

## Sex allocation in a group-living simultaneous hermaphrodite: effects of density at two different spatial scales

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### ABSTRACT

**Questions:** Is the effect of competitor density on the level of sperm competition a strong predictor of natural male gonadal allocation in a simultaneous hermaphroditic fish (*Serranus tortugarum*)? Is any such relationship consistent at different spatial scales? Does any variation in male gonadal allocation involve trade-offs in male and female gonadal investment previously proposed by sex-allocation theory?

**Data obtained:** Density, proximity of conspecifics during spawning, rate of male-role competitive intrusions, and gonadal allocation; collected from multiple locations, at two spatial scales, on Caribbean coral reefs of northwestern Panama.

**Research methods:** Data were obtained at two scales: (1) *fine-scale*: among different social groups within the same isolated population ( $n = 8$  and 12 groups, 2 populations); and (2) *large-scale*: among populations on different (isolated) reefs ( $n = 9$  reefs). Sex allocation (testes mass/total gonad mass, ovary/soma mass, and testes/soma mass) was compared with fish density and spawning behaviour in each social group and population.

**Conclusions:** Our comparative approach confirmed theoretical predictions about density effects on sex allocation in a simultaneous hermaphrodite in nature. The proximity of conspecifics during spawning, intrusive behaviours by sperm competitors, and male allocation (testes mass/gonad mass) all increased with increasing density. Increases in male allocation were accompanied by reductions in ovary/soma mass, while testes/soma mass remained unchanged. These patterns were consistent among isolated social groups within a single population, as well as among isolated populations.

**Keywords:** Caribbean, coral reefs, gonadal investment, mating systems, Panama, seabass, *Serranus tortugarum*, spawning behaviour, sperm competition, trade-offs.

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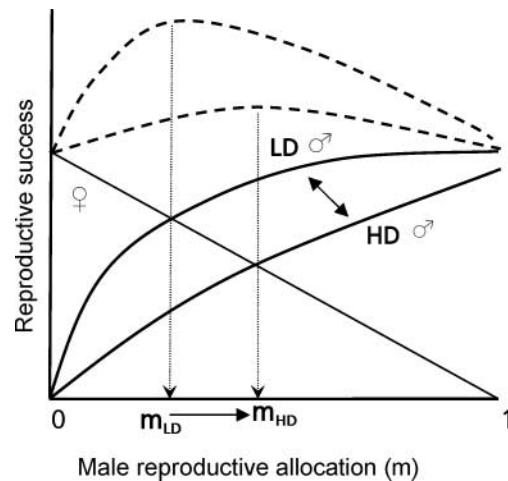
## INTRODUCTION

Sex-allocation theory predicts that both sperm competition and opportunities for multiple matings will lead to greater reproductive effort or allocation to male function (Charnov, 1982, 1996; Fischer, 1984b; Petersen, 1991). Across a wide range of taxa, species that breed in large groups have greater testes size than more solitary species found primarily in pairs [e.g. primates (Harcourt *et al.*, 1981, 1995); fishes (Stockley *et al.*, 1997); bats (Hosken, 1998); birds (Callim and Birkhead, 2007)]. In addition, many species have been shown to alter sex ratio or sex allocation in response to environmental factors (e.g. Herre, 1985; Clutton-Brock *et al.*, 1986; Brockmann and Grafen, 1992; Kruuk *et al.*, 1999). According to both theory and empirical evidence, group-living species subject to variable mating opportunities are expected to adjust their reproductive effort to each sex according to local conditions. The aim of this study was to improve our understanding of environmental, particularly density-related, effects on mating strategy by examining natural variation along a gradient, at different spatial scales.

Simultaneous hermaphrodites provide excellent model organisms for investigating environmental effects on sex allocation. In simultaneous hermaphrodites, individuals are expected to balance energetic investment between sex functions according to available mating opportunities. One can largely control for sex-specific differences in behaviour or physiology when examining individual gender allocation, especially for species that reproduce as both male and female with the same mating partner within a short time frame. Many simultaneously hermaphroditic animals have reproductive organs or tissues that are distinct or separable and can be used as a proxy for relative investment in each sex role (e.g. Fischer, 1981; Petersen, 1991; Raimondi and Martin, 1991; Schärer and Wedekind, 2001; Schärer and Ladurner, 2003; Tan *et al.*, 2004; Baeza, 2007). Furthermore, a considerable body of theory provides testable predictions for effects of mating group size on sex-allocation patterns (Charnov, 1982; Fischer, 1984b, Petersen, 1991; Petersen and Fischer, 1996).

The predictions of sex-allocation theory for mating strategies in simultaneous hermaphrodites can be visualized with male and female gain curves (Fig. 1) (see Charnov *et al.*, 1976; Fischer, 1981; Charnov, 1982; Petersen, 1991; Petersen and Fischer, 1996; Baeza, 2007; Schärer, 2009). For this model, female allocation,  $f$ , and male allocation,  $m$ , sum to one ( $f + m = 1$ ). Female reproductive success typically increases linearly with increasing female gonadal allocation, whereas male reproductive success typically increases with diminishing returns as male gonadal allocation increases, due to greater competition among self-sperm (Fig. 1) (see Fischer, 1981; Charnov, 1982; Petersen, 1991; Petersen and Fischer, 1996; Schärer, 2009). The optimal sex allocation is the point where the sum of the female and male gain curves is maximized; note that this is also where the rates of return on investment into each function,  $f$  and  $m$ , are equal. In pairs or small mating groups, a relatively small proportion of gonadal investment is required to fertilize eggs from available mating partner(s), beyond which an individual gains more though investing in female than in male function (Charnov, 1979, 1982; Fischer, 1981, 1984b; Petersen, 1991; Petersen and Fischer, 1996). Increases in mating group size and local sperm competition reduce self-sperm competition, which lowers the initial rate of return on male gonadal allocation and tends to straighten the male gain curve; this shifts the optimal sex allocation towards greater male function (Petersen, 1991; Yund, 1998; Schärer, 2009). If total allocation is constrained, increases in male allocation imply decreases in female allocation.

In addition to density, body size and resource availability are known to influence fitness gain curves and optimal sex-allocation patterns in simultaneous hermaphrodites (Petersen and Fischer, 1996; Klinkhammer *et al.*, 1997; St. Mary, 1997; Angeloni *et al.*, 2002). Increased body size and increased



**Fig. 1.** Predictions based on sex-allocation theory of increasing optimal male allocation with larger mating groups. Low-density (LD) mating groups yield higher male reproductive success per unit allocation with more rapidly diminishing returns than high-density (HD) mating groups. Thus an increase in density from low to high produces an increase in the ESS male allocation ( $m_{LD}$  shifts to the right along the x-axis to  $m_{HD}$ ) and a decline in overall reproductive success (compare the dashed curves peaks). Modified from Fischer (1981), Charnov (1982), Petersen (1991), and Schärer (2009).

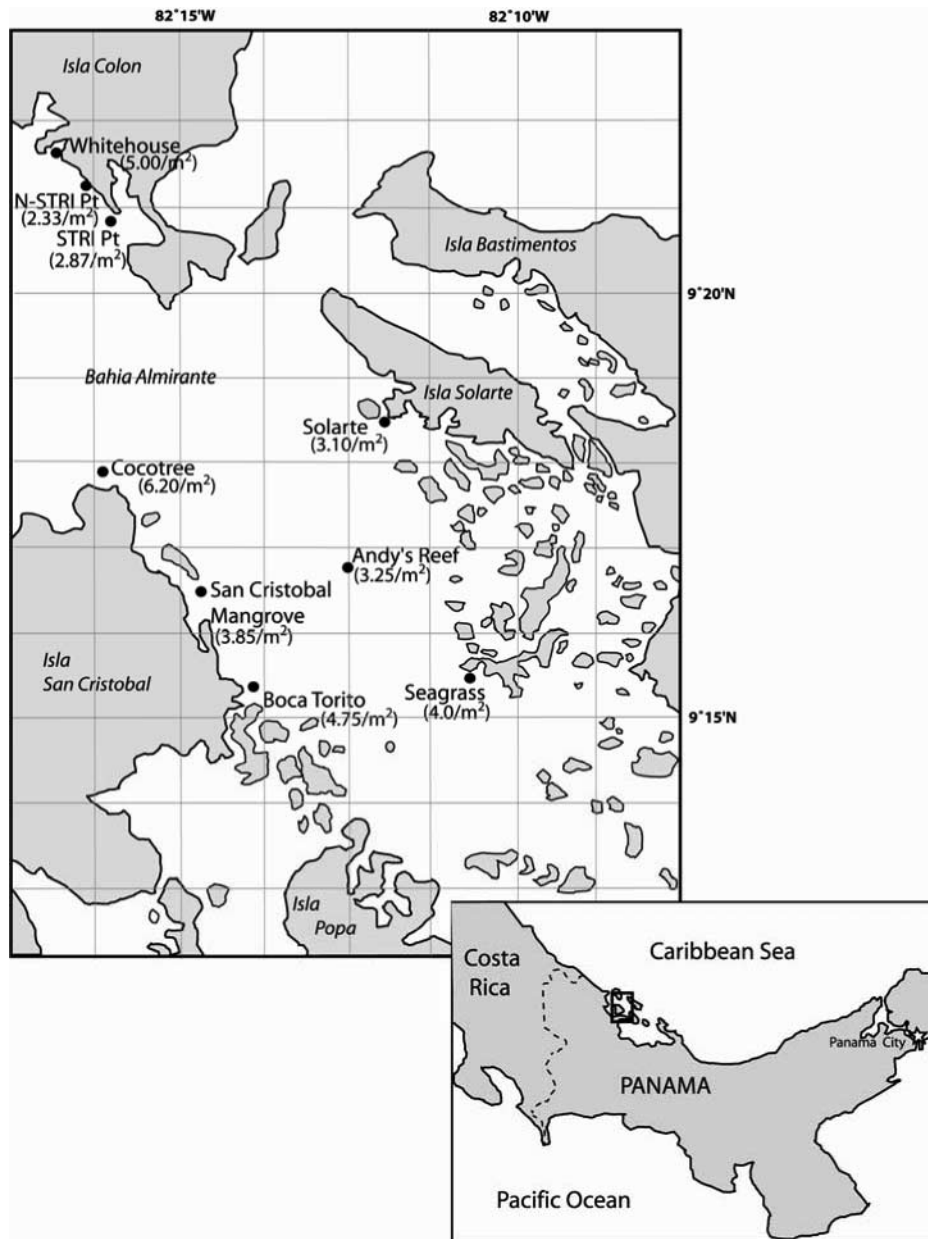
resource availability have been linked empirically with decreased male (and increased female) allocation in several taxa (e.g. Petersen and Fischer, 1996; Angeloni, 2003; Schärer *et al.*, 2001; Baeza, 2007; Vizoso and Schärer, 2007). For species in nature facing strong local sperm competition, density and mating group size should have an important and measurable effect. Yet as environmental heterogeneity increases, factors such as resources, habitat, and predator regime may obscure or interact with density effects (Wiens, 1989; Levin, 1992; White and Caselle, 2008). To address this, we examined the effects of density on sperm competition and sex allocation over two spatial scales: among different social groups within the same populations, and among well-separated populations. If confounding environmental effects are greater over larger spatial scales, we should expect that the relationship between density and sex allocation will be stronger within than among populations.

We expect that increasing densities will lead to closer proximity of sperm competitors and more intrusive behaviours on mating pairs. If sperm competition is more prevalent at higher densities, then male allocation should also be higher. A direct trade-off between sex functions will be indicated if female gonadal investment (ovary/soma mass) decreases as male gonadal investment (testes/soma mass) increases along the density gradient.

## METHODS

### Research location

Fieldwork for this study was conducted at the Smithsonian Tropical Research Institute's Bocas Research Station in Panama in 2005–2006. Study sites were located within 15 km of



**Fig. 2.** Map of nine study site locations. Densities for the among-site comparisons are denoted in parentheses under each study site name.

the station in the Bay of Almirante and ranged from 4 to 10 m in depth (Fig. 2). This large bay is sheltered by outer islands and features shallow, sloping reefs that progress from mangroves and seagrass beds along island perimeters.

### Study species

*Serranus tortugarum* (Serranidae: Serraninae) is highly site-attached in adulthood. Yet like many coral reef fishes, it spawns externally in the water column and has pelagic eggs and larvae that are typically transported away from the reef of origin. Populations thus have open gene flow but individuals must adapt to very local environmental processes for much of their lives, circumstances favouring phenotypic plasticity to facilitate local adaptation (Warner, 1991). *Serranus tortugarum*, or chalk bass, is found in localized aggregations of 20–500+ individuals over coral rubble and reef flats of the Caribbean and south Florida. Chalk bass spawn daily with same-sized mating partners in the 2 h before sunset (Fischer, 1984a). This species engages in egg trading, wherein partners alternate roles as male and female several times during a given spawning period, so that each individual has the opportunity to fertilize the other's eggs (Fischer, 1980, 1984a). Individuals frequently engage in 'streaking', regardless of pairing status, to gain more male-role fertilizations by intruding on other spawning pairs and releasing sperm (Fischer, 1984a). Chalk bass are found in a variety of local densities and habitat types near Bocas Research Station. Aggregations of chalk bass were localized, with stable abundances and typically separated by habitat discontinuities from other groups of conspecifics. Mark-resighting surveys on multiple reefs have shown that individuals were almost always observed within a 5-m radius of the aggregation from where they were captured (M.K. Hart, unpublished data).

### Study design

We included patterns from one large-scale comparison (among populations) and two small-scale comparisons (within populations) in our experimental designs and analysis (as the three Site-scale factors in our statistical analyses). For the large-scale comparison, study sites spanning approximately 1 ha were established on each of nine continuous reefs separated by 1.5–15 km (see Fig. 2). Within these nine study sites, data were collected in 2005–2006 and averaged from among multiple aggregations for each site. For examination of small-scale patterns in 2006, two reef study sites that had particularly wide ranges in aggregation densities were used; these two sites had already been sampled in 2005 as part of the large-scale comparison. In the fine-scale (or small-scale) comparison, eight aggregations were used from one study site (Seagrass), and twelve aggregations were selected from another (Cocotree). Data collected were specific to each aggregation within these two study sites. Cocotree had greater habitat complexity than Seagrass [i.e. substrate height and rugosity (M.K. Hart, unpublished data)] and the highest density of all the study sites used in the large-scale study (6.2 per m<sup>2</sup>); Seagrass had medium density among the study sites (4 per m<sup>2</sup>). Aggregations used in these comparisons were 10 m or more apart and considered socially isolated groups.

### Data collection

For each aggregation at the fine scale and for each study site at the large scale, means for density, number of streakers per spawn, and nearest neighbour distance among pairs (pair spread) were estimated. For the large-scale comparison, data were averaged from across 6–8 aggregations for each of the nine study sites.

Density and spawning distribution data were collected underwater using SCUBA between 16.30 and 18.40 h while fish are engaged in courtship and spawning. During

spawning, individuals within an aggregation are typically found in pairs, with fairly even spatial distribution among them. Visual estimates of the number of fish in a given area were translated to densities (number per square metre) by using measuring tape and flagging to ground-truth distance estimates. Neighbouring fish were the most likely candidates for sperm competitors, and so distance between spawning pairs (i.e. pair spread) was also estimated to contrast with density as another proxy for mating group size. Density provided a two-dimensional estimate, while pair spread was estimated in three-dimensional space, since spawning pairs were typically distributed from 10 to 100 cm above the substrate. Density and pair spread estimates for each study site or aggregation were averaged across 2–4 separate days, and each day estimates were recorded 2–4 times at 5- to 10-min intervals.

Video recording (with slow-motion playback) and visual estimates from random spawns were used to estimate mean number of streakers per spawn within an aggregation. Spawning events occur quite rapidly (<1 s), so slow-motion video playback was often necessary to assess the actual number of streakers, especially for high-density areas. For each study site or aggregation, 2–6 h of video was recorded, and from video and visual estimates, 30–125 random spawns were observed and used to calculate mean number of streakers per spawn.

In the gonad of the chalk bass, testicular tissue is a noticeably different texture and colour and was separated from ovarian tissue under a microscope; the separate tissues were then dried and weighed (following methods of Petersen, 1991; Petersen and Fischer, 1996). Fish were sub-sampled following collection of density and streaking data to compare body size (soma mass, dry weight in milligrams), male allocation (testes mass/total gonad mass  $\times$  100, dry weight in milligrams), and relative reproductive investments (ovary mass/soma mass vs. testes mass/soma mass, dry weight in milligrams) among aggregations and study sites. For the within-site comparisons, 9–12 fish were collected from each aggregation during August–October 2006. For the among-site comparison, 30–60+ fish were collected from 3 to 4 aggregations within the area of the study sites, in summer 2005 from Boca Torito, Cocotree, Seagrass, Solarte, STRI Pt, and White House and in summer 2006 from Andy's Reef, N-STRI Pt, and San Cristobal Mangrove. Fish were sampled over the representative range of body sizes for each aggregation or study site. A barrier net was used to encircle the fish; they were captured using hand nets against the larger net and euthanized with clove oil before placing in fixative (10% formalin solution).

### Statistical analysis

Relationships between pair spread and density and between number of streakers per spawn and density measured how effectively density served as a proxy for mating group size. Relationships between density and body (i.e. soma) mass and responses of male allocation, ovary, and testes mass to both density and soma mass were tested. Relationships between ovary mass and testes mass were divided by soma mass and examined across density and against each other to determine whether trade-offs existed in allocation patterns (as in Petersen, 1990b; Schärer *et al.*, 2005; Baeza, 2007).

A general linear model was used for each statistical test. Site-scale ( $n = 3$ ; Cocotree, Seagrass, Among-site) was treated as a fixed factor with density as a covariate to test effects on pair spread, number of streakers per spawn, soma mass, ovary/soma mass, and testes/soma mass:  $y = \text{Site-scale} + \text{Density} + \text{Site-scale} * \text{Density}$ . Ovary/soma mass was substituted for Density to test whether the relationship with testes/soma mass varied with Site-scale.

Soma mass was included as a covariate in the model testing effects on male allocation, ovary mass, and testes mass:  $y = \text{Site-scale} + \text{Density} + \text{Site-scale} \times \text{Density} + \text{Soma mass} + \text{Site-scale} \times \text{Density}$ . We used model II regression to test the relationship between residuals for log ovary mass and log testes mass corrected for soma mass because the variance around both the  $x$  and  $y$  variables was in question (see Ebert and Russell, 1994; Stolz *et al.*, 2005; Warton *et al.*, 2006).

Residuals for all dependent variables were tested for normality and homogeneity of variance. Data were log-transformed to examine allometric relationships between soma, ovary, and testes mass. Otherwise dependent variables were transformed only if necessary to reach assumptions of statistical tests. Statistical analyses were performed using SAS Jmp, version 8.0.1.

## RESULTS

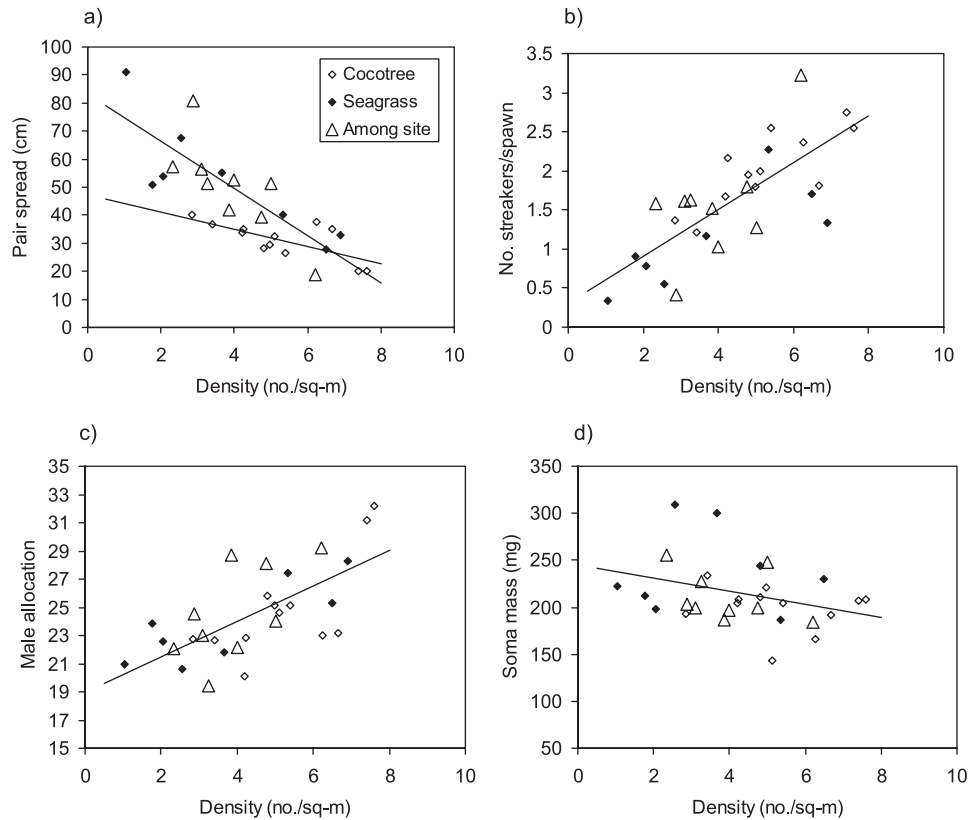
### Mating group size: density, pair spread, and streaking

Pair spread decreased significantly with increasing density (Whole-model  $R^2 = 0.7285$ ; Site-scale,  $F_{2,23} = 5.2280$ ,  $P = 0.0134$ ; Density,  $F_{1,23} = 37.2226$ ,  $P < 0.0001$ ; Site-scale  $\times$  Density,  $F_{2,23} = 3.4549$ ,  $P = 0.0480$ ; Fig. 3a), with a significant interaction and significantly different adjusted means between study sites. At Cocotree the relationship was not as strong as for Seagrass and the Among-site means, probably because pair spread was lower overall, and this led to the significant interaction. Strong and significant positive relationships were observed between density and streaking (Whole-model  $R^2 = 0.6626$ ; Site-scale,  $F_{2,23} = 3.1559$ ,  $P = 0.0615$ ; Density,  $F_{1,23} = 25.0694$ ,  $P < 0.0001$ ; Site-scale  $\times$  Density,  $F_{2,23} = 0.7142$ ,  $P = 0.5001$ ; Fig. 3b). The strong relationships between density and pair spread and density and streaking indicate a shift towards increased mate competition with higher local densities.

### Density and gonadal (sex) allocation

Density showed the expected positive effect on male allocation (Fig. 3c), while soma mass had no influence (square-root transformed male allocation: Whole-model  $R^2 = 0.5318$ ; Site-scale,  $F_{2,20} = 0.5047$ ,  $P = 0.6111$ ; Density,  $F_{1,20} = 18.3239$ ,  $P = 0.0004$ ; Site-scale  $\times$  Density,  $F_{2,20} = 0.8641$ ,  $P = 0.4366$ ; Soma mass,  $F_{1,20} = 0.3730$ ,  $P = 0.5482$ ; Site-scale  $\times$  Soma mass,  $F_{2,20} = 2.1708$ ,  $P = 0.1402$ ). Density did not affect soma mass (Whole-model  $R^2 = 0.2720$ ; Site-scale,  $F_{2,23} = 2.2720$ ,  $P = 0.1258$ ; Density,  $F_{1,23} = 1.5437$ ,  $P = 0.2266$ ; Site-scale  $\times$  Density,  $F_{2,23} = 0.1031$ ,  $P = 0.9024$ ; Fig. 3d). Log ovary mass decreased with density and increased with body size (Whole-model  $R^2 = 0.6811$ ; Site-scale,  $F_{2,20} = 9.9401$ ,  $P = 0.0010$ ; Density,  $F_{1,20} = 7.2118$ ,  $P = 0.0142$ ; Site-scale  $\times$  Density,  $F_{2,20} = 1.3290$ ,  $P = 0.2871$ ; Log soma mass,  $F_{1,20} = 10.1067$ ,  $P = 0.0047$ ; Site-scale  $\times$  Log soma mass,  $F_{2,20} = 1.5532$ ,  $P = 0.2360$ ), while log testes mass increased with body size but was unaffected by density (Whole-model  $R^2 = 0.5500$ ; Site-scale,  $F_{2,20} = 7.0767$ ,  $P = 0.0047$ ; Density,  $F_{1,20} = 0.2739$ ,  $P = 0.6064$ ; Site-scale  $\times$  Density,  $F_{2,20} = 0.6209$ ,  $P = 0.5475$ ; Log soma mass,  $F_{1,20} = 6.8278$ ,  $P = 0.0167$ ; Site-scale  $\times$  Log soma mass,  $F_{2,20} = 0.8161$ ,  $P = 0.4564$ ). Differences in Site-scale effects were caused by lower adjusted means for Among-site (large-scale) gonadal tissue masses (Tukey's HSD,  $P < 0.05$ ).

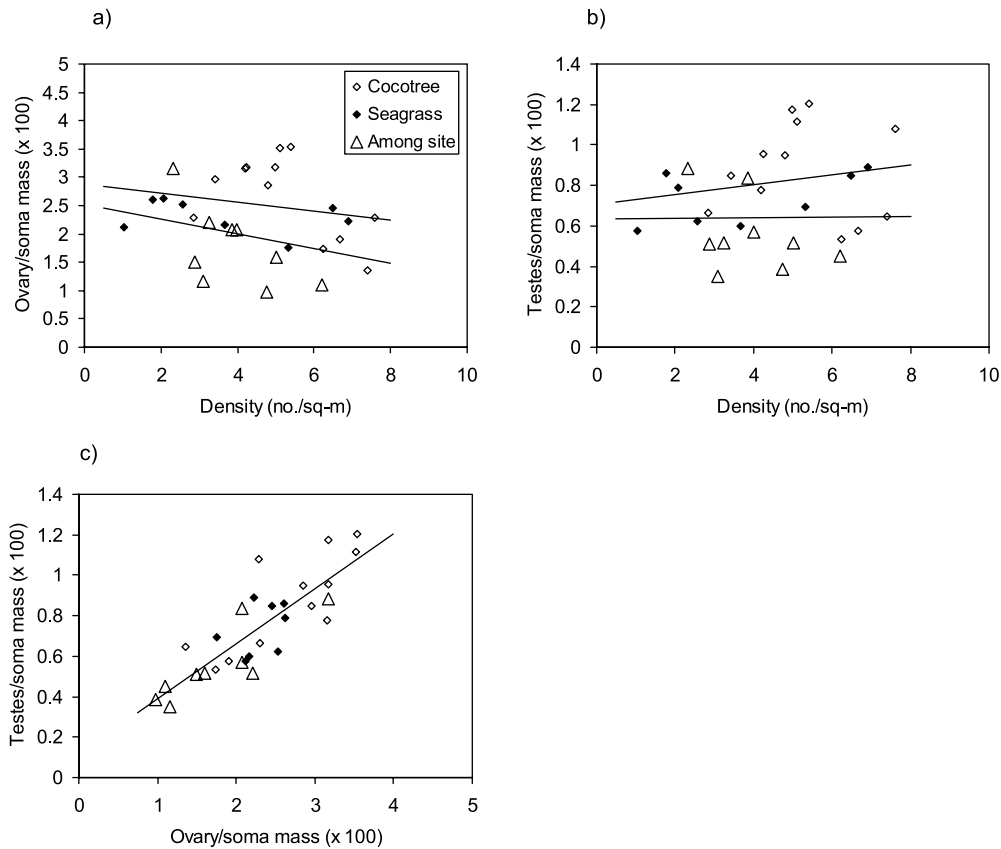
Ovary mass and testes mass were divided by soma mass for a more simplified analysis of changes in relative gonadal investments across densities. Ovary/soma mass showed



**Fig. 3.** (a) Relationship between density and pair spread. Model trends are depicted by lines for Seagrass and Among-site combined (upper trend line) and Cocotree alone (lower trend line) because Cocotree had a significantly different slope and adjusted mean from the other groups (Tukey's HSD,  $P < 0.05$ ). (b) Relationship between density and number of streakers per spawn. (c) Relationship between density and male allocation. (d) Relationship between density and body size. Trends for (b), (c), and (d) are depicted with a line through data from all groups (i.e. Among-site, Seagrass, and Cocotree).

a significant reduction with increasing density (Whole-model  $R^2 = 0.5129$ ; Site-scale,  $F_{2,23} = 11.3057$ ,  $P = 0.0004$ ; Density,  $F_{1,23} = 9.2906$ ,  $P = 0.0057$ ; Site-scale  $\times$  Density,  $F_{2,23} = 1.8657$ ,  $P = 0.1775$ ; Fig. 4a), while testes/soma mass remained unchanged (Whole-model  $R^2 = 0.3899$ ; Site-scale,  $F_{2,23} = 6.5922$ ,  $P = 0.0055$ ; Density,  $F_{1,23} = 0.3424$ ,  $P = 0.5641$ ; Site-scale  $\times$  Density,  $F_{2,23} = 0.9196$ ,  $P = 0.4128$ ; Fig. 4b). The relationship between ovary/soma mass and testes/soma mass was strongly significant and positive and this relationship was consistent across sites and scale (log-transformed data: Whole-model  $R^2 = 0.7311$ ; Site-scale,  $F_{2,23} = 1.0213$ ,  $P = 0.3759$ ; Log ovary/soma mass,  $F_{1,23} = 11.5761$ ,  $P = 0.0024$ ; Site-scale  $\times$  Log ovary/soma mass,  $F_{2,23} = 0.1693$ ,  $P = 0.8453$ ; Fig. 4c). The relationship between residuals for log ovary mass and log testes mass against log soma mass did not deviate significantly from isometry (model II regression across log-transformed data points: slope = 0.94, confidence interval = 0.73, 1.21).





**Fig. 4.** (a) Relationships between density and ovary/soma mass. (b) Relationships between density and testes/soma mass. (c) Relationships between ovary/soma mass and testes/soma mass. For (a) and (b), model trends are depicted by lines for Seagrass and Cocotree combined (upper trend line) and Seagrass and Among-site combined (lower trend line) because Cocotree had a significantly higher adjusted mean than Among-site, but the adjusted mean for Seagrass was not different from the other groups (Tukey's HSD,  $P < 0.05$ ). For (c), trends are depicted with a line through data from all groups (i.e. Among-site, Seagrass, and Cocotree) because density showed the only significant effect.

## DISCUSSION

We have used a much-needed comparative approach to show positive and consistent relationships between density, sperm competition, and sex allocation in a simultaneously hermaphroditic fish. Past research on related seabasses (*Serranus* spp. and *Hypoplectrus* spp.) suggested density-related variation in both intraspecific and interspecific mating patterns (Fischer and Petersen, 1987; Petersen, 1990a, 1990b, 1991) and implied that these species provided excellent model systems for further investigation of density effects on sex allocation and mating strategy. Recent experimental manipulations with simultaneously hermaphroditic invertebrates demonstrated greater male allocation with larger mating group sizes and explored trade-offs in relative gonadal investments across treatments (Raimondi and Martin, 1991; Trouvé *et al.*, 1999; Schärer and Ladurner, 2003; Tan *et al.*, 2004; Lorenzi *et al.*, 2005; but see Koene *et al.*, 2006). Such

experiments greatly advanced our progress in predicting how sex allocation responds to changes in density in a controlled environment. However, we lack a general knowledge of the range of intraspecific variation and the key ecological parameters that affect sex-allocation patterns in nature. By utilizing natural variation (i.e. a density gradient), we demonstrated intraspecific trends in sex allocation that suggest adaptive plasticity in response to local density. By including patterns at two spatial scales, we have also shown that density has predictive value regardless of the added environmental heterogeneity at the larger scale, at least among sites in this area of Panama.

The use of natural density gradients across multiple scales or locations provides the most relevant comparative approach to understanding sex allocation and reproductive investment patterns in species with sperm competition. A study with a gonochoristic species, the promiscuous rodent *Peromyscus maniculatus*, showed a positive relationship between testicular investment and density by utilizing temporal variation in population density (Long and Montgomerie, 2006). Comparisons across social systems at different densities have revealed a great deal of inter- and intraspecific variation in coral reef fish mating systems (Robertson and Warner, 1978; Warner and Robertson, 1978; Warner and Hoffman, 1980; Fischer and Petersen, 1987; Petersen, 1990a). In wrasse and parrotfish species with alternative male mating tactics, small 'initial' males were more abundant in larger social groups where large, territorial males had reduced mating success (Robertson and Warner, 1978; Warner and Robertson, 1978; Warner and Hoffman, 1980). In the small seabass, *Serranus psittacinus*, fish spawned in pairs at low density and formed harems that became increasingly complex at higher densities (Petersen, 1990a). Natural variation in density was recently used to compare sex ratios in a sequential, protogynous hermaphrodite (Allsop and West, 2004). In that study, the proportion of males in populations of the marine goby, *Coryphopterus personatus*, increased with increasing density among one set of study sites but not another, where density was higher overall. By exploring variation in natural patterns, we can better estimate the environmental parameters that are most relevant to life history and sexual selection.

We sought to establish that mating group density during spawning is a fair approximation of mating group size because theory predicts that sex allocation should respond to the actual number of competitors for matings (Charnov, 1982; Fischer, 1984b, Petersen, 1991). Furthermore, recent laboratory studies have indicated that mating group size may not necessarily be correlated with density (Schärer and Ladurner, 2003; Lorenzi *et al.*, 2005; Janicke and Schärer, 2009). However, these studies manipulated enclosure size under laboratory conditions while holding the number in the mating group constant, effectively altering density. In this study, we did not hold the mating group size constant and did not vary the amount of space occupied; this would have been virtually impossible under natural conditions. Rather, we included both the number of individuals and the amount of space occupied in our density measure. We were not able to assess actual mating group size in this study as number of individuals contributing to fertilization of each egg clutch *per se*. We reasoned that the strong negative relationship between pair spread and density and the strong positive relationship between streaking and density indicated that density served as a good proxy for conspecific mate competition in the chalk bass mating system. We chose to use density as the predictive variable, rather than pair spread or number of streakers per spawn, because density reflects multiple aspects of the social environment likely to influence sex allocation, such as proximity to alternative mates and potential streaking opportunities.

We found no evidence that body size interacted with density to influence sex allocation. A previous study with chalk bass did show an effect of size on male allocation within

another Panama population, about 400 km east of the present study area (Petersen and Fischer, 1996). However, fish for the previous study ( $n = 69$ ) were sampled from the same area on the collection reef, not from multiple aggregations (C.W. Petersen, personal communication). In our study design, variation among aggregations, coupled with strong density effects and relatively small sample sizes within each aggregation, may have obscured any local body size effects.

Chalk bass appeared, in general, to shift sex allocation by altering investment in ovarian and not testicular tissue. Several studies that manipulated densities have shown changes in investment patterns of one sex while the other remained unchanged. For instance, Lorenzi *et al.* (2005) found that *Ophryotrocha diadema*, a polychaete worm, reduced female investment with increased mating group size but did not alter sperm production. Male-oriented, aggressive behaviours increased, however, suggesting that increases in energetic resources to male function may be devoted to behaviour and not tissue (Lorenzi *et al.*, 2006). An opposite trend was found in *Macrostomum lignano*, a free-living flatworm, where increasing mating group size led to increased testicular volume but no change in ovarian volume or egg production (Schärer and Ladurner, 2003). By contrast, for the pond snail, *Lymnaea stagnalis*, greater mating frequency led to reduced prostate gland size and slower somatic growth coupled with higher egg production (Koene *et al.*, 2006). By examining sex-allocation trade-offs only in terms of investments in male and female reproductive tissue, we may be missing important shifts in life history or behavioural strategies.

A negative relationship between male and female gonadal investments can also indicate trade-offs in sex allocation and this can be detected through examining the relationship between residuals for male versus female investments corrected for body size (Petersen, 1990b; Schärer *et al.*, 2005; Baeza, 2007). By this metric, trade-offs in sex functions with increasing group size were not detected in flatworms, *Macrostomum lignano*, that were fed *ad libitum* (Schärer and Ladurner, 2003) and were only revealed when individuals were placed under specific experimental conditions that included a restricted food supply (Schärer *et al.*, 2005). But such trade-offs were also not found in a small fish and a shrimp species collected in the natural environment (Petersen, 1990b; Baeza, 2007) where food availability may not be unlimited. This method of analysis may not be appropriate for revealing how shifts in allocation occur in species that show strong positive allometric relationships, like the chalk bass, because body-size allometry can turn negative relationships that reflect trade-offs into positive ones. A contrast of male and female gonadal investments across environmental gradients (e.g. of density, resources, or predation risk), as we have done here, may be necessary to determine how gonadal allocation is adjusted and whether trade-offs exist with other life-history traits, namely survival and growth.

Density does appear to be shaping sex-allocation strategy in the chalk bass. Patterns of sex allocation across density follow trends predicted by theory (Fischer, 1981, 1984b; Charnov, 1982; Petersen, 1991) and empirical investigations specific to the chalk bass (Fischer, 1984b; Petersen, 1991; Petersen and Fischer, 1996). Nonetheless, the possibility exists that individual fish are choosing their own environs based on the sex-allocation strategy they prefer according to their condition or other requirements. However, pelagic spawning species like chalk bass typically spend 2–4 weeks floating in the plankton as eggs and then larvae, settling into a new area as juveniles (Leis and McCormick, 2002). While some larval fish have been shown to home in on reef acoustics to find settlement areas (Simpson *et al.*, 2005), it is unclear whether they would be able to choose a specialized environment without sampling sites across a large scale (multiple kilometres). Given that small fish are generally at high risk of predation and adults are extremely site-attached (Fischer, 1984a; M.K. Hart, personal observation), juvenile chalk bass are highly

unlikely to travel among reefs or for significant distances within reefs once they settle to the benthos. Thus it is probable that chalk bass individuals are constrained by the environment in which they initially settle and survive as a juvenile and then grow to adulthood.

The chalk bass model system provides an excellent opportunity to integrate life history and sexual conflict into sex-allocation theory. Experimental manipulations, such as transplants, should be performed to test how flexible sex allocation is for chalk bass and whether sex allocation responds to factors other than density. Comparisons of fecundity, sperm production, survival, and growth across study sites and experimental treatments will aid in revealing the costs and trade-offs involved in sex-allocation strategy. In addition, continued research should aim to identify whether individual behaviours, such as pairing and reciprocity in egg trading, can influence sex-allocation patterns.

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