

Home range ecology of *Naso unicornis* (Bluespine Unicornfish): Use of acoustic telemetry to define foraging interactions with an invasive alga, *Gracilaria salicornia*, in a marine reserve

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF
HAWAI'I AT HILO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL CONSERVATION BIOLOGY AND ENVIRONMENTAL SCIENCE

JUNE 2014

By

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Keywords: *Naso unicornis*, home range, *Gracilaria salicornia*, acoustic telemetry, invasive algae

(UMR/Proquest Publishing Page)

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Acknowledgements

I would like to express my sincere gratitude to my mentor, Carl Meyer, committee members Don Price and Adam Pack, and my sampling partners Luisa Quieroz, Mariana Azevedo, Kira Wong and Mark Royer. I would not have been able to complete and pursue my interests in research without these people. I would also like to extend special thanks to my friends and family who have given me encouragement to go after my passions.

Furthermore, I am thankful for the resources provided to me by University of Hawaii at Hilo, University of Hawaii Manoa, and the Hawaii Institute of Marine Biology (HIMB). The HIMB Pelagic Fish Research Lab, NSF Lab, and Haggerdorn Lab through HIMB gave me materials and were a constantly supportive resource for my research needs.

I would like to thank specific people who have contributed to field and laboratory data collection: Carl Meyer, Luisa Quieroz, Mariana Azevedo, Mark Royer, Ginnie Carter, Greta Aeby, Mark Heckman, Leon Weaver, Kim Holland, Don Price, Adam Pack, James Anderson, and Melanie Hutchinson.

This project is a small component of a larger research collaboration with funding from SeaGrant award number ORS 20101809.

Abstract

Blooms of non-indigenous marine macroalgae have been reported throughout the Hawaiian Islands over the past 30 years. Successful invaders have dominated regions on both the Eastern and Western shores of Oahu. In Kaneohe Bay, a particular species of rhodophyte, *Gracilaria salicornia*, has successfully monopolized macroalgal communities throughout the Southern portion and is currently distributed to the most Northern regions of the bay. In a healthy reef community, herbivorous grazers play a large role in maintaining coral dominance by containing overgrowth of indigenous algae by grazing. Because of this known association, other studies have suggested that marine protected areas may carry the capacity to suppress overgrowth of successful invaders via promoting increased biomass of herbivorous reef fish.

The interactions of *Naso unicornis*, bluespine unicornfish, with *G. salicornia* have never been addressed in a field capacity. By determining whether these fish select for habitats covered with *G. salicornia* and actively graze on the invasive macroalgae, we can determine whether or not this species-specific interaction exists. Recent studies also suggest that herbivorous reef fish contribute to the dispersal of some macroalgal species via gut passage. We used a combination of active tracking with underwater visual census to determine habitat selectivity and residence of *N. unicornis* in a marine protected area in Kaneohe Bay. After confirming that *N. unicornis* selects for and grazes *G. salicornia*, we collected the fecal fragments to determine regenerative capacity once passed through the gut. Using a pulse amplitude modulation fluorometer along with observation, we were able to determine fluorescence at different stages of growth post-egestion.

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CHAPTER I: LITERATURE REVIEW

Introduction:

An ongoing discussion is occurring within the scientific community regarding the most practical uses of marine protected areas and their support of coral reef ecosystems (Marshall et al. 2011). In order to optimize such management discussions and resultant strategies, we need to understand the interactions of organisms across trophic levels that influence sustainability of a healthy reef. The overall success of marine reserve design has been well documented and has been shown to increase resilience of reef communities (Halpern and Warner 2002). Reserves that reduce fishery exposure to protected areas as well as support biodiversity may also offer indirect benefits from species within the community whose populations have benefited from reserve design.

Large reef fish in Hawai'i are marked as an important topic of study for marine reserves due to reduction of biomass in commercial use in artisanal fisheries and the aquarium trade. Contributing to ecosystem resilience, herbivorous reef fish are integral to the structure and composition of a coral system. Reef dwelling herbivorous fish are attributed to suppression of overgrowth of algae which prevent phase shifts from coral to algal dominance (Chong-Sen et al. 2014). Because movements and area use of these fish are often species specific, defining home range ecology of marine organisms in a reef system is an important component in establishing the size and structure of a reserve. Many known factors play into home range selection of a particular species such as niche use, body size, life history movements, diel pattern, bathymetric composition, and structure of reef systems (Kramer and Chapman 1999; Ogden and Quinn 2002, Marshall et al. 2011). Research efforts that define home ranges of these species also are often

site-specific due to benthic composition of an area as well as environmental factors that are unique to a geographic location.

One of the more common ways to research home range selection is through tracking via acoustic telemetry in a near-shore area. Uses of acoustic tracking are increasing and data concerning movements of different species of reef fish are readily available (Arnold and Dewar 2001). Investigating site fidelity within herbivorous species is necessary to understand the relationships of a group of organisms and their benthic food source within a reef ecosystem.

While scientific research shows benefit to promotion of biomass in target species, few have investigated indirect bio-control of invasive organisms by protecting consumers through reserve design. Invasive algae are linked to trophic phase shifts in marine ecosystems worldwide and are an increasing problem in coastal communities (Chong-Seng et al. 2014). Few reports also address spread and dispersal of algae through gut passage of herbivorous grazers inclusive to reef fish. By identifying whether fish select for a species of algae, we can also examine viability of fecal fragments to determine potential for dispersal in a habitat.

This literature review provides a comprehensive background in the use of marine protected areas in conservation and the type of research that is necessary to demonstrate the efficacy of this style of management practice. Topics in this review delineate the trophic role of large herbivorous fish in reef communities and the style of data collection that is currently acceptable to accurately represent ecosystem function. Spatial distribution and success of nonindigenous macroalgae are also included as a background to support the importance of research in the interaction between consumers and producers in a reef system.

1. Marine Protected Areas in Conservation:

Marine protected areas are geographic locations designated to manage and protect resources in habitats. Marine reserves are a facet of marine protected areas defined through limitation of fishing activity via no-take zones (Halpern and Warner 2002). A principal function of reserves which limit collection of organisms is to conserve marine habitats and provide biomass to adjacent fisheries (Polunin 1990, Dugan & Davis 1993, Shackell and Willison 1995, Bohnsack & Ault 1996, Kramer & Chapman 1999, Eristhee & Oxenford 2001, Marshall et al. . 2011). Marine reserves assist in sustaining fisheries by allowing females to reach reproductive age as well as increase recruitment to nearby habitats and beyond (Beverton and Holt 1957, Dugan & Davis 1993, Roberts and Polunin 1993, Kramer & Chapman 1999, Eristhee & Oxenford 2001). Some argue that the most successful marine reserves are those that properly assess home ranges of target populations (Roberts and Polunin 1993, Kramer and Chapman 1999, Eristhee and Oxenford 2001). Shape and size of home ranges in reef dwelling fish change between and within species and are influenced by a number of environmental factors. The availability of resources, density of populations, size of fish, and varied habitat contribute to fish movements that define home range (Kramer and Chapman 1999, Marshall et al. 2010). For marine reserves to be effective, boundaries of the reserve must reflect and stay within the confines of selected habitat and movement of the target population or organism. Defining a reserve boundary also takes into account the assumption of spillover. Spillover is the capacity for a fish to move beyond the boundaries of the reserve once the primary period of growth (post-settlement) has passed (Kramer and Chapman 1999). To quantify the success of the primary function of reserves, scientists have attempted to most accurately reflect the movements of targeted populations within a reserve.

Another main argument directed towards marine reserves is the question of efficacy. A review of 80 different marine reserves found that every biological component measured within each study was greater in reserve sites compared to non-protected locations (Halpern and Warner 2002). Growth in density, average size, biomass, and diversity within reserves was supported by the review. This finding was also independent of the size of the reserve, supporting the idea that reserves are overwhelmingly effective in their design. One component of the review that showed variation between reserves is the temporal aspect (length of time it takes for reserves to become effective), which can be attributed to differences in growth rate and fecundity of varied species within reserves (Halpern and Warner 2002). The length of time to see improvement is often linked with the level of existing degradation to the structure and diversity of a reef.

It is necessary to understand influencing factors that affect relocation of an individual passing through reserve boundaries to non-protected areas. Models for reserve design and function are constantly addressed, but little weight has been placed on notable environmental fluctuations and behavior of organisms that may affect movement (Kramer and Chapman 1999).

2. Use of Acoustic Telemetry to Define Home Range:

Increasing resource availability and access of information allow design and management of marine reserves to improve estimation of home range in populations. A fish is said to possess a home range when movement and habitat use are repeatedly observed in the same area (Kramer and Chapman 1999). Data collection is increasing in efficiency as technology evolves. There are multiple methods for measuring population size and spatial distribution of marine organisms, but all share the commonality of assessing repeated observations of tagged individuals in the same area (Kramer and Chapman 1999, Arnold and Dewar 2001).

Traditionally, assessment of population size and distribution has used mark-release recapture (MRR) techniques. This method uses either natural markings or externally visible tags to mark sampled individuals and quantify the size and movement of populations based on the number of recaptured individuals at a certain location (Kramer and Chapman 1999; Marshall et al. 2011). The nominal cost of tags typically allows a researcher to tag a large number of target individuals. This can give true insight into the size of a population and species growth rates in a given area. Although functional, MRR is limited by home range interpretation from linear distances between points of capture to points of recapture (Bernard and Hansen 1992). High tag loss rates and improper representation of population size can lead to gross underestimation of home range of a population (Seber and Felton 1981). Newer technologies allow determination of home range to a much finer degree of accuracy. While mark release recapture is still seen as a viable research method, ultrasonic telemetry provides robust definition of movements within a sampling site.

Acoustic telemetry can provide detailed information on the fine-scale movements between relocations that estimate the full extent of a fish movements and residency in a habitat (Arnold and Dewar 2001). Behavioral ecology can also be determined from variable spatial and temporal structures based on the type of tag used (Clements et al. 2005, Heupel et al. 2006). Dependent on resources for a study, there are multiple options for a researcher wishing to execute a tracking regime for any particular species. Ultrasonic tags are available that once attached or implanted into a fish, can deliver continuous or coded pings to a receiver.

In the last twenty years, use of tags has extended to measuring habitat use and home ranges of coral reef fish (Holland et al. 1993). The most commonly used acoustic tracking for reef fish can be broken down into two types of internal tags; active and passive (Arnold and

Dewar 2001). These tags are either continuous or coded transmitters placed in the body cavity of individual fish. Acoustic pingers are tags set at different frequencies that transmit continuous signals which can be actively monitored from a hydrophone connected to a receiver when activated (Arnold and Dewar 2001). The receiver logs temporal coordinate data that can later be downloaded to a computer. Alternatively, coded transmitters are set at a single frequency with a unique coded ID that is logged from underwater receivers at a fixed position (Clements et al. 2005, Heupel et al. 2006). Temporal coordinates are logged each time a tagged fish passes within range of the receiver. It logs fixations passively over an extended period of time. The receiver can then be collected and downloaded to a computer.

Active tracking is completed over a short time frame that typically lasts no longer than 2-3 months and requires constant monitoring of each tagged fish during that time (Arnold and Dewar 2001). This style allows fine-scale movements to be quantified but can result in temporal gaps. Passive tracking allows a researcher to collect data over a much longer temporal scale ranging from months to years. Bathymetric composition and background noise can interfere with the signal strength of tags sent to either passive or active receivers. While there are benefits and disadvantages to both methods of tracking, it would be optimal to use a combination of both with each tracking study. Funding tends to limit this option in many cases. Either option can provide valuable spatial analysis for fish within a habitat and have been used to create and assess boundaries that define marine reserves.

Acoustic tracking of reef fish didn't become popular until the early 1990's and has since shaped how we manage reef space through marine reserves (Holland et al. 1993). Recent publications incorporate one or both styles of tracking in their study as an acceptable means for defining home ranges of reef fish. Many of the study sites track fish within a marine reserve and

have characterized movement patterns to a finer degree than previously seen. Sample size, methods, and time spent tracking can have an effect on the quality of data collected.

Recognition of the need to understand movements in a reserve area produced early studies that shaped the framework, methods, and scope of research projects incorporating acoustic tags. Some of the earlier designs included *Kyphosus sectarix* (Bermuda chub) in the West Indies and goatfish in Hawai'i (Holland et al. 1993, Eristhee & Oxenford 2001). In the West Indies, continuous acoustic pingers were implanted in 5 fish at one site, and 6 fish at the other (Eristhee & Oxenford 2001). The purpose was to compare home range movements between two marine reserves. They found that the home ranges were not significantly different between the sites and use of space often overlapped in individuals in the same reserve. Data suggested that one of the reserve boundaries did not fully encompass the size of the home range and recommended that it be increased based on their findings (Eristhee and Oxenford 2001).

In Hawai'i, two different species of goatfish movement patterns were examined in the same marine reserve in Kāne'ohe Bay, Oahu. To examine growth rates and movement patterns of *Mulloides flavolineatus* (white goatfish), four fish were followed continuously for 2-16 days where obvious daily movement patterns were recognized (Holland et al. 1993). Some years later, the same researchers participated in a study for a different species of goatfish; *Parupeneus porphyreus* (whitesaddle goatfish). Five fish were implanted with continuous acoustic pingers and tracked for a period of one month. Results supported that the fish stayed within the boundaries of the reserve at for the study period suggesting that even small areas can be used to protect a species as long as their energetic and reproductive needs are met by that particular habitat (Meyer et al. 2000).

Standard methods of tracking have changed minimally in the last 15 years, but more studies are incorporating use of remote underwater receivers in their tracking regime. The goal of combining active and passive tracking for a single species is to describe movement patterns and habitat selectivity with the confirmation of long-term site attachment. *Caulolatilus princeps* is a targeted fish for California fisheries and is managed in similar style to groundfish due to assumed sharing of behaviors (Bellquist et al. 2008). By using both active and passive tracking of 17 individuals for each method, home ranges and habitat use were quantified along sites selected from the Catalina Marine Life Refuge found within the Southern California Coastline. Each fish was actively tracked up to three 24 hour periods. They were found to be diurnally active with crepuscular movements displaying similar movements to other daytime foraging reef fish seen in Hawai'i (Bellquist et al. 2008).

Scarus rubroviolaceus (redlip parrotfish) is common to Hawai'i and are important for local fisheries and tourism. A recent study reported home range and movement patterns from a sample size of 21 individuals. Their active tracking substituted observations from snorkelers instead of implanting continuous pingers. A total of 17 coded tags were deployed for the passive tracking portion. Their findings suggested that while these fish display high site-attachment within Hawaiian MPA boundaries, more regard in design should be directed towards nighttime habitats in addition to daytime foraging areas to allow for full protection (Howard et al. 2013).

These studies substantiate claims that residency and site attachment of reef dwelling species are common. Both continuous and coded transmitters are ideal for reef fish based on the tendency to stay in a small area versus some of the difficulties of tracking larger, migratory pelagic fish.

3. Analysis of Acoustic Data

Calculation of home range is performed with different methods and the assessment of the optimal method is still under debate. There is no universal standard in place because home ranges are not only species specific, but can vary greatly based on how a habitat or function of that habitat shapes general movements of fish site attached to the area (Kramer and Chapman 1999). Sample size and number of fixations per animal can also affect estimation of home range of an entire population. In addition to outlining an area used by an individual with a minimum boundary, calculations need to account for random or exploratory movements outside the area of repeated use. Many times a fixed percent of activity of recorded movements (95% or 99%) are considered to be in the normal area of use for the study population. Accepted analyses typically include minimum convex polygons and kernel utilization distributions as they can limit outliers and smooth boundaries.

The equation for minimum convex polygons (MCP) developed by Mohr in 1947 connect the outermost points to make a polygon of at least four sides that is convex in nature. Because this form of calculation is non-parametric, it can be used with all types of data distribution, but is also limited by bounding geometry. Areas of high utilization within the boundary are calculated the same as areas of low utilization. For use in home range, the outermost points that have been connected enclose movements of an animal or population within a boundary created by the connecting segments between points (Mohr 1947). Kernel utilization distributions (KUD) use density probability as opposed to bounding geometry to estimate space utilization from a collection of points (Worton 1989). Working off non-parametric assumption, estimation of random points from locational observation is used to estimate the utilization distribution. Areas of high utilization are identified within the home range of an individual. Smoothing parameters

allow density to be understood through use of histograms. Using both types of calculation gives a more precise perspective of true home ranges in populations.

Spatial autocorrelation is the most common form of bias in home range study. Added mathematical weight is placed on neighboring regions of smaller distances than fixations that are farther away (Fartheringham and Rogerson 1993). During analysis, it is necessary to account for autocorrelation by performing analysis by individual which allows for independence among tracks. When combining fixations of the entire sample size, autocorrelation is more likely.

4. *Naso unicornis*

Classification: Actinopterygii; Perciformes; Acanthuridae; Nasinae; *Naso unicornis* (Randall 2001).

Commonly known in Hawai'i as Kala or the bluespine unicornfish, *Naso unicornis* is within the family Acanthuridae. Native ranges are from the Indo-West Pacific extending to the east coast of Africa inclusive of the Hawaiian archipelago (Borden 1998, Hardman et al. 2010). Known as a primary consumer, *Naso unicornis* is classified as an herbivorous grazer. Life history studies have shown that these fish are one of the larger grazers with a maximum length of 70cm and ages recorded up to 58 years (Choat and Axe 1996, Randall 2001, Eble et al. 2009, DeMartini et al. 2014).

Unicornfishes are named by the protruding tuberosity from the head of some species within the genus *Nasinae* (Borden 1998, Arai & Sato 2007). This protuberance is thought to be used predominately for sexual selection in mating as opposed to aggression based on results from an experimental study (Arai & Sato 2007). Little is known about sexual behavior and spawning in unicornfish, but most observations explain that spawning occurs near the surface and in

channels with current for better dispersal (Randall 2001, Arai & Sato 2007). As one of six genera in the suborder surgeonfishes of the family Acanthuridae, unicornfish are commonly associated with shallow reefs. Species within the family Acanthuridae also possess caudal plates that are used in dominance or defense against potential predators. *N. unicornis* have two anteriorly pointed blue caudal plates on either side of the body. The plates are typically larger and fin filaments longer in males of the species (Myers 1989, Arai & Sato 2007).

Until recently, limited data have been available for the life cycle and history of *N. unicornis*. Maximum age and size varied with each report. To date, no empirical peer-reviewed study has been published regarding the life history of the species. In 2009, a study prepared for the fisheries local action strategy for the Division of Aquatic Resources in Hawai'i examined the gonads and sagittal otoliths from a sample size of 197 *N. unicornis*. Reproductive size at maturity (L_{50}) and length-age relationships were quantified for the species. Annual rings were counted to assess individual age from the otoliths. This study estimates the L_{50} for females with a fork length (FL) of 37.8cm and males with a FL of 28.6cm (Eble et al. 2009, DeMartini et al 2014). FL is measured from the tip of the snout to the bifurcation of the caudal fin. Total length (measured to the end of the caudal fin) is not typically used due to varying lengths of fin filaments within the species.

Unicornfish are found in tropical regions throughout the Indo-Pacific and are a target group for artisanal fisheries. In Guam, total catch of unicornfish declined 86% from 1950-2002 (Marshall et al.2011). While the International Union for Conservation of Nature (IUCN) Red List states that there is not enough data to easily quantify catch declines, they are listed as least concern based on their abundance in well managed, protected areas. Even though record of catch decline has been reported, local stocks may be able to be sustained by continued management of

reserves based on positive response to protected areas (McIlwain et al. 2012). Well managed reserves contribute to the protection of *N. unicornis* as well as support their functional role in trophic control of reef ecosystems as algal grazers. They are also an ideal subject of study based on their size (Hardman et al. 2010, Hoey and Bellwood 2011). Specimens large enough can be tagged with sonic transmitters and previous ultrasonic telemetry work with *N. unicornis* have provided a basis for comparing behavior and movement in varied geographic locations.

One study used an outboard vessel to track seven *Naso unicornis* for a period of 13-21 days for active tracking. Their results supported the condition that closing the marine protected area to fishing would be beneficial as their study subject stayed within the confines of the reserve (Hardman et al. 2010).

In Guam, there is a high fisheries production rate from artisanal fishers for reef dwelling species. Marine reserves are the primary management tool for this fishery. Two commonly targeted species, *Naso unicornis* and *Naso lituratus*, were the subject of a passive tracking study. The design of the study addresses whether reserve size matches the movement of marine fishes. Twenty *N. lituratus* and ten *N. unicornis* were implanted with coded acoustic transmitters. The study found that all fish stayed close to the reef flats and 98% of the detections from their shallow reef receivers were from *N. unicornis*. Results showed that both species displayed site fidelity with home ranges of individuals from 0.005 to 10.82 ha. (Marshall et al.2011).

In Hawai‘i, movement patterns and home range size have been defined for *N. unicornis* in the Waikiki Marine Reserve on O‘ahu (Meyer and Holland 2005). Site-attachment was identified by a combination of MRR, underwater visual census, and active tracking in the study area. This particular study found bathymetric composition to be responsible for differences in

biomass and home range size of this species and is attributed to ontogenetic shifts in habitat use. Results from MCP and KUD showed that individual fish that were actively tracked varied in home range size, but all stayed within the boundaries of the marine reserve. The variance was explained by size of individuals and habitat composition (Meyer and Holland 2005). This site attachment is consistent with other studies that address movements within confines of a marine reserve and show that many surgeonfish among other reef fish display high site fidelity to each study area (Robertson and Gaines 1986, Hardman et al. 2010).

Site fidelity and habitat use can be attributed to many environmental factors, including foraging resources. The genera *Naso* exhibits diverse feeding preferences for a range of zooplankton and alga; where *Naso unicornis* predominantly exhibits herbivorous qualities preferring brown macroalgae (Robertson & Gaines 1986; Choat et al. 2002). *N. unicornis* are commonly described as marine algal grazers, playing a role in ecosystem function. *N. unicornis* is known for a preference for brown algae (phaeophytes). Other species are known for grazing on both filamentous and thallate rhodophytes (red algae) and chlorophytes (green algae). In Hawai‘i, one of the local names given to the phaeophyte *Sargassum echinocarpum* is “limu kala,” and is frequently used as bait for fishing to specifically target *N. unicornis*. (personal observation) Stomach contents of *N. unicornis* and *N. lituratus* caught in Indo-Pacific regions have been identified with high molar values of acetate from short chain fatty acid analysis. Acetate is the main product of fermentation and in *N. unicornis* specifically, is attributed to fleshy brown alga (Choat et al. 2002).

Resource competition and interactions with other species can also affect habitat use. In the Indian Ocean, a study found a 99% habitat overlap among *N. unicornis* and *N. brevirostris* where individuals within the habitat displayed non-territorial behavior. They were found to have

a large home range inclusive of multiple hectares of intertidal reef crest when foraging (Robertson & Gaines 1986).

5. Invasive Macroalgae

Natural geographic distribution of marine algae is commonly described by variation in sea temperature (Chapman 1943, Lewis 1964, Sousa et al. 1981). Tidal gradients and interspecific biological interactions are also thought to be influential in structuring the composition and distribution of algae in communities (Sousa et al. 1981). To the contrary, prevalence of invasive algae is the result of anthropogenic experimental manipulations, commercial production, and accidental introduction through sources such as fouling of ships (Russell 1992, Rodgers and Cox 1999). Hawaiian ecosystems are heavily impacted by alien species due to specialized endemic biota that have proven to be extremely susceptible to invasion (Eldredge and Smith 2001, Friedlander et al. 2002, Smith et al. 2004). Macroalgae can store nitrogen and phosphorous on a short term basis, and can switch energy investment from carbon to nitrogen to phosphorous acquisition. Invasive algae can be highly adapted for nutrient uptake and storage that carry potential to out-compete specialized endemics, creating phase shifts in algal communities (Andreakis and Shaffelke 2012, Gordillo 2012). Establishment of invasive macroalgae on O‘ahu, Hawai‘i is recognized and documented for at least 18 species (Eldredge and Smith 2001). Proliferation since the incidents of introduction vary with each species, but there are ones in particular that are prevalent throughout coastal regions of O‘ahu. *Gracilaria salicornia* and *Kappaphycus alvarezii* are two species that have spread in abundance in Kāne‘ohe Bay since the 1970s (Rodgers and Cox 1999). Both have the ability to reproduce asexually through vegetative fragmentation which is a primary reason for spread through a large

portion of South Kāneʻohe Bay. The spread of nonindigenous macroalgae has threatened reef structure in Hawaiʻi and has potential to monopolize community composition.

The increased distribution in Kāneʻohe Bay has prompted studies that encompass comprehension of life history and ecology of the invasive species commonly found in the area. Some species of nonindigenous macroalgae are extremely resilient to environmental changes. *Gracilaria salicornia* carry a wide range of traits that adapt for growth in harsh conditions. This species can tolerate high ranges of desiccation, light availability, salinity, temperature, and chemical treatment (Rodgers and Cox 1999, Smith et al. 2004, Nelson et al., 2009, Andreakis and Schaffelke 2012). Based on the dense matting in growth of this species, *G. salicornia* is also highly adapted to predatory resistance. In Kāneʻohe bay, distribution is increasing yearly and now extends to North Bay where it once only dominated areas in the South Bay (Smith et al 2004). Physical disturbance of any kind can generate viable fragments contributing to the spread and distribution of the species. Spread may also be caused by transport through guts of herbivorous fish based on new evidence of dispersal of macroalgae consumed by herbivorous reef fishes; although *G. salicornia* was not specifically measured in the report (Smith et al. 2004, Vermeij et al 2013). Mean specific growth rates were found to increase for both non-treatment control (0.0461g/g/day) and treatments with grazing pressure (0.0283g/g/day) for *G. salicornia*. The study concluded that manual removal would be the most practical means for controlling overgrowth of *G. salicornia* (Smith et al. 2004).

The idea that herbivorous grazers may hold the potential to disperse algae was initially suggested from a study by Breeman and Hoeksema (1987) suggesting that regenerative capacity of fecal fragments is high under a large range of temperature and light conditions, and if the apices remain intact, there is a greater regenerative capacity. Using amphipods and gastropods,

regenerative capacity of fecal algal fragments were interpreted. Another study that examined gastropod mollusk grazing on chlorophyta in Chile (Santelices and Paya 1989) suggested that fecal pellet adhesion capacities gave egested fragments an ecological advantage over free propagules. That same year a report was released showing that macroalgae survive digestion by fishes (Paya and Santelices 1989). Since then, little research interest has been placed in determining the capability of reef fish to disperse algae until recently. Herbivorous coral reef fish gut passage of algae was studied in the Caribbean confirming a mechanism for dispersal for chlorophyte, rhodophyte, and phaeophyte species that has rarely been described (Vermeij et al 2013).

One of the methods for assessing viability of fragments is through pulse amplitude modulation fluorometry (PAM). This method measures the excitation of chlorophyll molecules when a visible photon is absorbed promoting electrons of the absorbing molecule to a higher energy level (Kitajima and Butler 1975). The excitation returns to a ground state through emissions of three different competing pathways; (1) fluorescence, (2) heat radiation, and (3) photochemistry. Decreased rates of any pathway will increase emissions of the other competing pathways. So when heat radiation and photochemistry pathways are blocked, the highest fluorescence emission can be evaluated as F_m (Kitajima and Butler 1975, Genty et al. 1989). PAM fluorometers measure the height of fluorescence peaks of plant material caused by measuring pulses from the light source emitted through a fiber optic attachment. A saturating pulse is delivered to close any photochemical quenching before delivering actinic light that quickly opens photosystem II reaction centers where minimum and maximum fluorescence levels can be measured in the same sample (Genty et al. 1989). The ratio of F_v/F_m can be used to determine health and productivity of the sample by allowing the researcher to measure

electron transport and maximum photochemical quantum yield of photosystem II for study samples. This method for determining photosynthetic efficiency specifically for macroalgae isn't as common, but significant positive relationships between growth and electron transport in *G. salicornia* suggesting that photosynthetic rates can be used in place of growth if necessary (Beer and Axelsson 2004, Smith et al. 2004).

Measuring relationships between producers and consumers provide insight into sustainability of healthy reef ecosystems which can affect management measures and conservation of resources. Examining these relationships in an appropriate scope can lead to more efficient methods of quantifying dynamics, home range, and behavioral ecology of target populations.

CHAPTER II: MANUSCRIPT

Interactions of an herbivorous reef fish, *Naso unicornis* (Bluespine Unicornfish), with the invasive algae, *Gracilaria salicornia* in a Hawaiian Marine Reserve

To be formatted for Publication: *Coral Reefs*

1. Abstract

Invasive algae are a threat to coral reef ecosystems in Hawai‘i and elsewhere. In Hawai‘i, an invasive rhodophyte, *Gracilaria salicornia*, poses a significant threat due to its dense structure, resilience to a large range of temperature and salinity, and resistance to predators (Smith et al 2004). It has been proposed that marine protected areas may have the potential to control overgrowth of invasive algae due in large part to the increased abundance of herbivorous grazing fish. *Naso unicornis* is a large herbivorous grazing fish native to Hawai‘i and is abundant in Kaneohe Bay, Oahu. The goals of this study were to; (1) define the home range of *N. unicornis* in a marine reserve using acoustic telemetry to assess whether this particular species is (a) site-attached and (b) actively selects or avoids habitats covered with *G. salicornia*, (2) determine grazing intensity of *N. unicornis* (if any) on *G. salicornia*, and (3) assess whether gut passage of *G. salicornia* carry any regenerative capacity contributing to dispersal of the invasive algae. Home range was quantified for five herbivorous grazing fish (*N. unicornis*) fitted with internal acoustic tags by continuous tracking over a two month period for a marine reserve in Kaneohe Bay, Oahu. The mean home range (24.66 ha) in Kaneohe Bay is significantly larger than previously documented home ranges for the same species in other locations Hawaii and Guam. The species remained site attached to the study site and displayed predominantly diurnal activity with crepuscular migrations to and from nocturnal refuge areas. There significant correlation shown between tide level and location of individual within the reserve. Daily

foraging areas included the *G. salicornia* dominated reef flat, with evidence of grazing supported by fecal fragments collected from field captured individuals. Capacity for growth of *G. salicornia* through regenerative capacity was supported by ANOVAs for pulse amplitude fluorometry measurements. Strong site fidelity with habitat selectivity inclusive of *G. salicornia* dominated habitat suggests that *N. unicornis* are intentionally foraging for the invasive algae and hold the potential to prevent distribution to the coral-dominated reef crest. Regenerative capacity through gut passage of fecal fragments also suggests these fish could also be responsible in the dispersal of *G. salicornia*.

2. Introduction

Marine reserves are designed to conserve habitats by restricting collection of organisms within a set boundary and are commonly used as a tool for fishery management (Polunin 1990; Dugan and Davis 1993; Shackell and Wilson 1995; Bohnsack and Ault 1996; Kramer and Chapman 1999; Eristhee and Oxenford 2001; Marshall et al. 2010). Successful reserves are those that set boundaries within the home range of the target population and are large enough to account for ontogenetic shifts and limit spillover to nearby habitats. Coral reef ecosystems harbor an array of fishes that exhibit varying behavioral patterns. Movement is often species specific and many known factors play into home range size of a species. Niche use, body size, life history movements, diel rhythm, bathymetric composition, and reef system structure affect home range and habitat selectivity of fish in a coral reef community (Kramer and Chapman 1999; Ogden and Quin 2002; Marshall et al. 2011).

Home ranges of fish are quantified by assessment of repeated observations of tagged individuals in the same area (Kramer and Chapman 1999). The variability of home range

between species creates a necessity for site specific research of a population. Advancements in tagging technology have replaced mark release recapture (MRR) with acoustic telemetry as an accepted means for assessment of home range for target species (Clements et al. 2005). Acoustic telemetry interprets fish movement to a finer degree than MRR and can provide useful behavioral information linking movements and habitat use (Kramer and Chapman 1999; Marshall et al. 2011). Studies using this technology are often limited by funding, and while some of the more recent reports use both active and passive data to define home range in a species, most only incorporate one or the other (Arnold and Dewar 2001). There are a number of published works that report on the movement of animals in reserve boundaries, but very few address what environmental factors influence movement and home range size (Arnold and Dewar 2001).

Complex habitats are able to support a large number of species and are a fundamental property of any ecological system (MacArthur and MacArthur 1961; Huston 1979; Bell et al 1991; Hoey and Bellwood 2011). Rugosity of coral reefs support fish community composition by offering protective holes for nighttime refuge as well as sustaining a larger amount of resources (Friedlander and Parish 1998, Meyer et al. 2005). Negative outcomes have been demonstrated for the overall function of and complexity for a reef ecosystem through degradation in coral cover (Friedlander and Parish 1998; Graham et al 2006; Paddock et al. 2009; Hoey and Bellwood 2011). Decreases in coral cover are often paralleled by rapid colonization of algae that can dominate substratum (Norstrom et al. 2009). A healthy reef system can combat overgrowth of algae with herbivore abundance where overgrowth of algae is suppressed by foraging (Arthur et al. 2006; Hoey and Bellwood 2011).

Unicornfish are found in tropical regions throughout the Indo-Pacific and are a target group for artisanal fisheries (Randall 2001, Marshall et al. 2010; McIlwain et al. 2012). The bluespine unicornfish, *Naso unicornis*, is common in Hawaiian waters and has a maximum length of 70 cm and ages recorded up to 58 years confirmed by examination of saggital otoliths (Randall 2001, DeMartini et al. 2014). Well managed reserves contribute to the protection of *N. unicornis* as well as support their functional role in reef ecosystems as consumers. *N. unicornis* are also known as an ideal subject of study based on their size (Hardman et al. 2010; Hoey and Bellwood 2011). Individual *N. unicornis* large enough can be tagged with sonic transmitters and have provided a basis for comparison of behavior and movements in varied geographic locations. (Robertson and Gaines 1986, Meyer and Holland 2005, Hardman et al. 2010, Marshall et al. 2011). Observations using both active and passive tagging methods have shown that like many acanthurids, *N. unicornis* express high site fidelity and marine reserves are an ideal management tool for protection of this species.

N. unicornis are commonly described as marine algal grazers, playing a role in ecosystem function. They prefer fleshy phaeophyta such as Sargassum (Choat et al. 2002), and some have been documented grazing on rhodophytes, preferring the native Hawaiian *Gracilaria coronopifolia* to the invasive *Gracilaria salicornia* in experimental feeding trials (Smith et al. 2004). They tend to have a large foraging range inclusive of multiple hectares and are considered non-territorial when feeding (Robertson and Gaines 1986).

Increasing fishing pressure on herbivorous reef fish has limited suppression of phase shifts into algal dominated, positive feedback mechanisms (Scheffer et al. 2001; Mumby and Steneck 2008). Invasive algae are a threat to tropical ecosystems worldwide, and are particularly damaging in areas with highly specialized, endemic species (Eldredge and Smith 2001;

Friedlander et al. 2002; Smith et al. 2004). The Hawaiian Islands are the source of at least 18 algal invasions in the last 60 years as a result of intentional or accidental anthropogenic introduction (Russel 1992; Rodgers and Cox 1999; Eldredge and Smith 2001, Conklin and Smith 2005). It has been proposed that marine reserves hold the potential to mitigate excessive growth of algal communities by protecting herbivorous reef fish biomass (Williams and Polunin 2001).

To date there is little understanding of interactions of invasive algae and herbivorous reef fish in the Hawaiian Islands where *G. salicornia* has become heavily established. This species of algae has adapted to the Hawaiian coastline and is known for monopolization of any substrate that it inhabits and is resilient to a large range of temperatures and salinity (Rodgers and Cox 1999; Smith et al. 2004; Andreakis and Schaffelk 2012). Coral reefs are threatened by this species based on morphological and physiological properties. *G. salicornia* form dense mats that dominate nutrient acquisition from substrate and sunlight (Andreakis and Shaffelk 2012). Manual removal efforts are difficult due to fragility of branching filaments that easily break where the main form of reproduction in *G. salicornia* is vegetative fragmentation (Smith et al. 2004). Removal Efforts often result in increased distribution in shallow waters (Andreakis and Shaffelk 2012).

To date, no previous study has determined the interactions of reef fish and invasive algae in a marine reserve. The objective of this study is to determine whether marine reserves control overgrowth of invasive algae by protecting large herbivorous grazing fish. We used active acoustic telemetry to identify short-term, fine scale movement data to assess home range size and habitat selection of *Naso unicornis* in a no-take marine reserve. We used this data to compare to abundance and distribution of the invasive species, *Gracilaria salicornia*, to deduce whether the tagged individuals actively seek out or avoid areas covered by the algae. By confirming that *N.*

unicornis both forage on and select for habitats covered with *G. salicornia*, we determined whether or not marine reserves may be a method for indirect control of overgrowth in Hawai‘i. We also examined the ability of *N. unicornis* to disperse viable fragments of *G. salicornia* through gut passage and distribution onto the reef. While we recognize that *N. unicornis* holds potential to control overgrowth of the invasive algae, it is also necessary to consider the ability to distribute as well.

3. Methods and Materials

Data Collection

Study Site

Coconut Island is located in Kāne‘ohe Bay, O‘ahu (21°26’02.25”N, 157°47’16.95”W) encompassing 13.7 ha of shallow patch reef (Figure 1). The patch reef consists of large, algal dominated shallow reef flat (<1m) comprised mainly of sand and coral rubble extending into a coral dominated fringing reef crest that descends approximately 13m tapering into a fine silt floor. The waters surrounding Coconut Island have been established as a no-take marine reserve since 1953. The nearest patch reef is approximately 50 m from the Island and is separated by a deep channel. This site is impacted by invasive rhodophyte algae, *Gracilaria salicornia* and large herbivorous reef fish including *Naso unicornis* are resident to the area, shown to exhibit long-term site attachment (>1 years) (Meyer, not-published).

Acoustic Tracking

Acoustic telemetry was used to actively track *N. unicornis* movement patterns to determine behavioral ecology and home range size. Two separate tracking regimes were

executed from June-August 2013. Collection events occurred with the first in June and the second in July. Unicornfish were captured by free-diving at night using hand nets and flashlights from shallow sleeping and refuge areas of the reef (Figure 2). Captured individuals were transported by 17 foot outboard Boston Whaler in containers with fresh seawater into flow-through tanks with equipped with constant seawater pumps to be held overnight. The fish that appeared under the least stress (determined by pigmentation and free swimming) and of good size and health were chosen for surgery the following day. Vemco V9 Continuous Tags (V9-6L[®], 2s delay, 26-day battery life, Vemco, Nova Scotia, Canada) were surgically implanted in each individual. Based on the expected battery life of the tags, monitoring and tracking were split into two separate tagging events. Two individuals were tagged for the primary round of tracking in June and three were subsequently tagged for the secondary round in July for a total sample size of five *N. unicornis*. Captured fish encompassed a range of all size classes for sexual maturity.

Fish were placed in an aerated seawater bath with 0.2g/L⁻¹ MS-222 (tricaine mesylate) anesthesia to induce before the surgical tag implantation. Total surgery time for each fish was 7-15 minutes where transmitters were placed in the peritoneal cavity from a single 2-4cm incision posterior from the pelvic fin extending proximal to the ventral midline. Tags were disinfected with betadine antiseptic before placement in the gut cavity. The incision was closed with 3-5 interrupted stitches with 4/0 polydioxanone (PDO) absorbable suture using a traditional surgeon's knot. Fish were measured and returned to the holding tanks and monitored for up to 24 hours prior to release back into the habitat.

For tracking, an ocean kayak was equipped with a custom mount to house the Vemco VR100 receiver with a directional hydrophone built to stay perpendicular to water level at all

times for accuracy in receiving signals. The mount was adjustable to account for movement in shallow reef flats versus deeper reef crest areas and was interchangeable between the kayak and a 17' Boston Whaler. While both have limitations, the kayak was predominantly used based on maneuverability and limited effect on fish behavior (Meyer and Holland 2001). A recent study showed that kayaks are the best method in comparison to swimmers and powered vessels (Welsh & Bellwood 2012). They determined that a kayak can get up to 0.5m away from a fish before altering the behavior of the individual. At times a combination of the two was used with the boat as a central point in case movements were rapid and lengthy where the kayak was used for normal monitoring.

The first set of fish were tracked at frequencies of 63 and 75 kHz between 14 June and 1 July carried out for 19 days during that time period. The second set of fish were tracked at frequencies of 78, 81, and 84 kHz from 10 July to 15 August for 20 days. Fish were followed continuously from a minimum of 8-24 hours during each tracking day. At least every fifteen minutes during each tracking segment the kayak or boat was maneuvered directly over the individual to allow for maximum signal strength. All data below 70db were ignored in analysis due to uncertainty of fish position. Signals >70db were found in preliminary range testing of the tags to be within 5m of a fish in either shallow or deep habitats allowing for consistency when recording signals.

Ground Truthing and Habitat Selectivity

A combination of underwater visual census (UVC) and transecting was completed for analysis of substrate composition. When transecting, snorkelers laid measuring tape from 45-100m positioned shoreward in the reef flats extending out to the fringing reef crest. Percent cover

and grazing intensity of *G. salicornia* was recorded along with GPS fixations from a handheld Garmin[®]. UVC was completed by snorkeling above to generally assess habitat types and cover. Snorkelers were equipped with Go Pro[®] (HD Hero 2 and 3) cameras to record cover in designated encompassing both the reef flat, reef crest, and reef slope.

Camera mounts were fashioned to hold Go Pro[®] (HD Hero 2 and 3) units underwater at varied depths and locations. To determine use of habitat, a camera was placed in popular sites of *N. unicornis* determined from the tracking process for 2-4 hours recording video. These units were placed at areas on the reef flat, reef crest, and reef slope to observe behavior.

Algal Grazing

N. unicornis were captured using hand nets and flashlights in night refuge areas from surface swimming and free-diving snorkelers in shallow habitats. Fish were transported via surface snorkeling to a dock and were then transferred into 1000 gallon constant seawater flow through tanks in the Pelagic Fish Research lab at the Hawaii Institute of Marine Biology. Fecal samples were collected and recorded for up to three days following capture. Using a pulse amplitude modulation fluorometer (Walz Junior-PAM[®]), minimum fluorescence yield (Fv), maximum fluorescence yield (Fm), and photosynthetically active radiation (PAR) values were recorded using saturated pulse analysis (SAT) of the algal fragments filtered from the fecal matter. The fragments were then transferred into separate one gallon tanks for the grow-out portion of the experiment.

For one week following the three day initial fecal matter collection period, captive fish were offered *G. salicornia* by taking individual algal mats from the field and placing them in the tank for grazing. Using Go Pro[®] (HD Hero 2 and 3), grazing rates were recorded by counting

bite numbers per half hour, as well as taking wet weights of the algal mats every hour following initial introduction. After one week's time, each fish was transferred to a separate flow through tank empty of any *G. salicornia* to ensure that collected fecal fragments did not contain any viable fragments broken off in the grazing process. For the following three days, fecal samples were again collected and using the PAM, measured Fv, Fm, and PAR values and transferred into separate grow out tanks. This process was repeated twice one month apart during 1 January and 3 March.

As a control to determine fluorescence and photosynthetic efficiency in healthy viable fragments, ten algal mats were taken from the field where individual healthy branches were measured for PAR, Fv, and Fm values with the PAM from each mat. Ten measurements were taken from each mat, creating independent replicates with a sample size of n=10.

Regenerative Capacity of Algae

Fragments collected from fecal matter from both initial capture (field grazing) and experimental grazing were placed in replicate tanks on a shallow surface water table made of fiberglass. There was no way to differentiate which fragments came from individual fish, so the fragments were not separated once transferred to grow-out tanks. Instead, the tanks were separated by field grazing and experimental grazing per length of time. To minimize pseudoreplication, a minimum of 15 fecal samples filtered for *G. salicornia* fragments (up to 50 fragments per fecal sample) were placed in the tank so that each one gallon unit housed up to 750 fragments at a time. The water table is housed outdoors in a partially sheltered area that blocks rainfall but allows sunlight. A PVC unit connected to the water intake for the surface table was extended over each of the tanks with individual valves to keep the replicates independent, where

flow rates could be adjusted. This allowed the temperature to remain constant in each tank as fresh seawater is pumped in to the water table. Each one gallon unit was equipped with a sandy substrate (filtered) and bleached coral to mimic similar field conditions for growth. Lines extending from an air compressor pump were placed in each tank to allow for constant aeration of the sea water as well as create turbulence in the housing. A control tank with no algal fragments was also equipped with the same sandy substrate and coral to show there is no *G. salicornia* introduced from the seawater pumped in. Each month, fragments were measured again for Fv, Fm, and PAR.

Data Analysis

Home range size estimation

Minimum convex polygons (MCP) were used to calculate the boundaries of the home range, and kernel utilization distributions with Brownian bridges (KUD) were used to estimate the area use within the home range using *adehabitat* packages in R using both the *mcp* and *kernelUD* functions (Calenge 2006). MCP and KUD were calculated at 95 and 50% to eliminate random outliers and smooth densities. MCP home range was also plotted against the percent of relocations to show whether it is justified to eliminate outliers based on number of relocations. This function was performed with home range bootstrapping (nreps=100). Linear regression in R was used to test significant relationships between FL and home ranges of individuals.

Habitat Use

ArcMap 10.2 (ESRI) was used to visually represent fixations of individuals and behavioral movements by location on the Coconut Island Patch Reef. Percent algal cover raster from transecting on the Northward reef flat was generated using ArcMap. Underwater video

capture was used to interpret behavior by sites commonly visited within the home range. Tide level was quantified by location, and Student's t-test was used in R to examine relationships between location of fish and tide height. Relationships between time spent on algal-dominated reef flat and reef crest were also tested using paired t-test.

Experimental Grazing

Based on normality and equal variance, ANOVAs in R with Tukey's post-hoc test were used to test relationships in growth modules of fecal fragments measured from PAM Fv/Fm values of fluorescence.

4. Results

Acoustic Tagging

A total of five *N. unicornis* were captured at the study site and successfully tagged with internal continuous acoustic pingers. There were no mortalities from the point of capture through the duration of the study. Each fish was successfully released within the refuge by the end of the project. For those fish implanted with an acoustic transmitter, all were able to be located for the duration of battery life for each tag. Tagged *N. unicornis* ranged from 26-38cm fork length (FL) (n=5; \bar{x} =28.4cm). (Table 1).

Acoustic Tracking

All fish were located within the first hour of release. For the first 24 hours, position fixes were not recorded in tracking data set to allow tagged fish to return to normal behavior. Fish were then tracked daily from continuous periods of 8-24 hours. When tracking began for the day, a single fish was tracked for the designated time period (unless the fish were traveling together)

with re-positioning over the individual at least every 15 minutes to ensure GPS position reflected the true location of the track. The VR100 receiver logs a GPS position of the internal tag every 2 seconds allowing for a fine scale movement path to be mapped reflecting the position of the fish during each tracking segment. When multiple tagged fish are in the same area, the receiver is able to log the signal strength of each tag within range so the associations of individuals within a population were also determined.

Fish were commonly seen schooling together during the day moving from the edge of the reef crest inward onto the shallow reef flats. Each tagged individual held separate night refuge areas, but were all seen displaying crepuscular migrations between refuge areas and daily foraging areas. The highest density of fixations from fish seen together came from an area of convergence identified by Figure 22. This area was also a common access point for movement from the reef crest to the shallow reef flats.

Home range area

Home range estimates are described in Table 1 showing values for bounding geometry and centers of activity. There was no significant difference between these two home range estimation methods (paired t test: $p=0.111$, $df=4$). Fork length was also found to be unrelated to home range of the tagged individuals. The mean 95% home range for KUD was 11.26 ha and 24.66 ha for 95% MCP home range. Percent of time spent at each location by individual is also listed in Table 1.

Habitat Use

Home range of each individual included the reef flat extending outward to the fringing reef crest and slope. The percent of *N. unicornis* detections per individual (Table 1) by site did

not significantly differ in size between the reef flat and reef crest. Although there was no significant difference in number of detections, there were clear and direct movements by all individuals onto the algal-dominated reef flat (Figure 15). There was also significant relationship with tide height and presence on the reef flat ($t=p<.0001$, $df=144919.9$); (Figure 16). The mean tide height for fixations recorded on the reef flat was 0.95m versus the mean of 0.20m for reef crest.

There are overlapping areas where individuals were commonly observed foraging and swimming together on both the reef flat and reef crest (Figures 5-7) in schools consisting of *N. unicornis* and other acanthurids. Based on observations from snorkelers and UVC, reef crest areas with a greater depth (>3m) captured schools of *N. unicornis* from 4-70 individuals foraged from station to station at a steady pace (Figures 6 and 7). When on the reef flat, schools from 4-70 individuals were observed by direct rapid commutes between areas of high *G. salicornia* density (Figure 5). After arriving at an area of algal cover, the school would expand outward and forage on *G. salicornia* for 5-10 seconds, then tighten together and rapidly commute to another area on the flat. While the reef crest had a higher percentage of activity than the flat in all but one tagged individual, each fish consistently was seen on the flat. Algal distribution for areas within the home range of tagged individuals showed high algal cover on the shallow flats versus sparse to no cover on the crest (Figure 21).

From the two 24-hour tracking events, night tracking revealed little to no movement of fish after sunset. One 24-hour track was at the Eastern windward side during nocturnal hours and the other at the Western leeward side. Both areas displayed little activity with the target individual staying within 5 meters of nocturnal refuge area for the whole evening.

Both MCP and KUD values show site attachment to the Coconut Island Marine Laboratory refuge. While the 95% KUD stays within the reserve size (Table 1, Figure 3), the 95% MCP has a larger HR size than reserve size (Table 1, Figure 4). The bounding geometry extends past the edge of the reef crest into channels between reef patches, but does not extend to coral cover of other patch reef areas. Only one instance of extreme relocation of a fish outside the boundaries of the reserve to an adjacent reef patch was observed throughout the tracking study.

Algal Grazing

All fecal matter of captured individuals that were relocated to captive tanks was examined for content (n=20). Unidentifiable organic matter was filtered from the samples before assessing presence of *G. salicornia*. Figure 8 is an image of filtered fecal contents from individuals captured in the field. Every captured *N. unicornis* fecal fragment contained *G. salicornia*, ranging from 20-100 clipped fragments per individual. Experimental captive grazing was observed with all fish (Figure 9).

Regenerative Capacity of Algae

Samples tested using the PAM at one and two month stages were at significantly higher in fluorescence than initially egested fragments ($F= 404.5$, $df=3$ $p<0.001$). The relationship between healthy and initial egested fragment fluorescence were also significantly different ($F= 404.5$, $df=3$, $p<0.001$). The relationship between healthy fragments and fecal fragments at one month growth were of equal variance and not significantly different ($F= 404.5$, $df=3$, $p=0.127$). Fluorescence ratios were significantly higher in 2 month samples than measured ratios for

healthy fragments ($F=404.5$, $df=3$, $p<0.001$). Boxplots of each growth condition are displayed in Figure 17.

5. Discussion

This study is the first to address any association of *N. unicornis* with *G. salicornia* in the field. While there are similar behavioral and home range findings of this particular population with unicornfish and acanthurids at other locations, this study specifically addresses the interactions and potential bio-control of an invasive algal species through grazing pressure from herbivorous reef fish. This study is also the first to examine the viability of egested fecal fragments of a grazed invasive species by herbivorous fish. By looking at species-specific interactions of *N. unicornis* with *G. salicornia*, we can determine whether the mechanism of grazing that has been shown to suppress overgrowth of indigenous algae is also supported for invasive species as well. This study adds to the understanding of the ecological interactions between the two species while also adding to the growing pool of reports on home ranges and site fidelity of reef fish in a marine reserve.

The tracking of individual fish suggests strong site fidelity to Coconut Island MLCD. HR bootstrapping by random selection of fixations ($n_{reps}=100$) for all frequencies are consistent with acceptable measures for accurate estimation of home range (Springer 2003). The home range behavior of the *N. unicornis* at Coconut Island MLCD stayed homologous with other studies inclusive to surgeonfish (Robertson and Gaines 1986, Meyer and Holland 2005, Hardman et al. 2010, Marshall et al. 2011), but was highly variable among individuals. These results are parallel to observations of *N. unicornis* in Guam (Marshall et al. 2011). While our results are somewhat similar, the study in Guam found a relationship with HR size and body size (FL) of

tagged individuals, where we determined no significant association in our study. According to our results, our largest fish had the smallest home range size. This opposes the general notion that marine reserves must account for larger body size as it typically means relocations of larger distances (Kramer and Chapman 1999). It has yet to be determined whether body size or habitat availability and rugosity of a reef system has a larger influence on HR size. Theoretically if a habitat carries all the energetic needs for an individual, ontogenetic shifts to adjacent habitats may never be observed. The suggestion by Meyer and Holland (2005) that broad habitat characteristics could be a predictor for movement is more congruent with our findings. Based on bathymetry and consistency of home ranges seen along the reef crest, there is potential for models to characterize the movement of unicornfish.

Tagged individuals remained site-attached with crepuscular migrations between sleeping refuge areas and daily movement stations. This is consistent with surgeonfish behavior described previously (Kramer 2004). The foraging/commuting described by Meyer and Holland (2005) was commonly observed during the course of the study but varied by individual. One individual stayed within 20m of the night refuge area for 2 weeks, and then began to commute in daily migrations to distances up to 500m from the night refuge. During daily patterns, individuals were commonly seen solitary in the morning and in large numbers in the afternoon. This could be attributed to an area of high activity, diversity, and biomass for reef fish identified on the north side of the island. Tagged individuals were often observed together in schools moving on to the reef flats from this area of convergence. This area is protected from the deeper slope by a sandy plateau at approximately 5m depth off the fringing reef crest (Figure 6 and 21). Because this study showed a significant relationship with location by tide level, predictors for foraging behavior could change seasonally. Long-term researchers at Coconut Island have reported that

the distribution of *G. salicornia* changes drastically by season, where the cover is reduced in the summer compared to a higher distribution in the winter season (not-published). Whether these changes are due to differences in grazing rates or environmental shifts in wind and current are unsubstantiated to date.

N. unicornis are previously described to have a home range consistent with shallow areas (Robertson and Gaines 1986, Meyer et al. 2005, Hardman et al. 2010). The foraging behavior on the reef flats of Coconut Island MCLD suggest selectivity for an area that is dominated by *G. salicornia*. Based on movement patterns from the tracks, entry onto the flat often extends from a single location where individuals are staying in this area for multiple hours at a time foraging. This observation is in addition to confirmed reports and image capture of foraging observations with *G. salicornia*. This behavior is different from daily migrations to and from sleeping areas where individuals tracked displayed movement along the reef crest versus the reef flat. Because all previous reports show preference for fleshy brown algae (Robertson and Gaines 1986, Choat et al. 2002, Smith et al. 2004), this newly defined interaction with *G. salicornia* may be described as adaptive foraging optimization and niche occupation. For this to be confirmed, SCFA gut analysis against control areas that are not dominated with *G. salicornia* would be an ideal method for displaying differences in foraging behavior. It has recently been shown that *N. unicornis* have a higher capacity for foraging on macroalgal dominated reefs than other herbivorous reef fish (Chong-Seng et al. 2014).

This is the first study to support regenerative capacity of *G. salicornia* fragments passed through the gut of *N. unicornis*. We attempted to match field conditions as best as possible without method of culturing fragments as seen in other regenerative studies (Santelices and Paya 1987, Vermeij 2013). While regeneration portion of the study suggests that growth is possible

(Figure 18 and 19) in addition to the significant relationships seen at growth stages to healthy fragments (Figure 17), this study would need to be further replicated before showing support as a viable method of dispersal. Crude estimates from our study show fragment survival and growth at less than ten percent of total fragments collected. While this is a small value, dispersal via gut passage holds potential to further investigate based the increased distribution to northern areas of the bay area.

Change in distribution from 2009-2014 of *G. salicornia* within the Coconut Island MLCD is shown in Figures 20 and 21. Figure 15 also displays the majority of fixations of *N. unicornis*. While growth of *G. salicornia* has not been eliminated, spread of distribution onto the reef crest has been prevented in areas that reflect the home ranges of tagged individuals. This may not necessarily be attributed to grazing pressure. Reefs are maintained by a balance of limiting nutrients and promoting herbivore biomass (Littler 2006). To support statements regarding suppression of *G. salicornia* growth by grazing intensity, experimental trial with exclusion cages by season would be necessary to confirm efficacy of grazing.

In areas such as Kaneohe Bay that are subject to eutrophication from high variations in nutrients based on runoff, it is necessary to maintain an abundance of herbivorous grazers. Defining interactions between producers and consumers is an important aspect of examining the most effective ways to combat overgrowth of potentially devastating invasive species. These smaller mechanisms are often overlooked in large scale studies. By supporting concepts that herbivorous grazing fish can hold the potential to suppress overgrowth of invasive algae without examining species-specific interactions, pertinent details of the relationship could be overlooked. If an abundance of herbivorous grazers such as *N. unicornis* carry the potential to increase the distribution of invasive algae, then other management methods beyond bio-control

should be explored. The unique interaction defined through this study could potentially be used as a proof of concept to further examine space and habitat use of unicornfish within a reserve and their potential indirect role in maintaining or degrading a healthy reef system.

6. Tables

Table 1: Summary of tracking data from five individual *N. unicornis* captured and tracked within the Coconut Island Marine Refuge Laboratory

	Fish Number				
	1	2	3	4	5
Date of Tag Implantation	06/12/13	06/12/13	07/09/13	07/09/13	07/12/13
Start Date Track	06/14/13	06/14/13	07/10/13	07/11/13	07/14/13
V9 Tag ID	1162614	1162616	1162617	1162618	1162619
V9 Tag Frequency	63	75	78	81	84
External Tag ID	4173	4175	4180	4169	4168
Fork Length (cm)	30.5	26.0	27.0	25.0	33.5
Total Length (cm)	33.6	28.7	29.0	26.7	38.0
Total Fixations	196,406	265,354	284,753	156,887	142,216
Fixations >70db	93,204	63,180	44,535	33,078	23,816
Tracking Span (days)	19	19	20	20	18
Kernel Home Range- 50% (ha)	0.46	1.85	0.92	2.31	2.31
Kernel Home Range- 95% (ha)	6.31	15.54	8.31	13.85	12.31
MCP Home Range Area-95% (ha)	43.7	31.91	16.40	19.22	12.07
Average Distance (m²)	47.86	97.50	71.78	15.59	17.02
Percent Fixations (Reef Flat)	3.84	18.46	32.66	39.85	82.19
Percent Fixations (Reef Fringe)	96.16	81.54	67.34	60.15	17.81

Table 2: Estimation of age and weight of individuals from measured FL (fork length) *N. unicornis* based on the Von-Bertalanffy [$l(t) = 51.20(1 - e^{-0.167(t+0.5)})$] and Von-Bertalanffy LW equation [$0.026(FL)^{2.92}$] by (Eble et al 2009)

Fish	Fork Length (cm)	Age(y)	Weight (g)
1	30.5	4.92	561.21
2	26	3.74	352.12
3	27	3.99	393.15
4	25	3.51	314.02
5	33.5	5.86	738.08

7. Figures

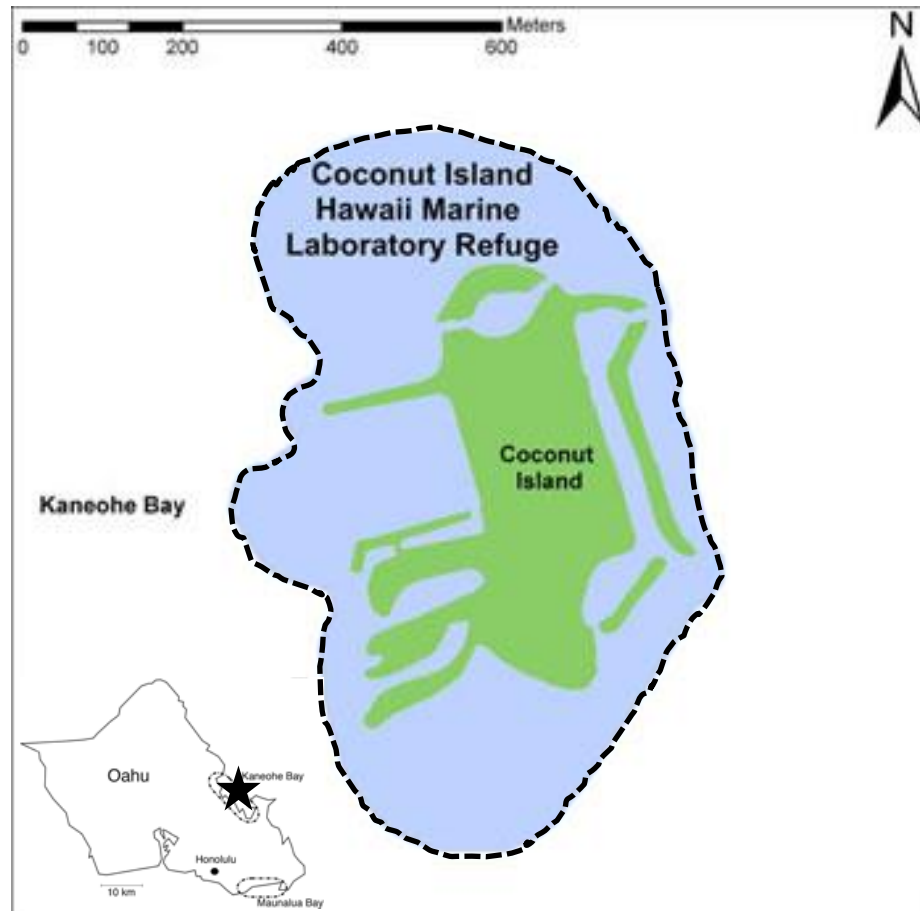


Figure 1: Extent of Coconut Island Hawaii Marine Laboratory Refuge , Kāne’ohe Bay, Oahu, Hawaii (21°26’02.25”N, 157°47’16.95”W)



Figure 2: Location of range of capture for *N. unicornis* during collections at frequencies: 63,75,78,81, and 84 kHz (21°26'02.25"N, 157°47'16.95"W)

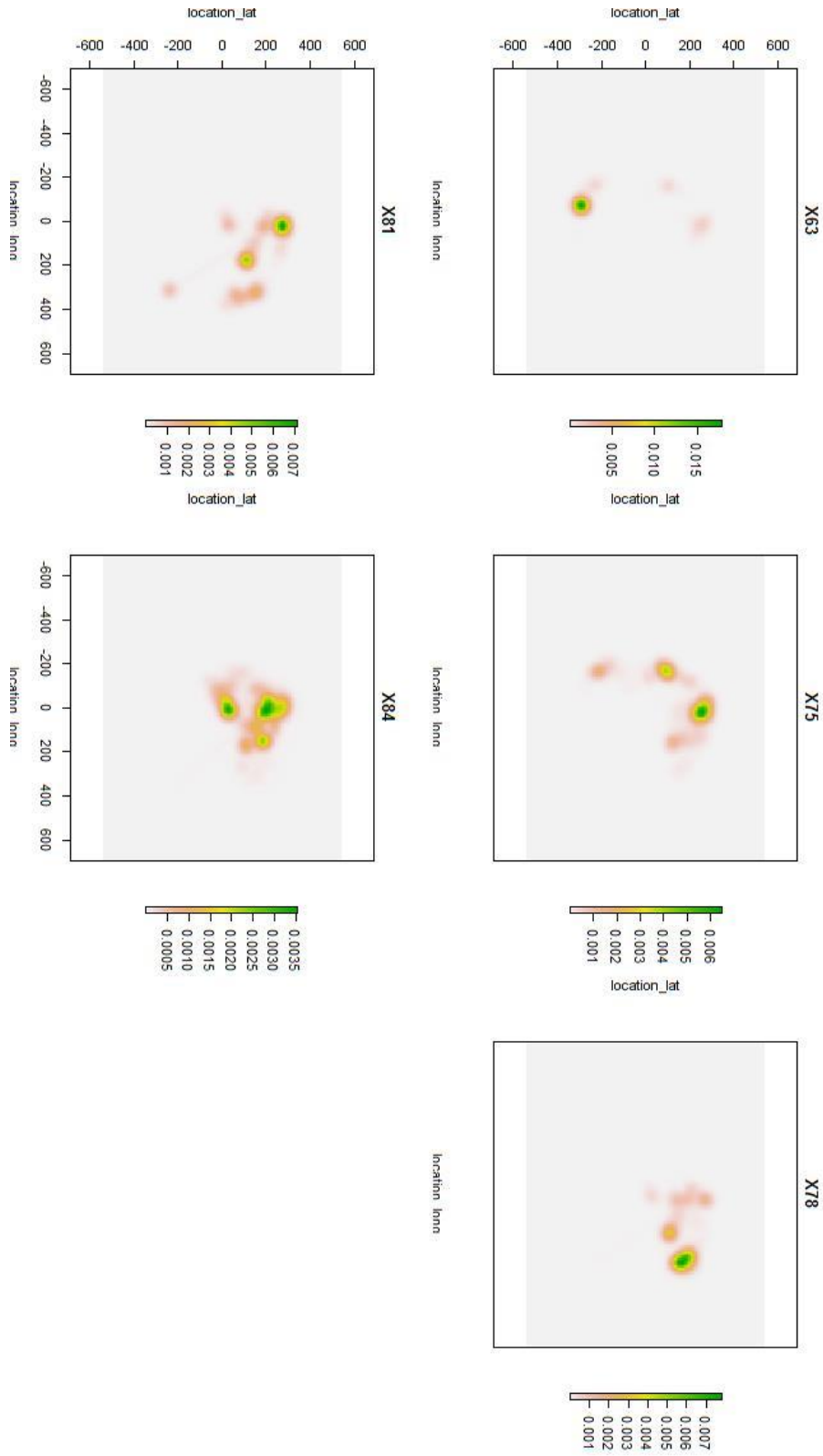


Figure 3: KUD at 95% with Brownian Bridges. Projection: UTM WSG 1984 4N

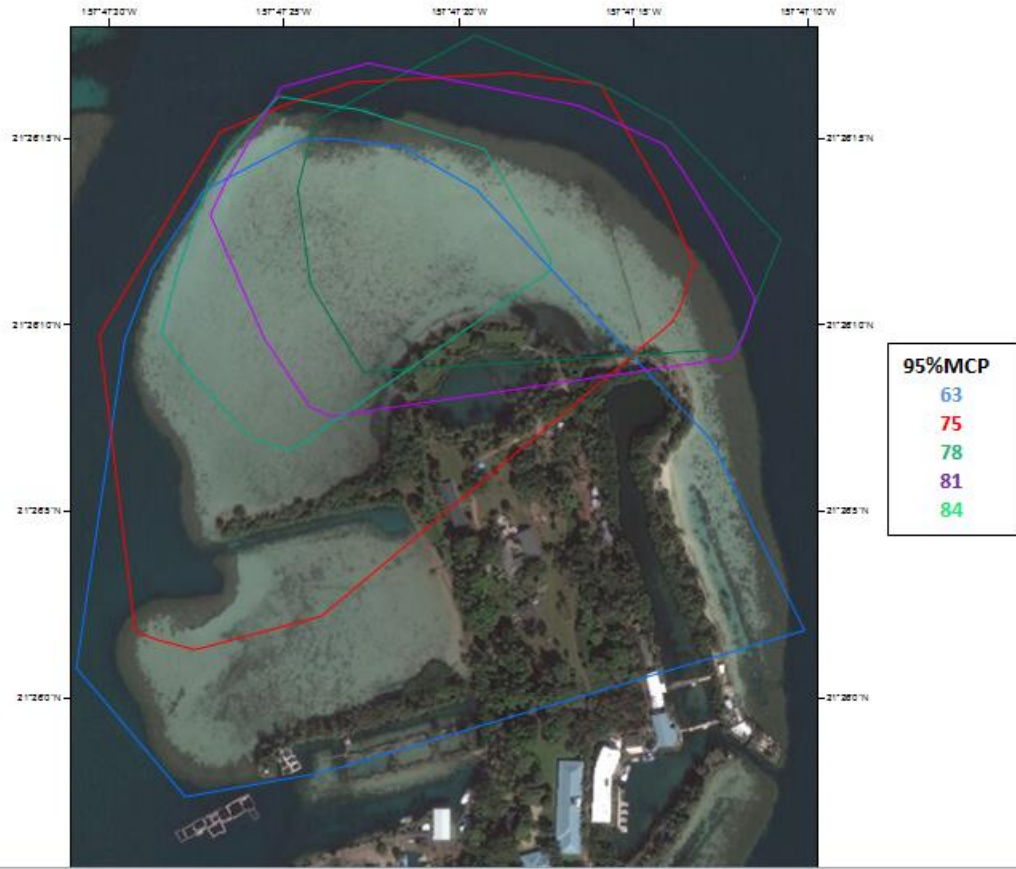


Figure 4: Image of 95% MCP Bounding Geometry by Frequency

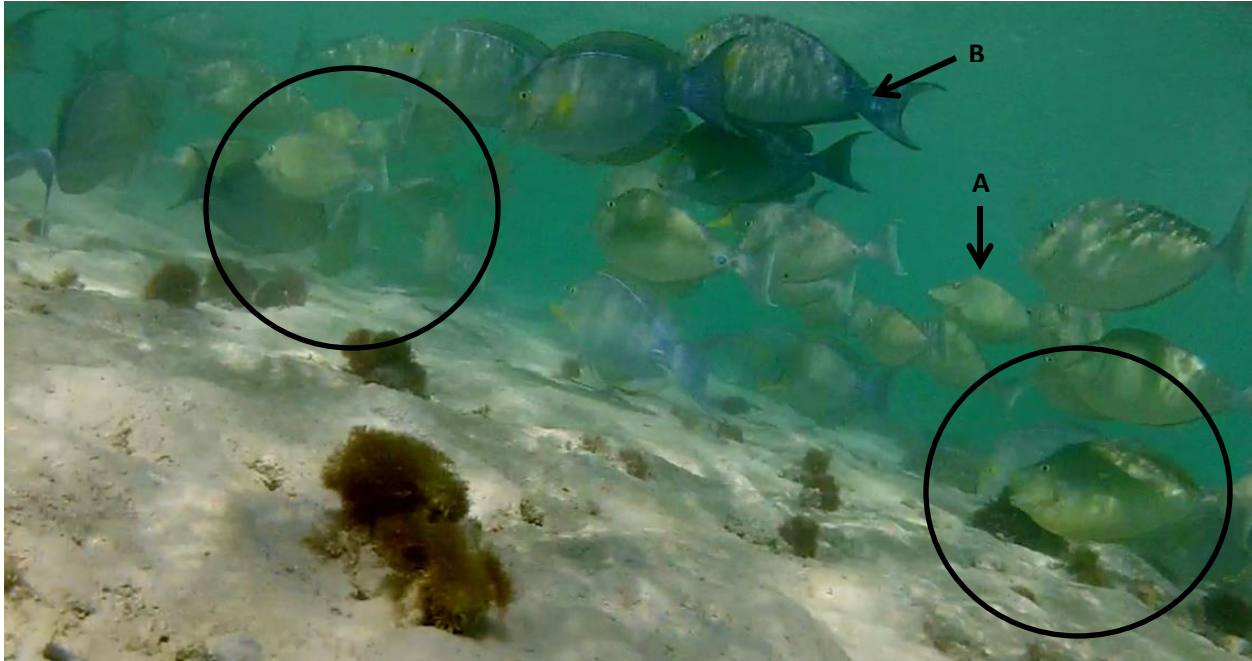


Figure 5: Still image capture of grazing behavior from field video collection on the algal dominated reef flat. Majority abundance is (A) *N. unicornis* (Hawaiian name: “Kala” and (B) *Acanthurus dussumieri* (Hawaiian name: “Palani”). Circled are captures of *G. salicornia* foraging.



Figure 6: Still image capture from underwater video record on the reef slope off the fringing reef of Moku o Lo‘e, Kāne‘ohe Bay, Oahu. Shown is a school of *N. unicornis*.



Figure 7: Still image capture from underwater video record on a location of the reef slope off the fringing reef of Moku o Lo'e, Kāne'ohe Bay, Oahu. Shown is a small group of *N. unicornis* with other herbivorous reef fish



Figure 8: Image of filtered sample fecal fragment of field captured individuals.



Figure 9: Still capture from video record of experimental feeding of *G. salicornia* to captive *N. unicornis*. Shown in photo is two individuals clipping apices of the algal mat while grazing.

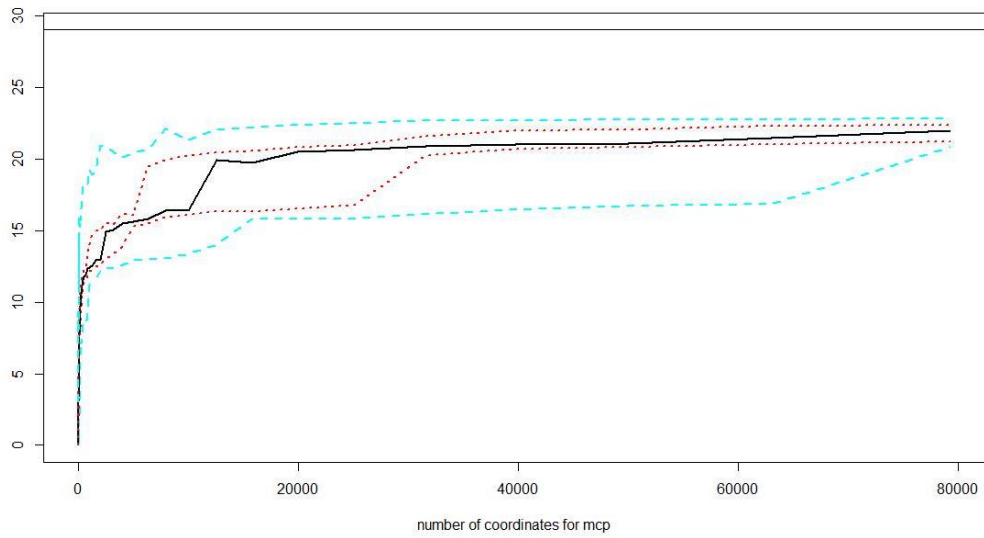


Figure 10: Home range bootstrapping (nreps=100) for fish at frequency 63. Number of fixations (x-axis) by home range size (y-axis, hectares).

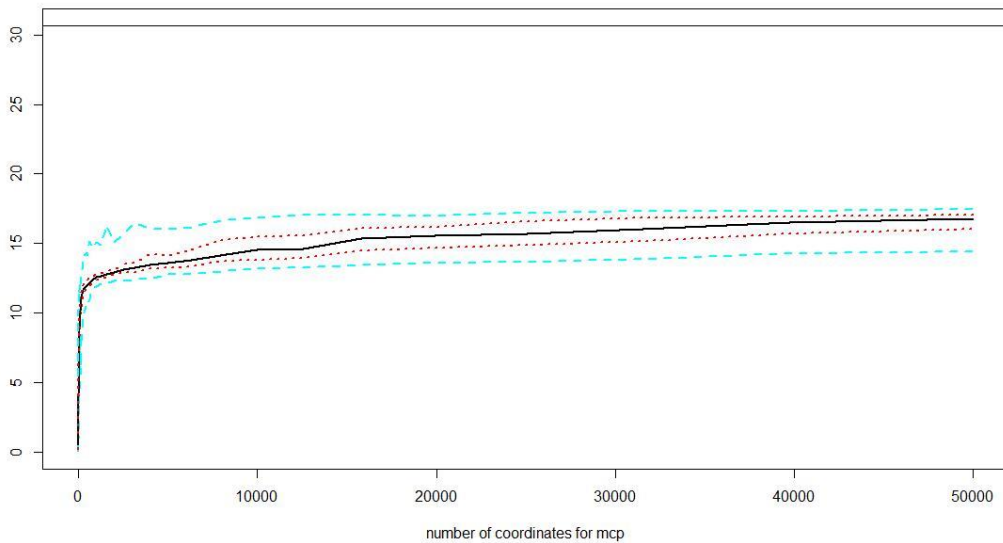


Figure 11: Home range bootstrapping (nreps=100) for fish at frequency 75. Number of fixations (x-axis) by home range size (y-axis, hectares).

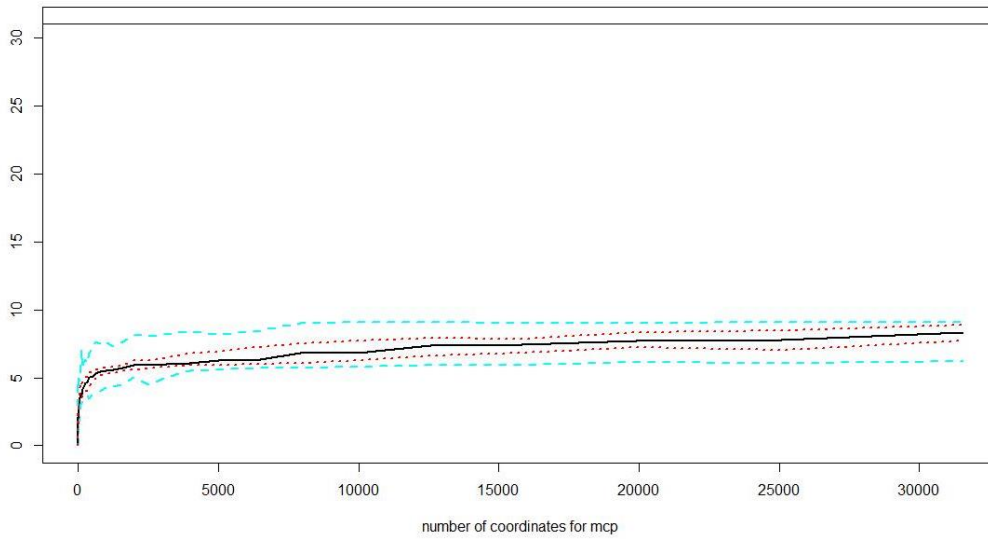


Figure 12: Home range bootstrapping (nreps=100) for fish at frequency 78. Number of fixations (x-axis) by home range size (y-axis, hectares).

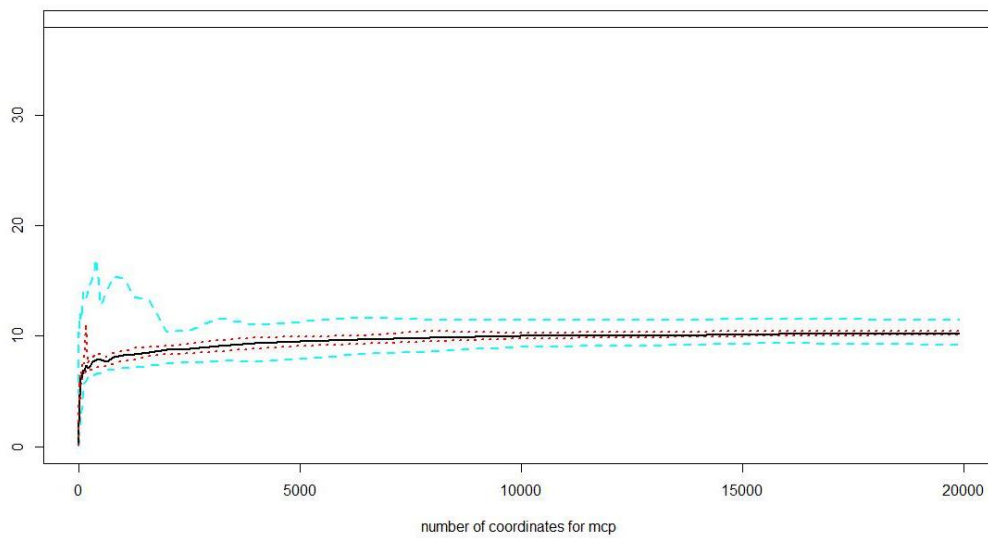


Figure 13: Home range bootstrapping (nreps=100) for fish at frequency 81. Number of fixations (x-axis) by home range size (y-axis, hectares).

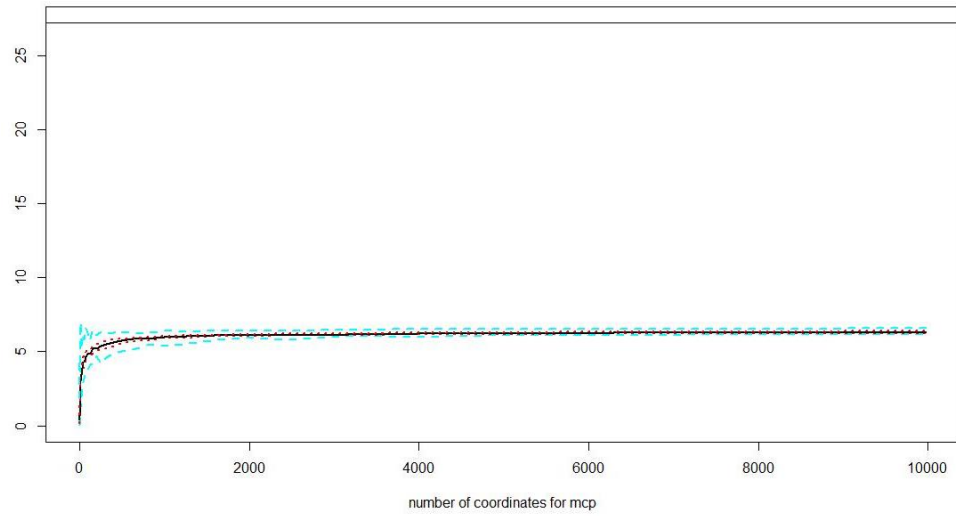


Figure 14: Home range bootstrapping (nreps=100) for fish at frequency 84. Number of fixations (x-axis) by home range size (y-axis, hectares).

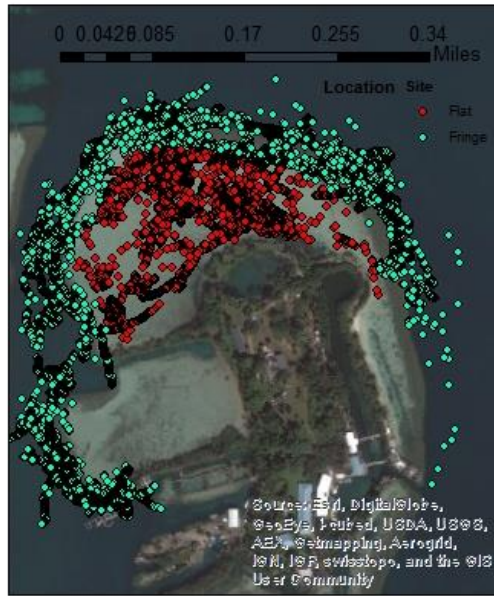


Figure 15: “Flat” summarizes geographic fixations within home range from tracking on the algal-dominated shallow region shoreward of the fringing reef. “Fringe” are the fixations of tagged individuals beyond the flat from the reef crest to the reef slope of Moku o Lo‘e.

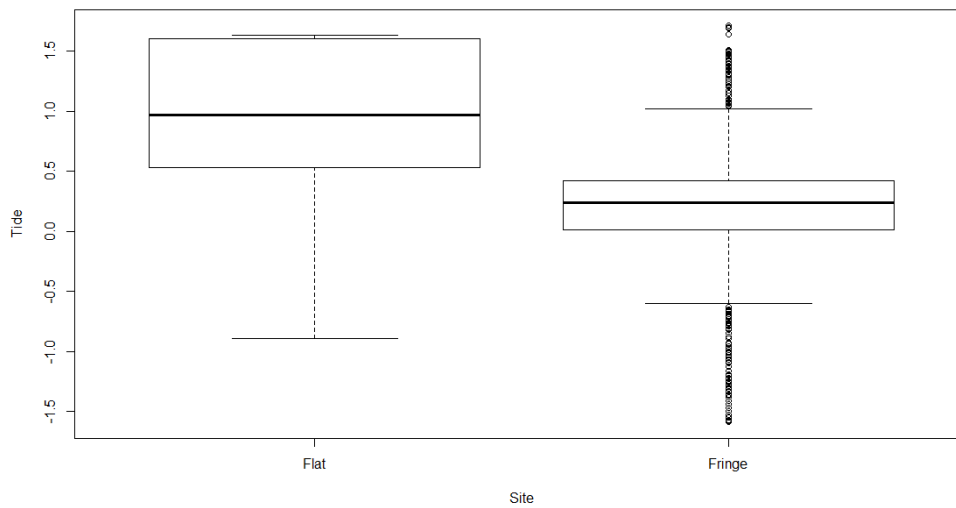


Figure 16: Boxplot of location of tagged fish based on tidal height. Tidal height is measured in meters.

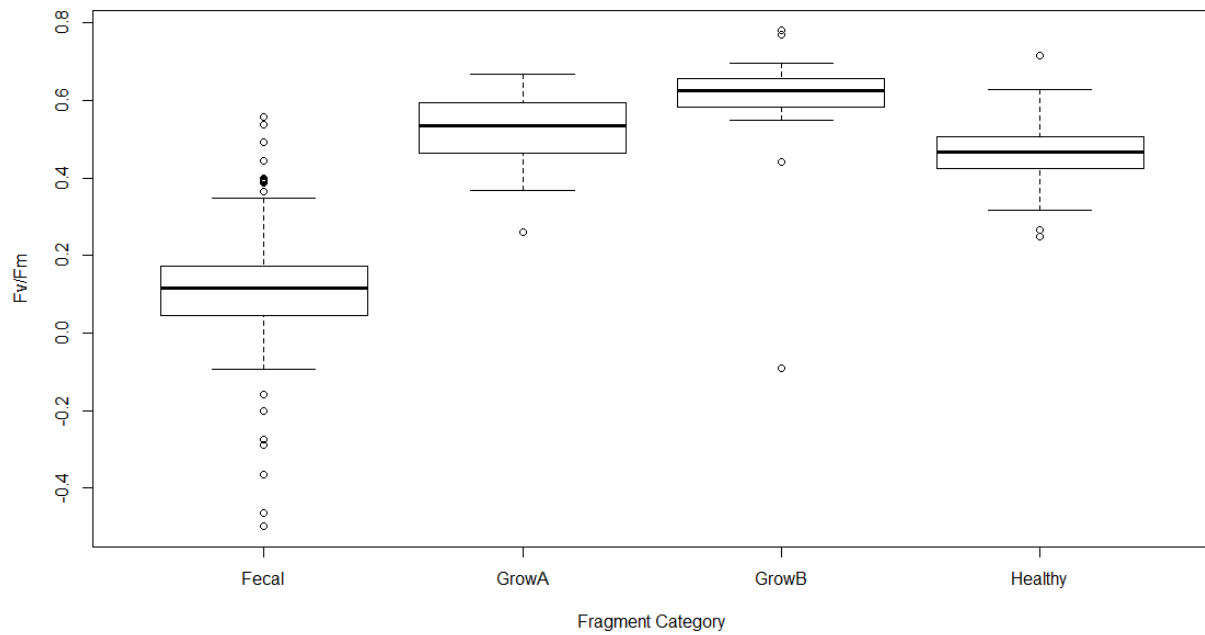


Figure 17: Boxplot of PAM measurements of *G. salicornia* fragment condition by Fv/Fm ratio “Fecal” are collected fragments immediately following egestion, “GrowA” are egested fragments after 1 month of growth, “GrowB” are egested fragments after 2 months of growth, “Healthy” are fragments measured from a healthy and viable *G. salicornia* mat collected from the field.

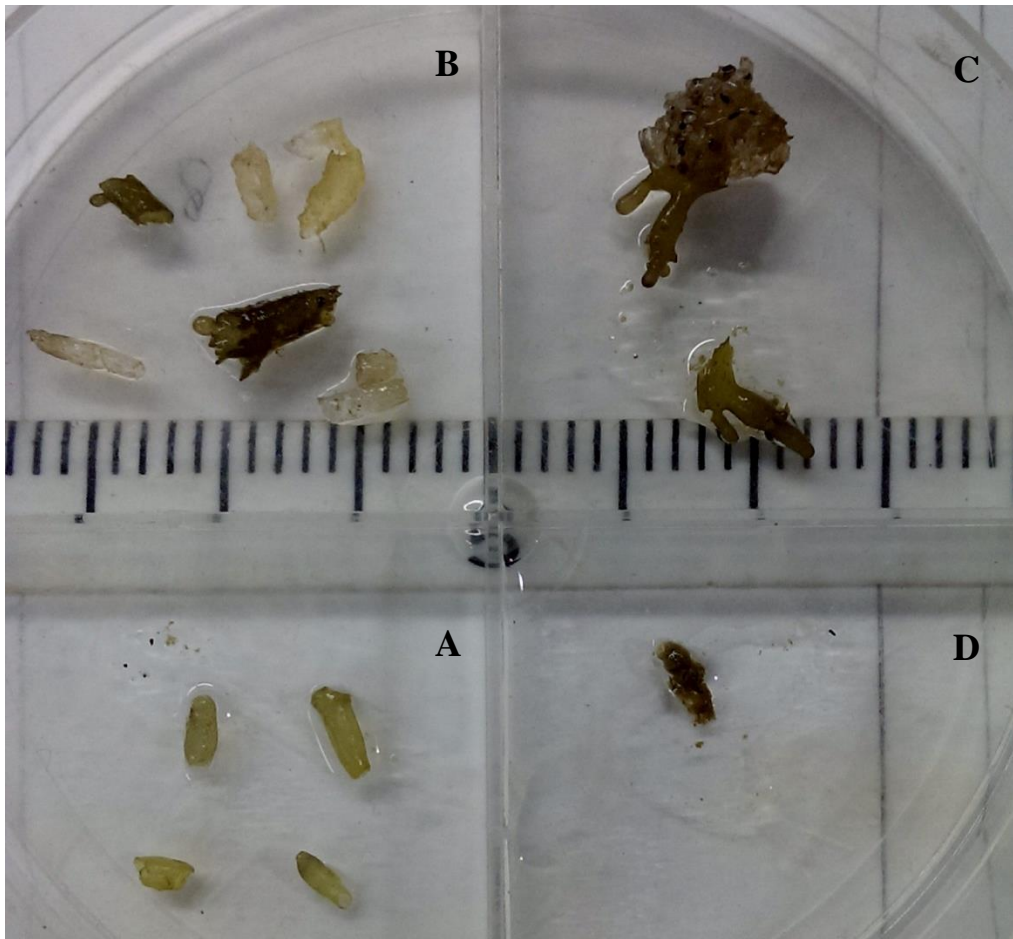


Figure 18: Egested fecal fragments at varying states of health: (A) Initial fecal fragments immediately following egestion, (B) Condition of fecal fragments at 1 month post egestion; evidence of budding and increased pigmentation, (C) fecal fragments at 2 months post egestion; evidence of attachment and budding from apices posterior to clipped area (D) example of decomposing fragment (sample taken from 2 month growth collection). Ruler ticks measured in millimeters for scale.

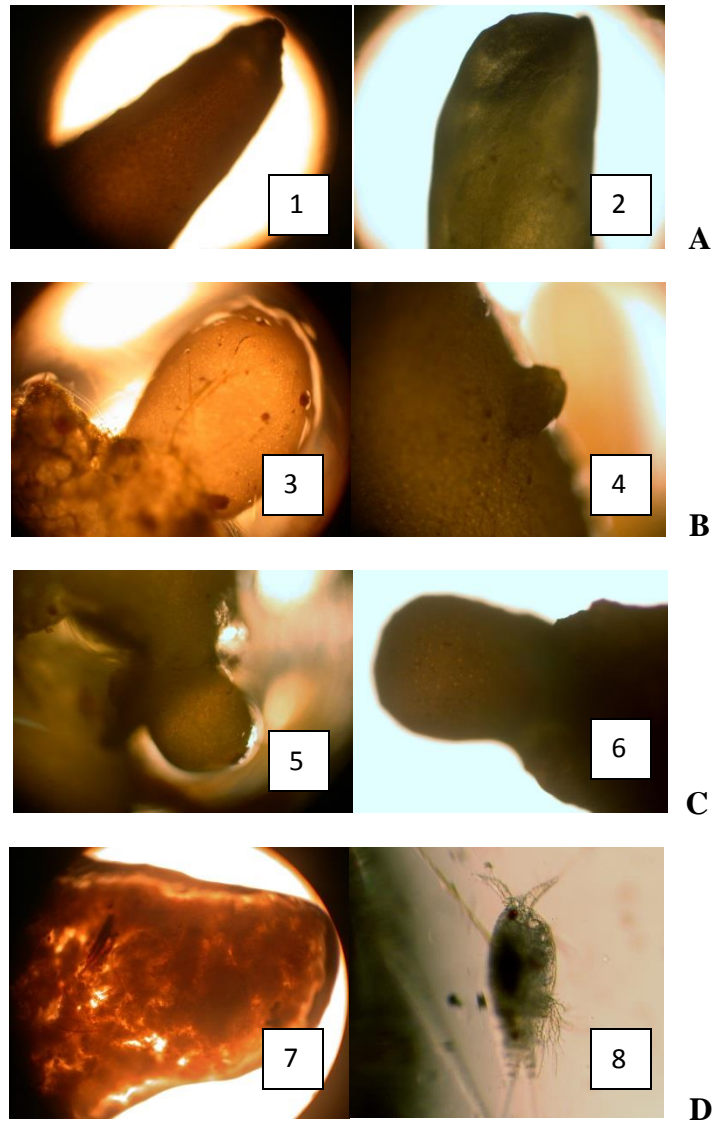


Figure 19: stills under 40x microscope view, A (1 & 2); Initial egested fecal fragments, B (3&4); 1 month growth (example of budding in both views); C(5 & 6) 2 month growth (example of budding and increased pigment); D (7) Decomposed fecal fragment from 2 months (no cell structure, houses many zooplankton) (8) Example of zooplankton identified with *G. salicornia* fecal fragments

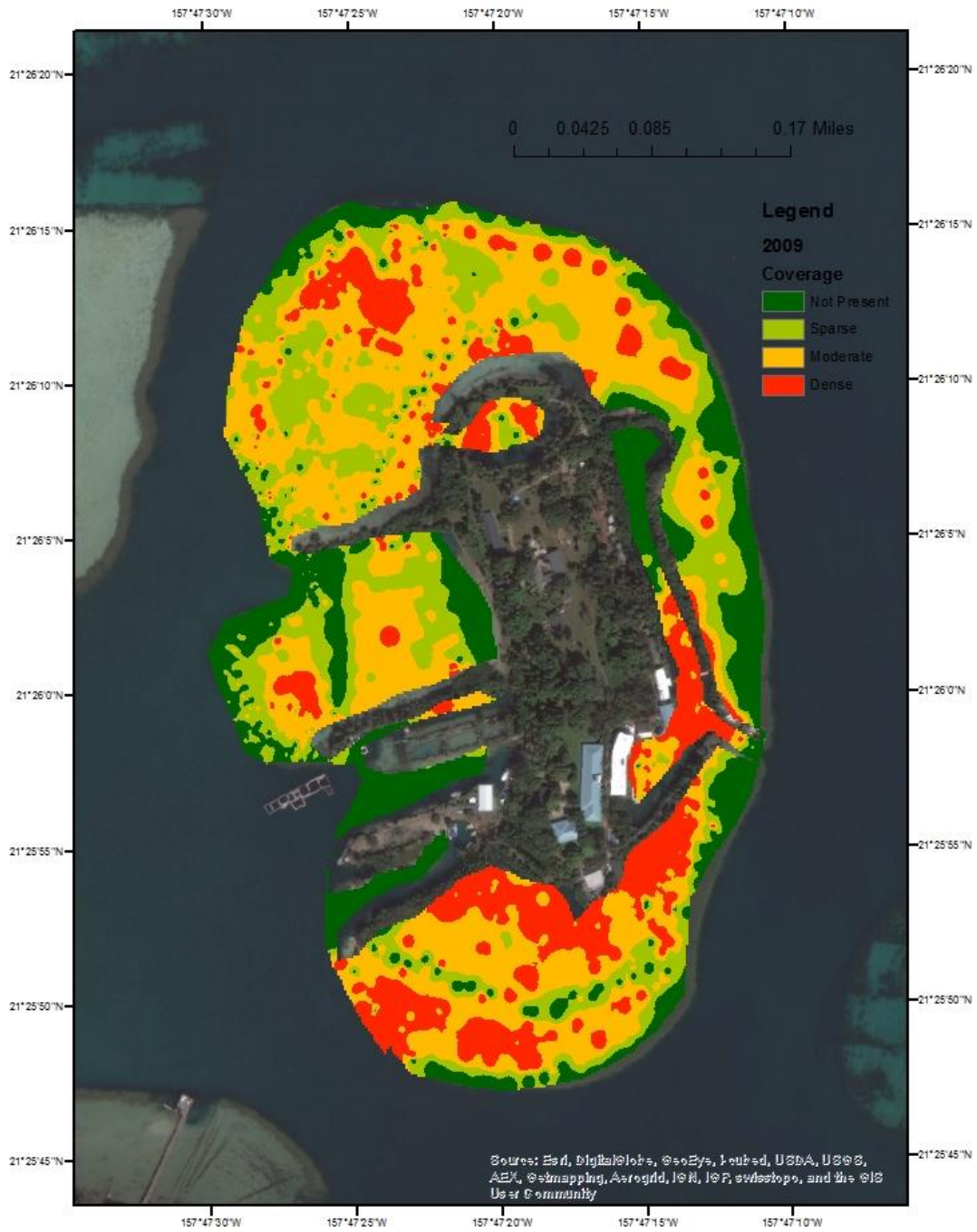


Figure 20: Distribution of *G. salicornia* in September 2009, (Raw Data Provided by DAR)

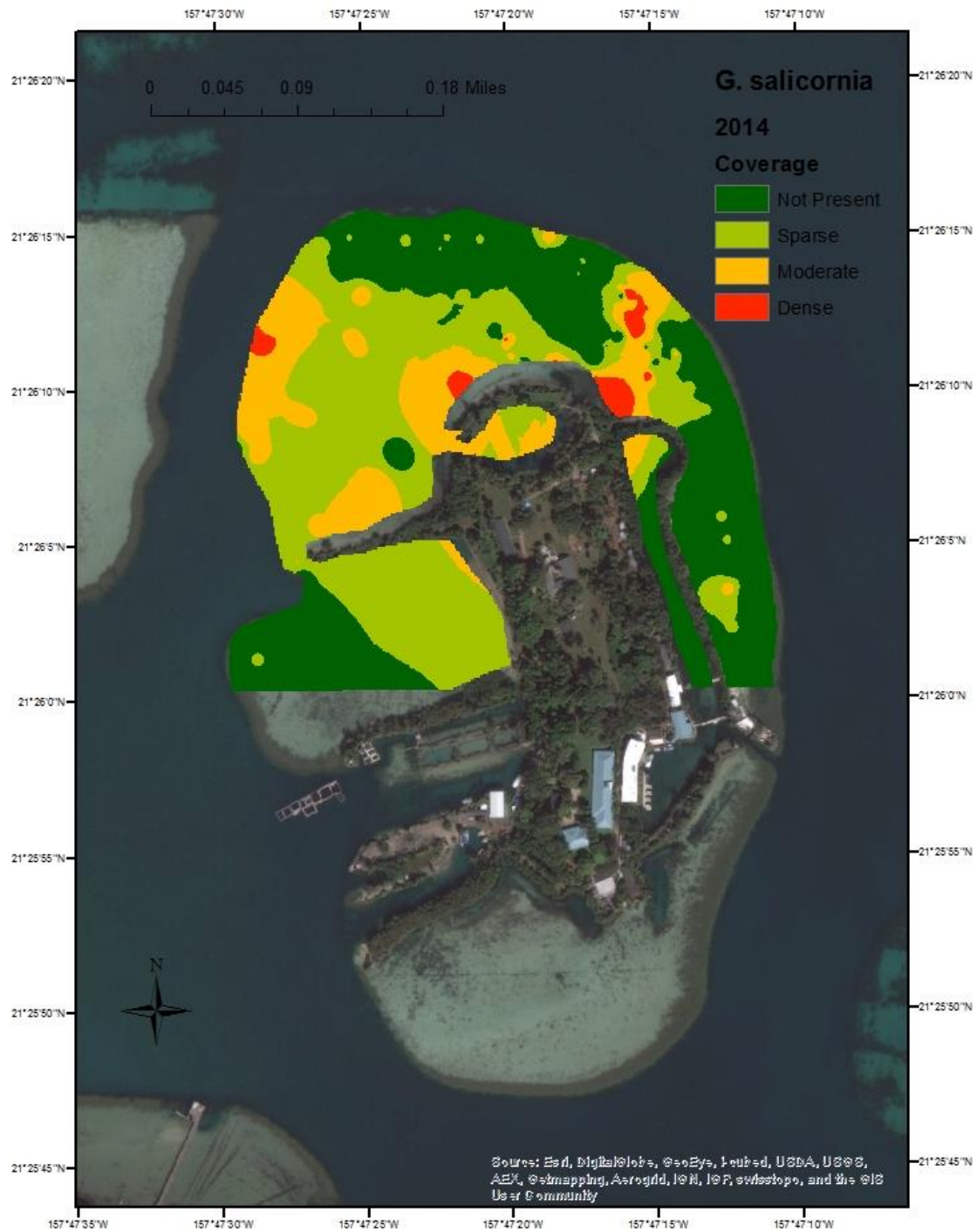


Figure 21: March 2014 *G. Salicornia* Cover

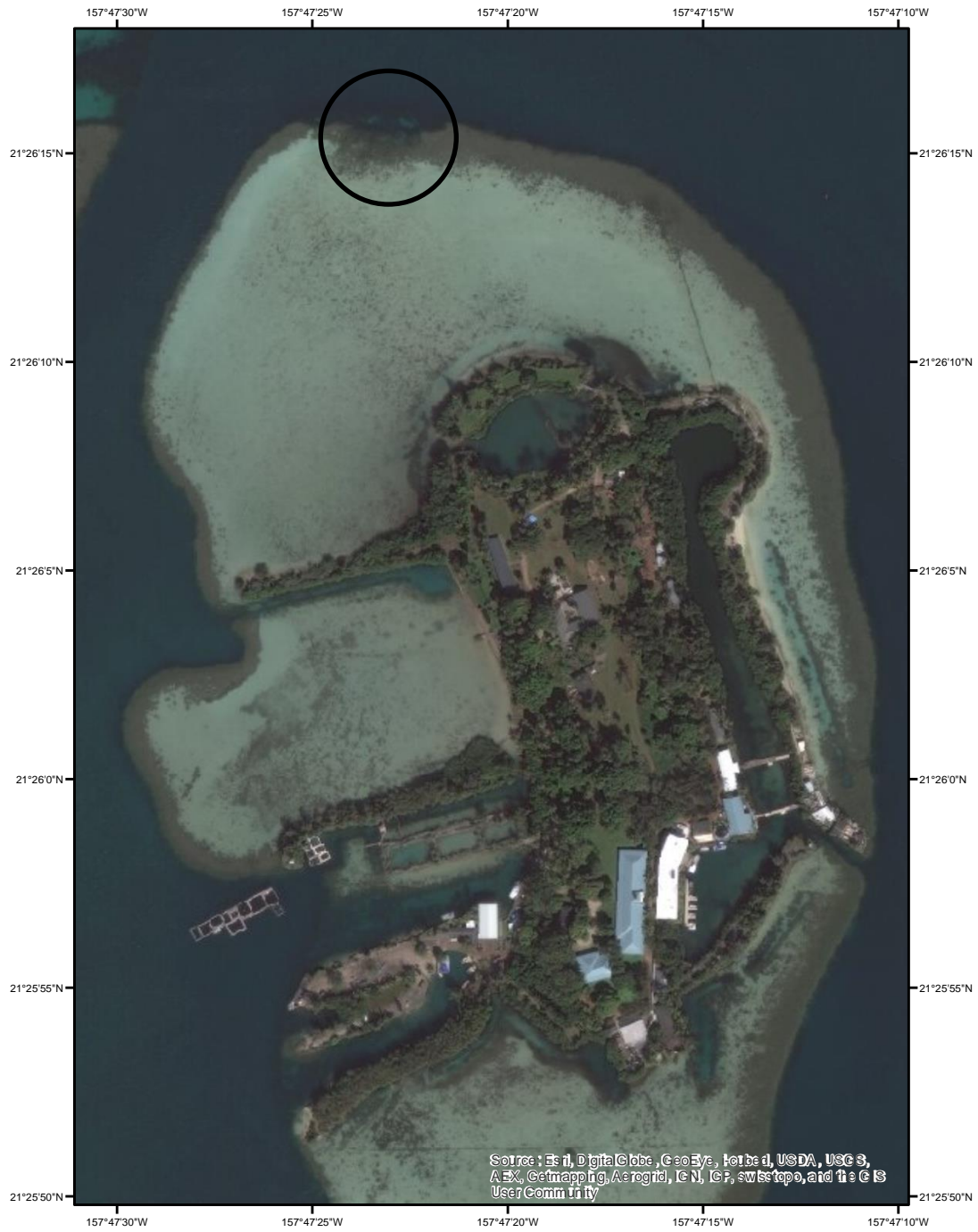


Figure 22: Area of convergence for tagged individuals. (Circled in Black Outline)

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