

SPERM COMPETITION AND SEX CHANGE: A COMPARATIVE ANALYSIS ACROSS FISHES

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Current theory to explain the adaptive significance of sex change over gonochorism predicts that female-first sex change could be adaptive when relative reproductive success increases at a faster rate with body size for males than for females. A faster rate of reproductive gain with body size can occur if larger males are more effective in controlling females and excluding competitors from fertilizations. The most simple consequence of this theoretical scenario, based on sexual allocation theory, is that natural breeding sex ratios are expected to be female biased in female-first sex changers, because average male fecundity will exceed that of females. A second prediction is that the intensity of sperm competition is expected to be lower in female-first sex-changing species because larger males should be able to more completely monopolize females and therefore reduce male–male competition during spawning. Relative testis size has been shown to be an indicator of the level of sperm competition, so we use this metric to examine evolutionary responses to selection from postcopulatory male–male competition. We used data from 116 comparable female-first sex-changing and nonhermaphroditic (gonochoristic) fish species to test these two predictions. In addition to cross-species analyses we also controlled for potential phylogenetic nonindependence by analyzing independent contrasts. As expected, breeding sex ratios were significantly more female biased in female-first sex-changing than nonhermaphroditic taxa. In addition, males in female-first sex changers had significantly smaller relative testis sizes that were one-fifth the size of those of nonhermaphroditic species, revealing a new evolutionary correlate of female-first sex change. These results, which are based on data from a wide range of taxa and across the same body-size range for either mode of reproduction, provide direct empirical support for current evolutionary theories regarding the benefits of female-first sex change.

KEY WORDS: Hermaphrodite, mating system, protogyny, reproductive model, sex allocation theory, size-advantage model, Teleostei.

Sex change is the sequential and functional expression of both sexes during a lifetime (Sadovy and Shapiro 1987; Wasson 1999) and can occur in either direction (male-first and female-first), and in some species it can be reversed (e.g., Munday et al. 1998). Sex change is exhibited by a wide range of animals including molluscs, crustaceans, and annelids (Policansky 1982). It is unusual in vertebrates, most of which have fixed, separate sexes (i.e., gonochorism or dioecy). Exceptions occur in teleost fishes where sex change is taxonomically widespread (Smith 1975; Policansky 1982).

Current theory regarding the adaptive benefit of sex change focuses on differences between males and females in their respective relationships between body size and reproductive success (Ghiselin 1969; Warner 1975; Warner et al. 1975; Leigh et al. 1976; Charnov 1982; Gardner et al. 2005). Specifically, sex change is thought to be beneficial when the size–success relationships differ between the sexes so that at small size it is most effective to reproduce as one sex, whereas at a larger size, individuals are more successful as the other sex. Female-first sex change is predicted to be adaptive when reproductive success increases with age/size faster in males than in females (Warner 1975; Leigh et al. 1976). Such a sexual asymmetry in size–success relationships could arise, for example, if males are able to monopolize access to females by guarding limited resources or groups of females and reducing the level of postcopulatory male–male competition (Taborsky 1998). In such mating systems, smaller males can be entirely excluded from reproductive attempts, resulting in highly skewed individual fertilization and reproductive success for large males.

Given that the relative number of males and females in a population should be inversely related to the relative reproductive success of each sex (Charnov 1993; Allsop and West 2004b), it follows that when second-sex (i.e., male) fertility exceeds first-sex (i.e., female) fertility, sex ratios should be female biased (Allsop and West 2004b). Female-biased sex ratios and the monopolization of access to females by large males should reduce the potential for sperm competition during spawning (Taborsky 1998) and bias fertilization success toward larger males. We test this prediction by measuring the intensity of sperm competition that a species sustains to determine whether there has been relaxed selection from male:male competition under female-first sex change, which would support the size-advantage model.

Support for current theory regarding the adaptive benefits of sex change is plentiful but is almost always specific to small taxonomic units and rarely considers phylogenetic nonindependence of data (e.g., Warner 1984; Cowen 1990; Wernerus and Tessari 1991; Tsai et al. 1999; Allsop and West 2004a; but see for a notable exception Allsop and West 2004b). Female-first sex change occurs in a taxonomically diverse array of ray-finned fishes (Actinopterygii) and, in several cases, closely related species have evolved either hermaphroditism or gonochorism (Smith 1975; Policansky

1982; Devlin and Nagahama 2002). Furthermore, the phylogeny of this class is becoming increasingly well resolved. The availability of phylogenetic as well as reproductive and life-history data on fishes makes this group exceedingly valuable for tackling evolutionary questions in a phylogenetic context (e.g., Goodwin et al. 1998, 2002; Reynolds et al. 2002, 2005; Allsop and West 2003; Kolm et al. 2006; Mank and Avise 2006a, 2006b; Mank et al. 2006). Here, we use Actinopterygii species to test two predicted correlates of female-first sex change. First, we predict that breeding sex ratios should be more female biased in populations of female-first sex changers than in gonochorists. Second, we predict that reduced postcopulatory male–male competition associated with a male body-size advantage in fertilization would be manifested as reduced selection on relative testis size (but not ovary size) in female-first sex changers.

Materials and Methods

CATEGORIZATION OF REPRODUCTIVE MODE

We performed an extensive literature search on fish reproductive mode using existing reviews of hermaphroditism and ISI Web of Knowledge searches. Reproductive mode was categorized as gonochorism or female-first sex change. We only categorized a species as the latter if sufficiently detailed histological and/or behavioral analyses had been performed to fulfill the criteria described by Sadovy and Shapiro (1987) (Table 1). We categorized sex changers as monandric (all individuals change sex) or diandric (some individuals mature directly as male) where these reproductive systems had been clearly identified. If a species was reported to be hermaphroditic, but the criteria of Sadovy and Shapiro (1987) had not been met, we did not include it in analyses. Gonochorism in fishes is not an exceptional discovery and is therefore rarely reported. We categorized a species as gonochoristic if histological analyses of gonads had been performed and (1) gonochorism was reported, (2) hermaphroditism was reported as abnormal, (3) hermaphroditism was not mentioned, or (4) in gobies (Perciformes: Gobiidae), the criteria of Cole (1990) for identifying gonochorism had been met (Table 1).

SEX RATIOS AND SPERM COMPETITION LEVELS

Sex ratios were recorded as the proportion of the mature population that is male, that is, males/(males + females). Because sexual allocation theory predicts breeding sex ratios, the ratio of mature males and females was recorded where possible. If sex ratios were available for several populations of the same species we calculated an average. For seasonal breeders, operational sex ratios were recorded as the average sex ratio during the breeding season. If a range was reported, we recorded the median.

Intensity of sperm competition can be measured via its correlation with relative male gametic/gonadal investment, which is

Table 1. Summary of the criteria suggested by Sadovy and Shapiro (1987) for identifying hermaphroditism in fishes, and the criteria suggested by Cole (1990) for identifying gonochorism in gobies (Perciformes: Gobiidae).

Reproductive mode	Criterion
Female-first sex change	
<i>Monandry</i> . One male type: all males previously functioned as females	Presence of a membrane-lined lumen within a testis that is not used for sperm transport and homologous to a similar lumen in females used to egress eggs. Or, in porgies (Sparidae), remnants of a former ovary attached to testis. Sperm ducts or sinuses run longitudinally through the gonad wall. Presence of stages 1, 2, or 3 atretic ova in testes. Presence of transitional individuals. Experimental, nonhormonal induction of sex change.
<i>Diandry</i> . Two male types: “secondary” males previously functioned as females; “primary” males mature as males	Secondary males as for “Monandric” above. Primary testes do not contain ovarian features and usually have sperm ducts that run laterally through the gonad wall.
Gonochorism in gobies (Cole 1990)	Ovarian tissue does not contain precursive accessory gonad structures (pAGS) accessory gonad structures (AGS) developing spermatogenic tissue pAGS are tissue masses that are associated with the ventral ovary wall. These develop into AGS, which are secretory structures on testes after sex change.

well established across species, populations, and even individuals (Stockley et al. 1997; Taborsky 1998; Weddell et al. 2002). The male gonadosomatic body plan is shaped by sperm competition with elevated levels of competition selecting for males to partition greater investment into gonadal tissue and gamete production. We recorded male gonad weight (GW) divided by total body weight (BW), to yield gonadosomatic investment (GSI). Gametic investment was occasionally reported as the ratio of gonad weight divided by somatic weight ($BW - GW$), GSR. We converted GSR to GSI using the formula $GSI = GSR / (1 + GSR)$. Other measures of reproductive investment could not be converted to GSI and were not used. To control for seasonal variation in reproductive investment, we recorded the seasonal peak mean GSI. If a range was reported, we recorded the median value. We did not include a species if a mean or range was not given.

Some sex-changing species, known as diandric species, contain two types of males: primary and secondary. Primary males never function as females; instead, they mature directly as males. As juveniles, these males may have possessed immature ovarian tissue, but this does not develop into functional gonads. Because “sex change” has a strictly functional definition (Sadovy and Shapiro 1987; Warner 1988; Francis 1992; Wasson 1999), these males are considered gonochoristic (Reinboth 1970; Warner 1975; Warner and Robertson 1978; Sadovy and Shapiro 1987; Francis 1992). To compare male gametic investment between sex

changers and non-sex changers (gonochorists), we only used GSI values for the males of diandric species that had changed sex (“secondary” males) ($n = 8$ species). We did not use male GSI for diandric species if the data did not differentiate between male types ($n = 1$). In some species, males exhibit two distinct color phases: initial and terminal. These phases are associated with different spawning strategies: initial-phase males usually group- or streak-spawn whereas terminal-phase males usually defend territories or harems and pair-spawn (Robertson and Warner 1978; Warner and Robertson 1978; Warner 1982; Warner and Lejeune 1985). In keeping with our predictions, terminal-phase males are usually secondary males and initial-phase males are usually primary males. However, because exceptions do occur, we included all available data on secondary (sex-changed) males, irrespective of color phase. Where mean male GSI was reported separately for secondary males of different color phases or sizes (e.g., Warner and Lejeune 1985), we calculated a mean using the average GSI for each size or morph weighted by the number of individuals of that size group or color phase.

To avoid any biases from uncontrolled allometric relationships between testis and body weights (Stockley et al. 1997), we also compared the relationship between body weight (\overline{BW}) and gonad weight (\overline{GW}) for an average-sized male of each species for which we had male GSI data. We predicted that male \overline{GW} in female-first sex changers would be lower than that of similarly

sized non-sex changers across all values of male \overline{BW} . Male \overline{GW} was calculated by multiplying male GSI by the average body weight of the males used in the study that reported GSI. When only male length was reported, we recorded average total length (cm) (converting from standard or fork length where necessary), from which we calculated average weight (g). Species-specific length-length and length-weight relationships were either taken from the same study as the original data or from Froese and Pauly (2005). If either relationship was not available for a species, we used the relationship from a similarly sized close relative ($n = 12$). When only a range of weights or lengths was presented, we recorded the median value. When only the frequency of males occurring in different length classes was presented, we calculated an overall mean using the median values of each size class weighted by the number of males in that size class. When only a length-frequency histogram was presented, we recorded the median value of the modal size class. As with our collection of male GSI, we only used data on secondary males of diandric species for this analysis.

To confirm the selective pressure on males toward decreased gametic production in female-first sex changers, we compared differences in gametic investment by males to that of females. Female GSI, female \overline{GW} , and \overline{BW} were recorded using the same methods described above for males.

ANALYSES

Initial analyses were performed treating species as independent data. Sex ratios were compared between gonochorists and female-first sex changers using a t -test. The distribution of male GSI data could not be normalized; therefore, nonparametric Mann-Whitney U -tests were used to investigate differences in GSI between males. Female GSI was arcsin square-root transformed (Zar 1999) and differences between reproductive modes were tested using a t -test. Male and female \overline{GW} and \overline{BW} were \log_{10} -transformed. We performed regression analyses to identify the specific relationship between $\log_{10}\overline{GW}$ and $\log_{10}\overline{BW}$ for each reproductive mode within each sex and t -tests to compare the slopes and intercepts between reproductive modes within each sex (Zar 1999).

Because lower gametic investment was observed in female-first sex changers than in non-sex changers for both sexes (see Results), we examined whether males showed a greater decline than females. To do this, we performed a two-way analysis of covariance (ANCOVA) with a full factorial design using $\log_{10}\overline{GW}$ as the independent variable, $\log_{10}\overline{BW}$ as the covariate, and both sex and reproductive mode as fixed factors (Field 2005).

In addition to our cross-species analyses, we performed two types of phylogenetically based tests to reduce potential phylogenetic nonindependence: analysis of phylogenetically indepen-

dent contrasts and analysis of higher taxonomic nodes (Harvey and Pagel 1991). For sex ratio, GSI and \overline{GW} we generated independent contrasts using sister terminal taxa (Maddison 2000). We identified closely related pairs of terminal taxa that differed in their reproductive mode (gonochorism or female-first sex change). To investigate whether body weight confounded comparisons of the gonad weights of sex changers and non-sex changers, contrasts of $\log_{10}\overline{GW}$ were regressed through the origin against contrasts of $\log_{10}\overline{BW}$ (Harvey and Pagel 1991; Garland et al. 1992). A significant positive relationship would indicate that differences between nonsex and sex-changing species in gonad weight were associated with, and therefore confounded by, a difference in body weight.

To identify paired contrasts, we followed nomenclature of Froese and Pauly (2005) and, when necessary, calculated genus means for species with the same reproductive mode. A composite phylogenetic tree was constructed in Mesquite (Maddison and Maddison 2003) based on published phylogenies (Craig et al. 2001; Wang et al. 2001; Carlin 2002; Streelman et al. 2002; Pondella II et al. 2003; Orrell and Carpenter 2004; Quenouille et al. 2004; Westneat and Alfaro 2005) (Fig. 1). Where phylogenetic information was not available, we used taxonomies (sensu Nelson 1994). Clades of taxa for which phylogenetic information was not available were grouped according to taxonomy, and set as soft multiple nodes (polytomies). Polytomies between families or genera with the same reproductive mode were collapsed to a single nested mean (e.g., the serranid species, Fig. 1). Polytomies between families and genera that differed in their reproductive modes were collapsed to two nested means, one for each reproductive mode (e.g., *Ctenolabrus* and *Tautoga* vs. *Labrus*). All remaining polytomies were resolved to zero-length branches. Because information on branch lengths was sparse we assumed all branch lengths were equal (i.e., an explicitly punctuational model of character evolution, Garland et al. 1992).

Terminal pairs were selected to maximize the number of phylogenetically independent contrasts using Figure 1. Female-first sex changers were paired with their most closely related nonsex-changing (gonochoristic) sister taxon for which data were available (see the appendix). Because the availability of data differed for each variable the number of contrasts and the pairing of taxa varied between tests (see Table A1). In all independent contrast analyses, wreckfish (Polyprionidae: *Polyprion* sp.) was paired with the groupers (Serranidae). However, because evidence in support of this pairing is weak (Carlin 2002), analyses were repeated without this contrast. We report results from both analyses (i.e., with and without the wreckfish-grouper contrast) when they supported different hypotheses; in all other cases, we only report results from analyses that included this contrast. Differences between reproductive modes in sex ratio, male and female GSI, \overline{GW} , and \overline{BW} were analyzed using paired-sample Wilcoxon

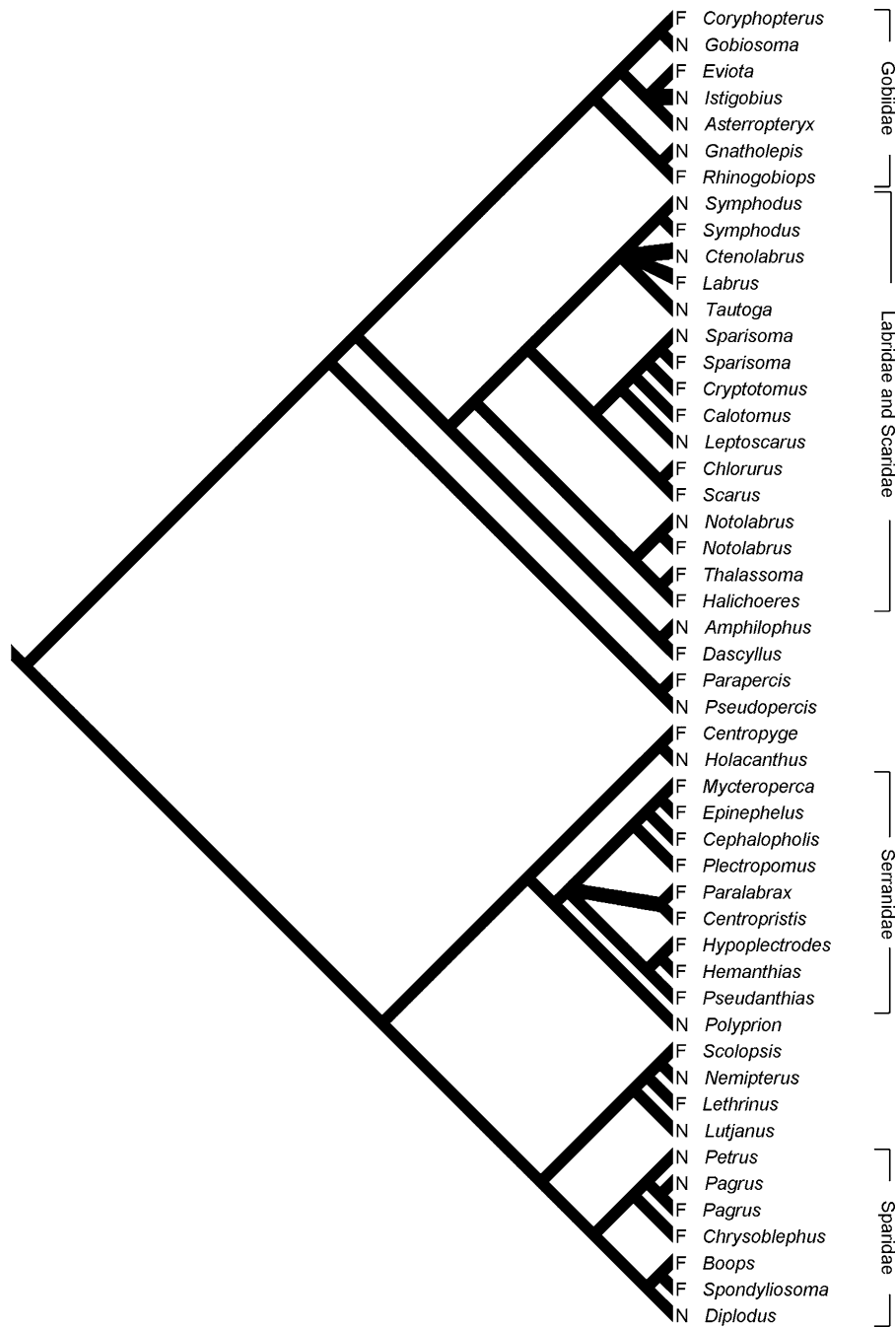


Figure 1. Composite tree of teleost fishes used to identify phylogenetically independent sister taxa for pairwise comparisons: N, non-sex changer; F, female-first sex changer. Position of major families is indicated. See Materials and Methods for further detail on tree construction and organization.

signed-rank tests. All contrasts were calculated as $X_S - X_G$, where X_S and X_G are the observed values for sex changers and gonochorists, respectively. Two-tailed, exact P -values were calculated for all Wilcoxon tests.

Because few contrasts of male and female GSI and \overline{GW} were available, we repeated our cross-species analyses on these variables using nested means at higher taxonomic levels (“analyses of higher taxonomic nodes,” Harvey and Pagel 1991). The aim of

these analyses was to reduce phylogenetic pseudoreplication that may exist in cross-species analysis while retaining greater statistical power than was possible using independent contrasts. Nested means were calculated for genera and families. When species with different reproductive modes were found within the same genus or family (e.g., *Symphodus* in Labridae), separate means were calculated for each mode. For all variables, variance in genus means was greater than in species-level data and reductions in samples

sizes at the genus level were not too great to severely weaken statistical analyses. Therefore, all higher node analyses were performed at the genus level. Nonparametric Mann–Whitney U -tests were used to compare male and female GSI because these were significantly skewed and could not be transformed. We used the same regression, t -test, and ANCOVA methods as employed in our cross-species analyses to investigate sex-specific differences in the relationship between $\log_{10}\overline{GW}$ and $\log_{10}\overline{BW}$ for genera with different reproductive modes. Because the results of our higher nodes analyses corroborated our species-level and independent contrast results, they are not presented here.

Results

SEX RATIOS

As predicted, the sex ratios of populations of female-first sex changers were significantly more female biased than those of non-sex changers, using species as independent data (mean \pm SD sex ratio: gonochorists = 0.46 ± 0.02 , female-first sex changers = 0.29 ± 0.05 ; $t_{105} = 6.57$, $P < 0.001$). This result was supported by the independent contrast analysis (positive contrasts = 4, negative contrasts = 13, paired-sample Wilcoxon signed-rank test $T = 23$, $z = -2.53$, $P = 0.009$).

SPERM COMPETITION

In cross-species analyses, male GSI was significantly lower in female-first sex changers than non-sex changers as predicted (Mann–Whitney $U = 44$, $P < 0.0001$, $n = 21$ gonochorists and 26 sex changers). On average, gametic investment by sex-changed males was only 20% of that of non-sex changers (mean \pm SD male GSI: female-first sex changers = 0.006 ± 0.01 ; gonochorists = 0.03 ± 0.02). Females of female-first sex-changing species also had significantly lower GSI than non-sex changers (back transformed mean \pm SD female GSI: gonochorists = 0.057 ± 0.006 , female-first sex changers = 0.032 ± 0.002 ; statistics on arcsin square-root data $t_{32} = 2.55$, $P = 0.02$). However, gametic investment by females of these sex changers was over half that of non-sex changers (mean \pm SD female GSI: female-first sex changers = 0.04 ± 0.02 ; gonochorists = 0.06 ± 0.04).

These results were supported by differences in the sex-specific relationships between $\log_{10}\overline{mean\ gonad\ weight}$ ($\log_{10}\overline{GW}$) and $\log_{10}\overline{mean\ body\ weight}$ ($\log_{10}\overline{BW}$) for each reproductive mode. For both sexes the regression slopes did not differ significantly between reproductive modes (slope \pm 95% CI; male sex changers = 0.91 ± 0.17 , male gonochorists = 1.04 ± 0.16 , $t_{39} = 1.17$, $P = 0.13$; females sex changers = 0.93 ± 0.14 , female gonochorists = 1.01 ± 0.08 , $t_{21} = 1.23$, $P = 0.13$; Fig. 2). The intercept was significantly lower for both sexes of female-first sex changers than for non-sex changers (intercept \pm 95% CI; male sex changers = -2.30 ± 0.37 , male gonochorists =

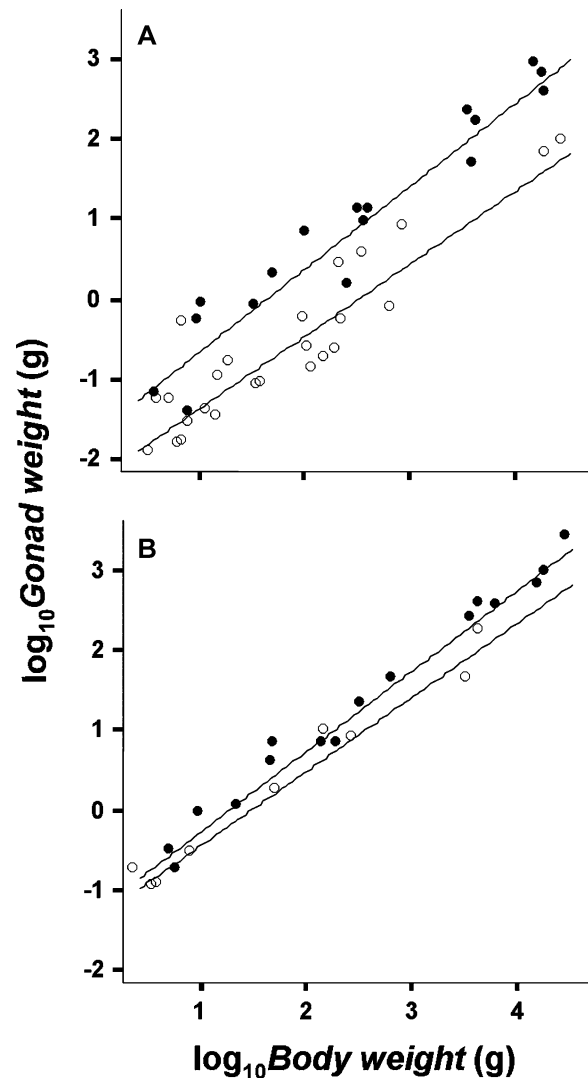


Figure 2. The relationship between body weight ($\log_{10}\overline{BW}$, g) and gonad weight ($\log_{10}\overline{GW}$, g) for (A) males and (B) females of non-sex-changing (gonochoristic, filled circles) and female-first sex-changing (open circles) fish species. Lines represent the best-fit linear regression for each sex of each reproductive mode separately.

-1.72 ± 0.44 , $t_{35} = 6.22$, $P < 0.0001$; female sex changers = -1.38 ± 0.30 , female gonochorists = -1.29 ± 0.22 ; $t_{22} = 3.14$, $P = 0.002$). These differences were reiterated by our cross-species ANCOVA: $\log_{10}\overline{BW}$ significantly predicting $\log_{10}\overline{GW}$ ($F_{1,62} = 745.68$, $P < 0.0001$, $\eta^2 = 0.92$); there were significant main effects of both reproductive mode and sex on $\log_{10}\overline{GW}$ (reproductive mode: $F_{1,62} = 37.22$, $P < 0.0001$, $\eta^2 = 0.38$; sex: $F_{1,62} = 53.78$, $P < 0.0001$, $\eta^2 = 0.47$). In addition, the interaction between reproductive mode and sex on $\log_{10}\overline{GW}$ was significant ($F_{1,62} = 11.77$, $P = 0.001$, $\eta^2 = 0.16$) indicating that the difference between reproductive modes in (relative) gametic investment was greater in males than in females (Fig. 3).

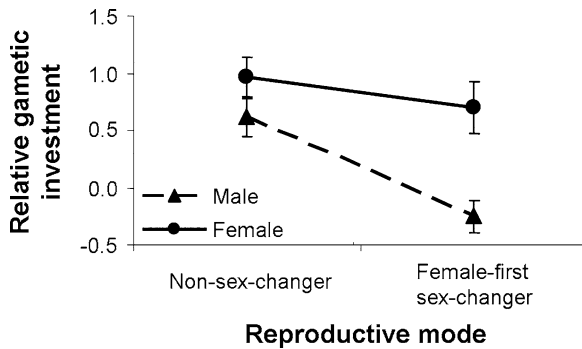


Figure 3. The mean gametic investment, controlled for body weight, of males and females of non-sex-changing (gonochoristic) and sex-changing fish species. Relative gametic investments are estimated marginal means \pm 95% CI of $\log_{10} \overline{GW}$ adjusted for effect of $\log_{10} \overline{BW}$ obtained from ANCOVA.

Independent contrast analyses of male and female GSI also revealed significantly lower gametic investment by sex-changed males than non-sex changers (for both sexes: positive contrasts = 0, negative contrasts = 6, $n = 6$ contrasts, paired-sample Wilcoxon signed-rank $T = 0$, $z = -2.20$, $P = 0.03$). Again, differences between reproductive modes were greater in males than females: on average, the GSI of female-first sex changers was 26% that of non-sex changers in males and 56% in females. Sex-changed males also had smaller absolute gonad weight than their non-sex-changing counterparts (positive contrasts = 0, negative contrasts = 6, $n = 6$ contrasts, paired-sample Wilcoxon signed-rank $T = 0$, $z = -2.20$, $P = 0.03$), which was not associated with consistently smaller body sizes in sex changers (regression through the origin: $F_{1,5} = 5.86$, $P = 0.06$, $r^2 = 0.54$). Exclusion of the wreckfish–grouper comparison in contrasts of male GSI and gonad weight reduced sample size to 5, thus precluding the possibility of obtaining a two-tailed P value of ≤ 0.05 using the Wilcoxon sign rank test (Zar 1999, table B.12). Nevertheless, in support of the analyses that included this comparison, all available contrasts of male GSI and gonad weight showed lower reproductive investment by sex-changed males than gonochorists (both tests: positive contrasts = 0, negative contrast = 5; $T = 0$, $z = -2.02$, $P = 0.06$). There was no significant difference between female-first sex changers and gonochorists in female gonad weight (positive contrasts = 1, negative contrasts = 4, $n = 5$ contrasts, paired-sample Wilcoxon signed-rank $T = 1$, $z = -1.73$, $P = 0.125$). Furthermore, for females, the relatively smaller gonads of sex changers was associated with smaller body weight compared to gonochorists (regression through the origin: $F_{1,4} = 267.13$, $P < 0.001$, $r^2 = 0.99$, slope = 1.01, SE = 0.07).

Discussion

We tested two predicted evolutionary correlates of female-first sex change: breeding sex ratios and the influence of male–male

sperm competition. As expected, sex changers had more female-biased sex ratios and a mating pattern that generated lower levels of sperm competition than nonhermaphroditic species. The use of ray-finned fishes as a model system allowed us to perform phylogenetically independent contrasts and analyses of higher nodes, to minimize the possibility that these results were confounded by shared ancestry. Our results clearly identify female-biased sex ratios and reduced sperm competition as two evolutionary correlates of female-first sex change. Thus, they offer support for current theory regarding the adaptive benefits of female-first sex change.

Theory predicts that female-first sex changers will have female-biased breeding sex ratios because population average male fecundity exceeds population average female fecundity (Warner 1975; Charnov 1982, 1993). Female-biased breeding sex ratios are therefore expected to be an evolutionary correlate of this reproductive mode. Indeed, female-first sex-changing fishes had populations with approximately twice as many females as males, whereas non-sex-changing populations usually exhibited sex ratios near unity. This result held in cross-species comparisons as well as in phylogenetically controlled analyses. Our results thus provide robust empirical support for the prediction of skewed sex ratios in sex changers, as was also observed by Allsop and West (2004b) in a comparison of female-first and male-first hermaphroditic animals.

Skews in breeding sex ratios may be driven by skews in overall sex ratios caused by size-related mortality. That is, in female-first sex changers males must always arise via females, and because some females die before changing sex, overall sex ratios may approach unity but cannot be male biased. This phenomenon may have contributed toward the observed differences in breeding sex ratios between female-first sex changers and non-sex changers. However, it is unlikely that this mechanism fully accounts for the extensive bias in the sex ratios of female-first sex changers, which was observed despite the fact that diandric species (which can, theoretically, have male-biased overall sex ratios) were included in analyses.

Irrespective of the cause, female-biased sex ratios will result in reduced levels of sperm competition because relative female availability is greater, and males may invest in monopolizing access to females. An indicator of the intensity of sperm competition (and hence the variance in individual male reproductive success) is relative investment in testes (e.g., Stockley et al. 1997). We found that relative testis mass was far lower, across all size classes, for sex-changed males compared to gonochoristic males. Furthermore, the difference between female-first and nonsex-changing fish in gonadal investment was far greater in males than for females. This evidence demonstrates that individual males in female-first sex-changing species are under reduced selection intensity from postcopulatory competition for fertilizations

compared with gonochoristic relatives (Warner 1975; Stockley et al. 1997; Taborsky 1998). This result therefore lends empirical cross-species support for the size-advantage model to explain the evolutionary benefits of female-first sex change (Warner 1975; Charnov 1982, 1993).

Our study focused on ray-finned fishes (class Actinopterygii), which constitute roughly half of all vertebrate species (Jonna 2004), and provide a suitable model system to address general evolutionary questions. The acquisition of more (comparable) data on reproductive investment by other groups of closely related female-first sex changers and gonochorists will allow our predictions to be tested across an even broader range of species. The generality of our results is nevertheless supported by previous studies that have described similarities in sex ratios, and hence extent of sperm competition experienced by breeding males, between taxonomically diverse species with the same reproductive mode (Charnov 1982; Allsop and West 2004b).

It is possible that our observation of low sperm production by sex-changed males is the result of a constraint associated with sex change. However, very rarely, sex-changed males in diandric species (i.e., secondary males) exhibit an initial color phase, which is associated with group- or streak-spawning, and high sperm competition and production (Warner and Robertson 1978). In this uncommon situation, sex-changed males do have large testes. This implies that the amount of sperm produced by secondary males is not constrained by an intrinsic limitation associated with sex change; rather, it has evolved in response to levels of sperm competition (Warner and Robertson 1978).

In summary, we confirm the hypothesis that female-biased sex ratios and risk of sperm competition, which is driven by the ability of males to monopolize access to females, are evolutionary correlates of female-first sex change. Although these findings support current theories to explain the evolution of sex change, a fruitful future direction could be to test directly for mating system and ecological correlates of sex change.

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Table A1. Genera used in phylogenetically independent contrasts. Asterisk indicates datum was not used because more closely related sister taxon was available. Reproductive mode: F, female-first sex changer; N, nonsex changer. Genera listed in the same order as they appear in Figure 1.

Genus	Reproductive mode	Sex ratio (prop. male)	Male GSI	Female GSI	Male \overline{GW}	Female \overline{GW}	Male \overline{BW}	Female \overline{BW}	Source(s)
<i>Coryphopterus</i>	F	0.25							Cole and Robertson 1988; Cole and Shapiro 1990
<i>Gobiosoma</i>	N	0.48							Cole et al. 1994
<i>Eviota</i>	F	0.34							Cole and Shapiro 1990
<i>Istigobius</i>	N	0.56							Cole and Shapiro 1990
<i>Asterropteryx</i>	N	0.36							Cole and Shapiro 1990
<i>Gnatholepis</i>	N	0.44							Cole and Shapiro 1990
<i>Rhinogobiops</i>	F	0.25							Kroon et al. 2000
<i>Bodianus</i>	F	0.11	0.001*		0.22		223.29		Warner and Robertson 1978
<i>Symphodus</i>	N	0.45	0.054	0.062	1.11	0.53	27.53	12.77	Dipper and Pullin 1979; Warner and Lejeune 1985; Sayer et al. 1996
<i>Symphodus</i>	F	0.52		0.028		0.11		4.00	Warner and Lejeune 1985
<i>Ctenolabrus</i>	N	0.63							Sayer et al. 1996
<i>Labrus</i>	F	0.17	0.006	0.065	6.98		1164.03		Dipper and Pullin 1979
<i>Tautoga</i>	N	0.44	0.033	0.080	161.13	385.81*	4882.73	4822.62*	White et al. 2003
<i>Sparisoma</i>	N	0.24							de Girolamo et al. 1999
<i>Sparisoma</i>	F	0.28	0.005*		0.77*		163.25*		Robertson and Warner 1978; Mumby and Wabnitz 2002; De Nóbrega and Villamizar 2004
<i>Cryptotomus</i>	F	0.23	0.007*		0.16*		22.87*		Robertson and Warner 1978
<i>Calotomus</i>	F	0.26							Robertson and Justines 1982
<i>Leptoscarus</i>	N	0.29							Robertson and Justines 1982
<i>Scarus</i>	F	0.21	0.002		0.41*		393.72*		Robertson and Warner 1978; Mumby and Wabnitz 2002
<i>Notolabrus</i>	N	0.39	0.036	0.042	13.11	6.83	364.15	162.69	Denny and Schiel 2002
<i>Notolabrus</i>	F	0.16							Jones 1980b
<i>Thalassoma</i>	F	0.49	0.003	0.027	0.03	0.12	5.00	4.41	Warner and Robertson 1978; Warner 1982
<i>Halichoeres</i>	F	0.34	0.003		0.07		38.83		Warner and Robertson 1978
<i>Amphilophus</i>	N	0.43							Francis and Barlow 1993
<i>Dascyllus</i>	F	0.47							Cole 2002; Asoh and Yoshikawa 2003; Asoh 2005
<i>Parapercis</i>	F	0.15	0.003*	0.031*	0.03*	0.29*	9.34*	9.34*	Walker and McCormick 2004
<i>Pseudopercis</i>	N	0.43							Macchi et al. 1995
<i>Centropyge</i>	F	0.29							Sakai and Kohda 1997
<i>Holacanthus</i>	N	0.4	0.029*	0.030*	13.29*	6.85*	458.33*	228.43*	Arellano-Martinez et al. 1999
<i>Mycteroperca</i>	F	0.23	0.003	0.031	80.09	176.53	26697.87	4903.62	Collins et al. 1992; Bullock and Murphy 1994; Collins et al. 1998; Crabtree and Bullock 1998

continued

Table A1. continued

Genus	Reproductive mode	Sex ratio (prop. male)	Male GSI	Female GSI	Male $\frac{GW}{BW}$	Female $\frac{GW}{BW}$	Male $\frac{BW}{BW}$	Female $\frac{BW}{BW}$	Source(s)
<i>Epinephelus</i>	F	0.29		0.022		44.43		3702.35	Bruslé and Bruslé 1975; Moore and Labisky 1984; Shapiro et al. 1993; Sadovy et al. 1994; Bullock et al. 1996; Beets and Friedlander 1998; Brulé et al. 1999; Mackie 2000; Wyanski et al. 2000; Fennessy and Sadovy 2002
<i>Cephalopholis</i>	F	0.37	0.005	0.031	0.56	1.81	111.18	58.24	Siau 1994; Chan and Sadovy 2002; Nakai and Sano 2002
<i>Plectropomus</i>	F	0.51							Adams 2003
<i>Paralabrax</i>	F	0.55							Hastings 1989; Hovey and Allen 2000; Baca Hovey et al. 2002
<i>Centropristis</i>	F	0.43							Cochran and Grier 1991; McGovern et al. 2002
<i>Hypoplectrodes</i>	F	0.41							Jones 1980a; Webb and Kingsford 1992
<i>Hemanthias</i>	F	0.37							Hastings 1981
<i>Pseudanthias</i>	F	0.13	0.064	0.066	0.51	0.18	8.03	2.67	Fishelson 1975; Shapiro 1986; Shapiro 1988
<i>Polyprion</i>	N	0.17	0.052	0.080	891.60	2660.19	17146.13	33252.35	Peres and Klippel 2003
<i>Scolopsis</i>	F	0.38							Young and Martin 1985
<i>Nemipterus</i>	N	0.7							Young and Martin 1985; Lau and Sadovy 2001
<i>Lethrinus</i>	F	0.38							Young and Martin 1982; Bean et al. 2003
<i>Lutjanus</i>	N	0.54	0.056	0.116	6.63	6.60	118.39	56.87	Kaunda-Arara and Ntiba 1997; Kamukuru and Mgaya 2004; Shimose and Tachihara 2005
<i>Petrus</i>	N	0.5	0.018	0.046	390.76	934.27	21708.77	20310.16	Smale 1988
<i>Pagrus</i>	N	0.44							Matsuyama et al. 1988
<i>Pagrus</i>	F	0.25	0.008	0.025	7.87	7.84	983.95	313.55	Lamrini 1986; Pajuelo and Lorenzo 1996; Kokokiris et al. 1999; Hood and Johnson 2000; Pajuelo and Lorenzo 2000
<i>Chrysoblephus</i>	F	0.36							Buxton 1990
<i>Boops</i>	F	0.5							Lo Gordo 1995
<i>SpondylIOSoma</i>	F	0.34	0.011	0.057	2.76	9.88	250.80	173.42	Pajuelo and Lorenzo 1999; Gonçlaves and Erzini 2000
<i>Diplodus</i>	N	0.55							Mann and Buxton 1998