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## Marine Reserves Promote Coral Reef Resilience by Mitigating Human Impacts Through the Restoration of Parrotfish Populations, Increasing Their Reproductive Output and Seeding Neighboring Overfished Reefs

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**MARINE RESERVES PROMOTE CORAL REEF RESILIENCE BY MITAGING HUMAN  
IMPACTS THROUGH THE RESTORATION OF PARROTFISH POPULATIONS, INCREASING  
THEIR REPODUCTIVE OUTPUT AND SEEDING NEIGHBORING OVERFISHED REEFS**

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## **ABSTRACT**

### **MARINE RESERVES PROMOTE CORAL REEF RESILIENCE BY MITIGATING HUMAN IMPACTS THROUGH THE RESTORATION OF PARROTFISH POPULATIONS, INCREASING THEIR REPRODUCTIVE OUTPUT AND SEEDING NEIGHBORING OVERFISHED REEFS**

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Old Dominion University, 2017  
Director: Dr. Kent E. Carpenter

Coral reefs are some of the most productive ecosystems on the planet, providing fisheries resources for over a billion people with billions of dollars in revenue from tourism for developing nations. Coral reefs are under threat from overfishing and water pollution, resulting in less productive algae dominated reefs. Marine reserves are widely expected to promote the resilience of reefs by protecting and increasing the abundance of herbivorous fishes that can graze on algae, therefore directly or indirectly preventing coral to algal phase shifts. However, the ability of marine reserves to mitigate human impacts, restore herbivorous fish populations and seed nearby reefs is still poorly known.

The study location was within the central Philippines wherein 58 reefs (30 marine reserves and 28 sites open to fishing) were surveyed and sampled. This study examined the relationships between anthropogenic stressors and reef health through regression analyses, determined shifts in sizes of reproductive female parrotfishes and how this restores reproductive output and utilized genetic techniques in detecting migration patterns. Results revealed that marine reserves were able to prevent the proliferation of algae and loss of coral in the face of increasing anthropogenic impacts, while the increase in parrotfish biomass within reserves was implicated in these benefits. Additionally reproductive female size and abundance increased within reserve boundaries compared to fished areas resulting in an exponential increase in egg production with increasing years of protection. Genetic analyses identified several related pairs of parrotfishes sampled at over 400kms apart, while migration analyses supported a north to south dispersal of immigrants. When coupled with ocean currents, reefs within the Sibuyan Sea

or further north were highlighted as potential sources for reefs to the south in the Bohol Sea and Sulu Sea.

These results are potentially encouraging for managers within the Philippines, indicating that marine reserves can promote the resiliency of coral reefs by preventing algal proliferation and promote coral growth. Additionally these reserves can restore the reproductive potential of ecologically important herbivorous fishes (i.e. parrotfishes) and seed nearby reefs.

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This dissertation is dedicated to Dr. Angel C. Alcala who inspired me to work with marine reserves.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 ABSTRACT

The connectivity of marine populations via larval dispersal is a key consideration in creating networks of marine protected areas (MPAs) that can improve fisheries, protect biodiversity and bolster the resilience of coral reefs to climate change. MPAs are also expected to impart resilience to coral reefs by enhancing populations of herbivorous reef fishes such as parrotfishes that may prevent “phase shifts” from coral to algal dominance. However, scientific understanding of the patterns of connectivity at spatial scales of 10’s to few 100’s of km that are relevant to networks of MPAs in the Philippines is still at an early stage. Furthermore, the idea that herbivorous reef fishes can impart greater reef resilience is largely untested. This project will use advanced genomic methods to investigate the patterns of larval connectivity among populations of reef fishes in the central Philippines within a 300 km-wide area encompassing 4 provinces in the Bohol Sea. The role of herbivorous reef fishes in relation to other natural and anthropogenic factors that can impact reefs negatively (e.g., eutrophication) or positively such as protection from fishing within MPAs, will also be investigated. Within the study area are over 100 existing MPAs, some of which are the most successful in the Philippines (e.g., Apo Island marine reserve). This project intends to provide empirical information on connectivity to decision-makers involved in local management and networking of MPAs. The objectives of this project are to: 1) advance the state of knowledge on reef fish connectivity and its implications to local population dynamics; 2) help improve the design of MPA networks by providing information supported by empirical data to managers and decision-makers; and 3) serve as a model for the biological networking of MPAs at spatial scales appropriate to levels of governance from within and between municipalities and provinces.

#### 1.2 BACKGROUND INFORMATION

##### *Current status and threats to Coral Reefs*

Coral reefs provide essential ecosystem services, especially in the Coral Triangle (CT) where millions of people rely heavily on reefs for food and livelihoods (Hoegh-Guldberg et al. 2009). However, reefs within the CT, particularly in Southeast Asia, have suffered extensive

degradation from the combined effects of overfishing and land-based anthropogenic activities (Burke et al. 2002). Indeed, some of the greatest threats to coral reefs are chronic disturbances such as poor water quality through increased sedimentation and reduced herbivory through overfishing (Knowlton 1992; Birkland 1997; McClanahan et al. 2002; Houk & Woesik 2010), all which are likely exacerbated by an increasing coastal population. In more recent decades, thermal stress brought about by global climate change has amplified the threats to reefs by causing episodes of coral bleaching, resulting in mortality of corals that can lead to phase shifts from coral to algal dominance over large spatial scales (Hughes et al. 2003, 2007). Coral reefs in the most biologically diverse parts of the CT, such as the Philippines, are currently in a deteriorated state (Burke et al. 2002, 2011; Nañola et al. 2010). These heavily degraded reefs provide fewer ecosystem goods and services to the human communities that depend on them, than healthy reefs, thus contributing to poverty (Alcala and Russ 2002).

#### *Anthropogenic Stressors on Coral Reefs*

Poor water quality through eutrophication and reduced herbivore biomass through over fishing represent some of the greatest threats to coral reefs (Birkeland 1997; McClanahan et al 2002; Houk and Van Woesik 2010). Most sediments are delivered to coral reefs via rivers, with > 95% of sediments being deposited within kilometers of river mouths (Golbuu et al. 2003), these increased sedimentation rates are directly linked to reduced coral health (reviewed in Fabricius 2005). In addition, particulate organic matter (POM) greatly contributes to the nutrient availability in coastal regions and a majority of these nutrients are also discharged through terrestrial run-off via rivers (Furnas, 2003). These increased nutrients result in a more eutrophic environment and have been implicated in the increased macro algae cover on coral reefs (Lapointe et al 2004; Burkepile and Hay 2009; Stuhldreier et al. 2015).

Coral reef fish stocks are negatively correlated with increasing human populations (Williams et al. 2008, Mora 2008, Hughes et al. 2017) and more specifically increased fishing pressure has led to declines in herbivorous fish stocks especially in developing nations (Comeros-Raynal et al. 2012; Edwards et al. 2014). Reductions in herbivorous fish populations on coral reefs have been implicated in increased algae cover (Hughes 1994; Mumby et al. 2006; Bonaldo et al. 2014).

The combination of increased nutrients and decreases in herbivory through over fishing may have synergistic effects on decreasing coral cover and increasing algae cover thus these stressors have been implicated as the primary drivers in decreased reef health (Hughes et al. 2017).

#### *The use of MPAs in restoring coral reefs*

Marine protected areas (MPAs), areas of the sea that are wholly or partially closed to any extractive activities by humans, are strongly advocated as an effective tool to conserve biodiversity, restore depleted populations and manage fisheries on coral reefs (Alcala 1988; Russ 2002; Sale et al. 2005). In developing countries, MPAs have become a popular reef management option because conventional marine resource management measures are often difficult to enforce (Russ 2002). For instance, in the Philippines, >1500 MPAs have been established on coral reefs to date (Aliño et al. 2002; Weeks et al. 2010; Horigue et al. 2014). Although MPAs are clearly not a panacea to the many problems threatening coral reefs, there is undeniable evidence for increased density, biomass and diversity of exploited reef species within the ‘no-take’ boundaries of MPAs (Roberts and Polunin 1991; Russ 2002; Halpern and Warner 2002; Gell and Roberts 2003; Lester et al. 2009). High biomass and diversity of key functional groups within MPAs, such as herbivorous reef fishes, may promote reef resilience against climate change by preventing or reversing coral-to-algal “phase shifts” (Stockwell et al. 2009; Hughes et al. 2010).

#### *Role of herbivorous reef fishes in reef resilience*

Few studies have shown evidence for reduction of macroalgal cover due to increased herbivore abundance within MPAs or similar management measures (McClanahan et al. 2001, Williams and Polunin 2001, Mumby et al. 2006). No studies to date have conclusively shown that an increase in herbivorous fish biomass will lead to coral recovery, however this might be a result of the slower recovery rates of corals compared to algae (Stockwell et al. 2009). It is not clear whether coral recovery is the inevitable outcome of a reduction of macroalgal cover (Newman et al. 2006; Mumby et al. 2007). Additionally, there is much debate whether reductions of parrotfish abundance has a top-down control on algae preventing benthic phase shifts (Hughes 1994, Bellwood et al. 2004; Mumby et al. 2006; Bonaldo et al. 2014) since the bottom-up control of algae on parrotfish abundance has been demonstrated by recent studies (Suchley et al. 2016; Russ et al. 2015).



The majority of studies on herbivore and algal community dynamics within MPAs have been done in the Caribbean and in Kenya. Few studies have investigated the dynamics between herbivores, algae and corals within MPAs in the western Pacific (Hughes et al. 2007; Stockwell et al. 2009). Studies are particularly lacking in the Coral Triangle (CT) region, where the diversity of corals and reef fishes is highest (Veron 2000; Carpenter & Springer 2005; Stockwell et al. 2009; Nañola et al. 2010) and where greater functional diversity due to higher species richness may promote reef resilience (Nyström 2006). Bellwood et al. (2004) argued that western Pacific reefs have a higher ‘ecological insurance’ compared to Caribbean reefs because of their higher functional diversity. Studies of herbivore-algal community dynamics within MPAs in the CT region are thus warranted.

#### *Connectivity of reef fish populations*

Significant build-up of biomass inside MPAs may positively affect neighbouring areas through spill-over of mobile adults (Roberts and Polunin 1991; Russ 2002; McClanahan and Mangi 2000; Abesamis and Russ 2005; Halpern et al. 2009). Additionally, greater spawning output per unit area within MPAs may result in a net export of larvae to neighbouring areas (Carr and Reed 1993; Russ 2002; Gell and Roberts 2003). However, adult reef fishes are known to be site attached (Mumby and Wabnitz 2002; Bonaldo et al. 2006; Afonso et al. 2008), with some herbivorous fishes having home ranges as small as 2.4 hectares (Welsh & Bellwood, 2012). Given that the average MPA size in the Philippines is around 8 hectares (Weeks et al. 2010), it is likely that adults remain within these protected areas. Thus the role of larval dispersal is likely to be more important in the recovery of networks of marine reserves than adult spill-over.

Demographic larval connectivity, or the linking of marine populations through the dispersal and recruitment of larvae within ecological (as opposed to evolutionary) time scales, is a key issue in using networks of MPAs on coral reefs to rejuvenate ecosystems, improve fisheries and buffer the effects of climate change (Sale et al. 2005; McLeod et al. 2009). Historically, reef fish populations were considered to be ‘open’ non-equilibria systems that relied on recruitment of larvae from sources external to local populations (Sale 1977, 1991). A paradigm shift began in the late 1990’s when two ground-breaking studies demonstrated that reef fish populations can exhibit self-recruitment to a significant degree (Jones et al. 1999; Swearer et al. 1999). These

were soon followed by other studies indicating that many reef fish populations may be more 'closed' than 'open' (Cowen et al. 2000; Jones et al. 2005; Almany et al. 2007; Zamborain-Mason et al. 2017). Factors that may promote self-recruitment in reef fish populations include larval fish swimming ability and behavior interacting with meso-scale physical oceanographic features such as eddies that can retain larvae close to natal sites (Cowen and Castro 1994; Stobutzki and Bellwood 1997; Wolanski et al. 1997; Cowen 2002; Leis and McCormick 2002; Paris and Cowen 2004; Leis 2006). In the last decade, a considerable amount of attention has been directed towards examining the extent of larval dispersal in reef fishes and its potential demographic consequences (Swearer et al. 2002; Mora and Sale 2002; Palumbi 2003; Sale et al. 2005; Cowen et al. 2006; Jones et al. 2009; Krueck et al. 2017). Much of the current interest in investigating ecological-scale larval dispersal in reef fishes is directed towards the utilization of MPAs as conservation and fisheries management tools (Sale et al. 2005; Almany et al. 2007; Planes et al. 2009; Magris et al. 2016).

The spatial scales of larval dispersal critical to the persistence of local reef populations must be known in order for managers to decide on the appropriate sizes, spacing and locations of individual MPAs (Crowder et al. 2000; Roberts 2000). For example, in overfished situations, MPAs within a network must have a high chance for supplementing recruitment to each other to hasten the recovery of protected populations because spawners are rare outside of MPAs (Roberts et al. 2001). Knowledge on the spatial patterns of demographically relevant larval dispersal is also needed to empirically measure net larval export by MPAs to reef fisheries (Pelc et al. 2010). Lastly, a reliable understanding of the spatial patterns of ecological scale larval connectivity is required to build MPA networks that can ensure adequate supply of larvae to reefs that are more vulnerable to climate-related disturbances, such as coral bleaching (Green and Bellwood 2009; McLeod et al. 2009).

A wide variety of methods have been used to investigate patterns of larval dispersal in reef fishes over ecological time scales (Jones et al. 2009). These methods include biophysical modelling (Paris and Cowen 2004; Paris et al. 2005; Cowen et al. 2006), molecular genetics (Taylor and Hellberg 2003; Planes et al. 2009; Christie et al. 2010; Liu et al. 2010; Saenz-Agudelo et al. 2011) and otolith microchemistry (Patterson et al. 2004, 2005; Almany et al. 2007; Gerlach et al. 2007; Patterson and Swearer 2007). Some of the most informative molecular methodologies are

parentage analyses, wherein mature individuals and recruits are sampled, genotyped and the likelihood of parentage is estimated (Almany et al. 2007; Jones et al. 2009; Jones 2015). These methods have allowed for the estimation of larval dispersal kernels (the probability of larvae settling at different distances from a source population). However, Pinsky et al. (2017) utilized a logistically less taxing method based on isolation-by distance (IBD) and was able to estimate similar dispersal distances for the same damselfish (*Amphiprion percula*). These studies have led to the development of dispersal kernels showing that dispersal distances occur at only 10s of kilometers (Jones et al. 2009, Harrison 2012; D'Aloia et al. 2015), which has led to the recommendations that marine reserves be placed no more than 15 km apart to enhance recruitment between reserves (Green et al. 2015). However, a more recent study (Williamson et al. 2016) of two coral grouper species with moderate pelagic larval durations (26 days) found median dispersal distances of a magnitude greater (100-200km) suggesting that recruitment subsidies between reserves can occur at much longer distances. Additionally studies are concluding that with the estimation of dispersal distances the complexity and patchiness of reef habitats needs to be considered (Krueck et al. 2017; Van Wynsberge et al. 2017).

Within the Philippines initial molecular studies examined phylogenetic breaks within the archipelago with little emphasis on local larval dispersal and population connectivity (reviewed in Carpenter et al. 2011). Most of these studies show evidence for genetic structuring at larger spatial scales (100's of km), which is likely to be indicative of larval dispersal patterns over longer time scales (Magsino et al. 2002; Juinio-Meñez et al. 2003; Ravago-Gotanco et al. 2007; Magsino and Juinio-Meñez 2008; Ravago-Gotanco and Juinio-Meñez, 2010). However, empirical studies examining larval connectivity are lacking within this region with just one known to date (Abesamis et al. 2017). This study utilized parentage analysis of a butterflyfish (*Chaetodon vagabundus*) sampled from a network of MPAs within the central Philippines and estimated that 50% of larvae settle within 30 km of parent stocks. This study corroborates previous studies showing dispersal distances of 10s of km, additionally this study supports the growing evidence that MPAs can replenish other MPAs and supply larvae to fished areas (Abesamis et al. 2017).

### 1.3 HYPOTHESES

The first aim of this study is to determine if marine reserves can mitigate detrimental effects of human impacts through the restoration of parrotfish populations. The specific hypotheses are whether marine reserves can:

*H1a: prevent the increase in macroalgae cover resulting from increasing levels of sedimentation and human population density,*

*H1b: promote or sustain a coral dominated system in the face of increasing levels of sedimentation and human population density,*

*H1c: and increase the biomass of parrotfishes within reserves resulting in a top-down effect on the reduction of macroalgae.*

The second aim of this study is to determine if the potential increase in parrotfish populations within marine reserves can function as net exporters of larvae. The specific hypotheses are that the reduction in fishing mortality within marine reserves:

*H2a: will increase the size at sex-change for parrotfishes resulting in larger females*

*H2b: and whether the combination of more abundant and larger females will result in an exponential increase in egg production.*

The third aim of this study is to examine the potential larval connectivity patterns of parrotfishes within the central Philippines, through the use of restriction-site-associated DNA sequencing (RADseq). The specific hypotheses are that the discovery of thousands of single-nucleotide-polymorphisms (SNPs) will reveal:

*H3a: fine scale (100s of km) population structuring,*

*H3b: more accurate estimates of population parameters such as relatedness and effective population size ( $N_e$ ) and*

*H3c: the direction and intensity of migratory gene flow and therefore the connectivity of parrotfish populations among reefs within the central Philippines, concordant with dominant currents.*

## 1.4 GENERALIZED METHODS

### *Role of MPAs in promoting reef resilience*

The focal area of this study was the northern boundary of the Bohol Sea (Figure 1). About 58 sites (30 of which are MPAs) were surveyed. All sites are situated on nearshore fringing reefs with similar slopes to minimize confounding habitat effects. Although actual fisher densities were not measured during this study coastal human population densities were used as proxies. Previous studies were able to estimate fishing pressure using GIS referenced based maps with coastal population densities and were able to show a negative correlation between increasing human population densities and decreasing fish populations on coral reefs (Mora 2008; Stewart et al. 2010). Additionally nutrient and sediment levels were not directly measured, but proxies using GIS data on annual sediment loads were used. Again previous studies on coral reefs were able to utilize GIS generated sediment data from streams to accurately estimated annual sediment loads (Fabricious et al 2013; Oleson et al 2017). For sites situated within an MPA, the history of protection of that site was researched and recorded.

Belt transects were utilized to estimate fish abundances (Green and Bellwood 2008). For each site, surveys were conducted along the reef slope (8-10m) and reef crest (2-4m). At each depth, three belt transects (10m x 50m) were laid parallel to the shore, wherein fish abundance and size (total length) will be estimated by a single observer (B. Stockwell).

Substrate cover was estimated using a modified point intercept method in which benthic organism types were recorded ever 0.5 m along each of the fish belt transects. A total of 100 intercept points were recorded per transect, giving a precision of 1%. Substrate cover functional groups were recorded as either: hard coral, soft coral, fleshy algae, coralline algae, crustose coralline algae, filamentous algae, dead coral, dead coral with algae, rock, or sand. Live coral was further classified as branching, massive, foliose, solitary, or encrusting. Fleshy algae was also further classed to at least the family level or genus level when possible.

Regression analyses were used to examine the correlation between algae cover and either increasing human population densities or increasing sediment loads. Similarly, regression analyses were conducted examining the relationship between hard coral cover and the same

stressors (human population and sedimentation). And lastly, regression analyses were used to examine the relationship between parrotfish biomass and algae cover.

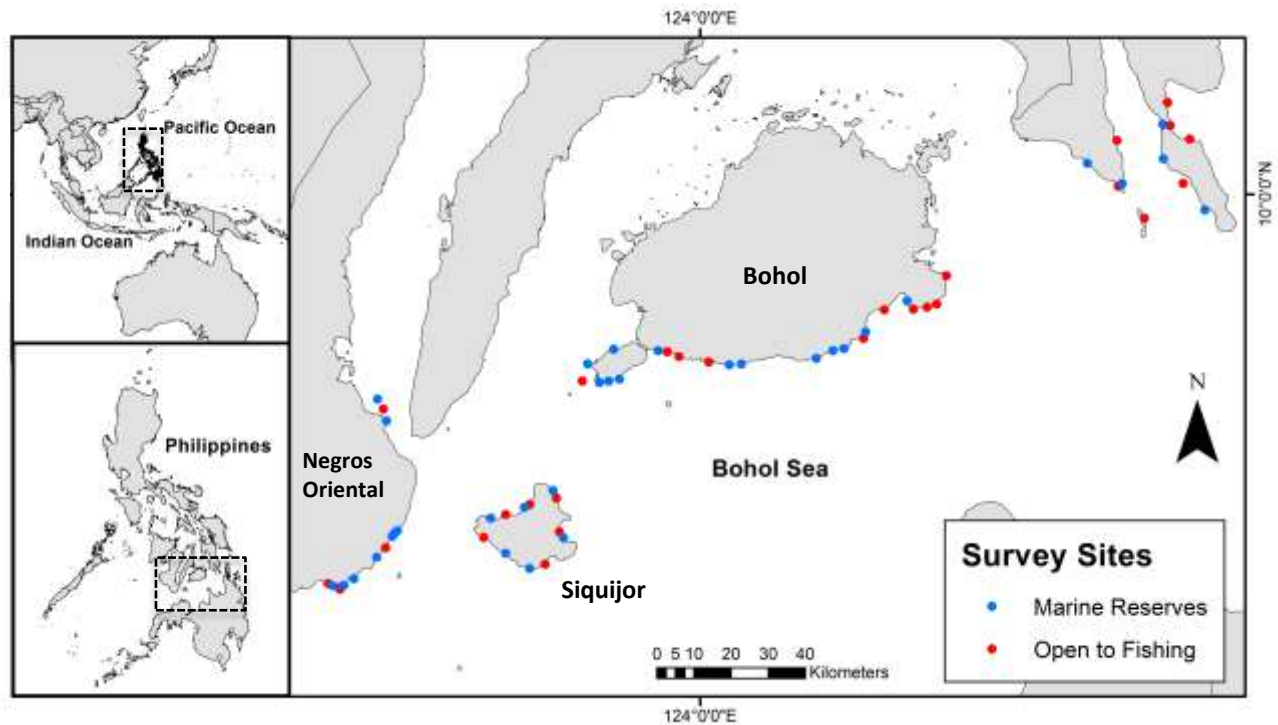
#### *Role of MPAs in restoring the reproductive potential of parrotfishes*

Parrotfish abundances and lengths for both initial phase (female) and terminal phase (male) categories were recorded both within sites open to fishing and within marine reserves. Further analyses were limited to the four most abundant sexually dimorphic species of parrotfishes (*Chlorurus bleekeri*, *Chlorurus spilurus*, *Scarus dimidiatus* and *Scarus rivulatus*). Marine reserves were further classified according to duration of protection as recently-established reserves (1-3 years), established reserves (4-6 years) and well-established reserves (> 6 years). These classifications were based on the short generational times for parrotfishes (5 to 14 yrs) resulting in rapid population turn-over rates (Choat et al. 1996; Choat and Robertson 2002). In addition, studies have recorded rapid initial increases in parrotfish biomass within 3-4 years of protection (Samoilys et al. 2007) and an asymptotic biomass after 6 years of protection (Stockwell et al. 2009). Logistic regression analyses were conducted on the percent composition of terminal phase (TP) individuals at specific lengths to determine the length at sex change. These length estimates were compared between both sites open to fishing and reserves. Histograms displaying the abundances of initial phase (IP) binned according to size classes were generated for the four species of parrotfishes for sites open to fishing, recently-established reserves, established reserves and well-established reserves. This allowed for the comparison of modal size class changes in IP (female) individuals. Lastly, total fecundity per unit area was calculated from the abundance and length data for on the four levels of protection.

#### *Connectivity within the central Philippines*

This study was conducted in the same region as the MPA studies described above (Fig. 1). Previous work by the investigator (Abesamis et al. 2016) suggested the presence of strong connectivity in this region that includes southern Leyte, southern Bohol, Siquijor and southern Negros (H = I, J in Fig. 2, left panel). Conversely, connectivity is also thought to be weak or absent between this Bohol Sea region and certain bays, channels and the adjacent Sulu Sea (A, B, C, E, F, G in Fig. 2). These hypothetical patterns were tested using population genetic methods applied to three species of herbivorous reef fish (*Chlorurus bleekeri*, *Scarus niger* and

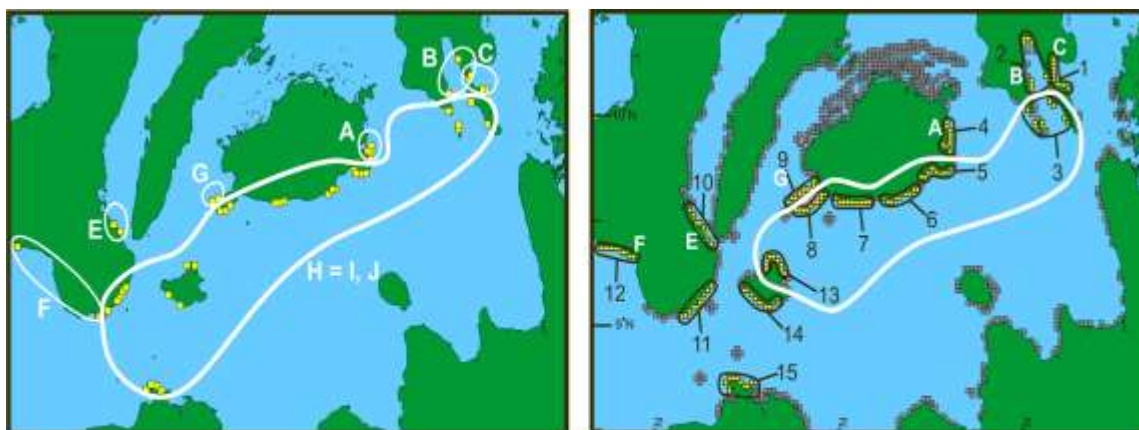
*Siganus guttatus*). Between 30 and 40 individuals were sampled from 5 sites (corresponding to A, B, C, E, F, G and H in Fig. 2). Since all three species are targeted by local fishers, samples were obtained from local fish markets and landing sites.



**Fig. 1** Location and distribution of sites for the biophysical surveys within the central Philippines.

For each species a minimum of 32 samples were collected from several sites within the Bohol Sea (2-3 sites) and one site outside of the region as an outlier. Of these 96 samples, RAD (Restriction-site Associated DNA) libraries were prepared following the methods of Everett et al. (2012) and sequenced on an Illumina HiSeq2000. The STACKS software package (Catchen et al. 2011) was used to discover and genotype SNPs (single nucleotide polymorphisms) from the sequenced RAD tags. Fixation indices ( $F_{ST}$  based analyses) were used to determine population structuring, while NeEstimator (Do et al. 2014) was used to estimate effective population sizes,

*related* (Pew et al. 2015) was used to estimate relatedness and Migrate-N (Beerli 2008) used to examine migration patterns.



**Fig. 2** Patterns of connectivity along the northern boundary of the Bohol Sea suggested by multivariate analysis of reef fish assemblage data (left) are supported by the results of larval dispersal modelling (right). Regions that were similar in their species composition or were predicted to be strongly connected by larval dispersal are enclosed by white ellipses (Modified from figure 3; Abesamis et al. 2016)

## 1.5 SUMMARY

MPAs are widely expected to promote the resilience of reefs by protecting and increasing the abundance of herbivorous fishes that can graze on algae, therefore directly or indirectly preventing coral to algal phase shifts (Bellwood et al. 2004; Hughes et al. 2007; Stockwell et al. 2009). This study aims to determine role of parrotfishes in reducing algal cover and promoting a coral dominated reef.

This project will advance the current state of knowledge on the patterns and mechanisms of reef fish larval dispersal at spatial scales relevant to networking of MPAs in the Philippines (10's to a few 100's of km). Output will contribute to a better understanding of the extent to which the dynamics and structure of local reef fish populations depend upon larval dispersal patterns. The project will also highlight the importance of protecting parrotfishes inside MPAs, which may



also spur local stakeholders to manage parrotfishes outside of MPAs (i.e., traditional fisheries management).

The empirical data on connectivity gathered by this project will be important specifically to MPA networks in the Philippines because the typical distances separating Philippine MPAs (<1-5 km) are within the expected spatial scales of demographically significant larval connectivity (10's of km) (Jones et al. 2009; Weeks et al. 2010). The results can help MPA managers, government officials at the municipal and provincial levels and their advisers from non-government and academic organisations decide on how to best structure MPA networks. Information generated by this project will be valuable especially in cases where ideas about larval connectivity are vague or not considered.

## CHAPTER 2: INCREASING PARROTFISH BIOMASS MITIGATES HUMAN IMPACTS WITHIN MARINE RESERVES

### 2.1 ABSTRACT

Marine reserves are an important tool in marine conservation but it is unclear if they directly mitigate effects from increased human population density such as sedimentation and overfishing of herbivorous fishes. These stressors are some of the greatest threats to coral reefs and have led to the proliferation of macroalgae, a reduction in coral cover and overall declines in reef health. One of the main objectives of marine reserves is to mitigate these stressors through the restoration of herbivorous fishes (mainly parrotfish). The aims of this study are to 1) determine if marine reserves can prevent the increase in macroalgae cover caused by increasing levels of siltation and human population density, 2) promote or sustain a coral dominated reef system in the face of increased siltation and human population density and 3) test if there is top-down control of algae by parrotfishes. Within the central Philippines, I surveyed fish biomass and benthic cover at 30 no-take marine reserves and 28 sites open to fishing. Sampling sites were then geo-referenced with population density and sedimentation data using GIS software, allowing for the generation of total population and sedimentation estimates for each site. Regression analyses revealed nearly flat, non-significant ( $p > 0.05$ ) relationships between human population and algae cover, or sedimentation and algae cover within marine reserves. However, there were positive exponential relationships between human population and algae ( $R^2 = 0.60$ ,  $p < 0.001$ ) and sedimentation and algae ( $R^2 = 0.56$ ,  $p < 0.001$ ) for sites open to fishing. No significant ( $p > 0.05$ ) relationships between human population and coral cover or sedimentation and coral cover were detected within marine reserves. However, significant exponential decreases were found between human population and coral ( $R^2 = 0.44$ ,  $p < 0.001$ ) and sedimentation and algae ( $R^2 = 0.52$ ,  $p < 0.001$ ) for sites open to fishing. The mean parrotfish biomass of  $35.96 \text{ g/m}^2$  ( $\pm 1.99 \text{ g SE}$ ) within marine reserves was significantly greater than that of sites open to fishing ( $11.15 \text{ g/m}^2$  [ $\pm 0.95 \text{ g SE}$ ]). A piecewise regression of parrotfish biomass and fleshy algae cover revealed a threshold value of  $41.8 \text{ g/m}^2$  at which the negative relationship reached an asymptote and algae cover remained below 10%. These results demonstrated that marine reserves can 1) prevent the proliferation of algae 2) sustain coral cover despite increasing

sedimentation and human population density and 3) restore parrotfish populations that have a top-down control on algae cover.

## 2.2 INTRODUCTION

Marine reserves are the most common means of ecosystem-based management for tropical nations (Gill et al. 2017) but is not clear if and how they mitigate against human population-based stressors (Bruno and Valdivia 2016). Some of the greatest threats to coral reefs are chronic disturbances such as poor water quality and reduced herbivory through overfishing that are inhibitors to coral reef recovery (Knowlton 1992; Birkland 1997; McClanahan et al. 2002; Houk & Woesik 2010). Coastal development is a leading cause of decreased water quality through increased nutrients and sediment loads (Taylor et al. 1995). These increased nutrients lead to eutrophication of the reef, which enhances primary productivity and increases algal growth (Smith et al. 1981; Lapointe et al. 2004). Additionally, increased sedimentation can suffocate coral and be detrimental to coral recruitment (Browne 2012) and increase the prevalence of coral disease (Pollock et al. 2014). When combined, these chronic stressors can alter the reef community from a coral dominated system to one dominated by macroalgae, bioeroders and suspension-feeders (Fabricius 2005). A major drawback to a macroalgae dominated system is that it produces less goods and services compared to a coral dominated system (Bellwood et al. 2004).

The relationship between poor water quality and increased macroalgae has been well documented but there is considerable debate about whether the recovery of herbivory (mainly parrotfish abundance) will reverse degraded, algae dominated reefs (Aronson & Precht 2006; Mumby et al. 2006; Hughes et al. 2007; 2010; Graham et al. 2015; McClanahan et al. 2011; Cheal et al. 2013; Toth et al. 2014). Initial support for the control of algae by herbivores came from caged exclusion experiments, which showed proliferation of macroalgae in the absence of herbivory (Lirman 2001; Hughes et al. 2007). Natural experiments have shown that reefs with higher abundance or biomass of herbivores (especially parrotfishes) also have lower macroalgae cover (Williams & Polunin 2001; Stockwell et al. 2009; Rasher et al. 2012). This has led to the widely held belief that fishing-induced reductions of parrotfish abundance causes benthic phase shifts from coral to macroalgal dominance (Hughes 1994, Bellwood et al. 2004; Mumby et al.

2006; Bonaldo et al. 2014), thus invoking top-down control of algae on the reef. However, recent studies have questioned the role of herbivory on algae and point to the bottom-up control of algae on parrotfish abundance (Suchley et al. 2016; Russ et al. 2015).

A main function of marine reserves is to restore reefs and make them more resilient (Selig & Bruno 2010; Heenan & Williams 2013). For coral reefs, resilience has been defined as the ability to return to a coral dominant state relatively quickly after an acute disturbance, or when faced with a chronic stressor (Scheffer et al 2001; Hughes et al. 2005; Diaz-Pulido et al. 2009). However, the ability of marine reserves to promote resilience and protect coral reefs from chronic stressors may be minimal (Hughes et al 2003; Halpern et al. 2013). Additionally, recent studies found conflicting results when examining the mitigating effects of marine reserves. Olds and colleagues (2014) indicated that marine reserves enhance the capability of reefs to recover after decreased water quality following a flooding event and attributed this to an increase in herbivore biomass. However, another study within Australia found conflicting results showing that reef health was not sustained inside marine reserves after a flooding event, in fact coral cover was significantly lower inside reserves compared to fished sites (Wenger et al 2015).

In many tropical countries parrotfish are heavily fished (Edwards et al. 2013), which has led to the widespread declines of these fishes in the last few decades (Comeros-Raynal et al. 2012; Edwards et al. 2014). Philippine coral reefs are not immune to this phenomenon and have some of the most heavily fished reefs in the world (Alcala & Russ 2002; Newton et al. 2007), with parrotfishes accounting for as much as 10% of total reef fish yields (Alcala & Russ 2002). Yet, parrotfish densities have seen recoveries within no-take marine reserves in the Philippines (Russ & Alcala 1998; Stockwell et al. 2009). In addition to overfishing, coral reefs in Southeast Asia are severely impacted by pollution (Knight et al. 2012), since many countries are lacking water treatment facilities, resulting in over 80% of untreated sewage being discharged onto neighboring reefs (Todd et al. 2010).

In this study I investigated the ability of marine reserves to mitigate chronic anthropogenic stressors such as decreased water quality and reduced herbivory due to overfishing in the central Philippines. Secondly it examines the increase in parrotfish biomass within marine reserves and

how this might impact macroalgae cover. Benthic habitat and parrotfish biomass was enumerated in 30 marine reserves and 28 sites open to fishing and were compared with both water quality measures (annual sediment load) and human population densities. Specifically the following questions were addressed:

1. Can marine reserves prevent the increase in macroalgae cover resulting from increasing levels of sedimentation and human population density?
2. Can marine reserves promote or sustain a coral dominated system in the face of increasing levels of sedimentation and human population density?
3. What is the evidence for parrotfish biomass top-down control of macroalgae levels?

## **2.3 METHODS AND MATERIALS**

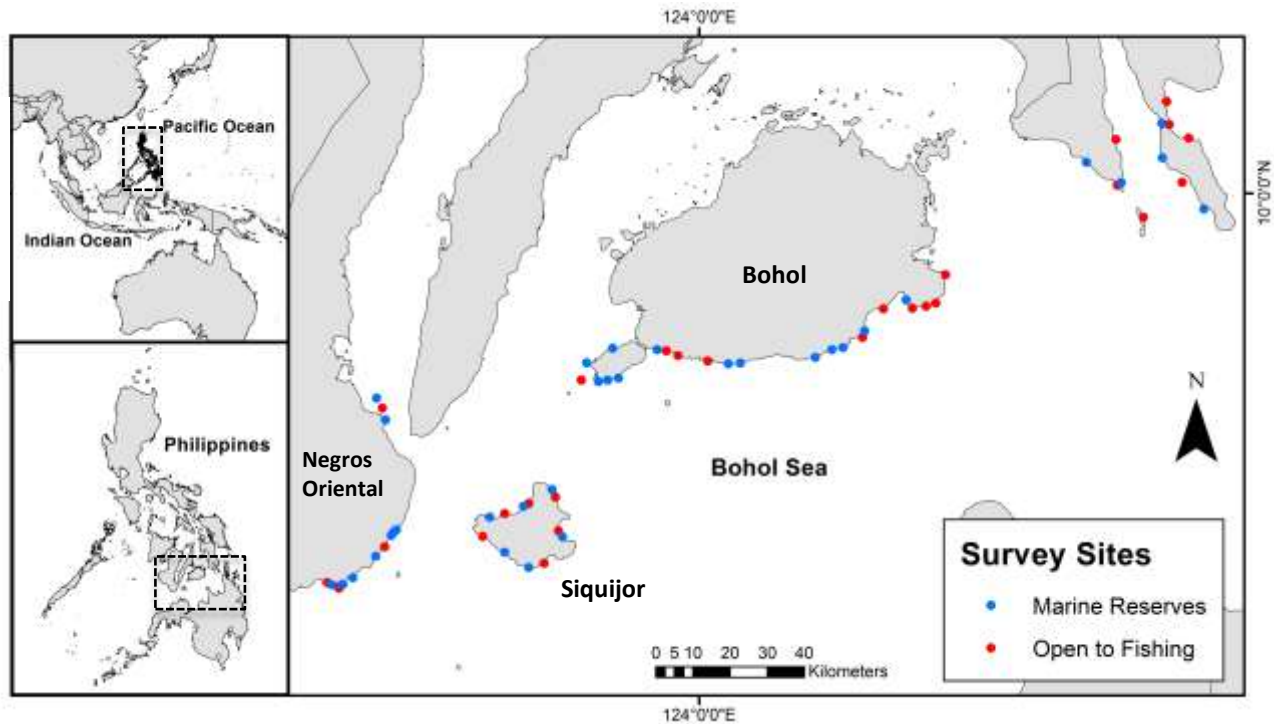
### *Study Location*

A total of 58 reefs sites were surveyed within the Bohol Sea, located in the central Philippines (Figure 3). Of the sites, 30 were within no-take marine reserves and 28 within reefs open to fishing. The surveys were conducted from March to June 2012. All sites were located within 1 km of the coast and thus classified as nearshore, fringing reefs.

### *Ecological Data*

Belt transects were utilized to estimate fish abundances (Green and Bellwood 2009) along the reef slope (10-12m) and reef flat (2-3m) at each site. At both locations (reef slope, reef flat), three belt transects (10m x 50m) were laid parallel to the shore, wherein fish abundance and size (total length) were estimated by a single observer. Fish biomass estimates were calculated from the length records using the formula  $W = a * L^b$  where  $W$  = weight (g),  $L$  = length (cm) and  $a$  and  $b$  = species-specific growth parameters obtained from Fishbase (<http://www.fishbase.org>).

Benthic cover was estimated using a modified point intercept method in which benthic organism types were recorded every 0.25m along each of the fish belt transects. A total of 200 points were recorded per transect, giving a precision of 0.5%. Substrate cover of functional groups was recorded as either live hard coral, soft coral, macroalgae, coralline algae, crustose coralline algae, filamentous algae, dead coral, rock, or sand. Live coral was further classified as branching,



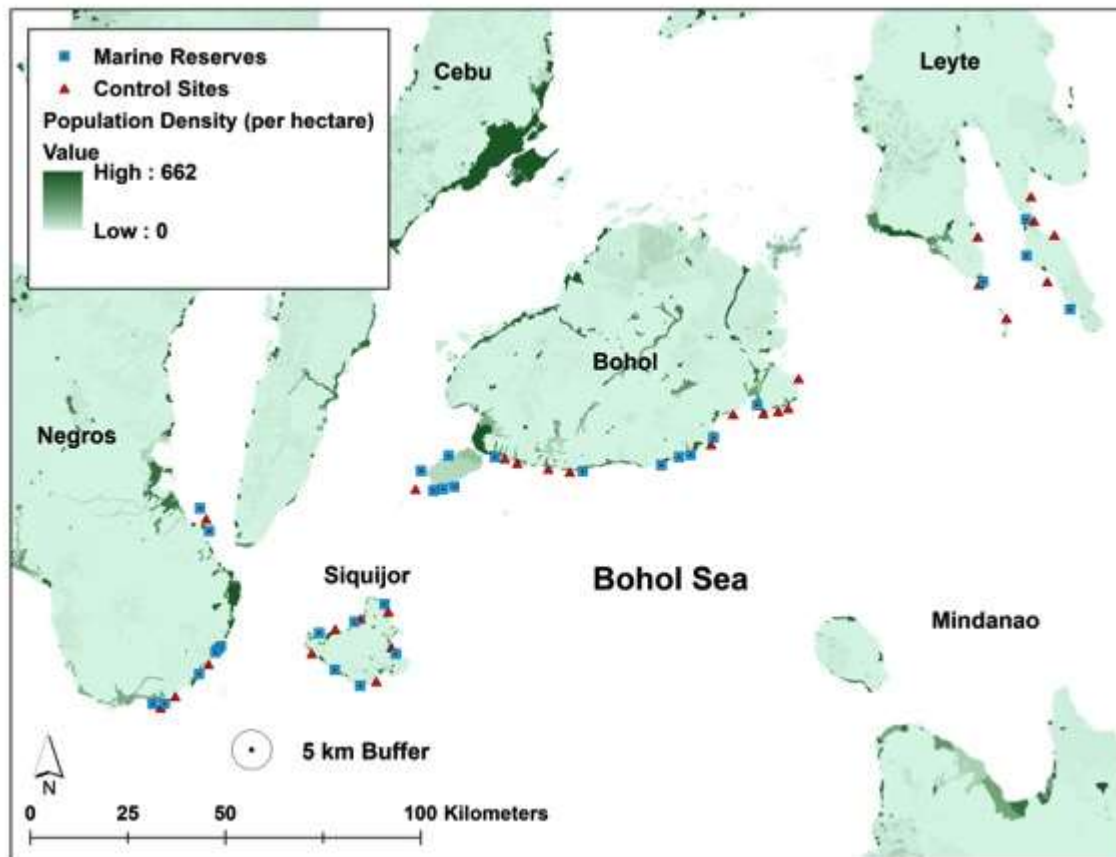
**Fig. 3** Location of the 58 reef sites (30 marine reserves and 28 sites open to fishing) surveyed within the Bohol

massive, foliose, solitary, or encrusting. And lastly the category of fleshy algae was specified as macroalgae or filamentous algae.

#### *Environmental Impact Data*

Human coastal population was used as a proxy for local fishing pressure. These data were generated by using a base map of human population densities for the year 2012 from the Philippine GIS Data Clearinghouse (<https://www.philgis.org>). The base map was a raster file containing human population counts within hectare-sized cells. This base map was imported into ArcGIS® with the 58 geo-referenced samples sites. A 5 km buffer was generated around each

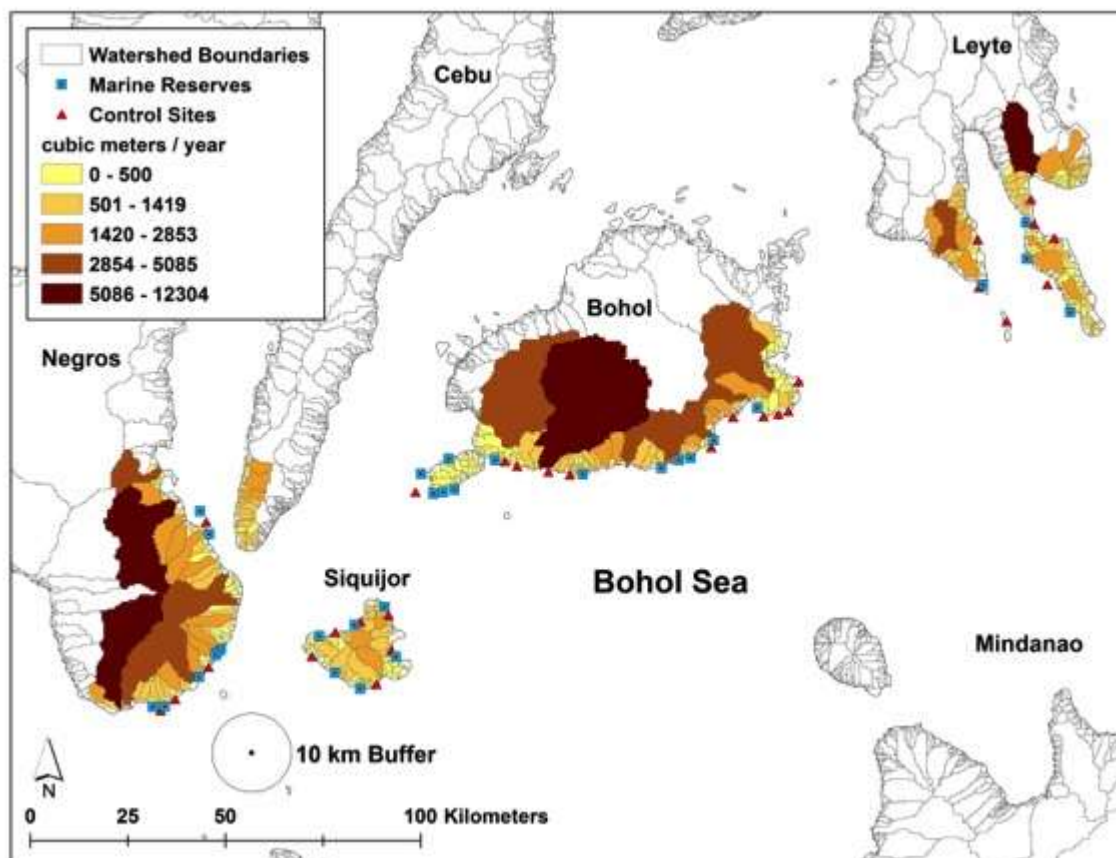
sampling site and clipped to the population base map allowing for an estimate of total human population within a 5 km radius for each reef site (Figure 2.) A 5 km radius was chosen since this is the average distance fishers in the region travel (Horigue et al. 2014).



**Fig. 4** Location of the sample sites in relation to population densities used in the analysis.

Total annual sedimentation from rivers and streams was used as a proxy for water quality. A base map incorporating the total annual sedimentation ( $m^3$ ) per drainage basin was downloaded from Reefs at Risk Revisited within the World Resources Institute (Reefs at Risk 2017). The same 58 sample sites were also geo-referenced to this base map in ArcGIS®. The oceanographic currents of the northern Bohol Sea are dominated by a strong current called the Bohol Sea Jet

(Han et al. 2009; Gordon et al. 2011), which runs at  $0.56\text{ms}^{-1}$  on average from east to west. Because of this strong current, a larger buffer with a radius of 10 km was used to estimate total annual sediment load received by each reef site. After the 10 km radius clip was applied to the reference map, total annual sedimentation was calculated by summing the output from the entire drainage basin of river mouths that were within the 10 km buffer (Figure 3). A potential weakness of these estimated values is that they do not take into account local site turbulence and assumed that sediment retention rates were equal for all sites. Although most sites were on relatively exposed coastlines, sites within Sogod Bay in Southern Leyte may have higher sediment loads due to embayment effects.



**Fig. 5** Location of the sample sites in relation to total annual sediment load from relevant watersheds within a 10 km buffer of each site.



### *Data Analysis*

To examine the relationship between sedimentation and fleshy algae cover and hard coral cover, linear regressions were conducted for both marine reserves and control sites for both fleshy algae and hard coral cover. Since the precision of total sediment load could not be determined to the level of transect, but only to the level of site, mean percent fleshy algae cover and live hard coral cover per site was used in the regression analyses. Exponential models describing both rapid algae growth and rapid coral decline were estimated to be optimal for reefs in this region (Stockwell et al. 2009). Therefore, macroalgae growth was modelled with exponential growth by applying a  $\log_{10}$  transformation. Coral cover loss was also modelled using an exponential decline function and was also  $\log_{10}$  transformed. Linear regression was then conducted on the transformed data in r (r-project.org). Plots of the residuals were viewed to determine if the assumption of equal variances were met. Lastly, final figures with the non-transformed data were fitted with the exponential regression models to allow for a better visualization of the results.

To examine the relationship between total coastal population (proxy for fishing pressure) and macroalgae cover and hard coral cover, linear regressions were also conducted for marine reserves and control sites for both macroalgae and hard coral cover. Similar methods used in comparing sedimentation relationships were utilized in these analyses.

For each transect, parrotfish biomass was converted to  $\text{gm}^{-2}$  for a total of 348 transects. Mean biomass for both reserves and non-reserves was calculated and a one-way ANOVA was run with parrotfish biomass as the response variable and protection (reserve or non-reserve) as the factor.

To determine the potential threshold at which parrotfish biomass may reduce fleshy algae cover, a piecewise linear (PL) analysis was conducted with the r package SiZer (r-project.org). This method of “significant zero crossings” (SiZer) applies a non-parametric smoother of the stressor response data and then identifies the existence of a threshold from the derivatives of the smoothed curve (Sonderegger et al. 2008). In this case parrotfish biomass is considered the stressor in relation to fleshy algae cover. Individual transect data was used with parrotfish biomass on the x-axis and percent fleshy algae cover on the y-axis. The threshold was defined where macroalgae cover becomes asymptotic (slope of 0). In the data set a piecewise linear

(Toms and Lesperance 2003) model was used to fit the data, since the PL model assumes an abrupt transition between linear sections (Sonderegger et al. 2008).

## 2.4 RESULTS

### *Fleshy Algae*

The regression analysis between fleshy algae and total human population within marine reserves was non-significant ( $p = 0.39$ ; Figure 4, Tables 1 & 2). However a significant relationship ( $p < 0.001$ ) with an adjusted  $R^2$  value of 0.60 was detected for the sites open to fishing (Figure 4, Tables 1 & 2). Thus a significant positive relationship was only found between human population and algae for sites open to fishing.

The regression analysis between fleshy algae and total annual sediment load within marine reserves was found to be non-significant ( $p = 0.057$ ; Figure 5, Tables 1 & 2). However a significant relationship ( $p < 0.001$ ) with an adjusted  $R^2$  value of 0.56 was detected for the sites open to fishing (Figure 5, Tables 1 & 2). This also indicates an exponential increase in fleshy algae cover with an increase in total annual sedimentation load only for reefs open to fishing.

### *Live Hard Coral*

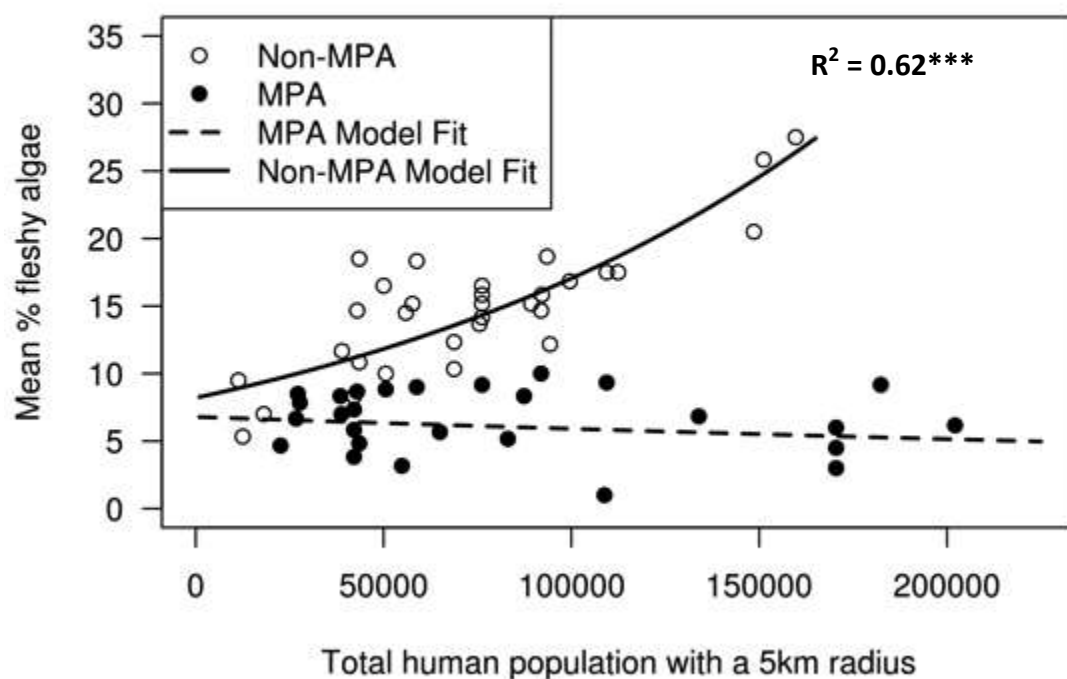
The regression analysis between hard coral cover and total human population within marine reserves was found to be non-significant ( $p = 0.89$ , Figure 6, Tables 3 & 4). However, a significant relationship ( $p < 0.001$ ) with an adjusted  $R^2$  value of 0.42 was detected for the sites open to fishing (Figure 6, Tables 3 & 4). This indicates an exponential decrease in hard coral cover with the total human population for reefs open to fishing only.

The regression analysis between hard coral cover and total annual sediment load within marine reserves was found to be non-significant ( $p = 0.53$ , Figure 7, Tables 3 & 4). However a significant relationship ( $p < 0.001$ ) with an adjusted  $R^2$  value of 0.52 was detected for the sites open to fishing (Figure 7, Tables 3 & 4). This again indicates an exponential decrease in hard coral cover with an increase in total annual sedimentation load only for reefs open to fishing.

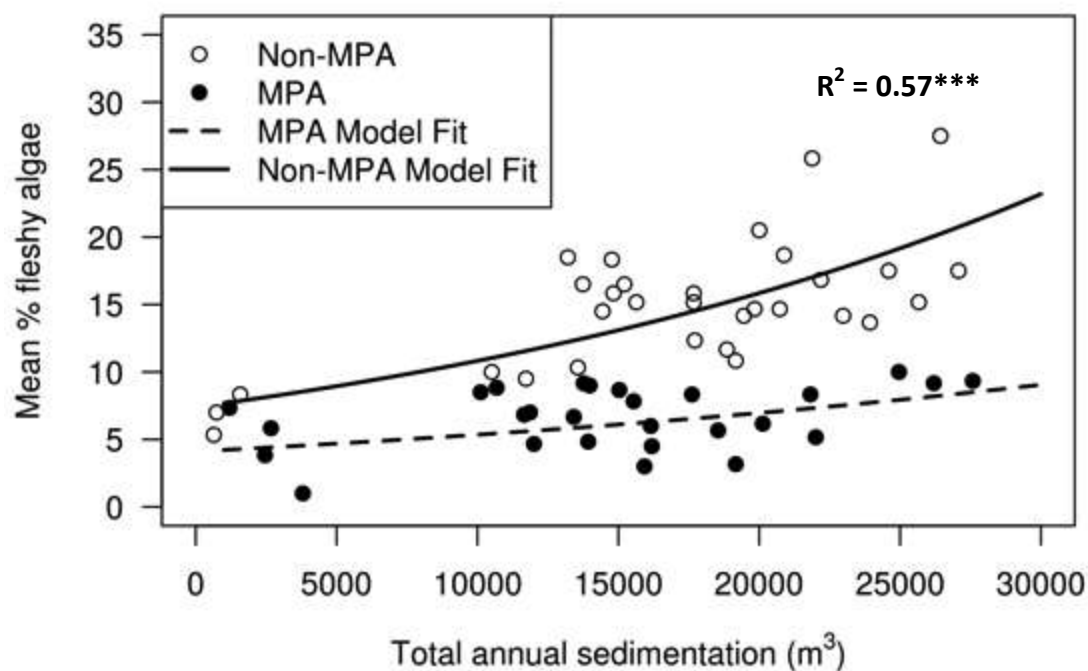
### Parrotfish Biomass

Mean parrotfish biomass within marine reserves was  $36.0 \text{ gm}^{-2}$  ( $\pm 2.0 \text{ g SE}$ ) while mean parrotfish biomass was  $11.2 \text{ gm}^{-2}$  in sites open to fishing ( $\pm 0.9 \text{ g SE}$ ). The ANOVA analysis revealed that these means were significantly different ( $p < 0.001$ , Table 5).

The piece-wise linear regression analysis revealed an initial negative trend between total parrotfish biomass and fleshy algae ( $y = 17.45 - 0.295x$ , Figure 8, Tables 6 & 7). A threshold of parrotfish biomass was detected at  $41.79 \text{ g}$  ( $34.78 - 48.80 \text{ CI}$ ) at which point the slope was determined to asymptote to 0. This indicates that once parrotfish biomass reached  $41 \text{ gm}^{-2}$  flesh algae cover tended to remain below 10% (Figure 8).



**Fig. 6** Regression analyses of mean percent fleshy algae vs. total human population within 5 km for 30 marine reserves (solid circles) and 28 sites open to fishing (open circles).



**Fig. 7** Regression analyses of mean percent fleshy algae vs. total annual sedimentation ( $m^3$ ) within 10 km for 30 marine reserves (solid circles) and 28 sites open to fishing (open circles).

## 2.5 DISCUSSION

### *Mitigating Effects of Marine Reserves*

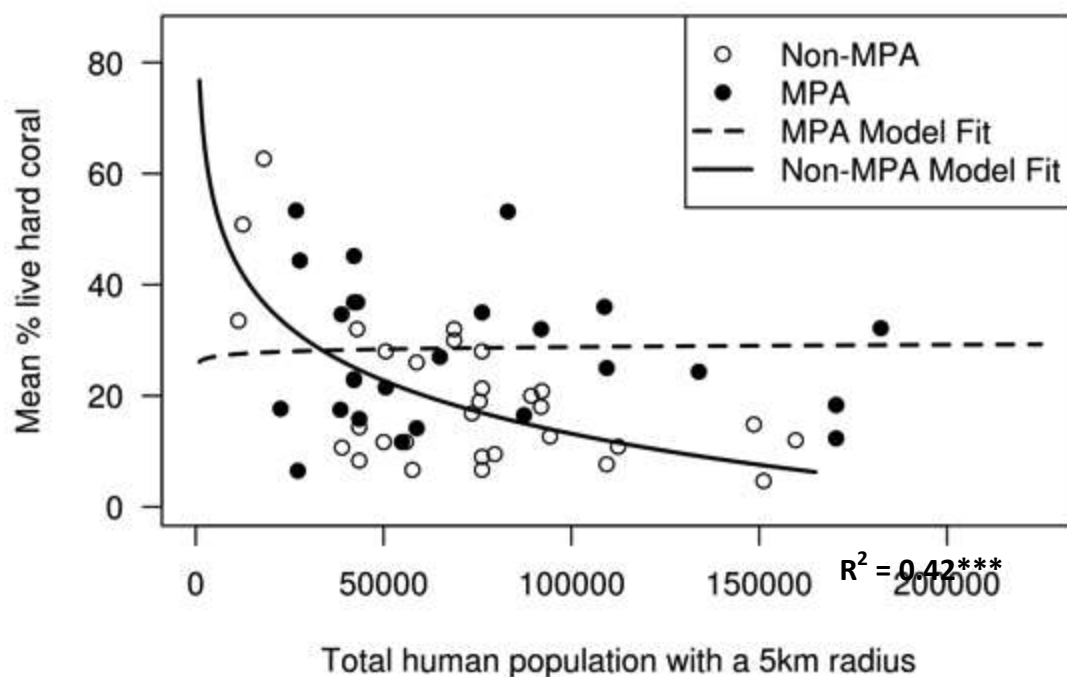
Marine reserves appear to mitigate coral reef degradation associated with increased human density effects such as increased fleshy algae cover. Fleshy algae cover was shown not to increase within marine reserves despite an increasing human population and actually remained below 10% cover. An exponential increase in fleshy algae occurred with an increasing human population for sites open to fishing, even to mean levels higher than 25% cover. Live hard coral

**Table 1** Summary of the coefficients of the regression analyses with fleshy algae (FA) as the dependent variable.

<b>Treatment</b>	<b>Coefficients</b>	<b>Estimate</b>	<b>SE</b>	<b>t</b>	<b>p</b>
<b>Reserves</b>	FA vs. Human Pop.				
	Intercept	1.990	0.1905	10.448	< <b>0.001</b>
	Population	-1.685e-06	1.941e-06	-0.868	0.394
	FA vs. Sedimentation				
	Intercept	1.414	0.2141	6.605	< <b>0.001</b>
	Sedimentation	2.630e-05	1.318e-05	1.995	0.057
<b>Fished Sites</b>	FA vs. Human Pop.				
	Intercept	2.105	0.08805	23.903	< <b>0.001</b>
	Population	7.313e-06	1.066e-06	6.843	< <b>0.001</b>
	FA vs. Sedimentation				
	Intercept	2.001	0.1123	17.826	< <b>0.001</b>
	Sedimentation	3.8409e-05	6.133e-06	6.211	< <b>0.001</b>

**Table 2** Summary of regression analyses with fleshy algae (FA) as the dependent variable for reserve and fished sites.

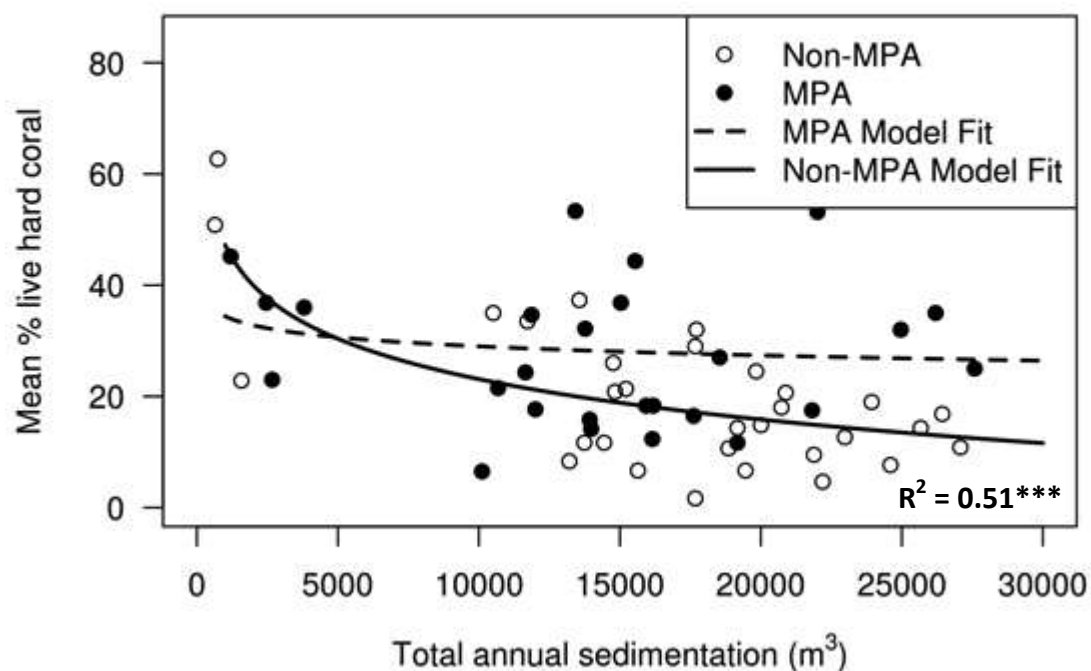
	<b>Model</b>	<b>Res. SE</b>	<b>df</b>	<b>Mult. R<sup>2</sup></b>	<b>Adj. R<sup>2</sup></b>	<b>F</b>	<b>df</b>	<b>p</b>
<b>Reserves</b>								
FA vs. Human Pop.	Exp.	0.5454	25	0.0292	-0.0096	0.7536	1,25	0.3936
FA vs. Sedimentation	Exp.	0.4656	25	0.1374	0.1028	3.9810	1,25	0.0570
<b>Fished Sites</b>								
FA vs. Human Pop.	Exp.	0.2186	29	0.6176	0.6044	46.83	1,29	< <b>0.001</b>
FA vs. Sedimentation	Exp.	0.2309	29	0.5708	0.5560	38.57	1,29	< <b>0.001</b>



**Fig. 8** Regression analyses of mean percent hard coral cover vs. total human population within 5 km for 30 marine reserves (solid circles) and 28 sites open to fishing (open circles).

did not decrease with an increase in human population inside marine reserves, but within sites open to fishing it declined exponentially. This demonstrates that marine reserves can prevent the accumulation of algae cover and prevent the loss of coral cover in the face of increasing stressors from human populations.

Marine reserves also appear to mitigate detrimental effects of increased sedimentation. Annual sedimentation load and the cover of fleshy algae were uncorrelated in marine reserves. However, an exponential increase in fleshy algae cover was detected within sites open to fishing. Coral cover also behaved in the same manner, with no significant decrease with increasing sediment loads in reserves, while in sites open to fishing coral cover declined exponentially with



**Fig. 9** Regression analyses of mean percent hard coral cover vs. total annual sedimentation ( $m^3$ ) within 10 km for 30 marine reserves (solid circles) and 28 sites open to fishing (open circles).

increasing sediments. This indicates that marine reserves within the central Philippines can prevent the accumulation of algae and the loss of coral cover with increasing sediments. Olds et al. (2014) also found that marine reserves enhance the resilience of corals to increased sedimentation, however this was following a single flood event. However, results by Wenger et al. (2016) revealed declines in coral cover within well-protected marine reserves after a severe flood event, indicating that the inherent resilience of the system may have a threshold.

This study may provide tools to predict the performance of marine reserves in protecting hard coral cover from human impact and fleshy algae proliferation in the particular habitat studied. A

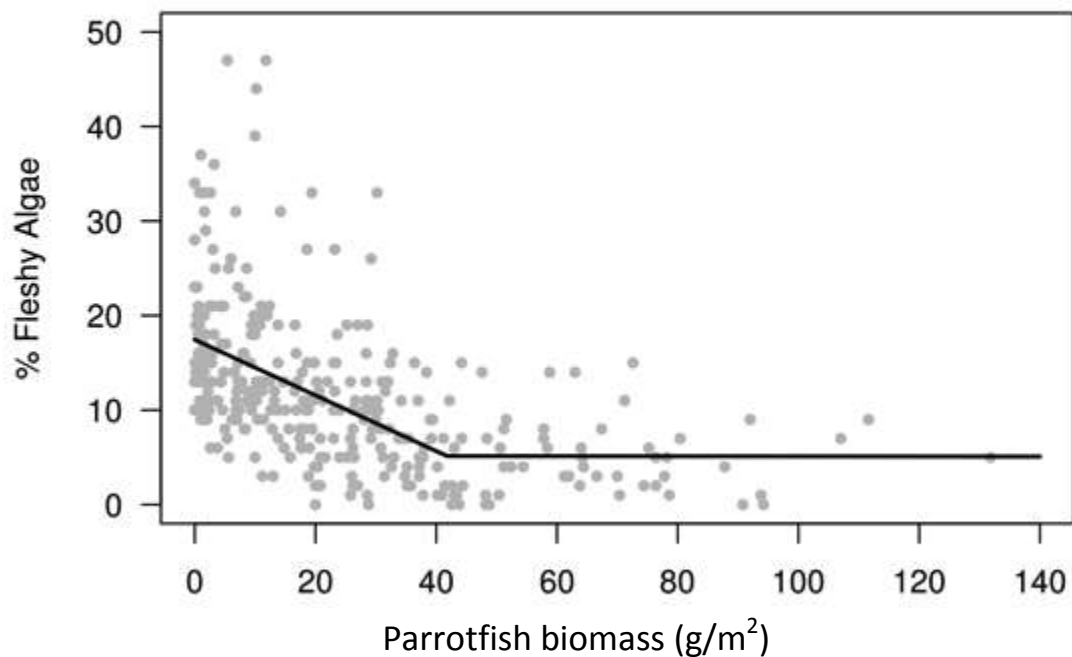
**Table 3** Summary of regression analyses with live hard coral cover (LHC) as the dependent variable for reserve and fished sites.

	<b>Model</b>	<b>Res. SE</b>	<b>df</b>	<b>Mult. R<sup>2</sup></b>	<b>Adj. R<sup>2</sup></b>	<b>F</b>	<b>df</b>	<b>p</b>
<b>Reserves</b>								
LHC vs. Human Pop.	Exp	14.47	29	0.00073	-0.03924	0.0182	1,29	0.893
LHC vs. Sedimentation	Exp	14.36	29	0.01609	-0.02327	0.4087	1,29	0.528
<b>Fished Sites</b>								
LHC vs. Human Pop.	Exp	9.934	27	0.4417	0.4225	22.95	1,27	< <b>0.001</b>
LHC vs. Sedimentation	Exp	9.567	27	0.5231	0.5067	31.81	1,27	< <b>0.001</b>

**Table 4** Summary of the coefficients of the regression analyses with live hard coral (LHC) as the dependent variable.

<b>Treatment</b>	<b>Coefficients</b>	<b>Estimate</b>	<b>SE</b>	<b>t</b>	<b>p</b>
<b>Reserves</b>	LHC vs. Human Pop.				
	Intercept	22.1171	47.6253	0.464	0.646
	Population	0.5789	4.2827	0.135	0.894
	LHC vs. Sedimentation				
	Intercept	50.632	34.662	1.461	0.157
	Sedimentation	-2.348	3.673	-0.639	0.528
<b>Fished Sites</b>	LHC vs. Human Pop.				
	Intercept	172.100	31.849	5.404	< <b>0.001</b>
	Population	-13.804	2.882	-4.790	< <b>0.001</b>
	LHC vs. Sedimentation				
	Intercept	119.610	17.764	6.733	< <b>0.001</b>
	Sedimentation	-10.478	1.858	-5.640	< <b>0.001</b>





**Fig. 10** Individual transect data comparing % fleshy algae cover and parrotfish biomass fit with a piecewise linear model (threshold = 41.79g, 95% CI = 34.78, 48.80).

**Table 5** Summary of the one-way ANOVA comparing parrotfish biomass within marine reserves and sites open to fishing.

Treatment	df	SS	MS	F	p
Protection	1	52232	52232	139.8	2e-16
Residuals	346	127025	374		

**Table 6** Model coefficients for the piecewise linear model

Beta[0]	Beta[1]	Beta[2]
Intercept	x	w
17.451	-0.295	-0.294

**Table 7** Change point (threshold) confidence limits and slopes from the piecewise linear model (threshold = 41.79).

C.I	Change point	Initial slope	Slope change	Second slope
2.5%	34.78	-0.487	0.218	-0.081
97.5%	48.80	-0.240	0.421	0.047

meta-analysis of 1708 reefs survey across the world found no correlation between human population and coral reef degradation (Bruno and Valdivia 2016) and suggested that local factors such as fishing pressure and pollution have minimal effects on coral reefs. However, Levin (1992) showed that when increasing the complexity and study designs, defined by the number, strength and scale of ecological interactions, contrasting patterns often become evident. Thus studies including wide geographical areas and multiple reef types are likely to encounter more confounding factors, which may produce conflicting results. The same can be applied to meta-analyses incorporating many different observers and sampling methodologies. The strength of this study is that it was conducted entirely on nearshore fringing reefs within a single sea (i.e. the Bohol Sea) in the central Philippines, which were surveyed with the same methodologies. Because of this, we believe our results may extend to other similar habitats facing similar threats.

#### *Effects of Parrotfish*

The cause of the decline in algae within marine reserves is likely to be the result of increasing parrotfish abundance, which would increase algae consumption. Along with this study, others

within the region (Russ & Alcala 1998; Stockwell et al. 2009) have found parrotfish biomass was significantly greater within marine reserves compared to sites open to fishing. To clarify the correlation between parrotfish abundances and algae cover, this study also found a threshold value of between 34g and 48gm<sup>-2</sup> beyond which algae cover remains suppressed at below 10%. It is interesting to note that the mean parrotfish biomass for marine reserves was 35.96 gm<sup>-2</sup> ( $\pm$  1.99g SE) well within the threshold range. This suggests that parrotfish biomass within marine reserves reaches levels high enough to prevent the proliferation of algae.

Other large herbivorous fishes such as surgeonfishes (Acanthuridae), batfishes (Ephippidae), rudderfishes (Kyphosidae) and rabbitfishes (Siganidae) occur on these reefs and have a potential role in controlling algae levels (Green et al. 2009). However of these families, batfishes, rudderfishes and rabbitfishes occurred at low abundances (current study) and are likely to have minor impacts on algae cover. Although surgeonfishes occur in greater abundances and can make up a majority of the herbivore biomass, previous studies (McClanahan et al. 2007; Stockwell et al. 2009) found that this was only the case for marine reserves with greater than 6 years of protection. Thus the initial rapid decline in algae within the first few years of protection is likely the result of the rapid increase in parrotfish abundance within these marine reserves (Stockwell et al. 2009).

There is considerable debate whether there is bottom-up control of parrotfish by food availability on the benthos (Russ 1984, Cheal et al. 2012; Tzadik and Appeldoorn 2013; Taylor et al. 2014) or top-down control of food such as algae on the benthos by parrotfish (Hughes 1994; Bellwood et al. 2004; Mumby et al. 2006; Bonaldo et al. 2014; Adam et al. 2015). Bottom-up control implies that if the habitat changes then fish abundance changes, which is a much simpler process to demonstrate (Russ et al. 2015; Suchley et al. 2016). Top-down control on the other hand, implies that parrotfish abundances have a direct effect on algae cover. Russ et al. (2015) proposed a four step proof of top-down control of parrotfishes on macroalgae cover and data in this study supports a critical step in this proof. The four steps in order are: (1) Fishing reduces parrotfish abundance, (2) this reduces grazing pressure, (3) this results in increased macroalgae cover, (4) lastly algae outcompetes coral reducing coral cover (Hughes 1994; Bonaldo et al. 2014). It is well established that parrotfish are heavily targeted and their abundances have been

in decline worldwide (Comeros-Raynal et al. 2012; Edwards et al. 2014). Reduced grazing pressure and increased algae cover have been demonstrated by several studies showing that a decline in grazing pressure has led to an increase in algae cover (Lewis 1986, Jompa and McCook 2002; Stockwell et al. 2009, Rasher and Hay 2010). However, it is step number 4, algae proliferation leading to a decrease in coral cover which has been more difficult to demonstrate given the comparably slower growth rates of coral compared to algae (Stockwell et al. 2009). To that end, this study overcomes this difficulty and found a decrease in coral cover with increasing algae in response to increases in human population and sediment load for reef sites open to fishing. Additionally, coral cover was sustained within marine reserves with a greater parrotfish biomass. This supports the top-down control of algae on these reefs by parrotfish rather than algae availability controlling abundance of herbivores.

There have been some conflicting studies demonstrating that algae cover on the benthos controls the abundance of parrotfish (Taylor et al. 2014, Russ et al. 2015, Suchley et al. 2016) but this may be a function of nutrient availability. A meta-analysis by Suchley et al. (2016) of 398 sites, studied a variety of reef types across the Mesoamerican Reef, including both nearshore and offshore reefs. Although a study by Russ and colleagues (2015) also surveyed reefs within the central Philippines, these reefs were on small, offshore islands. Offshore reefs tend to be nutrient poor (oligotrophic) while nearshore reefs have higher sediments and nutrient loads where eutrophication is more problematic (Hodgson and Dixon 2000). These nutrient rich reefs are likely to support more primary productivity and thus more algae. It is then more likely that herbivory is a more important process on nearshore eutrophic reefs, where top-down control may be more prevalent. These results highlight that one coral reef ecology does not fit all, thus one cannot make overarching statements about the effects of marine reserves on reefs without specifying reef type.

However, these findings should not be misinterpreted as promoting marine reserves as a cure all for the restoration of nearshore coral reefs. This was highlighted by Wenger et al. (2015), in which threshold stress levels were breached and protection from well-managed marine reserves was not enough to prevent coral loss. Instead, marine reserves may be seen as “buying-time”, allowing for the development of more long-term solutions such as reforestation, wastewater treatment plants and better fisheries management plans.

## **2.6 CONCLUSIONS**

This study demonstrated that marine reserves can prevent the proliferation of algae and sustain coral cover despite increasing sedimentation and human population density. The maintenance of low levels of algae and reduced coral loss in marine reserves, indicate that reserves can mitigate human stressors and promote reef resilience. This study also produced evidence of top-down control of algae by parrotfish. Marine reserves were able to sustain herbivore biomass to levels large enough to suppress the proliferation of fleshy algae.

## CHAPTER 3

### RESERVES RESTORE REPRODUCTIVE POTENTIAL BY DELAYING SEX CHANGE IN PROTOGYNOUS REEF FISHES

#### 3.1 ABSTRACT

No-take marine reserves are expected to enhance fisheries by becoming net exporters of fish larvae. However, few studies have investigated whether the reproductive potential of fish builds-up over time inside reserves relative to fished areas. Female size-at-sex-change (SSC) and female modal size were estimated for four targeted protogynous parrotfishes: *Chlorurus bleekeri*, *C. spilurus*, *Scarus dimidiatus* and *S. rivulatus* (Family Scaridae). For each species, the abundance and size of fishes were recorded in 30 no-take marine reserves (ranging between 1 and 13 years of protection) and 28 sites open to fishing within the central Philippines (Bohol Sea). Size-at-sex-change, female abundance and female modal size class was estimated and included in a space-for-time comparison among the 30 reserves. For each species, reproductive potential in terms of total egg production per unit area was estimated for each survey site. Size at sex change increased within reserves between 33 mm for *C. bleekeri* to 62 mm for *C. spilurus*, whereas female abundance and modal size class increased steadily with duration of protection from fishing. Egg production per unit area increased for all species by between 6 and 13 times in the older (> 6 yrs. of protection) reserves. These results indicate that no-take marine reserves can delay the size at sex change for overfished protogynous reef fishes, resulting in an exponential increase in fecundity. This surplus of egg production may seed neighboring fished reefs and replenish overfished populations.

#### 3.2 INTRODUCTION

No-take marine reserves are widely promoted as fisheries management tools because of a large body of evidence indicating that density and biomass of targeted fishes increases over time inside reserves (Russ 2002; Gell and Roberts 2003; Halpern et al. 2009; Babcock et al. 2010). Net export of adult fish (spillover) from reserves may positively impact fisheries but this effect may be limited and highly localized (Abesamis et al. 2006; Halpern et al. 2010). In contrast, enhanced production of fish eggs and larvae within reserves is expected to result in larval export and substantial subsidies of recruits to fished areas (Russ 2002; Gell and Roberts 2003; Evans et al.

2008; Pelc et al. 2010). This recruitment effect of reserves is based on the assumption that sexually mature females of targeted fishes inside reserves will attain greater densities and larger body sizes over time, resulting in higher fecundity per unit area compared to fishing grounds (Russ 2002; Carter et al. 2014, 2015). In other words, reserves are expected to increase the reproductive potential of fishes.

Theoretical studies indicate that reproductive potential must be exceptionally greater inside reserves than in fished areas for fishers to detect the recruitment effect amongst considerable natural variability in larval supply patterns (Halpern and Warner 2003; Pelc et al. 2010). However, empirical studies that have estimated the reproductive potential of targeted fishes inside relative to outside reserves are limited and available studies to date have dealt with temperate fishes and report a 3-18 fold greater reproductive potential in reserves after 4 to >20 years of full protection (Paddock and Estes 2000; Willis et al. 2003; Denny et al. 2004). Only one study has reported differences in reproductive potential for a coral reef fish between protected and non-protected sites (Evans et al. 2008). Specifically, that the reproductive output per unit area of a gonochoristic species (*Lutjanus carponotatus*) was on average 2.5 higher inside than outside reserves in Australia (Great Barrier Reef) after 14 years of full protection (Evans et al. 2008). Kamukuru and Mgaya (1997) attempted to compare the reproductive potential of another gonochoristic species of coral reef fish (*Lutjanus fulviflamma*) inside a marine park in Tanzania (Mafia Island) but found no mature females in fished areas due to high fishing pressure.

Sequential hermaphroditism is a common sexual life history trait in coral reef fishes with protogyny (sex change from female to male) prevalent in species commonly targeted by fisheries such as parrotfishes (Family Scaridae) (Choat and Robertson 1975). Size at sex change for parrotfishes are flexible and highly dependent on mortality rates. An increase in natural mortality through higher densities of predators has been shown to reduce the size at sex change (Gust 2004; De Martini and Howard 2016), while greater fishing pressure (i.e. removal of the larger terminal phase males) also leads to a decrease in the size at sex change (Hawkins and Roberts 2003; Taylor 2014). The short term benefit of a flexible size at sex change is that this allows for reproduction to continue at higher rates of mortality. However, the exponential relationship between fish size and the number of eggs produced suggests that removal of large females by

fishing, either directly or indirectly by forcing them to undergo sex change at a smaller size will lead to reduced total fecundity of the population (Adams et al. 2000). This begs the question of whether the reduction in fishing mortality in no-take marine reserves can reverse this effect by restoring the size at sex change to a greater size and thus result in an increase in reproductive output.

To date, however, no study has estimated the magnitude of potential differentials in reproductive output of such fishes inside versus outside reserves. It also remains unclear how the development of reproductive potential would differ among species of protogynous reef fish inside reserves through time. In general, the development of reproductive potential in fish populations within reserves may broadly reflect patterns of population recovery in the absence of fishing that are ultimately driven by life history characteristics (Jennings 2001; Abesamis et al. 2014).

The main goals of this paper are to compare the size at transition (sex change) of four species of parrotfishes (*Chlorurus bleekeri*, *C. spilurus*, *Scarus dimidiatus*, *S. rivulatus*) between reserves and fished areas and to describe the effects on reproductive output. I hypothesize that length at sex change would increase within reserves and that reproductive potential (egg production) would greatly increase within reserve sites with increasing years of protection.

### **3.3 MATERIALS AND METHODS**

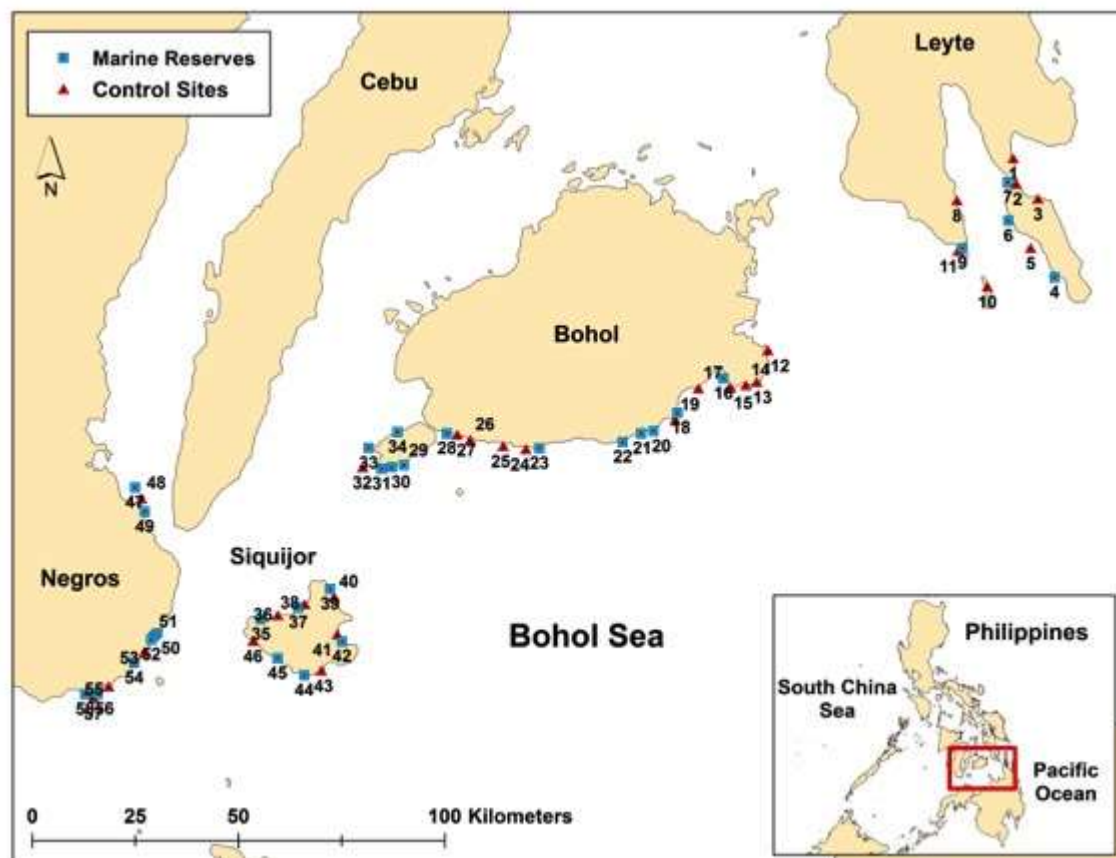
#### *Study Area*

Survey sites included 28 reefs open to fishing (control sites) and 30 reefs within no-take marine reserves across the Bohol Sea in central Philippines (Figure 1, Table 1 and 2). The surveys were conducted from March to June 2012.

#### *Sampling Design*

Belt transects were utilized to estimate fish abundances (Green and Bellwood 2009). For each site, surveys were conducted along the reef slope (10-12m) and reef crest (2-4m). At each depth, 3 belt transects (10m x 50m) were laid parallel to the shore, wherein fish abundance and size





**Fig. 11** Locations of marine reserves and control sites (open to fishing) included in this study. Numbers correspond to site names (see Table 1).

(total length, TL) was estimated by a single observer (B. Stockwell). Parrotfishes (Family, Scaridae) were recorded in 5 cm size classes up to 35 cm (seven total size class) and fishes greater than 35 cm were recorded to the nearest 1 cm, because biomass and fecundity increase disproportionality at these greater lengths. In addition, the color phase (initial or terminal) was recorded for each fish.

### *Study Species*

This study focuses on the four most abundant sexually dimorphic species of parrotfishes recorded during the surveys. All four species of parrotfishes (*Chlorurus bleekeri*, *C. spilurus*, *Scarus dimidiatus*, *S. rivulatus*) have a distinct female (initial phase - IP) and male (terminal phase - TP) color patterns; *C. bleekeri* attains the largest TL at around 490 mm and the other three reach a maximum length of around 400 mm (Table 3). The length at maturity for *C. spilurus* is 150 mm (Randall et al., 1990); however no information on female size at maturity is available for the other three species. All four species are common targets of artisanal reef fisheries in the Philippines (Abesamis et al. 2006; Stockwell et al. 2009).

### *Determination of size at sex change*

The estimation of size (total length, TL) at sex change was determined using logistic regression models and utilized mean percent composition of terminal phase males at specific size classes within and outside marine reserves. Mean percent composition of terminal phase males was calculated for each of the 5 cm size classes for fished sites (n = 28) and reserves sites (n = 30) and plotted as a scatterplot. Logistic regression was performed on this data to estimate length at transition or  $L_{m50}$  – the size at which 50% of individuals are terminal phase males. The form of the logistic model was:  $y = \frac{a}{1 + be^{cx}}$ , where  $y = \% \text{ TP males in a given size class}$ ;  $a = 100\%$ ;  $x = \text{midpoint of size class}$ ;  $b$  and  $c = \text{model parameters}$ .  $L_{m50}$  and  $L_{s50}$  are given by  $-b/c$ . Logistic modelling was performed using R v3.110 (R Core Development Team 2015).

### *Estimation of modal size classes*

Modal size classes of initial phase (IP) females was determined by plotting histograms of these individuals within sites open to fishing and reserves with increasing duration (years) of protection from fishing. Duration of protection from fishing was binned into three classes: newly established reserves (1-3 years, n = 11), established reserves (4-6 years, n = 11) and well established reserves (> 6 years, n = 8). Histograms of the mean frequencies of both IP and TP individuals were then plotted using 5 cm size classes for all four categories of protection and for all four species. Modal size classes for IP females were then noted.

**Table 8** List of the 30 marine reserves, the corresponding map ID numbers (**Fig. 11**) and years of protection (YOP) from fishing at the time of the survey.

<b>Province</b>	<b>Municipality</b>	<b>Site</b>	<b>Map #</b>	<b>YOP</b>
Southern Leyte	Pintuyan	Son-ok MR	4	6
	San Francisco	Napantao MR	6	8
	Lilo-an	Tabogon MR	7	4
	Padre Burgos	St. Sophia MR	9	5
Bohol	Guindulman	Basdio MR	16	8
	Jagna	Larapan MR	18	1
		Cantigay MR	20	5
		Garcia-Hernandez	Cayam MR	21
	Lila	Olbogan MR	22	2
		Lila MR	23	1
	Baclayon	Tagihon MR	28	4
	Panglao	Bolod MR	29	5
		Tawala MR	30	6
		Danao MR	31	3
		Doljo MR	33	3
		Dauis	Bingag MR	34
	Siquijor	Siquijor	Caticogan MR	35
Larena		Nonoc MR	37	2
Enrique Villanueva		Tulapos MR	39	11
Maria		Candaping MR	42	6
Lazi		Talayong MR	44	3
San Juan		Tubod MR	45	8
Negros Oriental	Amlan	Takot Diot MR	47	5
		Tandayag MR	49	6
	Dauin	Poblacion MR	50	8
		Masaplod Norte MR	51	12
		Masaplod Sur MR	52	7
	Zamboanguita	Maluay MR	54	3
	Siaton	Andulay MR	56	1
		Bon-Bon MR	58	2

**Table 9** List of the 28 control sites open to fishing and the corresponding map ID number (**Fig. 11**).

<b>Province</b>	<b>Municipality</b>	<b>Site</b>	<b>Map #</b>		
Southern Leyte	San Juan	Lepantao	1		
		Liloan	Molopolo	2	
			Estella	3	
	Padre Burgos	San Francisco	Nueva Estrella	5	
		Padre Burgos	Bunga	8	
			Adrian's Cove	10	
			Coral Garden	11	
Bohol	Anda	La Manok	12		
		Dap-Dap	13		
		Bacong	14		
		Blue Star	15		
	Guindulman	Guidulman Pt.	17		
		Jagna	Delila	19	
		Lila	Poblacion	24	
		Albur	Albur	25	
		Loay	Tayong Oriental	26	
		Baclayon	Montano	27	
		Panglao	Gakang	32	
		Siquijor	Siquijor	Candanay	36
				Larena	State College
Enrique Villanueva	Bino-ongan		40		
	Maria		Camanita	41	
	Lazi		Gabayan	43	
	San Juan		Coral Cay	46	
Negros Oriental	Amlan	Takot Dako	48		
	Dauin	Maayong Tubig	53		
	Zamboanguita	Lutoban	55		
	Siaton	Antulang Pt.	57		

**Table 10** Known maximum length ( $L_{\max}$ ) and mean length at maturity ( $L_m$ ) of the four species of parrotfishes in this study.

Species	$L_{\max}$	$L_m$	Reference
<i>Chlorurus bleekeri</i>	490 mm	unknown	Lieske & Myers, 1994
<i>C. spilurus</i>	450 mm	150 mm	Randall et al., 1990
<i>Scarus dimidiatus</i>	400 mm	unknown	Kiuter & Tonzuka, 2001
<i>S. rivulatus</i>	400 mm	unknown	Schroeder, 1980

#### *Estimation of total fecundity per unit area*

To estimate the reproductive potential for each of the species at each site, field data on female fish (IP) densities and sizes inside and outside reserves were converted to fecundity per unit area. Fecundity (number of eggs per individual) relationships for fish length (TL) were determined for only *Chlorurus bleekeri*  $F = 129e^{0.025L}$  and *Scarus dimidiatus*  $F = 4564e^{0.012L}$  where F = batch fecundity (number of eggs per individual) and L = total length (TL) (A. Bucol unpublished data). Because of time constraints fecundity parameters were not calculated for *C. spilurus* or *S. rivulatus*. However, the parameters calculated for their congeners were used as proxies due to their similar size ranges (Table 10). Of the four parrotfish species the only size at female maturity known is 15 cm for *C. spilurus* (Randal 1990), thus this was used as a proxy for the other three species. Therefore, total egg production per site was calculated by using the mid-point of each size class starting with the 15-20 cm-size class for all IP individuals and multiplying this by the number of individuals in each size class. Mean egg production per unit area (eggs / hectare) was calculated for the four protection categories from the previous analysis (years of protection = 0, 1-3, 4-6 and > 6).

### **3.4 RESULTS**

#### *Size at Sex Change*

For *C. bleekeri* the size at sex change (SSC) in sites open to fishing occurred at 269 mm and at 303 mm for reserves sites, indicating a 33 mm increase in *C. bleekeri* TL when protected from

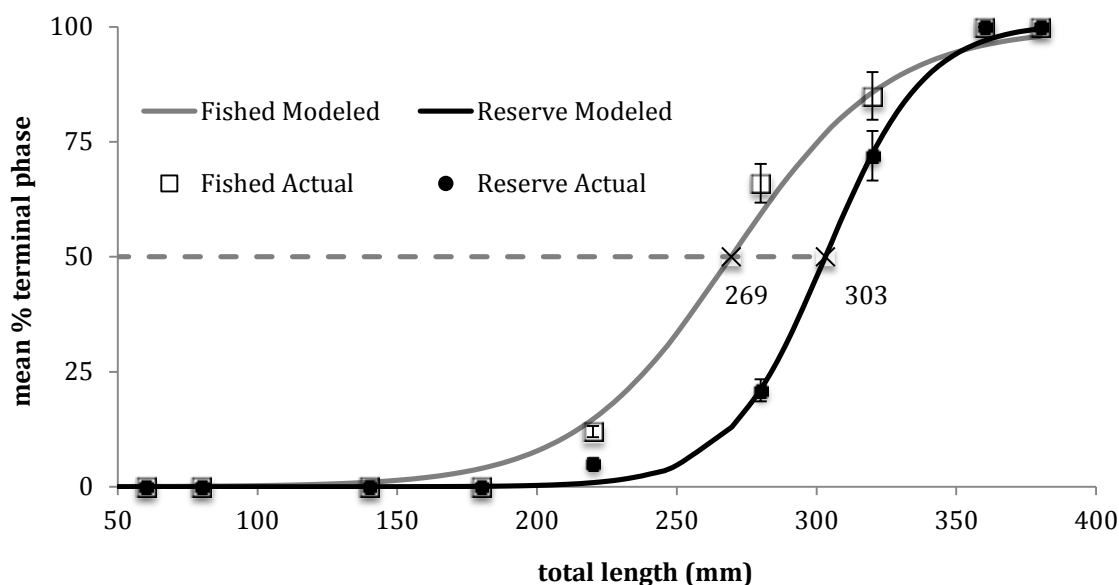
fishing within marine reserves (Figure 2, Table 4). For *C. spilurus* the SSC in sites open to fishing occurred at 234 mm but was 296 mm for fish in reserves sites, resulting in a 62 mm increase with protection (Figure 3, Table 4). For *S. dimidiatus* the SSC in sites open to fishing was 220 mm and 272 mm for reserves sites, resulting in a 52 mm increase with protection (Figure 3, Table 4). For *S. rivulatus* the SSC in sites open to fishing was 244 mm and 284 mm for reserves sites, resulting in a 40 mm increase with protection (Figure 5, Table 4).

**Table 11** Logistic model parameters used to determine size at sex change ( $L_{S50}$ ) of four species of parrotfishes inside reefs protected from fishing (Reserve) and those opened to fishing (Fished). The difference in length between reserves and fished reefs is presented as  $\Delta L_{S50}$ .

Species	Protectio n	a	b	c	$L_{S50}$ (mm)	$\Delta L_{S50}$ (mm)
<i>Chlorurus bleekeri</i>	Reserve	100	-17.151	0.056	303.2	33.7
	Fished	100	-9.595	0.036	269.5	
<i>C. spilurus</i>	Reserve	100	-14.494	0.049	296.5	62.2
	Fished	100	-26.538	0.113	234.3	
<i>Scarus dimidiatus</i>	Reserve	100	-13.505	0.050	272.5	52.5
	Fished	100	-8.280	0.038	220.0	
<i>S. rivulatus</i>	Reserve	100	-16.423	0.058	284.0	40.0
	Fished	100	-16.026	0.066	244.0	

#### *Modal Size Class Histograms*

For all four species of parrotfishes, both abundance and modal initial phase (IP) size classes increased with increasing years of protection. Modal IP size class increased at different rates for each of the species. For *C. bleekeri* the modal IP size class increased from the 11-15 cm size class to the 16-20 cm size class for new reserves (1-3 yrs) and increased further to the 21-25 cm size class for the established reserve (4-6 yrs) and remained the mode at well-established marine reserves (> 6 yrs) (Figure 6). For *C. spilurus* a modal IP size class increase was not detected until the establish reserves (4-6 yrs) from 21-25 cm to 26-30 cm, while a further modal size class increase was detected for the well-established reserves (31-35 cm)(Figure 7). For *S. dimidiatus* a

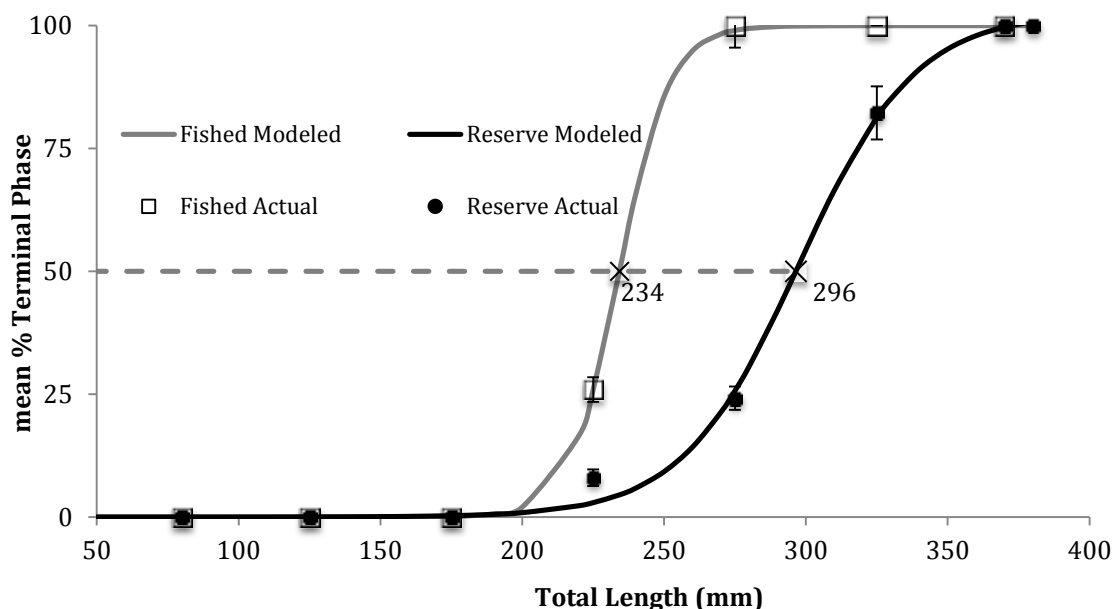


**Fig. 12** Mean per cent terminal phase per size class for *Chlorurus bleekeri* within reserves (solid circles) and sites open to fishing (open squares) and logistic regression curves (predicted fits) of percentage terminal phase for reserve and fished sites. Horizontal dashed line indicates length at which 50% of individuals are terminal phase  $L_{M50}$ .

modal size class increase was detected from 16-20 cm to 21-25 cm within newly established reserves and this mode remained for the established reserves. However, an increase to the next size class (26-30 cm) was detected in the well-established reserves (Figure 8). Modal IP size class in unprotected reefs was the lowest for *S. rivulatus* at 11-15 cm, but increased to 16-20 cm for newly established reserves. A second increase in modal IP size class was further detected in established reserves at 21-30 cm, but did not increase in the well-established reserves (Figure 9).

#### *Fecundity per unit area*

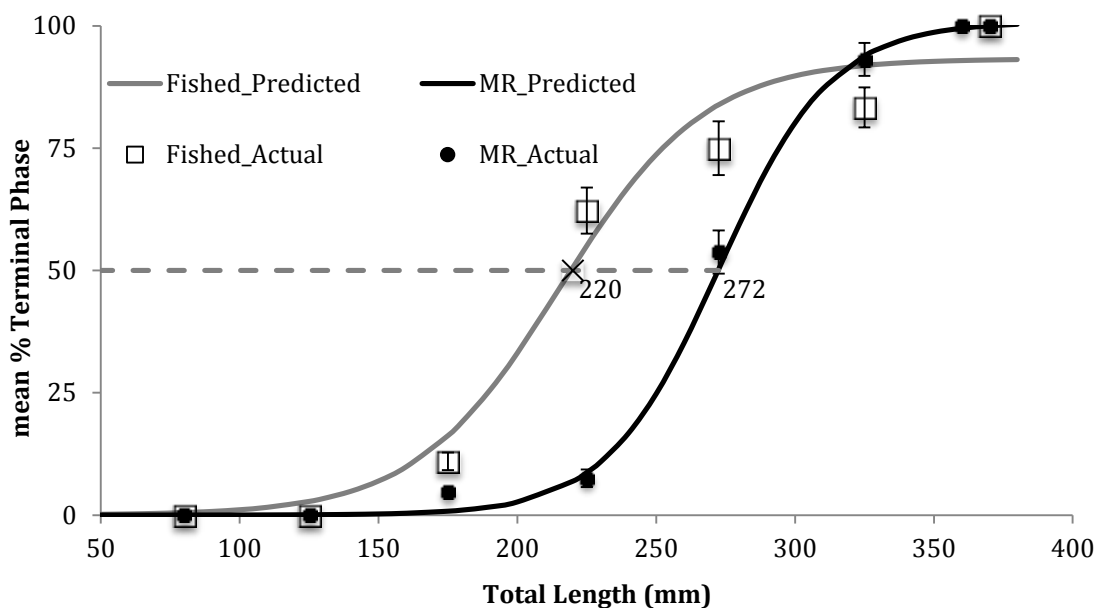
Egg production per hectare increased drastically for all four species with increasing years of protection, however two distinct patterns were detected. Both *C. bleekeri* and *S. dimidiatus* were



**Fig. 13** Mean per cent terminal phase per size class for *Chlorurus spilurus* within reserves (solid circles) and sites open to fishing (open squares) and logistic regression curves (predicted fits) of percentage terminal phase for reserve and fished sites. Horizontal dashed line indicates length at which 50% of individuals are terminal phase  $L_{M50}$ .

characterized by an initial rapid increases in newly established reserves (1-3 yrs) and established reserves (4-6 yrs) but appeared to have levelled off within well-established reserves (> 6 yrs) (Figures 10 and 11). However, *C. spilurus* and *S. rivulatus* showed more of an exponential increase, with a more gradual initial increase for newly established reserves, but a much more drastic increase for both establish and well-established reserves (Figures 10 and 11). Additionally, both *C. bleekeri* and *C. spilurus* had greater fecundities than the other two *Scarus spp.*, which is likely to their overall larger sizes.

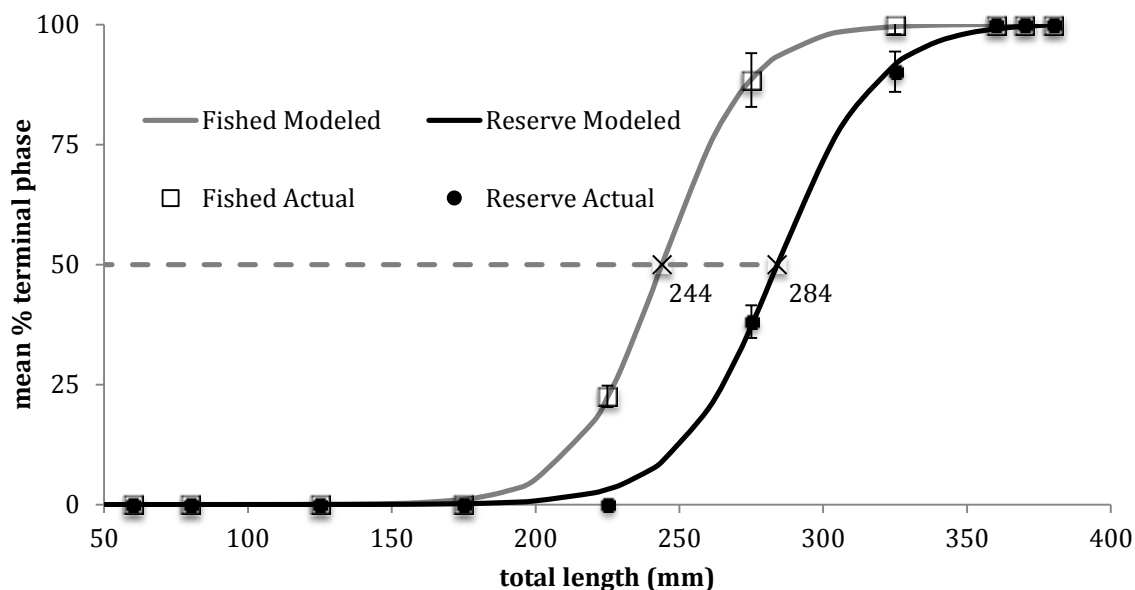




**Fig. 14** Mean per cent terminal phase per size class for *Scarus dimidiatus* within reserves (solid circles) and sites open to fishing (open squares) and logistic regression curves (predicted fits) of percentage terminal phase for reserve and fished sites. Horizontal dashed line indicates length at which 50% of individuals are terminal phase  $L_{M50}$ .

### 3.5 DISCUSSION

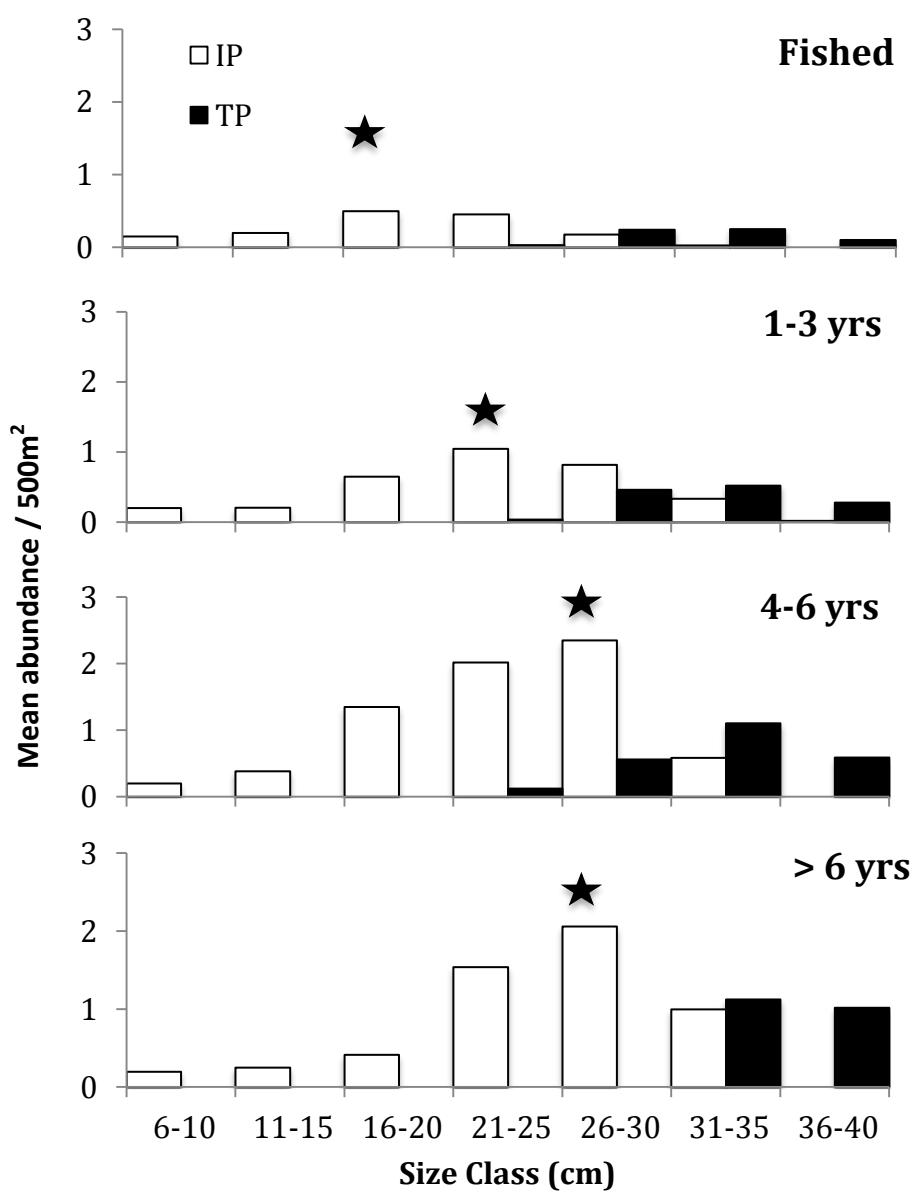
This study demonstrated that size at sex change (SSC) of four species of parrotfishes occurs at larger sizes within reserves protected from fishing in the central Philippines. These lengths at sex change increased between 33 mm and 62 mm for fished and unfished sites accounting for between a 13% to 26% increase. As with other studies, the modal size distribution analysis revealed increasing density with increasing years of protection (Russ 2002; Gell and Roberts 2003; Halpern et al. 2009; Babcock et al. 2010). However what makes this more important is that



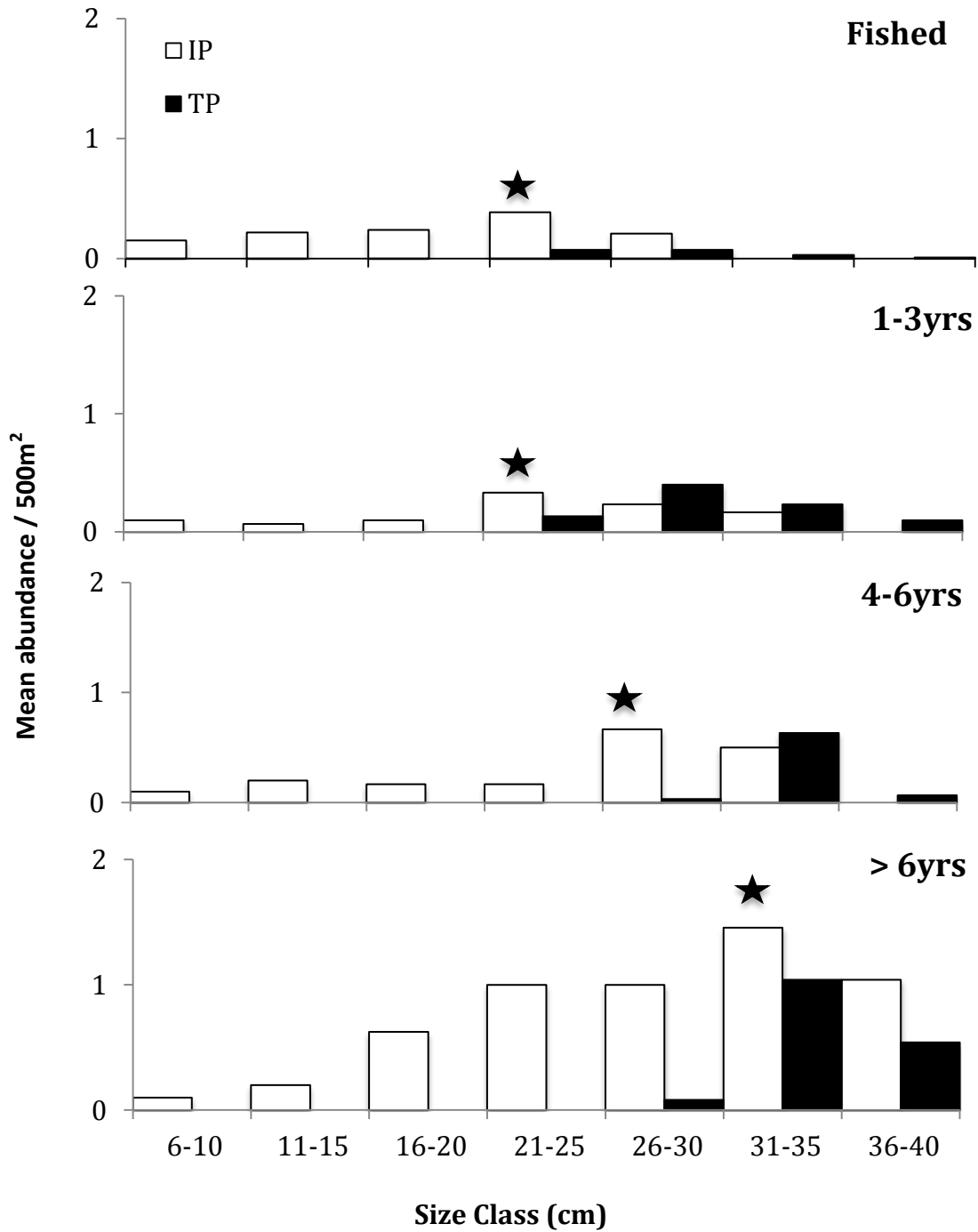
**Fig. 15** Mean per cent terminal phase per size class for *Scarus rivulatus* within reserves (solid circles) and sites open to fishing (open squares) and logistic regression curves (predicted fits) of percentage terminal phase for reserve and fished sites. Horizontal dashed line indicates length at which 50% of individuals are terminal phase  $L_{M50}$ .

initial phase (IP) female modal size classes also increase with increasing years of protection. Thus, not only are female fish becoming more abundant, they are also growing to larger sizes within reserves with increasing duration of protection.

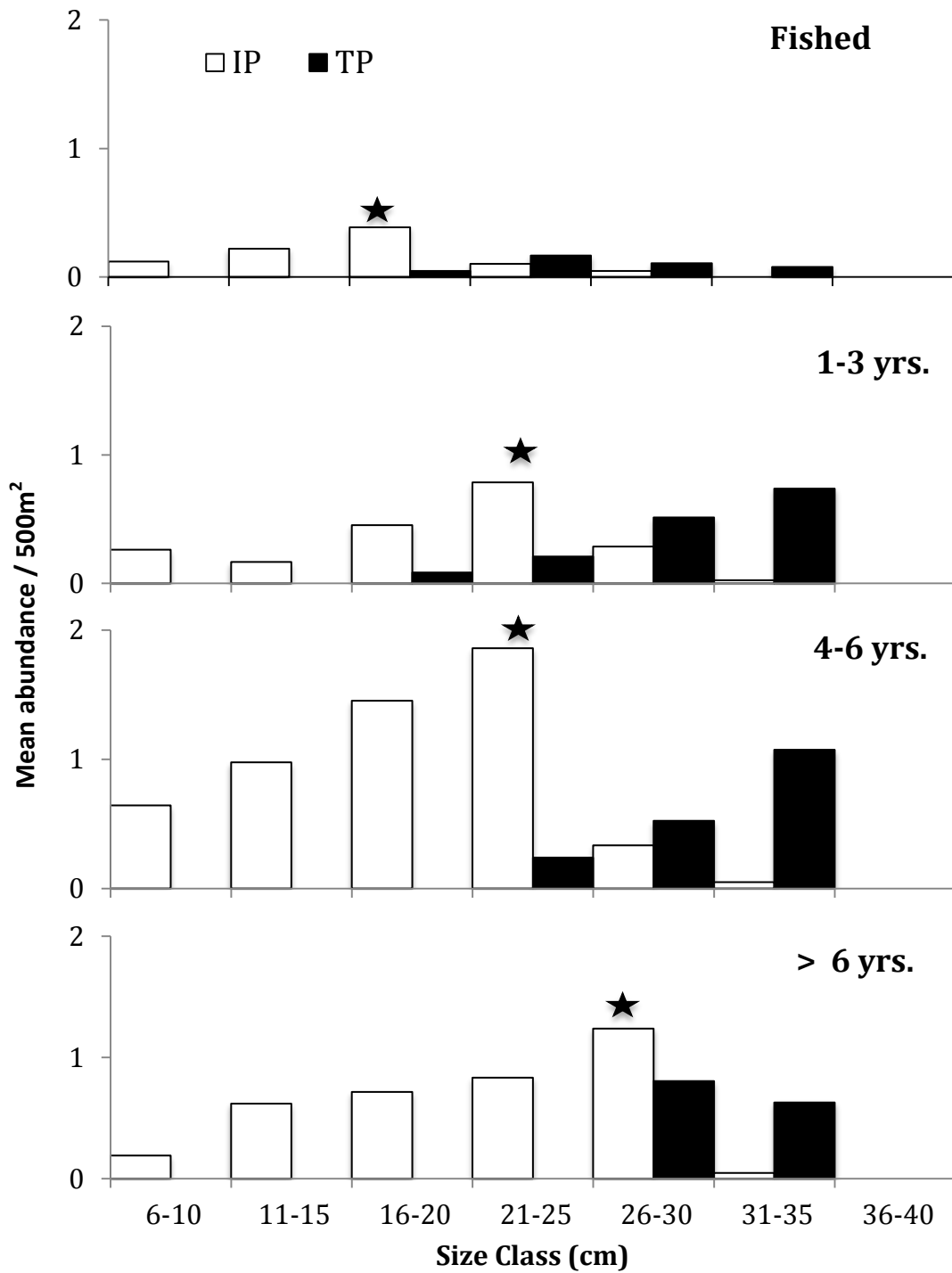
The increasing size of female parrotfishes with protection has drastic consequences on total egg production (fecundity). It is well documented that larger fish produce both larger clutch sizes and larger, more viable eggs (Evans et al. 2008; Hixon et al. 2014). Fecundity of reef fishes generally follow a rapid increase with body size (Evans et al. 2008; Sadovy 1996; Gust 2004). Therefore, the combination of both an increase in abundance and size of female parrotfishes is thus likely to



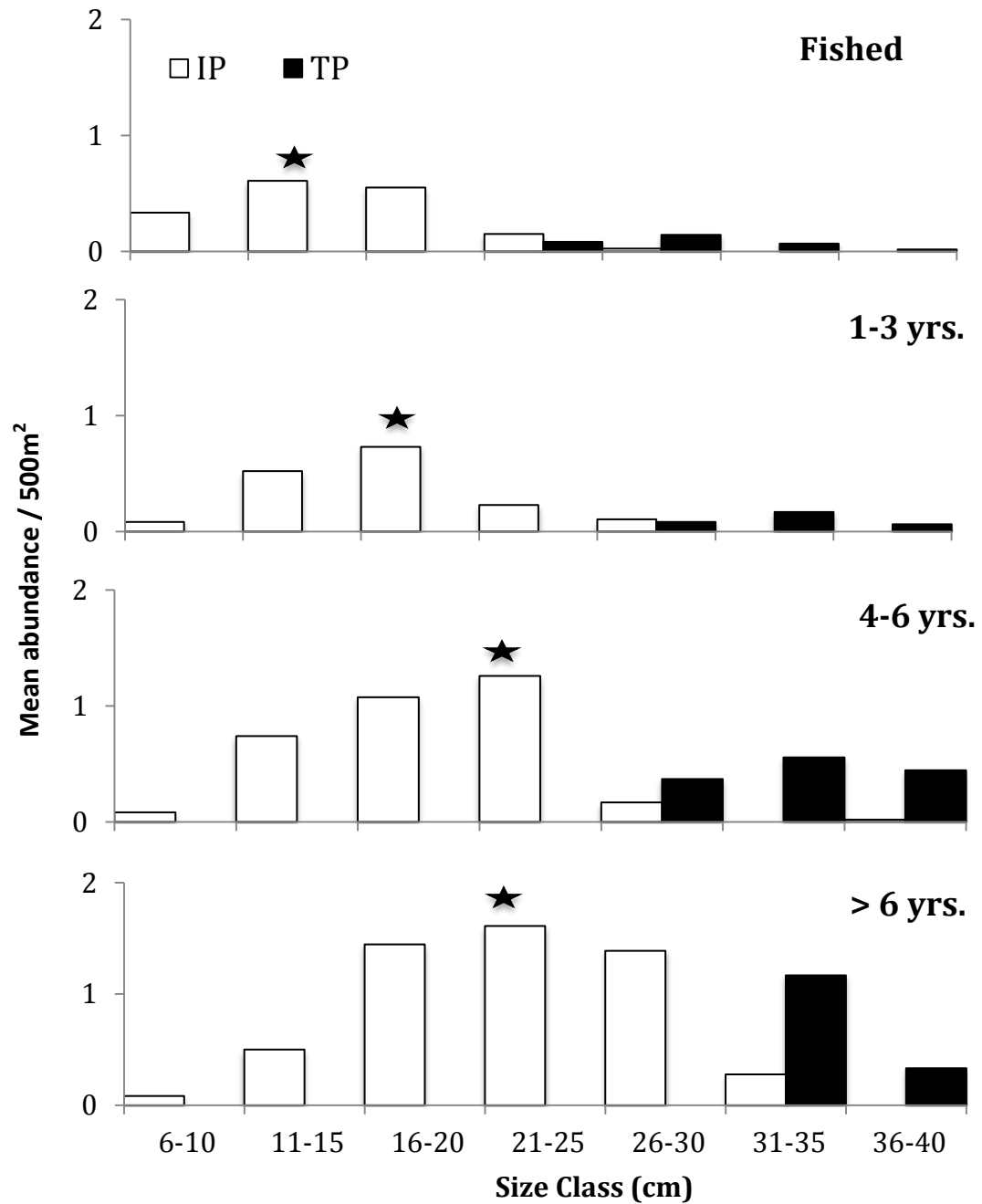
**Fig. 16** Body size (total length, TL) frequency distributions of initial phase (IP) and terminal phase (TP) individuals of *Chlorurus bleekeri* for sites open to fishing and reserve categories with increasing years of protection. The star indicates the modal size class for IP.



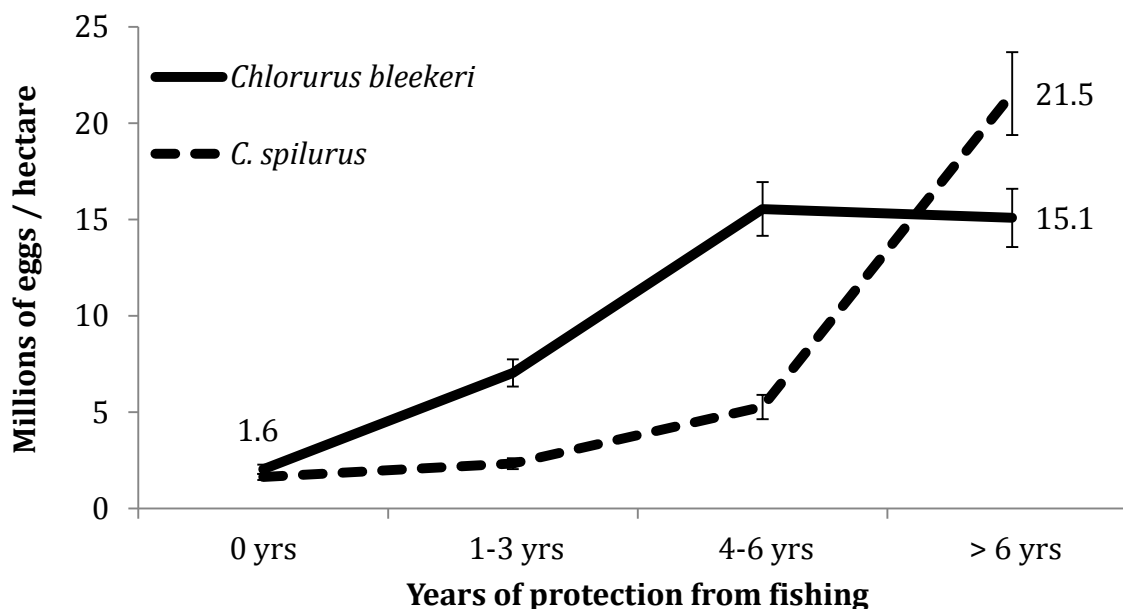
**Fig. 17** Body size (total length, TL) frequency distributions of initial phase (IP) and terminal phase (TP) individuals of *Chlorurus spilurus* for sites open to fishing and reserve categories with increasing years of protection. The star indicates the modal size class for IP.



**Fig. 18** Body size (total length, TL) frequency distributions of initial phase (IP) and terminal phase (TP) individuals of *Scarus dimidiatus* for sites open to fishing and reserve categories with increasing years of protection. The star indicates the modal size class for IP.

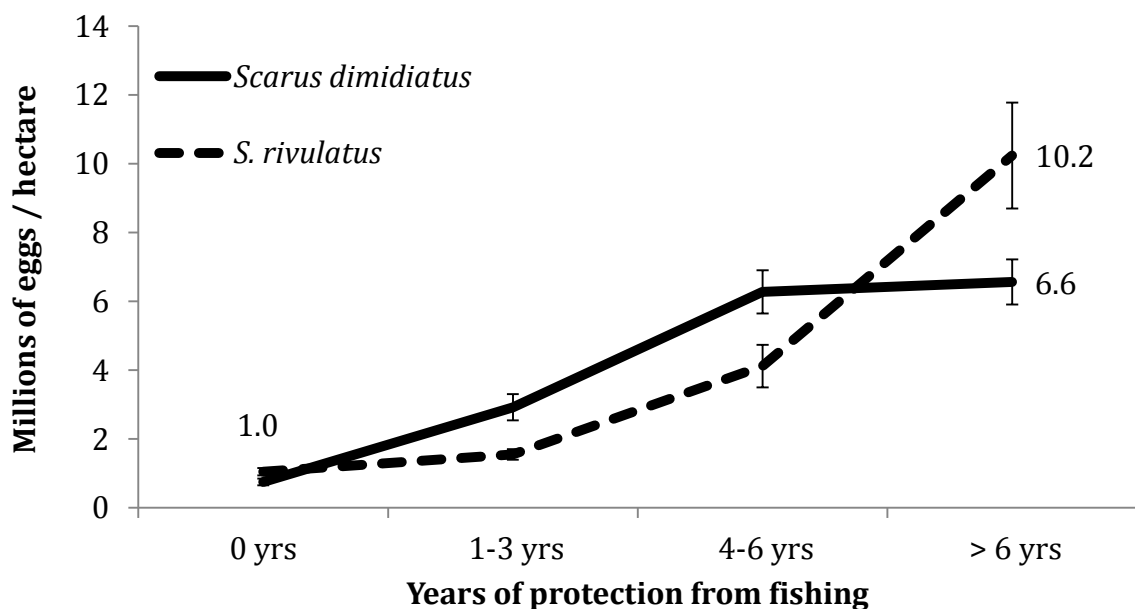


**Fig. 19** Body size (total length, TL) frequency distributions of initial phase (IP) and terminal phase (TP) individuals of *Scarus rivulatus* for sites open to fishing and reserve categories with increasing years of protection. The star indicates the modal size class for IP.



**Fig. 20** Mean egg production for sites with increasing years of protection from fishing calculated for *Chlorurus bleekeri* and *Chlorurus spilurus*. (error bars = 1 S.E.)

(Serranidae) will require a longer time (2-4 decades) to recover from heavy fishing while parrotfishes generally recover within 5 years of protection (Stockwell et al. 2009; Abesamis et al. 2014). Some parrotfish species, however, may show different recovery rates. For example, the parrotfish *Scarus tricolor* has a clear positive response to protection over 3-14 years and 8-12 years in Sumilon and Apo reserves, respectively (Babcock et al. 2010). Apparently, since FUA is dependent on size of the fish, protogynous fishes with different life history would respond to protection differently. This is the first study to date to have quantified FUA of targeted protogynous reef fishes and demonstrate an exponential increase in FUA with increasing duration of protection from fishing.



**Fig. 21** Mean egg production for sites with increasing years of protection from fishing calculated for *Scarus dimidiatus* and *Scarus rivulatus*. (error bars = 1 S.E.)

Evans et al. 2008 only reported a 1 to 4.2 fold FUA for *Lutjanus carponotatus* between no-take reserves and fished sites, which is far less than what was recorded in this study. In this study, FUA differentials of protogynous reef fishes were found to be much higher inside the reserves probably due to heavy fishing pressure in the fished sites. In developing countries, including the Philippines, where fishing pressure is extremely high, it is possible that FUA differences may be even higher inside marine reserves where sex change occurs and therefore females are larger and more fecund, compared to the fished reefs. Fishing of larger individuals may affect protogynous species in two ways. First via the removal of the larger males, which may affect the sex ratio. The second is the removal of larger females, which would affect reproductive output. In Micronesia, Taylor (2014) showed that selective removal of terminal phase (TP) individuals of



the parrotfish *Chlorurus spilurus* initiates sex change earlier in fished reefs compared to reefs with low fishing pressure. This finding corroborates with an earlier study by Barba (2010) who documented that in the fished areas of Guam, *C. spilurus* sexually matures at a younger age and undergoes sex change at smaller size and has reduced longevity and growth rate. DeMartini et al. (2016) confirmed that in most sites in the tropical Pacific, parrotfish species mature and change sex at smaller sizes compared to the populations in Hawaii, probably due to differences in water temperature, habitat productivity, conspecific density and fishing pressure. In Kenya, Locham et al. (2015) estimated fecundity and size at sex change of the parrotfish *Leptoscarus vaigiensis*, which was higher inside protected areas.

The results from this study provide further support for the protection of big old fat fecund female fish (BOFFF) and how they contribute disproportionately more to stock productivity than smaller females (Hixon et al. 2014). Several management approaches for the restoration of BOFFF have been recommend including: 1) greatly reducing fish mortality 2) slot size limits and 3) marine reserves, wherein old-growth age structure can be restored, spawning biomass increased and seed nearby fished areas (Berkeley et al. 2004). The latter option (establishment of marine reserves) has been the most effective method of restoring coral reef fish stocks in developing nations due to the lack of resources to enforce reduced fishing pressure and slot limits through conventional management plans (Russ 2002; Krueck et al. 2017) over the past few decades. This study demonstrated that within marine reserves the increase in BOFFF results in a greatly increased egg/larvae production. However, one of the proposed results of this increased propagule production within marine reserves is their ability to seed nearby fished reefs. This ability to supply neighboring reefs through larval connectivity is a fundamental proponent of marine reserves (Abesamis et al. 2017) and has been demonstrated to occur both between marine reserves and to nearby fished areas in coral reefs (Almany et al. 2017).

### **3.6 CONCLUSIONS**

This study has shown that 1) reserves can reverse the effects of overfishing and increase the size at sex change for parrotfishes and 2) FUA differentials (reserve vs. fished reef) increases exponentially with increasing years of protection. This is critical for parrotfishes, since

hermaphroditic sex-changing fish species are especially vulnerable to fishing because their fertilization is dependent on complex social structures such as harems (Provost and Jensen 2015).

## CHAPTER 4

### HERBIVOROUS REEF FISHES REVEAL HIGHLY CONNECTED REEFS IN THE CENTRAL PHILIPPINES

#### 4.1 ABSTRACT

Coral reefs within the Coral Triangle (CT) are home to the greatest marine diversity on the globe. Many coral reefs within the CT and around the world are under threat from over-exploitation. Marine protected areas (MPAs) have proven effective in restoring fish stocks within their boundaries. However, the role that MPAs play in promoting connectivity at greater distances through larval dispersal is still being investigated. Restriction-site-associated DNA (RADseq) was used to discover between 4K and 12K single nucleotide polymorphism (SNPs) from three species of herbivorous reef fishes (*Chlorurus bleekeri*, *Scarus niger* and *Siganus guttatus*) from four sites within the central Philippines. A sibling analysis revealed four pairs of full siblings ( $r = 0.949$  to  $0.501$ ), one pair of half siblings ( $r = 0.291$ ) and five pairs of first cousins ( $r = 0.184$  to  $0.129$ ) for *C. bleekeri*; 1 pair of full siblings ( $r = 0.773$ ) and one pair of half siblings ( $r = 0.191$ ) for *S. niger*; and no related pairs for *S. guttatus*. Of the 7 pairs of related individuals between sites for both parrotfish species, 6 of these pairs included sites separated by over 400km. Migration (through larval dispersal) models tested found north to south migration patterns to be the most likely direction of migration for both parrotfishes and *S. guttatus*. Estimates of  $N_e$  from linkage disequilibrium are relatively large for both *S. niger* (1,200- 2000) and *S. guttatus* (3,000 to 11,000), but alarmingly low for *C. bleekeri* (70 – 390). The strong north to south current patterns within the central Philippines are likely vectors by which larvae are transported between these sites, suggesting that reefs in the Sibuyan Sea are sources for reefs 100's of km to the south. Given the reliance of a vast majority of coral reef fishes on larval dispersal, this study reveals that MPAs established within the central Philippines can supply varying levels of larvae to overfished reefs.

#### 4.2 INTRODUCTION

The Coral Triangle (CT) encompasses Indonesia, Malaysia, Papua New Guinea, the Philippines, Solomon Islands and Timor-Leste, represents 30% of the world's coral reefs and is the global center of marine diversity (Veron 2000). These reefs also supply important marine resources for

over 370 million people, a third of which rely directly on fish and other marine resources for their livelihood (Hoegh-Guldberg et al. 2009). Large coastal populations expose the reefs to anthropogenic threats such as overfishing and habitat destruction, which can reduce species abundance and biodiversity and threaten the long-term sustainability of these important marine resources (Burke et al. 2012). Marine protected areas (MPAs), defined here as areas that are wholly or partially closed to extractive activities by humans, are strongly advocated as a tool to conserve biodiversity, restore depleted populations and manage fisheries on coral reefs (Alcala 1988; Sale et al. 2005; Mora et al. 2006). Although MPAs cannot completely mitigate human impacts, there is evidence for increased density, biomass and diversity of exploited reef species within the 'no-take' boundaries of MPAs (Russ 2002; Gell and Roberts 2003; Halpern et al. 2010). Additionally, greater spawning output per unit area developing over time within MPAs may result in larval export to surrounding areas (Jones et al. 2009; Pelc et al. 2010; Harrison et al. 2012). There are more than 900 MPAs that have been established in the CT (White et al. 2014), reflecting their perceived importance as a coral reef management strategy in the region.

Larval dispersal in coral reef fishes ranges from less than 1 km to several 100's of km (Cowen et al. 2006; Patterson and Swearer 2007; Jones et al. 2009; Hogan et al. 2012; Puebla et al. 2012). This large range in dispersive capability, even within species, has led to varying definitions of connectivity between populations. Demographic connectivity, which can alter the vital rates (i.e. population growth rate) of connected populations, is more likely to occur at shorter distances while connectivity via seeding dispersal, which can maintain the genetic cohesiveness of populations, is more likely to operate at longer distances (Kritzer and Sale 2004; Steneck et al. 2009). Genetic or reproductive connectivity on the other hand not only refers to the settlement of larvae to an area but their incorporation into the population through survival, growth and reproduction (Gaggiotti 2017). Initial studies using genetic parentage analysis and genetic isolation by distance approaches indicate that demographically relevant connectivity in reef fishes typically occurs within a few 10's of km to less than 10 km (Harrison et al. 2012; Puebla et al. 2012; Almany et al. 2013). Although recent studies have increased estimates of mean dispersal distances for a butterflyfish species to over 30 km (Abesamis et al 2017) and between 100 and 200 km for groupers (Williamson et al. 2017).

Despite relatively short average demographic dispersal distances, most population genetic studies within the Philippines do not show evidence of genetic structuring at fine scales. For example, allozyme analyses in a rabbitfish (*Siganus fuscescens*) demonstrated significant differentiation at large scales (100's of km) between and within regions defined by the bifurcating North Equatorial Current on the eastern side of the Philippines (Magsino and Juinio-Meñez 2008). Mitochondrial DNA (mtDNA, control region) analyses of a fusilier (*Caesio cuning*) including 13 sites spanning more than 1000 km revealed no structure within the Philippines (Ackiss et al. 2013). However, mtDNA markers have found patterns of structuring aligning with previously isolated basins across the region are also seen in several invertebrates (Barber *et al.* 2002, Kochzius *et al.* 2009) and vertebrates (Lourie *et al.* 2005, Raynal *et al.* 2013).

These previous studies have relied on only a handful of nuclear or mtDNA loci, possibly limiting their power to detect fine scale population structure. More recently, population genomics has been used to examine genetic variation at thousands of loci (Allendorf et al. 2010). This very high number of genetic markers can reveal structure that may have been undetectable using previous methods. For instance, genetic analyses using ~10,000 single-nucleotide polymorphisms (SNPs) were able to detect fine-scale population structure in Chinook salmon (*Oncorhynchus tshawytscha*) that could not be resolved with allozymes, microsatellites or small SNP panels (Larson et al. 2014). In addition to increasing power to detect population structure, the use of genomic data can facilitate more accurate parentage/sibling analyses and more accurate estimates of effective population size ( $N_e$ ) compared to traditional methods (Allendorf et al. 2010, Willette et al. 2014, Larson et al. 2014). Recently the discovery and utilization of thousands of SNPs has found fine population structuring where handfuls of markers have failed in a variety of marine organism such as blue crab (Plough 2017), Caribbean reef corals (Durante and Baums 2017) and grey reef sharks (Momigliano et al. 2017).

Large herbivorous reef fishes belonging to families such as Acanthuridae (surgeonfishes), Ehippidae (batfishes), Scaridae (parrotfishes) and Siganidae (rabbitfishes) are some of the most abundant (in terms of biomass) fishes on the reef and thus represent a major component of artisanal fisheries (Rhodes et al. 2007; Houk et al. 2011). Of these, parrotfishes are among the

most conspicuous and diverse groups of herbivorous fishes on coral reefs and have the ability to alter the reef substratum by scraping or excavating calcareous surfaces during feeding (Bellwood and Choat 1990). A negative correlation between parrotfish abundance and macroalgal cover indicates an important functional role performed by this taxonomic group through the top-down control of algal abundance, which may help to maintain a coral-dominated state (Bellwood et al. 2004; Stockwell et al. 2009; Williams and Polunin 2014). Recent studies, however, indicate a reduction of up to 50% of the herbivorous fish biomass in Indo-Pacific and Caribbean reefs that are subject to fishing (Edwards et al. 2014).

Previous studies examining the genetic structure of herbivorous reef fishes using traditional markers have failed to detect significant divisions at less than 1000km. Dudgeon et al. (2000) were not able to detect genetic divisions across the Great Barrier Reef in Australia for both *Chlorurus sordidus* and *Scarus frenatus*, while Bay et al. (2004) also examined the genetic structure of *C. sordidus*, but were only able to find divisions at oceanic scales. Visram et al. (2010) only found weak genetic structure within the Indian Ocean for *Scarus ghobban* by also using mitochondrial markers. And lastly, a study using 15 microsatellites across the entire range of *Scarus rubroviolaceus* (Fitzpatrick et al. 2011) detected genetic divisions only at oceanic scales. Iwamoto et al. (2012) found some structure across the western Pacific in rabbitfishes (Siganidae).

The overall aim of this study is to determine if the discovery and utilization of thousands of single nucleotide polymorphisms (SNPs) using restriction-site associated DNA RAD sequencing (RADseq) can reveal important population level characteristics of herbivorous reef fishes within the Philippines. It is hypothesized that with this greater number of polymorphic loci that (1) fine scale population structure (100's km) can be detected, (2) direction and intensity of gene flow through estimates of migration can be demonstrated and (3) accurate estimates of important population parameters such as relatedness and effective population size ( $N_e$ ) can be calculated. The ultimate goal is to apply these results in determining the connectivity of reef fish populations and aid managers in the better placement of MPAs.

### 4.3 MATERIALS AND METHODS

#### *Study Species*

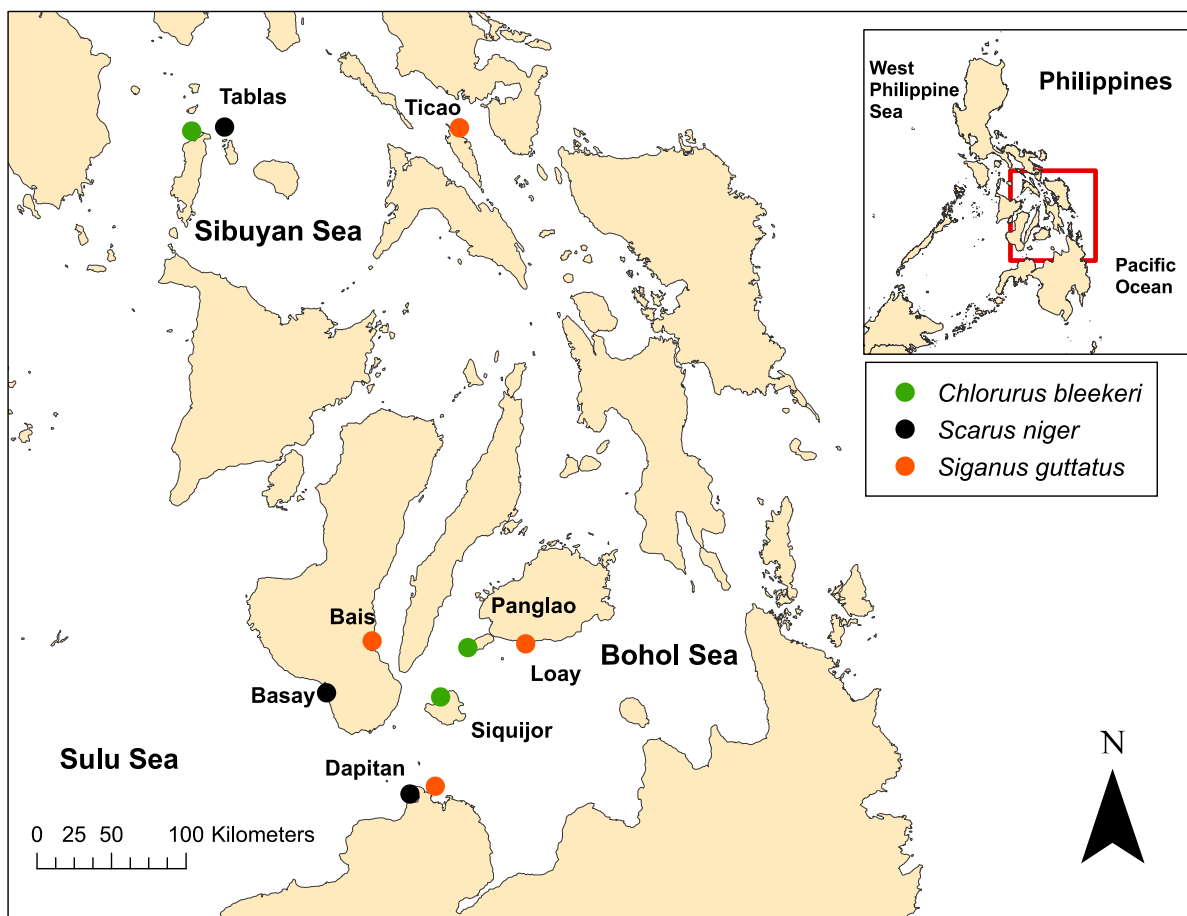
Three species of herbivorous reef fishes targeted by artisanal fisheries were chosen for this study because they span a variety of life history strategies. This included two species of parrotfishes (family: Scaridae), *Chlorurus bleekeri* and *Scarus niger* that are protogynous hermaphrodites, which is typical for the family (Chaot & Roberston 1975). They differ in that *S. niger* is sexually monomorphic and tends to breed in pairs whereas *C. bleekeri* is sexually dimorphic with the larger males maintaining a territory with a harem of females (Myers, 1991). The third species included was a rabbitfish (*Siganus guttatus*, Family: Siganidae), which are gonochoristic and breed in pairs (Bagarinao 1986).

#### *Tissue sampling and DNA extraction*

Between 32 and 40 tissue samples (fin clips) were collected from local landing sites in the central Philippines in 2012. For *C. bleekeri*, samples were collected from the islands of Tablas in the Sibuyan Sea and Pangalo and Siquijor in the western Bohol Sea; for *S. niger*: Tablas, Basay in the Sulu Sea and Dapitan in the western Bohol Sea; and for *S. guttatus*: Ticao in the Sibuyan Sea, Bais in the Tañon Strait, Loay in the western Bohol Sea and Dapitan (Figure 22, Table 12). For all four species sampled, specimen sizes (total length) ranged from small juveniles (< 15 cm) to large mature individuals (> 35 cm), thus spanning multiple generations. The shortest (over water) distance between sample sites was estimated by hand using ARCGIS 10 (ESRI, Inc.) (Tables 15 - 17). Tissues were preserved in 95% ethanol and stored at room temperature. DNA extractions were performed in a 96-well format using a Wizard® SV 96 Genomic DNA Purification System (Promega Inc., Madison, WI).

#### *RAD sequencing, SNP discovery and genotyping*

Restriction-site-associated DNA (RAD) libraries were prepared from the DNA extractions following the methods of Baird et al. (2008) and Everett et al. (2012). First, genomic DNA was digested with the *SbfI* restriction enzyme. The DNA was then randomly sheared using a Bioruptor (Diagenode, Inc., Denville, NJ) and fragments between 400-800 base pairs (bp) were gel-extracted. Finally, 6 bp barcodes and sequencing primers were ligated to the fragments. The RAD libraries for *S. niger* and *C. bleekeri* contained 48 individuals, while 96 individuals were



**Fig. 22** Locations of sample sites where tissues were collected for the three species of fishes in the study.

used for *Siganus guttatus*. The genome size of *S. guttatus* (0.67 Gb) is roughly half that of both parrotfishes (1.47 and 2.01 Gb; Ojima and Yamamoto 1990), allowing for twice as many individuals to be included in each library. Two libraries were prepared for each species with a total of 96 individuals for the two parrotfishes and 192 individuals for the rabbitfish. Libraries were sequenced with single-end chemistry (100 bp target length) on an Illumina HiSeq2000



**Table 12** Sample sites for each of the species included in this study, number of individuals collected per site ( $n_s$ ) and the number of individuals that were successfully genotyped ( $n_g$ ).

Site	Sea / Region	Species	$n_s$	$n_g$
Tablas	Sibuyan Sea	<i>Chlorurus bleekeri</i>	32	31
		<i>Scarus niger</i>	32	27
Ticao	Sibuyan Sea	<i>Siganus guttatus</i>	40	39
Bais	Tañon Strait	<i>Siganus guttatus</i>	40	40
Basay	Sulu Sea	<i>Scarus niger</i>	32	24
Panglao	Bohol Sea	<i>Chlorurus bleekeri</i>	32	29
Loay	Bohol Sea	<i>Siganus guttatus</i>	32	30
Siquijor	Bohol Sea	<i>Chlorurus bleekeri</i>	32	30
Dapitan	Bohol Sea	<i>Scarus niger</i>	32	30
		<i>Siganus guttatus</i>	32	32

(Illumina, Inc., San Diego, CA) at the University of Oregon Genomics Core Facility (one sequencing lane per library).

The STACKS software package, version 0.9999 (Catchen et al. 2011) and methods similar to Larson et al. (2014) and Gruenthal et al. (2014) were used to discover and genotype SNPs from the sequenced RAD tags. Quality filtering of raw reads and de-multiplexing based on barcode was conducted using *process\_radtags* with the default parameters. In addition the last 9 bp of each sequence was removed at this stage because Illumina sequencing is more error prone towards the terminal position of reads (Minoche et al. 2011), which could create false positive SNPs (Gruenthal et al. 2014). Stacks of similar sequences were assembled in each individual with *ustacks* and the default parameters with two exceptions: the deleveraging and removal algorithm was used to break up highly repetitive sequences and a genotyping model with a bounded error rate of 0 to 0.1 was selected. A catalogue of loci was then created with *cstacks* from the five individuals from each sample site with the greatest amount of sequence data. Finally, *sstacks* and *populations* were used to combine the genotypes from each individual into a single GENEPOP formatted file.

*SNP validation*

Putative SNPs discovered with *Stacks* were filtered to remove loci that could not be consistently genotyped, contained possible sequencing errors, or contained uninformative polymorphisms. First, any putative SNP that failed to genotype in > 90% of individuals was removed. Then those with a minor allele frequency < 0.1 in all sample sites were removed. These polymorphisms are likely to be uninformative, are difficult to distinguish from sequencing errors, can distort signals of selection and drift in natural populations and may bias tests for selection (Roesti et al. 2012). Additionally putative SNPs that displayed significant deviations from Hardy-Weinberg equilibrium (HWE) in at least two of the three sample sites for the two parrotfishes and three of the four sampling sites for *Siganus guttatus* ( $P < 0.05$ ) were removed. Tests for deviations from HWE for each locus were conducted in GENEPOP version 4 (Rousset 2008). Finally, only the putative SNP with the highest minor allele frequency from each RAD tag was retained to reduce linkage in this dataset. If minor allele frequencies were equal for multiple SNPs in a RAD tag, a randomly selected single SNP was retained. As a final filtration step, individuals missing genotypes at > 10% of filtered SNPs were removed.

#### *Population Structure ( $F_{ST}$ )*

Pairwise  $F_{ST}$  values (Weir and Cockerham 1984) were used to determine population structure. For each sample site pair and exact tests for significant genetic differentiation were calculated in GENEPOP with the default parameters ( $P < 0.05$ , Raymond and Rousset 1995; Goudet et al. 1996).

#### *Relatedness ( $r$ )*

Relationships between individuals were estimated with the R package *related* (Pew et al. 2015). Of the five moment estimators, the Lynch and Ritland (1999) estimator was chosen since it has the lowest misclassification rates (Csilléry et al. 2006). Both maximum likelihood methods, the dyadic likelihood estimator (Milligan 2003) and the triadic likelihood estimator (Wang, 2007), were used. For all three estimators 95% confidence intervals were calculated with 500 bootstrap events for each pairwise comparison. The complete data set of filtered SNPs were utilized for all three species.

### *Effective Population Size ( $N_e$ )*

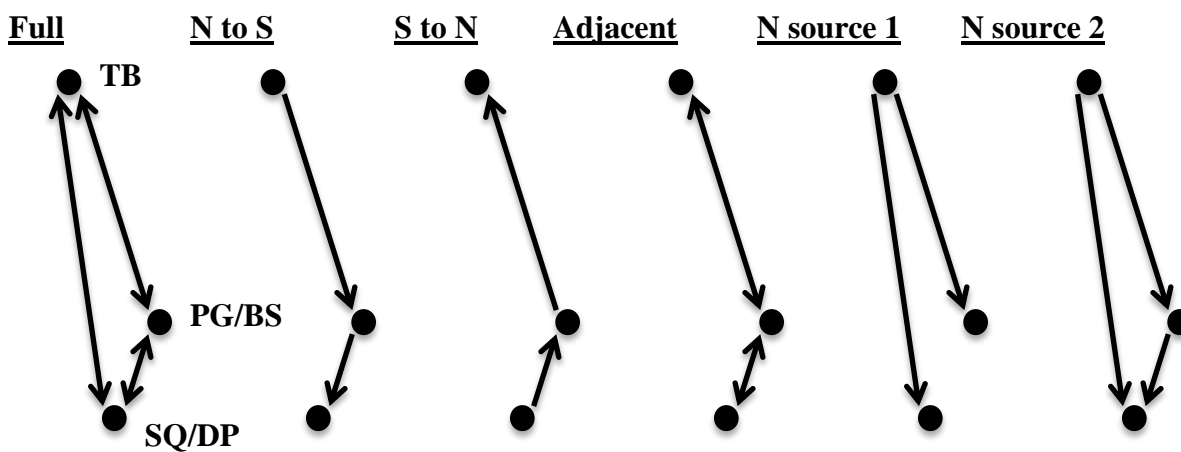
Estimates of effective population size ( $N_e$ ) were obtained with the linkage disequilibrium method (Hill 1981; Waples 2006) updated for missing data following Peel et al. (2013). Values of linkage disequilibrium ( $r^2$ ) for each locus pair were first generated with NeEstimator v2b (Do et al. 2014) with a minor allele frequency cutoff of 0.05. For each species, three data sets were used: (1) all individuals as one population, (2) all individuals from each site as a separate population and (3) one sibling from each sibling pair removed from within each population (when applicable).

### *Migration rates ( $M$ )*

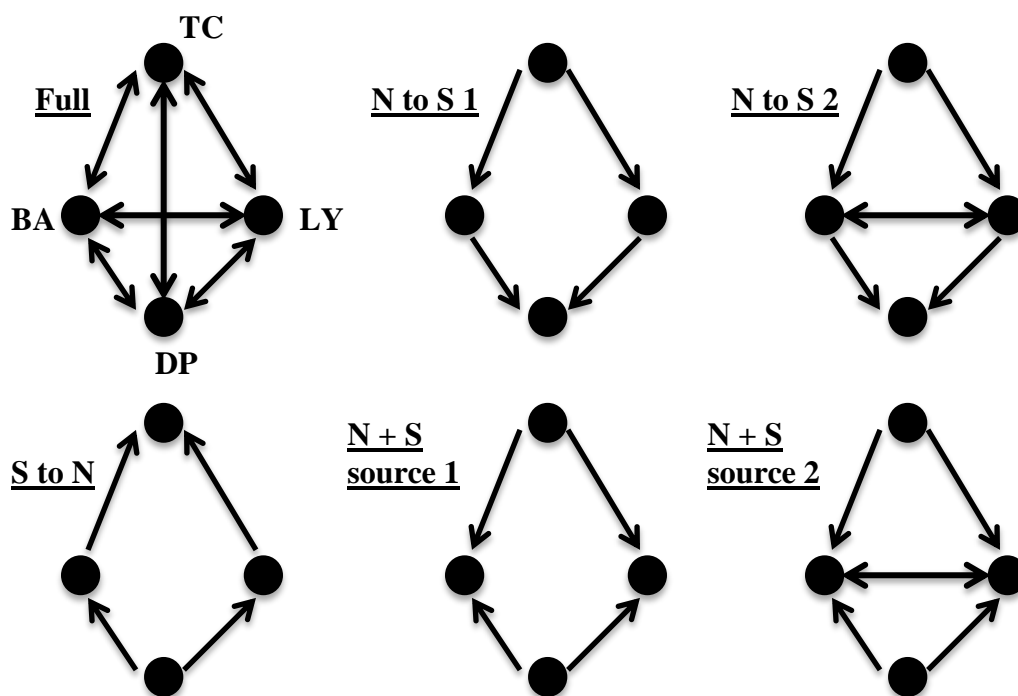
Migration rates between populations were calculated in Migrate-N Ver. 3.6.11 (Beerli 2008) to estimate patterns of historical population connectivity. To balance between adequate sampling and the computational effort needed, 100 random tags were selected for each species. The entire 90 bp RAD tag sequence was included in the analysis and only RAD tags that successfully sequenced for every individual of each species were included for a balanced matrix. For each analysis, initial runs were used to establish the priors for the mutation-scaled migration ( $M$ ) and mutation scaled populations size ( $\theta$ ) parameters. After the test runs, the settings for Migrate-N were set as follows: maximum  $\theta$  value = 2, Maximum  $M$  = 40,000 and metropolis sampling every 1000 steps for 10 million generations, with a burn-in period of 20 million steps. Four parallel chains were run with a static heating scheme and a swapping interval of 1.

Patterns of migration were calculated by comparing and ranking the marginal likelihood between different models of gene flow among the populations and calculating their specific probability based on the Bayes factor (Beerli & Palczewski 2010). The tested connectivity models were chosen based on the known sea surface current patterns in the Philippines (Han et al. 2009; Gordon et al. 2011). Given geographically similar sampling sites, identical models for both parrotfishes (*C. bleekeri* and *S. niger*) were developed. In all, six models were evaluated: (1) full migration, with all migration paths open; (2) north to south, with unidirectional migration from the Sibuyan Sea to the Bohol and Sulu Seas; (3) south to north, with unidirectional migration from the Sulu and Bohol Seas to the Sibuyan Sea; (4) adjacent, with bi-directional migration paths between adjacent sites only; (5) north source 1, with the Sibuyan Sea as a source to

southern reefs and with the Bohol Jet preventing migration between the two southern sites; and (6) north source 2, with the Sibuyan Sea as a source for both southern sites and the more northerly site of the southern sites also being a source (Figure 23). For *Siganus guttatus* six models were also evaluated: (1) full migration, with all migration paths open; (2) north to south 1, with migration from the Sibuyan Sea to the Bohol Sea; (3) north to south 2, as the previous but with reciprocal migration between the Bohol Sea and the Tañon Strait; (4) south to north, with migration from the Bohol Seas to the Sibuyan Sea; (5) north and south as sources 1, with migration from the Sibuyan Sea in the north and the Bohol Sea in the south to the two central sites; and (6) north and south sources 2, as the previous but with reciprocal migration between the Bohol Sea and the Tañon Strait (Figure 24).



**Fig. 23** Gene flow models compared in MIGRATE-N for *Chlorurus bleekeri* and *Scarus niger*. (TB = Tablas, PG = Panglao (*C. bleekeri* only), BS = Basay (*S. niger* only), SQ = Siquijor (*C. bleekeri* only) and DP = Dapitan (*S. niger* only)).



**Fig. 24** Gene flow models compared in MIGRATE-N for *Siganus guttatus*. (TC = Ticao, BA = Bais, LY = Loay and DP = Dapitan).

## 4.4 RESULTS

### *Sequencing, SNP discovery and filtration*

The mean number of reads per individual was greatest for *C. bleekeri* at around 4.6 million and just over 2 million for both *S. niger* and *S. guttatus* (Table 13). For *C. bleekeri* alignments, using STACKS revealed 44,027 putative SNPs that were genotyped in > 90% of individuals, of these 17,139 were removed because they had an allele frequency of < 0.1; 15,753 were removed due to deviations from HWE; with a total of 11,135 being retained. A total of 6 individuals with > 10% missing data across these filtered SNPs were removed: 1 from Tablas, 3 from Panglao and 2

from Siquijor (Table 14). For *Scarus niger* alignments using STACKS revealed 15,300 putative SNPs that were genotyped in > 90% of individuals and of these 9,738 were removed because they had a minor allele frequency of < 0.1, 1,187 were removed due to deviations from HWE, resulting in 4,253 retained SNPs.

**Table 13** Maximum, minimum, and mean (standard error) number of reads generated per individual after sequencing (Illumina HiSeq2000) for each of the three species.

Species	Max reads	Min Reads	Mean (SE)
<i>Chlorurus bleekeri</i>	9,865,293	1,416,078	4,652,123 (177,828)
<i>Scarus niger</i>	9,338,158	793,257	2,713,267 (124,087)
<i>Siganus guttatus</i>	6,801,248	495,637	2,355,481 (70,347)

**Table 14** Summary of total putative SNPs discovered in STACKS, SNPs eliminated through filtering by allelic frequency and deviation from Hardy-Wienberg Equilibrium (HWE), retained SNPs, and retained individuals for all three species.

	Species		
	<i>Chlorurus bleekeri</i>	<i>Scarus niger</i>	<i>Siganus guttatus</i>
Putative SNPs	44,027	15,300	29,266
Allele frequencies < 0.1	-17,139	-9,738	-9,906
Deviation from HWE	-15,753	-1,187	-6,741
Retained SNPs	<b>11,135</b>	<b>4,253</b>	<b>12,619</b>
Retained Individuals	90	81	141

A total of 14 individuals with > 10% missing data across these filtered SNPs were removed: 2 from Dapitan, 7 from Basay and 5 from Tablas. Finally, 1 individual from Basay that was heterozygous at 50% of loci was removed. This sample contained almost twice as many

heterozygous loci as the average individual indicating contamination. For *S. guttatus* alignments using STACKS revealed 29,266 putative SNPs that were genotyped in > 90% of individuals, of these 9,906 were removed because they had an allele frequency of < 0.1; 6,741 were removed due to deviations from HWE; with a total of 12,619 being retained. A total of 4 individuals with > 10% missing data across these filtered SNPs were removed: 1 from Ticao, 1 from Bais and 2 from Loay (Table 14).

#### *Population structure*

Pairwise  $F_{ST}$  values ranged from 0.0011 to 0.0035 for *C. bleekeri*, 0.0002 to 0.0014 for *S. niger* and 0.0006 to 0.0018 for *S. guttatus* (Tables 15 - 17). However, no significant genetic differentiation was found between sample site pairs for all three species ( $P > 0.9999$ ). Levels of observed heterozygosity for each sample site ranged from 0.291 to 0.298 and the proportion of polymorphic loci for each sample site ranged from 99.3% to 99.8%.

**Table 15** Pairwise  $F_{ST}$  values on for *Chlorurus bleekeri* on upper diagonal calculated using 11,135 SNPs. All pair-wise comparisons were not significantly different ( $p > 0.999$ ). Shortest over water distance (km) between sampling sites on lower diagonal.

	Tablas	Panglao	Siquijor
Tablas	-	0.0011	0.0035
Panglao	481	-	0.0022
Siquijor	475	34	-

Siquijor) (Table 18). For *Scarus niger* 1 pair each of putative full siblings and half siblings were identified between Tablas and Basay (Table 19). It is important to note that for the 7 pairs of related individuals between sites for both parrotfish species, 6 of these pairs include Tablas and

#### *Relatedness analysis*

Estimates for the dyadic likelihood estimator (Milligan 2003) and the triadic likelihood estimator these comparisons are separated by over 400 km (Figure 22; Tables 18 & 19).

**Table 16** Pairwise  $F_{ST}$  values on for *Scarus niger* on upper diagonal calculated using 4,253 SNPs. All pair-wise comparisons were not significantly different ( $p > 0.999$ ). Shortest over water distance (km) between sampling sites on lower diagonal.

	Tablas	Basay	Dapitan
Tablas	-	0.0002	0.0014
Basay	421	-	0.0006
Dapitan	511	104	-

**Table 17** Pairwise  $F_{ST}$  values on for *Siganus guttatus* on upper diagonal calculated using 12,619 SNPs. All pair-wise comparisons were not significantly different ( $p > 0.999$ ). Shortest over water distance (km) between sampling sites on lower diagonal.

	Ticao	Bais	Loay	Dapitan
Ticao	-	0.0006	0.0008	0.0008
Bais	373	-	0.0014	0.0012
Loay	405	104	-	0.0018
Dapitan	482	101	121	-

(Wang, 2007) resulted in nearly identical values for all pair-wise analyses and for all species, thus only the results for the triadic likelihood estimator are presented. In addition both the likelihood and moment estimators were in agreement when it came to identifying putative full siblings ( $r > 0.5$ ) half siblings ( $< 0.5 r \leq 0.25$ ) and first cousins ( $< 0.25 r \leq 0.125$ ) for both parrotfishes. No related individuals were identified for *Siganus guttatus*. For *Chlorurus bleekeri* 4 pairs of putative full siblings were identified from individuals within Squijor and 1 pair of putative half siblings was identified between Tablas and Panglao (Table 18). And a total of 5



pairs of putative first cousins were identified for *C. bleekeri* (1 pair within Tablas, 1 pair between Tablas and Siquijor, 2 pairs between Tablas and Panglao and 1 pair between Panglao and

#### *Effective population size ( $N_e$ )*

For *C. bleekeri*, when all individuals were analyzed together and all related individuals were included  $N_e$  was rather small at only 280 but increased fivefold to 1,006 when one related individual was removed from each related pair (Table 20). When each site was considered separately and all related individuals were included  $N_e$  was rather low at 390 for Tablas and 141 for Panglao, but extremely low for Siquijor at only 70 individuals (Table 4.7). The lower  $N_e$  estimate when all sites were considered versus Tablas alone, is the result of the larger number of individuals were included  $N_e$  was 1,208 but increased to 2,094 when one related individual was removed from each pair (Table 20). When each site was considered separately  $N_e$  was similar for all three sites and ranged from 2,320 in Tablas to 1,639 in Basay (Table 20). No related pairs were detected within each site, thus no further analyses were conducted. Effective population size estimates for *S. guttatus* were much larger at 8,479 when all individuals were analyzed together and ranged between 3,265 in Loay to 11,411 in Bais when each site was considered separately (Table 20). Since no related individuals were detected for *S. guttatus* no further analyses were conducted.

#### *Migration Rates ( $M$ )*

For both parrotfish species the N source 2 model (Fig. 23) showed the highest support, in which migration occurs from northern reefs of Tablas to the southern reefs and the more northerly site of the southern sites also being a source (Table 21). The mutation scaled population sizes ( $\theta$ ) and migration ( $M$ ) (Table 22) were used to calculate the total number of immigrants per generation by multiply the  $\theta$  value of the population receiving the immigrants by  $M$  for each pathway.

Immigrants per generation for both of the parrotfishes were between 900 and 700 individuals for almost all pathways (Table 23). However for *Scarus niger*, immigrants per generation was 10x greater (8,295) from Tablas to Basay. For *S. guttatus* the highest supported model was N & S sources 2 (Fig. 24) in which the northern and southern most sites act as sources of migrants to the two central sites, with reciprocal migration between these two central sites (Table 21).

Immigrants per generation rates (Table 23) for *S. guttatus* revealed a similar pattern with the greatest immigration occurring from a south to north direction (Dapitan to Bais) and north to south (Ticao to Loay).

**Table 18** Results of relatedness analyses for *Chlorurus bleekeri*. Coefficients of relatedness ( $r$ ) with 95 % confidence intervals in parentheses for both maximum likelihood estimator (Wang, 2007) and the moments estimator (Lynch & Ritland, 1999) and the most likely relationship (FS = full siblings, HS = half siblings, FC = first cousins). (TB = Tablas, PG = Panglao, SQ = Siquijor).

Ind.	Ind.	Site comp.	TrioML (Wang, 2007) $r$	Lynch & Ritland (1999) $r$	Relatedness Category
SQ_13	SQ_05	SQ-SQ	<b>0.949</b> (0.937 - 0.962)	<b>0.815</b> (0.803 - 0.827)	FS
SQ_15	SQ_07	SQ-SQ	<b>0.917</b> (0.899 - 0.932)	<b>0.767</b> (0.753 - 0.780)	FS
SQ_12	SQ_04	SQ-SQ	<b>0.831</b> (0.810 - 0.853)	<b>0.642</b> (0.625 - 0.658)	FS
SQ_17	SQ_09	SQ-SQ	<b>0.501</b> (0.476 - 0.527)	<b>0.410</b> (0.389 - 0.430)	FS
PG_32	TB_10	PG-TB	<b>0.291</b> (0.273 - 0.308)	<b>0.341</b> (0.322 - 0.362)	HS
SQ_28	PG_32	PG-SQ	<b>0.184</b> (0.168 - 0.202)	<b>0.222</b> (0.203 - 0.244)	FC
TB_10	TB_28	TB-TB	<b>0.153</b> (0.136 - 0.172)	<b>0.177</b> (0.154 - 0.198)	FC
TB_28	PG_32	PG-TB	<b>0.131</b> (0.115 - 0.149)	<b>0.158</b> (0.139 - 0.180)	FC
SQ_28	TB_10	SQ-TB	<b>0.131</b> (0.116 - 0.148)	<b>0.158</b> (0.135 - 0.178)	FC
PG_32	TB_11	PG-TB	<b>0.129</b> (0.113 - 0.146)	<b>0.160</b> (0.137 - 0.177)	FC

**Table 19** Results of relatedness analyses for *Scarus niger*. Coefficients of relatedness ( $r$ ) with 95 % confidence intervals in parentheses for both maximum likelihood estimator (Wang, 2007) and the moments estimator (Lynch & Ritland, 1999) and the most likely relationship (FS = full siblings, HS = half siblings). (TB = Tablas, BS = Basay)

Ind.	Ind.	Site comp.	Estimator: TrioML (Wang, 2007)	Estimator: Lynch & Ritland (1999)	Relatedness Category
TB_12	BS_24	TB-BS	<b>0.821</b> (0.787 - 0.853)	<b>0.763</b> (0.738 - 0.787)	FS
TB_15	BS_43	TB-BS	<b>0.209</b> (0.170 - 0.248)	<b>0.185</b> (0.148 - 0.219)	HS

## 4.5 DISCUSSION

Advanced genomic methods did not reveal fine-scale population structure in the central Philippines but did provide information on the influence of life history strategies on population structure and dynamics also important for resource management. In addition, relationship analyses revealed an unexpectedly large number of related individuals with four pairs of full siblings, one pair of half siblings and five pairs of first cousins for *Chlorurus bleekeri*, a pair of full siblings and a pair of half siblings for *Scarus niger*, but no related pairs for *Siganus guttatus*. Initially these results appeared enigmatic until ocean currents and migration patterns were included. The prevailing north to south currents (i.e., Mindoro-Panay throughflow) within the central Philippines is a potential vector for larvae dispersal, possibly explaining the prevalence of related pairs. Migration analyses supported this hypothesis by revealing north to south migration patterns for both parrotfish species. Estimates of  $N_e$  were very small for *C. bleekeri* when related individuals were retained ( $> 300$  for most data sets) and only slightly larger when related pairs were not included. Effective population size estimates for *S. niger* were moderate at around 1,500; while  $N_e$  estimates for *S. guttatus* were much larger (several thousand). Although specimens were not aged a wide range of size classes were sampled in this study, thus multiple age classes were likely included. Given the multiple age classes sampled the population parameter estimates are temporally stable and not typical of a single age class or a single reproductive event. Therefore, even though population differentiation was not detected, this study was able to provide evidence of directional gene flow for herbivorous reef fishes across the central Philippines. The consequences of these findings indicate that MPAs within this region have the potential of supplying significant amounts of larvae to distant reefs.

### *Population structure*

The fact that this study was unable to discover any population structuring within the central Philippines is not unexpected given the results of previous studies and the reproductive characteristics of these fishes. For parrotfishes, these findings are consistent with previous studies such as *Scarus ghobban* (Visram et al. 2010) and *S. rubroviolaceus* (Fitzpatrick et al. 2011), which detected genetic structure only at oceanic scales (1000s of km). For *Siganus guttatus*, pair-wise  $F_{ST}$  values ranging between 0.019 and 0.170 were detected between the

**Table 20** Estimates of effective population size ( $N_e$ ) calculated with the full set of filtered SNPs for each species. For the “All sites” data set all individuals from each sample site were included while data sets with specific site names only included individuals from that location. All related pairs were included in the related included column while one related individual was removed from each pair when applicable for the related removed column. An allele frequency cut off of 0.05 was used and 95% confidence intervals are in parentheses.

Species	Dataset	$N_e$ (related included)	$N_e$ (related removed)
<i>Chlorurus bleekeri</i>	All sites	280 (278 – 281)	1,006 (991-1,021)
	Tablas	390 (383 - 397)	615 (597 - 635)
	Panglao	141 (140 -142)	-
	Siquijor	70 (69 – 70)	697 (670 -727)
<i>Scarus niger</i>	All sites	1,208 (1,154 – 1,267)	2,094 (1,940 – 2,231)
	Tablas	2,320 (1,843 – 3,127)	-
	Basay	1,639 (1,321 – 2,158)	-
	Dapitan	1,720 (1,407 – 2,210)	-
<i>Siganus guttatus</i>	All sites	8,479 (8,018 – 8,995)	-
	Ticao	9,684 (7,505 – 13,644)	-
	Bais	11,411 (8,556 – 17,116)	-
	Loay	3,265 (2,964 – 3,634)	-
	Dapitan	5,575 (4,591 – 7,092)	-

Philippines, Taiwan and southern Japan (Iwamoto et al. 2012) suggesting population differentiation at distances greater than 500 km (greater than the range of this study). Gene flow across long distances for these fishes may not be surprising given these fishes are broadcast spawners with a relatively long pelagic larval duration (PLD) ranging from 29-42 days (Ishihara and Tachihara 2011; Juario et al. 1985). Therefore, it is not unlikely that studies have not been able to find population structuring for these fishes at the scales (> 500km) of this study.

**Table 21** Log probabilities of the data given the model (\*marginal likelihood, based on the Bezier approximation score) and corresponding Bayes factors (LBF) for the tested connectivity models for three species of herbivorous fishes in the central Philippines. For descriptions of the models see Figures 4.2 and 4.3.

Species		Migration Model					
		Full	N to S	S to N	Adjacent	N source 1	N source 2
<i>C. bleekeri</i>	ILm*	-14450.7	-14540.6	-14541.9	-14460.8	-14445.9	-14408.4
	LBF	-84.56	-264.42	-266.98	-104.78	-75.00	0
	Rank	3	5	6	4	2	1
<i>S. niger</i>	ILm*	-15949.4	-15827.5	-15851.8	-15965.3	-15790.3	-15724.3
	LBF	-450.18	-103.2	-127.45	-240.94	-65.93	0
	Rank	6	3	4	5	2	1

Species		Migration Model					
		Full	N to S1	N to S2	S to N	N+S source 1	N+S source 2
<i>S. guttatus</i>	ILm*	-18510.7	-18897.2	-18765.4	-18880.5	-17958.0	-17902.9
	LBF	-1215.7	-1988.6	-1725.1	-1955.2	-110.2	0
	Rank	3	6	4	5	2	1

### Relatedness

The strongly supported detection of multiple related pairs (10 pairs for *C. bleekeri* and 2 pairs for *S. niger*) was unexpected given the long distances (300 to 500km) between pairs and the relatively small sample sizes (i.e., 20 out of 90 individuals related for *C. bleekeri* and 4 out of 81 for *S. niger*). However, these findings were corroborated by all three estimators (Lynch & Ritland 1999; Milligan 2003; Wang 2007) using a large number of SNPs (11,135 for *C. bleekeri* and 4,235 for *S. niger*). The confidence in accurate detection of related individuals becomes increasingly more difficult with lesser degrees of relatedness. Thus, the accurate assignment of first cousins requires more resolution power (i.e., more SNPs) than full siblings. Initial model-

**Table 22** 95% confidence interval and mode for mutation-escalated population sizes ( $\theta$ ) and migrations (M) for each population and migration pathway for the most probable migration model for three species of herbivorous fishes in the central Philippines (PG = Panglao, SQ = Siquijor, TB = Tablas, DP = Dapitan, BS = Basay, LY = Loay, TC = Ticao. BA = Bais)

Species		2.50%	Mode	97.50%
<i>C. bleekeri</i>	Population			
	PG $\theta$	0.02367	0.02950	0.03320
	SQ $\theta$	0.02500	0.02943	0.04087
	TB $\theta$	0.00560	0.00743	0.00913
	Pathway			
	TB to PG	908.7	943.0	988.7
	PG to SQ	620.7	704.3	788.7
TB to SQ	865.3	914.3	969.3	
<i>S. niger</i>	Population			
	DP $\theta$	0.01467	0.01710	0.01947
	BS $\theta$	0.00773	0.00963	0.01160
	TB $\theta$	0.00447	0.00623	0.00800
	Pathway			
	BS to DP	682.0	717.0	884.7
	TB to DP	895.3	967.0	1000.0
TB to BS	928.7	981.0	1000.0	
<i>S. guttatus</i>	Population			
	DP $\theta$	0.00413	0.00590	0.00767
	LY $\theta$	0.00373	0.00557	0.00740
	TC $\theta$	0.00000	0.00037	0.00200
	BA $\theta$	0.00187	0.00390	0.00593
	Pathway			
	DP to LY	915.3	961.0	988.7
	TC to LY	0.0	16.3	32.7
BA to LY	552.7	649.0	768.0	
DP to BA	954.0	981.7	1000.0	
LY to BA	633.3	725.7	781.3	
TC to BA	0.0	6.3	23.3	

**Table 23** Number of migrants per generation for each population and migration pathway for the most probable migration model for the three species (PG = Panglao, SQ = Siquijor, TB = Tablas, DP = Dapitan, BS = Basay, LY = Loay, TC = Ticao, BA = Bais)

Species	Pathway	Migrants / Generation
<i>Chlorurus bleekeri</i>	TB to PG	900
	TB to SQ	991
	PG to SQ	930
<i>Scarus niger</i>	TB to BS	8,295
	TB to DP	724
	BS to DP	729
<i>Siganus guttatus</i>	TC to LY	399
	TC to BA	21
	BA to LY	626
	LY to BA	70
	DP to BA	3,373
	DP to LY	7

based investigations determined that a minimum of 1,600 and 3,200 SNPs were required to correctly assign full sibs and half sibs respectively, with 95% correction classification rates (Kopps et al. 2015); whereas a second study estimated that 127,491 and 1,858 SNPs were required to discriminate full siblings, half siblings and first cousins, respectively (Mo et al. 2016). The number of SNPs used in these analyses (4,000 to 12,000) far exceed these minimum requirements for accuracy.

The four pairs of full-siblings detected for *C. bleekeri* within Siquijor suggests a high rate of self-recruitment ( $8/29 = 27.6\%$ ) comparable to those recorded for other reef fishes (Berumen et al. 2012). However, more rigorous parentage analyses using methods not employed in this study are needed to support this hypothesis. An alternative explanation to self-recruitment is a common larval source “upstream” that may be possible given the prevailing currents toward Siquijor (Fig. 25). The Bohol Jet is a strong east to west current that enters in through the Pacific Ocean, runs through the northern Bohol Sea and exits into the Sulu Sea (Gordon et al. 2011). A study using

both larval dispersal models and species assemblages (Abesamis et al. 2016) demonstrated that larvae are likely transported along the Bohol Jet resulting in a network from southern Leyte, to southern Bohol, to Siquijor. Thus, it is likely that larvae produced along the reefs of southern Leyte and Bohol are sources for reefs in Siquijor. However, this can only be corroborated for the species in this study with more sampling from these potential source reefs.

Aside from within site sibling pairs, a combined total of 7 between site related pairs (Tables 18 & 19) were detected for both parrotfishes, setting up inferences for reef connectivity. It is interesting to note that of the 7 pairs, 6 pairs include an individual from Tablas and another from a site more than 400 km apart (Basay, Panglao, or Siquijor). Three different scenarios may explain these results. The first scenario is that there are source reefs other than those sampled that are providing recruits to all the sampled sites. A second possible scenario is that the southern sites (Basay, Panglao and Siquijor) are acting as sources and providing larvae to Tablas in the north. The third scenario is the opposite of the second, i.e., the reefs in the vicinity of Tablas were the sources to the southern sites. The presence of strong southward-flowing currents within the central seas (Figure 25; Han et al. 2009; Gordon et al. 2011) seems to favor the third scenario, whereby these major current transport fish larvae southward from Tablas to the southern sites. This third scenario is corroborated with analyses of gene flow and migration rates as discussed below.

#### *Migration Direction and Rates*

This third scenario mentioned above, which relied on north to south currents explaining the presence of distant sibling pairs, was corroborated by Migrate-N. Migration flow patterns (larval dispersal) for both parrotfishes showed a north to south flow in that migration occurs from Tablas to all southern sites with additional migration from the more central site to the southernmost site (N source 2 in Figure 23). The immigration rates (per generation) obtained from Migrate-N for *C. bleekeri* revealed roughly equal rates for all three pathways. Similar rates were found for *S. niger* for similar pathways, except between Tablas and Basay, in which rates were 10 greater than all other pathways. It is interesting to note, that one pair of full siblings and one pair of half siblings for *S. niger* were detected between these two sites. These results support scenario three stated in the “Relatedness” section above, indicating that the reefs surrounding



Tablas are a larval source for reefs in the south and are likely transported by the prevailing north to south currents in the central Philippines (Figure 25). However, since Migrate-N utilizes coalescent theory it tends to be better at estimating historical migration patterns ( $4N_e$  generations) (Beerli 2008). However a study compared Migrate-N to BayesAss (Faubet and Gaggiotti 2000) a disequilibrium-based method and determined that Migrate-N was better at estimating recent migration (50yrs), but still gave a warning that both methods tend to overestimate migration rates (Samarasin et al. 2017). Therefore the estimated numbers of migrants need to be interpreted with caution, but the relative values still indicate a strong north to south connection from Tablas to Basay.

The migration pattern detected by Migrate-N for *S. guttatus* was different from that of the parrotfishes, potentially reflecting differences in life history strategies. The northern most and southern most sites (Ticao and Dapitan respectively) were shown to be sources for the central sites (Bais and Loay) with reciprocal migration between these last two sites. However, when migration rates are considered (Table 22), the pathways involving Ticao are much smaller (2 magnitudes of order) than all other pathways, thus showing Dapitan as a source of larvae for Bais and Loay and reciprocal larval transport between Bais and Loay. In support of this, Abesamis and colleagues (2016) found greater levels of connectivity amongst reefs in the western Bohol Sea, which happen to include these three southern sampling sites. Although *S. guttatus* is also a broadcast spawner, the contrasting migration patterns for the parrotfishes and *S. guttatus* may be a result of other differing reproductive behaviors. Critical factors that may play a role are the timing of spawning and the associated oceanographic currents. Tropical reef fishes tend to have a more protracted spawning season, however Abesamis (et al. 2010) was able to detect seasonal peaks of recruitment in the western Bohol Sea, with varying current patterns. Thus, if the timing of spawning of these species differs and coincides with different currents, different migration patterns would likely be detected.

The overall temporal stability of the migration patterns selected by these analyses is supported by both the sampling effort and the assumptions of the software package, Migrate-N. As mentioned earlier a wide size selection of individuals was sampled. This wide range of sizes likely included multiple generations and cohorts, thus these patterns can be extended beyond a single generation

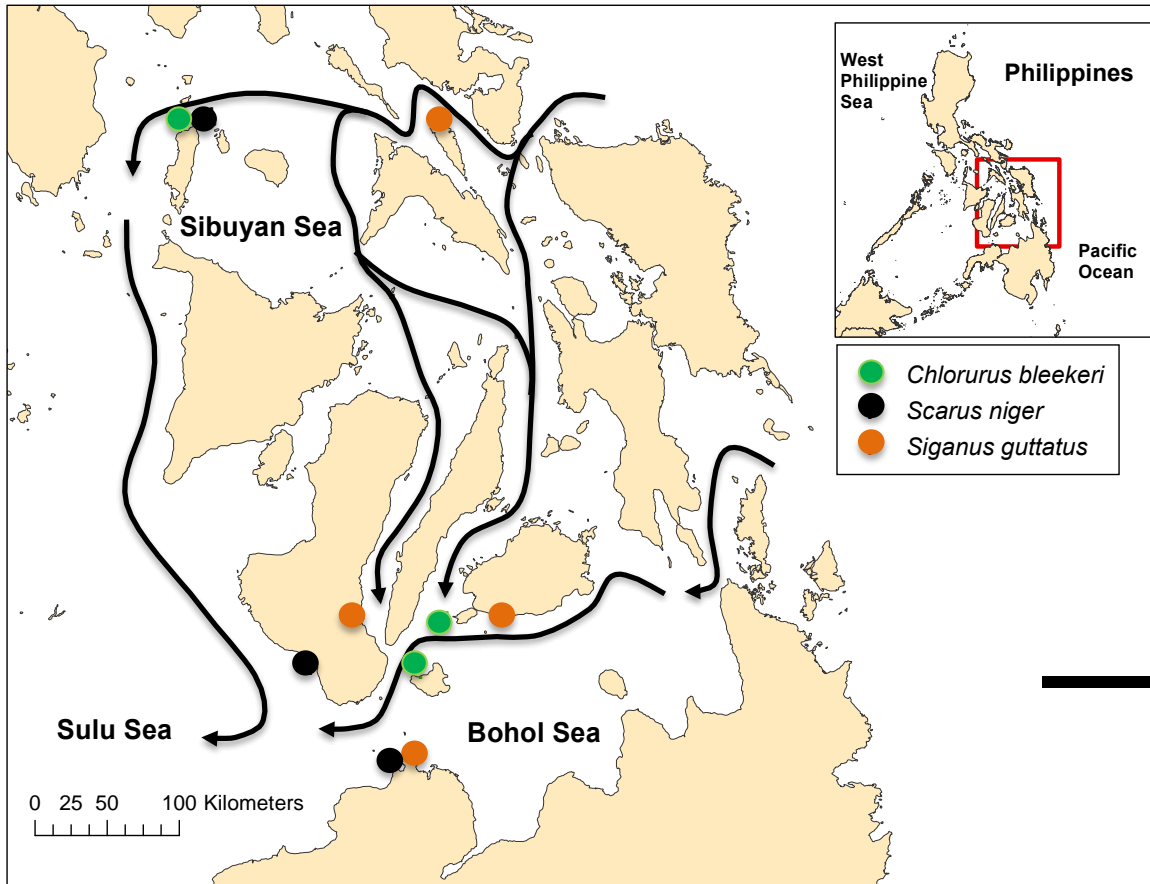


Figure 25. Predominant ocean currents for the central Philippines (Han et al. 2009; Gordon et al. 2011) in relation to the sample sites

or breeding cycle. In addition Migrate-N (Beerli 2008) estimates the historical or long-term migration rates of at least  $4N_e$  (effective population size) generations (Samarasin et al. 2017). Thus given the multiple generations within the samples and the time scale estimates of the analyses used these migration patterns are likely to be temporally stable and representative of many generations.

### *Seascape Genetics*

The synthesis of the genetic results of this study can be applied to seascape genetics, which combines measured genetic patterns with a spatial reference. Seascape genetics is a relatively new field of study and gains its origins from landscape genetics, however seascape genetics differs in that it examines the roles of ocean currents in generating spatial genetic patterns (Baums et al. 2006; Galindo et al. 2006). However, more recent studies have developed this further and are revealing differences in demographic, functional and neutral connectivity providing much needed insights into marine population connectivity and marine reserve network designs (Slekoe et al. 2016). With the combination of the relatedness analyses, migration patterns and rates with the ocean currents, this study was able to infer population connectivity for both parrotfish species. The relatively large percentage of related individuals between sites in the north (Tablas) and the southern sites suggests high rates of connectivity between these reefs. The migration analyses give further support to these results by identifying the model with the north to south migration pattern as having the best support for both parrotfish species, thus providing directionality. In addition, the estimated numbers of migrants per generation for these species were rather high between 700 and 900 between Tablas and the southern site, further supporting the northern sites as significant larval sources for southern sites. However, a value of roughly 8,000 individuals per generation was found between Tablas and Basay for *S. niger*. This value of a magnitude greater than all other estimates gives further support to the detection of related pairs at these great distances and gives even more support to Tablas as being an important source population for the southern reefs. Considering the predominantly north to south ocean currents within the central Philippines (Han et al. 2009; Gordon et al. 2011), it is likely that these currents are delivering larvae to the southern reefs. Thus, this study demonstrates how seascape genetics can identify source and sink populations and help in the planning of marine reserve networks.

### *Effective population size*

There was considerable variation in effective population sizes across the three species tested that is consistent with differences in life history strategies. Estimates of  $N_e$  for all datasets analyzed for both *S. niger* and *S. guttatus* were over 1,000 individuals, indicating that substantial genetic diversity is present in both these species across the central Philippines. Despite this consistent pattern, estimates for the full dataset that included the sibling pair for *S. niger* were substantially

lower than those that did not include the sibling pair (1,293 with the pair and 2,371 without the pair). Estimates of  $N_e$  for *C. bleekeri*, on the other hand, were very small (all sites below 500) when related individuals were included and slightly larger when related pairs were not included. The frequency of related pairs within a population is an important component of the signal of  $N_e$  and should be used in its estimation (Wang 2009). However, if the true proportion of siblings within a population is not estimated correctly,  $N_e$  estimates could be biased. There appears to be no reason to believe the proportion of siblings found in this study is not representative of the population, therefore the  $N_e$  estimates including the sibling pair should be used for future management decisions. Nevertheless, it would be desirable to sample additional individuals from these sites and conduct relationship analysis to ensure that the proportion of siblings found in this study is representative.

The existence of these small  $N_e$  estimates within highly fecund marine species is controversial (Waples 2016). Nunney (1993) pointed out that the  $N_e$  to census size ( $N$ ) ratio should not deviate from 0.5. Although estimates of  $N$  were not calculated for both parrotfish species, these parrotfishes have large population sizes typical of marine species ( $N \geq 10^6$ ) (Slatkin 1987). The  $N_e$  estimates for the entire sampled region in this study were roughly 1200 for *S. niger* and 300 for *C. bleekeri* thus resulting in very small ratios of  $10^{-3}$  and  $10^{-4}$  respectively. Such small  $N_e/N$  values in marine organisms have been shown to occur through theoretical models and empirical studies (Hedgecock & Pudovkin 2011; Hedrick 2005). There are several demographic characteristics of marine organisms that can allow for a small portion of the population to account for a disproportionately large part of the reproductive output and thus reduce  $N_e$ . One of which is sweepstakes reproductive success in which successful recruits from a few families by chance happen to end up in the right place at the right time (Hedgecock 1994). A second is the ‘big old fat fecund female fish’ (BOFFF) hypothesis (Hixon et al. 2014), in which older females are both more fecund and produce higher quality eggs, thus giving them a higher chance of producing viable offspring. And a third is the occurrence of a highly skewed sex ratio, which is common amongst protogynous reef fishes such as parrotfishes (Choat and Robertson 1975), although it requires a large departure from parity, such that  $N_e$  is reduced by 50% only when the least common sex is 15% of the total population (Allendorf et al. 2013).

There are several demographic characteristics of parrotfishes that may support these small  $N_e$  estimates reported in this study. First, results from chapter 3 in this study demonstrated that female fishes within marine reserves grew to larger sizes and produced a disproportionately larger number of eggs. According to the BOFFF hypothesis (Hixon et al. 2014) these fewer, but larger females, have a greater chance of producing recruits. Thus a reduced number of individuals may have a dominant impact on the population structure, resulting in a smaller  $N_e$ . And secondly, as mentioned earlier parrotfishes can have highly skewed sex ratios. Surveys from reef sites in Siquijor (Stockwell, unpublished data) recorded a mean female sex ratio of 4:1 (25%), with some sites as high as 7:1 (14.2%) for *C. bleekeri*. These female skewed sex ratios are likely to become more skewed for parrotfishes in which larger fish (i.e., terminal phase males) are more highly prized and thus selectively targeted. Additionally the high number of full siblings detected within Siquijor (8 out of 32 individuals) is likely a symptom of a reduced  $N_e$ . Although just one these demographic characteristics may not lead to a tiny  $N_e$  (Waples 2016) it is possible that synergistic effects may occur when both of these occur together. This is likely the case for *C. bleekeri* and thus these tiny estimates of  $N_e$  cannot be ignored.

### *Implications for Conservation*

The lack of genetic structure for all three species indicates high levels of gene flow between reefs within the central Philippines and suggests strong genetic connections between existing MPAs and reefs open to fishing within this region for these representative herbivorous fishes. This is initially encouraging for marine conservation and when this is coupled with both direction and strength of connectivity these results become a more powerful tool. The presence of highly related individuals between Tablas and southern sites and the combination of the predominant north to south currents suggests that the reefs around Tablas are a larvae source for these southern reefs. This directionality was corroborated with the Migrate-N analyses of both parrotfishes, thus strongly suggesting that the reefs of Tablas and the Sibuyan Sea are sources of larvae for the reefs of the Bohol Sea and Sulu Sea in the south. The strength of this connectivity can be inferred from both the high numbers of related individuals between Tablas and the southern sites (as high as 10% for some sites) and the greater migration rates between Tablas and the southern reefs than between the corresponding, but closer, southern sites. Given the distance of over 100 km between sites, this is likely the result of seeding dispersal (Puebla et al. 2012).

Additionally the relatively high number of related pairs sampled between these sites were related, suggesting that just a few individuals supply recruits to these reefs, which is in support of sweepstakes reproduction (Hedgecock 1994).

Thus for parrotfishes, the reefs of the Sibuyan Sea appear to be important sources of larvae for the Sulu and Bohol Sea. Therefore, larvae from MPAs within the Sibuyan Sea may be able to reseed reefs where stocks have been depleted (Harrison et al. 2012) due to severe overfishing or other disturbances (McLeod et al. 2009). A study from the neighbouring Bohol Sea (Stockwell et al. 2009) found the biomass of these parrotfish species much greater (5 times greater for *C. bleekeri*, 4 times for *S. niger*) inside well-established marine reserves. This increase in biomass within marine reserves will likely increase the reproductive output since an increase in biomass is strongly correlated with an increase in reproductive output (Evans et al. 2008). This suggests that efforts in establishment and enforcement need to be strengthened within the Sibuyan Sea to maintain the potential larval connectivity. In addition reefs within the Sulu and Bohol Seas also need to be protected through MPA establishment, since these reefs are likely larval sources for reefs further downstream.

Although conservation efforts may benefit from this high connectivity, the small  $N_e$  estimated for *Chlorurus bleekeri* is a warning for managers. All values, either as one single population or for each of the sample sites, were below 500. Additionally the presence of four pairs of putative full siblings within Siquijor further supports the small estimates of  $N_e$ . These small values important to note since according to the 50/500 rule proposed by Franklin (1980),  $N_e$  should not drop below 500 to prevent inbreeding depression. Although this has been considered an over simplistic estimation, it can still be a useful guiding principle to indicate genetic concerns are like to affect the long-term viability of the population (Jamieson and Allendorf 2012).

#### **4.6 CONCLUSIONS**

Willette et al. (2014) postulated how advanced genomics could be applied to marine organisms to generate better estimates of effective population size, to determine spatial connectivity of populations and to help position fisheries management zones or MPAs. RADseq and SNP analyses were able to provide estimates of  $N_e$  for three species of herbivorous reef fishes.

Additionally, the results presented here indicated significant levels of genetic connectivity within the central Philippines and provide evidence for demographic connectivity from the reefs in the Sibuyan Sea to reefs 100s of km to the south. This last finding suggests that the placement of a network of MPAs along this throughflow will likely facilitate larval connectivity between MPAs and neighboring fished reefs. Thus, even with the lack of population structure as indicated by  $F_{ST}$  analyses, the utilization of thousands of loci was able to detect both direction and strength of gene flow for reef fish populations within the central Philippines.

## CHAPTER 5

### SUMMARY

#### 5.1 INTRODUCTION

Some of the main expectations of marine protected areas are to promote resilience by mitigating human induced stressors (Hughes et al. 2003; Wenger et al. 2016), restore the reproductive potential of fish populations (Gell and Roberts 2003; Evan et al. 2008) and establish linkages among local populations through larval connectivity (Gaines et al. 2003; Grorud-Colvert et al. 2014). The three main chapters addressed these three key expectations and are summarized below.

#### 5.2 SUMMARY OF RESULTS

##### *Role of MPAs in promoting reef resilience*

The first aim of this study was to determine if marine reserves can mitigate detrimental effects of human impacts through the restoration of parrotfish populations. Regression analyses were able to demonstrate that sites within marine reserves showed no correlation between algae cover with either increasing population density or sediment loads, but in fact algae levels remained low (less than 10%). In contrast, algae cover in sites open to fishing was positively correlated with increasing human population density and sediment loads (Figures 6 and 7). In addition, coral cover was not correlated with human population densities or sedimentation within reserves, but declined exponentially with these same stressors on reefs exposed to fishing (Figures 8 and 9). Lastly, the greater biomass within marine reserves compared to fished sites was found to be correlated with decreasing algae cover (Figure 10). This finding suggests top-down control of algae by parrotfishes within these nearshore reefs. However, other studies seem to refute the top-down control of algae by parrotfishes (Russ et al. 2015; Suchley et al. 2016). These studies though, were conducted on offshore, more oligotrophic reefs where herbivory may be less of a factor. Therefore, the arguments of top-down vs. bottom-up control of algae may not be mutually exclusive but likely depends on the types of reefs being examined. In summary, this study demonstrated that for nearshore reefs within the Bohol Sea marine reserves appear to mitigate the effects of increasing human populations and sediment loads by increasing parrotfish biomass.



### *Role of MPAs in restoring the reproductive potential of parrotfishes*

The second aim of this study was to determine if the potential increase in parrotfish populations within marine reserves can function as net exporters of larvae. This was partly demonstrated by revealing a larger size at sex change within marine reserves (Figures 12 - 15) for the four most abundant sexually dimorphic parrotfish species (*Chlorurus bleekeri*, *Chlorurus spilurus*, *Scarus dimidiatus*, *Scarus rivulatus*). Histogram distributions of abundances of females (initial phase, IP) according to size classes not only showed increasing abundances with increased years of protections, but also revealed increasing modal size classes (Figures 16 - 19). These two analyses are in agreement and demonstrate that IP individuals within marine reserves become more abundant and delay sex change thus resulting in larger, more abundant females within these marine reserves. When combined with fecundity (number of eggs), these results demonstrated two patterns of recovery. Both *C. bleekeri* and *S. dimidiatus* showed an initially rapid 6 fold increase and 10 fold increase (respectively) within the 4-6 years category of marine reserves, while within marine reserves protected greater than 6 years *C. sordidus* and *S. rivulatus* showed delayed recovery with 13 fold and 10 fold increases respectively.

Few studies have demonstrated the effects of marine reserves on restoring fecundity of fishes, while those that have only revealed a slight increase of 4 fold (Evans et al. 2008). These lower rates of recovery may be the result of both differing fishing pressures and differences in life history traits. The study by Evans and colleagues (2008) was on a gonochoristic species (*Lutjanus carponotatus*), which has a roughly equal sex ratio. The parrotfishes in this study are all protogynous hermaphrodites with a harem breeding strategy with a highly skewed female ratio. Thus the recovery of larger more abundant females will likely have a larger impact on egg production. Additionally, the difference between fished and unfished sites may be more drastic since the Evans et al. (2008) study was conducted in Australia where fishing pressure is likely to be less than in the Philippines.

### *Connectivity within the central Philippines*

The third aim of this study was to examine the potential population structuring within the central Philippines, detect migration patterns and generate more accurate estimates of important population parameters (i.e. relatedness and effective population size) through the use of

restriction-site-associated DNA sequencing (RADseq). Even with the discovery of thousands of single nucleotide polymorphisms (SNPs), no significant ( $p < 0.05$ ) pair-wise  $F_{ST}$  values were detected between any of the sites for all three species (*Chlorurus bleekeri*, *Scarus niger* and *Siganus guttatus*). Although no related pairs were discovered for *Siganus guttatus*, several widely distant sibling and cousin pairs were found for both *C. bleekeri* and *S. niger*. For *C. bleekeri*, 4 pairs of putative full siblings were identified from individuals within Siquijor, 1 pair of putative half siblings was identified between Tablas and Panglao (Table 4.5) and a total of 5 pairs of putative first cousins were identified (1 pair within Tablas, 1 pair between Tablas and Siquijor, 2 pairs between Tablas and Panglao and 1 pair between Panglao and Siquijor) (Table 4.5). For *S. niger*, 1 pair each of putative full siblings and half siblings were identified between Tablas and Basay (Table 4.6). Combined with the six pairs of related individuals between Tablas and sites to the south and current patterns, it seems likely that the reefs of the Sibuyan Sea or reefs further north are larval sources for reefs to the south. This pattern of connectivity was corroborated by the migration analyses, which selected the north to south migration patterns for both *C. bleekeri* and *S. niger* to have the best support. Estimates of effective population size ( $N_e$ ) for both *S. niger* and *S. guttatus* were over 1,000 individuals, indicating that substantial genetic diversity is present in both these species across the central Philippines. Despite this consistent pattern, estimates for the full dataset that included the sibling pair for *S. niger* were substantially lower than those that did not include the sibling pair (1,293 with the pair and 2,371 without the pair). Estimates of  $N_e$  for *C. bleekeri*, on the other hand, were very small (all sites below 500) when related individuals were included and slightly larger when related pairs were not included.

Given the random sampling of individuals and relatively small sample sizes the number of related pairs seems rather high, but has implications for long range larval dispersal. When only including related pairs between sites, roughly 8% of *S. niger* individuals and 11% of *C. bleekeri* were related. This level of relatedness suggests high levels of connectivity between these sites, which is noteworthy at these distances of over 100s of kilometres. A study examining larval dispersal kernels from within the Bohol Sea estimated that most larvae settle within 10s of kilometers from their parents for a species of butterflyfish (Abesamis et al. 2017). Other studies on coral reef fishes have also found dispersal kernels of 10s of kilometers (Harisson et. al 2012; Almany et al. 2013), leading to the recommendation that marine reserves be spaced at no more

than 15 kilometers apart (Green et al. 2014). However, this study suggests that substantial recruitment can occur at much greater distances.

Although the effective population size ( $N_e$ ) estimates for *Siganus guttatus* was comparable with other marine fish species, the small  $N_e$  estimates for both parrotfishes is considered controversial, since this requires very few families to reproduce successfully (Waples 2016). However, very small effective population sizes in marine organisms have been shown to occur through theoretical models and empirical studies (Hedgecock and Pudovkin 2011; Hedrick 2005). Several demographic characteristics can allow for a small portion of the population to account for a disproportionately large part of the reproductive output, resulting in a small  $N_e$ . One of these is sweepstakes reproductive success, in which successful recruits from a few families by chance happen to end up in the right place at the right time (Hedgecock 1994). A second is the ‘big old fat fecund female fish’ hypothesis (Hixon et al. 2014), in which older females are both more fecund and produce higher quality eggs. A third is the occurrence of a highly skewed sex ratio, which is common among protogynous reef fishes such as parrotfishes (Choat & Robertson 1975). Although it requires a large departure from parity, such that  $N_e$  is reduced by 50% only when the least common sex is 15% of the total population (Alendorf et al. 2013), highly skewed sex ratios of this magnitude have been recorded for reef fishes. Surveys from reef sites in Siquijor (Stockwell, unpublished data) recorded a mean skewed female sex ratio of 4:1 (25%), with some sites as high as 7:1 (14.2%) for *C. bleekeri*. These female skewed sex ratios are likely to become more skewed for parrotfishes in which larger fish (i.e. terminal phase males) are more highly prized and thus selectively targeted. Although just one of these demographic characteristics may not lead to a tiny  $N_e$  (Waples 2016), it is possible that synergistic effects may occur when all three of these occur together. This is likely the case for *C. bleekeri*, so these tiny estimates of  $N_e$  cannot not be ignored.

### 5.3 CONCLUSIONS

This study demonstrated that marine reserves can promote the resilience of reefs by reducing the impacts from increasing human populations and sediment loads by protecting and increasing the abundance of parrotfishes. However, this may only be applicable for nearshore reefs with these specific human population densities and sediment load ranges. Secondly marine reserves were

also shown to restore the reproductive potential of these key parrotfishes and potentially operate as net exporters of larvae. Thirdly this study demonstrated that these larvae are likely to seed reefs within the central Philippines.

#### **5.4 FUTURE DIRECTIONS**

These findings raise further the question of which future studies are needed. Firstly, this study only included a moderate range in human population density and sediment loads. Therefore it would be beneficial to expand these methods to other reefs exposed to more urban areas and larger rivers to determine the thresholds of these mitigating effects. Secondly, with respect to marine reserves restoring the reproductive potential of parrotfishes, no asymptotic fecundity per unit area was reached for both *Chlorurus spilurus* and *Scarus rivulatus*. Future studies should thus include reserves with longer years of protection to determine this value for these parrotfishes. Additionally, the impacts of increased fecundity should also be explored for other targeted protogynous fishes such as groupers (Serranidae) and large wrasses (Labridae). The last section of this study examined the population structure of three herbivorous reef fishes, but failed to detect significant structuring for the sites sampled. However, by expanding the range of sampling sites, barriers to gene flow may be detected. Additionally, by expanding sampling efforts, more sources and sinks may be discovered. These additional migration patterns will greatly aid marine conservation managers in the establishment of marine reserve networks.

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### **EDUCATION**

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Bachelor of Science (Biology), Houghton College, Houghton, NY. 14474. 1996.

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### **EXPERIENCE**

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Grad. Research Assistant Old Dominion University Norfolk, VA 01/2017 - present

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Associate Researcher Silliman University Dumaguete, Philippines 01/2000 - 05/2010

### **PEER-REVIEWED PUBLICATIONS**

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#### Dissertation-related

- Stockwell BL, Larson WA, Waples RK, Abesamis RA, Seeb LW, Carpenter KE. 2016. The application of genomics to inform conservation of a functionally important reef fish (*Scarus niger*) in the Philippines. *Conservation genetics*. 2016 Feb 1;17(1):239-49.
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