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**THE EFFECT OF OPERATIONAL SEX RATIO ON THE
REPRODUCTIVE BIOLOGY OF TWO SEX-CHANGING FISH
(*RHINOGOBIOPS NICHOLSII* AND *LYTHRYPNUS DALLI*)**

A Thesis

Presented to the

Faculty of

Moss Landing Marine Laboratories

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Marine Science

by Stephen Pang

Spring 2019

CALIFORNIA STATE UNIVERSITY, MONTEREY BAY

The Undersigned Faculty Committee Approves the


Thesis of Stephen Pang:

THE EFFECT OF OPERATIONAL SEX RATIO ON THE REPRODUCTIVE BIOLOGY OF
TWO SEX-CHANGING FISH (*RHINOGOBIOPS NICHOLSII* AND *LYTHRYPNUS DALLI*)

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21 May 2019

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ABSTRACT**THE EFFECT OF OPERATIONAL SEX RATIO ON THE REPRODUCTIVE BIOLOGY OF TWO SEX-CHANGING FISH (*RHINOGOBIOPS NICHOLSII* AND *LYTHRYPNUS DALLI*)**

by

Stephen Pang

Master of Science in Marine Science
California State University Monterey Bay, 2019

By targeting the largest individuals in a population, size-selective fisheries can influence the life history traits and population parameters of exploited fish stocks. For protogynous (female-to-male) hermaphrodites, this type of harvest is also sex-selective since it preferentially removes males from the population. These differences in sex-specific survival can lead to populations that are heavily female-biased. While males historically have not been considered a limiting factor when assessing the health of gonochoristic populations, modeling work suggests that reduced male abundance and skewed sex ratios could cause a concomitant decline in the reproductive output of protogynous hermaphrodite populations. This study used two nest-brooding sex-changers, *Lythrypnus dalli* and *Rhinogobiops nicholsii*, to examine the effect of operational sex ratio on reproductive and nesting success, growth, and rates of sex change. Fish were outplanted on artificial patch reefs at varying sex ratios and their reproductive output was monitored by photographing eggs laid in artificial nests. Sex ratios ranged from 1:1 to 1:19 male:female. Fish were tagged so that growth and sex change could be determined upon recollection from the artificial reefs. For both *L. dalli* and *R. nicholsii*, total egg production, female per capita production, average production per nest, and the number of nests per reef were not affected by sex ratio. By contrast, male per capita production and the percentage of males that nested significantly increased as sex ratios became more female-biased. For *R. nicholsii*, growth rates were highest for individuals that completed sex change during the experimental period. During the breeding season, the frequency of sex change for *R. nicholsii* was highest on reefs that were strongly female-biased; there was no effect of sex ratio on the frequency of sex change during the non-breeding season. In *L. dalli* and *R. nicholsii*, it appears that males do not limit the reproductive output of heavily female-biased populations—as had been predicted by previous modeling work. Instead, for species that defend demersal nests, intrasexual competition between males (i.e., territory and mate monopolization) or females (i.e. competition for nest space) may limit total production when operational sex ratios are more balanced or more female-biased, respectively. As sex ratios became skewed in favor of females, male-male competition was relaxed and individual males became more reproductively successful; the discrepancy in per capita production between males and females at skewed sex ratios indicates that some females would increase their reproductive success by undergoing sex reversal (as demonstrated by *R. nicholsii* during the breeding season). It is possible that some of the results on reproductive success from this study are specific to nest-brooding or pair spawning species.

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INTRODUCTION

Naturally occurring size-selective mortality is prevalent in many aquatic systems. Gape limitation often constrains the diets of predators to prey that can be swallowed—leading to increased vulnerability of smaller individuals to predation (Leggett and DeBlois 1994; Hare and Cowen 1997). Conversely, zooplanktivorous fishes that forage visually often preferentially feed on larger prey items, resulting in increased survivorship of smaller individuals (Brooks and Dodson 1965; Janssen 1980; Newman and Waters 1984).

Fisheries attempting to target the largest individuals in a population can be a major source of size-selective mortality. In the commercial sector, this size-selective tendency (which may ultimately be driven by consumer demand or preference) is often due to the selectivity of the fishing gear (Millikin and Williams 1984; MacLennan 1992). Gear type allows fishermen to target specific size classes, e.g., large hook and mesh size have been shown to selectively remove larger individuals, which consequently increases the survivorship (and thus fitness) of smaller size classes (Ricker 1981; Russ 1991; Stergiou et al. 1997; Platten et al. 2002; Sutter et al. 2012). In the recreational sector, size-selectivity may be present if fishermen are limited by minimum size or bag limits; by targeting the largest individuals, anglers and spear fishermen are able to maximize their catch.

In the long-term, continuous size-selective mortality can reduce phenotypic and genetic variation (Stokes and Law 2000). Typically, natural selection selects for fast growing individuals because by attaining larger sizes more quickly, individuals are able to escape predation by avoiding predators with smaller gape sizes (Hare and Cowen 1997). Because of this increased susceptibility to predators, smaller and slower growing individuals naturally have the highest mortality (Leggett and DeBlois 1994). Directional selection against larger individuals, as

exhibited by fisheries-induced evolution, selects for slow-growing individuals, which results in a decline in length-at-age and body weight after years of exploitation (Ricker 1981; Conover and Munch 2002; Sinclair et al. 2002; Hawkins and Roberts 2003; Hamilton et al. 2007).

This decrease in average fish size has implications for the reproductive success of exploited populations. Removing the largest individuals in a population decreases the number of mature age classes, which can be particularly problematic for long-lived species if individuals are removed from the population before they mature, eliminating their ability to spawn (Kelly et al. 1999; Andrews et al. 2001). Older age classes also confer additional benefits to the reproductive success of populations. Big, old, fecund, fat females (BOFFFs) play a disproportionately important role during spawning. In addition to producing more eggs (gamete production is typically related to total fish length), these large females may produce eggs with a higher oil content, which leads to increased survivorship relative to eggs of smaller females (Berkeley et al. 2004).

While many fish are gonochoristic (i.e., sexes are separate; male and female reproductive organs are in different individuals), a number of commercially and recreationally important fish are hermaphroditic (i.e., individuals have male and female gonadal tissue, either at the same time or at different phases of the life cycle). This hermaphroditism is explained by the “size-advantage model” which predicts that if the amount of offspring produced at a given body size differs between the sexes, then a sex-changing individual can take advantage of these differences to produce more offspring than an individual limited to a single sex (Ghiselin 1969; Ghiselin 1975; Warner 1988; Munday et al. 2009). Differences in reproductive output between the sexes largely occur due to (1) the relative number of male and female gametes produced and (2) the mating strategy of the species (Warner 1970).

There are two major types of hermaphrodites: simultaneous and sequential. Simultaneous hermaphrodites have functional female (ovarian) and male (testicular) gonadal tissue concurrently whereas sequential hermaphrodites have only one gonadal tissue functioning at a time (although the type of tissue can switch during sex reversal) (Warner 1970; Sadovy and Shapiro 1987). Sequential hermaphroditism can be further divided into protogyny and protandry. Protogyny (female-to-male sex change) typically occurs when species exhibit a polygynous mating system (males mate with many females) while protandry (male-to-female sex change) occurs when species exhibit a monogamous or random mating system (Warner 1970; Alonzo and Mangel 2005; Munday et al. 2011). Sex change in sequential hermaphrodites is governed by endogenous and/or exogenous behavioral mechanisms (Warner 1970). Endogenous factors are static cues that trigger sex change once a certain threshold (e.g., absolute size or age) is reached; exogenous factors are external cues from the environment, such as the size of the individual relative to other members of its social group (Bullough 1947; Armsworth 2001; McErlean and Smith 1964; Ross et al. 1983; Shapiro 1987; Provost and Jensen 2015).

The life history of sex-changing fishes presents a unique management problem since most gonochoristic fisheries models cannot be applied to protogynous hermaphrodites (Bannerot et al. 1987; Russ 1991; Armsworth 2001). Traditionally, female biomass (and the spawning potential ratio) has been used as a proxy for population health in gonochoristic species, while the contribution of males has largely been ignored (Punt et al. 1993; Coleman et al. 2000; Alonzo and Mangel 2004). This is done because (1) sperm is energetically cheap to produce and (2) males are able to spawn with multiple females (Bateman 1948; Marconato et al. 1995; Heppell et al. 2006; Alonzo et al. 2008). In protogynous populations though, the possibility of reduced fertilization rates may necessitate using male biomass or the biomass of both sexes to accurately

estimate biological reference points (Brooks et al. 2008). Furthermore, these models for protogynous stocks need to incorporate sex change since populations will lose female biomass—but not total biomass—as females transition to males (Alonzo et al. 2008).

Most traditional models also ignore sex-specific characteristics and sex-specific survival (Heppell et al. 2006; Alonzo et al. 2008). In some protogynous species, males experience a burst of growth after sex change, which allows them to establish dominance over large females and to better compete with existing males; this accelerated growth may push males into size classes that are more vulnerable to fishing (therefore increasing their mortality when compared to non-transitioning individuals) (Aldenhoven 1986; Ross 1987; Warner 1991; Garratt et al. 1993; Walker and McCormick 2004). Similarly, protogynous males often have a higher likelihood of interacting with fishing gear due to their larger size, greater aggression, and higher energy requirements (Côté 2003; Sutter et al. 2012). Since spawner-recruit curves may depend on the relative abundance of females and males, differences in catchability and sex-specific mortality rates also need to be incorporated into assessments (Punt et al. 1993; Heppell et al. 2006).

Intensely harvested protogynous species routinely exhibit fishery-induced changes to life history traits. In exogenous sex-changing species, the timing of maturation and sex change can occur at younger ages and smaller sizes when populations are heavily harvested (Buxton 1990; Cowen 1990; Warner and Swearer 1991; Buxton 1992; Buxton 1993; Claro et al. 2001; Gust et al. 2001; Gust et al. 2002; Platten et al. 2002; Hawkins and Roberts 2003). In California sheephead (*Semicossyphus pulcher*), a heavily fished protogynous species, females matured earlier and underwent sex reversal at younger ages and smaller sizes after intense exploitation (Hamilton et al. 2007). A decreased mean size and age for females could lead to decreased egg production and reduced population fecundity. In species that change sex based on endogenous

cues, populations may be unable to compensate for intense harvest on ecological time scales; if individuals are removed before they are able to reach the age or size necessary for sex change, males could be completely removed from the population (Hawkins and Roberts 2003). Spatial variation in life history and population parameters, in response to spatial variation in fishing intensity and selectivity, can confound spatially based fisheries management and necessitate more localized management (Gust 2004).

In sequential hermaphrodites, size-selective fishing is also sex-selective by disproportionately removing a single sex from a harvested population (Alonzo et al. 2008). In the case of protogynous hermaphrodites, targeting the largest individuals in a population will preferentially remove males. Sex ratios in protogynous hermaphrodites are often already skewed in favor of females and this sex-selective fishing can often add even greater female bias in heavily fished populations (Garratt 1985; Beets and Frielanders 1992; Buxton 1992; Buxton 1993; Coleman et al. 1996; Wyanski et al. 2000; Hawkins and Roberts 2003; Hamilton et al. 2007; Shepherd et al. 2010). This trend is especially obvious in gag grouper (*Mycteroperca microlepis*), a protogynous species that aggregates to spawn, where the proportion of males fell from 14% to 1.9% after only 10 years of exploitation in the Gulf of Mexico (Hood and Schlieder 1992; Coleman et al. 1996; Coleman et al. 1998).

Severely skewed gender ratios in exploited protogynous populations can lead to male limitation and a reduction in reproductive output (even if female biomass remains high) (Alonzo et al. 2008). This decrease in reproductive output may be due to: (1) females being unable to find mates, (2) a male's inability to successfully defend their territories or nests, or (3) sperm limitation (Gray and Powell 1964; Powell et al. 1973; Nakatsura and Kramer 1982; Møller and Legendre 2001; Hines et al. 2003; Alonzo and Mangel 2004). Sperm limitation can occur if the

remaining males spawn repeatedly without sufficient time to rebuild sperm stores (Smith and Jamieson 1991; Marconato et al. 1995; Hines et al. 2003). Alternatively, males in exploited populations may be too immature or too small to produce a sufficient amount of sperm (Lovrich et al. 1995; Sainte-Marie et al. 1997). Both of these situations ultimately lead to an increase in the number of unfertilized eggs and a decrease in total population production.

Several modeling studies have examined how fishing might impact the productivity of protogynous stocks, with varying results. Early work indicated that protogynous populations were considerably more susceptible to harvest and crashed at lower fishing mortalities when compared to gonochoristic stocks (Bannerot et al. 1987; Huntsman and Schaaf 1994). In contrast, more recent work suggests that protogynous hermaphrodites should perform better than gonochoristic stocks when subjected to size-selective (i.e., large size classes) fishing, since fecundity and fertility (in the absence of sperm limitation) will remain high, whereas gonochoristic species would lose BOFFF's to fishing mortality (Alonzo and Mangel 2004; Alonzo and Mangel 2005). It is difficult to unequivocally say whether gonochoristic or protogynous stocks are more sensitive to fishing. With that said, male and sperm limitation are predicted to impact the reproductive success of protogynous species more significantly than comparable gonochoristic stocks (although gonochores may also be vulnerable to sperm limitation when spawning aggregations are very small) (Bannerot et al. 1987; Alonzo and Mangel 2004; Alonzo and Mangel 2005).

While past field surveys have documented the effect of male and sperm limitation in natural populations, they have largely focused on sessile and sedentary free-spawning invertebrates (Lessios 1988; Levitan 1992; Sewell 1994; Lasker 1996; Yund 2000; Hines et al. 2003). And while other work has focused more specifically on the effects of sex ratio skew in

protogynous hermaphrodite species, these investigations have largely been confined to computer simulations rather than manipulative field experiments in a natural setting (Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004). One field experimental study examined the relationship between sex ratio and reproductive output in a nest-brooding temperate goby species (*Lythrypnus dalli*; Kapus and Fong 2014), but focused primarily on individual reproductive success (i.e., male and female per capita production) as opposed to population-level reproductive success (i.e., total production).

In this study, I expanded on the work done by Kapus and Fong (2014) and investigated the influence of operational sex ratio on reproductive and nesting success, sex change, and growth in two species of temperate goby (family *Gobiidae*), *Lythrypnus dalli* and *Rhinogobiops nicholsii*. I hypothesized that as populations become more female-biased, there would be a decrease in the number of viable eggs produced (i.e., reproductive output) and that this reduction in reproduction would largely be due to sperm or male limitation. Similarly, I predicted that other reproductive metrics (e.g., the number of nests, the number of egg clutches in a population, etc.) would also be reduced as sex ratios became skewed in favor of females. I hypothesized that sex change would occur most frequently in populations that were strongly female-biased and that growth would be fastest in individuals that undergo sex reversal.

MATERIALS AND METHODS

Study Species

Lythrypnus dalli

The bluebanded goby (*Lythrypnus dalli*; Fig. 1a) is a subtropical and temperate zone fish that ranges from southern California, USA to Baja California, Mexico. It typically associates with rocky substrata—particularly vertical faces and rubble—and can be found from the shallow subtidal zone to depths up to 30 m, with densities exceeding 100 individuals/m² (Wiley 1976; Behrents 1983; St. Mary 1993; St. Mary 1994; Steele 1997; Kappus and Fong 2014). These small fish (adult standard length = 18-45 mm) are short lived, with a life expectancy of 1.5-2 years (St. Mary 1994).

Lythrypnus dalli is a hermaphroditic species that exhibits both sequential and simultaneous components (Ghiselin 1969). *L. dalli* individuals experience protogynous sex change (female-to-male) but occasionally also exhibits sex reversal (male to female). Based on gonadal allocation, individuals can be distinguished as: pure female (100% ovarian tissue), female-biased (>95% ovarian tissue), male-biased (>95% testicular tissue), and pure male (100% testicular tissue); but despite gonadal allocation often containing both ovarian and testicular tissue, individuals only adopt one behavioral gender (St. Mary 1994). While this type of life history provides plasticity and increased reproductive potential (e.g., unsuccessful males can revert back to females), less than 15% of males exhibit reverse sex change, despite only one male being necessary for fertilization and nest defense (St. Mary 1994). Male-to-female sex reversal may be limited to males that possess viable ovarian tissue, meaning that only male-biased individuals can reverse sex change from male to female (St. Mary 1994). Sex change in *L. dalli* typically takes approximately 2 weeks although in some strongly female-biased individuals, the

transition can take as little as 2 days (St. Mary 1994; Rodgers et al. 2007). Sex change appears to be governed by exogenous factors (such as social hierarchy) and seems to be consistent with the size-advantage model (Ghiselin 1969; Warner 1970; Warner 1988; Rodgers et al. 2005).

Lythrypnus dalli is a territorial nest-brooding species with a single socially dominant male spawning with a harem of females. Sex ratios in natural populations exhibit considerable temporal and spatial variation, with males becoming the predominant sex (>50% of the population) during the non-breeding season, but sex ratios being female biased during the breeding season, typically range from 1:2.5 to 1:5 males:females (Behrents 1983; Kappus 2012). Nests typically consist of rocky crevices, worm tubes, or snail shells (St. Mary 1993). Breeding typically occurs between April and October, during which individual males guard nests that females lay eggs in; multiple females may lay eggs within the same nest with individuals laying up to 1,300 eggs per clutch (St. Mary 1993; St. Mary 1994). Larvae hatch from eggs in 4-10 days with a planktonic phase of around 6-10 weeks (St. Mary 1993; St. Mary 1994; Shanks et al. 2003; Archambeault et al. 2015). Juveniles begin to settle in June, with settlers maturing within a month (St. Mary 1993; St. Mary 1994; Steele 1997).

Rhinogobiops nicholsii

The blackeye goby (*Rhinogobiops nicholsii*; Fig. 1b) is a cryptic fish found in temperate waters from Baja California, Mexico to British Columbia, Canada (Breitburg 1987; Kroon 1997; Kroon et al. 2000). They typically associate with rocky substrata on subtidal reefs and can be found from the shallow subtidal zone to depths of up to 100 m (Wiley 1973; Breitburg 1987). *R. nicholsii* is relatively small (maximum standard length = 100 mm) and has a life expectancy of up to 5 years (Wiley 1973; Cole 1983).

Rhinogobiops nicholsii is a sequential protogynous hermaphrodite that changes sex from female to male (although primary males do exist) (Cole 1983; Kroon 1997). This sex change is governed largely by exogenous cues, although endogenous factors are suspected to play a role. There is a distinct social organization/hierarchy that is somewhat, but not absolutely, correlated with size (Wiley 1973). The most aggressive behavior is exhibited between mature males of similar size with larger individuals winning a higher percentage of agonistic interactions (Wiley 1973; Kroon et al. 2000). Sex change in *R. nicholsii* takes a minimum of 4 weeks (Cole 1983; Kroon 1997).

Rhinogobiops nicholsii is a territorial (year-round defense) nest-brooding species with a single socially dominant male that spawns with a harem of females (Wiley 1973; Kroon 1997). The sex ratio in natural *R. nicholsii* populations varies both temporally and spatially but typically ranges from 1:1.7 to 1:6 male:females (Wiley 1973; Cole 1983; Breitburg 1987; Kroon et al. 2000). Males select and prepare a nest, which consists of a chamber under hard substrata (e.g., rocks, crevices, shells, or other hard objects) to which eggs are attached. Nest building is intermittent and alternates between courtship and other social behavior. Once eggs have been laid, the male defends, cleans, and fans the eggs; nests may contain eggs from multiple females (Wiley 1973). The breeding season typically occurs between March and July although the duration appears to vary by location with some sites spawning year-round if water temperatures remain cool enough (Wiley 1973; Cole 1983; Breitburg 1987; Kroon 1997; Kroon et al. 2000; Schram and Steele 2016). Eggs hatch after 1-3 weeks with individuals then entering a planktonic phase for around 10 weeks (Kroon 1997; Shanks et al. 2003; Schram and Steele 2016).

Study Site

Field work was conducted in Big Fisherman Cove (BFC), Santa Catalina Island, California, USA (33°26'41"N, 118°29'8"W, Fig. 2). This westerly facing cove is surrounded by rocky reefs on the south, east, and north sides, with a sandy bottom in the middle that ranges from approximately 5 m deep at the shallowest areas to 30 m deep in the middle of the cove. The sandy bottom is bordered by a small rocky reef at the southern and eastern ends of the cove. There are a number of mooring blocks and chains interspersed throughout the cove. The cove is moderately protected but is exposed to swells from the northwest during storm events. Water temperatures range from 10°C (late spring) to 21°C (late summer). Anchoring and fishing are prohibited within the cove, which helped prevent anthropogenic disturbances.

The state of California established BFC as a Marine Life Refuge in the mid-1980s and it is now part of the larger Blue Cavern State Marine Conservation Area. Fishes typically found within BFC include kelp bass (*Paralabrax clathratus*), barred sand bass (*Paralabrax nebulifer*), California sheephead (*Semicossyphus pulcher*), Garibaldi (*Hypsypops rubicundus*), halfmoon (*Medialuna californiensis*), señorita (*Oxyjulis californica*), rock wrasse (*Halichoeres semicinctus*), blacksmith (*Chromis punctipinnis*), ocean whitefish (*Caulolatilus princeps*), leopard sharks (*Triakis semifasciata*), and bat rays (*Myliobatis californica*) (Hobson and Chess 1976).

Experimental Design

This study examined how sex ratio affects the reproductive and nesting success, sex change, and growth of the protogynous hermaphrodites *L. dalli* and *R. nicholsii*, which were used as a model system (Fig. 1a and 1b). While *L. dalli* exhibit characteristics of protogyny and

simultaneous hermaphroditism (i.e., individuals that possess both ovarian and testicular tissue), they were considered to be functionally protogynous hermaphrodites for the scope of this study (St. Mary 1994; Reavis and Grober 1999). While the two study species have similar reproductive strategies, they were selected because differences in size, sex-change dynamics, fecundity, and nesting behavior may cause the reproductive output of each species to respond differently to skewed sex ratios. For example, a species with proportionally larger testes may be less affected by sperm limitation due to relatively greater gamete production. Furthermore, *R. nicholsii* nest sites typically cover a larger area than *L. dalli* nests. If males are limited to only one nest site during the breeding season (and male limitation is not due to sperm limitation), this increased nest size could allow for more egg clutches from mates, which might diminish the effects of male limitation. Finally, these species were selected because their small home ranges make them good candidates for field experiments.

To examine how male limitation affects the reproductive process of these sex-changing fishes, a total of 20 artificial reefs were constructed within BFC. Up to 40 individuals were used for *L. dalli* while only 20 fish were used for *R. nicholsii*. Ten different sex ratio treatments were used for *L. dalli* (ranging from 1:1 to 1:19 male:female and a 1:39 male:female treatment); 9 different sex ratio treatments were used for *R. nicholsii* (ranging from 1:1 to 1:19 male:female). The 9:11 male:female treatment was not used for either species. Reproductive output was then monitored for each local population (i.e., reef). The experiments for each species were conducted in separate field seasons, with *L. dalli* and *R. nicholsii* being used in 2016 and 2017, respectively. One round of the field experiments was performed for *L. dalli* while two rounds were performed for *R. nicholsii*.

Artificial reefs within BFC were separated into 2 blocks that were distributed between 2 rows (Fig. 2). Reefs were separated from adjacent reefs and other existing structures (e.g., mooring blocks) by 10 m of sand to discourage the movement of individuals between reefs. Reefs were 2x2 m in area and contained 4 separate sub-reefs in each corner. Sub-reefs consisted of a cinder block surrounded by small rocks and were established to prevent a single male from suppressing the reproduction of other males (Fig. 3b). Rocks were collected from a beach to the east of BFC known as “the Quarry” (33°26’31”N, 118°28’17”W). The cinder blocks and rocks were lowered to the seafloor using ropes and buckets. Free divers descended with the buckets before discharging the contents on the sea floor. Mesh was placed beneath the cinder blocks and rocks to prevent them from sinking into the sandy bottom. The reefs were arranged while on SCUBA after the necessary cinder blocks, rocks, and mesh had been placed on the substrate. After the reefs were arranged, wire mesh (1x1 in) cages were constructed and placed over the reefs to minimize predation and increase survivorship of *L. dalli* and *R. nicholsii* (Fig. 3a). Gill netting was sewn onto the wire mesh to further discourage predation by piscivorous fish. The cages were attached to the bottom mesh using reusable cable zip ties to reduce possible sources of entry.

To quantify reproductive output, nests were photographed so that the eggs could later be analyzed for a number of different metrics. To simplify the documentation process, artificial nests were placed on the reefs to promote spawning in specific sites (Fig. 4). Reefs that contained *L. dalli* had 16 polyvinyl chloride (PVC) tubes (4 per sub-reef) containing a rolled, sanded acetate sheet; each tube was 1.5 cm in inner diameter and 15.2 cm in length, and was sealed on one end with an end cap (Fig. 3b and 4a). *L. dalli* willingly spawn in these tubes on the acetate sheets, which can be removed to easily examine the egg clutches (St. Mary 1994; St. Mary

1996). *R. nicholsii* reefs had 5 overturned terra cotta saucers (1 per sub-reef and 1 at reef center) to serve as artificial nests (Fig. 4b); previous work has shown that *R. nicholsii* readily lay eggs on the underside of these saucers (Schram and Steele 2016). The PVC tubes and terra cotta saucers (for *L. dalli* and *R. nicholsii*, respectively) were examined every 7 days for eggs. When found, eggs were photographed. The terra cotta saucers on reefs with *R. nicholsii* were photographed directly whereas the acetate sheets from the PVC tubes *L. dalli* spawned in were placed upon a dive slate with a 1x1 cm grid (for scale) before being photographed.

Lythrypnus dalli and *R. nicholsii* that were used in the experiments were captured from nearby sites along the northwest end of Catalina Island. *L. dalli* were captured from Isthmus Reef (33°26'52"N, 118°29'23"W), and *R. nicholsii* were captured from Lion Head Point (33°27'12"N, 118°30'6"W), Big Geiger Cove (33°27'34"N, 118°31'4"W), Little Geiger Cove (33°27'27"N, 118°30'56"W), and the Quarry (33°26'31"N, 118°28'17"W). Fish were collected by SCUBA divers using handnets, placed into Nalgene bottles, and taken to the lab at Wrigley Marine Science Center (WMSC) where they were placed into holding tanks. Captured individuals were sedated with tricaine mesylate (MS-222) (0.07 gm/L and 0.1 gm/L for *L. dalli* and *R. nicholsii*, respectively) and the sex and size of each individual was determined. Sex was determined by examining the genital papilla of each individual under a microscope (Cole 1983; Drilling and Grober 2005). The standard length (SL) was determined by measuring each individual with a dial caliper. Each fish was then given 2 tags using visual implant elastomer (VIE) designating the individual's sex and size at the time of tagging (Fig. 5). After the fish were recaptured, these tags permitted the calculation of growth rates and were used to determine if sex change had occurred during the field experiment. VIE tags have been shown to have no effect on the growth or mortality of individuals (Malone et al. 1999).

Healthy, tagged, sexually mature fish were transplanted onto the artificial reefs the following morning at the desired operational sex ratios and densities. Fish were transported from the lab to the artificial reefs in plastic bags filled with seawater and were released while on SCUBA. Any fish that died during transplantation were immediately replaced with unused individuals from the lab. *R. nicholsii* reefs had 20 individuals on each reef and 9 different sex ratio treatments (ranging from 1:1 to 1:19 male:female). The 9:11 male:female sex ratio was not used as little change in reproductive output was expected at that end of the range. Instead, 4 artificial reefs had the 1:19 male:female treatment as male limitation was expected to be most extreme at this end of the spectrum. All other treatments had 2 replicates (i.e., reefs). Similar to the *R. nicholsii* reefs, the *L. dalli* reefs were stocked with 20 individuals, although two reefs were outplanted with 40 individuals. Forty individuals per reef was still representative of *L. dalli* densities in natural populations (although 40 fish would not have been appropriate for *R. nicholsii* densities). The *L. dalli* sex ratios ranged from 1:1 to 1:19 male:female with an additional 1:39 male:female treatment; the 9:11 male:female treatment was, again, not used. A 1:39 male:female ratio was used since an even more skewed sex ratio could provide results not seen in the *R. nicholsii* experiment if the skew in the 1:19 treatment was not extreme enough. Each block (10 reefs/block) received each sex ratio treatment except for the 1:19 male:female treatment on the *R. nicholsii* reefs, which was included twice per block. In addition to outplanting specific sex ratios on each reef, individuals were selected so that the average male and female size on each reef was similar (± 2 mm) across the whole block. The exception to this is that the average male size on the heavily female-biased treatments was typically higher than the other reefs. This was done to ensure that at least one male on each reef was larger than the

largest female, as is normally seen in nature, and reefs with many females included some large females.

The fish were left on the artificial reefs for a period of three and four weeks for *L. dalli* and *R. nicholsii*, respectively. Fish were re-collected at the end of the study period and returned to the lab at WMSC where they were again examined to determine their sex and size, allowing calculation of growth and sex change during the experiment.

Analyses

Comparing Natural to Experimental Populations

The size of *L. dalli* and *R. nicholsii* individuals collected throughout the sampling period were plotted on separate histograms to provide an estimate of the size structure in the natural populations surrounding the experimental site. The sex ratio for each collection dive was also calculated and plotted on histograms. To examine how collections may have impacted natural populations, a two-sample t-test was performed to determine if sex ratios in natural populations at the beginning of the collection period (July and June for *L. dalli* and *R. nicholsii*, respectively) was significantly different than the sex ratio at the end of the collection period (August and July for *L. dalli* and *R. nicholsii*, respectively). All analyses were completed using JMP Pro 13.

Quantifying Reproductive Output

The number of nests with eggs (i.e., broods), the total number of eggs per nest, the number of clutches per nest, and the number of unfertilized eggs were counted for each reef. A clutch was defined as a group of eggs from (presumably) a single female; a difference in developmental stage relative to the surrounding eggs was used to identify individual clutches.

The photographs of the egg broods for both species were examined with the ImageJ image processing program. *L. dalli* broods were manually counted by marking and enumerating individual eggs (Fig. 6a and 6b). Due to their larger size, *R. nicholsii* broods were subsampled to determine the number of eggs in each nest. First, the scale of the image was set in ImageJ before calculating the area of the brood (Fig. 7a). Next, a 1x1 cm grid was imposed on top of the picture and 5 randomly selected cells were counted to obtain the average egg density (Fig. 7b). *X* and *Y* coordinates were randomly generated to determine which cells to count. Only cells that were fully within the brood were counted; selected cells that were on the perimeter of the brood were discarded and a new cell was selected. The average egg density was determined from the 5 subsamples and brood size was calculated by multiplying this density by the area. The subsampling error for this method was an order of magnitude lower than the among-brood variability. If there were multiple stages of egg development within a nest, each stage was considered a single clutch (as long as all the eggs at that stage of development were adjacent).

Male Limitation and Reproductive Output Responses

For both species, models were produced to examine the relationship between the operational sex ratio (the percentage of the population on each reef that was male) and the (1) the proportion of fertilized eggs (i.e., fertilization rate) per reef, (2) the total number of eggs per reef, (3) the female per capita egg production per reef, (4) the male per capita egg production per reef, (5) the average number of eggs per nest, (6) the average number of eggs per clutch, (7) the number of nests per reef (i.e., the number of males that nested [assuming that each nest is only used by a single male]), (8) the percentage of males that nested per reef, (9) the number of clutches per reef, and (10) the average number of clutches per nest.

The reproductive output responses (metrics #2-6 above) were modeled using a Poisson generalized linear model with an overdispersion parameter. The models included sex ratio, a blocking factor, and the interaction between the two; the average female size (mm) and average male size (mm) on each reef were also added as covariates. Corrected Akaike's information criterion (AIC_c) was used to determine the best factors for each model with the caveat that sex ratio had to remain in the model (Burnham and Anderson 1998). Statistical significance was determined in cases where the difference in AIC_c values (ΔAIC_c) was greater than or equal to 2 (Burnham and Anderson 1998). Linear regressions were performed for the remaining reproduction responses to determine their relationship with sex ratio (metrics #7-10). All analyses were completed using JMP Pro 13.

Although egg production data was collected for both species throughout the monitoring period (3-4 weeks), only data from the first week were used. This was done because the populations deviated from the desired densities and sex ratios as time progressed; this was confirmed by survey data for *R. nicholsii* (during the monitoring period) and the number of individuals recollected for *L. dalli*. While it is not possible to know how representative the sex ratios were of the desired treatments after a week, surveys indicated that approximately 90% of the original tagged individuals were still on the reef. For *L. dalli*, both 1:39 male:female treatments were dropped from the analyses (but included in the figures) because it was difficult to disentangle the effects of sex ratio and fish density when examining differences in response variables among treatments (that had 20 individuals outplanted).

Growth and Sex Change Responses

Growth and sex change data were obtained from tagged individuals that were recollected at the end of the study period. Because few *L. dalli* individuals were recollected at the end of the monitoring period, the growth and sex change were only analyzed for *R. nicholsii*. Growth rate was determined by subtracting the initial length from the final length of each individual; this new length was then divided by the time spent by each individual on the artificial reef. Growth rate data were analyzed using a generalized linear model that included: block, type of sex change (female ‘no sex change’, female to transitional, female to male, or male ‘no sex change’), and the interaction between the two; the initial size of the individual and the sex ratio were added as covariates. Factors and covariates that did not contribute to the model were dropped. Transitional individuals were fish that were undergoing sex change at the time of recollection but were still not considered functionally male (based on the genital papilla). Additionally, linear regressions were performed to determine the relationship between growth rate and the initial size of the individuals. Both of these analyses (ANCOVA and linear regression) were performed twice: once for the first round (the breeding season at the beginning of the field season [June-July]) and once for the second round (the non-breeding season at the end of the field season [July-August]). The data used in the reproductive output analyses were from the first round because there was no reproduction during the second round.

Frequency of sex change was calculated by examining the proportion of females on each reef that changed sex (either were fully transitioned at the time of recollection or in the process of transitioning). Since *R. nicholsii* are not bi-directional sex changers, individuals that were male at the start of the experiment were excluded from the analyses. A linear regression examined the relationship between sex change and sex ratio. Again, two separate models

(breeding and non-breeding season) were run for the sex change analyses. Finally, a two-sample t -test was performed to compare the difference in the average frequency of sex change (without regard to sex ratio) between the breeding and non-breeding season. All analyses were completed using JMP Pro 13.

RESULTS

Size Distribution and Sex Ratios in Natural Populations

In natural populations, both *L. dalli* and *R. nicholsii* followed a distribution typical of a diandric sequential hermaphrodite—a female-bias (with small males present) in the smaller size classes prior to a shift to male predominance in the larger size classes (Fig. 8). The shift from female- to male-predominance was more distinct in *R. nicholsii* (67 mm) than in *L. dalli* (35 mm) and occurs earlier in the size range.

The sex ratio of natural *L. dalli* populations averaged 0.26 (male:female) and showed little variation throughout the collecting period (Fig. 9a). There was no significant difference in sex ratio between the beginning (June-July) and end (August) of the field season (two-sample t -test; $t_8=0.87$, $p=0.407$). By contrast, the sex ratio of *R. nicholsii* over the collecting period averaged 0.47 (male:female) but was significantly different between the beginning (0.37; June) and the end (0.58; July) of the sampling period, with populations becoming significantly more male-biased by the end of the summer (two-sample t -test; $t_8=6.54$, $p<0.001$) (Fig. 9b).

Male Limitation and Reproductive Output Responses

Lythrypnus dalli

There were no observed cases of unfertilized eggs during the *L. dalli* monitoring period; fertilization rate was 100% for all broods found (Fig. 10). The total number of eggs produced per reef did not differ significantly among sex ratios (Fig. 11a). Female per capita production, the average number of eggs produced per nest, and the average number of eggs produced per clutch exhibited a similar trend (Fig. 11b, 11d, 11e) and did not differ significantly as a function of sex ratio. Conversely, male per capita production increased exponentially as sex ratios became more female-biased with the most marked increase in egg production occurring around 0.2 male:female (Fig. 11c).

While most reefs typically had 1-2 nests with eggs. There were 2 reefs that never showed evidence of eggs in any of the artificial nests, and the most nests per reef at any given time was 3. There was no significant relationship between the number of nests on each reef and sex ratio (Fig. 12a; regression: $F_{1,16}=0.03$, $p=0.874$, $r^2<0.01$) although the percentage of males that nested significantly increased as the treatments became more female-biased (Fig. 12b; regression: $F_{1,16}=9.43$, $p=0.007$, $r^2=0.37$). There was no significant relationship between sex ratio and the number of clutches per reef (Fig. 13a; regression: $F_{1,16}=0.008$, $p=0.931$, $r^2<0.01$) or the average number of clutches per nest (Fig. 13b; regression: $F_{1,16}=0.94$, $p=0.35$, $r^2=0.06$).

Rhinogobiops nicholsii

The prevalence of unfertilized *R. nicholsii* eggs was low, with an average fertilization rate of 96% (Fig. 14). There were only three cases of broods with fertilization rates lower than 97% and two of these three reefs had the most female-biased sex ratio (Fig. 14). The total

number of eggs produced per reef did not change significantly with sex ratio but it tended to increase slightly as female-bias increased (Fig. 15a). Female per capita production and the average number of eggs produced per nest also exhibited a non-significant increase as sex ratios became more female-biased (Fig. 15b and 15d). Male per capita reproduction and the average number of eggs per clutch also increased as sex ratios became more female-biased, and did so significantly (Fig. 15c and 15e). The most marked increase in per capita male production occurred around 0.15 male:female.

Similar to *L. dalli*, most reefs typically had 1-2 nests with eggs although there were 4 reefs on which no eggs were found; the most nests found on a single reef on any day during the monitoring period was 3. There was no significant relationship between the number of nests on each reef and sex ratio (Figure 16a; regression: $F_{1,18}=0.01$, $p=0.915$, $r^2<0.01$) although the percentage of males that nested significantly increased as populations became more female biased (Figure 16b; regression: $F_{1,18}=8.88$, $p=0.008$, $r^2=0.33$). Sex ratio did not influence the number of clutches per reef (Figure 17a; regression: $F_{1,18}=0.31$, $p=0.587$, $r^2=0.02$) or the average number of clutches per nest (Figure 17b; regression: $F_{1,18}=0.012$, $p=0.736$, $r^2=0.01$).

Growth and Sex Change Responses

During the breeding season, *R. nicholsii* individuals that transitioned from female to male exhibited significantly faster growth rates than fish that did not change sex or that had not completed the change from female to male (Figure 18a; ANCOVA: $F_{4,144}=28.98$, $p<0.0001$, $r^2=0.46$). After controlling for initial size, females that did not transition had the slowest growth rates when compared to the other three treatments (Fig. 18a). Males and transitional individuals (fish that had initiated sex change but did not complete reversal) were not significantly different

from each other and exhibited growth rates slower than sex changing individuals, but faster than non-transitional females (Fig. 18a). During the non-breeding season, block 2 had significantly higher growth rates than block 1 (Figure 18b; ANCOVA: $F_{8,188}=7.57$, $p<0.0001$, $r^2=0.24$). Similar to the breeding season, females that underwent sex change exhibited the highest growth rates while females that did not change sex had the slowest growth rates. Initial size was a significant covariate for both the breeding (regression: $F_{1,147}=30.52$, $p<0.0001$, $r^2=0.17$) and non-breeding season (regression: $F_{1,195}=22.04$, $p<0.0001$, $r^2=0.10$) with smaller individuals growing significantly faster in length than large fish (Fig. 19).

During the breeding season, the proportion of *R. nicholsii* females that changed sex on each reef increased as sex ratios became more female-biased (Figure 20a; regression: $F_{1,17}=4.78$, $p=0.043$, $r^2=0.22$). By contrast, the proportion of females that changed sex during the non-breeding season did not differ significantly among sex ratios although it tended to increase as sex ratios became more balanced (Figure 20b; regression: $F_{1,18}=2.18$, $p=0.157$, $r^2=0.11$). When averaged across all treatments, the frequency of sex change was greater in the non-breeding (0.56) versus the breeding season (0.34) (two-sample t-test; $t_{38}=4.31$, $p<0.001$).

DISCUSSION

Comparing Natural to Experimental Populations

Both *L. dalli* and *R. nicholsii* appear to be diandric due to the presence of (primary) males in the smaller size classes (Fig. 8). For *L. dalli*, this finding is supported by the work of Drilling and Grober (2005) although their theory of a sneaker male alternative phenotype cannot be corroborated without observing mating behavior. Previous literature on the pattern of sexual

ontogeny in *R. nicholsii* has been less clear. Kroon (1991) speculated that this species may be diandric due to the presence of males much smaller than the smallest mature females (50 mm total length)—something supported by the present study (Fig. 8b; Kroon and Liley 2000). Unfortunately, the testes of *R. nicholsii* males do not reveal structures indicating any former ovarian function, which makes it impossible to determine the presence of primary males through histological examination (Cole 1983). There was a marked exponential decrease in the abundance of large *L. dalli* individuals (>31 mm) (Fig. 8a). Large *L. dalli* individuals were considerably harder to catch during collections, which makes it difficult to say whether the upper end of this size distribution is truly representative of natural populations. Furthermore, the *L. dalli* size distribution did not exhibit a distinct shift in gender predominance (from female to male) that was evident in the *R. nicholsii* size range (67mm) (Fig. 8b). This again may be attributed to the difficulty of collecting large (male) *L. dalli* individuals.

While *L. dalli* sex ratios remained consistent throughout the summer, *R. nicholsii* populations became significantly more male-biased between the beginning and end of the collection period. Since the breeding season of *R. nicholsii* typically ends in mid-July (although in some locations it extends as late as October), it is possible that sex change in this species occurs most frequently immediately after the end of the breeding season, something observed in temperate protogynous wrasses through year-round inspection of gonadal tissue (Warner 1975; Dipper et al. 1977; Dipper and Pullin 1979; Jones 1980; Cole 1983). The presence of many transitional individuals throughout the breeding season, however, indicates that sex change is not exclusive to the non-breeding season. Alternatively, Breitburg (1987) observed that areas with many suitable nest sites typically contained a higher proportion of males. Therefore, it is possible that collections earlier in the season increased nest availability, which influenced the frequency

of sex change in these local populations. Whether the increase in male abundance at the end of the collecting period is due to a temporal cue or an increase in available nests remains unclear.

Male Limitation and Reproductive Output

Sperm limitation was not present in either species (Fig. 10 and 14). While there were two *R. nicholsii* reefs at the most female-biased sex ratio (1:19 male:female) that had lower fertilization rates than the other treatments, the fertilization rates remained high (>76%) and the fit of statistical models was not impacted if unfertilized eggs were included when calculating reproductive output. Many of the previous studies documenting sperm limitation have focused on taxa with reproductive behavior different from the species studied here. For example, in the Caribbean, black sea urchin (*Diadema antillarum*) sex ratios were skewed heavily in favor of females after a mass mortality event (Lessios 1988). While females had larger body sizes and higher per capita egg production due to decreased competition, reproductive output did not increase due to reduced fertilization rates (Levitan 1992). In marine systems, the prevalence of sperm limitation in sessile and sedentary invertebrates highlights the importance of distance between spawning partners—something that is not an issue with mobile species that seek out mates.

Moreover, previous modeling studies that have predicted sperm limitation in populations of protogynous hermaphrodites have typically focused on species that aggregate to spawn. For these species, sperm competition between male conspecifics may encourage individuals to release high concentrations of sperm early in the spawning process, leading to a greater incidence of sperm depletion. Conversely, coral reef pair spawning species exhibit a strategy of “sperm economy” whereby they regulate the quantity of sperm released (Shapiro et al. 1994).

The quantity of sperm released by these males is dependent upon: (1) the size of the female mate, (2) the total number of spawning partners, (3) and whether the dominant male experiences sperm competition from “streaker” males (Shapiro et al. 1994; Marcanato et al. 1995; Warner et al. 1995; Marcanato and Shapiro 1996). Moreover, by economizing their sperm release, these males are able to maximize the number of matings that they are able to successfully participate in throughout the spawning period (Shapiro et al. 1994). While it is unclear if *L. dalli* and *R. nicholsii* employ sperm economy, their pair spawning mating strategy may make them less susceptible to sperm depletion than the aggregate spawning behavior characteristic of species like gag grouper (*Mycteroperca microlepis*) and coral trout (*Plectropomus leopardus*) (Bannerot et al. 1987; Armsworth 2001).

For both species, total egg production per reef, female per capita egg production, average egg production per nest, and the number of nests per reef remained consistent regardless of the operational sex ratio (Figs. 11 and 15). Kappus (2012) performed a similar manipulative study with *L. dalli* and also found no effect of sex ratio on total egg production, female per capita egg production, and the number of nests per reef (Kappus and Fong 2014). These results seem to indicate that these species are fairly resistant to sex ratio skew and male limitation. Since the average number of eggs per nest was not affected by sex ratios, the number of nests per reef was the main driver of variation in egg production among treatments (not including sex ratio for the per capita production metrics) (Figs. 11d and 15d). For both species, the number of nests that received eggs remained relatively constant among sex-ratio treatments; most reefs typically only had one or two dominant males that nested (i.e., nests with eggs) (Figs. 12a and 16a; Kappus 2012; Kappus and Fong 2014; Pang, pers. obs.). This trend and the trend observed with total egg

production might be attributed to: (1) intrasexual competition (both male and female), (2) mate preference/selection, or (3) an indifference or inability of males to occupy multiple nests.

The limited number of guarded nests for females to spawn in suggests that one or two dominant males were able to suppress other (subordinate) males from establishing breeding territories on most reefs. In territorial species, relative size can often determine the outcome of agonistic interactions between conspecific males for resources (e.g., nests), which consequently determines their reproductive success (Davis and Houston 1984; Stanley and Wootton 1986; Lindström 1988; Kvarnemo et al. 1995; van Rooij et al. 1996; Mumby and Wabnitz 2002). In both *L. dalli* and *R. nicholsii*, these aggressive interactions have been observed with males occupying nests being significantly larger and more reproductively successful than those that do not (Cole 1982; Breitburg 1987; St. Mary 1994). While one would expect intense intrasexual competition between males at balanced sex ratios, it is surprising that the number of nests per reef did not increase when female bias became extreme. Territory theory predicts that territory size should increase with a decreasing density of competitors (Warner and Hoffman 1980; Norman and Jones 1984), thus one might predict that dominant males could defend multiple nests at low male:female sex ratios, but there was no evidence of that. Surveys indicated that males did not occupy multiple nests on the same reef over the four-week monitoring period (Pang, pers. obs.). Males of *L. dalli* and *R. nicholsii* may only be able to defend a single nest site at a time, regardless of the density of other males nearby, possibly due to the need to defend eggs against egg predators.

In addition, it is possible that intrasexual competition between females further limited the number of eggs deposited and that reproductive output was controlled by a few, large dominant females. For both species, the number of clutches per reef remained fairly consistent (~2 clutches

per/reef) regardless of female density. Agonistic female-female interactions in mammals have been shown to reduce the number of offspring in subordinate individuals (Dublin 1983; Wasser 1983; Smuts 1987); similarly, in a harem angelfish, alpha females interfere with the mating of other females—resulting in reduced spawning time for subordinates (Lutnesky and Kosaki 1995). In this study, if there were female *L. dalli* and *R. nicholsii* that were unable to spawn due to intrasexual competition, they may have instead reabsorbed the eggs and allocated that energy to future egg production, growth, or sex change (Rideout et al. 2005; Rideout and Tomkiewicz 2011).

Although it is likely that dominant males suppressed reproduction by other males on reefs with balanced sex ratios, given that typically only one or two nests with eggs were found on reefs, it is also possible that mate preference by females prevented these subordinate males from receiving eggs (Sargent 1988; Forsgren 1992; Forsgren et al. 1996). Males were observed in nests without eggs, suggesting they had access to nest sites, but perhaps females instead chose dominant males. As outlined by the polygyny threshold model, a female that spawns with a male that has already mated gains a number of benefits including (1) a reduced risk of predation or filial cannibalism and (2) an increased parental investment (in broods that are larger and more valuable) (Rohwer 1978; Ridley and Rechten 1981; Coleman et al. 1985; Sargent 1988; Dugatkin 1992). For both study species, previous literature suggests that females typically prefer to spawn with the largest males available, regardless of male density (Breitburg 1987; St. Mary 1994). With that said, there was no evidence of males establishing multiple nests which suggests that on reefs with two nests, another “subordinate” male was still able to nest and court females successfully. This suggests that a single male was unable to monopolize the entire reef due to intrasexual competition and mate preference.

In both *L. dalli* and *R. nicholsii*, male per capita production increased significantly as sex ratios became skewed in favor of females. Previous literature has shown that as populations became more female-biased, intrasexual competition is relaxed, male reproductive variance was reduced, and individual males became more reproductively successful (despite total production remaining consistent across sex ratios) (Trivers 1972; Petrie 1983; Kodric-Brown 1988; Kvarnemo et al. 1995). Conversely, when operational sex ratios were more balanced (i.e., 30-40% male), males and females exhibited similar levels of per capita production. Itzkowitz (1990) and Kappus (2012) reported similar increases in male per capita production—in beaugregory damselfish and *L. dalli*, respectively—as sex ratios became more female-biased (Kappus and Fong 2014). Moreover, Kappus (2012) reported comparable levels of per capita production between males and females at similar sex ratios (30-40% male) to what was observed in this study. These results suggest that for both *L. dalli* and *R. nicholsii*, some females would likely gain a reproductive advantage if they underwent sex reversal when gender ratios are heavily female-biased.

It is possible that the design of the artificial nests and reefs may have also contributed to some of the unexpected results, relative to previous modeling work, seen in this study. Fertilization rates may have remained unnaturally high for both species if artificial nest space limited the number of eggs a male would typically fertilize. If this were true though, most (if not all) nests should have been completely filled with eggs, which was not the case. Alternatively, if the reefs had been larger, causing population densities to be lower, skewed sex ratios may have caused a significant decline in total production (and consequently, male and female per capita production) if females were unable to find mates. Moreover, larger reefs may have allowed more males to establish breeding territories when sex ratios were less female-biased—raising the total

number of eggs per reef. The population densities used in these experiments were representative of natural populations, though they may not reflect what happens in low density populations of the study species. In low-density populations, it is likely that males would become limiting in the manner predicted by previous work. Without further research, it is difficult to say how the results from this study might be extrapolated to the landscape-scale.

Growth and Sex Change

In *R. nicholsii*, growth rate was highest in individuals that completed sex reversal from female to male during the breeding and non-breeding season (Fig. 18). While females that had begun to transition at the time of recollection (but had not completed sex change) exhibited growth rates higher than females that did not change sex, they still had significantly lower growth rates than newly transitioned males. It appears that in *R. nicholsii*, transitional growth acceleration occurs towards the end of (or immediately following) sex change. Walker and McCormick (2004) found that cylindrical sandperch retained accelerated growth for up to 30 days after sex change at which time growth rate slowed. Without more closely monitoring the individuals that were outplanted in this study though, it is difficult to determine when exactly this burst in growth occurs. Accelerated growth due to sex change is well documented in marine species, and it allows the transitioned individual to establish dominance over females and to defend the harem from bachelor or roving males (Robertson 1972; Aldenhoven 1986; Ross 1987; Warner 1991; Garratt et al. 1993; Sakai 1997; Sakai and Kohda 1997; Walker and McCormick 2004; Lorenzi et al. 2006; Walker et al. 2007; Wong et al. 2007; Munday et al. 2009).

During the breeding season, the frequency of sex change in *R. nicholsii* females increased as sex ratios became more female-biased (Fig. 20a). This finding supports the previous literature

that sex reversal in this species is at least partly governed by exogenous cues (i.e., social hierarchy) (Kroon 1991; Kroon and Liley 2000). While the frequency of sex change during the non-breeding season did not exhibit the same trend with regard to sex ratio, it did have a higher rate of sex change across all treatments when compared to the breeding season (Fig. 20b). The increased proportion of females changing sex (regardless of sex ratio) may have been due to the number of *R. nicholsii* recruits found on the artificial reefs late in the field season. This influx of future females could indicate to existing mature females that sex change would increase their reproductive success in the next breeding season. Alternatively, the results on the artificial reefs during the non-breeding season mirror the trend found during the collections at natural reefs when sex ratios were significantly less female-biased in July (post-breeding season) than in June (breeding season). It is possible that while exogenous factors are the primary cue for sex change during the breeding season, there is an important temporal cue following the end of the breeding season as well. Without further sampling, it is unclear whether this increased frequency of sex change is maintained throughout the non-breeding season.

Fishery Management Implications

The findings from this study have important implications for fisheries management and highlight the importance of considering a species' mating system when managing a population. The evidence presented here shows that sex ratio does not influence the reproductive output (excluding per capita production of males) of *L. dalli* and *R. nicholsii*; for both species, total egg production and fertilization rates remained consistent regardless of the magnitude of female bias in sex ratio. It is possible that pair spawning species may be less susceptible to male limitation and sex ratio skew than the previous modeling work with sex-changing species implies.

It is important to note that the previous studies predicting sperm limitation have largely focused on species that aggregate to spawn (i.e., coral trout [*Plectropomus leopardus*], gag grouper [*Mycteroperca microlepis*], and graysby [*Epinephelus cruentatus*]) (Armsworth 2001; Huntsman and Schaff 1994; Heppell et al. 2006). It is possible that this mating strategy (and the sperm competition associated with it), encourages behavior that makes these species more susceptible to sperm depletion if they prioritize early mates. Conversely, pair spawning males that exhibit sperm economy may be considerably less susceptible to sperm limitation (Shapiro et al. 1994). By regulating the amount of sperm they release, males are able to maintain appropriate levels of fertilization while maximizing the number of successful spawns throughout the spawning period.

While female-biased populations of pair spawners may be less susceptible to sperm limitation, reduced male abundance (due to fishing pressure or natural mortality) can impact these populations in other ways. If mortality is constant (so that only a few males are ever present), females that respond to exogenous cues will undergo sex change at smaller sizes and younger ages leading to reduced stock fecundity (Buxton 1990; Cowen 1990; Warner and Swearer 1991; Platten et al. 2002; Hawkins and Roberts 2003; Hamilton et al. 2007). Moreover, these heavily skewed populations will be considerably more susceptible to population crashes if exploitation is intense enough that the frequency of sex change is unable to keep pace with fishing mortality and males are completely removed (Vincent and Sadovy 1998; Alonzo et al. 2008). Recruitment in these populations may become entirely dependent on external supply, further complicating management. With more balanced sex ratios, the role of large, dominant males would likely be assumed by subordinate males preventing any reduction in reproductive output.

The findings from this study corroborate the growing body of evidence that intraspecific competition, mate choice, and other components of mating systems can affect the operational sex ratio and reproductive output of a population and should be incorporated into population assessments if stocks (both gonochoristic and hermaphroditic) are to be managed appropriately (Pomiankowski 1988; Dugatkin 1992; Forsgren et al. 1996; Hutchings 1999; Côté 2003; Rowe and Hutchings 2003; Alonzo and Mangel 2004). For example, gag (*Mycteroperca microlepis*) and scamp grouper (*Mycteroperca phenax*), two protogynous species that aggregate to spawn, have experienced a significant decline in the proportion of males in exploited populations (Coleman et al. 1996). By contrast, red grouper (*Epinephelus morio*), which does not aggregate to spawn, has shown little change in sex ratio over the same time period and similar fishing pressure (Coleman et al. 1996; Brulé et al. 1999). Mating behaviors and systems need to be considered when assessing the resiliency of exploited marine fish populations.

CONCLUSIONS

In summary, male and sperm limitation does not appear to be a problem for *L. dalli* or *R. nicholsii* at heavily female-biased sex ratios. Total production remained consistent across the wide range of sex ratios tested here. Social and mating systems, such as the ability of individual males and females to exert dominance and suppress other conspecifics, seems to play a larger role in who is reproductively successful. At balanced sex ratios, where male abundance is high, intrasexual competition between males appears to play an important role in the establishment of viable nesting sites and mate monopolization. At sex ratios that are skewed heavily in favor of females, female-female competition for nest space may limit total population production. Why *L.*

dalli and *R. nicholsii* males did not appear to establish multiple nesting sites at heavily skewed sex ratios remains unclear. While previous models had predicted that males (and sperm) could be limiting to reproductive output, many of these models focused on broadcast spawners that aggregate to spawn in groups, and may not be applicable to nest-brooding or pair spawning species (Huntsman and Schaaf 1994; Heppell et al. 2006; Alonzo et al. 2008; Armsworth 2011). Ultimately, a stock's susceptibility to exploitation appears to be dependent on several factors that must be examined in the context of population dynamics, mating strategies, and reproductive behavior (Rowe and Hutchings 2003; Alonzo and Mangel 2004).

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TABLES

Table 1. Generalized linear model (GLM; Poisson with overdispersion) results testing the relationship between sex ratio and the per capita production of *Lythrypnus dalli* males. The ΔAICc shows the difference in AICc between the ‘best’ models (A and B) and the full model (C).

Model	Factor	df	χ^2	p	ΔAICc
A	Sex ratio	1, 17	14.38	<0.001	0
B	Sex ratio	1, 16	4.93	0.026	1.92
	Average male size (mm)	1, 16	0.02	0.877	
C	Sex ratio	1, 13	4.33	0.036	11.47
	Block	1, 13	0.13	0.724	
	Sex ratio * Block	1, 13	4.91	0.027	
	Average male size (mm)	1, 13	0.15	0.695	
	Average female size (mm)	1, 13	0.47	0.492	

Table 2. Generalized linear model (GLM; Poisson with overdispersion) results testing the relationship between sex ratio and the per capita production of *Rhinogobiops nicholsii* males. The ΔAICc shows the difference in AICc between the ‘best’ (A) and the full (B) model.

Model	Factor	df	χ^2	p	ΔAICc
A	Sex ratio	1, 17	18.53	<0.001	0
	Block	1, 17	3.66	0.056	
	Average female size (mm)	1, 17	4.88	0.027	
B	Sex ratio	1, 15	6.24	0.013	8.82
	Block	1, 15	3.79	0.052	
	Sex ratio * Block	1, 15	0.17	0.678	
	Average male size (mm)	1, 15	0.23	0.632	
	Average female size (mm)	1, 15	5.09	0.024	

Table 3. Generalized linear model (GLM; Poisson with overdispersion) results testing the relationship between sex ratio and the average production per clutch of *Rhinogobiops nicholsii*. The ΔAICc shows the difference in AICc between the ‘best’ models (A and B) and the full (C) model.

Model	Factor	df	χ^2	p	ΔAICc
A	Sex ratio	1, 19	4.75	0.029	0
B	Sex ratio	1, 18	4.59	0.032	1.77
	Average female size (mm)	1, 18	0.12	0.729	
C	Sex ratio	1, 15	8.17	0.004	10.68
	Block	1, 15	3.00	0.083	
	Sex ratio * Block	1, 15	0.14	0.705	
	Average male size (mm)	1, 15	2.01	0.156	
	Average female size (mm)	1, 15	1.87	0.172	

Table 4. Analysis of covariance (ANCOVA) results testing the relationship between the type of sex change and the growth rate of *Rhinogobiops nicholsii*.

Factor	df	SS	F	p
Breeding season				
Specific sex change	3, 144	14.08	23.75	< 0.001
Initial size (mm)	1, 144	13.05	68.32	< 0.001
Non-breeding season				
Specific sex change	3, 188	2.28	5.27	0.001
Block	1, 188	1.04	7.18	0.008
Sex change * Block	3, 188	0.65	1.51	0.213
Initial size (mm)	1, 188	3.63	25.19	< 0.001

FIGURES

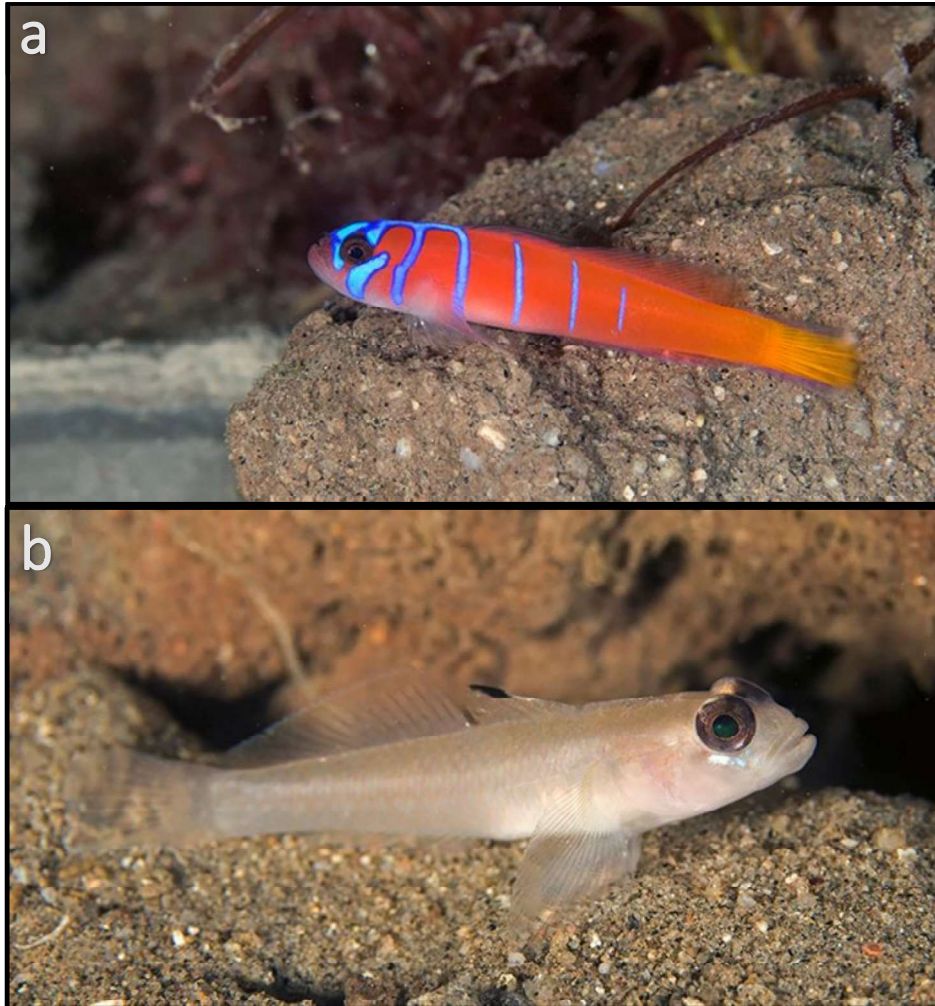


Figure 1. The two species used for the field manipulations in this study: (a) bluebanded goby (*Lythrypnus dalli*) and (b) blackeye goby (*Rhinogobiops nicholsii*). Source: Weiwei Gao (www.weiweigao.com).

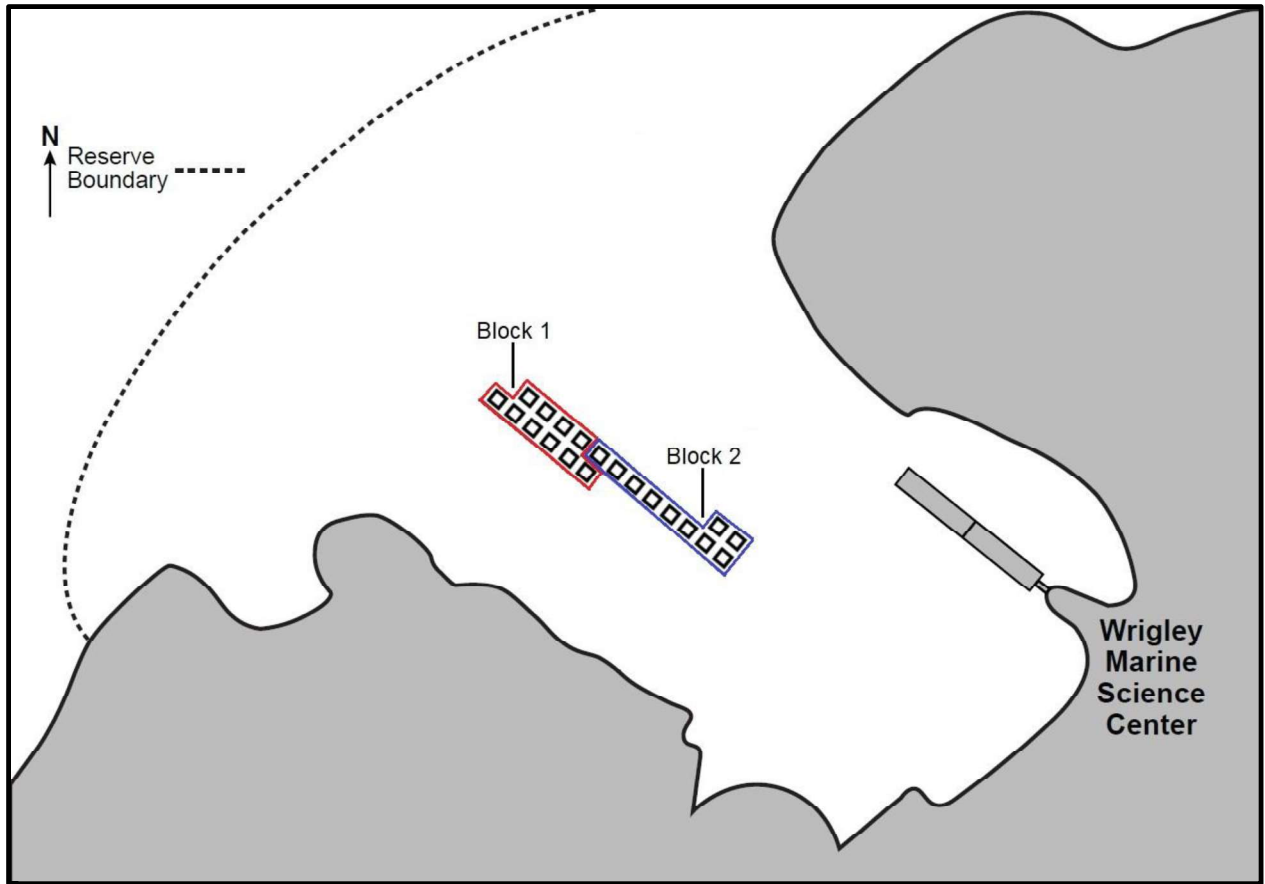


Figure 2. A map of the study site, Big Fisherman Cove, located on the western end of Santa Catalina Island and adjacent to the Wrigley Marine Science Center. The gray squares within the cove represent the artificial reefs used in this study. Block 1 and block 2 are outlined in red and blue, respectively. Map adapted from Schram and Steele 2016.

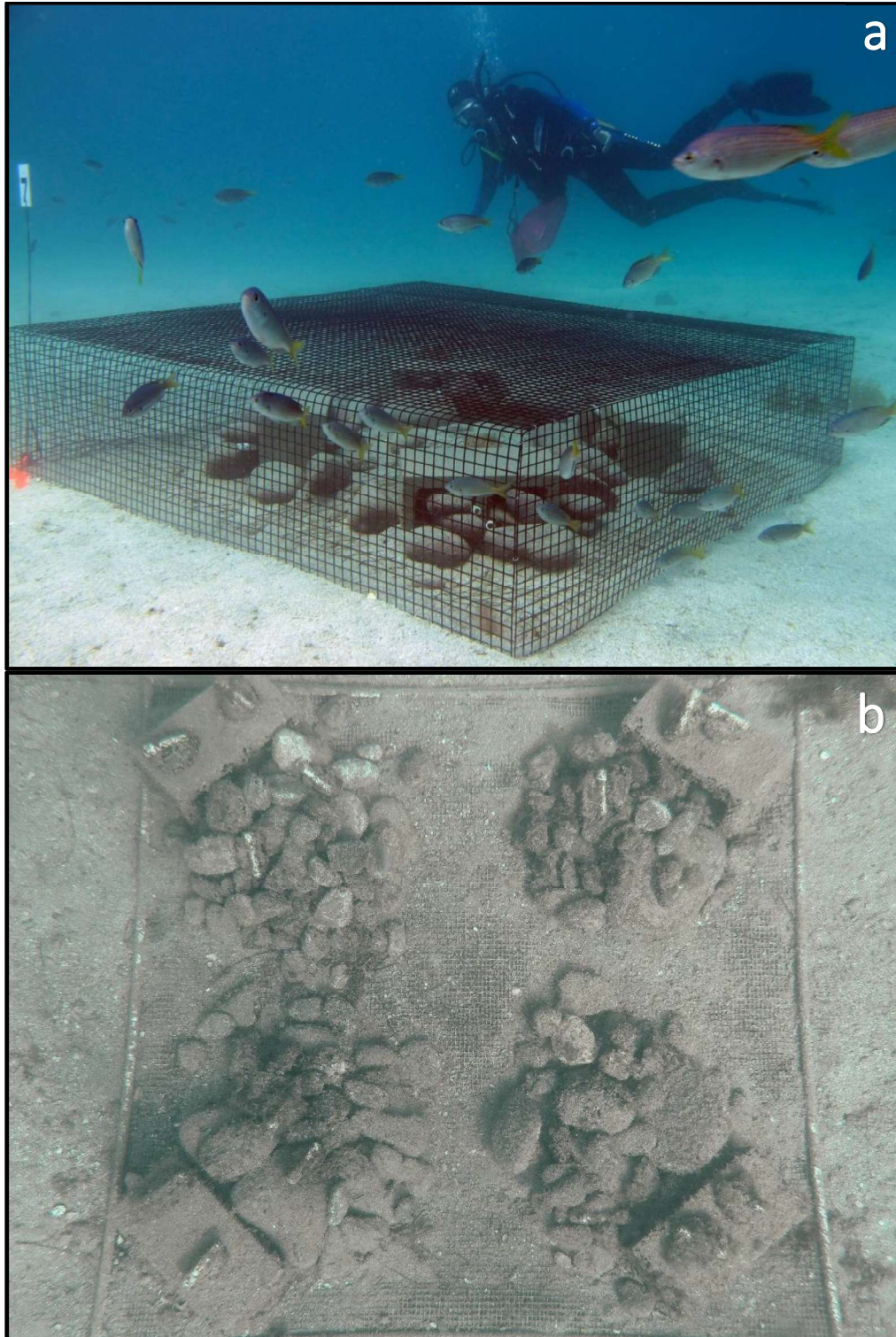


Figure 3. The artificial reefs located in Big Fisherman Cove, Santa Catalina Island, California. (a) Side view showing the cage covering an artificial reef, with a diver for scale. (b) Top-down view of a reef with the cage removed, showing the 4 subreefs within each reef.

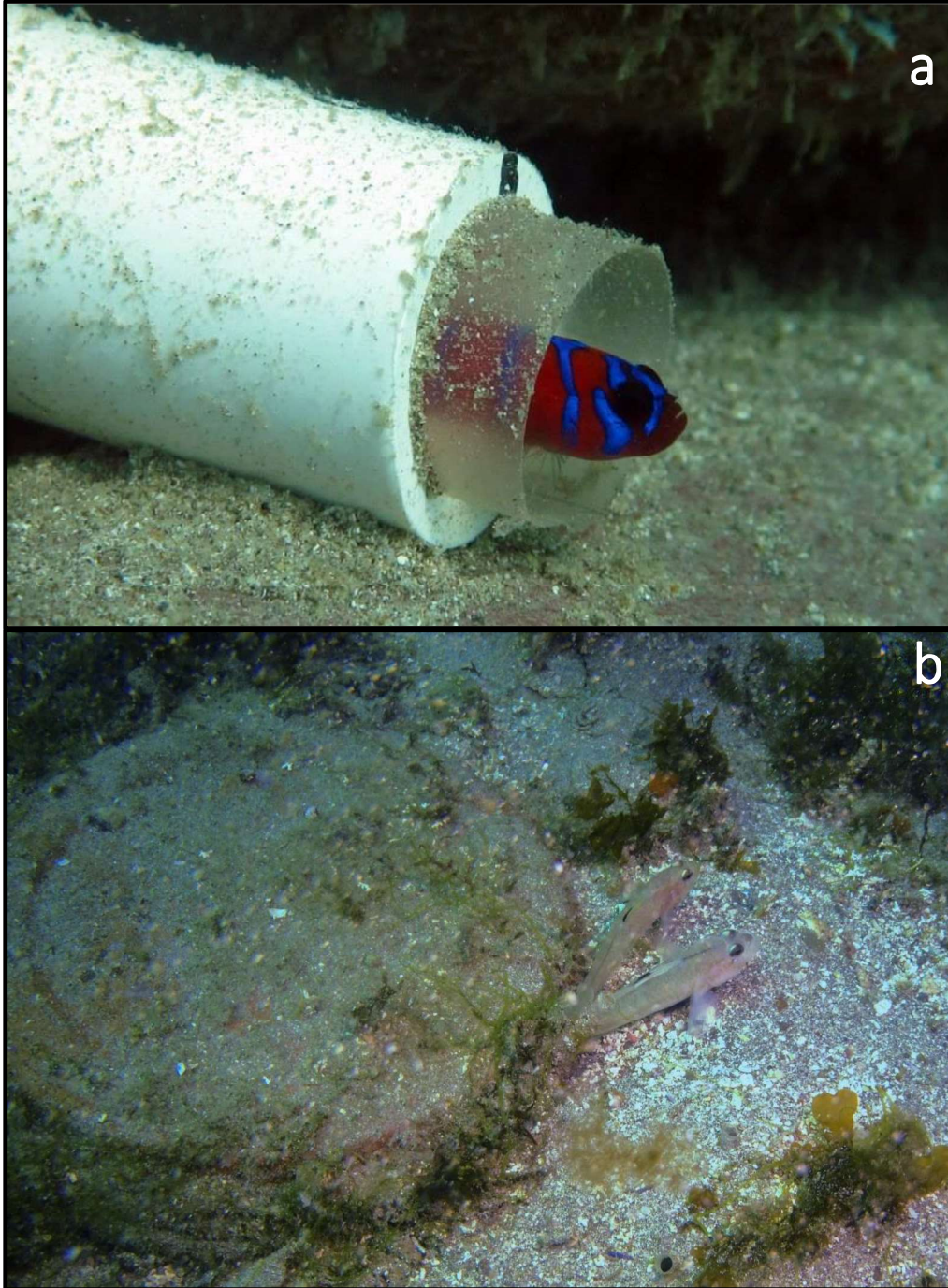


Figure 4. The artificial nests used for (a) *Lythrypnus dalli* and (b) *Rhinogobiops nicholsii*. *L. dalli* reefs were equipped with sixteen 15.2 cm PVC tubes while *R. nicholsii* reefs used five overturned terra cotta saucers.



Figure 5. The visual implant elastomer (VIE) tagging scheme used to mark the standard length and sex for *Lythrypnus dalli* individuals. Marks on (a) the left and (b) right side of the fish. A similar tagging scheme was used for *Rhinogobiops nicholsii*.

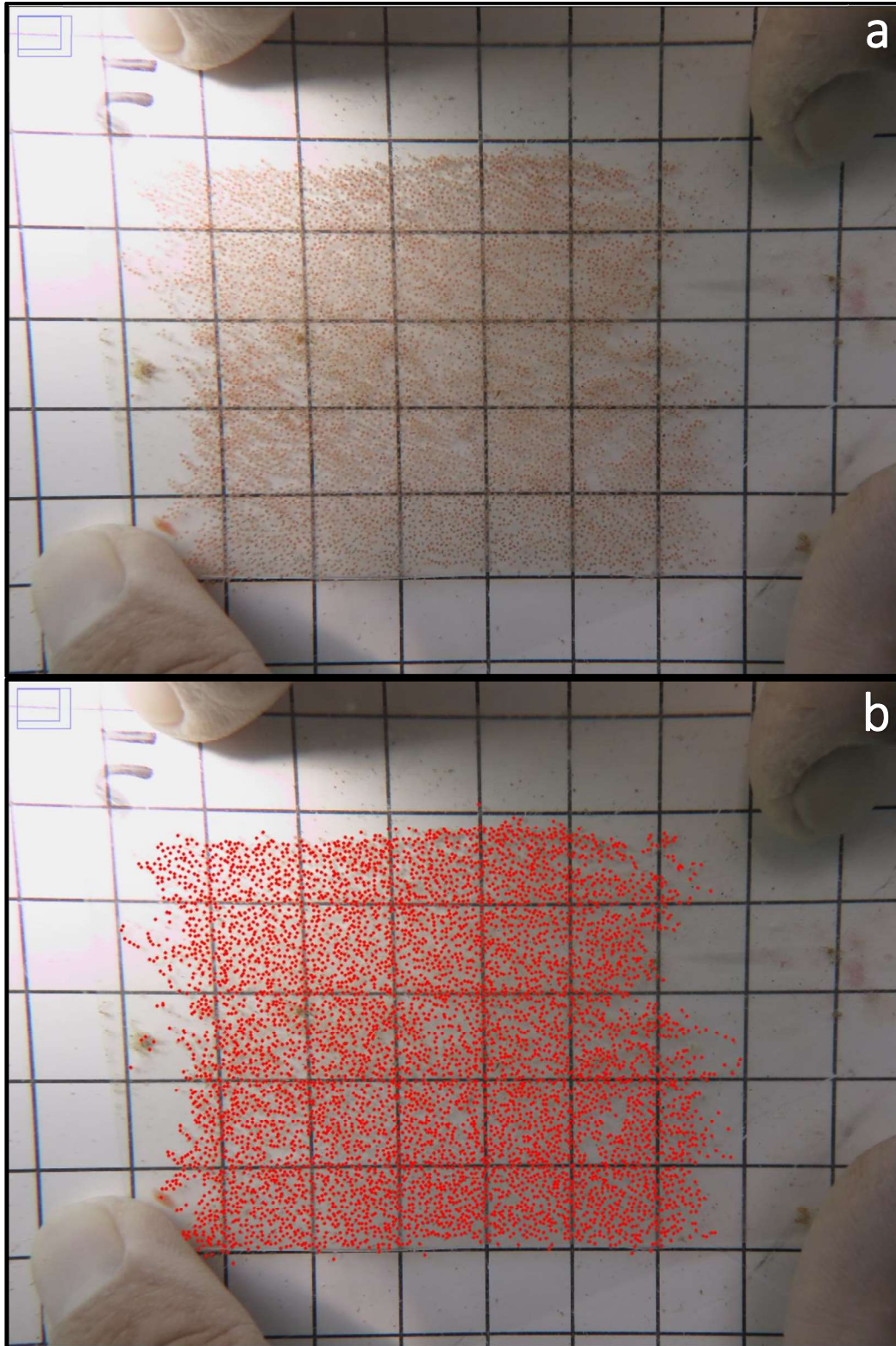


Figure 6. *Lythrypnus dalli* egg broods were manually counted using the ImageJ image processing program to determine brood size. (a) Unprocessed photo of an *L. dalli* egg brood and (b) the manually counted brood with a mark placed over each egg.

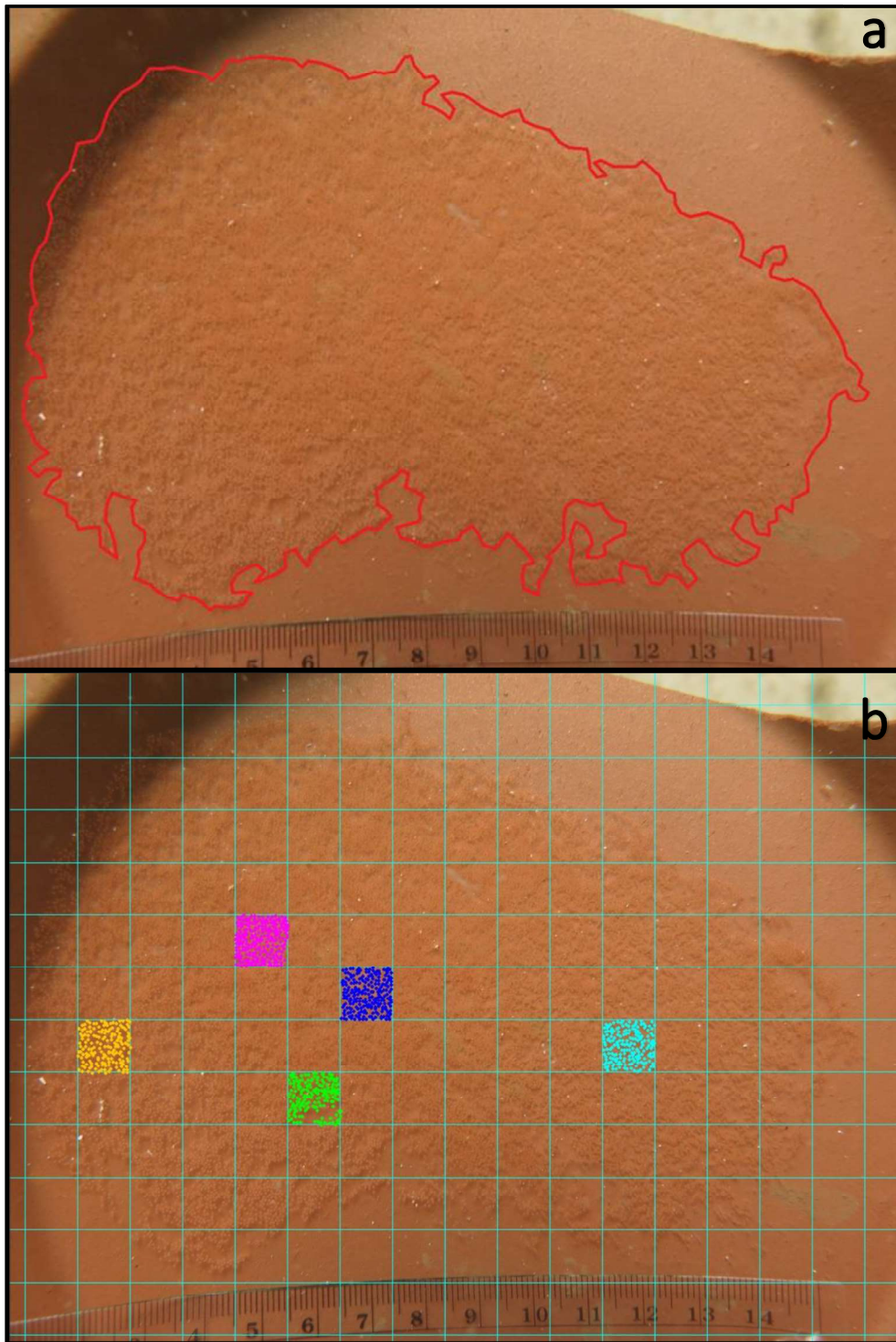


Figure 7. *Rhinogobiops nicholsii* egg broods were subsampled to determine brood size. First, the area of the brood was calculated by (a) outlining the brood in the ImageJ Processing Program. Second, (b) a 1x1 cm grid was imposed on top of the picture and 5 randomly selected cells were counted to obtain the average egg density. Brood size was calculated by multiplying the calculated area by the average egg density.

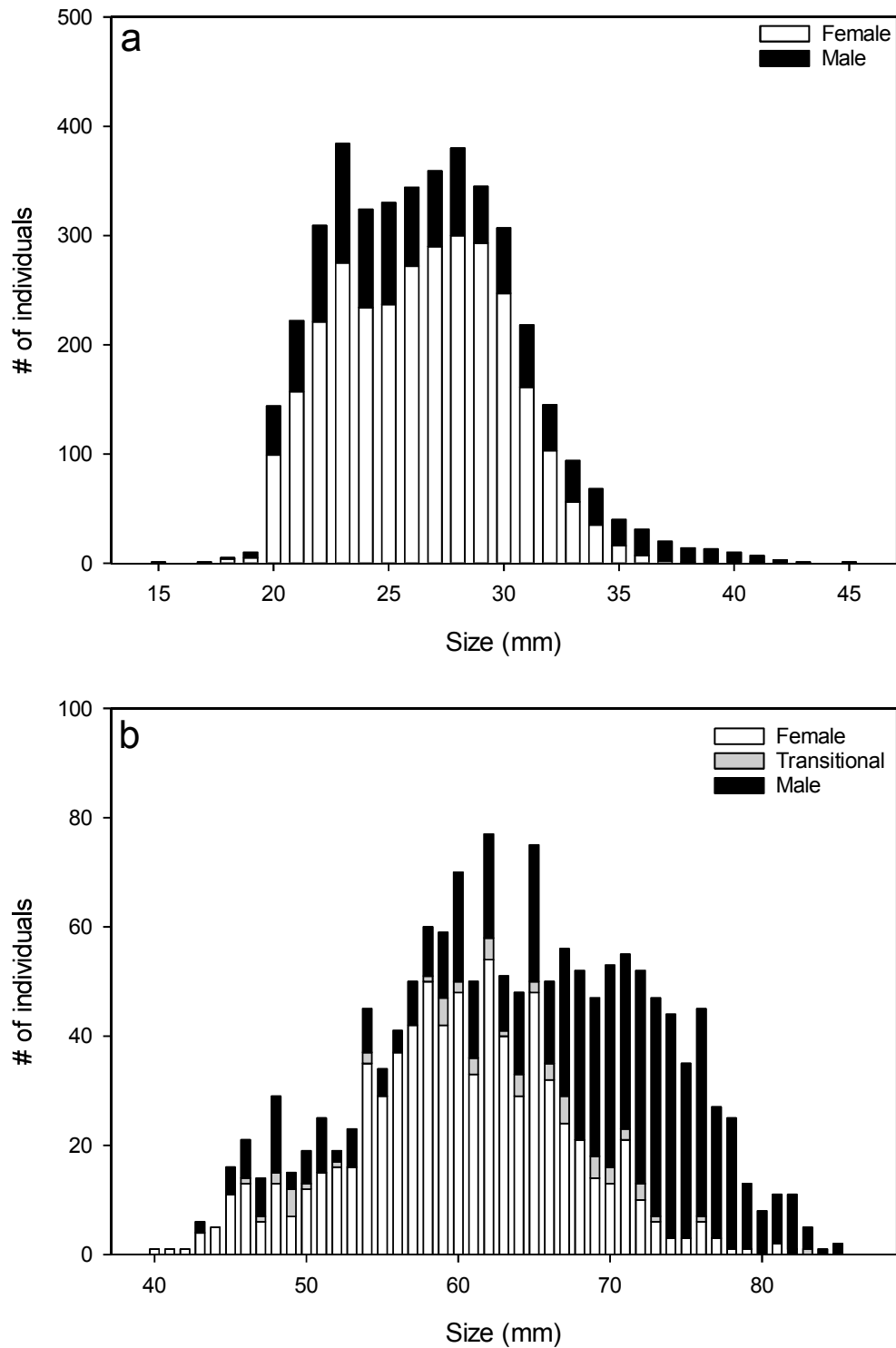


Figure 8. Size structure of all (a) *Lythrypnus dalli* and (b) *Rhinogobiops nicholsii* individuals collected from natural reefs throughout the summer field seasons (2015-2017).

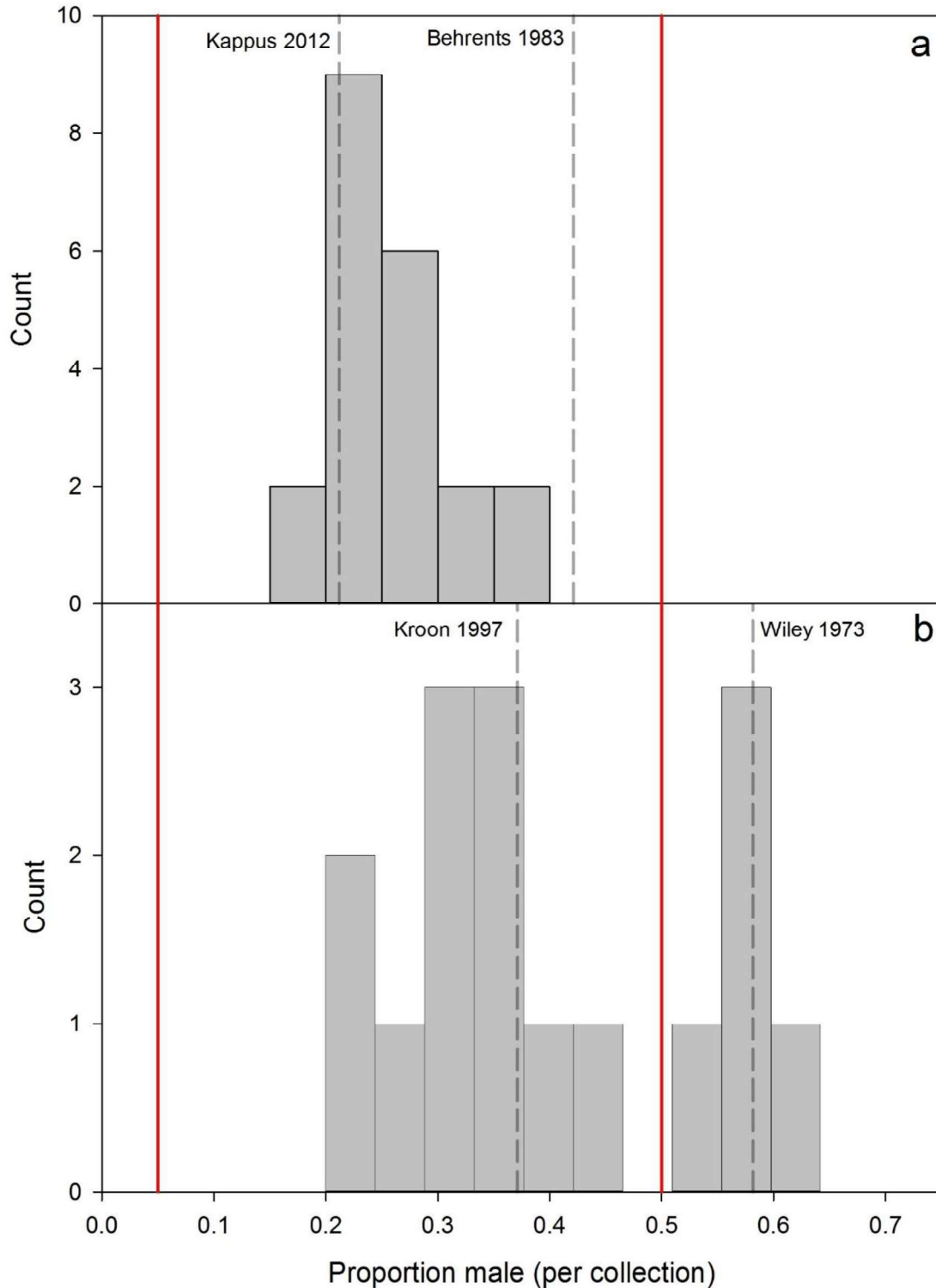


Figure 9. The proportion of males (i.e., sex ratio) of (a) *Lythrypnus dalli* and (b) *Rhinogobiops nicholsii* that were caught during each collection dive throughout the field season. Red lines represent the two extremes of the sex ratio treatments in the manipulative portion of this study. Dashed lines represent natural sex ratios reported in previous studies.

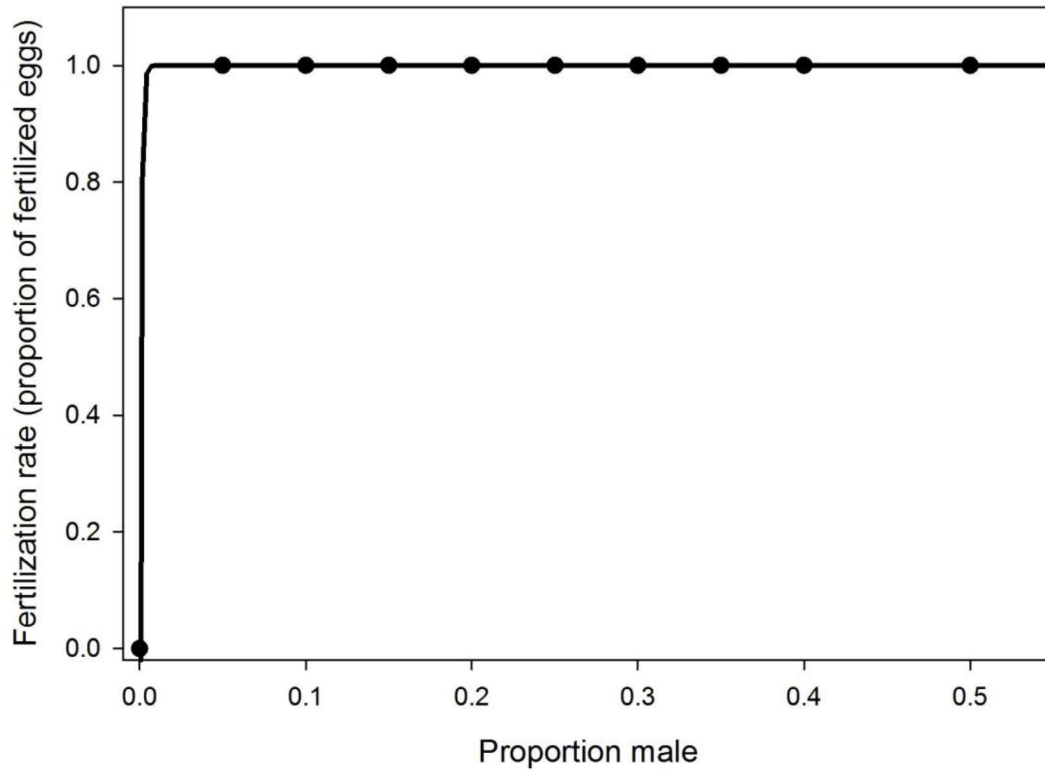


Figure 10. Fertilization function for bluebanded gobies. Shown is the relationship between the proportion of males (i.e., sex ratio) and the fertilization rate (proportion of fertilized eggs on each reef) in *Lythrypnus dalli*.

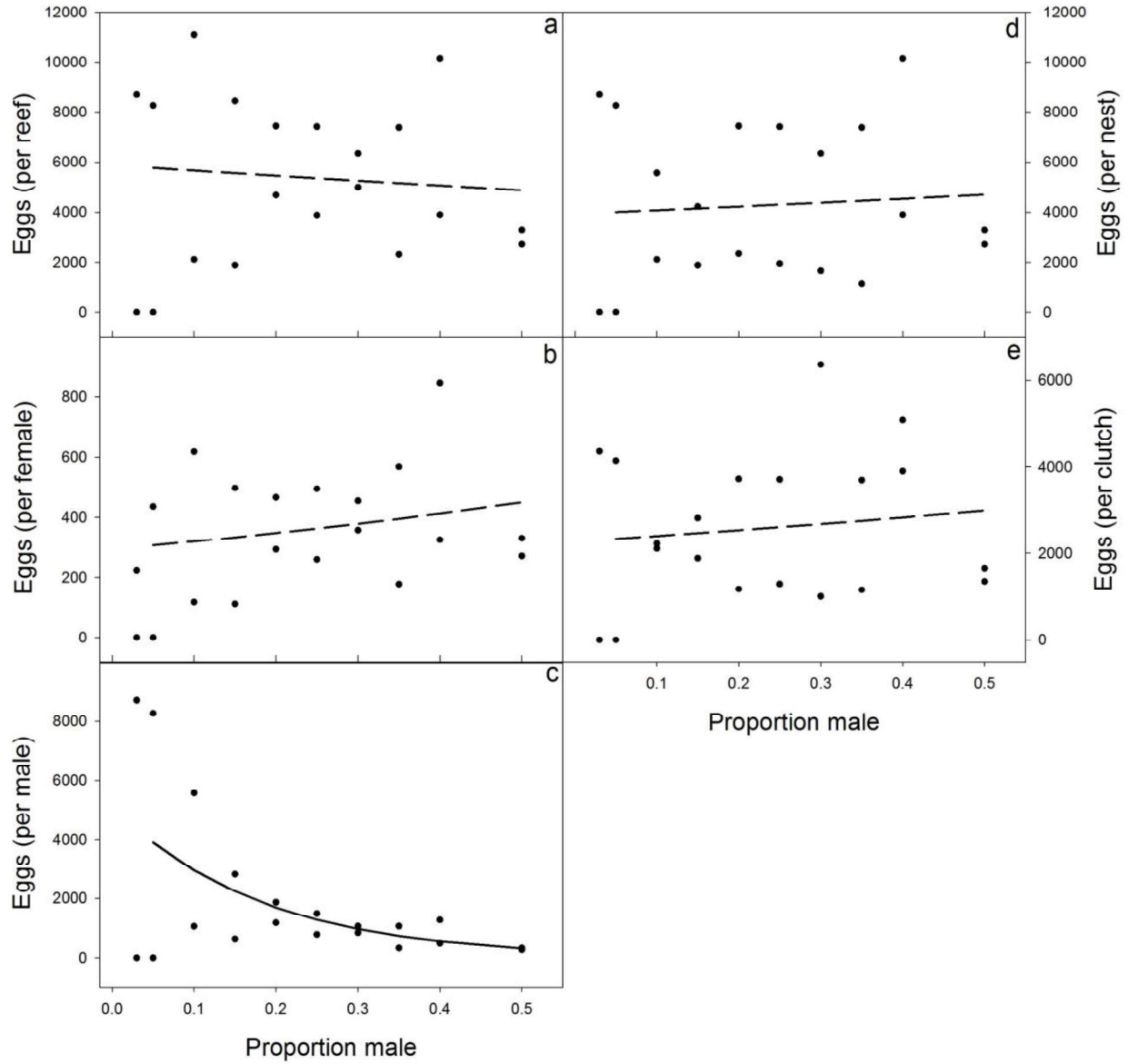


Figure 11. The relationship between sex ratio and the (a) total number of eggs per reef, (b) the female per capita production per reef, (c) the male per capita production per reef, (d) the average number of eggs per nest, and the (e) average number of eggs per clutch for *Lythrypnus dalli*.

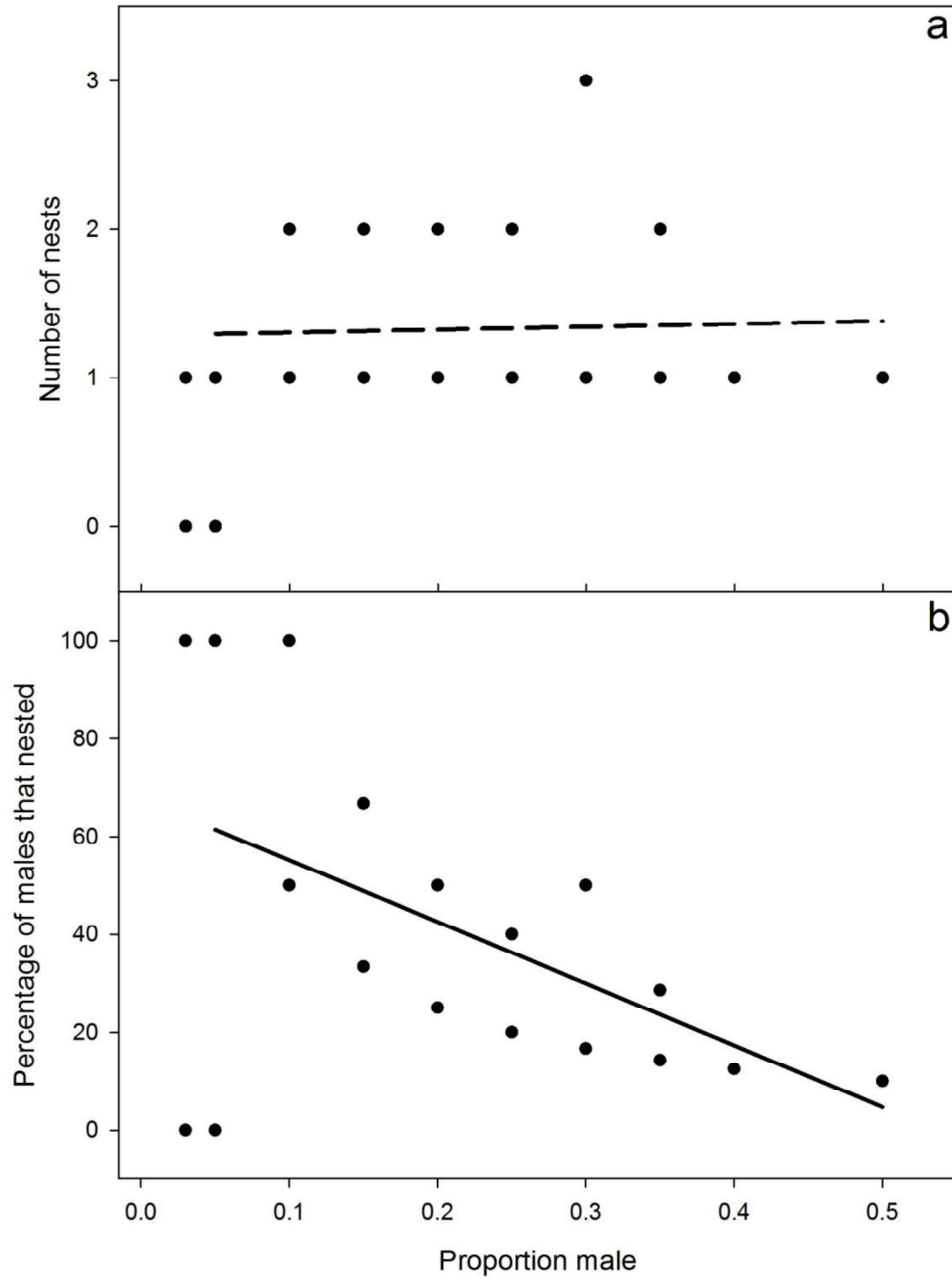


Figure 12. The relationship between sex ratio and the (a) number of nests per reef and the (b) percentage of males that nested on each reef in *Lythrypnus dalli*.

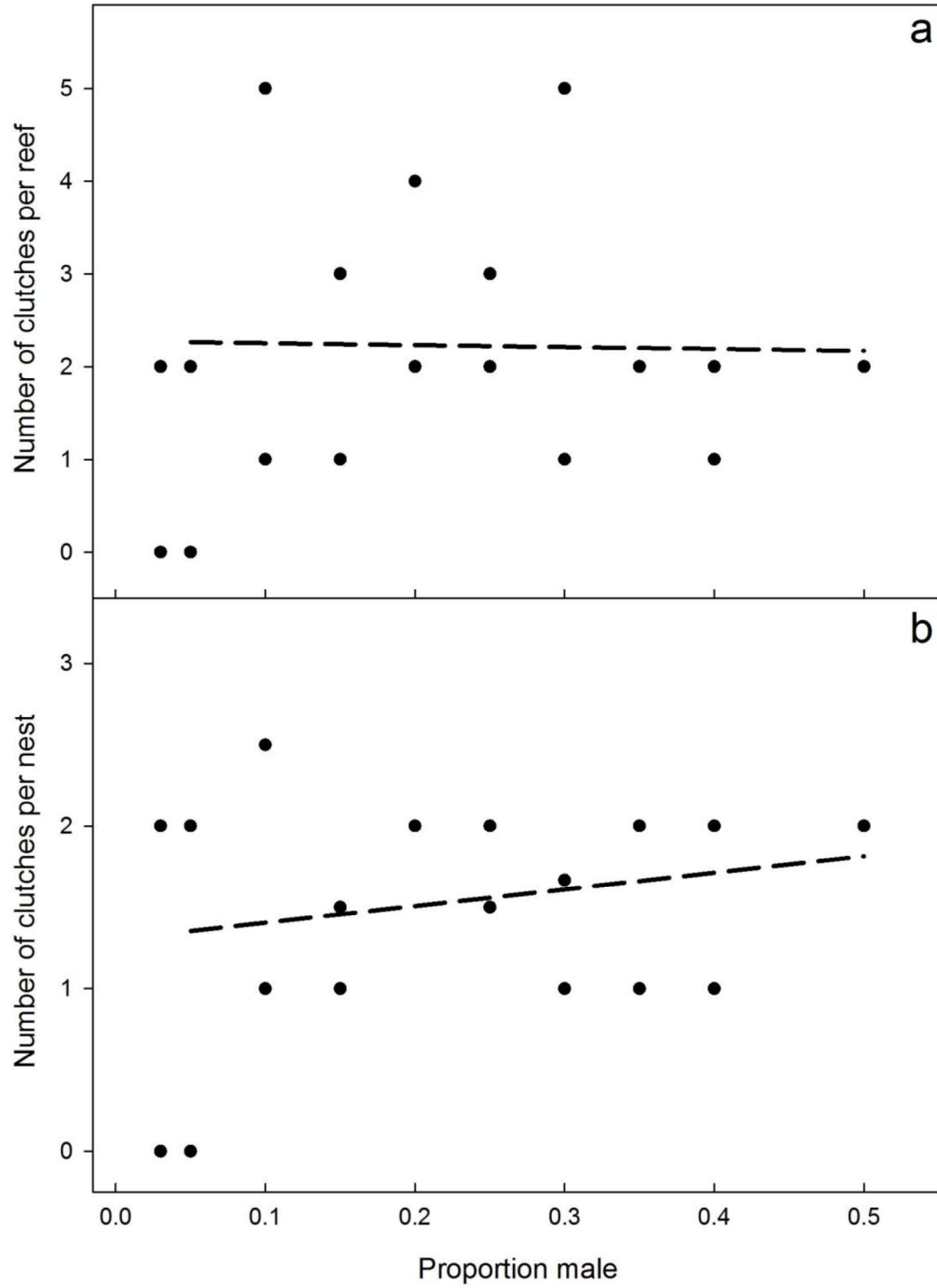


Figure 13. The relationship between sex ratio and the (a) number of clutches per reef and the (b) average number of clutches per nest in *Lythrypnus dalli*.

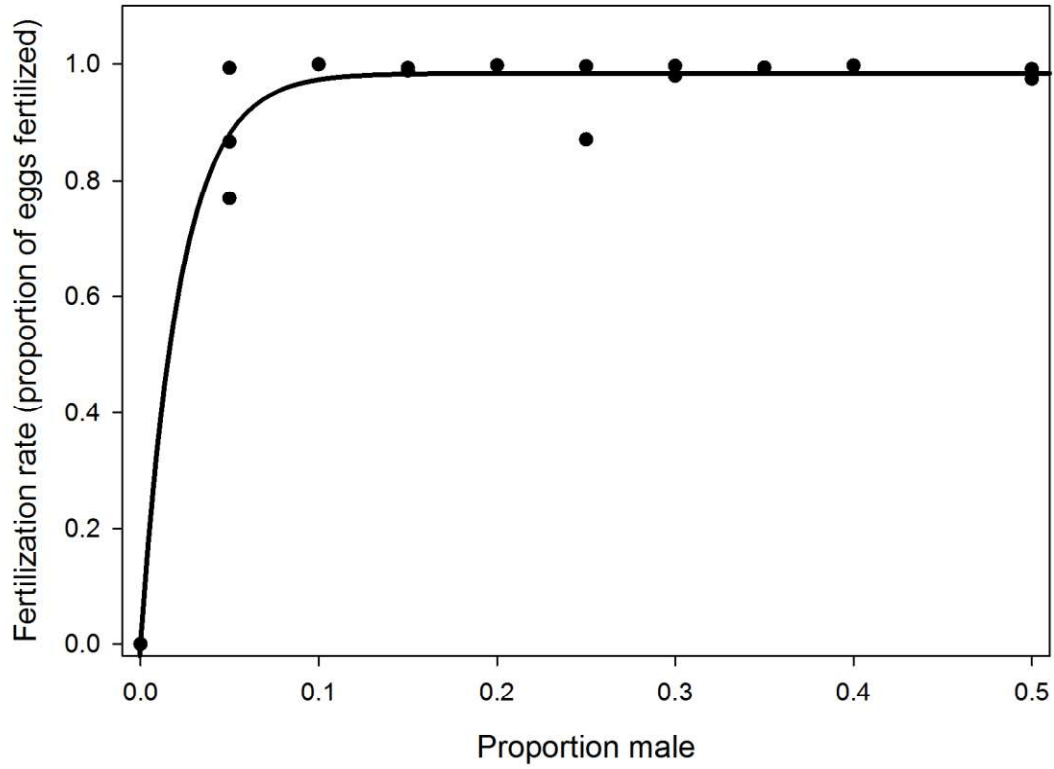


Figure 14. Fertilization function for *Rhinogobiops nicholsii*. Shown is the relationship between the proportion of males (i.e., sex ratio) and the fertilization rate (proportion of eggs in each nest fertilized) in *R. nicholsii*.

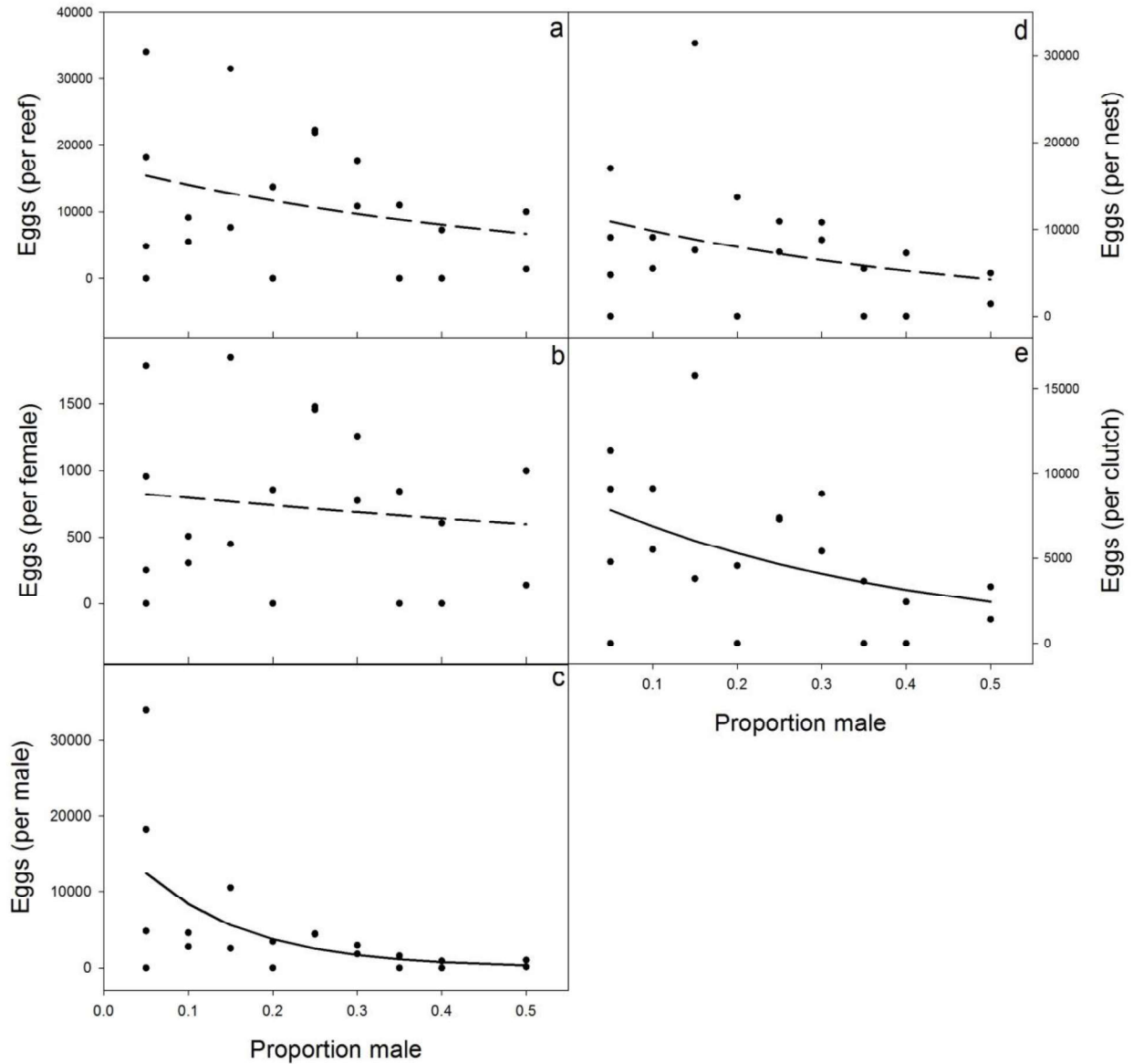


Figure 15. The relationship between sex ratio and the (a) total number of eggs per reef, (b) the female per capita production per reef, (c) the male per capita production per reef, (d) the average number of eggs per nest, and the (e) average number of eggs per clutch for *Rhinogobiops nicholsii*.

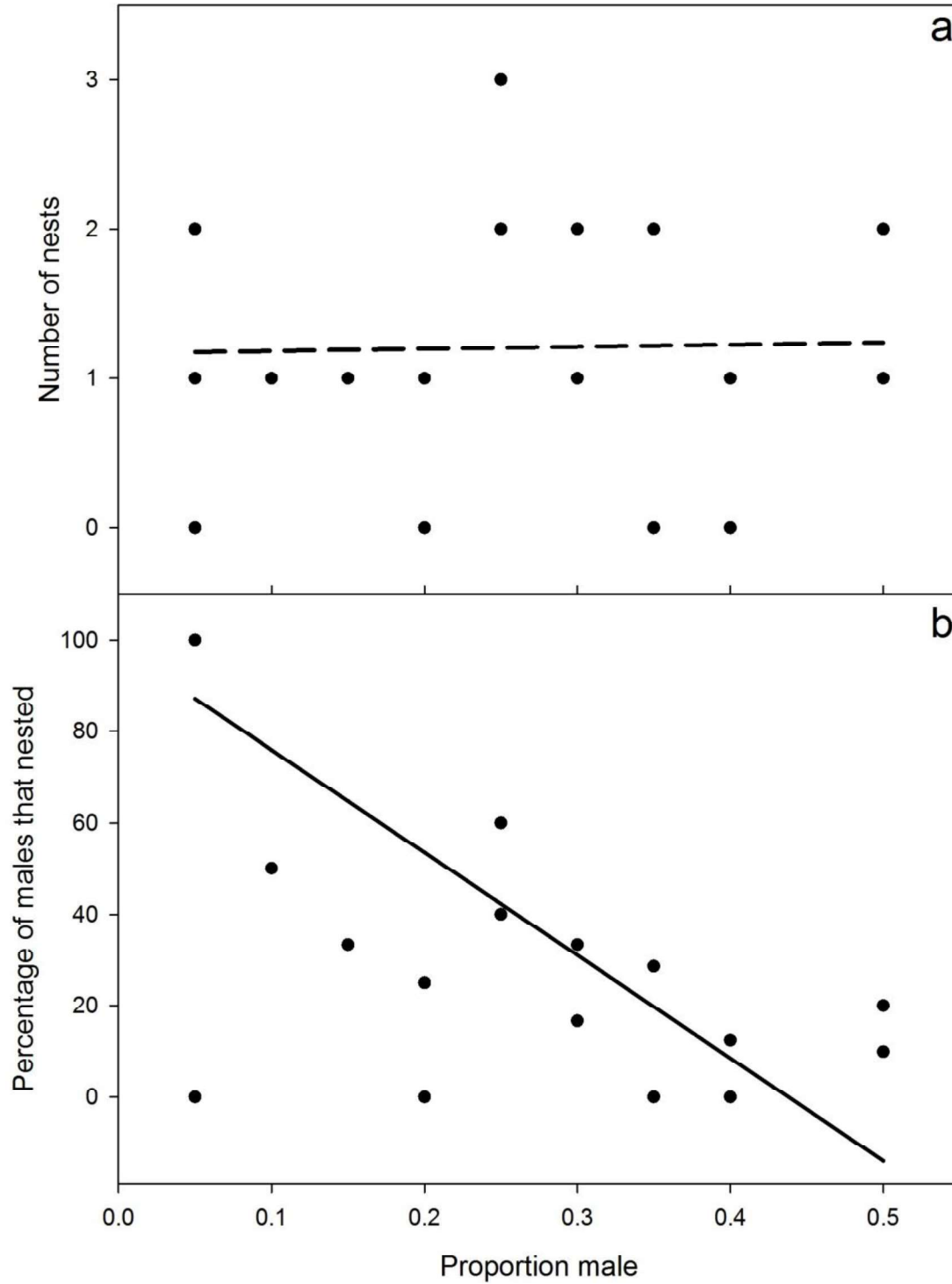


Figure 16. The relationship between sex ratio and the (a) number of nests per reef and the (b) percentage of males that nested on each reef in *Rhinogobiops nicholsii*.

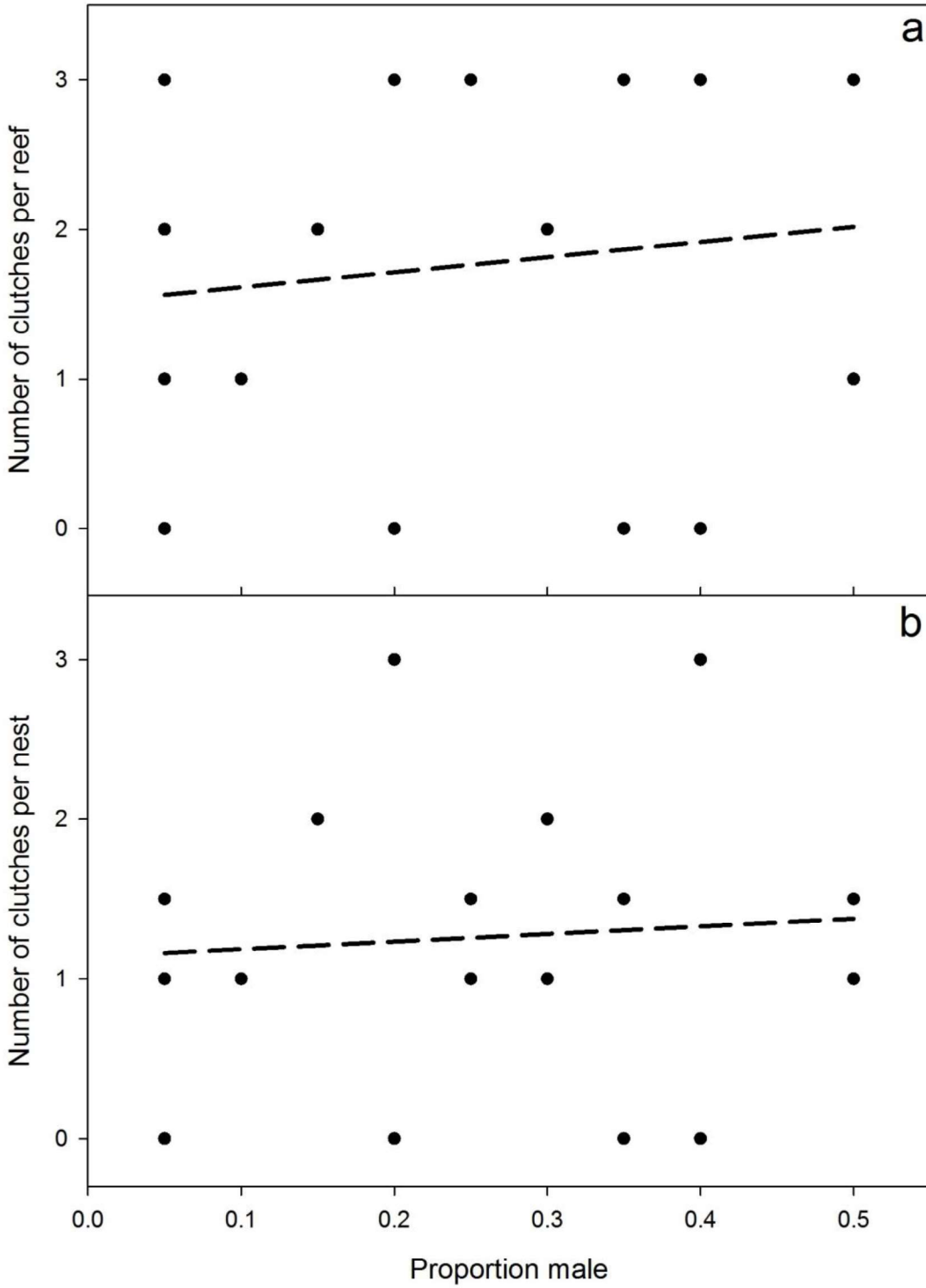


Figure 17. The relationship between sex ratio and the (a) number of clutches per reef and the (b) average number of clutches per nest in *Rhinogobiops nicholsii*.

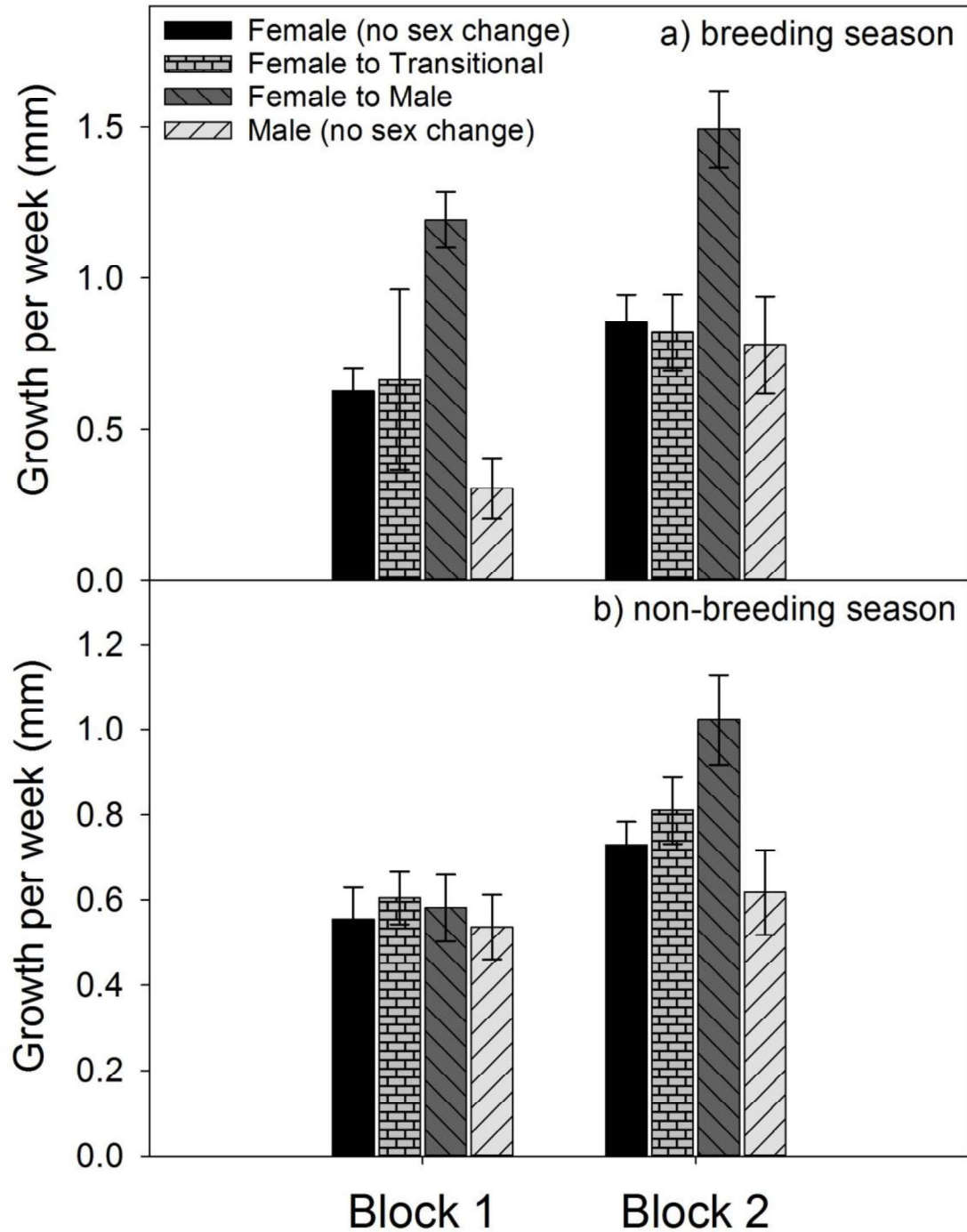


Figure 18. The growth per week of *Rhinogobiops nicholsii* individuals separated by block during the (a) breeding and (b) non-breeding season. The bars represent the sex of the individual at the time of placement on the reefs and the sex of the individual after recollection (~4 weeks). Transitional fish were females that were undergoing sex change to male but were not considered functionally male yet. Error bars are standard error.

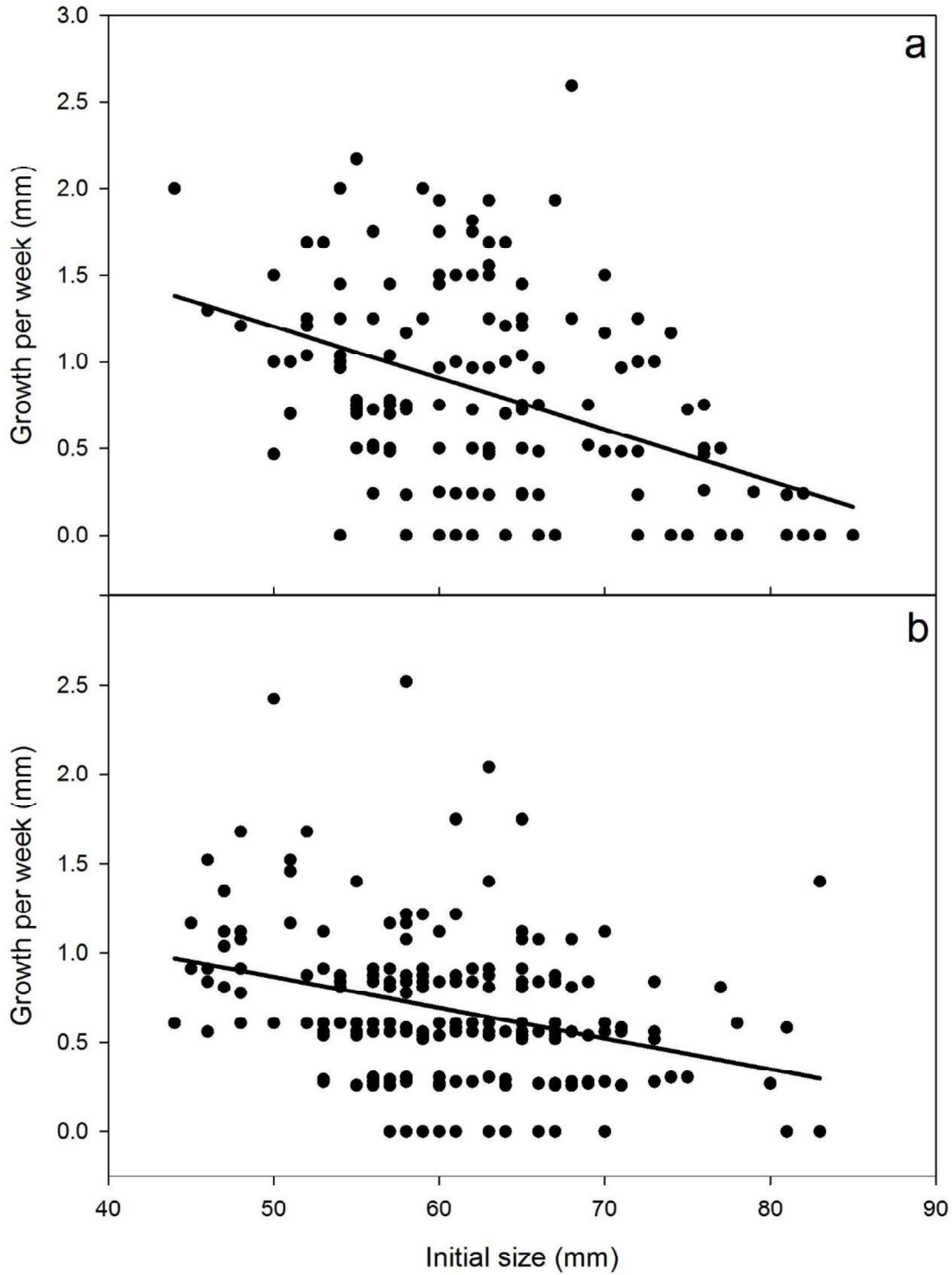


Figure 19. The relationship between growth and the initial size of *Rhinogobiops nicholsii* individuals during the (a) breeding and (b) non-breeding season.

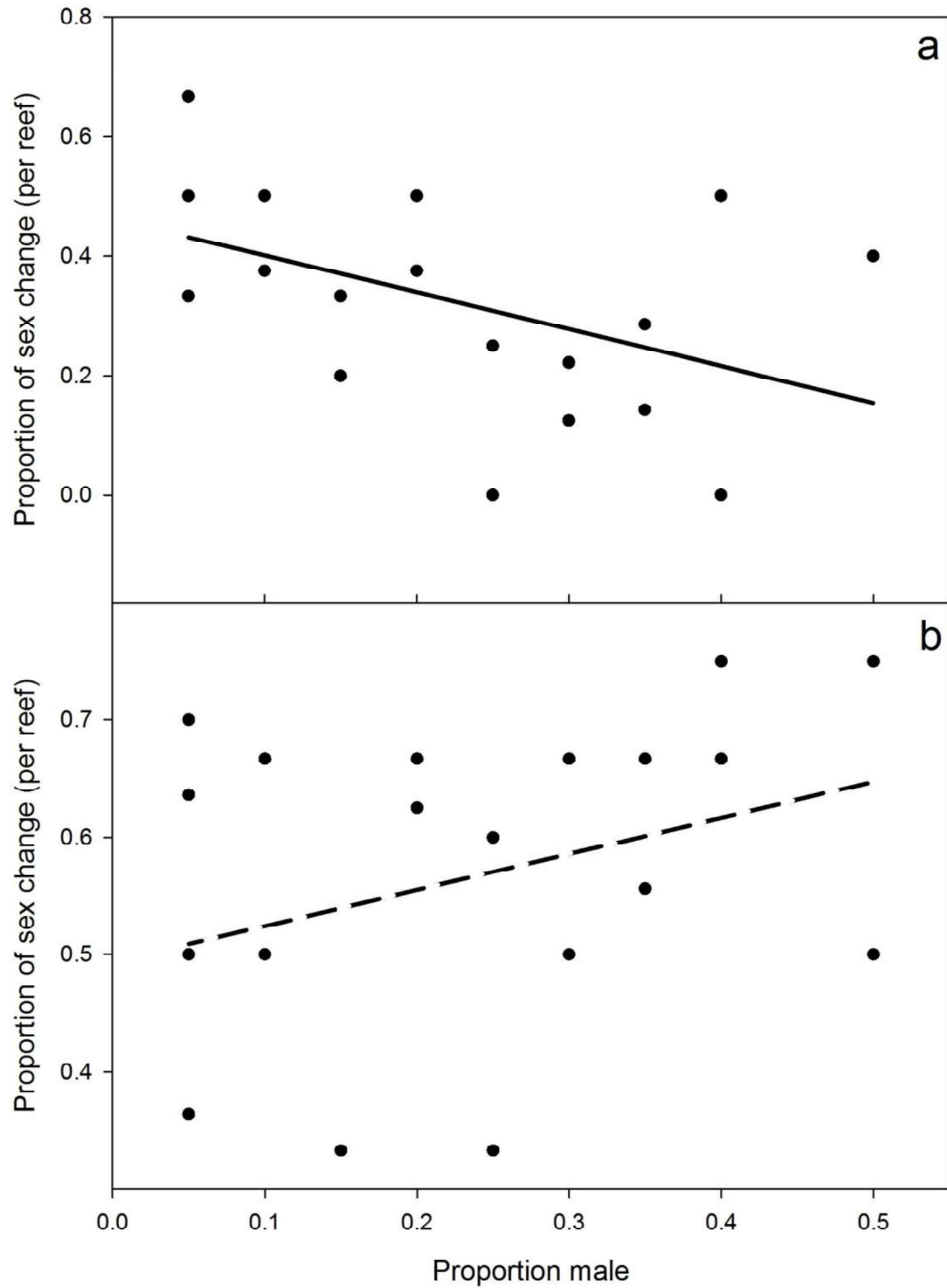


Figure 20. The relationship between sex ratio and the proportion of females on each reef that changed sex (from female to male) in *Rhinogobiops nicholsii* during the (a) breeding and (b) non-breeding season.