

AN ECOLOGICAL COMPARISON OF CEPHALOPHOLIS ARGUS BETWEEN
NATIVE AND INTRODUCED POPULATIONS

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ABSTRACT

Feeding Ecology and Diet Study

The blue-spotted grouper, *Cephalopholis argus*, was introduced to the Hawaiian islands from Moorea, French Polynesia in the late 1950s in an attempt to establish a grouper fishery in Hawaii (Randall 1987). *C. argus* was introduced from a region of high grouper diversity (14 species) into an environment with little competition from other large sedentary piscivorous species, and has flourished over the last 50 years. In these circumstances, changes in diet and habitat use, resulting from a reduction in interspecific competition, is known as competitive release (Larsen 1986). The present study found that total length, weight, and body condition were each significantly greater in introduced populations of *C. argus* in Hawaii than in native populations in Moorea. Both regions showed significant positive relationships between the total length of *C. argus* and: 1) the total length of prey, and 2) prey body depth. There were significant regional differences in diet; *C. argus* of a given length in Moorea consumed significantly deeper-bodied prey than their counterparts in Hawaii. These differences are consistent with competitive release experienced by *C. argus* in Hawaii.

Home Range Study

Cephalopholis argus was introduced to the Hawaiian islands from Moorea, French Polynesia, in an attempt to increase nearshore fisheries (Oda & Parrish 1981, Randall 1987). Despite its presence in Hawaii for over 50 years and recent population expansions along the shores of all of the Main Hawaiian Islands, there have been no investigations into the movements and use of space of *C. argus* in the Hawaiian islands or in its source locality. Active and passive tracking were used to determine the movement patterns,

home range size and utilization distribution of *C. argus* in both Moorea and the island of Hawaii. Minimum convex polygons (MCP) and 95% kernel utilization distributions (KUD) were used to define home range areas. Forty-three *C. argus* were tracked, and a geographic information system was used to calculate home range size and utilization distributions for each fish. Active and passive tracking confirmed that these predators are diurnal and highly site attached, patrolling home ranges by day and rarely moving at night. Home range size ranged from 230 to 1389 m², with a mean of 700 m² in Moorea, and 425 to 2300 m², with a mean of 1236 m² in Hawaii. There was a significant positive correlation between the size of *C. argus* and home range size, and *C. argus* in Hawaii held significantly larger home ranges than *C. argus* in Moorea. *C. argus* in both regions spent the majority of their time in a few small core use areas. These core use areas corresponded with areas of high rugosity and reef complexity, and were used as sheltering sites. *C. argus* in Hawaii and Moorea showed significant differences in the size of core use areas between the two regions. In Moorea, males held significantly larger home ranges than females. In Hawaii, male and female home range size did not differ significantly. Differences in home range area and habitat utilization between regions may be due to the lack of competing species, especially other groupers, in Hawaii.

Ciguatera Study

Today *Cephalopholis argus* is the most abundant large, nearshore predatory fish species on the reefs of the Main Hawaiian Islands (Dierking 2007). However, the species has failed to provide a fishery. *C. argus* is known in Hawaii for causing ciguatera fish

poisoning (CFP), and as a result there is not an active fishery for *C. argus* in Hawaii. Ciguatera fish poisoning (CFP) is caused by *Gambierdiscus toxicus*, a dinoflagellate that resides on nearshore algae and is inadvertently ingested by fish while feeding. The ciguatoxins (CTX) produced by *G. toxicus* are incorporated into the tissues of the fish (Randall 1987, Hokama & Yoshikawa-Ebesu 2001, Chateau-Degat et al. 2005, Lewis 2006, Darius et al. 2007). Humans and other mammals are highly sensitive to CTX and often acquire CFP when they consume contaminated fish. The present study is focused on CFP in Moorea, French Polynesia, using the Membrane Immunobead Assay (MIA) to determine whether there are “safe sizes” of *C. argus* that can be consumed, or “safe localities” from which to catch fish for food. Fish from Moorea were collected and tested for CTX, and the results for Moorea were compared to those found by Dierking (2007) in Hawaii.

Standard length of *C. argus* and CTX score were positively correlated ($p=0.045$), however, small fish could be strongly positive, and cause CFP. There were no clearly significant differences among sites (ANOVA, $p=0.064$). All sites tested contained some fish that scored 2 or higher and were considered to involve some hazard if eaten. At all sites, the majority of *C. argus* tested contained CTX levels at or above 0.32 ng/g, and were considered unsafe for consumption. When CTX scores were compared between Hawaii and Moorea, scores were significantly lower for *C. argus* in Hawaii. *C. argus* of a given length in Hawaii had lower CTX scores than fish of the same size in Moorea; (ANCOVA, $p<0.001$, $F=72.3$).

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CHAPTER 1

***Cephalopholis argus*: An Ecological Comparison of the Species in Introduced and Native Populations**

The Hawaiian islands support a unique assemblage of coral reef fish species, including 25% endemics among the shore and reef fishes (DeMartini & Friedlander 2004, Randall 2007). In comparison with most tropical Pacific localities, several fish families are underrepresented or completely missing from the shallow, inshore reefs of Hawaii; among these is the family Serranidae, the sea basses and groupers. Many species of serranids can be found elsewhere in the Indo-Pacific, where they are considered a prized food source. There are 159 species in 15 genera of groupers throughout the world (Heemstra & Randall 1993). Six genera and 57 species occur in the tropical Pacific, but there are only two naturally occurring groupers in Hawaii. These are a native deep-water grouper, *Epinephelus quernus*, commonly known as the hapu'u, and the rarely seen *E. lanceolatus*. There are no native, truly shallow-water groupers in Hawaii. The lack of inshore bottomfish species may be due to both the geographic and hydrographic isolation of the archipelago, preventing species from becoming naturally established (Randall 1987, Planes & Lecaillon 1998).

In the late 1950's the Bureau of Fisheries Management and the Division of Fish and Game of the State of Hawaii instituted an introduction program that would bring in 11 new species of groupers and snappers for fisheries purposes (Randall 1987, Planes & Lecaillon 1998). The blue-spotted grouper, *Cephalopholis argus*, was one of a number of

potential food fish introduced to Hawaiian waters from Moorea in the Society Islands (where they were known as "roi") in an attempt to increase nearshore fishery diversity in the state of Hawaii (Oda & Parrish 1981, Randall 1987). In two separate release events, in 1956 and 1961, a total of 2,385 *C. argus* were released, a portion in Kaneohe Bay on Oahu and the rest on the Kona coast of the island of Hawaii (Planes & Lecaillon 1998, Dierking 2007). Of the 11 species introduced, three have successfully become established: 1) *C. argus*, 2) the blacktail snapper, *Lutjanus fulvus*, and 3) the bluestripe snapper, *Lutjanus kasmira* (Oda & Parrish 1981, Randall 1987).

C. argus has a fairly widespread distribution, occurring throughout the tropical marine waters of the Indo-Pacific, from the east coast of Africa in the Red Sea, north to Japan, and south to Australia and French Polynesia (Randall 1987). *C. argus* is a bottom dwelling fish occupying the littoral zone, rarely rising more than two meters off the substrate to patrol its home range and hunt for food. This grouper is one of the top predatory fishes in its native habitat, and can be found in lagoons, surge zones, reef slopes, and to depths beyond 100 feet, on coral reefs or boulder piles, where caves and holes are plentiful (Randall 1987, Shpigel & Fishelson 1989b).

In their native habitat *C. argus* are polygamous, living in small social groups or harems, composed of one male and several females (Shpigel & Fishelson 1991b). Like most groupers, *C. argus* are protogynous hermaphrodites, changing sex from female to male when the proper social circumstances arise. Males are larger than females, reaching 52 cm in total length, and protect their harems by defending a home range that encompasses the smaller home ranges of the females in the harem. Groupers in the genus

Cephalopholis do not undertake massive spawning migrations like many other species in the subfamily Epinephelinae; instead they spawn year round in their home ranges (Donaldson 1995a).

These predators are highly site attached and defend their mates and feeding areas against conspecifics as well as other epinephelines (Shpigel & Fishelson 1991b). In the Red Sea males patrol their home ranges, which can be as large as 1,500 m², visiting each female within their harem (Shpigel & Fishelson 1991b). Female home ranges are contiguous within the male's home range, averaging 60 m² in size, with the largest and most dominant alpha female holding the largest home range, up to 100 m² (Shpigel & Fishelson 1991b).

Groupers in general have a very large buccal cavity, which allows them to feed using suction. A vacuum is created inside the buccal cavity so that when the grouper opens its mouth, its prey are sucked in and held by its pharyngeal teeth. The large mouth and flexible jaws of *C. argus* allow it to capture and consume its prey whole (Dierking 2007; Meyer unpublished). Shpigel and Fishelson (1989) reported that hunting activities of *C. argus* in the Red Sea have a bimodal pattern with peak activity during the morning and afternoon hours and a lull in the middle of the day. *C. argus* is an opportunistic feeder, feeding on the most common prey, and preying almost entirely on fishes (95%) (Shpigel & Fishelson 1989a, 1989b, Webster 2002, Webster & Almany 2002, Beukers-Stewart & Jones 2004). Consuming the most common prey can affect the recruitment and settlement of various fish species differently and can thereby affect the species diversity of coral reefs. In Australia the predation on cardinalfish by *Cephalopholis* sp. altered the

recruitment of butterflyfishes, surgeonfishes and rabbitfishes to patch reefs at Lizard Island (Webster & Almany 2002). Recent surveys of fish populations around Hawaii show that the number of *C. argus* is reaching alarmingly high levels in certain areas, and at these high levels this predator could have an impact on Hawaiian reef communities (pers. comm. Hawaii Division of Aquatic Resources).

Multiple studies have shown that groupers can have a large impact on the structure of reef fish communities by preying on both recruits and adult fish. Several studies have focused on the question of how the presence of and predation by groupers affect the recruitment of coral reef fishes (Randall & Brock 1960, Randall 1963, Parrish et al. 1985, Shpigel & Fishelson 1991a, Connell 1998, Planes & Lecaillon 2001, Steele & Forrester 2002, Webster 2002, Webster & Almany 2002, Beukers-Stewart & Jones 2004). Most coral reef fish species have a two-part life cycle; the adult phase occurs on the reef, and the larval portion occurs in the open ocean. The majority of coral reef fish spend their larval phase in the plankton and return to the reefs, metamorphose, and remain on the reef for the rest of their life cycle, where they are vulnerable to predation.

Webster (2002) found that predators have a strong negative impact on recruit survival. Groupers of the genus *Cephalopholis* were removed from nine patch reefs on Lizard Island, and similar groupers were added to nine patch reefs, to simulate various natural levels of predator densities. Webster found that the densities of several species of newly recruited fish decreased in the presence of abundant predatory groupers. In his study, predatory groupers were responsible for 62-90% of the mortality among new recruits across all species on experimental reefs, in contrast to reefs without predators. In a

second study involving high densities of cardinalfish recruits, Webster showed that groupers affect species diversity and richness by preying disproportionately on common species, thereby removing them from the community (Webster & Almany 2002). Once the common species was no longer plentiful, the groupers then switched to a new prey species (Webster & Almany 2002).

By using caging experiments Planes and Lecaillon (2001) showed that mortality of larval recruits was related to the abundance of resident predators. Large, size indiscriminant predators, such as groupers, which can feed on both small and large fish, were responsible for a 90% reduction in recruits as compared to control reefs. This reduction was shown to significantly influence community structure, species richness and diversity (Planes & Lecaillon 2001, Steele & Forrester 2002, Webster & Almany 2002).

By examining the stomach contents of speared groupers, scientists have found not only juvenile, but also mature adult reef fish in the stomach of *C. argus* (Randall 1987, Shpigel & Fishelson 1989a, 1991a, Dierking 2007). In their 1991 study, Shpigel and Fishelson removed predators from reefs and monitored the effects over 36 months. The elimination of entire social units of *C. argus* from their home ranges in the Gulf of Aqaba, Red Sea, was followed by an increase in abundance of fish as well as an increase in species diversity. The settlement and survival of new recruits increased, and this was accompanied by an increase in immigration of adults from several species (Shpigel & Fishelson 1991a). The release from predation pressure caused a change in the community structure, and a return to a balanced fish community with high species richness.

Since its introduction to Hawaii, *C. argus* has spread to all the Main Hawaiian Islands (pers. comm. Division of Aquatic Resources). Because of their social systems in native habitats, with small social groups, strong site attachment, and defense of home ranges, these groupers are prone to spread laterally as well as vertically across all suitable habitat as their numbers increase. However, the way these fish are responding since their introduction to a new environment remains an important question. How might the ecology of these fish in Hawaii differ from that of their native habitat in Moorea, French Polynesia?

The waters of Moorea are home to 14 species of grouper, and interspecific competition may influence the feeding ecology and habitat use of competing grouper species. The coral reef habitats of Hawaii and Moorea are similar, but the grouper species compositions are very different. Have introduced groupers undergone ecological character release in Hawaii due to the absence of predators and competition with other grouper species?

Little is known about the feeding ecology, movements, and behavior of *C. argus* in the Hawaiian Islands. In order to quantify the effects *C. argus* may be having on Hawaiian reefs, its ecology must be studied there. As an introduced piscivore that has few natural predators in Hawaii, its diet, movements, and habitat utilization in Hawaii may be different from those in its native habitat. Introduced *C. argus* might grow considerably larger than in their native habitat, dwarfing native Hawaiian benthic predators on the reefs, such as hawkfish and lizardfish. The endemic reef fishes of Hawaii have apparently evolved without large, shallow-water, serranid predators, such as *C. argus*. It

has been suggested that the degree of predation pressure can strongly influence the morphological and behavioral evolution of prey species (Hobson 1963, Shpigel & Fishelson 1991a). Most of the native, shallow water, benthic reef piscivores are much smaller than *C. argus*, and it was assumed that prey were safe once they reach a certain size because of the gape limitation of native predators. This is not the case with *C. argus*; this predator has a very large buccal cavity, enabling it to consume prey two thirds its own length (Meyer unpublished). Dierking (2007) reported large, mature reef fish in the stomachs of 50 cm groupers on the west coast of the island of Hawaii. This capacity may pose a threat to native reef fish communities, including mature adults as well as recruits and juveniles.

Unlike transient native Hawaiian piscivores such as jacks, *C. argus* forms harems, holding home ranges on coral reefs, showing high site fidelity over time (Shpigel & Fishelson 1991b), and seems to experience little competition for resources within its home range. This site fidelity may further influence the predation pressure exhibited by these predators on Hawaiian reef communities.

There is an ever-growing level of interest in Hawaii and elsewhere in the effects of introduced predatory fish on native species. The lack of basic knowledge of the ecology of *C. argus* in Hawaii and the unknown effects this predator may have on Hawaiian reef communities led to studies by Dierking (2007) on the diet and feasibility of a fishery for *C. argus* in the Main Hawaiian Islands. Dierking discovered that *C. argus* in Hawaii are opportunistic predators eating mainly fish and a small portion of crustaceans. With a moderate but unpredictable incidence of occurrence of ciguatera in the tissues of

C. argus, Dierking deemed the Hawaii fishery too risky to be viable (Dierking 2007).

C. argus were introduced to Hawaii in order to establish a fishery. Although the grouper has become established, its fishery has not.

Studies have shown that the removal of species by overfishing may cause changes in competition and predation in the remaining non targeted species (Chiappone et al. 2000). Changes in the abundance of one species may release a competing species, allowing it to increase in size and abundance due to an increase in available resources, and may change aspects of its ecology and the way it uses its resources (Chiappone et al. 2000, Dulvy et al. 2000). Competition for space and resources between ecologically similar coral reef fish has been seen to control the abundance and habitat use of competing species in shared habitat (Schmitt & Holbrook 1990, Robertson 1996, Dulvy et al. 2000).

Interspecific interactions, differences in body size, and aggression between groups of species often affect their patterns of habitat use (Robertson 1996).

Competitive release may also occur when a species is introduced into a new habitat where its natural competitors are absent. With no natural predators or competitors and no fishing pressure, has competitive release resulted in changes in aspects of the ecology of *C. argus* in Hawaii?

To investigate possible differences in the ecology of *C. argus* between its native and introduced habitats, ecological comparisons were carried out at the two locations, focusing on (1) the feeding ecology and diet of *C. argus*, (2) the home ranges, movements, and utilization distributions of *C. argus*, and (3) the occurrence and severity

of ciguatera in *C. argus*. Findings from these studies were compared to work on *C. argus* done by Dierking (2007) and Meyer (unpublished) in Hawaii.

Feeding Ecology and Diet Study

An increase in available resources resulting from competitive release can affect the growth, population structure and feeding ecology of a species (Schmitt & Holbrook 1990, Robertson 1996, Chiappone et al. 2000, Dulvy et al. 2000, Lohrer et al. 2000). Several studies of marine fishes have documented increases in abundance and changes in habitat utilization of a species following the removal of a competitor (Schmitt & Holbrook 1990, Robertson 1996, Dulvy et al. 2000). However, few studies have examined competitive release of a species introduced into a new environment (Lohrer et al. 2000).

The objectives of the feeding ecology and diet study were (1) to describe quantitatively the diet of *C. argus* in Moorea, French Polynesia, combining the methods of stomach content analysis and stable isotope analysis, and (2) to test for size-related ontogenetic shifts in feeding by *C. argus*. This work makes up the bulk of the chapter entitled “Dietary Analysis of the Blue-spotted Grouper, *Cephalopholis argus* in Moorea, French Polynesia”.

Once the diet of *C. argus* was quantified in Moorea, the feeding ecology, growth, and population structure of *C. argus* in Moorea were compared to *C. argus* in the introduced habitat in Hawaii. These data were analyzed in the context of ecological release in the chapter entitled “Differences in Size, Diet, Feeding Ecology and Body Condition of *Cephalopholis argus* in Native and Introduced Populations”.

Home Range Study

Patterns of movement and resource utilization are becoming increasingly important when studying population dynamics, community structure, habitat use and feeding of fishes (Zeller 1997, Meyer et al. 2000, Topping et al. 2005, Meyer et al. 2007). Movement patterns and spatial use are fundamental factors that can give insight into ecological processes at the population, community, and species levels (Zeller 1997). Competition for space and resources between ecologically similar coral reef fish can influence the abundance and habitat use of competing species in shared habitat (Robertson 1996, Dulvy et al. 2000). *C. argus* is one of many species of grouper on the reef in its native habitat in Moorea, and these species probably compete for resources, including prey and sheltering sites. In Hawaii there is a complete lack of native shallow-water groupers, but also few large, sedentary, benthic predators. The objective of the home range study, “Differences Between Hawaii and Moorea in Home Range and Resource Use of the Blue-spotted Grouper, *Cephalopholis argus*”, was to test the null hypothesis that the size of home ranges and the patterns of utilization within home ranges do not differ between Hawaii and Moorea, despite differences in the associated fish communities.

Ciguatera Study

Ciguatera fish poisoning (CFP) occurs pantropically, from 35° N to 35° S around the globe, and poses significant health, resource and economic problems in tropical and subtropical regions of the world (Randall 1980, Lewis 1986, Quod & Turquet 1996, Hokama & Yoshikawa-Ebesu 2001, Chateau-Degat et al. 2005). Annually human CFP

cases range from 25,000 to 50,000 worldwide (Lehane & Lewis 2000, Lewis 2006). Reports of ciguatera incidence in French Polynesia suggest that it is an especially serious problem there, with a higher rate of occurrence there than in most other regions of the Pacific (Lewis 1986). French Polynesia reported an annual incidence of 330 cases per 100,000 population in 1999 (Chateau-Degat et al. 2005). Hawaii has also experienced a series of ciguatera outbreaks with the consumption of both herbivores and carnivores (Randall 1980, Lewis 1986, Hokama et al. 1998), and several fisheries have been closed due to high incidence of CFP. The ciguatera study, “Ciguatera Levels in the Grouper *Cephalopholis argus* in Moorea, French Polynesia”, was focused on CFP in Moorea, to determine whether there are “safe sizes” of *C. argus* which can be consumed, or “safe localities” from which to catch fish for food. Fish from Moorea were collected and tested for ciguatoxin (CTX), and the results for Moorea were compared to those found by Dierking (2007) in Hawaii.

CHAPTER 2

Dietary Analysis of the Blue-spotted Grouper, *Cephalopholis argus* in Moorea, French Polynesia

Introduction

Groupers are members of the subfamily Epinephelinae, inhabiting coral reefs and rocky substrate of littoral zones in subtropical and tropical latitudes, feeding mainly on fish and crustaceans (Randall & Brock 1960, Harmelin-Vivien & Bouchon 1976, Shpigel & Fishelson 1989a, St. John 1999, Morato et al. 2000, St. John & Russ 2001, Webster 2002, Webster & Almany 2002). The blue-spotted grouper, *Cephalopholis argus*, has a widespread distribution, occurring throughout the tropical Indo-Pacific, from the east coast of Africa and the Red Sea, to Japan in the north, and Australia and French Polynesia in the south (Randall 1987). Because of their presence near the top of the food chain these predators can play an important trophic role in reef ecosystems (Shpigel & Fishelson 1989a), however, little is known about the diet of *C. argus* in its native habitat of Moorea French Polynesia.

The use of stable isotope analysis (SIA) in ecological studies is on the rise. The isotopic ratios of carbon and nitrogen in animal tissues can be used to reconstruct dietary history and elucidate trophic interactions (DeNiro & Epstein 1978, 1981, Sheaves & Molony 2000, Ben-David & Schell 2001, Renones et al. 2002, Graham et al. 2007, Hadwen et al. 2007, Ho et al. 2007, Lin et al. 2007, Reich et al. 2007, Takai et al. 2007). Carbon and nitrogen are among several elements that occur in more than one isotopic form; the isotopic composition of various substances, including soil and plant or animal tissue, can

be measured with great precision (Peterson & Fry 1987). Animals retain dietary ^{15}N and ^{13}C preferentially over dietary ^{14}N and ^{12}C , which are released from the body as respired CO_2 and as ammonium (DeNiro & Epstein 1978, 1981, Post 2002), leading to the enrichment of the heavier isotopes in the animal's tissues. The isotopic values of an individual animal's tissue generally reflects the isotopic values of its diet (DeNiro & Epstein 1978, 1981, Minagawa & Wada 1984, Peterson & Fry 1987, Ben-David & Schell 2001), with tissue that is enriched by 3‰ and “heavier” than dietary nitrogen, and enriched by 1‰ for carbon (Fry & Arnold 1982, Peterson & Fry 1987, Post 2002, Takai et al. 2007). Nitrogen isotopic values have been commonly used as an indicator of trophic level, while carbon values provide information about the base of the food web (DeNiro & Epstein 1978, 1981, Renones et al. 2002). ^{13}C and ^{15}N concentrations in muscle and protein are analyzed using a mass spectrometer, measuring “assimilated, not just ingested diet,” elucidating an animal's short-term and long-term diet, as well as dietary switching (Fry & Arnold 1982, Peterson & Fry 1987, Renones et al. 2002).

Stomach content analysis (SCA) is an effective method for estimating the range of diet of a particular predator, providing information on prey taxonomy, size composition, and possible ontogenetic shifts that may occur during the growth of the predator species, that cannot be derived from SIA (St. John 1999, Renones et al. 2002). Several species of grouper have been the subject of prey choice, stomach content analysis, and diet studies, the results of which show these predators near the top of the food chain, feeding mainly on fish and crustaceans (Harmelin-Vivien & Bouchon 1976, Hyslop 1980, Parrish et al. 1985, Kingsford 1992, St. John 1999, Morato et al. 2000, St. John & Russ 2001, Renones et al. 2002, Craig 2007, Graham et al. 2007, Hadwen et al. 2007, Ho et al. 2007, Lin et al.

2007). When used in conjunction, SCA and SIA can give a more comprehensive view of an animal's diet and trophic position. The objectives of the present study were to combine the methods of SCA and SIA to 1) describe quantitatively the diet of *C. argus* in Moorea, French Polynesia, and 2) test for size-related ontogenetic shifts in feeding by *C. argus*.

Methods

Stomach Content Analysis

Specimens of *Cephalopholis argus* were collected at 13 sites along the north and southwestern coasts of the island of Moorea (Fig. 2.1) in March and June of 2005, and used for both SCA and SIA. Moorea is a small island in the South Pacific, located 19 km west of the island of Tahiti in French Polynesia.

Fish were collected throughout the day on the reef slope from depths of 3-30 m by scuba divers using spears. Speared fish were placed immediately into Ziploc bags while under water to prevent loss of regurgitated stomach contents after capture. Stomach contents were analyzed using methods similar to those of Dierking (2007). Standard and total length (TL) of all *C. argus* specimens were measured to the nearest millimeter, and each specimen's sex and weight (to the nearest gram) were recorded. The mouth and gills of each specimen were inspected for regurgitated prey. The stomachs were removed and their contents noted as either "empty" or "full" (i.e. containing prey).

Vacuity rate, defined as the fraction of all stomachs examined that were empty, was determined. For “full” stomachs, the length and body-depth of each prey item was measured to the nearest millimeter and identified to the lowest taxon possible, following Randall (2005).

Stable Isotope Analysis

Tissue samples from *C. argus* were used in this study because it is impractical to determine isotopic ratios from the whole body of a large animal (DeNiro & Epstein 1978). White muscle tissue samples were taken from the head, middle (posterior of the pectoral fin), and tail (caudal peduncle) regions of the fish for processing at the University of Hawaii Manoa. White muscle tissue samples of all prey items present in the stomachs of *C. argus* specimens were also analyzed for carbon and nitrogen isotopic composition. Tissue samples were dried to a constant weight at 60° C for 72 hours and ground into a powder with a mortar and pestle. A 0.02 mg subsample of the powdered tissue was then analyzed on an isotope ratio mass spectrometer, and ratios of carbon and nitrogen were calculated. The resulting isotopic values from *C. argus* and prey samples were compared to international standards of V-PDB for carbon, and atmospheric N₂ for nitrogen. δC and δN values are expressed as parts per thousand (‰).

$$\Delta^{13}\text{C}(\text{in } \text{‰}) = \left[\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{sample}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{standard}}} - 1 \right] \times 1000$$

$$\Delta^{15}\text{N}(\text{in } \text{‰}) = \left[\frac{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}}}{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{standard}}} - 1 \right] \times 1000$$

Statistical Analysis

The types of prey items present in the stomachs were analyzed with numerical abundance and frequency of occurrence data expressed as percentages. Following Sudekum et al. (1991), numerical importance (defined as the number of individuals of one prey category divided by the total number of prey individuals found in all the sampled stomachs, multiplied by 100) and frequency of occurrence (defined as the number of stomachs containing prey items of one category divided by the total number of stomachs that contained any identifiable prey items, multiplied by 100) were calculated for prey types.

In the present study, several aspects of size related changes in feeding were examined to assess ontogenetic feeding patterns: 1) change in the proportion of full and empty stomachs as *C. argus* increases in size, 2) change in length (TL) and body-depth of prey consumed as *C. argus* increases in size, and 3) change in the proportions of fish and crustacean prey consumed as *C. argus* increases in size. Ontogenetic variations in diet were examined by grouping *Cephalopholis argus* by total length into five size classes (Morato et al. 2000): 1) 15-20 cm, 2) 20.5-25 cm, 3) 25.5-30 cm, 4) 30.5-35 cm, and 5)

35.5-40 cm. All statistical analyses were conducted with Minitab 14 with results considered significant at $p < 0.05$. Presence of prey items in the stomachs of the different size classes was analyzed by chi-squared test. Predator-prey size relationships were tested by linear regression and ANOVA between predator size (TL) and prey length (TL) and between predator size (TL) and prey body depth (D). Regressions were run to investigate the relationship between length of *C. argus* and isotopic signatures for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. An ANOVA and Tukey's comparison were run for size class and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Prey isotopic values were plotted against *C. argus* isotopic values to investigate the long-term diet of *C. argus*.

Results

Stomach Content Analysis

Diet Composition

A total of 166 *C. argus* were collected from the reef slopes of Moorea; 62 of the 166 had prey items in their stomachs. Fish were the dominant prey (by both numerical abundance and frequency of occurrence), accounting for 76% of the stomachs with prey; crustaceans accounted for 19%, and the remaining 5% of stomachs contained both fish and crustaceans. When broken down into numerical percent, fish accounted for 79% of *C. argus*' diet, and crustaceans for 21%. Of the 62 stomachs with prey, 89% contained only one prey item. The maximum number of prey were found in the stomach of a 22-cm fish which contained three prey fish. *C. argus* with multiple prey present in their stomachs ranged in size from 20.5 to 33 cm in total length, with a median length of 26 cm.

Diet Composition: Fish Families

Approximately 56% of the prey fish were not identifiable as a result of advanced stages of digestion; this was a higher percentage of prey in advanced stages of digestion than seen in other studies (Connell 1998, St. John & Russ 2001, Dierking 2007). The remaining prey fish could be classified to family, and many to genus and species. The fish portion of the diets of sampled *C. argus* included 10 families (Table 2.1), with a wide variety of species from different feeding guilds. Piscivores (Cirrhitidae, Epinephelinae, Labridae), herbivores (Acanthuridae, Scaridae), corallivores (Chaetodontidae, Monacanthidae), and planktivores (Pomacentridae, Holocentridae) occurred in the sampled stomachs. Small, cryptic, benthic species, such as gobies and blennies, were mostly absent from the diet. Three families -- Pomacentridae, Scaridae, and Monacanthidae -- accounted numerically for more than 60% of the fish prey consumed. Pomacentrids were taken in the highest numbers at 25%, followed by scarids at 22%.

Ontogenetic Shift

There was a significant difference in vacuity rate between the different size classes of *C. argus* (Chi-squared, $p=0.031$) (Fig. 2.2)

Cephalopholis argus in Moorea consumed prey of a wide range of sizes and shapes (Table 2.2). There was a significant positive relationship between *C. argus* total length and the total length of prey (Linear regression, $p<0.001$, $R^2=0.42$) (Fig. 2.3). The stomach of a 33-cm *C. argus* contained an 18-cm labrid, showing that *C. argus* can consume prey larger than half its body length.

Grouping *C. argus* into size classes allowed for analysis of the range of prey lengths and body-depths consumed by the different size classes of *C. argus* (Table 2.2, Fig. 2.4).

There were significant differences between the lengths of prey consumed by *C. argus* in size classes two and three (20.5-25 cm and 25.5-30 cm), versus those consumed by the larger predators in size class four (30.5-35 cm) (ANOVA, $p=0.002$, $R^2=0.46$) (Fig. 2.4).

As *C. argus* increased in length, they consumed deeper-bodied prey, with a significant positive relationship between predator TL and prey body-depth, D, (Linear regression, $p<0.001$, $R^2=0.54$) (Fig. 2.5). Prey body-depth varied significantly with *C. argus* size class; prey of *C. argus* in size classes one, two and three were significantly less deep-bodied than prey in the largest size class (ANOVA, $p=0.004$, $R^2=0.54$) (Fig. 2.6). Larger *C. argus* fed on longer and deeper-bodied prey as well a wider range of prey sizes than smaller *C. argus* (Table 2.2).

Ontogenetic Shift: Fish vs Crustacean

The diets of *C. argus* in size classes two, three, and four consisted of 81, 70, and 85% fish respectively (Fig. 2.7). There were no significant changes in the proportions of fish or crustaceans in the diets of *C. argus* as they increased in length (Chi-square, $p=0.373$).

The sample size of *C. argus* in size class one was small (only nine individuals), and only two of those contained prey: one contained a pomacentrid and the other, a crustacean.

This sample and the sample of size class five were too small to analyze for ontogenetic shifts.

Stable Isotope Analysis

The variability of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ within fish samples from head, body and tail was nominal in the five fish tested (Fig. 2.8a and Fig. 2.8b). SIA precision for *C. argus* tissue was high, and variability among tissue samples within the same fish fell below the Glycine standard and acceptable $\pm 0.3\text{‰}$ value (Table 2.3). These results show that SIA is applicable to *C. argus* tissue. Samples from the midsection of the body were chosen for all further analyses.

The $\delta^{15}\text{N}$ values of *C. argus* white muscle taken from the mid-body ranged from 11.06 to 14.10‰ with a mean of 12.41‰ (Fig. 2.9 & 2.13). There was a significant, gradual increase in $\delta^{15}\text{N}$ values as the total length of *C. argus* increased, (Linear regression $p < 0.001$, $R^2 = 0.21$) (Fig. 2.9). $\delta^{15}\text{N}$ values varied significantly by size class; the $\delta^{15}\text{N}$ values of size class one were lower and differed significantly from all larger size classes (ANOVA, $p < 0.001$, $R^2 = 0.24$) (Fig. 2.10).

The $\delta^{13}\text{C}$ values for *C. argus* muscle tissue ranged from -15.00‰ to -11.53‰ with a mean of -14.10‰ (Fig. 2.11 & 2.13). There was a significant positive relationship between $\delta^{13}\text{C}$ values and fish size (Linear regression, $p < 0.001$, $R^2 = 0.46$) (Fig. 2.11), and $\delta^{13}\text{C}$ values varied significantly by size class (ANOVA, $p < 0.001$, $R^2 = 0.55$) (Fig. 2.12). Values differed in a stepwise fashion with increasing size class: size class one differed from size classes four and five; size class two differed from size classes three, four, and five; size class three differed from size classes four and five, and size class four differed from size class five (Fig. 2.12). The scatterplot of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ values in Figure 2.13

shows that the isotopic values are independent of each other (Linear regression, $p=0.392$, $R^2=0.007$).

Discussion

Groupers are known to be highly picivorous predators (Randall & Brock 1960, Harmelin-Vivien & Bouchon 1976, Shpigel & Fishelson 1989a, Kingsford 1992, Connell 1998, St. John 1999, Morato et al. 2000, St. John 2001, St. John & Russ 2001, Renones et al. 2002, Craig 2007), and because of their large gape, they are able to consume a wide variety of shapes and sizes of prey (St. John 1999).

Stomach Content Analysis

Overall food was found in 37% of the stomachs of *C. argus* sampled; fish made up 79% and crustaceans 21% by numbers of the diet of *C. argus* in Moorea. These results agree with those in Tahiti (Harmelin-Vivien & Bouchon 1976), but differ from the Red Sea and some other parts of the world. Of the *C. argus* sampled in the Red Sea, 72% contained at least one prey item, and fish represented 95% of the prey (Shpigel & Fishelson 1989a).

In the Marshall Islands, the diet of *C. argus* was composed of 60% fish and 30% crustaceans (Harmelin-Vivien & Bouchon 1976). With a diet of 79% fish in Moorea and 95% in the Red Sea, *C. argus* is one of the more strongly picivorous species of grouper. Similar studies have shown that other species of serranids are also highly picivorous: 92-96% *Plectropomus leopardus* (Kingsford 1992, St. John 1999, St. John & Russ 2001), 95% *Mycteroperca microlepis* (Mullaney & Gale 1996), 50% *Epinephelus quoyanus*

(Connell 1998), 58% *Epinephelus marginatus* (Renones et al. 2002), 68% *Cephalopholis urodeta* (Nakai et al. 2001).

The species composition of reef fish and crustaceans in the diet of *C. argus* shows that this predator feeds on the reef, and not on adjacent sand areas or high in the water column. The dominant prey consumed were from the families Pomacentridae, Scaridae, and Monacanthidae, which could be categorized as living in the demersal reef habitat. The presence of these demersal fish families, coupled with the complete absence of clupeids and other pelagic and high-water-column species in the stomachs of sampled *C. argus*, indicates that *C. argus* feed close to the reef substrate, on demersal and low-water-column reef fish.

This high diversity of prey fish species indicates that *C. argus* are opportunistic and generalist predators, feeding on a wide variety of reef fish from all feeding guilds (Table 2.1). The members of the family Holocentridae found in the stomachs of *C. argus* were the only nocturnal prey consumed. Holocentrids shelter in a stupor-like state in large schools during the day, making them easy prey for large predators. The lack of other nocturnal prey, and tracking data from both Hawaii and Moorea (Meyer unpublished), indicate that *C. argus* in Moorea feed primarily during the day.

A large piece of tuna flesh was found in the stomach of a *C. argus* collected at a shark feeding site where dive shops regularly chum for sharks with tuna. This reinforces the impression that groupers in general, and *C. argus* in particular, will consume whatever prey that will fit into its buccal cavity. The occurrence of several picivorous species -- large wrasses, hawkfishes and even other groupers -- in the stomachs of *C. argus*

indicates the high trophic position of these groupers and indicates that they are one of the top predators on the reefs of Moorea.

Ontogenetic Shift: Prey Length and Body Depth

With increasing size, *C. argus* consumed a wider and more variable size range of prey. This trend has been seen in several piscivorous species, including other species in the family Serranidae (*Plectropomus leopardus*, Kingsford 1992, St. John 1999; *Epinephelus quoyanus*, Connell 1998). *C. argus* in Moorea seem to make a shift in feeding at about 30 cm total length, where they begin taking significantly larger and deeper-bodied prey. *C. argus* in size class four (30.5-35cm) consume prey that are significantly longer and deeper-bodied than prey consumed by *C. argus* in size classes two and three, 20.5-25 cm and 25.5-30 cm respectively. The results of prey length and body-depth analyses show that as *C. argus* grow larger, they utilize more of the available resources.

This trend was also observed in *P. leopardus* by Choat (1968), Kingsford (1992), and St. John (1999), and in *Serranus atricauda* by Marato et al. (2000). The diet of *P. leopardus* on the Great Barrier Reef varied with size until predators reached 35cm TL (St. John & Russ 2001). St. John (1999) noted that *P. leopardus* in smaller size classes consumed longer, more slender prey while they were more gape limited, and larger *P. leopardus* consumed significantly deeper-bodied prey.

The Moorea data show that there are increases in both the lower and upper size limit of prey that are consumed by *C. argus* (Fig. 2.4, Fig. 2.6), and despite their ability to consume larger prey, fish in the larger size classes of *C. argus* frequently fed on small

prey. Surprisingly large fish (up to 18 cm) were eaten by *C. argus* in this study. Adult fish from all feeding guilds were found in the stomachs of *C. argus*, ruling out the possibility of gape limitation protecting prey species once they reach maturity. *C. argus* have been seen in both Moorea and Hawaii with the tails of prey fish protruding from their mouths, showing that these predators are taking prey that are so large that they do not fit fully into the body cavity of the *C. argus* (Dierking 2007). *C. argus* were also captured that had prey that were partially digested; the portion of the prey that was in the stomach was in various stages of digestion, while the portion of the prey that was in the mouth was fresh.

Ontogenetic Shift: Fish and Crustaceans

Several grouper species show an increase in piscivory as they increase in size. Juvenile serranids feed on both invertebrates and fish, but as the predators grow, the proportion of invertebrate prey decreases, and the diet consists mostly of fish (Harmelin-Vivien & Bouchon 1976, Shpigel & Fishelson 1989a, Kingsford 1992, St. John 1999, Morato et al. 2000, Nakai et al. 2001, St. John & Russ 2001, Renones et al. 2002). In the present study there were no significant differences among size classes in the proportion of fish in the diet of *C. argus*.

Stable Isotope Analysis

The $\delta^{15}\text{N}$ values show the trophic level of an organism in a food chain, with primary, secondary, and tertiary consumers exhibiting step-wise enrichment of $\delta^{15}\text{N}$ with each

increasing trophic level (Minagawa and Wada 1984; Peterson and Fry 1987; Post 2002).

As a top predator, *C. argus* should have the $\delta^{15}\text{N}$ signature of a tertiary consumer, resulting from eating planktivores, herbivores, and other carnivores, including piscivorous fishes. Zooplanktivores are secondary consumers, and occupy a lower position on the trophic ladder; as a result, zooplanktivores generally have low $\delta^{15}\text{N}$ values (Fig. 2.15). Minagawa and Wada (1984) found that the $\delta^{15}\text{N}$ of planktivores ranged from 5 to 7‰. This is similar to, and slightly lower than the values for planktivores in this study, 8.5 to 9.5‰. Differences in $\delta^{15}\text{N}$ in similar feeding guilds are due to basal nitrogen values of the primary producers in the study area. Other secondary consumers include the corallivore *Chaetodon pelewensis* and *Pseudocheilinus hexataenia* which feed on benthic invertebrates and have lower $\delta^{15}\text{N}$ values (Fig. 2.14). Benthic omnivores consume both invertebrates and fishes, and occupy a higher trophic level than do species that consume only invertebrates. *Sargocentron microstoma*, *Epibulus insidiator*, and *Paracirrhites arcatus* are all members of the benthic omnivore feeding guild, and due to this high trophic level, they acquire correspondingly higher values of $\delta^{15}\text{N}$ (Fig. 2.14).

The carbon isotopic values of benthic herbivores are enriched in $\delta^{13}\text{C}$ when compared to planktivorous species, due to the basal difference of the carbon source; benthic algae are enriched in $\delta^{13}\text{C}$ compared to phytoplankton. The carbon signature of *C. argus* can be used to determine whether they are general and opportunistic predators, or if they focus on a particular feeding guild preferentially. For example, if *C. argus* feed more on benthic herbivores, their muscle tissue should be enriched in $\delta^{13}\text{C}$. Because of the basal difference in $\delta^{13}\text{C}$ values, fish that feed on zooplankton, such as *Chromis vanderbilti* and *Cirrhilabrus* sp., have lower $\delta^{13}\text{C}$ values than those that feed on benthic invertebrates,

typically falling between -19 and -16‰ (Takai et al. 2007) (Fig. 2.14). Benthic foragers have higher $\delta^{13}\text{C}$ values, ranging from -16 to -13‰ (Takai et al. 2007), and fall on the right side on the graph in Figure 2.14.

When $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for *C. argus* are plotted with isotopic signatures from prey, a pattern is clear, and *C. argus* cluster at the top of the graph, ranging from 11.06 to 14.10‰ (mean 12.4‰) for $\delta^{15}\text{N}$ and -15.0 to -11.5 ‰ (mean -14.1‰) for $\delta^{13}\text{C}$ (Fig. 2.15). The $\delta^{15}\text{N}$ values show *C. argus* as a tertiary consumer, and the $\delta^{13}\text{C}$ values place the group, as a whole, as benthic feeders. Similar results for nitrogen have been seen in other groupers, e.g. $\delta^{15}\text{N}$ values for *E. marginatus* ranged from 8.8 to 13.1‰ (Renones et al. 2002). In other tertiary consumers, *Thunnus albacares* had a mean of 10.2‰ (Graham et al. 2007), *Sebastes* sp. ranged from 11 to 13‰ (Minagawa & Wada 1984), and scorpaeniform species ranged from 10.2 to 13.1‰ (Takai et al. 2007). The carbon values of *C. argus* are characteristic of benthic foragers, suggesting that *C. argus* are primarily consuming benthic foraging prey, rarely venturing off the substrate and into the water column to feed. These are reef-dwelling predators, not pelagic foragers like jacks and tunas.

Several species of Epinephelinae undergo an ontogenetic feeding shift (St. John 1999, St. John & Russ 2001, Renones et al. 2002). If there is a feeding shift in *C. argus* as they increase in size, different size classes should show different isotopic signatures, i.e. changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Stable isotope analysis of *C. argus* tissue revealed an ontogenetic shift in feeding as *C. argus* reach larger sizes; this feeding shift is clear when isotopic values are grouped into size classes (Fig. 2.10 & 2.12). The $\delta^{15}\text{N}$ values of the smallest size class (size class one) are lower and differ significantly from all larger size classes when analyzed by ANOVA ($p < 0.001$). These results indicate that smaller *C.*

argus are eating prey that are primary consumers, and are lower on the trophic ladder.

The $\delta^{13}\text{C}$ values of size classes one, two and three all differ significantly from size classes four and five; indicating that smaller *C. argus* have a mixed diet, feeding on both planktivores and benthivores. As *C. argus* increase in size, they switch to feeding on benthic herbivores and benthic secondary consumers.

Conclusions

The combined diet analyses show that *Cephalopholis argus* is a generalist predator, feeding on a variety of prey from different trophic levels as well as different feeding guilds, including planktivores and benthic feeders. Data from both the SCA and SIA indicate that as *C. argus* increase in size, they consume larger prey and prey that occupy higher trophic levels. As *C. argus* grow, they feed more on secondary consumers and larger prey, both longer and deeper-bodied. The positive relationship between $\delta^{15}\text{N}$ and *C. argus* size concurs with information obtained from stomach content analysis and shows a gradual increase in trophic position by this species with age. $\delta^{15}\text{N}$ enrichment can be caused by several factors; two obvious factors are a shift in trophic feeding level and an increase in feeding breadth that occurs with an increase in predator size. As *C. argus* grow, there may be a shift away from preferred prey in lower trophic levels and low $\delta^{15}\text{N}$ values (crustaceans and planktivorous fishes) to prey of higher $\delta^{15}\text{N}$ values (benthic feeding carnivores) (Renones et al. 2002).

The $\delta^{13}\text{C}$ values of the smaller size classes all differed significantly from the larger size. These results indicate that smaller *C. argus* have a mixed diet, feeding on both

planktivores and benthivores, and that as they increase in size, they switch to feeding more heavily on benthic herbivores and benthic secondary consumers. The shift in feeding seems to occur at ~ 30.5-35 cm in total length, i.e. in size class four. Evidence of such an ontogenetic feeding shift at size class four was seen in the results of the stomach content analysis, where larger *C. argus* preyed upon significantly larger, deeper-bodied prey. Benthic foragers tend to be larger and deeper-bodied than planktivorous species. Because of their shape, benthic foragers may only be accessible as a prey source to larger predators.

Small, young individuals of many species undergo size-related feeding shifts as they grow (Renones et al. 2002, Ho et al. 2007). Feeding shifts may be caused by gape limitation, predator evasion, or a combination of the two. The carbon isotopic values, as well as the stomach content analysis of *C. argus* in Moorea, indicate that small *C. argus* are feeding on small planktivores such as *Chromis vanderbilti* that hover over the reef in large swarms. Smaller *C. argus* are more susceptible to predation and may use different habitats to avoid being eaten by larger predators. By focusing on schooling planktivores that are near the substrate and hover over coral heads, smaller *C. argus* decrease their own risk of being preyed upon. As *C. argus* grow, they become less susceptible to predation and their gape increases, enabling them to use different feeding habitats, switching to benthic consumers as their main form of prey.

Table 2.1. Frequency % of fish families found in the stomachs of *C. argus* at Moorea.

Fish Family	Number	%
Pomacentridae	7	25
Scaridae	6	22
Monacanthidae	4	15
Labridae	3	11
Holocentridae	2	7
Cirrhitidae	1	4
Chaetodontidae	1	4
Epinephelinae	1	4
Pomacanthidae	1	4
Acanthuridae	1	4

Table 2.2. Prey by *C. argus* size class. Basic statistics for total length and body-depth of prey fish consumed by *C. argus* in different size classes.

Size Class (TL)	Prey Length (cm)				Prey Body Depth (cm)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
1	5	2.1	3.5	6.5	1.8	1.0	1.0	2.5
2	4.5	2.4	2.0	10.0	1.7	0.7	1.0	3.0
3	6.2	2.0	4.0	8.5	2.3	0.6	1.5	3.0
4	11.25	4.6	5.5	18.5	3.8	1.2	2.0	5.5

Table 2.3. Stable isotope variation within a single individual. Standard deviations of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among tissue samples within the same individual *C. argus*. SIA precision for *C. argus* tissue was high, and variability fell below the Glycine standard and acceptable $\pm 0.3\text{‰}$ value.

Fish	Stdev $\delta^{15}\text{N}$	Stdev $\delta^{13}\text{C}$
M002	0.06	0.26
M013	0.09	0.28
M020	0.06	0.07
M026	0.28	0.11
M032	0.05	0.11

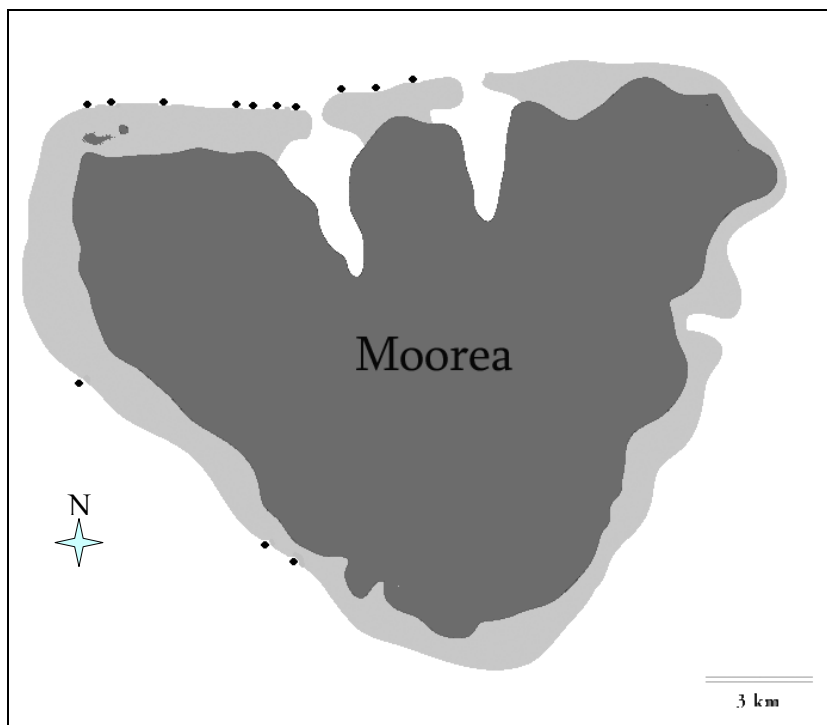


Figure 2.1. The island of Moorea. Black circles represent the 13 sampling sites for SCA and SIA.

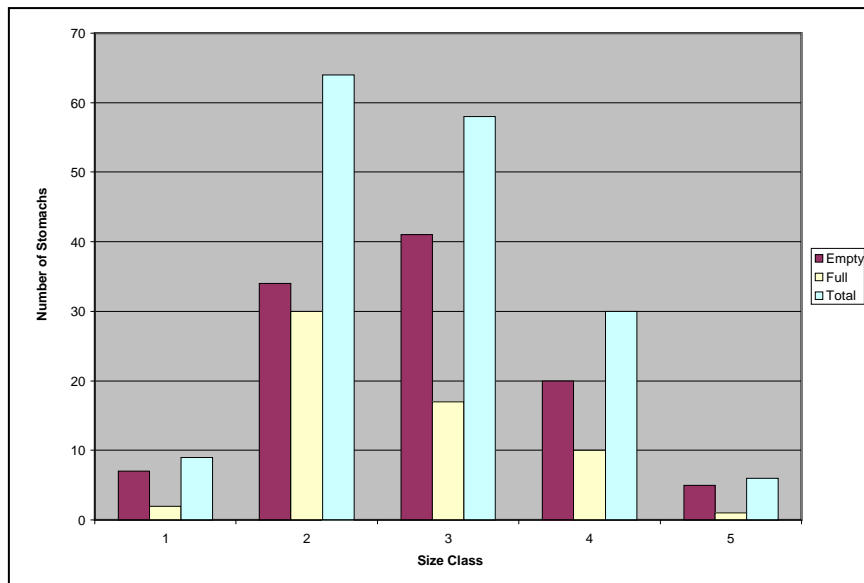


Figure 2.2. Number of empty and full stomachs by size class of *C. argus*. Fish were divided into size classes by total length (cm): 1) 15-20, 2) 20.5-25, 3) 25.5-30, 4) 30.5-35, and 5) 35.5-40. $p=0.031$.

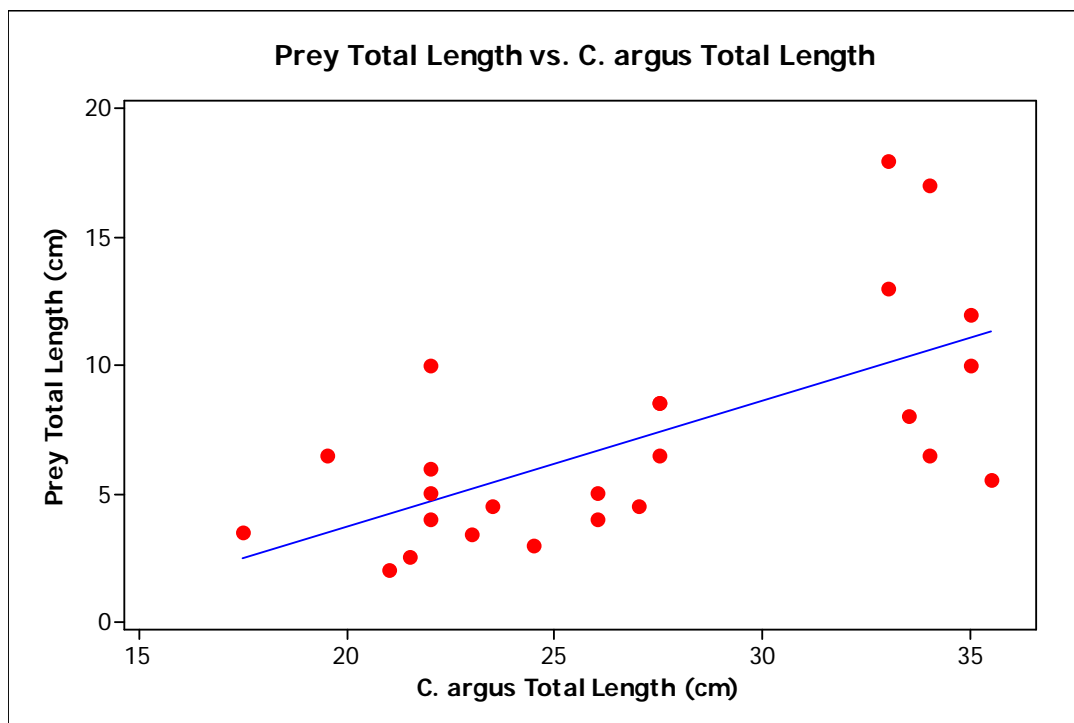


Figure 2.3. Scatterplot of prey total length and *C. argus* total length, $p < 0.001$, $R^2 = 0.42$.

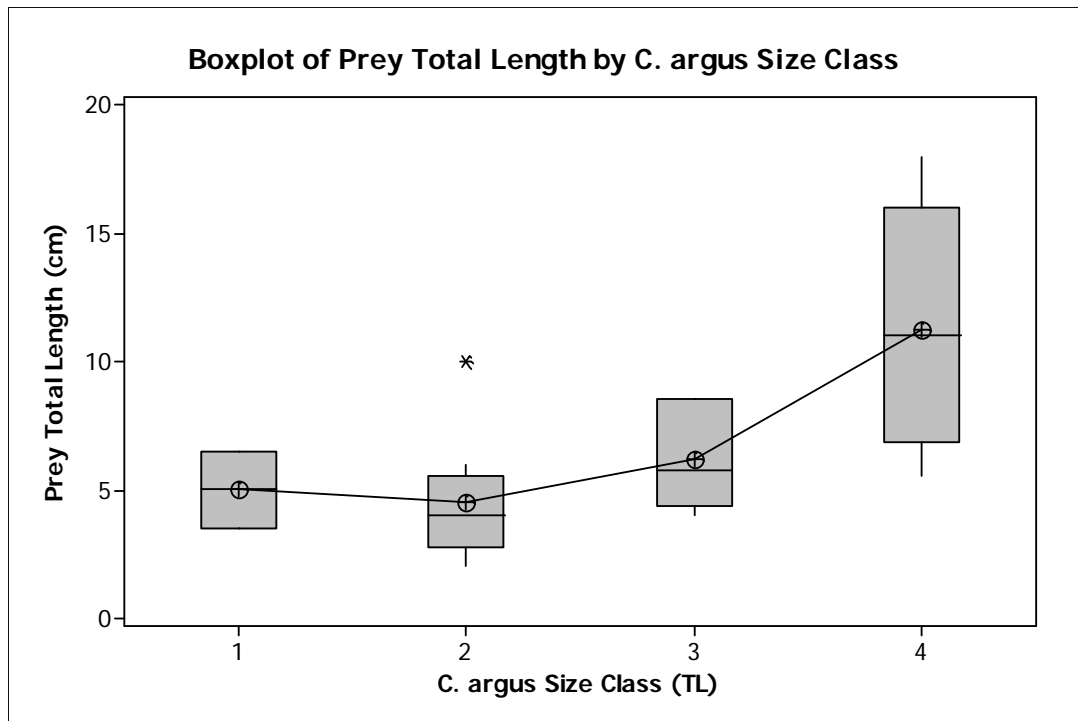


Figure 2.4. Boxplot of prey total length (cm) vs. *C. argus* size class. Sizes classes were grouped by total length: 1) 15-20 cm, 2) 20.5-25 cm, 3) 25.5-30 cm, 4) 30.5-35 cm. $p=0.002$, $R^2=0.49$.

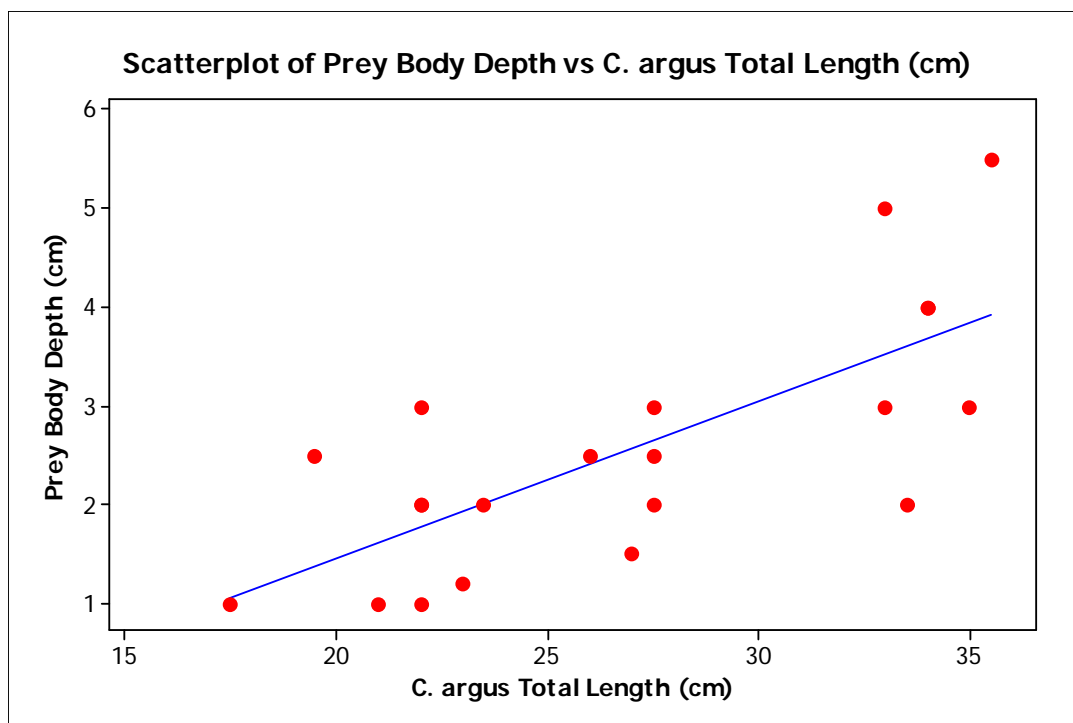


Figure 2.5. Scatterplot of prey body depth vs. *C. argus* total length, $p < 0.001$, $R^2 = 0.54$.

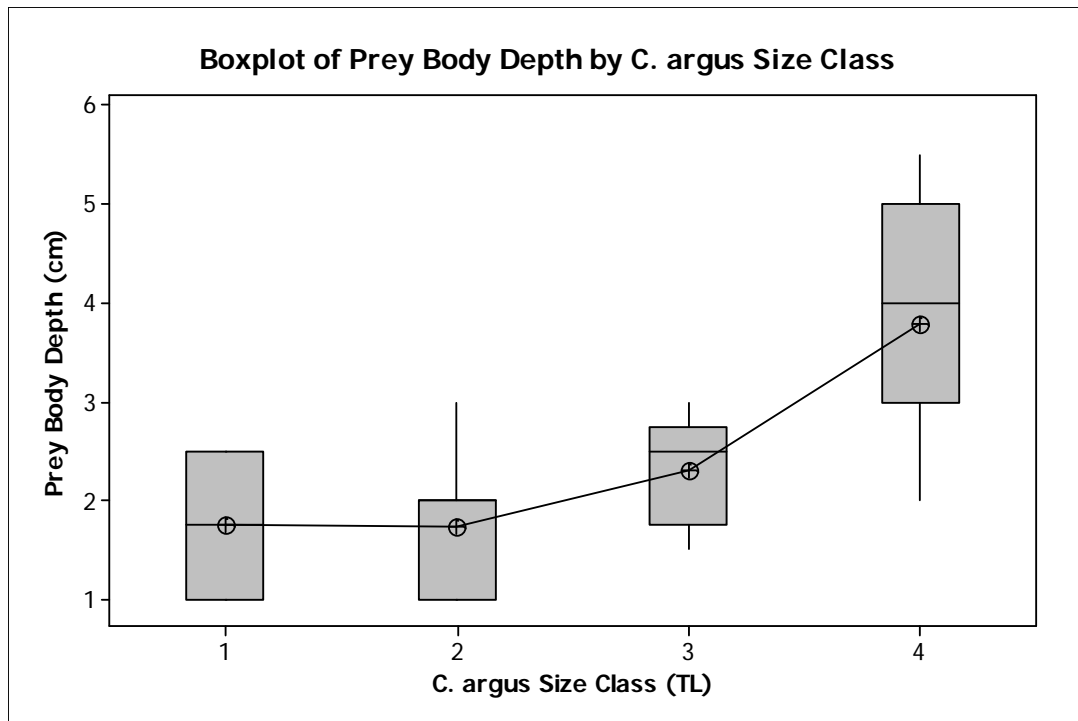


Figure 2.6. Boxplot of prey body depth (cm) by *C. argus* size class. Size classes were grouped by total length: 1) 15-20 cm, 2) 20.5-25 cm, 3) 25.5-30 cm, 4) 30.5-35 cm. $p=0.004$, $R^2=0.54$.

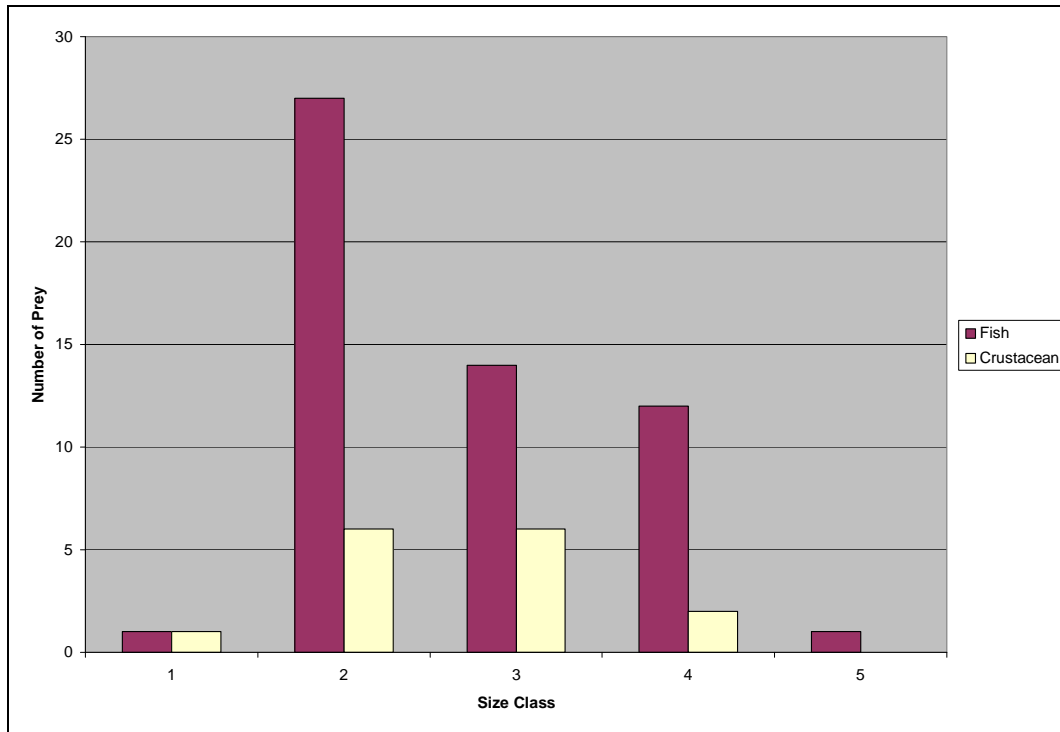


Figure 2.7. Stomach content of *C. argus* by size class. Dark bars represent the number of fish, and light bars represent the number of crustaceans. Size classes are grouped by total length (cm). Chi-square test, $p=0.373$.

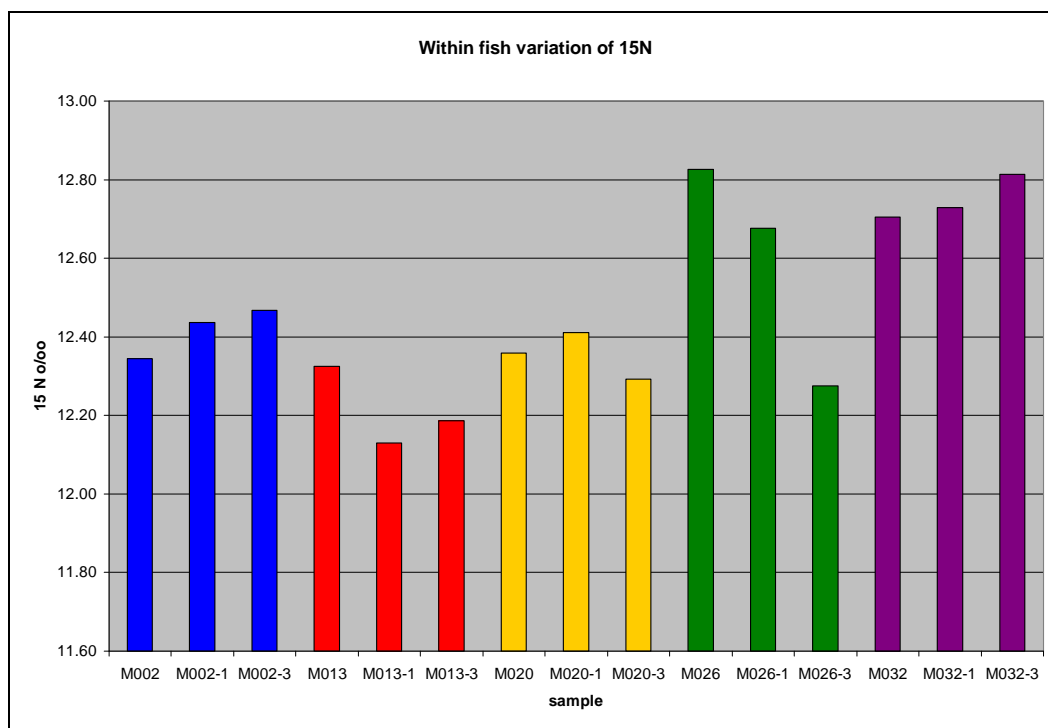


Figure 2.8a. Variability in $\delta^{15}\text{N}$ within an individual. Variability in $\delta^{15}\text{N}$ of samples from head (1), body (2), and tail (3) of same fish.

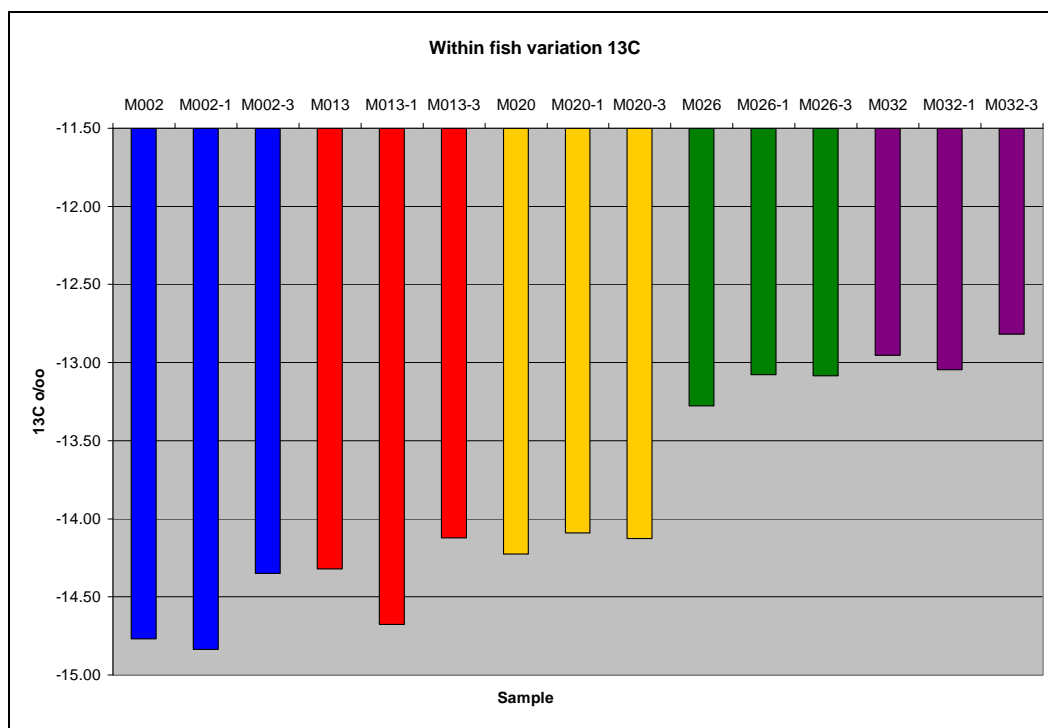


Figure 2.8b. Variability in $\delta^{13}\text{C}$ of samples from head (1), body (2), and tail (3) of same fish.

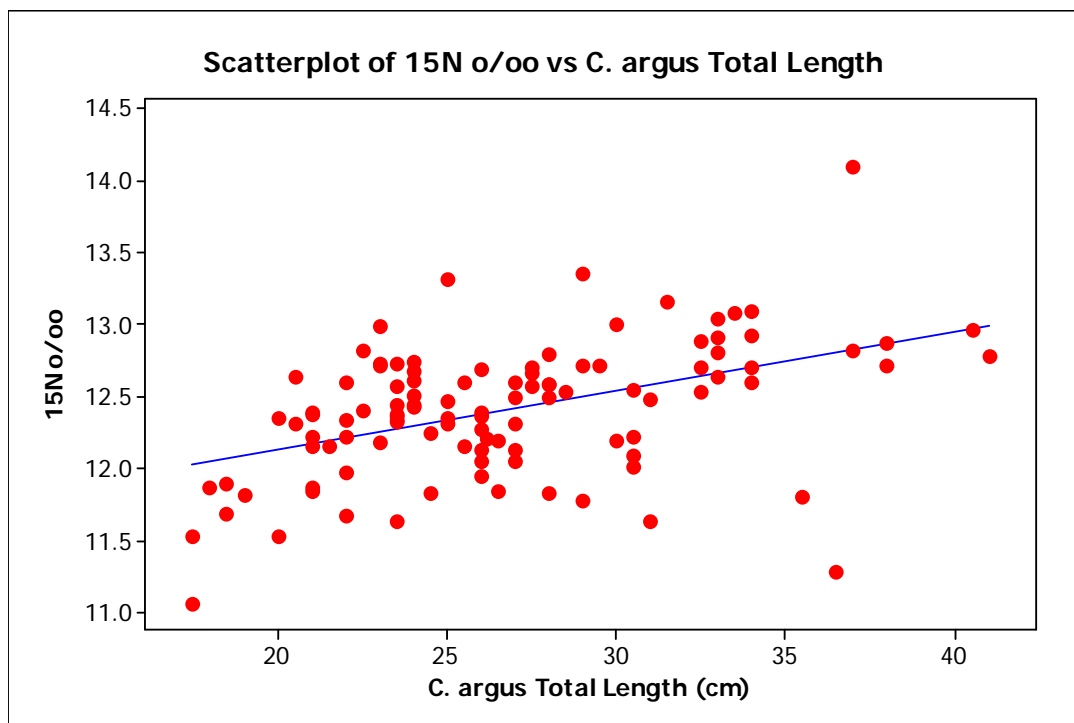


Figure 2.9. Scatterplot of $\delta^{15}\text{N}$ vs. *C. argus* total length (cm), $p < 0.001$ $R^2 = 0.21$.

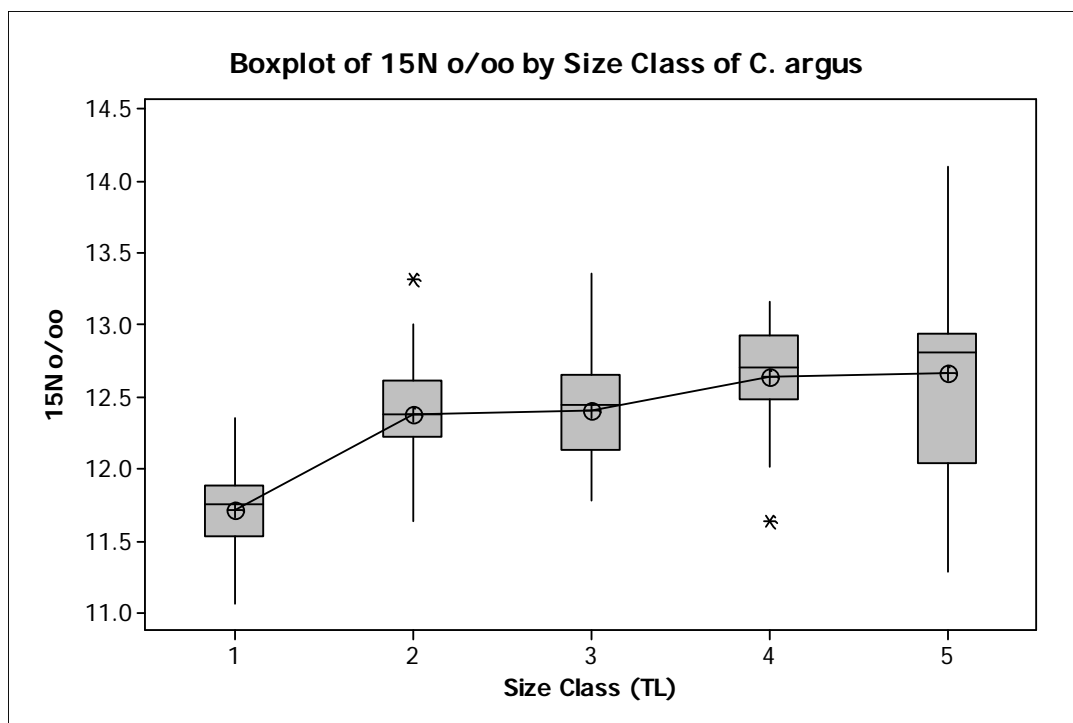


Figure 2.10. Boxplot of $\delta^{15}\text{N}$ values of *C. argus* in 5 size classes. $\delta^{15}\text{N}$ values varied significantly with size class of *C. argus*, $p < 0.001$, $R^2 = 0.24$. Size class 1 is significantly different from all other size classes; size class 2 is significantly different from size class 4. Size classes: 1) 10-15cm, 2) 15.5-20cm, 3) 20.5-25cm, 4) 25.5-30cm and 5) 30.5-35cm.

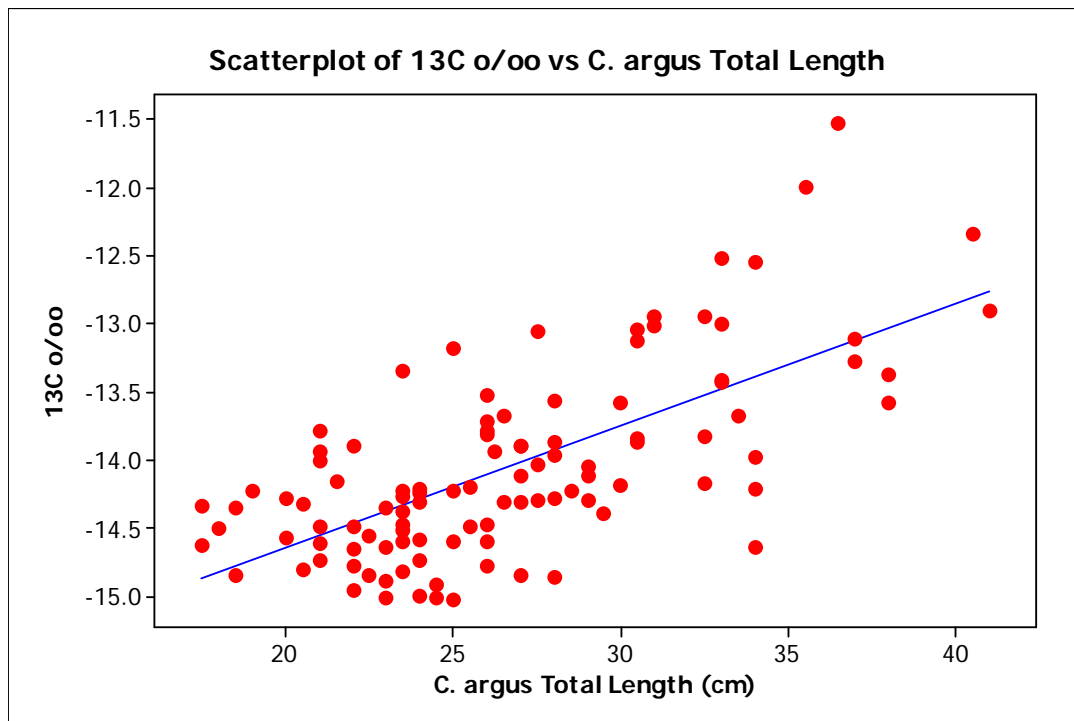


Figure 2.11. Scatterplot of $\delta^{13}\text{C}$ vs. *C. argus* total length (cm), $p < 0.001$, $R^2 = 0.46$.

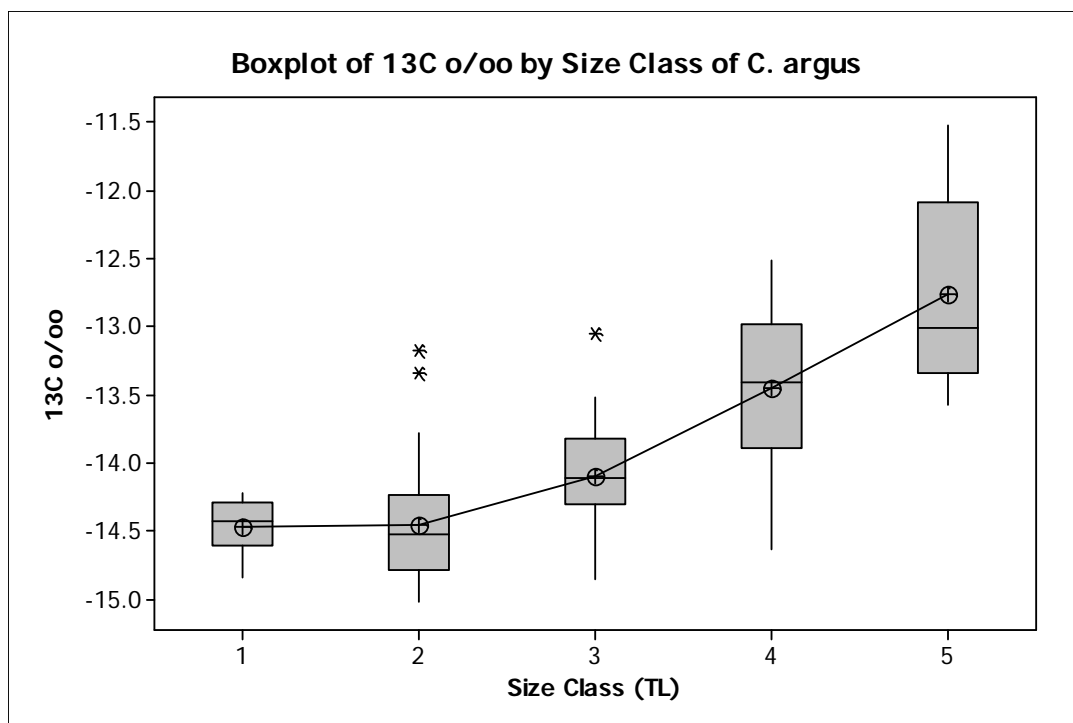


Figure 2.12. Boxplot of $\delta^{13}\text{C}$ values of *C. argus* in 5 size classes. Size class 1 is significantly different from size classes 4 and 5. Size class 2 is significantly different from 3, 4, and 5. Size class 3 is significantly different from 4 and 5, and size class 4 is significantly different from 5, $p < 0.001$, $R^2 = 0.55$.

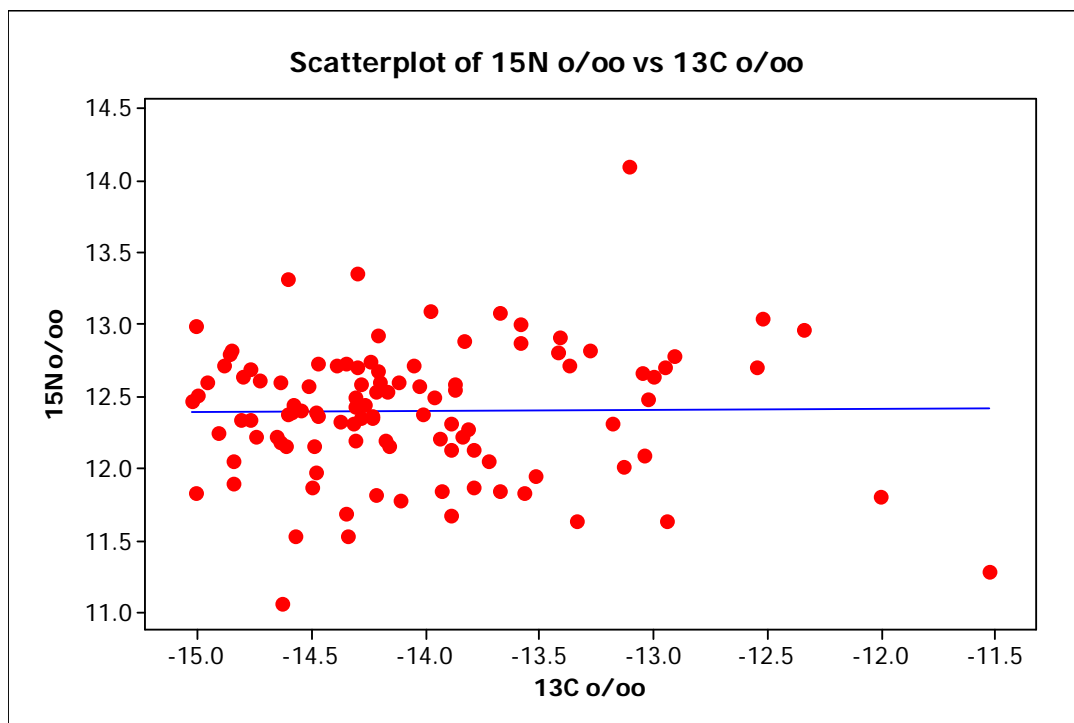


Figure 2.13. Scatterplot of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The isotopic values are relatively independent of each other, $p=0.392$, $R^2=0.007$.

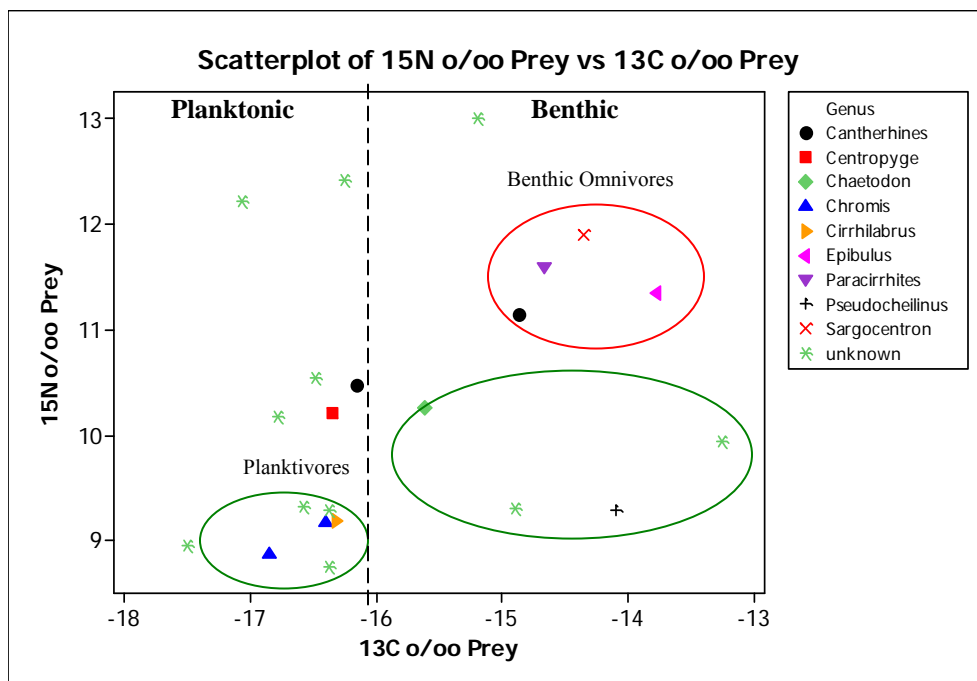


Figure 2.14. Scatterplot of $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ of prey species muscle tissue, grouped by genus of prey species. The dashed line indicates the break in $\delta^{13}\text{C}$ values between planktivores, to the left of -16‰ and benthic feeders to the right of -16‰ . Different trophic levels are signified by green circles for secondary consumers, and red circles for tertiary consumers. Green asterisks represent prey items that could be identified as fish, but not to family or genus.

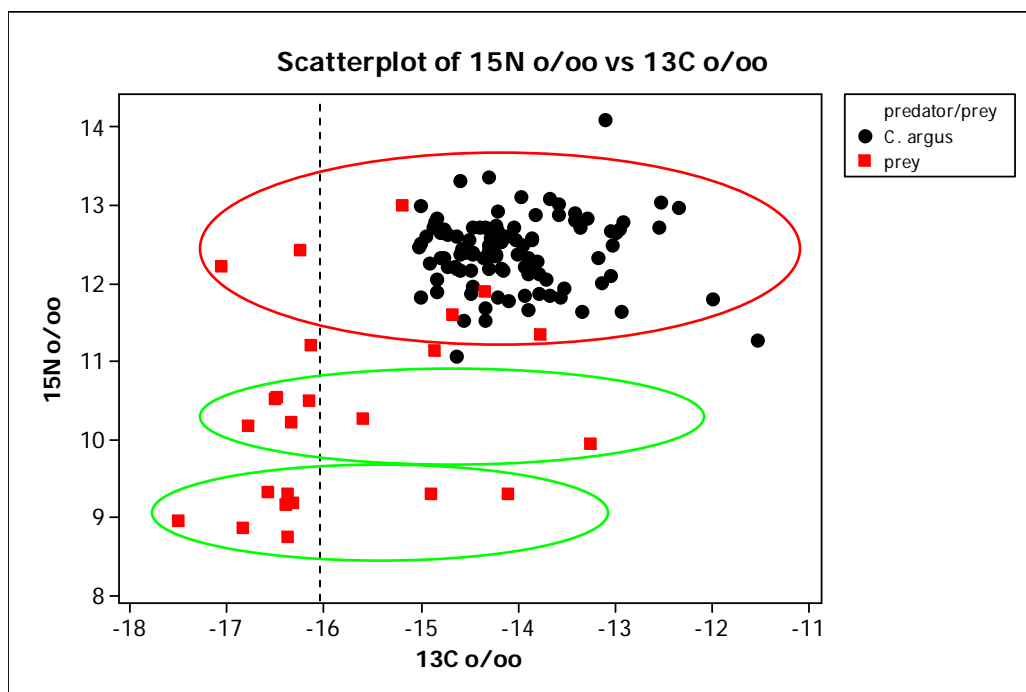


Figure 2.15. Scatterplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for *C. argus* and prey. Red squares represent the isotopic values of prey items found in the stomachs of *C. argus*. Different trophic levels are represented by the green ovals, secondary consumer, and red oval for tertiary consumers. The dashed line indicates the break in $\delta^{13}\text{C}$ values between planktivores, to the left of -16‰ and benthic feeders to the right of -16‰ .

CHAPTER 3

Differences in size, diet, feeding ecology and body condition of *Cephalopholis argus* in native and introduced populations

Introduction

Interactions, including competition, among ecologically similar species commonly affect patterns of habitat use and species abundance in areas of shared habitat (Robertson 1996). Competitive release can result from (1) the removal of a competitor due to over exploitation, thus freeing up previously restricted resources, or (2) the introduction of a species into an environment where there is little or no interspecific competition for available resources. An increase in available resources resulting from competitive release can affect the growth, population structure and feeding ecology of a species (Schmitt & Holbrook 1990, Robertson 1996, Chiappone et al. 2000, Dulvy et al. 2000, Lohrer et al. 2000). Several studies of marine fishes have documented increases in abundance and changes in habitat utilization of a species following the removal of a competitor (Schmitt & Holbrook 1990, Robertson 1996, Dulvy et al. 2000). However, few studies have examined competitive release of a species introduced into a new environment (Lohrer et al. 2000).

Because of the extreme isolation of the Hawaiian Archipelago, the Hawaiian islands have a unique assemblage of coral reef and shore fish species, with an estimated rate of endemism of 25%, which is one of the highest in the world (Randall 2007). In comparison with most tropical Pacific localities, several fish families are underrepresented or completely absent from the shallow, inshore reefs of Hawaii.

Among these is the family Serranidae, specifically the subfamily Epinephelinae, or groupers. There are only two native grouper species in Hawaii, both of which occur in deep water; these are *Epinephelus quernus*, commonly known as hapu'u and the rarely seen *E. lanceolatus* (Randall 1987).

The bluespotted grouper, *Cephalopholis argus*, was introduced to the Hawaiian islands as a potential food fish from Moorea, French Polynesia (where it is known as "roi") in the late 1950s in an attempt to increase nearshore fisheries (Oda & Parrish 1981, Randall 1987). Since its introduction, *C. argus* has spread to all the Main Hawaiian Islands, and can be found in large numbers along most shores of these islands (Division of Aquatic Resources unpublished data). The population increase and spread of *C. argus* have caused concerns about potential negative effects of this predator on populations of native species that have evolved without shallow water grouper predators, and on community structure in general. Despite its presence in Hawaii since the late 1950s and recent population expansions, there have been few investigations into the feeding ecology and diet of *C. argus* in Hawaii (Oda & Parrish 1981, Dierking 2007), and no comparisons of the ecology of this species in native and introduced habitats.

The coral reef habitats of Hawaii and Moorea are similar in age and distance from the equator, but the grouper species compositions are very different. The waters around Moorea are home to 14 species of Epinephelinae, and interspecific competition may influence the feeding ecology of competing grouper species. Differences in grouper species compositions between the reefs of Moorea and Hawaii suggest that competition may be more intense for *C. argus* in Moorea, and that the lower competition for resources

in Hawaii may have facilitated the initial establishment and recent rapid increase in the population density of this predator.

The ecology of *C. argus* may differ greatly between the two regions because of differences in resource availability and the effects of competition. The present study compared the feeding ecology, size, body condition, and population structure of *C. argus* in its native habitat of Moorea and in the introduced habitat in Hawaii. The data were then analyzed in the context of competitive release.

Methods

Study sites

Moorea is a small island located 19 km west of the island of Tahiti in French Polynesia. *C. argus* were collected from 13 sites along the coast of Moorea in March and June of 2005, between S 17° 28.442' W 149° 50.216', and S 17° 30.579' W 149° 55.593' (Fig. 3.1).

C. argus were collected in the Main Hawaiian Islands from a total of 19 sites in July 2003 (Dierking 2007). *C. argus* were collected from 12 sites along the west coast of the island of Hawaii, between N 20° 07.050' and N 19° 10.782', and 7 sites along the west, south, and east shores of the island of Oahu (Dierking 2007) (Fig. 3.2).

Stomach Content Analysis

Fish were collected in both Moorea and Hawaii during daytime on the reef slope from depths between 3 and 30 m by scuba divers using spears, following the methods of

Dierking (2007). Speared fish were placed immediately into Ziploc bags while under water to prevent loss of stomach contents due to regurgitation, and placed on ice after completion of the dive. Measurements were made to the nearest millimeter of (1) standard length (SL) and (2) total length (TL: defined as the distance from the tip of the snout to the longest part of the tail). Whole body wet weight (W) was measured to the nearest gram. Sex of all specimens was recorded. Stomachs of all specimens were removed and their contents noted as either “empty” or “full” (containing prey items). When stomachs were “full”, the total length (TL) and body depth (D; maximum vertical measurement, from dorsal to ventral, of the body of the prey) of prey items were measured to the nearest millimeter, and prey items were identified to the lowest possible taxon (Randall 1999, 2005). Following Hyslop (1980), numerical percent (%N) is defined as (the number of individuals of one prey category divided by the total number of prey individuals found in all the sampled stomachs) x 100, and frequency percent (%F) is defined as (the number of stomachs containing prey items of one category divided by the total number of stomachs that contained any identifiable prey items) x 100. %N and %F were calculated for all prey types.

Statistical analysis

The occurrence of “full” versus “empty” stomachs and differences in prey types occurring in the diet between regions were assessed using Chi-square tests. Body "condition", defined by the ratio of $\log_{10}W$ to $\log_{10}TL$, was calculated for *C. argus* in both regions and used as one measure of fitness, following Dierking (2007).

Predator-prey size relationships were tested by linear regression for predator TL and prey

D, and for predator TL and prey TL. ANCOVA was used to test for differences in predator-prey size relationships between regions. All statistical analyses were conducted with Minitab 14, with results considered significant at $p < 0.05$.

Results

Length and Weight

Mean *C. argus* TL was 32.0 cm (range 15.8 – 52.0 cm) in Hawaii (Dierking 2007) and 27.0 cm (range 17.5 – 41.0 cm) in Moorea. Similarly, mean *C. argus* W was greater in Hawaii (631.4 g, range 113.0 – 2098.0 g) (Dierking 2007) than in Moorea (mean 197.5 g, range 65.0 – 1020.0 g). Differences in both TL (t-test, $p < 0.001$) and W (t-test, $p < 0.001$) were highly significant between regions (Fig. 3.3a, 3.3b). When *C. argus* of the same length were compared between regions, W at a given length was significantly lower in Moorea, (ANCOVA; $F=86.6$, $p < 0.001$, $R^2=0.98$) (Fig. 3.4), indicating that Moorea fish are in poorer “condition” than Hawaii fish.

Stomach Content Analysis

The stomach vacuity rate (incidence of empty stomachs) was significantly higher in Moorea (63%) than in Hawaii (43%) (chi-square test, $p < 0.001$).

At the highest systematic levels, the proportion of different prey types in the diet did not differ significantly between the two regions, although fish made up a somewhat higher proportion of the diet in Hawaii (Dierking 2007) (Table 3.1). When fish prey composition was analyzed at the family level in Moorea, Monacanthidae, Pomacentridae

and Scaridae accounted for over 60% of the diet (by N%) (Fig. 3.5b). Pomacentrids were present in the stomachs in the highest numbers, accounting for 25%. These findings differ from those in Hawaii where Acanthuridae made up 22% of prey fish found in the full stomachs of *C. argus* (Fig. 3.5a) (Dierking 2007). Together Acanthuridae, Balistidae, and Chaetodontidae accounted for 48% of the prey fish found in the diet in Hawaii (Fig. 3.5a) (Dierking 2007). In both Moorea and Hawaii, *C. argus* consumed prey from all feeding guilds: planktivores, herbivores, omnivores and piscivores.

Prey Size and Shape

C. argus in both Hawaii and Moorea showed a significant increase in prey TL with an increase in *C. argus* TL (Linear regression; Hawaii $p < 0.001$, $R^2 = 0.17$, Moorea $p < 0.001$, $R^2 = 0.42$) (Fig. 3.6). An ANCOVA was run to investigate relations in prey TL between regions. There was no significant regional interaction, and *C. argus* of a given size consumed prey of similar TL in both regions ($df = 1$, $F = 2.72$, $p = 0.103$).

Figure 3.7 shows prey D vs *C. argus* TL for both regions. There was a significant positive correlation between prey D and *C. argus* TL in both Hawaii and Moorea (Linear regression; Hawaii: $p < 0.001$, $R^2 = 0.08$; Moorea: $p < 0.001$, $R^2 = 0.54$) (Fig. 3.7). An ANCOVA of prey D and *C. argus* TL between regions was statistically significant: ($df = 1$, $F = 6.63$, $p = 0.011$). The slopes of the regression in Figure 3.7 differed significantly, meaning that as *C. argus* in Moorea increase in length, the body depth of their prey relative to the length increases more rapidly than that of *C. argus* in Hawaii. When prey D for *C. argus* of a given length were compared between regions, *C. argus*

from Moorea consumed significantly deeper-bodied prey than *C. argus* of the same size in Hawaii (ANCOVA, $df=1$, $F=5.35$, $p=0.020$).

Discussion

Interspecific competition occurs most frequently among ecologically similar species with high overlap in diet and microhabitat use (Schoener 1983, Robertson 1996). Competition between similar species can affect the abundance and ecology of the species involved.

There are at least 14 species of groupers on the reefs of Moorea, and so competition for food and resources is probably high. In Moorea, *C. argus* were seen competing for food and in aggressive interactions with *C. urodeta*, *E. fasciatus*, *E. hexagonatus*, and *E. merra* (A. Meyer personal observation). *C. argus* from Moorea and Hawaii differed in their feeding ecology, range of body sizes, and their body condition in ways that seem best explained by the concept of competitive release.

Shifts in microhabitat use and feeding-associated resources following competitive release have been documented for territorial damselfishes (Robertson 1996), surfperch (Schmitt & Holbrook 1990), and the Asian shore crab (Lohrer et al. 2000). When the highly aggressive *Stegastes planifrons* was removed from reefs in the Caribbean, several sympatric species of *Stegastes* moved into areas left vacant by *S. planifrons*, expanding their range and diet (Robertson 1996).

In the early 1990's the Asian shore crab, *Hemigrapsus sanguineus*, invaded and established breeding populations along the northeastern coast of North America (Lohrer et al. 2000). Lohrer et al. (2000) showed that these crabs increased their diet breadth and

vertical distribution in the invaded habitat, with resulting significant differences in resource use between native and invaded regions. Throughout the invaded range, crab diversity was relatively low and there was little dietary and habitat overlap with resident crabs (Lohrer et al. 2000). Competitive release was a driving factor in the establishment of *H. sanguineus* and in the subsequent increase in diet breadth and habitat utilization.

Diet Composition

In both native and introduced habitats, the diet of *C. argus* was dominated by fish, with crustaceans making up the remainder of the diet. Cephalopods, which have been found in the diets of groupers elsewhere, were absent from the diet of *C. argus*. Regional differences in the relative importance of fish and crustaceans were not significant. The proportion of fish and crustaceans has been found to vary in other species of the genus *Cephalopholis*, e.g., the proportion of fish and crustaceans in the diet of *C. urodeta* in Japan differed significantly from that in French Polynesia (Nakai et al. 2001).

Dietary breadth was large for *C. argus* in both Hawaii and Moorea, including a wide variety of fish species from many feeding guilds, e.g. planktivores, benthic herbivores, corallivores, omnivores and other piscivores. Together Acanthuridae, Balistidae, and Chaetodontidae accounted for almost half the prey fish found in Hawaii (Dierking 2007) (Fig. 3.5a), while in Moorea, Pomacentridae, Scaridae and Monacanthidae made up the majority of prey (Fig. 3.5b). This demonstrates that *C. argus* is a generalist predator and opportunistic feeder, consuming the most abundant and available prey, as has been described for several species of groupers elsewhere (Shpigel & Fishelson 1989a, Webster & Almany 2002). Whereas the specific systematic composition of the diet differed

between regions, it appears likely that this results from differences in fish community composition and thus prey availability between regions. A prudent predator has been defined as one that preys upon the most common species available in its habitat (Shpigel & Fishelson 1989a). This was the case in the Red Sea where there was a high incidence of acanthurids in the stomachs of *C. argus*, accounting for 25% of all fish consumed. The high incidence of acanthurid prey items was correlated with a high abundance of these fish on the reef.

Competitive Release

Although *C. argus* was brought to Hawaii as a food fish, the fishery failed to develop because of concerns over the high incidence of ciguatera poisoning. *C. argus* was introduced into an environment with little competition from other large sedentary piscivorous species, and the population has persisted and flourished for the last 50 years. Other large apex predators present on Hawaiian reefs include jacks and sharks. Both of these highly mobile, roving, predatory groups have declined drastically due to overfishing throughout the 20th century (Shomura 1987, Harman & Katekaru 1988, Smith 1993, Zeller et al. 2005), further decreasing food competition for *C. argus*.

Competitive release of *C. argus* in Hawaii may be the driving force behind significant differences in body size range, feeding, and body condition between Hawaii and Moorea. In our collection in Hawaii, *C. argus* had a significantly higher incidence of full stomachs than in Moorea, indicating that more resources are available in Hawaii, allowing *C. argus* to feed more. In the Caribbean, Chiappone et al.(2000) showed that changes in the abundance of one species may release a competing species, allowing it to increase in size

due to an increase in available resources. In the present study, weight at a given length was significantly lower in Moorea. Body condition, measured as weight at a given length, can be a measure of individual fitness (Laidre et al. 2006). The clear and significant differences between weight at a given length in *C. argus* populations at Moorea and Hawaii indicate an overall higher fitness of individuals in the Hawaii population. More available resources and higher overall fitness in Hawaii may lead to faster growth rates. Growth data from J.H. Choat (pers. comm.) show that *C. argus* in Hawaii grow at a faster rate than those in Moorea, and obtain larger sizes at a younger age.

As fish increase in TL, they also increase volumetrically in size, and their gape increases, allowing them to consume a wider and more variable size range of prey. This has been reported in several piscivorous species, including other species in the serranid family: *Plectropomus leopardus* (Kingsford 1992, St. John 1999, St. John & Russ 2001) and *Epinephelus quoyanus* (Connell 1998). *C. argus* in both Hawaii and Moorea showed a significant positive correlation between fish size and prey length, where larger *C. argus* consumed longer prey. There were no statistically significant regional differences in the length of prey consumed, and *C. argus* of the same size in both regions consumed similar length prey. However, the slopes of the regressions in Figure 6 differ, and although the results were marginally significant ($p=0.103$), there appears to be a trend toward differences in prey length between regions. The R^2 value for Hawaii is lower than that for Moorea, 0.17 and 0.42 respectively, showing that the Moorea data fit the model better and that there is less variability.

There were significant positive linear correlations between *C. argus* TL and prey D in both regions, where larger *C. argus* consumed significantly deeper-bodied prey. There was also a significant regional difference; overall, *C. argus* in Moorea consumed significantly deeper-bodied prey than *C. argus* in Hawaii, and when prey body depth of *C. argus* of a given length were compared between regions, *C. argus* in Moorea consumed deeper-bodied prey than their Hawaiian counterparts of the same size. A possible explanation for regional difference might be the lack of competition from other grouper species. *C. argus* in Hawaii might not be forced to change their feeding habits as they grow. In Hawaii, large *C. argus* consume a wide range of prey body depths, but *C. argus* in the upper size classes are consuming relatively shallow bodied prey when compared to similar size fish in Moorea. In Moorea, a 35.0 cm TL *C. argus* consumed prey ranging from 3.0 to 5.5 cm in body depth, and its Hawaii counterpart consumed prey ranging from 1.0 to 3.0 cm in body depth. Interspecific competition in Moorea may force *C. argus* of larger sizes to consume deeper bodied prey, reducing competition with smaller conspecifics and other species of small groupers, partitioning prey resources into size groups. Smaller *C. argus* and other small grouper species can focus on small, slender prey, and larger *C. argus* can focus on deeper-bodied prey, allowing them to coexist on a crowded reef.

A change in diet with increase in size is known as an ontogenetic feeding shift. *C. argus* in both Hawaii and Moorea are undergoing ontogenetic feeding shifts in the length and shape of the prey they consume as they grow. The R^2 values for both prey length and prey body depth are higher for Moorea, showing a better fit of the data and less variability. The higher R^2 values and the larger slopes suggest a stronger ontogenetic

shift. *C. argus* in Moorea may be more constrained by competition and may rely more heavily on an increase in gape size to expand their ability to feed on deeper bodied prey, and perhaps ease competition, allowing the larger predators to utilize more of the available resources. Ontogenetic shifts such as this have been reported in *P. leopardus* by Choat (1968), Kingsford (1992), St. John (1999), and St. John and Russ (2001), and in *Serranus atricauda* (Morato et al. 2000).

The ontogenetic shift in feeding is not as pronounced in Hawaii. Larger *C. argus* in Hawaii continue to feed on small, slender prey even when they are capable of eating much larger, deeper-bodied prey, arguably because of the absence of competing grouper species. The possibility to exploit a wide size range without encountering interspecific competition may be one reason that stomach vacuity rates are significantly lower in Hawaii. Increased feeding success over time offers a possible explanation for the superior body condition in introduced compared to native habitats of *C. argus*.

Conclusion

We observed evidence of what appears to be competitive release, where *C. argus* in Hawaii attained larger size than in Moorea, achieved better body condition, and showed differences in diet, from Moorea fish. These results indicate that *C. argus* in Hawaii have higher fitness than *C. argus* in Moorea. Alternate explanations could include differences in primary productivity or ecosystem functions. However, we favor the explanation of competitive release because of multiple lines of evidence.

The coral reefs of Moorea and Hawaii are very similar in latitude, age, climate, and many

physical characteristics. Lower productivity could explain the smaller fish in Moorea, but would not account for the stronger correlation between prey body depth and prey length. As mentioned above, the high endemism in Hawaii indicates a unique ecosystem. Ecosystem differences could account for divergent prey composition but would not explain the superior body condition of *C. argus* in Hawaii. Fishing pressure could be another factor with different influence between regions. However, due to the potentially high risk of ciguatera in both native and introduced habitats, this species experiences very low fishing pressure in both regions.

The case of *C. argus* in Hawaii provided a rare opportunity to test aspects of ecological theory, that were formulated primarily in the terrestrial realm and given support by this research in the aquatic domain of coral reefs.

Table 3.1. Diet of *C. argus* in Moorea and Hawaii, expressed as number of occurrences and percent occurrence in stomachs of *C. argus*.

	Hawaii		Moorea	
Stomach Contents at highest systematic levels	Number	%	Number	%
Fish	144	85	47	76
Crustacean	16	9	12	19
Fish & Crustacean	10	6	3	5

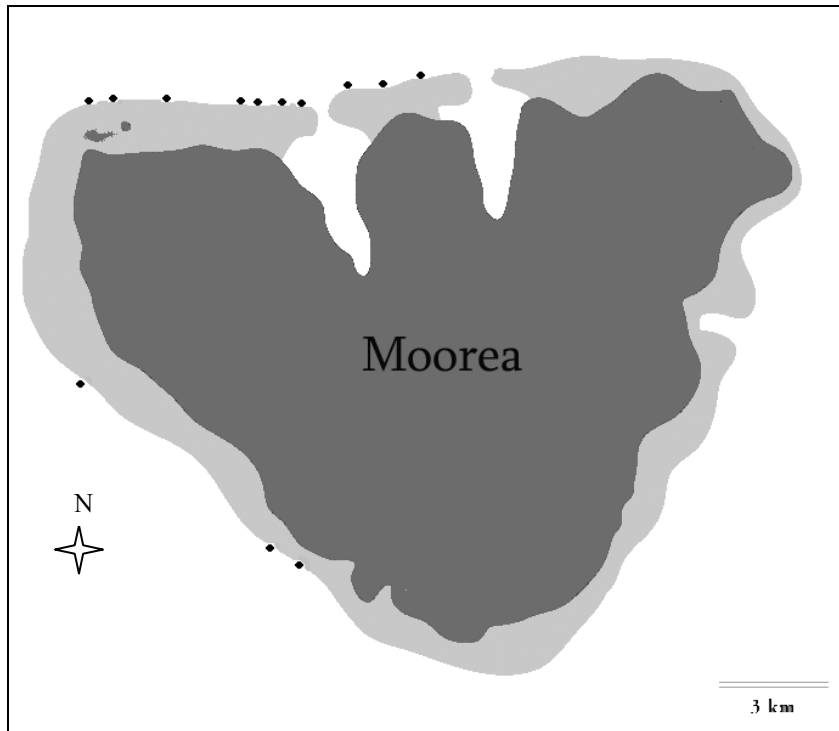


Figure 3.1. Collecting sites along the north and southwest coasts of the island of Moorea, French Polynesia. The collecting sites are represented by black circles. *C. argus* were collected from depths of 3 to 30 meters.

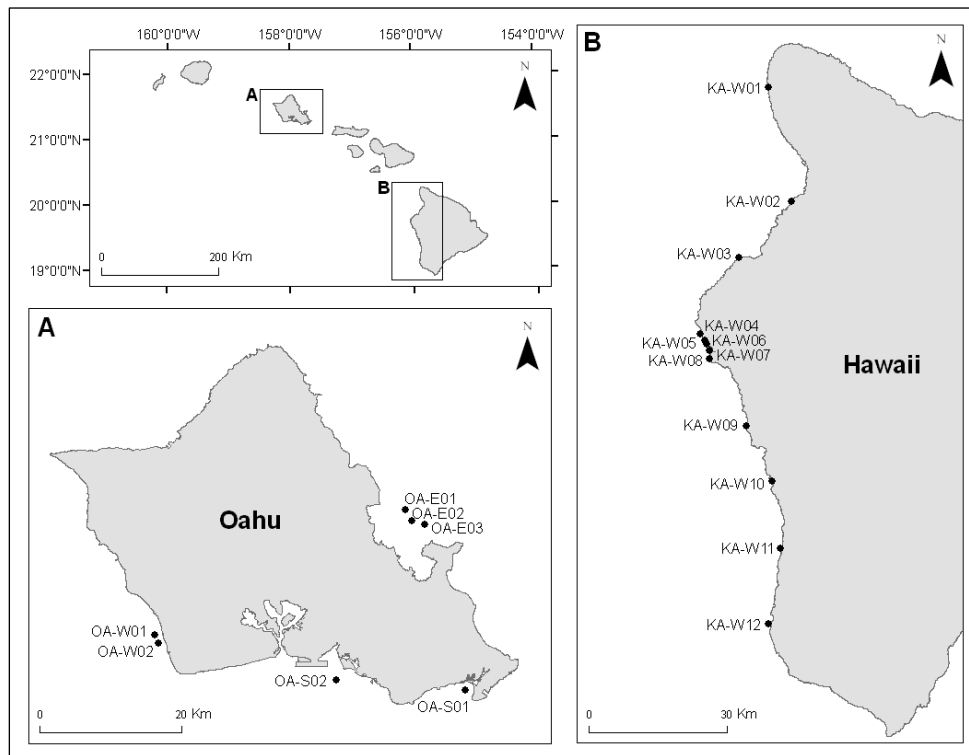


Figure 3.2. Collecting sites along the west coast of the island of Hawaii, and the south, east, and west coasts of the island of Oahu. Collecting sites are represented by black circles, *C. argus* were collected from depths of 3 to 30 meters.

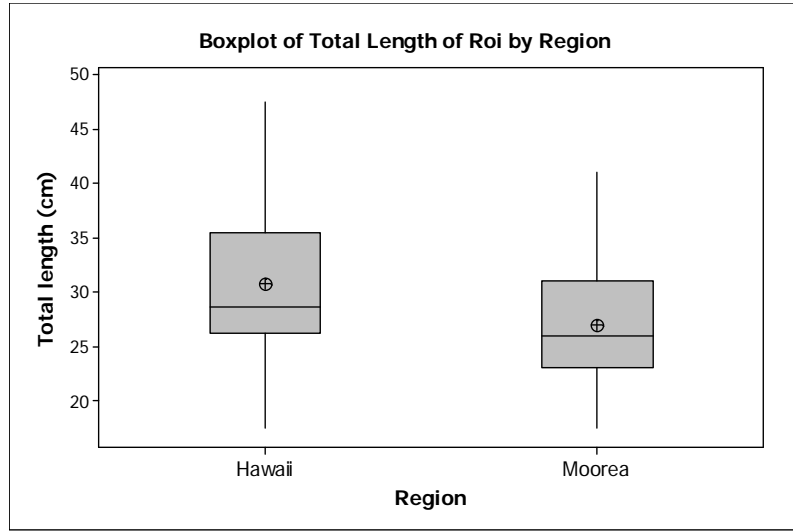


Figure 3.3a. Boxplot of TL (cm) of *C. argus* by region, t-test, $p < 0.001$.

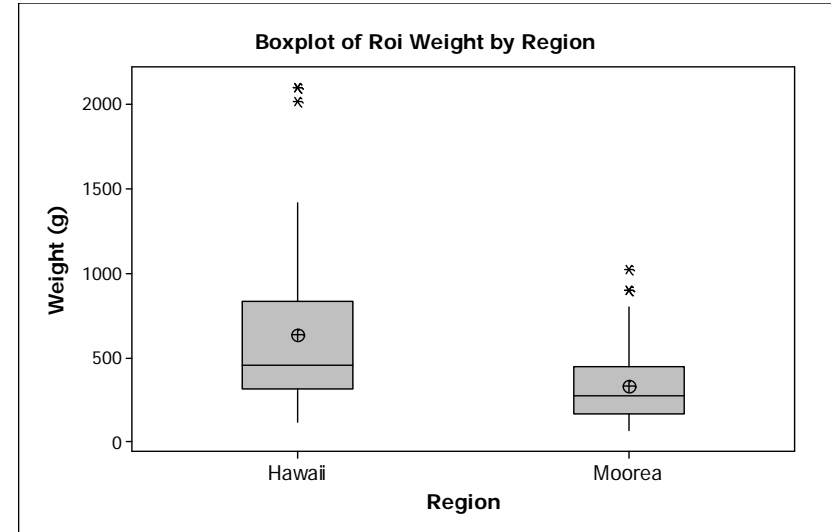


Figure 3.3b. Boxplot of weight (g) of *C. argus* by region, t-test, $p < 0.001$.

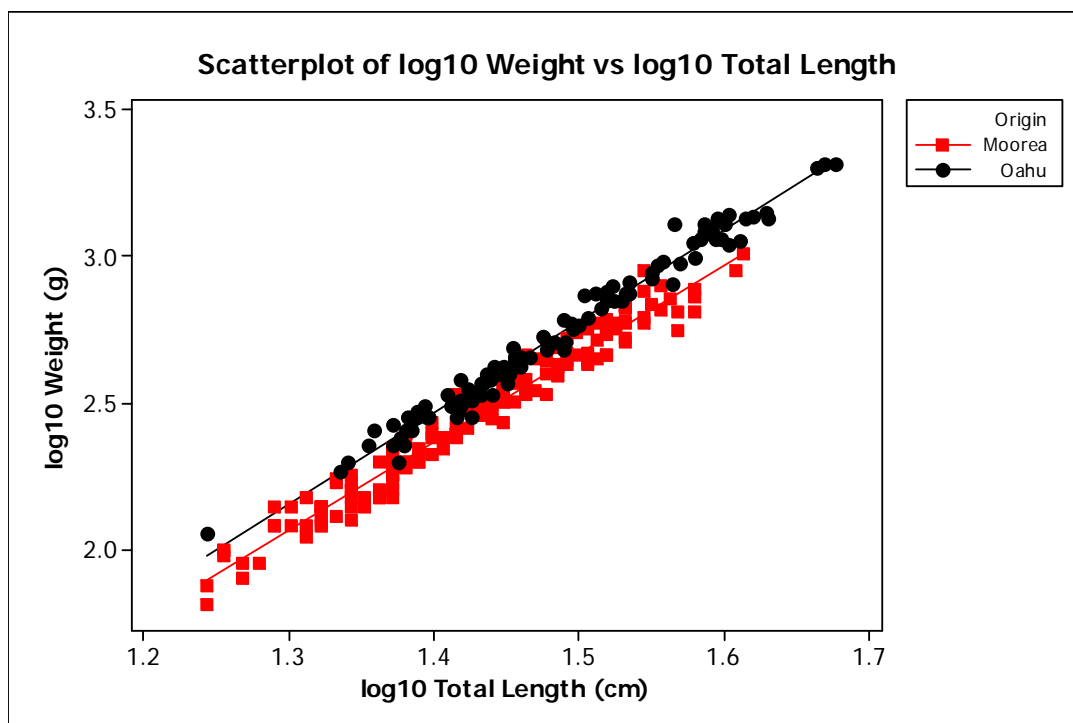


Figure 3.4. Scatterplot of log₁₀ W vs log₁₀ TL. Moorea is represented by red squares, and Oahu is represented by black circles. W at a given length was significantly lower in Moorea, ANCOVA $p < 0.001$, $F = 86.6$, $R^2 = 0.98$.

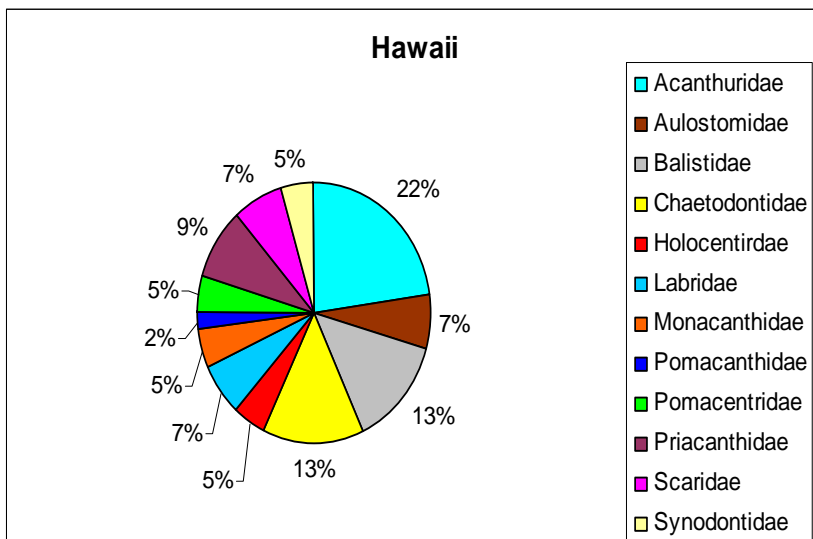


Figure 3.5a. Frequency % of prey fish families found in the stomachs of *C. argus* in Hawaii.

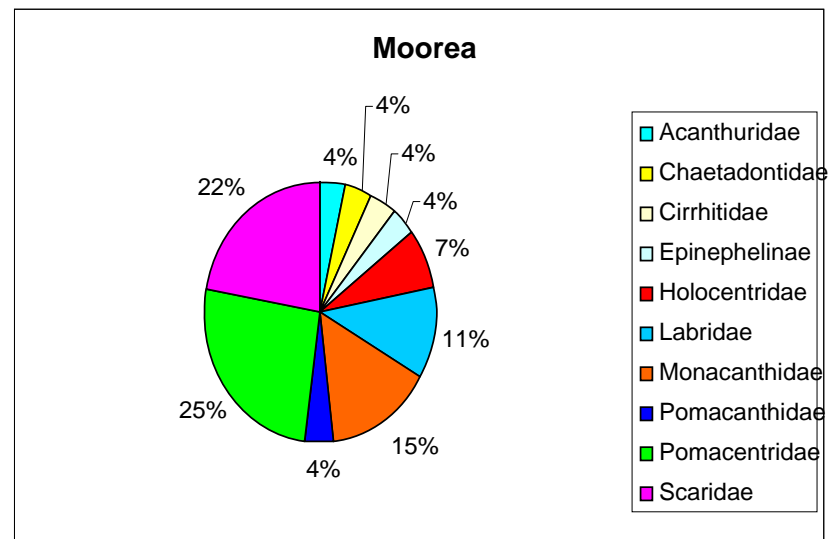


Figure 3.5b. Frequency % of prey fish families found in the stomachs of *C. argus* in Moorea.

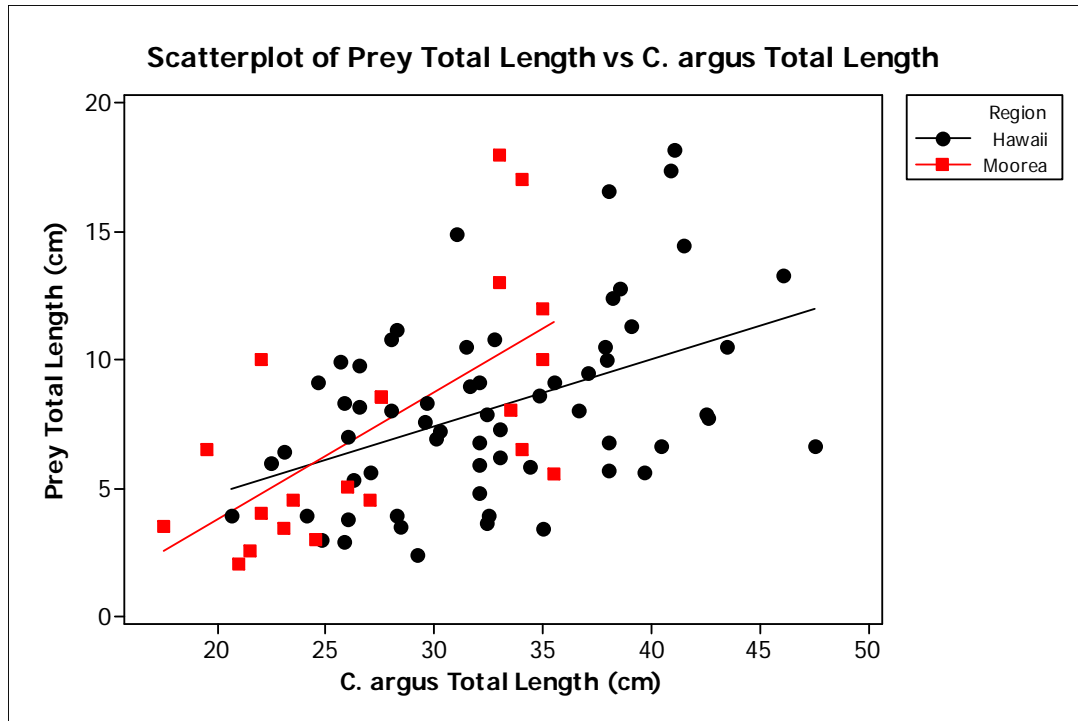


Figure 3.6. Scatterplot of *C. argus* TL (cm) vs prey TL (cm) for Hawaii and Moorea. The red squares represent Moorea and the black circles represent Hawaii. There is a positive linear correlation between length of *C. argus* and length of prey for both regions; Hawaii linear regression: $p < 0.001$, $R^2 = 0.17$; Moorea linear regression: $p < 0.001$, $R^2 = 0.42$. There were no significant differences in prey length between regions, ANCOVA, $df = 1$, $F = 2.27$, $p = 0.103$.

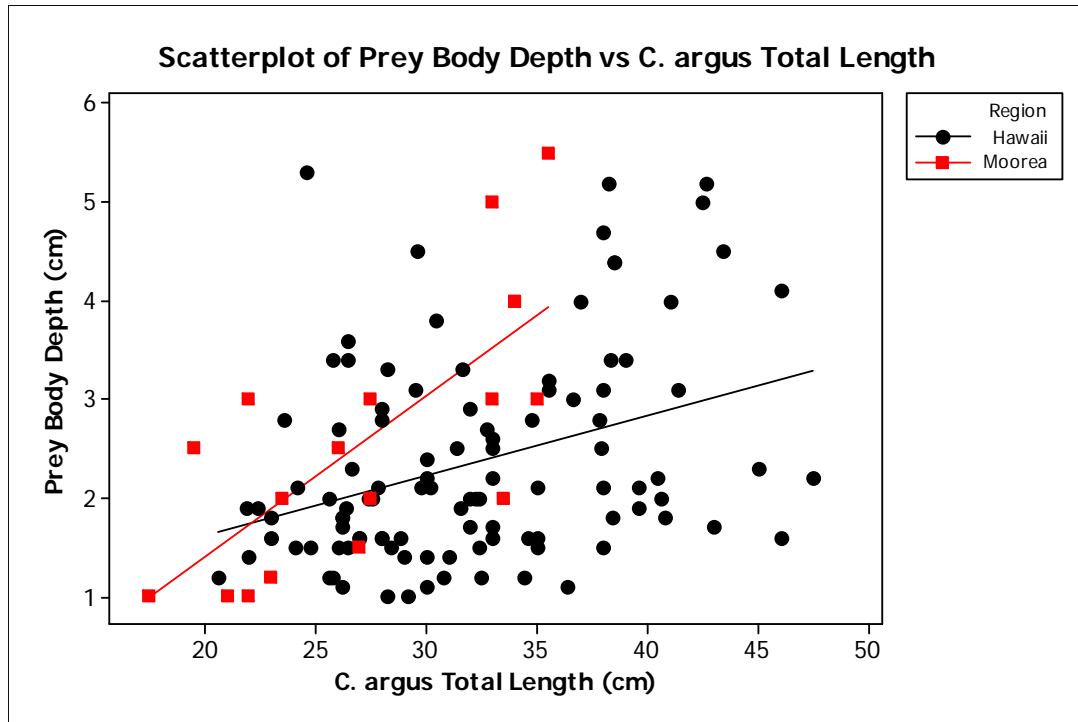


Figure 3.7. Scatterplot of *C. argus* TL (cm) vs prey D (cm) for Hawaii and Moorea. The red squares represent Moorea and the black circles represent Hawaii. There is a positive linear correlation between length of *C. argus* and body depth of prey for both regions; Hawaii linear regression, $p < 0.001$, $R^2 = 0.08$; Moorea linear regression, $p < 0.001$, $R^2 = 0.54$. An ANCOVA of prey D and *C. argus* TL between regions was significant, $df=1$, $F=6.63$, $p=0.011$. *C. argus* of a given length in Moorea consume significantly deeper-bodied prey than do *C. argus* in Hawaii, ANCOVA, $df=1$, $F=5.35$, $p=0.020$.

CHAPTER 4

Differences between Hawaii and Moorea in home range and use of spatial resources by the blue-spotted grouper, *Cephalopholis argus*

Introduction

Patterns of movement and resource utilization are becoming increasingly important when studying population dynamics, community structure, habitat use and feeding of fishes (Zeller 1997, Meyer et al. 2000, Topping et al. 2005, Meyer et al. 2007). Movement patterns and spatial use are fundamental factors that can give insight into ecological processes at the population, community, and species levels (Zeller 1997).

Hawaii is extremely isolated, and due to this isolation, has a unique assemblage of coral reef and shore fish species, with high endemism estimated at 25% (Randall 2007). In comparison with most tropical Pacific localities, several fish families are underrepresented or completely missing from the shallow, inshore reefs of Hawaii.

Among these is the family Serranidae, the sea basses and groupers. Members of the subfamily Epinephelinae, or groupers, are thought to be the most numerous large predatory fishes in the shallows of warm water regions, and often provide a major structuring component of the reef community (Randall 1963, Shpigel & Fishelson 1991b, Zeller 1997). However, there is a surprising absence of grouper species in the shallow, nearshore reef communities of Hawaii. There are only two naturally occurring groupers found in Hawaii; these are the native deep-water grouper, *Epinephelus quernus*, commonly known as the hapu'u, and the rarely seen *E. lanceolatus*. The blue-spotted

grouper, *Cephalopholis argus* was introduced to Hawaii from Moorea, French Polynesia, in the late 1950's in an attempt to increase nearshore fisheries (Oda & Parrish 1981, Randall 1987).

The coral reef habitats of Hawaii and Moorea, French Polynesia share many similarities, but the grouper species compositions are very different. The shallow waters of Moorea are home to 14 species of groupers, some of which are numerous and widespread around the island. Interspecific competition may influence the ecology of competing grouper species. Further, there are a larger number of potential predators on *C. argus* in Moorea, but not in Hawaii. Studies focusing on social organization and movement of fishes have shown that predation pressure and competition are factors that often influence social organization, movements, home ranges, and spatial use of habitat (Shpigel & Fishelson 1989b, 1991b, Shapiro et al. 1994). In recent years, the numbers and distribution of *C. argus* in Hawaii have increased alarmingly, and this predator has spread onto the reefs throughout much of the Main Hawaiian Islands (pers. com. Hawaii Division of Aquatic Resources).

Differences in species composition between the reefs of Moorea and Hawaii indicate that *C. argus* in Moorea may experience more intense competition and predation. The low level of competition and predation experienced by *C. argus* in Hawaii has facilitated the rapid increase in the population density of this predator. The population increase and spread of *C. argus* causes concern because of the unknown influences this predator may have on community structure, and the effects on native species that have evolved without shallow water grouper predators. Despite its presence in Hawaii since the late 1950's and recent population expansions, there have been no investigations into the movements and

spatial use of *C. argus* in the Hawaiian islands. The objective of the present study is to test the null hypothesis that the size of home ranges and the patterns of utilization within home ranges do not differ between Hawaii and Moorea, despite differences in the associated fish communities.

Methods

Active and passive tracking data were used to determine 1) home range areas, 2) utilization distributions within home ranges, 3) activity patterns, and 4) site fidelity of *Cephalopholis argus* in Hawaii and Moorea.

Study sites

This study was conducted along the western coast of the island of Hawaii and the northern coast of the island of Moorea, in French Polynesia. Twenty-three *C. argus* from four sites along the western coast of the island of Hawaii were tracked during the summer of 2006. Twenty *C. argus* from four sites on the northern fringing reef of Moorea were tracked during the summer of 2005. Sites were between 10 and 20 meters in depth, on the reef slope, and were chosen based on habitat type and accessibility by boat.

Active tracking

In order to study social groups or harems of *C. argus*, SCUBA divers followed the largest *C. argus* during two 2-hour scouting dives throughout the day, while dropping markers to

outline its home range (Shpigel & Fishelson 1991b). Once the home range of the largest fish was outlined with markers, divers caught and tagged fish within the marked area. Fish were caught in a barrier net, measured and tagged underwater. Standard and total lengths were recorded to the nearest millimeter, and colored t-bar tags were inserted into the dorsal musculature just below the dorsal fin on both sides of the fish for individual visual identification. Once tagged, the fish was released into the hole from which it was caught. As many fish as feasible from each social group were caught and tagged.

Divers followed tagged *C. argus* throughout the day over a three to five day period. In order to record the movements of the groupers, divers followed tagged *C. argus* while towing a Garmin Map76 GPS unit attached to a modified surface float. GPS units recorded the divers' position every 5 or 20 seconds. Divers hovered at 10 ft water depth, over or slightly behind tagged fish, and followed the fish as they swam. Tagged fish were followed for at least 40 minutes during three-hour dives. In order to sample grouper movements evenly throughout the day, dives were conducted during the intervals 7:00-10:00, 11:00-13:00, and 15:00-19:00. Behaviors and interactions among the tagged fish were recorded on an underwater slate. GPS data were downloaded using MapSource 6.0. Home ranges and fish movements were plotted and analyzed in ArcView GIS 3.2.

Passive acoustic monitoring

Long-term movement patterns of *C. argus* were investigated by passively tracking six individuals off the west coast of the island of Hawaii. *C. argus* were caught in July 2006 using the same methods as above, and brought to the surface for transmitter implantation.

Fish were anesthetized in a salt water solution of MS222 (0.75g/l) until loss of equilibrium and lack of reaction to handling occurred. Fish were removed from the anesthetic, and placed on a foam pad. A 1.5-cm incision was made approximately 1 cm below the pectoral fin on the left side of the body, between the pelvic fins and the vent, and a coded transmitter (VEMCO V8) was inserted into the peritoneal cavity. The incision was closed with three sutures. Standard and total lengths were measured to the nearest millimeter, and fish were tagged externally with colored t-bar tags in the dorsal musculature for visual identification. Fish were placed into aerated seawater for recovery. Once recovered, fish were transferred into a catch bucket and released in the area of capture.

Acoustic receivers (VEMCO VR1 and VR2) were placed in core use areas determined by previous active tracking. Receivers were secured to the reef substrate by a length of chain and suspended in the water column by a float. Data from receivers were downloaded after three months.

Data analysis

Home range areas were estimated by minimum convex polygons (MCP) and 95% kernel utilization distributions (KUD) (Kernohan et al. 1998). Home range estimates were calculated using ArcView GIS 3.2 with the Animal Movement Analyst Extension (AMAE) (Hooge & Eichenlaub 1997). The MCP produces an estimate of the total area used by an individual, and provides a non-statistical measure of dispersion over that area (Zeller 1997). MCP home range areas are the smallest convex polygon that can be drawn

around all the positions recorded for an individual. MCPs, as a measure of home range, are highly influenced by the number of position fixes recorded for an individual. Few positions recorded may cause the MCP to underrepresent the animals' home range area. MCP can overestimate an animal's home range area by recording seldom visited or rarely used areas. In this study, no less than 250 position points for each individual were used to calculate MCPs.

Kernel home range analysis was used to determine utilization distribution and habitat use. Least square cross validation was used to determine the smoothing factor (h) (Worton 1989). Kernel utilization distributions (KUD) are a more accurate measure of home range area because they do not overestimate home range by including seldom visited areas (Kernohan et al. 1998). Home range data were analyzed for 95%, 75% and 50% use kernels to investigate core use areas and key geographic locations within the home range. The 95% KUD represents an area where the individual in question can be located 95% of the time. The 75% and 50% KUD contours were used as indicators of core use areas within the home range (Zeller 1997).

Site fidelity was quantified by passive acoustic monitoring and active tracking. Site fidelity was measured by a linearity index calculated with the AMAE in ArcView GIS 3.2. The linearity index is the quotient of the straight line distance between the initial and final positional fixes divided by the total cumulative distance the fish traversed during the entire active track (Zeller 1997, Lowe et al. 2003). Linearity index values range from 0 to 1, with a value of 1 representing linear movement over an area (Lowe et al. 2003).

One hundred replicates of the site fidelity test were run for each fish to determine whether the active tracking data were random walks or directed movement resulting in site fidelity.

Results

Hawaii

Home Range Areas

A total of 23 *C. argus* ranging in size from 21 to 42 cm total length were tracked in Hawaii (Table 4.1).

Minimum Convex Polygon (MCP) home range areas ranged from 425 to 2300 m², with a mean of 1236 m². There was a statistically significant positive correlation between fish total length and MCP home range area: $p=0.046$, $R^2=0.18$ (Fig.4.1). Larger fish held marginally significantly larger MCP home range areas than did smaller fish.

The 95% KUDs ranged from 145 to 1235 m², with a mean of 598 m² (Table 4.1). There were no significant relationships between total length of *C. argus* and the 95%, 75% or 50% KUD home range areas: $p=0.79$, 0.45 and 0.38 respectively (Fig. 4.2).

Utilization Distribution

Figure 4.3 shows the 95%, 75% and 50% kernel utilization distributions of four *C. argus* tracked in Hawaii. KUDs of 75% and 50% were used to analyze core use areas. Core use areas were associated with crevices or coral heads, i.e. with areas used for sheltering.

Fish would settle on the substrate near shelter sites and remain there for prolonged periods of time, sometimes when displaced by other *C. argus*. Most *C. argus* observed in Hawaii had several shelter sites and core use areas within their home range areas, and they showed high site fidelity for shelter sites. *C. argus* in Hawaii held between 1 and 4 core use areas with a median of 3 and 2 for the 75% and 50% KUDs core use areas respectively.

MCP and core use areas overlapped for fish within harems. In general female home ranges were clustered within the male's home range, and core use areas of females within the harem overlapped with each other and the male.

Activity Patterns

All six of the *C. argus* that were implanted with transmitters in Hawaii showed similar diel activity patterns; fish were active during the day and quiescent at night. *C. argus* moved very little, if at all, during the hours from 19:00 to 05:30. Transmitter signals were present on receivers during the daylight hours starting around 06:00, shortly after sunrise, and ending around 19:00, sunset. When nighttime signals occurred they were constant, signifying that the tagged fish was stationary and sheltering in bottom cover very close to the receiver during the entire night. Figure 4.4 illustrates passive tracking data from fish WCg at receiver 1476.

Site Fidelity

C. argus in Hawaii showed high site fidelity. All six acoustically tagged fish remained in their home range areas for the duration of the battery life of their transmitter, which ranged from 1.5 to 3 months. All actively tracked fish consistently revisited the same core locations during each successive active track. Comparisons of the home ranges for each of the fish indicated almost complete overlap of ranges between tracking sessions. Eighteen tagged fish were seen within the same home range areas three years after a pilot tracking study in 2003. Two fish from the pilot study were re-tracked in the summer of 2006. Their MCP home range areas were in the same area on the reef, and the size and shape of the MCP had not changed significantly from data collected in 2003. *C. argus* in Hawaii are strongly site attached, using core areas within their home range, and spending large amounts of time in these specific core areas. *C. argus* in Hawaii showed low linearity index values, ranging from 0.001 to 0.041 with a mean of 0.013.

Moorea

Home Range Areas

A total of 20 *C. argus*, ranging in size from 20 to 35 cm total length, were tracked on the fringing reef of Moorea (Table 4.2). MCP home range areas ranged from 230 to 1389 m², with a mean of 700 m² (Table 4.2). There was a significant positive relationship between the total length of *C. argus* and MCP home range areas in Moorea (Linear regression, $p=0.001$, $R^2=0.52$) (Fig. 4.5).

The 95% KUD ranged from 73.5 to 854.1 m² (Table 4.2), with a mean of 393.4 m². A

trend toward larger fish utilizing larger areas was found. The relationship between the size of a fish and its 95% utilization distribution was marginally significant: $p=0.057$, $R^2=0.19$ (Fig.4.6). The 75% and 50% KUDs also varied significantly with body size, $p=0.017$ and $p=0.026$ respectively (Fig. 4.6), with larger fish holding significantly larger core use areas.

Utilization Distribution

C. argus in Moorea used coral heads, crevices, and areas of high rugosity for sheltering sites, and these sites corresponded to the 75% and 50% KUDs core use areas. *C. argus* in Moorea used between 1 and 5 core use areas with a median of 2 and 3 for the 50% and 75% KUDs respectively. Figure 4.7 shows the 95%, 75% and 50% KUDs of four *C. argus* in Moorea. *C. argus* in Moorea held several core use areas and spent prolonged amounts of time at sheltering sites. MCPs and core use areas of fish within social groups overlapped and fish shared common shelter sites.

Site Fidelity

C. argus in Moorea showed high site fidelity and were re-sighted in the same home ranges five months after the original tracking study. Comparisons of the home ranges for each fish indicated almost complete overlap of ranges between tracking sessions. *C. argus* in Moorea showed low linearity index values ranging from 0.0009 to 0.034 with a mean of 0.011.

Comparison

A total of 43 *C. argus* ranging from 20 to 42 cm total length, were tracked in Hawaii and Moorea (Tables 4.1 and 4.2). The size of *C. argus* in Moorea and Hawaii did not differ significantly between regions, $p=0.071$. In Hawaii, *C. argus* ranged from 21 to 42 cm, with a mean of 30.0 cm, and in Moorea *C. argus* ranged from 20 to 35 cm, with a mean of 26.6 cm.

MCP ranged from 230 m² for a 20-cm fish to 2300 m² for a 39-cm fish (Fig. 4.8). An ANOVA was run to test for interaction between total length of *C. argus* and region for both MCP and 95% KUD home range areas. No significant interaction was found. In both Hawaii and Moorea, as *C. argus* increased in total length, their MCP and 95% KUD home range areas increased in size. MCP and 95% KUD were both significantly different by region, with *C. argus* in Hawaii holding larger home ranges than *C. argus* in Moorea (t-test $p<0.001$). To further investigate the differences between Hawaii and Moorea home ranges, an ANOVA was run to test for differences in MCP and 95% KUD by fish size, between regions. The results showed that *C. argus* in Hawaii have significantly larger MCP and 95% KUD home range areas when compared to *C. argus* of the same size in Moorea (MCP: $p=0.001$, $F=11.67$; 95%KUD: $p=0.05$, $F=3.80$, Fig. 4.8).

Total length of individuals was compared for 75% and 50% KUDs to determine whether fish size is correlated with core area size. When core use areas of 75% and 50% KUD were analyzed, *C. argus* in Hawaii and *C. argus* in Moorea showed different trends. Hawaii showed a negative correlation between total length of *C. argus* and 75% KUD. As fish increased in total length, their 75% KUD home range decreased; with larger fish

holding smaller 75% KUD's than smaller fish. In Moorea there was a positive correlation between *C. argus* total length and 75% KUD, i.e. 75% KUD home range areas increased with an increase in fish length (Fig. 4.9). 50% KUD home range areas were similar for Moorea and Hawaii and did not differ significantly between regions.

Total lengths of individuals were compared between Hawaii and Moorea to determine whether fish size is correlated with region. In both Moorea and Hawaii, male *C. argus* were significantly larger than females ($p < 0.001$), with means of 24.6 cm and 32.6 cm respectively for females and males in Moorea, and means of 27.0 cm and 40.3 cm respectively for females and males in Hawaii.

Home range area was compared for the sexes, between Hawaii and Moorea to determine whether sex was a defining factor in home range size. In Moorea MCP and 95% KUD home range areas for *C. argus* differed significantly between the sexes (Fig. 4.10), with males holding larger home ranges than females (MCP $p = 0.001$, 95% KUD $p = 0.014$).

This was not the case for *C. argus* in Hawaii (Fig. 4.10), where MCP and 95% KUD home range areas did not differ significantly by sex: $p = 0.160$ and $p = 0.616$ respectively.

Female *C. argus* in Hawaii hold larger 95% KUD home range areas than females in Moorea when regions are compared: $p = 0.015$, $F = 6.65$. When females of the same size were compared between regions, *C. argus* females in Hawaii held larger 95% KUDs than females of the same size in Moorea, $p = 0.02$, $F = 6.04$ (Fig. 4.10). Male home ranges did not differ statistically by region.

Discussion

There have been few studies on the territoriality and social interactions of *C. argus* in its native habitat. Shpigel and Fishelson (1991b) examined territoriality, feeding, competition, and species interactions of three species of *Cephalopholis*. These studies took place in the Red Sea, and have provided the bulk of published knowledge about *C. argus* in its native habitat. Shpigel and Fishelson found these predators to be highly site attached, defending their harems and feeding territories against conspecifics as well as other predatory fish species. *C. argus* were polygamous, living in small social groups or harems composed of one male and four to six females (Shpigel & Fishelson 1991b). Males were larger than females, and protected their harems by defending a home range that generally encompassed the smaller home ranges of the females in the harem. Males patrolled home ranges, which were recorded as large as 1500 m², visiting each female within their social units several times throughout the day (Shpigel & Fishelson 1991b). Female territories were contiguous within the male's territory, averaging 60 m² in size, with the largest and most dominant (alpha) female holding the largest territory - up to 100 m² (Shpigel & Fishelson 1991b).

Home range areas

Home ranges in the present study, represented by MCP and 95% KUD, ranged from 230 to 2300 m² and 73 to 1235 m². When compared with other species of serranids, *C. argus* home ranges are small for the size of fish tracked. *Cephalopholis cruentata* between 27 and 33 cm total length in the Caribbean held larger home ranges than *C. argus*, with a

mean of 2120 m² and a range of 1200 to 4000 m² (Popple & Hunte 2005). Preliminary data for *Epinephelus marginatus* showed fish from 20.5 to 40 cm total length with 95% KUD home ranges from 1673 to 18,626 m² and a mean of 5312 m² (Lembo et al. 2002). *Paralabrax clathratus* from 25 to 40 cm standard length held 95% KUD home ranges of 33 to 11,244 m² with a mean of 3349 m² (Lowe et al. 2003). Zeller (1997) reported a mean MCP for *Plectropomus leopardus* of 10,485 m² for fish between 37.6 cm and 67.5 cm fork length. *Cephalopholis spiloparaea* also form small social groups with one male and several females, whose home ranges cluster within the home range of the dominant male, ranging in size from 6.6 to 73 m² (Donaldson 1995b). In the Red Sea, *C. argus* held similar size home ranges, with a maximum MCP of 2000 m², and *Cephalopholis miniata* showed smaller home ranges with a maximum MCP of 475 m² (Shpigel & Fishelson 1991b).

The differences in home range areas between some species may be related to the reproductive ecology of the species. *C. argus* do not undertake spawning migrations, remaining in their home ranges year round, defending females within their home range and social group. By remaining in home ranges year round, the dominant male secures the opportunity to mate with all or most of the females within his home range, and maintains control of his social group (Donaldson 1995b). Year round defense of home ranges may increase a male's mating success by excluding rivals in neighboring areas, and responding to females when they are receptive. The energy expended to defend mates may limit the home ranges of *C. argus*. Time spent in defense and maintenance of social hierarchies may reduce time available for foraging and other activities. Many of the above species undertake annual mass spawning migrations and do not defend mates

within their home ranges. The dominance hierarchy and social interactions within a social group may also restrict the home ranges of the subordinate females, causing smaller fish to use smaller home ranges.

In both Hawaii and Moorea there was a significant positive linear correlation between home range size and fish total length. Larger fish held larger home range areas, as seen with MCP and 95% KUD analysis. Positive correlations between fish size and home range size have been seen in other grouper species, including the congeners *Cephalopholis boenak* (Liu & Sadovy 2005) and *C. miniata* (Shpigel & Fishelson 1991b). Although several species of groupers, including *C. cruentata*, *P. clathratus*, *Epinephelus striatus* and *P. leopardus*, have shown no significant correlation between total length and home range size (Zeller 1997, 2002, Lowe et al. 2003, Popple & Hunte 2005, Starr et al. 2007), fish increasing in size require more resources, which may be provided by an increased density of food items, larger shelter sites, and/or a larger home range.

Utilization distribution

C. argus held large MCP and 95% KUD home range areas, but the majority of their time was spent within smaller core use areas. In both Hawaii and Moorea, core use areas corresponded to areas with high reef complexity and high rugosity, where holes and crevices were plentiful. *C. argus* showed a clear preference for these high rugosity sheltering sites and spent very little time in sandy or open habitat, crossing sand patches only to get to other high rugosity sites within home ranges. This behavior was also seen

in habitat utilization studies for *C. cruentata* (Poppo & Hunte 2005), and *P. clathratus* showed a distinct preference for high vertical relief and high rugosity areas with abundant shelter sites that were thought to facilitate prey capture (Lowe et al. 2003). *C. argus* remained at sheltering sites for extended periods of time (sometimes more than 30 minutes), and used 1 to 5 core areas throughout their home range, with a median of 2 and 3 core areas for the 50% and 75% use contours. Similar results were seen in *P. leopardus*, with 2 to 3 and 3 to 4 core areas providing the 50% and 75% use contours respectively (Zeller 1997). When successive tracks for each fish were analyzed, there was a complete overlap of core areas, indicating that *C. argus* in both regions have high site fidelity for core areas.

Specific locations within a home range are visited often. Repeated use of such locations, for example core use areas, may increase fitness because of the access to food and other resources, shelter and resting sites, and reduced predation or competition. Parrish (1987) suggested that, given the wide dietary variety and generalist nature of most serranids, the size and shape of home ranges may be determined by the availability of shelter sites rather than by prey abundance and densities. This fits well with the data collected for *C. argus*. Home ranges were based around core use areas, and the core use areas corresponded to areas of high rugosity that functioned as shelter sites.

In both Hawaii and Moorea, MCPs and core use areas of fish in harems overlapped, indicating that these fish use home ranges but do not defend territories against other fish within their social group. Home range areas of females were clustered within the male's home range, and many of the core use areas were shared by all the individuals within the

social group. Shpigel called overlapping home ranges clustered within the male's home range "compound home ranges" and reported them for *C. argus* and *C. miniata* in the Red Sea (Shpigel & Fishelson 1991b). Overlapping home ranges within social groups were also seen in the congeners *C. boenak* and *C. spioparaea* (Donaldson 1995b, Liu & Sadovy 2005). When *C. argus* from neighboring harems were encountered, aggressive interactions ensued, and posturing, biting and color fighting were observed. In the Red Sea, Shpigel & Fishelson (1991b) also found that female *C. argus* home ranges overlapped and clustered within a male's home range, and males defended home ranges from neighboring males, with biting and color fighting. The congener *C. cruentata* in the Caribbean showed overlapping home ranges within social units, but conspecifics did not share core use areas (Popple & Hunte 2005).

Activity patterns

The results from the passive acoustic data reveal that *C. argus* in Hawaii are diel predators with minimal nocturnal activity. Studies on the activity patterns of several grouper species have shown differing results. Several studies on grouper activity patterns have shown that groupers move during the day and are stationary at night (Carter et al. 1994, Zeller 1997, Liu & Sadovy 2005). *Cephalopholis boenak* was diurnally active, and all movement stopped just before sunset (Liu & Sadovy 2005). Zeller (1997) reported that of the 39 *P. leopardus* tracked, 35 of them did not change positions or move at night. *Cephalopholis cruentata* in the Caribbean (Popple & Hunte 2005) was significantly more active during the night than during daylight hours, and *Paralabrax clathratus* in the

eastern Pacific were reported to be active both day and night (Lowe et al. 2003).

Active tracking in both Hawaii and Moorea showed reduced activity in the late afternoons. As sunset approached, *C. argus* would settle into core areas and remain there for the duration of the tracking session. Focal *C. argus* individuals, once settled into a hole or crevice at dusk, were not observed to move.

Site fidelity

Groupers are known to be highly site attached predators, and several species have been reported in the same home ranges for long periods of time (*P. clathratus*, 3 years, Lowe 2003; *E. marginatus*, more than 40 days, Lembo 2002; *P. leopardus*, 12 months, Zeller 1997; *E. striatus*, 4 months, Starr 2007; *C. argus*, 3 months to 3 years, this study). The passive acoustic data were also used to examine site fidelity. All the implanted *C. argus* remained in the receiver area throughout the battery life of the transmitter, showing site fidelity over longer periods of time. Further evidence of high site fidelity and site attachment was obtained with the re-sighting, as well as recapture, of 22 tagged *C. argus* in the same home ranges several years after initial tracking studies were performed in Hawaii and five months after preliminary tracking ended in Moorea. There were no more opportunities for further tracking, recapture or re-sighting in Moorea after the five-month period. Comparisons of the home ranges for each *C. argus* showed almost complete overlap of ranges between tracking sessions. Successive overlap coupled with low linearity index values for both Hawaii and Moorea further indicate repeated use of the same area and high site fidelity. The low linearity index values indicate that *C. argus* in

both regions are not swimming in a straight line and continuing over large areas of reef in a unidirectional fashion. Their movements are directed and occur in short lengths. *C. argus* visit specific places, moving back and forth within their home ranges. *P. clathratus* and *P. leopardus* showed similar linearity index values ranging from 0.001 to 0.03 with a mean of 0.015 for *P. clathratus*, and values ranging from 0.001 to 0.11 with a mean of 0.02 for *P. leopardus*. Both of these groupers are thought to be highly site attached (Zeller 1997, Lowe et al. 2003).

Comparison

There were significant positive relationships between length of fish and size of MCP home range area in both Moorea and Hawaii, where larger fish generally held larger home ranges. The scatterplot in Figure 4.1 shows that, although there is a marginally significant correlation between fish total length and area of the MCP in Hawaii, the variability is high, $R^2 = 0.18$. For data from Moorea, the model showed less variability than Hawaii ($R^2 = 0.52$), and *C. argus* in Moorea showed a stronger correlation between fish total length and home range size (Fig. 4.5).

C. argus in Hawaii used significantly larger home range areas than fish of the same size in Moorea. This difference may be due to lower competition in Hawaii. Competition for space and resources between ecologically similar coral reef fish can influence the abundance and habitat use of competing species in shared habitat (Robertson 1996, Dulvy et al. 2000). *C. argus* is one of many species of grouper on the reef in its native habitat in Moorea, and these species probably compete for resources, including prey and

sheltering sites. In Hawaii there is a complete lack of native shallow-water groupers, and there are also few other large, sedentary, benthic predators. Hawaii is home to several species of jacks that are highly mobile and roam the reefs in search of prey, but these fish employ different predation tactics and may not be competing directly with *C. argus*. The major native, benthic, sedentary predators of Hawaii are lizardfish and hawkfish, that are dwarfed by the introduced predator and are known to be actively hunted and eaten by *C. argus* (Dierking 2007). When a competing species of territorial damselfish was removed from patch reefs in the Caribbean, the competitive release allowed a less aggressive species of damselfish to expand its territory and move into new microhabitats previously occupied by its competitor (Robertson 1996). The competitive release experienced by *C. argus* moved to Hawaii may have caused an increase in the amount of habitat used by *C. argus*, resulting in larger home ranges.

The 75 and 50% KUD's were used to define core use areas, and in Moorea larger fish had larger core use areas. When 75 and 50% KUD's were examined, only *C. argus* in Moorea showed a significant relationship between fish length and KUD size. When differences between regions were examined, *C. argus* in Hawaii had a negative relationship between total length and size of 75% KUD. Larger fish held smaller 75% KUD's than smaller fish in Hawaii. Large *C. argus* held larger 95% KUD's and spent time sheltering in smaller core use areas than smaller fish. This may be due to the dominance hierarchy observed in several species of groupers and documented in *C. argus* (Shpigel & Fishelson 1991b). Larger fish often displace smaller fish that are sheltering in crevices or under coral heads. Large fish may have smaller core use areas due to the fact that they only use a few prime sheltering sites, and use these sites exclusively. They do

not need to search for other shelter sites, and can merely displace subordinates if the site is occupied. Smaller fish that are lower in the dominance hierarchy may be displaced from sheltering sites and forced to use lower quality sites. This will lead to an increase in shelter sites and thus an increase in core use areas for smaller fish.

The opposite pattern was seen in Moorea, where there was a positive trend of increase in 75% KUDs with an increase in fish length. This difference may be due to competition for resources and shelter sites between grouper species in Moorea. Interspecific interactions, differences in body size, and aggressiveness between groups of species often affect their patterns of habitat use (Robertson 1996). Several species of groupers were seen interacting with *C. argus* at shelter sites: *C. urodeta*, *E. fasciatus*, *E. hexagonatus*, *E. merra*. On some occasions *C. argus* were displaced by other grouper species, and on other occasions *C. argus* displaced the other species. When displaced, *C. argus* would usually move to one of several nearby shelter sites within a core area, thus expanding 75% core use area.

Predation, presumably lower in Hawaii, may also be an influencing factor determining the size of core use areas. Large and small *C. argus* are preyed upon by several species of sharks on the reefs of Moorea (personal observation). An increase in the number or size of shelter sites in a localized area could potentially lead to a decrease in the risk of predation, by insuring more reliable and safe sheltering sites. Predation and competition on the reef of Moorea affect *C. argus* of all sizes.

Males have significantly larger home range areas than females in Moorea but not in Hawaii (Fig. 4.10). In the Red Sea, Shpigel (1991) reported that males of *C. argus* and

C. miniata held larger home ranges than females and suggested that patrolling the home range and defending females from neighboring males increased male home ranges.

Males of *C. boenak* also held larger home ranges than females (Liu & Sadovy 2005).

Males are also generally larger than females, and as reported above, *C. argus* size influences home range area.

The lower competition experienced by *C. argus* in Hawaii may cause a shift or breakdown in the size or sex related structuring of home ranges and resource use in Hawaii. The lack of difference between male and female home range sizes in Hawaii may also reflect the reduced competition from other species, as well as the overall difference in home range size between Hawaii and Moorea.

Conclusion

The coral reef habitats of Hawaii and Moorea are similar, and they are comparable in age and distance from the equator, but the grouper species compositions are very different.

The waters of Moorea are home to at least 14 species of Epinephelinae, and interspecific competition may influence the home ranges of competing grouper species. In their introduced environment, *C. argus* have few predators and can grow to total lengths of 62 cm, dwarfing native benthic predators on the reefs, such as hawkfish and lizardfish. Data collected on lengths and weights of *C. argus* in Moorea and Hawaii for a diet study show that *C. argus* in Hawaii grow to lengths and attain weights that are significantly greater than those of *C. argus* in Moorea, and this may influence home range sizes and the lateral spread of *C. argus* over Hawaiian reefs. In general, home range area was found to be

positively correlated with fish size, and larger fish hold larger home ranges for *C. argus* in both Moorea and Hawaii. In both regions, fish did not use their home ranges evenly, spending much of their time sheltering in several highly rugose core use areas. *C. argus* forms harems, holding overlapping home ranges on coral reefs, and has shown high site fidelity over time - on the order of months and years in both regions. *C. argus* in Hawaii seem to experience little competition for the resources within their home ranges, and fish show larger home ranges when compared to their counterparts in Moorea.

Table 4.1. Total lengths and home range areas of *C. argus* in Hawaii.

Total Length (cm)	MCP (m²)	KUD 95% (m²)	KUD 75% (m²)	KUD 50% (m²)	Sex
21.0	543.4	396.4	125.1	35.5	Female
23.0	545.8	260.5	97.5	49.8	Female
23.5	948.3	664.3	278.5	82.2	Female
23.5	1110.1	576.9	196.6	78.9	Female
23.5	1131.1	159.2	52.1	24.2	Female
24.0	425.2	229.3	125.9	42.4	Female
24.0	1003.3	501.5	165.0	56.3	Female
25.0	1260.9	762.3	176.2	50.1	Female
25.0	1293.7	780.6	294.5	121.7	Female
26.5	1098.0	825.1	220.6	56.4	Female
27.0	1489.8	981.4	221.9	68.0	Female
27.5	2031.4	1235.2	561.2	273.6	Female
29.0	738.5	480.6	120.6	37.7	Female
29.0	2259.6	947.7	453.9	135.0	Female
32.0	1523.3	978.6	284.4	69.6	Female
33.0	1052.2	144.9	53.1	19.7	Female
35.0	757.3	281.3	90.2	27.1	Female
36.0	1488.9	198.3	58.3	31.5	Female
39.0	2300.3	1116.1	276.9	59.0	Male
39.0	1014.0	203.7	71.0	29.7	Male
40.5	1723.3	796.0	164.2	47.5	Male
41.0	1388.0	738.6	193.0	63.6	Male
42.0	1310.3	488.9	76.3	39.2	Male

Table 4.2. Total lengths and home range areas of *C. argus* in Moorea.

Total Length (cm)	MCP (m²)	KUD 95% (m²)	KUD 75% (m²)	KUD 50% (m²)	Sex
20.0	230.7	73.5	19.1	11.0	Female
20.0	300.1	208.0	48.6	8.6	Female
21.0	341.8	244.2	66.6	14.9	Female
21.0	704.7	454.0	74.2	34.3	Female
22.0	484.6	343.6	106.7	42.8	Female
22.0	231.0	143.4	50.1	11.5	Female
22.5	1002.0	699.4	132.8	44.3	Female
23.5	603.8	355.6	115.6	47.5	Female
25.0	527.2	134.0	48.6	28.4	Female
26.0	487.1	194.3	41.3	12.4	Female
27.0	978.3	726.6	147.0	49.2	Female
27.0	532.3	453.3	227.3	61.2	Female
28.5	858.0	416.3	163.0	58.3	Female
30.0	781.2	385.1	99.7	40.7	Male
31.0	1165.3	700.5	183.4	42.4	Male
31.5	430.9	250.2	74.9	17.2	Female
32.0	856.2	178.8	55.5	22.3	Female
33.0	1017.5	854.1	441.8	152.7	Male
34.0	1389.6	591.9	166.8	50.4	Male
35.0	1094.8	460.4	158.8	59.7	Male

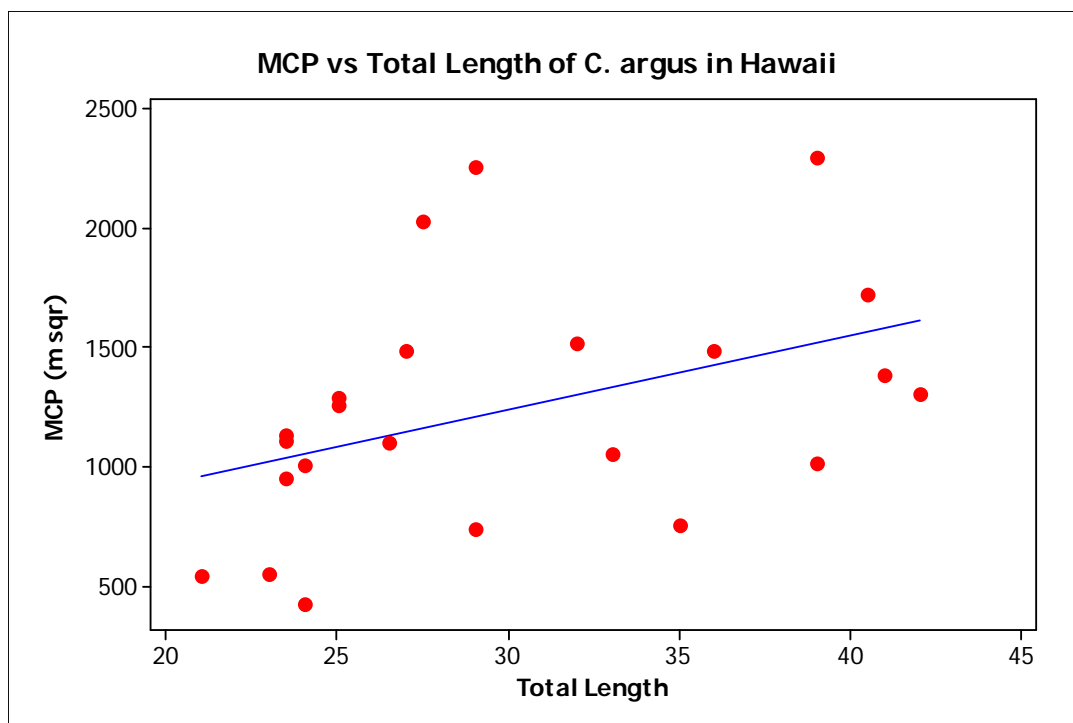


Figure 4.1. Scatterplot of Minimum Convex Polygon home range area (m²) vs. *C. argus* total length (cm) in Hawaii. Linear regression, $p=0.046$, $R^2=0.18$.

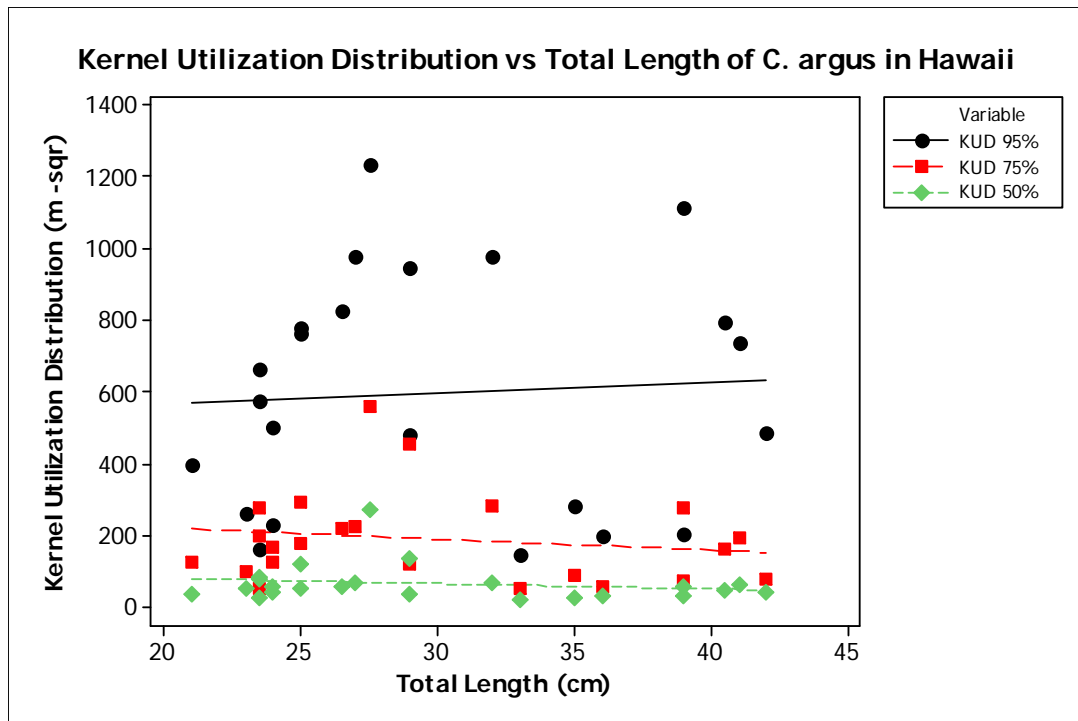
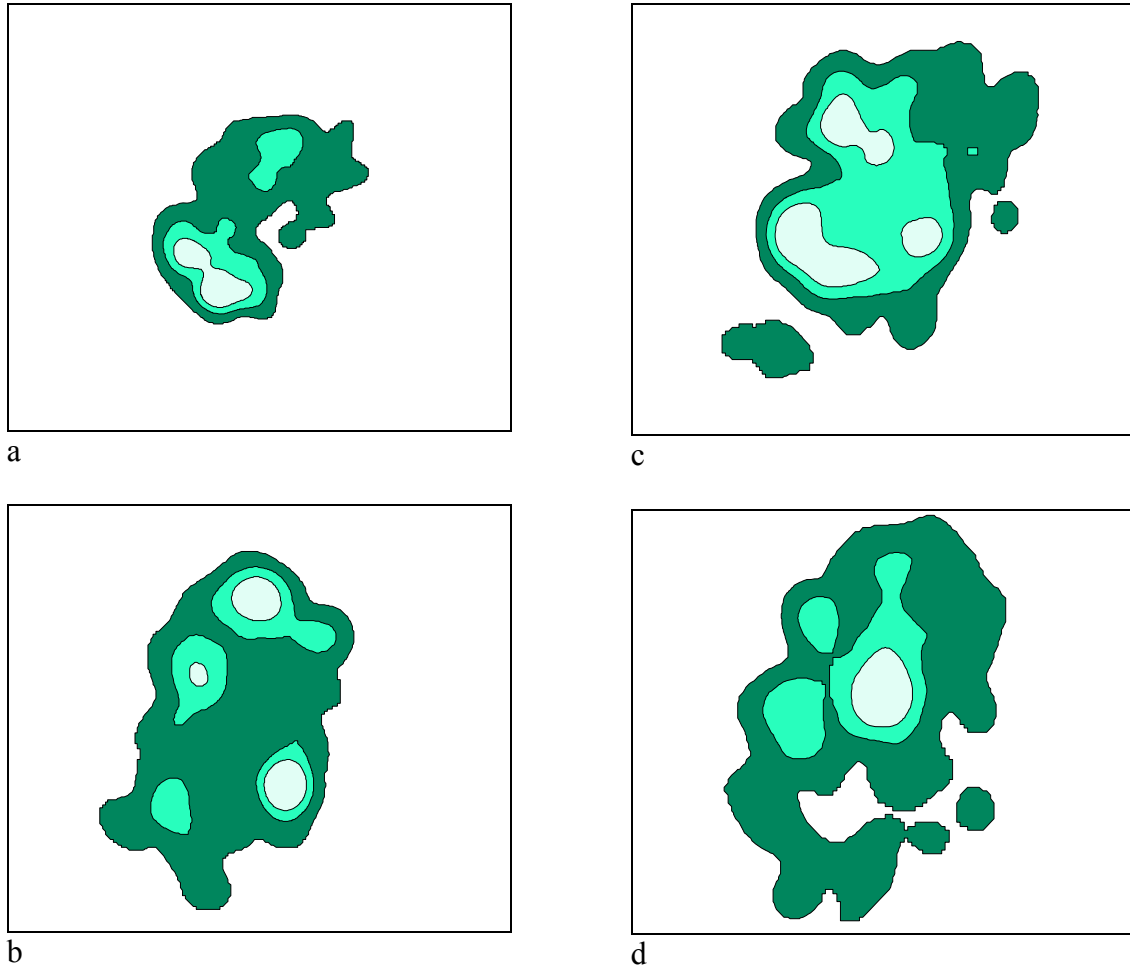


Figure 4.2. Scatterplot of kernel utilization distributions (m^2) vs. total length (cm) of *C. argus* in Hawaii. Black circles represent 95% KUD, $p=0.793$, $R^2=0.003$. Red squares represent 75% KUD, $p=0.455$, $R^2=0.03$. Green diamonds represent 50% KUD, $p=0.386$, $R^2=0.04$.



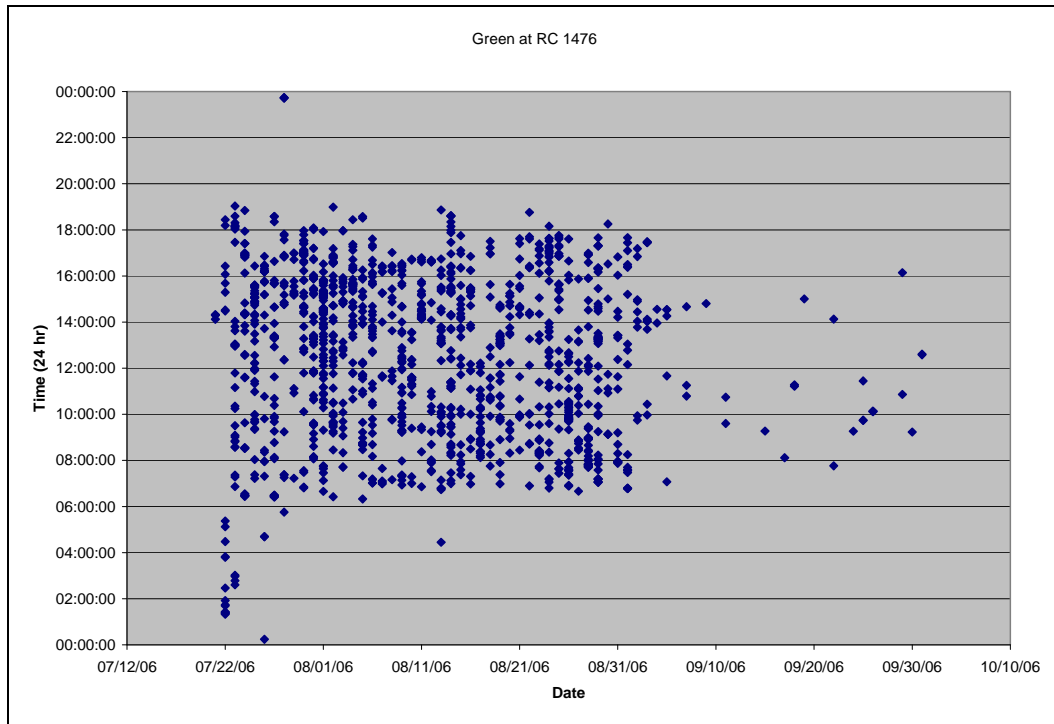


Figure 4.4. Scatterplot of transmitter signals for WCg fish at receiver 1476. Dots indicate when a fish passed by the receiver.

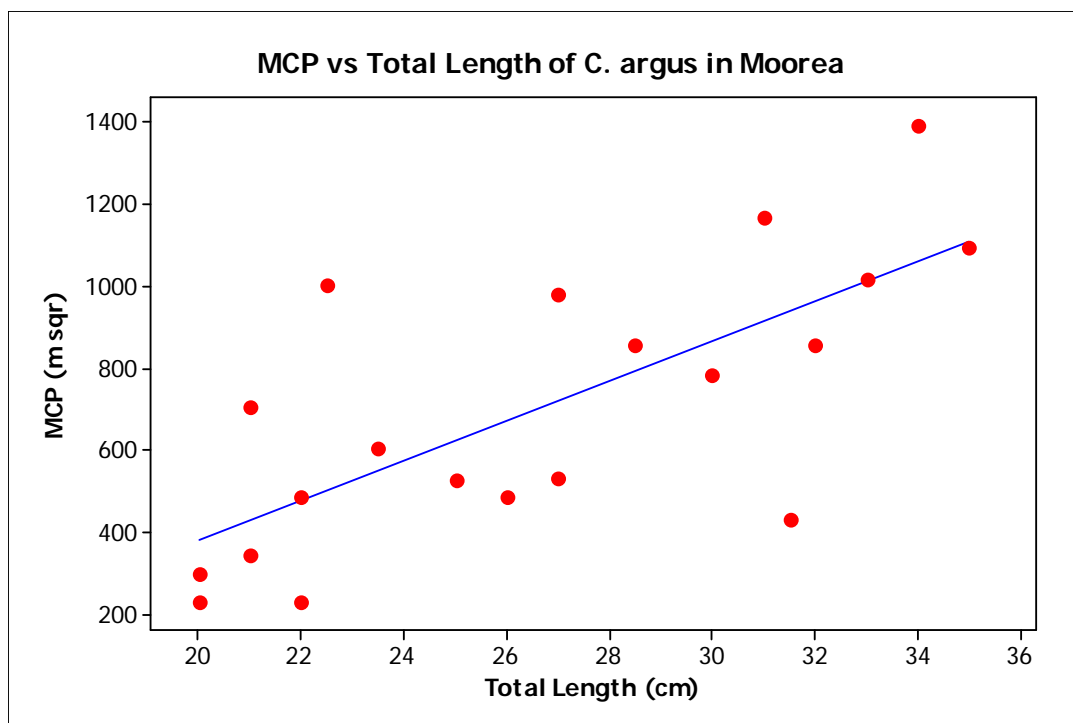


Figure 4.5. Scatterplot of Minimum Convex Polygon home range areas (m²) vs. *C. argus* total length (cm) in Moorea. Linear regression, $p < 0.001$, $R^2 = 0.52$.

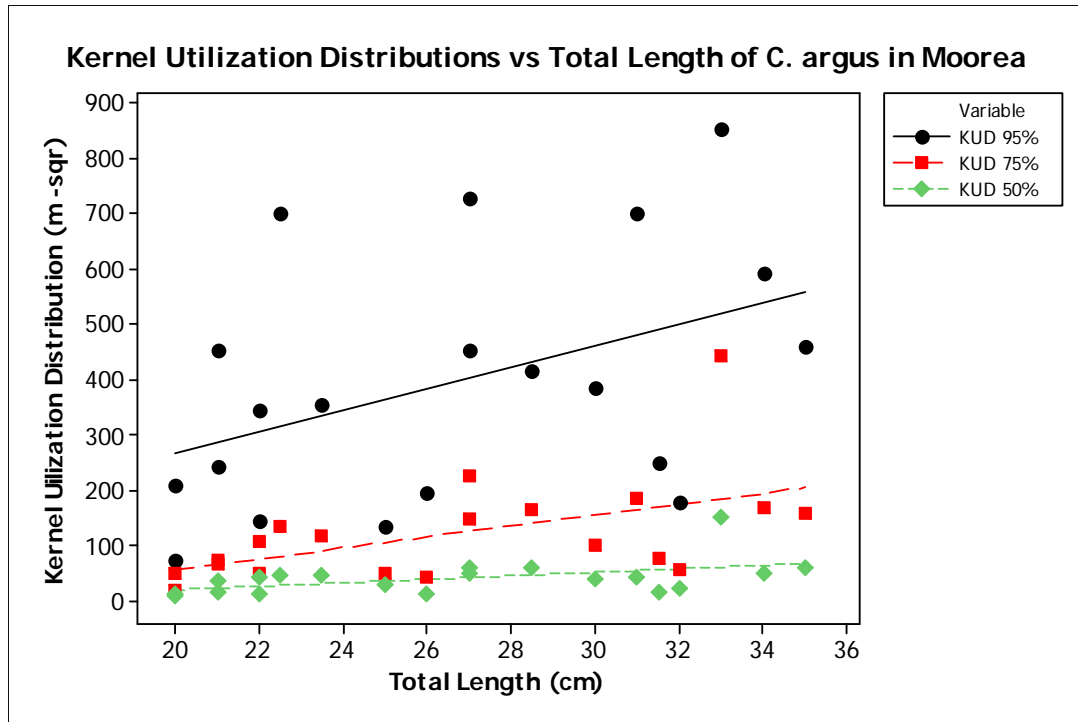


Figure 4.6. Scatterplot of kernel utilization distributions (m^2) vs. total length (cm) of *C. argus* in Moorea. Black circles represent 95% KUD, $p=0.057$, $R^2=0.19$. Red squares represent 75% KUD, $p=0.017$, $R^2=0.28$. Green diamonds represent 50% KUD, $p=0.026$, $R^2=0.25$.

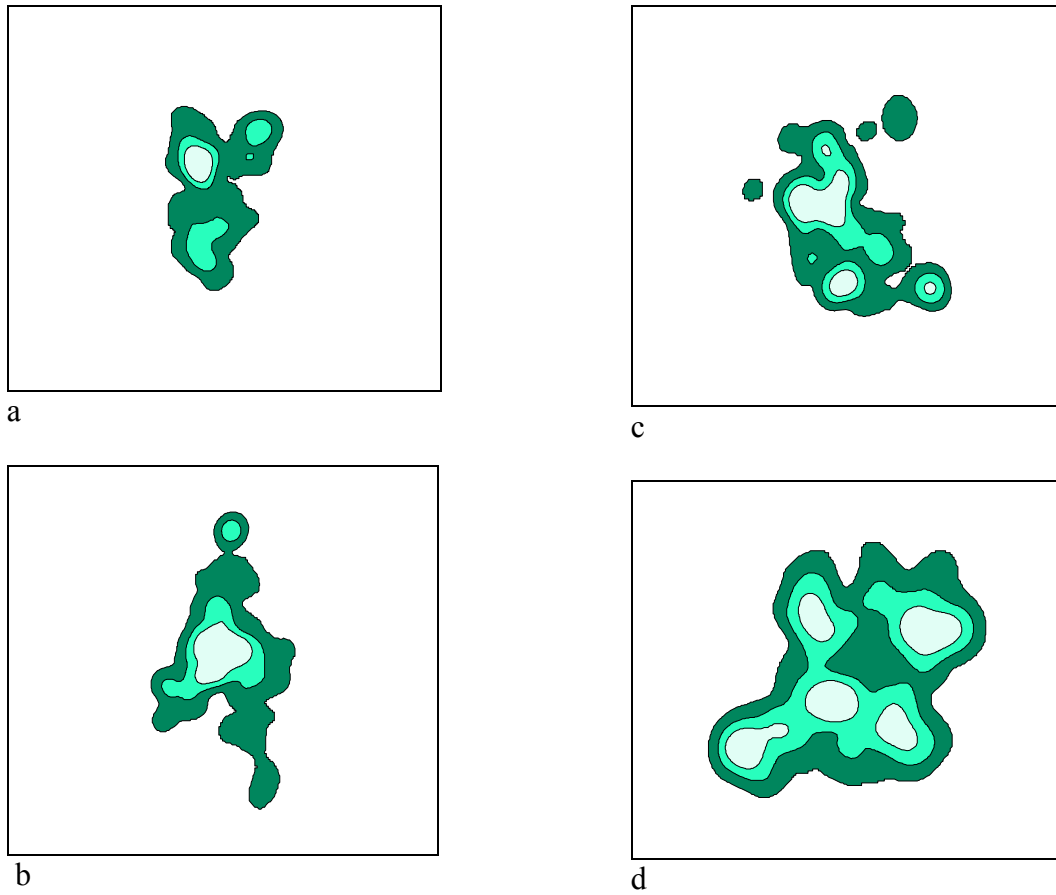


Figure 4.7. Kernel utilization distribution contours for four representative *C. argus* individuals in Moorea. Dark green represents 95% KUD, middle green represents 75% KUD, and light green represents 50% KUD. a) MT3b 21 cm total length, 95% KUD 244 m²; b) MT4w 23.5 cm total length, 95% KUD 355 m²; c) MT5t 28.5 cm total length, 95% KUD 416 m²; d) MT3y 33 cm total length, 95% KUD 854 m².

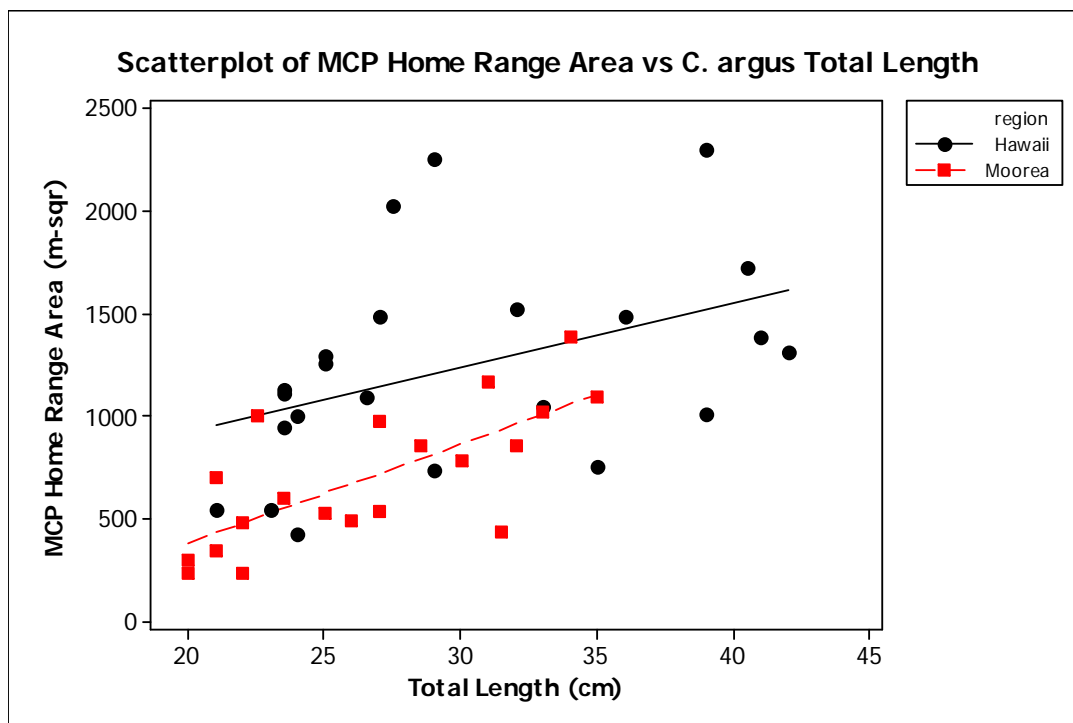


Figure 4.8. Scatterplot of Minimum Convex Polygon home range areas (m^2) vs. total length (cm) of *C. argus* by region. Black circles represent *C. argus* in Hawaii, and red squares represent *C. argus* in Moorea.

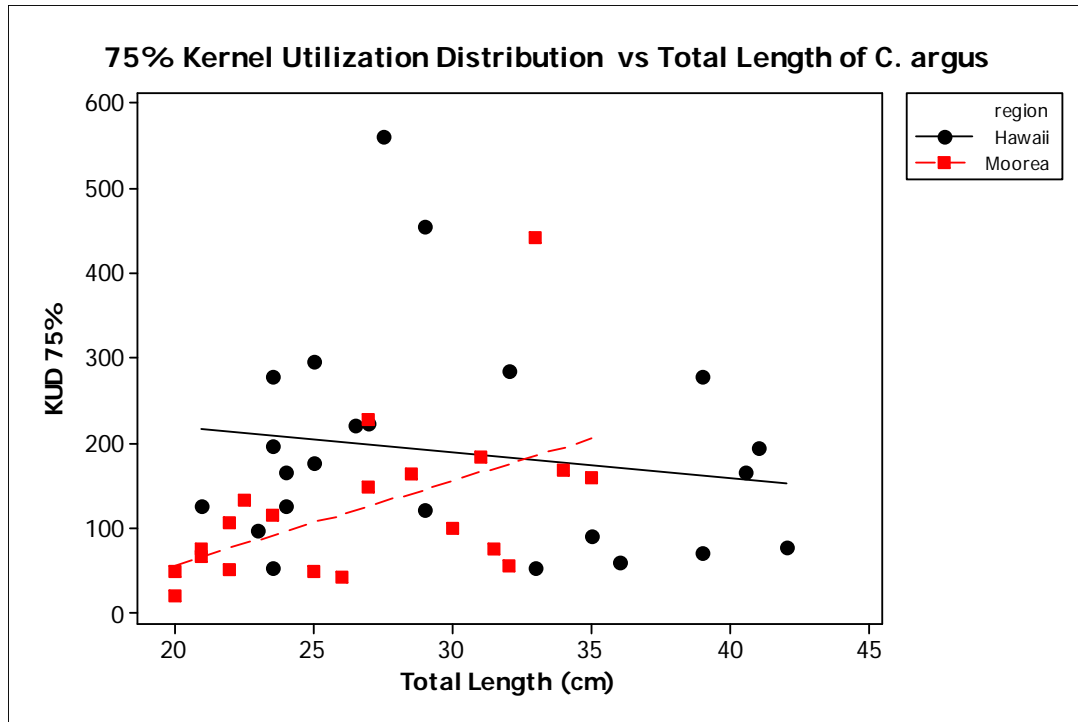


Figure 4.9. Scatterplot of 75% kernel utilization distribution home range area (m^2) by total length (cm) of *C. argus* by region. Black circles represent Hawaii and red squares represent Moorea. In Hawaii there is a small negative correlation between *C. argus* total length and 75%KUD. In Moorea there is a positive correlation between *C. argus* total length and 75% KUD. The difference between slopes between regions was statistically significant, $p=0.04$ $F=3.8$.

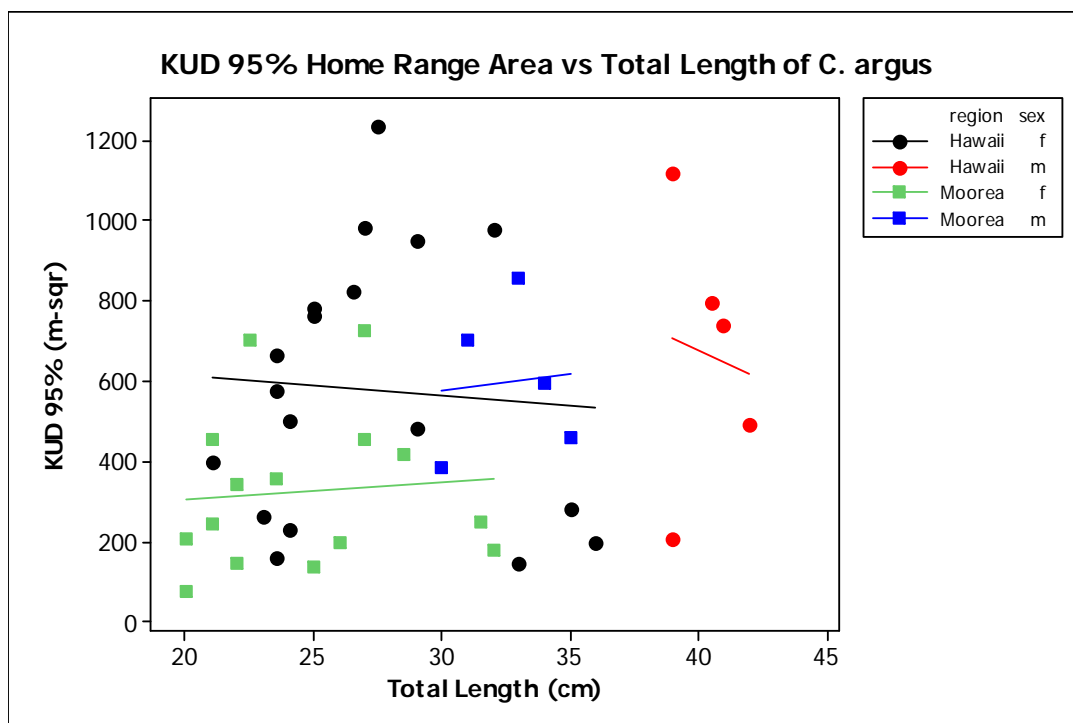


Figure 4.10. Scatterplot of kernel utilization distribution home range area (m^2) vs total length of *C. argus* (cm), by geographical region and sex of fish. Black circles represent Hawaiian females, red circles represent Hawaiian males, green squares represent Moorean females, and blue squares represent Moorean males. In Moorea male home range areas were significantly larger than female home range areas, $p < 0.001$. There was no significant difference in home range areas between sexes in Hawaii.

CHAPTER 5

Ciguatoxin levels in the grouper *Cephalopholis argus* in Moorea, French Polynesia

Introduction

The Hawaiian Islands are the most isolated geographic region in the world. Because of this extreme isolation, several fish families are underrepresented or completely absent from the shallow, inshore reefs of Hawaii. Among these is the family Serranidae, the sea basses and groupers. Many species of serranids can be found elsewhere on coral reefs throughout the Indo-Pacific, where they are considered a prized food source. However, there are no native groupers in Hawaii restricted to shallow waters.

In an attempt to increase nearshore fishery diversity in Hawaii, the Bureau of Fisheries Management and the Division of Fish and Game of the State of Hawaii instituted a bottomfish introduction program in the late 1950's (Randall 1987, Planes & Lecaillon 1998). The peacock grouper, *Cephalopholis argus*, was one of a number of potential food fish introduced to Hawaiian waters from Moorea in the Society Islands (where they were known as "roi") (Oda & Parrish 1981, Randall 1987). Today *C. argus* is the most abundant large, nearshore predatory fish species on the reefs of the Main Hawaiian Islands (Dierking 2007). However, the species has failed to provide a fishery. *C. argus* is known in Hawaii for causing ciguatera fish poisoning (CFP), and as a result there is not an active fishery for *C. argus* in Hawaii.

Ciguatera fish poisoning (CFP) is caused by *Gambierdiscus toxicus*, a dinoflagellate that resides on and attaches to benthic algae. *G. toxicus* is inadvertently ingested by

herbivores while they graze on algae, and the ciguatoxins (CTX) produced by *G. toxicus* are incorporated into the tissues of the fish (Randall 1987, Hokama & Yoshikawa-Ebesu 2001, Chateau-Degat et al. 2005, Lewis 2006, Darius et al. 2007). The polyether toxins derived from *G. toxicus* that cause CFP are not harmful to the fish that consume them, having little or no effect on the growth and condition of the host (Dierking 2007). When incorporated into an organism's tissue, CTX are accumulated up the food chain as carnivorous fish prey on herbivores, omnivores, and other carnivores. Worldwide, more than 400 species of herbivorous and carnivorous reef fish have been associated with ciguatera outbreaks; many of the predators are in the grouper (Serranidae), jack (Carangidae), and snapper (Lutjanidae) families (Lehane & Lewis 2000). Growth of *G. toxicus* can be influenced by gradual changes in environmental factors such as changes in sea water temperature, currents, nutrients, and salinity, or by abrupt environmental changes, such as severe storms, hurricanes, and runoff from flooding (Hokama & Yoshikawa-Ebesu 2001, Chateau-Degat et al. 2005). These environmental changes can cause increased growth and large blooms of *G. toxicus*, resulting in an increase in CFP. Humans and other mammals are highly sensitive to CTX and often acquire CFP when they consume contaminated fish. The presence of ciguatoxins causes the depolarization and destabilization of nerve cells and the release of neurotransmitters, thus prolonging the opening of voltage dependent sodium ion channels and thereby affecting neurological, gastrointestinal, musculoskeletal and cardiac systems (Hokama & Yoshikawa-Ebesu 2001, Palafox & Buenconsejo-Lum 2001, Chateau-Degat et al. 2005, Cruz-Rivera & Villareal 2006, Hokama et al. 2006, Lewis 2006, Darius et al. 2007). Symptoms include nausea, vomiting, diarrhea, stomach pains, alteration in temperature sensation, weakness,

sweating, myalgia, and arthralgia (Gollop & Pon 1992, Palafox & Buenconsejo-Lum 2001, Chateau-Degat et al. 2005, Cruz-Rivera & Villareal 2006, Lewis 2006, Darius et al. 2007).

Several tests have been designed to detect CTX in fish tissue. These tests were designed to be simple, yet selective and sensitive. Over the years, several very sensitive and accurate tests have been used, but have not been available to the public because of their complexity. Recently the Membrane Immunobead Assay (MIA), developed by Hokama, has been used as a simple way to detect CTX in reef fish (Hokama et al. 1998, Hokama et al. 2006, Lewis 2006, Dierking 2007). The MIA is composed of a hydrophobic membrane that is laminated to a solid plastic stick (Hokama et al. 1998, Hokama & Yoshikawa-Ebesu 2001, Hokama et al. 2006). Once sensitized, the MIA stick is dipped into the immunobead suspension that is made up of small polystyrene beads coated with monoclonal antibodies to CTX (Mab-CTX) (Hokama et al. 1998). The MIA uses colored immunobeads that bind with the CTX on the membrane. If CTX is present in the fish tissue (positive), the membrane portion of the test stick changes color (Hokama et al. 1998). The resulting color and the intensity of the color determine whether the fish tissue contains CTX.

Ciguatera fish poisoning occurs pantropically, from 35° N to 35° S around the globe, and poses significant health, resource and economic problems in tropical and subtropical regions of the world (Randall 1980, Lewis 1986, Quod & Turquet 1996, Hokama & Yoshikawa-Ebesu 2001, Chateau-Degat et al. 2005). CFP has recently become more widespread throughout the world because of the export of tropical fish to temperate regions (Lewis 2006). Annually human CFP cases range from 25,000 to 50,000

worldwide (Lehane & Lewis 2000, Lewis 2006). These figures are believed to underrepresent the total incidence worldwide because 1) CFP can be misdiagnosed (having some symptoms similar to influenza), and 2) it commonly occurs in remote tropical regions where many cases simply go undiagnosed or unreported (Darius et al. 2007). Reports of ciguatera incidence in French Polynesia suggest that it is an especially serious problem there, with a higher rate of occurrence there than in other regions of the Pacific (Lewis 1986). French Polynesia reported an annual incidence of 330 cases per 100,000 population in 1999 (Chateau-Degat et al. 2005). Hawaii has also experienced a series of ciguatera outbreaks linked with human consumption of both herbivores and carnivores (Randall 1980, Lewis 1986, Hokama et al. 1998), and several fisheries have been closed due to high incidence of CFP.

Randall (1960, 1987) showed that not all fish of the same species in a given area were carriers of CTX. In an attempt to determine whether a fishery for *C. argus* was viable in Hawaii, Jan Dierking collected and tested *C. argus* from locations around the Main Hawaiian Islands for CTX, to investigate the variability of ciguatera incidence with fish size and location (Dierking 2007). Throughout the history of ciguatera and *G. toxicus* research, Hawaii has reported relatively low incidences of CFP and CTX in fish relative to French Polynesia (Hokama & Yoshikawa-Ebesu 2001). The present study is focused on CFP in Moorea, French Polynesia, to determine whether there are “safe sizes” of *C. argus* that can be consumed, or “safe localities” from which to catch fish for food. Fish from Moorea were collected and tested for CTX, and the results for Moorea were compared to those found by Dierking (2007) in Hawaii.

Methods

Cephalopholis argus were collected by spearing at 13 sites off the fringing reef of Moorea, French Polynesia, at depths from 3 to 30 m (Fig. 5.1). Fish were measured for standard length (SL) and total length (TL), weighed, and tissue samples were taken from the head, middle (just posterior of the pectoral fin) and tail (caudal peduncle) of each fish. Tissue samples were frozen immediately and kept on ice for transfer to Hawaii. Ciguatera analysis of fish tissue took place at the University of Hawaii at Manoa in the Hokama laboratory, following methods provided by Hokama et al. (1998), using the Membrane Immunobead Assay (MIA). A sterile razor blade was used to remove sub-samples of flesh from each tissue sample. Tissue sub-samples and MIA sticks were soaked together in methanol for 20 minutes. The test stick was then removed from the methanol and allowed to dry completely. Once the MIA test stick was dry, it was placed into a test tube and the immunobead suspension was added. The MIA test stick was allowed to soak for no more than 10 minutes, when it was removed and examined and the color and intensity of color recorded. MIA test scores were interpreted based on the final color and intensity of color of the test stick, and the results were translated into CTX concentration in the fish tissue (Hokama & Yoshikawa-Ebesu 2001). MIA test sticks were compared to controls that were run without fish tissue as well as controls for standards of known CTX concentrations.

MIA interpretation

The result from each MIA stick was given a numerical score following the methods of Dierking (2007) (Table 5.1), ranging from 0 to 2. The scores from each of the three tissue samples, (head, mid body, and tail region) were then added together to provide a whole-fish CTX score ranging from 0 to 6 (Dierking 2007). Whole-fish CTX scores were ranked according to Table 5.2. Fish CTX scores were used to provide information about the overall CTX concentration of toxin in a fish, and to assess how safe the fish was for human consumption.

Statistical analysis

A regression was run to determine the relationship between SL of fish and CTX concentration. Fish were divided into five size classes by SL (1= 10-15 cm, 2= 15.5-20 cm, 3= 20.5-25 cm, 4= 25.5-30 cm, and 5= 30.5-35 cm), and an ANOVA was performed to further assess the relationship between size and CTX concentration. An ANOVA was run to investigate any links between capture site and CTX concentration, to determine whether sites differed in incidence of CTX present in fish. A regression was run to relate stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to CTX concentration, to search for food-chain effects. Lastly, Chi-square tests and a General Linear Model were performed to compare results from Moorea to those found by Dierking (2007) in Hawaii. All statistical analyses were performed with Minitab 14, and an *a priori* value for significance was set at $p < 0.05$.

Results

Length vs CTX Score

The SL of *C. argus* and CTX score were positively correlated, with marginal significance: $p=0.045$, $R^2=0.036$ (Fig. 5.2). When an extreme outlier was removed, $p=0.012$ ($N=109$). The outlier was a small (16-cm SL) fish in size class two, and had the highest obtainable CTX score of 5, which is considered strongly positive.

In the ANOVA of CTX concentration scores by standard length class, there were no significant differences when the entire sample was analyzed ($p=0.11$). However, when the same extreme outlier was removed, there were marginally significant differences in the CTX scores between the different size classes of *C. argus* ($p=0.049$) (Fig. 5.3). Size class 4 had the highest mean CTX score (2.8), which is considered positive and involves substantial risk if eaten. Size class 1 had the lowest mean CTX score (1.9), which is just below the positive ranking in the marginal range and can be considered at risk.

Between site variability

When sites were compared for differences in CTX scores, ANOVA showed that sites varied significantly: $p=0.049$. Site M1 had the lowest mean CTX score (1.7); this was the only site with a mean below 2. Site M2 had the highest mean CTX score (2.7). Tukey's comparison showed that site M1 was significantly different from M2. However, site M2 had a strong outlier, a 16-cm fish with a CTX score of 5 (same individual described above). When the outlier was removed, there were no clearly significant differences among all sites (ANOVA, $p=0.064$) (Fig. 5.4). All sites tested had fish that scored 2 or

higher and were considered to involve some hazard if eaten, and at all sites except M1, the majority of *C. argus* tested contained CTX levels at or above the cut-off range of 0.32 ng/g (Table 5.1).

Stable Isotope vs Ciguatera score

There were no significant relationships between ciguatera score and stable isotope value for either of the isotopes tested ($\delta^{15}\text{N}$ linear regression, $p=0.84$, $R^2=0.001$; $\delta^{13}\text{C}$ linear regression, $p=0.27$, $R^2=0.012$) (Fig. 5.5).

Hawaii-Moorea Comparison

Dierking (2007) sampled and tested 291 *C. argus* from the Main Hawaiian Islands. Of the 291 fish, 46% tested negative, 36% marginal, 14% positive, and 4% strongly positive (Fig. 5.6). These results differ significantly from the results found in Moorea in the current study. Of the 110 Moorea fish analyzed, 2% tested negative, 51% marginal, 46% positive, and 1% strongly positive (Fig. 5.6). The frequency of occurrence of toxic *C. argus* in Moorea was significantly greater than in Hawaii (Chi-square, $p<0.001$). The mean CTX scores were also significantly different between the two regions (t-test, $p<0.001$).

Overall CTX scores were significantly lower for *C. argus* in Hawaii. *C. argus* of a given length in Hawaii had lower CTX scores than fish of the same size in Moorea; (ANCOVA, $p<0.001$, $F=72.3$) (Fig. 5.7). Size classes also differed significantly between

regions, where Hawaii had significantly lower CTX scores than the fish in corresponding size classes in Moorea (ANCOVA, $p < 0.001$, $F = 73.5$) (Fig. 5.8). Figure 5.8 also shows strongly positive outliers for Hawaii that were generally not seen in Moorea, as well as the occurrence of more negative fish in several of the size classes.

Discussion

Because of the bioaccumulation of CTX, higher CTX scores, corresponding to higher concentrations of CTX, might be expected in larger *C. argus* individuals. On the basis of Pearson product-moment correlation analysis, the results of this study show that the SL of *C. argus* and CTX score were significantly positively correlated, with a gradual, slight increase of ciguatera toxicity as *C. argus* increased in SL. Banner et al. (1966) found similar results for the red snapper, *Lutjanus bohar*, where larger fish from a toxic area repeatedly had higher scores of toxicity than smaller fish.

Figure 5.3 illustrates the unpredictable nature of ciguatera, where even small fish, 16 cm SL, can have a CTX score of 5 and be very toxic. The ANOVA of CTX score among size class categories is only marginally significant if outliers are omitted. Although there is a slightly significant correlation between ciguatera score and size of fish, it is evident that small fish can be highly toxic and often are either marginal or positive and probably unsafe to eat. Only 2 individuals, out of the 110 fish tested at Moorea, fell within the negative range and were considered safe for consumption. All other fish ranked marginal or higher.

In a study by Banner et al. (1966), ciguatoxic red snapper were kept in captivity and fed a nontoxic diet for 3 to 30 months. At the end of this period, the fish did not show a statistically significant decrease in toxicity. The half life for CTX in moray eels is 264 days (Lewis 1992), and once CTX is incorporated into fish tissue it can be stored for a very long time. CTX accumulates over time, and a fish does not have to consume a steady diet of toxic food to acquire high levels of CTX. Fish with marginal CTX scores may be unsafe to eat because of personal, unpredictable sensitivity to CTX, as well as variability in the accumulation of CTX in human tissue. The consumption of several marginal fish may lead to the accumulation of enough CTX to cause ciguatera fish poisoning.

CTX by size class

In stomach content and stable isotope analyses performed on these same fish, there was evidence of an ontogenetic feeding shift as fish increased in size. Results from the stomach content analysis showed that *C. argus* of size class 4, (25.5-30 cm SL), feed on significantly larger prey than *C. argus* in smaller size classes. Stable isotope analysis, which measures assimilated and long term diet, revealed that *C. argus* in smaller size classes preyed on a mixture of planktivores and benthivores. *C. argus* in size class 4 shift their feeding and focus on benthic feeding prey. The life history of *Gambierdiscus toxicus* suggests that larger size classes of *C. argus* which feed primarily on benthic herbivores and omnivores would have higher ciguatera scores. This was the case when an extreme outlier was removed from the data set, and there were significant differences

in ciguatera scores between size classes (Fig. 5.3). The occasional outliers tend to mask the tendency for smaller fishes to have lower CTX values. The CTX scores of size classes 1, 2, and 3 were all significantly different from size class 4 (Fig. 5.3). Size class 5 had a small sample size, but a relatively high CTX mean score of 2.25.

CTX by site

Figure 5.4 shows that at each site sampled in Moorea, the mean ciguatera score was either near or above 2, which is on the high side of the marginal ranking. There was no “safe” site where negative (CTX free) *C. argus* could be consistently caught on the sampled reefs in Moorea. All sites had some positive or strongly positive fish, leading to a high probability of CFP at each site (Fig. 5.4).

Site M1 had the lowest ciguatera score mean (1.7), which is in the marginal range. Because of the variability among humans of susceptibility to ciguatera, and the bioaccumulation of CTX, consumption of marginal fish may cause poisoning in some individuals. Sites M2, M5 and M6 all had mean scores above 2.5 and were classified as positive for CTX.

Previous studies have shown that along coastlines or fringing reefs of many islands there were hot spots that consistently produced fish with high levels of CTX (Lewis 2001, 2006, Darius et al. 2007). Reefs adjacent to these hot spots were apparently safe, with a low incidence of ciguatoxic fish, and these hot spots and “clean” areas were predictable throughout time. In cases like these, local fishermen could avoid hot spots and fish at

clean reefs. In the present study, all the sample sites along the fringing reef of the north and southwestern coast of Moorea were considered unsafe.

CTX- Stable Isotope Analysis

Stable isotope analysis (SIA) uses nitrogen and carbon isotopes in animal tissue as tracers, providing information about an animal's diet and trophic level (DeNiro & Epstein 1981, Minagawa & Wada 1984). The highest values of $\delta^{15}\text{N}$ should be found at the highest trophic levels. The carbon isotopic signature of *C. argus* varies depending on their diet and where in the water column they feed. Benthic algae are enriched in $\delta^{13}\text{C}$ compared to planktonic primary producers, and these distinct $\delta^{13}\text{C}$ values are transferred up the food chain, where they can be used to trace where in the water column organisms are feeding. The results of the gut content and SIA showed an ontogenetic feeding shift at 20.5 to 25 cm SL. The SIA data show a spatial feeding shift wherein larger *C. argus* switch their feeding focus to benthic feeding prey items. Such a shift might accompany a positive correlation between $\delta^{13}\text{C}$ and CTX score, where *C. argus* that were enriched in $\delta^{13}\text{C}$, by feeding on benthic prey, would show a higher CTX score. This was not the case in this study, and there was no significant correlation between $\delta^{13}\text{C}$ and CTX score (Fig. 5.5b). SIA data suggested that *C. argus* in the larger size classes fed at a higher trophic level and were enriched in $\delta^{15}\text{N}$. Because of these previous feeding and SIA results, a positive correlation between $\delta^{15}\text{N}$ and CTX levels was expected. Two processes could lead to these results, 1) the bioaccumulation of CTX in fish tissue, and 2) the trophic

feeding shift of larger *C. argus*. However, this was not the case, and there was no significant statistical relationship between the two variables (Fig. 5.5a).

Neither the $\delta^{13}\text{C}$ nor the $\delta^{15}\text{N}$ results correlated with the CTX values for *C. argus* - where correlation might have been expected. This may have resulted from the frequent occurrence and high levels of CTX in the *C. argus* that were tested. Ontogenetic feeding shifts detected from stomach content and stable isotope analysis were coupled with an increase in fish size, yet it is clear that small fish can be highly ciguatoxic. The SIA data trends may not be detectable in the CTX “noise”, leading to no significant differences associated with the feeding shift for SIA data. The length and size class data show that CTX scores can be high for fish of all sizes. It can be concluded that CTX is very common in prey of all sizes eaten by *C. argus*, and CTX can accumulate to dangerous levels in small and large fish.

Hawaii-Moorea comparison

Hawaii and Moorea differed significantly in both the frequency of occurrence of CTX scores and the severity of toxicity scores (Fig. 5.6), as well as the overall mean CTX score. The mean CTX score for the entire Hawaii population sampled was 1.25 (Dierking 2007). This was significantly lower than the mean CTX score for Moorea, which was 2.33. The mean CTX scores for Hawaii and Moorea are on the low and high ends of the marginal ranking respectively (Table 5.2). Forty-seven percent of the *C. argus* population sampled in Moorea had CTX concentrations that were potentially harmful to humans. This is a much higher occurrence than the 18% in Hawaii (Dierking 2007), and

potentially harmful *C. argus* are almost 3 times as common in Moorea as in the Main Hawaiian Islands.

Positive and strongly positive fish were present at all sample sites in Hawaii, but in contrast to Moorea, the mean *C. argus* toxicity in Hawaii differed significantly between sites. This may be due to the lower occurrence of toxic individuals in Hawaii. The mean ciguatera score for all sites tested in the Main Hawaiian Islands, except site KA-W12 in Kona, was below 2, whereas the mean CTX scores for the Moorea sites were generally above 2. Overall CTX levels and incidences of ciguatera are significantly lower in Hawaii (Fig. 5.6) (Lewis 1986, Chateau-Degat et al. 2005, Darius et al. 2007, Dierking 2007).

When the interactions between size class of fish and CTX score were examined between the two regions, several trends appear. First, it is evident that not only were overall CTX scores lower for Hawaii (as discussed above), but when fish were grouped into size classes, all the fish size classes in Hawaii had significantly lower CTX scores than the fish in corresponding size classes in Moorea (Fig.5.7). Figure 5.7 also shows that there are strongly positive outliers present in Hawaii, with CTX scores of 6. Although there were some more strongly positive individuals in Hawaii than in Moorea, these fish did not affect the overall trend of higher CTX scores in Moorea.

Table 5.1. Interpretation of CTX scores from MIA test. Scale for determination and interpretation of CTX scores from MIA test sticks, following Dierking (2007).

Membrane coloration	Score	CTX (ng*g ⁻¹)
None	0	0
Faint	0.5	0.08
Distinct blue	1	0.16
Intense blue	2	0.32

Table 5.2. CTX fish score. Fish score is the result of adding the three tissue scores for each fish, following Dierking (2007). Fish scoring is used to determine relative CTX concentration and judge whether the fish is safe for consumption.

Fish Score Range	Score Class	Interpretation
0 - 0.5	Negative	Safe to eat
1 - 2	Marginal	Risk with frequent consumption
2.5 - 4	Positive	Medical incident possible
4.5 - 6	Strongly positive	Medical incident likely

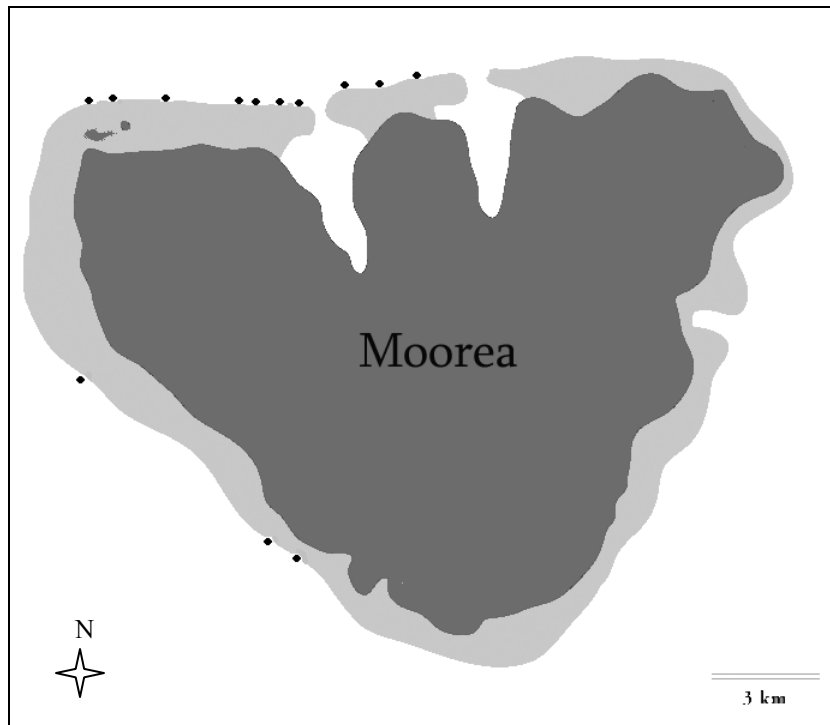


Figure 5.1. Island of Moorea collection sites. *C. argus* were collected from 13 sites, represented by black circles, off the northern and southwestern coasts of the island of Moorea in French Polynesia, at depths from 3 to 30 m.

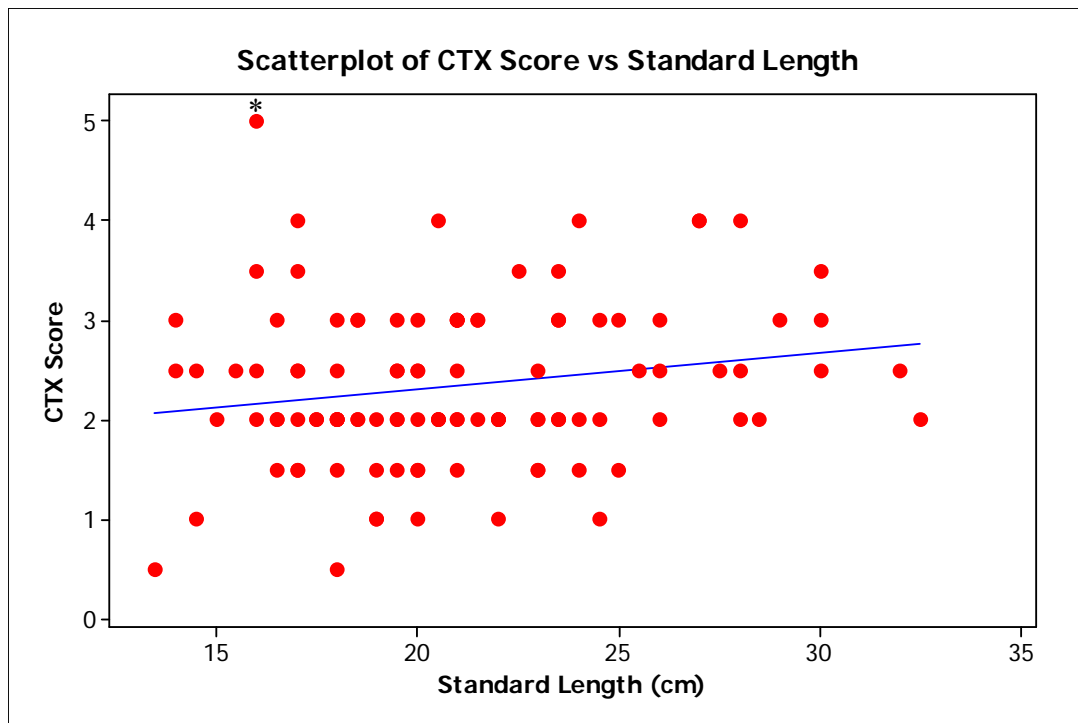


Figure 5.2. CTX score vs. standard length (cm) of *C. argus*. Linear regression, $p=0.045$, $R^2=0.036$. The outlier, a 16-cm fish with a CTX score of 5, is identified by an *.

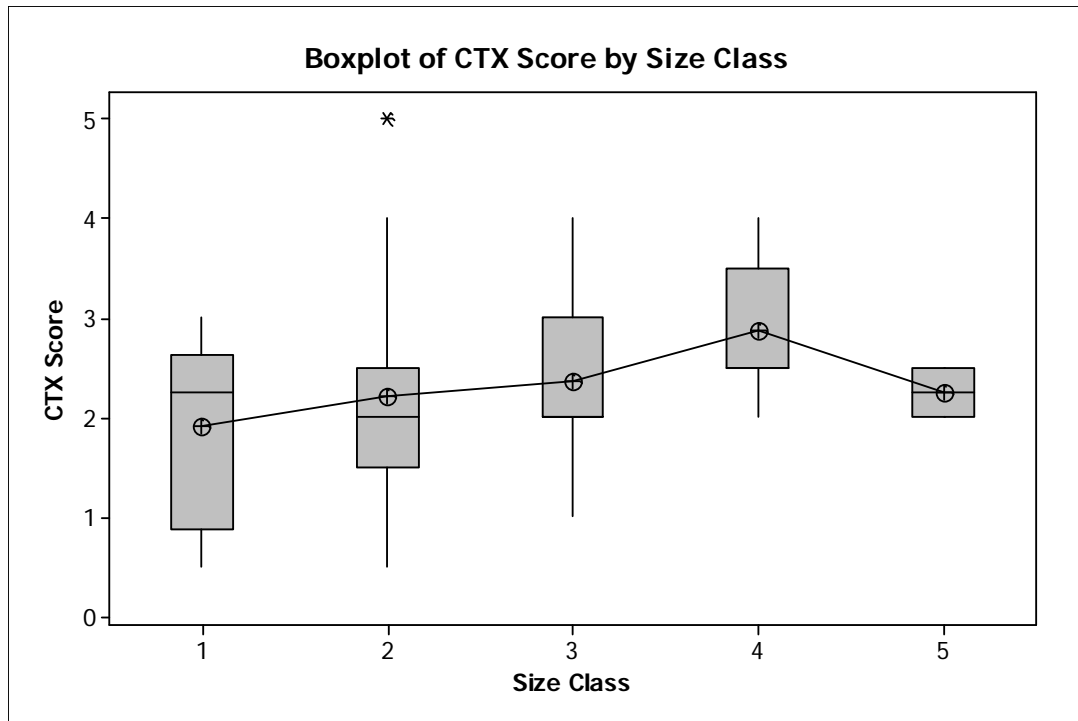


Figure 5.3. Boxplot of CTX score by size class of *C. argus*. Size classes were measured in SL: 1) 10-15cm, 2) 15.5-20cm, 3) 20.5-25cm, 4) 25.5-30cm, and 5) 30.5-35cm. ANOVA with outlier * removed from size class 2, $p=0.049$.

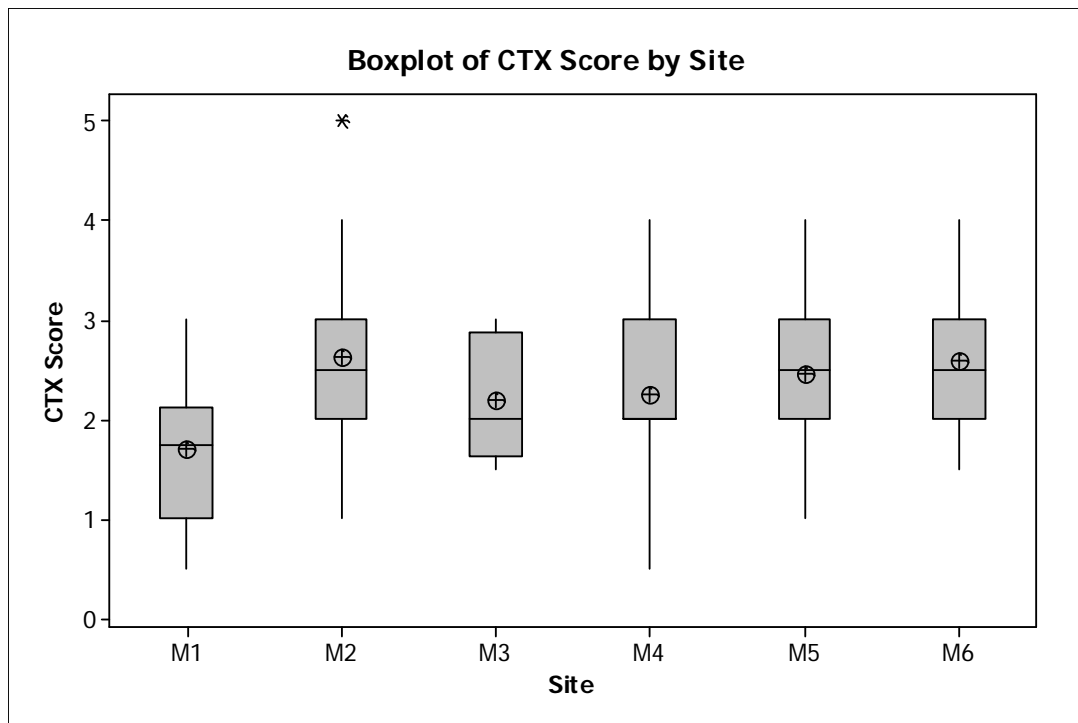


Figure 5.4. Boxplot of CTX score by sample site. Results of ANOVA with outlier * removed from site M2, $p=0.064$.

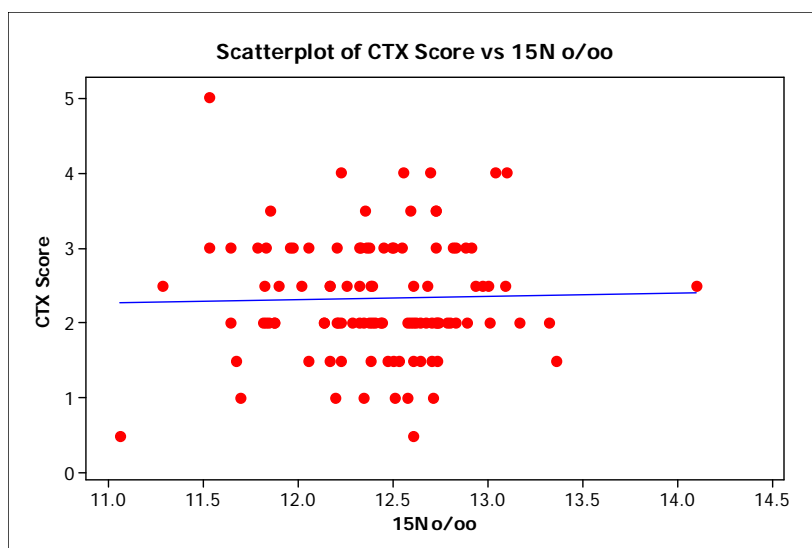


Figure 5.5a. Scatterplot of CTX score vs. $\delta^{15}\text{N}$. Linear regression, $p=0.84$, $R^2=0.001$.

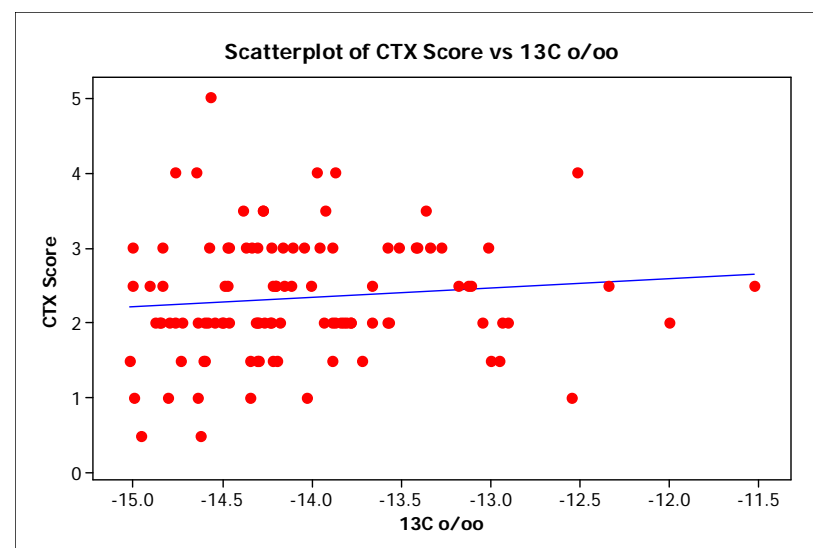


Figure 5.5b. Scatterplot of CTX score vs. $\delta^{13}\text{C}$. Linear regression $p=0.27$, $R^2=0.012$.

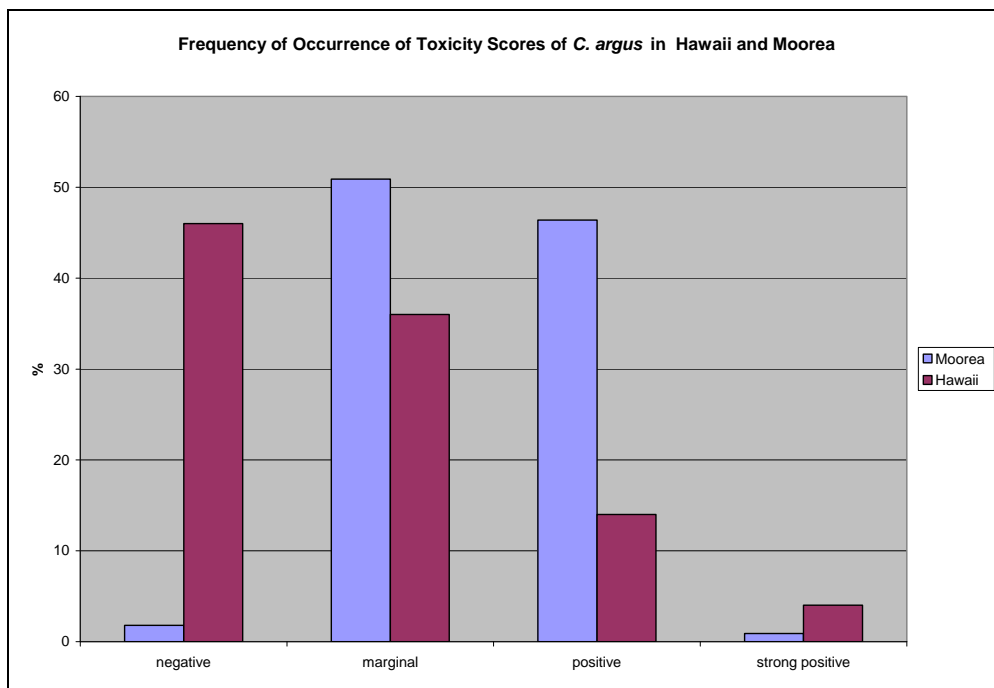


Figure 5.6. Frequency of occurrence of toxicity scores of *C. argus* in Hawaii and Moorea. Toxicity scores are negative (safe for consumption), marginal (may cause ciguatera), positive (CTX present in tissue and will cause ciguatera), and strongly positive (high levels of CTX and will cause ciguatera if consumed).

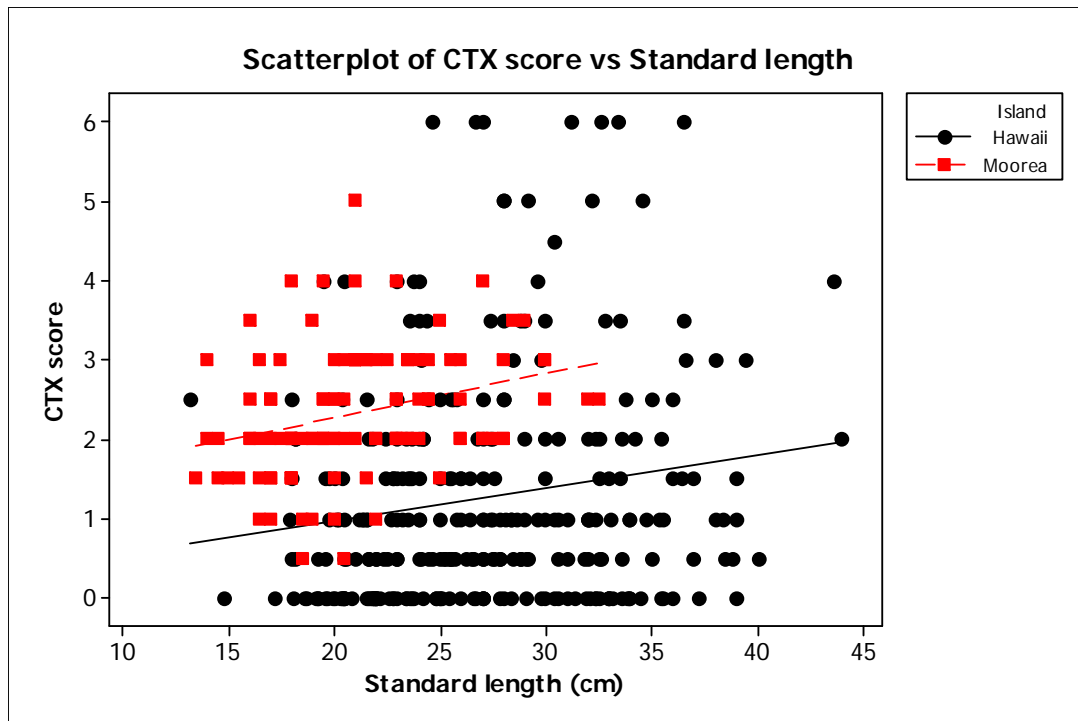


Figure 5.7. Scatterplot of CTX score vs. standard length by region. *C. argus* of a given length in Hawaii have significantly lower CTX scores than *C. argus* of the same size in Moorea, ANCOVA, $p < 0.001$, $F = 72.3$.



Figure 5.8a. Boxplot of CTX score by size class of *C. argus* in Moorea. Size classes were measured in SL: 1) 10-15cm, 2) 15.5-20cm, 3) 20.5-25cm, 4) 25.5-30cm, and 5) 30.5-35cm.

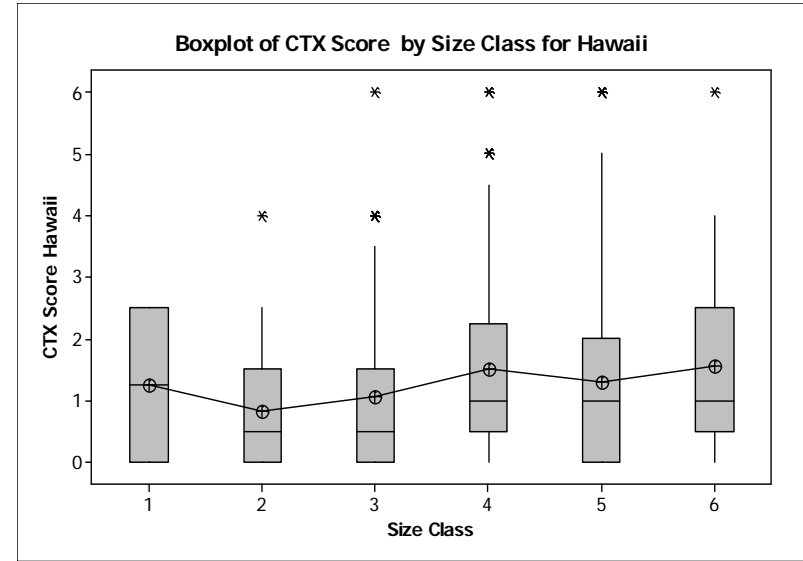


Figure 5.8b. Boxplot of CTX score by size class of *C. argus* in Hawaii (Data from Dierking 2007). Size classes were measured in SL: 1) 10-15cm, 2) 15.5-20cm, 3) 20.5-25cm, 4) 25.5-30cm, and 5) 30.5-35cm.

CTX scores of *C. argus* in a given size class in Hawaii were significantly lower than CTX scores of *C. argus* in the same size class in Moorea, ANCOVA, $p < 0.001$, $F = 73.5$.

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