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The influence of population density and sex ratio on reproduction  
and sex change of a temperate reef fish, *Lythrypnus dalli*

A dissertation submitted in partial satisfaction of the requirements  
for the degree Doctor of Philosophy in Biology

by

Sara M. Kappus

2012

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## ABSTRACT OF THE DISSERTATION

The influence of population density and sex ratio on reproduction  
and sex change of a temperate reef fish, *Lythrypnus dalli*

by

Sara M. Kappus

Doctor of Philosophy in Biology

University of California, Los Angeles, 2012

Professor Rick Vance, Co-chair

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Population size is ultimately determined by processes influencing reproductive success of individuals. While processes influencing recruitment, growth and survival have received much attention in marine populations, factors influencing reproductive rates are less well understood. We tested for an influence of population density and sex ratio on reproduction (egg production) of populations, per capita reproduction of individuals, and the frequency of sex change in the bi-directionally sex-changing goby *Lythrypnus dalli*. By manipulating populations of *L. dalli* on artificial reefs in the field and in laboratory aquaria, we provided evidence that reproduction was influenced by natural levels of variation in these two population characteristics and that these relationships differed between laboratory and field populations. While total egg production increased with increasing density in field populations, per capita reproduction declined. In

contrast, per capita reproduction was not density-dependent in a small-scale laboratory experiment. While, the frequency of sex change was density-dependent in the laboratory experiment it was not on reefs in the field. Together these results indicate that small scale laboratory studies may not be sufficient for evaluating factors causing variation in reproductive success of natural populations of *L. dalli*. We suggest that decreasing social stability in larger populations in more complex environment drive these differences in sex-change behavior and individual reproductive success. In a separate experiment, we found that per capita reproductive success of females did not decline in female-biased field populations and thus, for a given density, populations with the most females tended to produce the most eggs. Reproductive success of males, however, strongly increased in female-biased populations and was much higher than average reproductive success of females. Despite the influence of sex ratio on relative reproductive success of males and females, we found no evidence that individuals use sex ratio as a cue for sex change. Rather, sex change of females could be predicted from their body size, and this relationship did not vary with population density. Sex change of males was uncommon and not predicted by on body size, population density, or sex ratio. These results have deepened understanding of the factors that influence individual reproductive success and reproductive rates of populations.

The dissertation of Sara M. Kappus is approved.

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2012

In memory of my mother, Sue Ellen Painter. Without her support, love  
and encouragement I would never have journeyed so far.

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## INTRODUCTION

To understand and predict changes in the size of any population, we must first understand processes that influence birth and death rates. Variation in population characteristics, such as density or sex ratio, may influence population size through their effects on rates of mortality (Hille Ris Lambers et al. 2002, Webster 2003, Steele and Forrester 2005) or reproduction (Røed et al. 2002, Solberg et al. 2002). For instance density-dependent processes are known to influence the number of individuals entering populations through effects on settlement (Takashi et al. 1998, Webster 2002) and recruitment (Steele et al. 1998, Harms et al. 2000, Ribeiro et al. 2005). The sex ratio of adults may also influence the number of individuals entering populations through effects on reproductive behavior of individuals such as the intensity and direction of sexual selection (Kvarnemo and Ahnesjö 1996, Prohl 2002, Kasumovic et al. 2008, de Jong 2009). To better understand the dynamics of populations, we must understand how variation in population characteristics influences the ecology of individuals.

Processes that influence reproduction of populations have been under evaluated in most marine species. Per capita reproductive output of marine populations was long thought only to influence adult population size at the large metapopulation scale as planktonic larvae have the potential to disperse long distances from their natal populations (Scheltema 1986, Shanks et al. 2003), effectively decoupling local reproduction from settlement of new individuals. Consequently, most marine populations have long been considered demographically open (Hixon et al. 2002). As a result, local population size is believed to be determined by some combination of long-distance larval supply and by local post settlement processes such as competition and predation (Doherty and Fowler 1994, Caley et al. 1996, Armsworth 2002, Siegel et al. 2008). However, major advances in our understanding of the connectivity of marine

populations has revealed that some populations may be demographically closed on smaller scales than previously understood and therefore may, to some extent, rely on local reproductive output (reviewed in Swearer et al. 2002, Levin 2006). Evidence for retention of larvae to local populations comes from studies of ocean currents (Swearer et al. 1999), marked otoliths of developing embryos (Jones et al. 1999, Swearer et al. 2002, Morgan et al. 2009), and genetic dissimilarity between neighboring populations (Palumbi and Warner 2003, Taylor and Hellberg 2003). If a large portion of pelagic larvae return to settle in their natal populations then processes influencing the production of larvae may strongly contribute to changes in size and size structure of marine populations over time.

Sex change is prevalent among a wide range of taxa (reviewed in Policansky 1982, Munday et al. 2006), and variation in the frequency of sex change among populations may ultimately influence rates of reproduction. Sex change of marine fishes is a phenotypically plastic trait that is socially controlled (Warner and Swearer 1991, Munoz and Warner 2003, Liu and Sadovy 2004, Black et al. 2005), and variation in the timing of sex change is thought to allow individuals to maximize reproductive success under different social conditions (Warner 1988a, Munday et al. 2006). Changes in population characteristics, such as size structure, density or sex ratio may generate substantial variation in the frequency of sex change in natural populations. For instance, fluctuations in population size distribution were correlated to variation in the size at sex change in multiple taxa (Charnov and Anderson 1989, Guest 2004). The size advantage model has been successful in explaining the adaptive significance of sex change in many species (Warner 1975, 1988a), and relative body size is often proposed as the primary cue individuals use to determine social status and functional sex (Ghiselin 1969, Warner 1988a, b, Rodgers et al. 2007). However, whether the importance of relative body size in determining sex



change varies with population characteristics, such as density or sex ratio, is largely unknown. Currently we have little understanding of the population characteristics that cause variation in sex change and how such variation may ultimately influence reproductive rates.

Processes influencing reproduction may be density-dependent and such processes have received much interest in an attempt to understand the mechanisms by which populations are regulated (Brook and Bradshaw 2002, Hixon et al. 2002). Despite a thorough understanding of the processes causing density-dependent mortality in marine fishes (Shima and Osenberg 2003, Webster 2003, Steele and Forrester 2005, Schmitt and Holbrook 2007, Johnson 2008), little is known regarding the effects of density on reproduction (but see Forrester et al. 2010). Density-dependent reproduction may arise in females if high density increases competition for resources and increases stress levels resulting in reduced allocation of energy to egg production (Lindstrom et al. 2005, McCormick 2006), and it may arise in males through competition for mating sites resulting in limited mating opportunities (Lindstrom 1988, Jirotkul 1999). Variation in population density may also influence social interactions, reducing the stability and strength of dominance hierarchies at high densities (Alanara and Brannas 1996, Ang and Manica 2010a), ultimately influence the frequency of sex change in populations. The influence of population density on the frequency of sex change, the nature of this relationship or the impact to reproduction is largely unknown.

Similarly, the responses of individuals to variation in adult sex ratio are also poorly understood (Le Galliard et al. 2005). Variation in the adult sex ratio may arise via differences in the sexes in age at maturity, reproductive longevity, sex change behavior (in hermaphrodites), and differential mortality (Lovich and Gibbons 1990, Clutton-Brock and Parker 1992, Arnold et al. 2003, Donald 2007). Reproductive behavior in marine fishes has been shown to be strongly

influenced by the adult sex ratio (Kodric-Brown 1988, Itzkowitz 1990, Kvarnemo 1995, Le Galliard et al. 2005, de Jong 2009), and yet the corresponding consequences to reproductive rates of populations are largely unknown. Examinations of how variation in adult sex ratio influences reproductive behavior, and consequently reproductive success, will deepen our understanding of variation in reproduction among populations.

Small-scale laboratory studies are often used to determine factors important for influencing reproductive success and behavior in marine fishes (Stanley and Wootton 1986, Lindstrom 1988, Karino 2006, Rodgers et al. 2007, de Jong 2009), but for many species, it is unknown whether the importance of these factors similarly influence natural populations. I investigated the influence of two population characteristics (density and adult sex ratio) on reproductive rates of populations, per capita reproductive success, and frequency of sex-change in a temperate reef fish, *Lythrypnus dalli*, and evaluated the potential for density-dependent processes occurring in a laboratory studies to approximate processes occurring in natural field populations. *Lythrypnus dalli* are bi-directionally sex changing hermaphrodites common among rocky subtidal habitats in southern California. Bi-directional sex change (in which both males and females can change sex) is an extremely flexible reproductive strategy that is likely highly sensitive to the social environment and thus to changes in population density or adult sex ratio. Most of the research examining the proximate mechanisms governing sex change in this species focuses on small social groups and is conducted in laboratory aquaria (Rodgers et al. 2005, Lorenzi et al. 2006, Rodgers et al. 2007, Lorenzi et al. 2009). Little is known regarding the frequency of this behavior or its adaptive significance in natural populations. In chapter 1 and 3, I examine the relationship between population density and reproductive output and the frequency of sex change of *L. dalli* and compare the results of a small laboratory experiment with that of a

larger field experiment. In chapter 1, I quantify the influence of density on population egg production, per capita egg production, and reproductive success of males. In chapter 3, I tested for an influence of population density on 1) the frequency of sex change and 2) the relationship between body size and sex change. In chapter 2, I investigated the effect of adult sex ratio on reproduction and the frequency and direction of individual sex change. The results of these studies have deepened understanding of the factors that influence individual reproductive success and consequently cause variation in reproductive rates of populations.

## Chapter 1:

# POPULATION DENSITY INFLUENCES PER CAPITA EGG PRODUCTION IN A TEMPERATE REEF FISH

### **Abstract:**

Density-dependent processes have received much attention in an attempt to understand the mechanisms by which populations are regulated and to better predict and manage these populations. While the processes causing density-dependent mortality in marine fishes are well-studied, little is known regarding the effects of density on reproduction. We tested for density-dependent reproductive output in both field and laboratory populations of the temperate marine goby *Lythrypnus dalli*. The total number of eggs produced increased at high density in the field experiment yet per capita egg production was negatively density-dependent. In contrast, per capita reproduction did not significantly decline with density in the laboratory experiment suggesting that examinations of fish in the laboratory may not yield accurate approximations of the processes influencing reproduction of natural populations. Growth of fish in the laboratory aquaria was lower than fish in the field suggesting that conditions in captivity may have restricted energy available for reproduction and resulted in reduced per capita egg production at all densities. In both experiments the proportion of males that received eggs within a nesting site declined with density, yet the density of eggs within their nests increased, indicating that mating opportunities are restricted at high density to relatively fewer males that each receive higher average reproductive success. Such changes in the reproductive success of individuals may be driven by changes in social interactions with increasing density. At high density larger females

may express stronger preference for large males as mating partners and suppress mating by smaller subordinate females. Our research contributes to a limited body of evidence suggesting that reproduction of fishes may vary among natural populations of differing density. Such variation in reproduction may ultimately influence larval supply at the spatial scale over which populations become reproductively closed.

### **Introduction:**

Understanding the processes by which populations are regulated is essential to inform the conservation and management of many organisms. It is generally thought that most populations are regulated at some scale by density-dependent processes (Steele 1997, Brook and Bradshaw 2002, Hixon et al. 2002). Population regulation can occur through density-dependent mortality, density-dependent reproduction or some combination thereof. A multitude of life history characteristics have been shown to vary with population density including growth (Steele and Forrester 2002, Johnson 2008, Drago et al. 2010), sexual maturity (Booth 1995, Cooper et al. 2009) and mortality (Forrester and Steele 2000, Hille Ris Lambers et al. 2002, Webster 2003, Steele and Forrester 2005). In marine organisms density dependence has also been detected in settlement (Takashi et al. 1998, Webster 2002) and recruitment (Steele et al. 1998, Ribeiro et al. 2005) of juveniles to populations. These density-dependent processes act similarly to reduce population size when large by decreasing the number of individuals in the population. Despite our current understanding of how density may ultimately affect the longevity of marine organisms, the influence of density on the generation of new organisms is largely unknown (but see Forrester et al. 2010).

Unlike terrestrial or lacustrine systems where there are strong links between processes influencing reproductive output and abundance (Elliott 1984, Montgomery 1989, Johnson and Geupel 1996, Vandebos et al. 2006), it has long been assumed that processes influencing per capita reproductive output of marine populations were not strong contributors to the local abundance of adults. Many marine species have a bipartite life cycle in which relatively sedentary adults produce planktonic larvae that have the potential to disperse large distances from their natal populations. The number of settlers arriving at any suitable habitat may be largely influenced by environmental processes occurring during the larval stage (Siegel et al. 2008). The resulting abundance of adults would then be determined, not by local reproductive output, but by some combination of larval supply and post-settlement processes (Doherty and Fowler 1994, Caley et al. 1996, Armsworth 2002). As a result, factors influencing reproduction were thought only to influence population dynamics at the large, metapopulation scale. For this reason the potential for density-dependent processes to influence reproductive output and larval supply has not been evaluated for many marine organisms.

Recent studies of connectivity of marine populations have revealed that many marine populations may, to some extent, rely on local reproductive output (reviewed in Swearer et al. 2002, Levin 2006), and suggest that the historic lack of focus on the factors influencing reproduction may be limiting our understanding of population dynamics. Ocean currents have the potential to distribute larval fish over vast distances; however, in some cases they may actually retain juveniles near their natal populations (Swearer et al. 1999, Morgan et al. 2009). As a result, a significant portion of juveniles may return to settle in their natal populations (Jones et al. 1999, Swearer et al. 2002) resulting in demographic closure, or near closure, of populations at scales smaller than previously anticipated (Palumbi and Warner 2003, Taylor and Hellberg

2003). Thus, fluctuation in the number of larvae produced in local populations may produce considerable variation in local population size. Recognition that demographic closure of marine populations may occur at small spatial scales highlights the need to understand sources of variation in reproductive output of marine fishes.

Density-dependent reproduction is generally thought to arise in females when increasing competition for resources or increased stress result in reduced energy for egg production (Lindstrom et al. 2005, McCormick 2006), and in males when competition for mating sites results in limited mating opportunities (Lindstrom 1988, Jirotkul 1999). Female egg production may decline due to reduced fecundity, reduced spawning frequency, or some combination thereof (Forrester et al. 2010). Territorial behavior and competition for mates among males is known to increase with density and may limit opportunities for females to reproduce (Stanley and Wootton 1986). For instance, decreased per capita egg production in the zebrafish, *Danio rerio*, at high densities resulted from increased aggression of males interrupting female spawning attempts (Spence and Smith 2005). Per capita reproduction may also decline from the influence of density on other life-history characteristics such as growth. Individual growth rates are often lower at high densities and reproduction may be reduced or delayed at small body sizes. For example, spawning frequency in two species of anchovies was found to vary with body size, with large females spawning more frequently (Claramunt et al. 2007). It is unknown how prevalent these mechanisms are among natural populations or whether they act independently or interactively to cause density-dependent reproductive output.

Small-scale laboratory studies are often used to determine factors important for influencing reproductive success and behavior in marine fishes (Stanley and Wootton 1986, Lindstrom 1988, Karino 2006, Rodgers et al. 2007, de Jong 2009), yet for many species, it is

unknown whether the patterns discovered in the lab also occur in natural populations. We tested for and compared patterns in density-dependence in reproductive output of a temperate reef fish, *Lythrypnus dalli*, in both a field and laboratory experiment. *Lythrypnus dalli* are small site-attached gobies with polygynous mating systems. Females may spawn repeatedly over the breeding season, and multiple females may spawn simultaneously into cryptic nesting sites defended by males, that then guard the eggs until hatching. We manipulated goby density on artificial patch reefs in the field and in laboratory aquaria to determine the influence of population density on per capita egg production and reproductive success of males.

## **Methods:**

### *Study Species:*

*Lythrypnus dalli* are a brightly colored benthic fish that occupy physically complex rocky subtidal habitats along the coasts of Southern California and Baja California, Mexico. Populations are female biased (usually about 75% female) and densities commonly range from 24 to 58 individuals per square meter (Behrens 1983). Individuals primarily feed on free-swimming planktonic organisms by darting into the water column from a rocky perch (Wiley 1976). *Lythrypnus dalli* are bi-directionally sex changing hermaphrodites with socially controlled sex change. Although individuals maintain some allocation to both male and female gonad tissue, they exhibit only one functional sex at a time, reproducing as either male or female (St. Mary 1994). Reproduction is seasonal and females with developed eggs are commonly found from May to August (Wiley 1976, Behrens 1983). Males guard and defend nest sites into which multiple females lay their demersal eggs. Larvae hatch after 5-7 days and spend about 6 weeks in the plankton before settling on a rocky reef (St. Mary 1994). Life expectancy of *L. dalli* is short



(1.5-2 years) relative to the length of the reproductive and recruitment season and thus populations of *L. dalli* are dynamic, changing in size structure and density both seasonally and annually (Wiley 1976, Behrents 1983).

*Field Experiment:*

We explored the effects of population density on egg production in a field experiment during July and August 2009 by manipulating the density of adult fish on artificial patch reefs built in Big Fisherman Cove adjacent to Santa Catalina Island, California, U.S.A. (33°27'3" N; 118°29'15" W). We constructed 24 artificial patch reefs on a sandy bottom approximately 30 meters away from the nearest natural rocky reef. Reefs were built in 3 rows of 8, with 4 meters of sand separating each reef, following the depth contour of the bottom with each row at slightly different depths: 13, 15, and 17 meters. Each reef was composed of approximately the same number and size distribution of rocks collected from the nearby shore and was assembled by piling the rocks on top of plastic mesh netting (1.56 cm x 1.90 cm openings) stretched over a 1 m x 1 m polyvinyl chloride (PVC) frame. Each artificial reef measured 1 m<sup>2</sup> at the base and was approximately 0.3 m high at the center. On each reef we placed 8 artificial nesting sites (12.7 cm long 1.3 cm diameter piece of PVC with a cap on one end) each of which was lined with a sanded piece of acetate sheet that could be removed periodically to photograph the eggs (St. Mary 1994).

We anesthetized and collected *Lythrypnus dalli* from nearby natural reefs using squirt bottles containing a solution of 10% clove oil and 90% ethanol and transported them to the laboratory where we anesthetized each individual in a seawater solution containing tricaine methanesulfonate (MS-222). To ensure the size structure and sex ratio of fish stocked on artificial reefs matched that of natural reefs we measured the standard length of each fish to the

nearest 0.1 mm, and determined the sex through examination of the genital papilla under a dissecting microscope. Previous examinations found that fish containing a blunt shaped papilla spawn as female while fish with a long pointed papilla spawn as male (St. Mary 1993, Drilling and Grober 2005). We classified fish with blunt shaped papilla as female and those with a long pointed papilla as male while individuals with an ambiguous morphology (~1% of individuals) were not used in the experiment. Each fish was given two visible implanted elastomer tags, one to indicate their size to the nearest 1.0 mm and the other tag to indicate their initial sex. To ensure that only sexually mature adults were used for the experiment we did not include individuals less than 20 mm standard length.

Three density treatments (8 replicates of each) were randomly assigned to the 24 artificial patch reefs. We stocked three artificial reefs every 1-3 days with their pre-determined treatment density between July 3<sup>rd</sup> and July 20<sup>th</sup>, 2009. Because the experiment took place during the middle of the reproductive season we assumed that reproductive output was not significantly influenced by start date. Mortality of fish during transfer to the reef resulted in a slight variance within treatments. High density reefs were stocked with  $125.3 \pm 0.5$  fish (mean  $\pm$  standard error), mid with  $67.8 \pm 1.1$  fish and low with  $20.1 \pm 0.4$  fish, reflecting and exceeding the range of densities observed on natural reefs (Behrems 1983). Neither average size of fish nor variance in size differed between density treatments (ANOVA,  $F_{1,23}, p = 0.9814$ , Levene's test for equal variance  $p = 0.9721$ ).

Previous experiments found mortality of *L. dalli* to be strongly density-dependent and caused by predation (Steele 1998); thus, we used predator exclusion cages to maintain differences between experimental density treatments. The bottom of each cage was laid on the sea floor prior to the construction of the artificial reef (see above). Exclusion cages were

constructed using a 1 x 1 x 0.5 m (L x W x H) PVC pipe frame covered on 4 sides and the top with the same plastic mesh described above. The plastic mesh was small enough to exclude large predatory fish from entering the cage, but large enough that *L. dalli* could swim in and out freely. The exclusion cage was placed over the reef and attached to the 1m-square frame below each reef using cable ties. To minimize algal growth cages were regularly scrubbed by divers. However, regular scrubbing could not eliminate differences in algal growth so we built a second set of cages and exchanged them approximately every 10 days.

Despite the predator cages, mortality of fish on the reefs caused densities across all treatments to decline approximately 50% from their original stocking values (Figure 1). At the end of the experiment high, mid and low density reefs contained  $62.1 \pm 5.7$  fish (mean  $\pm$  standard error),  $34.1 \pm 3.4$  fish, and  $11.3 \pm 2.0$  fish, respectively, and density treatments still differed significantly (ANOVA,  $F_{1,23} = 57.34, p < 0.0001$ ). Furthermore, mortality did not differ between males and females and the sex ratio of fish on the reefs at the end of the experiment did not vary with density (Wilcoxon/Kruskal-Wallis test  $\chi^2 = 3.21, p = 0.2009$ ). In order to obtain an estimate of the density of fish on the reefs during the experiment we conducted a census of each reef at the midpoint of the experiment. Divers used the same clove oil solution described above to anesthetize and collect the fish while carefully pulling the reef apart one rock at a time. During the same dive we recorded the number of surviving fish, rebuilt the reef, and returned the fish. We did not observe a decrease or hiatus of reproduction following the census suggesting that it did not significantly disrupt mating behavior.

Reproduction was monitored during the experiment by photographing artificial nests containing eggs every three to four days (7 sampling times per reef). We found at least one artificial nest containing fish eggs on nearly every artificial reef. We never observed more than 6

of the 8 available nesting sites to contain eggs, indicating that nest availability probably did not limit reproduction. When eggs were found we gently removed the acetate insert and placed it on a 1-cm grid to be photographed. Because multiple females may lay eggs in each nest at the same time or in rapid succession we were not able to accurately identify separate clutches (eggs produced by a single female) nor were we able to reliably distinguish newer eggs from those laid a few days earlier. Thus, to prevent the possibility of photographing the same eggs on subsequent visits we wiped all eggs from the acetate sheet after each photo was taken. The number of artificial nests containing eggs was recorded for each reef during each sampling time.

We estimated the number of eggs in each photograph using the program ImageJ (US National Institute of Health). We first measured the area of the nest by drawing a circle around the perimeter. Next we counted the eggs within 5 representative 1-cm squares and used the average density multiplied by the total nest area to calculate the total number of eggs present in each nest. We never found any nests containing eggs on one low-density artificial reef during the experiment. Because we were interested in how density influences reproductive output, and not in the factors that influence whether or not reproduction occurs, we dropped this reef from the data analysis and consequently reduced the number of replicates to 7 in this treatment.

After 31 days we collected all surviving fish from the reefs and brought them back to the laboratory where we measured their standard length. Growth of each individual was determined by subtracting their initial standard length (as recorded by the elastomer tag) from their final standard length. Upon conclusion of the experiment all fish were returned to natural reefs from which they were collected.

### *Laboratory Experiment:*

Concurrent with the field experiment, we explored the effects of population density on egg production in a laboratory experiment. Twenty-four experimental populations were established in replicate small aquaria (43.8 cm long x 29.2 cm wide x 25.4 cm high) containing approximately 32.5 L of seawater. In each aquarium we placed several small rocks to provide refuges as would be found in natural environments and 3 artificial nests identical to those used in the field. We never observed more than 2 of the 3 available nests to contain eggs suggesting that nest availability did not limit reproduction of fish in the laboratory.

*Lythrypnus dalli* were collected from natural reefs and tagged using the same methods as described above. The sex ratio and size structure used for experimental laboratory populations matched those used for the field experiment; however, we could not use the same population densities as the field experiment as the laboratory aquaria were smaller than the field reefs (~1/8 the bottom surface area). Higher density treatments were used in the laboratory in order to create the same sex ratio as that used for the field experiment (85% female). The average number of fish used for each treatment was: high =  $27.6 \pm 0.2$  (mean  $\pm$  standard error), mid =  $13.0 \pm 0.0$ , and low =  $7.0 \pm 0.0$  individuals per aquarium which equates to a density of 208, 100 and 54 fish per square meter (the area of the field reefs). These densities were similar to those used in previous laboratory experiments with this and other similar species (Reavis and Grober 1999, Rodgers et al. 2007), but exceeded the range found in natural field populations. Density treatments were randomly assigned to aquaria and fish were stocked on the same days as the artificial reefs. Neither the average size of fish nor the variance in size differed between density treatments (ANOVA,  $F_{1,23}, p = 0.1847$ , Levene's test for equal variance  $p = 0.2627$ ). Unlike the field experiment, we did not census fish in the laboratory mid experiment because mortality in

the laboratory was observed to be very low ( $0.01\% \pm 0.02$ ) and thus density treatments remained stable. Furthermore, any dead fish that were found in the laboratory were replaced within 24 hours with a fish of equal size and sex.

Reproduction was monitored every 3-4 days (methods as above) for a total of 31 days resulting in 7 sampling times per aquarium. To compare the effect of density on growth rates of fish in the laboratory to that of fish on artificial reefs in the field we determined the growth of each individual as described above. Upon completion of the experiment fish were then returned to the natural reefs from which they had been collected.

#### *Analysis:*

To compare the effect of density on fish in the laboratory and field experiments we tested for density-dependent growth using one-way ANOVAs. Differences between experimental treatments were evaluated using Tukey's post-hoc tests. Temporal trends of per capita reproduction were analyzed for both experiments using repeated measures analysis of variance with density and sampling time as fixed effects. Because *L. dalli* are hermaphrodites and may have changed sex during the experiment, we could not reliably estimate the number of reproducing females and instead we quantified per capita reproduction as eggs per fish rather than eggs per female. For the field experiment per capita reproduction was calculated by dividing the total number of eggs on each reef by the number of fish estimated to be on the reef at each of the seven sampling times. The density of fish for each reef at each time period was determined from a logarithmic regression fitted to the three known densities (initial stocking number, mid experiment census, and final number collected). Per capita reproduction was calculated for laboratory populations as the total number of eggs produced in each aquarium divided by the number of fish in each aquarium for each of the seven sampling times.

We also evaluated the effect of density on male reproductive success. We never observed more than one male to occupy an artificial nest containing eggs at one time and thus we assumed the number of artificial nests containing eggs was an indication of the number of males reproducing at each sampling time. For both experiments we tested for the effect of population density on (1) the average number of nesting males (number of nests per reef or aquarium averaged over the 7 sampling times) (2) the proportion of males nesting (average number of nesting males divided by the average number of males present) and (3) the average number of eggs per artificial nest (average number of eggs within all nests on each reef or aquarium for each sampling time and then averaged over the 7 sampling times) using one factor ANOVA's. For both experiments we estimated the number of males present by calculating the average of the initial stocked number of males, mid-experiment census (field experiment only) and survivors collected at the end of the experiment. We did not estimate the number of males at each sampling time as done for total population size because the number of males present was determined by a combination of mortality and sex change of individuals and thus we could not assume a logarithmic decline in male density over the experiment.

Data were transformed when necessary to meet the assumptions of parametric statistics. All analyses were completed using JMP version 9.0 SAS Institute Inc.

## **Results:**

Growth rate of fish was density-dependent on field reefs (ANOVA,  $F_{1,23} = 11.92$ ,  $p = 0.0003$ ; Figure 2). The average growth of surviving individuals was greater on low-density reefs than on either the mid or high density reefs (Tukey's post hoc,  $\alpha < 0.05$ ). Average growth of fish in the laboratory, in contrast, was lower at all densities compared to growth in the field and was

not density-dependent (ANOVA,  $F_{1,23} = 0.59$ ,  $p = 0.5608$ ). Lower growth rates of fish in the laboratory at all densities suggest that there was a negative effect of captivity, which may also have influenced reproductive output.

Overall, per capita reproductive output over the seven sampling times was lower in the laboratory than in the field. On reefs in the field, we found a significant effect of density on per capita reproduction ( $F_{2,20} = 4.61$ ,  $p = 0.0226$ ) and no significant effect of time ( $F_{6,15} = 1.55$ ,  $p = 0.1686$ ) (Figure 3a). In the laboratory, in contrast, per capita reproduction declined over time ( $F_{6,16} = 3.98$ ,  $p = 0.0125$ ), and this pattern did not significantly differ between density treatments ( $F_{2,21} = 2.08$ ,  $p = 0.1502$ ) (Figure 3b). There was no significant interaction between density and sampling time in either experiment ( $F_{12,120} = 1.17$ ,  $p = 0.3109$  field,  $F_{12,126} = 1.23$ ,  $p = 0.2679$  lab).

We found evidence for an effect of fish density on male reproductive success. There was a positive effect of density on the average number of males guarding artificial nests containing eggs in the field experiment (ANOVA,  $F_{1,22} = 8.05$ ,  $p = 0.0027$ ) (Figure 4a). We found approximately half as many males guarding nests on the low-density reefs than on high-density reefs, but neither the low or high treatments differed significantly from the mid-density (Tukey's post hoc,  $\alpha < 0.05$ ). However, we detected a significant decline with density in the proportion of males that were nesting (ANOVA,  $F_{1,22} = 26.73$ ,  $p < 0.0001$ ) (Figure 4b). On low-density reefs in the field the proportion of nesting males was approximately double that found on high or mid density reefs (Tukey's post hoc,  $\alpha < 0.05$ ). Despite a decline in percent of males nesting, the average number of eggs within artificial nests increased with density (ANOVA,  $F_{1,22} = 5.12$ ,  $p = 0.0160$ ) (Figure 4c). Thus, at high population density, a smaller proportion of males were found guarding nests containing a higher number of eggs.



We found a similar influence of density on reproductive success of males in the laboratory. The number of nesting males in the laboratory increased with density (ANOVA,  $F_{1,23} = 6.35$ ,  $p = 0.0070$ ); however, this relationship was not as strong as in the field, with the number of nesting males in the high density treatments being only slightly greater than that in the low or mid density treatments (Tukey's post hoc,  $\alpha < 0.05$ ) (Figure 5a). The percentage of males that were nesting declined with density, just as in the field (ANOVA,  $F_{1,23} = 46.32$ ,  $p < 0.0001$ ). The percentage of nesting males in the low density treatment was more than double that in the mid or high density treatments (Tukey's post hoc,  $\alpha < 0.05$ ) (Figure 5b). Just as in the field, the average number of eggs per artificial nest was positively density-dependent (ANOVA,  $F_{1,23} = 25.20$ ,  $p < 0.0001$ ) and increased significantly with each increasing density treatment (Tukey's post hoc,  $\alpha < 0.05$ ) (Figure 5c).

### **Discussion:**

Our research contributes to a limited body of evidence suggesting that changes in population density may influence per capita reproductive rates of reef fishes, potentially affecting larval supply. We found direct evidence linking decreasing per capita egg production with increasing fish density in field populations of *Lythrypnus dalli*. Furthermore, the magnitude of density-dependent egg production was comparable to the magnitude of post settlement density-dependent mortality of juveniles in this species (Steele 1997). To date, few other studies report evidence for density-dependent reproduction in fishes. In the tropical goby, *Coryphopterus glaucofraenum* density-dependence was detected in both the rate of clutch production (eggs laid by individual females) and the number of eggs per clutch leading to reduced per capita egg production at high densities (Forrester et al. 2010). Similarly, in the

freshwater fathead minnow, *Pimephales promelas*, density-dependence was detected in both the rate of egg production and egg survival (Vandenbos et al. 2006). Density-dependent egg production may interact with density-dependence in other pre-recruitment characteristics such as hatching success, larval quality, or larval survival and ultimately lower recruitment to the next generation (Kynard et al. 2011). Correlations between population density and reproductive success have been reported in other fishes (Craig and Kipling 1983, Koslow et al. 1995, Abdoli et al. 2005) suggesting that variation in reproduction may ultimately contribute to variation in adult population size. For instance, processes occurring in the egg and larval stage, rather than post-recruitment density-dependent processes, determined cohort size of the woolly sculpin, *Clinocottus analis*, and were the primary contributor to changes in population size (Davis and Levin 2002). Together this evidence suggests that density-dependent reproduction may, to some extent, determine larval supply and resulting population size.

Differences in the effect of density on per capita reproduction in our laboratory and field experiments suggest that the processes that influence reproductive output of individuals in small social groups may not sufficiently approximate processes in larger field populations. Per capita egg production was lower in the laboratory and did not decline with density as found in the field experiment. Per capita reproduction may have been depressed in all laboratory aquaria because of the higher densities of fish used in this study. For instance, the low growth rates we found in our laboratory fish suggested that conditions in the laboratory may not have met energetic needs of reproduction. Alternatively, aggressive behavior between individuals of differing dominance status may have been more frequent in crowded laboratory conditions and interfered with natural mating behavior (Stanley and Wootton 1986, Spence and Smith 2005). Thus, laboratory studies

of small social groups may not be sufficient for elucidating the mechanisms that influence reproductive success and the potential role of density-dependence in early life stages.

Our results demonstrated that average egg production of females was reduced at high population density. Resources may become limiting at high density and reduce energy available for reproduction resulting in reduced egg production by each reproducing female (Lindstrom et al. 2005, McCormick 2006). Of the possible resources, nesting space is not a likely limiter of female egg production in this study as egg masses never completely filled the available area inside the artificial nests. Rather, territorial behavior of *L. dalli* suggests that feeding locations or sites used to shelter from predators may vary in quality and become limiting at high density, forcing some individuals to use low quality sites (Wiley 1976). If density-dependent egg production is driven by resource limitation in field populations then the strength and presence of this density-dependence may vary among habitats of different quality. Alternatively, social interactions may govern reproductive opportunities and reduce the fraction of females that reproduce at high density (Spence and Smith 2005). *Lythrypnus dalli* form dominance hierarchies in which large females exhibit aggressive behavior towards smaller subordinate females (Rodgers et al. 2007), potentially suppressing mating. Such behavior is known to occur in the polygynous angelfish, *Centropyge potteri*, where dominant females interfered with the mating of other females resulting in reduced spawning frequency of subordinates (Lutnesky and Kosaki 1995). Determining the relative role of each of these mechanisms will allow better understanding of how the strength or presence of density-dependent egg production may vary among populations.

Social interactions at different densities may drive the negative effect of density on the proportion of nesting males that we found in this study. We found proportionally fewer males

nesting at high density and that these males had higher average reproductive success than males at low density. Such changes in reproductive success of males may be caused by increased male-male competition at high density. For example, competition between male sand gobies for large nest sites may be density dependent (Lindstrom 1988). However, because there were always unused nests, and there was no variability in the artificial nests used in our experiment, the ability of a male to secure a nesting site seemed an unlikely limiter of male reproductive success. Rather, aggressive behavior of large dominant males may allow them to monopolize all mating opportunities in the vicinity (Peterson 1990, Rodgers et al. 2007). Furthermore, observations have demonstrated that female *L. dalli* prefer to mate with large males (St. Mary 1994) suggesting that strong female choice for the largest males drives density-dependence in the proportion of nesting males. The opportunity for choosiness may decrease at low density, when there are a limited number of males available. Overall these results suggest that changes in population density may influence the opportunity for female choice and thus the distribution of reproductive success among males.

Overall, our research contributes new understanding of factors that cause variation in reproduction of marine fishes, a subject that has received little attention. We found evidence suggesting negative density-dependence in per capita egg production of *Lythrypnus dalli* in field populations. Such density-dependence may ultimately influence larval supply to local populations (Jones et al. 1999, Swearer et al. 2002). Post-recruitment density-dependent mortality likely then further modifies the number of recruits that survive to adults (Myers and Cadigan 1993, Carr et al. 2002, White and Caselle 2008). Clearly, an understanding of the relative roles of density-dependent processes occurring in both pre-and post-recruitment life stages is needed to understand the processes shaping population dynamics.

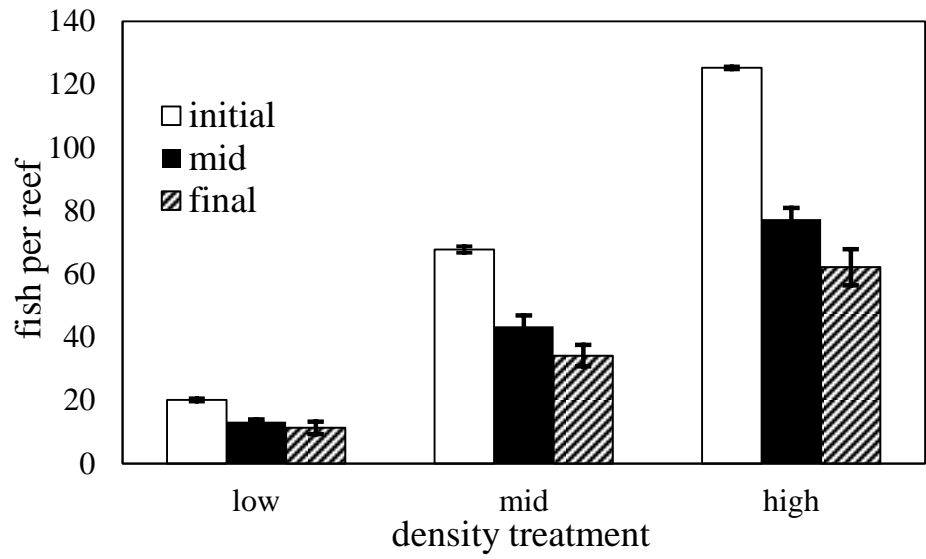


Figure 1.1: Number of fish (mean  $\pm$  standard error) on artificial reefs of different density at the time of initial stocking, mid experiment census, and of the final collection.

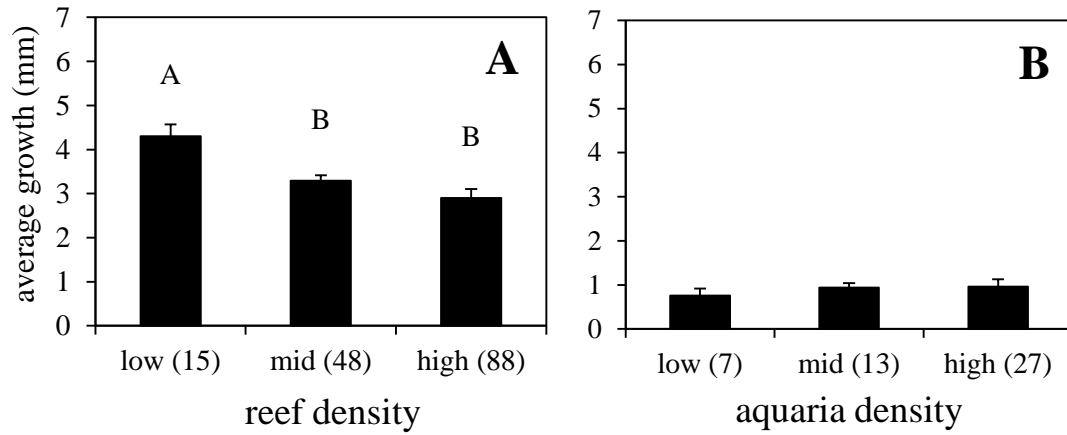


Figure 1.2: Growth (mean  $\pm$  standard error) of fish at different densities on (A) artificial reefs and in (B) laboratory aquaria. Numbers in parentheses represent the average number of fish in each treatment. Different letters designate significant statistical difference between treatments (Tukey's post-hoc  $\alpha < 0.05$ ).

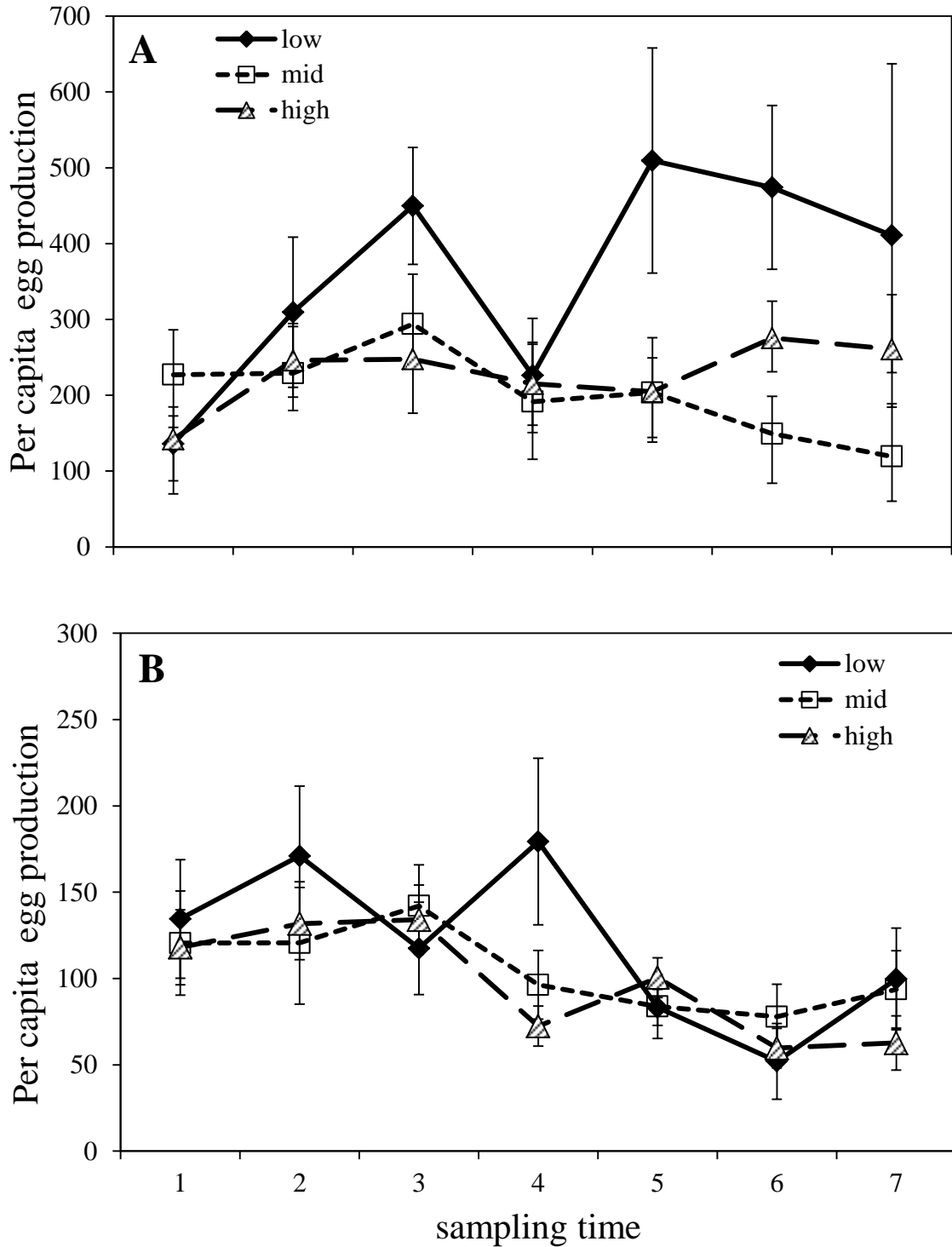


Figure 1.3: Per capita reproduction (mean  $\pm$  standard error) at low, mid and high density treatments for each sampling time on (A) field reefs and in (B) laboratory aquaria. Note the different scales on the y-axis.

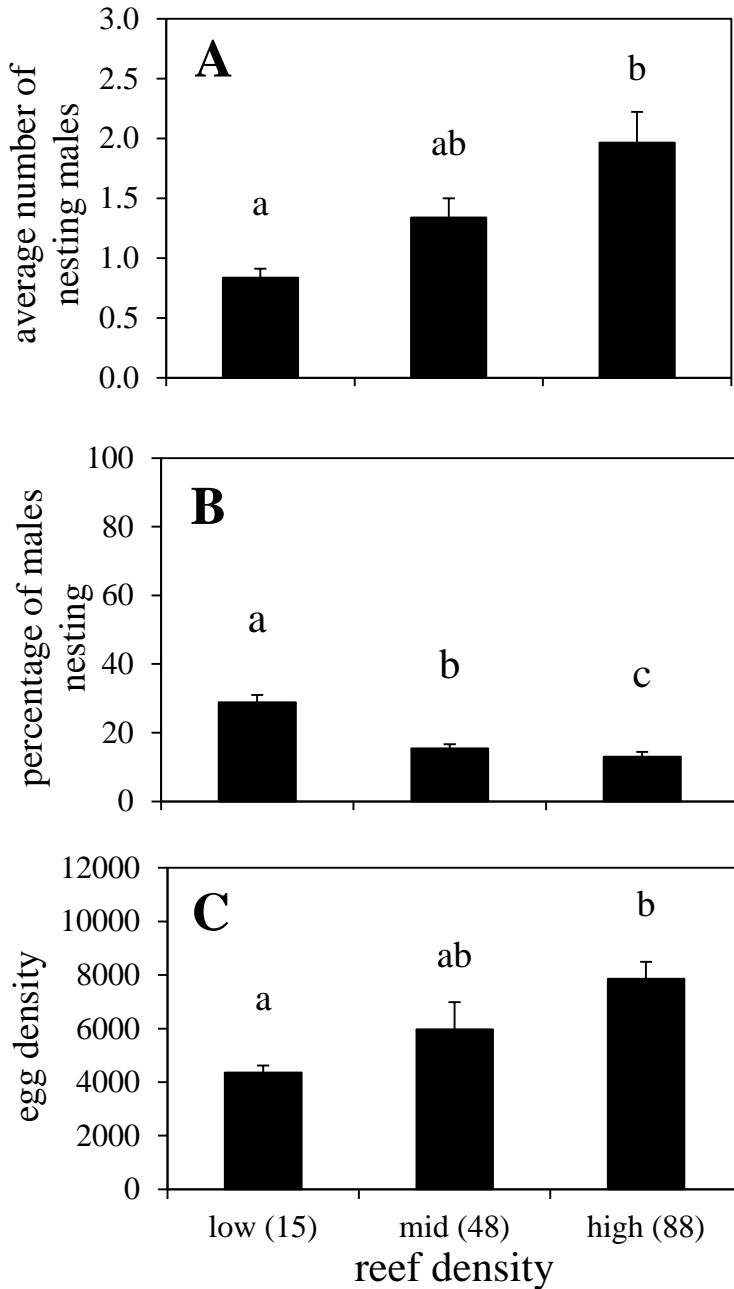


Figure 1.4: The relationship between fish density and (A) the number of nesting males, (B) the percentage of males nesting, and (C) the average number of eggs per nest on artificial patch reefs. Numbers in parentheses represent the average number of fish in each treatment. Bars represent means  $\pm$  standard error. Different letters designate significant statistical difference between treatments (Tukey's post-hoc  $\alpha < 0.05$ ).



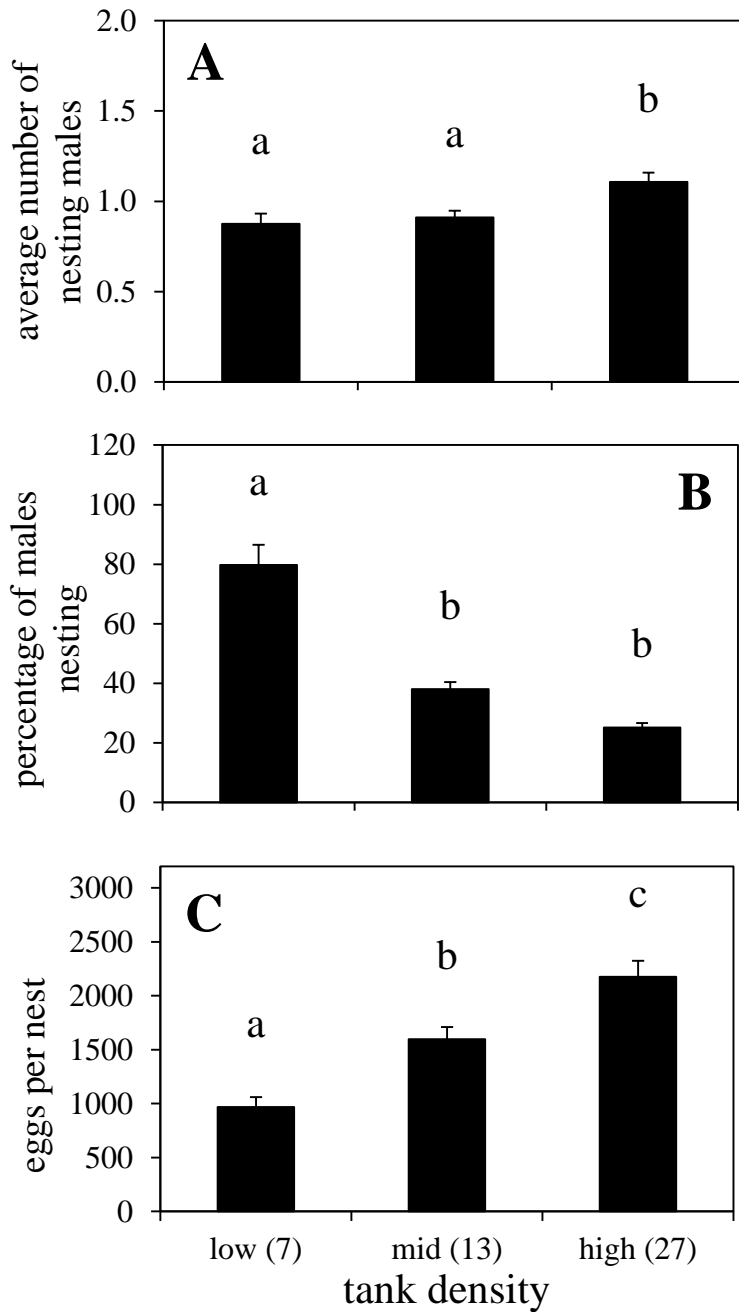


Figure 1.5: The relationship between fish density and (A) number of nesting males, (B) the percentage of males nesting, and (C) the average number of eggs per nest in laboratory aquaria. Numbers in parentheses represent the initial number of fish stocked in each treatment. Bars represent means  $\pm$  standard error. Different letters designate significant statistical difference between treatments (Tukey's post-hoc  $\alpha < 0.05$ ).

## Chapter 2

### SEX RATIO INFLUENCES RELATIVE REPRODUCTIVE SUCCESS OF MALES AND FEMALES BUT NOT SEX CHANGE OF *LYTHRYPNUS DALLI*

#### **Abstract**

Sex ratio of populations may vary over time and space, yet the response of individuals to this variation and its consequences to populations remain poorly understood. We tested for an effect of sex ratio on reproductive output and sex change of a temperate reef fish, *Lythrypnus dalli*. We varied the sex ratio of adult fish on artificial patch reefs and monitored reproductive output by photographing artificial nests containing eggs. We monitored sex change by tagging each individual fish to indicate their initial sex and then examined the sex of each individual again at the end of the experiment. Female per capita reproductive success did not decline in increasing female-biased populations or with female density, indicating that male availability did not limit female reproductive success. Reefs with the highest density of females tended to have higher total reproductive output, suggesting that for a given population density female biased populations may produce the most eggs. Correspondingly, per capita reproductive success of males increased in female biased populations. However, the number of nesting males did not vary in proportion to their abundance suggesting that the ability to monopolize mating with females may also determine reproductive success of males. Despite its influence on relative reproductive success of males and females, however, we found no evidence to suggest that individuals use sex ratio as a cue for sex change. Rather, for females, relative size was the best predictor of sex change. Sex change of males was rare, suggesting that, although physiologically

possible, this behavior may be uncommon in natural populations. Overall, we found that variation in the sex ratio, despite lacking influence on sex change, may be an important factor influencing relative reproductive success of males and female.

## **Introduction**

Sex ratio of adults often differs among populations or over time within populations (Grant and Mitton 1979, Behrents 1983, Cowen 1990, Berger and Gompper 1999), yet the effect of such variation on the reproductive success of individuals and the possible resulting feedbacks into population dynamics are poorly understood (Le Galliard et al. 2005). Factors that affect the sex ratio may include differences between the sexes in age at maturity, reproductive longevity, sex change behavior (in hermaphrodites), and differential mortality (Lovich and Gibbons 1990, Arnold et al. 2003, Donald 2007). Changes in the sex ratio likely influence the operational sex ratio (OSR) or ratio of males that are ready to mate to females that are ready to mate and thus, the direction and intensity of mating competition (Clutton-Brock and Parker 1992, Kvarnemo and Ahnesjo 1996). Variation in the OSR has been shown to influence aggression (Kvarnemo 1995, Weir et al. 2011) and the intensity of sexual selection (Prohl 2002, Kasumovic et al. 2008, de Jong 2009). In contrast, less is known regarding the influence of adult sex ratio on fecundity and reproductive success (but see Røed et al. 2002, Solberg et al. 2002). Examinations of how the effect of sex ratio on reproductive success of individuals may scale up to influence populations will deepen our understanding of and ability to predict population dynamics.

Variation in the sex ratio and reproductive success of individuals may ultimately influence the trajectory of populations. For example, a field experiment in the common lizard *Lacerta vivipara* showed that male-biased populations exhibited increased aggression towards

females causing reduced survival and fecundity (Le Galliard et al. 2005). Skew in the operational sex ratio may ultimately limit population growth if some individuals are not able to find mates (Steiffetten and Dale 2006). For instance, a laboratory experiment demonstrated that the spawning success of small male pupfish (*Cyprinodon pecosensis*) with no territories was positively related to the number of females available (Kodric-Brown 1988). Similarly, in the coral reef damselfish *Stegastes leucostictus*, males in female-biased field populations had an average reproductive success more than twice that of males in even sex ratio populations (Itzkowitz 1990). Although sex ratio clearly influences reproductive success of individuals, the corresponding consequences to populations are poorly understood.

Sequential hermaphroditism is a flexible reproductive strategy that may allow individuals to maximize reproductive success and, and sex ratio may be an important cue individuals use to determine if and when to change sex (Policansky 1982, Warner 1988a, Cowen 1990, Munday et al. 2006). Sex change has been studied in many species of reef fishes and is typically a socially controlled, phenotypic plastic response to the composition of the local mating group (Warner and Swearer 1991, Munoz and Warner 2003, Liu and Sadovy 2004, Black et al. 2005, Munday 2006). Relative body size is often proposed as the primary cue individuals use to determine social status and is predictive of sex change (Ghiselin 1969, Warner 1988a, b, Rodgers et al. 2007). However, whether individuals also use the sex ratio as a cue for sex change is unknown. The sex ratio of mature adults may vary with density or habitat, influencing reproductive success of individuals and the potential benefit of sex change. For instance, sex ratio was shown to influence sexual behavior and mate selection in sand gobies (Kvarnemo 1995, de Jong 2009). Furthermore, most research on sex change has focused on sequentially sex changing species, leaving bi-directional sex changing species (those physically capable of changing sex multiple

times) less well understood. For such species, the cues that govern sex change and the frequency of sex change in each direction have rarely been studied in natural field populations (but see Kuwamura et al. 1994, Munday et al. 1998).

We investigated the effect of adult sex ratio on reproduction and sex change in a temperate marine goby, *Lythrypnus dalli*, a bi-directionally sex changing hermaphrodite whose sex change is socially controlled. *L. dalli* is a small, site-attached reef fish that lives at high densities (~24 individuals per square meter) (Behrens 1983). In small experimental social groups, large individuals are behaviorally dominant and remain or change sex to male, while smaller individuals are submissive and remain or change sex to female (Reavis and Grober 1999, Rodgers et al. 2005, Lorenzi et al. 2006, Rodgers et al. 2007). Reproduction is seasonal and females spawn repeatedly into nesting sites where males guard and defend the eggs until hatching.

We manipulated the sex ratio of adult fish on artificial patch reefs and monitored reproductive output, as well as the frequency and direction of sex change. We used 5 metrics to measure reproduction: total reproductive output (number of eggs laid) per reef, average per capita egg production of males and of females, the number of nesting males, and the proportion of males that were nesting. To test for an effect of sex ratio itself on sex change, above and beyond the effects of relative body size of individuals, we compared the frequency of sex change of fish that were initially male and those that were initially female to rates of sex change predicted solely from relative body size.

## Methods

### *Study Species*

*Lythrypnus dalli* occupy subtidal rocky habitats along the coasts of Southern California and Baja California, Mexico. The sex ratio of natural populations varies over the reproductive season typically starting in May at around 65% female and peaking in July at 85% female and finally declining through late September (Behrents 1983). Whether these fluctuations in sex ratio result from sex-change behavior of individuals, differential mortality of males and females, maturation of juveniles, or some combination thereof is unknown. Although individuals typically maintain some allocation to both male and female gonad tissue, they exhibit only one functional sex at a time, reproducing as either male or female (St. Mary 1994). Individuals are capable of changing from fully functional females, to fully functional males (and vice versa) in as little as 15 days under the right social conditions (Rodgers et al. 2007). Multiple females lay their demersal eggs simultaneously within a nesting site guarded by a male. Larvae hatch from the eggs after 5-7 days and spend about 6 weeks in the plankton before settling on a rocky reef (St. Mary 1994). Life expectancy of *L. dalli* is short (1.5-2 years), relative to the length of the reproductive and recruitment season (~ 6 months each) and thus populations of *L. dalli* are dynamic changing in both size structure and density within and between years.

### *Experiment*

In the summer of 2008, we explored the effects of adult sex ratio on individual sex change and reproductive output in *Lythrypnus dalli* on Santa Catalina Island, California, U.S.A. (33°27'3" N; 118°29'15" W) by manipulating the sex ratio on artificial patch reefs. We constructed ten artificial rocky reefs in a sandy cove located approximately 2 km northwest of the University of Southern California Wrigley Institute for Environmental Studies. Each reef was

constructed with roughly the same number and size distribution of rocks collected from a nearby shore. The base of each artificial reef was 1m<sup>2</sup>, and the center was roughly 0.3 m high. Each artificial reef was constructed on top of a 1 x 1 m square polyvinyl chloride (PVC) frame with mesh netting (1.56 x 1.90 cm). This frame was used as the bottom of an anti-predator cage (described below). We built the reefs in a single row along the 10 meter depth contour. At least 10 meters of sand separated each reef from any other suitable *L. dalli* habitat to limit immigration/emigration, a practice employed in previous experiments of this kind (St. Mary 1994, Steele 1997, 1998). We placed 10 artificial nesting sites, constructed from a 12.7 cm long 1.27 cm diameter piece of PVC with a cap on one end, on each of the reefs. A rolled up piece of sanded transparent acetate sheet was placed inside each artificial nest in order to quantify egg production as described below.

We collected individual *L. dalli* from natural reefs and gave each individual 2 tags to indicate their initial size and sex. Fish were anesthetized and collected using hand nets and squirt bottles containing a solution of 10% clove oil and 90% ethanol and taken back to the laboratory. To measure standard length and determine functional sex, each individual was anesthetized by placing it in a solution of tricaine methanesulfonate (MS-222) and sea water. Only sexually mature individuals of at least 20 mm standard length were used for the experiment. We determined the functional sex of each individual by examining the shape of the genital papilla under a dissecting microscope. Previous histological examination of the gonads found that fish with a blunt shaped papilla allocate more than 95% of their gonad to ovarian tissue and spawn as female while fish with a long pointed papilla possess a male-typical accessory gonadal structure filled with mucus and sperm and spawn as male (St. Mary 1993, Drilling and Grober 2005). We classified fish with a blunt shaped papilla as female and those with a long pointed papilla as male

and gave each individual two visible implant elastomer tags, one to indicate initial size and one to indicate initial sex. Individuals with an ambiguous morphology (~5% of collected individuals) were not used in the experiment.

We stocked  $39.8 \pm 0.2$  adult gobies (variance due to handling losses during stocking) on the artificial reefs on August 25, 2008 where they remained for 20 days. We varied the sex ratio of stocked fish between 60% and 90% female, effectively spanning and slightly exceeding the range on natural reefs during the summer (Behrents 1983). Because body size and sex in *L. dalli* are interrelated, it was not possible represent males and females equally in each size class on the reefs (Figure 1). Rather, we stocked fish such that neither the average size ( $29.7 \pm 0.2$  mm) nor the variance in size differed between reefs (ANOVA,  $F_{1,9} = 0.16$ ,  $p = 0.9975$ , Levene's test  $p = 0.3691$ ). Thus, large size classes were initially dominated by females on female biased reefs, and by males on male biased reefs.

To minimize mortality from predators, each reef was enclosed in a cage to exclude large predatory fish. To construct the cage, a 1 x 1 x 0.5 m high PVC frame covered with mesh netting (1.6 x 1.9 cm openings) on all but one side was placed over the reef open side down and attached to the 1 x 1 meter PVC frame below the reef using cable ties. The mesh netting was small enough that large predatory fish could not enter the cage but large enough that the gobies and other small fish could move in and out of the cages freely.

Reproduction was measured every five days during the experiment. Eggs were found in at least some artificial nests on at least two occasions on every reef during the experiment. Because we never observed more than one male to occupy an artificial nest, we assumed the number of nests found containing eggs on any given day was a good approximation of the number of males that were successfully nesting. During each visit, divers carefully removed the



internal piece of acetate sheet from each artificial nest to check for the presence of eggs. If eggs were found, the transparent acetate sheet was placed on a 1 cm grid and photographed. We were not able to identify separate clutches of eggs reliably as multiple females may lay eggs in a nest in short succession. After each photograph was taken, the acetate sheet was wiped clean of all eggs to avoid the potential of photographing the same eggs during the next visit. Of the 10 artificial nests placed on each reef, we never observed more than 4 containing eggs, indicating that reproductive output was probably not limited by nest availability. The program ImageJ (NIH) was used to estimate the number of eggs present in each photograph. First we estimated the area of the nest by tracing the perimeter of the egg mass. This area was then multiplied by the average number of eggs within 5 representative one centimeter squares to estimate the total number of eggs present.

After 20 days, all surviving fish were collected from the reefs and taken back to the laboratory and final sex was recorded for each individual. To collect the surviving fish, divers used the same anesthetic described above and then systematically removed all rocks from the reef to ensure complete collection. No fish eggs were ever found on any of the rocks during dismantling, revealing that that the fish did not utilize any nesting sites other than the artificial nests provided. We recorded fish with an intermediate sex as the sex they were changing into as this status indicated that sex change had been initiated.

To determine if experimental treatments were maintained throughout the 20-day experiment, we quantified and analyzed the survival rates of the fish on each reef. Approximately half of the fish survived ( $53.2\% \pm 7.4\%$ ) over all treatments, and the surviving fraction on each reef did not vary with sex ratio (linear regression,  $r^2 = 0.03$ ,  $p = 0.6444$ ). There was also no difference between the proportion of initial females and that of initial males

surviving at the end of the experiment, (paired  $t$ -test:  $t_9 = 1.20$ ,  $p = 0.2577$ , males  $47.3\% \pm 7.7\%$ , females  $53.8\% \pm 7.5\%$ ). Neither the average size nor the variance in size of fish on each reef differed at the end of the experiment (ANOVA,  $F_{1,9} = 0.07$ ,  $p = 0.7959$ , Levene's test for equal variance  $p = 0.2299$ ).

### *Analysis*

We used regression analysis to determine the relationship between sex ratio (average percent female) and total reproductive output, per capita reproduction of males and females, number of nesting males, and percentage of males with artificial nests containing eggs. Because mortality during the experiment caused variation in the final density, we also used regression to test for an effect of final density on total reproductive output. Mortality also weakened the correlation between sex ratio and the density of females ( $r = 0.57$ ). To assess whether variation in sex ratio or female density were stronger predictors of changes in egg production we also tested for a relationship between female density and total egg production, and per capita reproductive success of females with linear regression. Total reproductive output for each reef was determined by adding together all the eggs that were photographed on each reef during the experiment. To evaluate the distribution of reproduction among individuals, we calculated per capita reproduction by dividing the total number of eggs produced by the average number of males and separately by the average number of females present. The number of males and females was estimated for each reef by averaging the number of each sex stocked at the beginning and the number of each sex collected at the end of the experiment. The average number of nesting males for each reef was estimated by the average number of nests containing eggs found during each visit. We estimated the percentage of nesting males by dividing the average number of nesting males by the average number of males present on each reef.

To examine the frequency of sex change among reefs with varying initial sex ratios, we compared expected with observed rates of sex change for males and females. Expected rates were based on the results of small-scale laboratory experiments that demonstrated a link in *L. dalli* between body size and the likelihood of sex change (Black et al. 2005, Rodgers et al. 2007). These studies found that in small social groups, the largest fish were always dominant and assumed the male sex. Thus, for each reef, we ranked fish by size and expected the largest individuals to remain or change to male and all smaller individuals to remain or change to female. We used the sex ratio (78.9% female) of *L. dalli* collected from natural field populations to determine the size at which sex change probably occurs under natural conditions. From this information we predicted that the largest 21.1% (body size 32 mm or greater) would be male, while the rest (body size 31 mm or less) would be female if relative size was the only cue individuals used for sex change. We used a goodness of fit test to compare observed rates of sex change to these expectations. On each reef, for both males and females, we calculated the difference between the observed and expected number of fish that changed to this sex. We then used regression analysis to determine if this difference varied with the initial sex ratio.

When necessary, data were square root transformed to meet the assumptions of parametric statistics. All analyses were completed using JMP version 9.0 SAS Institute Inc.

## **Results**

### *Reproduction distribution*

The initial sex ratio significantly affected the relative reproductive success of males and females and may have also influenced total reproductive output of the fish on each reef. On average,  $16,028 \pm 4,011$  eggs were produced per reef throughout the experiment.

We did not find a significant effect of sex ratio on total reproductive output (regression,  $df = 9$ ,  $r^2 = 0.25$ ,  $p = 0.1428$ ) (Figure 2a). However, the total number of eggs produced on artificial reefs tended to increase with female density (regression,  $df = 9$ ,  $r^2 = 0.34$ ,  $p = 0.0785$ ) and resulting per capita reproductive success of females did not decline with female density (regression,  $df = 9$ ,  $r^2 = 0.18$ ,  $p = 0.2269$ ) (Figure 3). We found no effect of the final density on reproductive output, suggesting that the variation in reproduction observed was caused by variation in the number of females rather than differences in overall density (data not shown, regression,  $df = 9$ ,  $r^2 = 0.22$ ,  $p = 0.1753$ ).

In fact, sex ratio of fish on the reefs exerted a strong influence on the per capita reproductive success of males but not females. Males displayed significantly higher per capita reproductive output when the sex ratio was strongly female biased (regression,  $df = 9$ ,  $r^2 = 0.47$ ,  $p = 0.0278$ ) (Figure 2b). In contrast, the sex ratio did not influence the per capita reproduction of females (regression,  $df = 9$ ,  $r^2 = 0.16$ ,  $p = 0.2609$ ). Averaged across all reefs, the total number of eggs per male ( $2,451 \pm 888$ ) was over three times greater than the total number of eggs per female ( $680 \pm 143$ ). However, the greater per capita reproduction of males appears to be driven by reefs that were strongly female biased. On reefs where sex ratio was between 60% and 70% female, males and females had similar levels of per capita reproduction.

The number of males guarding artificial nests containing eggs was not significantly influenced by the sex ratio (regression,  $df = 9$ ,  $r^2 = 0.03$ ,  $p = 0.6543$ ) (Figure 4a). In contrast, the percent of males that were nesting increased with the sex ratio, but this trend was not significant (regression,  $df = 9$ ,  $r^2 = 0.33$ ,  $p = 0.0805$ ) (Figure 4b). Thus, it appears that the increased production of eggs on strongly female biased reefs was experienced by only a few males.

### *Sex Change*

Sex change of initial males, but not initial females, differed from that which was expected based solely on relative body size. Of the 167 fish that were initially female, 26 changed sex to male during the experiment, which did not differ expected rates of female sex change based solely on relative body size (goodness of fit,  $df = 7$ ,  $\chi^2 = 4.93$   $p = 0.6685$ ). Also, the difference from expected sex change showed no relationship with the initial sex ratio (regression,  $df = 9$ ,  $r^2 = 0.11$ ,  $p = 0.3527$ ) (Figure 5). That is, sex ratio did not influence female sex change.

In contrast, observed sex change in males did differ from the expectation based solely on relative size (goodness of fit,  $df = 6$ ,  $\chi^2 = 15.33$ ,  $p = 0.0178$ ). Overall fewer males changed sex than expected, however, the difference from expected sex change was unrelated to the initial sex ratio (regression,  $df = 9$ ,  $r^2 = 0.21$ ,  $p = 0.1870$ ) (Figure 5). Of the 45 fish that were initially males, only 2 changed sex to female during the experiment. These results suggest that males do not use relative size or the sex ratio of the adults on the artificial reefs as a cue for sex change.

## **Discussion**

Results from our experiment indicate that resources affecting female reproductive output, such as nest space or male availability, were not limiting. Average per capita reproductive success of females did not decline with increasing female density or in strongly female biased populations. This result doesn't follow common hypotheses and evidence suggesting the availability of males should limit female reproduction in populations with female biased sex ratios or species that have male parental care (Berglund and Rosenqvist 1990, Forsgren et al. 2004, Mobley et al. 2009, Berger-Tal and Lubin 2011). Although it was not significant, the total number of eggs produced on field reefs tended to increase in female biased populations that contained a higher density of females. Resources may have not limited reproduction of females

in our study if the number of females that were reproducing did not vary with the sex ratio. For instance, large dominant females may have suppressed mating of smaller subordinate females. Such is the case for a polygynous angelfish where dominant females interfered with the mating of other females resulting in reduced spawning frequency of subordinates (Lutnesky and Kosaki 1995). In this case higher reproductive success may have been restricted to relatively few large dominant females. Few studies have examined the influence of sex ratio on reproduction and those few have focused on birds (Ewen et al. 2010) or mammals (Røed et al. 2002, Solberg et al. 2002). Our results indicate that for a given population density, female biased populations with a higher number of females, might similarly influence egg production in populations of marine fish.

The sex ratio also influenced the relative reproductive success of males versus females. We found, on average, that males had higher per capita reproductive output than females. A similar pattern was also found for a coral reef damselfish suggesting that this relationship may be common among nest guarding species (Itzkowitz 1990). Per capita reproductive success of males, but not females, increased with sex ratio resulting in a larger difference between male and female average reproductive success in strongly female biased populations. Number of nesting males did not vary in proportion to their abundance suggesting that the ability to monopolize mating with females may determine reproductive success of males. Female *L. dalli* prefer to mate with large males (St. Mary 1994) and may have chosen to mate only with the few largest males independent of male abundance. The strength of sexual selection is known to increase in strongly female biased populations (Bateman 1948, Emlen and Oring 1977, Jones et al. 2005) and may ultimately affect reproductive output of populations (Whitlock and Agrawal 2009).

These results indicate that the variation in reproductive success both between and within sexes may vary with the sex ratio.

Despite its influence on reproductive potential, we did not find evidence that individuals use the sex ratio as a cue for sex change. Neither male nor female sex change varied with the sex ratio. Instead, females changed sex as predicted by their body size. This finding suggests that females use relative body size as the sole cue for sex change, similar to patterns in sex change observed in smaller social groups (Black et al. 2005, Rodgers et al. 2007). Furthermore, this result supports the finding of previous studies on similar species, which indicate that social status, determined by relative size, is the most important determinant of female sex change (Ross et al. 1990, Liu and Sadovy 2004, Rodgers et al. 2005, Rodgers et al. 2007). In the saddleback wrasse, for example relative size was an important factor for both the stimulation and inhibition of sex change while sex ratio was not (Ross et al. 1983). It may be that simply the presence of a larger individual, regardless of sex, is sufficient to inhibit sex change of females. Because large fish in natural populations of *L. dalli* are nearly always male, size may be a sufficient cue to distinguish sex. Our results indicate that using relative size alone as a cue for sex change may provide sufficient information to maximize reproductive potential.

Our results suggest that sex change from male to female, while physiologically possible, may be unimportant in natural populations. Sex change of males was rare and the frequency of occurrence did not match that predicted by their relative size. This result correlates with earlier findings in this and other bi-directional sex changing fishes that sex change occurs more readily in the protogynous than in the proterandrous direction (St. Mary 1994, Munday 1998, Reavis and Grober 1999). It is not clear why small males do not change sex, as it appears that they would achieve higher reproductive success by doing so. Perhaps the social conditions that prompt male

sex change in the laboratory occur infrequently in field populations or in larger social groups. Alternatively, small males may not change sex if they are achieving reproductive success by utilizing a sneaking strategy. Such a strategy is generally only successful when there is a low abundance of sneakers and thus, provides an explanation for why small males change sex in small social groups but not in larger populations. In further support of this hypothesis, examination of the testicular development of these small males has shown them to contain gonads similar to that of larger nesting males (Cole 1982, Drilling and Grober 2005). Close behavioral observations, paternity genetics, and long term studies that examine lifetime reproductive success of different sex change strategies are needed to clarify why small males forgo sex change in field populations.

In summary, we found no evidence to suggest that individuals use the sex ratio as a cue to change sex despite its influence on per capita reproduction. This result does not support the widely-held belief that individuals should use the sex ratio as a cue for sex change in order to maximize reproductive success (Policansky 1982, Warner 1988a, Cowen 1990, Munday et al. 2006). Furthermore, we suggest that male sex change to female is probably rare in natural populations, and we emphasize the need to understand lifetime reproductive success of different sex-change strategies. Overall, we found that variation in the sex ratio, despite its lack of influence on sex change, might still be an important factor determining population level reproductive output and individual reproductive success.



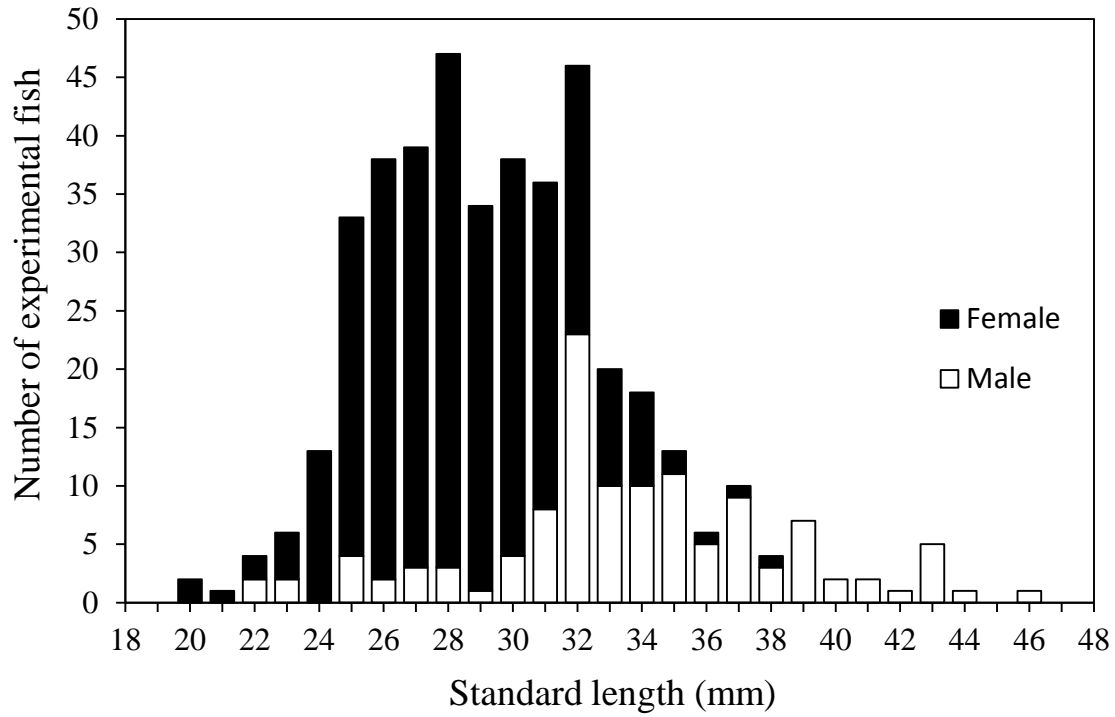


Figure 2.1: Size frequency distribution of all fish collected from natural reefs and used to create experimental populations. Bar height indicated the number of individuals in each size class. Males and females occur at equal frequency at 32 mm.

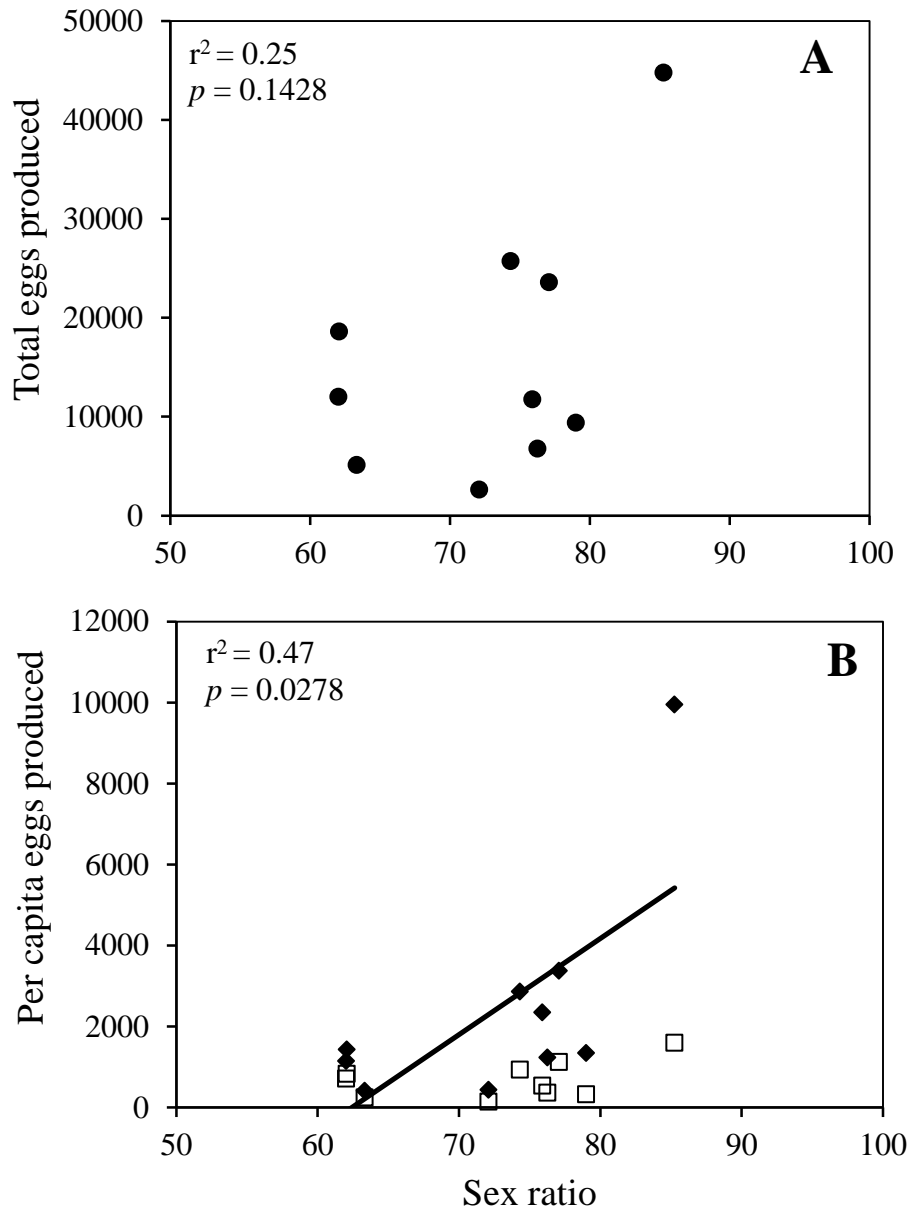


Figure 2.2: The relationship between the sex ratio (average percent female) and (a) total reproductive output (total number of eggs produced per reef) and (b) the per capita reproductive output of males (solid diamonds) and females (open squares). The solid line represents a significant linear relationship.

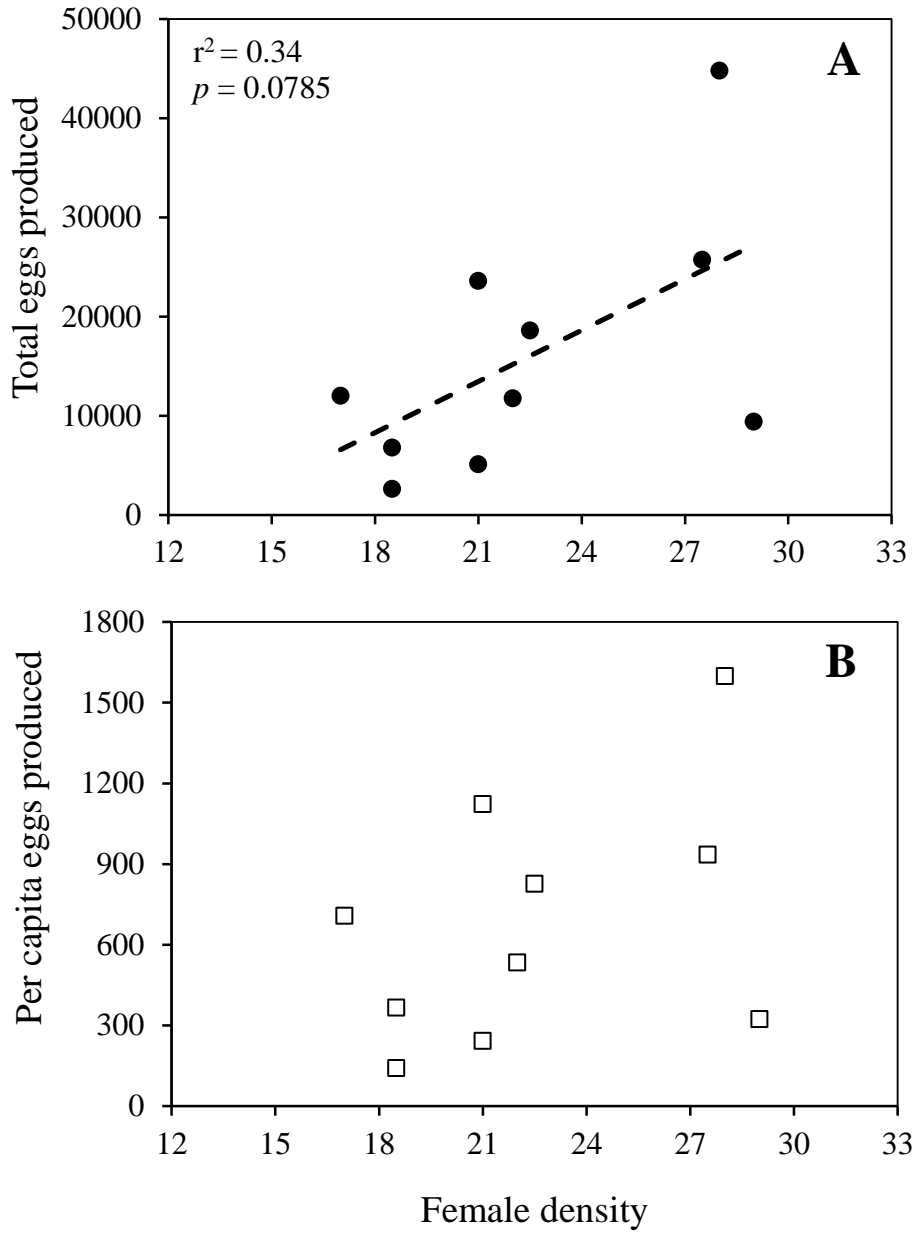


Figure 2.3: The relationship between female density (average number of females per reefs) and (a) total reproductive output (total number of eggs produced per reef) and (b) the per capita reproductive output of females. The dotted line represents a nearly significant trend.

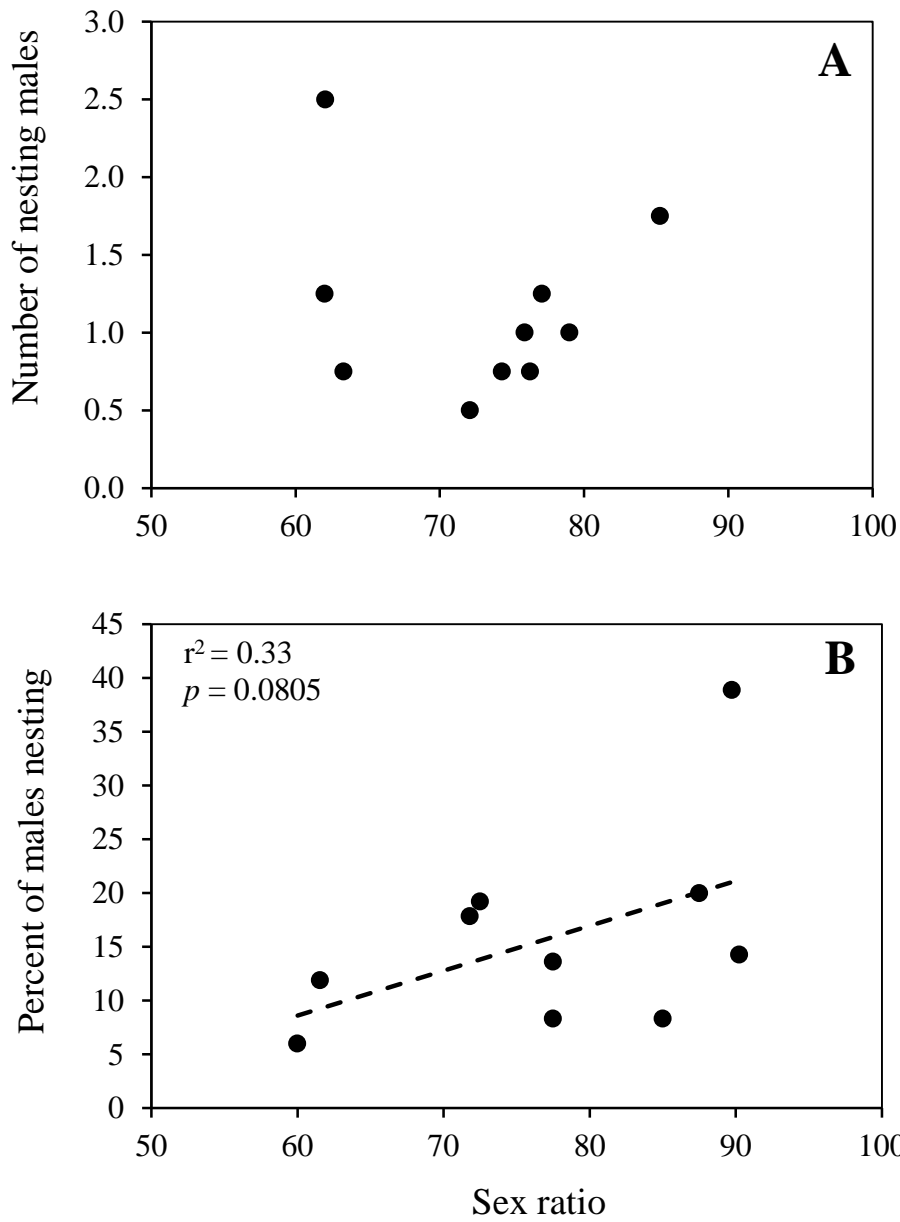


Figure 2.4: The relationship between the sex ratio (average of initial and final percent female) and (A) the number of nesting males (average number of nest found per visit) and (B) the percent of males nesting (average number of males with nests/average number of males present). The dotted line represents a nearly significant trend.

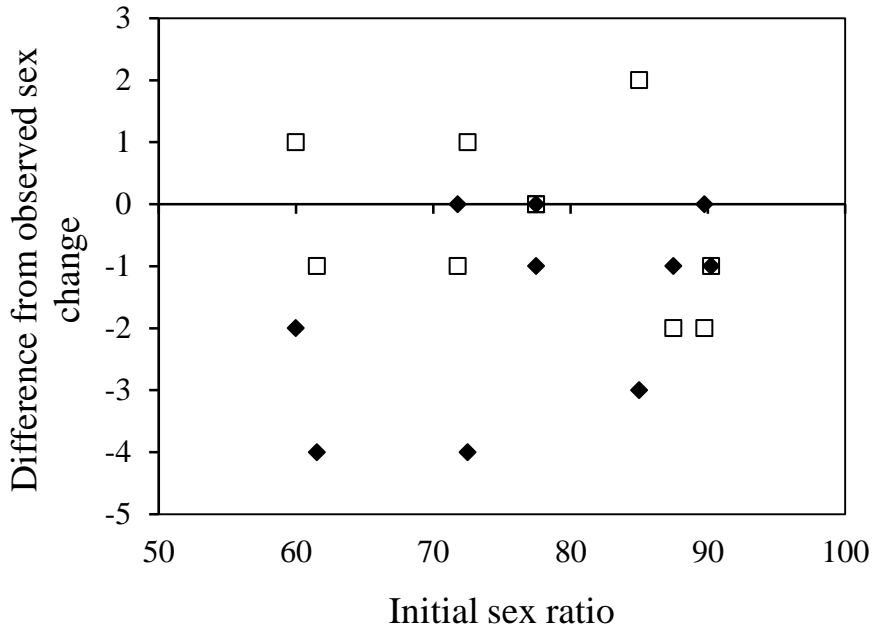


Figure 2.5: The relationship between initial sex ratio (percent female) and the difference from observed sex change (number observed minus expected number of individuals) for females (open squares) and males (solid diamonds). Negative numbers indicate fewer individuals changed sex than expected based solely on size, whereas positive numbers indicate that more individuals changed sex than expected. Note that sex change for neither sex varied consistently with sex ratio.

## Chapter 3

### SEX CHANGE IN FIELD POPULATIONS DIFFERS FROM LABORATORY POPULATIONS IN A BI-DIRECTIONALLY SEX CHANGING GOBY, *LYTHRYPNUS DALLI*

#### **Abstract:**

Bi-directional sex change (in which both males and females change sex) is an extremely flexible reproductive strategy that is likely highly sensitive to the social environment. Sex change is typically socially controlled; thus, changes in population characteristics, such as size-structure, density, or sex ratio may generate variation in the frequency or timing of sex change. We compared the results of a field and a laboratory experiment that tested for an effect of density and body size on the frequency of sex change in the bi-directionally sex changing reef fish, *Lythrypnus dalli*. We manipulated the density of fish on artificial patch reefs in the field and in laboratory aquaria and monitored the frequency of sex change and the body sizes of sex changing individuals. Sex change was density-dependent in the laboratory but not in the field, suggesting that factors influencing sex change of fish in the laboratory may not predict patterns of sex change in natural populations. Sex change of females was common in both experiments, causing sex ratios to become less female biased. However, differences in frequency of sex change did not result in a significant effect of density on the final sex ratio. The proportion of females that changed sex increased with body size and this relationship did not vary with population density in both experiments. We suggest decreasing social stability in larger populations and in more complex environments as a possible mechanism driving the differences in patterns of sex change between the field and laboratory experiments. Overall our results

indicate that laboratory examinations of sex change may overemphasize the importance of social group formation and size based hierarchies in governing this behavior.

### **Introduction:**

Sex change occurs in a wide range of taxa including many plants, invertebrates and fishes (reviewed in Policansky 1982), yet our understanding of the adaptive significance of the complex suite of sex change strategies that exist in nature is incomplete (Munday et al. 2010). A wide range of sex change strategies exist: from species that mature as one sex and change sex later under the right social conditions (sequential hermaphrodites), to species that can change sex in more than once (bi-directional hermaphrodites), and those that allocate reproductive tissue to both sexes (simultaneous hermaphrodites) (St. Mary 1997, Munday et al. 2006). For most fishes, sex change is a socially controlled, phenotypically plastic response to the composition of the local mating group (Warner and Swearer 1991, Munoz and Warner 2003, Munday et al. 2006, Rodgers et al. 2007). The size-advantage hypothesis has been successful in explaining the adaptive significance of this behavior in many species by observing that sex change is favored when reproductive success changes with size or age faster for one sex than the other (Warner 1975, 1988a). The size-advantage hypothesis may also be consistent with bi-directional sex change if changes in the composition of social groups occur commonly, or if the probability of finding a mating partner is unpredictable (Hobbs et al. 2004, Wong et al. 2005, Munday et al. 2010). However, little is known regarding how changes in social or environmental complexity may interact with body size to influence sex change in natural populations.

Despite extensive study on sex change in small social groups (Sakai et al. 2003, Wong et al. 2005, Rodgers et al. 2007, Munday et al. 2010), the influence of population characteristics, such as size structure, sex ratio or populations density, on sex change in natural populations is

largely unknown. The relationship between individual body size and reproductive success is likely influenced by changes in population characteristics and may ultimately affect the likelihood or timing of sex change. For example, fluctuations in population size distribution have been shown to be correlated to variation in the size at sex change in both the shrimp (*Pandalus jordani*) and reef fish (*Chlorurus sordidus* and *Scarus frenatus*) (Charnov and Anderson 1989, Guest 2004). High density was associated with earlier sex change and with a higher proportion of terminal phase males in the polygynous labrid *Coris julis* (Lejeune 1987). Understanding how population characteristics effect sex change may influence management decisions for such species (Alonzo et al. 2008, Ellis and Powers 2011). For instance, evidence suggests that sequential hermaphrodites may be more susceptible to size-selective fishing than other species (Hamilton et al. 2007). Currently we have little understanding of the population characteristics that cause variation in the frequency of sex change.

Studies have shown links between density and social interactions, which may in turn influence sex change. For example, changes in population density may change access to resources and thus potential benefits of being male or female (Borg et al. 2002, Vizoso and Scharer 2007). Variation in population density may also influence the structure or stability of social groups and thus social signals that either prompt or inhibit sex change. The stability of a size-based hierarchy is likely dependent upon the number of individuals in a social group and the average difference in size between individuals (Ang and Manica 2010b). For instance, high-ranking Arctic char and rainbow trout were less able to dominate food sources at high densities (Alanara and Brannas 1996). Changes in the stability or strength of dominance hierarchies due to variation in population density may alter social signals and ultimately influence the frequency of sex change in populations. Assuming that population density influences the rates of contact



between individuals and that rates of contact are cues for sex change, Lutnesky (1994) developed a model that predicts sex change should be inhibited at high, but not at low density. Despite the clear influence of population density on social behavior and the known influence of social behavior on sex change, the relationship between density and sex change has rarely been evaluated (but see Lejeune 1987, Wright 1989).

The adaptive significance and frequency of occurrence of bi-directional sex change in natural populations is less well understood than for sequentially sex changing species. Most of the research examining the proximate mechanisms governing bi-directional sex change and the adaptive significance of this behavior has concerned animals in captivity, over limited spatial and temporal scales, and focus on small social groups (Sunobe and Nakazono 1993, St. Mary 1994, Munday 2002, Sakai et al. 2003, Lorenzi et al. 2006, Rodgers et al. 2007). Although, these studies have increased our understanding they cannot provide an accurate description of the frequency of sex change in each direction in natural populations (but see Kuwamura et al. 1994, Munday 1998) or how population characteristics (such as density) influence this behavior. The limited spatial extent and environmental simplicity of laboratory studies may exaggerate the link between dominance and sex change. For example strong relationships between physical characteristics and social dominance that are prominent in laboratory studies often disappear in more complex environments (reviewed in Solman and Armstrong 2002). Furthermore, sex allocation theory models that adequately predicted sex change of a marine gastropod in small social groups did not predict sex change in natural populations (Collin 1995). A gap in our current understanding, then, is how sex change patterns observed in these small groups approximate patterns that occur in natural field populations.

To examine the influence of density on the frequency of sex change we manipulated population density of *Lythrypnus dalli*, a bi-directionally sex changing hermaphrodite, and monitored sex change in both a laboratory experiment with small social groups and in a field experiment with larger populations. Previous experiments with small social groups demonstrated that sex of *L. dalli* is socially controlled and determined by position within a size-based dominance hierarchy (Lorenzi et al. 2006, Rodgers et al. 2007). Although individuals maintain some allocation to both male and female gonad tissue, they exhibit only one functional sex at a time, reproducing as either male or female (St. Mary 1994). Our goals were to (1) test for an influence of population density on the frequency of sex change, (2) evaluate the influence of density on the relationship between body size and sex change, and (3) compare patterns of sex change in small laboratory social groups with those in larger field populations.

## **Methods:**

### *Study Species:*

*Lythrypnus dalli* is a brightly colored, site attached, benthic fish that occupies physically complex rocky habitats along the coasts of southern California and Baja California, Mexico. Populations are typically female biased and density typically ranges from 24 to 58 individuals per square meter (Behrents 1983). In small social groups sex is determined by rank in a dominance hierarchy where usually the largest individual is dominant, assuming the male sex, while smaller individuals are subordinate and assume the female sex (Reavis and Grober 1999, Black et al. 2005, Rodgers et al. 2005). Reproduction is seasonal and females with developed eggs are commonly found from May to August (Wiley 1976, Behrents 1983). Males guard and defend nest sites into which multiple females lay their demersal eggs. Life expectancy of *L. dalli*

is short (1.5-2 years) relative to the length of the reproductive and recruitment season (~6 months) and thus populations of *L. dalli* are dynamic, changing in both size structure and density within and between years (Wiley 1976, Behrents 1983).

*Field Experiment:*

We explored the effects of population density on the frequency of sex change in a field experiment in July and August of 2009. We manipulated the density of adult fish on artificial patch reefs built in Big Fisherman Cove on Santa Catalina Island, California, U.S.A. (33°27'3" N; 118°29'15" W). We constructed twenty-four artificial patch reefs on a sandy bottom approximately 30 meters away from the nearest natural rocky reef. Each artificial reef was assembled using approximately the same number and size distribution of rocks collected from a nearby shore. Each artificial reef was assembled by piling the rocks on a 1 x 1 m square polyvinyl chloride (PVC) pipe frame covered in plastic mesh netting (1.6 x 1.9 cm openings), measured 1m<sup>2</sup> at the base, and was approximately 0.3 m high at the center. The reefs were built in 3 rows of 8 following the contour of the bottom with rows at depths of 13, 15, and 17 m. Reefs were separated from each other and any other suitable habitat by a minimum of 4 m of sand eliminating movement of fish between reefs as done in previous field studies with this species (St. Mary 1994, Steele 1997).

We anesthetized and collected *L. dalli* from nearby natural reefs using squirt bottles containing a solution of 10% clove oil and 90% ethanol and transported them to the laboratory. We anesthetized each individual in a seawater solution containing tricaine methanesulfonate (MS-222), measured its standard length to the nearest 0.1 mm, and determined its sex by examination of the genital papilla. Fish with a blunt shaped papilla have previously been found to allocate more than 95% of their gonad to ovarian tissue and spawn as female while fish with a

long pointed papilla possess an accessory gonadal structure filled with mucus and sperm and spawn as male (St. Mary 1993, Drilling and Grober 2005). We classified fish with blunt shaped papilla as female and those with a long pointed papilla as male. Individuals with an ambiguous morphology (~5%) were not used in the experiment. Each fish was given a visual implant elastomer tag to indicate its size to the nearest 1 mm and another tag to indicate its initial sex. To ensure only sexually mature adults were used in the experiment we excluded individuals less than 20 mm standard length (Wiley 1976).

Three density treatments (8 replicates of each) were randomly assigned to the artificial patch reefs. We stocked 3 artificial reefs every 1-3 days with their pre-determined treatment densities between the 3<sup>rd</sup> and 20<sup>th</sup> of July, 2009. Anticipating some mortality, we stocked high density reefs with  $125 \pm 0.5$  fish, mid with  $68 \pm 1.1$  fish and low with  $20 \pm 0.4$  fish (variation due to handling loss), reflecting and exceeding the range of densities observed on natural reefs (Behrents 1983). Neither the average size of fish nor the variance in size differed among density treatments (ANOVA,  $F_{1,23}, p=0.9814$ , Levene's test for equal variance  $p = 0.9721$ ). The sex ratio of stocked fish (85% female) matched that of the fish collected from natural reefs and also did not vary between treatments.

Previous experiments found mortality of *L. dalli* to be density-dependent and caused by predation (Steele 1998) so we used predator exclusion cages to maintain differences between experimental density treatments. The bottom of each cage was laid on the sea floor prior to the construction of the artificial reef (see above). Exclusion cages were constructed using a 1 x 1 x 0.5 m (L x W x H) PVC box covered on 4 sides and the top with the same plastic mesh netting described above. The mesh netting was small enough to exclude large predatory fish but did not restrict movement of *L. dalli* in and out of the cages. The exclusion cage was placed over the reef

and attached to the 1m-square frame below each reef using cable ties. To minimize accumulation of algae, cages were regularly scrubbed and also exchanged approximately every 12 days with dry, relatively clean cages from the shore. Even with these predator cages loss of fish from the reefs was substantial and caused the densities to decline from their original stocking values. However, this loss was not density-dependent (ANOVA,  $F_{1,23} = 0.10, p = 0.9072$ ) and density treatments still differed significantly at the end of the experiment (ANOVA,  $F_{1,23} = 57.34, p < 0.0001$ ).

Thirty-one days after stocking, all surviving fish were collected from each reef and returned to the laboratory where their final sex was determined as before. By comparing the final sex of individuals to the initial sex indicated by the elastomer tag, we were able to identify individuals that had changed sex during the experiment. Fish with ambiguous papilla morphology were assumed to be in transition between sexes and thus were recorded as having changed sex. The prevalence of sex change was calculated as the proportion of survivors that changed sex on each reef. The initial body size of sex-changing individuals was also recorded. To determine the relationship between body size and the frequency of sex change we calculated the proportion of individuals that changed sex in each 1 mm size class.

#### *Laboratory Experiment:*

Concurrent with the field experiment we also examined the effects of population density on individual sex change in a laboratory experiment. Twenty-four experimental populations were established in replicate aquaria 43.8 x 29.2 x 25.4 cm (L x W x H) containing approximately 32.5 L of sea water. In each aquarium we placed several small rocks to provide refuges similar to the field experiment.

To create the experimental fish populations we collected *L. dalli* from natural reefs and tagged them as described above. The sex ratio and size structure used for experimental laboratory populations matched that used for the field experiment; however, we could not use the same population densities as the field experiment because the laboratory aquaria were smaller than the field reefs (~1/8 the bottom surface area). To create the same sex ratio as the field experiment (85% female), we had to use higher density treatments in the lab. The average numbers of fish used for each treatment were: high =  $28 \pm 0.2$ , mid =  $13 \pm 0.0$  and low =  $7 \pm 0.0$  individuals per aquaria which equate to densities of approximately 208, 100 and 54 fish per square meter respectively. These densities were similar to, and exceeded those used in previous laboratory experiments examining sex change in this species (Reavis and Grober 1999, Rodgers et al. 2007). Density treatments were randomly assigned to laboratory aquaria and laboratory populations were stocked on the same days as the artificial field reefs. Neither the average size of fish (ANOVA,  $F_{1,23} = 1.87, p = 0.1847$ ) nor the variance in size (Levene's test for equal variance,  $p = 0.2627$ ) differed among density treatments. Mortality of fish in the laboratory was low (7.1% of total fish) and any dead fish were replaced within 24 hours with a fish of equal size and sex, maintaining the density of the laboratory populations throughout the experiment. After 31 days all fish were collected from the aquaria and each individual was anesthetized in order to determine its final sex and record sex changing individuals as described above.

*Analysis:*

For both the laboratory and the field experiment we used a one-way ANOVA to test for the effect of fish density on the proportion of individuals that changed sex. We also used one-way ANOVA to determine if the final sex ratio differed among density treatments as a result of differences in frequency of sex change. We tested for an effect of density on the average change

in proportion female (final proportion females – initial proportion female) for both artificial reefs and laboratory aquaria. Data was transformed when necessary to meet the assumptions of parametric statistics.

We used logistic regression to test for an effect of initial size (independent) on the probability of female sex change (dependent) and included a density\*size interaction term to determine if the influence of body size varied with density. The low numbers of sex-changing males did not allow us to test for a similar relationship in male sex change. Sex change was binary, either change or no change. The contribution of initial size and density\*size interaction were evaluated using the likelihood-ratio test. The effect of density and the interaction between density and size was not significant in either of the models and so we re-ran the analysis without them. Data reported are for logistic regression with initial size as the only independent and the probability of sex change as the dependent. All data analysis was done in JMP (version 9.0; SAS Institute Inc.).

## **Results:**

The influence of density differed between field and laboratory populations. In the field 10.7% of survivors changed sex and sex changing individuals were found on every reef except for one. The proportion of individuals that changed sex was not significantly influenced by density (ANOVA,  $F_{1,23} = 0.67, p = 0.5233$ ) (Figure 1). Although the proportion of sex changing individuals declined with increasing density this pattern was not significant due to high variability. In contrast, sex change was density-dependent in small laboratory social groups. In the laboratory 8.1% of survivors changed sex and at least one individual changed sex in 16 of the 24 aquaria. The proportion of individuals that change sex was significantly affected by density

(ANOVA,  $F_{1,23} = 3.86, p = 0.0374$ ) (Figure 2). Only two of the eight low density laboratory tanks contained sex changing individuals which resulted in a marginally significant lower proportion of sex changing individuals in the low density treatment when compared to the mid ( $p = 0.0800$ ) and high ( $p = 0.0511$ ) density treatments (Tukey post-hoc test). Although the low density treatment deviated from a normal distribution this likely did not have a strong effect on the F-statistic as the other two treatments were normal and the variances of the transformed data were homogenous.

In both experiments, most of the sex-changing individuals were females. We observed 83 sex-changing females on artificial reefs and only 8 sex-changing males. Similarly in laboratory aquaria we observed 26 sex-changing females and only 3 sex-changing males. Averaged across all densities the proportion of females that changed sex exceeded the proportion of males that changed sex in both the field (Wilcoxon test  $\chi = 9.73, p = 0.0018$ ) and laboratory (Wilcoxon test  $\chi = 13.03, p = 0.0003$ ). In both experiments, the prevalence of female sex change caused most experimental populations to have a less female biased sex ratio at the end of the experiment. However, change in proportion female did not vary significantly with density for either experiment (Lab: ANOVA,  $F_{1,23} = 01.31, p = 0.2908$ , Field: Wilcoxon/Kruskal-Wallis,  $\chi^2 = 3.13, p = 0.2096$ ) (Figure 3). High variability in the final proportion female may have obscured the link between individual sex change and the final proportion female.

Sex change of females was significantly influenced by initial body size and this relationship did not vary with density in either experiment. The probability that females changed sex increased with body size on artificial reefs (logistic regression,  $\chi^2 = 55.15, p < 0.0001$ ) and in laboratory aquaria (logistic regression,  $\chi^2 = 48.70, p < 0.0001$ ) (Figure 4). The size at which 50%



of the females were expected to change sex was predicted from logistic regression models and found to be larger (35.2 millimeters) on field reefs than in laboratory aquaria (33.0 millimeters).

### **Discussion:**

We found the effect of density on the frequency of sex change to differ between laboratory populations and field populations suggesting that behavior patterns of fish in the laboratory may disappear in larger more natural populations. Sex change was density-dependent in our laboratory experiment, with the smallest social group having the lowest rates of sex change, but we found no effect of density on artificial patch reefs in the field. Our results contrast with the finding of Black et al. (2005), who found morphological and behavioral patterns in small (n=4) field and laboratory social groups of *L. dalli* were similar. In our experiment field populations contained more individuals than laboratory populations and we did not find similar patterns in behavior suggesting that population size may be driving these differences. Similarly, reproductive behavior of three-spined sticklebacks (*Gasterosteus aculeatus*) was found to vary between small and large pools of equal density (FitzGerald et al. 1992). In small social groups *L. dalli* are known to form stable dominance hierarchies dependent largely upon relative body size and maintained through social interactions (Black et al. 2005, Lorenzi et al. 2006, Rodgers et al. 2007). Consistent with these findings, the low frequency of sex change in our low-density social groups in laboratory aquaria suggested that individuals quickly formed stable social hierarchies in which the social status of each individual complemented its initial sex. Our results suggest that patterns in sex change of *L. dalli* may vary with population size and thus, patterns in sex change of the laboratory fish may not accurately approximate behavior in field populations.

Our findings suggest that results from laboratory studies may create false impressions regarding the importance of social group formation and size-based hierarchies in governing behavior of *L. dalli* in natural field populations. The differing effect of density on the frequency of sex change of fish in the laboratory and in the field may have been driven by changes in the stability of dominance hierarchies at these different scales. Size-based hierarchies, which determine dominance behavior and sex change, may become more ambiguous in larger populations as the average size difference between individuals decreases (Ang and Manica 2010b, Wong 2011). We found rates of sex change increased and were relatively similar when group sizes were larger ( $n > 13$ ) suggesting that when more individuals are present size hierarchies become less stable. Recognition of familiar individuals may also play an important role in social stability (Hojesjo et al. 1998) and become more difficult in larger populations. For instance, as group size increased in natural populations female guppies, *Poecilia reticulata*, were less likely to interact with familiar individuals (Griffiths and Magurran 1997). Together these results suggest that size-based hierarchies in natural populations may be dynamic and only loosely influence sex change of individuals.

Greater environmental complexity in field populations may also influence the frequency of sex change by decreasing the stability of social groups further. Compared to fish in the lab, fish on our field reefs experienced a more complex environment with variable food supply, changes in water temperature, higher number of refuges, nearby predators, and loss of individuals from their population. Increasing environmental complexity has been shown to influence social behaviors (Solman and Armstrong 2002) important for the formation of dominance hierarchies and determination of functional sex in *L. dalli* (Lorenzi et al. 2006). At larger spatial scales, like those of our field reefs, individuals may range over a larger area. High

spatial segregation between adjacently ranked *Centropyge bicolor*, a coral reef fish, was correlated to decreasing stability of size hierarchies (Ang and Manica 2010a). Furthermore, loss of some individuals (probably mortality if some fish swam out of the exclusion cages where they were not protected from predators) may have stimulated other individuals to change sex resulting in the higher rates of sex change. For instance, removal of a large dominant individual has been shown to stimulate sex change of one or more subordinates in a variety of sex-changing species (Sakai et al. 2003, Liu and Sadovy 2004, Black et al. 2005, Munday et al. 2006). Thus, loss of individuals from field reefs may have further disrupted social hierarchies and increased the frequency of sex change in field populations. Our findings suggest that environmental complexity may be interacting with the formation of social hierarchies to influence sex change of individuals in complex ways.

We found that sex change of female *L. dalli* may be common, predictable, and based on body size in natural field populations. In our experiment sex change of females was more prevalent than sex change of males as was found in natural populations of the bi-directional sex-changing coral gobies *Paragobiodon echinocephalus* and *Gobiodon histrio* (Kuwamura et al. 1994, Munday 1998). Sex change of females matched that predicted if dominance hierarchies based on relative size govern sex change (Black et al. 2005, Rodgers et al. 2007). The probability of female sex change increased with body size in both experiments and this relationship did not vary with density. This result suggests that the relationship between body size and reproductive success of *L. dalli* males and females does not vary with population density. Overall our results indicate that body size may be a sufficient predictor of female sex change in natural populations of bi-directionally sex-changing species.

In summary, sex change of fish in the laboratory was density-dependent while sex change of fish in the field was not and this difference suggests that social group formation and size-based hierarchies may be less important in governing sex change in natural field populations than previously thought. Increased population size and environmental complexity in natural populations may reduce social stability and result in higher frequency of sex change than might be predicted from laboratory observations alone.

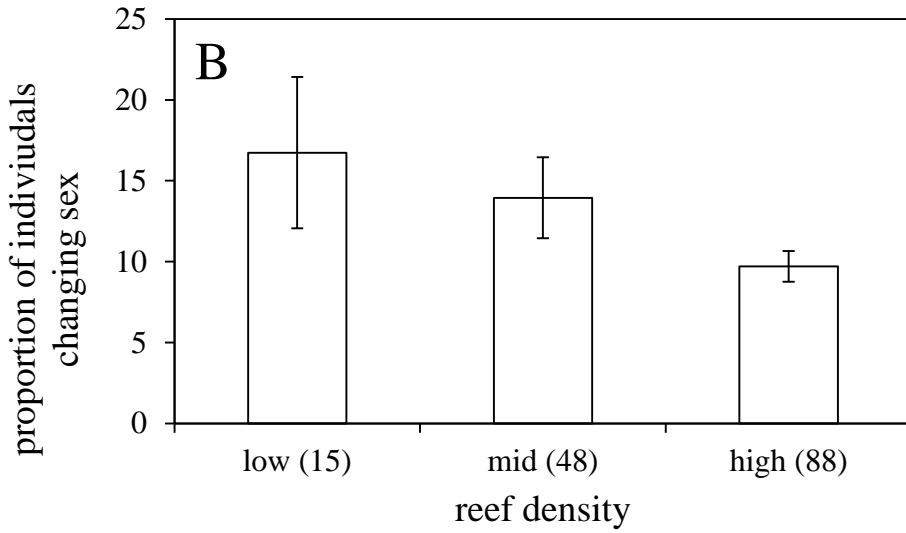


Figure 3.1: No significant effect of density on the proportion of individuals changing sex on artificial field reefs ( $p = 0.5233$ ). Numbers in parentheses are average number of fish on field reefs. Bars are means  $\pm$  standard error. Data was arcsine-root transformed before statistical analysis.

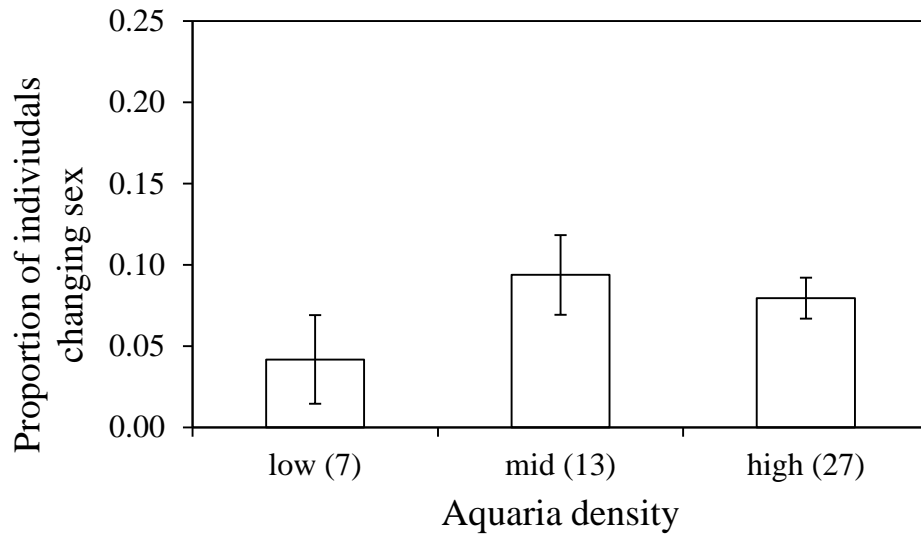


Figure 3.2: A significant effect of density on the proportion of individuals changing sex in laboratory aquaria ( $p = 0.0374$ ). Numbers in parentheses are the average number of fish in laboratory aquaria. Bars are means  $\pm$  standard error. Data was arcsine-root transformed before statistical analysis.

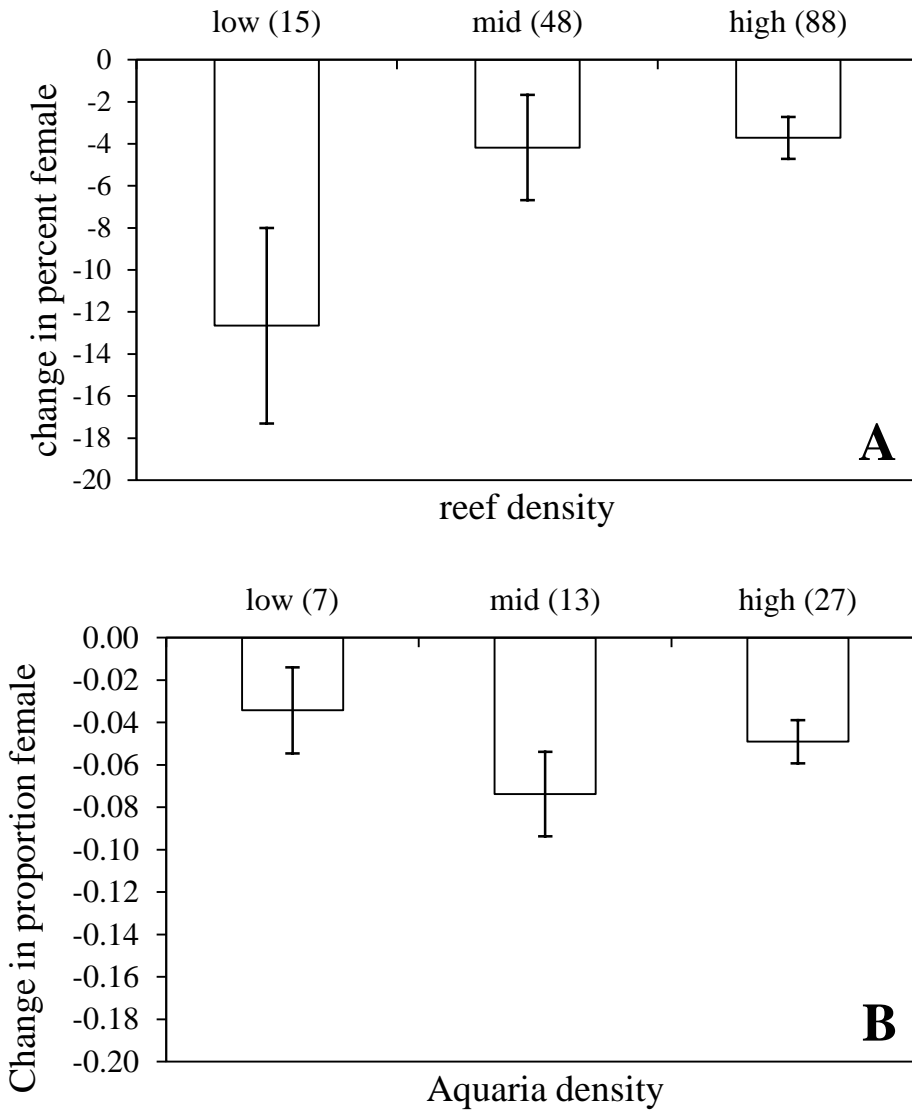


Figure 3.3: The relationship between the change in proportion female (final proportion female minus initial proportion female) and density treatment on (A) field reefs ( $p = 0.2096$ ) and in (B) laboratory aquaria ( $p = 0.2908$ ). Negative numbers indicate populations that became less strongly female biased during the experiments. Numbers in parentheses are average number of fish in each treatment. Bars are means  $\pm$  standard error. Wilcoxon non-parametric test was used for reef data as the data did not meet the homoscedasticity assumption.

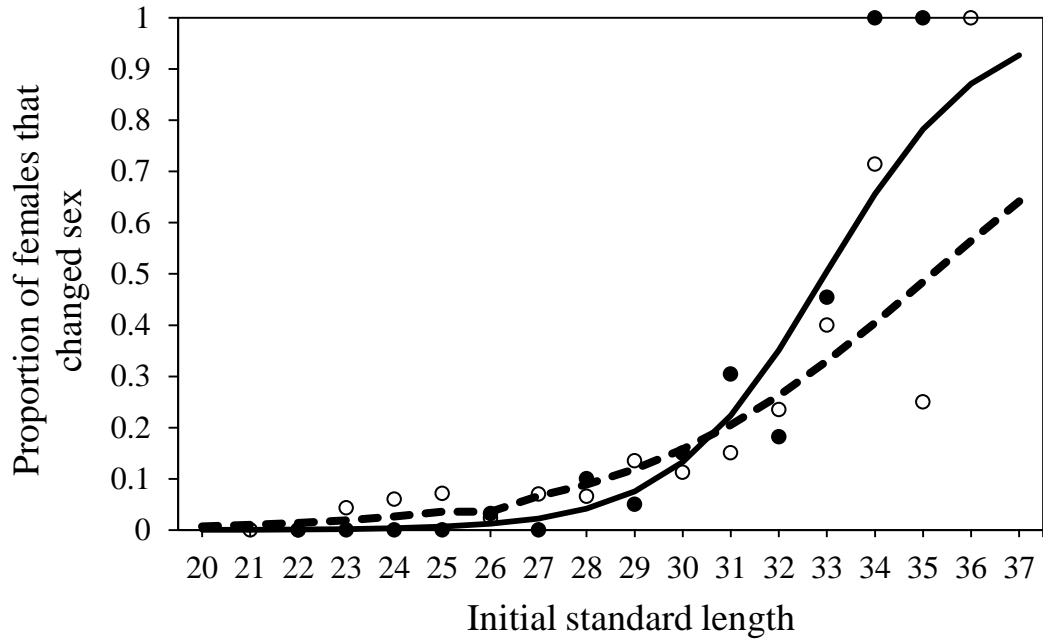


Figure 3.4: The increasing probability of sex change of females with initial body size in laboratory aquaria (solid line) ( $p < 0.0001$ ) and on artificial reefs (dotted line) ( $p < 0.0001$ ). Dots are the proportion of females in each 1mm size class that changed sex in laboratory aquaria (closed circles) and on artificial reefs (open circles).



## CONCLUSION

This study demonstrated the influence of two population characteristics, density and adult sex ratio, on individual sex change and reproductive success of the temperate reef fish *Lythrypnus dalli*. Population density may influence per capita egg production both directly, through competition for resources or by altering social structures and mating behavior (chapter 1), or indirectly through altering the frequency of sex change (chapter 3). High frequency of female to male sex change decreased the proportion of females in the population (chapter 3), and my results demonstrated reduced egg production when fewer females were present (chapter 2). The results from my dissertation suggest that changes in social structure, mating behavior and sex change may be the mechanisms reducing per capita reproduction at high density in marine fishes. The question still remains whether such variation in egg production influences adult population size across generations. A growing body of evidence suggests that populations of marine organisms may be demographically closed on smaller scales than previously understood, and therefore recruitment to local populations may, to some extent, rely on local reproductive output (Jones et al. 1999, Swearer et al. 1999, Swearer et al. 2002, Palumbi and Warner 2003, Taylor and Hellberg 2003, Levin 2006, Morgan et al. 2009). Together this evidence suggests that density-dependent reproduction may contribute to population regulation.

Results from this study suggest that egg production in nesting fishes may be influenced by variation in population characteristics such as density or sex ratio. We found that per capita reproduction in *L. dalli* declines with increasing density in field populations. Experimental evidence for density-dependent reproduction was also recently found in the tropical bridled goby, *Coryphopterus glaucofraenum* (Forrester et al. 2010) and in the freshwater fathead minnow, *Pimephales promelas* (Vandenbos et al. 2006), suggesting that these effects may be

common among benthic nesting fishes. Reduced per capita egg production of females at high density may occur if resources become limiting and reduce the amount of energy available for reproduction (Lindstrom et al. 2005, McCormick 2006), or if social behavior varies with density, reducing the proportion of individuals reproducing at high density (Lutnesky and Kosaki 1995, Spence and Smith 2005). Future research will be necessary to elucidate the mechanisms causing density-dependent reproduction and the degree to which they influence adult populations. We also found that populations with female-biased sex ratios tended to produce the most eggs. Similar results have been found for the influence of adult sex ratios on reproduction in birds (Ewen et al. 2010) and in mammals (Røed et al. 2002, Solberg et al. 2002). Invariance of per capita reproduction of females suggests that total reproductive output may be determined directly by the number of females present. A release from aggressive courting by males in female-biased populations may allow females to produce more eggs or develop them faster (Le Galliard et al. 2005, Spence and Smith 2005). Together this evidence suggests that variation in population characteristics may have strong influence on reproduction output of populations.

Variation in population characteristics may ultimately influence reproduction through their effects on social and reproductive behavior. Per capita reproduction of females declined in high density field populations. A potential mechanism may be that aggressive behavior of socially dominant individuals (Rodgers et al. 2007) suppresses mating of subordinate females (Lutnesky and Kosaki 1995, Spence and Smith 2005). We also found negative density-dependence in the proportion of males guarding nests with eggs. Female choice for, and aggressive behavior of, large dominant males (St. Mary 1994, Rodgers et al. 2007) may allow them to monopolize all mating opportunities in the vicinity (Peterson 1990). Furthermore, increased environmental complexity in natural environments may influence social behaviors

(Solman and Armstrong 2002) and alter the frequency of sex change (Rodgers 2003). Overall these results suggest that changes in population characteristics may influence the opportunity for female choice, the relative reproductive success of males and females and thus, the frequency of sex change.

This dissertation is one of a handful studies (Kuwamura et al. 1994, Munday 1998, Munday 2002, Manabe et al. 2007, Rodgers et al. 2007) that examine the adaptive significance and frequency of occurrence of bi-directional sex change in natural field populations. Relative body size proved to be the most important predictor of female sex change in this study, corroborating earlier results on this species (Black et al. 2005, Rodgers et al. 2007) and in other fish species (Ross et al. 1990, Liu and Sadovy 2004, Rodgers et al. 2005, Rodgers et al. 2007). Sex change of males, in contrast, was rare and unpredictable in natural populations. Few males changed sex in our experiments and, the few that did revealed, no clear relationship of sex change to body size, population density or the adult sex ratio. Previous documentation of male sex change in the laboratory (Rodgers et al. 2005, Rodgers et al. 2007) suggests that the social conditions that prompt male sex change in small social groups occur infrequently in field populations or in larger social groups. Additionally, small males may contain gonads similar in size to large dominant males (Cole 1982, Drilling and Grober 2005) suggesting that small males achieve reproductive success by utilizing a sneaking strategy. In such case there may no reproductive advantage of male sex change. We suggest that sex change from male to female, while physiologically possible, may be unimportant in natural populations. Together these findings emphasize our lack of understanding of the adaptive significance of bi-directional sex change in natural populations of *Lythrypnus dalli* and other bi-directional sex changing fishes.

Results from laboratory and field experiments suggest that processes that influence reproductive behavior of laboratory fish may differ from natural field populations. Differences in the effect of density on per capita reproduction and sex change in our laboratory and field experiment indicate the processes that influence reproductive behavior in small social groups may not approximate processes in larger field populations. We suggest that changes in individual social interactions between fish in small laboratory social groups and fish in larger field populations drive these differences in sex-change behavior and reproductive success. For instance, aggressive behavior between individuals of differing dominance status may be more frequent in large social groups and interfere with mating behavior (Stanley and Wootton 1986, Spence and Smith 2005). Female choice for large dominant males (St. Mary 1994) may become stronger in large populations in which more males are present, resulting in relatively few males that monopolize all mating opportunities (Peterson 1990). Recognition of familiar individuals may play an important role in social stability (Hojesjo et al. 1998) and become more difficult in larger populations (Griffiths and Magurran 1997). Furthermore, greater environmental complexity experienced by fish in natural populations may further decrease the stability of social groups. For instance, loss of individuals from the population may stimulate sex change in some remaining individuals (Sakai et al. 2003, Liu and Sadovy 2004, Black et al. 2005, Munday et al. 2006). Overall our results indicate laboratory studies of small social groups may not be sufficient for elucidating the mechanisms that influence reproductive success and reproductive behavior of *L. dalli* in natural field populations.

This work contributes to a limited understanding of the factors that may influence reproductive output in marine fish populations. We found evidence that both population density and the number of females in a population may influence total egg production and reproductive

success of individuals. Furthermore, decreasing social stability in high-density populations may also influence the frequency of sex change and the reproductive success of individuals. Future work will be necessary to determine the degree to which processes that influence individual reproductive success and per capita reproduction of populations contribute to changes in adult population size across generations.

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