

Phenotypic plasticity in sex allocation for a simultaneously hermaphroditic coral reef fish

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Abstract Phenotypic plasticity can facilitate reproductive strategies that maximize mating success in variable environments and lead to differences in sex allocation among populations. For simultaneous hermaphrodites with sperm competition, including *Serranus tortugarum* a small coral reef fish, proportional male allocation (testis in total gonad) is often greater where local density or mating group size is higher. We tested whether *S. tortugarum* reduced male allocation when transplanted from a higher density site to a lower density site. After 4 months, transplants mirrored the sex-allocation patterns of the resident population on their new reef. Transplants had significantly lower male allocation than representatives from their source population, largely as a result of reduced testis mass relative to body size.

Keywords Mating systems · Sperm competition · Density · Body size · Transplant experiment · Serranidae

Introduction

Environmental variation is a major factor contributing to the diversity of mating systems found within and among

species (Emlen and Oring 1977). Environmental heterogeneity can result in differences in mating opportunities and mate competition among populations, leading to different selective pressures that likely underlie variation in mating systems within and among populations (Warner 1984a, b, 1991, 1997). Phenotypic plasticity may play an important role in allowing individuals to optimize their fitness or reproductive success when encountering different social environments (West-Eberhard 1989, 2003). Phenotypic plasticity in sexual traits should be favored when the mating success gained from alternative mating tactics is variable across different environments, but individuals are not able to predict which environment they might experience (Taborsky 1998; Brockmann and Taborsky 2008).

Simultaneously hermaphroditic species provide excellent models for investigating plasticity in sex allocation and mating strategy because predictions about responses to changes in environment can be derived from theory. Simultaneous hermaphrodites are expected to adjust their behavior, reproductive anatomy, and energetic allocation in male and in female function according to available mating opportunities (Charnov 1979, 1982; Fischer 1981, 1984a; Petersen 1991). Individuals mating at low density should only produce enough sperm to fertilize the eggs of their partners and invest the remainder of their reproductive budget into female function (Charnov et al. 1976; Fischer 1981). However, when individuals face sperm competition with other male-role individuals, an increased investment in male function is expected (Charnov 1979, 1982; Fischer 1984a; Petersen 1991; Schärer 2009; Hart et al. 2010, Hart et al. in press).

Both field and laboratory experiments with simultaneously hermaphroditic invertebrates have demonstrated adjustments to sex allocation in response to altered mating

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group size and male-role mating opportunities (see review by Schärer 2009). The barnacle, *Catomerus polymerus*, the flatworm, *Macrostomum* sp., and the polychaete, *Ophryotrocha diadema*, all showed an increase in male allocation (i.e., male function relative to total reproductive investment) with increased sperm competition in larger experimental groups (Raimondi and Martin 1991; Schärer and Ladurner 2003; Lorenzi et al. 2005). An additional experiment with *Macrostomum lignano* revealed highly flexible sex allocation in adults (Brauer et al. 2007). Adjustments to proportional male allocation were effected through shifts in fecundity, sperm production, or both (Raimondi and Martin 1991; Schärer and Ladurner 2003; Lorenzi et al. 2005; Brauer et al. 2007). For this reason, comparisons of sex allocation across treatments should include not only proportional male allocation (the amount of testis or sperm produced divided by the total gonad), but also absolute measures of male and female function (Schärer 2009). A clear response to an increase in sperm competition should be indicated by a shift in sperm production, testis volume or mass, which may show a trade-off with female fecundity.

Multiple lines of evidence suggest that phenotypic plasticity in simultaneous hermaphrodites is adaptive, variable in form, and highly responsive to competition for male-role mating opportunities (Crean and Marshall 2008; Hoch 2009; Janicke and Schärer 2010). Yet, there is a general lack of field data on plasticity in reproductive traits across variable densities (Schärer 2009; but see Raimondi and Martin 1991; Crean and Marshall 2008), particularly for fishes.

For coral reef fish, population density is a key environmental factor that influences mating group size, mating opportunities, and sperm competition (Petersen 1990; Petersen and Warner 1998), and many hermaphroditic fish species have a more male-biased sex ratio or sex allocation at higher densities (Robertson and Warner 1978; Warner and Robertson 1978; Warner and Hoffman 1980; Allsop and West 2004; Hart et al. 2010).

In a previous study with the simultaneous hermaphrodite *Serranus tortugarum*, we illustrated that sperm competition and male allocation increase predictably with population density (Hart et al. 2010). Here, we test whether sex allocation is plastic in response to sperm competition, by transplanting fish from a high-density site to low-density site. We expected that if sex allocation is phenotypically plastic then proportional male allocation (testis in the total gonad) would be reduced in the transplants because of the reduction in sperm competition at the lower density study site. We also expected a reduction in male function (testis mass) for the transplants when size-related differences were controlled.

Materials and methods

Study species

The chalk bass, *Serranus tortugarum* (Serranidae: Serraninae), is planktivorous with pelagic eggs and larvae. They have a 1- to 1.5-year lifespan, are highly site-attached in adulthood, and found in localized aggregations of 20–500+ individuals over coral rubble and reef flats of the Caribbean and south Florida littoral zone (Fischer 1984b). Chalk bass spawn daily with mating partners of similar size in the 2 h before sunset. 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, 1984b). In addition, chalk bass use an alternative male-role tactic, termed "streaking" (Warner et al. 1975), to intrude upon neighboring pairs as they are spawning and release sperm. Streaking is frequently used by all individuals, even when paired, to gain extra male-role fertilizations (Fischer 1984b). The ovarian and testicular tissue in the chalk bass gonad can be cleanly separated, dried, and weighed to use as proxies for energetic allocation to each gender function (following Petersen 1991; Petersen and Fischer 1996; Hart et al. 2010).

Research location

This study was conducted at Smithsonian Tropical Research Institute's Bocas Research Station in Panama in 2006–2007. Study sites were located in the Bay of Almirante and separated by ca. 7 km and a deep-water channel. Each site occupied 1–2 hectares of continuous reef along mangrove-edged islands with depths ranging from 4–12 m. Because of logistical constraints, we were able to include only two sites that were selected based on statistically significant differences in density, sperm competition, and male allocation detected in a previous study (Hart et al. 2010; see Table 1).

Study design

The transplant experiment involved removing and marking fish from a high-density site, Cocotree (Ct), transplanting them to a low-density site, Solarte (Sol), and resampling marked transplants and unmarked fish from the two study sites after a four-month period. Approximately 400 live fish were captured from the Cocotree population and transplanted to Solarte over a 2-week period in October–November 2006. On each day of transplanting, fish were collected from Cocotree in the morning, anesthetized using a clove oil solution, and marked on each side with a small

Table 1 Significant differences between study sites in 2005 (n = sample size; Hart et al. 2010)

Reef study site	Density (no./m ²)	No. streakers/spawn	Male allocation (% testes/total gonad)
Solarte (n = 47)	3.1	1.5	22%
Cocotree (n = 53)	6.2	3.2	29%

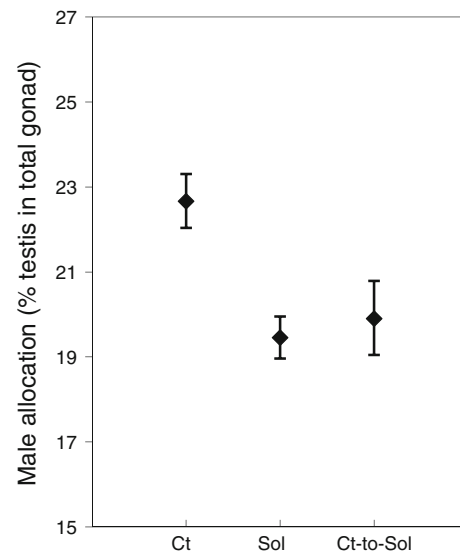
red dot of permanent nontoxic elastomer dye (Northwest Marine Technologies) injected under the skin. Fish were allowed to recover from anesthesia, transported from Cocotree to Solarte, and released underwater at different areas within the study site. Density at Solarte was not obviously increased by addition of the 400 fish from Cocotree. Surviving transplants were collected in March 2007 (n = 29) along with surrounding natural residents (n = 89) from Solarte. Fish were also subsampled from Cocotree during the same two-week time period in which the transplants were collected (n = 71). Fish collections did not noticeably reduce density at the sites.

Statistical analysis

Initially, we measured differences in soma mass (total dry body weight with the gonad removed) and male allocation (proportion testis in total gonad) among treatments (N = 3; Cocotree (Ct) population, Solarte (Sol) population, Transplants (Ct-to-Sol)) using *ANOVA*. We then statistically controlled for body size using a linear model with soma mass as a covariate to measure differences among treatments in proportional male allocation, testis mass, and ovary mass. We compared adjusted means among the treatments (Ct, Sol, and Ct-to-Sol) using Student–Newman–Keuls procedure (α = 0.05). Lastly, we conducted a post hoc comparison of the largest 30 fish sampled from Cocotree (approx. one-third of total fish sampled) with the 29 transplants, using a *t*-test. All variables were log-transformed, and statistical analyses were performed using JMP[®] 8.0.1. Residuals of dependent variables were examined for normality using probability plots and homogeneity of variances using Levene’s test, and the data met assumptions for parametric statistics.

Results and discussion

After 4 months, the proportional male allocation of transplants mirrored that of residents at their new reef (Fig. 1; $F_{2,185}$ = 8.64, P = 0.0003). However, average body size was higher for the Solarte populations (mean \pm SE: 294.1 \pm 7.7 mg, dry wt.) and the transplants (mean \pm SE: 285.3 \pm 13.5 mg, dry wt.) than for the Cocotree population (mean \pm SE: 217.0 \pm 8.6 mg, dry wt.;

**Fig. 1** Differences in proportional male allocation among the treatments (means \pm SE)

$F_{2,185}$ = 24.63, P < 0.0001). We did not anticipate the increase in body size of the transplants or the differences between body size in Cocotree and Solarte populations, because in 2005, no significant differences in size were found between Ct (mean \pm SE: 182.4 \pm 8.0 mg, dry wt.) and Sol populations (mean \pm SE: 200.4 \pm 11.4 mg, dry wt.; Hart et al. 2010).

Because body size has a positive influence on gonad size, we cannot exclude the possibility that, along with local density, larger body size and/or higher growth rate could have contributed to the transplants’ shift in sex allocation. This could result from indirect resource-related effects (i.e., budget effects, see Klinkhamer et al. 1997) that may be most evident when contrasted among populations, especially when the within-population relationship between size and sex allocation is weak (see Fig. 2a). However, male allocation was also significantly lower at Solarte than Cocotree in a previous year, when body sizes were similar among sites (Hart et al. 2010; see Table 1).

Another alternative explanation for our findings is that size-selective mortality led to survival of only the larger transplants, and this selectivity was somehow associated with lower proportional male allocation in the surviving transplants. However, body size and male allocation appear to be weakly related within these study populations (Hart

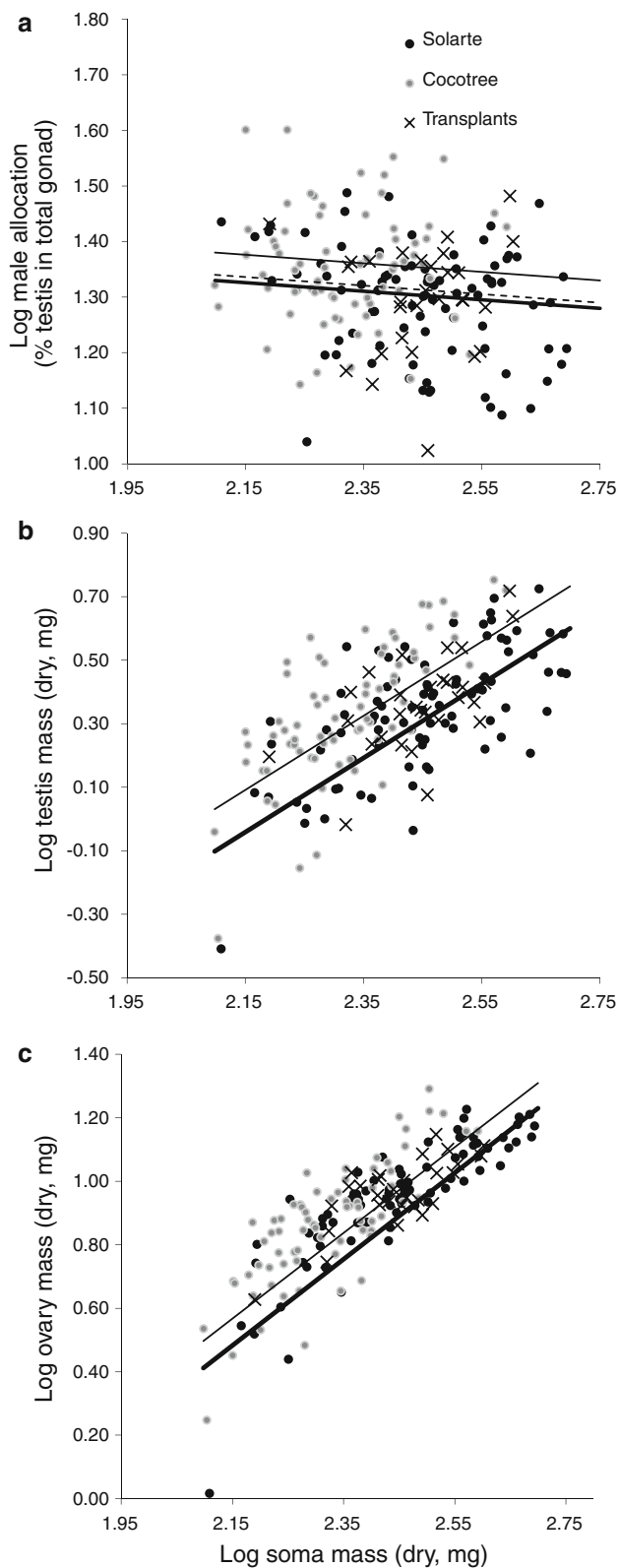


Fig. 2 Allometric relationships between body size and **a** male allocation, **b** testis mass, and **c** ovary mass. Trends from the general linear model (Table 2, reduced model) overlay the log-transformed data points. Cocotree trends are depicted by the upper solid line in each graph (not bold); in **a** Transplants are depicted with a dashed line and Solarte with bold; in **b** and **c** trends for Solarte and Transplants are depicted by the same bold line as these adjusted means are not significantly different

size range at Cocotree, and at the end of the experiment, transplants spanned the size range of resident fish collected at Solarte (see Fig. 2). This suggests that transplant survival was unrelated to body size and that higher growth rate led to larger body size at Solarte.

Our general linear model revealed a significant effect of the transplant treatment on proportional male allocation that was independent of body size (Table 2a; Fig. 2a). The adjusted mean (least squares) for male allocation was significantly higher for Cocotree than Solarte. The adjusted mean for the transplants was not significantly different from either Ct or Sol, though Solarte and the Transplants adjusted means were much more similar to each other than to the adjusted mean for Cocotree (see Fig. 2a). Body size had a significant positive effect on testis and ovary masses, and testis and ovary masses differed significantly between treatments (Table 2b, c; Fig. 2b, c). Adjusted means for both testis and ovary masses were significantly lower for the Solarte and the Transplants than for the Cocotree, and the magnitude of this difference was greatest for testis mass (compare Fig. 2b, c). Interactions between size and treatment were not significant for any tests ($P > 0.05$); however, the P -value for the testis mass response was <0.1 and

Table 2 Results from general linear model testing treatment ($N = 3$) and size effects for Cocotree (Ct; high-density population), Solarte (Sol; low-density population), and Transplants (Ct-to-Sol; high-to-low-density)

Source	DF	SS	F ratio	P -value
A. Log male allocation (% testis/total gonad)				
Treatment	2	0.0927	4.4574	0.0129
Soma mass	1	0.0261	2.5082	0.1150
Error	182	1.8922		
B. Log testis mass (dry, mg)				
Treatment	2	0.6078	14.1955	<0.0001
Soma mass	1	3.1081	145.1768	<0.0001
Error	182	3.8964		
C. Log ovary mass (dry, mg)				
Treatment	2	0.1500	6.7869	0.0014
Soma mass	1	3.8734	350.5554	<0.0001
Error	182	2.0120		

Bold font indicates statistically significant effects

et al. 2010; and see Fig. 2a), and we have no reason to suspect that larger transplants would have higher survival. Transplants were originally collected from the entire adult

Table 3 Means \pm standard errors for the largest fish sampled from the Cocotree population in 2007 ($n = 30$) and the Transplants ($n = 29$). Soma, ovary, and testis masses are in mg dry wt; male allocation refers to % testis in total gonad

Study site	Soma mass	Ovary mass	Testis mass ^a	Male allocation ^a
Cocotree	270.61 \pm 7.79	10.61 \pm 0.65	3.26 \pm 0.20	23.77 \pm 0.98
Transplants	285.30 \pm 10.41	9.50 \pm 0.39	2.45 \pm 0.16	20.38 \pm 0.79

^a Denotes variables with significant differences

suggestive of small differences in reproductive investment with size among populations.

In the final post hoc comparison, the largest 30 fish from Cocotree showed no significant differences from the 29 transplants in body size ($P = 0.36$) and ovary mass ($P = 0.26$). However, they had significantly greater testis mass ($P = 0.003$) and proportional male allocation ($P = 0.01$) than the transplants (Table 3). Though biased in sampling design, this statistical comparison suggests that any slight decrease in reproductive effort with size in the transplants resulted primarily in a reduction in male function, and not in female function, as would be expected in a new environment with lower sperm competition.

Density can influence sexual selection and the social environment and lead to variation in growth and reproductive investment patterns as has been illustrated in several protogynous coral reef fishes (Warner 1984b; Munday et al. 2006; Walker and McCormick 2009; McCormick et al. 2010). For instance, in the bluehead wrasse *Thalassoma bifasciatum*, reproductive success among competing males is more evenly spread on large, high-density reefs than on small reefs, where only a few large, dominant males gained most of the matings with females (Warner 1984b). On small reefs, males were more likely to defer reproduction to gain larger body size, and reciprocal transplants revealed flexible growth and reproductive investment patterns in response to different reef sizes (Warner 1984b). In the simultaneously hermaphroditic polychaete *Ophryotrocha diadema*, individuals that began reproducing as males before reaching full maturity as a simultaneous hermaphrodite had slower growth rates (Sella and Lorenzi 2003). In addition, hermaphrodites that mated in pairs rather than large groups had higher egg production and fewer aggressive behaviors among conspecifics (Lorenzi et al. 2005, 2006). It is conceivable that for chalk bass at lower density, where mating pairs can avoid heavy sperm competition, greater reproductive success may be gained from investing in absolute body size, which increases body-cavity space to enable greater ovary growth and egg production (often referred to as fecundity selection; Head 1995).

We contrasted populations based on differences in local density and sperm competition and provide preliminary evidence of phenotypic plasticity in sex allocation for *S. tortugarum* in relation to these factors. To our

knowledge, this is the first study to test whether adult simultaneously hermaphroditic fish from one population will shift sex allocation when introduced into another. We cannot exclude effects on sex allocation of unmeasured environmental factors, such as resource availability (Vizoso and Schärer 2007) and predation risk (Hart et al. in press). Nonetheless, the chalk bass mating system shows potential as an important model for investigating environmental effects on sex allocation and sexual selection in hermaphrodites. Because of logistical constraints, we did not mark residents as controls at the time of transplant or perform reciprocal, replicated transplants among the study sites. We also had high mortality in transplanted fish, with only a relatively small proportion of transplanted fish recaptured. Future work will seek to improve survival of transplants and to perform reciprocal transplants at a larger scale to reveal any constraints to flexible sex allocation for this species. A better understanding of how reproductive strategies respond to variation in sperm competition across environments will give greater insight into the evolutionary processes contributing to the diversity of coral reef fish mating systems found in nature.

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