Ecological niche metrics of coral reef piscivorous fishes: The effects of fishing revealed through stable isotope analyses

by

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Bachelor of Science, McGill University, 2011

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Abstract

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Coral reefs are highly complex and also highly threatened ecosystems. Population growth and the unsustainable use of coral reefs have resulted in 55% of the world's reefs being considered degraded. Fishing, the primary 'local' threat on most reefs, has altered the composition of most reef communities. As a result, very few pristine coral reefs remain. Typically, coral reef research is done via underwater visual censuses, providing abundance estimates but no indication of trophic interactions, therefore we know relatively little about the structure of intact reef food webs. Understanding how human activities affect trophic structure and feeding interactions among resident reef species may be important for coral reef conservation.

Here, I apply stable isotope analysis to coral reef piscivorous fishes from Kiritimati (Republic of Kiribati), the world's largest atoll. I examine dietary niche metrics of five focal species (*Cephalopholis argus, Cephalopholis urodeta, Aphareus furca, Lutjanus bohar,* and *Lutjanus fulvus*) and of the piscivore functional group as a whole, across an anthropogenic disturbance gradient that results from the atoll's heavily skewed geographic population distribution. Using bootstrapped stable carbon (δ13C) and nitrogen (δ15N) isotope values, controlled for body size effects and analysed with Bayesian methods using the SIAR (Stable Isotope Analysis in R) program, I provide evidence of isotopic niche differentiation in *C. argus* and *L. fulvus* relative to other sampled species in terms of niche width metrics and mean δ13C and δ15N values. I also analyse the effect of fishing pressure at an individual level (controlling for body size effects on stable isotope signatures for each species), population level (accounting for observed differences in body size distributions across the fishing pressure gradient for each species), and the 'community' level (accounting for body size and relative

abundance differences of the five piscivores across the fishing pressure gradient). These metrics reveal species-specific changes in niche metrics of three of the focal species at the individual level: *C. urodeta*, showed regionally distinct niche width metrics but no apparent correlation with fishing pressure, while *A. furca* and *L. bohar*, both had broader niche width metrics in heavily fished areas. No significant effect of fishing pressure was found at population or community levels. This study provides the first evidence using stable isotopes that fishing can alter the diets of coral reef fishes. The mechanism by which it can do so, while not entirely clear, would most likely be by expanding a given species' dietary diversity by either forcing it to switch to non-preferred prey items or changing the diet and/or body size of its prey items, both of which would reflect significant ecological changes within a community. This thesis provides evidence of the utility of stable isotope analyses in answering important ecological questions in coral reef food webs, and reveals that fishing can affect reef communities at the most fundamental level of trophic interactions.

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Introduction

Coral Reef Food Webs

Feeding interactions among individuals in an ecological community are increasingly recognized as important components of overall ecosystem function, yet are highly variable and complex (Vanderzanden et al. 1999; Carassou et al. 2008). These interactions, which together make up a community's trophic structure, are known to be particularly complex in tropical marine environments (Stevens 1989). In particular, coral reef trophic structure is thought to be highly complex, having many more components (feeding groups/types and individuals) and connections (feeding interactions between components) than temperate marine communities, despite being relatively understudied (Marguillier et al. 1997; Harmelin-Vivien 2002; Thompson et al. 2007).

Coral reefs are the most biologically diverse marine ecosystems in the world, but are also widely recognized as being highly threatened. The combination of global and local stressors has resulted in an estimated 75% of the world's reefs facing extinction risk (Burke et al. 2011), with 19% already considered lost (Hughes et al. 2010). By the year 2050, almost all of the world's coral reefs are expected to be in critical condition if current trends continue (Burke et al. 2011). Fishing, the primary 'local' threat on most reefs, has significantly altered the composition of most reef communities (Roberts 1995; Hodgson 1999), and as a result we know relatively little about the structure of intact reef food webs. This knowledge is foundational to understanding how anthropogenic disturbances alter feeding interactions between and within trophic guilds, and hence how these ecosystems function at the most fundamental level (Carassou et al. 2008). This problem is compounded by the fact that, due to human disturbances, very few pristine reefs remain, and baselines against which to evaluate these impacts are poorly defined (Carpenter et al. 2008). Worldwide, upwards of 275 million people depend on goods and services provided by coral reefs, and these ecosystem services in turn depend on the underlying food web structure and feeding interactions (Burke et al. 2011). Thus, knowing how human activities affect the feeding structure and relationships of reef species is important from management and conservation perspectives.

Stable Isotope Analysis

Stable Isotopes, Measurement and Notation

Atoms are composed of protons, neutrons and electrons. The atomic number of an element is defined by the number of protons in the nucleus, but the number of neutrons is slightly variable, giving rise to different forms of the same element, called isotopes (Fry 2006), which occupy the same position in the periodic table yet have different masses (Hoefs 2009). An element may have several isotopes, and additional neutrons tend to stabilize atoms (up to a point). While unstable, radioactive isotopes tend to 'decay', losing atomic particles and thereby becoming a new isotope or element, 'stable' isotopes do not. Multiple stable isotopes for an element, each with a different number of neutrons, may occur naturally in the environment (Fry 2006). Because they have different numbers of neutrons, different stable isotopes of the same element have different masses, and hence their relative abundance in a sample of material may be determined via mass spectrometry (Hoefs 2009).

Isotope chemistry is measured using mass spectrometry techniques, measuring mass to charge ratios of particles, which determines mass and elemental composition (Griffiths 1977). Mass spectrometers use gas chromatographs, and modern machines use lasers to analyze combusted samples, which are measured following a multi-step process separating ions across a magnetic field that are subsequently counted by a computer (Brand 1996). Prior to combustion, tissue samples must be dried or frozen, ground and pulverized as a pure powder, allowing for even combustion into a simple gas for analysis (Boutton 1991a).

Isotope signatures, most commonly denoted by δ notation, express a difference measurement made relative to internationally recognized standards. δ values are expressed as a ratio of a ratio, for example 13C/12C:15N/14N, and are multiplied by 1000 to amplify the very small differences between samples and standards (Hayes 1983). Thus, they are expressed in units of permil (‰) and range between -100 and 50‰ for most natural samples (Fry 2006). Higher δ values mean the sample is relatively enriched in the heavier isotope, and enrichment of the lighter isotope gives lower δ values (Hoefs 2009). These values are a relative ratio of the heavier to lighter isotope, and this allows them to be traced across a wide range of natural settings and to be used in many different

ways. Regulated by key enzymes in resource uptake, ecologists are concerned with hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S) stable isotopes; 1H, 13C, 15N, 17O, and 32S, respectively (Hoefs 2009).

Ecological Applications of Stable Isotope Analyses

Due to important relationships between stable isotope values and trophic structure, stable isotope analysis is a valuable tool in food web studies. Stable isotopes act as biological tracers and are used in many different applications, most notably 15N and 13C in food web analysis (Fry 2006), to evaluate management strategies and to answer important ecological questions such as:

- How do feeding strategies change under regime shifts?
- What plant resources are most important for supporting animal consumers?
- What are the effects of disturbance on food web structure, and how does this vary across different spatial scales? (Layman et al. 2007a; Post 2002a; Vander Zanden et al. 2004).

Stable isotope distributions of a population can be used to determine relative trophic relationships, providing an indication of energy flow among members of a food web (Vander Zanden et al. 1999). Together, δ13C and δ15N values of food web residents can reveal fundamental links and interactions of food webs (Post 2002; Layman et al. 2007b). Intensive stable isotope analyses have been successful in fields such as limnology and oceanography, where they have proved to be a highly valuable technique for studying community wide food web dynamics over time via careful interpretation of these two stable isotope metrics (Layman et al. 2007a).

δ15N and Trophic Level:

Through metabolic and excretory processes of all animals, 14N isotopes are lost at faster rates than 15N, resulting in higher levels of the heavier isotope, and as a result, δ15N values increase each trophic step (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003). The average trophic enrichment factor of 3.4‰ ± 1.1‰, found by Minagawa and Wada (1984) has generally been used as the standard value for the isotopic fractionation of δ15N, however enrichment factors can range between 2.2 and 3.5‰ Δ^{15}N (Galvan et al. 2012). This regular ratio increase provides the basis for estimating trophic levels in ecosystems (Post 2002a).

One of the most common descriptors used in food webs is trophic level, or the position within the food web occupied by a particular organism. In order to estimate trophic position of species at multiple levels, one first must obtain a general baseline of isotopic signatures representative of the base of the food web. Long-lived primary consumers, for example mussels or snails, are often used, but zooplankton has also served as a useful baseline (Vander Zanden and Rasmussen 1999; Post 2002a). Once such a baseline is established, accounting for isotopic variation and fractionation at the base of the food web, the average enrichment of δ15N values can be used to infer relative trophic positions of species or groups of individuals, and the total range of δ15N values, or the maximum occupied trophic position, is considered to be the food chain length of the food web (Post et al. 2000). While a long food chain does not necessarily indicate a healthy food web and food chain length is variable in nature (between two and six trophic levels; Post et al. 2000), it is known to influence trophic interactions and ecosystem function and is thus an important attribute of community structure (Post 2002b).

δ13C and Production Sources:

δ13C values provide an estimate of primary production sources (Vander Zanden and Rasmussen 1999). Trophic enrichment factors for δ13C are much lower than for δ15N (0 to 1.4‰ $\Delta^{13}C$; Galvan et al. 2012), and so δ13C values tend to be preserved in consumers at higher trophic levels (Hilting et al. 2013). However, due to different photosynthetic processes, isotope fractionation results in altered concentrations of isotopes in different types of primary producers, adapted to different environmental conditions (Pearcy et al. 1981; Tieszen et al. 1983). Specifically, phytoplankton tends to be less enriched in δ13C values than benthic algae, which has been shown in lake (~-32‰ $\Delta^{13}C$ versus ~-26‰ $\Delta^{13}C$), river (~-32‰ $\Delta^{13}C$ versus ~-29‰ $\Delta^{13}C$), and marine (~-22‰ $\Delta^{13}C$ versus ~-17‰ $\Delta^{13}C$) ecosystems (France 1995). Consequently, these distinctions allow for source mixing in many different ecological applications (Farquhar et al. 1989). Most notably, they allow for the determination of primary production sources in food webs, and can indicate shifts in carbon sourcing (*e.g.* Vander Zanden and Rasmussen 1999).

In natural food webs, there are often multiple sources of production, resulting in multiple energy pathways and dimensions across trophic levels. A main descriptor of trophic structure, therefore, is source of primary production, as indicated by relative δ13C

values (Vander Zanden and Rasmussen 1999). Traditional approaches have successfully differentiated between several types of plants in terrestrial ecosystems (ie; C3 vs. C4 plants) or between different zones of growth in marine ecosystems (ie; pelagic vs. littoral; Vander Zanden et al. 1999). However, these situations only apply to a limited number of habitats, and in recent years, mixing models have emerged as an effective way to examine all possible source combinations that could result in the observed isotope values of consumers in a variety of environments (Jackson et al. 2011). Stable isotope studies typically analyze these aspects of community trophic structure in intact ecosystems to evaluate the role of such things as body size (Layman et al. 2005), prevalence of omnivory (Thompson et al. 2007), or ecosystem size (Post et al. 2000) in food webs.

Niche Space:

Combining δ15N and δ13C values, plotted against each other in a bi-plot, has also been used as a valuable metric to describe what is called the "isotopic niche space" of a group of individuals (Layman et al. 2007a). Defined as the total plotted area of all the connected values for a group of individuals (e.g. all individuals of a species or a functional group), and recently characterized quantitatively to allow comparisons (Layman et al. 2011; Jackson et al. 2011), niche space is an excellent community level indicator of trophic diversity, food web stability and structure (Layman et al. 2007b).

In addition to niche space, Layman et al. (2007b) outlined a "toolset" for using stable isotope analysis to evaluate trophic structure of entire communities, that when viewed together under the right circumstances, provide meaningful insight into food web function and dynamics and how this changes with disturbance (Table 1). Since referred to as "Layman Metrics" (Jackson et al. 2011), these metrics are now generally recognized as valuable tools to help measure trophic interactions (Jackson et al. 2012).

Table 1: Descriptions of Layman metrics (Layman et al. 2007b).

Metric:	Description:	Measure of:
δ13C range	Max-min value	Basal resource diversity
δ15N range	Max-min value	Range of trophic positions/food chain length
Convex hull area	Area of polygon containing all points of a population in isotopic bi-plot space	Trophic diversity
Mean distance to centroid	Average Euclidean distance of each individual to the centroid (mean δ13C and δ15N value) in bi-plot space	More robust estimate of trophic diversity (less sensitive to outliers)
Mean nearest neighbour distance	Mean of all Euclidean distances to each individual's nearest neighbouring point in bi-plot space	Density of species packing (*ie;* trophic redundancy)
Standard deviation of nearest neighbour distance	Standard deviation of above metric	Evenness of species packing

More recently, stable isotope metrics have been improved upon using Bayesian methods for solving linear mixing models to infer diet composition in consumers and their food sources (Moore and Semmens 2008; Jackson et al. 2009). These methods have been developed in two separate formats, IsoSource (Philips and Gregg 2003), and more recently in the R statistical computing environment as a package called 'SIAR' (Stable Isotope Analysis In R) (Parnell et al. 2010). Given isotopic ratios of a consumer and a set of possible food sources, both these programs use Bayesian inference to solve for the most likely set of dietary proportions. Importantly for this thesis, SIAR can also take into account isotopic grouping structure (variability of isotope signatures in bi-plot space) and make comparisons between estimated diets of groups of individuals, for example a particular species or functional group, using a subset of functions referred to as SIBER (Stable Isotope Bayesian Ellipses in R). The SIBER functions developed by Jackson et al. (2011), for example, have the ability to calculate all Layman metrics, as well as a more robust measure of niche width, or trophic diversity, for a group of individuals using Bayesian methods. This method produces many iterations of a "standard ellipse", rather than a convex hull, and provides a mean value for ellipse area, producing a value for niche width that is much less sensitive to outliers and small sample sizes. Having many iterations of ellipses drawn based on Bayesian methods also provides a more quantitative

measurement of niche width, and SIBER can therefore test for significant differences in ellipse area between two groups.

Niche overlap:

Niche differentiation is one of many factors permitting coexistence between multiple species, which can arise by a number of different mechanisms, including differential habitat or resource use, or altered behaviour (Resetarits 1991). Stable isotope analysis has been used to detect differential resource use, and subsequently dietary niche partitioning/overlap, between species, which could potentially influence their coexistence in the same environment (Sepulveda et al. 2012).

There are now very useful and quantitative tools for measuring various aspects of the dietary niche using stable isotopes as a proxy for diet, both at the individual level and for comparing between groups, whether between different groups or before and after disturbance. Specifically, these analytical tools have also been widely used as an indicator of human influences, and how they alter food webs.

Detecting effects of human disturbances using stable isotope analysis

Importantly, the aforementioned stable isotope metrics can provide evidence of human impacts on trophic structure (Layman et al. 2007b). Distributions of δ13C and δ15N values over time or space can be used as a proxy to see how feeding relationships change with disturbance.

To evaluate these effects, certain studies test for changes in these metrics either spatially, in impacted and un-impacted sites, or temporally, before and after a disturbance. For example, Post (2002b) and Layman et al. (2005) have shown that when top predators are removed from lake ecosystems via fishing, a reduction in food chain length occurred. The same would be expected to occur with overexploitation of any functional feeding group, as it would lead to fewer available steps for trophic enrichment of nitrogen, and thus lower trophic positions in general. As for primary production sources, dietary shifts of consumers towards different resource pools following disturbance have been detected in lake ecosystems as well (Vanderzanden et al. 1999). In the case of a disturbance, such as the introduction of an invasive species resulting in the dominance of a single producer species, one would expect the number of source contributors to be reduced (Layman et al. 2011).

Following habitat fragmentation, niche width has been shown to shrink in size (with a decrease in trophic complexity with lower disturbance levels), to shift downward in nitrogen values (with decreased trophic positions as described above), and to have context dependent directional shifts in carbon values (Layman et al. 2007b). Alternatively, ecological release from intraspecific and interspecific competition has been linked with niche width expansion of consumers following fishing pressure, as previously monopolized resources become increasingly available as competitors are removed (Van Valen 1965, Bolnick et al. 2010). Over the past two decades, a number of studies have used stable isotope analysis to evaluate trophic effects of disturbance of various forms and mechanisms in a number of different ways (*e.g.* Table 2).

Table 2: Examples of stable isotope studies evaluating effects of various human disturbances on food web characteristics (SIA=Stable Isotope Analysis). Grouped by disturbance type.

Disturbance	Ecosystem	Finding	Study*
Species invasion	Lake	Native trout species underwent diet shift from littoral to pelagic zone following invasion. Reduced trophic position, altered diet composition	1
Species invasion	Lake	Reduction in prey populations, diet shift in native piscivores (trout) to invertebrate-based diets. Trophic position of trout depends on presence of invasive bass	2
Species invasion	Lake	Invasion-induced habitat alteration reduced dietary niche of crayfish by reducing the diversity of their prey	3
Fishing	Coastal Marine	Highly context dependent- generally, enhanced assimilation of kelp carbon in areas of overfishing	4
Fishing	Coastal Marine	Used SIA as an indicator for over-exploitation, found lowered trophic levels of impacted species due to exploitation of large top predators, and dominance of taxa with high growth rates and small body sizes	5
Fishing	Kelp forest	Expansion of dietary niche using SIA after recovery of population size structure post fishery exploitation	6
Wildlife provisioning	Tropical Marine	Provisioned sharks had higher δ15N values, but found no indication of altered behaviour	7
Ecosystem fragmentation	Mangrove tidal creeks	Niche width collapse, clear downward shifts in trophic positions, especially in top predators	8

* [1] Vanderzanden et al. 1999, [2] Vanderzanden et al. 2004, [3] Jackson et al. 2012, [4] Salomon et al. 2008, [5] Nam et al. 2011, [6] Hamilton et al. 2014, [7] Maljkovic and Cote 2011, [8] Layman et al. 2007b.

Limitations

Given the complexity of most food webs, there is understandably no known method for completely describing the trophic structure of any ecosystem (Layman et al. 2007a). From an ecosystem perspective, stable isotope analysis of many species is a broad approach, and some of the accuracy one has when studying fewer species is lost (Layman et al. 2007a). While it is arguably the most powerful current method for studying food webs, it has many assumptions and caveats that ecologists must be aware of (Gannes et al. 1997).

The primary concern with stable isotope analysis is variation in baseline values, and in fractionation values (Vander Zanden and Rasmussen 2001). In order to infer trophic structure, one must account for these isotopic patterns at the base of the food web by selecting appropriate representative species for the ecosystem in question (Vander Zanden and Rasmussen 1999). For example, in many limnological studies, long lived primary consumers have been used, since they best represent isotopic values of primary producers in those environments (Post 2002a). With baseline values taken under consideration, absolute trophic position of a consumer can be calculated, which is a significant improvement on previous techniques, such as gut content analysis. Stable isotope analysis is now among the most widely used techniques in food web ecology (Post 2002b; Greenwood et al. 2010). However, the vast majority of trophic studies using stable isotopes have been in limnological systems, as in the above examples, and less progress has been made in marine ecosystems, especially in coral reef environments.

Body Size, Productivity, and Stable Isotopes

Body size has very important consequences for an organism's ecology and behaviour, including foraging and feeding strategies and abilities (Schluter 2000). For example, for gape-limited fish species, larger bodied individuals are able to consume larger prey items (Vincent et al. 2005). As previously stated, stable isotope values, specifically δ13C and δ15N, are indicative of long-term dietary trends, and are thus affected by an individual consumer's diet. Therefore, a larger bodied individual that is capable of eating larger, and subsequently higher trophic level prey, is expected to have a higher trophic level itself by having a higher δ15N value (Jennings et al. 2008). While fractionation levels differ for δ13C, the same trend of increased values being associated with larger bodied individuals

has been observed, although to a lesser extent (Jennings et al. 2002). Thus, when considering stable isotope metrics, for example across fishing pressure regions, body size as a variable must be taken into consideration as a potential confounding variable, as any changes in stable isotope values between even the same species could be attributed to body size differences alone (Jennings et al. 2002).

Additionally, when considering stable isotope values of consumers, oceanographic productivity, is also a potentially confounding variable, mostly on δ13C, that needs to be taken into account. Production sources affect consumer stable isotope values by supporting the base of their diet's food web. Consumers with benthic production are generally more enriched in δ13C values, and pelagic production sources generally translate into less enriched δ13C values (Hobson et al. 1995). Therefore, since oceanographic productivity, which often is highest in areas of ocean upwelling, for example, mostly affects pelagic production, a species whose diet is based on pelagic production (*e.g.*; phytoplankton and diatoms) would be expected to have higher values of δ13C in regions with high levels of oceanographic productivity. This would not be the case for consumers with benthic production at the base of their food web, for example piscivores and apex predators in a coral reef environment (Hilting et al. 2013). Regardless, the role of oceanographic productivity as a potentially confounding variable in stable isotope analyses should always be evaluated.

Progress in coral reef ecosystems

There have been few stable isotope studies analysing coral reef communities at all, and even fewer analysing disturbance effects, despite the threatened state and complexity of these ecosystems (*e.g.* Table 3). This complexity creates a great deal of difficulty in studying coral reef food webs with stable isotopes, and very high levels of variation in trophic fractionation have been observed in coral reef fish across functional groups. This is particularly true with herbivores, which further complicates efforts to decipher trophic structure using stable isotope analysis in these communities (Mill et al. 2007). Previous work in coral reefs has had relative success in assigning species to distinct trophic groups using δ15N and δ13C values, but in many of these studies, few species were analyzed and with mixed results (*e.g.* Carrasou et al. 2008; Greenwood et al. 2010, Marguillier et al. 1997) (Table 3). In this thesis, I add to this limited knowledge on reef fish feeding

interactions with a case study using piscivorous fish in a minimally disturbed coral reef food web using stable isotope metrics, and assessing how they change across a significant human disturbance gradient.

Coral Reef Piscivorous Fish

This thesis focuses on stable isotope metrics of the five most abundant piscivorous reef fish species in Kiritimati Atoll. Here, I detail aspects of each species' natural history, in terms of relative body size and dietary components, both of which influence predictions of niche width shifts across a fishing pressure gradient: *Cephalopholis argus.* Common name peacock grouper, *C. argus* (Figure 1b) is a benthic piscivorous reef fish whose diet is comprised almost entirely of fishes, with a small portion of its prey being benthic crustaceans (Froese 2011). This species has been linked to ciguatera toxins in some regions of the Pacific, but not in Kiritimati, and is a subsistence fishing target. Its body size reaches 60cm total length and is on average around 40cm total length (Randall 2005).

Cephalopholis urodeta. Common name flagtail (or darkfin) grouper, *C. urodeta* (Figure 1c) is a benthic piscivorous reef fish. Like *C. argus*, the majority of its diet is comprised of fishes, with the remainder being crustaceans (Randall 2005). This species is also a target for subsistence fisheries, yet is smaller in body size, with the greatest observed total length being 26cm (Froese 2011).

Aphareus furca. Common name small-toothed jobfish, *A. furca* (Figure 1a) is a primarily open water reef piscivorous fish, yet also feeds on small benthic invertebrates, for example crustacean larvae (Randall 2005). *A. furca* is described as both pelagic and benthopelagic, occurring in primarily open waters either singly or in small groups. This species is used for both commercial and subsistence fisheries. *A. furca* reaches 55cm total length and is on average around 25cm standard length (Froese 2011).

Table 3: Summary of major studies applying stable isotope analysis (SIA) to coral reef/mangrove environment. Grouped by habitat type and ordered by total sample size. Functional group codes: POM= Primary Organic Matter, PP= Primary Producer, He= Herbivore, Pi= Piscivore, Om= Omnivore, AP= Apex Predator, BI= Benthic Invertivore, ZP= Zooplanktivore, De= Detritivore, Pl=Zooplankton.

Habitat	Region	Total no. of species (& no. by functional group)	Total Sample Size	Findings	Limitations	Study†
Coral reef	Red Sea	7 (1 POM, 1 He, 1 Pi, 1 ZP, 1 Coral, 1 Bivalve, 1 Pl)	677	Isotopic niches of feeding guilds significantly correlated with environmental factors at latitudinal scale	Extensive study, but did not evaluate disturbance effects	1
Coral Reef	Oman	23 (3 Pi, 4 ZP, 3 He, 5 Om, 8 PP)	506	Use of diet specific trophic step fractionation values and sulfur isotopes improve resolution of food web models	Serious implications of enrichment, conditions, and accuracy for estimating TLs in coral reefs	2
Coral Reef	Indo-Pacific and Caribbean	1 (combined coral/zoox-anthellae)	246	High inter-reef variation in SI values worldwide	Only analyzed coral tissue	3
Coral Reef	Western Australia	26 (4 He, 11 ZP, 2 De, 9 Pi)	156	High intra-specific variability in trophic fractionation, making it difficult to assign trophic positions	Only analyzed fish, not a food web study	4
Coral Reef	New Caledonia	33 (11 He, 7 ZP, 10 De, 5 Pi)	141	Carnivores/piscivores feed over broader range of TLs than implied by gut analysis	High omnivory in coral reef environments	5
Coral Reef	French Polynesia	5 (1 He, 1 Om, 1 Pi, 1 PP, 1POM)	120	Found significant differences in trophic position between species within and between regions of differing levels of run-off	Simplified model given complexity of system, few species used	6

† [1] Kurten et al. 2014, [2] Mill 2011 (Phd Thesis), [3] Heikoop et al. 2000, [4] Wyatt et al. 2010, [5] Carassou et al. 2008, [6] Letourneur et al. 2013, [7] Speed et al. 2012, [8] Greenwood et al. 2010, [9] Yamamuro et al. 1995, [10] Mill et al. 2007, [11] De la Moriniere et al. 2003, [12] Marguillier et al. 1997, [13] Thimdee et al. 2004, [14] Kieckbush et al. 2004, [15] Behringer and Butler 2006.

Coral Reef	Australia	7 (4 AP, 3 He)	119	Similar δ13C values for all species, and found an effect of species and size on δ15N	Only analyzed apex predators	7
Coral Reef	Solomon Islands	4 (1 He, 1 ZP, 1 PI, 1 De)	85	Distinct isotopic niches of trophic guilds, but also common production sources	Few species, small sample size	8
Coral Reef	Western Pacific	16 (1 POM, 1 He, 14 PP)	56	Found N sources for corals and high C values of macrophyta	Early study, only looked at coral and macrophyta, did not evaluate feeding interactions	9
Coral Reef	Oman	8 (5 PP, 3 He,	54	Higher trophic fractionation in herbivorous fish		10
Mangrove-sea grass-coral reef	Caribbean (Curacao)	9 (4 He, 4 Om, 1 ZP)	344	Spatial and ecological separation of juveniles and adults using SIA	Had mostly species from mangrove/sea grass, few from coral reef	11
Mangrove-sea grass	Kenyan Coast	27 (13 PP, 5 He, 1 Pi, 6 Om, 1 AP, 1 BI)	194	Identified three feeding groups using SIA of C and N	Found high level of omnivory within feeding groups	12
Mangrove	Thailand	40 (16 PP, 3 BI, 7 ZP, 3 De, 7 He, 4 Om)	162	Distinct carbon values of primary producers and enrichment of N values	Mostly looked at plants, and in mangrove environment only	13
Mangrove	Caribbean (Bahamas and Florida)	58 (27 PP, 2 Pi, 15 He, 9 De, 5 Om	137	Analyzed trophic linkages, found majority of primary production was algal based	As above	14
Tropical hard-bottom	Caribbean (Florida)	9 (4 PP, 2 POM, 3 He)	27	No differences in TPs across areas of varying disturbance	Focused on primary production, did not sample fish	15

Lutjanus bohar. Common name red (or twinspot) snapper, *L. bohar* (Figure 1d) is large benthopelagic piscivorous reef fish whose diet is comprised of reef fish and crustaceans, namely crabs and shrimp (Kulbicki et al. 2005; Froese 2011). Like *C. argus*, this species has been linked to ciguatera poisoning in many regions of the Pacific, but not in Kiritimati, and is used as gamefish and in subsistence fisheries. Adult *L. bohar* reach 90cm total length, and is on average around 70cm total length (Randall 2005).

Lutjanus fulvus. Common name blacktail snapper, *L. fulvus* (Figure 1e) is a medium sized benthopelagic snapper species, with a body size reaching 40cm maximum total length, and an average of 25cm (Froese 2011). Its diet is more evenly split between fish and crustacean prey items, and also feeds on holothurians and cephalopods, making its diet more diverse than all other focal species in this study (Kulbicki et al. 2005). *L. fulvus* is identified as a gamefish, and as a target for subsistence fisheries (Randall 2005).

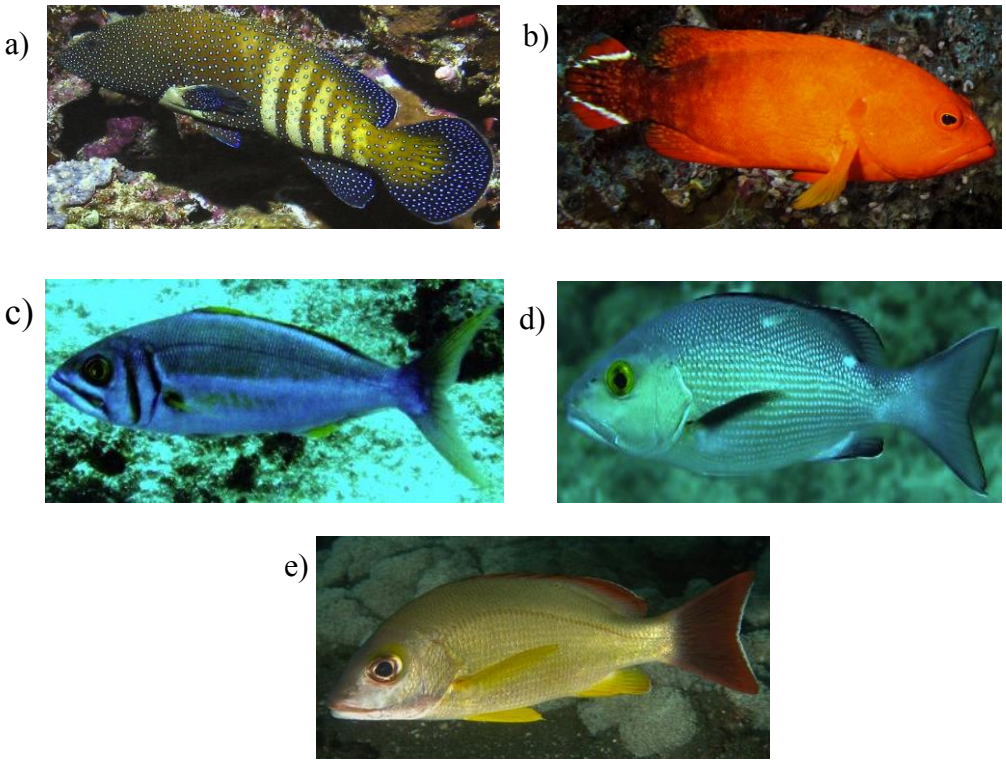

Figure 1: The five most abundant piscivorous reef fish species from Kiritimati Atoll, Republic of Kiribati: (a) *Cephalopholis argus* (peacock grouper), (b) *Cephalopholis urodeta* (flagtail grouper), (c) *Aphareus furca* (small-toothed jobfish), (d) *Lutjanus bohar* (red/twinspot snapper), e) *Lutjanus fulvus* (blacktail snapper).

Objectives and Hypotheses

The overall objectives of this thesis are 1) to determine the dietary niches of five focal Pacific coral reef piscivores, and 2) to quantify if, and how, fishing pressure alters these niches. Here, I detail these two objectives and the associated hypotheses:

Objective 1

My first objective is to examine the dietary niches of the five most common piscivorous fishes on a minimally disturbed Pacific coral reef. Focal species for this study, as described above, are two snapper species, *Lutjanus bohar* and *Lutjanus fulvus*, two grouper species, *Cephalopholis argus* and *Cephalopholis urodeta*, and one jobfish, *Aphareus furca*. I will compare inter- and intra-specific variation in Nitrogen and Carbon stable isotopes (δ13C and δ15N), as well as Layman (Layman et al. 2007b) and SIBER (Jackson et al. 2011) niche metrics. In order to differentiate between isotopic niches and compare metrics between different species with different body sizes and diets, relative trophic position (*e.g.* comparing amongst species) must be calculated using δ15N (a proxy for trophic position), which requires baseline isotope values, often reflected in long lived secondary consumers or primary producers to reflect the base of a given food web as a whole (Kurten et al. 2014). Not having these values, I cannot calculate absolute trophic positions of a given species, nor can I truly detect niche differentiation amongst species. However, given body size and dietary information I can make predictions of how each species' raw stable isotope values will compare to one another.

Based on dietary descriptions from stomach content analyses, the majority of the diet of *C. argus, C. urodeta* and *A. furca* is composed of fishes (of unspecified taxa), and any interspecific differences in stable isotope values across these species are expected to arise from body size differences and taxonomic differences of dietary items. However, the dietary composition of the two *Lutjanus* species, in particular *L. fulvus,* is more diverse, with a lesser proportion of its reported diet being fishes and including cephalopods and crustaceans, which I would expect to have different stable isotope values. Therefore, I expect the dietary niche widths, reflected by variation of stable isotope values (greater δ13C and δ15N range, assuming the dietary items vary in δ13C and δ15N values), of *L. fulvus* and *L. bohar* to be significantly greater than all other focal species.

Considering body size effects, based on differences between the two *Lutjanus* species, I expect *L. bohar* to be more enriched in δ15N, as it is the larger of the two. Both are large piscivorous bentho-pelagic consumers and so I would expect them to both have less enriched δ13C values compared to the two grouper species, which are more benthic consumers (Randall 2005). This is because of benthic sources being generally more enriched in δ13C as compared to pelagic sources (Hilting et al. 2013). On the same note, I would expect similarly enriched δ13C values for the two *Cephalopholis* species. However, because *C. argus* is generally much larger than *C. urodeta*, I expect *C. argus* to be more enriched in δ15N. Finally, *A. furca* is a more pelagic consumer than these other four piscivorous species, but with an overall smaller body size than the other focal species (Randall 2005), and so I would expect less enriched δ13C and δ15N values.

Objective 2

My second objective is to determine if, and how, fishing changes the stable isotope signatures and dietary niches of these five piscivorous coral reef fish species at the individual, population, and/or overall community levels. Changes in isotope signatures can arise from changes in prey or from changes in the predator itself due to fishing pressure. I examine three distinct ways these changes could arise:

Mechanism 1 – Individual-level changes: Fishing may change components of the coral reef prey base, and such changes may translate into altered stable isotope signatures (for δ13C or δ15N individually, or together for isotopic niche space) for individual piscivores of each species. This could arise in one of two ways, via an expansion or reduction in overall dietary niche width:

- Niche shifts or expansion: If fishing removes preferred, or typical prey items of a predator, then the predator would be forced to feed on non-preferred prey items (with potentially different stable isotope signatures themselves), and therefore potentially expand its niche width (akin to a Type III functional response; Holling 1973). Conversely, the predator could also simply switch to feeding on different prey items at similar trophic levels, so niche width might not change in total area, but could move in niche space. Changes in niche width metrics could also arise due to decreased relative strengths of intraspecific (thought to be a diversifying force), and interspecific (thought to be a

constraining force) competition as a result of fishing impacting the predatory fish populations themselves. This 'ecological release' would allow species to feed at trophic levels and on resources that otherwise may have been unavailable or controlled by competitors (Van Valen 1965; Grant and Price 1981; Bolnick et al. 2010).

- Niche reduction: If fishing greatly simplifies the prey community, there will generally be less for a given predator to prey on in terms of dietary diversity, which would therefore cause a niche width reduction.

Given how stable isotope analysis is only a proxy for dietary diversity, there is no way to determine exact mechanisms of how stable isotope metrics change, but using basic dietary information for each species, predictions can be made. I expect that there will be species-specific changes in stable isotope metrics. For species that have very narrow niche widths as per Objective 1, I expect a niche width expansion or shift in stable isotope values, reflecting a dietary shift due to limited availability of preferred prey items. For species that have broader niche widths, as per Objective 1 analyses, I expect a niche width reduction due to a reduction in the number or abundance of prey items available.

To separate this mechanism from fishery-induced changes to piscivore body size (described in Mechanism 2), I will control for body size. Thus, to examine evidence for this mechanism for each focal piscivore species, I will compare its stable isotope signatures and niche across the fishing pressure gradient, by selecting an equal number of individuals (from our total sample size for that species), from each of several body size classes that will be held constant in each fishing pressure region.

Mechanism 2 – Population-level changes: Fishing could also change the size-structure of the piscivore populations, which might alter their dietary niches, irrespective of changes to their prey base. Specifically, if fishing targets the largest individuals of a given piscivore species, shifts in niche metrics could simply arise because fished areas have smaller-bodied fish, which I would expect to have less enriched stable isotope values. To test this hypothesis, I will first have to determine if this is the case, by analysing body size distributions from underwater visual census data from the same sites on Kiritimati. If body size distributions for a given species are significantly different amongst regions with differing fishing pressure, then I will select samples for stable

isotope analysis so that they match the observed size frequency data of each species within each region to better represent the species' population in terms of body size in each region.

Mechanism 3 – Community-level changes: In addition to changing the body size distribution of individual populations, fishing also may change the relative abundance of piscivore species within the community as a whole, if for example, one species is more heavily fished than another. If the relative abundance of a certain species or multiple species is greater or lower in a given region, any changes in stable isotope values between regions at this level could simply be because of differences in community composition (different proportions of species and/or body sizes with potentially different stable isotope values). Specifically, if fishing targets the largest species in a community, at the functional group level, niche metrics would shift because fished areas would have a greater abundance of smaller-bodied species, which I would expect to have less enriched isotope values, or would at least be influenced by the stable isotope values of these more abundant species.

To test this hypothesis, community-level (all focal species combined) stable isotope analyses will be run using a subset of samples that reflect observed frequencies, relative to the other focal species, of each species at each fishing pressure region, as well as its observed body size distribution. I will select samples of each species that are proportional to observations of the other focal species, so that community-level comparisons of isotopic niche width will accurately reflect the community compositions that have been observed across the fishing pressure gradient. For example, if the high fishing pressure region had a significantly lower abundance of *L. bohar* than the low fishing pressure region, I would select samples to reflect this disparity (and any differences in body size, as described above), choosing samples of the same proportion for stable isotope analysis.

Methods

Study Site

Kiritimati Atoll (01°52'N 157°24'W), Republic of Kiribati, Northern Line Islands, is a remote central Pacific atoll (Figure 2) with approximately 5,500 residents (Walsh et al. 2012). It is the world's largest atoll by land area at 363.4 km^2, with a perimeter of ~150 km for the atoll and over 48 km of shoreline in the lagoon (Figure 2, Kiritimati Climate Report 2012). The primary human disturbances on Kiritimati are subsistence fishing and an aquarium export trade (Burke et al. 2011). Importantly, the only agricultural activity on the island is for copra, which requires no fertilizer, thus there is little run-off of nutrients into the surrounding waters apart from local sewage. The vast majority of Kiritimati's population is concentrated on the atoll's northwest coast, such that 60% of its coastline is uninhabited and experiences little to no fishing. Of the 5,500 people on the atoll, ~4,100 reside in two villages on the northwest peninsula (London, 1,879 and Tabwakea, 2,311, Figure 2; Kiritimati Climate Report 2012). This has resulted in a significant gradient of fishing around the atoll, from coral reefs near these villages in the northwest that are heavily impacted by fishing, to those in remote areas, which are considered near-pristine to the southeast (Walsh 2011). The atoll thus presents an excellent opportunity for testing the effects of human disturbance on ecosystem dynamics at a regional scale.

Across Kiritimati's coast, reef regions were selected and categorized into six levels of fishing pressure (Very high (sites 26, 27, 30, 33, and 40), High (sites 3, 24, and 25), High medium (sites 6, 8, 9, 14, 34, and 35), Low medium (sites 1, 2, 22, 23), Low (sites 7 and 13), and Very low (sites 10, 15, and 19); Figure 2), based on village size and information on subsistence fishing activity collected from household surveys conducted by Walsh (2011). Note that site numbers are based upon a broader monitoring scheme and are not ordered geographically (but rather by the original order of monitoring). The role of oceanographic productivity on stable isotope signatures was evaluated (see below under "Oceanographic productivity and year effects"), but no effect was detected, and thus only fishing pressure was used to categorize regions around the atoll's coast. No regions from the southern coast were sampled due to inaccessibility.

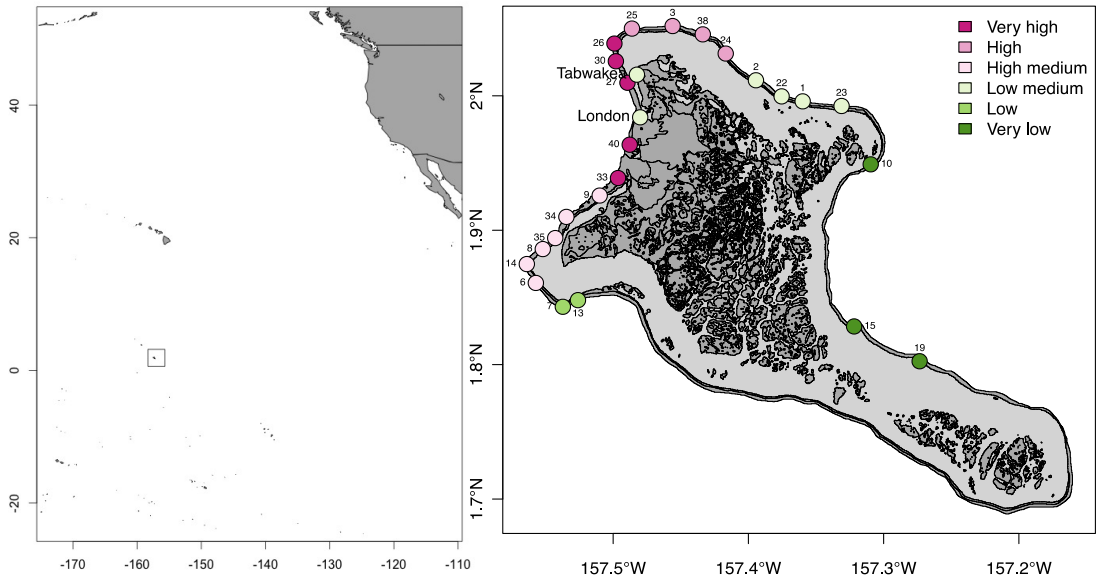

Figure 2: Location of Kiritimati Atoll, Northern Line Islands, Republic of Kiribati, and sampling sites across six levels of fishing pressure.

Sampling Design

Target species were chosen as representatives of the putative piscivore community of Kiritimati's reef fish community. We designed our sampling protocol to concentrate on the atoll's most abundant piscivorous species, as determined by underwater visual censuses (UVC) conducted at >25 sites around the atoll in both 2009 and 2011 (Baum and Walsh, unpublished data). We also aimed to sample the full size range of each species, as much as possible. All sampled species of piscivorous reef fish were collected over three field seasons, at 2 to 6 sites in each of the six fishing pressure regions (n=24 collection sites in total; Figure 2) and across a range of body sizes in May of 2010 and July/August, in 2011, and 2012 (Table 4). *Aphareus furca, Cephalopholis argus, Cephalopholis urodeta, Lutjanus bohar,* and *Lutjanus fulvus* were deemed to be the most abundant piscivores across all sites, and were therefore chosen as focal species for all species level analyses. Other, less abundant piscivore species were sampled opportunistically for functional group level analyses (Table 4). These species, *Caranx melampygus* and *Variola louti,* were only included in functional group wide exploratory plots and were not analysed for fishing effects.

All fish were collected using SCUBA and a variety of spearing equipment. Smaller bodied fish were collected using a banded micro-spear (approximately one meter in

length), while larger bodied fish were collected using a combination of a pneumatic spear gun and a pole spear (approximately two meters in length, three prong tip). Fish specimens were immediately put on ice after capture and dissected later that evening. All muscle tissue samples were frozen on site at -20°C and preserved for shipment to Victoria, BC, using dry ice (solid carbon dioxide). Samples were immediately stored at -20°C at the University of Victoria after shipment.

Sample Preparation

For each individual fish used in analysis, three samples (of approximately 10 g each) of dorso-lateral white muscle tissue were taken. We processed one sample from each fish for stable isotope analysis; the remaining two samples from each are frozen at -20°C as archives at the University of Victoria. Muscle tissue was chosen because it shows less variability in isotopic composition than other body parts and is more reflective of diet and growth as compared to other tissues, for example bone and lipids (Bearhop et al. 2004; Layman et al. 2011). Each sample was double rinsed in de-ionized water so as to minimize contamination from non-muscle tissue and scales. Samples were then dried at 60°C in a drying oven for 48 hours, and subsequently ground with a mortar and pestle. Samples were pulverized until further grinding would not make the resulting powder any more fine. Each sample was packed in aluminum capsules and weight calibrated to 1 mg (Sartorius ME microbalance, 1μg readability) to permit comparisons with the standard reference materials used with the mass spectrometer (see below).

Stable Isotope Analysis

Dual analyses of carbon and nitrogen stable isotope abundances were conducted in the Mazumder Laboratory in the Department of Biology, University of Victoria, British Columbia. Samples were run through an elemental combustion system (Costech Instruments) coupled to an isotope ratio mass spectrometer (ThermoFinnigan CONFLO III Delta V Advantage, Thermo Electron Corporation). Values of δ15N and δ13C were reported relative to internal standards, atmospheric N^2 and virtual PeeDee Belemnite (vPDB).

Table 4: Summary of piscivorous reef fish collections by fishing pressure region (highest to lowest for each species) from Kiritimati, Republic of Kiritibati including two non-focal species, *Caranx melampygus* and *Variola louti*.

Family	Species	Common name	Fishing pressure	Samples collected	Min size sampled (g)	Max size sampled (g)
Lutjanidae	Aphareus furca	Small-toothed jobfish	Very high	4	200	260
			High	12	200	440
			High medium	12	250	640
			Very low	9	210	420
Lutjanidae	Lutjanus bohar	Red snapper	Very high	18	15.3	4540
			High	13	129	593.9
			High medium	5	91.1	757.7
			Low medium	1	354.7	354.7
			Very low	17	176.9	3178
Lutjanidae	Lutjanus fulvus	Blacktail snapper	Very high	13	83.5	229.1
			High medium	21	56.2	308.9
			Low medium	39	75	182.8
			Low	4	189.7	229.3
Serranidae	Cephalopholis argus	Peacock grouper	Very high	39	169	1193.2
			High	24	212	1100
			High medium	29	212	946
			Low medium	25	194	1099.92
			Low	26	115	995
			Very low	13	184	758
Serranidae	Cephalopholis urodeta	Flagtail grouper	Very high	22	6.8	169
			High	15	74	151.1
			High medium	15	12.12	139
			Low medium	3	58.6	91.3
			Very low	20	27.1	145.9
Serranidae	Variola louti	Yellow-edged lyretail	Very high	3	530	820
			High	4	127.7	3405
			Very low	2	1589	3178
Carangidae	Caranx melampygus	Bluefin trevally	Very high	3	263.3	1054.9
			High	3	620	2833.1
			High medium	4	1816	2678.6
			Very low	6	126.6	3722.8
			TOTAL	424		

Increase or decrease in δ13C or δ15N indicates enrichment or depletion of the heavy isotope compared to the lighter isotope relative to the internal standard. Calibration of equipment and monitoring the long-term performance of the mass spectrometer was conducted with reference material (acetanilide and caffeine) from the National Institute of Standards and Technology (NIST). 10% of all piscivore samples were run as randomly selected double blinds (n=42; δ13C: mean difference between blinds=0.63, median=0.33, standard deviation=1.09; δ15N: mean difference between blinds=0.32, median=0.16, standard deviation=0.46). Duplicate samples were not included in analyses and were only used to assess the precision of the mass spectrometer used (Appendix D). Mean δ13C and δ15N sample groups (blinds) were not significantly different from each other (p=0.5337 and p=0.5426, respectively; paired t-test), and samples that were found to have a difference between blinds greater than three per mil (n=2 for δ13C only) were excluded from analysis, as it implies impurity within the muscle tissue sample.

Testing for Influences on Stable Isotope Signatures

Prior to addressing Objectives 1 and 2, I first examined if there was evidence that body size, region of the atoll, or oceanographic productivity influenced the δ13C and δ15N values of each species, in order to evaluate their role and to validate controlling for these factors in later analyses. To do this, I performed univariate regressions and linear mixed effects models for the piscivore functional group as a whole and on each focal species. Univariate regressions were run with both weight (grams) and standard length (mm) of collected samples on a logarithmic scale (log10) evaluated with both δ13C and δ15N. A logarithmic scale was chosen since piscivore body sizes cover a large range of values. Linear mixed effects models were performed with log10 weight as the fixed effect for each fish sample for both δ13C and δ15N, with species as the random effect at the functional group level, and with site as the random effect at the species level.

Finally, to evaluate all effects together, for both δ13C and δ15N, I ran a multiple regression for each of the five focal species, including fishing pressure region, body size (weight), and productivity, using mean chlorophyll-*a* values from the Bio-ORACLE (Ocean Raster for Analysis of Climate and Environment) open source dataset of worldwide GIS rasters of marine environmental information (Tyberghein et al. 2011).

Evaluating the role of oceanographic productivity on stable isotope values is covered in more detail later in this thesis ("Oceanographic productivity and year effects").

Data Analysis for Objective 1: Niche differentiation of piscivorous reef fish

To assess the level of niche differentiation between species, baseline values of primary producers or long-lived secondary consumer stable isotope signatures, representative of the base of the food web, must be used to calculate trophic positions. This permits comparison between members of different taxa with potential differences in body size and feeding behaviours, as it provides a reference point upon which comparisons of trophic position can be made (Kurten et al. 2014). Given how this does not include baseline values, and the focal species all differ in body size and feeding behaviour, we cannot calculate definitive trophic positions, however we can still use these known differences to make predictions and try to detect measurable differences amongst species' stable isotope values.

As an exploratory analysis of potential differences in isotopic niches of the five focal species, I first plotted the stable isotope data in δ13C vs. δ15N bi-plot (for all fishing pressure regions combined and separately) with both convex hull total area and SIBER-calculated standard ellipses shown for each focal species.

To examine if there are detectable niche differences amongst the five focal species, separate from any effect of either fishing or body size, one would ideally examine samples for each species from a single body size class and a single area of minimal fishing pressure. I did not, however, have sufficient data to conduct such an analysis. Instead, I attempted to control for these two potentially important effects by sub-sampling (from my overall stable isotope sample set) the same number and distribution of body sizes for each species in each region. While effects of body size cannot be controlled when comparing species of differing body size distributions, using the same range of body sizes for each species in each region ensured that similar comparisons among species were being made within each region. An equal number of samples were selected from body size classes for each region for each focal species based on the least observed body size class for each species (Table 5). To increase the number of samples used in each analysis, only regions with at least two samples of each species in each body size class were used. Body size classes were based on the range of collected samples' weight

in grams of each focal species and chosen to maximize the range of body sizes used while maintaining a sufficiently large sample size, in this case at least two samples per body size class per focal species. With these evenly selected samples of focal species across their respective size range, stable isotope metrics were compared across species in each region. All analyses were conducted separately in each region to eliminate any potential effect of fishing pressure. The "Low" and "Low medium" regions only had samples for *L. fulvus* and *C. argus*, and were thus excluded from all Objective 1 analyses. Every other region contained enough samples for comparison among four species of varying composition (Table 5).

Table 5: Objective 1 sample selections by region and body size class for stable isotope analysis of the five focal species.

Species	Fishing pressure region					
A. furca	Size class (g):		High	High medium		Very low
	200-299		7	3		4
	300-399		2	3		2
	400-499		3	2		2
	Select per bin:		2	2		2
L. bohar	Size class (g):	Very high	High			Very low
	0-199	9	4			2
	200-399	5	4			4
	400-799	2	5			2
	Select per bin:	2	2			2
L. fulvus	Size class (g):	Very high		High medium	Low medium	
	0-99	5		6	14	
	100-199	4		6	15	
	200-300	4		7	10	
	Select per bin:	4		4	4	
C. argus	Size class (g):	Very high	High	High medium	Low medium	Low	Very low
	100-299	6	5	2	8	15	2
	300-499	19	15	10	9	8	8
	500-699	8	3	12	4	3	3
	Select per bin:	2	2	2	2	2	2
C. urodeta	Size class (g):	Very high	High	High medium			Very low
	0-99	11	7	9			16
	100-199	11	8	6			4
	Select per bin:	4	4	4			4

All samples were randomly selected from their specified range in weight (Table 5), and within each region with enough species and samples (*i.e.* Very high, High, High medium, and Very low), for both δ13C and δ15N individual isotopes, the mean, standard deviation, median, minimum and maximum values were calculated for each species present in a given region. The previously described Layman metrics (*i.e.* TA, CD, MNND, SDNND; Layman et al. 2007b), and the SIBER metrics Standard Ellipse Area (SEA) and Standard Ellipse Area corrected for small sample size (SEAc) were also calculated for each species. To better represent all collected samples and to improve the statistical power of the results, selections were re-sampled and all metrics were bootstrapped with 1000 iterations, with replacement, and a mean value of each metric was calculated.

To test for significant differences in stable isotope values, for each iteration an ANOVA was run on mean δ13C and δ15N values followed by a post-hoc TukeyHSD (honestly significant difference) test for pair-wise comparisons among all species in a given region. A p-value was reported for each pair-wise species comparison for each iteration, and I decided that a significant difference in mean individual stable isotope values required at least 900 iterations (90% of the total) with a p-value less than or equal to 0.05.

For the SIBER-calculated metric for niche width (SEA), within each iteration for each species, as described above, 1000 posterior draws of an ellipse were made, and the proportion of ellipses greater than another for each pair-wise species comparison was determined using SIBER. A proportion greater than or equal to 0.95 or less than or equal to 0.05 was interpreted as there being a significant difference between the two species' SEA. As with δ13C and δ15N means, if at least 900 iterations (90% of the total) returned a significant result, the SEAs between the two species at hand were deemed to be significantly different. As for the remaining Layman metrics (*i.e.* TA, CD, MNND, and SDNND), mean values from the 1000 iterations were reported and tested for significant differences using ANOVAs by focal species for each region followed by post-hoc TukeyHSD pair-wise comparisons. All bootstrapped hypothesis testing methods were modified from a similar approach of bootstrapped hypothesis testing in Efron and Tibshirani (1993). Finally, sample ellipses were plotted for each species in each of the

four regions used that best represent the bootstrapped results, to help visualize any putative differences in stable isotope values and/or niche width metrics among focal species.

All sample selections, plots and analyses were performed in the statistical program R, available from the Comprehensive R Archive Network (CRAN), http://cran.r-project.org/, and stable isotope metrics and ellipses done using the package SIAR (Stable Isotope Analysis in R, Parnell et al. 2010), using the subset of functions under SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al. 2011).

Data Analysis for Objective 2: Stable isotope metrics across fishing pressure regions

To assess the effects of fishing, all analyses described above (Data Analysis for Objective 1) were done using fishing pressure region (Very high, High, High medium, Low medium, Low, and Very low) as the grouping variable instead of species. This was done separately for each species to isolate any potential species level effects, as determined by Objective 1. All analyses were first performed using raw data of all samples collected, without controlling for body size or abundances, and then using sampled data to control for body size and observed relative abundances between focal species, as per the three mechanisms outlined above ("Objectives and Hypotheses"):

Mechanism 1: Individual-level changes

As with Objective 1, all data were first plotted in a δ13C vs. δ15N bi-plot by fishing pressure region for each focal species separately with both convex hull total area and SIBER-calculated standard ellipses drawn for each focal region. These plots were used as exploratory analyses to gauge putative differences in isotopic niches of a given species across the different fishing pressure regions.

Samples were selected using the same proportions and body size classes as in Objective 1 so as to randomly select an equal number of samples from the body size classes for each species for each focal species to ensure equal representation of body sizes across all regions. Only regions with enough samples were included in each species' analyses (Table 6). Having controlled for body size effects, the same metrics used in Objective 1 (mean, SD, median, minimum and maximum δ13C and δ15N values, as well as SEA, SEAc, TA, CD, MNND, and SDNND) were calculated for each region with enough

samples. The same hypothesis tests (ANOVA followed by TukeyHSD pair-wise comparisons for δ13C and δ15N mean values, and SIBER calculated ellipse proportions) as Objective 1 were performed to produce a p-value for each pair-wise comparison between regions.

The same number of samples was randomly re-sampled (with replacement) from the assigned body size classes from each region for each species (Table 6) 1000 times, as before, with the same metrics and hypothesis tests being completed each iteration. Mean values for each bootstrapped metric were calculated and as with Objective 1, a significant difference between regions was considered to be two regions with at least 900 iterations with a significant result for a given metric ($p<=0.05$ for mean δ13C or δ15N and $0.05>=N>=0.95$ for SEA proportions). As with Objective 1, the remaining Layman metrics (*i.e.* TA, CD, MNND, and SDNND) mean values from the 1000 iterations were reported and tested for significant differences using ANOVAs by region for each focal species followed by post-hoc TukeyHSD pair-wise comparisons. Sample ellipses were plotted in SIBER for each of the five focal species that best represent the bootstrapped results to visualize putative differences in stable isotope values and/or niche width metrics among fishing pressure regions.

Mechanism 2: Population-level changes

Species level body size distributions across the three fishing pressure regions were analysed to test the hypothesis that fishing could have an effect on the overall size structure of each piscivore population in a given region (Mechanism 2, "Objectives and Hypotheses"). This was to investigate whether any observed changes in isotopic niche metrics are due to altered body sizes (which I expect to be correlated to stable isotope values, particularly δ15N, as described above in "Body Size, Productivity, and Stable Isotopes") of an entire species as a result of fishing rather than altered feeding interactions.

To do this, I analysed unpublished underwater visual census data of the fish community at sites around Kiritimati from 2007, 2009, 2011, and 2013. Data were collected by a pair of divers along three 25m transects, separated by 10m, on isobaths between 10-12 m depth at each site across all regions.

Table 6: Sample selections for bootstrapping per focal species per body size for mechanism 1. Note: not all samples are reported here; body size classes for each species without representation in a region were not considered (see text for details).

Species	*Aphareus furca*			Total to be selected per region per
Size class (g)	200-299	300-399	400-499	
High	7	2	3	
High medium	3	3	2	
Very low	4	2	2	
Choose per region:	2	2	2	6
Species	*Lutjanus bohar*			
Size class (g)	0-199	200-399	400-799	
Very high	9	5	2	
High	4	4	5	
Very low	2	4	2	
Choose per region:	2	2	2	6
Species	*Lutjanus fulvus*			
Size class (g)	0-99	100-199	200-300	
Very high	5	4	4	
High medium	6	6	7	
Low medium	14	15	10	
Choose per region:	4	4	4	12
Species	*Cephalopholis argus*			
Size class (g)	100-299	300-499	500-699	
Very high	6	19	8	
High	5	15	3	
High medium	2	10	12	
Low medium	8	9	4	
Low	15	8	3	
Very low	2	8	3	
Choose per region:	2	2	2	6
Species	*Cephalopholis urodeta*			
Size class (g)	0-99	100-199		
Very high	11	11		
High	7	8		
High medium	9	6		
Very low	16	4		
Choose per region:	4	4		8

Fishes greater than or equal to 20 cm total length were counted in an 8m wide strip (total surveyed area per site of 600 m^2) as the transect was laid, while fish smaller than 20 cm total length were counted in a 4 m wide strip (total surveyed area per site of 300 m^2) in the reverse direction along the transect. Survey methodology was performed consistently across all years. The 2007 data have been made available courtesy of Sheila

Walsh (The Nature Conservancy), 2009 data courtesy of Sheila Walsh and Julia Baum (University of Victoria), and the 2011 and 2013 data were made available by Julia Baum.

Body size data were analysed using ANCOVAs of linear models of body size (total length in centimeters) for each species and the piscivore functional group as a whole across high, medium and low fishing regions, and using a Tukey HSD test to determine any significant differences in mean total length between regions for each focal species. For each species in each region, mean, standard deviation, median and range of total lengths were considered as well. Data from each year were analysed separately, and then combined so as to maximize the number of observations for each species across all sites.

Out of the five focal species tested, all except *Cephalopholis argus* had significant differences in body size between the six fishing pressure regions tested (see Mechanism 2 Results). For these species (*i.e. C. urodeta, A. furca, L. bohar,* and *L. fulvus*), samples were selected for analysis based on relative observed (from aforementioned surveys) proportions of body size (total length) bins (chosen to maximize range of samples used while still keeping sample size high in each bin) from the six fishing pressure regions where they were sampled in high enough numbers (*A. furca*: High, High medium, and Very low; *L. bohar*: Very high, High, and Very low; *L. fulvus*: Very high, High medium, Low medium; Table 7; *C. urodeta* Very high, High, High medium, and Very low; Table 8). These selections, chosen to reflect observed body size distributions of each species within each region, were also proportional so that equal numbers of samples were used from each region for each species' analyses (using the least sampled region for each species; Table 7, Table 8). Occasionally, observed body sizes from the surveys did not match the collected samples, and sample selections were adjusted accordingly using the next available body size class. Thus, certain regions' selections are not fully reflective of observed body size distributions (denoted with an asterisk, Table 7, Table 8).

Selections were randomly sampled and all metrics and hypothesis tests previously described under Objective 1 and Objective 2, Mechanism 1 were performed on these samples. All analyses and tests were bootstrapped by re-sampling with replacement 1000 times in the same way as before.

Table 7: Sample selections for mechanism 2 Lutjanidae focal species to reflect observed body size distributions in sufficiently sampled fishing pressure regions (*=observed body size distribution did not fully match available samples, and samples were taken from the next available body size classes).

Aphareus furca	High fishing pressure						
	TL Size bins (cm)	Observations	Proportions	Samples collected	Based on highest proportion	Based on least sampled region	Rounded/ availability
	25-30	21	0.618	6	7.412	4.941	5
	30-35	9	0.265	3	3.176	2.118	2
	35-40	4	0.118	3	1.412	0.941	1
	TOTAL	34	1	12	12	8	8
	High medium fishing pressure *						
	25-30	22	0.595	1	6.541	4.757	*1
	30-35	10	0.270	3	2.973	2.162	3
	35-40	5	0.135	7	1.486	1.081	*4
	TOTAL	37	1	11	11	8	8
	Very low fishing pressure *						
	25-30	107	0.386	6	3.090	3.090	*6
	30-35	150	0.542	2	4.332	4.332	*2
	35-40	20	0.072	0	0.578	0.578	0
	TOTAL	277	1	8	8	8	8
Lutjanus bohar	Very high fishing pressure						
	0-20	1	0.143	8	2.571	1.857	4
	20-40	5	0.714	8	12.857	9.286	8
	40-60	1	0.143	1	2.571	1.857	1
	60-80	0	0	1	0	0	0
	TOTAL	7	1	18	18	13	13
	High fishing pressure *						
	0-20	6	0.286	0	3.714	3.714	0
	20-40	11	0.524	13	6.810	6.810	*13
	40-60	3	0.143	0	1.857	1.857	0
	60-80	1	0.048	0	0.619	0.619	0
	TOTAL	21	1	13	13	13	13
	Very low fishing pressure						
	0-20	19	0.039	0	0.666	0.509	0
	20-40	215	0.443	8	7.536	5.763	6
	40-60	201	0.414	8	7.045	5.388	6
	60-80	50	0.103	1	1.753	1.340	1
	TOTAL	485	1	17	17	13	13
Lutjanus fulvus	Very high fishing pressure						
	15-20	30	0.566	5	7.358	7.358	5
	20-25	19	0.358	7	4.660	4.660	5
	25-30	4	0.075	1	0.981	0.981	1
	TOTAL	53	1	13	13	13	11
	High medium fishing pressure						
	15-20	32	0.356	6	7.467	4.622	4
	20-25	8	0.089	8	1.867	1.156	1
	25-30	50	0.556	7	11.667	7.222	6
	TOTAL	90	1	21	21	13	11
	Low medium fishing pressure*						
	15-20	1	0.026	18	1.026	0.342	1
	20-25	18	0.474	19	18.474	6.158	8
	25-30	19	0.500	2	19.500	6.500	*2
	TOTAL	38	1	39	39	13	11

Table 8: Sample selections for mechanism 2 Serranidae focal species to reflect observed body size distributions in sufficiently sampled fishing pressure regions (*=observed body size distribution did not fully match available samples).

Cephalopholis urodeta	Very high fishing pressure						
	TL Size bins (cm)	Observations	Proportions	Samples collected	Based on highest proportion	Based on least sampled region	Rounded/ availability
	10-12.5	163	0.454	3	7.719	5.448	3
	12.5-15	43	0.120	1	2.036	1.437	1
	15-17.5	83	0.231	5	3.930	2.774	3
	17.5-20	70	0.194	8	3.314	2.340	3
	TOTAL	359	1	17	17	12	10
	High fishing pressure*						
	10-12.5	159	0.546	0	7.103	6.557	*0
	12.5-15	36	0.124	0	1.608	1.485	*0
	15-17.5	67	0.230	4	2.993	2.763	*4
	17.5-20	29	0.100	9	1.296	1.196	*6
	TOTAL	291	1	13	13	12	10
	High medium fishing pressure*						
	10-12.5	216	0.494	0	5.931	5.931	*0
	12.5-15	59	0.135	0	1.620	1.620	*0
	15-17.5	101	0.231	6	2.773	2.773	*5
	17.5-20	61	0.140	6	1.675	1.675	*5
	TOTAL	437	1	12	12	12	10
	Very low fishing pressure						
	10-12.5	24	0.179	2	3.582	2.149	2
	12.5-15	30	0.224	4	4.478	2.687	2
	15-17.5	69	0.515	10	10.299	6.179	6
	17.5-20	11	0.082	4	1.642	0.985	0
	TOTAL	134	1	20	20	12	10

Mechanism 3: Community-level changes

In addition to potentially impacting community body size distributions, fishing could also impact relative abundances of species within the piscivore community. Therefore, any observed changes in stable isotope metrics between regions of differing fishing pressure could be solely due to altered community composition, revealed through changes in relative species abundances (which would have different proportions of species with potentially different body sizes which I would expect to have potentially different stable isotope values) as a result of fishing rather than altered feeding interactions. Thus, this had to be treated as a potential influence on observed stable

isotope values. Relative abundances of each focal species across the six fishing pressure regions were analysed to select samples of each species in each region that are reflective of their observed abundances, as well as their body size distribution (*i.e.* Mechanism 2 analysis). In a sense, this analysis combines measures to control body size effects and to reflect naturally occurring abundances of each region, to get samples that most accurately represent the piscivore community.

I used the same data set as in mechanism 2 for analysing body size distributions (transect surveys in 2007, 2009, 2011 and 2013), but analysed frequency (total number of observations by region) distributions of each focal species across the fishing pressure gradient as a proxy for their relative abundances to one another in each region. Random sample selection was done in the same way as Mechanism 2 selections, but for each region, instead of body size classes, proportions of species observations were used to determine selections (Table 9). Not enough samples were collected in the 'Low medium' or the 'Low' fishing pressure region, so these two regions were excluded from this analysis.

From these selections, to additionally control for body size, samples were randomly selected within a body size range of the mean observed total length +/- one standard deviation to additionally reflect observed body sizes of each species in a given region. In the rare case where the required number of samples did not fit in this body size range, the range was increased by 1 cm until enough samples fit in the range to satisfy the observed proportional abundances relative to the other focal species. This was required for *C. urodeta* in the Very high, High, and High medium regions (by 20 cm, 30 cm, and 20 cm respectively), for *A. furca* for the Very high (20 cm) and High (30 cm), and *L. bohar* in the Very low region (10 cm).

With these random selections of samples from the focal species in each region, the same metrics and hypothesis tests as described above in Objective 1 and Objective 2, Mechanism 1 and 2, were calculated in the same way, although at the piscivore community-level with fishing pressure region as the grouping variable. The selections were randomly re-sampled as before (with replacement) 1000 times, and mean values for all metrics, as well as p-values for all ANOVAs of mean δ13C and δ15N and the SIBER SEA proportions metric, were reported. The same criteria were used to determine

significant differences between regions as in Mechanism 1 and 2 (at least 900 of 1000 iterations with a significant result for the given metrics: p<=0.05 for mean δ13C or δ15N and 0.05>=N>=0.95 for SEA proportions). Finally, the remaining Layman metric (*i.e.* TA, CD, MNND, and SDNND) mean values from the 1000 iterations were reported and tested for significant differences using ANOVAs by region for all species combined followed by post-hoc TukeyHSD pair-wise comparisons.

Oceanographic productivity and year effects

Although all the focal species analysed are mostly reef-associated piscivores, the potential effect of oceanographic productivity on stable isotope values was evaluated. Island-wake upwelling areas off Kiritimati (Figure 3) create an oceanographic productivity gradient across its coast. I used chlorophyll-*a* values from three different sources, all taken from remote sensing satellite data, as proxies for oceanographic productivity changes due to the upwelling zones. These sources include mean, minimum, and maximum chlorophyll-*a* values from Bio-ORACLE (Ocean Rasters for Analysis of Climate and Environment), an open-source dataset of worldwide GIS rasters of marine environmental information (Tyberghein et al. 2011), and unpublished remote sensing satellite data from collaborators Chelsea Wood (University of Colorado, Boulder) and Sheila Walsh (The Nature Conservancy), both of whom have previously worked on Kiritimati Atoll. All chlorophyll *a* values used were for the nearest pixel to each sampled site (Figure 4), depending on resolution. In total, five chlorophyll-*a* values from three sources were assessed across all sites (shown in geographical order, clockwise beginning in the southwest corner of the atoll in Figure 5). These values show a clear increase in productivity for all sources in sites along the west coast of the atoll, particularly near the lagoon mouth, coinciding with the large island-wake upwelling area in that region.

Table 9: Focal piscivore species samples selected for mechanism three analysis, reflecting observed abundances of each species in each region with enough samples (very high, high, high medium, and very low) from transect surveys taken in 2007, 2009, 2011 and 2013. Based on the lowest number of collected samples in the medium fishing pressure region, 21 samples were selected from each region.

Very high fishing pressure										
Species	Observations	Proportions	Samples collected	Based on highest proportion	Based on least sampled region	Rounded/ availability	SD TL observed (cm)	Mean TL observed (cm)	Min selected: (Mean-1SD)*10 (mm)	Max selected: (Mean+1SD)*10 (mm)
Cephalopholis argus	28	0.045	39	1.34	0.94	1	6.242	28	217.580	342.420
Cephalopholis urodeta	461	0.736	22	22.00	15.46	15	4.631	12.989	83.577	176.206
Lutjanus bohar	7	0.011	18	0.33	0.23	0	13.363	30.714	173.512	440.774
Lutjanus fulvus	114	0.182	13	5.44	3.82	4	7.711	12.009	42.977	197.198
Aphareus furca	16	0.026	4	0.76	0.54	1	7.958	15	70.418	229.582
TOTAL	626	1.000	96	29.87	21	21				
High fishing pressure										
Species										
Cephalopholis argus	47	0.108	24	2.33	2.26	3	6.697	31.128	244.304	378.249
Cephalopholis urodeta	303	0.695	15	15.00	14.59	15	3.428	12.878	94.501	163.057
Lutjanus bohar	23	0.053	13	1.14	1.11	1	19.345	31.652	123.070	509.974
Lutjanus fulvus	11	0.025	0	0.54	0.53	0	2.656	26.636	239.803	292.924
Aphareus furca	52	0.119	12	2.57	2.50	2	8.835	23.442	146.074	322.772
TOTAL	436	1.000	64	21.58	21	21				
High medium fishing pressure										
Species										
Cephalopholis argus	40	0.053	29	1.14	1.12	1	6.492	30.575	240.828	370.672
Cephalopholis urodeta	527	0.704	15	15.00	14.78	15	4.078	12.670	85.920	167.476
Lutjanus bohar	27	0.036	5	0.77	0.76	1	8.187	23.889	157.020	320.758
Lutjanus fulvus	106	0.142	21	3.02	2.97	3	5.382	23.340	179.573	287.219
Aphareus furca	49	0.065	12	1.39	1.37	1	5.236	27.531	222.941	327.671
TOTAL	749	1.000	82	21.32	21	21				

Very low fishing pressure										
Species										
Cephalopholis argus	150	0.136	13	5.26	2.86	3	7.743	29.040	212.967	367.833
Cephalopholis urodeta	138	0.125	20	4.84	2.63	3	2.641	14.696	120.551	173.362
Lutjanus bohar	485	0.440	17	17.00	9.24	9	13.362	38.845	254.838	522.069
Lutjanus fulvus	12	0.011	0	0.42	0.23	0	5.300	22.500	171.999	278.001
Aphareus furca	317	0.288	9	11.11	6.04	6	4.586	28.830	242.440	334.153
TOTAL	1102	1.000	59	38.63	21	21				

Linear mixed effects models and univariate regressions were run to evaluate the potential effect of chlorophyll-*a* values at each site on δ13C and δ15N of the five focal piscivorous fish species, each analysed separately. Both δ13C and δ15N data for each species were plotted against chlorophyll-*a* values from all three sources (mean values were used from the Bio-ORACLE dataset), and univariate regressions run, calculating R^2 values. While data from all three sources were fairly well correlated, albeit individually different, data from the Bio-ORACLE dataset have the best resolution of 9.2km and were considered to be the most reliable. Therefore, mean chlorophyll-*a* values from this dataset were used in all models testing for the effect of productivity on carbon and nitrogen stable isotope values of all five focal species. Linear mixed effects models at the functional group level (containing all piscivore samples) used the same mean chlorophyll-*a* values as a fixed effect and species as random effect, and at the species level, site was used as the random effect. All models were run with δ13C and then again with δ15N, using the lme4 package in R.

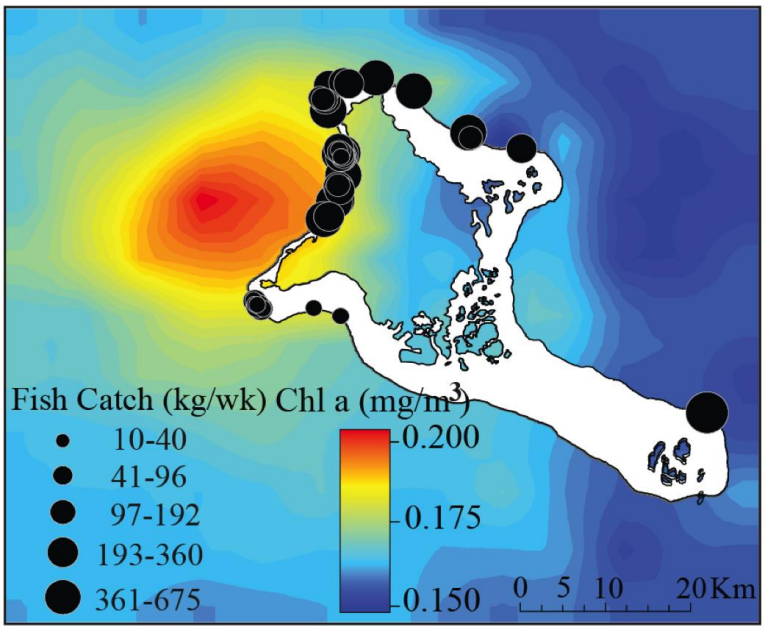

Figure 3: Distribution of island-wake upwelling areas off the coast of Kiritimati atoll, as determined by remote sensing data, as well as reports on fish catch across the region in kg/wk based on household surveys (Walsh 2011).

The potential impacts of productivity on stable isotope values of zooplanktivorous fish was also investigated, at the functional group level using 235 samples of seven species (*Caesio teres, Chromis vanderbilti, Melichthys niger, Pseudanthias bartlettorum, Pseudanthias dispar, Pseudanthias olivaceus,* and *Pterocaesio tile*) and at the species level, using the two most sampled species, specifically 83 *Pseudanthias olivaceus,* and 32 *Chromis vanderbilti.* All zooplanktivore samples were collected simultaneously with piscivorous samples using similar methods for a separate project. All analyses were identical to piscivore samples, with the intention being to compare against a trophic guild with a more pelagic production source, reflective of oceanographic productivity, as opposed to the supposedly (in reference to their close association with the reef) benthic reef production of piscivorous reef fish.

As samples were collected across a three-year span (2010, 2011, and 2012), the effect of year on stable isotope values was also evaluated using similar linear mixed effects models for each species and the piscivore functional group as a whole. In all models, year was used as the fixed effect, while species was used as the random effect at the functional group level, and site was used as the random effect at the species level. All models were run with δ13C and then again with δ15N.

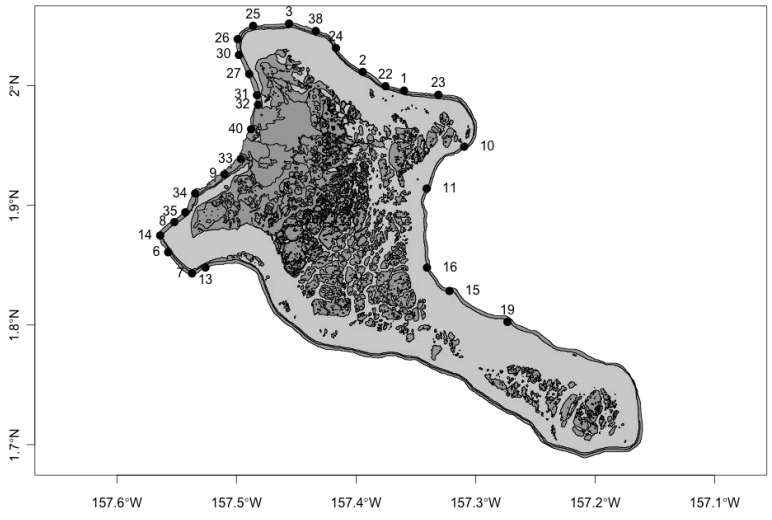

Figure 4: Names and locations of sampling sites across Kiritimati atoll.

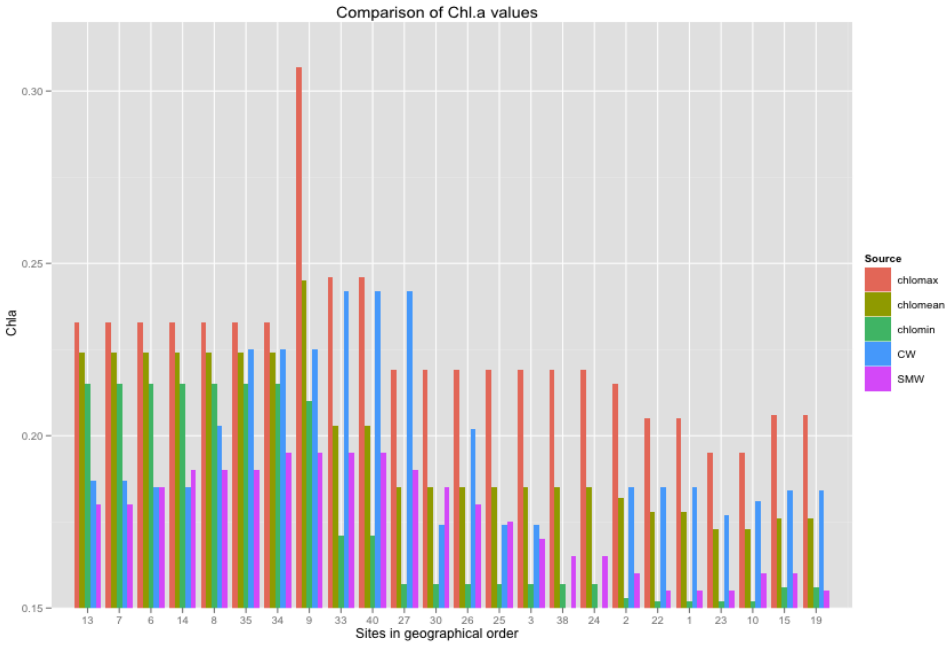

Figure 5: Chlorophyll *a* (mg/m^3) ranges from five different sources across all sites sampled, in geographical order clockwise starting in southwest region (chlomax, chlomean, chlomin=maximum, mean, and minimum chlorophyll *a* values from the Bio-Oracle database, CW=remote sensing data courtesy of Chelsea Wood, SMW=remote sensing data courtesy of Sheila M. Walsh).

Results

Testing for Influences on Stable Isotope Signatures

Prior to testing for differences in stable isotope values across species and the fishing pressure gradient, I evaluated the potential effects of body size and oceanographic productivity. Considering all raw data in exploratory δ13C vs. δ15N bi-plots (Figure 6) and body size plots for all focal species combined and separately for both δ13C (Figure 7) and δ15N (Figure 8), there appears to be a positive relationship between body size and both stable isotope values. Examining this further and testing for overall patterns, the multiple regressions revealed significant ($p<0.05$) effects of body weight (g) on all three Lutjanidae species, but not on either of the Serranidae species or the piscivore functional group as a whole (δ13C: Table 10, δ15N: Table 11). No effect of chlorophyll-*a* mean values (using mean values from the Bio-ORACLE dataset) on any of the species combined or separately for δ13C (Table 10) or δ15N (Table 11) were found, with the sole exception of mean chlorophyll-*a* showing a significant effect on *L. fulvus* δ15N.

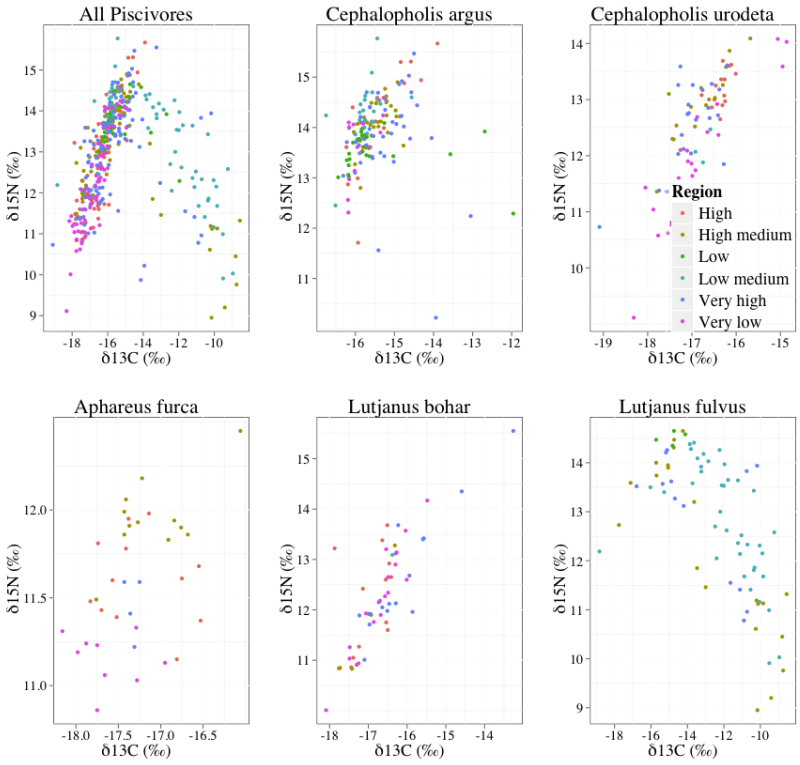

Figure 6: δ13C vs. δ15N exploratory bi-plots for all focal piscivore species combined and separately, by fishing pressure region.

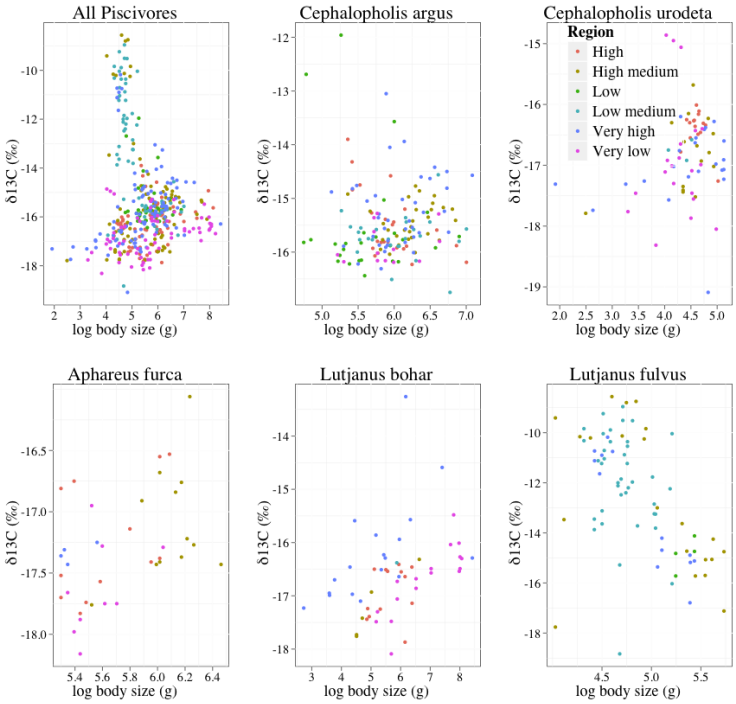

Figure 7: Log body size (g) vs. δ13C for all focal piscivore species combined and separately, by fishing pressure region.

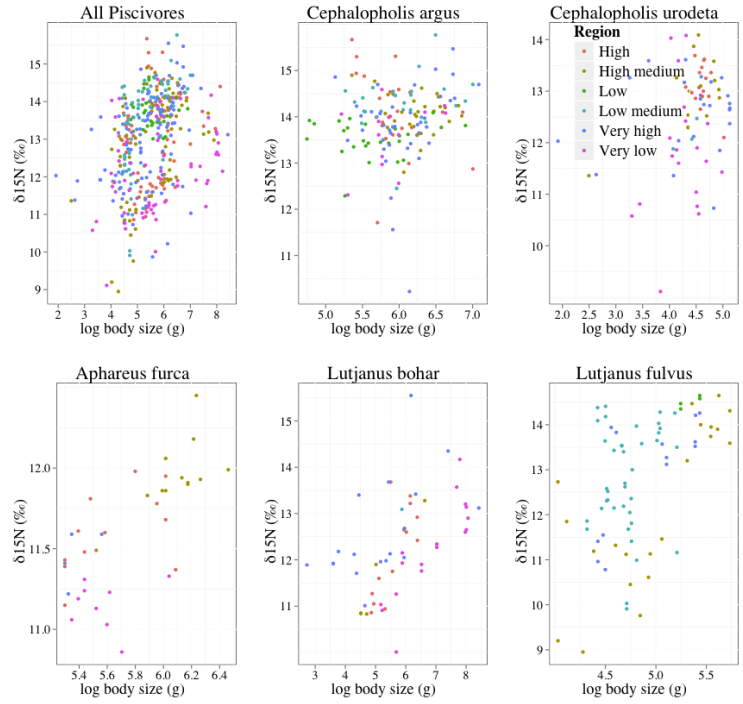

Figure 8: Log body size (g) vs. δ15N for all focal piscivore species combined and separately, by fishing pressure region.

Table 10: ANOVA table for multiple regressions on each focal species combined and separately testing the effects of chlorophyll-*a*, body size (weight in grams), and fishing pressure region on δ13C, Df= degrees of freedom, Sum Sq= sum of squares, Mean Sq=mean squares.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
All species	Region	5	395.686	79.137	26.745	0.000
	Chlmean	1	0.095	0.095	0.032	0.858
	weight (g)	1	0.157	0.157	0.053	0.818
	Residuals	424	1230.916	2.959		
		Df	Sum Sq	Mean Sq	F value	Pr(>F)
C. argus	Region	5	5.692	1.138	2.567	0.029
	Chlmean	1	0.016	0.016	0.035	0.851
	weight (g)	1	0.002	0.002	0.003	0.953
	Residuals	156	65.629	0.443		
C. urodeta	Region	4	3.859	0.965	2.152	0.084
	Chlmean	1	2.079	2.079	4.637	0.348
	weight (g)	1	0.077	0.077	0.172	0.679
	Residuals	75	30.489	0.448		
		Df	Sum Sq	Mean Sq	F value	Pr(>F)
A. furca	Region	3	1.547	0.516	3.042	0.044
	Chlmean	1	0.001	0.001	0.006	0.939
	weight (g)	1	0.760	0.760	4.481	0.042
	Residuals	37	5.256	0.170		
L. bohar	Region	4	6.735	1.684	3.640	0.012
	Chlmean	1	1.009	1.009	2.180	0.146
	weight (g)	1	4.573	4.573	9.885	0.003
	Residuals	56	21.744	0.463		
L. fulvus	Region	3	42.155	14.052	3.596	0.018
	Chlmean	1	8.163	8.163	2.089	0.153
	weight (g)	1	114.868	114.868	29.393	0.000
	Residuals	77	277.466	3.908		

Table 11: ANOVA table for multiple regressions on each focal species combined and separately, testing the effects of chlorophyll-*a*, body size (weight in grams), and fishing pressure region on δ15N, Df= degrees of freedom, Sum Sq= sum of squares, Mean Sq=mean squares.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
All species	Region	5	61.256	12.251	9.107	0.000
	chlmean	1	6.832	6.832	5.079	0.055
	weight (g)	1	31.264	31.264	23.241	0.000
	Residuals	424	559.606	1.345		
		Df	Sum Sq	Mean Sq	F value	Pr(>F)
C. argus	Region	5	10.906	2.181	4.504	0.001
	chlmean	1	0.226	0.226	0.466	0.496
	weight (g)	1	0.907	0.907	1.872	0.173
	Residuals	156	71.667	0.484		
C. urodeta	Region	4	17.127	4.282	5.872	0.000
	chlmean	1	0.184	0.184	0.252	0.617
	weight (g)	1	0.663	0.663	0.909	0.344
	Residuals	75	49.582	0.729		
		Df	Sum Sq	Mean Sq	F value	Pr(>F)
A. furca	Region	3	3.349	1.116	31.251	0.000
	chlmean	1	0.072	0.072	2.018	0.165
	weight (g)	1	0.351	0.351	9.836	0.004
	Residuals	37	1.107	0.036		
L. bohar	Region	4	5.587	1.397	1.650	0.177
	chlmean	1	0.369	0.369	0.436	0.512
	weight (g)	1	14.728	14.728	17.401	0.000
	Residuals	56	39.779	0.846		
L. fulvus	Region	3	19.956	6.652	5.426	0.002
	chlmean	1	5.759	5.759	4.698	0.034
	weight (g)	1	46.314	46.314	37.780	0.000
	Residuals	77	87.039	1.226		

The results of analyses on the effect of body size, both weight and standard length (both at logarithmic scales), varied by species and stable isotope, with body size having a significant effect ($p<0.05$) on almost all species, as shown by ANOVA results of linear mixed effects models (Table 12) and univariate linear regressions (plots for both standard length and weight in Appendix C). Fish weight (in grams) had a significant positive effect on δ15N of piscivores at the functional group level and individually for all focal species except *Cephalopholis argus* (Table 12). As for δ13C, no significant effect was found for body size at the functional group level, or for *Cephalopholis argus* or *Cephalopholis urodeta,* but it had a significant effect for *Lutjanus bohar, Lutjanus fulvus* and *Aphareus furca* (Table 12, Appendix C Body Size Effects). Therefore, no significant effect of body size was found for any stable isotope for *Cephalopholis argus,* but it did

have an effect on at least one of two isotopes for all other species and at the functional group level, justifying the body size control measures taken (as described above under "Mechanism 1 ").

Table 12: Linear mixed effects model results for the piscivore functional group and each focal species, assessing the effect of body size (log weight in grams) on δ13C and δ15N. Species was used as the random effect at the functional group level and site was used at the species level (*=significant effect, Df= degress of freedom, ΔAIC= difference in AIC between a given model and the model and the model with the minimum AIC).

	p-value	AIC	ΔAIC	Df	Chisq
Taxon			δ13C		
Piscivore functional group	0.5342	1397	1.5	1	0.4756
Aphareus furca	0.0153*	45.109	4.542	1	6.5421
Cephalopholis argus	0.7686	326.65	1.91	1	0.0866
Cephalopholis urodeta	0.061	141.18	1.57	1	3.5637
Lutjanus bohar	6.544e-06*	113.52	18.32	1	20.322
Lutjanus fulvus	2.213e-06*	333.73	20.4	1	22.401
			δ15N		
Piscivore functional group	4.203e-12*	1165.2	47.1	1	49.06
Aphareus furca	0.0073*	1.7119	6.711	1	8.7109
Cephalopholis argus	0.1954	330.59	0.33	1	1.6767
Cephalopholis urodeta	0.0083*	178.06	4.96	1	6.9608
Lutjanus bohar	9.777e-07*	141.51	21.97	1	23.972
Lutjanus fulvus	1.515e-05*	251.34	17.68	1	18.719

Objective 1: Niche differentiation of piscivorous reef fish

To evaluate the extent of isotopic "niche differentiation" based on known body size differences and dietary information amongst the five focal species, I first plotted exploratory SIBER ellipses on all samples collected, with all regions combined, and then split by region (using only species that were collected in each region), looking for trends in individual δ13C and δ15N values and Standard Ellipse Area (SEA), thought to be reflective of primary production source, trophic position, and niche width, respectively (Figure 9). Initial observations showed *Lutjanus fulvus* (light blue points and ellipses, Figure 9), at least in the regions where it was collected (*i.e.* Very high and High medium fishing pressure), having a seemingly larger SEA in isotopic niche space, and more enriched (less negative) δ13C values. *Cephalopholis argus* (red points and ellipses,

Figure 9), which was collected in all analysed regions, appeared to be generally more enriched in δ15N than all other focal species, and slightly more enriched in δ13C than *C. urodeta* (green dots and ellipses), *L. bohar* (dark blue), and *A. furca* (black). These latter three species (*C. urodeta*, *L. bohar*, *A. furca*) all showed no signs of distinctly different individual δ13C or δ15N values, however *A. furca* appeared to have smaller SEA areas in each region.

Taken together, these initial observations suggest distinct dietary niches for *L. fulvus* and *C. argus* relative to the other sampled species, while *L. bohar*, *C. urodeta*, and *A. furca* showed no signs of being significantly more or less enriched in either stable isotope value than each other, implying higher levels of niche redundancy (isotopic niche overlap). In terms of niche width metrics, these observations imply that *L. fulvus* has the largest isotopic niche width, and *A. furca* has the narrowest width.

Following 1000 iterations of re-sampling body size-controlled samples and bootstrapping mean, standard deviation, median, minimum and maximum values of δ13C and δ15N values, as well as SEA, SEAc, TA, CD, MNND, and SDNND, these observed trends from the exploratory plots were generally upheld (Figure 10, Table 13, Table 14).

Firstly in terms of mean δ13C, in the regions where *L. fulvus* was sampled and analysed (Very high and High medium), it had a significantly more enriched mean δ13C than the other species in the respective region (Very high: mean -13.388, High medium: mean -12.408; Table 13), with the exception of *C. argus* in the Very high region (Mean -15.251, 92/1000 iterations with significantly larger mean; Table 14). In the High and Very low regions, *C. argus* was found to have significantly more enriched δ13C values than all other species except *C. urodeta* in the Very low region, in that at least 900 iterations were found to have significant results ($p<=0.05$) for all pair-wise comparisons between *C. argus* and all other species (Table 14). All these trends were generally reflected in median, minimum and maximum δ13C values, however no ANOVAs were performed on these metrics (Table 13).

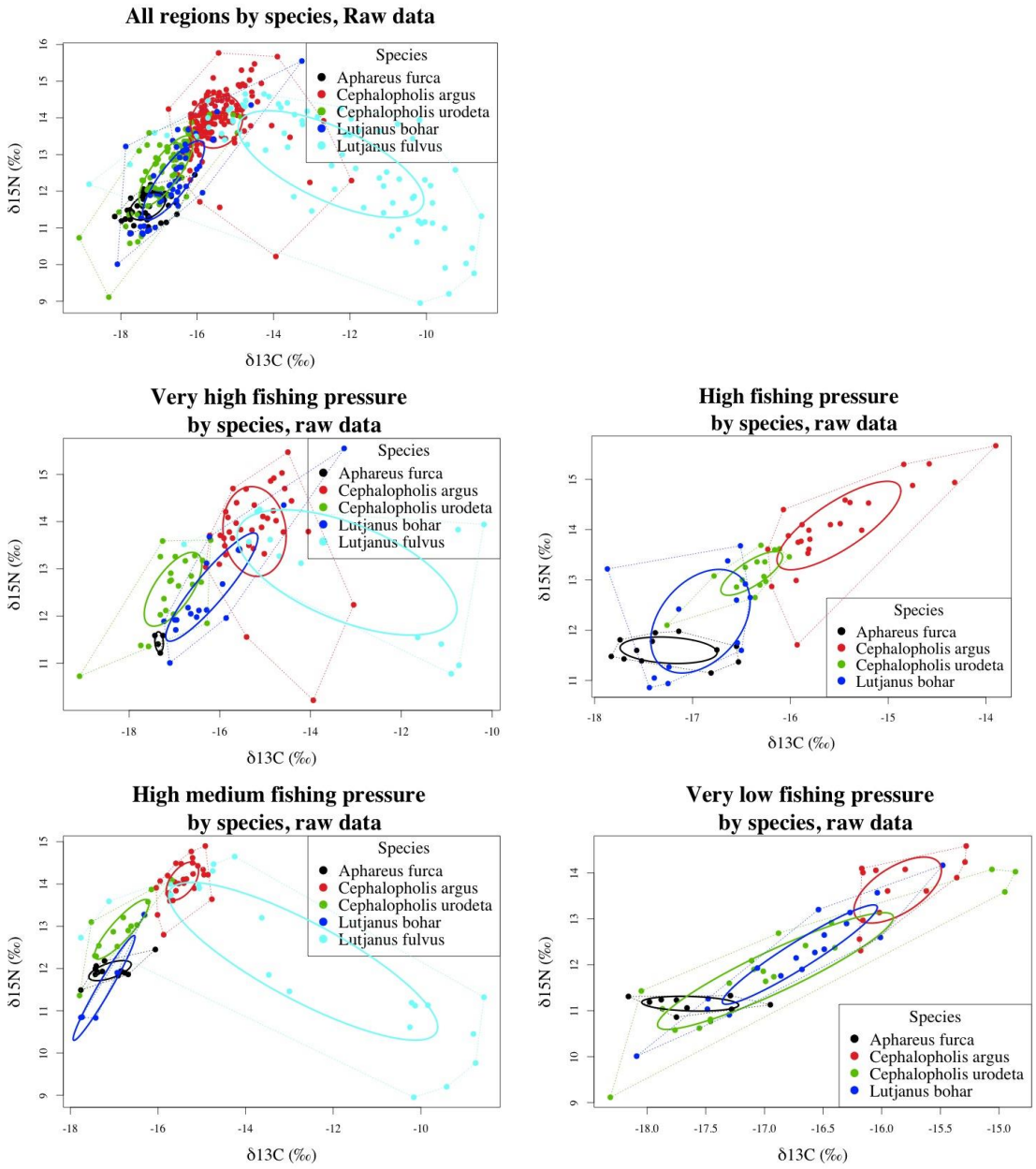

Figure 9: Standard ellipse areas (SEA) of each focal piscivore species for all fishing pressure regions combined using all samples, and by fishing pressure region separately, without body size corrections. Ellipses drawn (solid lines) portray the best estimate based on 10^4 posterior draws using SIBER. Dotted lines portray convex hull used for calculating total area.

In terms of mean δ15N values, there were not as clearly distinct results, with more similarity between species across the regions. In the Very high region, no significant differences were detected, however in the other three regions that were analysed (High,

High medium, and Very low), *C. argus* δ15N mean values were the most enriched out of the other species, as was seen in the exploratory plots, however *C. urodeta* also shared this status in the High and High medium region (Table 14, Figure 10). As with δ13C, these trends were consistent in median, minimum values of δ15N, however no ANOVAs were run (Table 13).

In terms of SEAs of each species across the four regions analysed, as was observed in the exploratory plots, *L. fulvus* was shown to have a significantly greater area in almost every iteration in the two regions where the species was included in analyses (Very high and High medium, Figure 10, Table 14). In contrast, in the regions where *L. fulvus* was not included, there were no clear trends, with no significant differences among species at all in the High region, and significantly greater SEA for *C. urodeta* in the Very low region.

Finally, analyzing the additional Layman metrics that were bootstrapped (TA, CD, MNND and SDNND), all mean metrics for each species in each region were found to be significantly different from each other after 1000 iterations ($p<0.05$, ANOVA followed by Tukey HSD). That is, calculated metrics for each species in each region were all significantly different from one another (Table 13). For TA and CD, which are both measures of niche width (the latter being more robust as it is less sensitive to outliers), for each region, they ranked in the same order of species as SEA, which agrees with the above findings. MNND and SDNND, however, which represent the density and evenness of species packing (a measure of trophic redundancy), respectively, did not follow the niche width metrics as closely in each region. Generally, species with larger niche width metrics also showed greater levels of trophic redundancy via MNND and SDNND, but the trend was not consistent across all regions (Table 13).

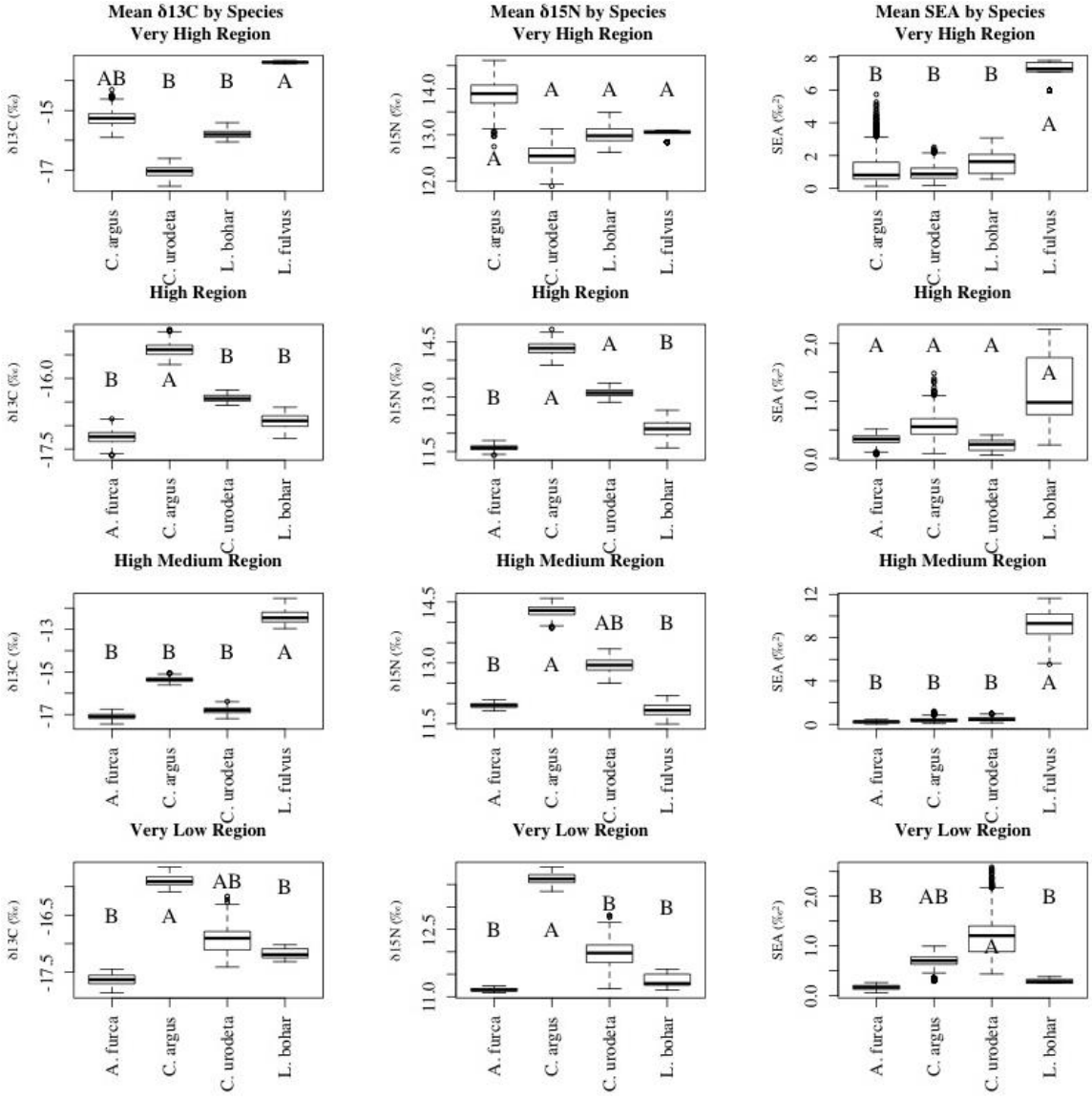

Figure 10: Distribution of mean δ13C, δ15N, and SEA values from 1000 iterations using body size controlled samples for focal species in each region with samples. Groups with the same letter represent mean values that are not significantly different from each other in at least 90% of the iterations. Note that species with larger variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples.

Table 13: Stable isotope analysis results by species for all regions with samples of at least four focal species, including niche width metrics (SEA=Standard Ellipse Area, SEAc=Standard Ellipse Area corrected for small sample size, TA=Total Area of convex hull), and Layman metrics (CD=Centroid Distance, MNND=Mean Nearest Neighbour Distance, SDNND=Standard Deviation of Nearest Neighbour Distance).

	Region	Very high				High			
	Species	*C. argus*	*C. urodeta*	*L. bohar*	*L. fulvus*	*A. furca*	*C. argus*	*C. urodeta*	*L. bohar*
δ13C (‰)	Mean	-15.251	-17.048	-15.794	-13.388	-17.245	-15.388	-16.421	-16.914
	SD	0.613	0.574	1.347	2.307	0.467	0.708	0.277	0.470
	Median	-15.281	-17.031	-16.143	-14.450	-17.317	-15.570	-16.338	-16.833
	Min	-16.004	-18.089	-16.998	-16.790	-17.763	-16.132	-17.003	-17.582
	Max	-14.383	-16.319	-13.260	-10.300	-16.602	-14.284	-16.146	-16.449
	Range	1.621	1.770	3.738	6.490	1.161	1.848	0.857	1.132
δ15N (‰)	Mean	13.868	12.546	13.003	13.022	11.605	14.324	13.109	12.124
	SD	0.764	0.743	1.438	1.220	0.244	0.687	0.413	1.010
	Median	13.917	12.661	12.619	13.512	11.598	14.182	13.112	12.097
	Min	12.710	11.280	11.699	10.817	11.278	13.564	12.381	10.951
	Max	14.796	13.470	15.550	14.260	11.929	15.366	13.644	13.426
	Range	2.086	2.190	3.851	3.443	0.650	1.802	1.263	2.474
SIBER	SEA (‰²)	1.219	0.965	1.583	7.143	0.333	0.580	0.233	1.193
	SEAc (‰²)	1.524	1.126	1.979	7.857	0.416	0.725	0.272	1.491
Layman	TA (‰²)	1.490	1.605	2.011	12.140	0.425	0.764	0.378	1.433
	CD	0.801	0.749	1.438	2.365	0.457	0.793	0.388	0.956
	MNND	0.602	0.483	0.953	0.528	0.301	0.491	0.248	0.521
	SDNND	0.452	0.353	1.123	0.426	0.129	0.289	0.217	0.363
	Region	High medium				Very low			
	Species	*A. furca*	*C. argus*	*C. urodeta*	*L. fulvus*	*A. furca*	*C. argus*	*C. urodeta*	*L. bohar*
δ13C (‰)	Mean	-17.091	-15.357	-16.802	-12.408	-17.629	-15.900	-16.926	-17.170
	SD	0.447	0.376	0.555	2.904	0.380	0.339	0.861	0.433
	Median	-17.169	-15.333	-16.781	-12.142	-17.683	-16.017	-16.974	-17.147
	Min	-17.588	-15.864	-17.611	-17.346	-18.086	-16.183	-18.109	-17.791
	Max	-16.392	-14.893	-15.973	-8.809	-17.063	-15.365	-15.492	-16.680
	Range	1.196	0.971	1.639	8.537	1.024	0.818	2.617	1.111
δ15N (‰)	Mean	11.952	14.276	12.942	11.840	11.153	13.627	11.956	11.377
	SD	0.215	0.533	0.616	1.856	0.149	0.788	1.014	0.618
	Median	11.925	14.263	12.971	11.473	11.177	13.940	11.883	11.459
	Min	11.671	13.527	11.904	9.002	10.917	12.310	10.425	10.456
	Max	12.286	14.900	13.854	14.488	11.318	14.302	13.550	12.034
	Range	0.615	1.373	1.950	5.487	0.401	1.992	3.125	1.577
SIBER	SEA (‰²)	0.223	0.410	0.486	9.095	0.167	0.677	1.224	0.291
	SEAc (‰²)	0.279	0.512	0.567	10.004	0.208	0.847	1.428	0.363
Layman	TA (‰²)	0.274	0.531	0.780	18.227	0.216	0.734	2.068	0.390
	CD	0.391	0.544	0.650	3.083	0.338	0.696	1.044	0.622
	MNND	0.256	0.375	0.399	0.791	0.253	0.409	0.581	0.312
	SDNND	0.226	0.173	0.253	0.625	0.104	0.325	0.432	0.205

Table 14: Testing effects of fishing on interspecific isotope patterns. Objective one bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise species comparison within each region. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between species for each comparison.

		δ13C		δ15N		SEA	
Region	Species comparison	mean p-value	N<= 0.05/1000	mean p-value	N<= 0.05/1000	mean p-value	0.05>N>0.95
Very high	*C. urodeta-C. argus*	0.211	32	0.207	260	0.501	0
	L. bohar-C. argus	0.907	0	0.531	2	0.500	0
	L. bohar-C. urodeta	0.486	0	0.796	0	0.500	0
	L. fulvus-C. argus	0.137	92	0.448	9	0.042	915
	L. fulvus-C. urodeta	0.000	1000	0.724	0	0.003	1000
	L. fulvus-L. bohar	0.030	949	0.975	0	0.038	970
High	*C. argus-A. furca*	0.000	1000	0.000	1000	0.674	125
	C. urodeta-A. furca	0.057	642	0.003	1000	0.480	34
	C. urodeta-C. argus	0.008	994	0.022	893	0.500	0
	L. bohar-A. furca	0.630	26	0.539	11	0.746	0
	L. bohar-C. argus	0.000	1000	0.000	1000	0.067	269
	L. bohar-C. urodeta	0.329	75	0.095	538	0.066	267
High medium	*C. argus-A. furca*	0.387	0	0.018	975	0.500	0
	C. urodeta-A. furca	0.979	0	0.458	0	0.597	0
	C. urodeta-C. argus	0.490	0	0.238	14	0.174	210
	L. fulvus-A. furca	0.000	1000	0.986	0	0.976	1000
	L. fulvus-C. argus	0.025	915	0.004	1000	0.975	999
	L. fulvus-C. urodeta	0.000	1000	0.253	20	0.977	985
Very low	*C. argus-A. furca*	0.001	1000	0.000	1000	0.053	427
	C. urodeta-A. furca	0.209	184	0.304	182	0.982	1000
	C. urodeta-C. argus	0.078	630	0.012	937	0.499	0
	L. bohar-A. furca	0.522	17	0.893	0	0.759	0
	L. bohar-C. argus	0.012	965	0.001	1000	0.053	427
	L. bohar-C. urodeta	0.779	1	0.501	57	0.982	1000

To visualize the results of these analyses on isotopic niche differentiation among species in each region, I plotted sample ellipses using an ideal set of samples from the 1000 iterations that best represent the overall bootstrapped results (Figure 11). These ellipses clearly show the more enriched δ13C values of *L. fulvus* (Very high and High medium regions), the more enriched δ13C values of *C. argus* (High and Very low

regions), the more enriched δ15N values of the *Cephalopholis* genus in all analysed regions, and the significantly larger SEA of *L. fulvus* in the regions where it was sampled.

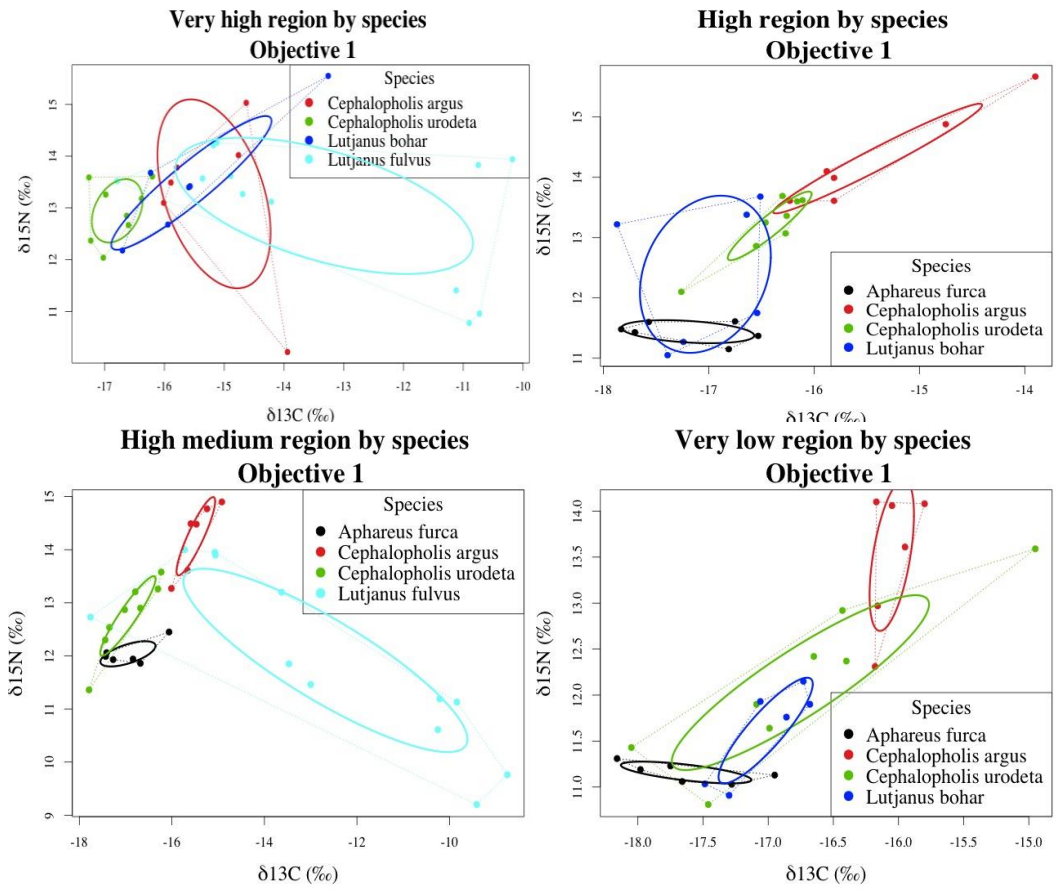

Figure 11: Sample Standard Ellipse Areas (SEA) of each focal piscivore species by region with sufficient samples, corrected for body size. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

Objective 2: Stable isotope metrics across fishing pressure regions

Prior to any body size controlling or sample selections for bootstrapping to assess the effects of fishing on each of the five focal species, I first plotted exploratory SIBER ellipses for all piscivore samples collected, looking for general trends in δ13C, δ15N, and SEA as was done above for Objective 1. These plots, solely for visualization purposes, are split by family; Serranidae (*C. argus* and *C. urodeta*; Figure 12) and Lutjanidae (*A. furca, L. bohar,* and *L. fulvus*; Figure 13). For each species, only regions with large

enough sample sizes were used, as described above for sample selections in Objective 1 and Mechanism 1 and 2 analyses.

Analysing Serranidae raw data plots first, no clear trends in shifts of δ13C or δ15N are clear in either *C. argus* or *C. urodeta* among the various fishing pressure regions that were sampled. However, it does appear that the Very high region has a larger SEA than other regions for *C. argus*, and for *C. urodeta*, the Very low region seems to be largest in this metric (Figure 12).

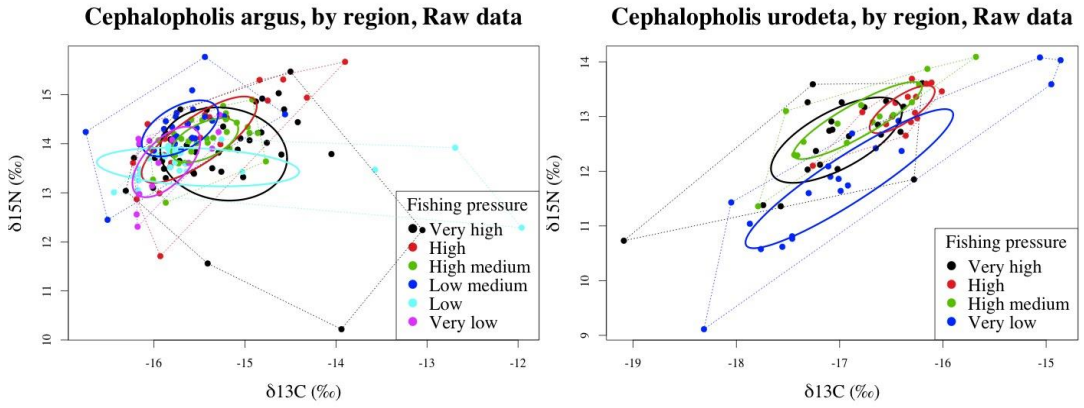

Figure 12: Standard ellipse areas (SEA) by region for the two Serranidae species using all samples. Ellipses drawn (solid lines) portray the best estimate based on 10^4 posterior draws using SIBER. Dotted lines portray convex hull used for calculating total area.

As for Lutjanidae exploratory plots, potential trends vary by species (Figure 13). *A. furca*, while showing consistent SEAs, appears to have distinctly lower δ15N values in the Very low region, with the other two sampled regions (High and High medium) being more enriched. No major differences in δ13C are evident, so any niche differentiation across regions would be from δ15N values. Finally, for the two *Lutjanus* species, no changes among regions are evident for individual δ13C or δ15N, however the exploratory plots suggest that the lowest fishing pressure sampled for both species (green ellipses in both plots) have smaller SEAs, suggestive of more narrow niche widths.

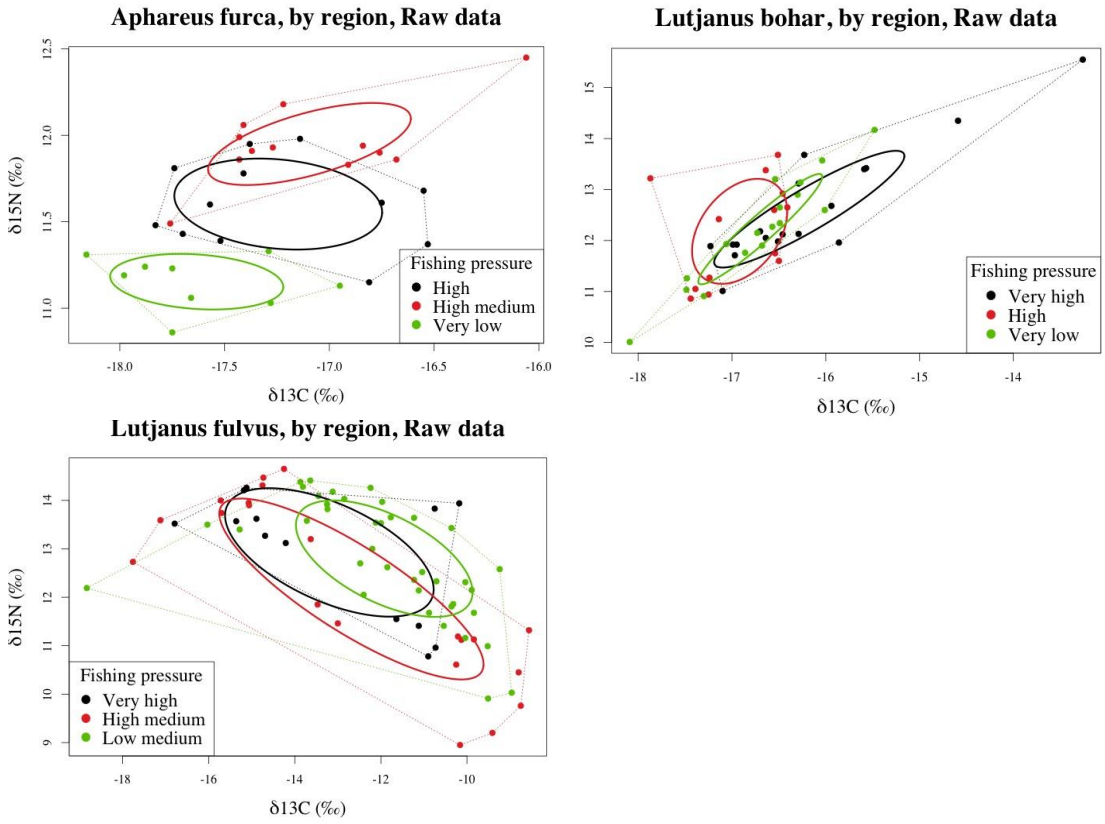

Figure 13: Standard ellipse areas (SEA) by region for the three Lutjanidae species using all samples. Ellipses drawn (solid lines) portray the best estimate based on 10^4 posterior draws using SIBER. Dotted lines portray convex hull used for calculating total area.

Mechanism 1: Individual-level changes

Following 1000 iterations of re-sampling body size controlled samples as per the previously described methods for Mechanism 1 and bootstrapping mean, standard deviation, median, minimum and maximum values of δ13C and δ15N values, as well as

SEA, SEAc, TA, CD, MNND, and SDNND, most of the initially observed trends (Figure 12, Figure 13) were upheld (Figure 14, Figure 15). Specifically, there were no significant differences in terms of δ13C or δ15N mean values for either of the Serranidae species (*C. argus, C. urodeta*) across fishing regions (Figure 14, Table 15). Very few significant results were found from the 1000 ANOVAs that were run, the largest numbers being associated with comparisons between the most and the least disturbed regions (Very high-Very low), with 120 p-values less than 0.05 for δ13C for *C. argus* and 147 significant p-values for δ15N for *C. urodeta* (Table 16). Comparing SEA among regions for these two species, no significant difference was found for *C. argus*, and the High and High medium regions for *C. urodeta* had significantly lower SEAs than the Very high and Very low regions, implying regional differences in this niche width metric, but not necessarily due to fishing pressure.

Considering the three Lutjanidae family species (*A. furca, L. bohar,* and *L. fulvus*), there were also not very many significant differences amongst these regions' metrics, however the initially observed trends from the raw data do hold true following body size controlling and bootstrapping all analyses (Figure 15, Table 15, Table 17). Firstly, as was seen in the earlier plots, *A. furca*'s mean δ13C showed no significant difference among the three regions in which it was sampled (High, High medium, and Very low), however its mean δ15N values were significantly less enriched in the Very low region (936 and 1000 iterations with a significant result for High and High medium, respectively), and the SEA for the Very low region was significantly smaller then the High (1000 significant results), and High medium (999 significant results).

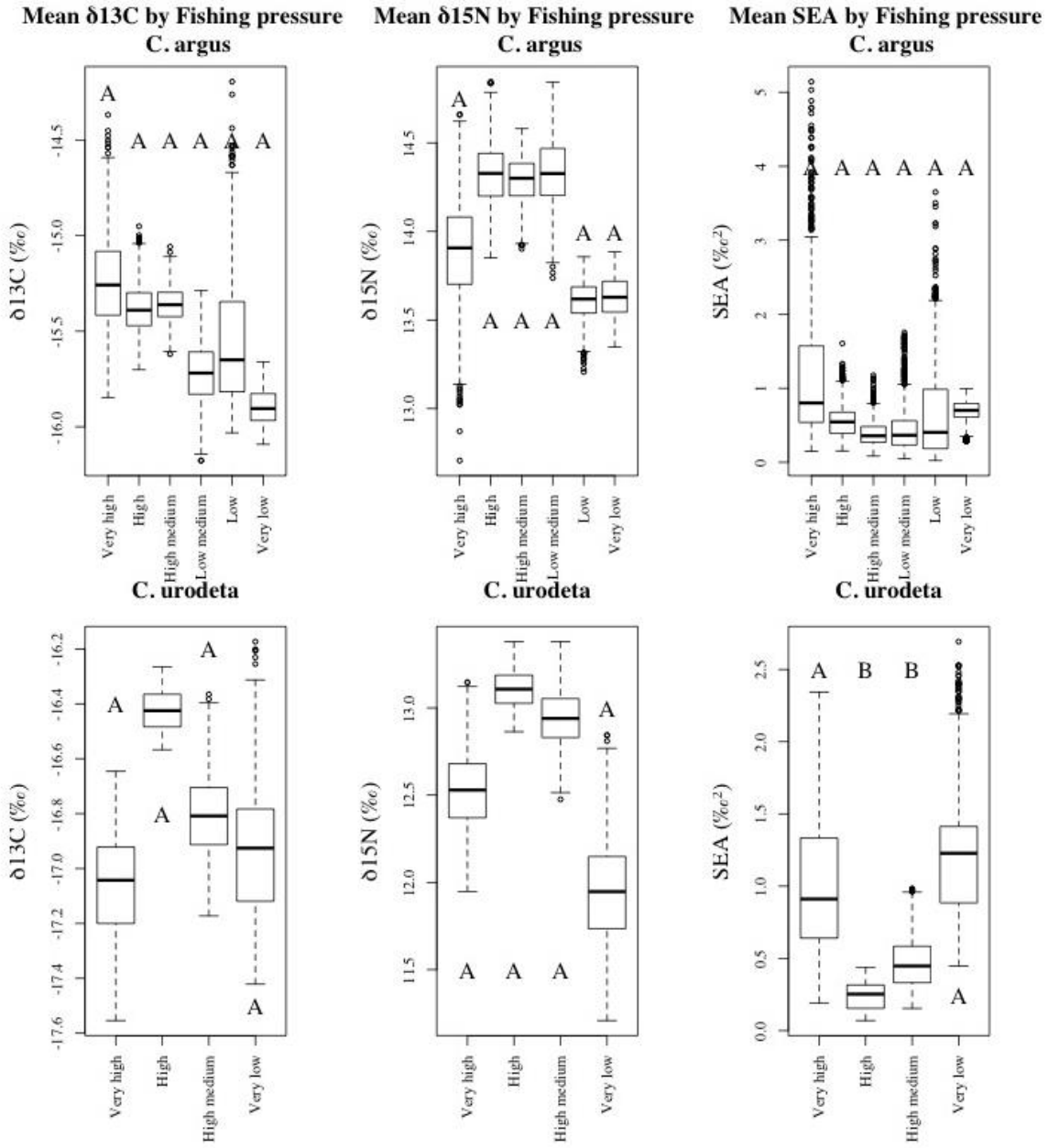

Figure 14: Mean δ13C, δ15N, and SEA values of Serranidae species from 1000 iterations of analyses by fishing pressure region for mechanism 1. Groups with the same letter had fewer than 900 iterations with significant results separating it from other groups (ANOVA and Tukey HSD for δ13C and δ15N and SIBER analysis for SEA. Note that regions with more variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples themselves.

The same was true for *L. bohar*, where its Very low SEA was significantly lower than the Very high region 931 times and 948 times for High region. As for mean δ13C values for *L. bohar*, the Very high region was significantly more enriched than the High and Very low regions for 912 and 901 iterations, respectively. Finally, *L. fulvus* showed no significant differences in any of the bootstrapped metrics (Figure 15, Table 15, Table 17).

Finally, analyzing the additional Layman metrics that were bootstrapped (TA, CD, MNND and SDNND), all mean metrics for each species in each region were found to be significantly different from each other after 1000 iterations ($p<0.05$, ANOVA followed by TukeyHSD test), with the exception of certain pair-wise regional comparisons for *C. argus* (MNND for Low medium-Very low: p=0.804, SDNND for Low medium-Very low: p=0.567, TA for Low-High p=0.538, Very low-High p=0.999, and Very low-Low p=0.362, and CD for Very high-High: p=0.983) and *L. fulvus* (MNND for High medium-Low medium: p=0.995). That is, almost all calculated metrics for each species in each region did not vary substantially amongst iterations, and thus almost all means were different from one another (Table 15). For TA and CD, which are both measures of niche width/trophic diversity (the latter being more robust as it is less sensitive to outliers), for each species, they ranked in the same regional order as SEA, which agrees with the above findings. MNND and SDNND, however, which represent the density and evenness of species packing (a measure of trophic redundancy), respectively, did not follow the niche width metrics as closely in each region. Generally, regions with larger niche width metrics also showed greater levels of trophic redundancy via MNND and SDNND, but the trend was not consistent across all species (Table 15).

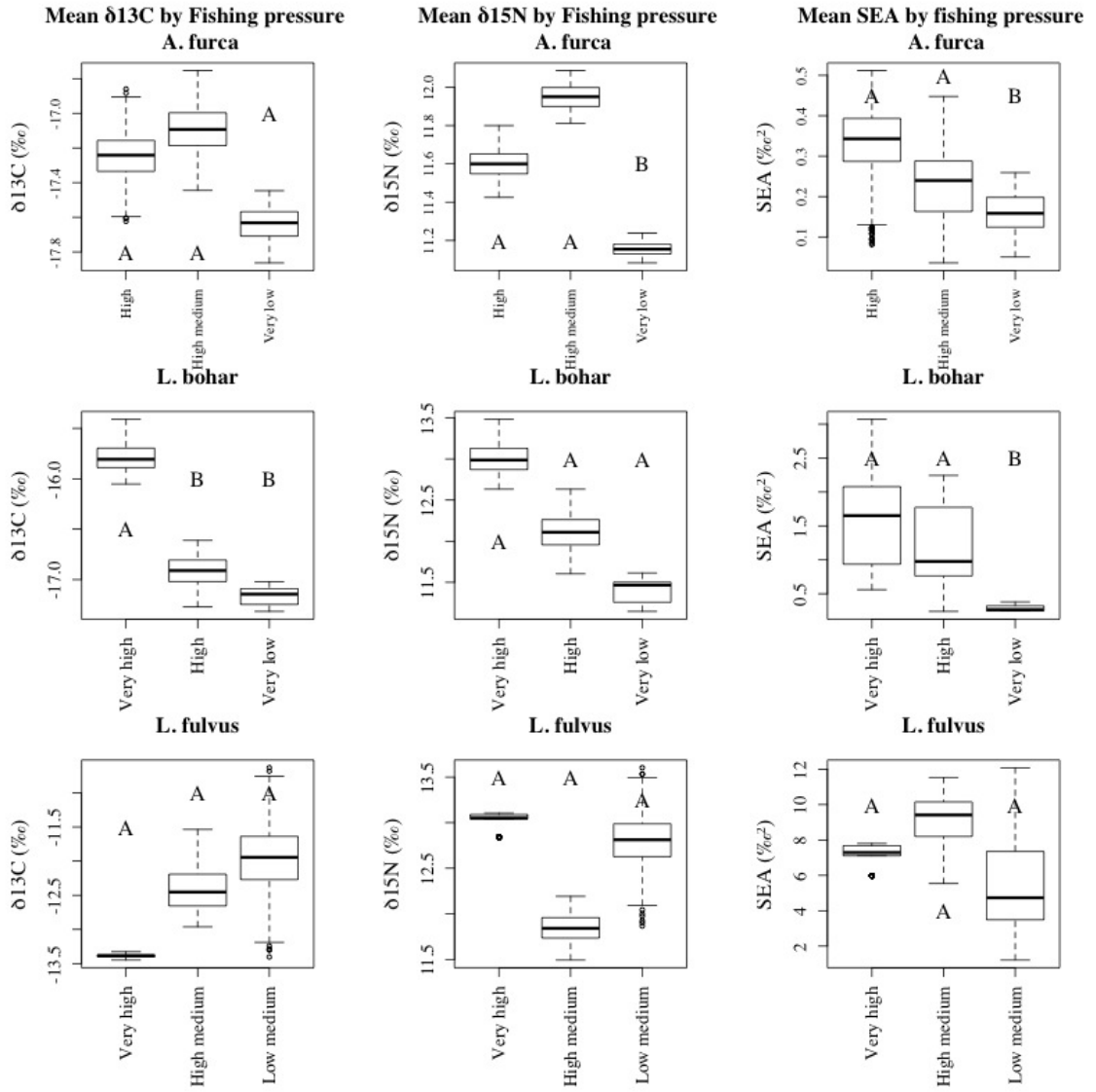

Figure 15: Mean δ13C, δ15N, and SEA values of Lutjanidae species from 1000 iterations of analyses by fishing pressure region for mechanism 1. Groups with the same letter had fewer than 900 iterations with significant results separating it from other groups (ANOVA and TukeyHSD for δ13C and δ15N and SIBER analysis for SEA. Note that regions with more variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples themselves.

Table 15: Stable isotope analysis summary statistics by fishing pressure region, grouped by species for comparison across fishing pressure gradient. Includes niche width metrics (SEA=Standard Ellipse Area, SEAc=Standard Ellipse Area corrected for small sample size, TA=Total Area of convex hull), and Layman metrics (CD=Centroid Distance, MNND=Mean Nearest Neighbour Distance, SDNND=Standard Deviation of Nearest Neighbour Distance), along with mean, SD, median, minimum and maximum δ13C and δ15N values.

	Family	Serranidae									
	Species	*Cephalopholis argus*						*Cephalopholis urodeta*			
	Region	Very high	High	High medium	Low medium	Low	Very low	Very high	High	High medium	Very low
δ13C (‰)	Mean	-15.242	-15.383	-15.359	-15.719	-15.548	-15.898	-17.059	-16.423	-16.805	-16.932
	SD	0.615	0.711	0.377	0.399	0.735	0.340	0.594	0.279	0.554	0.862
	Median	-15.264	-15.566	-15.333	-15.706	-15.780	-16.016	-17.027	-16.340	-16.784	-16.976
	Min	-16.001	-16.130	-15.869	-16.290	-16.158	-16.182	-18.159	-17.011	-17.605	-18.118
	Max	-14.376	-14.274	-14.894	-15.176	-14.213	-15.367	-16.326	-16.147	-15.979	-15.510
	Range	1.624	1.855	0.975	1.114	1.945	0.815	1.834	0.864	1.625	2.608
δ15N (‰)	Mean	13.878	14.322	14.288	14.322	13.608	13.634	12.530	13.108	12.943	11.941
	SD	0.747	0.677	0.524	0.506	0.347	0.784	0.761	0.414	0.613	1.028
	Median	13.922	14.185	14.279	14.306	13.657	13.947	12.651	13.113	12.972	11.881
	Min	12.750	13.570	13.551	13.644	13.083	12.310	11.240	12.380	11.927	10.371
	Max	14.794	15.348	14.900	15.050	14.014	14.299	13.475	13.642	13.854	13.538
	Range	2.043	1.778	1.349	1.406	0.931	1.989	2.235	1.263	1.927	3.168
SIBER	SEA (‰²)	1.197	0.567	0.401	0.477	0.651	0.682	1.010	0.236	0.480	1.246
	SEAc (‰²)	1.496	0.709	0.501	0.596	0.814	0.853	1.178	0.275	0.561	1.454
Layman	TA (‰²)	1.468	0.745	0.517	0.600	0.792	0.738	1.688	0.382	0.771	2.100
	CD	0.795	0.788	0.539	0.511	0.632	0.692	0.770	0.390	0.649	1.052
	MNND	0.598	0.487	0.374	0.421	0.450	0.411	0.493	0.248	0.394	0.584
	SDNND	0.435	0.288	0.164	0.353	0.499	0.334	0.364	0.218	0.249	0.435

	Family	Lutjanidae								
	Species	*Lutjanus bohar*			*Lutjanus fulvus*			*Aphareus furca*		
	Region	Very high	High	Very low	Very high	High medium	Low medium	High	High medium	Very low
δ13C (‰)	Mean	-15.790	-16.924	-17.165	-13.388	-12.396	-11.964	-17.245	-17.095	-17.636
	SD	1.343	0.472	0.429	2.307	2.891	1.981	0.468	0.450	0.378
	Median	-16.141	-16.853	-17.142	-14.450	-12.120	-11.764	-17.318	-17.173	-17.689
	Min	-16.984	-17.584	-17.776	-16.790	-17.305	-16.135	-17.763	-17.597	-18.089
	Max	-13.260	-16.450	-16.680	-10.292	-8.808	-9.390	-16.601	-16.390	-17.074
	Range	3.724	1.133	1.096	6.498	8.497	6.746	1.162	1.207	1.015
δ15N (‰)	Mean	13.010	12.112	11.385	13.026	11.843	12.805	11.601	11.949	11.156
	SD	1.436	1.011	0.613	1.218	1.857	1.171	0.247	0.218	0.144
	Median	12.632	12.082	11.468	13.515	11.473	12.934	11.597	11.924	11.178
	Min	11.699	10.943	10.478	10.817	9.004	10.599	11.268	11.660	10.930
	Max	15.550	13.420	12.038	14.260	14.508	14.284	11.925	12.284	11.318
	Range	3.851	2.477	1.560	3.443	5.505	3.685	0.657	0.624	0.388
SIBER	SEA (‰²)	1.601	1.195	0.288	7.157	9.049	5.462	0.336	0.226	0.160
	SEAc (‰²)	2.001	1.494	0.360	7.873	9.953	6.008	0.419	0.283	0.199
Layman	TA (‰²)	2.039	1.443	0.387	12.152	18.167	11.313	0.428	0.278	0.208
	CD	1.433	0.958	0.617	2.364	3.075	1.903	0.458	0.393	0.334
	MNND	0.955	0.529	0.309	0.527	0.794	0.795	0.303	0.258	0.248
	SDNND	1.122	0.355	0.196	0.421	0.624	0.690	0.132	0.231	0.104

Table 16: The effect of fishing pressure on isotopes within the Serranidae species. Mechanism 1 bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise fishing pressure region comparison within each focal Serranidae species. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between regions for each comparison.

		Serranidae family					
		d13C		d15N		SEA	
Species	Region comparison	mean p-value	N<= 0.05/ 1000	mean p-value	N<= 0.05/ 1000	mean p-value	0.05>N> 0.95
Cephalopholis argus	High medium-High	0.974	0	0.969	0	0.331	0
	Low medium-High	0.815	4	0.950	0	0.342	0
	Low medium-High medium	0.790	4	0.965	0	0.506	0
	Low medium-Low	0.868	1	0.441	53	0.455	27
	Low-High	0.776	6	0.443	43	0.403	2
	Low-High medium	0.750	7	0.474	22	0.550	16
	Very high-High	0.889	4	0.740	7	0.513	17
	Very high-High medium	0.916	1	0.772	4	0.667	65
	Very high-Low	0.655	64	0.823	3	0.601	80
	Very high-Low medium	0.654	57	0.729	9	0.656	80
	Very low-High	0.616	22	0.471	52	0.442	0
	Very low-High medium	0.567	19	0.504	30	0.619	0
	Very low-Low	0.811	8	0.986	0	0.552	1
	Very low-Low medium	0.929	0	0.468	55	0.609	0
	Very low-Very high	0.449	120	0.832	2	0.435	16
Cephalopholis urodeta	High medium-High	0.585	11	0.885	0	0.731	0
	Very high-High	0.255	137	0.472	52	0.988	1000
	Very high-High medium	0.757	3	0.656	13	0.968	905
	Very low-High	0.412	210	0.072	668	0.988	1000
	Very low-High medium	0.766	13	0.154	483	0.968	908
	Very low-Very high	0.792	2	0.449	147	0.500	0

To visualize the results of this first group of analyses on the effects of fishing pressure on stable isotope values of the five focal species, I plotted sample ellipses as above for Objective 1 using an ideal set of samples from the 1000 iterations that best represent the overall bootstrapped results (Serranidae, Figure 16; Lutjanidae, Figure 17). These ellipses show the complete lack of any pattern or trend in *C. argus* or *L. fulvus*, the significantly larger SEA in the Very high and Very low regions, the less enriched δ15N and smaller SEA of *A. furca*, and the more enriched δ13C values in the Very high region for *L. bohar,* as well as its significantly smaller SEA in the Very low region.

Table 17: The effects of fishing pressure on isotopes within the Lutjanidae species. Mechanism 1 bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise fishing pressure region comparison within each focal Lutjanidae species. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between regions for each comparison.

		Lutjanidae family					
		d13C		d15N		SEA	
Species	Region comparison	mean p-value	N<=0 .05/1000	mean p-value	N<=0 .05/1000	mean p-value	0.05>N >0.95
Aphareus furca	High medium-High	0.694	9	0.073	608	0.483	0
	Very low-High	0.357	71	0.015	936	0.975	1000
	Very low-High medium	0.166	226	0.000	1000	0.976	999
Lutjanus bohar	Very high-High	0.045	912	0.397	11	0.910	54
	Very low-High	0.833	0	0.498	0	0.959	948
	Very low-Very high	0.046	901	0.066	481	0.962	931
Lutjanus fulvus	Low medium-High medium	0.775	0	0.317	71	0.490	0
	Very high-High medium	0.585	0	0.144	49	0.500	0
	Very high-Low medium	0.364	22	0.822	0	0.510	0

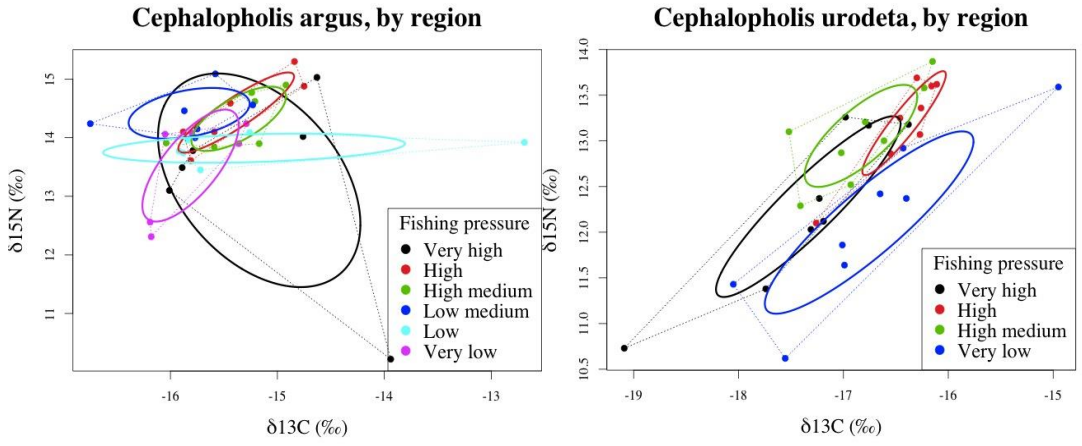

Figure 16: Sample Standard Ellipse Areas (SEA) for the two Serranidae focal piscivore species by fishing pressure region using Mechanism 1 body size controlled data. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

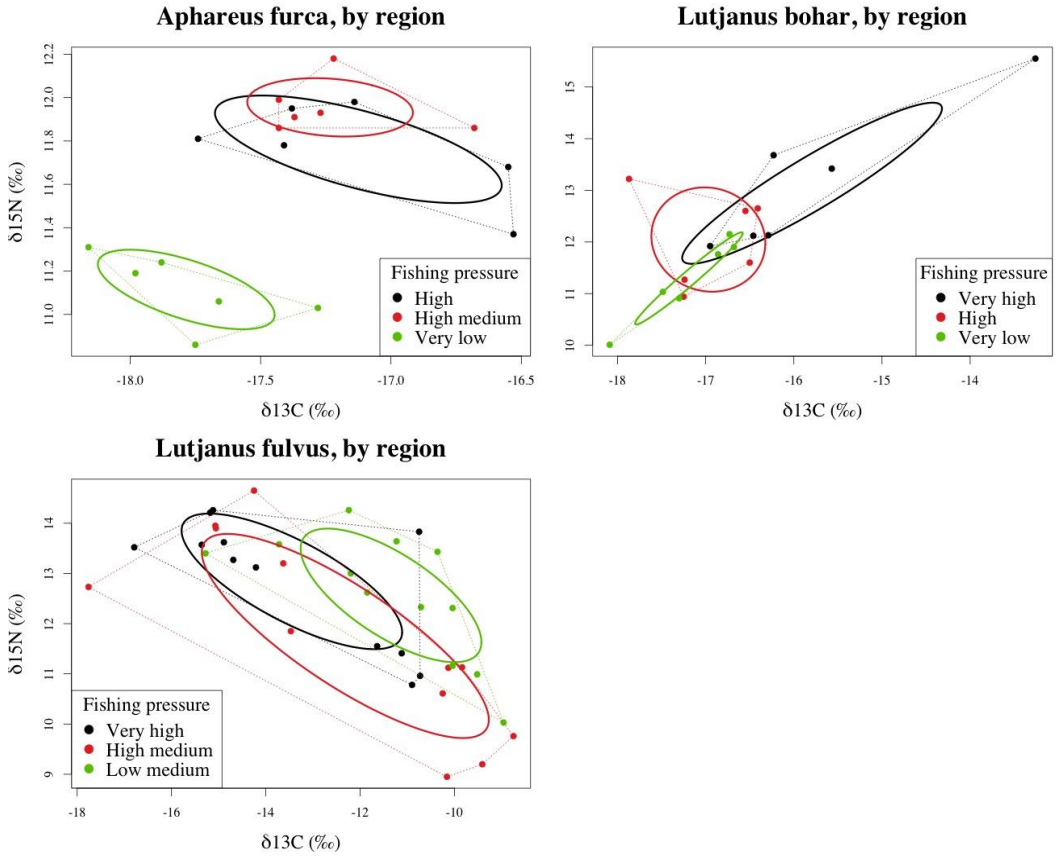

Figure 17: Sample Standard Ellipse Areas (SEA) for the three Lutjanidae focal piscivore species by fishing pressure region using Mechanism 1 body size controlled data. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

Mechanism 2: Population-level changes

All fish survey distribution plots at the piscivore functional group level and the species level for each focal species were analysed (Appendix B Survey Analysis). All analyses were done for each year the survey was performed (2007, 2009, 2011, and 2013), however all reported plots and analyses use combined data for all three years so as to increase the number of observations for each species and region. All observed general

trends were consistent across years analysed. From an exploratory perspective, for all species except *Lutjanus bohar*, relative body size distributions were comparable across the categorized fishing pressure regions, however sample sizes (frequency, or number of observations) were higher in the low fishing pressure region for *Cephalopholis argus* and in the medium fishing pressure region for *Lutjanus fulvus.*

Fishing reduced the average body size of most species. All species except for *C. argus* showed significant differences in mean body size (observed total length in cm, $p<0.05$) amongst the six fishing pressure regions, with the general trend showing significantly larger mean body size for these species in the lower fishing pressure regions, and a significantly smaller body size in the higher fishing pressure regions (Table 18, Figure 18, Figure 19). Specifically, *C. urodeta* observed mean total length was significantly greater in the Low (mean of 14.457 cm) and Very low (mean of 14.696 cm) regions than all other regions ($p<0.05$) (Figure 18). *A. furca* observed mean total length was significantly lowest in the Very high region at 15 cm, compared to its Very low mean value of 28.830 cm (Figure 19). *L. fulvus* had a much lower mean observed total length in the Very high region (12.009 cm) than all the other regions where it was observed (Figure 19). Finally, *L. bohar* observed total length by region had the least dramatic differences, however there were significant differences between them, with the Very low region being the largest at 38.845 cm and significantly larger than the High medium region at 23.889 cm, but neither of these two regions showed significant differences between any of the other regions, Very high, High, Low medium, or Low (Figure 19, Table 18).

Given these observed differences in observed body sizes among regions for all species except *C. argus*, the sample selections for stable isotope analysis outlined in the methods for Mechanism 2, to reflect observed body size distributions in each region, are warranted. These selections, which were re-sampled 1000 times as before and are meant to more accurately portray the isotopic niche of the population, are presented here and compared with results from Mechanism 1, which reflect isotopic niches of the individual.

Table 18: Summary statistics of survey data across fishing pressure regions for the five focal species. Data taken from survey analyses from 2007, 2009, 2011, and 2013. Significance groups for each species with the same letter are not significantly different from each other.

Species	Sig. groups	Region	MeanTL	N observed	SD	SE	Min TL observed	Max TL observed	Range
C. argus	A	Very high	28.000	28	6.242	1.180	15	40	25
	A	High	31.128	47	6.697	0.977	15	45	30
	A	High medium	30.575	40	6.492	1.026	20	50	30
	A	Low medium	30.105	38	6.555	1.063	18	43	25
	A	Low	30.055	55	8.629	1.164	15	60	45
	A	Very low	29.040	150	7.743	0.632	8	50	42
C. urodeta	B	Very high	12.989	461	4.631	0.216	4	25	21
	B	High	12.878	303	3.428	0.197	8	25	17
	B	High medium	12.670	527	4.078	0.178	3	27	24
	B	Low medium	12.474	190	2.958	0.215	5	20	15
	A	Low	14.457	70	4.593	0.549	9	42	33
	A	Very low	14.696	138	2.641	0.225	8	22	14
A. furca	B	Very high	15.000	16	7.958	1.990	10	30	20
	A	High	23.442	52	8.835	1.225	8	40	32
	AB	High medium	27.531	49	5.236	0.748	18	45	27
	A	Low medium	24.758	33	5.674	0.988	10	40	30
	AB	Low	27.161	62	4.046	0.514	20	45	25
	A	Very low	28.830	317	4.586	0.258	13	43	30
L. bohar	AB	Very high	30.714	7	13.363	5.051	20	60	40
	AB	High	31.652	23	19.345	4.034	10	70	60
	B	High medium	23.889	27	8.187	1.576	13	55	42
	AB	Low medium	30.489	47	9.952	1.452	12	55	43
	AB	Low	30.803	61	10.767	1.379	15	65	50
	A	Very low	38.845	485	13.362	0.607	5	80	75
L. fulvus	B	Very high	12.009	114	7.711	0.722	5	40	35
	A	High	26.636	11	2.656	0.801	21	30	9
	A	High medium	23.340	106	5.382	0.523	15	43	28
	A	Low medium	23.684	38	3.618	0.587	14	35	21
	A	Low	24.133	15	2.200	0.568	20	27	7
	A	Very low	22.500	12	5.300	1.530	16	30	14

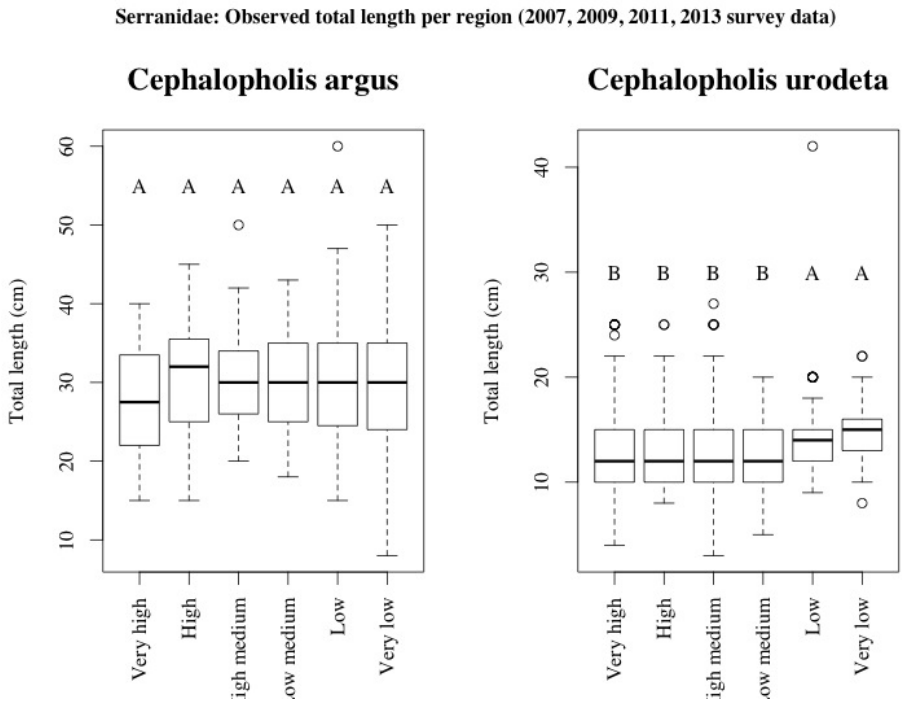

Figure 18: Observed body size (total length in cm) distribution by fishing pressure region for the two Serranidae species. Regions labeled with the same letter are not significantly different from each other.

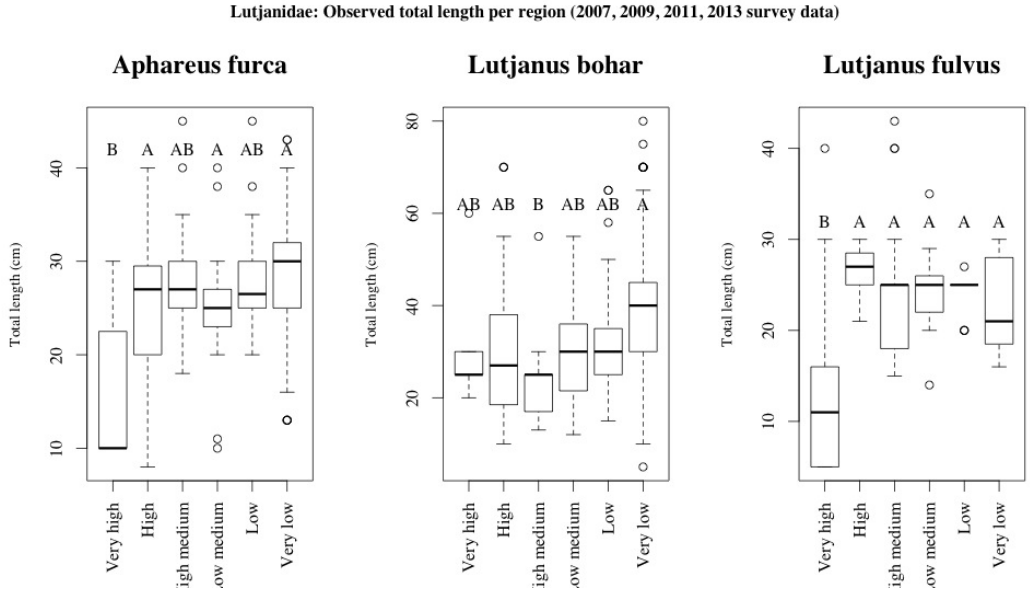

Figure 19: Observed body size (total length in cm) distribution by fishing pressure region for the three Lutjanidae species. Regions labeled with the same letter are not significantly different from each other.

Using Mechanism 2 sample selections, which reflect observed body size distributions of the focal species that have significantly different body sizes across the fishing pressure gradient, the exact same analyses as with Mechanism 1 were performed to see if any patterns changed. Despite theoretically being more representative of the naturally occurring populations of each of these species across the various fishing pressure regions, almost all results and trends from Mechanism 1 remained the same (Figure 20, Figure 21). The one exception was *C. urodeta* which had a significantly less enriched mean δ15N in the Very low region compared to the Very high region once body size differences were taken into account (985/1000 iterations with $p<=0.05$; Table 20) and the High region (926/1000 iterations; Table 20; Figure 20). All other species analyzed returned the same results as before, with *L. fulvus* showing no significant differences between any regions for any metric used, *A. furca* having less enriched mean δ15N values and smaller SEA in the Very low region compared to all other regions uses, and *L. bohar* having more enriched δ13C mean values in the Very high region and smaller SEA in the Very low region (Figure 21, Table 19, Table 21).

Finally, analyzing the additional Layman metrics that were bootstrapped (TA, CD, MNND and SDNND), all mean metrics for each species in each region were found to be significantly different from each other after 1000 iterations ($p<0.05$, ANOVA followed by Tukey HSD), with the exception of one pair-wise regional comparison for *A. furca* (MNND for High medium-Very low: $p=0.804$). That is, almost all calculated metrics for each species in each region were different from one another (Table 19). For TA and CD, which are both measures of niche width/trophic diversity (the latter being more robust as it is less sensitive to outliers), for each species, they ranked in the same regional order as SEA, which agrees with the above findings. MNND and SDNND, however, which represent the density and evenness of species packing (a measure of trophic redundancy), respectively, did not follow the niche width metrics as closely in each region. Generally, regions with larger niche width metrics also showed greater levels of trophic redundancy via MNND and SDNND, but the trend was not consistent across all species (Table 19).

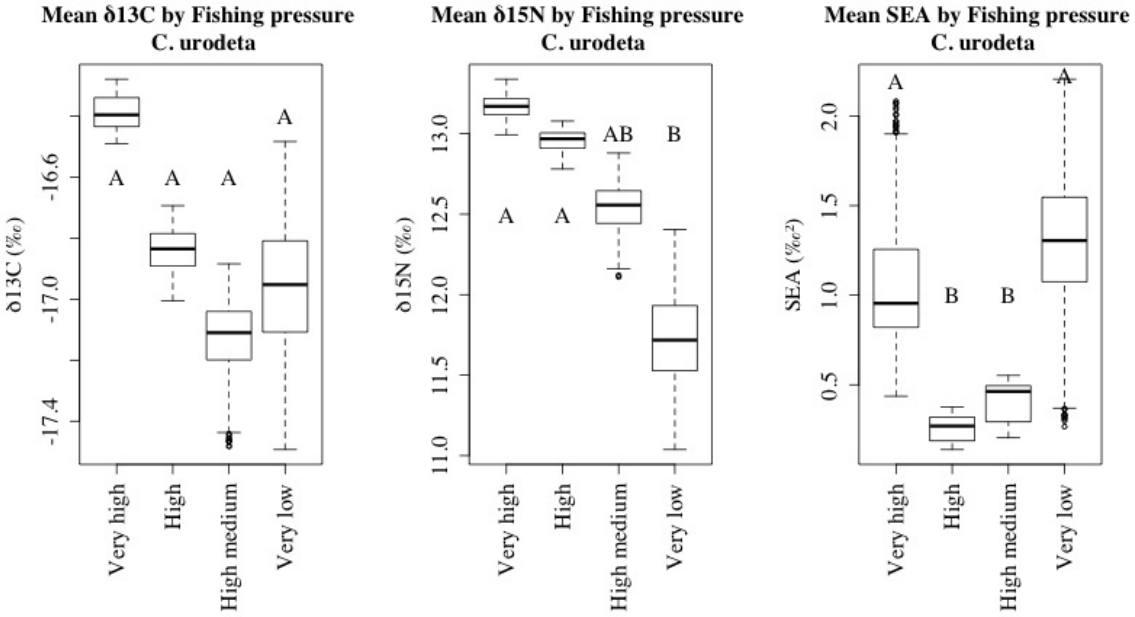

Figure 20: Mean δ13C, δ15N, and SEA values of *C. urodeta* (*C. argus* did not show body size differences across regions, nor any effects of fishing in mechanism 1 and is therefore excluded from this analysis) from 1000 iterations of analyses by fishing pressure region for mechanism 2. Groups with the same letter had fewer than 900 iterations with significant results separating it from other groups (ANOVA and TukeyHSD for δ13C and δ15N and SIBER analysis for SEA. Note that regions with more variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples themselves.

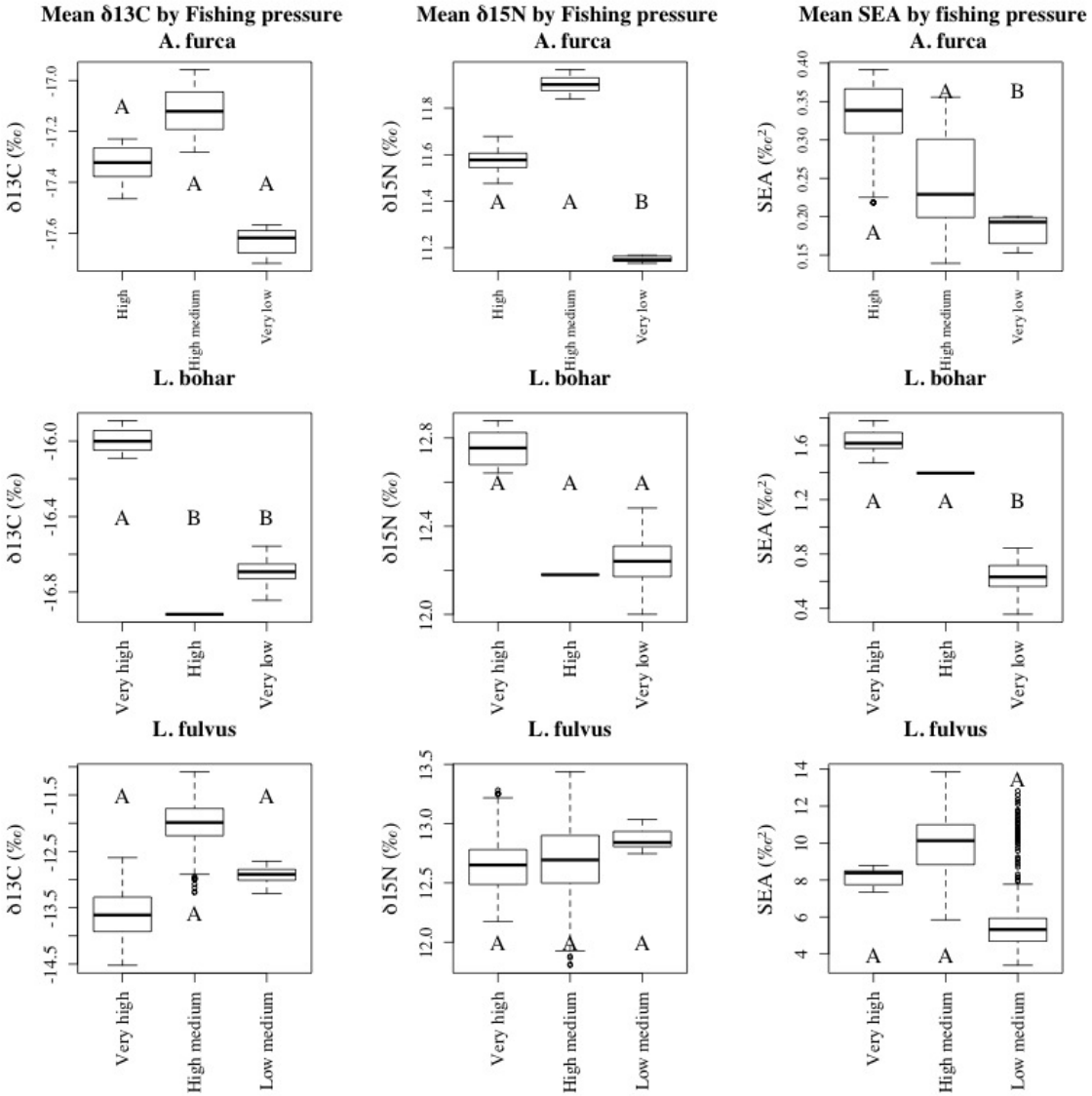

Figure 21: The effects of fishing on isotopic niches of Lutjanidae species. Mean δ13C, δ15N, and SEA values of Lutjanidae species from 1000 iterations of analyses by fishing pressure region for mechanism 2. Groups with the same letter had fewer than 900 iterations with significant results separating it from other groups (ANOVA and TukeyHSD for δ13C and δ15N and SIBER analysis for SEA. Note that regions with more variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples themselves.

Table 19: Stable isotope analysis summary statistics by fishing pressure region, grouped by species for comparison across fishing pressure gradient for the four focal species used in Mechanism 2 analyses. Includes niche width metrics (SEA=Standard Ellipse Area, SEAc=Standard Ellipse Area corrected for small sample size, TA=Total Area of convex hull), and Layman metrics (CD=Centroid Distance, MNND=Mean Nearest Neighbour Distance, SDNND=Standard Deviation of Nearest Neighbour Distance), along with mean, SD, median, minimum and maximum δ13C and δ15N values.

	Species	*Lutjanus bohar*			Lutjanus fulvus			*Aphareus furca*		
	Region	Very high	High	Very low	Very high	High medium	Low medium	High	High medium	Very low
δ13C (‰)	Mean	-15.995	-16.918	-16.691	-13.616	-11.997	-12.927	-17.324	-17.121	-17.629
	SD	1.069	0.486	0.636	2.959	1.995	2.338	0.449	0.451	0.381
	Median	-16.262	-16.64	-16.56	-14.69	-11.888	-12.394	-17.465	-17.184	-17.725
	Min	-17.144	-17.87	-17.927	-17.475	-16.209	-16.372	-17.814	-17.76	-18.134
	Max	-13.26	-16.41	-15.608	-8.803	-9.355	-10.18	-16.614	-16.379	-16.999
	Range	3.884	1.46	2.319	8.672	6.854	6.192	1.2	1.381	1.135
δ15N (‰)	Mean	12.755	12.18	12.24	12.65	12.69	12.866	11.578	11.902	11.151
	SD	1.206	0.987	1.044	1.851	1.332	1.335	0.247	0.226	0.156
	Median	12.133	12.42	12.303	13.635	13.062	13.463	11.571	11.885	11.185
	Min	11.386	10.86	10.258	9.148	10.333	10.78	11.189	11.49	10.86
	Max	15.55	13.68	14.015	14.598	14.202	14.26	11.932	12.281	11.33
	Range	4.164	2.82	3.757	5.45	3.869	3.48	0.743	0.791	0.47
SIBER	SEA (‰²)	1.626	1.395	0.634	8.187	10.034	5.575	0.331	0.247	0.184
	SEAc (‰²)	1.774	1.522	0.692	9.097	11.149	6.195	0.386	0.288	0.215
Layman	TA (‰²)	4.297	2.817	1.582	11.827	19.957	10.167	0.524	0.388	0.309
	CD	1.251	0.987	0.961	2.496	3.034	1.99	0.452	0.405	0.348
	MNND	0.531	0.312	0.372	0.498	0.87	0.814	0.261	0.225	0.226
	SDNND	0.528	0.265	0.281	0.337	0.736	0.659	0.111	0.203	0.089

	Species	*Cephalopholis urodeta*			
	Region	Very high	High	High medium	Very low
δ13C (‰)	Mean	-17.132	-16.389	-16.84	-16.953
	SD	0.507	0.288	0.488	0.984
	Median	-17.191	-16.309	-16.801	-17.204
	Min	-18.037	-17.043	-17.495	-18.067
	Max	-16.316	-16.057	-16.166	-15.043
	Range	1.721	0.986	1.329	3.024
δ15N (‰)	Mean	13.17	12.956	12.543	11.724
	SD	0.407	0.481	0.832	1.288
	Median	13.159	12.98	12.622	11.595
	Min	12.391	12.292	11.227	9.852
	Max	13.69	13.809	13.602	13.903
	Range	1.299	1.518	2.374	4.051
SIBER	SEA (‰²)	1.039	0.256	0.418	1.309
	SEAc (‰²)	1.169	0.288	0.47	1.473
Layman	TA (‰²)	1.879	0.501	0.783	2.418
	CD	0.825	0.404	0.575	1.261
	MNND	0.365	0.223	0.262	0.412
	SDNND	0.235	0.199	0.155	0.428

Table 20: Mechanism 2 bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise fishing pressure region comparison within *C. urodeta*, the only Serranidae family member used in this analysis. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between regions for each comparison.

		Serranidae family					
		δ13C		δ15N		SEA	
Species	Region comparison	mean p-value	N<=0.05/ 1000	mean p-value	N<=0.05/ 1000	mean p-value	0.05>N >0.95
Cephalopholis urodeta	High medium-High	0.400	20	0.908	0	0.682	0
	Very high-High	0.087	446	0.380	25	0.994	1000
	Very high-High medium	0.699	5	0.671	0	0.987	1000
	Very low-High	0.325	277	0.013	926	0.994	1000
	Very low-High medium	0.800	31	0.043	756	0.987	1000
	Very low-Very high	0.751	0	0.026	985	0.500	0

Table 21: Mechanism 2 bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise fishing pressure region comparison within each focal Lutjanidae species. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between regions for each comparison.

		Lutjanidae family					
		δ13C		δ15N		SEA	
Species	Region comparison	mean p-value	N<=0.05/ 1000	mean p-value	N<=0.05/ 1000	mean p-value	0.05>N >0.95
Lutjanus bohar	Very high-High	0.013	1000	0.374	0	0.500	0
	Very low-High	0.721	0	0.966	0	0.953	703
	Very low-Very high	0.077	326	0.455	0	0.953	726
Lutjanus fulvus	Low medium-High medium	0.335	55	0.860	0	0.520	0
	Very high-High medium	0.743	0	0.895	0	0.501	0
	Very high-Low medium	0.643	0	0.868	0	0.481	0
Aphareus furca	High medium-High	0.620	0	0.028	842	0.492	0
	Very low-High	0.380	1	0.003	1000	0.989	1000
	Very low-High medium	0.087	306	0.000	1000	0.989	1000

To visualize these results, which were consistent with all major findings at the individual level, I again plotted sample ellipses for each focal species used in Mechanism 2 that best represent the observed trends in the bootstrapped results. The same trends as in Mechanism 1 are evident for the three Lutjanidae species (Figure 23), and as for *C. urodeta,* the same trends are also seen, as well as the significantly less enriched mean δ15N values for the Very low region (dark blue ellipses, Figure 22).

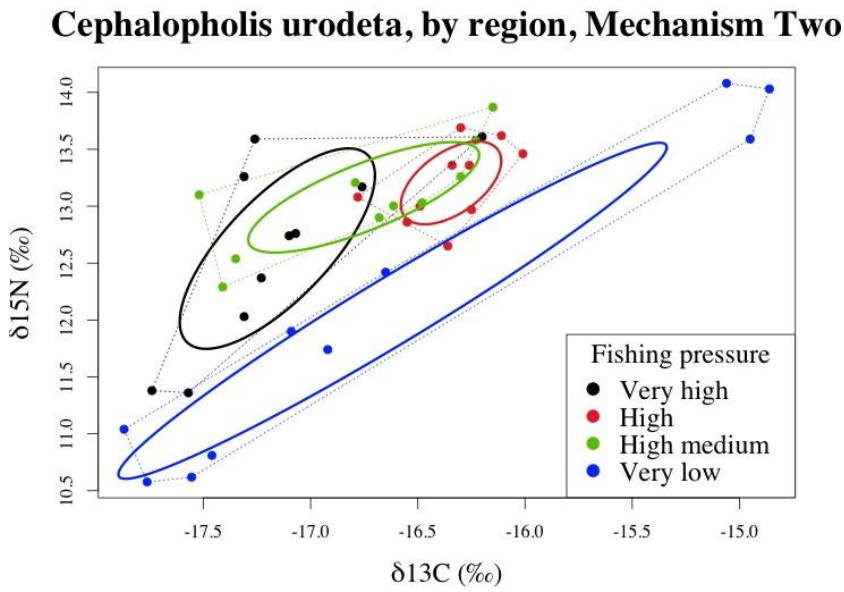

Figure 22: Sample Standard Ellipse Areas (SEA) for *C. urodeta* by fishing pressure region using Mechanism 2 controlled data. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

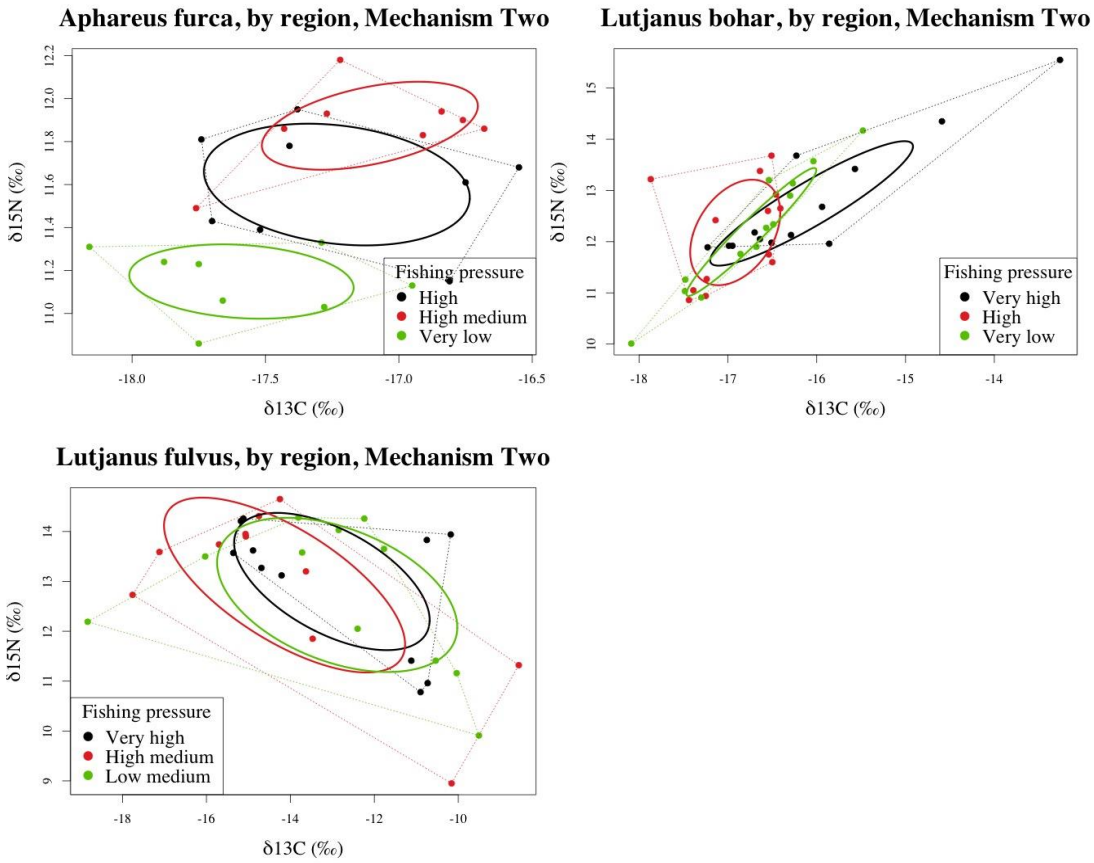

Figure 23: Sample Standard Ellipse Areas (SEA) for the three Lutjanidae focal species by fishing pressure region using Mechanism 2 controlled data. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

Mechanism 3: Community-level changes

Analyzing Mechanism 3 results, controlling for both observed body size distributions and relative abundances at the piscivore community-level, overall, no clear trends, in terms of any effects of fishing, are apparent. Firstly, for δ13C mean values, no significant differences were found for any pair-wise comparisons between regions (Figure 24, Table 22, Table 23). As for δ15N mean values, while the Very low region was found to be significantly lower than the High fishing pressure region (981/1000 iterations with $p<=0.05$), there was no such difference between the Very low region and the Very high region (19 significant iterations), or the High medium region (311 significant iterations) (Table 23). This implies that there are potential regional differences in mean δ15N values

when considering the piscivore functional group as a whole, but these differences, or the reasons for them, are certainly not clear.

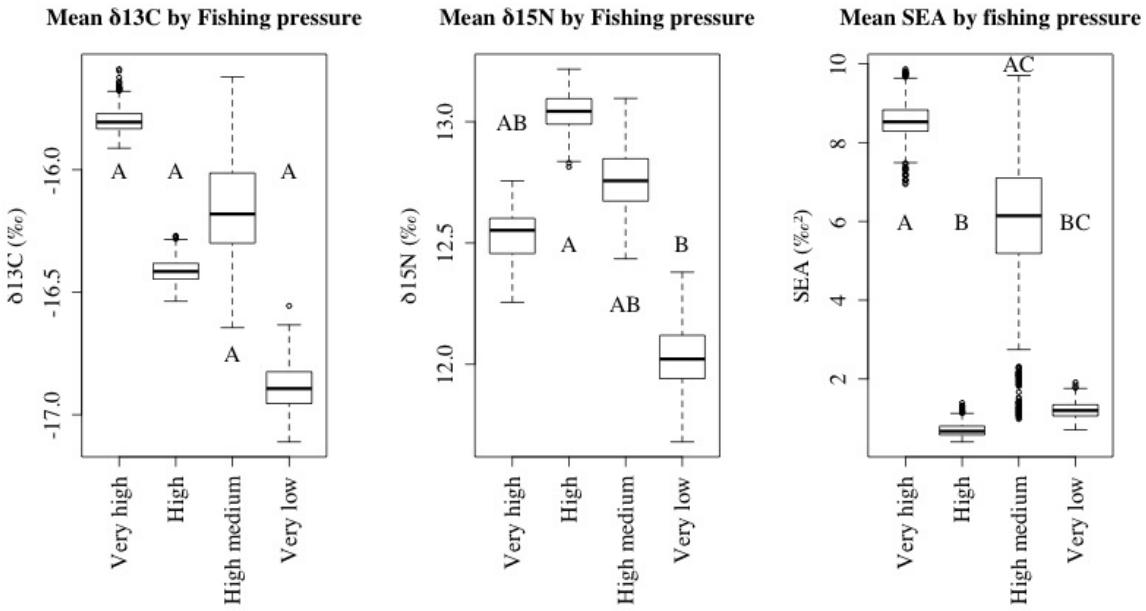

Figure 24: Mean δ13C, δ15N, and SEA values of the piscivore functional group from 1000 iterations of analyses by fishing pressure region for Mechanism 3. Groups with the same letter had fewer than 900 iterations with significant results separating it from other groups (ANOVA and TukeyHSD for δ13C and δ15N and SIBER analysis for SEA. Note that regions with more variation (longer whiskers) can be a result of re-sampling from a larger pool of samples as well as there being more variability within the samples themselves.

A similar result was found for bootstrapped SEA values, where the Very high region was significantly larger than the both the High (1000 significant iterations), and Very low (999 significant iterations), however there was no significant difference between it and the High medium region (36 significant iterations) (Table 23). Again, this implies some sort of unknown regional effect, but no clear trend on the effect of fishing pressure on these metrics is clear.

Table 22: Stable isotope analysis summary statistics by fishing pressure region for comparison across fishing pressure gradient for all piscivore samples used in Mechanism 3 analyses. Includes niche width metrics (SEA=Standard Ellipse Area, SEAc=Standard Ellipse Area corrected for small sample size, TA=Total Area of convex hull), and Layman metrics (CD=Centroid Distance, MNND=Mean Nearest Neighbour Distance, SDNND=Standard Deviation of Nearest Neighbour Distance), along with mean, SD, median, minimum and maximum δ13C and δ15N values.

	Species	All piscivores			
	Region	Very high	High	High medium	Very low
δ13C (‰)	Mean	-15.798	-16.412	-16.158	-16.886
	SD	2.618	0.539	1.973	0.861
	Median	-16.843	-16.31	-16.68	-16.935
	Min	-19.004	-17.701	-17.79	-18.170
	Max	-10.297	-15.362	-9.767	-15.161
	Range	8.70725	2.338	8.022	3.008
δ15N (‰)	Mean	12.535	13.040	12.757	12.028
	SD	1.049	0.798	1.014	1.200
	Median	12.830	13.103	12.942	11.848
	Min	10.715	11.245	10.568	9.887
	Max	14.056	14.452	14.354	14.284
	Range	3.341	3.206	3.786	4.396
SIBER	SEA (‰²)	8.554	0.706	5.923	1.210
	SEAc (‰²)	9.005	0.743	6.235	1.274
Layman	TA (‰²)	23.164	2.458	16.798	3.756
	CD	2.243	0.718	1.594	1.201
	MNND	0.430	0.255	0.489	0.292
	SDNND	0.455	0.246	0.747	0.211

Table 23: The effect of fishing pressure on isotopes at the functional group level. Mechanism 3 bootstrapped hypothesis testing results for 1000 iterations of ANOVAs of mean δ13C and δ15N values and SEA proportions for each pair-wise fishing pressure region comparison within the piscivore functional group. Mean p-values from all iterations are reported, along with the total number of iterations that returned a significant difference between regions for each comparison.

		Mechanism three					
		δ13C		δ15N		SEA	
	Region comparison	mean p-value	N<=0.05/ 1000	mean p-value	N<=0.05/ 1000	mean p-value	0.05>N >0.95
All piscivores	High medium-High	0.928	0	0.758	0	0.994	967
	Very high-High	0.651	0	0.419	4	0.990	1000
	Very high-High medium	0.839	0	0.834	0	0.662	36
	Very low-High	0.794	0	0.022	981	0.499	0
	Very low-High medium	0.532	0	0.167	311	0.662	38
	Very low-Very high	0.190	15	0.404	19	0.990	999

Finally, analyzing the additional Layman metrics that were bootstrapped (TA, CD, MNND and SDNND), all mean metrics for each species in each region were found to be significantly different from each other after 1000 iterations ($p<0.05$, ANOVA followed by Tukey HSD). That is, all calculated metrics for each region did not vary substantially amongst iterations, and thus all means were different from one another (Table 22). For TA and CD, which are both measures of niche width/trophic diversity (the latter being more robust as it is less sensitive to outliers), for each species, they ranked in the same regional order as SEA, which agrees with the above findings. MNND and SDNND, however, which represent the density and evenness of species packing (a measure of trophic redundancy), respectively, did not follow the niche width metrics, and did not appear to show any effect of fishing pressure. MNND was largest in the High medium region, followed by the Very high, Very low, and High regions, while SDNND was largest in the High medium region, followed by Very high, High, High, and then Very low (Table 22), and thus not appearing to show any trends with respect to fishing pressure effects. Finally, to visualize these results, as before I plotted sample ellipses for each fishing pressure region using an ideal sample from the 1000 iterations that illustrates these results (Figure 25).

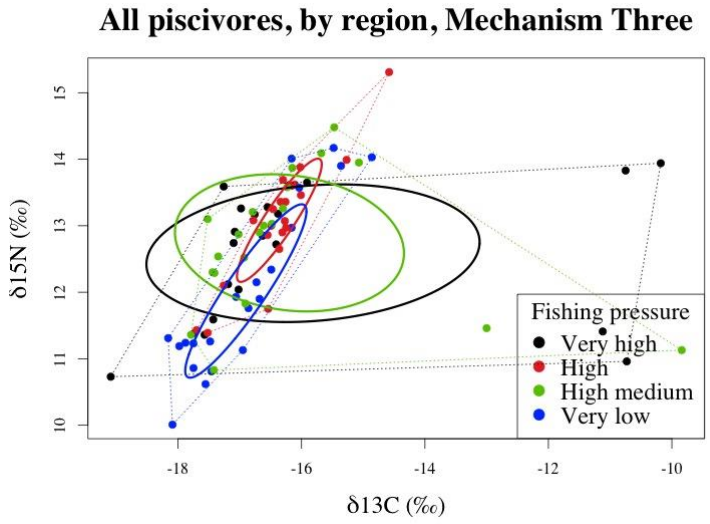

Figure 25: Sample Standard Ellipse Areas (SEA) for all piscivore focal species combined by fishing pressure region using Mechanism 3 controlled data. Ellipses drawn (solid lines) portray the best estimate based on 10^3 posterior draws using SIBER based on one sample of the 1000 bootstrapped iterations. Dotted lines portray convex hull used for calculating total area.

Oceanographic productivity and year effects

All analyses and models analysing the effect of chlorophyll *a* values from various sources on δ13C and δ15N values of all species of piscivores and zooplanktivores showed no significant effect (Table 24), with very low corresponding R-squared values for each source of productivity analysed (Appendix A Productivity Analyses), indicating no correlation. This was with one exception, in that chlorophyll *a* did show a significant relationship with δ15N for *Aphareus furca* (p=0.0093), however this species had the lowest sample size out of all focal species (n=10 for sites with both samples and a mean chlorophyll *a* value from the Bio-ORACLE dataset), and it had a reported R-squared value of 0.599, so this model result cannot be fully trusted. It must also be noted that the effect of year returned no significant effect on stable isotope values, validating the combination of data from all years for stable isotope analysis.

Table 24: Linear mixed effects model results for each focal piscivore species and two zooplanktivore species for comparison, assessing the effect of chlorophyll-*a* (mean mg*m^{-3}, taken from remote sensing data) on δ13C and δ15N. Species was used as the random effect at the functional group level and site was used at the species level (*=significant effect, Df= degrees of freedom, ΔAIC= difference in AIC between a given model and the model with the minimum AIC).

	p-value	AIC	ΔAIC	Df	Chisq
Taxon		δ13C			
Piscivore functional group	0.4507	1397	1.7	1	0.3422
Aphareus furca	0.3628	48.921	0.73	1	2.7302
Cephalopholis argus	0.2033	326.65	0.38	1	1.618
Cephalopholis urodeta	0.6437	142.75	1.78	1	0.2139
Lutjanus bohar	0.5841	131.84	1.7	1	0.2997
Lutjanus fulvus	0.2951	354.13	0.9	1	1.0963
Chromis vanderbilti	0.9563	22.419	1.97	1	0.003
Pseudanthias olivaceus	0.5855	48.873	1.703	1	0.2974
		δ15N			
Piscivore functional group	0.5585	1212.3	1.6	1	0.4516
Aphareus furca	0.0093*	2.0166	6.4062	1	8.4062
Cephalopholis argus	0.9468	330.59	2	1	0.0045
Cephalopholis urodeta	0.0873	182.1	0.92	1	2.9226
Lutjanus bohar	0.3579	163.48	1.16	1	0.8451
Lutjanus fulvus	0.9365	268.06	1.99	1	0.0063
Chromis vanderbilti	0.5423	53.914	1.628	1	0.3714
Pseudanthias olivaceus	0.6461	170.66	1.79	1	0.2108

Discussion

Niche differentiation of piscivorous fish

Dietary niche differentiation among taxa has been well documented across ecosystems, and has been suggested as a contributing mechanism by which ecological speciation occurs (Bolnick et al. 2003; Rundle and Nosil 2005). A reflection of resource partitioning between individuals of different populations, niche differentiation can be shown using stable isotope analysis by differing isotope values, niche width metrics, and low levels of overlap in niche width (Sepulveda et al. 2012). Such a result would be expected across functional groups, which often feed at different trophic levels and have altogether different dietary make-up, but even species of the same functional group can show significantly distinct isotopic niches (Polacik et al. 2014). This can arise from species having highly specialized diets, or even feeding in regions with differing sources of production (Reichard and Polacik 2010; Hilting et al. 2013).

In this thesis, I use stable isotope analyses to examine evidence of isotopic niche differentiation amongst piscivorous coral reef fish species. Taken together, the results of Objective 1 suggest that of the five focal species, *C. argus* and *L. fulvus* are the only two species to show signs of isotopic niche differentiation consistently in each region analysed. Across all analysed regions, *Lutjanus fulvus* consistently showed more enriched δ13C values, reflective of benthic production (Hilting et al. 2013). *L. fulvus* also consistently showed greater niche width (SEA) than all other species, suggesting its dietary diversity as a species exceeds those of the other focal species in this study. The two *Cephalopholis* species (Serranidae family) had more enriched δ15N values than the three other species (Lutjanidae family), which supports the idea of species-specific, or at least genus-specific dietary composition. However, only *C. argus*, and not *C. urodeta*, was significantly more enriched in δ13C than the other species when *L. fulvus* was not sampled. Why *L. fulvus* and *C. argus* differ from the other three species in their dietary niches is unclear, as a literature search on each focal species' diet reveals no large difference in either diet or region occupied (Froese 2011; Randall 1995). Because body size was not controlled in Objective 1 (each species has its own body size distribution), observed differences could also be due to body size differences, however *L. bohar*, for

example, is typically larger than either *L. fulvus* or *C. argus*, yet was not more enriched, so this theory does not hold altogether. Considering dietary differences, both grouper and both snapper species were identified as being largely benthic dwellers, while *A. furca* is described as bentho-pelagic. This could potentially explain why *A. furca* appeared to have less enriched values of δ13C, being more pelagic than the other species, but this result was not significant. Furthermore, *L. fulvus* being more enriched in δ13C than the other species does not have any backing in the literature based on gut content analyses. Its result of larger niche width metrics suggests it has a wider variety of prey items or prey sizes (or alternatively their prey items have larger dietary diversity; there is no way to be certain using stable isotopes alone), which is supported by gut content analyses (Kulbicki et al. 2005), and more enriched δ13C values suggest they feed more exclusively in a benthic zone.

As mentioned before for all Objective 1 methods and results, these analyses are not true calculations of niche differentiation, since isotopic baseline values of a food web (which are a requirement for calculating trophic position when dealing with multiple species of differing life histories, body sizes, etc. (Kurten et al. 2014)) were not available. Having baseline isotopic values would represent the base of the food web as a whole, which would then be incorporated into calculations of trophic positions that would be relative to a baseline, allowing comparisons across taxa. Given dietary and body size differences alone, however, I sought to simply determine whether or not it was possible to detect any differences in niche metrics of these species using stable isotope analysis, an approach that has been used infrequently in a coral reef setting. Given my samples, showing these differences in stable isotope values, while potentially and partially a result of body size differences, still potentially reflects differing dietary diversity amongst species. Additionally, to determine these answers in a more concrete way, since stable isotope analysis is only a proxy for diet, stomach content analysis would have to be conducted for cross-reference, which would at least confirm putative changes in prey items or prey size (De la Moriniere et al. 2003). Stomach contents have been retained for each sample, however they are not visually identifiable to species and so prey items would require DNA barcoding to determine species composition (Harrigan et al. 1989). Regardless, for one of the first times using stable isotope analysis in a coral reef

environment, I have been able to show a certain level of niche segregation among piscivorous species, representing a substantial step forward for both stable isotope/trophic ecology and coral reef ecology, as this approach, while imperfect, could be applied to many ecological questions regarding feeding interactions among species.

Effect of Fishing on Niche Width Metrics

Effects of disturbance, and specifically of fishing, on stable isotope metrics of consumers have been documented in previous studies (Table 2) with examples of niche width collapse following disturbance (*e.g.;* Jackson et al. 2012), and even re-expansion following time for recovery (*e.g.;* Hamilton et al. 2013). Such changes are implied to be an indirect result of a limitation of resources/prey items or changes in relative strengths of interspecific and intraspecific competition as a result of fishing changing the structure of the ecological community (Van Valen 1965). In this thesis, two distinct hypotheses were outlined to explain potential changes in niche metrics of a given species and a given body size. Firstly, fishing could remove preferred prey items of a particular predator, forcing the predator to expand their diet to non-preferred prey items (which potentially occupy different trophic positions/dietary niches). Niche width expansion also could arise from relaxed competition if fishing reduces abundance of the predators themselves, allowing the remaining individuals to feed on previously unavailable resources, thus expanding their dietary diversity (*sensu* the 'Niche Variation Hypothesis', Van Valen 1965; Rothstein 1973, Bolnick et al. 2010). Secondly, fishing may simplify the community in such a way that there is much less for a particular predator to feed on, and thus their niche width/dietary diversity would be reduced. Proving what mechanism is at work if a given species' dietary diversity, revealed through isotopic niche width metrics, changes between areas of differing disturbance, is not fully possible in this study without further information about stomach contents and therefore specific prey items and diversity measures of these prey items across all regions. However, using carefully selected samples to control for body size, samples to reflect observed body size distributions, and samples to reflect observed species composition, abundances and body sizes, I have analysed the effects of fishing at the individual, species population, and overall piscivore community-level.

At the individual level, selecting samples so as to compare the same number of fish that are each the same species and the same body size, five focal pisicivorous reef fish species were assessed for changes in stable isotope niche metrics across a fishing pressure gradient. Of these five, significant effects in three species were found. *C. urodeta* revealed regional differences in mean isotope values and niche width metrics, however no clear trends were evident in terms of fishing effects (*e.g.* smaller niche width in the High and the High medium region, however no difference between the most and least disturbed regions). As for the Lutjanidae family member species, two of the three saw significant changes in isotopic niche metrics, with both *A. furca* and *L. bohar* having smaller niche widths in the Very low region and larger values in the two other, more disturbed regions. This result suggests that in regions of high fishing pressure, these species either are forced to eat a wider range of prey items, the same prey items which themselves have had their diet altered or have switched to different production sources, different prey items all together, or any combination of these hypotheses. As stated, these results were all at the individual scale (Mechanism 1). However, following selections so as to compare fish across regions of a given species but of different body size classes to reflect observed body size distributions (Mechanism 2), essentially no changes in the results were found (with the exception of having less enriched δ13C for *C. urodeta*).

Since stable isotope analysis is strictly a proxy for long-term dietary composition, reasons, or mechanisms, for the observed expansion or reduction in niche metrics of these two species can only be speculative. It could even be noted that the two species that did not show any changes in isotopic niche metrics across all regions (*C. argus, L. fulvus*), were the two that also had the most differentiated "niches" from Objective 1, which might suggest that having differentiated niches, or perhaps high relative niche widths, could prevent any drastic dietary shifts arising due to fishing disturbance. However, it must be noted that this study only includes five of hundreds of coral reef fish species, tempering our ability to make general conclusions. Despite this limitation, in one way or another the observed shifts in niche width metrics between regions in this study must reflect some type of change in feeding dynamics within the ecological community, and could provide valuable insight on a largely understudied area of coral reef research.

At the piscivore-community level (Mechanism 3), selecting samples to reflect observed body size distributions and relative species abundances, significant differences in niche width metrics and some differences in mean isotope values were found, but there were no apparent trends or evidence that these differences were due to fishing pressure. Therefore, there is a large possibility that unknown regional factors, of which there could be multiple, are impacting these stable isotope values. For example, niche width metrics at the community level were highest (although not statistically significant) at the Very high and High medium region, both of which are at the lagoon mouth of the atoll, compared to the North coast regions of High and Very low fishing pressure. Given how the lagoon mouth is the location of the upwelling area (see "Oceanographic Productivity and Year Effects"), this would suggest a regional oceanographic effect. I have extensively analysed oceanographic productivity and found no significant effect on stable isotope values, but this does not rule out other factors that could potentially cause this trend to occur. I have taken careful measures to control for body size effects and evaluated the few known potentially confounding factors, but there could still be more unknown regional level effects at play.

The overall goal of this project was to explore stable isotope analysis as a tool to detect possible niche metric changes due to fishing pressure at the individual, population, and community level. As already mentioned, no trends regarding the effects of fishing pressure at the functional group level after controlling for species abundances and body size (Mechanism 3) were found. Therefore, only species-level shifts in isotopic niche metrics were found in this study, but this information is valuable when considering how little is known about coral reef trophic structure. However, as stated, this approach is far from perfect, as it can at best provide a reflection of an organism's diet over time, and there are several areas that could either be improved or expanded upon to improve the scientific accuracy of these findings.

Limitations and Future Directions

The largest concern with the analyses presented in this thesis has to do with the number of samples eventually used after body size was controlled. As explained, body size has been shown to have an effect on stable isotope signatures and it was found to have a significant effect on $\delta 15N$ of all species and on $\delta 13C$ of most of the species studied in

this thesis. Therefore, body size had to be controlled in order to report meaningful results on the effect of fishing on stable isotope signatures, and as explained under the methods section of this thesis, body size was controlled by selecting equal numbers of individuals from equal body size ranges for each focal species (Mechanism 1) and bootstrapping all analyses 1000 times with re-sampling. This way, any observed changes in niche metrics across fishing pressure regions would be much more likely attributable to altered diets of the group of organisms.

However, as alluded to multiple times in this thesis, not all body sizes were well represented, and the collection of samples did not match observed body size distributions and species abundances, leading to smaller sample sizes being used. It is worth noting, however, that bootstrapping to a certain extent takes care of this issue, and SIBER does take small sample sizes into account in its Bayesian analyses by giving more weight to ellipses based on fewer samples, and thus this issue is partially satisfied by considering this metric (SEAc; Jackson et al. 2011). Additionally, had body size effects not been taken into account, the results of these analyses could not fully be attributable to fishing pressure changes due to the clear effect of body size that was found in this thesis and in stable isotope literature (*e.g.* Bearhop et al. 2004, Sweeting et al. 2007). Future work using stable isotopes to solve similar problems should aim to collect more samples, however this is a very hard thing to plan, as samples were collected with the aim of obtaining all possible body size classes, regardless of what was observed (a sampling design for a separate project), and with only a rough idea of body size range prior to catching a given fish. Regardless, sample size issues are important considerations when interpreting any stable isotope results (Syvaranta et al. 2013).

Another interesting addition to this study would have been other study sites, introducing other elements of scale. It would have been beneficial to have samples from other reefs with varying levels of fishing pressure, even at whole island scales and across larger geographic areas for comparative purposes. At the same time, however, this could also introduce many more confounding variables, for example run-off nutrients or commercial fishing presence, which would affect the interpretability of the results as well. This case study, while being very local and therefore not immediately generalizable to reef systems elsewhere, has as a strength no known (significant) confounding

variables, due to a lack of fertilizer use or a large presence of a commercial fishery on Kiritimati, making the results as least scientifically more sound.

Having more samples and replicates, and performing these analyses over different scales would all be beneficial for the interpretability and scope of the project, however these things were not feasible for the amount of time spent sampling. Nonetheless, there are realistic additions to the study that would provide valuable insight on the presented findings. Having all the methods sorted for investigating the role of body size, productivity, and controlling for variables as needed, it would be very interesting to see if these patterns are consistent across different species. The focus of this particular study was piscivores, but samples of other functional groups, including benthic invertivores and piscivores, were collected, and so research using those other samples, done with as much detail as is presented here, would be an important next step in determining the effect of fishing on trophic structure. Additionally, samples of primary producers were collected from Kiritimati as well, but proved to be highly variable, not informative, and I am not confident that they support the diet and food web of my focal species. While I excluded these results from this thesis for these reasons, another important step would be to sample other primary producers that most likely support these consumers in order to incorporate these sources into mixing models. SIAR is well equipped to solve mixing models using as many as four production sources, and is capable of determining dietary source contributions of consumers (Parnell et al. 2010; Layman et al. 2011). This would be valuable in seeing how these contributions change across fishing pressure regions, and would provide another partial solution to the problem of trophic structure of these species. Finally, as mentioned before, cross-referencing stable isotope results with stomach contents, which are on hand at the University of Victoria but not identified to species, would be very informative, as it would allow interpretation of stable isotope results to go beyond speculation, linking an indirect proxy to the physical contents of diet composition.

Population metrics, as described in this thesis, with stomach content analysis and source contributions as determined by mixing models, taken together would provide a more complete picture of trophic structure of piscivorous fishes and how it changes with fishing pressure (Jackson et al. 2011). However, with the data made available from

sampling in this very regional case study, this thesis provides evidence of subsistence fishing effects on the dietary niche of three piscivorous fish species in a coral reef setting. Coral reef trophic structure is highly complex and ecologically important, and the successful application of stable isotope analysis in this system has been largely underused (Carassou et al. 2008). Thus, this study has provided an important step in the fields of both stable isotope and coral reef ecology, and provides valuable information on coral reef trophic structure for Kiritimati and other reef dependent nations around the world.

Trophic structure, comprised of feeding interactions between members of an ecological community, is a fundamental aspect of a functioning ecosystem, and therefore influences the goods and services they provide (Layman et al. 2011). With millions of people worldwide relying on coral reef ecosystems for these goods and services, and with their ecological baselines shifting, understanding these relationships is becoming urgently relevant and important (Knowlton and Jackson 2008). This project has made a substantial step in using stable isotope analysis as an approach for studying trophic structure in coral reef environments, and is important for the people of Kiritimati, who depend on the reef for their livelihood (Kiritimati Climate Report 2012).

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Appendix A
Productivity Analyses

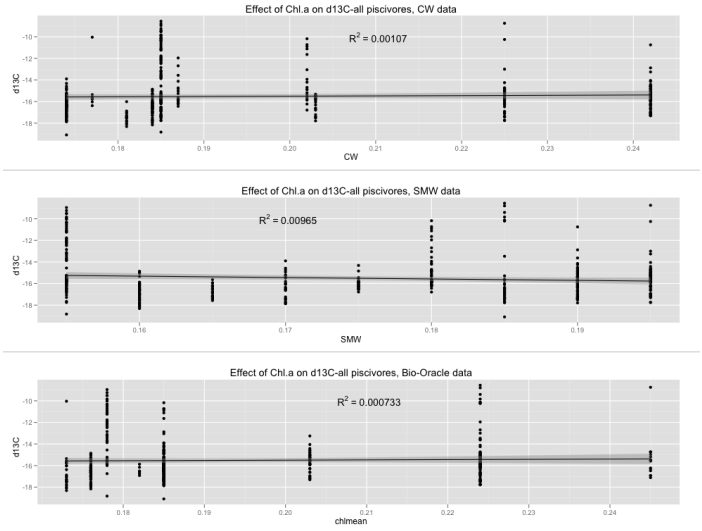

Figure 26: Regression analyses of chlorophyll-*a* effects on δ13C of all piscivore sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

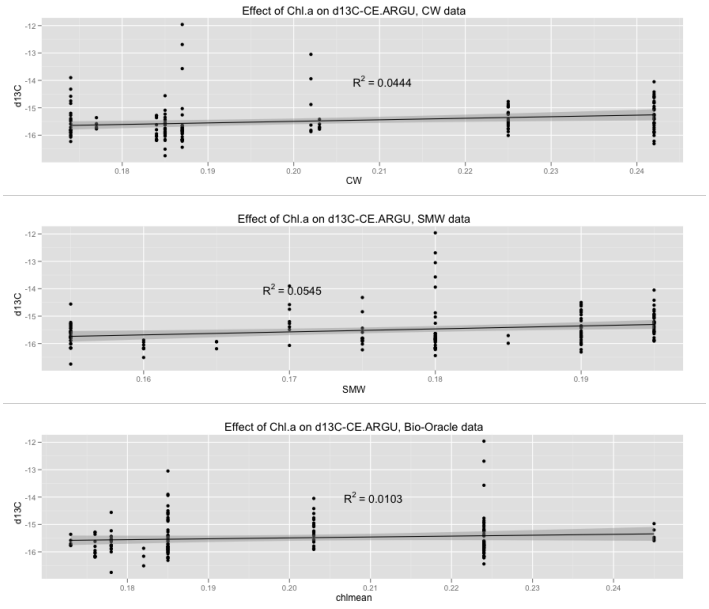

Figure 27: Regression analyses of chlorophyll-*a* effects on δ13C of *Cephalopholis argus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

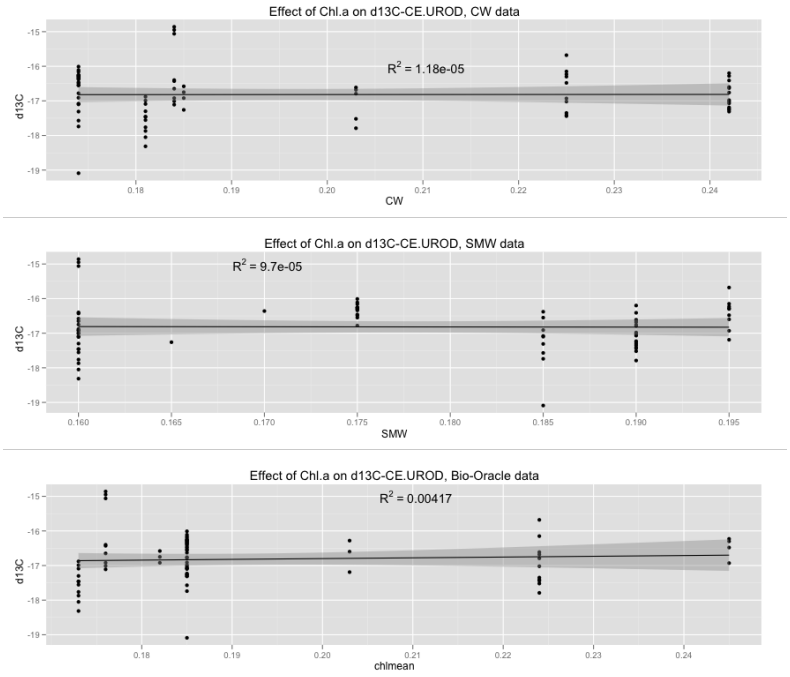

Figure 28: Regression analyses of chlorophyll-*a* effects on δ13C of *Cephalopholis urodeta* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

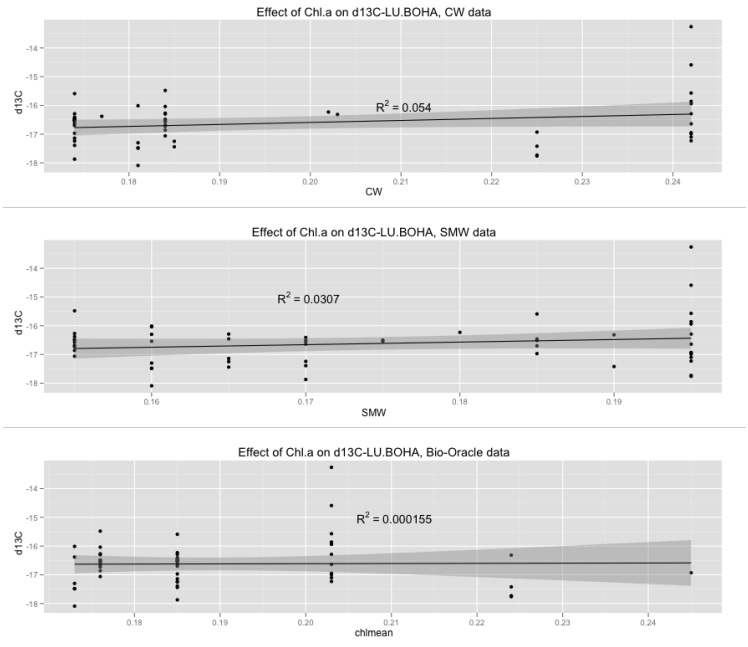

Figure 29: Regression analyses of chlorophyll-*a* effects on δ13C of *Lutjanus bohar* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

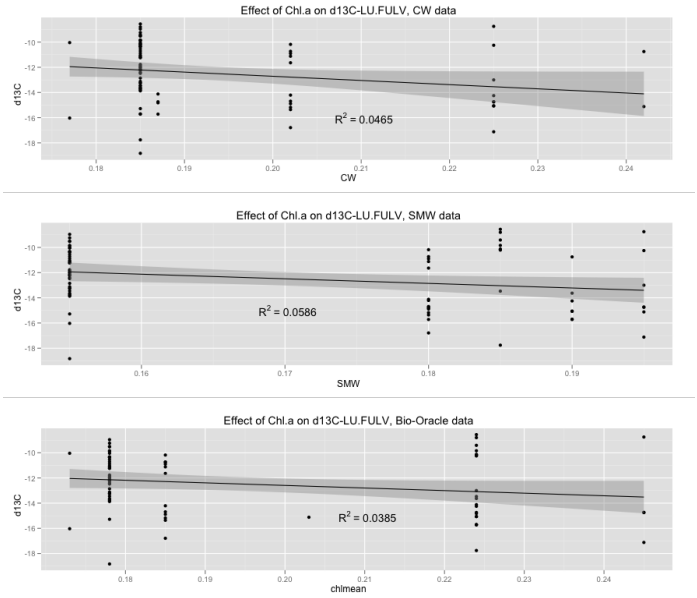

Figure 30: Regression analyses of chlorophyll-*a* effects on δ13C of *Lutjanus fulvus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

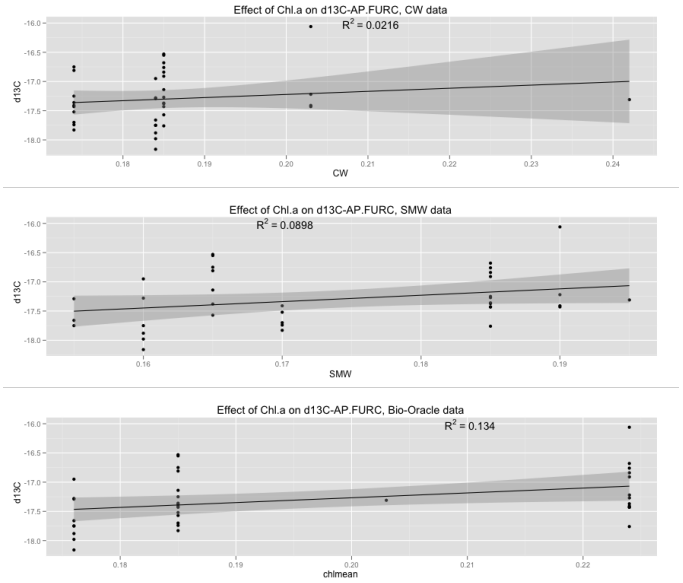

Figure 31: Regression analyses of chlorophyll-*a* effects on δ13C of *Aphareus furca* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

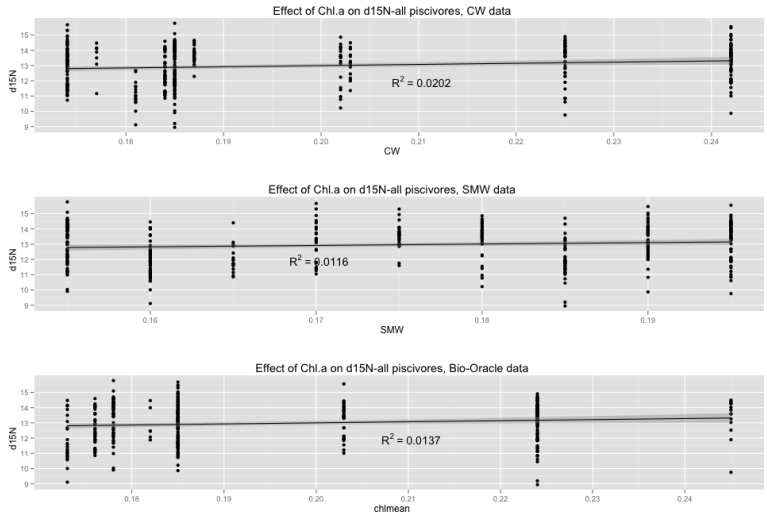

Figure 32: Regression analyses of chlorophyll-*a* effects on δ15N of all piscivore sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

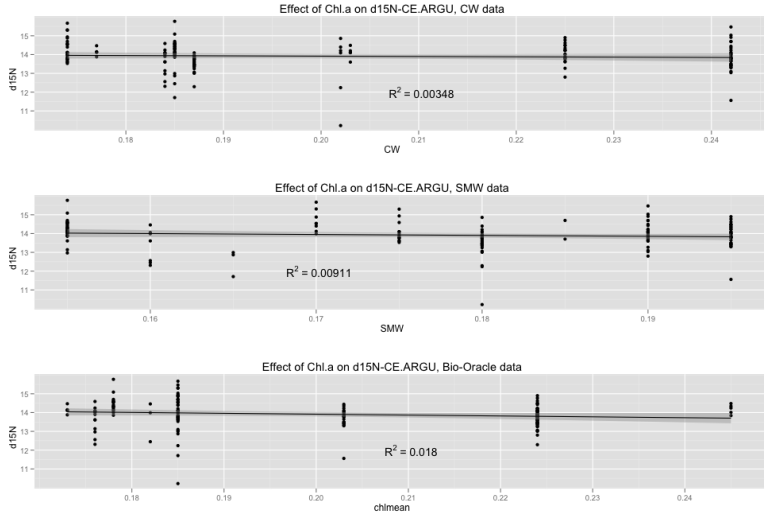

Figure 33: Regression analyses of chlorophyll-*a* effects on δ15N of *Cephalopholis argus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

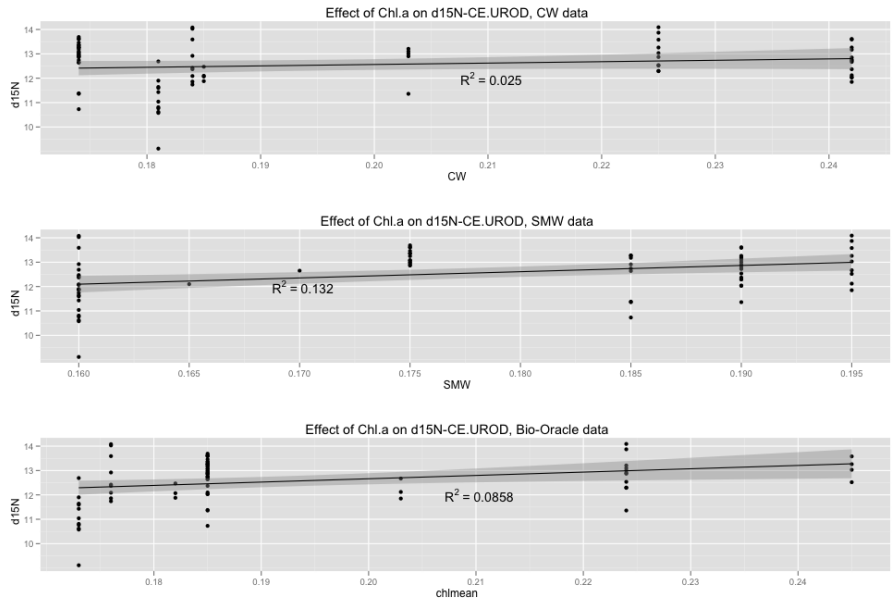

Figure 34: Regression analyses of chlorophyll-*a* effects on δ15N of *Cephalopholis urodeta* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

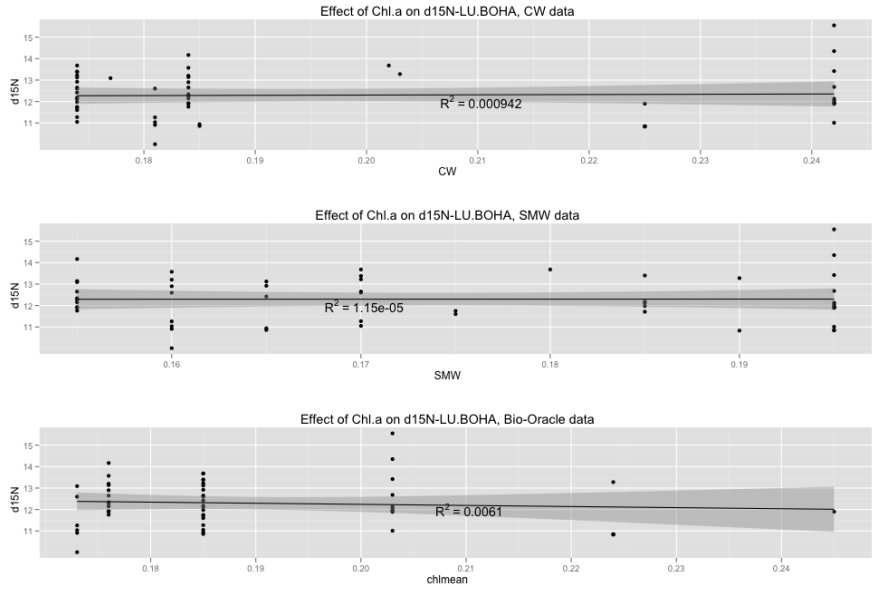

Figure 35: Regression analyses of chlorophyll-*a* effects on δ15N of *Lutjanus bohar* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

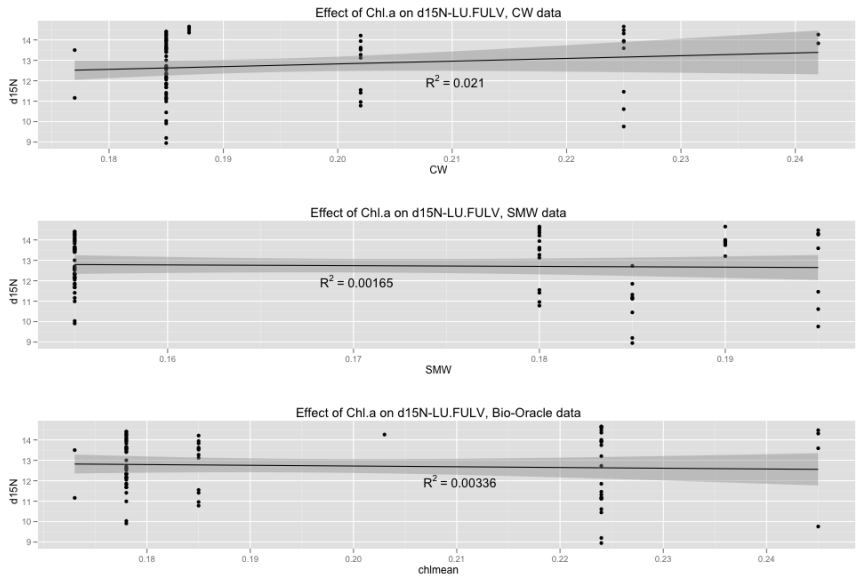

Figure 36: Regression analyses of chlorophyll-*a* effects on δ15N of *Lutjanus fulvus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

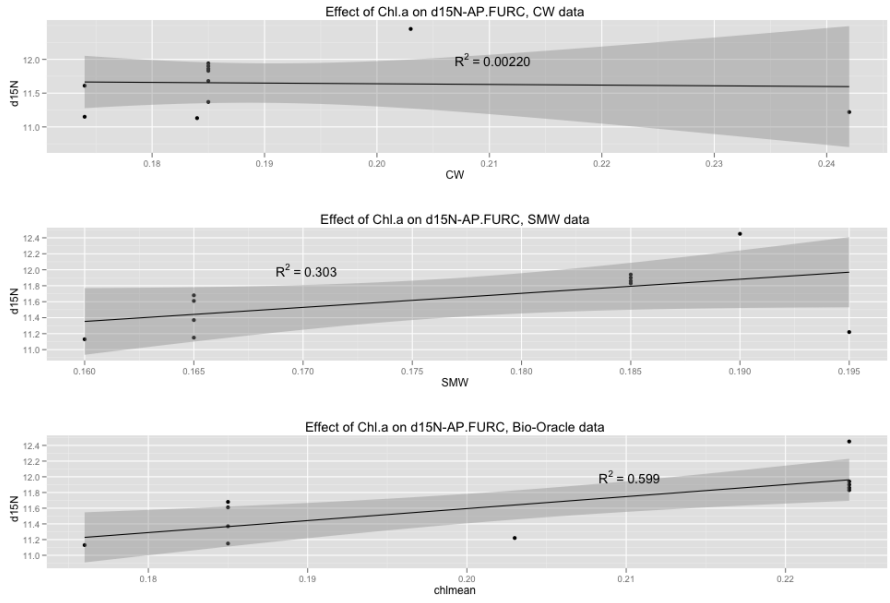

Figure 37: Regression analyses of chlorophyll-*a* effects on δ15N of *Aphareus furca* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

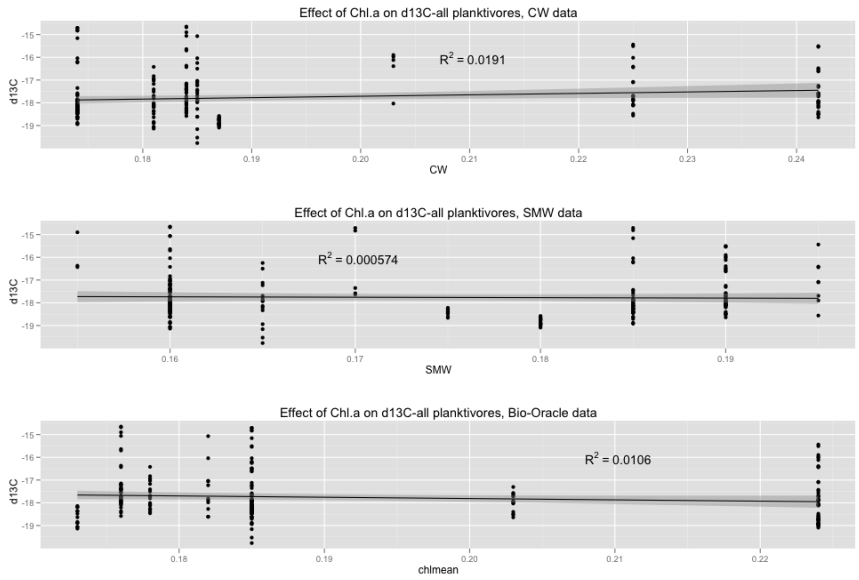

Figure 38: Regression analyses of chlorophyll-*a* effects on δ13C of all planktivores sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

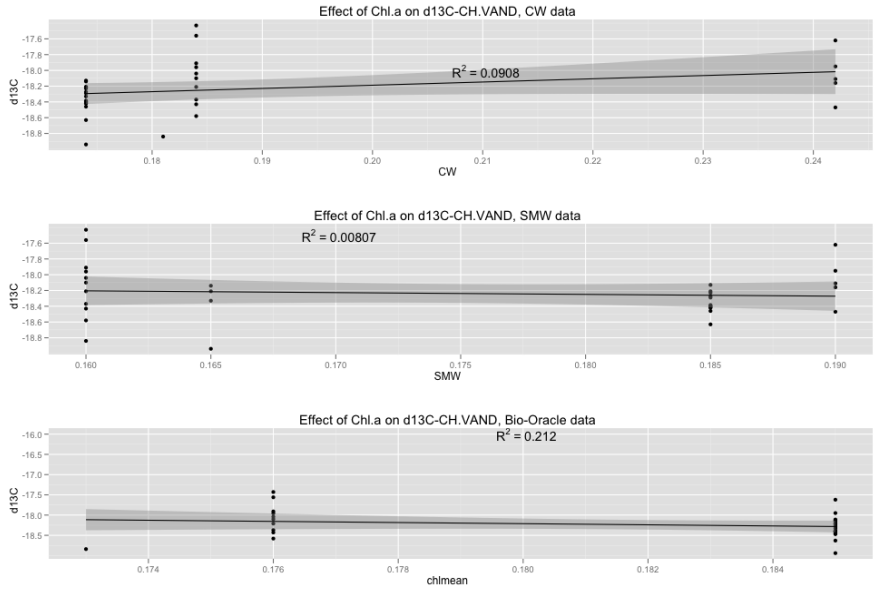

Figure 39: Regression analyses of chlorophyll-*a* effects on δ13C of *Chromis vanderbilti* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

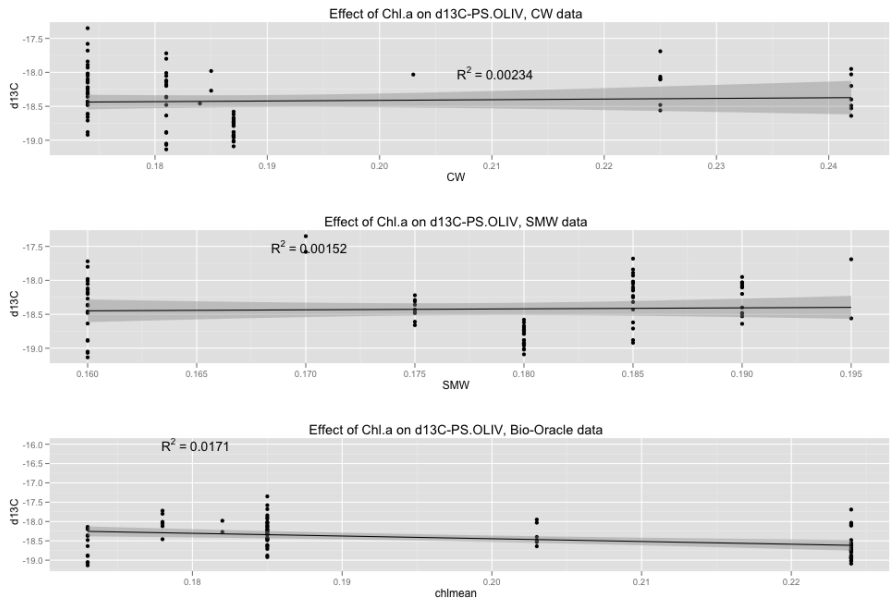

Figure 40: Regression analyses of chlorophyll-*a* effects on δ13C of *Pseudanthias olivaceus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

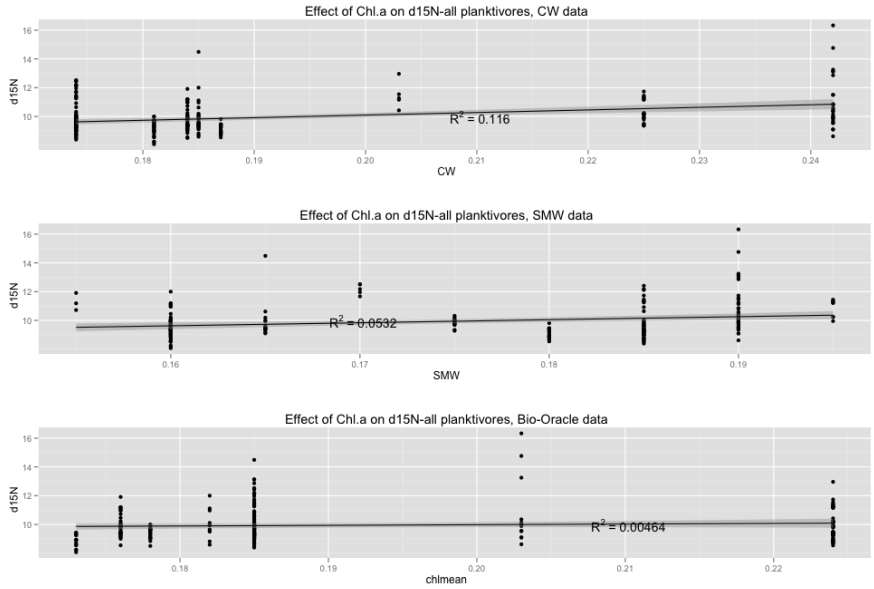

Figure 41: Regression analyses of chlorophyll-*a* effects on δ15N of all planktivore sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

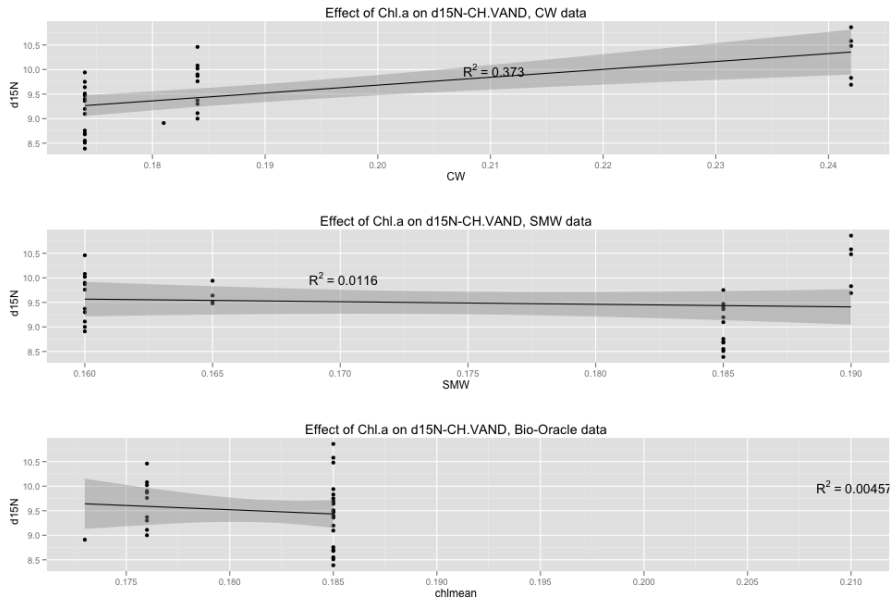

Figure 42: Regression analyses of chlorophyll-*a* effects on δ15N of *Chromis vanderbilti* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

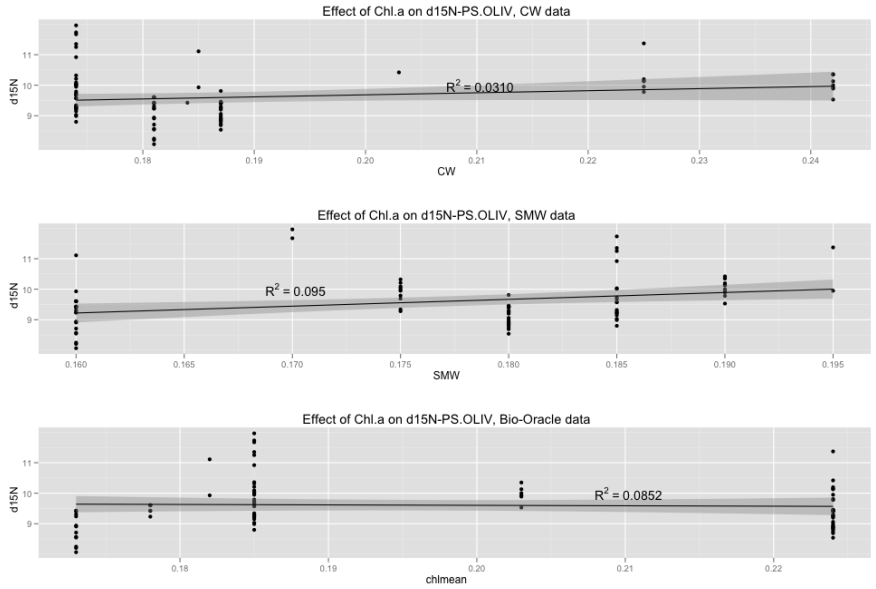

Figure 43: Regression analyses of chlorophyll-*a* effects on δ15N of *Pseudanthias olivaceus* sample values using chlorophyll-*a* data from three separate sources (CW: Chelsea Wood, SMW: Sheila M. Walsh, and Bio-Oracle oceanographic data).

Appendix B
Survey Analysis

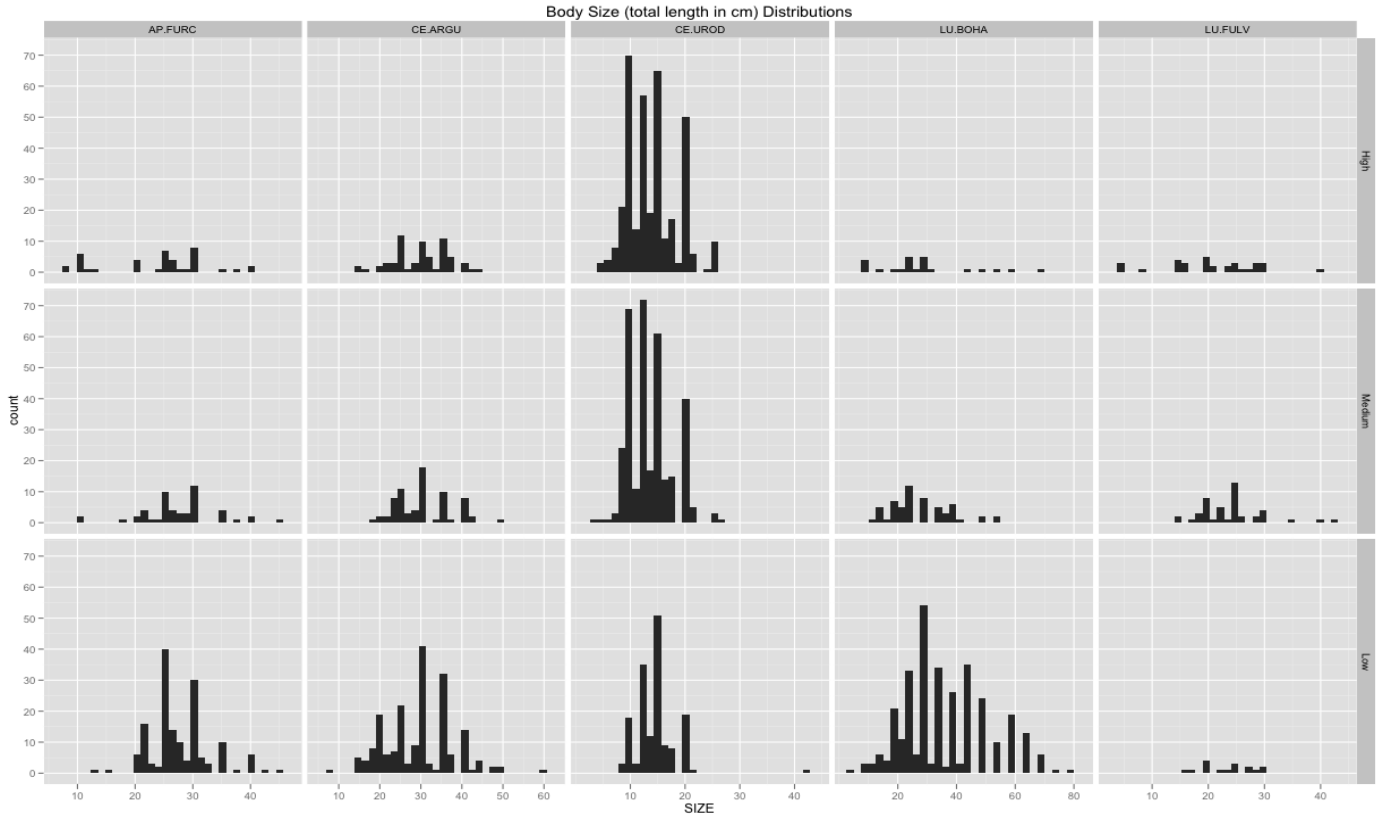

Figure 44: Body size distributions (total length in cm) of all focal species (AP.FURC= *Aphareus furca*, CE.ARGU= *Cephalopholis argus*, CE.UROD= *Cephalopholis urodeta*, LU.BOHA= *Lutjanus* bohar, LU.FULV= *Lutjanus fulvus*) across the three fishing pressure regions (high, medium, low).

Appendix C
Body Size Effects

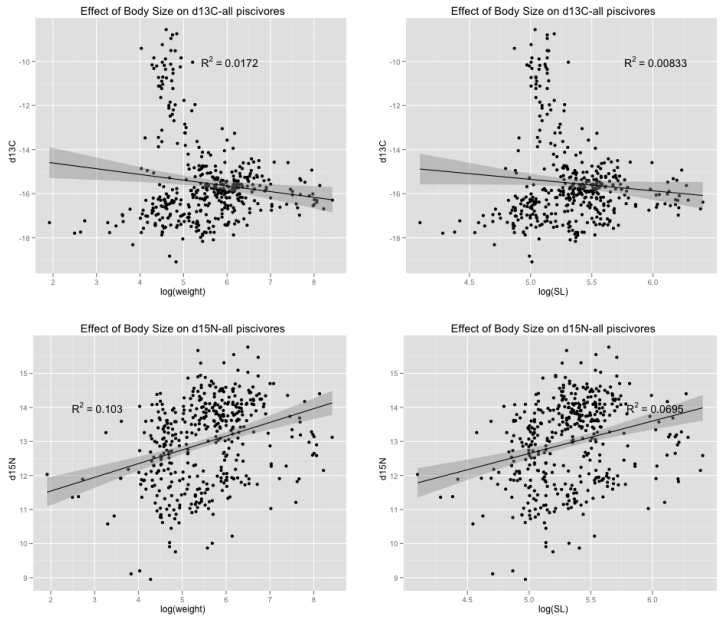

Figure 45: Body size effects plots showing regression analysis for all piscivores using log weight in grams and log standard length in mm for both δ13C and δ15N.

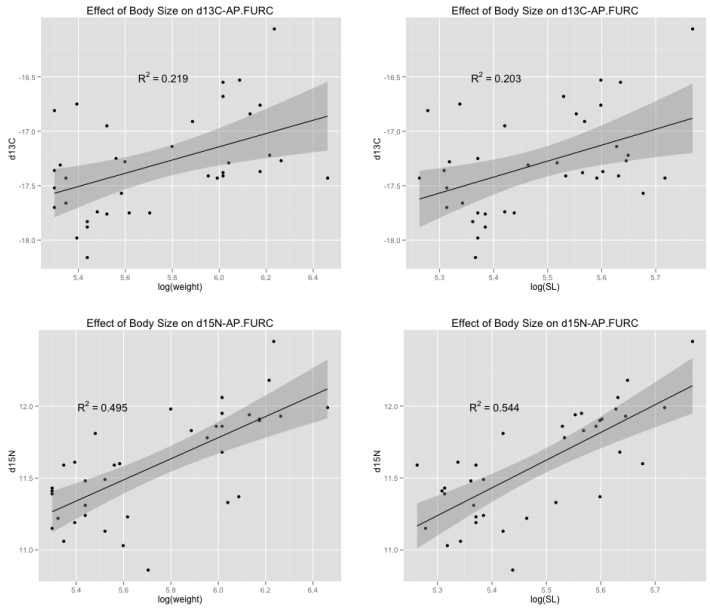

Figure 46: Body size effects plots showing regression analysis for *Aphareus furca* using log weight in grams and log standard length in mm for both δ13C and δ15N.

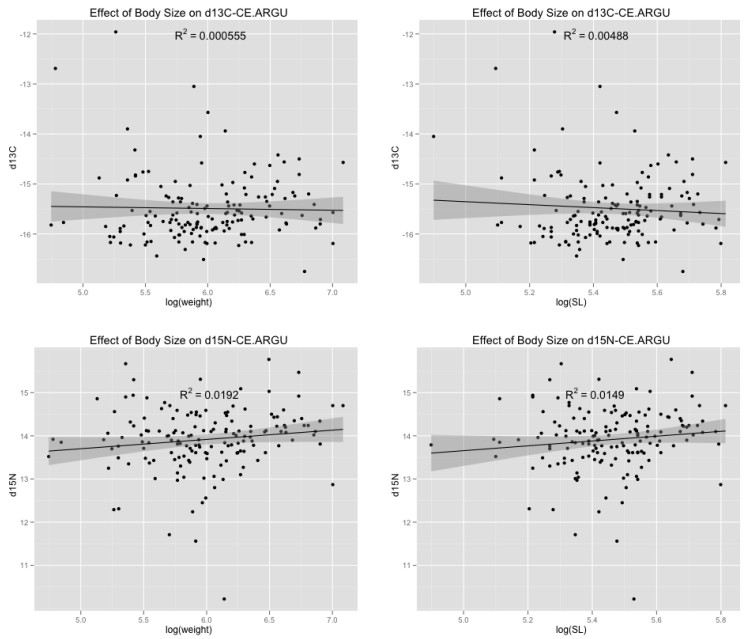

Figure 47: Body size effects plots showing regression analysis for *Cephalopholis argus* using log weight in grams and log standard length in mm for both δ13C and δ15N.

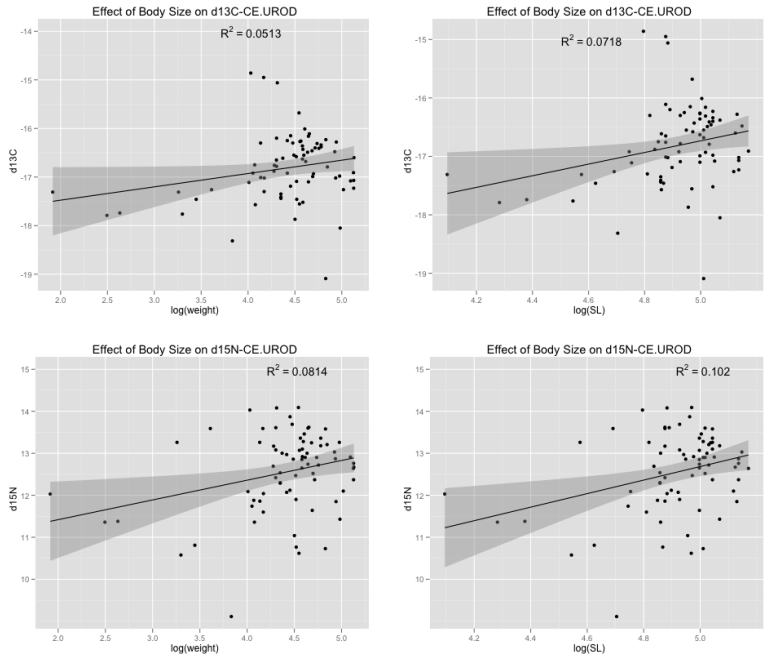

Figure 48: Body size effects plots showing regression analysis for *Cephalopholis urodeta* using log weight in grams and log standard length in mm for both δ13C and δ15N.

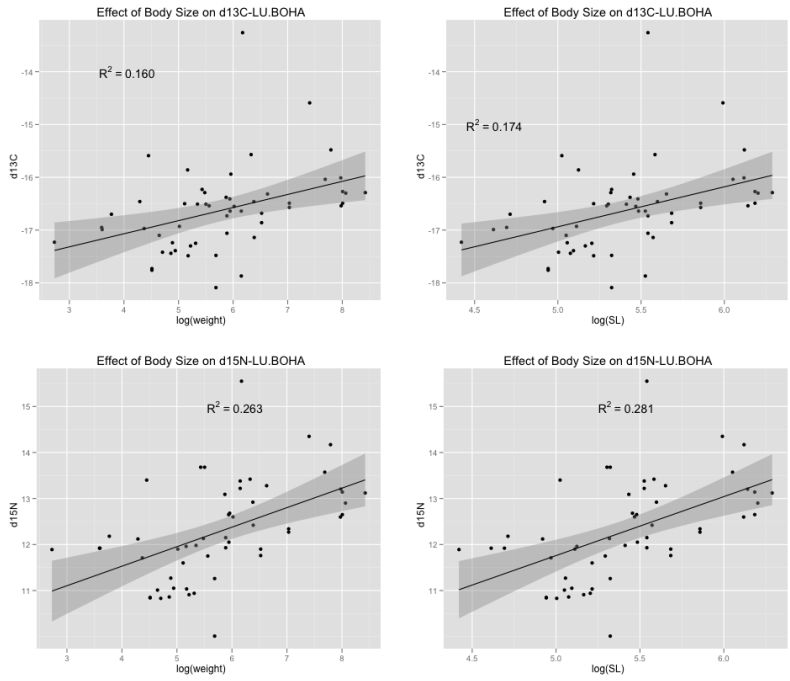

Figure 49: Body size effects plots showing regression analysis for *Lutjanus bohar* using log weight in grams and log standard length in mm for both δ13C and δ15N.

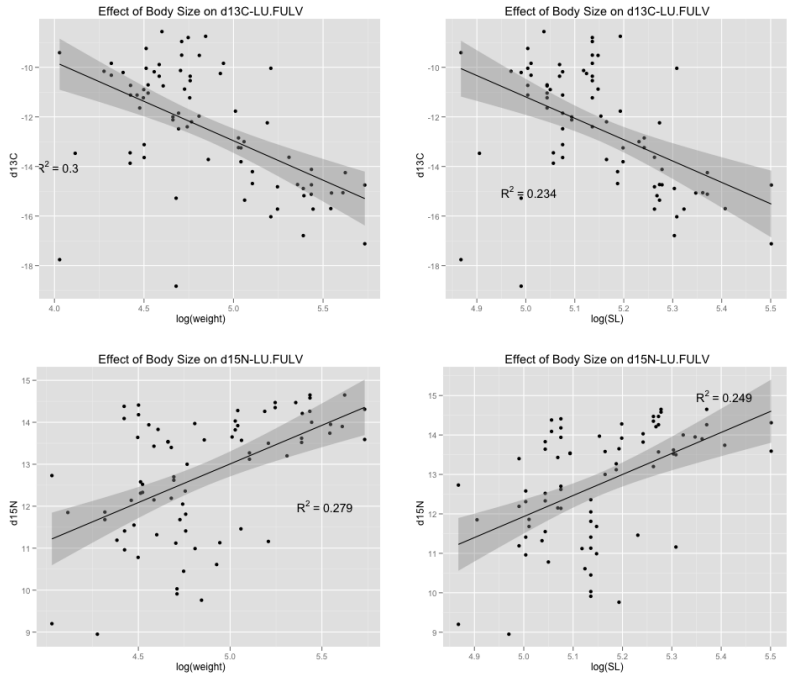

Figure 50: Body size effects plots showing regression analysis for *Lutjanus fulvus* using log weight in grams and log standard length in mm for both δ13C and δ15N.

Appendix D
Double blind analysis

Table 25: Double blind raw data for both Carbon and Nitrogen isotopes of randomly selected samples representing 10% of the entire population. Each row represents data for one δ13C and one δ15N sample, each analyzed twice with the same machine to assess its precision (Δδ13C or Δδ15N= difference between the two results).

δ13C1	δ13C2	Δδ13C	δ15N1	δ15N2	Δδ15N
-17.73	-17.76	0.03	10.85	10.84	0.01
-16.27	-16.49	0.22	13.14	12.65	0.49
-14.81	-14.50	0.31	14.92	15.47	0.55
-17.23	-17.07	0.16	12.37	12.76	0.39
-16.01	-15.72	0.29	13.10	13.65	0.55
-17.48	-18.09	0.61	11.26	10.01	1.25
-17.41	-17.44	0.03	12.29	12.30	0.01
-16.16	-16.02	0.14	12.97	13.14	0.17
-16.73	-17.06	0.33	12.15	11.93	0.22
-15.62	-15.36	0.26	13.61	13.90	0.29
-16.68	-16.86	0.18	11.90	11.76	0.14
-16.49	-16.57	0.08	12.34	12.27	0.07
-16.64	-17.87	1.23	13.38	13.22	0.16
-14.12	-14.74	0.62	14.58	14.65	0.07
-13.25	-13.81	0.56	13.92	14.28	0.36
-13.45	-13.87	0.42	14.09	14.38	0.29
-12.12	-12.00	0.12	13.54	13.53	0.01
-10.73	-11.12	0.39	10.96	11.41	0.45
-12.85	-13.24	0.39	14.03	13.82	0.21
-10.71	-11.04	0.33	12.33	12.52	0.19
-13.12	-13.64	0.52	14.18	14.41	0.23
-10.54	-10.36	0.18	11.41	11.81	0.40
-9.51	-8.96	0.55	9.91	10.03	0.12
-9.84	-10.32	0.48	11.68	11.86	0.18
-18.83	-15.28	3.55	12.19	13.40	1.21
-17.12	-14.75	2.37	13.59	14.31	0.72
-10.04	-16.03	5.99	11.16	13.50	2.34
-9.89	-10.36	0.47	12.15	13.43	1.28
-14.21	-14.69	0.48	13.12	13.27	0.15
-14.82	-15.72	0.90	14.35	14.47	0.12
-11.85	-12.48	0.63	12.62	12.70	0.08
-16.79	-14.89	1.90	13.52	13.62	0.10
-9.24	-10.04	0.80	12.58	12.31	0.27
-16.44	-16.41	0.03	11.22	11.22	0.00
-17.10	-17.08	0.02	10.23	10.25	0.02
-17.73	-17.70	0.03	10.90	10.92	0.02
-16.62	-16.59	0.03	13.14	13.11	0.03
-16.51	-16.48	0.03	10.82	10.82	0.00
-17.90	-17.87	0.03	12.35	12.38	0.03
-18.12	-18.09	0.03	11.46	11.49	0.03
-18.16	-17.62	0.54	9.69	9.83	0.14
-15.54	-15.51	0.03	11.50	11.50	0.00

Double blinds analysis comprised of paired t-tests for both groups of carbon and nitrogen samples, testing for significant differences between mean values of each. Both analyses showed each group's mean to be not significantly different from each other ($p=0.5337$ for δ13C and $p=0.5426$ for δ15N, 41 degrees of freedom for both). Samples with a difference between blinds greater than 3 were excluded from analyses, which only occurred twice (for difference of 3.55 and 5.99; Figure 51).

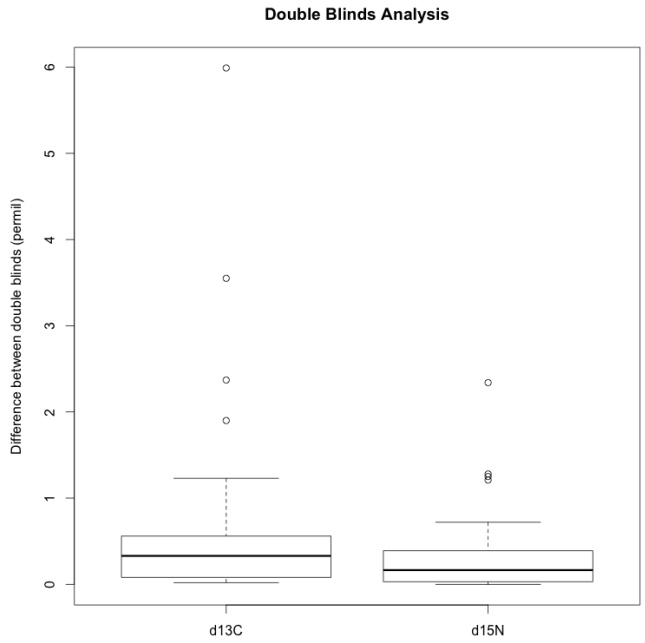

Figure 51: Boxplots displaying the differences between double blind samples for both δ13C and δ15N values (n=42 for both stable isotopes).

Table 26: Summary statistics for double blinds analysis, showing the sample size (N), mean, median, minimum and maximum values, and standard deviation (SD) of the differences between each double blind sample pair.

Isotope	N	Mean	Med	Min	Max	SD
Δδ13C	42	0.63	0.33	0.02	5.99	1.09
Δδ15N	42	0.32	0.16	0	2.34	0.46