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ORIGINAL PAPER

Phenotypic plasticity in sex allocation and body size leads to trade-offs between male function and growth in a simultaneously hermaphroditic fish

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Abstract Phenotypic plasticity in sex allocation enables organisms to maximize reproductive success in variable environments, and thus may generate different sex allocation patterns among populations that experience different mating opportunities. In this experiment, I test whether sex allocation is phenotypically plastic in *Serranus tortugarum*, a simultaneously hermaphroditic fish, by using reciprocal transplants among four reef study sites with populations at high and low densities and significant differences in sex allocation. Fish transplanted across different densities were predicted to alter sex allocation and body size through trade-offs in investments to somatic growth and male and/or female reproduction. As a control for effects of transplanting, I also transplanted fish across study sites with the same densities and marked and returned fish to their original study sites. As predicted, sex allocation and body size shifted significantly for fish transplanted across different densities but not for those transplanted across the same densities. Separate analyses revealed that the treatment effect on sex allocation was driven strongly by a reduction in male investment by fish transplanted from high to low density, and this reduction in male investment was accompanied by an increase in body size. Fish transplanted from low to high density did not appear to change either male or female investments, but they were smaller than transplants from low to low density. A trade-off between male and female function was not evident, but phenotypic plasticity in body size suggested a trade-off between growth and male function when sex allocation is adjusted. Large-scale empirical tests of sex allocation in the field are relatively rare, and the results of this experiment give novel insights into how animals respond to a change in mating opportunities under natural conditions. The effects of logistical problems associated with fieldwork, such as mortality of experimental animals, are considered in the discussion.

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Introduction

Phenotypic plasticity allows an organism to maximize fitness in a variety of social settings, and differences in reproductive traits among populations can reveal how mating systems are shaped by the environment. In addition, natural variation in mating opportunities can provide a background for experiments that improve our understanding of how individuals adjust reproductive and other life-history traits to maximize reproductive success (Warner 1991, 1997; Piersma and Drent 2003; Crean and Marshall 2008; Hoch 2009; Hart et al. 2010, 2011a). Many simultaneous hermaphrodites move through different environments in their lifetimes or experience changes in environmental conditions. For such animals, phenotypic plasticity may be advantageous for adapting to new locations (West-Eberhard 1989; Piersma and Drent 2003) and may result in different sex-allocation patterns among populations (see Schärer 2009). Sex-allocation theory predicts that simultaneous hermaphrodites should invest proportionally greater reproductive resources in male function where mating opportunities and competition for fertilizations are higher (Charnov 1979, 1982; Fischer 1981, 1984a; Petersen 1991). Implicit in this argument is the assumption of a trade-off between male and female function when limited resources are available to devote to reproduction (Charnov et al. 1976, Charnov 1979, 1982; Schärer 2009). That is, theory assumes that under most natural conditions investment in one sex function will necessarily result in a decrease in the other. However, it is not always clear whether this trade-off applies in situations where sex allocation changes with social conditions and mating opportunities.

Empirical evidence from simultaneously hermaphroditic invertebrates suggests that sex allocation is plastic and, generally becomes more male-biased with greater mate competition (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). Evidence for the assumed trade-off in male and female function as sex allocation changes is mixed (see Schärer 2009 for recent review). For example, in an experiment with *Macrostomum lignano*, a simultaneously hermaphroditic flatworm, reciprocal transplants across small and large mating groups demonstrated facultative adjustments to sex allocation (Brauer et al. 2007). However, in larger groups, this species increased sperm production and testis volume without altering ovary size (Schärer and Ladurner 2003; Janicke et al. 2013; but see Schärer et al. 2005) suggesting that changes in reproductive investment in male function drove the shift in sex allocation. In contrast, the simultaneously hermaphroditic polychaete *Ophryotrocha diadema* did not alter sperm production, but drastically reduced egg production as experimental mating group size increased (Lorenzi et al. 2005, 2006; Schleicherová et al. 2014), suggesting that changes in female investment drove the shift in sex allocation for this species. In addition, empirical evidence shows that in *O. diadema*, increased male investment during the adolescent protandrous stage is linked with a reduction in body growth and lifespan (Sella and Lorenzi 2003; Lorenzi et al. 2006). These mixed results suggest that phenotypic plasticity in sex allocation may occur without trade-offs between male and female investment. Furthermore, other life history trade-offs, such as the trade-

off between somatic and reproductive investments, should be considered to fully understand how individuals maximize reproductive fitness in different mating environments.

Certain difficulties exist for determining trade-offs in mating systems of simultaneous hermaphrodite. For one, selection may not always be acting independently on life history traits such as body size, growth rate, or male and female function (Yund et al. 1997), and positive correlations among such traits can obscure trade-offs (Zhang and Jiang 2002). Secondly, our measures of sex allocation (e.g., dry testis and ovary mass, testis and ovary area, number of eggs, number of sperm; see Schärer 2009 for summary of measures) do not always estimate the actual energetic investment contained in those traits with complete accuracy. They also typically do not include energetic investment in behaviors that contribute to success in each sex role (but see Lorenzi et al. 2006). Thirdly, trade-offs are expected to be found when an animal's total resource budget is limited (Charnov 1982). In cases where food resources are ad libitum, a "budget effect" (see Klinkhammer et al. 1997; Cadet et al. 2004, Vizoso and Schärer 2007) may conceal certain trade-offs that would be evident under resource limitation (see Schärer et al. 2005). Nevertheless, the degree of plasticity found in simultaneously hermaphroditic mating systems and the varied ways that different species adjust their sex allocation suggests that these study models can provide important information about reproductive investment decisions and life history trade-offs across different environments.

In this experiment, I evaluate the effect of changes in local spawning density on a species that shows considerable behavioral plasticity in its reproductive system, the simultaneously hermaphroditic chalk bass (*Serranus tortugarum*) (Hart et al. 2010, 2011a). In the chalk bass system, local density during spawning is closely correlated with both the availability of extra-pair male-role mating opportunities and the number of male-role mate competitors on pair spawns (Hart et al. 2010). Individuals spawn primarily in pairs but also use an alternative male-role mating tactic to intrude on the spawns of neighboring pairs and release sperm, a behavior called "streaking" (Warner et al. 1975; Fischer 1984b). Spawning events occur very rapidly (<1 s), and the rapid timing of pair spawns restricts the opportunity to streak successfully. Therefore, streaking is more likely to be successful when a streaker is closer to a pair that is spawning. Indeed, where spawning densities are higher, conspecifics have closer proximity to neighbors, more streaking occurs, and sex allocation is more male-biased than at lower spawning densities (Hart et al. 2010).

The Hart et al. (2010) study exploited natural variation and used regression to compare the effects of spawning density on streaking and sex allocation at 2 scales: within reef study sites spanning 1 ha of continuous reef and among reef study sites spread kilometers apart. The study showed that sex allocation (measured as proportion testis in total gonad) became more male-biased at higher densities as a result of a reduction in female investment (ovary mass/soma mass). Neither male investment (testis mass/soma mass) nor body size changed with density in this comparative study. In contrast, a recent partial transplant experiment showed that fish moved from a high to a low density reef study site decreased sex allocation as a result of a reduction in male investment (Hart et al. 2011a). However, in that study, fish at the low density reef were larger on average and had lower investments in both male and female function than fish from the high density reef. The reduction in sex allocation resulted because of a greater reduction in male investment than female investment. Combined these two studies provide evidence that sex allocation responds to the social environment and that adjustment may involve changes in both somatic and reproductive investments.

To test the hypotheses that sex allocation is phenotypically plastic and adjusted by trade-offs in reproductive and somatic investments, I use reciprocal transplants among two

high and two low density study sites with populations that are known to differ significantly in sex allocation (Hart et al. 2010, 2011a). If sex allocation is phenotypically plastic and responds to local density, then fish will alter sex allocation when transplanted across different densities but not when transplanted between sites with the same density. Body size varies among the study sites, giving opportunity to examine trade-offs between somatic and reproductive investments in this mating system. To account for variation within and among populations, I statistically control for body size (soma mass) when measuring changes in sex allocation. If sex allocation is phenotypically plastic, it will increase for fish transplanted from low to high density and decrease for fish transplanted from high to low density. Further, if the assumed trade-off between investments in male and female function holds, an increase in male investment will be associated with a decrease in female investment for fish transplanted from low to high density, and vice versa for fish transplanted from high to low density. Trade-offs between somatic and reproductive investments will be demonstrated if fish transplanted from low to high density reduce growth and increase male and/or female investment and if fish transplanted from high to low density increase growth and reduce male and/or female investment.

Materials and methods

Study system and experimental design

The zooplanktivorous chalk bass are highly site-attached in adulthood and live in discrete aggregations of 20–500 individuals that feed and spawn in the same area (Fischer 1984b). Mark-resighting studies have shown that individuals are typically found within 5 m of their original location (Hart M.K., unpublished data). Chalk bass mate daily in size-assortative pairings, releasing gametes in rapid upward spawns in which each partner releases either eggs or sperm with alternation of gender roles (i.e., egg parceling) (Fischer 1980, 1984b). Streaking occurs when an individual rushes over to a neighboring pair that is spawning and releases sperm (Warner et al. 1975). All individuals may streak on other pairs, although each typically has one mating partner for pair spawning. Streaking is not related to body size for the individuals that streak or those that are streaked upon (Fischer 1984b; Petersen and Fischer 1996).

Field research was conducted at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station, on Isla Colon in Panama where chalk bass are common on sheltered, laguna reefs of coral rubble, at depths of 3–20 m. Four reef study sites (2 at each density, High and Low) were selected from among nine sites near Bocas Research Station where local density, proximity to neighbors while spawning, streaking, and sex allocation (proportion male in total gonad) had previously been measured and were known to have significant positive relationships (Hart et al. 2010). The nine reef study sites were established in 2005–2006 (Hart et al. 2010, see Fig. 1 map therein) and estimates during 2007–2008 suggest that local densities and size distributions are fairly stable at the sites (Hart M.K., unpublished data). Each reef study site spanned approximately 1 ha of continuous reef and the sites were chosen because they contained high abundances of chalk bass, albeit at different local densities. The sites were well-separated (3–5 km part), and it is highly unlikely that adult chalk bass ever traverse the distance between them. Higher density sites had aggregations that numbered from 150 to 500+ individuals, each spread over an area ≥ 25 m^2, and lower density sites had aggregations that numbered from 20 to 175 spread over approximately 16–25 m^2. Local density estimates were made by repeated

counts during the spawning period at multiple discrete aggregations (usually 3–6) from each reef study site. Aggregations were site-attached and locations were marked and re-visited to account for variable conditions; local spawning density estimates were made for each aggregation by repeatedly counting the number of fish within a defined area of 4–25 m^2 over the course of a spawning period for multiple days, weeks, or months.

In this study system, local spawning density appears to represent a fair proxy of mating group size, and sex allocation patterns suggest a response to the change in mating group size across densities (Hart et al. 2010, 2011a). Counts over multiple aggregations and days were averaged for a spawning density estimate for each reef study site during 2005–2006 for Hart et al. (2010) and are reported herein for the four study sites chosen for this experiment, along with sex allocation measures from that study: the two low density study sites had spawning densities of mean $\pm$ SE = 3.1 $\pm$ 0.4 m^2 for Solarte (LD1) and mean $\pm$ SE = 3.3 $\pm$ 0.5 m^2 for Andy's Reef (LD2), and the two high density study sites had spawning densities of mean $\pm$ SE = 6.2 $\pm$ 1.3 m^2 for Cocotree (HD1) and mean $\pm$ SE = 3.8 $\pm$ 0.4 m^2 for San Cristobal Mangrove (HD2). For the low density study sites, sex allocation was mean $\pm$ SE = 0.23 $\pm$ 0.03 for LD1 and mean $\pm$ SE = 0.20 $\pm$ 0.03 for LD2. For the high density study sites, sex allocation was mean $\pm$ SE = 0.29 $\pm$ 0.04 for HD1 and mean $\pm$ SE = 0.29 $\pm$ 0.05 for HD2.

To test for phenotypic plasticity in sex allocation, I used replicated (n = 2), reciprocal transplants among the 2 high density and 2 low density reef study sites using three treatments (return home, same density transplant, and different density transplant). There were 4 reciprocal transplants: 2 among reefs of the same density and 2 among reefs of different densities (see Fig. 1). For each reciprocal transplant, 150–200 fish from each reef study site were collected, marked, and transplanted across the reefs to either the same or different density treatment (Fig. 1). To avoid altering density with the manipulation, I collected approximately the same number of fish from each of two origin reefs to mark and reciprocally transplant to their destination reefs on the next day. Each reciprocal transplant took 2–4 days of collecting, marking, and transplanting fish, which included marking and returning additional fish to their home reef for the return home treatment (67–135 per reef, see Fig. 1).
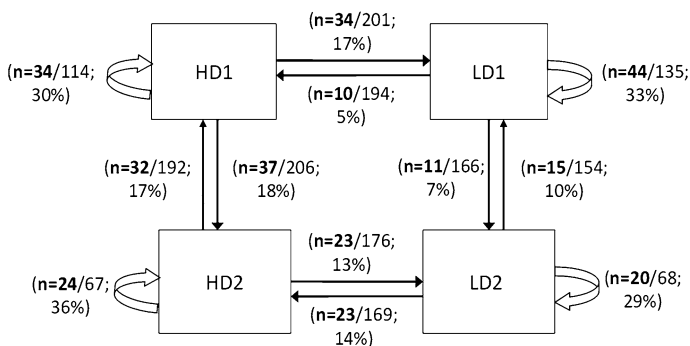

Fig. 1 Experimental design. *Boxes* represent the two high density (HD1 and HD2) and the two low density (LD1 and LD2) reef study sites. *Arrows in black* indicate the direction of reciprocal transplant treatments across the reef sites. *Open arrows* indicate return home treatments in which fish were marked and replaced in their original populations. Sample sizes (n) for fish collected after 4–5 months are noted *parenthetically* and in *bold* next to each arrow. Following the sample size and *backslash* is the number of fish originally collected, marked and transplanted; the percentage of those recovered at the end of the experiment is shown *below*

Fish were originally collected from their reefs of origin, marked, and returned or transplanted to a different reef between 25 January and 15 February 2008. To accomplish this, I collected fish with a hand net against a large barrier net and transported them in 5-gallon buckets filled with saltwater to the wet lab at Bocas Research Station. They were anesthetized (with 1–3 ml of 2 % clove oil per bucket) and each fish was given a treatment-specific subcutaneous marking using a non-toxic elastomer dye (Northwest Marine Technologies). Fish were held overnight in a large holding tank with running seawater to allow them to fully recover from anesthesia and handling before release early the next morning (<1 % failed to recover from anesthesia and handling). Marked fish were left on the experimental reef sites for 4–5 months after initiating the treatments and were collected at the end of the experiment between 28 May and 14 June 2008. I searched exhaustively for marked experimental fish over multiple days at each reef study site to ensure that all were collected. After collection, fish were euthanized by slowly increasing the dosage of clove oil until they were not breathing; fish were kept on ice for transport and later preserved in formalin and stored in ethanol.

Effects of transplanting on local density

Chalk bass on the reef study sites used in this experiment occupied 30–50 % of the total area at each site. Since sites spanned about 1 ha (=10,000 m^2), these study sites contained at least 10,000–15,000 fish each (at local densities of 3 per m^2). Chalk bass were spread out among discrete aggregations on the reefs and my experiments and observations interacted with about 25 % of the fish on the occupied area at each site, including many that were not captured and marked. Therefore, my collections and manipulations in this experiment included only about 10–20 % of the 2500–4000 chalk bass that I encountered on each reef study site where marking and transplanting took place. Marked experimental fish were collected and released at multiple locations on the reef study sites and were well-dispersed among their unmarked conspecific neighbors. The mortality experienced by experimental fish took place at a fairly gradual rate after the initial period immediately following the transplant when many transplanted fish (about 50 %) disappeared, and the experimental manipulation did not noticeably affect local densities on the reef study sites.

Recovery of experimental fish

For each reef study site and treatment, the number of marked fish recovered at the end of the experiment divided by the number originally captured, marked, and released served as a measure of survival over the duration of the experimental period (see Fig. 1). To examine differences in survival among experimental fish, nominal logistic regression was used to generate four separate likelihood ratio tests, with the dependent variable being whether originally marked fish were present or absent at the experiment's end. (1) I compared recovery among the four study sites of fish in the return home treatments, which effectively tested for any differences amongst the reefs in survival. (2) I compared recovery of same and different density transplants combined to recovery of fish in the return home treatment to assess the impact of transplanting fish across reef sites. (3) I compared recovery of same versus different density transplants to assess the impact of transplanting to a different density. (4) I compared recovery between all transplants from high-density reef origins and all transplants from low-density reef origins to test whether fish from either reef origin density appeared more or less likely to die or disappear over the 4-month experimental period.

Growth of experimental fish

During collection of marked experimental fish, a subsample from a representative size range of the surrounding populations (non-experimental fish) were collected and measured (using standard length, SL in mm) for use in estimating how much the experimental fish had grown past the expected original population average; experimental fish were also measured before preserving in formalin.

Measures of sex allocation and body size

The mature ovarian and testicular tissues in the gonad of chalk bass and related species are of a distinctly different texture and color and can be easily separated under a dissection microscope (as in Petersen 1991; Petersen and Fischer 1996; Hart et al. 2010, 2011a). The testis and ovary can then be dried and weighed to serve as proxies for energetic investment in male and female function. Sex allocation and male and female investments were calculated from these measures, and body size was compared using soma mass (body with gonad removed, dried and weighed). Previous studies indicate that while soma mass is positively correlated with both ovary and testis mass (Hart et al. 2010, 2011a; and Hart M.K. additional unpublished data), sex allocation does not vary significantly with body size in these study populations (but see Petersen and Fischer 1996 who sampled in another region of Panama with a broader adult size range). However, body size does vary among populations with a general trend toward larger body size at lower density reefs (see Hart et al. 2010, 2011a, and this study). For this reason, I controlled statistically for any differences in body size across reef study sites when measuring treatment effects on sex allocation. Body size was also controlled when examining responses in testis mass and ovary mass to permit comparisons of male and female investment, respectively.

Statistical analyses

Sex allocation and body size responses to treatments

Full general linear model: The full statistical analysis involved accounting for which reef density (High and Low) and which reef study site (2 at each density) fish in each treatment originated. Reef study site was treated as a nested effect within reef origin density: Cocotree (HD1) and San Cristobal Mangrove (HD2) were nested within High density, and Solarte (LD1) and Andy's Reef (LD2) were nested within Low density. Soma mass was included as a covariate in tests for changes in ovary mass, testis mass, and sex allocation. The model statement was: y = Reef origin density + Reef study site (Reef origin density) + Treatment + Treatment × Reef origin density + log Soma mass + error. The response variables were sex allocation (log testis mass/gonad mass), male investment (log testis mass), female investment (log ovary mass). Body size (log soma mass) was also analyzed separately using the same model, albeit with the covariate removed.

The analysis was performed separately for the two key comparisons of interest. Firstly, I compared the same density transplant to the different density transplant treatments to test for phenotypic plasticity in response to a change in density. In these analyses, significant interaction effects in the same versus different density transplant treatment comparisons indicate phenotypic plasticity in the trait measured by that response variable. Trade-offs in male and female investments are indicated by significant interaction effects for both

response variables, coupled with a significant interaction effect for sex allocation. This is because fish originating from High density and fish originating from Low density are expected to respond in the opposite manner to the Different density transplant treatment. Secondly, I compared the return home and same density treatments to test for any unexpected changes in sex allocation or body size caused by the transplanting of fish to a new reef study site with similar density. No significant interaction effects were expected in the return home versus same density transplant comparison, but reef origin density was expected to have a significant effect on sex allocation, male investment, body size and female investment, reflecting the density-related differences that existed on these reefs before the experiment was set up.

I separately analyzed the responses of fish that originated from Low and High density reefs to further resolve phenotypically plastic responses and to determine whether fish from different reef origin densities responded more or less strongly to transplant treatments. These analyses were divided in the same way as for the full general linear model: same and different density transplant treatments were compared and return home and same density transplant treatments were compared. I used the model: y = Reef study site + Treatment + Treatment × Reef study site + log Soma mass + error, removing the interaction term where non-significant. In the same versus different density transplant treatment comparison, a significant response to treatment in sex allocation, male or female investments, and/or body size indicates phenotypic plasticity in those traits. Significant interaction effects were not expected but would indicate differences in the strength of response to treatment among reef study sites within a given reef origin density. No treatment or interaction effects were expected in the return home versus same density transplant analysis.

All response variables were log-transformed to achieve normality of model residuals and homogeneity of variances. Additionally, removal of three outliers from the dataset of 307 experimental samples was necessary to achieve the normal distribution required for the statistical analyses. The outliers skewed the residuals because of unusually small testis weights which sometimes resulted from tissue being lost during weighing or another part of the dissection process. The reef origin and treatment(s) for each excluded sample are as follows: from Cocotree, one sample in the return home treatment; from Solarte, one sample in the Different density transplant treatment; and from Andy's Reef, one sample in the Different density transplant treatment. Exclusion of these outliers did not change patterns of significance or interpretation of the results. All statistical analyses were performed using JMP[®] 10.

The nature of the experimental design introduced statistical issues that were an unavoidable outcome of the logistics of this field environment and study system. This study depended on collecting marked individual fish after 4 months, which I knew would be difficult based on recovery of transplanted fish in a previous study (Hart et al. 2011b). Therefore, I limited the number of reef study sites used in the experiment and devoted extra effort to the experimental set-up to ensure that I marked a sufficient number of fish in all three treatments at all study sites. Sample replication in the statistical analyses was at the level of the individual fish that were marked and placed in their different treatments or controls, and later recovered. Therefore, the fish in each treatment were not truly independent from each other because they came from the same reefs and were moved together to other locations where they intermingled. However, experimental fish were well-dispersed among the non-manipulated conspecifics and were obviously surrounded by and interacting with a large number individuals that were not marked or moved for the experiment.

Results

Differential survival of experimental fish

Monthly observations over the course of the experiment confirmed that the majority of fish in the same and different transplant treatments were lost within the first month of the experiment. Afterward, surviving transplants were site-attached, and abundance estimates in the month prior to collection were very similar to the number of marked fish in the final collection (data not shown). Therefore, recovery in the final collection likely provides an accurate estimate of survival for experimental fish. No differences in survival of fish in the return home treatment were found among the four reef study sites (see Fig. 1; Likelihood ratio Chi-square = 0.92, $p = 0.82$, df = 3, n = 384); of fish that were marked and returned to their resident reef, between 29 and 36 % were alive after the 4.5 month experimental period. The proportion of return home individuals recovered served as a benchmark to compare recovery of individuals that were captured and marked but also transplanted to a new reef.

The overall lower recovery of fish in the transplant treatments versus the return home treatment (see Fig. 1) indicates vulnerability of transplanted fish to new environments, as may be expected for a species that is highly site attached in adulthood: 13 % of all transplants survived and 32 % of all return home fish survived (Likelihood ratio Chi-square = 70.45, $p < 0.0001$, df = 1, n = 1842). Transplanted fish did not differ in survival whether they were transplanted to a reef of the same or different density; 13 % of same density transplants survived and 12 % of Different density transplants survived (Likelihood ratio Chi-square = 0.38, $p = 0.54$, df = 1, n = 1458). However, fish transplanted from Low density origin reefs had significantly lower survival (9 % survived) than fish transplanted from High density origin reefs (16 % survived) (Likelihood ratio Chi-square = 19.51, $p < 0.0001$, df = 1, n = 1458).

Growth and body size of experimental fish

When fish were collected at the end of the experiment, I measured how much experimental fish had grown relative to the estimated original size distribution. My best estimate of the original size distribution was the size distribution of the non-experimental fish on the reef study sites at the end of the experiment. The adult growth rate at these study sites is roughly 1 mm a month (Hart M.K., unpublished data); thus all experimental fish were expected to increase in size over the course of the 4-month experiment. I found that, on average, all experimental fish had consistently larger body size (by about 4 mm) than fish sampled from the surrounding non-experimental population (experimental fish mean ± SE = 37.35 ± 0.22; non-experimental fish mean ± SE = 33.54 ± 0.23; $F_{1,581} = 142.6$, $p < 0.0001$). The average body size was larger at low density than at high density for both experimental and non-experimental fish (Experimental fish: low density mean ± SE = 38.61 ± 0.21, high density mean ± SE = 36.07 ± 0.21, $F_{1,299} = 72.56$, $p < 0.0001$; non-experimental fish low density mean ± SE = 34.30 ± 0.37, high density mean ± SE = 32.65 ± 0.41, $F_{1,280} = 8.97$, $p = 0.003$), suggesting consistency in density-related differences in size distributions. Experimental fish grew, as expected, over the course of the experiment, suggesting that across-density transplants adjusted their growth pattern to match residents on their new reefs. However, I cannot rule out any effects of body size on survival of experimental fish.

Evidence for phenotypic plasticity and trade-offs

Full general linear model: The significant effects of reef origin density on sex allocation, male investment, and body size in the return home and same density transplant treatment comparisons (see Table 1) were expected because of the original known differences in sex allocation and body size among the experimental reefs (see Hart et al. 2010, 2011a). For the same versus different density transplant treatment comparisons, a phenotypically plastic response was indicated by significant interaction effects between treatment and reef origin density. Indeed, the interaction effect between treatment and reef origin density was significant in the same versus different density transplant treatment comparisons for all variables that showed significant responses to reef origin density in the return home versus same density transplant treatments: sex allocation, male investment, and body size (see Table 1). This means that the traits that originally differed among densities were those that responded when fish were transplanted across density, as would be expected in phenotypically plastic traits that are responsive to a change in social environment. Furthermore, both male investment and body size showed a plastic response, indicating a potential trade-off between male function and body growth. Female investment was not affected by reef origin density in comparisons of the return home versus same density transplant treatment or the interaction between treatment and reef origin density in the comparison of same versus different density transplant treatments (Table 1). Therefore, the hypothesized trade-off between male and female investments with shifts in sex allocation was not evident.

Separating effects for high and low reef origin densities

Separate analyses were necessary to uncover the underlying patterns leading to the observed interaction effects in the same versus different density transplant treatments. These analyses revealed that transplants from high to low density had stronger responses than transplants from low to high density (see Tables 2, 3; Fig. 2).

High density origin reefs: Individuals transplanted from high density to low density significantly reduced sex allocation and male investment in comparison to fish transplanted to another high density reef, but female investment did not respond to the treatment (same vs. different density transplants; Table 2; Fig. 2a, c, e). Furthermore, transplants from high to low densities had a significantly larger body size than transplants from high to high densities indicating they increased body growth relative to their counterparts (Table 2; Fig. 2g). Experimental fish from one high density origin reef (HD1) were larger than those from the other (HD2) (see Fig. 2g), hence the significant interaction between reef study site and treatment (Table 2).

Low density origin reefs: Individuals that were transplanted from low to high densities did not alter sex allocation or male and female investments significantly (same vs. different; Table 3; Fig. 2b, d, f). Body size was significantly smaller for transplants from low to high density than for transplants from low to low density (Fig. 2h), but the treatment effect was evident in fish from one reef (LD2) but not the other (LD1), hence the significant interaction effect (Table 3).

For both High and Low reef origin densities, no significant effects of treatment were found in the analyses of same density transplant versus return home (Tables 2, 3; Fig. 2).

Table 1 Results of nested general linear model

Full model (includes high and low density reef origins)	Effect	Return home versus same density transplant			Same versus different density transplants		
		df	F-ratio	p value	df	F-ratio	p value
Sex allocation (log testis mass/gonad mass)	Reef study site (reef origin density)	2, 205	1.99	0.14	2, 178	1.66	0.19
	Reef origin density	1, 205	**25.26**	**<0.0001**	1, 178	0.77	0.38
	Treatment	1, 205	0.15	0.70	1, 178	1.71	0.19
	Reef origin density × treatment	1, 205	0.12	0.73	1, 178	**16.03**	**<0.0001**
	Log soma mass	1, 205	0.02	0.88	1, 178	0.21	0.65
Male investment (log testis mass)	Reef study site (reef origin density)	2, 205	0.72	0.49	2, 178	0.67	0.51
	Reef origin density	1, 205	**13.53**	**0.0003**	1, 178	1.21	0.27
	Treatment	1, 205	0.30	0.58	1, 178	1.28	0.26
	Reef origin density × treatment	1, 205	0.75	0.39	1, 178	**8.24**	**0.0046**
	Log soma mass	1, 205	**60.40**	**<0.0001**	1, 178	**43.03**	**<0.0001**
Female investment (log ovary mass)	Reef study site (reef origin density)	2, 205	1.21	0.30	2, 178	1.04	0.35
	Reef origin density	1, 205	2.44	0.12	1, 178	0.07	0.80
	Treatment	1, 205	1.41	0.24	1, 178	0.04	0.84
	Reef origin density × treatment	1, 205	0.48	0.49	1, 178	1.98	0.16
	Log soma mass	1, 205	**86.95**	**<0.0001**	1, 178	**61.03**	**<0.0001**
Body size (log soma mass)	Reef study site (reef origin density)	2, 206	**6.91**	**0.0012**	2, 179	**4.61**	**0.01**
	Reef origin density	1, 206	**63.94**	**<0.0001**	1, 179	2.74	0.10
	Treatment	1, 206	0.08	0.78	1, 179	0.59	0.44
	Reef origin density × treatment	1, 206	0.03	0.87	1, 179	**29.00**	**<0.0001**

Comparisons are between return home and same density transplant treatments, and between same and different density transplant treatments. Bold indicates significant effects ($p < 0.05$). Non-significant interaction terms were retained in these models for interpretive value only; the removal of non-significant interaction terms does not alter the significance of main effects

Table 2 High density origin reefs

| High density origin reefs	Effect	Return home versus same density transplant			Same versus different density transplants		
		df	F-ratio	p value	df	F-ratio	p value
Sex allocation (log testis mass/gonad mass)	Reef study site	1, 119	0.39	0.53	1, 121	**4.63**	**0.033**
	Treatment	1, 119	0.24	0.62	1, 121	**17.78**	**<0.0001**
	Log soma mass	1, 119	1.79	0.18	1, 121	0.20	0.66
	Reef study site × treatment	**1, 119**	**4.50**	**0.04**			
Male investment (log testis mass)	Reef study site	1, 120	0.18	0.68	1, 121	0.73	0.39
	Treatment	1, 120	0.07	0.80	1, 121	**7.58**	**0.007**
	Log soma mass	1, 120	**28.28**	**<0.0001**	1, 121	**25.07**	**<0.0001**
Female investment (log ovary mass)	Reef study site	1, 120	0.53	0.47	1, 121	1.94	0.17
	Treatment	1, 120	0.15	0.69	1, 121	1.47	0.23
	Log soma mass	1, 120	**64.47**	**<0.0001**	1, 121	**44.96**	**<0.0001**
Body size (log soma mass)	Reef study site	1, 121	**13.95**	**0.0003**	1, 122	**8.56**	**0.004**
	Treatment	1, 121	0.01	0.92	1, 122	**19.80**	**<0.0001**

Comparisons are between return home and same density transplant (high to high density) treatments, and between same and different density transplant (high to low density) treatments. Non-significant interaction terms with $p \geq 0.10$ were excluded from the models. Bold indicates significant effects ($p < 0.05$)

Table 3 Low density origin reefs

Low density origin reefs	Effect	Return home versus same density transplant			Same versus different density transplants		
		df	F-ratio	p value	df	F-ratio	p value
Sex allocation (log testis mass/gonad mass)	Reef study site	1, 84	2.63	0.11	1, 54	0.18	0.67
	Treatment	1, 84	0.0004	0.98	1, 54	3.58	0.06
	Log soma mass	1, 84	2.71	0.10	1, 54	0.43	0.52
Male investment (log testis mass)	Reef study site	1, 84	1.52	0.22	1, 54	0.94	0.34
	Treatment	1, 84	0.92	0.34	1, 54	1.74	0.19
	Log soma mass	1, 84	**34.03**	**<0.0001**	1, 54	**25.09**	**<0.0001**
Female investment (log ovary mass)	Reef study site	1, 84	1.05	0.31	1, 54	0.32	0.57
	Treatment	1, 84	1.70	0.20	1, 54	0.93	0.34
	Log soma mass	1, 84	**24.72**	**<0.0001**	1, 54	**16.19**	**0.0002**
Body size (log soma mass)	Reef study site	1, 85	0.0004	0.98	1, 54	1.00	0.32
	Treatment	1, 85	0.08	0.78	1, 54	**7.06**	**0.01**
	Reef study site × treatment				1, 54	**7.44**	**0.009**

Comparisons are between return home and same density transplant (low to low density) treatments, and between different and same density transplant (low to high density) treatments. Non-significant interaction terms with $p \geq 0.10$ were excluded from the models. Bold indicates significant effects ($p < 0.05$)

Fig. 2 Results of reciprocal transplant experiment. Responses to treatment for **a**, **b** sex allocation (percentage testis in total gonad); **c**, **d** male investment (testis mass/soma mass × 100); **e**, **f** female investment (ovary mass/soma mass × 100); and **g**, **h** body size (soma mass, mg dry weight) for fish originating from high (*right-hand panels*: **a**, **c**, **e**, **g**) and low density reefs (*left-hand panels*: **b**, **d**, **f**, **h**). Return home treatments are designated as high origin and low origin; same density transplant treatments are designated as high-to-high and low-to-low; and different density transplant treatments are designated as high-to-low and low-to-high. Statistics are reported in Tables 1, 2 and 3
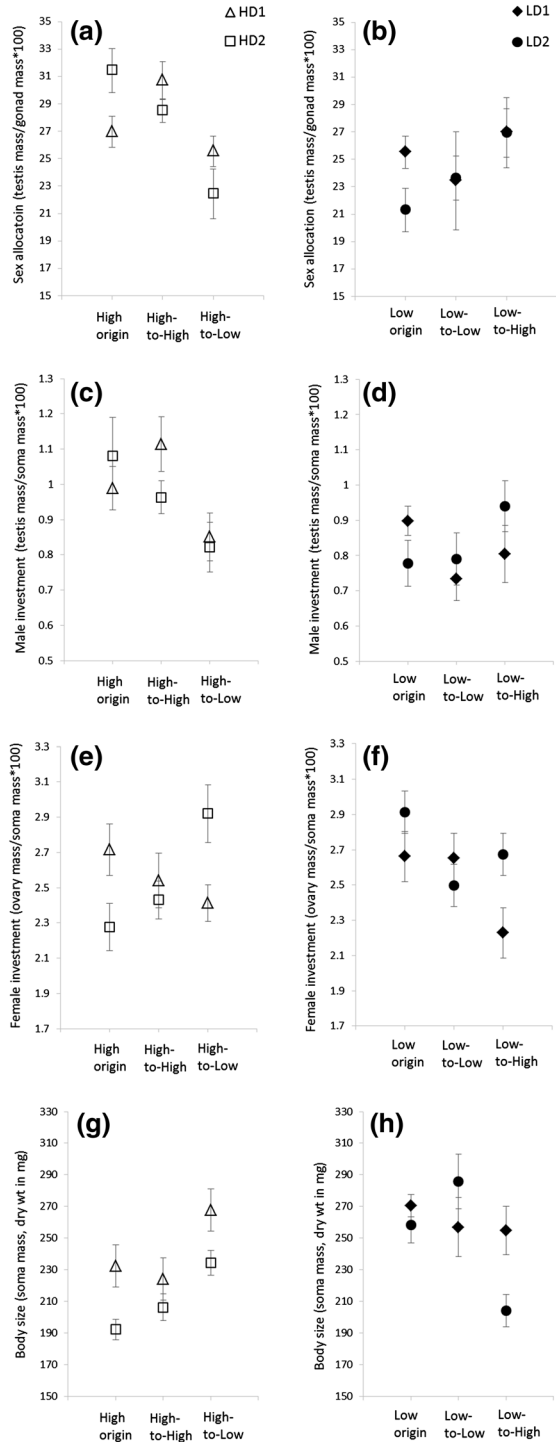
Discussion

Despite the low recovery of experimental fish (see Fig. 1), transplants across density showed a measurable response. The responses of surviving transplants indicate that sex allocation, male investment, and body size are phenotypically plastic and responsive to a change in local density (Table 1). However, female investment did not respond to the treatment, and the assumed trade-off between male and female investments with shifts in sex allocation was not evident. The response of transplants from high to low density was largely as predicted. As in the Hart et al. (2011a, b) study, transplants from high to low density reduced sex allocation and male investment and increased body size, suggesting a trade-off between investment in soma and male function (see Table 2; Fig. 2a, c, g). The response was not as strong in fish transplanted from low to high density (see Table 3; Fig. 2b, d, h). For low-to-high density transplants, body size was generally smaller than for low-to-low density transplants (see Fig. 2h), but the slight increase in sex allocation was non-significant and not clearly related to an increase in male investment or a reduction in female investment (Table 3; Fig. 2b, d, f).

A key limitation of this field experiment was the low replication at the reef study site level. The results indicate that replicates responded differently to the across-density transplants, but the non-significance of some of these patterns leaves questions about the consistency or predictability of these responses (Fig. 2b, d, e, f, h). For instance, low-to-high density transplants showed a marginal trend toward an increase in sex allocation (see Table 3; Fig. 2b) that appeared to be driven by different responses by the 2 replicates to the across-density treatment (Fig. 2d, f). For low-to-high samples in one replicate (LD2), male investment was slightly higher (see Fig. 2d) than for the low-to-low counterparts, and in the other replicate (LD1) female investment was slightly lower (see Fig. 2f). In addition, body size did not differ between low-to-high and low-to-low transplants in the LD1 replicate in which female investment was reduced, but body size was lower for low-to-high transplants in the LD2 replicate in which male investment increased (see significant interaction effect in Table 3 and Fig. 2h). While these patterns are not statistically significant, together they correspond with the predictions and corroborate evidence from previous studies (Hart et al. 2010, 2011a) that sex allocation can be adjusted by changes in either male or female investments.

High-to-low transplants clearly reduced male investment and increased body size relative to high-to-high transplants in both replicates (Fig. 2c, g), while low-to-high transplants merely trended toward a smaller size and more male-biased sex allocation patterns than low-to-low transplants (Fig. 2b, h). At higher densities, where male mating opportunities and competition are greater, the advantages of gaining a large body size may be overshadowed by the more immediate benefits of investment in male function rather than somatic growth. An increase in male function may come in the form of investment in behaviors that increase success in male-role extra-pair matings and may not necessarily be associated with an increased investment in testis mass. In another example, *O. diadema* individuals spent more time, and presumably more energy, engaging in aggressive, male-role behaviors with mate competitors when in large versus small mating groups (Lorenzi et al. 2006). They also reduced egg production significantly, indicating a trade-off in resources expending for male-role aggressive behaviors versus resources devoted to female function (Lorenzi et al. 2006).

Chalk bass spawn less frequently in pairs and more often as streakers at high density than at low density, and the daily number of male-role spawns for individuals mating at

high versus low density is much higher. At high density individuals streak, on average, 12 times during a daily spawning period and spawn in pairs 10 times; at low density individuals streak an average of 3 times during a daily spawning period and spawn in pairs 15 times (Hart and Kratter unpublished data). Just as for *O. diadema*, the male-role mating success of an individual chalk bass depends on how many of a partners' eggs are fertilized and the number of eggs it fertilizes in the streaker role. In this study, the non-significant trend toward an increase in sex allocation for transplants from low to high density likely reflects both the risk of sperm competition during pair spawns and the benefits of gaining extra-pair paternity by streaking. The value of fertilizing a partners' egg clutch is diminished when paternity is diluted by male-role mate competitors (streakers) (Hart et al. 2011b). In turn, the proximity to neighbors and increased potential for success as a streaker greatly increase the expected rewards for extra-pair streaking. Thus where male-role mating opportunities are at a premium, sexual selection on male function increases, and investment in male gonadal tissue or male-role mating behaviors over body growth may be a necessary trade-off. The reduction in body size for low to high density transplants where male-role spawning is higher—coupled with the increase in body size for high to low density transplants associated with a reduction in male investment—is certainly suggestive of a trade-off between body growth and costly male function.

Interestingly, individuals locally adapted to high density were both more responsive and better able to survive a change in environment than those from low density no matter which density they were transplanted to. This may be connected to differences in within-pair dynamics across densities and/or an inability of transplants from low density to adjust because of constraints to plasticity (e.g., maintenance costs, see DeWitt et al. 1998). At low density, mating partners show long-term fidelity and closely match egg production, with individuals spending much of the spawning period in close proximity with their primary partners (Hart and Kratter unpublished data). The relatively poorer survival of fish transplanted from low density suggests that they may pay a heavier cost for losing a mate and searching for a new one, particularly in a novel environment. In addition, sex allocation would not be expected to respond quickly if low-to-high transplants preferentially put effort into soliciting new mating partners by producing eggs for exchange in pair spawns rather than in seeking opportunities for male-role extra-pair spawns.

At high density mating partners have lower fidelity; individuals switch mating partners more often during a spawning period, and partners do not closely coordinate egg production (Hart M.K., unpublished data). Mating dynamics may also play a role in maintaining plasticity in fish from high density sites. Phenotypic plasticity in sex allocation and body size gives individuals the opportunity to adjust to the greater variation of mating situations they may be confronted with at higher densities. For instance, when individuals are able to find a high-quality mating partner, they may gain higher reproductive success in pair spawns than by streaking, and an immediate increase in female investment and/or body growth would be favored; but if they stand a good chance of losing that partner, selection should favor the flexibility to quickly take advantage of streaking opportunities. These hypotheses merit testing in future research because behaviors of transplants were not recorded during this experiment and no attempt was made to identify or keep spawning partners together. Future studies should also include paternity analysis of eggs spawned with varying numbers of streakers to provide insight into the advantages of alternative male-role strategies across different densities.

The results of this large-scale reciprocal transplant experiment have implications for theory and empirical research on sex allocation in simultaneous hermaphrodites. For one, it is clear that phenotypic plasticity in sex allocation and body size is important for shaping

natural mating systems. That both sex allocation and body size are responsive to the environment suggests that trade-offs in somatic versus reproductive investments are important in determining mating strategy. As in other studies (detailed in the review by Schärer 2009), I did not find a trade-off between male and female investment. In addition, male function appears more flexible and responsive than female function in the chalk bass system. These results suggest that the trade-offs between male and female investments assumed by sex-allocation theory do not always exist in nature. They also suggest that we need to consider the phenotypic plasticity of multiple life history traits when considering how organisms maximize fitness across environments with different mating opportunities.

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