EFFECTS OF THE INTRODUCED PREDATORY FISH *Cephalopholis argus* ON NATIVE REEF FISH POPULATIONS IN HAWAII

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Signature page

I dedicate this dissertation to my parents, Barbara and Jürgen Dierking.
Thank you for supporting me in all my endeavors!

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ABSTRACT

The grouper species roi (*Cephalopholis argus*) was introduced to Hawaii in 1956 to create a new fishery. It has become the dominant predator on coral reefs in the Main Hawaiian Islands, but the envisioned fishery failed due to concerns about ciguatera fish poisoning from roi consumption. This situation raises resource management and ecological questions, including (1) How much prey does the alien species roi consume, and what are the ecological effects on native species? and (2) Is roi as ciguatoxic as its reputation suggests, and could better understanding of factors associated with toxicity lead to a safe fishery for roi?

My dissertation addresses these questions, based on gastric evacuation rate experiments with roi, stomach content analysis, assays of ciguatoxin concentrations in roi tissue, and on the analysis of benthic habitat maps and underwater visual censuses of roi and prey fish densities.

Regarding (1), the estimated roi population size in 7.8 km^2 of reef habitat along the West coast of Hawaii Island was 56,290 individuals (biomass 34 metric tons (t)), with an annual prey consumption of 93.7 t of fish (equivalent to 8.2 million individuals) and 5.5 t of crustaceans. Consumption equaled 11.2% of the reef fish community standing stock biomass, and was focused on early life history stages. Compared to the aquarium fish industry, the most important fishery targeting small reef fish in Hawaii, roi consumption was focused on a wider variety of fishes and on smaller individuals (mean weight 11.4 g versus 25 g). It removed fewer individuals (73% of industry take) of yellow tang (*Zebrasoma flavescens*), the main species collected by the industry, but

removed 24 times more fish overall. The large scale of consumption suggested that roi may play a substantial role in shaping native reef fish communities.

Regarding (2), ciguatoxin concentrations in 18.2% of roi exceeded acceptable levels for human consumption. Toxicity was highly variable on all analyzed geographic scales, very weakly correlated with roi size, and not correlated with fitness and length-at-age of individuals. I concluded that a safe fishery for roi was not feasible in Hawaii due to poor predictability of toxicity.

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CHAPTER 1:

GENERAL INTRODUCTION

The subject of this dissertation is the predatory coral reef fish roi (*Cephalopholis argus* Bloch & Schneider, 1801) in the main Hawaiian Islands (MHI). Roi does not naturally occur in the MHI, but in 1956 and 1961, 2385 roi individuals from Moorea in French Polynesia were released along the South and West coast of the island of Oahu, and the West ("Kona") coast of the island of Hawaii, as part of a species introduction program to create new fisheries (Randall 1987). Roi became established and has become the most dominant nearshore reef fish predator in the MHI (Hawaii Division of Aquatic Resources (DAR) unpublished data). However, despite its abundance, a commercial fishery for roi never succeeded after it turned out to be more prone to cause ciguatera fish poisoning incidents in humans than other reef fishes in Hawaii (Afton 2003), and was henceforth rejected by consumers (Earle 2005).

This situation raises important resource management related questions, including:

(1) What are the ecological effects of roi predation on native species? and

(2) Could better understanding of ciguatera in roi lead to a safe fishery? (e.g., if roi individuals from some areas or of a certain size were predictably safe for consumption)

An answer to (1) would allow resource managers to address potential impacts of roi on native species in resource management plans for reefs in Hawaii. Regarding (2), if a fishery could be established in the future, it would offer economic and recreational benefits, but may also have the potential to control roi abundance, as groupers are

generally vulnerable to overfishing due to their life history characteristics (Morris et al. 2000). Standing in the way of satisfactory answers to the above questions has been the scarcity of published information on the subject. My doctoral research addresses the most prevalent knowledge gaps about roi in Hawaii. I present the results of this work in Chapters 2, 3 and 4, before returning to Questions (1) and (2) in the Summary and Conclusions section, Chapter 5.

Specifically, in Chapter 2, I address the feeding biology and ecology of roi. The first section of the chapter contains a thorough characterization of the feeding habits, including the diet composition and ontogenetic patterns, of roi. This section of the chapter is interesting in its own right because few previous studies have analyzed the feeding biology of groupers in sufficient detail to accurately characterize the full breadth of diet (Parrish 1987, Ferry and Cailliet 1996, St. John and Russ 2001). In addition, diet composition is an essential component of estimates of prey consumption by fish populations (see Bromley 1994).

The second part of Chapter 2 concentrates on the estimation of the prey consumption by roi populations along the Kona coast of the island of Hawaii. The estimate provided here is one of a small number of quantitative assessments of consumption of reef fish predators published to date (Bromley 1994), and only the second detailed discussion of consumption by populations of grouper species after Beukers-Stewart and Jones (2004) work on the consumption by populations of the grouper species *Cephalopholis cyanostigma* and *Cephalopholis boenak* on reefs at Lizard Island, Australia.

For the estimation of roi and prey fish population sizes, as well as the calculation of available reef habitat along the Kona coast of the Big Island, I collaborated with Ivor D. Williams, Charles Birkeland, William J. Walsh and Kostantinos A. Stamoulis of the DAR Division in Kona. The content of Chapter 2 will be submitted for publication in slightly modified form by myself (as first author) and my collaborators.

Next, in Chapter 3, I focus on ciguatera in roi in Hawaii. Ciguatera is a neurological disease with severe clinical symptoms in humans (Bagnis 1968, Palafox and Buenconsejo-Lum 2001). It is caused by ingestion of ciguatoxin, a natural polyether toxin that is produced by the benthic dinoflagellate *Gambierdiscus toxicus* (Yasumoto et al. 1980). Ciguatoxin enters the marine foodchain when herbivores consume benthic macroalgae with attached *G. toxicus* (Campbell et al. 1987). The lipid soluble ciguatoxin can be incorporated into fatty tissue of animal consumers, and is passed on to higher trophic levels when predators such as roi feed on toxic herbivores (Banner 1974).

Ciguatera appears to be the main issue standing in the way of a commercial fishery for roi in Hawaii. This is indicated by the economic value of roi in several locations to which it is native, and in which it is not a known ciguatera offender (Morris et al. 2000). E.g., roi obtains a wholesale price of up to $20/kg in the life reef fish trade in Hong Kong (Lee and Sadovy 1998). Consequently, market demand for non-toxic roi would likely exist in Hawaii. A safe roi fishery to address this demand would be feasible if certain roi, e.g., from reefs around certain islands, along certain coastlines, or certain size classes, were consistently below acceptable toxicity levels for safe consumption.

Chapter 3 contains the results of a large-scale screening of 291 roi from 17 sites around the islands of Oahu and Hawaii for toxicity, and analyzes processes that make individual fish toxic. It closes by addressing the question whether a safe fishery for roi, if limited in scope, is feasible. In addition to its relevance for resource management, Chapter 3 has the potential to add to our understanding of the biology of ciguatera. When seen in the light of high site fidelity of roi (Amanda Meyer, unpublished data), patterns of toxicity in roi may reveal underlying patterns of the distribution of *G. toxicus*, which are difficult to sample directly (Hokama et al. 1996) and incompletely understood to date (Lehane and Lewis 2000). I have submitted Chapter 3 for publication to the journal "Pacific Science", with myself as sole author.

Chapter 4 maintains the focus on ciguatera. It addresses the question whether ciguatoxin has negative physiological effects on roi individuals in the wild. All previous studies of the effects of ciguatoxin on marine fishes were based on tank or enclosure experiments (see reviews by Brusle 1995, Lehane 2000). In addition, in experiments that found negative effects, including erratic swimming, inactivity, loss of appetite, and death, unnaturally high concentrations of ciguatoxin were commonly used (reviewed by Landsberg 2002). Other researchers found little or no effect of ciguatoxin on reef fishes, and have proposed that marine fish possess evolutionary adaptations that allow them to sequester or inactivate ciguatoxin molecules (e.g., Hahn et al. 1992). The question of whether fish in wild populations are negatively affected by ciguatoxin has thus been intensively discussed, but has not been resolved to the present (Lewis 1992, Landsberg 2002). The systematic analysis of the effects of ciguatoxin on roi in the wild in Chapter 4

is the first field study on the subject that I know of, and therefore adds much needed scientific information to the ongoing discussion. I have submitted this chapter for publication to the journal “Fish biology”, with myself as sole author.

CHAPTER 2:

ECOLOGICAL IMPACTS OF PREY CONSUMPTION BY POPULATIONS OF THE INTRODUCED GROUPER *Cephalopholis argus* IN HAWAII

Manuscript

In preparation for publication.

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ABSTRACT

The non-native grouper species roi (*Cephalopholis argus*) has become the most abundant nearshore predatory reef fish in the Main Hawaiian Islands (MHI) since its introduction in 1956. Our goal was to determine the magnitude, focus, and ecological impact of predation by roi on native reef fishes. For this purpose, we calculated the annual prey consumption by roi populations in 63 km^2 of nearshore habitat along the west coast of the island of Hawaii, and compared it with the standing stock of prey and the take by the aquarium fish industry in this area. The calculation was based on the diet composition, daily ration, and population size of roi, which we determined from stomach content analysis of 286 roi collected at 17 sites in the MHI in 2003, a gastric evacuation rate experiment with 16 live roi in outdoor tanks, and analysis of benthic habitat maps and underwater visual censuses of roi and prey fish densities at 23 sites along the West coast of Hawaii.

Census data availability was best for reef habitat in the depth zone 10-17 m, which covered 7.8 km^2 in the area of interest. The roi population size in this area was 56,290 individuals with a total biomass of 34 metric tons (t). At a mean daily ration of 0.8% of own body weight, annual prey consumption by roi was 8.2 million reef fishes with a total biomass of 93.7 t, and 5.5 t of crustaceans. This was equivalent to 11.2% of total fish community standing stock biomass.

Consumption was characterized by (1) the major importance of fish prey relative to crustacean prey, (2) the large diversity of fish prey (16 families and 24 species identified from 286 roi samples), but at the same time, dietary importance of a limited number of families (> 85% of the diet consisted of 5 families), (3) the disproportionate

dietary importance of some families relative to their abundance in the wild, and (4) the small average size of fish in the diet relative to fish in the wild (mean weight 11.4 g versus 73.5 g).

Relative to take by the aquarium fish industry, roi consumption in areas open to aquarium fish collections removed larger numbers of fish (24 times the industry take), but was focused on smaller individuals (11.4 g versus 25 g). Roi consumption of yellow tang (*Zebrasoma flavescens*), the main species taken by the industry, was lower than industry take (33% of take by biomass and 73% by number), and again focused on smaller sized specimens.

Our results suggest that predation by roi is likely to be an important factor shaping reef fish communities, and a particularly important source of mortality for the early life history stages of prey fish populations, and raise the question how prey communities can be resilient to such large-scale predation pressure.

INTRODUCTION

Fishes of the grouper family (Serranidae) are among the most common predators on coral reefs worldwide (Parrish 1987), and play an important role in shaping reef fish communities (e.g., Goeden 1982, Macpherson et al. 1997, Hixon and Jones 2005, Mumby et al. 2006). Groupers support important commercial, artisanal and recreational fisheries over much of the tropics and subtropics, and are among the most commercially valuable species on coral reefs (Heemstra and Randall 1993). Due to their life history characteristics and high market value, e.g., in the live reef fish trade (Lee and Sadovy 1998), many grouper populations are today overfished or depleted (Morris et al. 2000).

In contrast to the worldwide pattern, the Main Hawaiian Islands (MHI) have only two native grouper species, both of which occur at low densities. The divergent pattern is due to the extreme geographic isolation of the islands, which has resulted in a reef fish community characterized by the lack or limited presence of fish families with short to medium larval durations and late maturity, which includes the groupers (Randall 1987, 1996). In the 1950s, when potential dangers of species introductions where not yet fully understood, the State of Hawaii initiated a reef fish introduction program intended to create new fisheries, in the course of which four snapper (Lutjanidae) and six grouper species, as well as two species of the emperor family Lethrinidae, were released on reefs in the MHI (Randall 1987). Only the grouper species roi (*Cephalopholis argus*) and two snapper species became established (Eldredge 1994).

In total, 2385 roi individuals were introduced to the MHI from Moorea in 1956 and 1961 (Oda and Parrish 1981). After a time lag of limited population growth, as indicated by reports from the 1970s and 1980s that considered it to be a rare species (e.g., Hobson 1974, Randall 1987), roi increased strongly in abundance and became the dominant resident predator on many nearshore reefs in Hawaii (Hoover 1993). Between 1999 and 2005, roi populations along the west coast of the Island of Hawaii (referred to as Kona coast hereafter) (see Figure 2.1, insert B) grew by another 22% (our data). Despite the abundance of roi, and the fact that the species is economically valuable in many of its native habitats (Heemstra and Randall 1993), the envisioned fishery for it did not develop in Hawaii because roi turned out to be prone to accumulate ciguatoxin, a marine toxin causing ciguatera fish poisoning in humans, and was therefore rejected by consumers (Bruno and Effler 2001b, Chapter 3 of this dissertation). In contrast to

originally envisioned benefits of the introduction, roi abundance has led to concerns about potential negative effects of increased predation pressure on native reef fishes and existing fisheries (Earle 2005). This situation is the direct opposite of the more common pattern worldwide, where fishing-induced declines of native groupers including roi, even up to local extinctions, have led to concerns about destabilization of reef fish communities from a decrease in predation (Chiappone et al. 2000, Morris et al. 2000, Jerry Davis, personal communication). The unifying factor is that strong changes in predation pressure could have top-down cascading effects on the reef system in both cases (Hixon 1997, Mumby et al. 2006). Consequently, assessments of prey consumption by grouper populations are important for our understanding of reef ecosystems both in Hawaii and elsewhere, and for the successful management of these systems.

The relevance of estimates of annual prey consumption by predator populations is well understood (Cortes 1996), which is mirrored in their availability for important freshwater (Bromley 1994, Jensen et al. 2006), and marine pelagic and bottom-fish predators (e.g., Overholtz et al. 2000, Olson and Galvan-Magana 2002). However, due to a historical lack of information on population sizes and daily rations of reef fishes (Levin 1992, Nasby-Lucas et al. 2002), studies of consumption by populations of coral reef fishes have been rare (Bromley 1994). In particular, we know of few estimates of grouper population sizes across spatial scales larger than transects or patch reefs (Zeller and Russ 2000, Friedlander and DeMartini 2002), and of only one published study that determined the daily ration and population consumption of a wild grouper population (Beukers-Stewart and Jones 2004). No estimates of the population size and prey consumption of roi in Hawaii or elsewhere existed prior to our study.

Considering the lack of population consumption estimates for groupers, and the importance of such information for general reef fish ecology and for the assessment of the role of the introduced species roi in Hawaii, our goal was to estimate population consumption by the grouper species roi in Hawaii, and to compare consumption with the population sizes of native reef fishes and the take by the aquarium fish industry (AFI), which is the main fishery targeting small reef fishes in Hawaii and the only nearshore fishery for which sufficient data for the analysis were available. For this purpose, we calculated (1) the population sizes of roi and prey fishes, (2) the daily ration of individual roi, and (3) the prey composition of roi, for an area spanning 2/3^{rds} of the Kona coast. Regarding (1), we benefited from recent advances in benthic habitat mapping (e.g., Chauvaud et al. 1998, Stanbury and Starr 1999, Monaco et al. 2001), which allowed us to integrate coral reef fish density and large-scale habitat information following the approach by Friedlander and Brown (2003).

This study is one of few studies that integrate all steps of a population consumption estimate without borrowing model parameters from other taxa, and only the second study to provide detailed information of quantitative population level consumption of a coral reef fish predator of the grouper family (Beukers-Stewart and Jones 2004). This information is particularly timely and relevant considering the current shift in fisheries management from species level to multi-species and ecosystem level management (Pauly et al. 2002), which in contrast to traditional individual based models require data on the food habits, daily ration, and total consumption of the major predators in the ecosystem (Olson and Galvan-Magana 2002, Pauly and Christensen 2002).

MATERIALS AND METHODS

Study organism and area of interest

Roi (*Cephalopholis argus*) naturally occurs throughout most of the Indo-Pacific. Hawaii is the only location where it is an introduced, alien species. To our knowledge, this is the only documented case of the successful establishment of a grouper species in a non-native habitat anywhere in the world. Roi is particularly abundant along the Kona coast of the Big Island. Understanding the magnitude of prey consumption by roi is of general ecological interest because consumption estimates for predatory coral reef fishes including groupers are rare. This information is of additional local importance for resource management in Hawaii, because, depending on the magnitude of effects on native species, roi may have to be included in management considerations, particularly in the light of potential overlap of roi consumption with the take by the AFI and other fisheries, which have caused declines of reef fishes in Hawaii (Friedlander and DeMartini 2002, Tissot and Hallacher 2003).

The 167 km long Kona coast (Figure 2.1, insert B) harbors some of the least disturbed reefs in the MHI. Dive tourism and the AFI along this coast generate significant economic revenues (Cesar et al. 2002). At the same time, the Kona coast has been the focus of intensive resource management efforts to sustain reef fish populations, in particular, addressing concerns about overharvesting of reef fishes by the AFI. Since 2000, when existing protected areas where supplemented by 9 Fish Replenishment Areas (FRAs) closed to take of reef fishes by the AFI, a total of 35.2% of the reef area along the Kona coast has been protected from aquarium collections (Tissot et al. 2004).

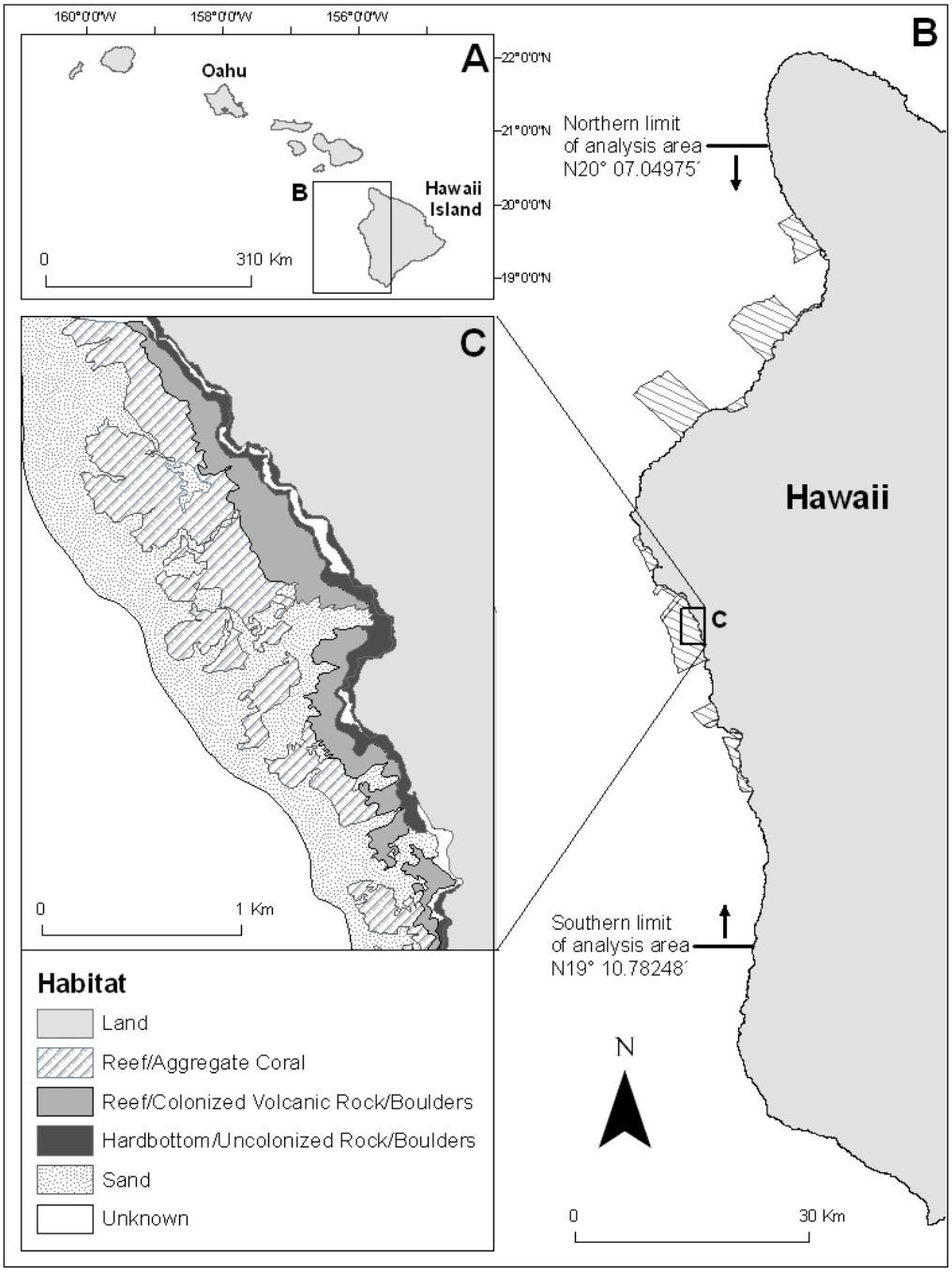

Figure 2.1. Insert A: Map of the Main Hawaiian Islands. Roi sampling took place on Oahu and along the Kona coast of Hawaii Island. Insert B: The Kona coast with the analysis area of the roi population consumption estimate. Areas closed to aquarium collections are marked by cross-hatching. Insert C: Example of the information available from NOAA benthic habitat maps. We used similar maps that incorporated bathymetric data not included here in ArcGIS 9.0 to quantify the spatial extent of habitats in the analysis area. WHAP surveys were done in the habitat types "Reef/Aggregate Coral" and "Reef/Colonized Volcanic Rock/Boulders", which represent the majority of suitable habitat for roi (see insert C).

Fish densities in FRAs and areas open to collections have been surveyed 4 – 6 times a year at 23 sites along the Kona coast since 1999. The survey program was conducted initially by the West Hawaii Aquarium Project (WHAP), a collaboration between Hawaii's Division of Aquatic Resources (HDAR), the University of Hawaii at Hilo and the University of Washington. Since 2005 the program has been run by the HDAR.

The availability of longterm fish monitoring, habitat distribution, and fisheries take data from the HDAR's commercial catch reporting program (aquarium collectors are required to file monthly catch reports), make the Kona coast ideal for a population level consumption study, even more so when considering the scarcity or lack of such data for most other reef locations in Hawaii and worldwide (e.g., Russ 1991). We limited our analysis to the area between N 20º 07.050' and N 19º 10.782', which has been intensively surveyed, and coincides with aquarium fish collection reporting zones.

Roi sampling

We sampled 286 roi from the Kona coast (n = 180, 11 sites) and Oahu (n = 100, 6 sites) by spearfishing on scuba in July and August 2003. Collection dives took place between 9:24 am and 3:22 pm. We attempted to capture each roi encountered during a dive, regardless of size or behavior (e.g., active swimming or resting). As divers perceived success rates of capture after sighting as equal for all roi, our sample was approximately representative of the size and stomach fullness distribution of the sampled roi population. We immediately sealed speared roi underwater in plastic bags to avoid loss of stomach contents from regurgitation after capture, which has been commonly observed in previous grouper studies (e.g., Renones et al. 2002).

We recorded standard length (SL) and total length (TL) of roi to the nearest mm, and wet weight (W) of Oahu roi to the nearest 5 g. Due to scale malfunctioning during Hawaii Island fieldwork, we estimated the W (in g) of Hawaii individuals from their TL (in cm), using the L-W equation described in Chapter 3 of this dissertation for roi in Hawaii:

$$W = 0.013 * TL^{3.122} \quad (1)$$

Stomach content analysis

We analyzed the stomach contents of all roi samples following Hyslop (1980). For the overall sample, we determined the vacuity rate, i.e., the proportion of empty stomachs relative to the total number of stomachs. For full stomachs, we determined the SL and TL of fish prey, and carapace length of crustacean prey to the nearest mm where digestion state allowed reliable measurements. For fishes, we were able to determine SL more often than TL due to the low digestibility of skeletons. In these cases, we estimated TL from SL based on the TL to SL ratio in less digested specimens of the same fish family. We determined the W (to the nearest mg) of all prey specimens, classified their digestion status based on a 5-step scale, and estimated their undigested W using Table 2.1.

We identified prey items to the lowest possible taxonomic level, using Randall's (1996) and Hoover's (1993) keys, and used cumulative prey curves (Ferry and Cailliet 1996) to assess the sufficiency of sample sizes for an accurate characterization of the diet. These curves reach an asymptote as the sample size becomes sufficient to describe the entire breadth of the diet.

Table 2.1. Prey digestion status classification, with the average proportion of undigested prey weight remaining at each status.

Digestion status	Description	Undigested prey W remaining (proportion)
1	Undigested to slightly digested; skin intact, coloration mostly intact, shape not distorted, flesh not digested, fin spines present	95%
2	Little digested; skin mostly intact, discoloration possible, signs of tissue digestion in head and tail region, fin spines present	85%
3	Medium digested; skin partly gone, tissue partly digested, head and tail often heavily digested, body shape still recognizable, fin spines often present	70%
4	Well digested; no skin, tissue heavily digested, bones/skeleton partly visible; fin spines often gone	50%
5	Strongly digested; body shape unrecognizable, mucus-tissue mix; sometimes only skeletons left	10%

To determine the dietary importance of prey taxa, we calculated gravimetric importance of each taxon (%W) as percentage of the overall weight of all stomach contents consisting of the taxon, numerical importance (%N) as percentage of the total count of all prey items consisting of the taxon, frequency of occurrence (%O) as the percentage of roi stomachs that contained the taxon, and the index of relative importance (%IRI) as the product of the frequency of occurrence and the sum of gravimetric importance and numerical importance. The %IRI is a compound index, and may provide a more accurate description of dietary importance than any of its three individual components alone by canceling out individual biases in these components (Cortes 1996).

Because all four indices indicated that fish prey dominated the diet of roi (see Results section), we recalculated them with only fish prey included in the analysis, and concentrated further dietary analyses on fish. Using the recalculated %N, we determined whether the numeric importance of the 7 most important fish families in roi diet reflected

their numerical importance in reef habitat in the wild (using survey data for 2003). To assess whether roi preferentially consumed certain prey sizes, we compared the mean W and the length-frequency distributions of the 7 most important fish families in roi diet (by %IRI) and in the wild using 2-sample Kolmogorov-Smirnov tests (Siegel and Castellan 1988). We excluded the Priacanthidae, which were abundant in roi stomachs, but did not occur in underwater survey counts commonly enough for the analysis.

To assess ontogenetic feeding patterns (i.e., changes in diet with increasing roi size) of Kona roi, we used simple linear regressions of the TL of prey, the relative length of prey (TL of prey divided by TL of roi), the number of prey items per stomach, and the relative stomach content W (total initial prey W per W of roi) on the TL of roi, and compared stomach vacuity rates between roi of different sizes using a chi-square test with the grouping categories small (< 25cm TL), medium (25 cm – 30 cm TL), medium-large (> 30 – 35cm TL) and large (> 35cm TL). Sample sizes for the analyses varied, because prey length could not be determined for highly digested prey specimens.

Daily ration of roi (C_{24})

We estimated daily ration (C_{24}, defined here as the amount of food consumed per day) of roi in Hawaii using Eggers' modification (1977, 1979) of the Bajkov method (1935), which Cortes (1996) considered one of the most suitable methods for analysis of daily ration in larger predators. Specifically,

$$C_{24} = 24 * R * \bar{S} \qquad (2)$$

where R is the hourly gastric evacuation rate (defined here as the proportion of stomach contents ejected from the stomach per hour), $\bar{S}$ is the mean mass of stomach contents over the 24 hour day (in W of prey per W of roi), and 24 is the number of hours in the day. The Eggers model assumes that gastric evacuation is exponential. We determined best, high and low estimates of C_{24} by using the mean value and upper (mean + S.E.) and lower (mean – S.E.) limits of the parameters R and $\bar{S}$ in Equation 2. The calculation of high and low estimates provides a measure of variability, but does not represent a formal statistical approach, due to the deterministic nature of models in the estimation of daily ration (Cortes 1996). As neither $\bar{S}$ nor R changed significantly with roi size (see Results section), C_{24} applied to roi of all sizes.

Components of the daily ration model

AVERAGE DAILY STOMACH CONTENT WEIGHT $\bar{S}$: We calculated $\bar{S}$ as weighted average of daytime and nighttime stomach content W ($\bar{S}_{\text{Day}}$ and $\bar{S}_{\text{Night}}$, respectively) of Kona roi. The average duration of daytime (defined here as the period from sunrise to sunset plus one hour to account for feeding in the crepuscular period) and nighttime (period of the full 24 hour day minus the duration of the daytime period), in Hawaii is 13h and 11h, respectively (Juvik and Juvik 1998).

We determined $\bar{S}_{\text{Day}}$ from the average W of prey per W of roi. To assess the validity of extrapolating from samples collected during a limited time period to the entire daytime period, we analyzed variation in stomach contents over the day based on Oahu and Hawaii samples grouped by time of sample collection (before 10am; 10am – 1pm;

after 1pm). We used ANOVA to test for differences in average stomach W, and a chi-square test to test for differences in the frequency of occurrence of empty versus full stomachs (Cortes 1996). Because both tests indicated the lack of significant differences of stomach contents between time periods (see Results section), we considered stomach fullness as constant over the daytime period.

From $\bar{S}_{\text{Day}}$ we extrapolated to nighttime hours using Equation 6, under the assumption that roi did not feed during nighttime. This is supported (1) by Hobson's finding (1974) that roi feeding in Hawaii is exclusively diurnal, (2) by the large importance of fish prey and minor importance of crustacean prey in roi diet in Hawaii (Table 2.3) which is characteristic of a daytime feeding pattern for groupers (Harmelin-Vivien and Bouchon 1976, Parrish 1987), and (3) by acoustic tracking of roi in Hawaii, which showed very little nocturnal movement by roi (Amanda Meyer, unpublished data). We used regression analysis to assess whether average stomach content W was associated with roi size (see section "Diet composition of roi" above).

GASTRIC EVACUATION RATE R: The choice of a suitable model of gastric evacuation, and the subsequent determination of R, are important for the calculation of C_{24}, as small changes in R can result in large changes of the C_{24} estimate. Following Bromley (1994), we experimentally determined R by tracing the proportion of initial stomach contents remaining in the stomachs of live fish after increasing time intervals.

For this purpose, in June 2004 we obtained 15 live roi (mean TL 28.0 cm, range 23 cm – 38.0 cm) from sites in Kaneohe Bay, Oahu, using monofilament barrier nets

(13 x 2 m, mesh size 5 cm) with two-diver teams on scuba. We placed captured roi individually in 1 m^3 circular outdoor tanks (solid plastic bottom and sides, open top) equipped with flow-through seawater systems (4 l/minute) and aeration stones at facilities of the Hawaii Institute of Marine Biology (HIMB), Coconut Island, Oahu. We placed open-ended PVC pipes (diameter 12.5 cm, length 40 cm) on the bottom of each tank as cover for roi. Tanks were protected from UV-light and elevated temperatures by shade netting. Water temperature in the tanks was 27.7 ± 0.56 ºC (mean ± 1 SD) over the 34-day holding period, and 26.9 ºC on the day of the gastric evacuation experiment, which is within the range of water temperatures observed on reefs in Hawaii (Juvik and Juvik 1998).

Subsequently, the handling of fish and the procedure to determine the gastric evacuation rate followed Elliott (1972, 1991), with modifications to account for the biology of roi. We accustomed roi to feeding by offering food at two-day intervals. By the end of the first week in captivity, all individuals were accepting food. For the rest of the holding period, all fish maintained regular feeding and showed no sign of disease or abnormal behavior. Food over the first 3 weeks consisted of small pieces of tilapia filet, and thereafter of whole palenose parrotfish (*Scarus psittacus*) that were collected on patch reefs in Kaneohe Bay and killed immediately prior to feeding. This species is one of the most common species in the diet of roi on reefs in Hawaii (Table 2.3).

Prior to the onset of the gastric evacuation experiment, we deprived all roi of food for 96 h to ensure that stomachs were empty. We then fed each roi individual a meal consisting of 1 dead freshly caught palenose parrotfish, based on the consideration that the majority of roi in Hawaii had only a single prey item in their stomachs (76% of all

full stomachs analyzed). The meal size was 0.06 g ± 0.019 g per g of roi (absolute wet weight after blotting with tissue paper 20.58 g ± 8.66 g) (mean ± 1 SD), which is within the range of weight of prey per weight of roi found in the wild (Figure 2.3). After individuals had ingested the meal, we left them undisturbed for pre-determined randomly assigned intervals ranging from 1 h to 72 h. At the end of the digestion periods, we killed individuals with an overdose of MS-222 (Ohr 1976), and immediately extracted and re-weighed the remainders of the meals to determine the percentage of initial stomach contents (in terms of wet W) remaining. In addition, we classified the digestion status of items based on a 5-step scale of external characteristics (Table 2.1, column "Description"), and added the column "% of initial undigested W remaining" to the table based on the average proportion of W remaining at each digestion status.

We then calculated R as follows: first, we plotted the proportion of initial stomach contents remaining versus the time passed in the stomachs after ingestion. Following Olson & Mullen (1986), we excluded feeding trials that resulted in complete gastric evacuation from the plot, because it is unclear when full evacuation was reached in these samples (Figure 2.7). Next, we assessed the suitability of a linear and an exponential regression model, which are the two models most commonly used in gastric evacuation studies (Bromley 1994), to describe the gastric evacuation data, and determined R directly from the equation of the more suitable model.

The linear model has the general form $Y_x = -RX + a$, and the exponential model has the general form $Y_x = a \exp(-RX)$, where Y_x is the proportion (in %) of the initial stomach content W remaining after time X, the constant R is the rate of gastric

evacuation (proportion of stomach contents ejected from the stomach per hour), X is the number of hours after the consumption of the initial meal, and the constant a is the amount of prey predicted by the model at the onset of gastric evacuation (ideally 100%) (Elliott 1972). Following Cortes (1996), we evaluated the models based on their adjusted r^2 values, and on their fit to the data based on the significance of the fit and on graphic and formal residual analysis assessing normality and homoscedacity (Ruggerone 1989).

Roi and prey fish population size estimate

Because coral reefs are characterized by large habitat variability, and densities of reef fishes in part depend on habitat type (Williams 1991, Connell and Kingsford 1998, Friedlander and Parrish 1998, Sluka et al. 2000), methods commonly used in pelagic and bottomfish stock assessments, such as Virtual Population Analysis (VPA) and mark-recapture techniques, have limited applicability in the reef setting (Cappo and Brown 1996, Zeller and Russ 2000). Following Nasby-Lucas (2002) and Friedlander and Brown (2003), we therefore used an alternative approach and determined population sizes of fish taxa of interest by coupling the spatial extent of different habitat types with the fish density in these habitats, using the equation

$$N_{\text{Area of interest}} = \sum_{i=1}^{n} \sum_{k=1}^{m} (D_{ik} * A_{ik}) \qquad (3)$$

where $N_{\text{Area of interest}}$ is the total abundance of a taxon in an area of interest, D_{ik} is the average density of the taxon in habitat type i within depth-zone k (in individuals per ha), A_{ik} is the total area size of habitat type i within depth-zone k (in ha), and n and m are the

number of different habitat types and depth zones occurring in the area of interest, respectively. In addition to the population size in terms of the number of individuals, we also determined the population biomass from the sum of Ws of all individuals, as calculated from their TLs using Equation 1 for roi, and L-W equations for prey fishes taken either from FishBase (Froese and Pauly 2006) or unpublished records of the Hawaii Cooperative Fisheries Research Unit at the University of Hawaii at Manoa.

Quantification of the spatial extent of habitats

We determined the spatial extent of the 16 most important habitat types in the depth zones 0 – 5 m, 5 – 10 m, 10 – 17 m, 17 – 23 m, and 23 – 42 m (Table 2.2) in the area of interest (Figure 2.1, insert B) using ArcGIS 9.0. We calculated sub-estimates for zones open and for zones closed to aquarium collections, which add up to the total estimate for the analysis area. Habitat categories and calculations were based on NOAA benthic habitat maps of the Kona coast (Monaco et al. 2001, Coyne et al. 2003) (see Figure 2.1, insert C, for example), and depth calculations on Light Detection and Ranging Technology (LIDAR) bathymetry data (Irish et al. 2000). We derived the habitat extent per depth zone by converting both the NOAA habitat map polygon shapefile and the LIDAR point shapefile into raster surfaces with identical grid sizes (5 m^2), which we merged in a single file in which each cell had a value for depth zone (1-5) and habitat type (1-16). For direct comparability of roi consumption with aquarium take, we filled a 5 km gap in NOAA maps of the analysis area by extrapolation from average habitat distributions of the adjacent 2.5 km of coastline North and South of the gap.

Table 2.2. Top: The 16 benthic habitat type categories (category 8 is land, and therefore not included) used in NOAA benthic habitat maps of Kona reefs, with relative spatial importance of each habitat type in our analysis area (proportion of total area across all depths, and of area in the depth zone 10 – 17 m, and presence/absence and relative density (D) estimates for roi. Bottom: The 5 depth zones used in our estimate, with spatial extent of total habitat in each zone in our analysis area (in ha and % of total area), and D estimates for roi. We calculated spatial extent and relative importance of habitats based on NOAA habitat maps of the Kona coast using ArcGIS 9.0. Fish D data were based on underwater visual (UV) surveys in habitat types 2 and 3, depth zone 10-17m (in bold print). Ds in unsurveyed habitats and depths were estimated relative to surveyed habitat Ds by experts. An adjustment of "1" means that the estimated D is equal to that in surveyed habitats, of "0" that no roi are present.

Categories from NOAA maps		Area (% of total)		Roi abundance	
#	Habitat types	All depths	10-17m	Presence	Density adjustment relative to UV Surveys
1	Unknown	23.3	6.5	Y	weighted average of known habitats
2	**Reef/Colonized Rock/Boulders**	19.1	**15.4**	**Y**	**1.0**
3	**Reef/Aggregate Coral**	25.0	**47.1**	**Y**	**1.0**
4	Sand	22.3	25.9	N	0.0
5	Hardbottom/Uncolonized Rock/Boulders	6.5	0.2	Y	0.1
6	Artificial/Fish Ponds	0.1	0.0	N	0.0
7	Artificial/Other Man Made Structures	0.0	0.0	N	0.0
9	Mud	0.5	1.8	N	0.0
10	Hardbottom/Reef Rubble	0.3	0.8	Y	0.1
11	Reef/Scattered Coral-Rock	0.2	0.0	Y	0.3
12	Encrusting Coralline Algae/10-50%	0.2	0.0	N	0.0
13	Encrusting Coralline Algae/50-90%	0.2	0.0	N	0.0
14	Reef/Colonized Pavement w. Channels	0.8	0.0	Y	1.0
15	Encrusting Coralline Algae/90-100%	0.0	0.0	N	0.0
16	Reef/Colonized Pavement	0.0	0.0	Y	1.0
17	Reef/Spur and Groove Reef	1.5	2.4	Y	1.0

#	Depth zones	Area (ha)	% of total	Presence	Adjustment
1	0-5m	1,205	19.1	Y	0.10
2	5-10m	998	15.8	Y	0.67
3	**10-17m**	**1,254**	**19.9**	**Y**	**1.00**
4	17-23m	773	12.2	Y	0.67
5	23m+	2,087	33.0	Y	0.33
	Total	6,317	100.0		

Roi and prey fish densities

Since 1999, the densities of reef fishes at 23 sites at depths between 10 m and 17 m in open and closed zones along the Kona coast have been monitored by means of underwater visual censuses using scuba. The surveys were initially conducted by the West Hawaii Aquarium Project (WHAP), and will therefore be called "WHAP surveys" in this paper. Since 2005, the surveys have been conducted by the HDAR. All survey sites are located in the dominant reef habitats on the Kona coast, NOAA habitat types 2 and 3 (2 = ’Reef/Colonized Rock/Boulders’; and 3 = ’Reef/Aggregate Coral’, Table 2.2). Those habitat types are henceforth referred to as ‘WHAP habitat’, and fish density in WHAP habitats is referred to as ‘WHAP density’. Spatial replication ranged from 3 to 7 sites per category (categories being: habitat 2 open; habitat 2 closed; habitat 3 open; and habitat 3 closed), and temporal replication (sampling rounds per site per year) from 4 to 6. The surveys were designed to accurately monitor the abundance of reef fishes that are part of the standing stock (defined for the purposes of this paper as the total stock of a fish population in the wild excluding settlers and early recruits up to several months of age), but strongly underestimate the abundance of fish of the life history stages settler and early recruit, in part because higher temporal replication would be necessary for this purpose. Details of the sampling regime are described in Tissot et al. (2004).

We used WHAP densities from the year 2003 for the estimate of roi and prey fish population sizes, and WHAP densities over the years 1999 – 2005 for the analysis of trends in roi population size over time. For habitats and depth zones not covered by the WHAP surveys, we estimated roi densities using adjustment factors based on personal

observations gained from several hundred dives on the Kona coast by authors since 1999 (Table 2.2).

Spatial scope of the analysis

We calculated population sizes of roi and other fish taxa for our area of analysis along the Kona coast on three spatial levels:

(i) NOAA habitat types 2 and 3 in the depth zone 10-17 m.

(ii) All habitat types in the depth zone 10-17 m.

(iii) All habitat types at all depths.

For each level, we calculated sub-estimates for zones open (protected) and closed (FRAs) to aquarium collections, and for the total area (= open + closed zone combined).

WHAP density data applied directly to (i), but density and consumption estimates for spatial scales (ii) and (iii) could only be done by applying the adjustment factors described in the previous section. Accuracy of estimates consequently declines from (i) to (iii), and therefore we based conclusions in this paper on data from spatial scale (i) (in the following referred to as the 'main analysis area') wherever possible.

Population consumption estimate

OVERALL CONSUMPTION: Following recent publications on consumption by fish populations (e.g., Overholtz et al. 2000, Olson and Galvan-Magana 2002, Jensen et al. 2006, Overholtz 2006), we calculated annual consumption by roi as

$$C_{\text{annual}} = N_{\text{roi}} * \overline{W}_{\text{roi}} * C_{24} * 365 \qquad (4)$$

where C_{annual} is the annual consumption (in wet W of prey) by the entire roi population in the area of interest, N_{roi} is the size of this population (from Equation 3), $\overline{W}_{roi}$ is the mean W of roi individuals, C_{24} is the daily ration of roi (in % of own body W) (from Equation 2), and 365 is the number of days in a year. The output of Equation 4 is annual consumption in terms of biomass, from which we determined consumption in terms of numbers by dividing biomass by the average weight of undigested prey items in roi diet.

We calculated annual consumption for the year 2003 by roi populations on the three spatial levels introduced in the previous section, with a focus on the main analysis area (NOAA habitat types 2 and 3 in the depth zone 10-17).

CONSUMPTION BY TAXON: For the main analysis area, we calculated the annual roi consumption by taxon in terms of biomass by multiplying overall roi consumption with the %W of the respective taxon in roi diet, and in terms of number by dividing the biomass of the taxon consumed by its mean undigested W in roi stomachs.

Assessment of population consumption

Consumption estimates, whether by biomass or number, only gain meaning when they are placed in a framework providing a measure of scale. For the assessment of the role of roi consumption and its potential effect on native fish communities in Kona, we therefore compared the magnitude and focus of roi consumption with the size and composition of prey fish populations, and the take of reef fishes by the AFI. We based the comparisons on results derived from the main analysis area only, due to the higher accuracy of the

population size and consumption estimates for this area compared to the estimates encompassing more habitat types and depths.

Roi consumption of reef fishes relative to their population sizes

Following Overholtz et al. (2000), we put the magnitude of predator consumption in relation to population sizes of its prey. Specifically, for the year 2003, we compared roi consumption of the 7 most important prey families (by %IRI), and of the Pomacentridae (included due to their large numerical importance in the wild), and of yellow tang (included due to its importance for the AFI), with the population sizes of these families.

Overlap of roi consumption and aquarium fish industry take

The comparison of consumption by a predator with fishery take can be used to assess the potential effects of predation (Overholtz et al. 2000). In addition, determination of combined extraction levels from predation and fishing may be important for the understanding of patterns in prey populations over time, and for ecosystem level management approaches (Pauly et al. 2000).

The important nearshore fisheries targeting reef fishes in the MHI are the commercial nearshore fishery, the recreational fishery, and the AFI (Everson 1995, Cesar et al. 2002, Tissot and Hallacher 2003). Due to the lack of reliable catch information for the commercial and recreational fisheries (e.g., Everson 1995, Anonymus 2003, 2004), we concentrated on the comparison of roi consumption with take by the AFI, for which species level catch information by zone of capture is available from HDAR records.

To determine aquarium take in our main analysis area from take reported in reporting zones 101 and 102 (i.e., our total analysis area), we applied two adjustment factors. First, we adjusted reported take upward (average of years 2001 – 2005 from HDAR unpublished records) by a factor of 1.5 to obtain estimated true take, based on considerations on underreporting by the AFI in Hawaii made by Dierking (2005) and Cesar et al. (2005). Second, we adjusted estimated true take downward by a factor of $7/12^{th}$, because the main analysis area consists of coral habitat between 10 and 17 m whereas reported take stems from coral habitat between 10 and 22 m (Dierking 2005). Aquarium take is reported in terms of numbers of fish. We also estimated take by W by multiplying take by number with the average W of collected fish, which we determined from the average length of collected specimens (mean 9 cm, range 4 – 13 cm) (from Dierking 2005 and Dierking, unpublished data) and L-W equations from Parrish and Claisse (2005).

Specifically, we calculated the take to consumption ratio (Overholtz et al. 2000) for open zones in the main analysis area in terms of biomass and number for yellow tang, all Acanthuridae, and the entire fish community. Choice of taxa for the analysis was based on the large importance of the family Acanthuridae in general (95% of take) and of the species yellow tang (81% of take) for the AFI.

We used Sigmaplot 9.0 for non-linear regression fitting and Minitab 14 for all other statistical analyses, and considered results significant at $p < 0.05$.

RESULTS

Diet composition of roi

In the 55.1% of stomachs that did contain at least one prey item, reef fishes were the principal diet component (97.7% by %IRI, Figure 2.2 A). Crustaceans were the only other higher taxonomic group in the diet, but were of minor importance (2.3% by %IRI). Roi diet in our survey included reef fishes from 16 families and 24 species, but the 5 most important families alone made up 86.4% and the 8 most important families more than 95% of the total diet (by %IRI). In order of importance, these families were Scaridae, Acanthuridae, Holocentridae (exclusively of the subfamily Holocentrinae, the Squirrelfishes), Monacanthidae, Priacanthidae, Chaetodontidae, Aulostomidae and Cirrhitidae (Table 2.3, Figure 2.2 B).

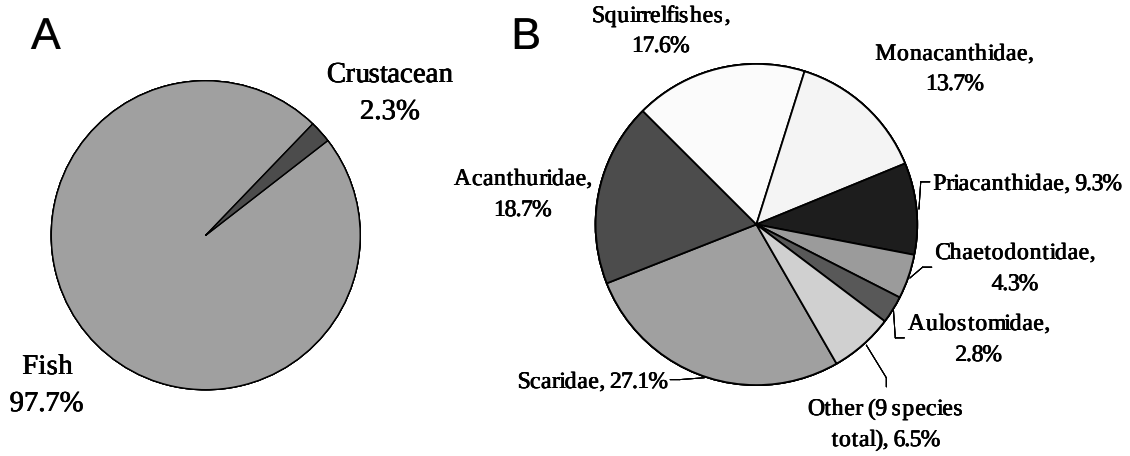

Figure 2.2. Diet composition from stomach content analysis of roi in Hawaii (n = 286). A. Importance of fish versus crustacean prey. B. Importance of prey fish families (%IRI calculated with crustaceans excluded from the sample; all fish families with a %IRI < 2% lumped in the category “other”).

Table 2.3. Importance of prey taxa in roi diet based on stomach content analysis of 286 roi specimens (156 full stomachs, 218 prey items, total prey W 1,418 g). W, wet weight; %N, percent by number; %W, percent by weight; %O, non-added percent frequency of occurrence; %IRI, percent index of relative importance. Unidentified prey (in parentheses) was not used in analyses at lower taxonomic levels. % in brackets are based on samples identified to the species level and were not included in the calculation of the %IRI.

Prey taxon	**% W**		**%N**		**%O**		**%IRI**
Fish	94.5		84.4		50.0		97.7
Acanthuridae	12.0		12.4		4.5		17.3
Acanthurus nigrofuscus		[1.4]		[5.4]		[1.0]	
Acanthurus nigroris		[11.4]		[3.6]		[0.7]	
Zebrasoma flavescens		[2.4]		[3.6]		[0.7]	
Apogonidae	0.7		1.8		0.7		0.3
Aulostomidae	2.6		5.3		2.1		2.6
Aulostomus chinensis		[2.9]		[8.9]		[1.7]	
Balistidae	5.5		2.7		1.0		1.3
Xanthichthys auromarginatus		[6.8]		[1.8]		[0.3]	
Chaetodontidae	6.8		5.3		2.1		4.0
Chaetodon multicinctus		[2.2]		[1.8]		[0.3]	
Forcipiger flavissimus		[2.8]		5.4		[1.0]	
Hemitaurichthys polylepis		[3.2]		[1.8]		[0.3]	
Cirrhitidae	3.3		3.5		1.4		1.5
Amblycirrhitus bimacula		[0.6]		[1.8]		[0.3]	
Kuhliidae	1.5		0.9		0.3		0.1
Kuhlia sandvicensis		[2.2]		[1.8]		[0.3]	
Labridae	2.6		1.8		0.7		0.5
Pseudocheilinus tetrataenia		[0.2]		[1.8]		[0.3]	
Stethojulis balteata		[3.5]		[1.8]		[0.3]	
Monacanthidae	4.6		13.3		4.5		12.7
Pervagor aspricaudus		[2.5]		[3.6]		[0.7]	
Mullidae	3.5		2.7		1.0		1.0
Pomacanthidae	1.7		0.9		0.3		0.1
Centropyge potteri		[2.5]		[1.8]		[0.3]	
Pomacentridae	3.1		2.7		1.0		0.9
Stegastes fasciolatus		[3.7]		[3.6]		[0.7]	
Priacanthidae	10.9		8.8		2.8		8.6
Heteropriacanthus cruentatus		[15.6]		[16.1]		[2.4]	
Scaridae	27.8		10.6		4.2		25.2
Calotomus carolinus		[9.2]		[1.8]		[0.3]	
Scarus psittacus		[13.6]		[7.1]		[1.4]	
Squirrelfishes	4.6		14.2		5.6		16.4
Sargocentron punctatissimum		[0.5]		[1.8]		[0.3]	
Synodontidae	3.6		0.9		0.3		0.2
Saurida gracilis		[5.3]		[1.8]		[0.3]	
Unidentified fish	(17.7)		(39.0)		(35.7)		
Crustaceans	5.5		15.6		10.1		2.3
Grapsidae	0.8		0.9		0.3		0.1
Plagusia depressa tuberculata		[1.1]		[1.8]		[0.3]	
Hippolytidae	3.3		8.8		3.5		6.7
Saron marmoratus		[4.8]		[17.9]		[3.5]	
Portunidae	1.0		1.8		0.7		0.3
Charybdis spec.		[1.6]		[3.6]		[0.6]	
Rhynchocinetidae	0.0		0.9		0.3		0.0
Unidentified Crustacean	(1.4)		(9.2)		(9.2)		

The analysis of cumulative prey curves indicated that stomach content sample size was sufficiently large for an accurate description of diet composition and patterns on the family and overall community level (Figure 2.6 A: clear asymptotic behavior of the curve), but that species level descriptions may be somewhat incomplete and should be treated with caution (Figure 2.6 B: slight asymptotic behavior of the curve).

Predation by roi focused on small prey fishes and targeted the smaller size classes of prey fish families in the wild. Specifically, 83.5% of prey fish were smaller than 10 cm TL (mean TL 7.2 cm) and 76.2% weighed less than 15 g (mean undigested W 11.4 g) (Table 2.4, Figure 2.3 A, B). The overall, and all of the family-level mean Ws were lower in the diet than in the wild population (Table 2.8, column W_P/W_W), and for several families, individuals in small size categories that were rarely encountered in reef fish surveys dominated in diet (Figure 2.4: Aulostomidae, Squirrelfishes, Monacanthidae).

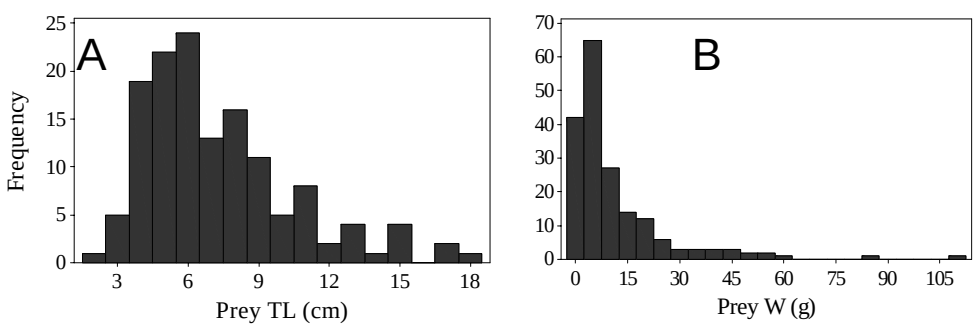

Figure 2.3. Size and weight distribution of fish prey in the diet of roi, based on the combined Oahu and Kona stomach content samples. A. TL (n = 138). B. Undigested W (n = 185).

Variable	Mean	SE of $\bar{x}$	StDev	Minimum	Median	Maximum
TL (cm)	7.2	0.74	3.21	2.4	6.2	18.2
SL (cm)	6.0	0.23	2.74	2.0	5.1	14.0
W (g)	11.4	1.12	15.25	0.34	5.2	111.0

Table 2.4. TL and SL of prey fish in the diet of roi (n = 140) and W (n = 185), from stomach content analysis of Kona and Oahu roi.

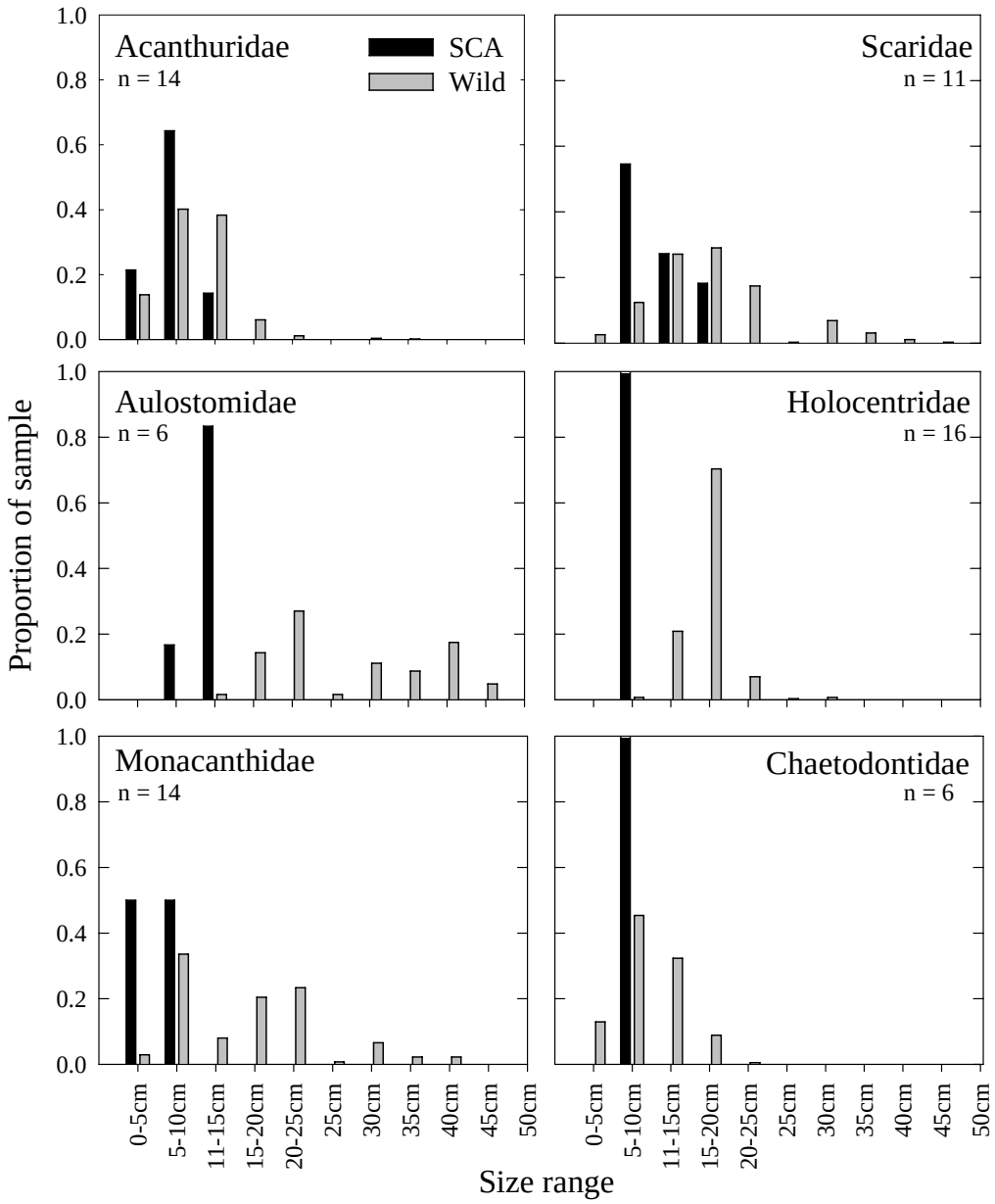

Figure 2.4. Size distribution of fish prey in the diet of roi in Hawaii and on reefs in Kona. "n" refers to the number of prey samples from roi stomachs available for the analysis. 5 cm size slots were used because underwater visual surveys did not allow a more precise determination of fish sizes.

Further indication of disproportionately larger predation pressure on small prey came from length frequency distributions of fish prey in roi stomachs and in the wild (Figure 2.4), which differed significantly both on the level of the overall lumped sample and on the family level (Kolmogorov-Smirnov tests: $D_{max\ overall} = 0.11$, $p = 0.03$; $D_{max\ Scaridae} = 0.42$, $p = 0.02$; $D_{max\ Acanthuridae} = 0.32$, $p = 0.05$); $D_{max\ Squirrelfishes} = 0.99$, $p < 0.001$; $D_{max\ Monacanthidae} = 0.47$, $p = 0.004$; $D_{max\ Chaetodontidae} = 0.55$, $p = 0.03$; $D_{max\ Aulostomidae} = 0.82$, $p < 0.001$).

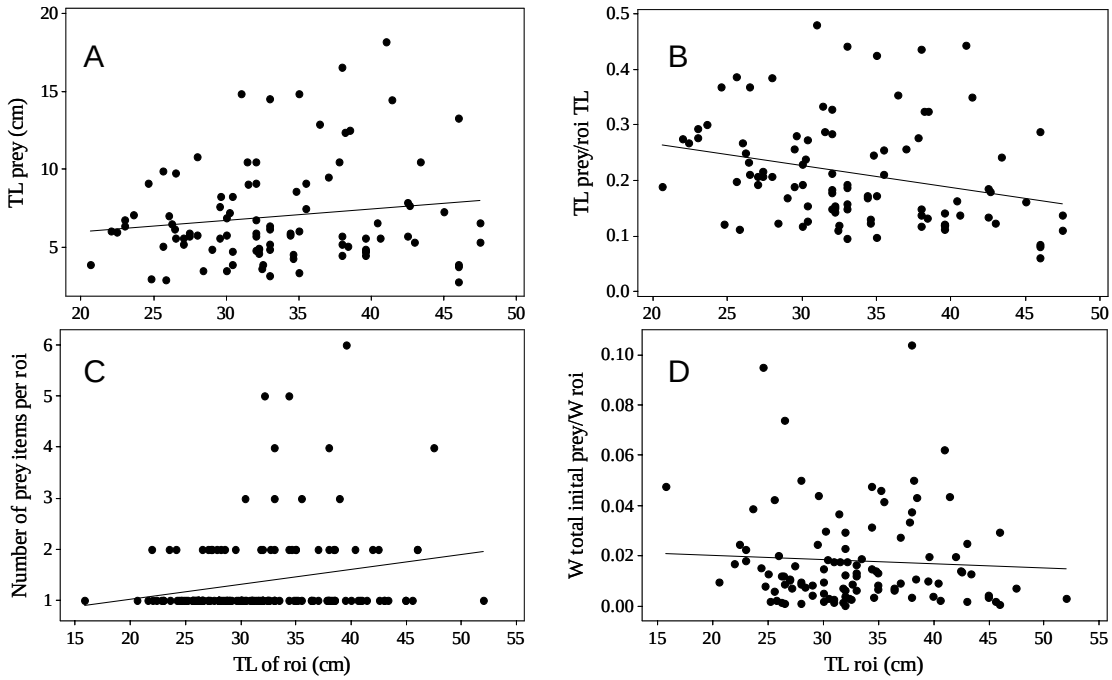

Figure 2.5. Ontogenetic feeding patterns of Kona roi. A. TL of fish prey in roi stomachs versus roi TL (n = 95). B. Relative length of fish prey (prey TL divided by roi TL) versus roi TL (n = 95). C. Number of prey items per stomach versus roi TL (n = 105). D. Relative W of fish prey (sum of the undigested prey Ws per stomach divided by the W of the roi) versus roi TL (n = 105). The analyses are based on roi with full stomachs only.

With increasing roi size, the range of prey sizes present in the diet expanded (Figure 2.5A), indicating that larger roi had the ability to consume larger prey items than

small roi. However, even large roi commonly fed on small reef fishes, and there was no significant relationship between roi size and prey size (n = 95, t = 1.39, p = 0.17, r^2 = 2%), which was expressed in a significant decrease in relative prey size (i.e., prey TL relative to roi TL) with increasing roi size (Figure 2.5 B; regression equation: $TL_{prey}/TL_{roi} = 0.345 - 0.004*TL_{roi}$; n = 95, t = -2.67, p = 0.009, r^2 = 6.1%). The decrease in relative prey size went along with an increase in the number of prey items taken by larger roi (Figure 2.5 C; regression equation: $N_{prey}/roi = 0.304 + 0.033*TL_{roi}$; n = 105, t = 2.56, p = 0.01, r^2 = 6.0%). The stomach vacuity rate did not differ significantly for roi of different sizes (Pearson chi-square = 5.94, df = 3, p = 0.114).

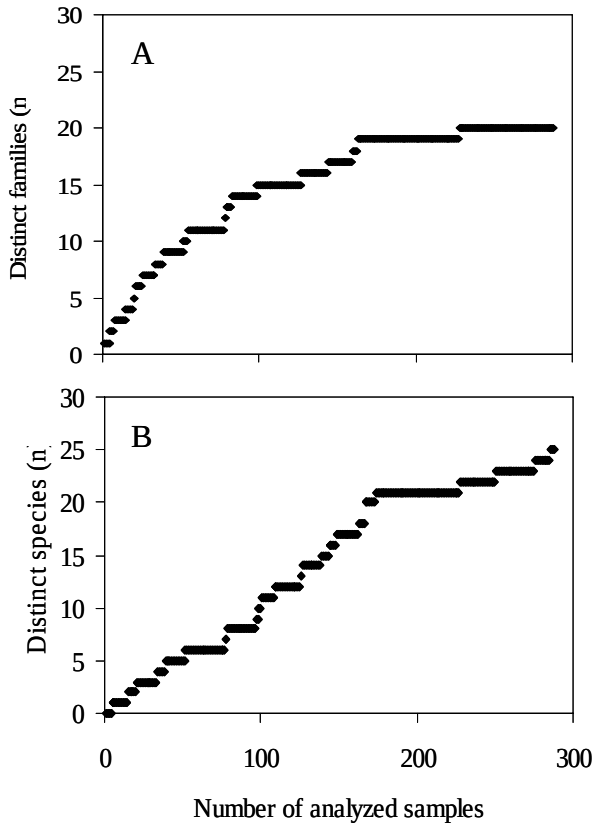

Figure 2.6. Cumulative prey curves based on stomach content analysis of 286 roi. The number of distinct taxa identified is plotted against the number of analyzed samples. Sample order was randomized prior for the nalysis. A. Family level. B. Species level.

In terms of biomass of prey consumed by roi of different sizes, all of these patterns resulted in a near constant amount of the relative stomach content W (sum of Ws of all prey items in a stomach per roi W) for roi of different sizes (Figure 2.5 D; regression equation: $W_{prey}/W_{roi} = 0.024 – 0.0002*TL_{roi}$, n = 105, t = -0.62, p = 0.54, $r^2 = 0.4\%$).

Daily ration of roi

Components of the daily ration model

AVERAGE DAILY STOMACH CONTENT WEIGHT $\bar{S}$: $\bar{S}$ of roi along the Kona coast in the summer of 2003 was 0.65% ± 0.09% of own body weight (mean ± 1 S.E.). This value is applicable to roi of all sizes, as average relative stomach content W and roi size were not significantly related (see the previous section). Non-significant differences of the average stomach content W and the frequency of occurrence of empty versus full stomachs between time periods of day (weight: $ANOVA_{df\ 2,\ 284}$, F = 1.05, p = 0.350, $r^2 = 0.74\%$; empty versus full stomachs: chi-square statistic = 6.547, df = 4, p = 0.162) indicated that the average stomach content W of Kona roi, though based on a limited sampling period, was a good estimator of $\bar{S}_{Day}$.

GASTRIC EVACUATION RATE R: We chose the exponential model with the equation

$$Y_x = 105.4 \exp(-0.0515X) \tag{5}$$

as suitable model to describe gastric evacuation in roi. It provided a better fit to the gastric evacuation data and explained a larger proportion of variability than the linear

model (distribution of model residuals: approximately normal for the exponential model, non-normal for the linear model; adjusted r^2: 98.4% versus 92.5%), had a highly significant fit ($p < 0.0001$), and caused no violations of model assumptions (normality test passed, $p = 0.84$, homoscedacity of variances test passed, $p = 0.62$) (Figure 2.7).

As shown in Equation 5, the value for R was $0.0515*h^{-1}$. The lack of systematic differences between residuals relating to small, medium and large roi, and to roi fed larger and smaller than average meals in the gastric evacuation experiment (data not shown), indicated that predator and meal size had at most minor effects on evacuation rates. R was therefore applicable to roi of different sizes.

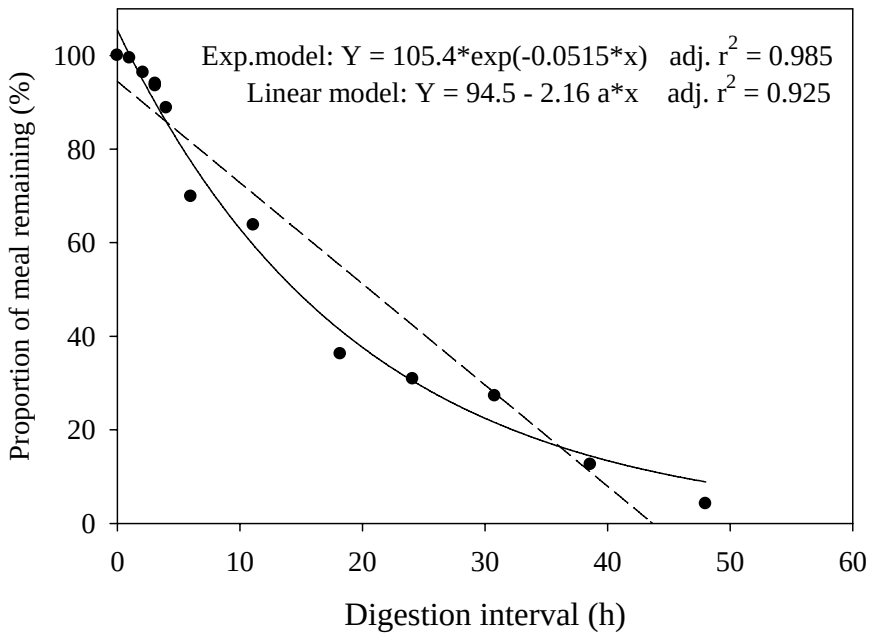

Figure 2.7. Scatterplot of the proportion of the undigested initial meal remaining (% of wet W) and digestion time (in h), with linear and exponential regressions fitted to the data. The exponential model was fitted with the regression fitting function in Sigmaplot 9.0, and the linear model was fitted using least squares regression. Meals consisted of a single whole palenose parrotfish per roi. Water temperature during trials was 26.9 ºC. Feeding trials in which full gastric evacuation was reached (45.2 h, 54.7 h, 72 h) were excluded from the analysis and are not shown.

Parameter	Equation	Mean	S.E.	Upper/lower limit
C_{24}	$C_{24} = 24*R*\bar{S}$	0.80%	n/a	0.65% - 0.97%
$\bar{S}$		0.65%	0.09%	
R		0.0515	0.0033	

Table 2.5. The calculation and parameters of roi daily ration (C_{24}) in Hawaii. Upper and lower limits of C_{24} are based on the upper (mean + S.E.) and lower (mean – S.E.) limits of $\bar{S}$ and R.

Daily ration C_{24}

C_{24} of roi in Kona was 0.80% of own body weight (C.I. 0.65% – 0.97%), based on the use of the mean values for $\bar{S}$ and R presented in this section (+ and – 1 S.E. for the calculation of the C.I.) in Equation 4 (Table 2.5). This value for C_{24} corresponds to a mean consumption of 0.4 prey fish weighing 4.8 g per average 600 g size roi per day, which equals 146 fish weighing 1,763 g per roi per year, and a Q (total annual prey consumption in terms of own body W) of roi of 3.10.

Roi and prey fish population sizes

Size of habitats

Total habitat extent in the entire analysis area (Figure 2.1) was 6,317 ha (= 63.17 km^2), of which 1,245 ha was in the 10 – 17 m depth zone. NOAA habitat types 2 and 3, in which WHAP sites were located, were the prevalent types of suitable roi habitat (i.e., total habitat minus habitat types in which roi is absent, e.g. “sand” or “mud”) in all depth zones. Specifically, in the 10 – 17 m zone, 62.5% of total and 86.4% of suitable habitat consisted of habitat types 2 and 3. Across all depths, the proportions were 47.3% and 57.6%. In both cases, most of the remainder was ‘unknown habitat’, which in part could

also have been coral habitat. Habitat types 2 and 3 in the depth zone 10 – 17m (i.e., the main analysis area) covered a total area of 784 ha. Table 2.2 summarizes all calculated habitat sizes.

Fish density and population size

ROI: The mean roi density in the 784 ha main analysis area in 2003 was 71.8 individuals/ha (mean W of individuals 600 g), which corresponds to a total population size of 56,292 individuals (C.I. 41,500 – 71,084) with a biomass of 33.8 t (Table 2.6, "2&3").

In the same year, mean density was 49.8 individuals/ha (estimated population size 62,388 individuals) across all habitats between 10 and 17 m (Table 2.6: "All"), and 22.1 individuals/ha (estimated population size 139,449) in the entire 6,317 ha analysis area. Table 2.7 summarizes results for all spatial levels.

Over the 6 year period 1999 to 2005, roi density and population size have increased by 22% on average in the main analysis area. At the same time, although FRAs provided no protection for roi, its density in areas closed and open to aquarium fish collections developed differently (Figure 2.8), with a large and significant increase by 51% in closed areas (regression analysis: $D_{closed} = 51\ ind*ha^{-1} + 5.7\ ind*ha^{-1}*$ years; n = 6, p = 0.009, $r^2 = 78\%$), and a slight non-significant decrease by 6% in open areas (regression analysis: $D_{open} = 58\ ind*ha^{-1} + 0.1\ ind*ha^{-1}*$years; n = 6, p = 0.97, $r^2 = 0\%$).

Roi density and population size in open and closed areas increased in 2004 and 2005 over the 2003 values that we used for calculations in this paper. Consumption by roi in these more recent years was therefore likely higher than our estimate for the year 2003.

Table 2.6. Population size calculation for roi in the depth zone 10 – 17m in the analysis area for the year 2003 (A, area; D, density; N, abundance; M, biomass). Calculations for zones open and closed to aquarium collections are shown individually, because Ds of roi differed between the 2 zones (see Figure 2.8). Open and closed zones add up to the total A. Roi Ds for habitats 2 and 3 are 2003 were determined by underwater visual surveys in these habitat types. Ds in other habitat types were calculated from the average of Ds for habitats 2 and 3, adjusted by the factors presented in Table 2.5. N and biomass in the row "2&3" are the roi population size estimate for the main analysis area, on which most further analyses in the paper were based.

10-17m	Closed					Open					Total		
Habitat	A (ha)	D (n/ha)	D (kg/ha)	N	M (kg)	A (ha)	D (n/ha)	D (kg/ha)	N	M (kg)	A (ha)	N	M (kg)
1	35.7	56.5	33	2,014	1,188	45.6	42.9	26.3	1,956	1,198	81.2	3,970	2,386
2	150.1	84.9	47.4	12,745	7,124	43.1	43.8	21.2	1,885	914	193.2	14,631	8,037
3	294.7	66.1	40.4	19,469	11,891	295.9	75.0	47.0	22,192	13,920	590.6	41,661	25,811
4	127.6	0.0	0.0	0	0	197.2	0.0	0.0	0	0	324.8	0	0
5	0.8	7.5	4.4	6	4	1.1	5.9	2.9	7	3	2.0	13	7
6	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0	0	0.0	0	0
7	0.0	0.0	0.0	0	0	0.4	0.0	0.0	0	0	0.4	0	0
8	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0	0	0.0	0	0
9	0.0	0.0	0.0	0	0	22.0	0.0	0.0	0	0	22.0	0	0
10	3.8	7.5	4.4	29	17	6.6	5.9	2.9	39	19	10.4	68	36
11	0.0	18.9	11.0	0	0	0.0	14.8	7.3	0	0	0.0	0	0
12	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0	0	0.0	0	0
13	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0	0	0.0	0	0
14	0.0	75.5	43.9	0	0	0.0	59.4	29.3	0	0	0.0	0	0
15	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0	0	0.0	0	0
16	0.0	75.5	43.9	0	0	0.0	59.4	29.3	0	0	0.0	0	0
17	18.0	75.5	43.9	1,362	792	11.5	59.4	29.3	683	337	29.5	2,045	1,129
2&3	444.8	72.4	44.0	32,215	19,014	339.0	71.0	29.3	24,077	14,834	783.8	56,292	33,848
All	630.8	56.5	33.3	35,626	21,015	623.3	42.9	26.3	26,762	16,391	1254.1	62,388	37,406

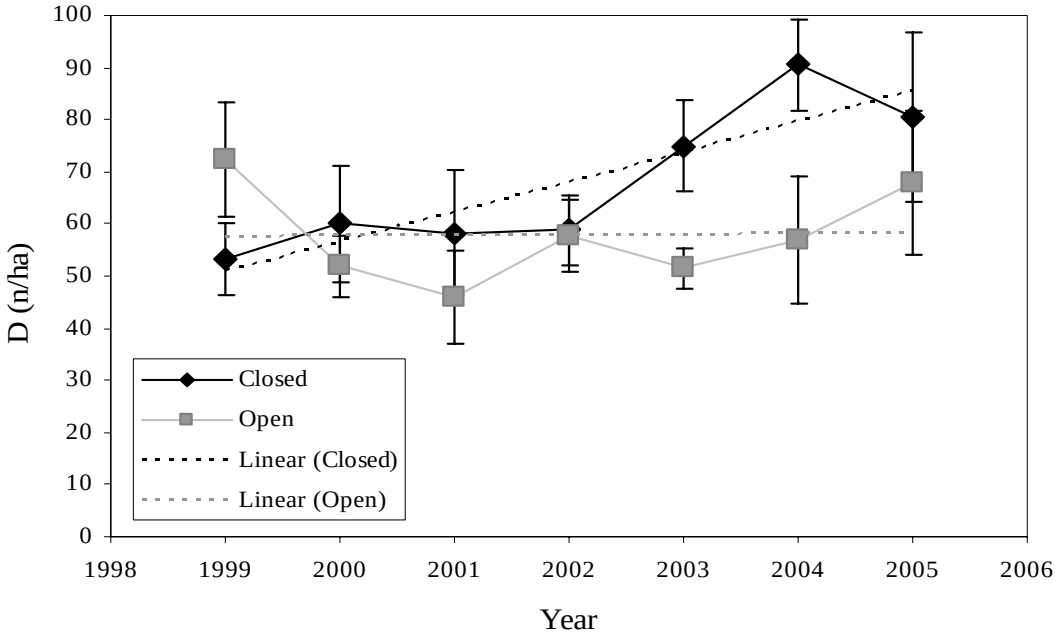

Figure 2.8. Average annual density (D) of roi in open and closed areas in the years 1999 till 2005. D is based on average D in NOAA habitat types 2 and 3 between 10 and 17 m, as determined by underwater visual surveys. The positive regression slope for closed areas was significantly different from 0 ($p = 0.009$, $r^2 = 77.5\%$), whereas the regression slope for open areas did not differ significantly from 0 ($p = 0.97$, $r^2 = 0\%$).

OVERALL FISH COMMUNITY AND INDIVIDUAL TAXA: The 2003 density of fish of all families combined in the main analysis area was 14,503 individuals/ha, with a total population size in the 784 ha area of 11.4 million individuals and a biomass of 835.5 t. This means that on average, 1 in 200 fish on reefs in Kona was a roi. By biomass, roi made up 1/25th of the total community. Population size estimates for individual families of interest and for the yellow tang are shown in Table 2.8.

Table 2.7. Roi population size (by number N, and biomass M (t = metric ton)) and consumption by roi populations (C_{annual}, in terms of biomass consumed) in the year 2003, with estimates for the main analysis area, the area 10 to 17m across all habitats, and the total analysis area, with separate estimates for open, closed, and combined open and closed (total) zones in each area. The individual components of the roi population size estimate (area size A, and roi density D) and the C_{annual} calculation (population size N, roi mean W, and roi daily ration C_{24}) for the three analysis areas are included. C_{annual} is the total prey consumption by roi. For the main analysis area, consumption by taxon is shown in Table 2.8.

Annual consumption $C_{annual} = N_{roi}$ x mean W_{roi} x C_{24} x 365

Area of estimate		A (ha)	D (ind/ha)	N (n)	M (t)	Mean W (kg)	C_{24} (% BW)	C_{annual} (t)
Main analysis area (10-17m, habitats 2&3)	Total	784	71.8	56,292	33.8	0.60	0.8	99
	Open	339	71.0	24,077	14.8	0.62		43
	Closed	445	72.4	32,215	19.0	0.59		56
10-17m, All habitats	Total	1,254	49.8	62,388	37.4	0.60	0.8	110
	Open	623	42.9	26,762	16.4	0.61		48
	Closed	631	56.5	35,626	21.0	0.59		62
Total analysis area (All depths & habitats)	Total	6,317	22.1	139,449	83.8	0.60	0.8	246
	Open	2,945	20.7	60,857	37.8	0.62		111
	Closed	3,371	23.3	78,592	46.1	0.59		135

Table 2.8. Annual roi population consumption, prey population standing stock, and relative roi consumption in terms of standing stock of prey for the main analysis area in 2003. In addition to the 7 most important families in roi diet (by %IRI), we included the Pomacentridae due to their large abundance in the wild, and the yellow tang due to its abundance in the wild and its importance in the AFI. C_M is roi annual consumption in terms of biomass (t = metric tons) and C_N is consumption in terms of numbers of prey taken. W_P is the average W of prey individuals in roi diet and W_W is the average W of individuals of the taxa on reefs in Kona. M is the total standing stock biomass, and N the total number of individuals in the standing stock of prey populations (the life history stages settler and early juveniles are not included in M and N values). Relative consumption measures the annual consumption of taxa by roi in terms of their standing stock in the wild in terms of biomass (C_M/M) and number (C_N/N). W_P/W_W is the average W of prey individuals in the diet of roi relative to their average W in the wild (i.e., a W_P/W_W below 100% indicates that roi feeding is focused on smaller than average individuals). (* Relative consumption values (in particular by number) can be large if predation focuses on recruits and early juveniles, because these life history stages can occur in very large numbers on reefs and in the diet without being counted in standing stock estimates. # Prey populations of nocturnally active taxa are underestimated by daytime WHAP surveys. We therefore did not determine population size and relative consumption by roi for these taxa (indicated by "n/a").

		Roi consumption (C_{annual})			Prey populations			Relative consumption *		
Prey types		C_M (t)	C_N (n*10^3)	W_P (g)	M (t)	N (n*10^3)	W_W (g)	C_M/M (%)	C_N/N (%)	W_P/W_W
Higher taxa	All	99.2								
	Crustacean	5.5	1,689	3.3						
	Fish	93.7	8,202	11.4	835.5	11,367	73.5	11%	72%	16%
Families #	Scaridae	26.0	745	35.0	87.7	316	277.1	30%	235%	13%
	Acanthuridae	11.3	997	11.3	422.5	5,146	82.1	3%	19%	14%
	Priacanthidae	10.2	652	15.7	n/a	n/a	n/a	n/a	n/a	n/a
	Chaetodontidae	6.4	427	15.0	37.0	535	69.0	17%	79%	22%
	Squirrelfishes	4.3	1,061	4.0	n/a	n/a	n/a	n/a	n/a	n/a
	Monacanthidae	4.3	847	5.1	5.1	16	316.2	84%	5,225%	2%
	Pomacentridae	2.9	218	13.3	59.6	3,144	19.0	5%	6%	70%
	Aulostomidae	2.4	297	8.1	1.3	11	112.1	191%	2,650%	7%
Species	Yellow Tang	2.2	203	11.0	106.1	1,824	58.2	2.1%	11.1%	19%

Roi population consumption

Integration of the results presented up this point (i.e., dietary composition, daily ration, and population size of roi) in one model leads to the centerpiece of this paper, the estimate of annual prey consumption by roi populations in Kona.

Total 2003 annual consumption by roi in the main analysis area was 99.2 t of prey (C.I. 59.2 t – 151.3 t) (Table 2.7), of which 93.7 t (C.I. 56.7 – 141.8 t) consisted of fish and 5.5 t of crustaceans (Table 2.8). Based on the average undigested prey weights of fish and crustaceans in roi diet, which were 11.4 g and 3.3 g respectively, consumption in terms of number of prey taken was 8.2 million reef fish and 1.7 million crustaceans (Table 2.8).

On the larger spatial scales, estimated consumption by roi was 9.1 million reef fish with a biomass of 110 t across all habitats between 10 and 17 m, and 20.3 million reef fish with a biomass of 246 t in the entire analysis area.

Biomass consumption by roi in open and closed zones on the three spatial scales is shown in Table 2.7.

Assessment of roi consumption

Roi consumption relative to reef fish population sizes

Consumption by roi was equal to 11% of the total reef fish community standing stock by weight and to 72% of standing stock by number.

The taxonomic composition of the consumed prey was not proportionate to that of the fish community in the wild. In particular, Scaridae (12.1% in the diet versus 1.2% in

the wild by numerical importance), Monacanthidae (15.2% versus 0.1%), Aulostomidae (6.1% versus 0.1%), Cirrhitidae (4.0% versus 0.6%) and Chaetodontidae (6.1% versus 4.6%) were more common in the diet than their abundance in the wild would suggest, and Acanthuridae (14.1% versus 38.9%) and Pomacentridae (3.0% versus 34.8%), the two most abundant families in the wild, were less common in the diet than their abundance would suggest. Roi consumption of fish in families in terms of their standing stock consequently varied strongly, ranging from a low of 3% for the Acanthuridae (which were underrepresented in the diet) to a high of 191% for the Aulostomidae (which had disproportionally large dietary importance) by biomass, and from 6% for the Pomacentridae to 5,225% for the Monacanthidae by number (Table 2.8).

Importantly, as described in detail in the section "Diet composition", the mean size of fish in roi diet was much lower than the mean size of fish in the standing stock on reefs in Kona (11.4 g versus 73.5 g, or 16% of the mean size in the standing stock). By family, the ratios of mean W in roi diet to mean W in the standing stock ranged from 2% for the Monacanthidae and 7% for the Aulostomidae to 22% for the Chaetodontidae and 79% for the Pomacentridae (Table 2.8 and Figure 2.4). For all common fish families in the diet of roi, the sizes consumed represented at least in part fish of the life history stages settler and early recruit (Figure 2.4).

Roi consumption relative to take by the AFI

In open zones in the main analysis area in 2003, the consumption-to-take ratio for the species most important for the AFI, the yellow tang, was 33% in terms of biomass. This means that roi removed 1/3rd as much yellow tang biomass as the AFI (1 t versus 3 t,

equivalent to 3% of standing stock biomass versus 9%). The consumption-to-take ratio by number was 73% (89,000 fish consumed by roi versus 122,000 taken by the AFI) (Table 2.9).

Table 2.9. Comparison of total 2003 roi consumption (C_{roi}) of yellow tang, surgeonfishes, and fish in general with take of these taxa by the aquarium fish industry ($Take_{AFI}$) in open zones of the main analysis area. We calculated consumption-to-take ratios ($C_{roi}/Take_{AFI}$) based on biomass and number of prey fishes removed (i.e., $C_{roi}/Take_{AFI}$ below 100% indicate that roi consumption was lower than AFI take, and vice versa). In addition, we compared sizes of fish removed by roi and the AFI (W_{prey}/W_{Take}: values below 100% indicate that prey sizes targeted by roi are lower than those taken by the AFI). Percentages in brackets represent proportions of prey standing stock size.

C_{roi} versus $Take_{AFI}$		Yellow Tang	Acanthuridae	All fishes
By biomass (t)	Population size	33.1	164.0	300.0
	C_{roi}	1.0 (3%)	4.9 (3%)	41.1 (14%)
	$Take_{AFI}$	3.0 (9%)	3.6 (2%)	3.7 (1%)
	$C_{roi}/Take_{AFI}$	33%	137%	1,101%
By number ($n*10^3$)	Population size	526	1,896	4,108
	C_{roi}	89 (17%)	437 (23%)	3,595 (88%)
	$Take_{AFI}$	122 (23%)	143 (8%)	149 (4%)
	$C_{roi}/Take_{AFI}$	73%	306%	2,408%
By mean W (g)	Kona population	63.1	86.7	72.9
	Roi prey	11.0	11.3	11.4
	$Take_{AFI}$	25.0	25.0	25.0
	W_{prey}/W_{Take}	44%	45%	46%

On the family level, the situation was different. For the Acanthuridae, the consumption-to-take ratios by biomass and number were 137% and 306%, i.e., roi consumption removed 37% more biomass than AFI take (4.9 t versus 3.6 t), and 3 times more surgeonfish individuals than AFI take (437,000 versus 143,000 individuals).

On the most inclusive taxonomic level, all families of the entire fish community, the consumption-to-take ratios by biomass and number were 1,101% and 2,408%, i.e., total roi consumption was 11 times higher than the total AFI take in terms of reef fish

biomass (41.1 t versus 3.7 t), and 24 times higher in terms of the number of individuals taken (3.6 million versus 149,000 individuals).

Importantly, there were strong differences in the focus in size of roi consumption versus AFI collections on all taxonomic levels of analysis. In particular, the mean W of yellow tang consumed by roi was only 11.0 g, which was much lower than the mean W of yellow tang in the standing stock (63.1g), and of yellow tang taken by the AFI (25 g). On the higher taxonomic levels, the mean W of surgeonfishes in the diet of roi was only 45% of the mean W of surgeonfishes taken by the AFI, and the overall mean W of fishes in the diet was only 46% of the overall mean W of fish taken by the AFI (Table 2.9).

DISCUSSION

Despite the importance of consumption rate and annual consumption information of major predators to the understanding of community structure, such data are woefully lacking for coral reef fish predators (Bromley 1994). Following work on the groupers *Cephalopholis cyanostigma* and *Cephalopholis boenak* in Australia by Beukers-Stewart and Jones in 2004, this study provides only the second estimate of prey consumption by grouper populations. The successful calculation of roi and prey fish population sizes along the Kona coast by coupling of electronic habitat maps (Monaco et al. 2001) with benthic fish survey data makes our study one of a small but growing number of examples (e.g., Nasby-Lucas et al. 2002, Friedlander and Brown 2003) of the applicability and suitability of this recent method to address questions in reef fish ecology that were previously difficult to approach. The sample size we had available for stomach content

analysis was among the largest of published studies on grouper feeding (Parrish 1987, St. John 1999). Results presented here are therefore not only important for the consumption estimate itself, but are of interest in their own right as a thorough characterization of the feeding patterns of a grouper.

On a local scale, our results allow a first assessment of the effects of the introduced grouper species roi on native fishes in Hawaii. This is relevant, because previously, resource managers could not consider roi in management plans. There is a common tendency for groups of extractive users of reef resources, such as commercial and amateur fishers or net and spearfishers, to blame each other for reductions in catches (Goeden, 1975, Ruello & Henry 1977). As a variation on this theme, in Hawaii various user groups have tended to reject responsibility for resource declines by putting the blame on roi as a “scapegoat” (personal observation consistently made by all of the authors). Resource managers will now be better able to respond to those claims.

More generally, our study provides baseline values of daily ration and gastric evacuation rate, which can be applied in grouper consumption studies elsewhere. Such studies are urgently needed to better assess the ecosystem level effects of declines in grouper populations worldwide. While ideally, population consumption parameters would be calculated for each grouper species and location, as long as published values are scarce, application of parameters for roi to other grouper species would be preferable to having no information at all.

We first discuss the results of the consumption estimate in the light of existing studies on fish predation, and attempt to draw conclusions about the potential ecological effects of roi in Hawaii by a comparison of consumption with prey fish standing stocks

and take by the AFI. We then briefly discuss potential sources of error that could have influenced the estimate.

ASSESSMENT OF THE EFFECTS OF ROI POPULATION CONSUMPTION: The main results on which large parts of the discussion will be based were (1) the large absolute number of reef fish and biomass consumed by roi per km^2 of reef (Table 2.8: 8.2 million reef fish with a weight of 93.7 t in the 7.8 km^2 main analysis area), (2) the substantial relative consumption in terms of prey fish standing stock biomass (Table 2.8: 11.4% of biomass per year), and extremely large relative consumption in terms of prey fish standing stock by number (Table 2.8: 72%), (3) the small mean size of prey fishes relative to their mean size in the standing stock, representing size classes belonging to the settlers and early juveniles for most species (Figures 2.3, 2.4), and (4) the large dietary importance of a relatively small number of families, with a numerical importance in roi diet that was out of proportion to their relative abundance in the wild and thus indicated that roi consumption was preferential.

The outstanding characteristic of consumption by roi populations was its magnitude. This finding may appear surprising, considering the low metabolic rates and slow growth of groupers (Morris et al. 2000), and their generally low prey consumption in terms of own body weight compared to more active, roving predators (Birkeland 1997). Roi fit this pattern with a gastric evacuation rate R of $0.0515*h^{-1}$, an individual consumption rate C_{24} of 0.8%, and an annual individual consumption rate (Q) of 3.10, all of which are low compared to published rates for piscivores (see e.g. Olson and Mullen

1986, Pauly 1989, Ruggerone 1989, Olson and Galvan-Magana 2002). However, roi abundance on reefs in Kona was high, with an average density in terms of biomass per area that was similar to that of groupers on coral reefs elsewhere that are considered to have large effects on prey species, e.g., the grouper *Plectropomus leopardus* in Australia (St. John 1999), and the entire guild of groupers at sites on the Great Barrier Reef (Goeden 1982). The combination of low individual consumption rate but large population size of roi (Table 2.7) resulted in substantial net consumption in terms of biomass and number of prey fish consumed by roi in Kona (Table 2.8). Considering that consumption in terms of the standing stock of prey fish populations by roi was on par with that of piscivores thought to be keystone predators in other systems (e.g., Beets 1997, Overholtz et al. 2000, St. John and Russ 2001, Olson and Galvan-Magana 2002, Read and Brownstein 2003, Jensen et al. 2006, Overholtz 2006), it is likely that roi consumption plays an important ecological role for the reef fish community in Kona. This confirms the findings of the only other published study on population consumption by groupers by Beukers-Stewart and Jones (2004), who found that on reefs around Lizard Island, Australia, the combined populations of the grouper species *Cephalopholis cyanostigma* and *Cephalopholis boenak* consumed slightly more prey fish biomass per area than roi in Hawaii, and had large impacts on populations of their prey.

At first sight, roi consumption in terms of numbers of reef fish appeared unrealistically high, since it far surpassed the total number of fish in the standing stock for some families (see Table 2.8). However, the preferential consumption of prey fish of early life history stages (settlers and early juveniles), by roi of all sizes (Figure 2.4) offers

an explanation. On coral reefs, the number of settlers arriving annually is commonly much larger than the total number of fish in the standing stock (Macpherson et al 1997). At the same time, predation induced mortality rates of settlers are extremely high (e.g., van der Veer & Bergman 1987, Ellis & Gibson 1995, Connell 1997, Steele 1997). For example, only about 5% of yellow tangs that initially settle on reefs in Hawaii are estimated to survive after one year (Jeremy Claisse, personal communication), and for most coral reef fish species, only between 1% and 10% of settlers eventually recruit to the standing stock (Doherty et al. 2004). Even in cases where pulses of large numbers of recruits arrive on reefs, complete recruitment failure can occur if predation levels are high (Webster 2002). Since roi consumes mostly small fish that are not part of the standing stock, whereas the underwater visual surveys on which standing stock estimates of reef fishes in this study were based were designed to monitor late recruit, juvenile and adult abundances, but undercount the abundance of settlers and recent recruits, the ratio of fish consumed by roi and standing stock size by number can result in seemingly high values. At the same time, the feeding focus entails the important caveat that the magnitude of consumption by roi is not necessarily equal to the ecological effect on the standing stock of fishes. In spite of this distinction, the large scale of roi consumption, even though it was focused on early life history stages, is likely to indirectly affect standing stocks by influencing recruitment success (see Shulman and Ogden 1987).

One factor that could mitigate effects of roi on individual taxa to some degree was the overall diversity of roi diet. However, the large dietary importance of only a limited number of families (see Figure 2.2), and the asymmetry of relative feeding pressure (i.e.,

the strong differences in relative consumption in terms of standing stock of families, Table 2.8) meant that fishes of some families were consumed in large numbers. Prey composition of roi showed that feeding focused on benthic reef fishes (Table 2.3), which is common for groupers, because they tend to hunt close to the reef and do not commonly target planktivores (Parrish 1987). Roi preferentially consumed fishes belonging to the families Monacanthidae, Aulostomidae, Scaridae, Cirrhitidiae and Chaetodontidae, whereas the Pomacentridae (note: this family includes planktivorous species) and Acanthuridae were less important in the diet than their abundance in the wild would suggest. Where preferential consumption by piscivores including groupers (e.g., Goeden 1982) occurs, it commonly affects the relative abundance of reef fishes (i.e., community structure) (Shulman and Ogden 1987). It therefore appears likely that the large-scale preferential consumption by roi affects reef fish community structure in Hawaii.

Table 2.10. Average change in density for different taxa in areas closed and open to aquarium fish collections along the Kona coast between 1999 and 2005 (from WHAP unpublished data). FRAs were created in 2000. Symbols: “++”: > +25%, “+”: > +5%, “+-“: > -5% and < +5%, “-“ < -5%, “--“: < -25%.

Taxon	Closed	Open
Total fish community	++	+/-
Zebrasoma flavescens	++	-
Cephalopholis argus	++	-
Acanthurus nigrofuscus	--	+
Chaetodontidae	+-	+
Aulostomidae	--	++

Evidence for such effects may come from the population development of roi and of prey fishes in areas open and closed to aquarium fish collections since the creation of

the closed areas along the Kona coast in 2000 (Table 2.10). Although roi is not protected from fishing in open or closed areas, roi population size significantly increased by 51% in closed areas, and decreased by 6% in open areas between 1999 and 2005 (Figure 2.8). The overall prey fish community size also increased strongly in closed areas, but stagnated in open areas during this time. These patterns suggest that roi abundance may have depended on resource availability. Presence of such bottom up effects (see Halpern et al. 2006) would explain the divergent trends in roi populations between open and closed areas. Secondly, against the trend of increasing reef fish populations in closed areas, some species and families, including the lavender tang (*Acanthurus nigrofuscus*), the Chaetodontidae, and the Aulostomidae, declined in number in the closed areas, but had stable or even increasing populations in the open areas (Table 2.10). This seemingly non-intuitive pattern could be explained by roi consumption, since the taxa that declined in closed areas belonged to the taxa that roi consumed preferentially (see above). Further research is needed to better elucidate these ecological patterns.

CONSUMPTION BY ROI RELATIVE TO TAKE OF THE AFI: This comparison puts consumption by roi into perspective, as the approximate magnitude of reef fish take by the AFI is known and the effects of this extraction are partly understood. The AFI has significant impacts on reef fish populations in open areas in Hawaii, most importantly in the form of significant depression of the standing stock size of fishes commonly collected, including the yellow tang (Tissot and Hallacher 2003, Tissot et al. 2004). It is important to recall that the direct comparison of roi consumption with AFI take is difficult, because the AFI removes older juveniles and young adults and has a direct effect on standing stocks,

while roi commonly feeds on settlers and early juveniles and has a more indirect effect on standing stocks (see above) (Table 2.9). In this context, some prey species, e.g., *Ctenochaetus strigosus* in the family Acanthuridae, are targeted by roi, the AFI, and by food fisheries (not analyzed in this paper), with roi consuming the smallest individuals, the AFI taking medium sizes, and food fisheries taking the adults (this study, DAR unpublished data, Anonymus 2003). Such sequential extraction seems more likely to affect prey fish populations than extraction on only one level.

Despite of the different focus in size, we attempted to draw some careful conclusions from the comparison of biomass consumed by roi with take by the AFI. Compared to AFI take, roi consumption of the yellow tang was lower but not insignificant (33% of the take by the AFI by biomass, Table 2.9), and consumption of surgeonfishes in general, and of fishes of all families overall was much higher (137% and 1101% of the take by the AFI in terms of biomass). Although the ecological implications of consumption and take differed, the very large consumption by roi relative to the AFI take is another indication that roi was a relevant factor in the reef ecosystems.

FURTHER ECOLOGICAL CONSIDERATIONS: In spite of the large absolute and relative consumption by roi, and the indications that reef fish community structure may be affected by consumption, the total number of fish in the analysis area increased over the years 1999 to 2005 (HDAR, unpublished data). Somehow, prey fish communities therefore must have been able to cope with predation pressure by roi. One of the big questions coming out of this paper is how communities can be so resilient to large removal of biomass, and astonishingly high pressure on young life history stages. In other

words, considering the magnitude of consumption by roi (and the additional effects from other predators and human extractions), the questions is not “is there an effect of roi predation on the reef fish community?”, but rather, “why do we not see more of an effect of roi predation on the reef fish community?”. Different hypotheses exist about the processes regulating reef fish populations, including competition, predation, and recruitment limitation (Hixon 1991). While for some reef fish families in Kona, roi predation was very large, and may have affected recruitment success strongly enough to cause declines in relative importance of these families in the long term (see above), overall, the increase in size of many reef fish populations indicated that sufficient numbers of recruits survived to enter the standing stock, despite roi consumption.

A last question regarding the effects of roi is whether roi consumption is really a new, previously not experienced impact on native species by an introduced predator, or whether it may in reality be functionally replacing other piscivorous predators. Large predators used to be much more common in the MHI prior to large-scale human exploitation of reefs than they are today (Shomura 1987, Friedlander and DeMartini 2002). In addition, smaller piscivores such as the Aulostomidae and Cirrhitidae (Hobson 1974) have been declining in Kona (HDAR unpublished data) in parallel with the roi increase, which may represent the reverse side of the commonly observed increase of smaller predatory fishes going along with fishing induced declines in native groupers elsewhere (e.g., Macpherson et al. 1997, Chiappone et al. 2000). In the light of declines in populations of other predators, the possibility that roi consumption is not a new effect on native reef fishes but replaces consumption by other predators should be kept in mind.

CONSUMPTION MODEL VALIDITY AND DISCUSSION OF POTENTIAL SOURCES OF ERROR: Population consumption estimates are strongly dependent on the choice of parameters (Overholtz et al. 2000). Generally, our study represents the optimum case of consumption studies, since all parameters (daily ration C_{24}, with its components average stomach fullness and gastric evacuation rate R; population size of roi) were determined for roi in Hawaii, and not borrowed from other taxa or locations. In the few instances that required assumptions, we chose the more conservative of the available options (e.g., in the determination of average stomach fullness, for which we did not have nighttime stomach samples available, we assumed that no nighttime feeding occurred, which led to a conservatively low average daily stomach fullness), which means that our consumption estimate may be an underestimation.

Due to the lack of published R and C_{24} values for groupers, our parameter estimates for roi were of particular importance. A detailed discussion of the derivation of the parameters will be published separately (Dierking, in preparation). R and C_{24} of roi lay on the low end of the range of published values for other fish predators (e.g., Elliott 1991, Bromley 1994, Jensen et al. 2006), i.e., individual consumption of roi was relatively low, which was explained by the fact that most published values of R and C_{24} are for more active predators with higher metabolisms than groupers (see Bromley 1994). The C_{24} value of 0.8% also lay between the average *ad libidum* feeding rate of roi of 1.3% of own body weight per day obtained in tank experiments (Dierking unpublished data), and grouper maintenance rations (i.e., the minimum ration required to avoid starvation and to maintain body weight over time) of 0.25% of own body weight per day

(Luo et al. 2006). This indicates that our estimates lay in the correct range, since C_{24} should be higher than maintenance rations considering the increase of roi population size in Hawaii in recent years, but should be lower than rations reached in tank experiments in which food is provided without limitations. Further support for our C_{24} estimate comes from a study by Olson and Galvan-Magana (2002), who found that time till full digestion in yellowfin tuna, a pelagic, high-performance species, ranged from 6–18 hours, and estimated that similar-size low performance teleost fishes require about five times longer than yellowfin tuna to evacuate a meal. This fits well with our estimate of time till full digestion of roughly 50 hours for roi, based on the gastric evacuation rate for roi determined in this study.

Our considerations about diet composition and consumption on the family and species level were based on the assumption that diet was accurately described by the stomach content analysis sample available in this study. Cumulative prey curves indicated that on the family level, this was clearly the case (Figure 2.6 A, clear asymptote showing in the curve), whereas on the species level, a larger sample size would have been preferable (Figure 2.6 B, slightly decreasing slope, but no clear asymptote). This means that considerations on the species level, which in this paper were limited to the yellow tang, should be treated with some caution. This result is interesting from a methodological point of view, as published studies of diet composition of groupers that include species level analyses have generally been based on much smaller sample sizes than the one available here (Parrish 1987).

An important potential source of error to consider is that roi samples in this study were collected in the summer months only, when settlers on reefs in Hawaii are more common than over the rest of the year (Walsh 1987). Average prey weight at other times of the year could therefore presumably be higher. Beukers-Stewart and Jones (2004) detected such changes in the prey of the grouper species *Cephalopholis cyanostigma* by annual sampling of stomachs. Prey weight was lowest during peak settlement periods over the summer months, and higher between such peaks (in the winter). However, although the average prey weight fluctuated, the biomass taken by *C. cyanostigma* did not change significantly between seasons (i.e., the lower number of prey taken in winter was compensated by the higher average W of prey). For our study, this suggests that the consumption estimate by number may be conservatively high, whereas the estimate by biomass would be less affected by potential seasonal patterns in prey sizes taken. Overall, these changes would not make a difference in our interpretation of results, as long as the increase in size is not so large that the focus of roi consumption shifts from settlers and early juveniles to larger size classes that are part of the standing stock.

Our estimate of roi population size was based on underwater visual surveys that took place in NOAA habitat types 2 and 3 between 10 – 17 m (Tissot et al. 2004). This is equivalent to the habitat types and depth zone of the main analysis area of our population consumption estimate, on which the discussion in this paper was based. As surveys applied directly to the main analysis area, no assumptions about densities were necessary, and serious errors could only be introduced by the survey methodology, for example if surveys systematically over- or undercounted roi. Cappo and Brown (1996) showed that

sufficiently replicated visual censuses are a suitable method for obtaining precise and relatively accurate estimates of actual grouper densities, with a slight tendency to underestimate densities. Based on the large number of survey replicates that we had available, we have confidence in the accuracy of our estimate of population size and population consumption.

CONCLUSIONS

The results presented in this paper allow an improved assessment of the effects of the introduced grouper species roi on the coral reef fish community in Hawaii. Specifically,

(1) Roi consumption was focused on settlers and early juveniles of prey fishes, which made the assessment of effects of roi on prey fish standing stocks difficult.

(2) The magnitude of consumption by roi relative to the size of reef fish standing stocks and take by the AFI, as well as the high density of roi, which was on the level of that of groupers elsewhere that are considered keystone predators, suggested that substantial effects of roi on native fish communities are likely.

(3) Preferential feeding by roi indicated that consumption was likely to affect prey fish community structure; such effects on communities may be visible in divergent development of prey fish populations between open and closed areas.

(4) The case of roi in Hawaii raises questions about the factors causing resilience to high predation pressure in reef fish communities, including the question whether roi may be filling the ecological role of several piscivore species that used to be more common in the MHI prior to the advent of large-scale human exploitation of reefs.

CHAPTER 3:

PATTERNS OF CIGUATOXICITY IN THE INTRODUCED GROUPER *Cephalopholis argus* IN HAWAII, AND IMPLICATIONS FOR FISHERY MANAGEMENT

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ABSTRACT

The grouper species roi (*Cephalopholis argus*) was intentionally introduced to the main Hawaiian Islands (MHI) from Moorea in 1956 to create a new fishery. It has become established and abundant, but the fishery never developed due to market concern about ciguatera (*Gambierdiscus toxicus*) incidents from roi consumption. My goal was to determine the predictability of the toxicity of roi individuals in Hawaii based on geographic patterns of ciguatoxin concentrations and on the association of roi morphometrics and toxicity. Good predictability could lead to avoidance of toxic roi and thus to a safe fishery. Using the Membrane Immunobead Assay (MIA), I analyzed toxin concentrations in 291 roi specimens collected by spearfishing at 17 Oahu and Hawaii Island sites. Overall, toxicity of 18.2% of individuals was too high for safe human consumption. Oahu roi populations were significantly less ciguatoxic than Hawaii Island roi, but highly toxic individuals did occur at five of six Oahu sites. Within islands, site populations differed strongly in average toxicity, but no gradients or other geographic patterns in toxicity were evident. Within-site variation in toxicity was the dominant source of variation (87.8% of total variation versus only 12.2% of from between-site variation). Roi length and ciguatoxicity were significantly positively associated, but length explained only 1.5% of variation in toxicity. Condition and ciguatoxicity of roi individuals were not significantly associated. I concluded that the analyzed factors were not associated strongly enough with ciguatoxicity to allow reliable prediction of roi toxicity, and that a safe roi fishery in the MHI was therefore not feasible.

INTRODUCTION

Due to their extreme geographic isolation, the Main Hawaiian Islands (MHI) have reef fish communities characterized by the lack or limited presence of fish families with short to medium larval durations, which include the commercially important snappers (Lutjanidae) and groupers (Serranidae), and by high rates of endemism in the extant fish families (Randall 1987, 1996).

In 1956 and 1961, 2385 individuals of the grouper species roi (*Cephalopholis argus*) were released in the MHI from Moorea as part of a species introduction program of snappers, groupers, and emperors (Lethrinidae) intended to create new fisheries (Randall 1987). Of 12 species introduced overall, only two snapper species and roi as the only grouper became established (Oda and Parrish 1981). After an initial time-lag in population growth, roi populations have grown strongly since the 1980s, and roi has become the dominant large nearshore predatory reef fish in the MHI, far surpassing the two rare native Hawaiian grouper species in abundance (Hoover 1993). The envisioned fishery nevertheless did not develop after roi caused a number of ciguatera fish poisoning incidents in Hawaii and was rejected by consumers thereafter (Bruno and Effler 2001b, Earle 2005). In spite of the low resulting consumption of roi by humans compared to popular market reef fishes in Hawaii (Anonymus 2001), 21 incidents of ciguatera from roi consumption were reported in Hawaii between 1996 and 2000, representing 17% of all reported incidents, more than for any other reef fish in the State (Bruno and Effler 2001b).

Ciguatera is a neurological disease with potentially severe clinical symptoms in humans (Bagnis 1968, Palafox and Buenconsejo-Lum 2001). It is caused by ingestion of

ciguatoxin (CTX), a natural polyether toxin that is produced by the benthic dinoflagellate *Gambierdiscus toxicus* (Yasumoto et al. 1980). CTX enters the marine foodchain when herbivores consume benthic macroalgae with attached *G. toxicus* (Campbell et al. 1987). After ingestion, CTX is stored in body tissues, and can subsequently be transferred to higher trophic levels of the foodweb when predators prey on toxic herbivores (Banner 1974). Ciguatera is confined to the tropics and subtropics, where it has important public health and economic implications, especially in island nations in the Caribbean and the Pacific (Lewis 2001). Conservative estimates of the annual number of cases range from 25,000 – 50,000 worldwide (Lehane and Lewis 2000), and from 35 – 70 in Hawaii (Afton 2003).

In contrast to originally envisioned commercial benefits of the introduction of roi, prey consumption by roi populations is today considered a potential threat to native reef fish communities and existing fisheries in the MHI (Earle 2005). The potential of roi predation to have substantial effects on native communities is emphasized by the large role that other groupers play in shaping reef fish communities in their native habitats (e.g., Macpherson et al. 1997, Webster 2002, Beukers-Stewart and Jones 2004, Hixon and Jones 2005). The situation in Hawaii is thus an anomaly: here, roi is considered too abundant, and is of no commercial value, whereas elsewhere, groupers are generally declining in numbers, because they are commercially valuable foodfish and targeted by fishermen, and because their life history traits render them vulnerable to fishing pressure (Heemstra and Randall 1993, Morris et al. 2000). In several of its native habitats, roi falls under the latter pattern as well (Heemstra and Randall 1993). In Guam, where roi is not considered to be ciguatoxic, it is overfished (Jerry Davis, personal communication), and

in the Asian live reef fish trade, it is sold at up to $20/kg (Lee and Sadovy 1998, Lau and Parry-Jones 1999).

In this light, ciguatera in roi in Hawaii is of great importance for management considerations. Understanding the factors correlated with it could allow resource managers and fishermen to predict if certain roi, e.g., individuals below a certain size limit or from a particular geographic area, are predictably free of CTX and safe for consumption. Such understanding could lead to a safe and profitable roi fishery that would have the potential to control the abundance of roi.

The problem is that while the general principle of CTX accumulation is understood (e.g., Banner 1974), the presence and distribution of ciguatoxic fish individuals often appears random and unpredictable, and the patterns behind CTX accumulation remain unknown (Lewis 2001). In some locations, certain stretches of coastline or specific reefs were found to consistently produce ciguatoxic fish, while neighbor coastlines or reefs were safe (Lewis 2001). Regarding predictable fish characters associated with ciguatera, one hypothesis is that the size of fish is positively correlated with ciguatoxicity (Randall 1958); more controversial is the effect of CTX on fish fitness or condition (e.g., Capra et al. 1988, Lewis 1992). However, few studies have explicitly looked at the role of geographic origin or morphometric characters of a fish in ciguatoxicity, partly because of the reliable quantitative tests of ciguatoxicity did not become available until recently (Lewis 1986, Lehane and Lewis 2000).

Identification and isolation of the CTX molecule by Scheuer et al. (1967) and Murata et al. (1989) led to the development of the Membrane Immunobead Assay (MIA) for detection of CTX, based on the specific binding of a monoclonal antibody (MAb-

CTX) to the CTX molecule (Hokama et al. 1998). The MIA has a sensitivity of 92.3% and a specificity of 85.7%, which is considered acceptable for biological test systems (Lehane and Lewis 2000). It was applied extensively in screenings of wild fish populations for ciguatera (Hokama and Ebesu 2001), and is the standard method used by the Hawaii Department of Health for post-hoc validation of ciguatoxicity of fishes implicated in ciguatera incidents (Bruno and Effler 2001a).

My goal was to assess patterns of CTX occurrence in roi populations in the MHI, with a focus on patterns that could lead to predictability of the ciguatoxicity of roi individuals in the field in the future. The main objectives of this study were (1) to determine the overall prevalence of toxin in roi in the MHI to assess the risk of an unlimited fishery, (2) to analyze geographic patterns in roi toxicity in the MHI to answer the question if roi from certain coastlines or parts of coastlines are free of CTX, and (3), to analyze the association of ciguatoxicity with morphometric characters of roi to assess their use as predictors of toxicity. To fulfill these goals, I measured toxin concentrations in 291 roi specimens from 17 sites on Oahu and Hawaii Islands using the MIA, and analyzed spatial patterns in toxicity as well as the relationship of toxicity with roi length and condition.

MATERIAL AND METHODS

Choice of sample sites

Oahu is the human population center of the MHI, and has the highest number of recreational fishermen of any Hawaiian island. Hawaii Island has a much lower population, but still experiences fish capture for home consumption on large scales

(Schmitt 2002). The West coasts of the islands of Oahu and Hawaii are coastlines with relatively high numbers of ciguatera incidents from locally caught fishes (Bruno and Effler 2001a). Roi densities on reefs along the West coast of Hawaii Island are the highest in the MHI, with on average 6.7 individuals/1000 m^2 in 2005 (Hawaii Division of Aquatic Resources (HDAR) unpublished data). This coastline has been the focus of intensive resource management efforts to sustain reef fish populations (Tissot et al. 2004).

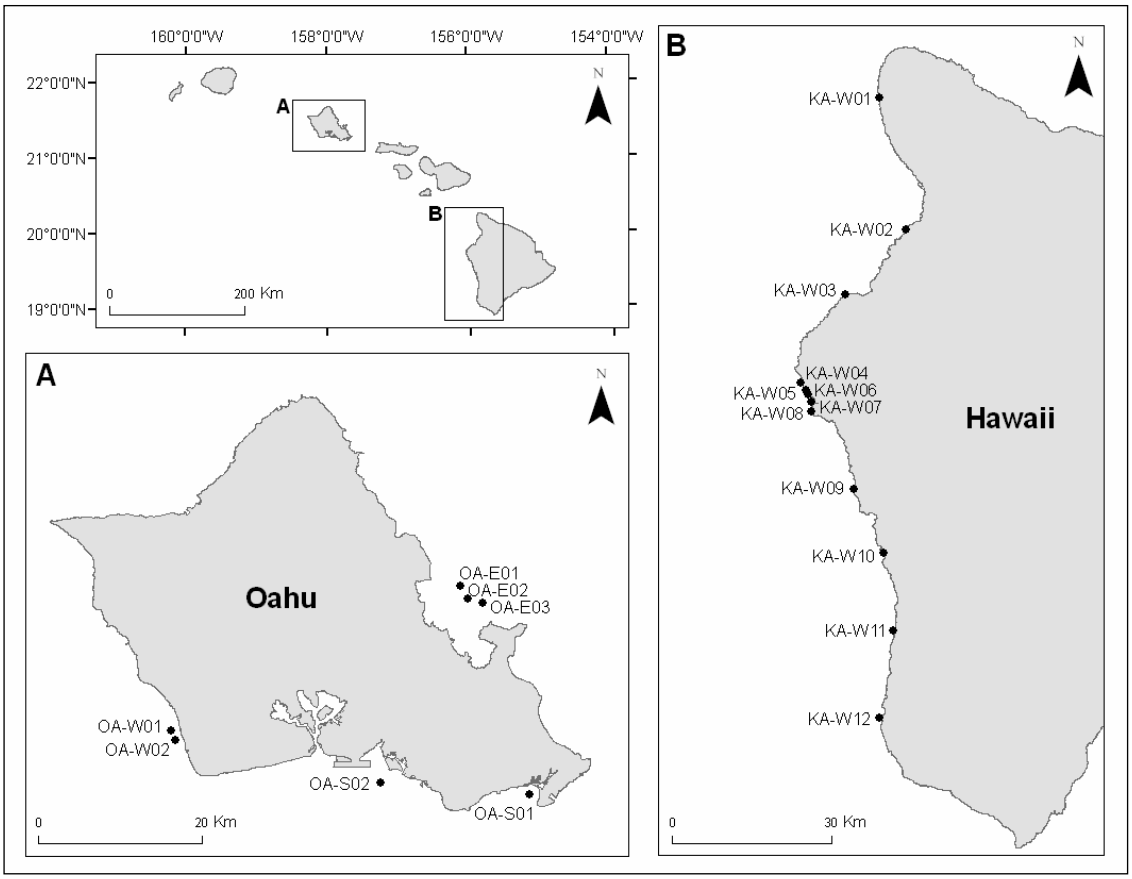

Figure 3.1. Map of the Main Hawaiian Islands (inset) and the the islands of Oahu (A) and Hawaii (B). Black dots mark sample sites. Site designators: OA = Oahu, KA = Kona; W = West, S = South, E = East. Note: no roi were caught at site OA-S01.

To determine geographic patterns in ciguatoxicity within these regions, I sampled a total of 17 sites along the West coast of Hawaii Island, and the East, South and West coast of Oahu (Figure 3.1). Sites had an average estimated area of 100 x 100 m (10,000 m^2). I designated a relative position indicator to each site for later analysis of geographic patterns (1. island abbreviation (Oahu = OA, Kona = KA), 2. coastline orientation (West (W), South (S), East (E)), and 3. relative order of sites along a coastline (numerical position indicator, “1”, “2”, “3”, etc.) (Figure 3.1).

Sampling

With a dive team consisting of five divers, I obtained 291 roi specimens (Oahu: 6 sites, n = 106; Hawaii Island: 11 sites, n = 185) by spearfishing on scuba over a 4-week period in July and August 2003. We attempted to capture each roi encountered during a dive, regardless of size. All divers perceived success rates of capture after sighting as equal for all roi sizes. Roi samples were therefore approximately random samples of populations at the sample sites. For each specimen, I recorded standard length (SL) and total length (TL) to the nearest mm, weight (W) to the nearest 5 g (for Oahu roi only, due to scale malfunctioning during Kona fieldwork), and sex based on visual inspection of gonads. SL ranged from 13.2 cm to 44.0 cm and W from 0.185 kg to 2.100 kg. I removed three samples of white muscle tissue (0.5 cm^3) from the right side of each specimen (one each from the head, body and tail region) and froze the samples at -20 ºC for ciguatera analysis.

Fish condition

Fish condition factors are based on the assumption that heavier fish of a given length are in better condition (Bolger and Connolly 1989). As measure of fish condition, I used the relative condition factor K_n (Le Cren 1951), with $K_n = W/aTL^n$, where a and n are the intercept and slope of the exponential form of the length-weight equation of the population of interest. I determined the length-weight equation for Oahu roi by least-squares regression of $\log_{10}W$ versus $\log_{10}TL$, where W is fish wet weight (g) and TL is total fish length (cm). The calculation of K_n was limited to Oahu roi because of the lack of W data on the Kona sample (see previous section).

Ciguatera analysis

I used the Membrane Immunobead Assay (MIA) for the detection of CTX following standard procedures established by Hokama et al. (1998) and Hokama and Ebesu (2001), with the following small modifications:

MIA TEST SCORES AND CTX CONCENTRATIONS: MIA scores are obtained based on the visual comparison of the coloration of test sticks with a standard scale of colorations from MIAs run on samples of known CTX concentration (Hokama et al. 1998). However, whereas Hokama et al. (1998) used a 3-step scale for the conversion of colorations into MIA scores, I used a scale consisting of 4 categories (Table 3.1) that was based on more recent work by Hokama and Ebesu (2001). The 4-step scale offers a better resolution than the previously used test.

MIA FISH SCORES AND PRACTICAL INTERPRETATION: Muscle tissue from different body regions of a fish can contain varying concentrations of CTX (Kimura et al. 1982). I determined the average CTX concentration of specimens by testing three samples of white muscle tissue (one each from the head, body and tail region) per specimen. I combined the three resulting test scores (each with a possible range of 0 – 2) to one overall fish score (possible range 0 – 6). Based on classification in one of four score classes, overall fish score can be interpreted regarding the toxicity of the fish for human consumption (Table 3.2).

Table 3.1. Scale for the determination of CTX scores from the coloration of the MIA membrane. Concentrations are rough estimates only, MIA scores were therefore used in all further analyses.

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 3.2. Conversion scale of overall fish scores (possible range: 0 (3 x 0) to 6 (3 x 2)) to relative CTX concentration score classes and practical interpretations.

Fish score range	Score class	Interpretation
0 – 0.5	Negative	Safe to eat
1 – 2	Marginal	Safe; small risk with frequent consumption
2.5 – 4	Positive	Incident possible
4.5 – 6	Strong positive	Incident likely

Score classes and interpretations are based on expert estimates on the basis of Hawaii DOH ciguatera incident records and MIAs of fish implicated in the incidents, again as previously described by Hokama et al. (1998).

Association of ciguatoxicity with geographic factors and roi morphometry

For the analysis of the relationship between fish condition and CTX score, I calculated the Pearson correlation. I assessed differences in toxicity between islands with a two sample t-test. For the analysis of the relationship of geographic location and roi size and ciguatoxicity, I used an Analysis of Covariance (ANCOVA) type general linear model (GLM) (Rutherford 2001) with site as explanatory variable, CTX score as response, and roi SL as covariate. The GLM allowed me to first test for the hypothesis of no relationship between roi size and ciguatoxicity while controlling for site differences in average ciguatoxicity, and to secondly analyze site differences in average toxicity while controlling for the potential influence of roi size as covariate to toxicity. Since *C. argus* is a protogynous hermaphrodite, I could not assess sex related differences in ciguatoxicity separately from size-related differences. I graphically analyzed site CTX means along coastlines for large-scale geographic patterns using side-by-side boxplots of site toxicity, adjusted for variability introduced by roi SL as covariate to toxicity (method of adjusted y-values as described by Lison (1968)). I used Minitab version 14 as statistical software for all analyses, and considered statistical analyses significant at $p < 0.05$.

RESULTS

Overall sample description

I screened the entire sample of 291 specimens for toxicity to gain a general overview of ciguatera in roi in the MHI. Forty-six percent of fish tested negative, 36% marginal, 14% positive and 4% strongly positive for CTX (Figure 3.2). The average fish toxicity score

was 1.25. Score variability was large and specimens with much larger than average scores occurred regularly (Table 3.3).

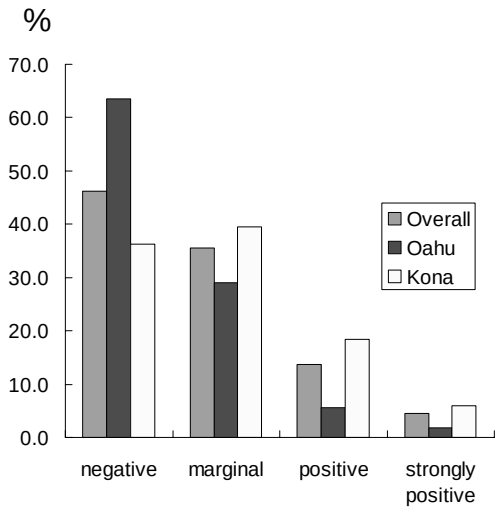

Figure 3.2. Frequency of occurrence of toxicity score classes.

Table 3.3. Roi population MIA score means ($\bar{x}$ ± 1 Standard Deviation), 95% confidence intervals (CI) and ranges in different regions in the MHI.

Region	n	$\bar{x}$	95% CI	Range
Overall	291	1.25 ± 1.40	0 – 4.0	0 – 6
Oahu	106	0.73 ± 1.07	0 – 2.8	0 – 6
Hawaii	185	1.55 ± 1.48	0 – 4.4	0 – 6

Large-scale geographic patterns in ciguatoxicity

Mean toxicity was significantly lower in the Oahu subsample compared to the Hawaii Island subsample (Table 3.3) (t-test for equality of means with sample site mean as sampling unit; DF = 10, t = 3.82, p = 0.003). Despite the low average toxicity of the Oahu sample, 7.5 % of samples fell into the positive and strong positive score categories. In the more toxic Hawaii Island sample, close to every fourth sample (24.3% of the total) fell

into the two highest toxicity categories (Figure 3.2). Boxplots of site ciguatoxicity in order of their relative geographic position did not reveal large-scale geographic gradients or patterns in toxicity (Figure 3.3).

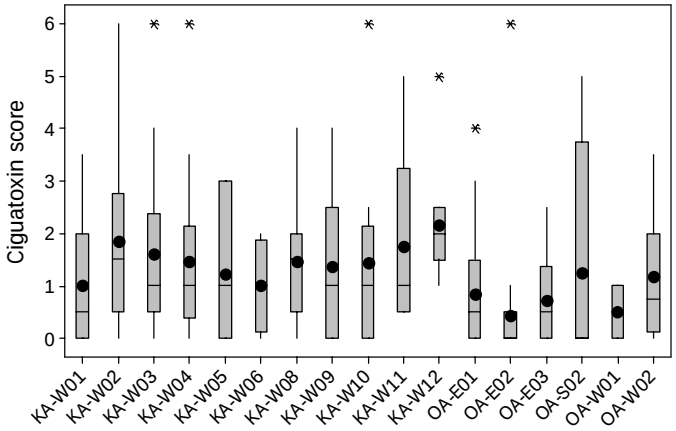

Figure 3.3. Boxplots of the ciguatoxicity of roi subpopulations, in order of the occurrence of sample sites along coastlines. Boxes represent the middle 50% of data, lines through the boxes the median, black dots the mean, and lines extending from the boxes the quartiles. Outliers are represented by asterisks (*).

Site differences, roi length and ciguatoxicity

On the next lower spatial level, I analyzed between-site and within-site patterns in toxicity. Because roi length covaries with roi toxicity, I analyzed the roles of site and roi SL in explaining toxicity in an ANCOVA type GLM (Table 3.4). Mean ciguatoxicity differed significantly between sites ($p = 0.006$), and roi SL and ciguatoxicity were significantly positively associated (toxicity = 0.032 SL, S.E. of slope = 0.015, $p = 0.030$) (Table 3.4). The interaction of site and SL was clearly non-significant (main effects GLM with added interaction term “site * roi SL”, $F = 0.72$, $p = 0.77$), which means that slopes of the regression of roi SL and ciguatoxicity did not differ significantly between sites,

and that the general process of increasing toxicity with roi size occurred consistently across sites. The total amount of variation explained by the association of ciguatoxicity with site and roi SL was 13.7%. Partitioning of variation showed that the association of ciguatoxicity and sites explained 12.2%, and the association of ciguatoxicity and roi SL 1.5% of total variation in ciguatoxicity (Table 3.4). The 87.8% of variation in toxicity that were not explained by between-site variation represent within-site variation.

Table 3.4. Analysis of variance table of the main effects GLM of site (fixed factor) and ciguatoxicity score (dependent variable) with roi SL as covariate. Adjusted mean squares are based on adjusted sums of squares calculated with all model terms included in the model.

Source of variation	Degrees of freedom	Sums of squares	Adj. mean squares	F value	p	% of variation explained
Roi SL	1	15.66	8.52	4.74	0.030	1.5
Site	16	62.38	3.89	2.17	0.006	12.2
Error	273	490.65	1.79			
Total	290	568.68				
r^2 = 13.72%						

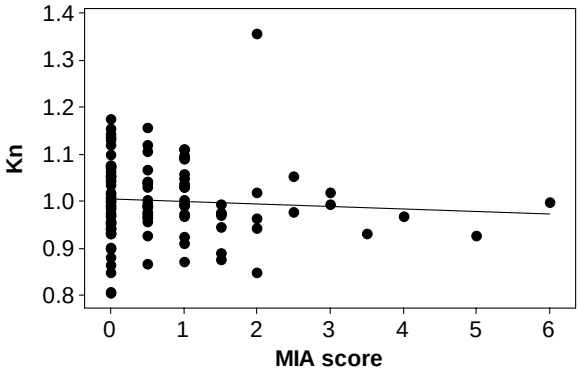

Figure 3.4. Oahu roi condition K_n plotted versus ciguatoxicity.

Table 3.5. Length-weight relationships and the relative condition factor for Oahu roi in July 2003.

N	Logarithmic L-W	Exponential L-W	K_n
110	$\log_{10}W = 3.122 \log_{10}TL - 1.9015$	$W = 0.013\ TL^{3.122}$	$K_n = W/(0.013\ TL^{3.122})$

Roi condition and toxicity

Roi condition was not significantly correlated with ciguatoxicity (Pearson correlation factor $r = -0.069$, $p = 0.480$) (Figure 3.4). The derivation of the parameters of the relative condition factor equation for the calculation of roi condition is summarized in Table 3.5.

DISCUSSION

Ciguatera in roi in Hawaii has been a biological riddle and an economic and public health problem. My results shed light on patterns of ciguatera in roi and thus offer the chance to elucidate the biological processes behind it, and to evaluate the potential of a roi fishery in Hawaii. Insight into ciguatera gained from this casestudy may be applicable to fisheries elsewhere.

The initial focus of this study lay on a general screening of roi populations in the MHI for ciguatoxicity to assess the human health risk of an unlimited roi fishery. The mean CTX score of the entire population was 1.25, on the lower end of the "marginal" category of the MIA (Table 3.2), which is interpreted as generally safe for human consumption. However, despite the relatively low average toxicity, 18.2% of roi contained CTX at concentrations potentially harmful to humans (positive and strong positive scores, Figure 3.2). How many of the toxic individuals would actually have caused ciguatera incidents is difficult to answer, because human susceptibility to ciguatera varies (Lewis et al. 1999), and because the borders for interpretation of the MIA are placed conservatively low to avoid false negative tests (i.e., classifying a toxic fish as edible) in human health studies (Hokama and Ebesu 2001). Our numbers therefore

represent conservatively high estimates. In another 35.6% of roi, CTX occurred in measurable but low concentrations (marginal category). While marginally tested fish would be generally safe for consumption, CTX can bioaccumulate in human tissue, which could lead to ciguatera incidents after frequent consumption of marginally toxic reef fish (Lehane and Lewis 2000).

The frequency of occurrence of toxic individuals in roi was much higher than the frequency observed for Hawaiian reef fishes in general (MIA, n = 25: 4% positive) in 1998 by Hokama et al.. Compared with other reef fishes that have the reputation of being ciguatoxic, the frequency of occurrence of toxic roi was slightly higher than for ulua *Caranx* spec. in Hawaii (MIA test, n = 26; 15.4% of tests positive (equals the combined positive and strong positive categories in this study) (Hokama et al. 1998)) and for amberjack *Seriola dumerili* in Hawaii (precursor of the MIA, n = 5,596; 14.7% positive and borderline (Kimura et al. 1982)). Amberjack has not been sold in fishmarkets in Hawaii since the early 1980s, because the risk of ciguatoxicity is considered too high (Shirai et al. 1991). In Florida, Bottein Dechraoui et al. (2005) detected CTX in low concentrations in 60%, and in concentrations potentially harmful to humans in 30% of great barracuda *Sphyraena barracuda*. Sale of great barracuda was prohibited in Florida due to concern about ciguatera (De Sylva 1994). Roi toxicity in my sample was thus comparable to that of fishes in Hawaii and Florida that are considered dangerous for human consumption. Considering the potential severity of ciguatera fish poisoning in humans (Bagnis 1968), a large-scale fishery for roi does not appear feasible.

GEOGRAPHIC PATTERNS IN CIGUATOXICITY: Understanding of geographic patterns in roi toxicity in Hawaii might still lead to safe fisheries of limited geographic extent. I concentrated on three spatial scales within the Hawaiian Islands: (i), on the scale of islands, (ii), on patterns along the coasts of islands, and (iii), on the scale of sites. Between islands, mean toxicity of roi differed significantly (Table 3.3). The mean toxicity of Oahu roi ($\bar{x}$ = 0.73) was near the upper limit of the "negative" MIA score class (Table 3.2), which is considered absolutely safe for consumption. Even the much higher Hawaii Island mean toxicity ($\bar{x}$ = 1.55) still falls into the "marginal" score class generally considered as safe. However, variability in toxicity was large in both island roi populations (Table 3.3). As a consequence, on Oahu, despite the low mean toxicity, toxic (outlier) scores occurred regularly, and 7.5% of roi contained toxin at concentrations potentially harmful for consumption (Figure 3.2). The risk of acquiring ciguatera from Oahu roi appears unacceptably large for a fishery. For Hawaii Island, the case is even more unambiguous, as potentially harmful roi individuals were more than three times as common as on Oahu (24.3% of cases).

Large-scale spatial gradients in toxicity along coastlines were not present (Figure 3.3: no evidence of regular changes in mean toxicity). Furthermore, no coastline or part thereof was completely free of ciguatoxic roi (Figure 3.3), and of all sites, only site OA-W01, on the West coast of Oahu, did not have positive or strong positive individuals. However, the sample size for this site was very small (n = 3), and the site did not represent a larger geographic trend, as toxic roi did occur at the neighbor site OA-W02. These results agree with the general pattern of ciguatera incidents in the MHI, which have been caused by fish from various islands, coastlines, and regions (Bruno and Effler

2001b, Afton 2003), but stand in contrast to the pattern described by Lewis (2001) in the Republic of Kiribati, where the southern reef of the atoll of Tarawa and the western reef of the atoll of Maraki have a high risk of ciguatera, whereas all remaining reefs are low risk areas. The Kiribati pattern offers the resource management solution of closing only the toxic reef or coastline to fishing. My findings indicate that such a solution is not feasible for roi in Hawaii.

Despite the lack of regular patterns across sites, mean roi toxicity differed significantly between sites (Table 3.4, Figure 3.3). At the same time, independent of mean toxicity, all sites were characterized by large variability in toxicity and by common occurrence of toxic outliers (Figure 3.3: note the broad spread of scores and the regular presence of positive outliers). This situation is reflected in the large importance of within-site variation for overall variation in toxicity (Table 3.4: between-site variation explained only 12.2% of total variation, whereas the remaining 87.8% represent within-site variation), and has the corollary that even full understanding of factors causing differences in ciguatoxicity between sites would not be sufficient for good understanding of patterns leading to the toxicity of roi. Regarding resource management, the consistent occurrence of toxic roi even at sites with low average toxicity indicates that safe sites for roi fishing are rare.

ROI MORPHOMETRICS AND CIGUATOXICITY: A strong correlation between morphometrics and ciguatoxicity would strongly enhance our ability to predict the toxicity of roi caught in the wild. In this section, I address the associations of (1) roi size and ciguatoxicity, and (2) roi condition and ciguatoxicity.

Regarding (1), since roi length varied strongly within roi subpopulations at each site, could the high variability of toxin concentrations within subpopulations be linked to variation in roi size? My data suggest that while a link did exist, it did not explain much of the variation in toxicity. On average, large roi were slightly more toxic than small roi, but there were many exceptions to this pattern, including large roi that were completely free of CTX, and small roi that were highly toxic. This is reflected in the weakness of the (significant) correlation of ciguatoxicity with roi SL (r^2 = 1.5%, Table 3.4).

In contrast to the situation in roi, Helfrich et al. (1968) found strong bioaccumulation of CTX with age in *Lutjanus bohar* in the Line Islands; furthermore, Lehane and Lewis (2000) reviewed the literature on the subject and state that for most fish species, ciguatoxicity increases with length due to bioaccumulation over the lifespan of fish. They suggest marketing only fish below a "cut-off size" for these species. However, Lewis and Holmes (1993) cautioned that bioaccumulation does not necessarily occur in all cases, based on the finding that the half-life of CTX (i.e., the time required for toxin concentration to decrease to 50% of its initial value, e.g., due to excretion or breakdown of toxin molecules) in moray eel tissue is only 264 days (Lewis et al. 1992) and that *G. toxicus* blooms are not continuous in time (Gillespie et al. 1985).

Considering that the MHI are one of the less ciguatoxic island groups in the Pacific (Lewis 1986), it seems likely that *G. toxicus* blooms in Hawaii are rare compared to more toxic locations. Consequently, a decrease of CTX in roi tissue could occur between blooms independently of roi size as long as the CTX half-life is in the general range of the one found for moray eels. In view of the longevity of roi of up to 16 years in Hawaii (Howard Choat, unpublished data), the resulting scenario would be repeated

cycles of high toxicity after *G. toxicus* blooms and decreasing toxicity in the periods between blooms over the lifespan of a roi, which would explain the weakness of the association of toxicity and size that I observed in roi. A 4-year timeseries of ciguatoxicity of the roi population at Kukio Bay, Hawaii Island, supports this line of thought, as it showed large interannual fluctuations in ciguatoxicity (HDAR, unpublished data).

Species differences in CTX half-life or differences in frequency of *G. toxicus* blooms could explain the stark contrast between previous reports of strong bioaccumulation of CTX with age and the lack of bioaccumulation observed by Lewis and Holmes (1993) and me. Specifically, bioaccumulation would occur if half-lifes are long or if blooms are frequent, but not if half-lifes are short or blooms infrequent.

The significance, if very weak, of the association of toxicity and roi length may be due to dietary patterns of roi: large roi in the MHI fed on carnivorous fishes more commonly than small roi (Dierking, unpublished data), and carnivorous fishes are on average more ciguatoxic than herbivorous fishes (Lehane and Lewis 2000). Large roi would therefore on average take up slightly more CTX than small roi, but fluctuations in toxicity over the life-span of roi would occur independently of these differences.

In summary, high variability in roi toxicity within sites was only in small parts linked to size distributions of roi at the sites. Consistent bioaccumulation of toxin over the lifespan of roi did therefore not occur. Consequently, roi length is not a suitable indicator of roi toxicity, and size limits below which fish are safe to eat cannot be applied in fishery management of roi.

As second morphometric character, I considered roi condition. CTX has negative effects on most mammals, as well as species from orders of several different phyla including freshwater fishes (Hokama and Ebesu 2001). Regarding marine fishes, while some species show no negative effects from CTX in their tissue (Helfrich and Banner 1963), other species can be impacted by CTX (Capra et al. 1988, Lewis 1992). I tested the hypothesis of the presence of a negative correlation of ciguatoxicity and roi condition. In contrast to this hypothesis, roi condition was clearly independent of CTX concentration in roi tissue ($p = 0.480$; also see Figure 3.4). Since the condition of roi was not influenced by CTX, the ciguatoxic roi cannot be distinguished from non-ciguatoxic individuals based on their weight-at-length (i.e., on their K_n value).

THE PATTERNS BEHIND THE PATTERNS: The foodchain concept of ciguatera (Randall 1958) suggests that patterns of fish toxicity are linked to prior variation in *G. toxicus* abundance. Patterns of *G. toxicus* blooms are to date not well understood. In this section, I attempt to increase our understanding of blooms in Hawaii, by deducting underlying patterns of *G. toxicus* blooms from patterns of toxicity in roi. The outstanding pattern of roi toxicity in the MHI was the large amount of unexplained within-site variability, which made the occurrence of toxic individuals appear unpredictable. Which hypothetical pattern of *G. toxicus* blooms could account for this pattern?

Roi live in harems occupying home ranges with distinct spatial boundaries, which are maintained by active defense against intrusion from neighbor harems. Home ranges are distinct in space and continuous in space over time, and much smaller (100 to 1,000 m^2 depending on roi sex and size) (Amanda Meyer, unpublished data) than the extent of

my sample sites (10,000 m^2). Each sample site therefore encompassed multiple non-overlapping roi territories. As described above, CTX was present at all sites, but at highly variable concentrations between individuals at each site. Assuming that *G. toxicus* blooms were spatially heterogeneous on scales smaller than my sample sites, only some of the roi territories within each sample site would overlap with blooms at any given time, which could lead to spatially highly variable (point) uptake of toxin into roi populations. Researchers in Hawaii (Hokama et al. 1996) and elsewhere (Gillespie et al. 1985) found that *G. toxicus* may occur in small, localized patches that are highly heterogeneous on larger spatial scales, supporting my hypothesis.

The point uptake model provides a possible explanation of why two roi of equal size from the same sample site commonly differ strongly in toxicity. I suggest that high spatial variability of *G. toxicus* blooms is present in Hawaii, and that this pattern is a decisive factor causing the large variability of toxicity within roi populations observed in this study.

CONCLUSIONS

In summary, I assessed patterns of CTX occurrence in roi populations in the MHI to enhance scientific understanding of ciguatera in roi, and to determine whether a safe roi fishery could be established in Hawaii. I found that overall occurrence of toxic roi individuals was too frequent to establish such a fishery. Island and site populations of roi differed significantly in mean toxicity, with Oahu roi on average safer for consumption than Hawaii Island roi. However, variability in ciguatoxicity was large on all spatial scales that I analyzed, no regular geographic patterns or gradients were apparent, and no

coastline or site tested was completely safe. This means that geographically limited fisheries for roi are not risk-free, either. The condition of roi individuals was not related to their toxicity, and while small roi were on average safer for consumption than large roi, this pattern was too weak to allow use of roi size as an indicator of roi toxicity. Consequently, roi morphometrics cannot be applied in fishery management. Regarding biological patterns causing toxicity in roi, my data suggest that *G. toxicus* blooms in Hawaii must be spatially highly heterogeneous on scales smaller than my sample sites. I conclude that the factors analyzed here are associated too weakly with ciguatoxicity to allow reliable prediction of the toxicity of roi individuals in the future. A safe roi fishery based on simple factors is therefore not feasible.

CHAPTER 4:

THE EFFECTS OF CIGUATERA ON FITNESS AND GROWTH OF THE GROUPER *Cephalopholis argus* IN THE WILD.

Manuscript

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ABSTRACT

The neurological disease ciguatera is acquired by the consumption of reef fish containing ciguatoxin (CTX). CTX is produced by the benthic marine dinoflagellate *Gambierdiscus toxicus*, and accumulates through the marine foodchain after initial uptake by herbivores through grazing on benthic macroalgae with attached *G. toxicus*. CTX negatively affects humans and other mammals, as well as species from diverse orders in different animal phyla. In contrast, it is commonly thought that marine fishes are not negatively affected by CTX because they can act as carriers of CTX without apparent symptoms of disease. This is put in doubt by studies that found negative impacts of CTX on marine fishes in experimental settings. Field studies on the effects of CTX on marine fishes in the natural environment are needed to complete the picture, but such studies have been scarce or non-existent. To provide one case study, I analyzed the association of CTX concentrations with fitness and growth in a wild-caught sample of 292 specimens of the grouper species roi (*Cephalopholis argus*) from Hawaii, where the species is a common ciguatera carrier. I used the Monoclonal Immunobead Assay (MIA) for the detection of CTX, length-weight equations and the relative condition factor as measure of roi fitness, and length-at-age as measure of the long-term growth of roi. No significant associations were detectable, which led me to conclude that CTX does not negatively affect roi fitness or growth at concentrations that naturally occur in wild roi in Hawaii.

INTRODUCTION

Ciguatera is a neurological disease with potentially severe clinical symptoms in humans (Palafox and Buenconsejo-Lum 2001) that is acquired by consumption of ciguatoxic reef fish (Bagnis et al. 1979). It is confined to the tropics and subtropics, where it has important public health and economic implications (Lewis 2001). The annual number of human ciguatera cases range from 25,000 to 50,000 worldwide (Lehane and Lewis 2000).

The physiological mechanism behind the disease is a destabilization of the sodium ion (Na^+) balance across cell membranes due to the induction of active sodium ion channels by ciguatoxin (CTX) molecules (Hokama and Ebesu 2001). CTX is a polyether toxin produced by the benthic marine dinoflagellate *Gambierdiscus toxicus* (Yasumoto et al. 1980). It enters the marine foodchain when herbivores consume benthic macroalgae with attached *G. toxicus* (Campbell et al. 1987), is stored in body tissues, and is transferred to higher trophic levels of the foodweb when predators prey on toxic herbivores (Banner 1974). Groupers (Serranidae) and jacks (Carangidae) are the fish families most commonly associated with ciguatera incidents in humans, but a total of over 400 species has been implicated in ciguatera outbreaks (Lehane and Lewis 2000).

Negative effects of CTX are not confined to humans. They have been observed in most mammals species, and in species from various phyla, including freshwater fishes (Hokama and Ebesu 2001). In contrast, the effect of CTX on marine fishes is controversial. It is commonly assumed that negative effects of CTX do not occur in wild fish populations, based on the observation that ciguatoxic marine fishes can act as carriers of CTX without apparent symptoms of disease (e.g., Banner 1974). At the same time, neurophysiological (in vitro) Na^+ efflux studies indicate that the Na^+ channels of marine

fishes are susceptible to CTX, and that effects are similar to those found in mammalian Na^+ channels (Capra et al. 1987). In addition, laboratory and mesocosm studies in vivo have found negative effects of CTX on marine fishes, ranging in intensity from behavioral changes to morphological changes and death (e.g., Davin et al. 1986, Capra et al. 1988, Davin et al. 1988, Lewis 1992, Gonzalez et al. 1994).

While negative effects of CTX on marine fishes are thus inducible in experimental settings, expansion of conclusions to natural food chains may not be justified, in part because naturally occurring toxin concentrations may be lower than experimental ones (Brusle 1995). Case studies on the effects of CTX in wild fish populations are needed to expand the picture, but such studies have been rare or non-existent (Brusle 1995, Lehane and Lewis 2000, Landsberg 2002).

In this setting, the goal of this study was to determine the effects of CTX on individuals of a wild fish population known to contain ciguatoxic individuals. I obtained a sample of 292 specimens of the grouper species roi (*Cephalopholis argus*) from the Main Hawaiian Islands (MHI), where the species is highly ciguatoxic with large variability in toxicity between individuals (Chapter 3 of this dissertation), and consistently among the top species associated with human ciguatera incidents (Afton 2003). I determined CTX concentrations in muscle tissue of all specimens with the Membrane Immunobead Assay (MIA) (Hokama et al. 1998), and analyzed the association of CTX concentrations with the fitness of individuals, as measured by length-weight relationships and the relative condition factor (Bolger and Connolly 1989), and with growth over time, as measured by intra-annual variation in length-at-age data (Moreau 1987).

MATERIAL AND METHODS

Choice of sample locations and study organism

I chose *C. argus* as study organism because it is one of the most consistently ciguatoxic fish species in the MHI (Afton 2003). The species is of special interest in Hawaii because it is an introduced species that may threaten native species (Randall 1987). Groupers in general are among the fish families most commonly associated with ciguatera in worldwide comparison, and are thus of special interest in ciguatera studies (Lehane and Lewis 2000). I collected roi samples from Oahu and Hawaii Island, where roi regularly cause ciguatera incidents (Bruno and Effler 2001a).

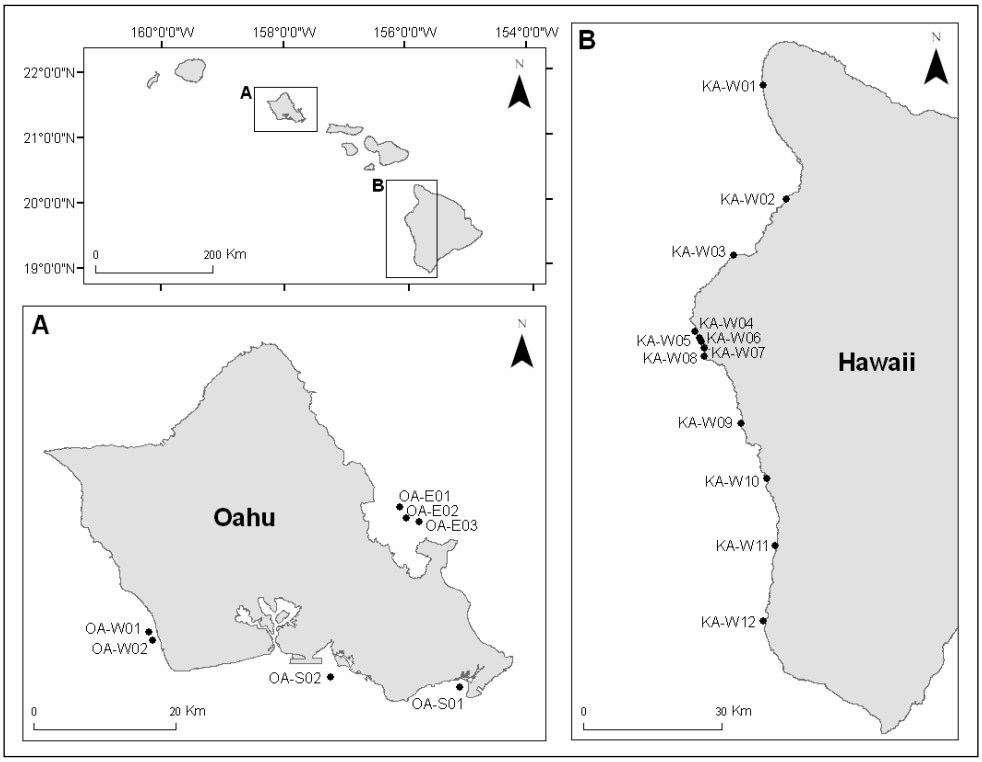

Figure 4.1. Map of the Main Hawaiian Islands (inset) and the the islands of Oahu (A) and Hawaii (B), with sample sites marked by black dots.

Sampling

With a team of five divers, I obtained 291 roi specimens (Oahu: 6 sites, n = 107; Hawaii Island: 11 sites, n = 185) (Figure 4.1) by spearfishing on scuba over a 4-week period in July and August 2003. We attempted to capture each roi encountered during a dive, and all divers perceived success rates of capture after sighting as equal for roi of different sizes and behavior patterns. For all specimens, I recorded standard length (SL) and total length (TL) to the nearest mm. For Oahu roi, I also recorded weight (W) to the nearest 5 g. No Ws were obtained for Kona roi due to scale malfunctioning during Kona fieldwork. Roi SL ranged from 13.2 cm to 44.0 cm and W from 0.185 kg to 2.100 kg. I took samples of white muscle tissue from each specimen and froze them at -20ºC for ciguatera analysis. Otoliths of all specimens were removed for age analysis.

Ciguatera analysis

I tested a total of 291 roi with the Membrane Immunobead Assay (MIA) for the detection of CTX following standard procedures as described by Hokama et al. (1998) and Hokama and Ebesu (2001), with slight modifications as described in Chapter 3 of this dissertation. CTX concentration in muscle tissue of fish individuals is expressed as an MIA score ranging from 0 (no CTX detectable) to 6 (very high CTX concentration) in steps of 0.5. For analytical purposes, I grouped MIA scores into three score classes (scores 0 - 0.5 = “low”, 1 - 2 = “medium”, 2.5 - 6 = “high”) in some of the analyses. The classes are based on score categories used in the practical interpretation of the MIA when testing fish for human consumption, with fish in the low score category considered safe for consumption,

fish in the medium a potential risk, and fish in the high category likely to cause ciguatera in humans (Hokama et al. 1998).

Age analysis

The age of 193 roi haphazardly chosen from my sample was determined by otolith analysis in the laboratory of Howard Choat, using the following methodology: roi sagittae were removed, cleaned, washed in ethanol and stored dry. One sagitta from each pair was weighed to the nearest 0.1 mg. This was mounted on the edge of a glass slide in thermoplastic glue and ground to the core using 800 or 1200-grade wet and dry sandpaper. Subsequently, the glue was re-heated, the otolith placed with the core flat against the glass slide and the opposite end ground, leaving a thin section through the core mounted in the glue on the glass slide. The thermoplastic glue was then spread thinly across the section to increase the clarity of the increments. Sectioned otoliths were examined under dissecting microscopes using transmitted light. Counts were made along a consistent axis following Choat and Axe (1996). Each otolith was read at least 2 times for annuli. Any discrepancy resulted in a 3rd reading. Any sagittae that were obviously 0+ age categories were polished further and counts of presumed daily rings made under high power microscopes using transmitted light.

Fish condition and growth

Fish condition factors are based on the assumption that heavier fish of a given length are in better condition (Bolger and Connolly 1989). As measure of fish condition in the Oahu roi population, I used the relative condition factor $K_n = W/aTL^n$, where a and n are the

intercept and slope of the exponential form of the length-weight equation of the population of interest (Le Cren 1951). I determined the length-weight equation for Oahu roi by least-squares regression of $\log_{10}W$ versus $\log_{10}TL$, where W is fish wet weight (g) and TL is total fish length (cm). I previously described the derivation of the K_n-equation for roi from the logarithmic length-weight equation (Table 4.1) in Chapter 3 of this dissertation.

Table 4.1. Derivation of the relative condition factor for Oahu roi.

N	Logarithmic L-W	Exponential L-W	K_n
110	$\log_{10}W = 3.122 \log_{10}TL - 1.9015$	$W = 0.013\ TL^{3.122}$	$K_n = W/(0.013\ TL^{3.122})$

I used length-at-age as measure of long-term growth. I obtained length-age curves for the Oahu and the Hawaii Island roi populations by linear regression of TL versus $\log_{10}$ of age. The age data transformation resulted in linearized length-at-age curves, due to declining growth rates with increasing age in, which are typical for most fishes (Moreau 1987). The linear form of the regression facilitated residual analysis. For the length-age range present in my sample, the regression fit was equal to the fit obtained using the more complicated von Bertalanffy model commonly applied to describe fish growth (Moreau 1987).

Analysis of the effect of CTX on roi fitness and growth

For the analysis of the effect of CTX on fish fitness, I compared logarithmic length-weight regressions of roi of low, medium and high toxicity for differences in intercept or slope by graphic analysis and by use of an ANCOVA (analysis of covariance) type

General Linear Model (GLM). I used a full GLM (with interaction term) with $\log_{10}W$ as response variable, toxicity class as explanatory variable, and $\log_{10}TL$ as covariate. Secondly, I used a one-way ANOVA to test for differences in mean relative condition factors of roi of low, medium and high toxicity. Due to differences in sample sizes and variances for roi of different toxicity, I took random subsamples from roi of low and medium toxicity to obtain a balanced statistical design (Underwood 1997).

For the analysis of the effect of CTX on long-term growth, I used the relationship of TL versus $\log_{10}Age$. I graphically analyzed Oahu and Hawaii Island roi populations separately, because length-at-age differed significantly between islands (ANCOVA with TL as response variable, Island and Age as explanatory variables, $df_{Island} = 1$, $df_{total} = 192$, $F_{Island} = 32.51$, $p_{Island} < 0.001$). In addition, I previously found that the Hawaii Island roi population is two times more ciguatoxic than the Oahu population (Chapter 3 of this dissertation), providing the opportunity to test for the role of CTX in a weakly and a highly toxic roi population. My measure of the potential influence of CTX on long-term growth was intra-annual (within year class) variation in length-at-age, based on the premise that individuals that are consistently affected by a toxin would attain a smaller length in a given time than individuals that are growing unimpededly. I grouped data in both island datasets by toxicity (low, medium, high) and analyzed length-at-age scatterplots for systematic intra-annual differences in length between toxicity classes. I further analyzed residuals of the length-at-age regressions plotted versus MIA scores.

I used Minitab 14 for all statistical analyses, and considered results significant at $p<0.05$.

RESULTS

Effect of CTX on fitness

LENGTH-WEIGHT CURVES BY CIGUATOXICITY: My results show that mean weight-at-length did not differ significantly between toxicity classes (Table 4.3: $p_{toxicity} = 0.828$). Furthermore, differences in regression slopes and intercepts for roi of low, medium and high toxicity were small (Table 4.2) and insignificant (Table 4.3: $p_{toxicity*logTL} = 0.830$), leading to strong overlap of the regression lines (Figure 4.2).

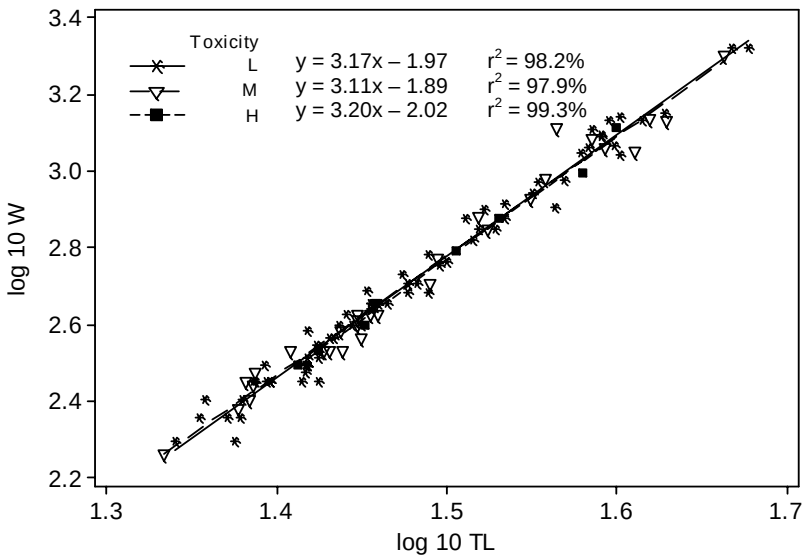

Figure 4.2. Logarithmic W-TL regressions, based on 106 Oahu roi. Data are grouped by toxicity, with separate regression lines for the roi of low (L), medium (M) and high (H) toxicity.

Table 4.2. Slopes and intercepts of the logarithmic L-W equation, and mean condition factors (±1SE) for Oahu roi of different toxicities.

Toxicity	N	L-W slope	L-W intercept	K_n
Low	68	3.17±0.05	-1.97±0.08	1.00±0.01
Medium	31	3.11±0.08	-1.89±0.13	1.00±0.02
High	8	3.20±0.11	-2.02±0.16	0.98±0.02

Table 4.3. Analysis of variance table of the full GLM of $\log_{10}W$ (dependent variable), toxicity (fixed variable) and $\log_{10}TL$ as covariate. The interaction term toxicity*$\log_{10}TL$ is an indicator of differences between regression slopes. Adjusted mean squares are based on adjusted sums of squares calculated with all model terms included in the model.

Source of variation	Degrees of freedom	Sums of squares	Adj. mean squares	F value	p
$\log_{10}TL$	1	7.1758	2.5341	1879.61	<0.001
Toxicity	2	0.0005	0.0003	0.19	0.828
Toxicity*$\log_{10}TL$	2	0.0005	0.0003	0.19	0.830
Error	101	0.1362	0.0013		
Total	106	7.3130			
r^2 = 98.14%					

RELATIVE CONDITION AND CIGUATOXICITY: Mean relative condition of Oahu roi of low, medium and high toxicity did not differ significantly (ANOVA $_{df = 2, 23}$, $F < 0.13$, $p = 0.88$). Condition could not be calculated for Kona roi, due to the lack of W data for that population (see Materials and Methods).

Effect of CTX on growth

Linear regressions of TL versus $\log_{10}$ of age for Oahu and Hawaii Island roi provided a good fit to the data (Figure 4.3a, b), and residuals of the regressions plotted versus observation order showed no systematic deviation from the regression over the dataset range (figures not shown). At the same time, r^2 values of the regressions (r^2_{Oahu} = 68.2%, $r^2_{Hawaii\ Island}$ = 63.3%) indicate that a significant amount of scatter occurred around both length-age regression lines. Differences in the distribution of scatter for roi of low, medium and high toxicity around the regression lines were not apparent in scatterplots of the grouped data (Figure 4.3a, b). Plots of residuals of the length-age regression versus

toxicity (Figure 4.4a, b) further confirmed the lack of systematic deviations from the regression related to ciguatoxicity.

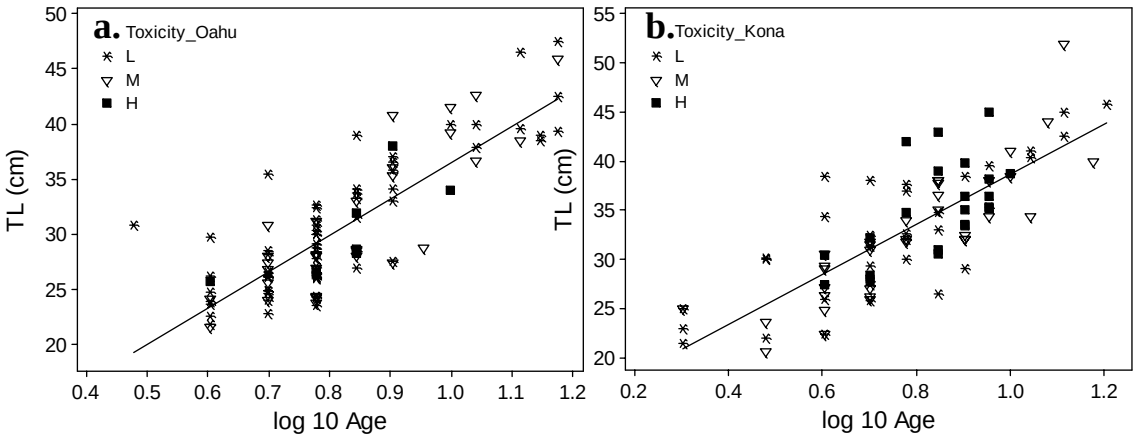

Figure 4.3. Total length plotted against $\log_{10}$age, grouped by toxicity. The regression lines are based on the ungrouped total samples. a. Oahu; b. Hawaii Island

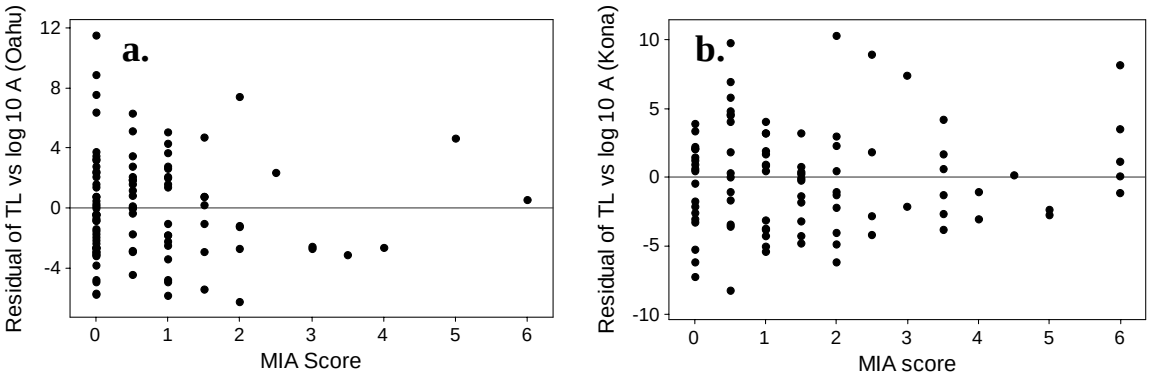

Figure 4.4. Residuals of the regression TL-$\log_{10}$Age vs. ciguatoxicity. a. Oahu; b. Hawaii Island

DISCUSSION

Whether negative effects of CTX occur in marine fish populations in the wild has been a controversial question (e.g., Brusle 1995, Lehane and Lewis 2000, Landsberg 2002). My results indicate that in roi in the wild in Hawaii, CTX did not have an effect on either the fitness or on the long-term growth of individuals.

Regarding the effect of CTX on fitness, analysis of the length-weight relationships of roi of low, medium and high ciguatoxicity showed that length-weight equations for the three groups were very similar (Figure 4.2). The insignificant difference in mean weight-at-length of roi of different ciguatoxicity (Table 4.3: row "Toxicity") indicated that toxic roi were not generally depressed in fitness. Similarly, the insignificant differences in slopes of the length-weight regressions for roi of different toxicity classes (Table 4.3: "row Toxicity*$\log_{10}$TL") indicated that fitness of toxic roi followed the same pattern across the range of body sizes in my sample as non-toxic roi. More evidence against a negative effect of CTX on roi comes from the non-significant difference between the mean relative condition factors of roi of different toxicities (ANOVA, $p = 0.88$), which indicated that on average, roi of low, medium and high toxicity were equally fit.

Regarding the effect of CTX on growth, analysis of length-at-age relationships showed that roi of low, medium and high ciguatoxicity did not differ systematically in length at a given age and therefore growth rates over time in either the Hawaii Island or the Oahu population (Figure 4.3a, b: lack of patterns in intra-annual variation in length grouped by toxicity). In particular, highly toxic roi did not appear more frequently below the common length-age regression line than above, and roi with low toxicity did not appear more frequently above the regression line than below, which should have been the case if CTX had had an impact on growth rates.

The graphic analysis was supported by the analysis of residuals of the length-age regressions, which were independent of toxicity (Figure 4.4a, b). The apparent decrease

in variability of residuals with increasing toxicity likely is an artifact of the decreasing sample sizes for roi of higher toxicity due to the infrequent presence of highly toxic roi individuals in nature. This interpretation is supported by the fact that for the Hawaii Island sample, which had a higher average toxicity and more toxic samples than the Oahu sample, variability in residuals was much more balanced than for the Oahu sample.

In summary, roi with high CTX concentrations in their tissue did not grow more slowly over time than roi with low CTX concentrations.

Due to the lack of quantitative work on the effects of CTX on marine fish populations in the wild, including studies using quantitative measures of fish health such as the length-weight and length-age relationships presented here (e.g., Landsberg 2002), the direct comparison of my results with previous fieldstudies is not possible. Experimental work on CTX in marine fishes and previously stated hypotheses of the role of CTX in natural settings nevertheless provide a framework for discussion.

Lewis (1992) proposed that negative effects of CTX on wild fish populations could lead to impairment and subsequent vulnerability to predation or to death by disease. He suggested that this mechanism was the explanation for the rarity of human deaths from ciguatera after fish consumption, because highly toxic fish would not reach human consumption. However, neither Lewis nor other researchers have presented evidence for (or against) this hypothesis. My results contradict Lewis' hypothesis, with an important caveat: in worldwide comparison, Hawaii is on the low end of ciguatera incident rates (Lewis 1986). According to Lewis, incident rates in several locations in the Pacific, e.g., in French Polynesia, are more than 100 times higher than in Hawaii (> 500 versus < 5

cases per year per 100,000 inhabitants), which suggests that CTX concentrations in wild fish populations can be much higher than in Hawaii. While even the most toxic individuals in my sample of 291 roi did not show signs of diminished fitness or lowered growth rates, my results do not exclude the possibility that negative effects of CTX set in above a large threshold concentration that is not reached in Hawaii. The experimental studies showing negative effects up to death of fishes from large doses of CTX (e.g., Davin et al. 1988, Landsberg 2002) support the possibility of such a threshold level occurring. At the same time, my study shows that concerns about the indiscriminate expansion of results of experimental work on CTX in captive fish to the natural environment (e.g., Brusle 1995) were justified.

My results raise an additional question: how could CTX have no detectable effect on roi, although Capra et al. (1987) consistently detected physiological activity of the CTX molecule on marine fish Na^+ channels in vitro? In the same context, how can highly toxic specimens in my sample show no negative effects of CTX, even though they would have been likely to cause ciguatera incidents upon consumption by humans (see the Material and Methods section for the practical meaning of score classes)? These discrepancies suggest the existence of a raised physiological tolerance to CTX of roi compared to humans. Hahn et al. (1992) demonstrated that CTX associates with a monomeric soluble protein in toxic muscle of the narrow-barred Spanish mackerel. Capra (1997) suggested that this association may be the basis of a sequestration mechanism that diminishes the binding of CTX to the target sites of the Na+ channels. Although my

results do not implicate a specific mechanism, they do support Capra's general hypothesis of the existence of a sequestration mechanism in marine fishes.

Because *G. toxicus* and thus CTX have been present in marine systems since at least prehistoric times (Hokama and Ebesu 2001), mechanisms leading to immunity to CTX may be an evolutionary adaptation to selective pressure imposed by the negative effects of CTX. In contrast, the selective value of toxicity to *G. toxicus* is less clear. Since herbivore consumers of macroalgae are not thought to distinguish between algae settled or not settled by *G. toxicus*, CTX may be an accidental byproduct and not a defense.

To conclude, I found no effects of CTX on either fitness or growth of roi in Hawaii, which suggests that in moderately ciguatoxic locations, marine fishes may be immune to detrimental effects of CTX at the concentrations naturally encountered in the wild.

CHAPTER 5:

SUMMARY AND CONCLUSIONS

Results of my doctoral research advance our understanding of the feeding ecology of roi and of ciguatera in roi. To avoid redundancy, I will not repeat conclusions specific to individual chapters here. Instead, I will use the newly available information from Chapters 2, 3 and 4 to address the two questions that I posed in the General Introduction (Chapter 1), namely,

(1) What are the ecological effects of roi predation on native species? and

(2) Could better understanding of ciguatera in roi lead to a safe fishery for roi?

I will close with a discussion of new questions that were raised in the course of my work, and of the future research directions that appear most promising to me.

FEEDING ECOLOGY

In Chapter 2, I showed that the magnitude of roi consumption was on the scale of consumption reported for the groupers *Cephalopholis cyanostigma* and *Cephalopholis boenak* on reefs at Lizard Island, Australia, by Beukers-Stewart and Jones (2004). In this only other published study on consumption by grouper populations to date, the authors concluded that the effect of the two groupers on prey fish populations was large. Regarding the first question that I posed above, the similar scale of consumption suggests that roi may play an important ecological role in Hawaii. This conclusion is supported by the population density of roi in Hawaii, which was on the scale of densities of groupers

elsewhere that are considered keystone predators (e.g., Goeden 1982, Chiappone et al. 2000, Sluka et al. 2000, Zeller and Russ 2000).

Surprisingly, many questions about the effects of predation remain unanswered in the field of reef fish ecology (reviewed by Hixon 1991, Hixon and Webster 2002). Most evidence to date for the importance of predation as a process regulating reef fish abundances stems either from experimental (e.g., Hixon and Webster 2002) or correlative (e.g., Hixon and Beets 1989) studies on predator-prey assemblages on small artificial reefs, or from studies that followed cohorts of recruits in the wild through time, assessing mortality rates (reviewed by Hixon 1991). Survivorship typically followed type III survivorship curves (Deevey 1947), with steep initial declines in abundance after settlement that indicates high mortality. While the observed patterns have commonly been attributed to predation (e.g., Connell 1996, Doherty et al. 2004), little specific evidence implicating predation has been presented to date (Hixon 1991). The approach that I took in Chapter 2 was different. Here, the large magnitude of consumption by roi and the focus of roi predation on small prey individuals indicate that predation is likely to play an important role. The observed patterns of predation fit the general patterns of mortality of reef fishes observed in previous studies (see above), and thus provide circumstantial evidence for the important role of predation in causing high mortality rates in recent settlers and recruits.

CIGUATERA

Chapter 3 showed that levels of ciguatoxin in 18.2% of roi were potentially dangerous for human consumption, and that roi from all tested locations, and of all sizes, could be toxic.

Regarding Question (2) posed above, this suggests that a safe fishery for roi, even on a limited geographic scale or for a limited size range of roi, is not feasible at present.

It should be noted that in recent years, a small number of fishermen has been selling a limited number of roi to restaurants, and catching roi for home consumption without conducting prior tests for ciguatoxicity (Shawn Fujimoto, personal communication; Derek LeVault, personal communication). If – as these reports suggest – roi is regularly consumed, the relatively low number of 4 – 5 reported ciguatera incidents per year attributed to consumption of toxic roi (Bruno and Effler 2001a) seems lower than my findings on the high prevalence of toxic roi would suggest. This may indicate that either, ciguatera incidents from roi consumption are underreported in Hawaii, or, that the Membrane Immunobead Assay (MIA) (Hokama et al. 1998) that I used to determine ciguatoxin concentrations in roi overestimates concentrations of ciguatoxin. Both explanations may hold some truth, as studies of the epidemiology of ciguatera have indicated that the disease is often misdiagnosed and commonly not reported (Lehane and Lewis 2000), and as the MIA test boundaries indicating dangerous toxin concentrations were set conservatively low on purpose to avoid false negative results (i.e., fish that test negative for toxicity, but in reality are toxic enough to cause ciguatera in humans), due to its application in the screening of fish intended for consumption (Yoshitsugi Hokama, personal communication).

In any case, levels of toxicity considered unsafe in Hawaii may be considered acceptable in locations that have higher incident rates of ciguatera, or a different standard of living, than Hawaii, including in many areas in the South Pacific (compare Dalzell 1993, Lehane and Lewis 2000, with Afton 2003). While "safety" is thus a term with a

relative meaning, the potential for a fishery for roi needs to be assessed in the context of the situation in Hawaii, not elsewhere. Considering the availability of alternative, less toxic reef fish for consumption (Bruno and Effler 2001a, Afton 2003), and the abundance of other affordable sources of protein in Hawaii, my assessment stands that a large-scale fishery for roi is not feasible at this time.

FUTURE DIRECTIONS

Feeding ecology

Fish communities in Kona have thrived in recent years in spite of increases in roi population size and consumption (DAR unpublished data). How have prey communities been able to resist the strong predation pressure from roi? Also, roi in Hawaii is an alien predator that only arrived in 1956 – how could the community of native fish species be resilient to this new and large source of predation? At the same time, can we really assume that consumption by roi has increased the overall feeding pressure from all predators present on reefs in Hawaii above levels that occurred prior to its arrival? Or does it in reality only replace predation by other predators in Hawaii that have declined since the arrival of roi? Last of all, recruitment of reef fishes has been high for the last several years in Kona (DAR unpublished data). Will effects of roi consumption be different in periods of low recruitment that may occur in the future? In particular, if roi was able to switch to a diet more strongly based on older, larger prey fish, this could lead to declines in the standing stock size of prey fishes. However, if prey fish find safety in

size (see Stewart and Jones 2001), declines in roi density could be expected during times of low resource availability.

These issues clearly indicate that while the island of knowledge on the feeding biology of roi has grown through work presented in Chapter 2, the shoreline of the unknown has grown with it. To me, particularly promising lines of work to address the new questions include:

(i) The continuation of underwater visual surveys monitoring roi and prey fish species abundances along the Kona coast that have been carried out by the West Hawaii Aquarium Project and by the Hawaii Division of Aquatic Resources since 1999. The surveys would provide data on the development of prey and roi populations during cycles of lower recruitment in the future. In addition, evidence for selective effects of roi on certain prey fish species and families that I addressed in Chapter 2 may strengthen, if divergent trends in roi density between areas open and closed to aquarium fish collections hold.

(ii) The design and execution of controlled large-scale roi removal experiments looking at changes in populations of prey fish species and competitors of roi in reaction to changes in roi abundance (Note: logistical problems may prevent this line of research, as most previous attempts to manipulate predator densities in the field have been unsuccessful (reviewed by Hixon 1991), including attempts to remove predators from patch reefs in Hawaii (e.g., Stimson et al. 1982, e.g., Schroeder 1989) and an attempt to increase roi densities on patch reefs in Kaneohe Bay, Oahu, by stocking treatment reefs with roi caught outside of Kaneohe Bay (Amanda Meyer, personal communication)).

(iii) Seasonal sampling of roi stomach contents, to better understand seasonal shifts in the roi diet and to answer the question what happens during periods of the year in which no new recruits are available for consumption. Does roi consume less prey and biomass, or does it adjust by consuming larger prey, as the work by Beukers-Stewart and Jones (2004) appears to suggests?

Ciguatera

In spite of better understanding of factors correlated with toxicity in roi that came out of the work presented in Chapters 3 and 4, in many ways, ciguatera remains an enigma. Particularly puzzling is the large variability of toxicity – even between individuals of the same size and age and in the same general area of a reef – that is only in part explained by the factors that I analyzed in Chapters 3. The issue becomes even more intriguing when considering results of stable isotope analysis of roi which showed that one of the remaining factors that could have explained the observed patterns, long-term diet composition, was not significantly related with toxicity (Dierking, unpublished data).

The apparent randomness of toxicity in roi led me to the hypothesis that ciguatera blooms on reefs may be small in scale and short in duration (see Chapter 3). Are blooms of *G. toxicus* so localized that in territorial fish such as roi, a roi individual with a home range coinciding with a bloom can be exposed to ciguatoxin from the uptake of toxic prey, while another roi nearby may inhabit a stretch of reef without *G. toxicus* and feed on non-toxic prey? A future line of research that could answer this question would be the analysis of the toxicity of fish on small spatial scales, ideally fish occupying overlapping home ranges, such as roi that belong to the same harem.

Chapter 4 showed succinctly that ciguatoxin did not have a negative effect on roi fitness or growth in Hawaii. A remaining question is whether the lack of effects of ciguatoxin on fish fitness and growth can be confirmed in roi or other fishes in locations that are more highly ciguatoxic than Hawaii. This question could be addressed by copying the approach that I used in Chapter 4 to analyze wild fish population in locations known for higher ciguatera incident rates than Hawaii.

One conclusion from my work on ciguatera is that the availability of an easy-to-use fully quantitative test of ciguatoxin concentrations, instead of the semi-quantitative test used in this dissertation, could lead to more powerful and effective analyses. The development of an Enzyme-Linked ImmunoSorbent Assay (ELIZA) (Engvall and Perlman 1971) that uses monoclonal antibodies to ciguatoxin, and has the potential to quantitatively detect ciguatoxin concentrations, is underway in the laboratory of Yoshitsugi Hokama at the University of Hawaii (Yoshitsugi Hokama, personal communication). An alternative approach using mass spectrometry has been successfully used by Lewis et al. (1999), but still lacks ease of use. Hopefully, new and improved tools for work on ciguatera will therefore soon be available, which would facilitate future research on this topic.

CONCLUSION

The casestudy of roi in Hawaii provided in this dissertation served to advance our understanding of the role of predators in coral reef systems, and of the biology of ciguatera. From a resource management perspective, my findings suggest that roi is unlikely to fulfill the original goal of its introduction, i.e., the creation of a new

commercial fishery. Moreover, effects of roi on native species remain a potential cause for concern, although a clear verdict still stands out – on the one hand, roi population consumption is of large magnitude, but on the other hand, prey fish communities have shown surprising resilience to roi predation pressure over the past six years.

Many important questions remain, and new questions were raised in the course of my work. Roi in Hawaii may be an ideal subject to address these questions in the future, since it is an introduced species occurring in large abundance. In particular, field studies involving destructive sampling or manipulation of grouper abundances will be easier to accomplish, and easier to defend from an ethical standpoint, in this setting than in the many locations worldwide where groupers have become rare or endangered, and may have protected status.

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