

## Methods

### *Data collection*

Building on a previous study investigating the existence of life history invariants in fish<sup>1</sup>, we expanded our dataset to incorporate the known diversity of sex changing organisms. We located relevant studies by: (1) searching the ISI Web of Science database, using the key words “sex change” + “population” + “size” NOT “fish”; (2) searching citations in all papers found as well as reviews on the topic; (3) directly contacting researchers. We collected data on: (a) the size at sex change, and (b) the maximum body size achieved in a population. The size at sex change ( $L_{50}$  = size at which 50% are the second sex (male for protogynous animals, female for protandrous animals)) was calculated for each population using the logistic regression of the proportion of second sex breeders against the population body size distribution. We used the sigmoidal logistic regression curve as it is consistent with the method used to develop the theory that we are testing, on the basis that it provides a rigorous and quantitatively consistent estimation of the population average size at sex change<sup>1</sup>. Our criteria for inclusion of a species in the study were as described in Allsop & West<sup>1</sup>. Our complete dataset is available upon request.

### *Statistical analyses*

We tested whether the relative size at sex change ( $L_{50}/L_{\max}$ ) was invariant across all populations and species studied, using a log-log regression of  $L_{50}$  against  $L_{\max}$ . If the data fit an invariant life history rule for governing the size at which to change sex, such a regression would give a slope of 1.0. We carried out these regressions in two ways: (1) a

regression using species as independent data points, and (2) an independent contrasts analysis across species employing a phylogeny for sex changing animals presented elsewhere <sup>2</sup>. Species may not be independent data points because they are phylogenetically related <sup>3</sup>, and thus may share derived traits through a variety of processes, including phylogenetic niche conservatism, phylogenetic time lags and through similar adaptive responses <sup>4</sup>. This leads to problems if a trait is evolutionary conserved or highly correlated with an unknown phylogenetically inert third variable. A formal method to control for this is to carry out an analysis with independent contrasts. Independent contrasts are derived by calculating the difference in the response and explanatory variables across pairs of species or higher nodes that share a common ancestor <sup>3,4</sup>. These contrasts can then be analysed by a regression through the origin; the expected value of the slope through the origin equals the true relationship between the variables in the absence of the effects of evolutionary relatedness <sup>5</sup>. We carried out such an analysis with the CAIC statistical package <sup>6</sup>, assuming equal branch lengths.

We carried out regression analyses using two methods. The use of standard Ordinary Least Squares (OLS) regression is based upon the assumption that the  $x$  variable is fixed or controlled by the observer (i.e. no error in the  $x$  term). This is the most commonly used method of estimating the functional relationship between biological variables, and yet its assumptions are often violated, leading to a biased slope estimate. Although we may often be more confident of our estimates of the  $x$  variables in our analyses, they are clearly subject to measurement error. A possible solution to this problem is to carry out a Major Axis (MA) regression <sup>7</sup>. MA (or Model II) regression is used when there may be no causal structure between the  $y$  and the  $x$  variable and they are both measured with equal error. The Major Axis produces a line that minimises the sum of the squared deviations perpendicular to itself. In order to examine any affect of error

assumptions, we repeated all analyses using both OLS and MA regression<sup>7</sup>, using SYSTAT 10.2.

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