SEX ALLOCATION IN A SIMULTANEOUSLY HERMAPHRODITIC MARINE SHRIMP

J. Antonio Baeza[1,2]

[1] Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Balboa, Ancón, Republic of Panama

   E-mail: baezaa@si.edu

[2] Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949

Two fundamental questions dealing with simultaneous hermaphrodites are how resources are optimally allocated to the male and female function and what conditions determine shifts in optimal sex allocation with age or size. In this study, I explored multiple factors that theoretically affect fitness gain curves (that depict the relationship between sex-specific investment and fitness gains) to predict and test the overall and size-dependent sex allocation in a simultaneously hermaphroditic brooding shrimp with an early male phase. In *Lysmata wurdemanni*, sperm competition is absent as hermaphrodites reproducing in the female role invariably mated only once with a single other shrimp. Shrimps acting as females preferred small over large shrimps as male mating partners, male mating ability was greater for small compared to large hermaphrodites, and adolescent males were predominant in the population during the breeding season. In addition, brooding constraints were not severe and varied linearly with body size whereas the ability to acquire resources increased markedly with body size. Using sex allocation theory as a framework, the findings above permitted to infer the shape of the male and female fitness gain curves for the hermaphrodites. The absence of sperm competition and the almost unconstrained brooding capacity imply that both curves saturate, however the male curve levels off much more quickly than the female curve with increasing level of investment. In turn, the predominance of adolescent males in the population implies that the absolute gain of the female curve is greater than that of the male curve. Last, the size-dependent female preference and male mating ability of hermaphrodites determines that the absolute gain of the male curve is greater for small than for large hermaphrodites. Taking into consideration the inferred shape of the fitness gain curves, two predictions with respect to the optimal sex allocation were formulated. First, overall sex allocation should be female biased; it permits hermaphrodites to profit from the female function that provides a greater fitness return than the male function. Second, sex allocation should be size-dependent with smaller hermaphrodites allocating more than proportionally resources to male reproduction than larger ones. This size-dependent sex allocation permits hermaphrodites to profit from male mating opportunities that are the greatest at small body sizes. Size-dependent sex allocation is also expected because the male fitness gain curve decelerates more quickly than the female gain curve and experiments indicated that resources are greater for large than small hermaphrodites. These two predictions were tested when determining the sex allocation of hermaphrodites by dissecting their gonad and quantifying ovaries versus testes mass. Supporting the predictions above, hermaphrodites allocated, on average, 118 times more to the female than to the male gonad and the proportion of resources devoted to male function was higher in small than in large hermaphrodites. A trade-off between male and female allocation is assumed by theory but no negative correlation between male and female reproductive investment was observed. In *L. wurdemanni*, the relationship between sex-specific investment and fitness changes during ontogeny in a way that is consistent with an adjustment of sex allocation to improve size-specific reproductive success.

KEY WORDS: Fitness-gain curves, hermaphrodite, sex allocation, shrimp, size dependent.

In simultaneous hermaphrodites, individuals allocate resources to both male and female function and reproduce as both sexes at the same time. Two current outstanding questions in sex allocation theory dealing with simultaneous hermaphrodites are how resources are optimally allocated to the male and female function (overall sex allocation; Charnov 1982) and what factors determine shifts in this optimal sex allocation with age or size (size-dependent sex allocation; Klinkhamer et al. 1997; Hardy 2002; de Jong and Klinkhamer 2005). Attempts to explore these two questions provide an opportunity to test predictions fundamental to sex allocation theory.

Fitness gain curves that relate investment in male or female reproduction to fitness gains derived from these investments are central to most models predicting optimal sex allocation (Charnov 1982). Gain curves are often represented as power functions with the exponent determining if the fitness gain is a function of investment (Fig. 1A). If the exponent is one, greater than one, or less than one, the gain curve is linear, accelerating, or decelerating, respectively, and fitness increases at a constant, increasing, or decreasing rate with a unit increase in investment (Klinkhamer et al. 1997). The optimal sex allocation is the value that maximizes the sum of the fitness derived from both male and female investments (Charnov 1982) (Fig. 1B). Because male and female fitness gain curves are generally not of the same shape, sex allocation is expected to be biased, with more resources invested in the sex function with the larger fitness returns (Charnov 1982). When the fitness gain curves are nonlinear or when the gain curves (when plotted for absolute investment) are different for small and large individuals, size-dependent sex allocation should evolve (Klinkhamer et al. 1997).

Various conditions affect the shape of the fitness gain curves, ultimately determining the overall and size-dependent sex allocation. The effect of these conditions may depend directly or indirectly on the body size of hermaphrodites (Klinkhamer et al. 1997; Cadet et al. 2004). Direct effects of body size on sex allocation occur when fitness returns for a given absolute amount of resources invested in one sex function differ for small and large hermaphrodites. For instance, in benthic free-spawning hermaphrodites, the sperm from large individuals with their gamete-shedding structures (e.g., siphons) positioned tall in the water column might disperse over larger distances and be more successful in fertilizing eggs than the sperm from small individuals with their siphons close to the bottom. All else being equal, hermaphrodites are expected to adjust sex allocation to their size (Klinkhamer et al. 1997). In this particular case, large individuals should invest proportionally more to male function compared to small individuals.

In turn, body size may indirectly affect sex allocation because large individuals generally have more resources, and hence, will produce more gametes and offspring than small conspecifics
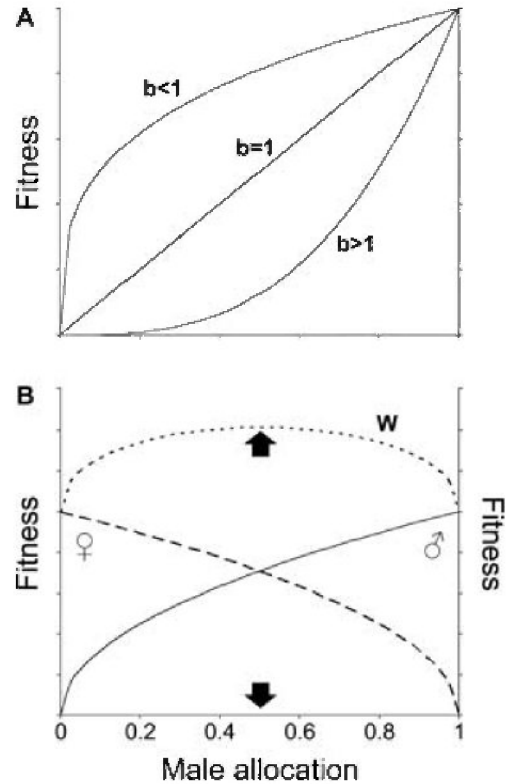
Figure 1. (A) Fitness gain curves that relate investments in a particular reproductive function (e.g., male allocation) to fitness gains derived from these investments. Curves are represented as power functions ($y = ax^b$) where the exponent (b) determines their shape and whether the reproductive success is a linear ($b = 1$), accelerating ($b > 1$), or diminishing ($b < 1$) function of investment. (B) The optimal sex allocation in simultaneous hermaphrodites depends on the value (see arrows) that maximizes the sum of the fitness derived from the male and female investment, denoted by the curve W in the figure. In the x-axis, a value of zero indicates that an individual is allocating 0% of its resources to the male function, and subsequently, 100% to the female function.

(i.e., the budget effect of size on fitness gains sensu Klinkhamer et al. 1997; see also Cadet et al. 2004; de Jong and Klinkhamer 2005). For example, if small and large hermaphrodites invest the same absolute amount of resources in eggs (or sperm), they have an equal female (or male) fitness gain. Nonetheless, because large hermaphrodites have a larger reproductive budget than small hermaphrodites, with an equal proportion of resources invested in eggs, a large hermaphrodite produces more offspring than a small hermaphrodite (Klinkhamer et al. 1997). Under these circumstances, fitness returns per unit of investment are determined by the level of investment. Allocation to female (or male) function is expected to increase with increasing body size if the male (or female) gain curve decelerates more quickly than the female (or male) gain curve (de Jong and Klinkhamer 2005). Overall, the interplay between the direct and indirect effects of size on the

fitness gain curves will determine the overall and size-dependent sex allocation of simultaneous hermaphrodites.

Among those conditions affecting the shape of the male fitness gain curve, sperm competition is considered important (Petersen 1991; Yund 1998). If the female gain curve is linear and sperm competition is intense, hermaphrodites are expected to invest heavily in male function (up to 50% of total reproductive investment) because fitness gains increase proportionally with male allocation. This occurs because each time eggs become available for fertilization, most or all of the other hermaphrodites in the population attempt to fertilize them. Therefore, hermaphrodites producing large amounts of sperm cope well with competition among ejaculates and profit from increased paternity. In turn, sex allocation should be female biased when sperm competition is weak and the female gain curve is linear. All else being equal, with low intensity of sperm competition there is low risk of loosing paternity due to the infrequent multimale copulation behavior of female-role hermaphrodites. Hermaphrodites investing large amounts of resources in sperm will be wasting energy compared to hermaphrodites producing few sperm to fertilize eggs when available. The idea of sex allocation biased toward the female function with weak sperm competition has received increasing empirical support (e.g., Fischer 1984; Schärer and Ladurner 2003; Tan et al. 2004; Lorenzi et al. 2006; Brauer et al. 2007). It must be mentioned that other optimal patterns of sex allocation with a fixed intensity of sperm competition are expected when the female fitness gain curve is nonlinear. For instance, if the male curve is linear (because of intense sperm competition) but the female curve is decelerating, the optimal sex allocation should be male biased (see also Greeff and Michiels 1999).

Other aspects of sexual selection that affect the shape of the male fitness gain curve and that might favor biased and size-dependent sex allocation patterns include male mating ability and female mating preferences (Michiels 1998; Angeloni and Bradbury 1999). If hermaphrodites reproducing in the female role prefer small conspecifics as male mating partners or if small hermaphrodites are more successful in mating in the male role than large conspecifics, then for a given absolute investment the male fitness returns are greater for small than for large hermaphrodites. Thus, all else being equal, small individuals should allocate more than proportionally to the male function compared to large individuals. Last, in simultaneous hermaphrodites with an early male phase (e.g., some polychaete worms and caridean shrimps; Sella 1990; Baeza 2007) the relative abundance of the adolescent males should affect the optimal sex allocation of the simultaneous hermaphrodites (Baeza 2006). The existence of functional males should result in female-biased sex allocation in hermaphrodites because part of the hermaphrodites reproducing as females will mate with males instead of with other male-role hermaphrodites. The fact that female-role hermaphrodites mate with males in-

stead of male-role hermaphrodites indicates lower absolute fitness gains for the male function compared to the female function in hermaphrodites (see also Ghiselin 1974; Petraitis 1990; Allsop and West 2004).

There are at least two situations affecting the shape of the female fitness gain curve. This curve should saturate quickly when limited dispersal of the offspring leads to sibling competition (i.e., local resource competition; Maynard Smith 1978), or with constraints on the number of brooded offspring in species with parental care (Heath 1979). For instance, limited brood capacity causes diminishing female fitness returns because sheltering space for offspring is gradually constrained as allocation to the female function increases. In this situation, reproductive effort that would otherwise be allocated to female reproduction should be allocated to male reproduction (Heath 1979; Strathmann et al. 1984).

Biased sex allocation patterns and shifts in sex allocation with body size have been reported for a few simultaneous hermaphrodites (biased overall sex allocation: Fischer 1981; Strathmann et al. 1984; Sella 1990; Petersen 1991; Locher and Baur 2000; size-dependent sex allocation: St. Mary 1994; Petersen and Fischer 1996; Trouvé et al. 1999; Schärer et al. 2001; Vizoso and Schärer 2007). Studies that have first used experimental manipulations to explore factors that might be important in determining the overall and size-dependent sex allocation of hermaphroditic animals, and which have then used these results to predict and test for optimal sex allocation are rare (for exceptions see Petersen and Fischer 1996; St. Mary 1997; Vizoso and Schärer 2007). This approach represents an ideal opportunity to test predictions important to sex allocation theory.

In this study, I tested for the optimal sex allocation of a simultaneously hermaphroditic animal with an early male phase. First, through field observations and experimental manipulations, I examined various factors (i.e., sperm competition, male mating ability, population structure, female mate choice, brooding constraints, and resource [food] holding potential) that are considered important in affecting the relationship between sex-specific reproductive investment and fitness returns in simultaneous hermaphrodites. Second, after inferring the combined effect of all these factors on the shape of the fitness gain curves, a prediction of the overall and size-dependent sex allocation pattern was formulated. Third, I tested this prediction by measuring investment in male and female gametes in hermaphroditic individuals of different body sizes. For this purpose, I used the marine shrimp *Lysmata wurdemanni* as a model.

Material and Methods
THE MODEL ORGANISM
Lysmata wurdemanni (Caridea: Hippolytidae) is a simultaneous hermaphrodite with an early protandric phase. Juveniles

consistently mature as male individuals bearing typical caridean male characters (i.e., coupling hoods on pleopod 1 and appendix masculina on pleopod 2) and are only capable of reproducing as males (Bauer and Holt 1998). These adolescent males later attain female sexual function and develop into functional simultaneous hermaphrodites (hereafter hermaphrodites). After maturation, hermaphrodites resemble females of caridean gonochoric species, and brood embryos in an abdominal chamber. Hermaphrodites retain testicular tissue, male ducts, and gonopores, and can reproduce as both male and female (Bauer and Holt 1998). Copulation between hermaphrodites is not reciprocal. A newly molted prespawning hermaphrodite copulates as a female with a male or another hermaphrodite exclusively acting as a male at that time. During copulation, which lasts a few seconds, a sperm mass is attached to the underside of the prespawning hermaphrodite by the mating partner. There is no long-term sperm storage in this shrimp and sperm from a mating is used to fertilize only the eggs released during the spawning event that occurs 2–3 h later (Bauer and Holt 1998). After becoming hermaphrodites, individuals do not revert to males and no self-fertilization has been observed (Bauer 2002a). In previous studies, I examined why this simultaneously hermaphroditic shrimp features an early protandric phase (Baeza 2006) and those conditions determining the moment at which males mature as hermaphrodites (Baeza and Bauer 2004; Baeza 2007). In the present study, I focus on those conditions affecting the sex allocation pattern of the simultaneously hermaphroditic phase of this shrimp.

COLLECTION AND MAINTENANCE OF SHRIMPS

Individuals of *L. wurdemanni* were collected with a long-handled dip net from the rock jetty at Port Aransas, Mustang Island, Texas (27°50′N, 97°03′W) from May 2003 to September 2005. Collected specimens were placed in large plastic bags containing aerated seawater, and transported to the laboratory at the University of Louisiana at Lafayette. Prior to the experiments, individuals were maintained in 114-L recirculating aquaria at a water temperature of 24°C, 34–35 ppt salinity, and a light:dark cycle of 14 h:10 h, and were fed daily (1/2 food pellet per shrimp, Wardley® Shrimp Pellets; Hartz Mountain, Secaucus, NJ).

FACTORS AFFECTING THE SHAPE OF THE MALE FITNESS GAIN CURVE

I tested the null hypotheses of no multimale mating per female reproductive event because the absence of sperm competition would cause the male fitness gain curve to saturate. I tested the null hypotheses of no overt preference of "parturial" hermaphrodites for small or large shrimps and no differences in male mating ability between small and large hermaphrodites because mate choice by female-role hermaphrodites and male mating ability should cause the male fitness gain curve to differ between large and small hermaphrodites. In addition, I examined the ratio of males to hermaphrodites during the main breeding season in the field because the relative abundance of adolescent males in the population should cause the absolute gain of the male curve to be low.

The risk of sperm competition due to multiple inseminations

I examined the null hypothesis of no multiple mating per reproductive event (i.e., no risk of sperm competition) in *L. wurdemanni* while maintaining shrimp aggregations in the laboratory and recording the number of times a parturial hermaphrodite was inseminated by different shrimps acting as males. Parturial hermaphrodites are prespawning hermaphrodites close to molting and reproducing in the female role (Bauer 2002a). During the main reproductive season of *L. wurdemanni*, the relative proportion of small males (shrimps < 6.0 mm carapace length, CL) to large males (shrimps > 6.0 mm CL) to small hermaphrodites (shrimps < 9.0 mm CL) to large hermaphrodites (shrimps > 9.0 mm CL) in the field is 4:3:2:1, respectively (see Bauer 2002b). This relative proportion was emulated in the laboratory by placing combinations of 10 shrimps of the different sizes and sexual phases (4:3:2:1) into 20-L aquaria (40 × 20 × 25 cm). After an initial acclimatization period of at least 8 h, one parturial hermaphrodite was introduced into the same aquarium. Molting and the subsequent number of inseminations experienced by this parturial hermaphrodite were counted in each of 20 replicates. Recording was conducted with time-lapse video (GYRR model TLC1400; GYYR; Anaheim, CA) at a speed of five frames per second (24-h recording mode). A Cohu CCD surveillance video camera equipped with a 6-mm lens (Cohu; San Diego, CA) sensitive to both low-intensity white and infrared light was used. Fluorescent and infrared (880 nm) lamps provided day and night illumination, respectively. If the frequency of multimale copulation by hermaphrodites reproducing as females is high, then the risk of sperm competition should be high in *L. wurdemanni*.

Mate choice by parturial hermaphrodites

I tested the null hypothesis of no overt preference of parturial hermaphrodites for large (>9.0 mm CL) or small (<9.0 mm CL) shrimps by exposing parturial hermaphrodites simultaneously to a small and large nonparturial hermaphrodite. Each of the nonparturial hermaphrodites was tethered to the opposite side of an aquarium (20 L). Human hair was used because it caused no harm to the shrimps and did not appear to negatively affect swimming and crawling. One of the extremes of the thread was knotted at the junction of the abdomen and cephalothorax of each nonparturial shrimp whereas the other extreme was fixed to the top of one of the ends of the aquarium. After an initial acclimatization period of at least 8 h, one parturial hermaphrodite was introduced into the same aquarium. Because the length of the thread allowed tethered

shrimps to swim and crawl freely up to the middle portion of the aquarium but not beyond, potential competitive interactions between shrimps acting as males as well as any long-term pursuing of the parturial hermaphrodite by these shrimps were precluded. In addition, this procedure permitted free visits of the parturial hermaphrodite to either of the tethered shrimps. The identity of the shrimp with which the parturial hermaphrodite copulated was recorded with time-lapse video as above for each of 30 replicates. To determine preferences by parturial hermaphrodites for small or large male mating partners, an exact chi-square test of goodness of fit was used to test for significant differences between the observed distribution of inseminations by shrimps of the different size classes and the binomial random distribution using procedure FREQ as implemented in the software SAS version 9.1 (SAS Institute 2004). Significant differences would indicate female preference.

Male mating ability of hermaphrodites

I tested the null hypothesis of no differences in male mating ability between small (<9.0 mm CL) and large (>9.0 mm CL) hermaphrodites by recording the behavior of pairs of shrimps competing for inseminating a parturial hermaphrodite in the laboratory. One small and one large hermaphrodites were first placed into an aquarium (20 L). In contrast to the previous experiment, these shrimps moved freely in the aquarium, allowing competitive interactions between them. After an initial acclimatization period of at least 8 h, one parturial hermaphrodite was introduced into the aquarium. The identity of the shrimp inseminating this parturial hermaphrodite was recorded with time-lapse video as above for each of 30 replicates. To determine differences in male mating ability between small and large hermaphrodites, I compared the observed distribution of inseminations by shrimps of the different size classes with the binomial random distribution using an exact chi-square test of goodness of fit (SAS Institute 2004). If male mating ability of larger hermaphrodites is greater than that of smaller hermaphrodites, then parturial hermaphrodites will be inseminated by larger shrimps more frequently than expected by chance alone.

Natural abundance and sex ratio in L. wurdemanni

To determine the abundance and sex ratio (adolescent males to hermaphrodites) of *L. wurdemanni* during the main breeding season, I collected shrimps between May and October, 2004 from Port Aransas, Texas. I recorded the number of shrimps captured per sampling night and identified their sexual phase according to the presence/absence of external traits (coupling hoods on pleopod 1 and appendix masculina on pleopod 2 both present in males but absent or reduced in hermaphrodites) (Bauer and Holt 1998). The sampling effort (total number of hours spent collecting shrimps) was calculated for each sampling night (one night each during

May and October and two nights per month from June to September). Abundance of shrimps was estimated by dividing the sample abundance (number of shrimps captured) by the sampling effort. I compared the observed proportion of males to hermaphrodites and tested for deviations from a 1:1 sex ratio using a binomial test (Zar 1999, Wilson and Hardy 2002).

FACTORS AFFECTING THE SHAPE OF THE FEMALE FITNESS GAIN CURVE

Brood capacity of hermaphrodites

I tested the null hypotheses of no limitation on brood capacity with size of brooding hermaphrodites because brooding constraints would cause the female fitness gain curve to saturate.

In caridean shrimps, embryo volume increases throughout the development and loss of crowded embryos from the brooding chamber may occur when they reach their maximum size (close to hatching) (see Kuris 1991). If hermaphrodites suffer brooding space constraints, fitness returns to the female function should decelerate with increasing allocation to the female function (Heath 1979). All else being equal, sex allocation should be biased toward the male function (Charnov 1982). Additionally, egg production generally scales to the cube whereas brood capacity scales to the square of body size in marine invertebrates (Strathmann et al. 1984). This differential scaling usually determines a disproportionate increase in brood loss with increasing body size of individuals because of increasing brooding space constraints (Strathmann et al. 1984). If brooding capacity constraints increase with body size of shrimps, fitness returns to the female function should decelerate more quickly for large than for small shrimps. All else being equal, large hermaphrodites should allocate disproportionately more into the male function compared to small hermaphrodites (Charnov 1982).

I tested the null hypothesis of no size constraints on brooding capacity by comparing the relationship between fecundity and body size (CL) of individuals brooding early and late embryos. If brooding capacity is not constrained, the intercept of the curve describing the relationship between fecundity and body size of shrimps carrying early should not differ from that of shrimps carrying late embryos. In turn, if brood capacity is constrained with body size of shrimps, the slope of the curves describing the relationship between fecundity and body size should differ between shrimps carrying early and late embryos. For instance, if brooding space constraints are greater for large than for small shrimps, the slope of the curve describing the relationship between fecundity and body size of shrimps carrying early embryos should be steeper than that of shrimps carrying late embryos (Strathmann et al. 1984).

To explore the relationship between fecundity and body size in *L. wurdemanni*, I collected 50 hermaphrodites covering most of the range in body size reported for the species (about 7.0- to

14.0-mm CL—Bauer 2000b) carrying early (newly spawned) and late (close to hatching) embryos. Each shrimp was fixed in 10% seawater formalin and transferred after washing to 70% alcohol for preservation. In the laboratory, embryos were gently collected with forceps and counted under a stereomicroscope (Wild Co. Model M5A). The CL of each hermaphrodite was measured under the stereomicroscope to the nearest 0.1 mm. The curve denoting the relationship between fecundity and body size (CL) of shrimps was compared between hermaphrodites brooding early and late embryos (25 shrimps per stage) with a one-way ANCOVA using embryo stage as the independent variable, shrimp size as covariate, and fecundity as the dependent variable (SAS Institute 2004).

FOOD-RESOURCE HOLDING POTENTIAL OF HERMAPHRODITES

I tested the null hypothesis of no differences in resource holding potential between small and large hermaphrodites because differing resource (food) availability among shrimps of different body sizes coupled with nonlinear fitness gain curves determines size-dependent sex allocation.

I tested the null hypothesis of no differences in resource holding potential between small (<9.0 mm CL) and large (>9.0 mm CL) hermaphrodites while recording the behavior of shrimps competing for food in the laboratory. One small and one large hermaphrodites were placed in separated containers (1 L) and not fed for three days. After an initial acclimatization period of 30 min in a 20-L aquarium, a single food pellet was provided by dropping it equidistant between them. Most commonly, shrimps move toward pellets immediately after they are provided, especially after short periods without food (J. A. Baeza, pers. obs.). Behavioral interactions (i.e., approach, striking at opponent with maxillipeds and pereiopods, retreat) between shrimps for the pellet were recorded for 30 min using time-lapse video as above for each of 30 replicates. The shrimp possessing and feeding on the pellet after any interaction was declared the winner. The null hypothesis of no association between shrimp size (large vs. small) and interaction outcome (winner vs. loser) categories was tested with an exact chi-square test of goodness of fit (SAS Institute 2004). If food-resource holding potential increases with size of shrimps, then large hermaphrodites will monopolize food more frequently than expected by chance alone.

Field observations indicate that *L. wurdemanni* feed on small distinct clumps of algae that represent discrete resources similar to food pellets in this experiment. Therefore, the experiment above does emulate potential (agonistic) interactions among shrimps while feeding under natural conditions (Baeza 2006).

PREDICTING SEX ALLOCATION IN *L. WURDEMANNI*

The exact shape of the fitness gain curves is difficult to estimate because of conceptual and empirical problems (i.e., lack of vari-

ation in allocation to the sex functions within a population, difficulties in measuring paternity; Klinkhamer et al. 1997). Animals are not particularly suited to overcome these problems, unless they feature a modular body plan (Yund 1998 and references therein). Nonetheless, predictions on the optimal sex allocation of hermaphrodites can be formulated and tested even if the exact shape of these curves is not known due to our present knowledge of the processes governing these curves (Klinkhamer et al. 1997). For instance, if we explore the intensity of sperm competition in a population of hermaphrodites, it is possible to predict a priori that each hermaphrodite in the population should allocate more to ova than to sperm if sperm competition is weak (assuming a linear female gain curve). Conversely, if sperm competition is intense and the female gain curve is linear, each hermaphrodite in the population should allocate equally to sperm and ova (Charnov 1982). Here, my first goal was to infer the combined effect of the above conditions on the shape of the fitness gain curves for large and small hermaphrodites. After inferring the shape of the fitness gain curves, I formulated a qualitative prediction on the overall and size-dependent sex allocation of *L. wurdemanni*.

ESTIMATING SEX ALLOCATION IN *L. WURDEMANNI*

To quantify sex allocation, I collected, during 2005, a total of 44 hermaphrodite shrimps with mature ovaries covering most of the range in body sizes reported for the species and transported them alive to the laboratory. Sperm mass, testes mass, and ovaries mass were measured. Sperm mass contained in the ejaculatory ducts (that serve as reservoirs of their own sperm in shrimps) was retrieved by electro-ejaculation. Short electric shocks at 10–12 volts and 1.5 amp were applied for about 2–5 sec near the male gonopores. Next, each shrimp was dissected to extract the ovarian and testicular portions of its ovotestes. Finally, shrimps and their ovaries, sperm mass, and testes were dried for 48 h at 70°C in an oven (Grieve Co., LO–201C), and weighed to the nearest 0.01 mg with an analytical balance (Mettler AE163).

From the measurements above, I calculated four different estimates of reproductive allocation. Allocations to female and male function were estimated, respectively, as the mass of ovaries and the mass of sperm contained in the ejaculatory ducts plus the mass of the testes. Total reproductive investment was estimated as the sum of the male and female allocations and represents the absolute amount of resources that individuals invest in reproduction. Sex allocation was estimated as the ratio of female to male allocation and represents the relative proportion of resources that individuals invest in functioning as the different sexes.

TESTING SEX ALLOCATION IN *L. WURDEMANNI*

I tested whether total reproductive investment in simultaneous hermaphrodites increases linearly with body size. The relationship between total reproductive investment and body dry mass of

hermaphrodites was examined using the allometric model $y = ax^b$ (Klinkhamer et al. 1997). The slope b of the log-log least-squares linear regression represents the rate of exponential increase ($b > 1$) or decrease ($b < 1$) of the total reproductive investment with shrimp dry mass (Schärer et al. 2001). To determine if the relationship deviates from linearity, an F-test was used to test if the estimated slope b deviates from the expected slope of unity (SAS Institute 2004). If total reproductive investment per body mass neither increases nor decreases with body size in *L. wurdemanni*, then the relationship should be linear.

The same approach was used to determine if the relationship between male allocation (sperm plus testes dry mass), female allocation (ovaries dry mass), and sex allocation (ovaries dry mass/sperm plus testes dry mass) and body dry mass were all linear. If male allocation, female allocation, or sex allocation does not vary linearly with body size, then the slope of the different curves should differ significantly from unity.

Last, I tested for a phenotypic trade-off between the two sex functions by calculating the partial correlation between male and female allocation while controlling for shrimp body size (SAS Institute 2004).

Results

FACTORS AFFECTING THE SHAPE OF THE MALE FITNESS GAIN CURVE

The risk of sperm competition due to multiple inseminations

Sperm competition is absent in the mating system of *L. wurdemanni*. Hermaphrodites reproducing in the female role copulated only once, and successfully resisted any subsequent approaches by shrimps mating in the male role. Males and hermaphrodites roamed freely in the aquarium when maintained in aggregations. No agonistic interactions for access to or defense of the single available parturial hermaphrodite were observed among potential male mating partners. In nine of the 20 replicates (45%), insemination occurred at the moment of molting (i.e., when the parturial hermaphrodite was restricted in its movements while emerging from its older molt skin), and in the remaining 11 replicates, mating occurred while the parturial hermaphrodite was swimming (i.e., seconds after molting). Notwithstanding this variation in the moment at which insemination occurred, all prespawning hermaphrodites copulated only once with either a male or hermaphrodite, in each of the 20 trials. In those replicates in which copulations occurred after molting (55% of the trials), the prespawning hermaphrodites consistently resisted mating by performing tail flips to flee from potential male mating partners, which attempted to seize them to transfer a spermatophore. After these prespawning hermaphrodites copulated, they intensively rejected other potential sperm donors as indicated by the significantly greater total number of tail flips observed 5 min after mating
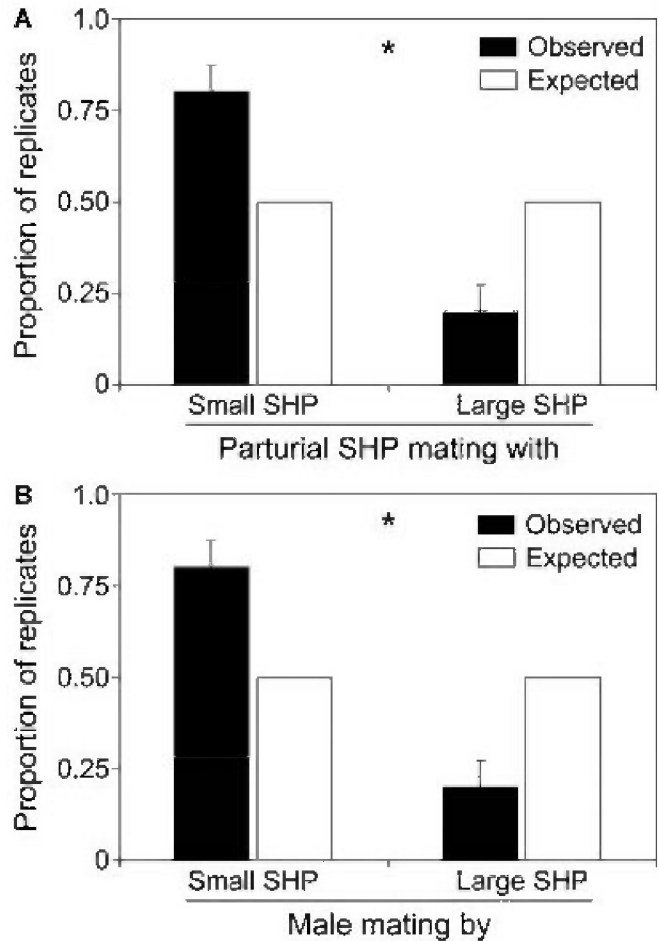
Figure 2. Female mating preferences and male mating ability in *Lysmata wurdemanni*. **(A)** Observed and expected proportion of the replicates in which a parturial hermaphrodite copulated with a large or small tethered hermaphrodite. **(B)** Observed and expected proportion of the replicates in which a large or small nontethered hermaphrodite acting as male inseminated a parturial hermaphrodite. The asterisk indicates significant differences (*P* < 0.05). Bars in A and B are binomial standard error bars (see Methods for details).

compared to those that occurred between molting and insemination (29.09 ± 12.05 vs. 4.91 ± 6.16, mean ± SD, respectively, paired *t*-test; $t_{10} = 5.49, P = 0.0003$).

Mate choice by parturial hermaphrodites

Parturial hermaphrodites given a choice between tethered large and small shrimps mated more frequently with small male mating partners. In all of the trials, shrimps acting as males reacted as they would have reacted if they were not tethered when the parturial hermaphrodite molted, by attempting to grasp and seize the recently molted hermaphrodite. Smaller shrimps inseminated the parturial hermaphrodites more often (24 out of 30 trials) than expected by chance alone ($\chi_1^2 = 10.8, N = 30, P_{exact} < 0.0014$) (Fig. 2A).

Table 1. Population dynamics of *Lysmata wurdemanni* during the main breeding season at Port Aransas, Texas. The total number of shrimps collected (sample size), the ratio of hermaphrodites to males (sex ratio), and the number of shrimps retrieved per hour of collection (abundance, mean and SD) are provided for each month. Also, the corresponding *P* values obtained when testing for a significant difference between the observed sex ratio and an expected sex ratio of 1:1 with the binomial test are presented.

Month	Sample size	Sex ratio	P	Abundance	
				Mean	SD
May	33	0.88	$\ll 0.0001$	39.60	–
June	126	0.23	$\ll 0.0001$	39.75	25.10
July	425	0.14	$\ll 0.0001$	106.25	83.79
August	637	0.25	$\ll 0.0001$	159.25	26.52
September	246	0.32	$\ll 0.0001$	87.05	0.78
October	47	0.13	$\ll 0.0001$	56.40	–

Male mating ability of hermaphrodites

Observations of unrestrained shrimps showed that male mating ability of small hermaphrodites is greater than that of large hermaphrodites. Small hermaphrodites inseminated hermaphrodites reproducing as females in 24 of the 30 trials which was higher than expected by chance alone ($\chi_1^2 = 10.8, N = 30, P_{\mathrm{exact}} <$ 0.0014). Small individuals had higher mating success regardless whether insemination occurred at molting (11 out of 14 observations) or while the parturial hermaphrodite was swimming (13 out of 16 replicates). In general, smaller hermaphrodites were four times more successful than larger hermaphrodites in mating in the male role (Fig. 2B).

Natural abundance and sex ratio in L. wurdemanni

Except in May, when shrimp numbers were low and the proportion of hermaphrodites composing the population was high, shrimp abundance was high and the population was significantly male biased during the reproductive season (Table 1). Shrimps live in aggregations as numerous shrimps were collected from some refuges (ledges) but not from others.

FACTORS AFFECTING THE SHAPE OF THE FEMALE FITNESS GAIN CURVE

Brood capacity of hermaphrodites

Brood capacity is constrained, but not severely, and increases with the cube of carapace length in *L. wurdemanni*. There was no significant differences between the slopes of the curves depicting the relationship between fecundity and body size for shrimps carrying early and late embryos (one-way ANCOVA after log-log transformation; interaction term effect $F_{1,46} = 1.42, P = 0.2399$; Fig. 3A). Fecundity varied between 407 and 1493 (807.9 ± 324.9, mean $\pm$ SD) in shrimps brooding early embryos and between 382 and 1367
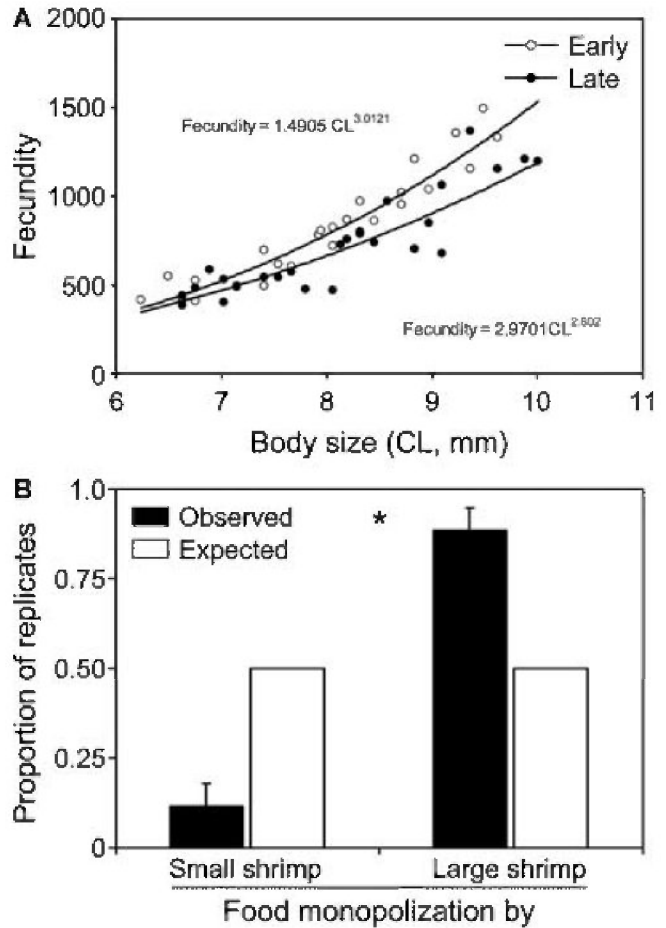
Figure 3. Brood capacity and resource holding potential in shrimps of different body sizes of *Lysmata wurdemanni*. (A) Fecundity of shrimps brooding early (recently spawned) and late (close to hatching) embryos. (B) Observed and expected proportion of the replicates in which large or small hermaphrodites retained a food item after 30 min of observation. Only those replicates in which agonistic interactions were observed were considered. The asterisk indicates significant differences (*P* < 0.05). Bars in A are binomial standard error bars (see Methods for details).

(726.9 ± 284.8) in shrimps brooding late embryos suggesting that shrimps lose 7.14% of their brood during development.

FOOD-RESOURCE HOLDING POTENTIAL OF HERMAPHRODITES

The ability to monopolize food is greater for large compared to small hermaphrodites. When one large and one small hermaphrodites competed for food in the laboratory, agonistic behaviors between the two shrimps for access to the food item were observed in 26 of 30 replicates. Agonistic interactions usually lasted only a few seconds. When the small hermaphrodite was the first to encounter the pellet (20 out of the 26 replicates with agonistic interactions), the larger hermaphrodite took the pellet from it seconds later by repeatedly striking at its body with the

third maxillipeds and/or the clawed first pereiopods. This fighting behavior did not appear to cause injuries to the small recipient shrimp and was repeated until the smaller shrimp retreated from the pellet. Conversely, on the few occasions in which the large hermaphrodite reached the pellet first (six out of 26 replicates with agonistic interactions), this hermaphrodite secured and kept the pellet out of reach of the smaller shrimp simply by directing short antennal taps at it. Large hermaphrodites monopolized food items more often than expected by chance alone in replicates with agonistic interactions ($\chi_1^2 = 15.38, N = 26, P_{exact} = 8.79 \times 10^{-5}$; Fig. 3B).

PREDICTING SEX ALLOCATION IN *L. WURDEMANNI*

In light of the preceding results, in this section I formulated two predictions about the overall and size-dependent sex allocation in *L. wurdemanni*.

In terms of the male fitness gain curve, the monoandrous behavior of hermaphrodites reproducing in the female role indicates that there is no risk of sperm competition for male-role hermaphrodites. This absence of sperm competition implies that the number of sperm produced by hermaphrodites that is successfully used in fertilizing eggs from hermaphrodites reproducing as females decreases more than proportionally with an increasing proportion of resources allocated to sperm. The relationship above determines a male fitness gain curve that follows the law of diminishing returns (fitness gains decrease more than proportionally with increasing level of male investment) independent of body size of hermaphrodites (Fig. 4). In addition, the predominance of males in the population indicates that a certain proportion of hermaphrodites acting as females are mating with males instead of with male-role hermaphrodites. This reduced male mating opportunity for hermaphrodites due to the presence of adolescent males in the population determines that the absolute gain of the male fitness gain curve is lower than that of the female fitness gain curve. This difference in absolute gains is depicted in Figure 4 by the steeper female fitness gain curve compared to the male fitness gain curve, both in large and small hermaphrodites. Furthermore, recent experiments have demonstrated that adolescent males are more successful in mating as males than are hermaphrodites (Baeza 2007 and references therein). This greater male mating ability of males compared to that of hermaphrodites further argues in favor of an absolute gain of the male fitness gain curve lower than that of the female fitness gain curve, independent of the body size of hermaphrodites (Fig. 4). Additionally, the size-dependent female preference and size-dependent male mating ability of hermaphrodites implies greater male mating opportunities for small than for large hermaphrodites. This size-dependent male mating opportunity determines that the absolute gain of the male curve is greater for small than for large hermaphrodites (Fig. 4).
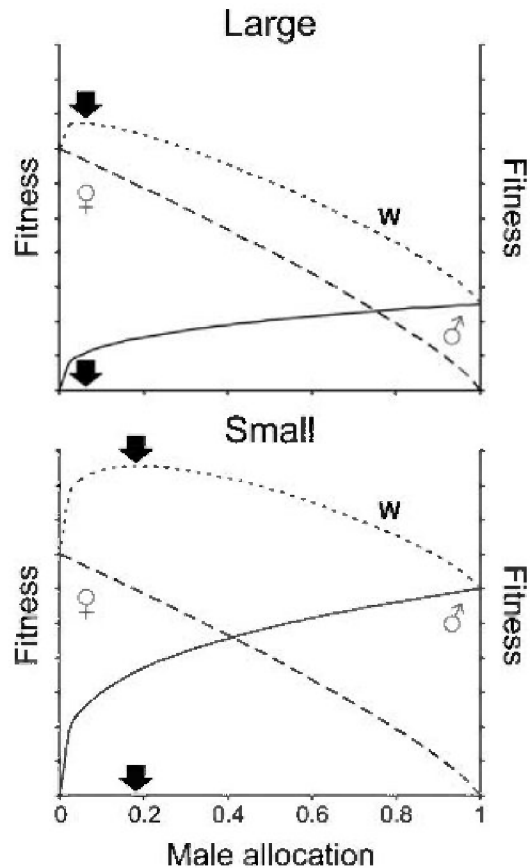
Figure 4. Qualitative predictions for the fitness gain curves in hermaphroditic individuals of different body sizes (small and large) of *Lysmata wurdemanni*. The depicted scenario predicts that *L. wurdemanni* should have a female-biased overall sex allocation and that small hermaphrodites should allocate proportionally more to the male function compared to larger shrimps (i.e., size-dependent sex allocation) (see text for details).

In terms of the female fitness gain curve, the constraints in brood capacity indicate that this fitness gain curve also saturates somewhat (not heavily) with increasing level of investment (Heath 1979). This occurs because some eggs are lost during incubation; the proportion of eggs that hatch successfully as larvae decreases more than proportionally with increasing level of investment. Importantly, because brood constraints did not increase more than proportionally with shrimp body size, the female fitness gain curve should saturate equally in both large and small hermaphrodites (Fig. 4). Furthermore, the fact that brood capacity is not severely constrained and that sperm competition is absent indicates that, irrespective of size, the male fitness gain curve decelerates more quickly than the female gain curve with increasing level of investment (Fig. 4).

Last, because the fitness gain curves are nonlinear and because food availability differs between large and small hermaphrodites, size-dependent sex allocation should be expected

in *L. wurdemanni*. Because large hermaphrodites have more resources due to their larger resource (food) holding potential and because the male fitness gain curve decelerates more quickly than the female gain curve, allocation to the female function should increase more than proportionally with increasing body size (Klinkhamer et al. 1997; de Jong and Klinkhamer 2005).

Overall, the combined effect of all the studied conditions (sperm competition, male mating ability, population structure coupled with greater male mating ability of adolescent males, female mate choice, brooding constraints, and resource [food] holding potential) on the theoretical relationship between investment and fitness gains indicates (1) that the absolute gain of the female curve is greater than that of the male curve, (2) that the male gain curve saturates more quickly than the female gain curve with increasing level of investment for both small and large hermaphrodites, and (3) that the absolute gain of the male curve is greater in small than in large hermaphrodites (Fig. 4).

What do these results mean for understanding the optimal allocation of resources to the male and female function in *L. wurdemanni* and shifts in this optimal sex allocation with body size? Considering the male and female fitness gain curves here inferred for *L. wurdemanni*, the following two predictions can be made. First, the overall sex allocation should be female biased in *L. wurdemanni*. Second, sex allocation should be size dependent with large shrimps allocating relatively less to the male than to the female function compared to small shrimps (see arrows in Fig. 4). In general, to optimize lifetime reproductive success, each hermaphrodite in the population should produce few sperm; only enough to fertilize eggs on the few occasions these become available for fertilization (Charnov 1982). Any hermaphrodite producing copious amount of sperm would be wasting energy because these male gametes most likely will not be used. As discussed above, this overall female-biased sex allocation is driven by the absence of sperm competition, the predominance of competitively superior adolescent males in the population, and because of the almost unconstrained brooding capacity (Heath 1979; Charnov 1982). In addition, each hermaphrodite in the population should produce proportionally more sperm than eggs when small than when large. This size-dependent sex allocation permits hermaphrodites to profit from male mating opportunities that are the greatest at small body sizes. As discussed above, this size-dependent sex allocation is a consequence of the size-dependent male mating ability and mating preferences by female-role hermaphrodites that determine greater male mating opportunities for small than for large hermaphrodites (Michiels 1998; Angeloni and Bradbury 1999; Cadet et al. 2004). This size-dependent sex allocation also occurs because the male fitness gain curve decelerates more quickly than the female gain curve and resources are greater for large than small hermaphrodites (Klinkhamer et al. 1997; de Jong and Klinkhamer 2005).

TESTING SEX ALLOCATION IN *L. WURDEMANNI*

Total reproductive investment is a linear function of body size and overall sex allocation is highly female biased in *L. wurdemanni*. Hermaphrodites allocated proportionately to reproduction (ovaries, stored sperm, and testes dry mass) with increasing body size; the slope of the relationship between shrimp dry mass and total reproductive investment dry mass did not differed significantly from unity ($b = 1.04$, $P = 0.6472$; Table 2; Fig. 5A). When the effect of shrimp body size was not considered, the ratio of female to male dry reproductive tissue mass varied between 49.4 and 219.2 with an average ($\pm$SD) of 117.9 ($\pm$38.9). Therefore, hermaphrodites invested about 118 times more to the female than to the male function independent of body size.

Sex allocation is also size dependent in *L. wurdemanni* with small hermaphrodites allocating proportionally more resources to male function compared to large hermaphrodites. Hermaphrodites allocated proportionately to female reproduction with increasing body size as the slope of the relationship between shrimp dry mass and ovaries dry mass did not deviate significantly from unity ($b = 1.05$, $P = 0.6243$: Table 2; Fig. 5B). In contrast, hermaphrodites allocated proportionately less to male reproduction with increasing body size as the slope of the relationship between shrimp dry mass and stored sperm plus testes dry mass was significantly smaller than unity ($b = 0.65$, $P = 0.0001$; Table 2; Fig. 5C). The slope of the relationship between shrimp dry mass and sex allocation was significantly smaller than unity indicating a size-dependent sex allocation ($b = 0.40$; $P < 0.0001$; Table 2; Fig. 5D). Smaller hermaphrodites (<9.0 mm CL) invested on average 105.5 ± 33.9 (mean $\pm$ SD, range 49.4–187.5, $N = 18$) times more to female than to male function whereas larger shrimps (>9.0 mm CL) invested on average 126.6 ± 40.4 (range 70.4–219.2, $N = 26$) times more to female than to male function. Thus, even though overall sex allocation was highly female biased, smaller hermaphrodites invested a comparatively larger amount of resources to male reproduction than larger hermaphrodites.

Last, there was no phenotypic trade-off between male and female allocation in *L. wurdemanni* as female allocation was not negatively correlated with male allocation when shrimp body weight was accounted for ($r = -0.11$, $F_{1,42} = 0.52$, $P < 0.4754$) (Fig. 6).

Discussion

This study examined multiple conditions affecting the relationship between sex-specific investments and fitness gains derived from these investments in a simultaneous hermaphrodite. By considering the effect of sperm competition, male mating ability, natural abundance and sex ratio, female mating preferences, brooding constraints, and food-holding potential on the relationship between investment and fitness returns, specific predictions on the

Table 2. Reproductive measurements and their variation with body size in *Lysmata wurdemanni*. The adjusted coefficient of determination (r^2), the slope (*b*) of the curve denoting the relationship between a particular measurement and body size of shrimps, and the standard error (SE) of the estimated slope are provided for each specific reproductive measure. Also, the *F*-statistic and the corresponding *P* value obtained when testing for a positive correlation and for linearity between a particular reproductive measurement and body size are presented.

Dependent variable	Model			Test H_o: $b = 0$		Test H_o: $b = 1$	
	r^2	b	SE	$F_{1,42}$	P	$F_{1,42}$	P
Reproduction investment	0.70	1.04	0.104	100.9	<0.0001	0.21	0.6472
Female allocation	0.69	1.05	0.105	99.60	<0.0001	0.24	0.6243
Male allocation	0.61	0.65	0.081	65.54	<0.0001	18.31	0.0001
Sex allocation	0.14	0.40	0.139	8.11	0.0068	18.57	<0.0001

overall and size-dependent sex allocation were formulated. First, overall sex allocation was predicted to be female biased in *L. wurdemanni* (prediction one). Second, sex allocation was predicted to be size-dependent; small shrimps were expected to allocate proportionately more to the male function and proportionately less to the female function compared to large shrimps (prediction two). These two predictions were tested by measurements of the amount of resources devoted to male and female gonad in individuals of different body sizes.

Supporting the first prediction, hermaphroditic shrimps allocated to the female function 118 times more than to the male function. To my knowledge, this is the most extreme case of female-biased overall sex allocation ever reported from a simultaneously hermaphroditic animal. Other species with a less extremely female-biased sex allocation include the reef fish *Hypoplectus nigricans* (ovary comprises 88.6% of the total gamete crop biomass; Fischer 1981), the marine worm *Ophryotrocha diadema* (i.e., ovarian biomass four times greater than testicular biomass; Sella 1990), the tapeworm *Schistocephalus solidus* (i.e., investment in female gonad products 2.6 times greater than in male products; Schärer et al. 2001), and the land snail *Arianta arbustorum* (egg production is about 95% of reproductive investment; Locher and Baur 2000).

The sex allocation estimated for *L. wurdemanni* (based solely on the biomass of reproductive structures) may not be accurate if the energetic or temporal costs of sperm and egg production differ. The few available studies on the caloric content of male and female gonad products in hermaphrodites indicate that energy per unit mass of sperm is slightly lower than that in the lipid-rich oocytes (i.e., 16% less energy in sperm than in eggs in the sequentially hermaphroditic fish *Thalassoma bifasciatum*; Schärer and Robertson 1999). If the same is true for *L. wurdemanni*, correcting for such an energetic difference between eggs and sperm would result in a slightly more female-biased sex allocation. Conversely, the time commitment of the female function is comparatively longer than that of the male function in this species (i.e., hermaphrodites replenish their sperm reservoirs within two days after mating as

males but they take 11 days or more to refill their ovaries after spawning; Baeza 2006). Hence, a shrimp that reproduces successfully as male every two days would produce five times more sperm than that retrieved from the ejaculatory duct in this study. Thus, if the original male allocation estimate is multiplied by five, this new value will correspond to the amount of sperm produced in the same time period as that of a single batch of eggs. This correction implies that *L. wurdemanni* allocates about 23 times more to the female than to the male function. This new estimate is not as extreme as the original, but still highly female biased.

Supporting the second prediction formulated during the present study, *L. wurdemanni* was found to feature a size-dependent sex allocation with shrimps investing proportionately less to the male function with increasing body size. A similar size-dependent sex allocation pattern has also been shown for other simultaneously hermaphroditic animals (i.e., Petersen and Fischer 1996; Schärer et al. 2001). In *L. wurdemanni*, the observed size-dependent sex allocation pattern mostly resulted from female preferences and male mating ability that varied with shrimp size. Nonetheless, a simpler set of conditions might explain the same observed size-dependent sex allocation pattern; the pattern could simply be the result of male mating ability decreasing with body size. At present, it is not possible to distinguish between these competing hypotheses. Future quantitative predictions from mathematical models should allow distinguishing among competing hypotheses determining qualitatively similar size-dependent sex allocation patterns (St. Mary 1997; Munday et al. 2006).

On the other hand, this study highlights the importance of examining not a single but various factors that altogether shape the fitness gain curves to predict the optimal size-dependent sex allocation of hermaphrodites. For instance, if only two conditions, sperm competition and brood constraints, had been examined during this study, an overall female biased but not a size-dependent sex allocation would have been predicted as sperm competition was absent and brood constraints were shown to increase linearly with body size. The observed size-dependent sex allocation of *L. wurdemanni* had not been in agreement with this prediction.
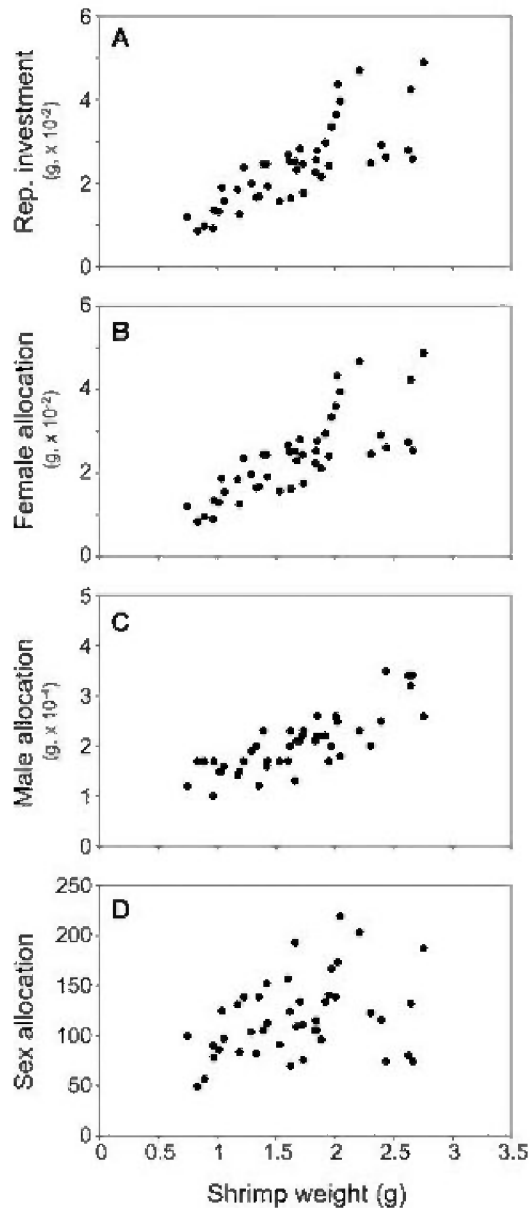
Figure 5. Reproductive measurements and their variation with body size in *Lysmata wurdemanni*. (A) Total reproductive investment. (B) Female allocation. (C) Male allocation. (D) Sex allocation. All measurements are in grams (g) of dry mass (see Table 2 for statistics and details).

The accuracy with which overall and size-dependent sex allocation is predicted in future studies should improve by examining the effect of multiple factors on the fitness gain curves and by exploring whether these factors change with age or size of simultaneous hermaphrodites.

Overall, in the marine shrimp *L. wurdemanni*, the relationship between sex-specific investment and fitness changes with body size in a way that is consistent with an adaptive adjustment of sex allocation to improve age-specific reproductive success.
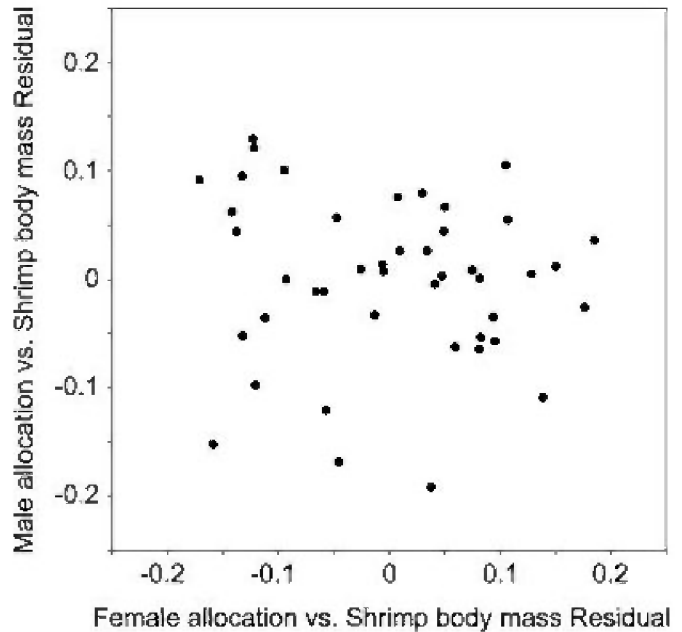
Figure 6. Relationship between male and female allocation in *Lysmata wurdemanni*. All measurements are in grams (g) of dry mass.

Because sex allocation theory assumes a direct trade-off between male and female allocation, a more than proportional increase in female allocation is expected when a more than proportional decrease in male allocation is found (Charnov 1982; Schärer et al. 2005). However, female allocation increased linearly with body size in *L. wurdemanni*. The lack of a negative correlation between female and male allocation further argues against the theoretical phenotypic trade-off between the male and female function in the studied species. A trade-off between the sex functions has been documented for some hermaphrodites (Raimondi and Martin 1991; De Visser et al. 1994; Trouvé et al. 1999; Schärer et al. 2005), but not for others (Petersen 1990; Locher and Baur 2000; Schärer and Ladurner 2003). In *L. wurdemanni*, the apparent lack of trade-off between the sex functions may arise if variation in both reproductive resource budget and sex allocation are large (the van Noordwijk and de Jong's effects; van Noordwijk and de Jong 1986; de Jong 1993; Schärer et al. 2005). Large shrimps of *L. wurdemanni* were more successful than small shrimps in monopolizing food, indicating high resource budget variability within the population. Similarly, variation in sex allocation appeared also to vary widely as the ratio of female to male gonad dry mass varied between 49 and 219 in the studied shrimps. Alternatively, the lack of phenotypic correlation between the male and female allocation in this species may also be explained if male and female allocation does not trade-off with each other, but with other life-history traits (Koene and ter Maat 2004; Schärer et al. 2005). Indeed, female allocation impacts growth in *L. wurdemanni* as indicated by experiments showing a decrease in growth

rate of hermaphroditic shrimps reproducing as female compared to that of shrimps not reproducing as females (Baeza 2006). Experiments that minimize variation in reproductive resource budget are needed to test whether a phenotypic correlation between male and female allocation in *L. wurdemanni* exists (see Schärer et al. 2005).

Although this study did not find the theoretically expected phenotypic trade-off between the male and female function in *L. wurdemanni*, a phenotypic trade-off between male and female allocation may still exist and go undetected if, concomitantly with the less than proportional increase in male gonad tissue, there is a more than proportional increase in female allocation components other than egg production that were not considered in this study. For instance, the frequency or intensity of female behaviors such as oxygen provision to, and grooming of, brooded embryos might increase more than proportionally with age or size of individuals (Baeza and Fernandez 2002). Nonetheless, similar (not quantified) aspects of male behavior should exist. For instance, roaming, considered a male activity because male mating success depends on the ability to search for receptive females in species living in aggregation such as *L. wurdemanni* (Andersson 1994), may decrease more than proportionally with increasing body size. Components of sex allocation other than gonad tissue (i.e., physiological, anatomical, and behavioral investments) should be included in future studies measuring investment to the male and female function and in experiments testing for a trade-off between the sex functions in this and other simultaneous hermaphrodites (Lorenzi et al. 2006).

OUTLOOK

Lysmata wurdemanni features an unusual sex allocation pattern with younger individuals investing all of their resources in male reproduction and older shrimps investing in both male and female reproduction. The moment at which males mature as hermaphrodites, a form of sex allocation, is influenced by both abiotic (Bauer 2002a) and biotic (social) conditions (Baeza and Bauer 2004; Baeza 2007). I have shown here that sex allocation of larger hermaphroditic shrimps is highly female biased, and that this sex allocation also varies with body size of these hermaphrodites. Size-dependent sex allocation has been shown for other simultaneously hermaphroditic animals (St. Mary 1994; Petersen and Fischer 1996; Trouvé et al. 1999; Schärer et al. 2001). Recent studies have also reported animal species featuring "mixed" sexual systems (i.e., males coexisting with simultaneous hermaphrodites in androdioecious clam shrimps; Zucker et al. 1997: bidirectional sex change in gobiid fish; St. Mary 1994; Munday et al. 1998). Sex allocation theory is in need of a single comprehensive model predicting the evolution of "pure" and "mixed" sexual systems and size-dependent sex allocation may be considered the theoretical framework on which to build this model. Future models consider-ing differences on the shape of the fitness gain curves with size or age of individuals should allow improving our understanding of the evolution of both "pure" and "mixed" sex allocation patterns.

LITERATURE CITED
Allsop, D. J., and S. A. West. 2004. Sex-ratio evolution in sex changing animals. Evolution 58:1019–1027.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

Angeloni, L., and J. W. Bradbury. 1999. Body size influences mating strategies in a simultaneously hermaphroditic sea slug, *Aplysia vaccaria*. Ethol. Ecol. Evol. 11:187–195.

Baeza, J. A. 2006. Testing three models on the adaptive significance of protandric simultaneous hermaphroditism in a marine shrimp. Evolution 60:1840–1850.

———. 2007. Male mating opportunities affect sex allocation in a protandric simultaneous hermaphroditic marine shrimp. Behav. Ecol. Sociobiol. 61:365–370.

Baeza, J. A., and R. T. Bauer. 2004. Experimental test of socially mediated sex change in a protandric simultaneous hermaphrodite, the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). Behav. Ecol. Sociobiol. 55:544–550.

Baeza, J. A., and M. Fernández. 2002. Active brood care in *Cancer setosus* (Crustacea: Decapoda: Cancridae): the relationship between female behaviour, embryo oxygen consumption, and the cost of brooding. Funct. Ecol. 16:241–251.

Bauer, R. T. 2002a. Test of hypotheses on the adaptive value of an extended male phase in the hermaphroditic shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). Biol. Bull. 203:347–357.

———. 2002b. Reproductive ecology of a protandric simultaneous hermaphrodite, the shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). J. Crust. Biol. 22:742–749.

Bauer, R. T., and G. J. Holt. 1998. Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. Mar. Biol. 132:223–235.

Brauer, V. S., L. Schärer, and N. K. Michiels. 2007. Phenotipically flexible sex allocation in a simultaneous hermaphrodite. Evolution 61:216–222.

Cadet, C., J. A. J. Metz, and P. G. L. Klinkhamer. 2004. Size and the not-no-single sex: disentangling the effects of size and budget on sex allocation in hermaphrodites. Am. Nat. 164:779–792.

Charnov, E. L. 1982. The theory of sex allocation. Princeton Univ. Press, Princeton, NJ.

De Visser, J. A. G. M., A. Ter Maat, and C. Zonneveld. 1994. Energy budgets and reproductive allocation in the simultaneous hermaphrodite pond snail, *Lymnaea stagnalis* (L.): a trade-off between male and female function. Am. Nat. 144:861–867.

Fischer, E. A. 1981. Sexual allocation in a simultaneously hermaphroditic coral reef fish. Am. Nat. 117:64–82.

———. 1984. Local mate competition and sex allocation in simultaneous hermaphrodites. Am. Nat. 124:590–596.

Ghiselin, M. T. 1974. The economy of nature and the evolution of sex. Univ. of California Press, Berkeley, CA.

Greeff, J. M., and N. K. Michiels. 1999. Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality. Am. Nat. 153:421–430.

Hardy, I. C. W. 2002. Sex Ratios: concepts and research methods. Cambridge Univ. Press, Cambridge, U.K.

Heath, D. J. 1979. Brooding and the evolution of hermaphroditism. J. Theor. Biol. 81:151–155.

de Jong, T. J. 1993. Covariances between traits deriving from successive allocations of a resource. Funct. Ecol. 7:75–83.

de Jong, T. J., and P. G. L. Klinkhamer. 2005. Evolutionary ecology of plant reproductive strategies. Cambridge Univ. Press, Cambridge, U.K.

Klinkhamer, P. G. L., T. J. de Jong, and H. Metz. 1997. Sex and size in cosexual plants. Trends Ecol. Evol. 12:260–365.

Koene J. M., and A. ter Maat. 2004. Energy budget in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis*: a trade-off between growth and reproduction during development. Belgian J. Zool. 134(Suppl. 1):41–45.

Kuris, A. M. 1991. A review of patterns and causes of crustacean brood mortality. Pp. 117–141 *in* A. Wenner and A. Kuris, eds. Crustacean egg production. Crustacean issues Vol. 7. Balkema, Rotterdam, The Netherlands.

Locher, R., and B. Baur. 2000. Mating frequency and resource allocation to male and female function in the simultaneous hermaphrodite land snail *Arianta arbustorum*. J. Evol. Biol. 13:607–614.

Lorenzi, M. C., D. Schleicherová, and G. Sella. 2006. Life history and sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*: the role of sperm competition. Integr. Comp. Biol. 46:381–389.

Maynard-Smith, J. 1978. The evolution of sex change. Cambridge Univ. Press, Cambridge, U.K.

Michiels, N. K. 1998. Mating conflicts and sperm competition in simultaneous hermaphrodites. Pp. 219–254 *in* T. R. Birkhead and A. P. Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Munday, P. L., P. M. Buston, and R. R. Wagner. 2006. Diversity and flexibility of sex-change strategies in animals. Trends Ecol. Evol. 21:89–95.

Munday, P. L., M. J. Caley, and G. P. Jones. 1998. Bi-directional sex change in a coral-dwelling goby. Behav. Ecol. Sociobiol. 43:371–377.

Petersen, C. W. 1990. Variation in reproductive success and gonadal allocation in the simultaneous hermaphrodite, *Serranus fasciatus*. Oecologia 83:62–67.

———. 1991. Sex allocation in hermaphroditic sea basses. Am. Nat. 138:650–667.

Petersen, C. W., and E. A. Fischer. 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: the effect of individual size. Evolution 50:636–645.

Petraitis, P. S. 1990. Dynamic of sex change in a capitellid polychaete. Pp. 127–154 *in* M. Mangel, ed. Sex allocation and sex change: experiments and models. The American Mathematical Society, Providence, RI.

Raimondi, P. T., and J. E. Martin. 1991. Evidence that mating group size affects allocation of reproductive resources in a simultaneous hermaphrodite. Am. Nat. 138:1206–1217.

SAS. 2004. SAS/STAT software; version 9.1. SAS Institute, Cary, NC.

Schärer, L., and P. Ladurner. 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proc. R. Soc., London B. 270:935–941.

Schärer, L., L. M. Karlsson, M. Christen, and C. Wedekind. 2001. Size-dependent sex allocation in a simultaneous hermaphrodite parasite. J. Evol. Biol. 14:55–67.

Schärer, L., and D. R. Robertson. 1999. Sperm and milt characteristics and male *v*. female gametic investment in the Caribbean reef fish, *Thalassoman bifasciatum*. J. Fish Biol. 55:329–343.

Schärer, L., P. Sandner, and N. K. Michiels. 2005. Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm *Macrostomum* sp. J. Evol. Biol. 18:396–404.

Sella, G. 1990. Sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*. Ecology 71:27–32.

Strathmann, R. R., M. F. Strathmann, and R. H. Emson. 1984. Does limited brood capacity link adult size, brooding, and simultaneous hermaphroditism? A test with the starfish *Asterina phylactica*. Am. Nat. 123:796–818.

St. Mary, C. M. 1994. Sex allocation in a simultaneous hermaphrodite, the blue-banded goby (*Lythrypnus dalli*): the effects of body size and behavioral gender and the consequences for reproduction. Behav. Ecol. 5:304–313.

———. 1997. Sequential patterns of sex allocation in simultaneous hermaphrodites: do we need models that specifically incorporate this complexity? Am. Nat. 150:73–97.

Tan, G. N., F. R. Govedich, and M. Burd. 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, *Helobdella papillornata* (Euhirudinea: Glossiphoniidae). J. Evol. Biol. 17:574–580.

Trouvé, S., J. Jourdane, F. Renaud, P. Durand, and S. Morand. 1999. Adaptive sex allocation in a simultaneous hermaphrodite. Evolution 53:1599–1604.

van Noordwijk, A J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128:137–142.

Vizoso, D. B., and L. Schärer. 2007. Resource-dependent sex-allocation in a simultaneous hermaphrodite. J. Evol. Biol. 20:1046–1055.

Wilson, K., and I. C. W. Hardy. 2002. Statistical analysis of sex ratios: an introduction. Pp. 48–92 *in* I. C. W. Hardy, ed. Sex ratios: Concepts and research methods. Cambridge Univ. Press, Cambridge, U.K.

Yund, P. O. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology 79:328–339.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, New Jersey, NJ.

Zucker, N., M. Cunningham, and H. P. Adams. 1997. Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*. Hydrobiologia 359:171–175.

Associate Editor: T. Day