ENVIRONMENTAL FACTORS INFLUENCING AND BEHAVIORAL CHANGES ASSOCIATED WITH REPRODUCTION AND THE IMPACTS OF SIMULATED SIZE-SELECTIVE HARVESTING ON A SEX-CHANGING FISH

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

By

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ABSTRACT

ENVIRONMENTAL FACTORS INFLUENCING AND BEHAVIORAL CHANGES ASSOCIATED WITH REPRODUCTION AND THE IMPACTS OF SIMULATED SIZE-SELECTIVE HARVESTING ON A SEX-CHANGING FISH

By

Michael John Schram

Master of Science in Biology

Overfishing has led to significant declines in worldwide fish stocks, particularly large bodied species. Contemporary management attempts to mitigate fishing effects to ensure the sustainability and accessibility of future fish stocks. A typical approach in fisheries management is to set size-limits, which focus fishing pressure on specific size-classes, a practice known as size-selective harvesting. Minimum size-limits are common, placing fishing pressure on the largest most fecund size-classes. In many sex-changing species, the largest size-classes are composed of a single sex. In the case of protogynous hermaphrodite species (those with female to male sex change), a few large males dominate harems of females. Size-selective harvesting therefore becomes sex-selective and has the potential for putting these types of species at risk for overfishing.

Observational studies have noted life history changes in harvested protogynous species that correlated with fishing pressure, but controlled manipulative studies demonstrating causal effects of size-selective mortality have not been conducted. I used the blackeye goby (*Rhinogobiops nicholsii*) as a model species to test the effects of size-
selective mortality. Because harvested species are not amenable to controlled manipulation of replicate populations in the field, use of similar model species may be the best approach to gain an in depth understanding of the effects of size-selective mortality on the ecology, reproductive characteristics, behavior, and life history of sex-changing species. Several aspects of the reproductive ecology of the blackeye goby, however, were unknown. My work aimed to (1) identify the factors influencing the reproductive patterns of the blackeye goby and (2) test the effects of simulated size-selective harvesting on their reproduction and growth using a field experiment.

To conduct this work, twenty 2.25 m² artificial reefs were built and stocked with natural densities of blackeye gobies. To determine the environmental characteristics influencing and behavioral changes associated with reproduction in blackeye gobies, I measured reproduction in artificial nests from weekly photographs. Photographs were taken for two months to test for the presence of a lunar cycle in blackeye goby reproduction. Temperature loggers were used to determine the effect of temperature on blackeye goby reproduction. Behavioral observations were made to explore whether behavior of three size-classes (male, female, and juvenile) changed when eggs were present on a reef versus when they were absent. Neither lunar phase nor water temperature were found to influence reproduction of the blackeye goby. The lengthy pelagic larval duration of the blackeye goby likely minimizes any potential fitness gains that could be obtained by timing reproduction with small-scale local variation in environmental factors. Rates of behavioral expression were unaffected by the presence of eggs on the reef. The persistent social dominance hierarchy exhibited by blackeye gobies
may allow dominant, nest-guarding males to maximize their reproductive output without any behavioral changes.

To test the effects of size-selective removals, populations of blackeye gobies were subjected to one of three size-selective removal treatments that reflected typical size-based management protocols (minimum, slot, and maximum size-limits) and these were compared to a control treatment with no removals. Reproductive output was measured on each reef, and individuals were tagged to track growth and sex change over the study period. Population densities were reduced by ~25%, which is a conservative reduction relative to known effects of fishing on population densities, but which ensured that not all individuals of a single sex were removed from any reef. The size-selective removals had no effect on reproductive output, growth, or incidence of sex change of blackeye gobies. The flexibility in social dynamics likely provides protogynous species the capacity to buffer modest losses of individuals from their populations. Future research incorporating additional species and a wider range of removal intensities and frequencies would provide additional insight into the impacts of size-selective harvesting on protogynous species.
Chapter 1

General Introduction

The exploitation and sustainability of marine resources has been a topic of great concern in recent years (Bohnsack and Ault 1996). Anthropogenic impacts on those resources, through direct exploitation and climatological alterations, have increased with modern industrialization; however, concomitant technological advances have improved our capability to understand and predict those impacts. A variety of approaches have been used to understand current trends and predict future change in impacted species and ecosystems including literature reviews and mathematical modeling (e.g. Warner et al. 1975, Warner 1975a, Dayton et al. 1995, 2002, Jackson 1997, Jackson et al. 2001, Pauly et al. 2002, Essington et al. 2006, Sanchirico et al. 2006, Byers and Noonburg 2007, Starr 2008), citizen science (e.g. Delaney et al. 2007, Pita et al. 2010, Murphy 2012, Rio et al. 2013, Martin 2013), and contemporary research (e.g. Roberts et al. 2003, Halpern 2003, Gerber 2005, Munch et al. 2005, Froeschke et al. 2006, Williams et al. 2006, Swain 2007, Hamilton et al. 2007, 2011, O’Connor et al. 2007, Conover et al. 2009, Walraven and Mollet 2010, Wilson et al. 2010, Rijnsdorp et al. 2010, Caselle et al. 2011, Mariani et al. 2013, van Wijk et al. 2013). These studies highlight the impacts of exploitation and disruption, such as overfishing or invasive species, within marine ecosystems as well as the efficacy of programs, such as marine reserves or community based monitoring, intended to mitigate those impacts. The contributions provided by these research projects are important for maintaining the sustainability of resources deemed ecologically, economically, or culturally important in an ever-changing, anthropocentric world.
Research on harvested species, or functionally similar taxa, is used to establish guidelines and restrictions aimed at maintaining the sustainability of fish stocks. Size-limits are commonly used tactics in fisheries management. Maximum size-limits are set to protect the largest, most fecund individuals while minimum size-limits ensure any harvested individual has had at least one opportunity to reproduce. Size-slot limits attempt to offer the benefits of both maximum and minimum limits by focusing on intermediate size-classes. Despite the management approach used, however, the focus is on specific size-ranges within a population, a practice known as size-selective fishing. Minimum size-limits are the most common, used in an attempt to maintain an effective, yet suboptimal, breeding stock; however fishing pressure then lies almost exclusively on the largest most fecund size-classes (Hutchings 2005, Hamilton et al. 2007, Conover et al. 2009).

Prior to contemporary fisheries management, regional fishing practices typically lead to substantial declines in fish populations (Hutchings and Myers 1994, Jackson 1997, Hutchings 2000, 2005, Jackson et al. 2001, Hutchings and Reynolds 2004). Jackson and colleagues (2001) noted significant reductions in the abundances of long-lived, large-bodied species compared to historical records. Similarly, the significant addition of fisheries targeting mid- and low-trophic-levels worldwide (Essington et al. 2006) has added extra complexity to management as a number of species occupying these trophic levels are hermaphroditic (Robertson and Choat 1974, Warner 1975a, Warner et al. 1975, DeMartini and Sikkel 2006).

Protogynous hermaphroditism, or female to male sex change, is the most common form of hermaphroditism in fishes. Populations of protogynous species typically have sex
ratios that are skewed toward females, with few large, dominant males (Webb and Kingsford 1992, DeMartini and Sikkel 2006, Molloy et al. 2008, Helfman et al. 2009). Shifts in fishing focus to mid-size species, combined with size-selectivity for larger individuals within those species, has the potential to place them at increased risk for overfishing. Reductions in mean body size, size at maturity, and size at sex change have been observed in heavily fished populations of protogynous species, (Hamilton et al. 2007, 2011, Caselle et al. 2011, Mariani et al. 2013); however, the causal links have not been demonstrated in these correlative studies. A controlled, manipulative study would elucidate the effects of size-selectively harvesting protogynous species and help inform future management techniques to maintain sustainable future fish stocks.

The use of model study species is a widely used approach for conducting research that may otherwise be logistically challenging or impractical to conduct on the species of direct interest. I chose to use the blackeye goby (*Rhinogobiops nicholsii*, Bean) as a model species to experimentally evaluate the effects of size-selective harvesting. This species is a small, protogynous, reef-associated fish found from British Columbia, Canada to Baja California, Mexico. The small size, high abundance, small home-range size, and wide distribution of this species has made it a popular model study species in temperate marine science (e.g., Steele 1996, Steele et al. 1998, Malone et al. 1999, Steele and Forrester 2002a, 2002b, Andrews and Anderson 2004, Froeschke et al. 2006, Starr 2008, Laidig et al. 2009, Floyd and Anderson 2010, Zalmon et al. 2010, Block 2011, Yong and Grober 2013). Despite the extensive literature that has accumulated on this species over the past 50 years (e.g., Ebert and Turner 1962, Wiley 1973, Cole 1983, 1984, Breitburg 1987, Watson 1996, Kroon 1997, Steele 1997a, 1997b, Csepp and Wing
1999, Love and Schroeder 2006, Love 2011), important aspects of the reproductive ecology of this species are unknown. Specifically, whether reproduction varies seasonally, with lunar period, or is related to water temperature is not known. Nor is it known whether the presence of egg clutches on a reef affects the behavior of blackeye gobies in the vicinity. The first part of my thesis explores these unknown aspects of the reproductive ecology of the blackeye goby. The second part of my thesis describes a field experiment that uses the blackeye goby as a model species to investigate how simulated size-selective fishing affects reproduction and growth in protogynous fishes.
Chapter 2

Environmental Factors Influencing and Behavioral Changes Associated with Reproduction in a Temperate Marine Reef Goby, *Rhinogobiops nicholsii*

Introduction

For species exhibiting a bipartite lifecycle, population growth is driven by reproduction and subsequent larval recruitment. The reproductive output of a population is linked with average adult body size (Duarte and Alcaraz 1989, Bohnsack and Ault 1996, Berkeley *et al.* 2004, Gherard *et al.* 2013). Recruitment rates may be more variable over space and time than is reproductive output (Sale *et al.* 1984, Victor 1986, Doherty and Williams 1988, Choat *et al.* 1988, McCormick 1999) because the environmental characteristics experienced during the pelagic phase influence larval survivorship (Meekan and Carleton 2003, Green and Fisher 2004, Bergenius *et al.* 2005, Sponaugle and Grorud-Colvert 2006). Timing reproduction with favorable environmental conditions therefore has the potential to provide significant fitness gains.

Reproductive lunar synchrony has been observed in a wide array of marine taxa including corals, polychaetes, and fishes (Middaugh 1981, Robertson *et al.* 1990, Levitan *et al.* 2004, Fox 2013). Pelagic larvae are thought to be more conspicuous to visual predators during full moons when lunar radiance is greatest (Hobson *et al.* 1981); however, photopositive larvae may swim toward the surface during full moons to avoid predator filled reefs (Thresher 1984). Additionally, internal waves, associated with changes in tidal amplitude driven by lunar phase, are thought to influence larval transport (Shanks 1983, 1986, Thresher 1984, Kingsford and Choat 1986, Sponaugle and Cowen 1997). Changes in larval transport influence the probability of finding suitable settlement
habitat and intraspecific competition upon settlement (Levinton 2009a). Consequently, timing larval release with particular lunar phases may ultimately provide fitness benefits.

In addition to lunar phase, temperature is known to influence reproduction in marine fishes (Gerking et al. 1979, Pankhurst and Munday 2011). Faster development occurs in warmer waters (Pauly and Pullin 1988, McCormick and Molony 1995), reducing incubation time (Lasker 1964, Ryland and Nichols 1975, Miranda et al. 1990) and pelagic larval duration (O’Connor et al. 2007). Reproduction during periods of warm water ultimately improves larval survivorship by increasing larval growth rates and shifting prey out of size ranges that predators are capable of consuming, an idea known as the “growth-mortality” hypothesis (Anderson 1988).

Alongside abiotic factors, behavior often plays a significant role in reproductive success. Demersal eggs are typically defended by the male (DeMartini and Sikkel 2006), and less often by the female or both parents. Behavioral changes, such as increased aggression and nest maintenance, improve the proportion of eggs that hatch. Increased aggression and nesting may occur at the cost of foraging, predatory avoidance, or further courtship. In addition, the majority of protogynous species exhibit a size-based social dominance hierarchy whereby dominant individuals influence the behavior of subordinate individuals (Ross 1990, Munday et al. 2009). Specifically, alpha males dominate and socially repress growth of nearby individuals, through increased energetic expenditure and decreased foraging rates of subordinates, to maximize their competitive edge and reproductive opportunities (DeMartini and Sikkel 2006, Helfman et al. 2009, Munday et al. 2009). While altered behavior by the nesting male may improve egg
survivorship, it may detract from future mating opportunities by allowing increased
courtship and foraging in competing subordinate individuals.

The blackeye goby (*Rhinogobiops nicholsii*, Bean) is a protogynous temperate
fish that is closely associated with rocky reef habitat (Miller and Lea 1972, Love 2011)
and is common in the waters of Southern California. The ubiquity and small size of this
species has resulted in its widespread use as a model study species to address ecological
questions (e.g. Steele 1996, Steele *et al.* 1998, Malone *et al.* 1999, Steele and Forrester
al.* 2009, Floyd and Anderson 2010, Zalmon *et al.* 2010, Block 2011, Yong and Grober
2013). Numerous studies have characterized their ecology (e.g. Ebert and Turner 1962,
and Wing 1999, Love and Schroeder 2006). Despite the extensive body of knowledge
compiled on this species over the past 50 years, aspects of the reproductive ecology of
this species are still unknown. Ebert and Turner (1962) briefly described blackeye goby
reproduction; Wiley (1973) documented food and nesting habits, morphometrics,
population structure, and social behavior; and Cole (1983) determined that this species
exhibits protogynous hermaphroditism, later confirmed by Kroon (1997).

Male blackeye gobies establish and defend a nest within their territory by digging
a burrow in sand under overhead rock (Ebert and Turner 1962, Wiley 1973). Females lay
adhesive eggs overhead and the male defends and maintains that clutch of eggs until they
hatch (Ebert and Turner 1962). Larvae then drift in the plankton for 40-76 days (Block
2011) before settling to suitable reef habitat.
Reproductive patterns of fishes with pelagic larval phases are often reconstructed from recruitment surveys and otolith-based aging. This approach, however, can mask the actual patterns of reproductive output due to high and variable mortality rates during the larval phase (Carr and Syms 2006, Johnson 2008). I directly measured reproductive output using methods similar to those used in other studies on reproductive output of small, reef-dwelling fishes (DeMartini 1988, 1991, Knapp and Sargent 1989, Knapp 1993, Knapp et al. 1995, Samhouri 2009, Forrester et al. 2010). Through the application of this seldom-used method, my study aimed to (1) determine whether blackeye gobies time reproduction with lunar phase, (2) investigate the effects of temperature on reproductive timing, and (3) determine whether the presence of eggs on a reef influences the behavior of nearby blackeye gobies.

**Materials and Methods**

**General Methods**

To evaluate factors influencing reproduction in blackeye gobies, experimental populations were established on twenty rock rubble reefs in Big Fisherman Cove, Santa Catalina Island, USA (33°26’42”N, 118°29’8”W) during the summers of 2012 and 2013 (Fig 1A). Each reef was 2.25 m² and constructed of ~ 60 L of rock placed on a PVC frame lined with plastic mesh to keep the rocks from sinking into the sand. Reefs were built on a sand plane at 10-13 m depth, and were 10 m from any other reef structure to minimize movement of fish among reefs. To minimize mortality, each reef was covered with a predator exclusion cage (3.4 and 3.8-cm plastic mesh in 2012 and 2013, respectively). To investigate the influence of temperature on reproduction, six iButton
temperature loggers were deployed throughout the reef array. These recorded bottom
temperature hourly from July 19th – September 13th, 2013.

Each reef was stocked with a standard density, size distribution, and sex ratio of
blackeye gobies as determined from natural reef surveys. Sex was determined by genital
papillae and external morphology (Wiley 1973). The black pelvic discs and greater length
of the second dorsal and anal fins in males makes them easy to distinguish from females
(Wiley 1973). The small size of juveniles and undefined morphology of their genital
papillae made them easy to distinguish from sexually mature individuals (personal
observations). Gobies used were collected with dip nets by SCUBA divers from natural
reefs between Lion’s Head Point (33°27’08”N, 118°30’05”W) and Little Geiger Cove
(33°27’32”N, 118°30’59”W).

Reproductive Output

Reproductive output was quantified via photographs of egg clutches in artificial
nests, using methods similar to those of Forrester et al. (2010) who studied a similar
species of goby. Inverted terracotta potting saucers were used as artificial nest sites. A
small slot was cut along one edge of each saucer (Fig. 2A), which provided easy access
for males and promoted their use over natural rocks. Three saucers were placed on each
reef (Fig 1B). Saucers were checked weekly for the presence of clutches of eggs, and all
clutches were digitally photographed. The digital images were analyzed in Image J
(Abràmoff et al. 2004) to estimate the number of eggs in each clutch. Images were set to
scale, total clutch area was measured, and egg counts were made in five 1-cm²
subsamples. Egg density appeared to be uniform, and therefore the total number of eggs
in each clutch was estimated as the product of the average density of eggs in the five subsamples and total clutch area.

**Behavior**

Behavioral observations were conducted weekly for 7 weeks beginning on July 8th, 2012 to determine behaviors of three classes: male, female, and juvenile. Two divers visited each reef for 6 minutes and observed focal fish in each class. The first minute was spent identifying focal individuals and allowed fish to acclimate to diver presence, and the remaining 5 minutes were spent observing behavior. Due to the sedentary nature of the study species, it was not difficult for each diver independently to monitor all three focal fish simultaneously. Large males were specifically targeted because of their social dominance and influence on conspecific behavior. They were easily identified by their size [>7.5 cm standard length (SL)], black pelvic disc, and close proximity to a nesting saucer. Females were smaller (4.0 – 7.5 cm SL) and lacked the obvious black on their pelvic disc. Juveniles were noticeably smaller than adults (<4.0 cm SL) and were often found at reef margins, likely displaced from optimal reef habitat by territorial adults. The frequency of four focal behaviors defined by Wiley (1973; Table 1), were recorded: aggression, courtship, nesting, and foraging. Divers were unable to communicate to each other which fish they observed, therefore to avoid pseudoreplication, mean behavioral frequencies were used for statistical analyses.
Statistical Analyses

To test for any lunar pattern in blackeye goby reproduction, weekly nest photos from two full lunar cycles during July and August, 2012 were analyzed. Data met the parametric assumptions of normality and homoscedasticity, therefore an analysis of variance (ANOVA) was used to determine whether the number of eggs per reef differed among the four lunar phases. The categorical variable was lunar phase and total egg count per reef was the response variable. An *a priori* comparison using spring (full and new moons) and neap (first and last quarter moons) tides was conducted to test for any influence of tidal amplitude.

I could not test directly for an effect of temperature on reproduction at each reef because I did not have enough temperature loggers to place one at each reef. Instead, I tested for differences in temperature between reefs in different positions relative to the back of the cove (near, middle, and far) and at different depths (shallow or deep; Fig. 1C) and then evaluated whether any position or depth related differences in temperature were mirrored in reproductive output. A two-way ANOVA was used to test for differences in daily bottom temperature between depths and among positions. Mean daily bottom temperature data were normal and homoscedastic. Repeated measures ANOVA was used to determine the influence of reef position and depth on weekly reproduction over 8 weeks. Although the reproductive data failed to meet parametric assumptions, I used ANOVA because it is statistically robust to these violations.

I tested whether blackeye goby behavior differed when a clutch of eggs was present on a reef. Egg presence was based on nest photos taken 2-3 days prior to the behavioral observations. Multivariate analysis of variance (MANOVA) was used to test
for differences in behavior, with egg presence in a nest and focal fish sex as the predictor variables and frequencies of aggression, courtship, nesting, and foraging as the response variables. Behavioral data failed to meet the assumptions of normality; however, MANOVAs are statistically robust to parametric assumptions.

Results

Reproduction occurred in all months that populations of blackeye gobies were on the reefs, from July to March, with eggs clearly visible within saucer nests (Fig. 2B). Average clutch size was 42.2 ± 2.0 cm² with 210.9 ± 4.6 eggs cm⁻² (mean ± SE, n = 210). As previously described by Ebert and Turner (1962), recently laid eggs appear pink/orange (Fig. 2C) transitioning to fully transparent as they develop (Fig. 2D). The eyes of developed embryos are visible in late-stage eggs, allowing the differentiation of several clutches in a single nest. The same clutch was observed in sequential photos on several occasions (Fig. 2C and 2D), establishing a minimum incubation period of 7 days. Assuming eggs hatched within 24 hours after the second photograph of the sequence, or 24 hours prior to the third photograph, blackeye goby egg incubation was estimated to be 8-13 days.

Blackeye gobies did not appear to time reproductive effort with any particular lunar phase ($F_{3,156} = 0.50, p = 0.69$, Fig. 3). Reproductive output was also unrelated to tidal amplitude ($F_{1,156} = 1.21, p = 0.27$).

Reproduction did not differ statistically between depths ($F_{1,14} = 2.70, p = 0.12$) or among positions ($F_{2,14} = 0.29, p = 0.75$; Fig. 4); however, deeper reefs were colder on average than shallower reefs ($F_{1,336} = 24.55, p < 0.001$), as were reefs closer to the mouth.
the cove compared to those in the middle or near the back \( (F_{2,336} = 13.77, p < 0.001; \) Fig. 5).

The presence of a nest with eggs did not alter behavior of fish living on that reef (Egg Presence \( \times \) Focal Fish Sex interaction: Wilks’s \( \Lambda = 1.00, F_{8,822} = 0.18, p = 0.99, \) Fig. 6) nor did nest activity have an effect on the overall expression of the four behaviors independently (Wilks’s \( \Lambda = 0.99 \) \( F_{4,411} = 1.09, p = 0.36 \)). Behavioral rates varied among focal fish (Wilks’s \( \Lambda = 0.38, F_{8,822} = 64.66, p < 0.001 \)). Males were the most aggressive, females were intermediate, and juveniles were the least aggressive. Feeding rates were the opposite, with juveniles feeding the most and males the least. Males courted and nested, whereas females and juveniles did not (Fig. 6).

**Discussion**

Active nesting was documented each month from July through March. When coupled with Ebert and Turner’s (1962) account of nesting from April through October, there is documented evidence for year-round reproduction by the blackeye goby in Southern California, supporting claims by Love (2011). Estimates of reproductive output were much greater than the 1700 eggs in a single nest reported by Ebert and Turner (1962). Based on Wiley’s (1973) estimation of female fecundity ranging from 3274 to 4788 eggs \( (n = 4 \) females), a minimum of two females would be necessary to account for the \( \sim 8860 \) eggs per nest recorded in this study; however, female sizes were not included with Wiley’s estimates. Love (2011) stated males may defend the clutches of up to 6 females; however, one or two females appears to be the average, which is in line with reported population sex ratios of 1.7 females per male (Wiley 1973). Although multiple
clutches were observed on several occasions, visual determination of egg developmental stage suggests the majority of nests were comprised of a single clutch. Since female fecundity is known to increase exponentially with body size (Duarte and Alcaraz 1989, Bohnsack and Ault 1996, Berkeley et al. 2004, Gherard et al. 2013), it is possible that a single large female could produce the average number of eggs observed here. Conversely, female garibaldi (*Hypsypops rubicundus*) preferentially approach and lay eggs in nests containing early stage eggs over those with late stage or no eggs (Sikkel 1989). It has also been shown that female damselfish would deposit new eggs contiguous to early stage eggs and noncontiguous to late stage eggs (Knapp et al. 1995). If female blackeye gobies exhibit similar nesting preference and habits, numerous females could be courted into a single nest over a short period to produce a seemingly singular clutch.

The exact incubation period could not be determined from once-a-week photos, but it must be in the 8-13 day range based on the nest photographs. While increasing the frequency of nest visits could provide more accurate estimates, consistent disruption might also result in nest abandonment by guarding males, and bias estimates low. An attempt was made to passively observe reproduction in raised clear acrylic aquaria equipped with flow through sea water systems, but no eggs were produced in either of two lab populations after a month. Aquaria were set up similar to field plots (rock rubble collected under water and artificial nesting saucers), but sand was not included as it would obscure the view of the nest.

Wiley (1973) concluded that peak reproduction occurs between February and October based on the proportion of females with ripe eggs. Steele (1997a, 1997b), estimated that peak settlement occurs June to July, which would coincide with a
reproductive peak in April and May. Methodological limitations of the present study precluded any determination of reproductive peaks. The rock rubble used to construct artificial reefs was initially bare, simulating basic rocky reef structure. As time progressed, the increased abundance of fouling organisms such as barnacles, small fleshy algae, and tubeworms fundamentally altered reef structure, community composition, and nesting space. DeMartini (1991) showed that plainfin midshipman (*Porichthys notatus*), another temperate nesting fish, produced 75% greater embryo cover in experimental nests where fouling organisms were removed compared to control nests. Although blackeye goby eggs were identified on the surface of fouling organisms in a small proportion of nests, the vast majority of eggs within a clutch were found on bare space. Males may clear space adequate to accommodate an average clutch, reducing unnecessary energetic expenditure. Changes in the abundance of fouling organisms, coupled with variable densities of blackeye gobies due to natural settlement and immigration, made it impossible to obtain unbiased estimates of peak reproduction. Increasing the frequency of data collection on egg presence, coupled with detailed information on population size structure, density, and number of breeding adults would allow for accurate documentation of seasonal fluctuations in reproduction to definitively determine if there is a peak breeding season.

Blackeye gobies did not exhibit any noticeable lunar cycle in their reproductive activities. Thresher (1984) noted several hypotheses that would select for lunar reproductive patterns, two of which, involving reproduction in hetero- or conspecifics, may explain the lack of synchrony in blackeye gobies. The first hypothesis involves timing reproduction to coincide with spawning efforts of larval prey such that larvae
would hatch at a time when food densities are the highest. The second hypothesis suggests that lunar phase may act as a convenient way to synchronize reproductive efforts among numerous individuals or species, resulting in a larval dilution effect to reduce predatory risk and larval mortality. While support for either of these methods to select for lunar synchrony is weak (Thresher 1984), they could just as well explain a lack of lunar synchrony. If other species within the habitat do not exhibit lunar synchrony than it would negate the hypothesized benefits of synchronized reproduction and selection would favor continuous reproduction to maximize lifetime fitness. Although literature investigating lunar reproductive synchrony in Southern California fishes is limited, it is already known that kelp bass (Paralabrax clathratus; Erisman and Allen 2006) and California sheephead (Semicossyphus pulcher; Adreani et al. 2004), abundant mesopredators at Santa Catalina Island, do not exhibit a lunar cycle in their reproductive patterns. If predatory species are reproducing continuously throughout a season but other prey species are not than there may be no benefit for blackeye gobies to adapt a cyclical pattern of reproduction. Further research on the reproductive patterns of other Southern California reef fish with respect to lunar phase would provide additional insight.

The absence of lunar synchrony or influence of temperature on reproduction might also be attributable to the long and variable larval duration of the blackeye goby. During their roughly two-month-long pelagic phase (Block 2011), larvae would experience several lunar cycles and temperature fluctuations. Timing reproductive effort with local conditions instead of annual or seasonal changes in currents and average sea surface temperature is likely to result in negligible fitness gains. Transformation size in blackeye goby larvae is thought to be between 16 to 25 mm SL (Watson 1996) and this
range of settlement size, coupled with a variable pelagic duration, suggests variation or flexibility in larval growth rates and settlement potential. Larvae have been known to delay metamorphosis (McCormick 1999) despite having reached adequate settlement size (Victor 1986), presumably increasing exposure to potential settlement habitat. A similar process may exist in blackeye gobies, promoting continuous reproduction to supply a pelagic larval stock with the capability for differential or selective settlement, distributing risk over larger areas and time periods.

Behavioral rates of males, females, and juveniles were unaltered by the presence of eggs on the reef. Large males were the most aggressive, a trend observed in this species (Cole 1984) as well as other protogynous species (Warner and Schultz 1992) as a form of social dominance and territorial defense. Courtship and nesting were rarely, if ever, exhibited by females or juveniles, although their foraging rates were much higher than was observed in males, as would be expected by the ecology of a sex-changing fish (Wiley 1973, Ross 1990, Rodgers et al. 2007).

Somewhat surprisingly, rates of male aggression and nesting behavior did not change despite their control of a nest containing eggs. Multiple clutches, presumably from numerous females, were noted in a single nest on several occasions and were often in close proximity, if not overlapping, within the nest. Males continually court additional females, despite the potential for damaging or dislodging eggs, presumably to maximize reproduction. Typical male behavior such as maintaining nest control, dominating nearby subordinates, nest cleaning, and egg fanning may eliminate the need for increased nest defense. Warner and Hoffman (1980), however, showed that population density significantly influences the allocation of time and energy devoted to territorial defense.
and courtship in a protogynous labrid, the bluehead wrasse \textit{(Thalassoma bifasciatum)}. Although artificial reefs were stocked with standard densities reflecting those of natural reefs at Catalina (~ 6 ind\textbullet m\textsuperscript{-2}, Cole 1984, \textit{personal observation}), densities of ~ 9 ind\textbullet m\textsuperscript{-2} (Love 2011) and even as high as ~20 ind\textbullet m\textsuperscript{-2} (Steele, unpublished data) have been noted. Similarly, nest sites were abundantly available to nesting males on artificial reefs, which could alleviate density-dependent competition for optimal nesting space. Increasing adult densities or decreasing the number of suitable nesting sites would increase the frequency and intensity of competitive interactions among territorial adults, ultimately increasing energetic demands for territorial defense.

Results from this study suggest reproduction comes at a low cost to blackeye gobies. Selection therefore appears to favor year-round reproduction without any lunar patterns; however, it is still unknown whether a reproductive peak occurs throughout the year. Temperature did not affect reproduction although seasonal temperature shifts may influence mean incubation time which could have reproductive implications. If female blackeye gobies exhibit a preference for nests containing early stages eggs, than the rate at which nests cycle to a new clutch could regulate reproduction. Incubation time has been shown to decrease with increasing temperature (Lasker 1964, Miranda \textit{et al.} 1990), which would reduce cycling time and ultimately increase reproductive output.
Tables and Figures

Table 1. Focal behaviors as described by Wiley (1973).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
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| Aggression | Flaring fins and/or gaping mouth  
Chasing |
| Courtship | Flaring fins while undulating body outside nest opening  
Quick rushes in and out of nest |
| Nesting | Removing sediment or growth from inside nest  
Body undulations within nest entrance aerating eggs |
| Foraging | Short bursts into the water column picking at detritus  
Sifting mouthfuls of sediment for infaunal organisms |
Figure 1. Artificial reef design. A) The reef array was placed along the southern side of Big Fisherman Cove in two parallel lines. B) Each artificial reef was composed of four interconnected sub-reefs positioned evenly within the 2.25-m² plot to maximize habitable space. Three inverted terracotta potting saucers served as artificial nests on each reef. C) Reefs were categorized by depth, shallow (~ 10 m) or deep (~ 13 m) and position relative to the back of the cove.
Figure 2. A) Terracotta nesting saucer. A small slot along one edge provides easy access for males, promoting artificial nest use over natural rock. B) Close-up photo of a single clutch. Individual eggs are easily identifiable. C) A recently laid clutch of eggs. D) The same clutch 7 days later. Notable changes in coloration occur as well as the visible presence of developed embryos within eggs.
Figure 3. The average number of eggs produced by reef populations (solid) relative to lunar phase (dashed) during two months in 2012. Cyclical patterns, coinciding with lunar phase or tidal amplitude were not apparent (see Results). Error bars represent ± 1SE.
Figure 4. Average egg production pooled over eight weeks for each depth and position combination during 2013. Despite temperature differences throughout the reef array, blackeye goby reproduction was not statistically different between depths or among positions (see Results). Error bars represent ± 1SE based on $n = 3$ or 4 per week for each group.
Figure 5. Mean daily temperature for each depth and position during two months in 2013. Deep reefs (solid) were colder on average than shallow (dashed) reefs. Reefs farther from the back of the cove were also colder on average than those near or in the middle (see Results for statistical details). Error bars omitted for clarity.
Figure 6. Behavioral expression rates among the three classes of blackeye goby: male, female, and juvenile. The presence of eggs in a reef nest did not influence behavior among focal fish (see Results). Courtship and nesting by females or juveniles were never detected during behavioral observations. Error bars represent ± 1SE.
Chapter 3

The Effects of Size-Selective Harvesting on the Growth and Reproduction of a Protogynous Temperate Reef Fish, *Rhinogobiops nicholsii*

Introduction

The exploitation of marine fishes has increased as technology has improved, ultimately reducing stocks of fishes worldwide (Jackson 1997, Pauly *et al.* 1998, 2002, Hutchings 2000, Jackson *et al.* 2001, Dayton *et al.* 2002, Pondella and Allen 2008). The sustainability of marine resources has become a hot topic in recent years, driven heavily by calls to understand species and ecosystem level effects of exploitation (e.g., Dayton *et al.* 1995). Understanding the impacts humans have on particular species and populations is vital for the maintenance of sustainable stocks for a continually growing world population.

It is well known that body size influences various aspects of life for fishes. Larger bodied individuals reproduce exponentially more (Duarte and Alcaraz 1989, Bohnsack 1990, Berkeley *et al.* 2004, Gherard *et al.* 2013) and are more resilient to periods when food or shelter may be scarce (Schultz and Conover 1997, Sogard and Olla 2000). Body size also influences courtship potential or reproductive success (Warner and Schultz 1992, Danylchuk and Fox 1996, Markert and Arnegard 2007), as well as mortality risk (Brunton and Booth 2003, van Kooten *et al.* 2007).

enough time, these harvesting practices can cause population-level life history changes (Hamilton et al. 2007, Conover et al. 2009), which may cause dramatic changes in sustainable yield (Law 2000, Conover et al. 2009).

Maximum size-limits protect highly fecund adults and attempt to maintain a large breeding stock, whereas size-slot limits focus on intermediate sizes. Minimum size-limits, the most common approach, typically ensure that harvested individuals have had at least one opportunity to reproduce. This approach, however, promotes removal of the largest size-classes. Persistent removal of the largest individuals reduces average body size and may cause a directly proportional shift in size at maturity (Hutchings 2005, Hamilton et al. 2007, Conover et al. 2009). Smaller adult body size, while maintaining the effective breeding stock, reduces gross reproductive output (Conover et al. 2009). Growth rates are also expected to decrease after maturity as energetic allocation shifts to incorporate reproduction and other aspects of adulthood (Enberg et al. 2012). The resulting reductions in population and per capita growth rates make impacted populations less resilient to future harvesting or environmental variation.

Sequential hermaphroditism is a common reproductive pattern in fishes. It is predicted to arise when there is a difference in the relative reproductive value of each sex over an individual’s lifetime (Ghiselin 1969, Warner 1975b). Sequential hermaphrodites are either protogynous (female first) or protandrous (male first). Marine fishes exhibiting protogyny, the more common form (DeMartini and Sikkel 2006), are often characterized by having large males that monopolize a group of females within an established territory. Dominant males are superior competitors, have greater experience and success at eliciting courtship, and maintain larger territories than smaller males (Munday et al. 2009). The
mating success of smaller males is therefore lower than similar sized females, selecting for a life history whereby juveniles initially mature as females, reducing mating competition at small sizes, and later change to male at competitively effective sizes.

Alpha males of protogynous species maintain dominance by aggressively chasing transient competitor males and reducing energetic intake of subordinates within their territory (Koebele 1985). These agonistic interactions limit growth that would otherwise allow subordinates to compete for dominant status (Munday et al. 2009). If the alpha male is lost, through natural mortality or harvesting, growth repression is alleviated and the next largest individual, or sometimes second largest (Cole and Shapiro 1995, Munday et al. 2009), typically takes the dominant position. During this period, female growth rates are expected to increase, providing new males the ability to defend harems more effectively and ward off transient competitors (Munday et al. 2009). This produces a system that favors individuals who reach competitively effective sizes quickly and change sex to male (Munday et al. 2009, Walker and McCormick 2009) while size-selective removals targeting large individuals creates a counter pressure against those same size-classes. In addition, large-size-selective harvesting in sex-changing species often results in the disproportionate removal of the terminal sex, further skewing an already skewed sex ratio (Webb and Kingsford 1992, Hamilton et al. 2007, Munday et al. 2009), increasing the potential of gamete limitation (Molloy et al. 2008) and a population crash (Alonzo and Mangel 2005).

Controlled manipulative studies are necessary to establish the causal links between size-selective removals and changes in life history and demographic rates. Such experiments are difficult because most harvested species are slow growing, mobile,
sparse, and have long generation times, making manipulative studies unreasonable at ecologically relevant scales. Observational studies have assessed harvesting impacts on protogynous fish populations (Hamilton et al. 2007, 2011, Caselle et al. 2011, Mariani et al. 2013) and have found that heavily harvested populations have reduced size at maturity and size at sex change, although the magnitude of those changes are regionally distinct. While these studies provide compelling evidence for the potential impacts of size-selective harvesting on protogynous fishes, inherent differences among sites, resource availability, habitat degradation associated with fishing tactics (Hamilton et al. 2007, 2011), and density- or temperature-dependent factors (Caselle et al. 2011) could create some of the patterns that seem to be attributable to size-selective harvesting.

Using the small, protogynous, reef-associated blackeye goby, *Rhinogobiops nicholsii* (Bean), as a model study species, this study is the first to measure the direct impacts of size-selective harvesting on protogynous hermaphrodites. By selectively manipulating the density and size structure of controlled blackeye goby populations, reflecting typical fisheries management, the goals of my study were to identify the effects of size-selective harvesting on the (1) growth, (2) incidence of sex change, and (3) reproductive output of a protogynous hermaphrodite.

**Materials and Methods**

**Study Species**

The blackeye goby (*Rhinogobiops nicholsii*) is a small, protogynous hermaphrodite that is typically associated with rocky reefs in temperate waters along the coast of North America. It lives at depths of 6 to 60 m (Wiley 1973) along the sand-reef interface (Love 2011). The small size, short reproductive cycle, and small home range of
this fish make it ideal for manipulative studies. Adult blackeye gobies generally occur in densities of ~ 6 individuals per m² (Cole 1984) and peak spawning is speculated to occur between February and October (Wiley 1973, Love 2011); however they are known to spawn year-round in Southern California (Chapter 2). Planktonic larvae recruit to rocky reefs after a pelagic larval period that averages ~ 59 days (Block 2011) at 15-25 mm standard length (SL) (Steele, unpublished data). Females mature two to three months after settlement, at roughly 4.5 cm SL (Wiley 1973), with some females later changing sex to male at ~ 7.5 cm SL (Wiley 1973, Cole 1983). Males establish nests by burrowing under overhead rock where females lay a clutch of adhesive eggs that the male will defend (Ebert and Turner 1962). Males will dominate subordinate individuals within their territory, which is up to 1.2 m² (Cole 1984), and they will defend the clutches of eggs laid by up to 6 females (Love 2011) until they hatch. Estimated incubation time is 1-2 weeks (Chapter 2).

**Study System**

From July to September, 2013, I conducted a field experiment to evaluate the impacts of size-selective harvesting of populations of blackeye gobies on twenty 2.25-m² artificial rock rubble reefs constructed in Big Fisherman Cove, Santa Catalina Island, California (33°26’42”N, 118°29’8”W) (Fig. 1). Reefs were built in two parallel lines of ten at ~ 10 and 13 m depth, with 10 m spacing between each reef and any nearby natural reef structure to minimize movement of fish among reefs. Each reef was comprised of ~ 60 L of rocks, 5-40 cm in size, placed on a square PVC frame lined with plastic mesh to prevent the rocks from sinking into the sand over the study period. Piscivorous fishes
are abundant in Big Fisherman Cove, so each reef was encompassed in an exclosure cage (3.8-cm plastic mesh) to reduce mortality rates.

Gobies were collected by SCUBA divers with dip nets from natural reefs between Lion’s Head Point (33°27’08”N, 118°30’05”W) and Little Geiger Cove (33°27’32”N, 118°30’59”W). Sex was determined via examination of the genital papillae (Wiley 1973, Cole 1983), a reliable indicator of sex in many gobiid species (Cole and Robertson 1988), and length (mm SL) was measured. Each individual was injected with two subcutaneous, colored, elastomer tags (Northwest Marine Technology Inc.) indicating sex and size (Fig. 2).

**Experimental Treatments**

Reefs were stocked with a standard density and size-distribution of blackeye gobies as estimated from surveys conducted at collection sites. Three size-selective removal treatments, reflecting typical size-based management protocols (minimum, slot, and maximum size), were used to evaluate the effects on reproduction and growth. A control group was used to represent no harvest. Because protogynous species are typically sexually dimorphic in size, removal of large (>7.5 cm SL) individuals primarily reduced the density of males, removal of medium (4.0 – 7.5 cm SL) sized individuals primarily impacted females, and removal of small (<4.0 cm SL) individuals impacted juveniles. Control populations were caught and handled in the same way as treatment populations but all individuals were placed back on the reef.

Population densities were visually estimated regularly using the tags of fish, which were visible to divers, to identify the sex and size of all individuals on
experimental reefs. Time-averaged densities were calculated from the presence/absence data to estimate the number of males, females, and juveniles present on each experimental reef during the study period. If an individual was seen during two consecutive observations, it was assumed to have been present on all days between the observation dates. If a fish was observed one day but not the next, it was counted as present on half of the days between the two observations. If there were gaps between sightings of a fish, it was assumed to be present during the gap but missed by the observers. The time-averaged density of each size class was calculated as the sum of all days present for all individuals in a size class, divided by the duration of the experiment.

Multivariate analysis of variance (MANOVA) comparing the densities of the three size-classes (males, females, and juveniles) present on each reef was used to test whether the size-selective removal treatments were successfully implemented and maintained. The three response variables were the time-averaged densities of each of the three size-classes per reef with removal treatment as the categorical predictor variable.

**Growth**

Fifty-five days after the removal treatments were imposed, all remaining fish were collected, sexed, and remeasured. Length measurements from day 0 and day 55 were converted to mass using the following length-to-weight equation:

\[
\text{Mass}(\text{g}) = 0.00002 \cdot \text{SL}(\text{mm})^{3.0078}
\]

\((n = 76, r^2 = 0.95, p < 0.0001, \text{Schram unpublished})\). Using these two estimates of weight, growth over the course of the experiment was calculated and expressed as daily per capita growth \((\text{g} \cdot \text{day}^{-1})\).
One-way analysis of variance (ANOVA) was used to test for differences in growth rate among the four treatment groups using average per capita daily growth on each reef as the response variable. Growth data met parametric assumption of normality and homogeneity of variance. Growth regulation is socially influenced in many protogynous species, which could result in high rates of growth for some size-classes and low rates for others within the same population, which would not be evident in average growth rates. Therefore, MANOVA was also used to test whether the treatments affected the three size-classes differently. The average per capita growth rates of each size class on each replicate reef were the response variables and treatment was the categorical variable.

Sex change

Incidence of sex change was determined by recording the sex of all fish after recapture. A contingency table was used to determine whether there were differences in the number of females that changed among the four treatment groups.

Reproduction

Each experimental reef had three artificial nests made from inverted terracotta potting saucers (Forrester et al. 2010, Fig. 3A). Males preferred these saucers over natural rocks as nest sites (Fig. 3B). Nesting saucers were checked weekly for clutches of eggs, and digital photographs of all clutches found were taken for 8 weeks, 3 weeks before and 5 weeks after density adjustments (Fig. 3C). The three weeks prior to removals were used to ensure individuals were established (i.e. males defending and
maintaining nests) and reproductively active while the five weeks after removals were used to test for the effects of removals on reproductive output. Clutch margins were outlined on the saucer to distinguish old clutches from new clutches. Photos were digitally analyzed using Image J (Abràmoff et al. 2004) to estimate the total number of eggs in each clutch. Using saucer diameter as a known value, photos of the entire saucer were set to scale. A higher resolution close-up photograph, used to conduct egg counts, was scaled using conspicuous points present in both photos. Total clutch area was measured and egg densities in five randomly selected 1-cm² subsamples were recorded. Density was assumed to be uniform throughout the clutch, and therefore the average density of the five subsamples was multiplied by total clutch area to calculate the total number of eggs in each clutch.

Differences in total egg production among treatments were tested for with one-way ANOVA. Reefs were treated as replicates and the response variable was the total number of eggs produced on that reef with treatment group as the categorical variable. One-way ANOVA was favored over repeated measures ANOVA because the 8-13 day incubation time of blackeye goby eggs (Chapter 2) could produce fluctuating weekly counts, directly confounding any “time” effect. Three a priori comparisons were also made: control vs large removal, control vs medium removal, and control vs small removal. Gobies on two of the twenty experimental reefs, a control and a small removal treatment, produced no eggs during the 8 weeks and were deemed fundamentally different than all other replicates. Those two replicates were dropped from statistical analyses of reproduction. The data met the parametric assumptions of ANOVA.
Results

The size-selective removal treatments were successful in altering the size structure of the replicate populations as intended. Throughout the experimental period removal treatment densities were ~ 25% lower than control densities (MANOVA, $F_{9,34} = 4.45, p = 0.001$, Fig. 4). Male densities in the large-removal treatment were significantly lower than on all other reefs ($F_{1,16} = 16.71, p = 0.001$), as were female densities in the medium-removal treatment ($F_{1,16} = 7.17, p = 0.02$), and juvenile densities in the small-removal treatment ($F_{1,16} = 6.82, p = 0.02$).

Growth rates of individuals (g\cdot day$^{-1}$) were statistically similar among the four treatments (ANOVA, $F_{3,16} = 0.22, p = 0.88$, Fig. 5). Despite social growth regulation, growth rates of each size class were unaffected by the size-selective removal treatments (MANOVA, $F_{9,34} = 0.94, p = 0.50$, Fig. 6). Incidence of sex change was statistically independent of treatment group ($\chi^2 = 2.71, df = 3, p = 0.44$, Table 1). The average number of juveniles that matured was low and relatively similar among treatment groups as well (Fig. 7). The population structure of treatment groups did not appear to converge on either that of the control group or their respective predisturbance status (Fig. 8). Size distribution patterns were, however, similar among all four groups after final collection with the majority of individuals centered in the 51-60 mm SL size range. The final male:female sex ratios of treatment populations were closer to 1:1 than that of the control group.

While the control treatment populations tended to have the highest reproductive output, there were no statistically significant differences among treatment groups ($F_{3,14} = 1.11, p = 0.38$, Fig. 9). A priori comparisons showed no statistical difference in
the reproductive output of control treatment populations versus large removal 
\( (F_{1,14} = 3.13, p = 0.10) \), medium removal \( (F_{1,14} = 1.76, p = 0.21) \), or small removal 
treatment populations \( (F_{1,14} = 1.44, p = 0.25) \).

**Discussion**

This experiment revealed no significant effects of size-selective removals on the growth or reproduction of blackeye gobies, implying that this species is resilient to moderate intensities of size-selective harvesting. Although the findings in other systems suggest size-selective fishing practices have impacted target populations (i.e. truncated size-structure, reduced size-at-maturity, reduced size-at-sex-change, altered growth rates etc.: Sinclair 2002, Conover and Munch 2002, Hutchings 2005, Hamilton *et al.* 2007, Caselle *et al.* 2011, Mariani *et al.* 2013), the majority of those studies have only demonstrated correlative results. In those studies, the intensity of fishing pressure could only be implied from fishery-dependent catch data in the respective regions of study. The present work was the first attempt to measure direct changes in reproduction and growth of protogynous hermaphrodites caused by controlled size-selective removals.

Conover and colleagues (2009) conducted a similar experiment on Atlantic silverside (*Menidia menidia*) and showed a significant reduction in the mean weight of populations subjected to large size-selective removals over multiple generations; however, the impacted populations were able to rebound relatively quickly after removals stopped. Conover and colleagues (2009) reduced *M. menidia* densities by 90% in each generation, significantly more than the ~25% density reductions in the current study. Although *M. menidia* do not change sex, the study highlights a genetic capacity for the
species to recover despite artificial selective pressure induced by size-selective fishing over generations (Conover et al. 2009). A similar capacity may be present in blackeye gobies, and perhaps other protogynous hermaphrodites. The ecology of protogynous hermaphrodites, along with the evolution of a protogynous mode of reproduction based on the size-advantage model (Ghiselin 1969, Warner 1975a), already selects for increased growth rates and larger body size, suggesting the presence of some genetic component that could persist in the population (Munday et al. 2009, Walker and McCormick 2009). Studies investigating populations of sex-changing species under variable harvesting pressure have also noted flexibility in the size at sex-change among those populations (Linde and Palmer 2008, Mariani et al. 2013). These results suggest that protogynous species have the flexibility to respond to changes in their population structure, whether it is from natural or anthropogenic sources, to maintain functional sex ratios and moderate levels of reproduction. Protogynous species, therefore, likely maintain some capacity to buffer removals to a certain degree, and the extent by which they can buffer those removals, or even recover upon cessation, is likely a complex function of harvesting history, relative body size, life history, and population dynamics.

A post-hoc power analysis revealed only a ~30% chance to detect a 50% decrease in reproductive output due to removals, primarily due to the high variability in reproductive output among replicate reefs. During any given collection period, a single reef could have no clutch or a number of clutches totaling as many as 30,000 eggs, introducing considerable variance. While increasing the number of replicate reefs per treatment would address the large variance, it would be logistically challenging to achieve, and it would not address natural variability in reproductive output. Natural
variation could be caused, for example, by variation in the abundance of predators, or female body size. The presence of predators, despite the use of exclusion cages, has been shown to alter the behavior of prey through visually perceived risk (Magnhagen 1990, Forsgren 1992, Candolin 1998). Piscivorous predators are abundant in Big Fisherman Cove and were often found near artificial plots which could result in differential impacts on reproduction among replicate reefs over time. Similarly, individuals on replicate reefs were actively growing and body size ultimately influences the number of eggs produced (Duarte and Alcaraz 1989, Gherard et al. 2013). As reproductively active individuals increased in size, the potential number of eggs produced would increase (e.g., 0 – 10,000 eggs vs 0 – 30,000 eggs), introducing greater variance in reproductive estimates over time. While normalizing reproductive estimates to account for average adult body size may address variable reproductive output with growth, it may also introduce bias as there is no simple method to identify which females produced any given clutch.

In my experiment, growth rates were unaffected by size-selective removals. Although treatments were successful as intended, social hierarchies were not necessarily disrupted. While size has particular implications for social hierarchy position, differences between an alpha and beta male was as little as 2-3 mm. Removal of the alpha male still left a male of relatively similar biological or social equivalence, maintaining a functional social hierarchy and social derived growth repression. Growth rates were also calculated from final size measurements taken 55 days after initial removals. While recording growth rates at two points in time reduced handling stress on the study populations fine scale variations in growth could not be addressed. Daily increments in otoliths could be used to explore short-term responses in somatic growth relative to population
manipulations (Oozeki and Watanabe 2000, Thorrold and Hare 2002, Molony and Choat 2006, Munday et al. 2009), but these bands are difficult to read accurately in otoliths of blackeye gobies from adults and older juveniles (Block 2011, personal observation). This approach, however, may be useful in other species. Growth differences in larger longer-lived species could also be easier to detect, through direct measurements or seasonal variations in otolith banding patterns, and thus provide better estimates of biologically relevant effects.

Blackeye gobies are significantly smaller than most harvested protogynous species. They reproduce year-round (Chapter 2) and quantifying reproduction is relatively easy, making them a good model species; however, their small size also influences aspects of their biology. Smaller bodied species tend to be $r$ selected, maturing at young ages with fast growth rates (Adams 1980), decreasing the time required to reach sexual maturity relative to the more desirable larger bodied species. Smaller bodied species are therefore likely able to replace individuals with high reproductive value at a greater rate than larger species under similar harvesting pressure. The impacts of size-selective removals on reproduction or growth may therefore be a function of harvesting frequency and intensity relative to the average body size of target taxa.

Unlike many existing fisheries, this experiment only imposed a single removal event at a relatively low intensity. Population densities were reduced by ~ 25%, however many fisheries aim to reduce population densities by 50% (Levinton 2009b), the point at which the theoretical maximum sustainable yield (MSY) can be achieved. Moreover, many fisheries reduce populations by much more than 50%, commonly by as much as 90% (Myers and Worm 2003). Reductions of that magnitude coupled with size-selective
practices would be expected to have a greater impact on harvested populations than did the simulated harvesting imposed in the present experiment. Future research incorporating a wider range of species, removal intensities, and frequencies would provide additional insight into the impacts size-selective harvesting has on protogynous species. That information could then be used to improve fisheries management tactics to optimize production and sustainability of yields of fisheries for protogynous marine fishes.
Tables and Figures

Table 1 – The number of females that changed sex pooled across replicate reefs. Incidence of sex change was not statistically different among treatment groups (see Results).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Females that Changed Sex</th>
<th>Total</th>
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<td></td>
</tr>
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<tr>
<td>Small</td>
<td>16</td>
<td>2</td>
<td>18</td>
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</table>
Figure 1. Study site map. The experimental reef array was constructed along the western edge of Big Fisherman Cove in two parallel lines of ten at 10 and 13 m depth. Gobies were collected by SCUBA divers from reefs at and around Lion’s Head point.
Figure 2. A) Each blackeye goby received two, colored, elastomer tags indicating sex and size. A sex tag was injected near the head on the left side of the body. A size tag was injected in one of ten unique positions along body. B) The color of the size tag corresponded to a 10-mm size range unique to each size-class, and tag position indicated the size of that individual to the nearest mm. C) This fish is a male (blue) that is 66-mm long (orange).
Figure 3. Artificial reef design. A) Predator exclusion cages encompassed each 2.25 m$^2$ reef to minimize mortality caused by predators. Three nesting saucers were spread out across the reef. B) One of the three artificial nests. A small slot cut along one edge served as a nest entrance. C) A single clutch of eggs in a saucer nest. This clutch, which is $\sim$ 21 cm$^2$, contains roughly 4800 eggs.
Figure 4. Average densities of each sex stage over the duration of the experiment. Population structures differed among the four treatments as intended. Specifically, male densities were significantly lower in the large-removal treatment compared to all other treatments, as were female densities in the medium-removal treatment, and juvenile densities in the small-removal treatment (see Results). Error bars represent ± 1SE based on $n = 5$ for each bar.
Figure 5. Average growth of blackeye gobies in four size-selective removal treatments. Growth rates did not differ significantly among the treatments (see Results). Error bars represent ± 1SE based on \( n = 5 \) for all treatments.
Figure 6. Average per capita growth of three size-classes of fish in each size-selective removal treatment. Treatments did not differentially influence growth rates among the three size-classes (see Results). Error bars represent ± 1SE based on $n = 5$ for all treatments.
Figure 7. Average number of juveniles that matured among treatments. Maturation counts were relatively low; however, the recovery of juveniles at the end of the study was also limited.
Figure 8. Population structure of treatment groups pooled across replicate reefs. Before removal (Left) – Prior to implementing removals. After removal (Middle) – 2-4 days after removals were implemented. Final collection (Right) – All remaining individuals were collected 55 days after initial removals.
Figure 9. Total numbers of eggs produced per reef over 5 weeks after the size-selective removals. Size-selective removals had no impact on reproduction (see Results). Error bars represent ± 1SE based on $n = 4$ or 5 for all treatments.
Chapter 4

Conclusions

My thesis examined previously unaddressed factors influencing blackeye goby reproduction and used this species as a model study species to investigate the direct effects of size-selectively harvesting protogynous fishes. Results from the first part of my work indicate that blackeye gobies reproduce year-round and do not time reproductive efforts with any particular lunar phase, nor does temperature influence reproduction. Behavioral changes that would presumably increase the proportion of hatched eggs were also not apparent. Aspects of blackeye goby ecology, particularly their variable and length pelagic larval duration and size-based social dominance hierarchy, likely minimize any fitness benefits that could be gained by responding to short-term, local-scale shifts in environmental conditions.

Forrester et al. (2010) demonstrated the use of artificial nests to directly measure reproduction of a tropical goby in the field; however, that particular methodology had not yet been demonstrated to work with the blackeye goby. Bluebanded goby (Lythrypnus dalli) and zebra goby (Lythrypnus zebra) reproduction has been directly recorded in the field around the site studied for my work (St. Mary 1994, Kappus 2012); however, the bi-directional nature of their sex change adds complexity to manipulative methods such as those used here. Blackeye gobies are protogynous, representing the most common sequentially hermaphroditic fish. Likewise, they readily used artificial nest saucers over natural reef in the field and digital analyses of clutches were successful.
The second part of my thesis research revealed that simulated size-selective harvesting had no effect on the reproductive output or growth of blackeye gobies. This result implies that populations of protogynous species may have the capacity to buffer moderate losses. In my experiment, size-selective removals were imposed only once and were relatively conservative, at ~ 25%, to ensure that not all individuals of a given sex were removed from any population. Compared to the typical 50% harvest recommended to achieve maximum sustainable yield or much larger reductions caused by modern fisheries (Myer and Worm 2003), the 25% reduction in my experiment was modest. Whether more intense and sustained removals might generate the changes observed in heavily fished populations of protogynous fish (e.g., Hamilton et al. 2007, 2011, Caselle et al. 2011) remains to be investigated. Experimental populations exposed to a range of size-selective removal intensities and frequencies would elucidate the cause of discrepancies between manipulative and observational work.

Although the extent to which the results of my small-scale experiment on the blackeye goby can be applied to address broad scale effects of size-selective harvesting on protogynous fishes is uncertain, this work does serve as a proof of concept. The ability to directly measure reproduction, eliminating uncontrolled variation associated with conventional recruitment surveys, coupled with the success of short and long-term monitoring provides a foundation for more in-depth research using this model study species aimed at determining the direct effects of size-selective harvesting on protogynous hermaphrodites.
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