
Acanthurus	nigrofuscus	Olowalu, Maui	19.5	111	78
Acanthurus	nigrofuscus	Sand Island, Oahu	32.5	110	93
Acanthurus	nigrofuscus	Sand Island, Oahu	15.9	86	74
Acanthurus	nigrofuscus	Sand Island, Oahu	38.8	122	110

Acanthurus	nigrofuscus	Sand Island, Oahu	11.6	81	68
Acanthurus	nigrofuscus	SW Maui	74.4	165	123
Acanthurus	nigrofuscus	SW Maui	28.3	121	86
Acanthurus	nigrofuscus	SW Maui	36.5	125	91
Acanthurus	nigrofuscus	SW Maui	33.2	116	96
Acanthurus	nigrofuscus	SW Maui	32.7	116	95
Acanthurus	nigrofuscus	SW Maui	27.6	123	86
Acanthurus	nigrofuscus	SW Maui	29.8	127	91
Acanthurus	nigrofuscus	SW Maui	43.9	150	114
Acanthurus	nigrofuscus	SW Maui	43.4	138	112
Acanthurus	nigrofuscus	SW Maui	29.6	126	88
Acanthurus	nigrofuscus	SW Maui	36.2	118	99
Acanthurus	nigrofuscus	SW Maui	17.4	92	80
Acanthurus	nigroris	Olowalu, Maui	115.8	187	136
Acanthurus	nigroris	Sand Island, Oahu	23.7	105	88
Acanthurus	nigroris	Sand Island, Oahu	12.9	87	72
Acanthurus	nigroris	Sand Island, Oahu	-	-	-
Acanthurus	olivaceous	Olowalu, Maui	38.3	122	100
Acanthurus	olivaceous	Olowalu, Maui	82.9	148	124
Acanthurus	olivaceous	Olowalu, Maui	57.9	143	118
Acanthurus	olivaceous	Olowalu, Maui	53.6	133	110
Acanthurus	olivaceous	SW Maui	54	140	112
Acanthurus	thompsoni	Marnies Rock, Oahu	46.5	137	121
Acanthurus	thompsoni	SW Maui	79.4	153	128
Acanthurus	thompsoni	SW Maui	86.3	159	133
Acanthurus	thompsoni	SW Maui	87.4	163	138
Acanthurus	thompsoni	SW Maui	73	149	123
Catherhines	verecundus	Waianae, Oahu	44	135	110
Cephalopholis	argus	Ala Moana, Oahu	983.2	430	360
Cephalopholis	argus	Sand Island, Oahu	901.7	349	289
Cephalopholis	argus	Sand Island, Oahu	527.9	305	255

Cephalopholis	argus	Sand Island, Oahu	1297.8	70	305
Cephalopholis	argus	SW Maui	202.9	220	185
Cephalopholis	argus	SW Maui	251	253	210
Cephalopholis	argus	SW Maui	346.9	285	235
Cephalopholis	argus	SW Maui	264	247	206
Cephalopholis	argus	SW Maui	368.5	268	229
Cephalopholis	argus	SW Maui	759.3	316	267
Cephalopholis	argus	SW Maui	533.7	304	252
Cephalopholis	argus	SW Maui	375.9	265	224
Cephalopholis	argus	SW Maui	1098.4	408	342
Chlorurus	sordidus	SW Maui	190.2	217	183
Chlorurus	sordidus	SW Maui	60	149	122
Chlorurus	sordidus	SW Maui	109.1	187	153
Chromis	agilis	Olowalu, Maui	14.9	78	64
Ctenochaetus	strigosus	Marnies Rock, Oahu	68.8	133	117
Ctenochaetus	strigosus	Marnies Rock, Oahu	38.7	112	90
Ctenochaetus	strigosus	Marnies Rock, Oahu	110.1	155	124
Ctenochaetus	strigosus	Marnies Rock, Oahu	31.4	113	85
Ctenochaetus	strigosus	Marnies Rock, Oahu	52.7	125	95
Ctenochaetus	strigosus	Marnies Rock, Oahu	41.8	112	90
Ctenochaetus	strigosus	Marnies Rock, Oahu	35.9	105	85
Ctenochaetus	strigosus	Marnies Rock, Oahu	46.7	116	94
Ctenochaetus	strigosus	Marnies Rock, Oahu	37.4	108	84
Ctenochaetus	strigosus	Marnies Rock, Oahu	39	106	86
Ctenochaetus	strigosus	Marnies Rock, Oahu	41.9	128	83
Ctenochaetus	strigosus	Marnies Rock, Oahu	27.2	96	78
Ctenochaetus	strigosus	Marnies Rock, Oahu	51.3	130	96
Ctenochaetus	strigosus	Marnies Rock, Oahu	37.3	110	86
Ctenochaetus	strigosus	Marnies Rock, Oahu	40.9	119	86
Ctenochaetus	strigosus	Marnies Rock, Oahu	14.3	80	65
Ctenochaetus	strigosus	Marnies Rock, Oahu	48.6	129	95

Ctenochaetus	strigosus	Marnies Rock, Oahu	14.9	90	69
Ctenochaetus	strigosus	Marnies Rock, Oahu	26.9	104	76
Ctenochaetus	strigosus	Marnies Rock, Oahu	-	-	-
Ctenochaetus	strigosus	Olowalu, Maui	36.6	115	95
Ctenochaetus	strigosus	Olowalu, Maui	29.7	102	82
Ctenochaetus	strigosus	Olowalu, Maui	33.7	106	87
Ctenochaetus	strigosus	Olowalu, Maui	28.8	95	78
Ctenochaetus	strigosus	Olowalu, Maui	59.5	145	104
Ctenochaetus	strigosus	Olowalu, Maui	56.9	140	100
Ctenochaetus	strigosus	Olowalu, Maui	57.6	142	104
Ctenochaetus	strigosus	Olowalu, Maui	63.4	147	105
Ctenochaetus	strigosus	Olowalu, Maui	32.3	112	81
Ctenochaetus	strigosus	Olowalu, Maui	20.4	97	70
Ctenochaetus	strigosus	Olowalu, Maui	24.9	98	73
Ctenochaetus	strigosus	Olowalu, Maui	37.9	119	89
Ctenochaetus	strigosus	Olowalu, Maui	70.2	147	106
Ctenochaetus	strigosus	Olowalu, Maui	20.6	103	73
Ctenochaetus	strigosus	Olowalu, Maui	68.1	156	112
Ctenochaetus	strigosus	Olowalu, Maui	73.3	158	111
Ctenochaetus	strigosus	Olowalu, Maui	37.8	134	95
Ctenochaetus	strigosus	Sand Island, Oahu	132.7	-	-
Ctenochaetus	strigosus	Sand Island, Oahu	140	163	136
Ctenochaetus	strigosus	Sand Island, Oahu	139.5	161	128
Ctenochaetus	strigosus	Sand Island, Oahu	128.6	160	126
Ctenochaetus	strigosus	Sand Island, Oahu	113.4	153	125
Ctenochaetus	strigosus	Sand Island, Oahu	83.2	140	115
Ctenochaetus	strigosus	Sand Island, Oahu	21.9	90	73
Ctenochaetus	strigosus	Sand Island, Oahu	22	93	77
Ctenochaetus	strigosus	Sand Island, Oahu	23.1	94	75
Ctenochaetus	strigosus	Sand Island, Oahu	18.4	87	70.5
Ctenochaetus	strigosus	Sand Island, Oahu	71.2	124	105

Ctenochaetus	strigosus	Sand Island, Oahu	49.4	113	91
Ctenochaetus	strigosus	Sand Island, Oahu	28.2	95	79
Ctenochaetus	strigosus	Sand Island, Oahu	34.9	107	87
Ctenochaetus	strigosus	Sand Island, Oahu	27.1	101	83
Ctenochaetus	strigosus	Sand Island, Oahu	-	-	-
Ctenochaetus	strigosus	Sand Island, Oahu	-	-	-
Ctenochaetus	strigosus	Sand Island, Oahu	19.4	84	67
Ctenochaetus	strigosus	SW Maui	28.8	106	76
Ctenochaetus	strigosus	SW Maui	27	99	73
Ctenochaetus	strigosus	SW Maui	42.9	120	93
Ctenochaetus	strigosus	SW Maui	30.7	104	82
Ctenochaetus	strigosus	SW Maui	30.1	101	77
Ctenochaetus	strigosus	SW Maui	23.3	99	72
Ctenochaetus	strigosus	SW Maui	20.8	89	72
Ctenochaetus	strigosus	SW Maui	27.1	100	81
Ctenochaetus	strigosus	SW Maui	67.7	130	104
Ctenochaetus	strigosus	SW Maui	77.7	140	111
Ctenochaetus	strigosus	SW Maui	50.1	122	97
Ctenochaetus	strigosus	SW Maui	47.9	117	92
Ctenochaetus	strigosus	SW Maui	48.6	118	94
Ctenochaetus	strigosus	SW Maui	57.5	125	101
Ctenochaetus	strigosus	SW Maui	50.3	121	97
Ctenochaetus	strigosus	SW Maui	67	129	101
Ctenochaetus	strigosus	SW Maui	59.8	121	102
Ctenochaetus	strigosus	SW Maui	63.5	153	115
Ctenochaetus	strigosus	Waianae, Oahu	26.3	94	72
Ctenochaetus	strigosus	Waianae, Oahu	35.1	107	85
Ctenochaetus	strigosus	Waianae, Oahu	22.1	89	73
Ctenochaetus	strigosus	Waianae, Oahu	27.9	81	100
Ctenochaetus	strigosus	Waianae, Oahu	44.3	114	89
Ctenochaetus	strigosus	Waianae, Oahu	48.3	115	93

Ctenochaetus	strigosus	Waianae, Oahu	55.3	117	97
Ctenochaetus	strigosus	Waianae, Oahu	35.1	102	85
Ctenochaetus	strigosus	Waianae, Oahu	42.3	110	88
Ctenochaetus	strigosus	Waianae, Oahu	51.1	113	95
Ctenochaetus	strigosus	Waianae, Oahu	323.6	102	74
Ctenochaetus	strigosus	Waianae, Oahu	30.9	99	80
Ctenochaetus	strigosus	Waianae, Oahu	40.3	105	88
Ctenochaetus	strigosus	-	13.1	87	65
Gomphosus	varius	-	30.2	176	143
Heteropriacanthus	cruentas	Makai pier, Oahu	159.9	215	175
Heteropriacanthus	cruentas	Makai pier, Oahu	179.9	224	184
Heteropriacanthus	cruentas	Makai pier, Oahu	139.9	205	165
Heteropriacanthus	cruentas	Makai pier, Oahu	142.6	205	170
Heteropriacanthus	cruentas	Makai pier, Oahu	149.2	210	170
Heteropriacanthus	cruentas	Makai pier, Oahu	166.3	220	176
Naso	hexacanthus	SW Maui	124.1	190	166
Pervagor	aspricaudus	SW Maui	22.8	100	85
Pervagor	aspricaudus	Waianae, Oahu	6.5	67	58
Priacanthus	meeki	SW Maui	238.2	249	207
Priacanthus	meeki	SW Maui	137	211	169
Sargocentron	diadema	Sand Island, Oahu	42.9	139	115
Sargocentron	diadema	Sand Island, Oahu	51.9	149	122
Sargocentron	diadema	Sand Island, Oahu	77.2	167	133
Sargocentron	xantherythrum	Olowalu, Maui	28.9	121	99
Sargocentron	xantherythrum	Olowalu, Maui	19.3	111	90
Sargocentron	xantherythrum	Olowalu, Maui	19.4	110	88
Sargocentron	xantherythrum	Olowalu, Maui	25.2	120	97
Sargocentron	xantherythrum	Olowalu, Maui	31.1	128	104
Sargocentron	xantherythrum	Olowalu, Maui	35	134	109
Scarus	dubius	SW Maui	152.4	196	159
Scarus	dubius	SW Maui	101.7	170	139

Scarus	dubius	SW Maui	60.9	156	127
Scarus	psittacus	Marnies Rock, Oahu	105.2	164	145
Scarus	psittacus	Marnies Rock, Oahu	67.2	150	132
Scarus	psittacus	Marnies Rock, Oahu	83.1	161	140
Scarus	psittacus	Marnies Rock, Oahu	38.9	120	110
Scarus	psittacus	Marnies Rock, Oahu	49.4	135	118
Scarus	psittacus	Olowalu, Maui	105.5	180	142
Scarus	psittacus	Olowalu, Maui	41.4	124	98
Scarus	psittacus	Olowalu, Maui	57.4	142	114
Sufflamen	bursa	Marnies Rock, Oahu	72	149	120
Sufflamen	bursa	Marnies Rock, Oahu	116.3	181	147
Thalassoma	duperry	Olowalu, Maui	82	175	142
Thalassoma	duperry	Olowalu, Maui	30.2	122	104
Xanthichthys	auromarginatus	Marnies Rock, Oahu	69.8	143	120
Zebrasoma	flavescens	Olowalu, Maui	14.6	82	70
Zebrasoma	flavescens	Olowalu, Maui	16.1	84	71
Zebrasoma	flavescens	Olowalu, Maui	26.1	98	82
Zebrasoma	flavescens	Olowalu, Maui	16.5	88	69
Zebrasoma	flavescens	Sand Island, Oahu	17.2	86	72
Zebrasoma	flavescens	Sand Island, Oahu	8.7	72	58
Zebrasoma	flavescens	Sand Island, Oahu	6.6	66	54
Zebrasoma	flavescens	Sand Island, Oahu	16.4	81	69
Zebrasoma	flavescens	Sand Island, Oahu	-	-	-
Zebrasoma	flavescens	SW Maui	17.7	86	62
Zebrasoma	flavescens	SW Maui	38.1	113	93
Zebrasoma	flavescens	SW Maui	30.4	106	88
Zebrasoma	flavescens	SW Maui	36.9	114	92
Zebrasoma	flavescens	SW Maui	38.5	117	93

Table 2. Ciguatoxin metadata. Information including genus, species, catch location by island and ciguatoxin (CTX) concentration for samples that tested above the limit of detection (limit of detection is estimated to be about 0.07 pg/mg).

Genus	Species	Location	CTX (pg/mg)
Acanthurus	*nigrofuscus*	Maui	7.94
Acanthurus	*nigrofuscus*	Maui	2.96
Acanthurus	*nigrofuscus*	Maui	2.23
Acanthurus	*nigrofuscus*	Maui	5.05
Acanthurus	*nigrofuscus*	Maui	2.58
Acanthurus	*nigrofuscus*	Maui	0.98
Acanthurus	*nigrofuscus*	Maui	4.76
Acanthurus	*nigrofuscus*	Maui	2.72
Acanthurus	*nigrofuscus*	Maui	5.04
Acanthurus	*nigrofuscus*	Maui	-
Acanthurus	*nigrofuscus*	Maui	-
Acanthurus	*nigrofuscus*	Maui	-
Acanthurus	*nigrofuscus*	Maui	-
Acanthurus	*nigrofuscus*	Oahu	5.02
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigrofuscus*	Oahu	-
Acanthurus	*nigroris*	Maui	-
Acanthurus	*olivaceous*	Maui	-
Acanthurus	*olivaceous*	Maui	-
Acanthurus	*olivaceous*	Maui	-
Acanthurus	*olivaceous*	Maui	-
Acanthurus	*olivaceous*	Maui	-
Acanthurus	*thompsoni*	Maui	0.83
Acanthurus	*thompsoni*	Maui	-
Acanthurus	*thompsoni*	Maui	-
Acanthurus	*thompsoni*	Maui	-
Acanthurus	*thompsoni*	Oahu	-
Catherhines	*verecundus*	Oahu	-
Chlorurus	*sordidus*	Maui	1.70
Chlorurus	*sordidus*	Maui	0.35
Chlorurus	*sordidus*	Maui	-
Chromis	*agilis*	Oahu	-
Ctenochaetus	*strigosus*	Maui	7.07
Ctenochaetus	*strigosus*	Maui	2.55
Ctenochaetus	*strigosus*	Maui	1.90
Ctenochaetus	*strigosus*	Maui	1.27
Ctenochaetus	*strigosus*	Maui	1.15

Ctenochaetus	*strigosus*	Maui	1.04
Ctenochaetus	*strigosus*	Maui	0.82
Ctenochaetus	*strigosus*	Maui	0.73
Ctenochaetus	*strigosus*	Maui	0.61
Ctenochaetus	*strigosus*	Maui	2.93
Ctenochaetus	*strigosus*	Maui	1.32
Ctenochaetus	*strigosus*	Maui	5.05
Ctenochaetus	*strigosus*	Maui	3.22
Ctenochaetus	*strigosus*	Maui	7.13
Ctenochaetus	*strigosus*	Maui	1.60
Ctenochaetus	*strigosus*	Maui	1.87
Ctenochaetus	*strigosus*	Maui	1.05
Ctenochaetus	*strigosus*	Maui	0.43
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Maui	-
Ctenochaetus	*strigosus*	Oahu	6.00
Ctenochaetus	*strigosus*	Oahu	3.42
Ctenochaetus	*strigosus*	Oahu	1.31
Ctenochaetus	*strigosus*	Oahu	4.14
Ctenochaetus	*strigosus*	Oahu	9.48
Ctenochaetus	*strigosus*	Oahu	514.13
Ctenochaetus	*strigosus*	Oahu	12.78
Ctenochaetus	*strigosus*	Oahu	3.95
Ctenochaetus	*strigosus*	Oahu	67.18
Ctenochaetus	*strigosus*	Oahu	11.98
Ctenochaetus	*strigosus*	Oahu	21.27
Ctenochaetus	*strigosus*	Oahu	58.03
Ctenochaetus	*strigosus*	Oahu	1.05
Ctenochaetus	*strigosus*	Oahu	0.77
Ctenochaetus	*strigosus*	Oahu	2.81
Ctenochaetus	*strigosus*	Oahu	1.20
Ctenochaetus	*strigosus*	Oahu	12.41
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-

Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Ctenochaetus	*strigosus*	Oahu	-
Gomphosus	*varius*	Maui	38.30
Heteropriacanthus	*cruentatus*	Maui	2.29
Heteropriacanthus	*cruentatus*	Oahu	23.21
Heteropriacanthus	*cruentatus*	Oahu	3.55
Heteropriacanthus	*cruentatus*	Oahu	-
Heteropriacanthus	*cruentatus*	Oahu	-
Heteropriacanthus	*cruentatus*	Oahu	-
Naso	*hexacanthus*	Maui	-
Pervagor	*aspricaudus*	Maui	-
Pervagor	*aspricaudus*	Oahu	-
Priacanthus	*meeki*	Maui	-
Priacanthus	*meeki*	Maui	-
Sargocentron	*diadema*	Oahu	-
Sargocentron	*diadema*	Oahu	-
Sargocentron	*diadema*	Oahu	-
Sargocentron	*xantherythrum*	Maui	34.01
Sargocentron	*xantherythrum*	Maui	-
Sargocentron	*xantherythrum*	Maui	-
Sargocentron	*xantherythrum*	Maui	-
Sargocentron	*xantherythrum*	Maui	-
Sargocentron	*xantherythrum*	Maui	-
Scarus	*dubius*	Maui	0.97
Scarus	*dubius*	Maui	-
Scarus	*dubius*	Maui	-
Scarus	*psittacus*	Maui	-
Scarus	*psittacus*	Maui	-
Scarus	*psittacus*	Maui	-
Sufflamen	*bursa*	Oahu	9.70
Sufflamen	*bursa*	Oahu	-

Thalassoma	*duperrey*	Maui	-
Thalassoma	*duperrey*	Maui	-
Xanthichthys	*auromarginatus*	Oahu	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Maui	-
Zebrasoma	*flavescens*	Oahu	-
Zebrasoma	*flavescens*	Oahu	-
Zebrasoma	*flavescens*	Oahu	-
Zebrasoma	*flavescens*	Oahu	-

Table 3. Amino acid compound specific metadata; information including species, catch location by island, nitrogen isotope value for glutamic acid (δ^{15}N Glu) and standard deviation (Stdev) of the measured value (each value is the average of 3 analyses for each sample), nitrogen isotope value for phenylalanine (δ^{15}N Phe) and standard deviation of the measured value, trophic position as reported on the website www.fishbase.org (TP_{FB}) and associated error, trophic position calculate from the δ^{15}N value of glutamic acid and phenylalanine using a trophic enrichment factor of 7.6 (see text) and propagated error, trophic position calculate from the δ^{15}N value of glutamic acid and phenylalanine using a trophic enrichment factor of 7.6 for the increase in associate with the change in trophic position 1 and 2 and a trophic enrichment factor of 3.9 for all trophic positions greater than 2 (see text) and propagated error.

Species	Island	δ^{15}N Glu (‰)	δ^{15}N Glu Stdev	δ^{15}N Phe (‰)	δ^{15}N Phe Stdev	TP_{FB}	TP_{FB} error	$TP_{TEF=7.6}$	$TP_{TEF=7.6}$ Error	$TP_{TEF=3.9}$	$TP_{TEF=3.9}$ Error
A. nigrofuscus	Maui	13.64	0.28	3.70	0.46	2.00	0.00	1.86	0.02	1.73	0.16
A. nigrofuscus	Maui	13.39	0.24	2.65	0.87	2.00	0.00	1.97	0.03	1.93	0.19
A. nigrofuscus	Oahu	13.13	0.21	1.65	0.32	2.00	0.00	2.06	0.02	2.12	0.15
A. nigroris	Oahu	14.29	0.60	0.65	0.57	2.00	0.00	2.35	0.03	2.68	0.18
A. nigroris	Oahu	12.96	0.51	1.11	0.61	2.00	0.00	2.11	0.03	2.22	0.18
A. thompsoni	Maui	15.95	0.29	-3.09	0.88	3.41	0.40	3.06	0.03	4.06	0.20
A. triostegus	Oahu	13.58	0.37	1.17	0.99	2.78	0.35	2.19	0.03	2.36	0.21
C. argus	Maui	20.40	0.36	3.88	0.39	4.48	0.79	2.73	0.02	3.41	0.16
C. argus	Maui	20.02	0.41	4.64	0.22	4.48	0.79	2.58	0.02	3.12	0.15
C. argus	Maui	20.68	0.25	6.56	0.13	4.48	0.79	2.41	0.02	2.80	0.15
C. argus	Maui	20.59	0.45	3.74	0.50	4.48	0.79	2.77	0.02	3.50	0.17
C. argus	Maui	19.90	0.59	1.40	0.17	4.48	0.79	2.99	0.02	3.92	0.17
C. argus	Maui	19.93	0.33	1.36	0.15	4.48	0.79	3.00	0.02	3.94	0.15
C. argus	Maui	18.90	0.49	1.29	0.12	4.48	0.79	2.87	0.02	3.69	0.16
C. argus	Maui	20.31	0.25	4.02	0.20	4.48	0.79	2.70	0.02	3.36	0.15
C. argus	Maui	19.78	0.14	0.67	0.44	4.48	0.79	3.07	0.02	4.08	0.15
C. argus	Maui	20.30	0.04	2.41	0.39	4.48	0.79	2.91	0.02	3.77	0.15
C. argus	Oahu	22.41	0.42	1.73	0.23	4.48	0.79	3.27	0.02	4.48	0.16
C. argus	Oahu	19.89	0.16	1.57	0.09	4.48	0.79	2.96	0.01	3.88	0.14
C. argus	Oahu	23.66	0.48	3.16	0.13	4.48	0.79	3.25	0.02	4.43	0.16
C. argus	Oahu	21.68	0.45	1.59	0.22	4.48	0.79	3.20	0.02	4.33	0.16

C. argus	Oahu	22.75	0.07	1.00	0.20	4.48	0.79	3.41	0.02	4.75	0.14
C. argus	Oahu	19.58	0.66	2.45	0.36	4.48	0.79	2.81	0.02	3.57	0.18
C. argus	Oahu	20.35	0.23	1.94	0.19	4.48	0.79	2.98	0.02	3.90	0.15
C. argus	Oahu	19.18	0.33	2.10	0.36	4.48	0.79	2.80	0.02	3.56	0.16
C. argus	Oahu	21.97	0.41	4.51	0.15	4.48	0.79	2.85	0.02	3.66	0.15
C. argus	Oahu	20.95	0.43	2.07	0.11	4.48	0.79	3.04	0.02	4.02	0.15
C. argus	Oahu	19.25	0.59	1.74	0.26	4.48	0.79	2.86	0.02	3.67	0.17
C. argus	Oahu	23.78	0.23	3.49	0.58	4.48	0.79	3.22	0.02	4.38	0.17
C. argus	Oahu	21.64	0.51	2.84	0.95	4.48	0.79	3.03	0.03	4.00	0.22
C. strigosus	Oahu	12.68	0.73	2.51	0.40	2.00	0.00	1.89	0.03	1.79	0.19
C. strigosus	Oahu	12.12	0.44	1.79	0.53	2.00	0.00	1.91	0.02	1.83	0.17
C. strigosus	Oahu	12.09	0.82	0.90	0.73	2.00	0.00	2.02	0.04	2.05	0.22
C. strigosus	Oahu	13.32	0.19	3.17	0.15	2.00	0.00	1.89	0.02	1.78	0.14
C. strigosus	Oahu	11.49	0.49	2.18	0.61	2.00	0.00	1.78	0.03	1.57	0.18
C. strigosus	Oahu	13.22	0.14	3.53	0.93	2.00	0.00	1.83	0.03	1.66	0.20
C. strigosus	Oahu	12.75	0.53	1.67	0.40	2.00	0.00	2.01	0.02	2.02	0.17
C.strigosus	Maui	13.27	0.14	2.58	0.69	2.00	0.00	1.96	0.02	1.92	0.17
C.strigosus	Maui	11.17	0.24	2.78	0.61	2.00	0.00	1.66	0.02	1.33	0.17
C.strigosus	Maui	12.20	0.26	2.52	0.27	2.00	0.00	1.83	0.02	1.66	0.15
C.strigosus	Oahu	12.02	0.48	1.02	0.48	2.00	0.00	2.00	0.02	2.00	0.17
H. cruentatus	Oahu	16.26	0.34	0.47	0.20	3.75	0.47	2.63	0.02	3.23	0.15
H. cruentatus	Oahu	16.89	0.40	0.05	0.22	3.75	0.47	2.77	0.02	3.50	0.15
H. cruentatus	Oahu	16.50	0.26	-0.70	0.22	3.75	0.47	2.81	0.02	3.59	0.15
H. cruentatus	Oahu	17.07	0.19	0.74	0.05	3.75	0.47	2.70	0.02	3.37	0.14
S. bursa	Oahu	17.03	0.29	1.56	0.71	3.10	0.38	2.59	0.02	3.14	0.18
S. dubius	Maui	11.80	0.25	3.28	0.92	-	-	1.67	0.03	1.36	0.20
S. psittacus	Oahu	11.32	0.49	0.91	0.55	2.00	0.00	1.92	0.02	1.85	0.18
S. psittacus	Oahu	11.76	0.33	1.84	0.04	2.00	0.00	1.86	0.02	1.72	0.15
S. psittacus	Oahu	10.54	0.16	0.51	0.24	2.00	0.00	1.87	0.02	1.75	0.15
S. xantherythrum	Maui	21.06	0.25	2.31	0.28	3.46	0.54	3.02	0.02	3.98	0.15
S. xantherythrum	Oahu	19.05	0.95	3.78	0.36	3.46	0.54	2.56	0.03	3.09	0.21

Table 4. Metadata associated with trophic position calculation using the average of "trophic" amino acids and average of "source" amino acids (see text); information including the average nitrogen isotope ($\delta^{15}N$) value of "trophic" amino acids (alanine, valine, leucine, isoleucine, praline and glutamic acid) and the standard deviation (Stdev), average $\delta^{15}N$ value of "source" amino acids (glycine, serine and phenylalanine) and the standard deviation, and trophic position calculated using this method (see text) with propagated error.

Species	$\delta^{15}N$ Trophic (‰)	Trophic Stdev	$\delta^{15}N$ Source (‰)	Source Stdev	TP_{Tr-Sr}	TP_{Tr-Sr} Error
C.strigosus	10.73	1.60	2.90	1.01	1.79	1.14
C.strigosus	12.06	1.09	2.55	1.01	2.09	1.10
C.strigosus	12.28	2.02	2.04	1.38	2.22	1.49
C.strigosus	10.84	2.06	0.22	1.12	2.29	1.23
C.strigosus	10.14	0.61	1.85	0.29	1.87	0.35
A. nigrofuscus	13.62	1.56	2.44	0.94	2.39	1.04
A. thompsoni	14.09	1.44	-0.99	0.95	3.09	1.04
S. xantherythrum	19.47	1.56	1.96	0.21	3.52	0.31
C. argus	22.56	3.64	1.57	0.77	4.14	0.93
C. argus	18.61	1.06	1.15	0.55	3.51	0.63
C. argus	19.52	1.52	2.50	0.65	3.43	0.74
C. argus	20.16	0.82	1.81	1.24	3.67	1.31

Table 5. Bulk isotope metadata. Information including nitrogen isotope value ($\delta^{15}N$), carbon isotope value ($\delta^{13}C$) and molar carbon to nitrogen ratio (Molar C:N).

Species	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Molar C:N
A. nigrofuscus	6.90	-17.69	3.74
A. nigrofuscus	5.80	-18.10	3.87
A. nigrofuscus	6.70	-18.30	3.83
A. nigrofuscus	6.10	-18.20	3.81
A. nigrofuscus	6.82	-16.85	3.63
A. nigrofuscus	6.65	-17.59	3.77
A. nigrofuscus	6.52	-17.47	3.82
A. nigrofuscus	7.40	-16.96	3.69
A. nigrofuscus	7.23	-17.21	3.74
A. nigrofuscus	7.41	-17.45	3.84
A. nigrofuscus	6.37	-18.13	3.70
A. nigrofuscus	7.04	-17.88	3.72
A. nigrofuscus	6.90	-17.08	3.92
A. nigrofuscus	6.66	-16.86	3.85
A. nigrofuscus	6.56	-19.14	3.92
A. nigrofuscus	6.77	-17.12	3.94
A. nigrofuscus	6.47	-17.09	4.00
A. nigrofuscus	6.56	-18.03	3.84
A. nigrofuscus	7.39	-15.81	3.86
A. nigrofuscus	7.39	-16.65	3.90
A. nigrofuscus	6.47	-18.15	3.84
A. nigrofuscus	7.33	-17.59	3.78
A. nigrofuscus	7.37	-17.21	3.84
A. nigrofuscus	7.50	-16.54	3.89
A. nigrofuscus	6.33	-18.10	3.69
A. nigrofuscus	7.09	-15.72	3.88
A. nigrofuscus	7.51	-17.07	3.73
A. nigrofuscus	7.30	-16.90	3.81
A. nigrofuscus	7.90	-17.38	3.65
A. nigrofuscus	7.30	-17.85	3.73
A. nigrofuscus	6.79	-18.11	3.78
A. nigrofuscus	7.74	-17.26	3.74
A. nigrofuscus	6.90	-17.34	3.68
C. argus	9.56	-15.18	3.86
C. argus	10.27	-13.72	3.61
C. argus	8.95	-13.90	3.72
C. argus	10.25	-13.77	3.56
C. argus	10.95	-13.77	3.68
C. argus	10.42	-14.11	3.68
C. argus	10.32	-13.70	3.57
C. argus	10.01	-13.13	3.63
C. argus	9.72	-13.07	3.67
C. argus	9.41	-13.65	3.68
C. argus	10.12	-12.88	3.65

C. argus	9.12	-14.11	3.64
C. argus	9.75	-14.82	3.63
C. argus	9.83	-14.37	3.68
C. argus	9.95	-16.88	5.73
C. argus	9.13	-14.96	3.79
C. argus	10.37	-14.41	3.88
C. argus	9.76	-13.84	4.00
C. argus	9.60	-13.92	5.06
C. argus	9.91	-16.03	6.57
C. argus	8.98	-13.34	3.71
C. argus	9.34	-13.34	3.67
C. argus	9.17	-13.82	3.62
C. argus	9.25	-12.99	4.00
C. argus	9.75	-13.46	3.74
C. argus	8.90	-13.61	3.66
C. argus	9.23	-13.85	4.02
C. argus	9.57	-14.23	4.77
C. argus	9.68	-13.06	3.67
C. argus	9.67	-13.02	3.77
C. argus	10.19	-16.53	5.68
C. argus	8.92	-17.95	6.34
C. argus	9.32	-14.41	3.68
C. argus	9.74	-14.31	3.72
C. argus	9.41	-13.37	3.69
C. argus	10.02	-12.75	3.73
C. argus	9.78	-12.92	3.69
C. argus	9.26	-13.80	3.61
C. argus	9.18	-13.36	3.61
C. argus	9.74	-13.05	3.71
C. argus	9.50	-12.93	3.68
C. argus	9.16	-14.98	3.67
C. argus	9.12	-15.09	3.66
C. argus	9.57	-13.91	3.72
C. argus	10.14	-13.24	3.67
C. argus	9.65	-14.63	3.60
C. argus	9.12	-14.16	3.63
C. argus	9.54	-14.02	4.07
C. argus	9.20	-13.87	3.63
C. argus	10.17	-13.83	3.68
C. argus	10.14	-13.33	3.65
C. argus	9.09	-14.30	3.64
C. argus	10.11	-14.04	3.58
C. argus	9.27	-14.17	3.70
C. argus	9.30	-13.82	3.69
C. argus	9.89	-14.52	3.80
C. argus	9.87	-14.38	3.72
C. argus	9.16	-14.18	3.72
C. argus	9.20	-13.70	3.68
C. argus	10.04	-13.68	3.67

C. argus	9.67	-13.07	3.86
C. argus	9.62	-13.68	3.68
C. argus	10.24	-14.50	3.88
C. argus	9.30	-14.90	3.58
C. argus	9.30	-13.91	3.58
C. argus	9.11	-13.28	3.57
C. argus	9.41	-14.80	3.68
C. argus	10.08	-14.26	3.72
C. argus	9.32	-14.14	3.61
C. argus	9.20	-14.14	3.64
C. argus	9.32	-14.06	3.57
C. argus	9.05	-13.77	3.60
C. argus	9.65	-14.46	3.66
C. argus	9.07	-14.27	3.58
C. argus	9.35	-14.06	3.81
C. argus	9.49	-14.07	3.75
C. argus	10.00	-13.79	3.68
C. argus	9.54	-14.63	3.68
C. argus	8.85	-14.29	3.80
C. argus	10.16	-14.00	3.55
C. argus	9.56	-14.09	3.79
C. argus	9.20	-13.89	3.59
C. argus	9.47	-14.26	3.67
C. argus	9.40	-13.96	3.66
C. argus	9.89	-14.87	4.00
C. argus	10.81	-14.70	4.06
C. argus	9.07	-13.58	3.65
C. argus	8.34	-13.50	3.59
C. argus	9.96	-13.97	3.74
C. argus	9.16	-14.24	3.78
C. argus	9.76	-14.68	3.95
C. argus	9.21	-14.11	3.51
C. argus	9.21	-14.46	3.76
C. argus	10.60	-13.68	3.56
C. argus	9.25	-14.64	3.77
C. argus	9.50	-14.16	3.85
C. argus	9.81	-14.36	3.95
C. argus	9.64	-14.03	3.59
C. argus	9.86	-14.19	3.77
C. argus	8.86	-14.48	3.68
C. argus	10.03	-13.88	3.67
C. argus	9.76	-14.93	3.98
C. argus	10.55	-13.67	3.68
C. argus	9.86	-14.16	3.72
C. argus	9.92	-15.14	4.26
C. argus	9.66	-13.68	3.62
C. argus	9.12	-13.79	3.64
C. argus	9.40	-13.77	3.75
C. nigrofuscus	6.86	-17.37	3.73

C. sordidus	4.60	-10.70	3.69
C. sordidus	5.50	-13.30	3.74
C. strigosus	6.70	-14.20	3.75
C. strigosus	6.77	-14.20	3.71
C. strigosus	5.10	-12.90	3.74
C. strigosus	4.90	-12.40	3.78
C. strigosus	5.90	-12.50	3.84
C. strigosus	6.00	-13.80	3.82
C. strigosus	5.70	-14.50	3.81
C. strigosus	6.20	-11.80	3.79
C. strigosus	6.00	-13.30	3.78
C. strigosus	6.30	-12.80	3.99
C. strigosus	6.20	-14.60	3.76
C. strigosus	5.90	-13.60	3.91
C. strigosus	6.30	-12.90	3.83
C. strigosus	6.30	-14.50	3.94
C. strigosus	6.08	-13.13	3.87
C. strigosus	6.12	-14.45	3.90
C. strigosus	6.68	-12.86	3.87
C. strigosus	6.20	-13.78	3.95
C. strigosus	7.15	-13.12	3.87
C. strigosus	6.17	-13.86	3.94
C. strigosus	5.81	-13.38	3.71
C. strigosus	5.51	-13.37	3.91
C. strigosus	5.86	-13.77	3.97
C. strigosus	6.83	-13.31	3.65
C. strigosus	5.83	-14.18	3.73
C. strigosus	6.29	-14.43	3.77
C. strigosus	6.63	-14.11	3.93
C. strigosus	6.91	-13.27	3.83
C. strigosus	7.38	-13.15	3.87
C. strigosus	7.33	-13.24	3.84
C. strigosus	6.86	-14.49	3.85
C. strigosus	6.02	-12.47	3.82
C. strigosus	6.73	-12.71	3.81
C. strigosus	6.64	-13.91	4.13
C. strigosus	6.93	-13.83	3.84
C. strigosus	6.42	-13.81	3.77
C. strigosus	6.67	-13.37	3.91
C. strigosus	7.08	-14.13	3.91
C. strigosus	6.18	-13.79	3.81
C. strigosus	6.22	-13.90	3.76
C. strigosus	5.79	-13.19	3.86
C. strigosus	5.75	-11.88	3.73
C. strigosus	6.95	-13.14	3.81
C. strigosus	6.72	-13.56	3.91
C. strigosus	7.03	-11.85	3.56
C. strigosus	6.81	-14.01	3.79
C. strigosus	6.80	-13.14	3.83

C. strigosus	6.97	-14.37	4.60
C. strigosus	6.44	-13.48	3.88
C. strigosus	7.13	-13.37	3.81
C. strigosus	6.50	-11.99	3.71
C. strigosus	6.48	-12.05	3.60
C. strigosus	7.18	-13.14	4.13
C. strigosus	5.90	-12.33	3.83
C. strigosus	5.34	-12.52	3.96
C. strigosus	7.58	-11.87	3.56
C. strigosus	10.13	-13.62	3.81
C. strigosus	9.35	-14.73	3.79
C. strigosus	9.76	-15.06	3.73
C. strigosus	6.85	-13.67	3.74
C. strigosus	6.58	-14.08	3.78
C. strigosus	6.21	-14.15	3.77
C. strigosus	7.01	-13.77	3.76
C. strigosus	6.51	-11.94	3.71
C. strigosus	6.94	-13.76	3.84
C. strigosus	6.41	-13.17	3.78
C. strigosus	5.87	-12.62	3.90
C. strigosus	6.78	-13.91	3.82
C. strigosus	7.03	-14.26	3.79
C. verecundus	7.68	-17.51	3.65
H. cruentas	7.64	-16.80	3.69
H. cruentas	7.00	-16.44	3.67
H. cruentas	7.08	-16.81	3.69
H. cruentas	7.15	-16.32	3.66
H. cruentas	7.13	-16.75	3.63
H. cruentatus	6.74	-16.67	3.66
H. cruentatus	7.42	-16.66	3.62
P. aspricaudus	7.20	-16.53	3.75
S. psittacus	5.09	-12.76	3.56
S. psittacus	5.42	-11.96	3.58
S. psittacus	6.06	-12.23	3.63
S. psittacus	4.92	-12.48	3.72
S. psittacus	6.44	-14.12	3.76
S. psittacus	4.69	-12.28	3.61
S. psittacus	4.61	-12.47	3.70
Z. flavescens	6.06	-19.06	3.70
Z. flavescens	6.96	-18.50	3.70
Z. flavescens	5.93	-18.42	3.75
Z. flavescens	5.73	-19.05	3.73

APPENDIX B

Trophic Position Propagated Error Calculations from Amino Acid Isotopic Analyses Using the New TEF for TP>2

$$TP = 2 + \frac{(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - \beta - \Delta)}{TEF}$$

$$TEF = 3.9 \pm 1.3$$

$$\sigma^2_{TP} = \left(\frac{\partial TP}{\partial \delta^{15}N_{GLU}}\right)^2 \sigma^2_{\delta^{15}N_{GLU}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{PHE}}\right)^2 \sigma^2_{\delta^{15}N_{PHE}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial \Delta}\right)^2 \sigma^2_{\Delta} + \left(\frac{\partial TP}{\partial TEF}\right)^2 \sigma^2_{TEF}$$

To Calculate:

$$\sigma^2_{TP} = \left(\frac{\partial TP}{\partial \delta^{15}N_{GLU}}\right)^2 \sigma^2_{\delta^{15}N_{GLU}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{PHE}}\right)^2 \sigma^2_{\delta^{15}N_{PHE}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial \Delta}\right)^2 \sigma^2_{\Delta} + \left(\frac{\partial TP}{\partial TEF}\right)^2 \sigma^2_{TEF}$$

$$\frac{\partial TP}{\partial \delta^{15}N_{GLU}} = \frac{1}{TEF} = \frac{1}{3.9}$$

$$\sigma^2_{\delta^{15}N_{GLU}} = (\text{stdev } \delta^{15}N_{GLU})^2$$

$$\frac{\partial TP}{\partial \delta^{15}N_{PHE}} = \frac{-1}{TEF} = \frac{-1}{3.9}$$

$$\sigma^2_{\delta^{15}N_{PHE}} = (\text{stdev } \delta^{15}N_{PHE})^2$$

$$\frac{\partial TP}{\partial \beta} = \frac{-1}{TEF} = \frac{-1}{3.9}$$

$$\sigma^2_{\beta} = (0.9)^2$$

$$\frac{\partial TP}{\partial \Delta} = \frac{-1}{\Delta} = \frac{-1}{7.6}$$

$$\sigma^2_{\Delta} = (1.2)^2$$

$$\frac{\partial TP}{\partial TEF} =$$

$$\frac{-1}{TEF^2}\left(\partial\delta^{15}N_{GLU} - \partial\delta^{15}N_{PHE} - 3.4 - 7.6\right) = \frac{-1}{15.21}\left(\partial\delta^{15}N_{GLU} - \partial\delta^{15}N_{PHE} - 3.4 - 7.6\right)$$

$$\sigma^2_{TEF} = (1.3)^2$$

$$\sigma^2_{TP} = \left(\left(\frac{1}{3.9}\right)^2 * (\text{stdev } \delta^{15}N_{GLU})^2\right) + \left(\left(\frac{-1}{3.9}\right)^2 * (\text{stdev } \delta^{15}N_{PHE})^2\right) + \left(\left(\frac{-1}{3.9}\right)^2 * (0.9)^2\right)$$

$$+ \left(\left(\frac{-1}{7.6}\right)^2 * (1.2)^2\right) + \left(\left(\frac{-1}{15.21}\left(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - 3.4 - 7.6\right)\right)^2 * (1.3)^2\right)$$

Trophic Position Propagated Error Calculations from Amino Acid Isotopic Analyses Using TEF = 7.6

$$TP = 1 + \frac{(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - \beta)}{TEF}$$

$$TEF = 7.6 \pm 1.2$$

To Calculate:

$$\sigma^2_{TP} = \left(\frac{\partial TP}{\partial \delta^{15}N_{GLU}}\right)^2 \sigma^2_{\delta^{15}N_{GLU}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{PHE}}\right)^2 \sigma^2_{\delta^{15}N_{PHE}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial TEF}\right)^2 \sigma^2_{\Delta}$$

$$\frac{\partial TP}{\partial \delta^{15}N_{GLU}} = \frac{1}{7.6}$$

$$\sigma^2_{\delta^{15}N_{GLU}} = (\text{stdev } \delta^{15}N_{GLU})^2$$

$$\frac{\partial TP}{\partial \delta^{15}N_{PHE}} = \frac{-1}{7.6}$$

$$\sigma^2_{\delta^{15}N_{PHE}} = (\text{stdev } \delta^{15}N_{PHE})^2$$

$$\frac{\partial TP}{\partial \beta} = \frac{-1}{7.6}$$

$$\sigma^2_{\beta} = (0.9)^2$$

$$\frac{\partial TP}{\partial TEF} = \frac{-1}{57.76}\left(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - 3.4\right)$$

$$\sigma^2_{TEF} = (1.2)^2$$

$$\sigma^2_{TP} = \left(\left(\frac{1}{7.6}\right)^2 * (\text{stdev } \delta^{15}N_{GLU})^2\right) + \left(\left(\frac{-1}{7.6}\right)^2 * (\text{stdev } \delta^{15}N_{PHE})^2\right) + \left(\left(\frac{-1}{7.6}\right)^2 * (0.9)^2\right)$$

$$+ \left(\left(\frac{-1}{57.76}\left(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - 3.4\right)\right)^2 * (1.2)^2\right)$$

Trophic Position Propagated Error Calculations from Amino Acid Isotopic Analyses Using Trophic and Source AAs

$$TP_{Tr-Sr} = 1 + \frac{(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Sr-AA} - \beta)}{TEF}$$

$$TEF = 5.6 \pm 0.7$$

$$\sigma^2_{TP} = \left(\frac{\partial TP}{\partial \delta^{15}N_{Tr-AA}}\right)^2 \sigma^2_{\delta^{15}N_{Tr-AA}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{Sr-AA}}\right)^2 \sigma^2_{\delta^{15}N_{Sr-AA}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial TEF_2}\right)^2 \sigma^2_{TEF_2}$$

$$\frac{\partial TP}{\partial \delta^{15}N_{Tr-AA}} = \frac{1}{7.6}$$

$$\sigma_{\delta^{15}N_{Tr-AA}} = \sqrt{\sigma^2_{\delta^{15}N_{Ala}} + \sigma^2_{\delta^{15}N_{Val}} + \sigma^2_{\delta^{15}N_{Leu}} + \sigma^2_{\delta^{15}N_{Iso}} + \sigma^2_{\delta^{15}N_{Pro}} + \sigma^2_{\delta^{15}N_{Glu}}}$$

$$\frac{\partial TP}{\partial \delta^{15}N_{Sr-AA}} = \frac{-1}{7.6}$$

$$\sigma_{\delta^{15}N_{Sr-AA}} = \sqrt{\sigma^2_{\delta^{15}N_{Gly}} + \sigma^2_{\delta^{15}N_{Ser}} + \sigma^2_{\delta^{15}N_{Phe}}}$$

$$\frac{\partial TP}{\partial \beta} = \frac{-1}{7.6}$$

$$\sigma^2_{\beta} = (0.9)^2$$

$$\frac{\partial TP}{\partial TEF} = \frac{-1}{57.76}\left(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Sr-AA} - 3.4\right)$$

$$\sigma^2_{TEF} = (1.2)^2$$

$$\sigma^2_{TP} = \left(\left(\frac{1}{7.6}\right)^2 * (\text{stdev } \delta^{15}N_{GLU})^2\right) + \left(\left(\frac{-1}{7.6}\right)^2 * (\text{stdev } \delta^{15}N_{PHE})^2\right) + \left(\left(\frac{-1}{7.6}\right)^2 * (0.9)^2\right)$$

$$+ \left(\left(\frac{-1}{57.76}\left(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Sr-AA} - 3.4\right)\right)^2 * (1.2)^2\right)$$

REFERENCES

Bagnis, R., Chanteu, S., Chungue, E., Hurtel, J.M., Yasumoto, T., Inoue, A. 1980. Origins of ciguatera fish poisoning: a new dinoflagellate, *Gambierdiscus toxicus* Adachi and Fukuyo, definitively involved as a causal agent. *Toxicon* **18**: 199-208

Banner A.H., Scheuer P., Sasaki S., Helfrich P., Alender C. 1960. Observations on ciguatera-type toxin in fish. *Ann. NY Acad. Sci*. **90**:770–787

Banner A.H., Helfrich P. 1964. The distribution of ciguatera in the tropical Pacific. Hawaii Institute of Marine Biology, Honolulu, Hawaii.

Banner A.H., Helfrich P., Piyakarn T. 1966. Retention of ciguatera toxin by Red Snapper Lutjanus Bohar. *Copeia* **2**:297–301

Bienfang, P., Oben, B., DeFelice, S., Moeller, P., Huncik, K. Oben, P., Toonen, R., Daly-Engel, T., Bowen, B. 2008. Ciguatera: the detection of neurotoxins in carnivorous reef fish from the coast of Cameroon, West Africa. *African Journal of Marine Science* **30**: 533–540

Bienfang, P.K., Trapido-Rosenthal, H., Laws, E. A. 2011. Magnification of Risks: Bioaccumulation/Biomagnifications in Food Chains. *In* Meyers, R.A., Encyclopedia of Sustainability Science and Technology. Springer Science and Business Media.

Bomber, J.W., Aikman, K.E., 1989. The ciguatera dinoflagellates. *Biol. Oceanogr.* **6**: 291-311

Chateau-Degat, M.L., Chinain, M., Cerf, N., Gingras, S., Hubert, B., and Dewailly, E., 2005. Seawater temperature, *Gambierdiscus spp*. variability and incidence of ciguatera poisoning in French Polynesia. *Harmful Algae* **4**: 1053-1062

Chikaraishi, Y., Kashiyama, N.O., Ogawa, N.O., Kitazato, H., Ohkouchi, N., 2007. Biosynthetic and metabolic controls of nitrogen isotopic composition of amino acids in marine macroalgae and gastropods: implications for aquatic food web studies. *Mar. Ecol. Prog. Ser*. **342**: 85-90

Chikaraishi, Y., Ogawa, N.O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., Miyashita, H., Kitazato, H., and Ohkouchi, N. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol. Oceanogr.: Methods,* **7**: 740–750

Chikaraishi, Y, Ogawa, N.O., Ohkouchi, N. 2010. Further evaluation of the trophic level estimation based on nitrogen isotopic composition of amino acids. *Earth, Life and Isotopes.*

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

Christensen, V., Pauly, D. 1992. Ecopath II - a software for balancing steady-state ecosystem models and calculating network characteristics. *Ecol. Modeling* **61**:169-185.

Christensen, V., Pauly, D., Editors. 1993. Trophic models of aquatic ecosystems. *ICLARM Conf. Proc.* **26**: 390

Christensen, V., Pauly, D. 1995. Fish production, catches and the carrying capacity of the world oceans. Naga, *ICLARM Q.* **18**:34-40.

Dale, J.J., Wallsgrove, N.J. Popp, B.N., Holland, K.N. 2011. Nursery habitat use and foraging ecology of the brown stingray *Dasyatis lata* determined from stomach contents, bulk and amino acid stable isotopes. *Mar. Ecol. Prog. Ser.* **433**:211-236

Dickey, R.W., Plakas, S.M., 2010. Ciguatera: a public health perspective. *Toxicon* **56**: 123-136

Dierking, J. 2007. Effects of the introduced predatory fish, *Cephalopholis argus*, on native reef fish populations in Hawaii. Unpublished PhD Dissertation.

Edwars, T.W. and Horn, M.H. 1982. Assimilation efficiency of a temperate-zone intertidal fish (*Cebidichthys violaceus*) fed diets of macroalgae. *Marine Biology* **67**: 247-253

Fry, B., 2006. Stable isotope ecology. Springer, New York

Hannides, C.C.S., Popp, B.N., Landry, M.R., Graham, B.S., 2009. Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes. *Limnol. Oceanogr.* **54**: 50-61

Honebrink, R. 1990. Fishing in Hawaii: a student manual. Education Program, Division of Aquatic Resources, Honolulu, Hawaii. 79 p.

Horn, M.H., 1989. Biology of marine herbivorous fishes. *Oceanography and Marine Biology: An Annual Review* **27**:167–272.

Hyslop, E.J. 1980. Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology* **17**: 411-429

Isaacs, J. D. 1972. Unstructured marine food webs and 'pollutant analogues'. *Fishery Bulletin* **70**: 1053-1059

Julsrud E.A., Walsh P.J., Anderson P.M. 1998. *N*-acetyl-Lglutamate and the urea cycle in gulf toadfish (*Opsanusbeta*) and other fish. *Arch Biochem Biophys* **350**:55–60

Lartigue, J., Jester, E.L.E., Dickey, R.W., Villareal, T.A., 2009. Nitrogen source effects on the growth and toxicity of two strains of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus*. *Harmful Algae* **8**: 781-791

Lassuy, D.R. 1984. Diet, intestinal morphology, and nitrogen assimilation efficiency in the damselfish, *Stegastes lividus*, in Guam. *Environmental Biology of Fishes* **10**: 183-193.

Lehane, L., Lewis, R.J. 2000. Ciguatera: recent advances but the risk remains. *National Office of Animal and Plant Health, Agriculture, Fisheries and Forestry*. Australia GPO

Lewis, R.J., Holmes, M.J., 1993. Origin and transfer of toxins involved in ciguatera. *Compar. Biochem. Physiol.* **106**: 615-628

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

Macko, S.A., Uhle, M.E. 1997. Stable nitrogen isotope analysis of amino acid enantiomers by gas chromatography combustion/isotope ratio mass spectrometry. *Anal. Chem.* **69**: 926–929.

Martinez del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* **84**: 91-111

Mazeroll, A.I. and Montgomery, W.L. 1998. Daily migrations of a coral reef fish in the Red Sea (Gulf of Aqaba, Israel): initiation and orientation. *Copeia*, **4**: 893-905

McClelland, J.W., and Montoya, J.P. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology,* **83**: 2173-2180

McClelland, J.W., Holl, C.M., Montoya, J.P. 2003. Relating low d15N values of zooplankton to N2-fixation in the tropical North Atlantic: Insights provided by stable isotope ratios of amino acids. *Deep-Sea Res. I* **50**: 849–861.

McCarthy, M.D., Benner, R., Lee, C., Fogel, M.L. 2007. Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochim. Cosmochim. Acta* **71**: 4727–4744.

McCutchan, J.H., Lewis, W.M. Jr., Kendall, C., McGrath, C.C. 2003. Variation in Trophic Shift for Stable Isotope Ratios of Carbon, Nitrogen, and Sulfur. *Oikos* **102**:378-390

Meyer, C.G. and Holland, K.N. 2005. Movement patterns, home ranges and habitat utilization of the bluespine unicornfish, *Naso unicornis* (Acanthuridae) in a Hawaiian marine reserve. *Environmental Biology of Fishes*, **73**: 201-210

O'Reilly, C.M., Hecky, R.E., Cohen, A.S., Plisnier, P.D., 2002. Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnol. Oceanogr.* **47**: 306-309

Pauly, D., Christensen, V. 1993. Stratified models of large marine ecosystems: a general approach and an application to the South China Sea, p. 148-174. *In* K. Sherman, L.M. Alexander and B.D. Gold (eds.) Large marine ecosystems: stress, mitigation and sustainability. AAAS Publication, Washington, DC. 376 p.

Pearn, J. 2001. Neurology of ciguatera. *Neurology, Neurosurgery, and Psychiatry* **70**: 4-8

Pilos, G.A., Strong, D.R., 1996. Food web complexity and community dynamics. *The American Naturalist* **147**: 813-846

Pinkas L., Oliphant M.S., Iverson I.L.K. 1971. Food habits of albacore, bluefin tuna and bonito in Californian waters. *Calif Dept Fish Game Fish Bull **152,*** Sacramento, CA

Polovina, J.J. 1984. Model of a coral reef ecosystem. I. The Ecopath model and its application to French Frigate Shoals. *Coral Reefs* **3**:1-11

Popp, B. N., Graham, B. S., Olson, R. J., Hannides, C. C. S., Lott, M. J., Lopez-Ibarra, G. A., Galvan-Magana, F., Fry, B. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares,* from compound specific nitrogen isotope analysis of proteinaceous amino acids, p. 173-190. *In* T. Dawson and R. Siegwolf [eds.], Stable Isotopes as Indicators of Ecological Change. *Terrestrial Ecology*

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**: 703-718

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi and C. G. Montana. 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* **152**: 179-189

Randall, J.E., 1958. A review of ciguatera, tropical fish poisoning, with a tentative explanation of its cause. *Bull. Mar. Sci.* **8**: 236-267

Randall, J.E, Clements K.D. 2001. Second revision of the surgeonfish genus *Ctenochaetus* (Perciformes: Acanthuridae), with descriptions of two new species. *Indo-Pacific Fishes* (32):33

Rolff, C., 2000. Seasonal variation in δ13C and δ15N of size-fractionated plankton at a coastal station in the northern Baltic proper. *Mar. Ecol. Prog. Ser.* **203**: 47-65

Sachs, L. 1984. Applied statistics. A handbook of techniques. 2nd ed. 707 p. Springer-Verlag, Inc., New York.

Sano, M., Shimizu, M. and Nose, Y. 1984. Food habits of teleostean reef fishes in Okinawa Island, southern Japan. University of Tokyo Press, Tokyo, Japan. 128 p.

Sherwood, O.A., Lehmann, M.F., Schubert, C.J., Scott, D.B., McCarthy, M.D. 2010, Nutrient regime shift in the western North Atlantic indicated by compound-specific δ15N of deep-sea gorgonian corals. *PNAS* **108**: 1011-1015

Shpigel, M., Fishelson, L. 1989. Food habits and prey selection of three species of groupers from the genus *Cephalopholis* (Serranidae: Teleostei). Environmental *Biology of Fishes* **24**: 67-73

Shpigel, M., Fishelson, L. 1991. Habitat partitioning between species of the genus *Cephalopholis* (Pisces: Serranidae) across the fringing reef of the Gulf of Aqaba (Red Sea). *Marine Ecology Progress Series* **58**: 17-22.

Sommer, C., W. Schneider and J.-M. Poutiers. 1996. FAO species identification field guide for fishery purposes. The living marine resources of Somalia. FAO, Rome. 376

Speers-Roesch, B., Ip, Y.K., Ballantyne, J.S. 2006. Metabolic organization of freshwater, euryhaline, and marine elasmobranchs: implications for the evolution of energy metabolism in sharks and rays. *J Exp Biol* **209**:2495–2508

Ueda et al. 1989. D-Alanine as a chemical marker for the determination of streptococcal cell wall levels in mammalian tissues by gas chromatography/negative ion chemical ionisation mass spectrometry. *Anal. Chem*. **61**: 265–270.

Vanderklift, M.A., Ponsard, S. 2003. Sources of variation in consumer-diet d15N enrichment: a meta-analysis. *Oecologia* **136**:169–182

Yasumoto, T., Nakajima, I., Bagnis, F., Adachi, R., 1977. Finding of a dinoflagellate as a likely culprit of ciguatera. *Bull. Jap. Soc. Sci. Fish* **43**: 1021-1026