

NOAA Project Final Report

- I. Project Title:** Assessing the outcomes of the west Hawai'i roi (*Cephalopholis argus*) project
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II. Executive Summary

Introduced predators can reduce biodiversity and abundance of native species by predation or by out-competition for resources. This research focused on the ecological effects of the introduced grouper roi (*Cephalopholis argus*) and native reef fish populations. The objectives of this research were to 1) involve the local community in testing the effects of roi on fish assemblage characteristics by a predator removal experiment and 2) to assess the feasibility of roi removal as a management tool in west Hawai'i

Differences for overall assemblage metrics (numerical abundance and biomass) and potential competitor species of roi were not significantly different by location six-months after roi removal. However, one-year after roi removal, the numerical abundance of all fish species decreased significantly less at the treatment site compared to both control locations. This pattern of numerical abundance was not apparent in total species biomass one-year after roi removal. For potential competitor species, there was no difference detected in biomass, however, by numbers of individuals, the treatment site decreased significantly less relative to one of two control sites.

Numbers of small-sized (≤ 15 cm TL) fishes increased by 18% percent at the treatment site and decreased by 2% at control locations six-months after roi removal. A recruitment pulse of the gold-ringed surgeonfish (*Naso lituratus*) at all study sites was largest in magnitude at the treatment site by six-fold. However, it is necessary to exclude the recruits and young-of year from the analysis (fishes ≤ 5 cm TL) in order to allow for the inherent spatial heterogeneity of reef fish recruitment. In so doing, the above pattern was not detectable in the narrow size-range and abundances of small ($5 \leq 15$ cm TL) select prey species did not appear to differ among locations one-year after roi removal.

Distributions and movements of roi in response to the 3-acre depletion experiment were mapped and spatially analyzed in ArcGIS10. Roi population density decreased overall, but remained similar in mean size and range. The movements of roi were monitored through a mark and re-capture program where tagged roi were found to travel distances between ~50-150 m from the periphery of the removal area toward its center at a rate of ~ 3 in 6 months' time.

Seventeen project presentations occurred over the first year of the project (August 2010-August 2011), including two international conferences. During year-two (September 2011 – September 2012) eleven project presentations were shared including one international conference in Cairns Australia and six community outreach events. Further work includes bi-annual monitoring of reef fish communities and continued collaboration and strengthened partnerships with managers, scientists, and community members through education and outreach.

III. Purpose

The purpose of the project is to examine the results of the field manipulative experiment that has been set up to test the ecological effects of the introduced grouper roi (*Cephalopholis argus*) on reef fish associations in West Hawai'i. This on-going research project, which began in September 2010, evaluates the impact of roi removal by collaborating with local fishers to remove >90% of the roi from a patch reef in Puakō, West Hawai'i for the Hawai'i Coral Reef Initiative Research Program. Our specific goals were to 1) assess the effect that experimental roi removal has had on the native reef fish assemblage 2) map distributions and movements of roi in response to the 3-acre depletion experiment and 3) promote collaboration between coral reef managers, researchers, and the fishing community through workshops, public outreach, education, and agency partnering.

Management problem addressed

Introduced predators can lead to biodiversity and abundance reduction via predation or outcompeting native species for resources (Balon & Bruten 1986, Faush 1988, Ross 1991). Roi were introduced to Hawai'i from Moorea, French Polynesia, in the 1950s and 60s in order to fill a sport game fishing niche (Randall 1963), however, due to the prevalence of ciguatera fish poisoning associated with roi (Dierking 2007), the intended benefits of this food-fish

introduction program were not obtained. Without fishing pressure, populations of roi continued to expand, and they are now the dominant marine predator on many Hawaii's reefs (Dierking 2008).

The estimated roi population size in 7.8 km² of reef habitat along the west coast of Hawai'i was recently 56,290 individuals with an annual prey consumption of 93.7 t (tons) of fish and 5.5 t of crustaceans (Dierking 2007). This large-scale consumption rate indicates that roi may play a prominent role in shaping native reef fish communities in Hawai'i (Randall & Brock 1960; Randall 1963; Parrish et al. 1985; Webster 2002). However, Hawaiian reef fish populations exhibit an apparent resilience to the predation pressure of roi.

Based on data from long-term reef fish monitoring conducted by the Hawai'i Division of Aquatic Resources (HDAR), Walsh (Walsh, Kona IEA 2011) suggested that roi do not show a detectible impact on aquarium species of concern or on total reef fish abundance. Aquarium industry fishers are concerned that roi may reduce numbers of valuable catch (West Hawai'i Fishers Council, 2011). While species of concern in the aquarium fishery are (among others) show significant declines over time, analysis of monitoring data have demonstrated that there are no significant relationships between roi density and the density of each population parameter examined, including the density of total reef fish populations, small fish species, or piscivores in West Hawai'i (Walsh, Kona IEA 2011). Furthermore, roi density does not appear to negatively affect the population size of the two most heavily collected aquarium species (*Zebrazoma flavesense* and *Ctenochaetus strigosus*) (Walsh, Kona IEA, 2011). Furthermore, trends in roi populations indicate a 50% decrease since the peak year (2004). This decline is likely due to a die-off in summer of 2006 (Walsh Kona IEA 2011).

Prey size and prey family electivity

The ecological effect of roi on fish communities may be a result of predation by roi structuring prey populations, or as a result of out-competing other predatory fishes for resources (Meyer 2008). Dierking and Meyer (2011) demonstrated that prey size of roi differs between Moorea and Hawai'i, where Hawai'i roi consume smaller sized fishes. This contrasts the pattern of prey availability in the wild (Dierking et al. 2008), suggesting that roi preferentially choose smaller fish. The question arises whether this observed dynamic results from the ease of capture of digestive processing of smaller sized fishes alone, or whether the efficiency of foraging at the

individual level translates to the population and ecosystem levels of organization. Taking smaller sized fish is more efficient according to the turnover rates of production in marine systems (Helfman et al. 2009).

In marine environments, young fishes display a greater growth curve and exponentially declining mortality, and therefore higher production at the early stages of development occurs (Ricker 1975; Ross 1997). The preferential consumption of intermediate-sized fishes might contribute to the effect of maintaining high turnover and high productivity in the system. Taking the small fast-growing fishes instead of the large slow-growing fishes maintains higher yields of prey biomass. In the relaxed competitive environment of Hawai'i, roi select smaller-sized fishes compared to Moorea where roi display resource partitioning. In response to greater competition and a reduction in optimal prey availability, an ontogenetic niche shift occurs to diversify the feeding strategy of Moorea roi populations (Dierking and Meyer 2011).

The possible effects of roi in Hawai'i are likely to differ among taxa, depending on the size at reproduction of the prey species, because of the gape-limitation in fishes (Wainright and Richards 1995). Also, prey vulnerability to predation by roi is likely to differ among feeding guilds because of the diel cycles of behavior determining the likelihood of encounter between predator and prey.

Predation

The influence of roi predation on prey fishes in Hawai'i may be determined by measuring the differential effects of roi removal on different size classes of prey fishes. Fish that commonly grow larger than 13-15 cm (such as larger wrasses and parrotfishes) may find refuge in size and therefore may remain resilient with a reproductive stock that is able to escape predation by roi. In contrast, fishes with smaller maximum size, such as the butterflyfishes and smaller wrasses may show a decrease in areas where roi are abundant.

Competition

A major change in the prey community may not be seen as a response to roi removal if roi are successfully out-competing other native predators for prey. A predator removal experiment in the Red Sea by Shpigel and Fishelson (1991) showed that prey populations were not significantly altered following the removal of three species of groupers. Instead, increases in the populations

of potentially competing predators such as lionfishes, lizardfishes, and hawkfishes were observed.

Objectives

- 1) Assess the actual affect that experimental removal of roi has had on the native reef fish assemblage.
- 2) Map distributions and movements of roi in response to the 3-acre depletion experiment.
- 3) Promote collaboration between coral reef managers, researchers, and the fishing community through workshops, public outreach, education, and agency partnering

Null hypotheses:

H₀₁ – Roi removal will yield no significant effect on reef fish assemblage characteristics in the removal area after one year.

H₀₂ – Roi removal will yeild no significant change in population density of fish species between 5 cm and 13 – 15 cm TL in the removal area after one year.

H₀₃ – Roi removal will yield no significant change in population densities of other reef predators that may be competitors of roi in the removal area after one year.

H₀₄ – Roi removal will be yeild no significant change in population densities of species more than 13 – 15 cm TL in the removal area after one year.

IV. Approach

Experimental design

Movements of roi

Prior to removal activities, local fishers were contracted to assist with a roi tagging study. Tagging was implemented in order to assess the response of surrounding roi populations to the

removal of roi at the treatment reef. Three buffer zones of 250-m concentric rings were designated with a specific tag type, color, and anatomical position on captured roi in order to facilitate underwater recognition of place of origin during subsequent underwater monitoring programs (Figure 2). Sixty-seven roi from the areas adjacent to the defined removal sites were captured in nets with diver-deployed handline and barrier nets, and tagged with a standard Hallprint tagging gun and t-bar tag. Tagging events were opportunistic from February to October 2011. Movements of tagged roi into the cleared area were noted during subsequent monitoring events, ranging from June 2011 – July 2012. The geographic coordinates of tagged and recaptured roi were obtained by matching the recorded time of capture/re-sight with the logged GPS time-track. The spatial distribution of all roi tagged and re-sighted or collected were mapped in ArcGIS10.

Roi removal

Local fishers were contracted to assist with roi removal at the treatment reef (13,366 m²) during April and May of 2011. Spear-fishing for roi occurred over the course of 11 days. Fishers removed roi from the treatment reef with the number of fishers and hours fished recorded. Roi were collected, counted, sized, and weighed on site. Each diver's individual effort in dive time was recorded, along with the number of roi dispatched. Dive times ranged from 37 min to 56 min, and number of dives ranged from 1-3 per day.

Fish assemblage monitoring

A Before-After-Control-Impact experimental design was used to assess changes in reef fish assemblages associated with roi removal activities (Green 1979). Forty-seven permanent transect were established at randomly selected sites of similar habitat in removal areas and adjacent control sites (Figure 3). The study area for each of three study sites lie on *Porites compressa* dominated habitat types with similar habitat complexity $F(2, 22) = 3.2, p = 0.06$ Table 3).

At these sites, visual fish transects were conducted following West Hawai'i Aquarium Project (WHAP) survey protocols for 5 m x 25 m fish transects (Tissot et al. 2004), whereby two scuba divers swim in tandem along 25-m fixed replicate transect at a constant speed (c. 15 minutes per transect). All fishes observed within the transect were identified to the lowest possible taxon and placed in size bins (0.0-4.9 cm TL, 5.0-9.9 cm TL, 10.0-14.9 cm TL, etc.).

Divers were previously trained in visual underwater size estimation techniques based on practice with various lengths of fish models that were later collected and measured.

Two rounds of data were collected from the suite of removal and control sites during November 2010 and March/April 2011, before any roi removal was implemented in order to establish baseline conditions at these sites. Surveys were conducted again one month after roi were removed from the experimental sites during June and November 2011 and then again in June 2012, six-months and one-year after roi removal. This quarterly monitoring schedule was implemented for the first year, and surveys will be conducted bi-annually for the duration of the project, which is expected to continue through 2015. The power for this design was 0.877 for a one-way ANOVA for the prey taxa abundance following roi removal after six-month. Power was calculated in Minitab16 using the maximum difference between means, standard deviation, and sample size (each replicate transect) to calculate the power of the data set. With additional post-removal surveys in the coming years, temporal changes on the individual transects will become the replication of this repeated measures BACI design.

Roi distribution monitoring

In order to assess roi population distributions throughout the wider region, a fish census focusing solely on roi was conducted bi-annually in removal and control areas (Figure 4). This census was conducted using tow-boards following NOAA Coral Reef Ecosystem Division (CRED) protocols, and followed isobaths from 10 m-30 m, covering a total area of 0.4 km². These census rounds took place in April, June, and November 2011 and in June 2012. Two divers were deployed along a 50-m tow line, each with a planning board; one diver had a telegraph system to communicate with the boat. Divers were towed at a constant speed along a set bearing. Maintaining depth c. 3 m from the benthos, divers recorded each roi sighting of within 5 m either side of the straight line trajectory. Communication was maintained between diver pairs both above water and during the survey, to ensure that roi were not double counted. Each roi sighting was recorded along with the roi body-size and time of sighting. The roi sight time was later matched to the boat GPS log of time at geographic location. The GPS fix of roi locations were entered into ArcGIS10 for mapping and spatial analysis. The offset from the boat GPS location to diver location along the tow line was calculated by the formula $a^2 + b^2 = c^2$, where a=depth, c=tow-line length, and b=distance from the boat GPS location.

Data analyses

Statistical analyses were performed in Minitab 16 and JMP 10 statistical software (2010) programs as well as ArcGIS10 spatial analysis software. Significance of all tests were evaluated at the $p=0.05$ level. All statistical tests were parametric using log-transformed fish count data or Asinh transformed data where noted in the text.

Reef fish assemblage and size structure

In order to assess the reef fish assemblage and size structure at Puakō, each replicate transect was surveyed by a buddy pair. The fish count observations from each diver were first averaged between replicate transects to obtain the sample ($n=1$) for that transect. Therefore, each transect analyzed is the mean number of fish observations recorded by one buddy pair. The two survey rounds conducted before roi removal (in November 2010 and March/April 2011) were pooled to obtain a mean value of fish observations for each transect before roi removal. The surveys after roi removal (November 2011 and May 2012) were analyzed separately, one for November 2011 (six-months after roi removal) and one for May 2012 (one-year after roi removal). The transect locations are not independent samples, but are repeatedly measured with each sampling occasion. In order to deal with the non-independence of the repeated measures, the difference between before and after fish observations per transect were first calculated. A one-way ANOVA was run on the differences in fish transect observations to assess whether changes in fish assemblage characteristics and size structure varied by location after roi removal. The impact of roi presence on reef fish assemblage structure is assessed by the difference in pairs of samples between sample periods:

$$D_{ik} = X_{iCj} - X_{iLk} = X + \eta_i + \varepsilon_{ik}$$

Where X is the mean difference between control and treatment, η_i is the change in the difference from before to after, and ε_{ik} is the error associated with the difference (Smith 2002). Here X is the assemblage characteristic in question, such as numerical abundance, diversity, biomass, and prey size distribution.

Roi distribution

ArcGIS10 Spatial Analysis tools were used to measure and determine patterns in roi distribution. First, a nearest neighbor analysis was used to determine whether roi were clustered

or spatially dispersed. Secondly, a Geographically Weighted Regression analysis (Charlton 2009) was used to determine possible predictive relationships between independent variables of depth and slope of slope (a measure of habitat complexity) and roi body-size.

Roi immigration rates

In order to assess movements and immigration rates of roi following roi removal at the treatment reef, tagged and re-sighted/re-captured roi were mapped in ArcGIS10. Time of sight/re-capture was matched with the GPS time-track and location log in order to obtain geographic coordinates. In ArcGIS10, distances and directions of movement between roi tag and recapture locations were measured using the spatial analysis measuring tool. Time duration between roi removal and re-sight in the treatment zone was used to calculate the immigration rate of surrounding populations of roi 6 months after initial roi removal.

Cost-benefit analysis of roi removal

The effort of roi removal was assessed by calculating Catch-per-Unit-Effort of roi removal at the treatment site:

$$CPUE = ((h - \ln(h)) * 24)$$

Where h=hours, weighted by total effort per day. Effort per day was recorded as each individual fisher dive time.

Costs of scuba supplies such as enriched air nitrox, dive-team hours, and boat operation costs were included to obtain a total cost of roi removal per unit area. Costs of operation were recorded in budget log and summed at the end of the removal period for total roi removal cost estimate.

Project management:

Organizations performing the work include The Nature Conservancy and the University of Hawai'i. The project was managed by Chad Wiggins of TNC and data was analyzed by Jonatha Giddens of UH. Eric Conklin and Chuck Birkeland served as PI's on the project for TNC and UH respectively. The TNC marine team and Jonatha Giddens performed all fish count surveys. Four local fishermen were contracted with the project and were directly involved with

the roi tagging and clearing portions fieldwork.

V. Findings

Objective 1) Assess the actual affect that experimental removal of roi has had on the native reef fish assemblage.

A total of 228 visual surveys (each 5X25 m=125 m²) were conducted during 5 survey periods to quantify the reef fish assemblage at three study sites at Puakō from November 2010 to June 2012. One treatment site (13,366 m²) and two controls (mean 13,493 m²) were surveyed during each of the quarterly periods. Two survey periods occurred before roi removal; one in November of 2010 and the second just before the fish-down experiment in March and April 2011. Roi removal at the treatment site took place over the course of 11 days in late April and early May 2011. Three fish assemblage survey rounds occurred after roi removal; one-month, six-months, and one-year following roi removal.

Roi were present at all three study sites before removal, with a pooled population density of 735.7 g/ 125 m² and 0.61 individuals per 125 m². Roi occurred in 66% of the surveys conducted at the treatment site before removal and accounted for 14.3 % of the total fish biomass and 0.81 % of the complete assemblage numerical density at the treatment site. Whereas roi ranked second in density at the treatment site by biomass (897.2 g/125 m²), this species was not present in the top ten abundant species by numerical density (Table1) and instead ranked twentieth by numerical abundance. Thirty-one kg of roi were removed from the treatment site during the fish-down experiment in late April and early May 2010.

Comparison of complete assemblage metrics

Habitat complexity and fish assemblage metrics

The sample means for habitat complexity, as measured by a $2.17607 + 1.56574 * \ln(X - 0.37)$ transformed rugosity ratio, were not significantly different at the $\alpha=0.05$ level ($F(2, 22) = 3.2, p = 0.06$). Habitat complexity was not correlated with biomass, numerical abundance,

species richness, or diversity at the three study sites (Table 2). Power for the rugosity analysis was 0.200.

Biomass and numerical density

The overall trend in reef fish abundance at Puakō from November 2010-June 2012 generally decreased in fish density at all sites (Figure 5). Both the reference and the treatment site decreased in fish density during the summer months and increased again during the winter survey, whereas the control site increased in fish density and then decreased during the winter months. The greatest rate of change in fish density occurred during the summer months when the control site spiked in fish biomass (8004.7 g/125 m²), and then decreased again (3789.2 g/125 m²) for a net decrease in fish biomass of 64 % for the year.

In June, one-month after roi removal, there was a significant increase in log-transformed fish biomass data at the control site relative to the treatment and reference site in June ($F(2, 37) = 3.33, p = 0.047$). By November, six months after roi removal, the difference in log transformed fish biomass detected during June was no longer significantly different by location ($F(2, 43) = 0.06, p = 0.945$), nor was numerical density significantly different by location ($F(2, 44) = 2.43, p = 0.100$). All sites decreased in fish biomass during the November survey. The dominant species by biomass, *Acanthurus olivaceus*, decreased from 1240 g/125 m² to 961 g/125 m² while *Cephalopholis argus* continued to increase in biomass from 643 g/125m² to 730 g/125 m². The general trend at the control site during the November survey was a decrease in large-sized fish, yet, densities of roi continued to increase despite this overall site trend.

One-year after roi removal the treatment site decreased significantly less in total fish biomass compared to the reference site, however not compared to the control site ($F(2, 44) = 3.94, p = 0.027$) (Figure 7). Excluding roi from the analysis, roi decreased significantly less at the treatment site compared to the control site, but not the reference site, one year after roi removal ($F(2,44) = 3.90, p = 0.027$). In contrast, for log-transformed numerical abundance, the treatment site decreased significantly less compared to both control locations ($F(2, 44) = 5.22, p = 0.009$) (Figure 8).

Density of species ≤ 15 cm TL

All sites decreased in the density of species below 15 cm TL one month after roi removal with a mean decrease of $8.4 (\pm 24.3)$ individuals per 125m^2 . The magnitude of this decrease was similar among all study sites ($F(2, 38) = 0.82, p = 0.447$). Six months after roi removal, the treatment site increased by 18% percent in numbers of small-sized fishes and decreased by 11% in numbers of large-sized fishes. This decrease of large fish observed at the treatment site is inclusive of the roi removed through the fish-down experiment. The increase of small-sized fish within the size range of vulnerability to predation by roi (≤ 15 cm TL) increased in numerical abundance by an average of $15.14 (\pm 28.22)$ numbers per 125 m^2 six months after roi removal. Meanwhile, the control and reference site both decreased in the numerical abundance of this size range an average of $1.26 (\pm 14.15)$ per 125 m^2 and $2.12 (\pm 27.31)$ per 125 m^2 respectively. Though this divergence in fish density trends among treatment levels is not significant at the $\alpha = 0.05$ level $F(2, 44) = 2.54, p = 0.083$, with a power of 0.779, sample distributions approach the significant limit and warrant further exploration.

The gold-ring surgeonfish (*Ctenochaetus strigosus*) was the dominant small fish (≤ 15 cm TL) observed at the treatment site before and after roi removal at the treatment site. However, after roi removal there were less counts of this species, decreasing from 945 to 769 for the entire site. There were also less of the second most dominant small fish counted, the yellow tang (*Zebrasoma flavescens*), which decreased from 514 to 455 counts for the entire site. The lavender tang (*Acanthurus nigrofusus*) increased from 219 to 265 for the entire treatment site. It was the orange-spine unicornfish (*Naso lituratus*) that increased the most of all small-sized (≤ 15 cm TL) species at the treatment site after roi removal. The orange-spine unicornfish was among the top ten species in numerical abundance before roi removal, averaging $29.5 (\pm 0.4)$ for the two pre-removal surveys combined. In November, six months after roi removal there were 204 orange-spine unicornfish counted, ranking fourth in numerical density at the treatment site. The orange-spine unicornfish also increased in numerical abundance at the control (468%) and reference (171%) sites. However, at the treatment site the orange-spine unicornfish increased nearly seven-fold (5147%). There was a recruitment pulse of orange-spine unicornfish that was detected at all study sites during the November 2011 survey, however, the pulse was largest in magnitude at the treatment site.

Changes in the numerical abundance of large-sized fishes were significantly different by location for $0.585408 + 1.13731 * \sinh((x - 4.00655) / 11.3593)$ transformed data one month after roi removal ($F(2, 38) = 3.93$, $p = 0.028$). During the June survey, the control site had increased with large fishes while the treatment and reference site decreased in the numerical abundance of this large-size class (>15 cm TL). In June 2011, the control site increased in numerical abundance on average 2.66 individual fish per transect (± 11.06). Bullethead parrotfish and gold-ringed surgeonfish increased in abundance the most from 35 to 45 and from 9.5 to 22.5, respectively. The treatment site decreased an average of 6.22 fish per 125m^2 (± 18.94). Roi removed through the fish-down experiment conducted in April and May 2011 was included in this analysis. In spite of the manipulative experiment removing roi at the treatment site, the reference site actually decreased the most in the numerical abundance of large sized fishes ($<15\text{cm TL}$), an average of 19.58 fishes (± 23.05). However, six months after roi removal all three study sites were similar in the numerical abundance of large fishes ($F(2, 44) = 0.04$, $p = 0.956$) (Figure 9). By November 2011, all study sites had decreased in numerical abundance of this large size class (>15 cm TL) an average of 4.05 (± 7.9) per 125m^2 (Figure 10). The reference site decreased by the most, 8.1 per 125m^2 , but also had the largest \pm of 22.36 per 125m^2 . Roi increased the most at the control site from an average of 11 (± 0.25) individuals during the surveys before roi removal at the treatment site to a total of 15 individuals counted at the control site six months after roi removal.

Selected size class and vulnerable taxa

Abundances of the select size range ($5 \leq 15$ cm TL) and taxa vulnerable to roi predation (Table 3 from Dierking et al. 2009) differ between the treatment and control site six-months after roi removal, but are similar between the treatment and the reference site ($F(2,44)=6.07, p=0.005$) (Figure 11). The greatest increase in the numerical abundance of small select prey species occurred at the treatment site six-months after roi removal. One-year after roi removal, log-transformed differences in numerical abundance of small select species are not significantly different by location ($F(2,44)=2.96, p=0.062$) (Figure 12).

Aggregated analysis of potential competitor species

Potential competitors of roi are listed in Table 4. There was no difference in potential competitor biomass by location one-year after roi removal ($F(2, 43) = 2.70, p=0.079$) (Figure 13).

All sites decreased in the numerical abundance of these competitor species one year after roi removal. The treatment site decreased least of all, and significantly so compared to the control site ($F(2, 43) = 4.71, p=0.014$) (Figure 14).

Ratio of native/non-native species

The ratio of native to non-native (*Cephalopholis argus*, *Lutjanis fulvus*, *Lutjanus kasmira*) increased at all study sites one-year after roi removal (Figure 15). The treatment site ratio increased by 185 % while the control and reference site increased by 154 % and 213 % respectively.

Objective 2) Map distributions and movements of roi in response to the 3-acre depletion experiment.

Roi distribution

A census of the size structure and distribution of the roi population in a 1 km² area surrounding treatment and reference sites was established before and after roi removal. Census rounds occurred before roi removal in October 2010 and April 2011. Distributions of roi are significantly clustered by distance for census round 1 ($z = -6.4, p = 0.00$) (Figure 16, Table 5) and for census round 2 (Figure 17, Table 5) ($z = -3.8, p = 0.0004$) by Nearest Neighbor spatial analysis before roi removal. However, roi were not clustered by size (Table 6), depth (Figure 18), or habitat complexity (Figure 19) based on Geographically Weighted Regression results. Census rounds occurred after roi removal during November 2011 and June 2012. Overall, roi populations decreased in density (from $\sim 9 \text{ ha}^{-1}$ to $\sim 7 \text{ ha}^{-1}$) though remained similar in size range one-year after roi removal (Figure 20, Table 7).

Roi immigration rates

Sixty-seven roi were externally tagged in three distance buffer zones (250 m) surrounding the treatment reef (Figure 21). Six roi have been re-captured and six roi have been re-sighted in sixteen months of monitoring. Distances of roi travel averaged ~ 94 m and generally occurred from the periphery of the removal reef towards the center, and also in a northward direction from the northern inner buffer zone (Table 8). Three roi were observed to immigrate into the treatment reef in 6 months of monitoring after roi removal (Figure 25).

Objective 3) Promote collaboration between coral reef managers, researchers, and the fishing community through workshops, public outreach, education, and agency partnering.

The third project goal was to collaborate with the fishing, research, and scientific communities to strengthen partnerships in an issue deemed critically important by the fishing community. The project has been a success in that we were able to engage the local community in our study. Four local fishers are contracted to assist with tagging and fishing efforts. Partners have been assisting with fieldwork since February 2011. Additionally, the Hawai'i DAR engaged providing research boat and captain support in 2011. Additionally, private donors (Michael Morris memorial fund), as well as the NSF and NOAA support this research. Partnerships have been established with dive clubs in Hilo, Maui, and Oahu. Many volunteer hours have been recorded during the project, including a large-scale community roi removal and research effort conducted in partnership with TNC during July 2012. Over two days, 21 divers from multiple islands caught 353 roi in the area surrounding the Puakō study site. With community support, volunteer hours totaled 280 for the research effort. During that time spearfishermen and community members worked together to catch, weigh, measure, and collect ciguatera samples for on-going roi research in west Hawai'i. Kayakers and paddlers collected gps data on caught tagged fish to assist with the study of roi movements in response to the removal experiment.

Seventeen project presentations occurred over the first year of the project (August 2010-August 2011), including two international conferences, summarized in Table 9. During year two (September 2011 – September 2012) eleven project presentations were shared including one international conference in Cairns Australia and six community events. One HCRI meeting is

scheduled for September 2012 and several dive club presentations will be scheduled within the next month as a partner through the NOAA NMFS extension program.

Further work

Assemblage level effects of predation

We expected that the assemblage-level effects of predator removal would take multiple seasons to manifest based on the inherent spatial and temporal heterogeneity of reef fish recruitment patterns. In Hawai‘i, there are two recruitment pulses: one large peak of new recruits settle to the Kona reefs in the summer months during June and July, and one secondary, generally smaller peak in recruitment occurs during February and March (Walsh 1987). We expected that it would take several recruitment pulses to detect the effects of roi predation upon survivorship of these new cohorts that settled after roi removal. A predator removal experiments in the Red Sea (Shpigel and Fishelson 1991) took three years monitoring the effects of predator removal on the prey species assemblage.

As expected, our results testing the differences in complete assemblage-level metrics did not show a direct effect of predator removal. Six months after roi removal, there was no significant difference in the biomass ($F(2, 43) = 0.06, p = 0.945$) or numerical density ($F(2, 44) = 2.43, p = 0.100$) of fish species abundance by location. However, one year after roi removal there was a significant difference in numerical abundance ($F(2, 44) = 5.22, p = 0.009$), but not biomass ($F(2, 44) = 3.94, p = 0.027$) at the treatment site compared to both control sites.

The influence of competing predators may dilute the initial response of an increased prey population over time. Shpigel and Fishelson (1991) documented an increase in competing species following grouper removal in the Red Sea, rather than an increase in prey species after 36 months. Therefore, predation as a structuring force might not be seen as a long-standing effect, but may be seen as inflection points within the larger time scales where the dynamics between competition and predation unfold in response to resource limitation and carrying capacity. Six months after roi removal, with an increase in numbers of small individuals, the reef ecosystem at Puakō may not be limited by recruitment, but limited instead by the effects of predation on early post-settling larvae. If predation by roi is the dominant process structuring this reef fish assemblage, then survivorship of these recruits will increase and there would be an

increase in the density of fish species over time. If predation by fishes other than roi predominate structuring processes, then we may see an increase in other piscivore species and a sustained decrease in small recruits. Finally, if it is competition that limits prey species below their carry capacity, then the numbers of small fish will remain low even without an increase in competitor species of predators.

Selected and vulnerable taxa

We expected that selected taxa, based on gut content analysis by Dierking (2008) and Meyer (2008) may be the first to respond to predator removal. At the six-month time scale, for the narrow band of select taxa within the size range most vulnerable to predation by roi, there is evidence that predation is a structuring force that directly affects the abundance of prey populations. In the absence of roi, numbers of small-sized vulnerable fishes increased. However, this analysis is preliminary, as it includes a small subset of the prey families potentially vulnerable to roi predation and it includes all sizes of fish below 15 cm TL, including recruit-sized fishes, which are themselves spatially variable even without the effects of predation. By excluding the recruit and young-of-year (≤ 5 cm TL) from the analysis, differences in small prey species abundance were not significant relative to both control locations six-months and one-year after roi removal. Further, the question remains whether these fishes that increased in abundance of small-sized fishes will survive to adulthood. These species would be susceptible to competing piscivores that may fill the niche of roi (Shpigel and Fishelson 1991). Therefore, predation may be the force keeping reef fish below carrying capacity even in the absence of roi. Further monitoring of reef fish abundance and size structure over larger time scales will elucidate this question.

One year following roi removal, populations of competing piscivores did not increase at the treatment site relative to the two control sites. However, a lag in the response of competing species population abundance would be expected even if the predation hypothesis were true. First there would be an increase in prey, and then following, competitor populations may respond to the increased prey abundance by lessening competition and increasing competitor species population density (Hixon 1991). Together, these processes invoke the predation hypothesis as

the dominant structuring process keeping prey populations below carrying capacity, whereas predation is mediated by competition among predators.

The experimental design is sensitive to the decrease in biomass with the removal of 31 kg of roi during the fish-down experiment. During the summer there was an increase in numerical abundance, yet a decrease in biomass at the treatment site if roi is included in the analysis. By excluding roi from the analysis, this decrease was not significant. This pilot study indicates that assemblage level differences will be detected with this experimental design.

Continued bi-annual monitoring will be required for the next several years in order to differentiate the inherent spatial heterogeneity of reef fish recruitment from predation effects. In particular, a roi population census round in November 2012, following the large-scale community roi removal event of July 2012, would show the effect of large-scale removal on actual populations of roi. Continued collaboration with the fishing community will continue through NOAA NMF partnership meetings, dive-club presentations, education, and public outreach events, as well as academic conference presentations.

VII. Applications

Outputs of the project for year two include 1) data on community effects of roi removal reported through an MS thesis that will be published with Proquest, and made available through four academic conferences, including one international symposium. All talks were recorded and made available online during the ICRS 2012 symposium. Secondly, information is provided on the cost of roi removal efforts and this information is made available to managers for a cost-benefit analysis of roi removal. New tagging methods were developed in order to more efficiently tag roi, and these may be used in future studies of reef fish movements. Finally, by presenting the project at six community events, include the Scientists And Fishers Exchange in Honolulu during August 2012, partnerships were strengthened through communication and sharing among the scientific and fishing community.

The outcomes of this project during year two include improved communication between fishers, fishers, scientists, and managers regarding the ecological effects of introduced roi. Year two has provided information on the effects of roi removal on the native reef fish assemblage at one-month, six-months, and one-year intervals. This information, along with the

cost estimate of roi removal via spearing, will aid resource managers assess the feasibility of roi removal as a long-term management tool. Furthermore this community-driven project serves as a possible model for habitat restoration, if decreasing the population of roi is shown to benefit native reef fish populations over time.

VIII. Evaluation

The project goals and objectives were obtained within the time frame available, one-year following roi removal and partnerships with local stakeholders, managers, and collaborating scientists have been formed and strengthened. We were able to meet each goal with the outputs and management outcomes specified at the start of the project. Namely, an estimation of costs of roi removal and roi immigration rates into a cleared area are quantified. This year of study provided data for effects of roi removal one-month, six-months, and one-year following roi removal. The groundwork is in place to conduct this predator removal experiment and determine the impacts of roi on the native Hawaiian reef fish assemblage over the long-term. More time is needed in order to decipher the effects of predation in contrast to recruitment spatial variability. The project is ready for year three, and we are actively seeking funding to continue the work.

IX. Acknowledgements

We gratefully acknowledge Chad Wiggins for coordinating this project from its inception and through every detail of design, fieldwork, and presentation. Thanks to Eric Conklin the TNC Marine Team: Russell Amimoto, Zach Caldwell, and Kydd Pollock. Thanks to Alan Friedlander for his help with research design and for supporting the essential contributions of HCFRU researchers Kosta Stamulous, Paulo Usseglio, and Mary Donovan. Thank you Evelyn Wight for your expert contributions to our effective project communications and community outreach. Thank you to Kawika Auld, Brian Thomas, Jake Merkel, and Rhinehardt Jensen of Kohala and Kekaulike Tomich of North Kona for sharing your fishing expertise and accomplishing the task of roi removal. Thank you to Amanda Meyer and Joe Laughlin for help developing field methods. Thanks to DAR Kona for boat support and council with the project design. Thank you to our funders, HCRI and NOAA, for making this work possible, it is a privilege to be a part of the initiative. Thank you to the National Science Foundation for supporting the development of innovative science with broad community impacts. Thank you to Andy Taylor, Pat Hart, and Ivor Williams for discussions and guidance on statistical analysis. The Puakō region is a very special place to work, thanks to the involvement and support of the volunteers and the Puakō Community Association. A very special and heartfelt thank you to Liz and Michael Morris family and friends; their love for the ocean inspires our shared commitment to care for the Hawaii's reefs, and resounds through every aspect of this project.

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TABLES

Table 1. Treatment site top ten species ranked by (A) biomass (g/125 m²) and (B) numerical abundance (number/125 m²) before and after roi removal.

(A)

Before roi removal		After roi removal	
Species	Biomass	Species	Biomass
<i>Chlorurus spilurus</i>	1171.2 ± 376.9	<i>Chlorurus spilurus</i>	560.2 ± 293.3
<i>Cephalopholis argus</i>	897.2 ± 506.4	<i>Naso lituratus</i>	379.4 ± 172.7
<i>Lutjanus kasmira</i>	565.7 ± 3178.1	<i>Naso brevirostris</i>	342.9 ± 1267.0
<i>Ctenochaetus strigosus</i>	518.5 ± 228.1	<i>Ctenochaetus strigosus</i>	300.7 ± 142.0
<i>Zebrasoma flavescens</i>	343.9 ± 163.3	<i>Zebrasoma flavescens</i>	234.2 ± 126.4
<i>Scarus psittacus</i>	324.9 ± 389.1	<i>Oxycheilinus unifasciatus</i>	186.0 ± 93.8
<i>Bodianus albotaeniatus</i>	301.2 ± 661.3	<i>Thalassoma duperrey</i>	159.4 ± 71.2
<i>Naso lituratus</i>	298.9 ± 177.3	<i>Scarus rubroviolaceus</i>	132.4 ± 743.2
<i>Thalassoma duperrey</i>	209.4 ± 70.3	<i>Scarus psittacus</i>	131.8 ± 164.4
<i>Oxycheilinus unifasciatus</i>	160.1 ± 90.5	<i>Acanthurus olivaceus</i>	123.4 ± 567.3

(B)

Before roi removal			Six-months after roi removal		
Species	Number		Species	Number	
<i>Ctenochaetus strigosus</i>	27.4	±7.9	<i>Ctenochaetus strigosus</i>	19.7	±6.1
<i>Zebrasoma flavescens</i>	14.7	±5.5	<i>Zebrasoma flavescens</i>	11.6	±4.1
<i>Thalassoma duperrey</i>	7.1	±1.7	<i>Thalassoma duperrey</i>	7.1	±1.4
<i>Chlorurus spilurus</i>	6.7	±2.2	<i>Naso lituratus</i>	7.0	±4.9
<i>Acanthurus nigrofuscus</i>	6.2	±2.6	<i>Acanthurus nigrofuscus</i>	6.7	±2.8
<i>Scarus psittacus</i>	5.1	±3.6	<i>Dascyllus albisella</i>	3.8	±10
<i>Dascyllus albisella</i>	4.3	±9.3	<i>Pseudocheilinus evanidus</i>	3.7	±1.7
<i>Pseudocheilinus evanidus</i>	2.9	±2.0	<i>Chlorurus spilurus</i>	3.1	±1.5
<i>Lutjanus kasmira</i>	2.9	±12	<i>Stegastes marginatus</i>	2.9	±4.3
<i>Naso lituratus</i>	2.7	±0.8	<i>Scarus psittacus</i>	2.8	±3.2

Table 2. Results of regression of assemblage metrics as a function of rugosity at aggregated study sites.

Rugosity	DF	F	P
Log Biomass	1,23	0	0.972
Numerical abundance	1,23	0.09	0.763

Table 3. Summary of selected prey species from Hawai‘i Island from Dierking et al. 2008

Table 3								
Composition of the fish portion of the diet of peacock hind (<i>Cephalopholis argus</i>) from Hawaii Island ($n=179$) and Oahu ($n=106$), based on fish prey identified to at least the family level. Crustaceans were excluded from the analysis because of their relatively minor dietary importance for <i>C. argus</i> from both islands (% <i>IRI</i> = 1.9% and 3.3%, respectively). Dietary importance is indicated by percent by number (% <i>N</i>), percent by occurrence (% <i>O</i>) (calculated on the basis of full stomachs), percent by mass (% <i>M</i>), and percent index of relative importance (% <i>IRI</i>).								
Family	Island							
	Hawaii				Oahu			
	% <i>N</i>	% <i>O</i>	% <i>M</i>	% <i>IRI</i>	% <i>N</i>	% <i>O</i>	% <i>M</i>	% <i>IRI</i>
Acanthuridae	16.9	9.4	13.0	20.9	8.6	5.7	11.9	7.1
Apogonidae	3.1	1.9	1.2	0.6	2.9	1.9	1.7	0.5
Aulostomidae	6.2	3.8	3.1	2.6	5.7	3.8	2.0	1.8
Balistidae	4.6	2.8	9.1	2.9	—	—	—	—
Chaetodontidae	7.7	4.7	10.1	6.2	2.9	1.9	2.1	0.6
Cirrhitidae	3.1	1.9	0.9	0.6	5.7	3.8	7.9	3.2
Holocentridae	24.6	15.1	7.5	35.8	—	—	—	—
Kuhliidae	1.5	0.9	2.5	0.3	—	—	—	—
Labridae	1.5	0.9	0.2	0.1	2.9	1.9	7.0	1.1
Monacanthidae	6.2	2.8	1.6	1.6	31.4	18.9	10.3	48.6
Mullidae	4.6	2.8	5.8	2.2	—	—	—	—
Pomacanthidae	—	—	—	—	2.9	1.9	5.0	0.9
Pomacentridae	1.5	0.9	0.9	0.2	5.7	3.8	7.3	3.0
Priacanthidae	9.2	5.7	9.2	7.7	11.4	3.8	15.2	6.2
Scaridae	9.2	5.7	34.8	18.4	17.1	11.3	19.1	25.3
Synodontidae	—	—	—	—	2.9	1.9	10.5	1.6

Table 4. Potential competitor species of roi in west Hawai‘i

Taxa	Common name
Carangidae	Jacks
Chanidae	Milkfishes
Cirrhitidae	Hawkfishes
Lethrinidae	Emperorfishes
Lutjanidae	Snappers
Mullidae	Goatfishes
Muraenidae	Eels
Scorpaenidae	Scorpionfishes
Synodontidae	Lizardfishes
Oxycheilinusunifasciatus	Ringtail wrasse

Table 5. Results of nearest neighbor analysis for roi distribution.

	Observed Mean Distance (m)	Expected Mean Distance (m)	Nearest neighbor ratio	z-score	p-value
Census 1	19.337497	27.298119	0.708382	-6.457992	0.000000
Census 2	25.859037	31.014489	0.833773	-3.483563	0.000495

Table 6. Results of geographically weighted regression for roi distribution by body-size.

Census	Size roi	Observed Mean Distance (m)	Expected Mean Distance (m)	Nearest Neighbor Ratio	z-score	p-value
March 2011	L(>40)	38.868689	43.638942	0.890688	-1.59262	0.11124
March 2011	S(≤40)	30.362354	34.089634	0.890662	-1.82350	0.06822
June 2011	L(>40)	47.485639	42.142428	1.126789	1.715136	0.08632
June 2011	S(≤40)	35.591354	40.104517	0.887465	-1.80122	0.07166

Table 7. Results of 4 roi population census rounds from April 2010 to June 2012.

	Apr-11	Jun-11	Nov-11	Jun-12
Number sighted	184	120	110	147
Density	~ 9 ha-1	~ 6 ha-1	~ 5 ha-1	~7 ha-1
Mean size (cm)	41.3 (\pm 8.2)	40 (\pm 7.9)	38 (\pm 8.8)	38 (\pm 9.5)
Size range (cm)	18-60	25-60	25-60	23-60

Table 8. Results of roi movements from tagged and resampled individuals.

	Tag zone	Sight zone	Distance ~(m)	Direction
Capture (n=6)	2	2	52	W
	2	2	172	WSW
	2	2	78	W
	3	3	48	SE
	4	4	67	N
	6	6	74	NE
Sight (n=6)	2	3	155	N
	3	3	109	N
	3	3	85	N
	1	1	85	SE
	2	2	85	N
	2	2	120	N

Table 9. Project year-one presentations (August 2010-August 2011)

Date	Place	Meeting	Audience
Aug - 2010	Bishop Museum, Honolulu	HCRI trimester 1	Scientists/managers
Dec - 2010	Blue Dragon, Kawaihae	TNC fundraiser	Public, ocean community
Feb - 2011	UH Hilo	TCBES Research symposium	Students/scientists
Feb - 2011	Bishop Museum, Honolulu	HCRI trimester 2	Scientists/managers
Feb- 2011	Puakō, Hawai'i	Roi talk-story/tagging	Fishers/community
Mar - 2011	UH Hilo	Water resource meeting	Scientists/managers
Apr - 2011	PACRAC, Hilo	Ocean day	Public/community
Apr - 2011	Kona, Hawai'i	Earth and Ocean day	Public/community
May - 2011	Vancouver, BC	Int' Marine Conservation Conference	Scientists/managers
May - 2011	East-west center	(poster) Smithsonian Evolution Conference	Scientists/students
May - 2011	Puakō, Hawai'i	Roi talk-story/fishing	Fishers/community
Jul - 2011	UH Hilo	Hawai'i Ecosystems	Scientists/students
Aug - 2011	Hawai'i Convention Center, Honolulu	Hawai'i Conservation Conference	Scientists/managers
Aug - 2011	Bishop Museum, Honolulu	HCRI trimester 3	Scientists/managers
Aug - 2011	Kawaihae	Roi-round-up tournament	Fishers/community
Aug - 2011	Puakō, Hawai'i	Roi-round-up	Fishers/community

Table 10. Project year-two presentations (Sept 2011-Sept 2012)

Date	Place	Meeting	Audience
Nov - 2011	Puakō	Makai watch	Ocean community
Jan - 2012	Waimea	Ocean film festival	Public outreach
Feb - 2012	Kawaihae	Ocean festival	Public outreach
Feb - 2012	Honolulu	DAR/HCRI sharing	Scientists/managers
Feb - 2012	Hilo	Ocean Day	Public outreach
Apr - 2012	UH Hilo	TCBES Symposium	Scientists/managers
Apr - 2012	Kona	Earth and Ocean Festival	Public outreach
Jun - 2012	UH Hilo	Thesis presentation	Scientists/public
Jul - 2012	Cairns, Australia	ICRS	Scientists/managers
Jul - 2012	Puakō, Hawai'i	Community roi removal	Community/fishers
August 2012	Honolulu	NOAA SAFE	Managers/fishers



Figure 1. Map of study site in Puakō, west Hawai'i. Map by K. Stamoulis

Puako Roi Tag, Recapture and Re-sight Locations

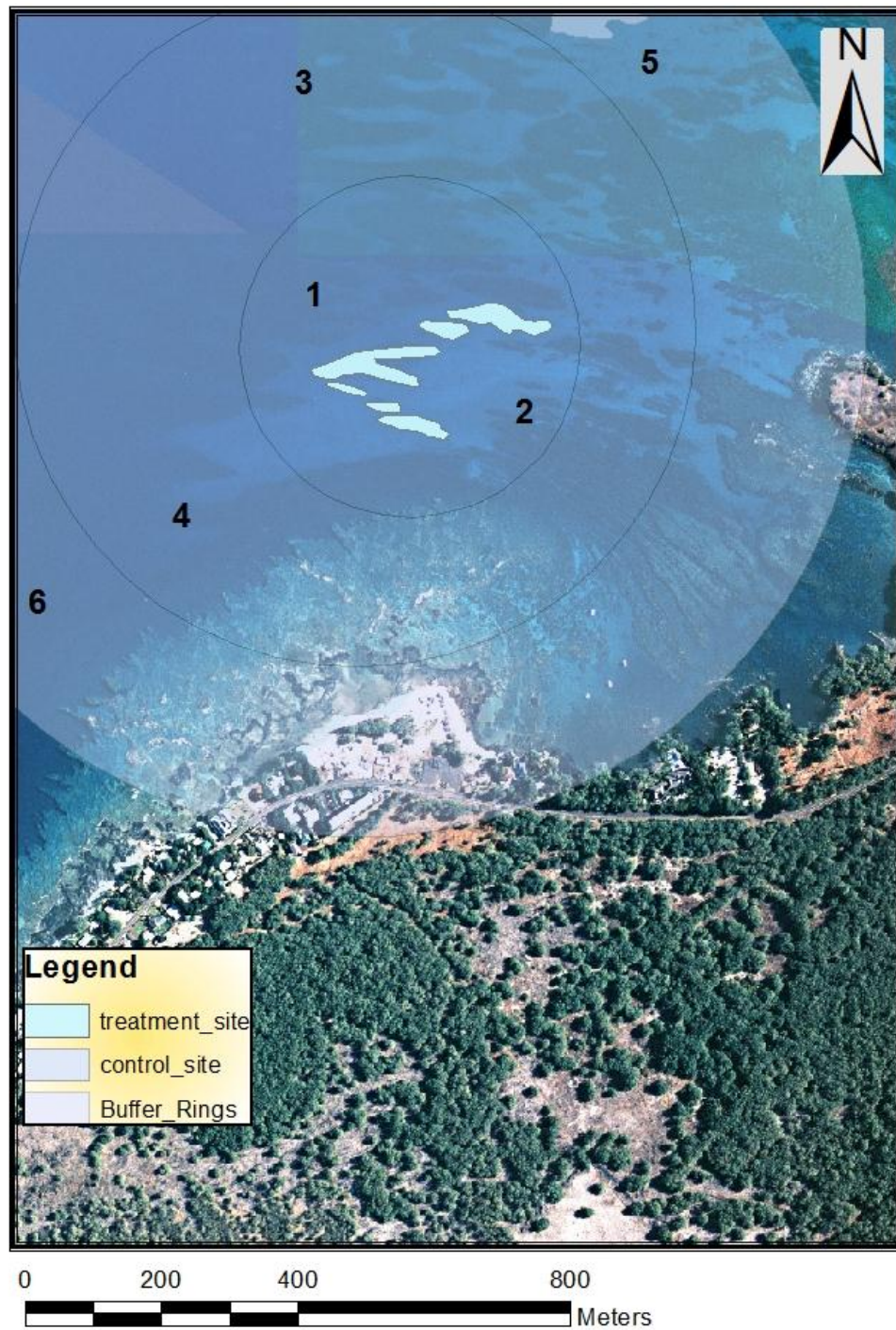


Figure 2. Map of roi tagging zones with concentric 250m rings around the treatment reef

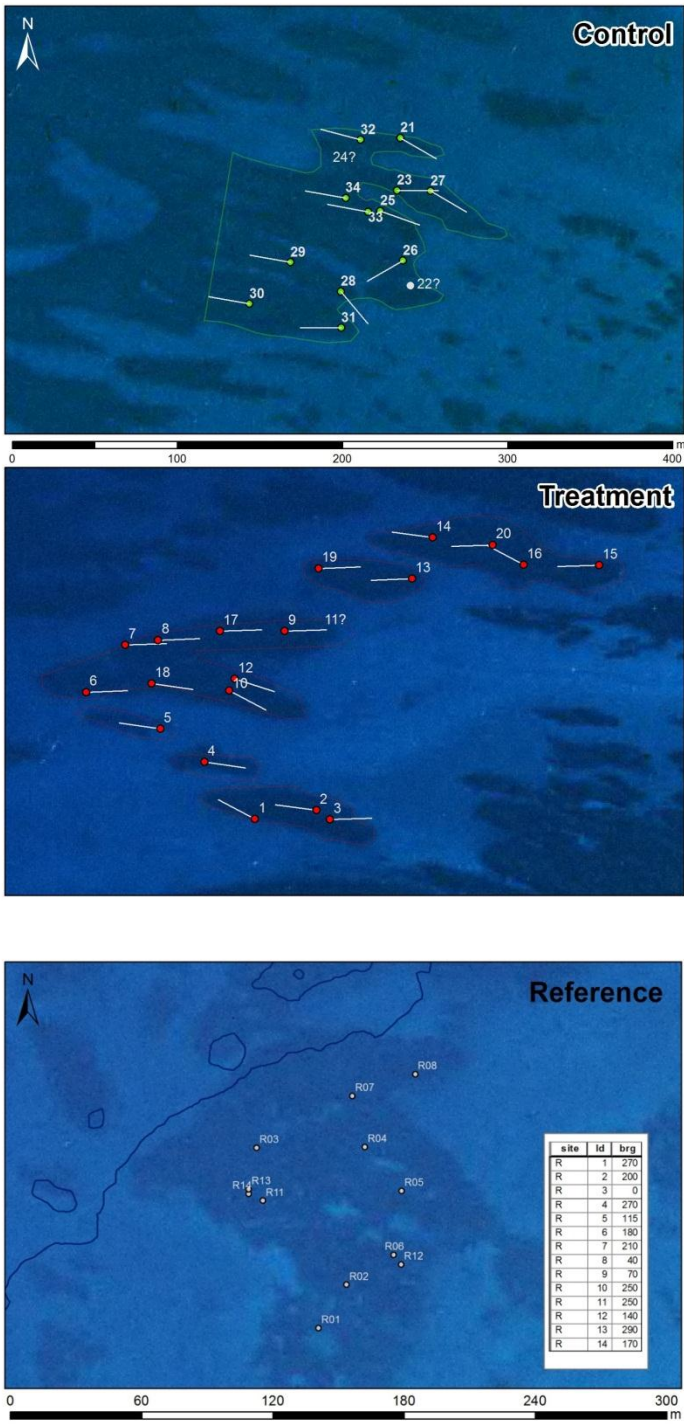


Figure 3. Location of forty-eight transects at treatment and control and reference study sites (map by K. Stamoulis).

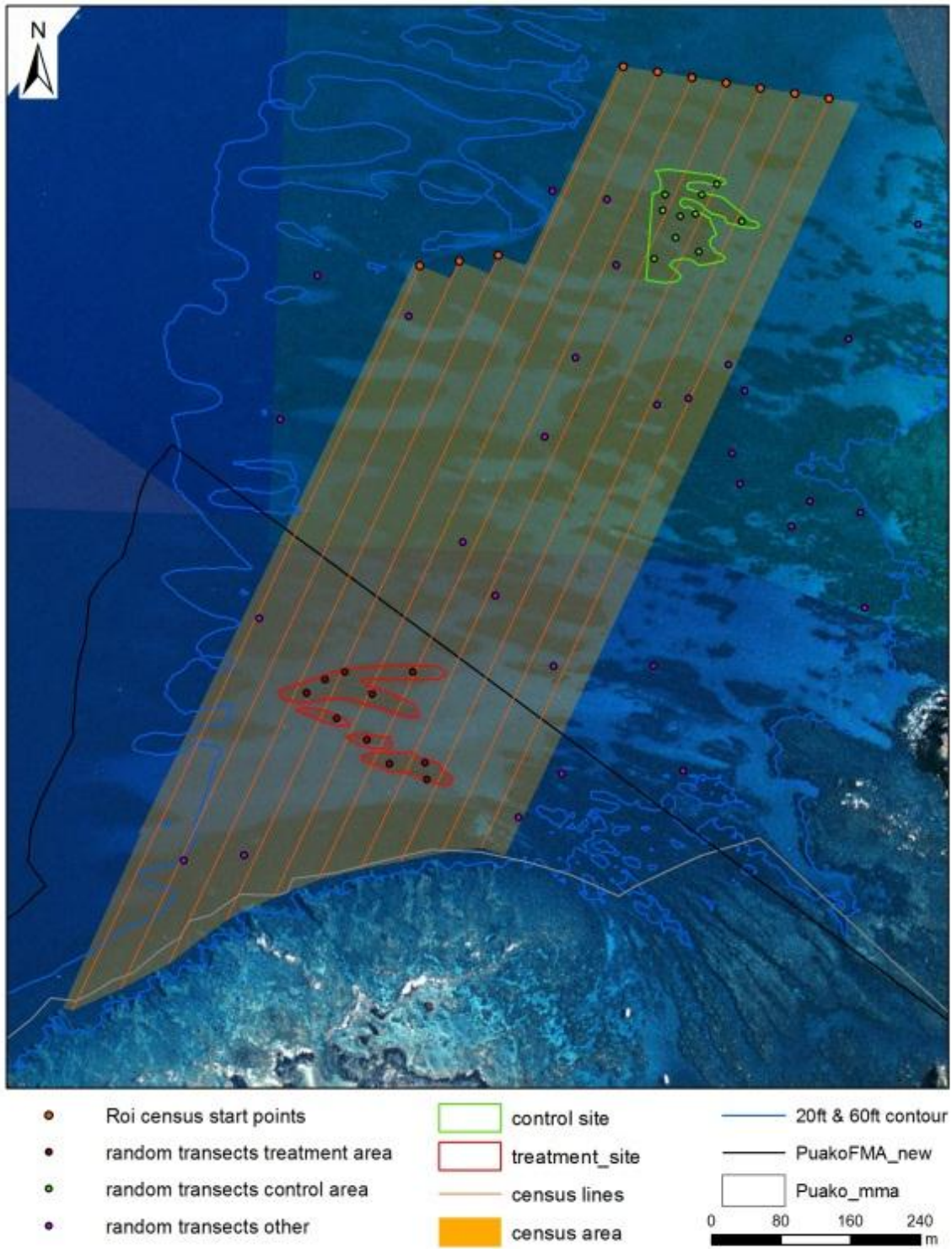


Figure 4. Location of roi census tracks for treatment and control reefs (map by K. Stamoulis).

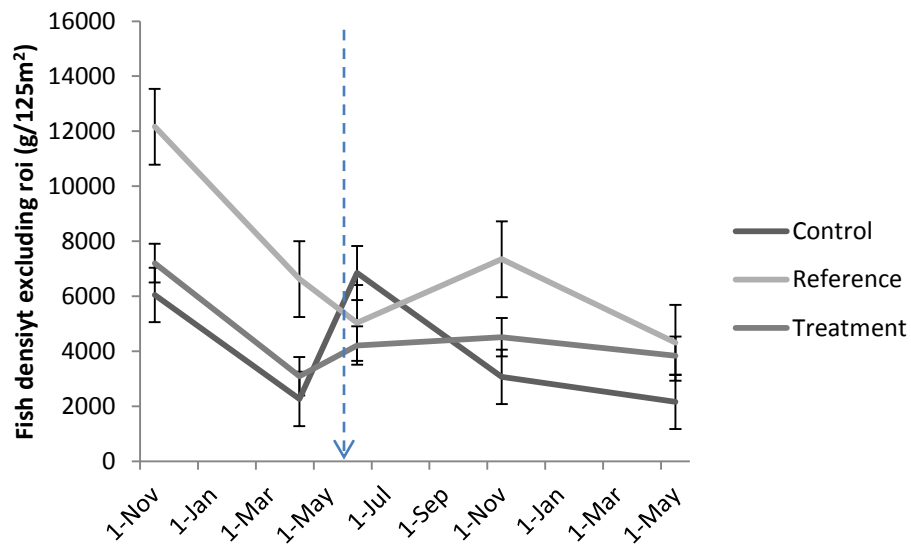
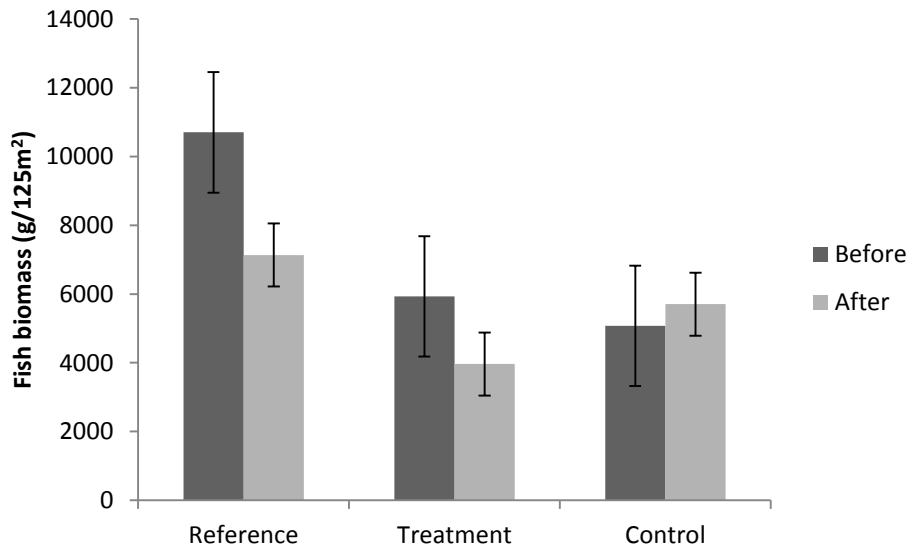


Figure 5. Trendline for total fish biomass excluding roi from November 2010- May 2012. Error bars are standard error of the mean. Fish down event is marked with the dotted line.

(A) Biomass



(B) Abundance

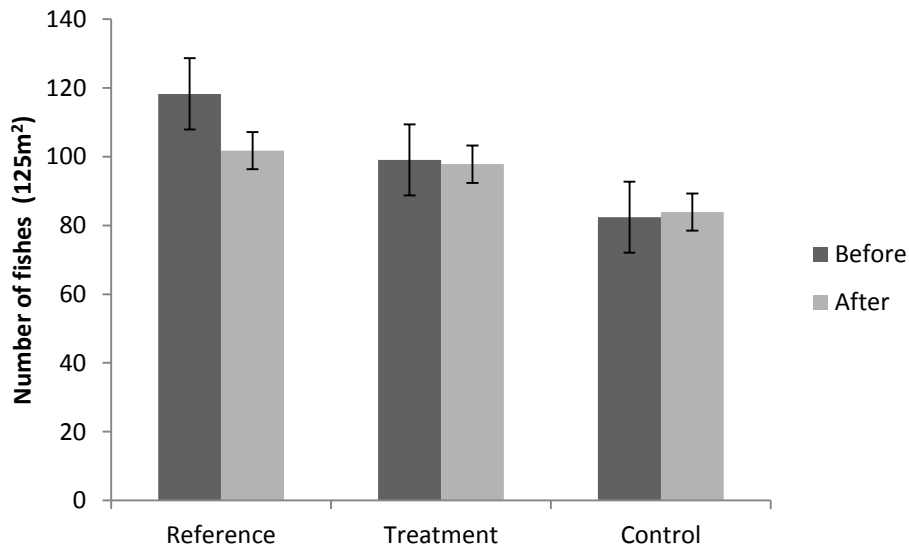


Figure 6. Fish assemblage metrics at three study sites before and six-months after roi removal for (A) biomass and (B) numerical density. Error bars are standard error of the mean.

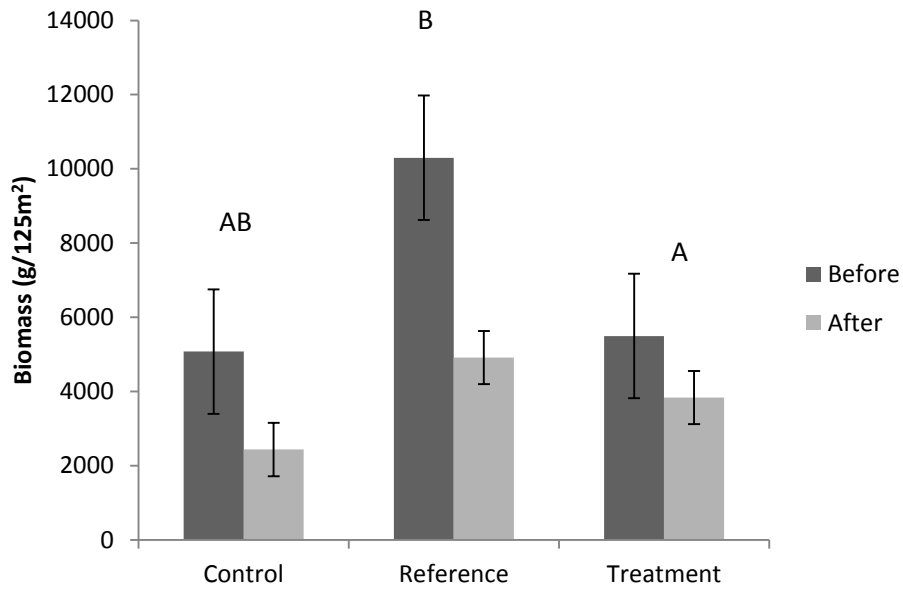


Figure 7. Total fish biomass before and one-year after (May 2012) roi removal. Error bars are standard error of the mean. Letters represent significant differences.

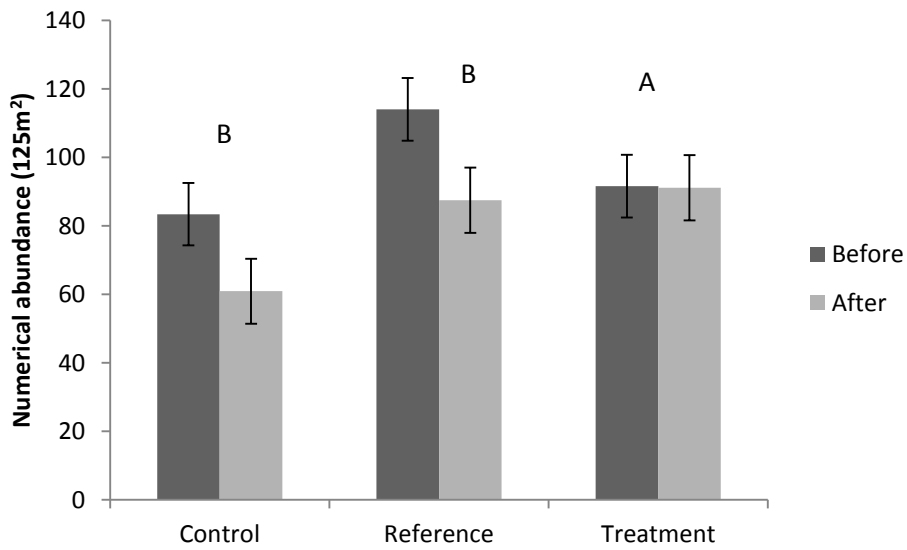


Figure 8. Total numerical abundance before and one-year after (May 2012) roi removal. Error bars are standard error of the mean. Letters represent significant differences.

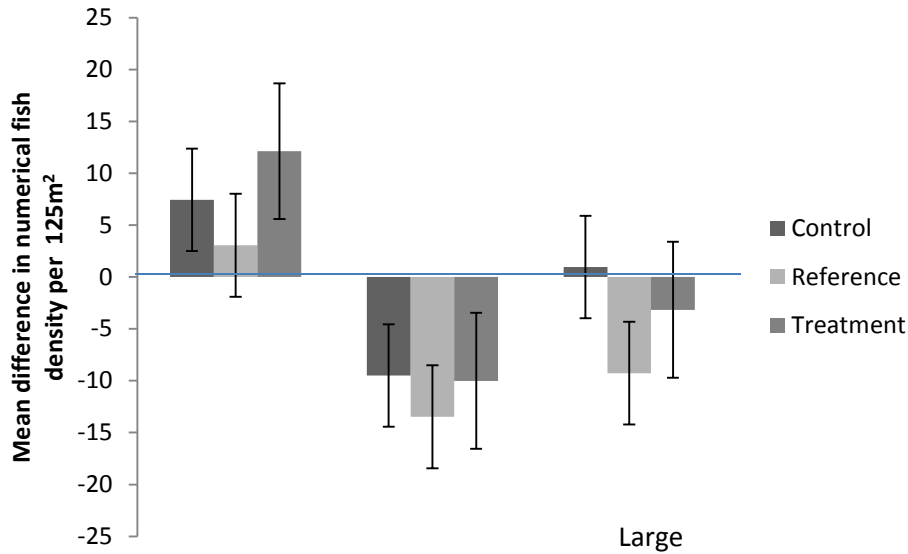


Figure 9. Mean difference in fish size structure at three study sites six-months after roi removal for small (≤ 10 cm TL) medium ($>10\text{cm} \leq 20\text{cm TL}$), and large ($>20\text{cm TL}$) fishes. Error bars are standard error of the mean.

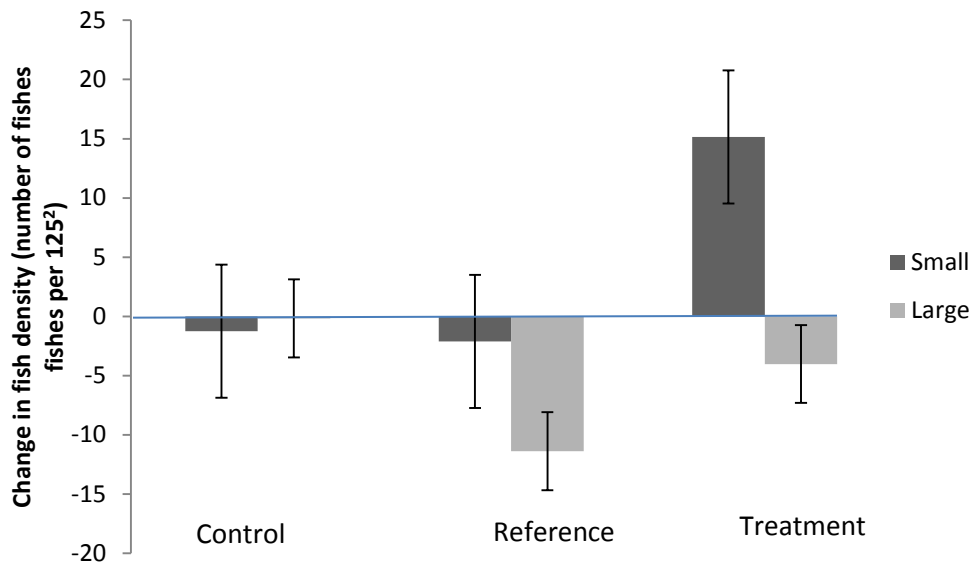


Figure 10. Change in numerical abundance of small (≤ 15 cm TL) and large (>15 cm TL) fishes six-months after roi removal. Error bar are standard error of the mean.

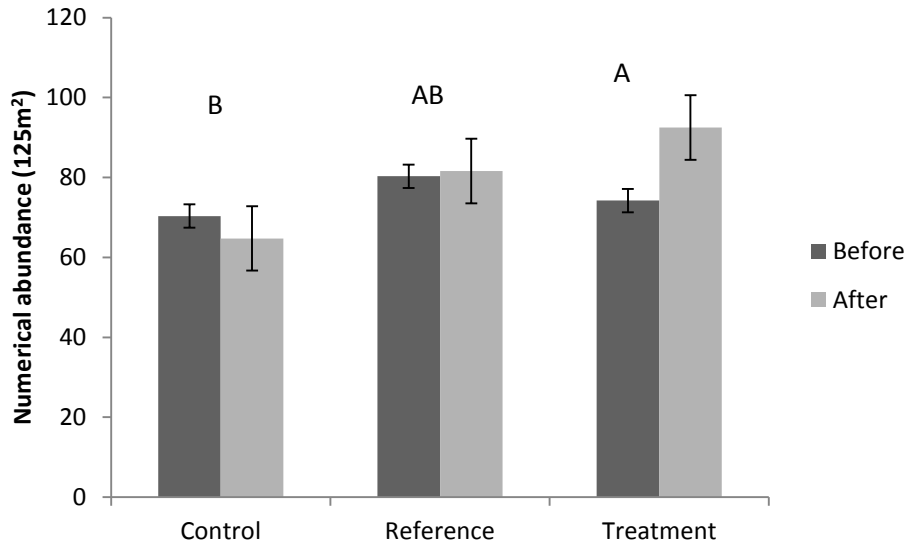


Figure 11. Numerical abundance of small ($5 \leq 15$ cm TL) select prey species before and six-months after roi removal. Error bars are standard error of the mean. Letters represent significant differences.

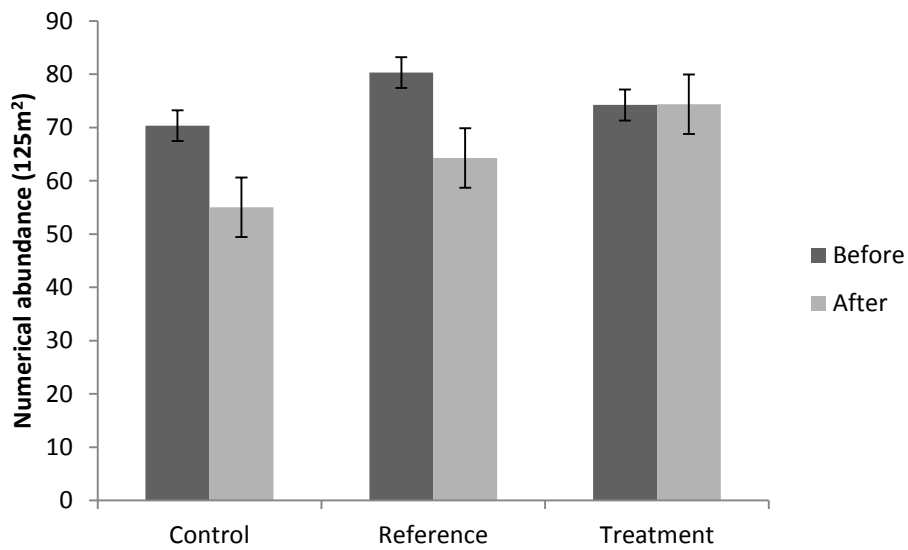


Figure 12. Numerical abundance of small (5-15 cm TL) select prey species before and 1-year after roi removal. Error bars are standard error of the mean. Letters represent significant differences.

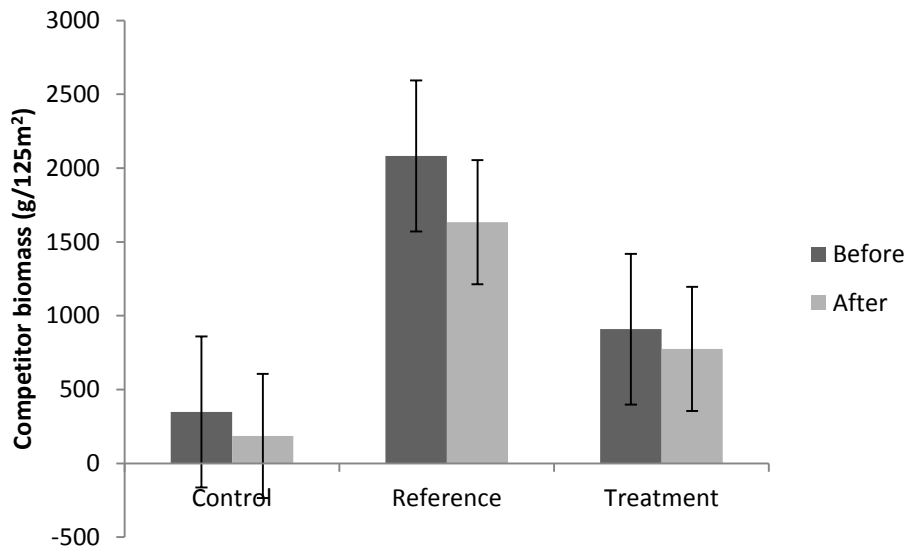


Figure 13. Biomass of potential competitor species before and 1-year after roi removal. Error bars are standard error of the mean.

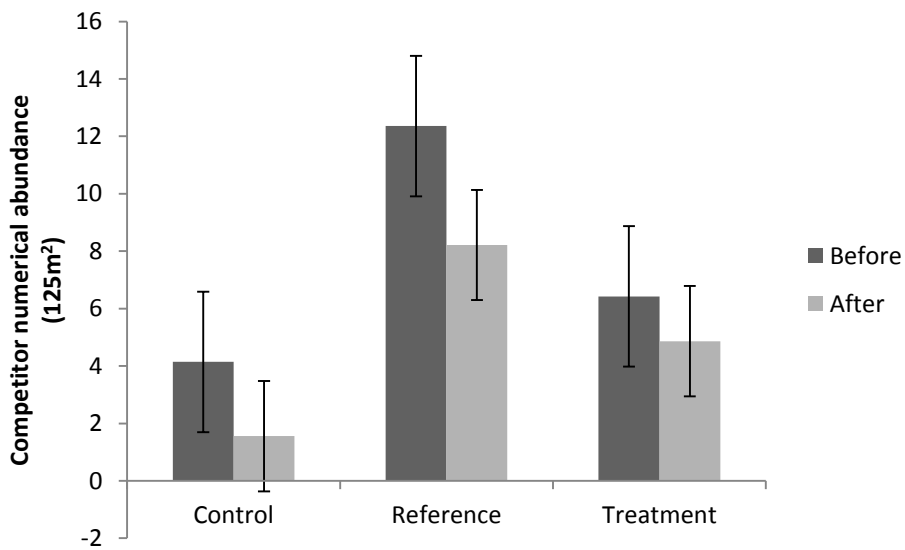


Figure 14. Numerical abundance of potential competitor species before and one-year after roi removal. Error bars are standard error of the mean. Letters represent significant differences.

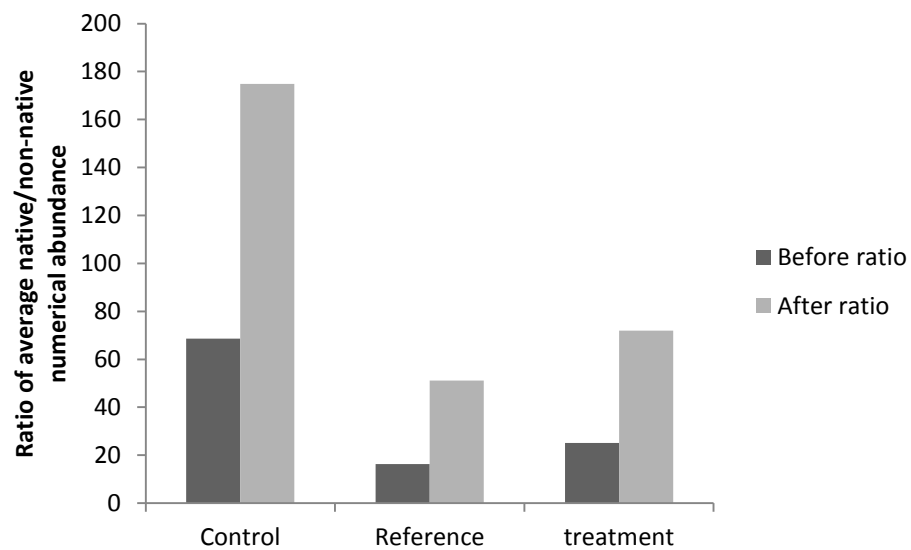


Figure 15. Ratio of native to non-native fish species before and one-year after roi removal.

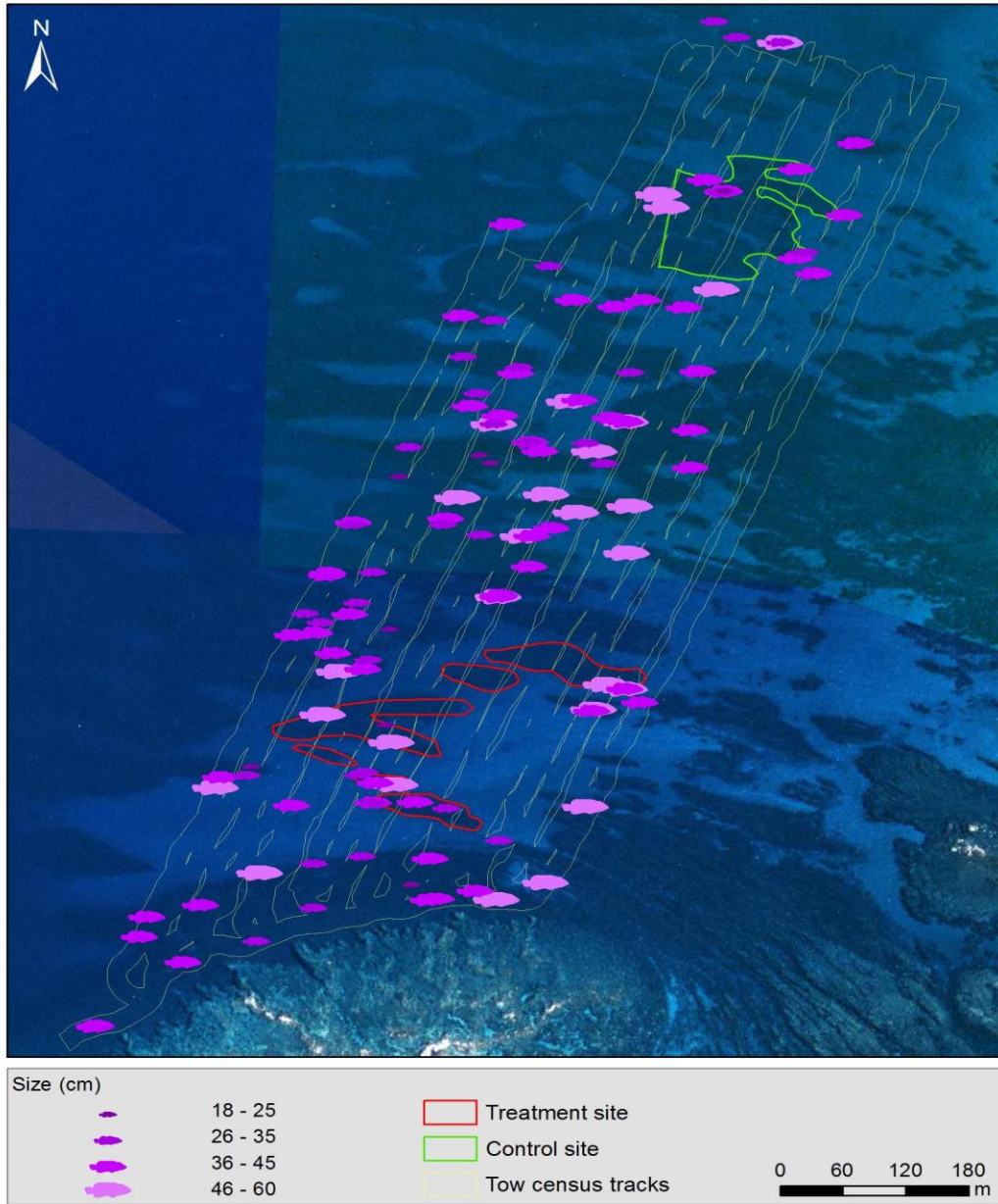


Figure 16. Map of roi distributions for census 1 before roi removal at the treatment site (April 2011) (K. Stamoulis).

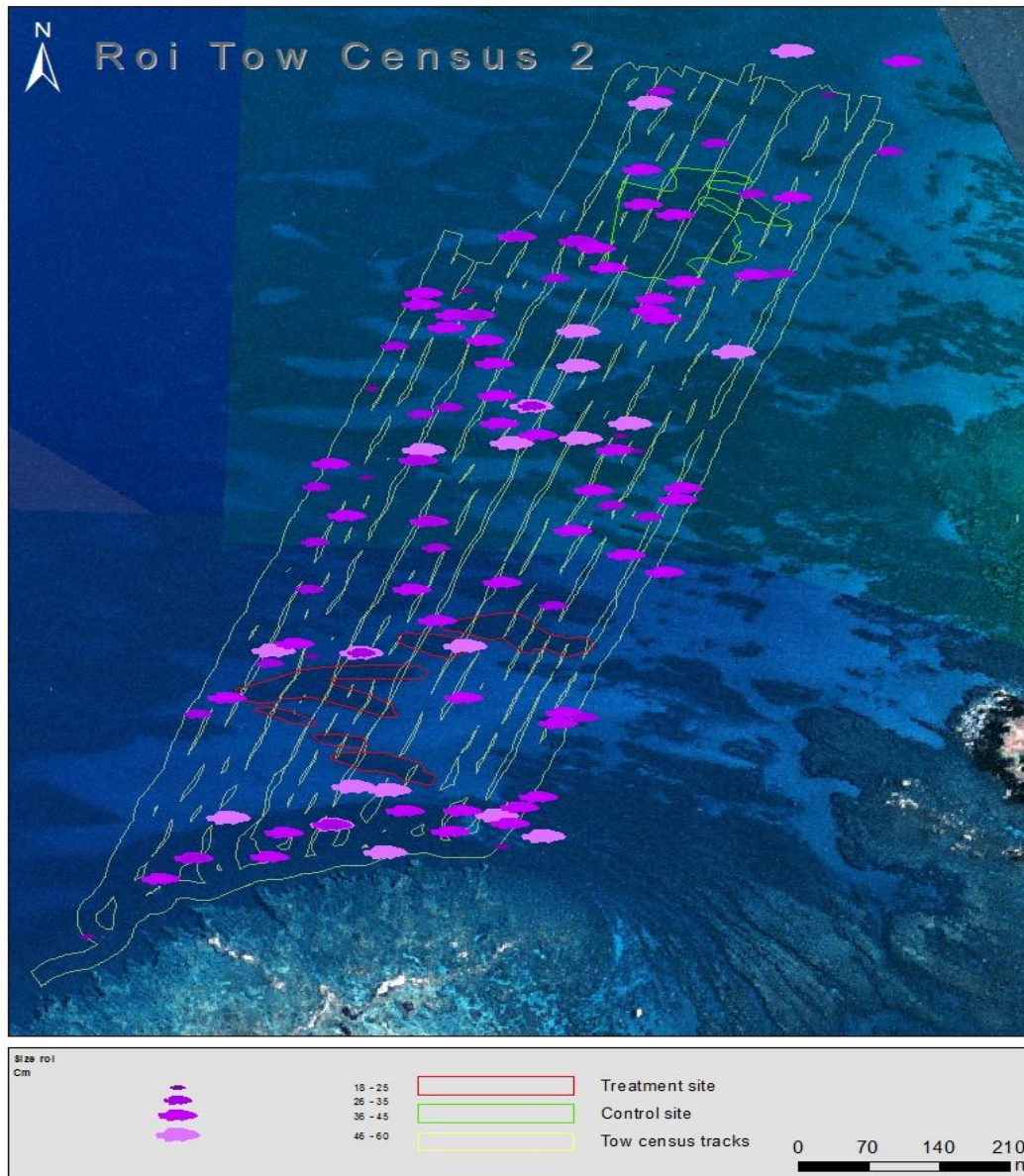


Figure 17. Map of roi distribution for census 2 after roi removal at the treatment site (June 2011). (Adapted from original map by K.Stamoulis).

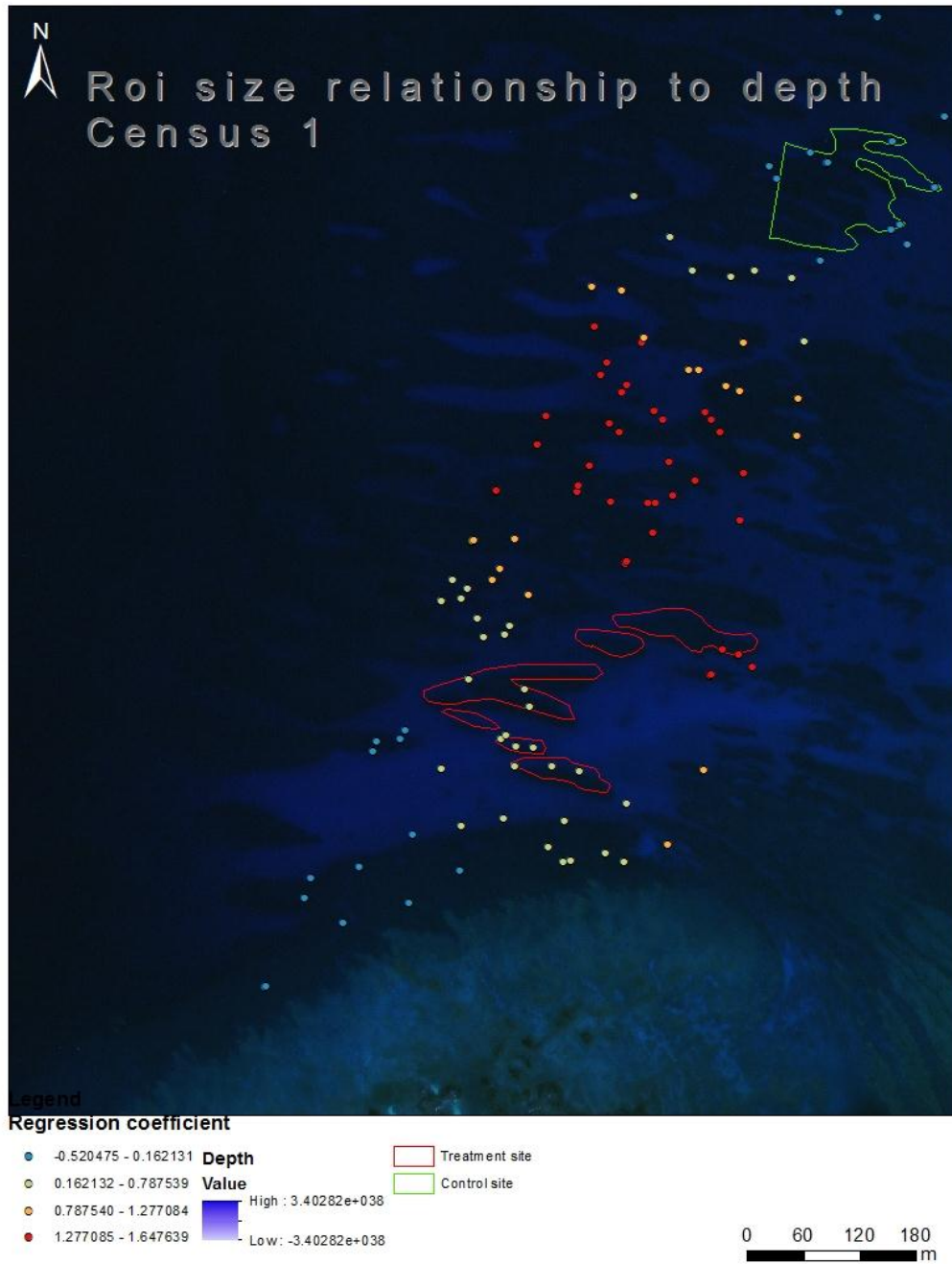


Figure 18. Map of geographically-weighted regression for roi body-size distribution by depth.

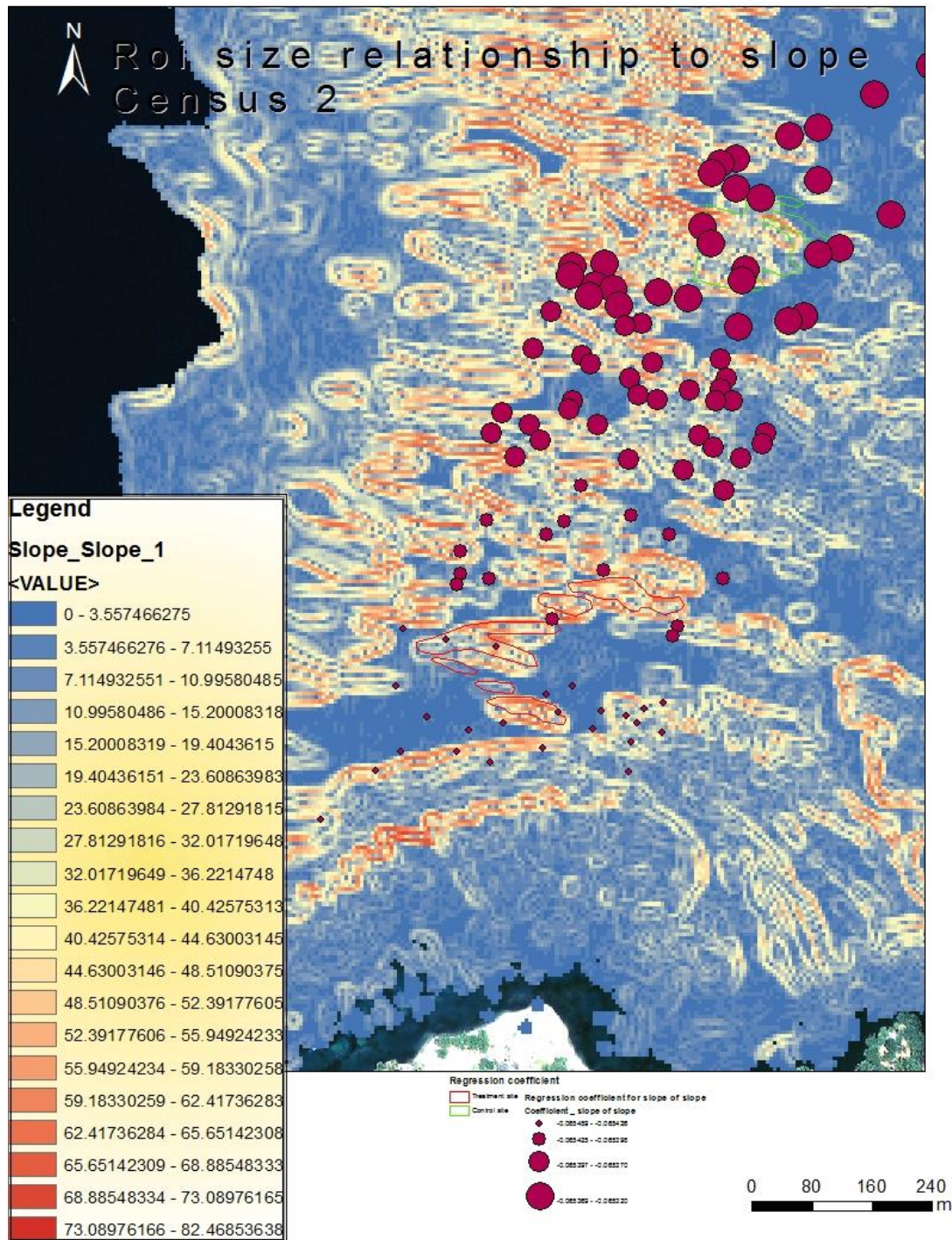


Figure 19. Map of geographically-weighted regression for roi body-size distribution by slope of slope (a measure of habitat complexity).

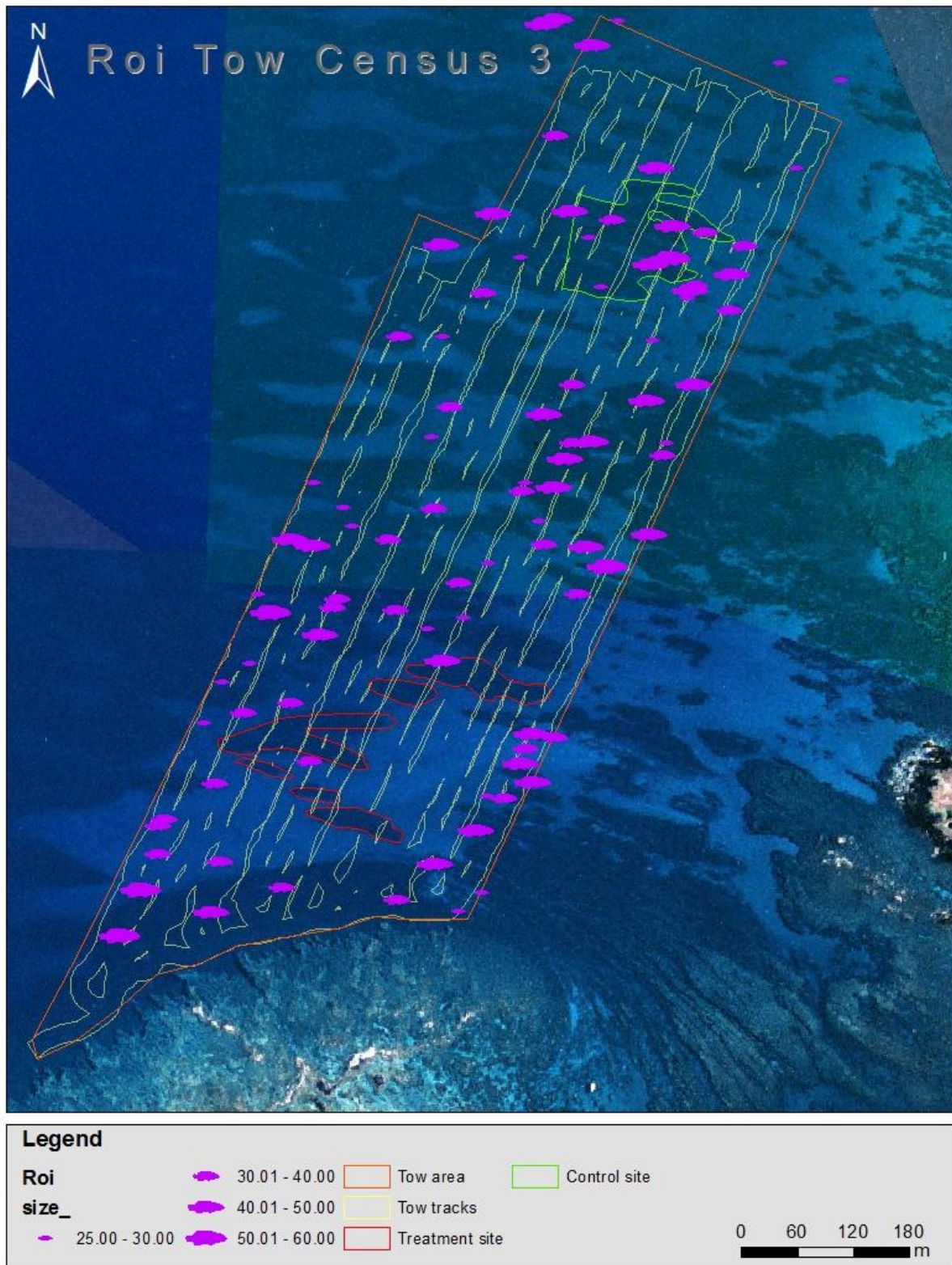


Figure 20. Map of roi distribution for census 3 after roi removal at the treatment site (Nov 2011). (Adapted from original map by K.Stamoulis).

Puako Roi Tag, Recapture and Re-sight Locations

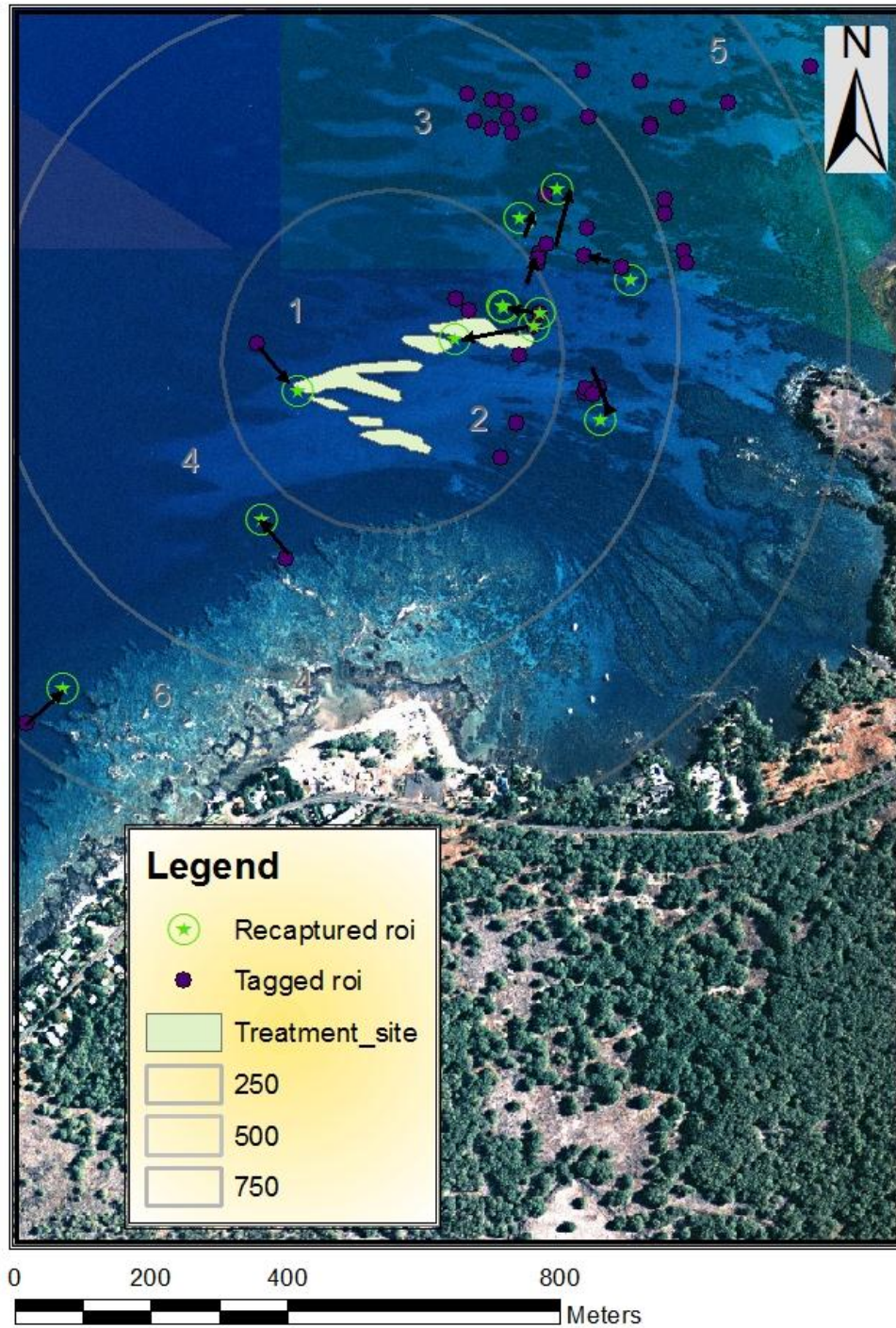


Figure 21. Map of roi tag and re-sight locations.

Appendix I. Cost of roi removal in Puakō

Recurring (by dive day)	
Tanks 3 @ \$10	\$30
Time 7 hr @ \$13/hr	\$91
Cost per diver/day	\$121
Additional Costs	
Fuel ~2gal/day @ \$5	\$10
Recurring summary:	
\$131X2 divers	\$262/day
\$262X11 days	\$2882/3 acres
\$2882/3 acres	~ \$960.66/acre

Appendix II. Cost estimate for roi removal in west Hawai'i. Estimate = Hard bottom reef habitat for west Hawai'i (excluding sand, including boulder habitat).

Depth range (feet)	Acres in west Hawai'i	Cost
30'-60'	4,316.3	\$4,147,964
10'-100'	12,595	\$12,103,795

Prepared By: Jonatha Giddens

A handwritten signature in black ink that reads "Charles Birkeland". The script is cursive and fluid, with the first name "Charles" and last name "Birkeland" clearly legible.

Signature of Principal Investigator

Date 29 August 2012