SEX RATIO, LIFE-HISTORY INVARIANTS, AND PATTERNS OF SEX CHANGE IN A FAMILY OF PROTANDROUS GASTROPODS

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Abstract.—Application of optimality theory to the evolution of life histories has been broadly successful in predicting the conditions favoring sex change, the type of change, and the timing of such changes. The size advantage hypothesis predicts that the optimal size at which an individual should change sex is a function of its size and the size and sex of its potential mates. I collected data on the size, sex, and grouping of individuals of 27 populations of 19 species of the calyptraeids, a family of protandrous marine gastropods that includes *Crepidula*. These data are used to test the following predictions about variation in size at sex change: (1) sex ratio is biased toward the first sex; (2) the ratio of the size at sex change to the maximum size is a life-history invariant; and (3) species that form variable-sized groups or stacks have more variation in size at sex change than species that show less variation in stack formation. Across all 19 species, sex ratio was not significantly more often biased toward the first sex than it was toward second sex, although sex ratios were significantly male biased more often than they were significantly female biased. Sex ratios ranged from 0.05 to 0.91, and this variation was related to mode of development, skew in size distribution, and frequency of stacking, but not with maximum body size. There was little evidence that the ratio of size at sex change and maximum size is invariant. There is evidence that one of the main underlying assumptions of this life-history invariant, that male fertility increases with the same function of size in all species, is invalid for calyptraeids and probably for other animals. Finally, species that form larger stacks or mating groups had more variation in size at sex change within a population than species that were generally solitary. These results suggest that information about individual groupings should be included in predictions of life-history theory and that more information about the relationship between male fitness and size is also needed.

Key words.—*Bostrycapulus*, Calyptraeidae, *Crepidula*, protandry.

Received April 12, 2005.   Accepted January 27, 2006.

Sex change is a widespread phenomenon that has been the subject of a variety of theoretical and empirical evolutionary studies (e.g., Warner 1975; Leigh et al. 1976; Hoagland 1978; Charnov 1979, 1982; Shapiro and Lubbock 1980; Policansky 1981). Predictions of the conditions that favor sex change and of the size/age at sex change are often cited as successful examples of the application of optimality theory to the evolution of life histories (Charnov 1982; Hardy 2002). Because detailed demographic and breeding data are difficult and time-consuming to obtain, detailed studies of these predictions have usually been limited to single model species (e.g., bluehead wrasse, *Thalassoma bifasciatum*: Warner and Hoffman 1980; Warner 1987; Schultz and Warner 1989, 1990; Warner and Swearer 1991; Warner and Schultz 1992; Peterson et al. 2001; peppermint shrimp, *Lysmata wurdemanni*: Bauer and Holt 1998; Lin and Zhang 2001; Bauer 2002a,b, 2005; Baldwin and Bauer 2003; Baeza and Bauer 2004).

Recent theoretical developments have used simplifying assumptions to make general predictions that can be applied across disparate taxa (see below). This allows comparative studies to use compilations of published data from distantly related species to test generalizations about patterns of sex change (e.g., all sex changing animals in Allsop and West 2003a). When strong support for predicted patterns is found, the simplifying assumptions of these studies appear to be justified. Because these studies have tended to combine a haphazard assortment of disparate taxa with few species drawn from a single genus or family, it is difficult to determine if the commonly observed high levels of variation and deviations from expectations are due to a general failure of the theory, or if there are differences among clades that each independently conform to theoretical expectations. For ex-

ample, a recently compilation of sex ratio for 40 protandrous animals (drawn from five phyla) demonstrated large range in sex ratio (0.11–0.89; Allsop and West 2004). More detailed sampling within more closely related monophyletic groups is necessary to determine whether such large variation is due to the differences in natural history between animals as different as sea cucumbers, marine isopods, and fishes, or if such variation is also typical of more closely related species. Here I use data from 27 populations of 19 species of calyptraeid gastropods to evaluate three predictions of sex change theory and compare the patterns observed in this monophyletic family to those of a compilation of animals from the literature (Allsop and West 2003a, 2004).

Background Theory

The size-advantage hypothesis predicts that sex change is favored when reproductive output increases more quickly with size or age in one sex than in the other (Warner 1975; Leigh et al. 1976; Charnov 1979, 1982). The optimal size at sex change occurs when the potential subsequent lifetime reproductive output as the second sex exceeds that of remaining as the first sex. Because the expected reproductive success depends on both size and/or age and the pool of each animal's potential mates, the optimal size at sex change should vary in response to the size distribution or age structure of the population. Modifications of the size at sex change in response to changes in demographics have been demonstrated in pandalid shrimp and certain fishes after fisheries have significantly decreased survival probabilities (e.g., Charnov 1981; Hannah and Jones 1991; Buxton 1993; Charnov and Hannah 2002).

Extension of the optimality approach shows that the sex ratio in sex-changing species should be biased toward the first sex. At the evolutionary stable size at sex change the adult sex ratio (r; males/total) is given by the equation (eq. 4 of Charnov and Bull 1989):

$$r/(1 - r) = [F/f(t)]/[M/m(t)], \qquad (1)$$

where F is average female fertility, M is average male fertility, $f(t)$ is the fertility of a female at the size of sex change, and $m(t)$ is the fertility of a male at the size of sex change. If it is assumed that both male and female fitness increase with size (as is often the case in protandry) then $M/m(t) < 1 < F/f(t)$ (eq. 5 of Charnov and Bull 1989). As a result $r/(1 - r) > 1$ and therefore sex ratio is expected to be greater than 0.5. For protogyny sex ratio is predicted to be less than 0.5. Data from protogynous coral reef fishes show more species and populations with female-biased than male-biased sex ratios (data from various sources reviewed in Charnov 1993). Statistical deviations from 0.5 for each species were not examined, but the overall interspecific distribution appeared to be biased toward the first sex. A recent comparative analysis of published studies of various marine animals demonstrates that, despite a wide range of sex ratios, protogynous species are generally female biased and that protandrous species are generally male biased (Allsop and West 2004).

A life-history invariant approach (Charnov 1993) has also been applied to the study of sex-changing animals (Charnov 1993; Charnov and Skúladóttir 2000; Allsop and West 2003a,b). This approach uses comparisons of dimensionless ratios to search for constant relationships between traits that might shed light on underlying organizing principles of evolution. Optimality theory has been applied to sex change to predict the following dimensionless life-history invariants (Charnov and Skúladóttir 2000): (size at sex change)/(maximum size) = constant and (age at maturity)/(age at sex change) = constant. This theory appeared to receive strong empirical support for marine animals on the basis of regression analyses (Allsop and West 2003a,b). Regression analyses of data where the range of the dependent variable is bounded by the independent variable (e.g., size at sex change can never exceed maximum size) have since been shown to be flawed (Buston et al. 2004; Cipriani and Collin 2005; Nee et al. 2005) because the pattern predicted by the null hypothesis is the same as predicted by the hypothesis of life-history invariants (Cipriani and Collin 2005). Gardner et al. (2005) conducted sensitivity analyses that held constant two of the three values that are assumed by life-history invariance theory to be constant (k/M, δ, and αM, where k = von Bertalanffy growth coefficient, M = instantaneous mortality rate, α = age at maturity, and δ = exponential scaling coefficient of male reproductive success with size; Charnov and Skúladóttir 2000) and varied the third parameter. Their subsequent sensitivity analyses were also based on the flawed regression analyses (Gardner et al. 2005, p. 563) and therefore suffer from the same weakness as the previous, less sophisticated analyses.

The incorrect application of statistical analyses to test for invariants does not, however, invalidate the general idea or hypotheses put forward by advocates of life-history invariants or the usefulness of the dimensionless approach. Cipriani and Collin (2005) suggested that simulation approaches could be used to directly examine the variation in the observed ratios with the idea that ratios showing less variation than expected at random would offer some support of an underlying relationship between the variables. Such a result could possibly be interpreted as support for life-history invariants. Application of this test to previously published datasets showed that several of them indeed had less variation in such dimensionless ratios than is expected at random (Cirpriani and Collin 2005).

Predictions of sex ratio and life-history invariants are derived using a number of basic simplifying assumptions. For example, they assume a stable age distribution (see Charnov 1982) and equivalent mortality rates for males and females (Charnov 1982, 1986). Life-history invariant theory makes other more complicated assumptions about demographics (e.g., k/M, δ, and αM are invariant, Charnov and Skúladóttir 2000). For most groups of sex-changing animals, data are not available to test these assumptions.

Studies seeking to test these predictions often make another assumption for which data may more often be available. The most common assumption is that the individuals sampled represent a single population and that there is a single optimal size at sex change across that population. However sex-changing species often have populations with structured mating groups. For example, labrid fishes often form harems, anemone fish live in breeding pairs, and calyptraeid gastropods often form clusters or stacks. Therefore, a large sample of individuals, as would be collected to assess population sex ratio, probably represents a pool of several groups. If the individual composition of such groups varies, the optimal size of sex change should also vary among the groups. In species with labile sex change, where animals change sex in response to cues from conspecifics, a population sample that includes several groups would be expected to show considerable within-population variation in size at sex change. Such variation in size at sex change is common among calyptraeid gastropods (e.g., Collin 1995, 2000).

Here I use comparative data on size-sex distributions of calyptraeid gastropods to test the following predictions of sex allocation theory: (1) sex ratios are biased toward the first sex; (2) size at sex change/maximum size is less variable than expected at random; and (3) intraspecific variation in size at sex change is associated with social environment, such that species with more variation in the social environments to which different individuals are exposed show more variation in size at sex change than species in which all individuals are exposed to similar social environments.

MATERIALS AND METHODS

Natural History of Calyptraeids

Calyptraeid gastropods, or slipper limpets, are protandrous, filter-feeding limpets that occur commonly in the intertidal and shallow subtidal. The snails reproduce via copulation and females can store sperm. The females brood embryos that hatch as crawling juveniles in some species and planktonic larvae in other species (Collin 2003a). Several species form stacks of two or more individuals, where a small male usually attaches to the shell of a larger female. Large stacks

TABLE 1. Results of CONTINUOUS likelihood-ratio tests for significant differences between the maximum likelihood (ML) value of λ and the null hypothesis.

	Null hypothesis			
Variable	κ (branch length scaling)	$\lambda = 0$ (no phylogenetic effect)	$\lambda = 1$	ML estimate of λ
Sex ratio	0	$P = 1$	$P = 0.01$	0
	1	$P = 1$	$P = 1.5 \times 10^{-5}$	0
L_{50} and L_{max}	0	$P = 0.47$	$P = 0.002$	0.14
	1	$P = 0.83$	$P = 3.3 \times 10^{-7}$	0.18
L_{50}/L_{max}	0	$P = 0.89$	$P = 0.011$	0.43
	1	$P = 0.52$	$P = 0.006$	0.04
Proportion stacked	0	$P = 1$	$P = 0.021$	0
	1	$P = 1$	$P = 0.0035$	0
Relative size overlap	0	$P = 1$	$P = 0.03$	0
	1	$P = 1$	$P = 0.0032$	0
Proportion stacked and relative overlap	0	$P = 1$	$P = 0.005$	0
	1	$P = 1$	$P = 3.12 \times 10^{-5}$	0

of up to 10 or 12 individuals are known from several species, most notably *Crepidula fornicata*. These groups are often attached to limited substrates (e.g., shells or small cobbles) in muddy habitats and to other gastropods in rocky habitats. There are sometimes several stacks on each of these small substrates. Other species more commonly occur in monospecific layers or individually on any hard substrate.

Calyptraeids are generally sedentary. Small individuals are relatively mobile, but larger ones grow to fit the substrate to which they are attached and seldom move. This suggests that large males can only copulate with females in their immediate vicinity (i.e., adjacent individuals on the same substrate or stack-mates). Smaller males can move over hard substrates in the laboratory, but do not tend to move unless disturbed. The differences in potential mobility have been used to support the idea that small males may have an advantage over larger males by moving between females and may monopolize matings at the expense of the large males. However, there is no direct evidence that this occurs in the field. Individuals of most species live for several years, but the average life span or growth rate in the field are not know for any species.

Experimental studies of sex change in calyptraeids have demonstrated that the size at sex change is labile and is influenced by other animals in the immediate vicinity (Gould 1917, 1919, 1952; Coe 1938, 1948, 1953; Collin 1995, 2000; Warner et al. 1996; Collin et al. 2005). Small males grown alone or housed with another male changed sex more quickly and at smaller sizes than those housed with females (Collin et al. 2005). A modeling study concluded that models based on simple assumptions about the relationship between male size and reproductive output do not accurately predict the size at sex change in *C. fornicata* (Collin 1995). The size at sex change was consistently underestimated, suggesting that there is either an advantage to being male, such as higher growth rate or lower mortality rate, or that, because sex change is irreversible, the snails were bet hedging by changing later than appeared to be optimal (Collin 1995). There is some evidence that male *C. fornicata* grow faster than females of comparable size in caged field experiments; however, laboratory experiments did not find a similar relationship for three other species (Collin et al. 2005).

Data Collection

Samples of 19 species of abundant calyptraeids were collected from areas of less than 15 m^2 in the intertidal or subtidal from North and South America, New Zealand, and South Africa (see Appendix, available online only at http://dx.doi.org/10.1554/05-210.1.s1). For six of the species (*Crepidula atrasolea*, *Crepipatella dilatata*, *C. fornicata*, *Crepidula norrisiarum*, *Crepidula ustulatulina*, and *Bostrycapulus calyptraeformis*) two or three spatially distinct populations were sampled. In addition, *B. calyptraeformis* were sampled from two separate quadrats in a single location (Playa Chumical, Panama). All animals that were encountered from each sampled area were collected. Species that live attached to the shells of other gastropods were collected with their hosts, those on cobbles were collected attached to their substrates, and those that attach to large rocks were removed from the substrate in the field.

Shell-length of all animals collected from each site was measured to the nearest 0.1 mm with vernier calipers. Sex was determined by viewing each animal under a dissecting microscope and recording the presence or absences of a penis and/or a female genital papilla. Species of *Crepipatella* and *Bostrycapulus*, genera in which animals do not form a female genital papilla, were removed from the shell and the presence or absence of the female capsule gland and albumin gland were recorded. Small juveniles lacking both penis and female reproductive structures and transitional animals with both or neither structure were observed in most species. Since they do not contribute to the current breeding population, both juveniles and transitionals were excluded from subsequent analyses. For most species, transitional individuals accounted for less than 5% of the population (Table 1); however, transitional animals reached up to 22% of one population of *C. dilatata*. Populations of all the species except for *C. fornicata* (which were collected in the winter) included brooding females, indicating that the size and sex distributions represent snap-shots of a breeding population.

Analyses were conducted including all sampled populations because different populations within a species often differed significantly from each other and because most of the predictions should hold for all populations of all sex-

changing species. All analyses were repeated with only a single sample (that with the largest sample size) representing each species (Table 1). The results did not vary.

ANALYSIS

Sex Ratios

Sex ratios were calculated as the number of males divided by the total number of males and females. Significant deviations from 0.5 were tested for in each population using a χ^2-test. The overall bias toward the first sex was tested for in the datasets of 27 populations or 19 species by comparison to a binomial distribution. Logistic regression was used to test for associations between sex ratio and other population parameters (Wilson and Hardy 2002).

Size at Sex Change/Maximum Size

The size at which 50% of the animals were female was considered to be the size at sex change (referred to here as L_{50}). This was estimated using probit analysis (which gives identical results to the logistic regression approach used by Allsop and West 2003a,b). Maximum size was the size of the largest individual in the samples collected from that location.

Previous studies seeking to find the invariant relationship between size at sex change and maximum size (L_{50}/L_{max}) have relied on a regression approach. If the slope of the regression of $\ln(L_{50})$ on $\ln(L_{max})$ is equal to one, the ratio has been considered invariant because the relationship between the two variables is linear (Charnov 1993; Allsop and West 2003a,b). However, the same relationship is expected from random data for data bounded by $Y < X$, and therefore the recovery of a slope of one is not informative (Cipriani and Collin 2005). Cipriani and Collin (2005) suggested that variables with an underlying linear relationship would be expected to produce a regression with a higher r^2-value than random data and that the r^2 rather than the slope of the regression could be used to determine if there is an underlying relationship. This approach may be sensitive to the choice of the null model (Buston et al. 2004), and the regression model varies significantly when the range of the Y-values is smaller than the range in X or when the ranges only partially overlap (Cipriani and Collin 2005). These problems can be circumvented by comparing the variance in the observed L_{50}/L_{max} to the variance of bootstap datasets generated from the observed values of L_{50} and L_{max} (Cipriani and Collin 2005).

Here I use three approaches to generate null expectations of the variation in L_{50}/L_{max}. I generated null expectations by producing 1000 datasets of 19 and 27 pairs of randomly generated L_{50} and L_{max} drawn from a uniform distribution, with L_{max} constrained to vary within the observed range of L_{max} and L_{50} free to range between L_{max} and zero. Size at maturity could increase with maximum size and therefore further increase the strength of association between randomly derived expectations (Buston et al. 2004). Therefore, these analyses were repeated with the additional constraint that $L_{50} > 0.18L_{max}$ (the average size at maturity for the calyptraeid species used here is 18% of L_{max}). Finally, I generated 1000 datasets of 19 and 27 L_{50}/L_{max} subject to $Y < X$ by drawing each L_{50}-value from the list of observed L_{50}-values and each L_{max} from the observed L_{max}-values. For each randomly generated dataset of 19 or 27 values, I calculated the mean and standard deviation of the L_{50}/L_{max}. The grand means and two-tailed 95% confidence intervals for the mean L_{50}/L_{max} and standard deviations of L_{50}/L_{max} were calculated from the 1000 samples from each simulation and compared to the observed mean and standard deviations of L_{50}/L_{max}.

There is an error associated with the estimates of both L_{50} and L_{max} that is not usually taken into account in studies of life-history invariants. To determine if the observed L_{50}/L_{max} differed significantly between species, the error of the estimate of L_{50}/L_{max} was calculated for each species. Such differences would provide evidence that the differences between ratios are not statistical errors, but real differences between species. Because there is no adequate method for calculating the error on an extreme value (i.e., maximum size) from a single sample, errors on this ratio were calculated in two ways. One method included only an error on the size at sex change estimated by the probit analysis. This is an underestimate of the actual error because it does not include the error on the maximum size. Second, a program in R (written by R. Cipriani, Universidad Simone Bolivar) was used to calculate the size at sex change and L_{50}/L_{max} from 5000 bootstrap analyses of the data for each species. This has the advantage of calculating a compound error including both the error on the size at sex change and the error on the estimate of maximum size. The value of the maximum size is necessarily slightly underestimated in the bootstrap samples, and therefore the calculated values and errors will be biased upward. However, the uncertainty in the estimate due to differences in sample size will be captured. In practice there was little difference between the two estimates or the size of their errors.

Characterization of Sociality and Size Overlap

I used a correlation approach to determine if intraspecific variation in size at sex change is associated with intraspecific variation in social environment. The standardized size overlap between males and females was used as a proxy for variation in size at sex change. This was calculated for each species as the difference in size between the largest male and smallest female divided by the average size. Variation in social environment was assessed by four different measures because it is not known how calyptreaids assess their social situation. The population that was part of a stack would reflect the animals' perception of their social situation if snails assess their situation as a simple distinction between being stacked or unstacked. If the stack size is important, then the average number of snails in a stack, the range in stack size, and the standard deviation in stack size, would all reflect different aspects of the social environment (see Table 3). Because solitary individuals were collected in all species, the maximum number of individuals in a stack is equal to the range of stack size. Multiple regression analysis with stepwise removal of independent variables and a P-to-remove of 0.05 (Sokal and Rohlf 1995) was used to determine which of the four variables is most closely associated with variation in size at sex change.

Logistic regression was used to determine if animals in stacks changed sex at a different size than animals that lived alone. The significance of effects of body length and stacking status on sex were tested.

Phylogenetic Effects

Because shared evolutionary history may cause close relatives to be more similar than expected, it has become a common practice to correct for such phylogenetic effects by using methods such as independent contrasts. However, these procedures should not be employed if there are no phylogenetic effects in the data. Here I looked for phylogenetic effects by conducting a phylogenetic generalized least squares analysis with CONTINUOUS (Pagel 1997, 1999) and using a likelihood ratio test to test for a significant phylogenetic correlation (λ) and by examining the data to determine how the within-species variation compares to variation among species. CONTINUOUS analysis was based on a neighbor-joining tree of the 19 species that showed the same topological relationships as a pruned version of a larger analysis of calyptraeids (Collin 2003b). For traits evolving along branches following a Brownian motion model of evolution, the phylogentic correction (λ) of the trait values is expected to be one, while traits that are independent of the phylogeny show a phylogentic correlation (λ) of zero. The maximum likelihood value of λ was estimated using CONTINUOUS and likelihood ratio tests were used to test for significant differences from both $\lambda = 0$ and $\lambda = 1$, as recommended by Freckleton et al. (2002). Freckleton et al. (2002) demonstrated that for phylogenies of 20 or more taxa, the power of λ to detect phylogenetic dependence is greater than 90%. Therefore this test should have sufficient power to detect phylogenetic correlation for the datasets examined here.

RESULTS

Phylogenetic Effects

There were no statistically significant phylogenetic correlations for sex ratio, L_{max}, L_{50}, L_{50}/L_{max}, or stacking behavior (Table 1). In all cases the inclusion/exclusion of branch length information did not change the results. There was always enough power to reject the hypothesis that the phylogenetic correlation was as expected from a Brownian motion model of evolution along the tree topology (i.e., $\lambda = 1$). In most cases the maximum likelihood estimate of λ was zero (Table 1), showing that there is no evidence for a phylogenetic effect. In addition, these traits often varied among populations of the same species (Table 2), as would be expected for a labile trait. Therefore, there is little evidence to justify the use of phylogenetically corrected data, and all subsequent analyses treated each species as an independent datapoint.

Sex Ratios

Thirteen of the 27 populations had sex ratios that could not be distinguished from 0.5 on the basis of a χ^2-test (Table 2). Eight populations (seven species including two populations of C. fornicata) had a statistically significant male (first sex) bias, and six populations (four species including three

populations of C. dilatata) had significant female bias. Examination of all populations or a single population per species shows that sex ratios across calyptraeids are not significantly more biased toward the first sex than expected at random (P > 0.05 for binomial expectation for 13 vs. 12 for 25 populations or 10 vs. eight for 18 species). Samples with a 0.5 sex ratio were excluded from the analysis. The range and extremes of observed sex ratios (0.05–0.91; Fig. 1, Table 2) was greater for these calyptraeids than for the 40 species of various protandrous fish and invertebrates included in a recent review of sex-changing animals (0.11–0.89; Allsop and West 2004).

Different populations from seven of the eight species for which there were multiple samples showed the same bias. The single exception was for B. calyptraeformis, which had one significantly male-biased population and two populations in which there was no significant bias. Populations of C. ustulatulina and C. fornicata had sex ratios that differed significantly from each other, while showing the same direction of bias. In addition, one of the pairwise comparisons among three C. dilatata populations and one among three B. calyptraeformis populations showed significantly different sex ratios (P < 0.05).

Logistic regression across all populations showed that sex ratio was significantly associated with proportion stacked (P < 0.0001), skew in the size distribution (P < 0.0001), mode of development (P < 0.0001), L_{50}/L_{max} (P < 0.0001), but not with maximum body size (P > 0.1). Skew in the size distribution and L_{50}/L_{max} had the largest effect (Fig. 2), but all of the effects remained significant according to likelihood ratio tests with stepwise addition or subtraction from the multiple regression model. When only a single population for each species was included, sex ratio was significantly associated with proportion stacked, skew in the size distribution, mode of development, L_{50}/L_{max}, and maximum body size (P < 0.001).

Size at Sex Change/Maximum Size

The range of L_{50}/L_{max} values for calyptraeids (0.33–0.72; Table 2; Fig. 3) is lower and slightly less variable than that reported for 77 sex-changing animals (0.47–0.96) and for 52 fishes (0.52–0.95; data from Allsop and West 2003a,b).

Comparisons of the observed data with randomly generated data and the bootstrap datasets made from randomizations of the observed L_{50}- and L_{max}-values shows that the mean of L_{50}/L_{max} for calyptraeids does not differ from the random expectations, but the standard deviation of these values is significantly smaller than expected (Table 3). Repeating the analysis for all 104 animals for which data are currently available (calyptraeids plus the 77 other animals from Allsop and West 2003) shows that mean L_{50}/L_{max} is significantly larger than random and the standard deviation is significantly smaller than random (Table 3). The significantly small standard deviation suggests that L_{50} and L_{max} may have an underlying relationship; however, the standard deviation is only slightly smaller than expected, suggesting that the effect is minimal. If L_{50} was further constrained to be less than 85% or 90% of the maximum recorded size (a realistic assumption), the observed ratios would be difficult to distinguish

TABLE 2. Summary of the sex ratio, size at sex change, and size distribution of males and females for 27 populations of calyptraeid gastropods. Names in bold indicate the populations with the largest samples sizes, which were retained in the among-species analyses.

Species	Number of breeding animals	Number of transitional animals	Sex ratio (male/total)	L_{50} (CI)		Smallest male	Largest male	Smallest female	Largest individual (L_{max})	L_{50}/L_{max}	Proportion stacked	Largest stack	Average stack size	Standard deviation of stack size
Bostrycapulus aculeatus	256	8	0.64**	13.6	(13.1–14.2)	4.3	17.8	9.7	27.2	0.50	0.12	3	1.1	0.28
Crepidula adunca	570	10	0.64****	9.7	(9.5–10.0)	4.2	10.7	7.0	19.0	0.51	0.59	3	1.3	0.59
Crepidula atrasolea 1	212	19	0.48	7.34	(7.0–7.8)	2.2	8.2	5.7	21.6	0.34	0.38	3	1.2	0.50
C. atrasolea 2	200	27	0.54	7.3	(6.8–7.9)	2.8	9.5	3.9	16.9	0.43	0.31	3	1.2	—
Bostrycapulus calyptraeformis 1	174	5	0.60	15.3	(14.7–16.0)	7.0	19.7	12.7	23.1	0.66	NA	3	—	—
B. calyptraeformis 2	104	0	0.50	15.3	(14.7–15.9)	4.9	17.8	14.3	23.0	0.66	NA	3	—	—
B. calyptraeformis 3	224	12	0.64**	13.7	(13.1–14.3)	4.9	17.7	10.0	21.9	0.63	NA	3	—	—
Crepidula complanata	152	11	0.49	14.7	(13.9–15.5)	4.0	17.8	11.7	31.7	0.46	0.65	10	1.7	1.41
Crepidula depressa	135	36	0.67**	9.9	(8.9–10.9)	2.4	13.4	8.2	29.9	0.33	0.45	5	1.2	0.69
Crepipatella dilatata 1	199	56	0.05***	10.8	(9.1–11.5)	5.9	12.1	10.4	25.7	0.42	NA	—	—	—
C. dilatata 2	317	47	0.10***	14.6	(11.5–15.7)	6.0	15.9	14.4	33.6	0.44	0.06	2	1.0	0.23
C. dilatata 3	373	11	0.07***	18.4	(17.1–19.3)	11.6	24.5	15.0	35.8	0.51	0.29	6	1.6	0.87
Crepipatella fecunda	217	5	0.50	36.6	(34.4–38.6)	7.9	51.7	27.9	58.7	0.62	0.81	4	1.8	0.83
Crepidula fornicata 1	688	16	0.59**	30.6	(29.8–31.4)	6.6	40.8	20.7	45.4	0.67	0.98	7	3.2	1.36
C. fornicata 2	198	10	0.70***	19.6	(18.5–20.9)	6.8	31.7	14.8	43.1	0.45	0.96	13	2.8	2.18
Crepidula incurva	327	11	0.40*	8.1	(7.8–8.3)	3.1	9.8	6.3	18.1	0.45	0.54	3	1.4	0.58
Crepipatella lingulata	652	5	0.48	11.5	(11.2–11.8)	3.4	15.6	7.9	26.8	0.43	0.02	2	1.0	0.14
Crepidula naticarum	301	4	0.91***	13.0	(12.0–14.5)	3.5	16.7	7.7	28.5	0.46	0.44	10	1.4	1.25
Crepidula norrisiarum 1	108	0	0.47	20.1	(18.6–21.7)	5.1	27.7	12.3	39.9	0.51	0.57	4	1.5	0.58
C. norrisiarum 2	114	3	0.53	12.8	(12.0–13.7)	5.7	15.2	7.1	24.9	0.51	0.48	3	1.4	0.46
Crepidula onyx	89	14	0.65*	14.7	(13.4–16.1)	2.7	18.2	13.1	35.8	0.41	0.04	2	1.0	0.16
C. cf. onyx	91	16	0.21***	13.8§		3.8	13.9	13.3	29.6	0.47	0	1	1	0
Crepidula ustulatulina 1	106	4	0.58	6.8	(6.4–7.2)	3.1	8.3	5.9	11.6	0.59	0.54	4	1.5	0.78
C. ustulatulina 2	108	3	0.43	5.5	(5.2–5.6)	3.1	6.1	5.0	9.4	0.58	0.48	3	1.3	0.52
Crucibulum personatum	181	18	0.54	18.0	(17.5–18.5)	5.4	21.1	16.4	25.9	0.70	0.01	2	1.0	0.07
Maoricrypta monoxyla	267	7	0.54	11.7	(11.2–12.1)	3.1	13.1	10.3	21.1	0.56	0.71	3	1.6	0.57
Zeaglarus tenuis	63	4	0.25**	6.5§		4.0	6.2	6.8	12.1	0.54	0	1	1	0

$* \; P < 0.05$; $** \; P < 0.01$; $*** \; P < 0.005$.

§ Likelihood estimate did not converge and therefore confidence interval was not calculated.
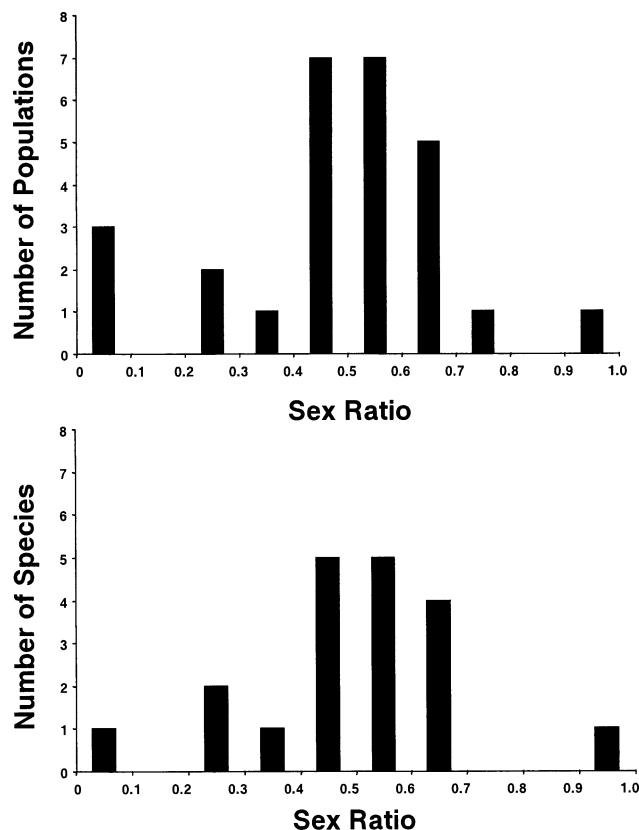
FIG. 1. Histogram showing the distribution of sex ratios of (A) all 27 populations examined and (B) the 19 species. Data are from Table 1. Sex ratios >0.5 are male bias and those <0.5 are female biased.
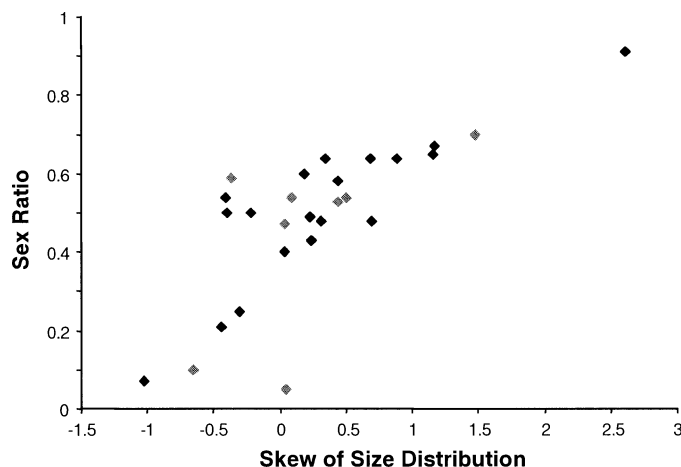

FIG. 2. Scatter plot of skew of the size distribution for each population versus sex ratio for that population. See text for details of logistic regression analysis. The black points are the values for single populations chosen to represent each species.

from random. Further evidence that statistical invariance is minimal comes from the combined dataset of calyptraeids and other animals. There is a significant difference in L_{50}/L_{max} between protandrous and protogynous species, with protandrous species changing sex at a smaller size relative to their maximum size (protandrous L_{50}/L_{max} = 0.61, protogynous L_{50}/L_{max} = 0.78, t-test, P = 0.0001). This difference was predicted by Gardner et al. (2005).

Confidence intervals estimated on the basis of probit analysis alone showed that many of the L_{50}/L_{max} values are significantly different from each other (Fig. 4). Bootstrap analysis of the same data gave slightly higher values for each ratio (as expected from the bootstrap replicates slightly underestimating the maximum size) and the confidence intervals were slightly larger. In a few cases where several taxa had similar values of L_{50}/L_{max}, the rank order of the taxa changed, but the overall pattern remained the same (Fig. 4). There are numerous values of L_{50}/L_{max} for which the confidence intervals do not overlap and in the cases of *C. atrasolea*, *C. dilatata*, and *C. fornicata* the values for different populations are significantly different. Again, this provides support for the idea that L_{50}/L_{max} varies within and between calyptraeid species.

Social Structure

Variation in size at sex change as measured by size overlap between males and females was correlated with social struc-

ture. All four variables reflecting intraspecific variation in social experience showed a significant association with this measure of variation in size at sex change when examined independently across all populations (proportion stacked: r^2 = 0.23, P < 0.05; range in stack size: r^2 = 0.43, P < 0.0005; average number in a stack: r^2 = 0.21, P < 0.05; standard deviation of stack size: r^2 = 0.42, P < 0.001). Multiple regression with stepwise removal of nonsignificant factors showed that only range in stack size had a significant effect in combination with the other factors (Fig. 5). When only a single population was included for each species, range in stack size and standard deviation in stack size were the only factors that were significant alone, and range in stack size was the only significant factor in the multiple regression (range in stack size: r^2 = 0.36, P < 0.01; standard deviation of stack size: r^2 = 0.42, P < 0.05).
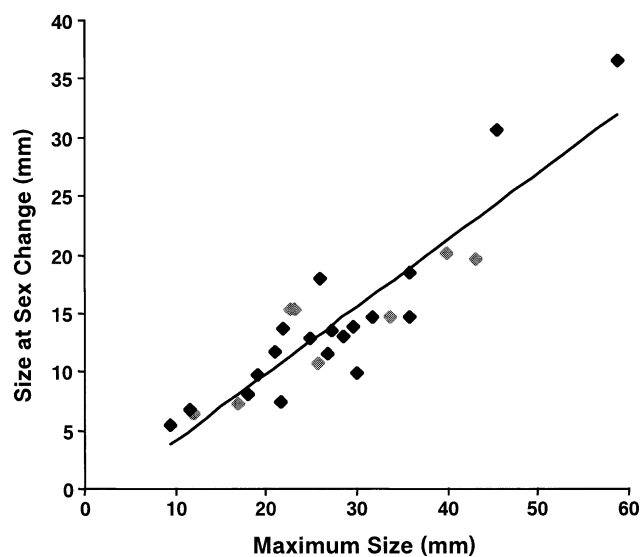

FIG. 3. Scatter plot of size at sex change versus maximum size. OLS r^2 = 0.82, P = 0.005, n = 27. The black points are the values for the single populations chosen to represent each species.

TABLE 3. L_{50}/L_{max} for 27 populations or 19 species of calyptraeids compared to random expectations.

Dataset	Sample size	L_{50}/L_{max} mean (CI)	L_{50}/L_{max} SD (CI)
Calyptraeid data			
Bootstrapped observed data	27	0.47 (0.398, 0.552)	0.20 (0.151, 0.247)
	19	0.47 (0.389, 0.567)	0.20 (0.143, 0.253)
	104	0.31 (0.256, 0.331)	0.27 (0.235, 0.293)
Random simulated data	27	0.51 (0.405, 0.615)	0.28 (0.233, 0.330)
	19	0.51 (0.384, 0.635)	0.28 (0.217, 0.342)
	104	0.51 (0.459, 0.563)	0.28 (0.258, 0.306)
Random simulated data with size at maturity	27	0.59 (0.499, 0.678)	0.23 (0.192, 0.278)
	19	0.59 (0.481, 0.699)	0.23 (0.184, 0.284)
Populations of calyptraeids	27	0.51	0.10*
Species of calyptraeids	19	0.51	0.10*
All animals	104	0.68*	0.16*

* Significantly different ($P < 0.05$) from random expectations.

Analyses treating stacked and unstacked individuals separately showed that there is often (nine of 20 populations) a significant effect of stacking on sex change (Table 4). Of the 20 populations for which appropriate data were available, five showed a significant difference in sex ratio between stacked and unstacked animals. There was no consistent direction of change with *C. adunca* and *C. naticarum* having higher sex ratios in the unstacked animals and *C. incurva*, *C. norissiarum*, and *C. ustulatulina* having higher sex ratios in stacked animals (Table 4). Logistic regression detected an effect of stacking on size at sex change (L_{50}) in six of 20 populations. In this case, all populations with significant differences showed a larger size at sex change in stacked than in solitary individuals animals. The power to detect a difference was low for several of the datasets, where no effect was detected because there were few animals in one of the categories (Table 4).

DISCUSSION

Variation in Sex Ratio and L_{50}/L_{max}

Compilations of data from the literature on sex-changing fishes, molluscs, crustaceans and echinoderms show large variation in sex ratio and L_{50}/L_{max} (Allsop and West 2003a,b, 2004). It could be argued that this variation is due largely to combining protandrous with protogynous, sedentary with mobile, and social with solitary organisms and that restricting the data to animals from a single clade would improve the fit to theory. This idea receives some support from the significant difference in L_{50}/L_{max} for protandrous and protogynous species recorded here and from the difference in sex ratio between the two types of sex change (Allsop and West 2004).

Even when protandrous and protogynous species are treated separately, sex ratio and L_{50}/L_{max} are still highly variable. When the analysis is limited to calyptraeids, a monophyletic
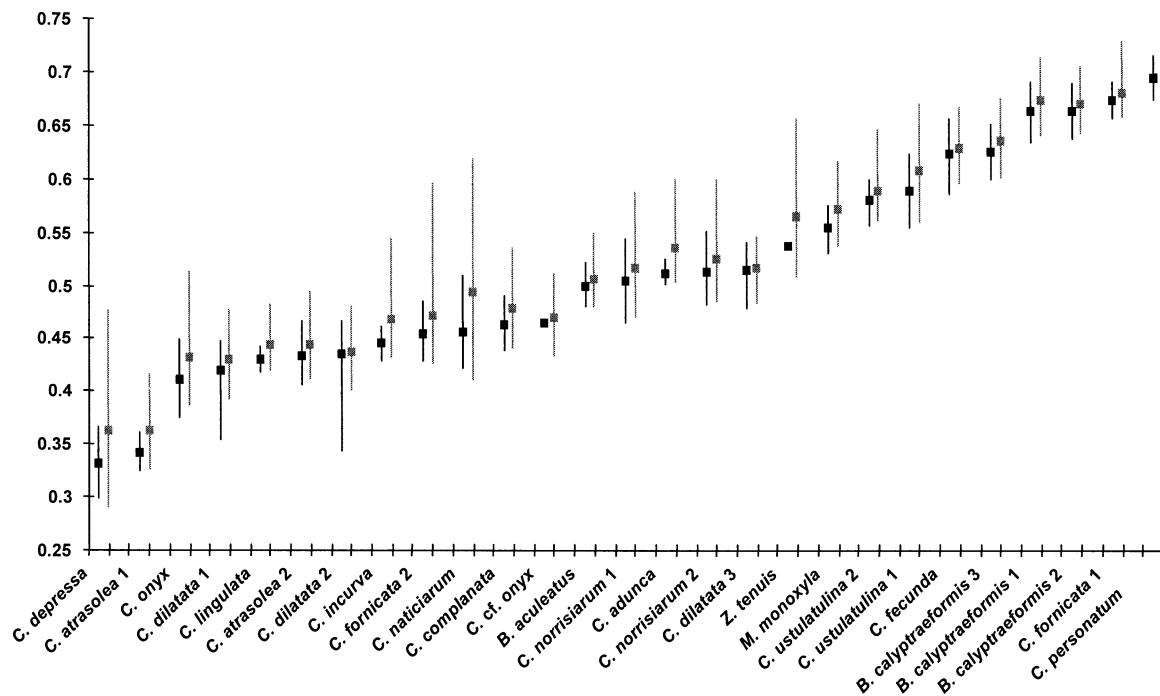

FIG. 4. L_{50}/L_{max} for all 27 populations of calyptraeids. Error bars indicate 95% confidence intervals. Points in black show the error of the results using probit analysis, and points in gray show the estimate and compound error based on resampling.
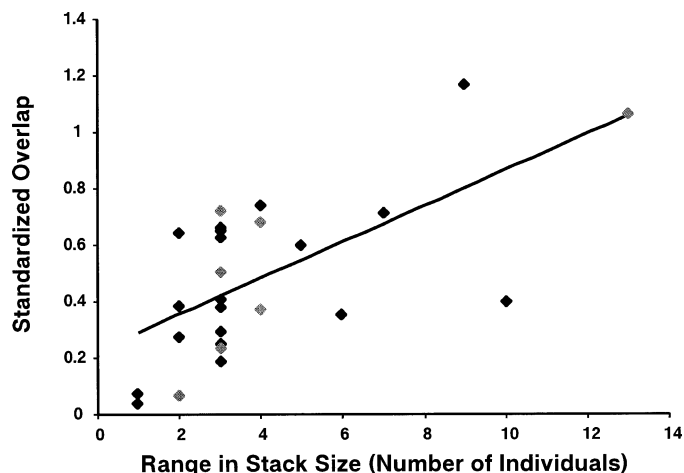
FIG. 5. Scatter plot of size overlap between males and females as a proxy for variation in size at sex change versus the range in stack size; OLS regression $r^2 = 0.40$, $P = 0.005$, $n = 26$. The black points are the values for the single populations chosen to represent each species.

family in which all species are sedentary and protandrous, both quantities remain highly variable. In fact, the range of sex ratios is larger for the 27 observed populations of calyptraeids than it is for 121 sex-changing animals (Allsop and West 2004). Because the sex ratios reported by Allsop and West were averages of available populations, the range is likely to be slightly underestimated (the original raw data are not given). Data from different calyptraeid populations were not averaged here, since one of the basic assumptions of sex allocation theory for environmentally mediated sex change is that sex can change at different sizes or ages in response to environmental conditions. Therefore, there is not a single sex ratio or L_{50}/L_{max} characteristic of each species. The idea that these values can vary intraspecifically is supported by the calyptraeid data that show significant differences among populations for sex ratios and L_{50}/L_{max}.

Assumptions of the Theory

The considerable, unpredicted variation in sex ratio and L_{50}/L_{max} in samples from a single taxon suggests that calyptraeids violate the basic assumptions of sex allocation theory. The prediction that sex ratio should be biased toward the first

sex is based on minimal assumptions and is not altered by size-specific mortality or growth rates (Charnov and Bull 1989). The main underlying assumption is that both male and female fertility increases with size (Charnov and Bull 1989). Data on egg production for several species of calyptreaids make it clear that female fertility increases with size (e.g., Collin 1995, 2000; Chaparro et al. 1999; Chaparro and Flores 2002). Unfortunately no data are available for male fertility. A male-biased sex ratio, consistent with the idea that both sexes have increasing fertility with size, is observed for several calyptraeids. The female-biased sex ratios observed for other species imply that male fertility at the size of sex change is less than average male fitness. Therefore, it is likely that male fertility decreases with size in these species. Such a decrease could be explained by the difference in mobility between large and small males that has been suggested for some species. Whatever the mechanism, it is clear that the shape of the relationship between male fertility and size probably differs between species, increasing in some and decreasing in others. The predictions of first-sex-biased sex ratios could also be altered by high mortality during the transition phase. This is unlikely to explain the lack of fit for calyptraeids, as laboratory studies have not found different mortality rates in transitional animals (Collin et al. 2005).

The theoretical derivation of L_{50}/L_{max} as an invariant relies on the assumptions that k/M, αM, and δ are constant across the species examined. The large variation in L_{50}/L_{max} among calyptraeids suggests that at least one of these three values is not constant across species. Unfortunately, there are no data currently available on natural mortality rate (M), von Bertalanffy growth coefficients (k), or age at maturity (α) for calyptraeids. There are also no data pertaining directly to δ values for calyptraeids. However, the sex ratio data reported here indicates that there are differences in the shape of the male fertility curves, providing indirect evidence that one of the basic assumptions of the original theoretical derivation of L_{50}/L_{max} as a life-history invariant is violated. The simulations of Gardner et al. (2005) suggested that the derivation of L_{50}/L_{max} as a life-history invariant is relatively robust to variation in δ. However, a number of their simulations used the flawed regression approach to the analysis of bounded variables. In addition, their sensitivity analyses follow their formal model and varied each presumed constant (k/M, αM, and δ) one at a time. Because the formal derivation that

TABLE 4. Propensity of calyptraeids to form stacks and aggregations.[1]

Species	Sex ratio of stacked vs. unstacked animals (P of χ^2-test)	L_{50} unstacked vs. L_{50} stacked (P of logistic regression)
Crepidula adunca	0.53 vs. 0.80 ($P < 0.0001$)	ns
Crepipatella dilatata 3	ns	16.7 vs. 19.8 ($P = 0.04$)
Crepipatella fecunda	ns	29.1 vs. 37.5 ($P = 0.002$)
Crepidula fornicata 1	ns	23.9 vs. 30.8 ($P = 0.0035$)
Crepidula incurva	0.53 vs. 0.23 ($P < 0.001$)	7.1 vs. 8.5 ($P < 0.0001$)
Crepipatella lingulata	ns	$P = 0.048$[2]
Crepidula naticarum	0.81 vs. 0.99 ($P < 0.001$)	ns
Crepidula norrisiarum 1	0.56 vs. 0.37 ($P = 0.04$)	ns
Crepidula ustulatulina 2	0.51 vs. 0.33 ($P = 0.05$)	5.2 vs. 5.6 ($P = 0.07$)

[1] Species with appropriate data but no significant results: *Bostrycapulus aculeatus*, *Crepidula atrasolea* 1, *Crepidula atrasolea* 2, *Crepidula complanata*, *Crepidula depressa*, *Crepipatella dilatata* 2, *Crepidula fornicata* 2, *Crepidula norrisiarum* 2, *Crepidula onyx*, *Crepidula ustulatulina* 1, *Maoricrypta monoxyla*.
[2] Sample of stacked too small to estimate L_{50} separately.

produced the prediction of an invariant L_{50}/L_{max} also predicted an invariant breeding sex ratio (Gardner et al. 2005, p. 564), which is a demonstrably highly variable in both calyptraeids (this paper) and across sex-changing animals (Allsop and West 2004), something about the model clearly does not accurately capture what is going on in nature. Studies designed to examine the relationship between male size and fertility directly, as well as real demographic data and not derived averages from a few distantly related species, are necessary before the interspecific variation in k/M, αM, and δ can be assessed and included in simulations of size at sex change.

Life-History Invariants in Sex Changing Animals

Life-history invariants are based on the idea that two features of life histories may have a relationship that is constant under a variety of transformations. This is similar to familiar physical or physiological allometries such as the scaling between body size and metabolic rate. Body size explains a lot of the variation in metabolic rate despite variation in, for example, color, family, or brood size. The invariants specifically examined by Charnov (1993) are dimensionless ratios (implying that the relationship between the two is linear) such as yearly clutch size/instantaneous mortality. Just as physical scaling relationships may explain a large or small portion of the variance in the data, the proposed life-history invariants may be tight or noisy. In cases where the variables are mutually unconstrained the r^2-value reflects the strength of the relationship, and high r^2-values reflect a good fit to the theory if the relationship is a linear.

Allsop and West (2003a) interpreted a log-log slope of one and an r^2 of 0.98 as evidence of universal invariance of L_{50}/L_{max}. They comment how amazing it is that this rule holds over all animals and conclude that k/M, αM, and δ must therefore also be invariant across all the taxa examined. As described above, linear regression statistics of bounded variables cannot be interpreted literally, but it is true that L_{50}/L_{max} is less variable than expected at random (Cipriani and Collin 2005; and above), suggesting some underlying relationship between the two variables. However, there are several lines of evidence that question the interpretation of this ratio as an invariant, not only for calyptraeids, but for the dataset examined by Allsop and West (2003a,b). First, if the same invariant relationship holds for both protandrous and protogynous species there should not be a significant difference in L_{50}/L_{max} between species with each strategy. However, both Allsop and West's (2003a,b) original data and their data combined with the calyptraeid data show significant differences in L_{50}/L_{max} between protandry and protogyny (original data: 0.78 vs. 0.67; $P = 0.0001$; $n = 77$). Secondly, the same theory from which the invariance of L_{50}/L_{max} is derived also predicts an invariant sex ratio. Allsop and West (2004) demonstrated that there is significant variation in sex ratio across the same sex-changing animals for which they believe L_{50}/L_{max} is invariant and sex ratio also varies significantly in calyptraeids. It will be difficult to identify the causes of these inconsistencies, until explicit measures of k, α, M, and δ are available, and it will indeed be surprising if they do not vary among all sex-changing animals. The significant differences

in L_{50}/L_{max} between different populations of the same species of calyptraeids suggests that these values may not even be constant across different populations of the same species. With so much variation in L_{50}/L_{max}, the faint signal of an underlying relationship might more reasonable be considered a curiosity rather than a biological law.

Sociality and Variation in Size at Sex Change

Experimental evidence shows that associations with conspecifics affects the size at sex change in several calyptraeid species (Gould 1917, 1919, 1952; Coe 1938, 1948, 1953; Collin 1995, 2000; Warner et al. 1996; Collin et al. 2005). Males experimentally placed with females change sex at larger sizes than those placed with other males or those alone. The field-collected data presented here is consistent with this, showing that stacked individuals change sex at larger sizes than solitary animals of the same species, and that sex ratios often differ for stacked and unstacked animals. There is also a correlation between the amount of stacking and variation in size at sex change, with up to 40% of the interspecific variation in variation in the size at sex change being explained by range in stack size. All of these lines of evidence support the idea that animals in the field experience difference social environments and alter the size at sex change in response to their interactions with conspecifics. Therefore, it seems likely that populationwide analyses may obscure interesting patterns and that analyses on the level of aggregations or stacks may be a fruitful direction for future research on sex change in calyptraeids.

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Corresponding Editor: D. Promislow