Vol. 471: 151–163, 2012
doi: 10.3354/meps10028

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 19

Measuring selective mortality from otoliths and similar structures: a practical guide for describing multivariate selection from cross-sectional data

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ABSTRACT: Selective mortality is an important process influencing both the dynamics of marine populations and the evolution of their life histories. Despite a large and growing interest in measuring selective mortality, studies of marine species can face some serious methodological and analytical challenges. In particular, many studies of selection in marine environments use a cross-sectional approach in which fates of individuals are unknown but the distributions of trait values before and after a period of selective mortality may be compared. This approach is often used because many marine species have morphological structures (e.g. otoliths in fishes, statoliths in some invertebrates) that contain a permanent record of trait values. Although these structures often contain information on multiple, related traits, interpretation of selection measures has been limited because most studies of selection based on cross-sectional data consider selection 1 trait at a time, despite known problems with trait correlations. Here, we detail how cross-sectional data can be analyzed within a multivariate framework and provide a practical guide for conducting these types of analyses. We illustrate these methods by applying them to empirical studies of selective mortality on early life history traits in 2 species of reef fish. These examples demonstrate that analyzing selective mortality in a multivariate framework can vastly improve estimates of selection and yield new insight into how combinations of traits can interact to influence survival. Accompanying the paper are 2 R scripts that can be used to perform the calculations described here and assist with visualizing selection on multiple traits.

KEY WORDS: Collinearity · Correlated traits · Larval survival · Natural selection · Selection gradients · *Stegastes partitus* · *Thalassoma bifasciatum*

INTRODUCTION

Quantifying selective mortality is central to many fundamental questions concerning the ecology and evolution of marine species. Measuring the relationships between phenotypic characteristics (including physical, physiological, and behavioral traits) and survival can yield insights into which traits are important for survival, and how variation in these traits can influence both the dynamics of populations and the life-history evolution of species. For example, measuring how the size and growth of larvae influences their survival and subsequent recruitment to juvenile and adult populations has led to major

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advances in understanding mechanisms driving recruitment variability in marine populations (e.g. Hovenkamp 1992, Meekan & Fortier 1996, Hare & Cowen 1997, Searcy & Sponaugle 2001, Steer et al. 2003, Takasuka et al. 2004). Quantifying selective mortality can also play a crucial role in identifying the evolutionary forces that constrain the body size of both larvae (Johnson et al. 2010, Marshall & Monro 2012) and adults (Warner & Schultz 1992, Roff et al. 2006, Johnson & Hixon 2011) of marine species. In addition to advancing our understanding of basic ecological and evolutionary processes, the measurement of selective mortality is becoming increasingly important in an applied context, as investigators seek to understand how size-selective fishing may disrupt natural patterns of selection (Rijnsdorp 1993, Sinclair et al. 2002, Carlson et al. 2007) and lead to complex evolutionary responses (Swain et al. 2007, Johnson et al. 2011).

Selective mortality is a widespread and important phenomenon that needs to be understood in greater detail. It is also clear that such selection can be particularly influential during the early lives of marine species (reviews by Gosselin & Qian 1997, Sogard 1997). Mortality during the larval and early juvenile phases is commonly high, and studies of selective mortality during these phases provide some of the strongest measurements of selection recorded (e.g. Perez & Munch 2010). Yet despite a large and growing interest in measuring selective mortality, studies of marine species can face some serious methodological and analytical challenges. In particular, for many species (or life stages), it is impossible to study selection by identifying individuals and collecting longitudinal data to track their fates over time. Such longitudinal studies are often precluded by biological details such as high rates of natural mortality, a high degree of dispersal, or sensitivity to tagging procedures. Rather, most studies of selection in the marine realm employ a cross-sectional approach in which distributions of traits are compared before and after a period of selection.

One reason that cross-sectional analyses of selection are relatively common for marine species is that the otoliths (ear stones) of fish, and similar structures for other taxa (e.g. statoliths, spines), can provide a permanent record of traits such as size, age, and growth (e.g. Campana & Neilson 1985, Jackson 1994). This feature makes it possible to measure selective mortality in the absence of information about individual fates. Selection can be measured by comparing the distributions of traits in before-selection samples (e.g. fish caught as larvae) to the distributions of traits in after-selection samples (e.g. groups of fish captured as surviving juveniles). Directional selection is commonly inferred by comparing whether the mean values of traits change during a period of selection, and aspects of disruptive or stabilizing selection can be inferred (in part) from changes in the variance of a trait during selection (reviewed by Manly 1985, Endler 1986).

Most cross-sectional studies in the marine literature that have quantified selection have used these measures (or very similar versions). A major shortcoming of this approach is that measurements of selection are taken 1 trait at a time. When traits are correlated (as is often the case for traits such as size, growth, and duration of life stages), single-trait measures of selection confound direct and correlated effects, and often do not provide an accurate description of selection (Lande & Arnold 1983). Furthermore, single-trait measures of selection provide no means of evaluating both the independent and interactive relationships between trait values and survival. In contrast, multivariate analyses of selection can parse out the direct effects of selection and evaluate how trait combinations can affect survival above and beyond the sum of their independent effects (Lande & Arnold 1983).

Here we review how cross-sectional data can be analyzed within a multivariate framework and provide a practical guide for computing these types of analyses. Accompanying the paper are 2 R scripts that were written to perform these calculations and assist with visualizing selection on multiple traits. We wrote the paper with otolith-based studies in mind, but we note that the analytical procedures we outline are general and can be used to analyze selection on any set of quantitative traits (i.e. continuous variables) in a cross-sectional study. Various features of the multivariate analyses and the types of new, biological insight that multivariate analyses can yield are illustrated through empirical examples of selection on some early life history traits of reef fishes. Our hope is that greater use of multivariate analyses of selection for cross-sectional data will help provide more accurate measures of selective mortality and accelerate our understanding of natural selection in the marine environment.

METHODS

By definition, mortality that is selective results in qualitative changes in the distribution of phenotypes. Comparing the distribution of phenotypes before and

after a period of selective mortality can therefore provide useful summaries of selection. In this section, we review the estimation and interpretation of statistics that describe the direction, strength, and form of selection. We begin with selection differentials, i.e. single-trait measures of selection that are commonly used in cross-sectional studies but have several shortcomings. Next we describe selection gradients, which are multivariate measures of selection that are much more useful because they describe the direct relationship between trait values and relative fitness. Moreover, a multivariate framework allows one to estimate whether traits affect relative fitness independently or in combination. Finally, we detail how changes in the distribution of phenotypes can be used to estimate and visualize multivariate selection (via analyses of selection gradients) even when data are in a cross-sectional format (i.e. samples are taken before and after a period of selective mortality, but fates of individuals are unknown).

Selection measurements

In the univariate case, directional (linear) selection can be described by the selection differential, i.e. the change in mean phenotype $\bar{z}$ before and after selection. Often it is useful to calculate the standardized selection differential:

$$S = \frac{(\bar{z}_{after} - \bar{z}_{before})}{SD_{before}} \quad (1)$$

Dividing by the standard deviation (SD) of the population before selection produces a standardized measure that facilitates interpretation and comparison among different traits, species, and places (see Kingsolver et al. 2001, Perez & Munch 2010 for examples of among-study comparisons). In the context of selective mortality, selection differentials also describe the covariance between relative survival and phenotypic value (reviewed by Brodie et al. 1995; see Price 1970 for a mathematical proof). Selection differentials are therefore very interpretable and useful measures of how trait variation translates to relative survival within populations. Another useful statistic is the nonlinear selection differential:

$$C = var_{after} - var_{before} + S^2 \quad (2)$$

which describes how the variance of the trait distribution changes during selection, independent of changes in variance brought about by directional selection (S). Again, nonlinear selection differentials can be standardized by dividing Eq. (2) by the variance in the population before selection. In the context of selective mortality, nonlinear selection differentials represent the covariance between relative survival and the squared deviations from the mean trait value (Brodie et al. 1995).

To our knowledge, most of the cross-sectional studies of selection in the marine literature that have been published to date and have quantified selection on multiple traits have calculated a series of selection differentials (e.g. Hare & Cowen 1997, Searcy & Sponaugle 2001, Sinclair et al. 2002, Gagliano et al. 2007, Swain et al. 2007, Vigliola et al. 2007, Hamilton et al. 2008, Samhouri et al. 2009, Grorud-Colvert & Sponaugle 2011, Rankin & Sponaugle 2011, but see Podolsky 2001). Although selection differentials provide useful information, they represent the sum of both direct selection (i.e. the direct covariance between relative survival and trait value) and indirect selection (an apparent association between relative survival and trait value that is mediated by correlated traits; Fig. 1). Consequently, the direct nature of selection on a particular trait may not be accurately represented by the selection differential, particularly when other, correlated traits are also important to survival (as is often the case with multi-trait studies). Another shortcoming of measuring selection on single traits in isolation is that the relationships between relative survival and trait combinations are unknown.

When data on multiple traits are available, we recommend that investigators measure selection in a multivariate framework and calculate selection gradients in addition to selection differentials. Directional selection gradients on a set of n traits (denoted by the $n \times 1$ vector $\boldsymbol{\beta}$) measure the direct, linear asso-

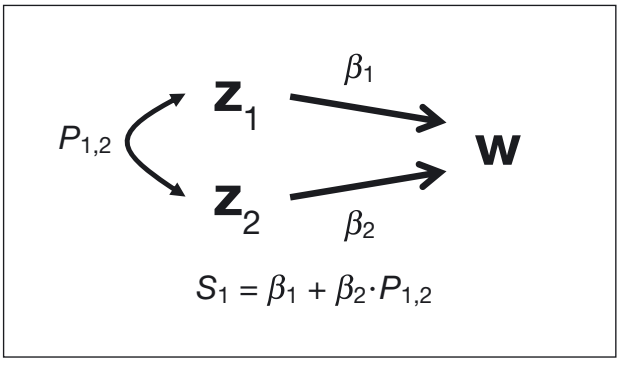

Fig. 1. Schematic of selection on multiple traits. Each trait ($\mathbf{z}_i$) affects relative fitness (w) through direct effects (β_i), as well as through its correlations with other traits that affect fitness ($P_{i,j}$ indicates correlation values). Single-trait measures of selection (selection differentials, S_i) include the sum of both direct and correlated effects

ciations between trait values and relative survival (i.e. β_i measures the linear relationship between trait i and relative survival, holding values of all other traits constant). Assuming traits follow a multivariate normal distribution (or can be appropriately transformed), $\boldsymbol{\beta}$ is a vector of partial regression coefficients relating relative survival to trait values. Similarly, nonlinear (quadratic) selection gradients (denoted by $n \times n$ matrix $\boldsymbol{\gamma}$) are partial regression coefficients relating relative survival to pairwise products of trait deviations from their mean values, holding all other traits constant. Nonlinear selection gradients also describe the partial changes in (co)variances that occur during selection (Kendall & Stuart 1973, Lande & Arnold 1983). Importantly, selection gradients summarize key features of the individual selection surface — the relationship between the expected value of relative fitness (≈ relative survival in the cases considered here) and an individual's phenotypic trait values. Again, assuming multivariate normality before selection, the selection gradients describe the average slope of the selection surface, and the nonlinear selection gradients describe the average curvature and shape of the surface (Phillips & Arnold 1989).

The interpretation of selection gradients as the direct relationship between trait value and relative fitness assumes that all correlated variables that appreciably affect relative fitness have been measured and are included in the analysis. We caution that when conducting a selection gradient analysis, as in any analysis of real data, one can never be entirely sure that all relevant variables have been included. Relevant variables include both phenotypic traits as well as environmental variables that may covary with both trait value and relative fitness (e.g. Price et al. 1988, Rausher 1992). Our view is that investigators should strive to conduct studies that resolve the patterns of selection in as much detail as is practical. To that end, a multivariate approach will be a large step forward.

Estimating selection gradients from cross-sectional data

When individual fates are known (e.g. in tagging studies), $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$ can be conveniently estimated by multiple regression of relative fitness on phenotypic trait values (Lande & Arnold 1983). When data are in a cross-sectional format (e.g. samples of phenotypes are available before and after selection), a less intuitive, but related method can be used to estimate selection gradients (Lande & Arnold 1983). Specifically, linear selection gradients can be estimated by calculating selection differentials (S) for all traits as in Eq. (1) and then multiplying this vector of selection differentials by the inverse of the phenotypic variance–covariance matrix of the traits in the population before selection ($\mathbf{P}$):

$$\boldsymbol{\beta} = \mathbf{P}^{-1}\mathbf{S} \tag{3}$$

Nonlinear selection gradients can be estimated in a similar manner. First, one needs to calculate a matrix of nonlinear selection differentials:

$$\mathbf{C} = \mathbf{P}^{\star} - \mathbf{P} + \mathbf{S}\mathbf{S}^{\mathrm{T}} \tag{4}$$

Here $\mathbf{P}^{\star}$ is the phenotypic variance–covariance matrix after selection and T represents the matrix transpose operation. Importantly, values in this matrix must be on the same scale as those in $\mathbf{P}$. For example if one is interested in calculating standardized selection gradients (as in Eq. 1), values used to calculate $\mathbf{P}^{\star}$ must be scaled by the SD in the population before selection (rather than after, which would produce errors). Estimates of nonlinear selection differentials can be further combined with the inverse of $\mathbf{P}$ to estimate the matrix of nonlinear selection gradients:

$$\boldsymbol{\gamma} = \mathbf{P}^{-1}\mathbf{C}\mathbf{P}^{-1} \tag{5}$$

The diagonal elements of $\boldsymbol{\gamma}$ represent nonlinear (quadratic) selection on each trait and the off-diagonal elements represent correlational selection on pairs of traits.

A few practical details about this method deserve mention. (1) This method assumes that the distribution of traits before selection is multivariate normal. Our experience suggests that most samples of otolith-derived traits meet this assumption or that traits can be appropriately transformed. (2) Estimates of variability in $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$ values can be obtained by resampling. For example, for each of 1000 iterations, n_1 individuals can be drawn with replacement from the n_1 individuals in the before-selection sample, and n_2 individuals can be drawn with replacement from the n_2 individuals in the before-selection sample. The estimation procedure (Eqs. 1 to 5) can be repeated during each iteration and the distribution of values within all iterations used to estimate standard errors and confidence intervals. More information regarding this procedure is provided in Supplement 1 at www.int-res.com/articles/suppl/m471p151_supp/. (3) Perhaps most importantly, is that this procedure can be seriously affected by a high degree of (multi) collinearity in the data (e.g. when 2 or more of the phenotypic traits are highly correlated). As an evalu-

ation of whether collinearity is a problem, we suggest calculating the condition number of the **P** matrix. Because phenotypic variance-covariance matrices (**P** matrices) contain real numbers, and are square and symmetric, condition numbers can be calculated as:

$$\kappa(\mathrm{P}) = \left| \frac{\lambda_{\max}(\mathrm{P})}{\lambda_{\min}(\mathrm{P})} \right| \quad (6)$$

where different λ represent the eigenvalues of the **P** matrix. Note that calculating condition numbers requires that one specify a matrix norm. Eq. (6) considers **P** in Euclidean space, which is often the default in such calculations (e.g. in the kappa function in R, where it is referred to as the 2-norm). Heuristically, the condition number provides a measure of how unstable the inverse of a matrix is and the degree to which small variations in the original matrix values (e.g. due to sampling variability) will be amplified by the process of matrix inversion. Larger numbers indicate greater problems. Collinearity is a matter of degrees, but as an informal guideline, we suggest that condition numbers of <10 are acceptable for calculating gradients, >20 indicate severe problems, and those in between are within the 'proceed with caution' realm.

Dealing with multicollinearity

When there is a high degree of multicollinearity in the data (e.g. when 2 or more of the phenotypic traits are tightly correlated), the mathematical procedure used to estimate selection gradients becomes unreliable. Matrix inversions become unstable, and we often have little confidence in our estimates of selection gradients. To circumvent this problem, we suggest reducing the number of traits considered in the selection analysis. Note that this procedure results in a loss of information and can change the estimated values of the selection gradients. However, for highly correlated traits, the effects of collinearity can be so pathological that it is worth sacrificing some information to gain stability in the computational procedure. In any case, just as in multiple regression analysis, the estimated selection gradients should always be interpreted in light of which variables were included in the model, and which were not.

One option for reducing variables is to drop 1 or more traits from the analysis until collinearity is minimized and then proceed as described above. Which variable(s) to drop is ultimately up to the discretion of the investigator, and should be based on the aims of the study and on biological information. We suggest a 2-stage procedure. First, to identify which variables are problematic, we suggest calculating the variance inflation factor (VIF) associated with each variable in the data set:

$$\mathrm{VIF} = \frac{1}{(1-\mathrm{R}^2_i)} \quad (7)$$

Here, R^2_i is the coefficient of determination for the regression of variable *i* on all other variables in the data set. The VIF values provide a measure of the degree to which including the variable in the dataset contributes to collinearity. In the context of selection analyses, larger VIF values indicate that a particular variable covaries so strongly with other variables in the set that the analysis cannot accurately estimate the (partial) relationships between trait values and relative fitness. Once the set of problematic variables has been identified, 1 or more variables should be dropped from the analysis. In many cases, the aims of the study and associated biological information (e.g. causal relationships among traits, natural history, logistics) will suggest which variable(s) to exclude. However, if there is no *a priori* reason to exclude particular variables, dropping the variable with the largest VIF will provide the greatest reduction in collinearity. Again, we suggest a sequential procedure. If dropping a single variable sufficiently minimizes collinearity (as evaluated by the condition number of the **P** matrix), then gradients can be calculated. If not, more variables will likely need to be dropped.

Another option for reducing the number of traits considered in the selection analysis is to redefine the traits such that they are less correlated. For example, one could average or sum 2 or more of the highly correlated traits into a single measure. Similarly, one could perform a principal components analysis on the traits. This analysis will produce a number of principal components (linear combinations of the original traits) equal to the number of original traits. One can then conduct a selection gradient analysis on a reduced subset of the principal components, treating each as a redefined trait. A benefit of this approach is that by definition, the principal components will be uncorrelated and the selection differentials will be the same as the selection gradients. A drawback is that it is somewhat difficult to interpret selection on linear combinations of traits.

Visualizing multivariate selection surfaces

Visual representations of the individual selection surface can be extremely helpful for interpreting and

analyzing selective mortality. When considering selection on 2 or fewer traits, we recommend using the procedure outlined by Anderson (1995), with slight modifications, where necessary. Briefly, you create a 'response' variable coded as 0 for those individuals in the before-selection sample, and as 1 for those in the after-selection sample. Using a generalized additive (or linear) model and a logit link, you can then estimate h_z, the conditional probability that an individual with phenotype(s) z was found in the after-selection sample, given that it was sampled at all. Specifically,

$$h_z = \frac{e^{u_z}}{(1 + e^{u_z})} \tag{8}$$

where u_z is a function that describes the logit of h_z. u_z can be described as a smooth function (e.g. as a spline in a generalized additive model, GAM) or as a parametric function (e.g. as a quadratic equation in a generalized linear model). Although originally proposed in a univariate context, note that u_z can easily be formulated as a function of multiple phenotypic traits (including multiplicative interaction terms).

Anderson (1995) demonstrated that relative survival, w_z, could be expressed as a function of phenotype and in terms of h_z:

$$\begin{aligned} w_z &= \left(\frac{n_{\text{before}}}{n_{\text{after}}}\right)\left(\frac{h_z}{1-h_z}\right) \\ &= \left(\frac{n_{\text{before}}}{n_{\text{after}}}\right)e^{u_z} \end{aligned} \tag{9}$$

Predicted values of u_z (based on the multivariate phenotype) can be inserted into Eq. (9) to plot the individual selection surface. In Supplement 2, we provide options for describing u_z using thin plate splines (estimated via GAMs in the R package mgcv; R Development Core Team 2012). It is possible to visualize the individual selection surface by fitting parametric models to the data (e.g. by representing u_z as a quadratic function), but we recommend the use of less restrictive, 'nonparametric' functions such as splines. Although nonlinear selection gradients are based on a quadratic approximation to the individual selection surface, these statistics are sample averages and best represent the slope and curvature near the mean value (Phillips & Arnold 1989). Parametric models (especially simple ones) are often too constrained to accurately represent the individual selection surface throughout the full range of phenotypic values within the sample. In contrast, splines provide more flexibility to represent expected values of fitness, particularly at the extremes of the distribution of trait values.

To visualize selection, we suggest that investigators start by estimating u_z as a smooth function of both variables of interest. Although many options are available to model smooth functions, we recommend the use of thin plate splines. When trait values are on a similar scale (e.g. standardized trait values), isotropic smoothers such as thin plate splines may be most appropriate (Wood 2006). Another consideration is that unlike other smoothing functions, thin plate splines do not require users to specify technical information that affects model fitting (e.g. basis functions or knot positions) and can therefore be more straightforward to fit to data. Finally, our experience suggests that compared to other approaches (e.g. building smooth functions from each of several covariates and their tensor products), thin plate splines are less likely to overfit the data. This is particularly important when one considers the nature of cross-sectional data (i.e. that the data are actually a combination of 2 samples) and that one is usually concerned with the pattern of selection in the broader population, rather than within the sample. Additional details regarding how to estimate u_z using GAMs are provided in the R code supplied in the supplement.

Visually representing selection on >2 traits is difficult and necessarily requires some compromises. We refer the reader to Box & Draper (1987) for a thorough treatment of visualizing data relationships in higher dimensions and to Phillips & Arnold (1989) for examples specific to natural selection. Here we concentrate on cases where the data visualizations can be reduced to 2 or fewer traits. In particular, if there is no strong evidence for any correlational selection on combinations of traits, then the selection surface can be adequately approximated by piecewise, single-trait representations of the relationship between relative fitness and trait value. If there is evidence for correlational selection between 2 traits, however, a 3-dimensional plot is required to adequately represent the selection surface. In the case where >2 traits are considered and there is correlational selection between some (but not all) traits, the fitness surface may be represented by a number of 2-trait, 3-dimensional plots, provided that there is no correlational selection between traits that are plotted and those that are not. When >2 traits are considered and correlational selection is strong and widespread, it may be most useful to concentrate on selection gradients and forgo visualizations of the selection surface.

RESULTS AND DISCUSSION

Example 1. Direct and indirect measurements of selective mortality

Our first example concerns the measurement of selection on correlated traits. The data come from Rankin & Sponaugle's (2011) study of early life history traits and selective mortality in the coral reef damselfish *Stegastes partitus*. This study focused on evaluating how certain traits of larvae affected their survival during the early juvenile phase (i.e. during the first few weeks of reef-associated life). The before-selection sample represents fish that were captured as late-stage larvae in light traps. The after-selection sample represents fish of the same cohort that were captured as surviving juveniles (15 to 21 d post-settlement, after appreciable selection had occurred). Here we measured selection on pelagic larval duration (PLD) and mean larval growth, 2 traits that were calculated from otolith records and were negatively correlated (r = −0.59). We used data from cohort 'B' in the original study, in which 176 fish were sampled before selection and 90 were sampled after selection (Rankin & Sponaugle 2011).

Longer development in the plankton (greater PLD) may be associated with greater cumulative growth and a larger size when transitioning to the reef habitat. Faster larval growth may also lead to larger size and may reflect genetic qualities or environmental effects on development that carry over to affect post-settlement survival. However, larvae with relatively high growth rates may also take less time to develop the morphological and physiological capacity to transition to reef-associated habitats, leading to a strong, negative correlation between larval growth and PLD. As a first step in evaluating the relationships between these traits and relative survival, we calculated the standardized selection differentials (Eq. 1 above) to be 1.11 (SE = 0.148) for PLD and −0.421 (SE = 0.136) for mean larval growth (SEs were calculated with a resampling procedure). These values suggest that slow-growing fish that had a long larval duration survived better as juveniles. However, calculating selection gradients (described in detail below) revealed a strikingly different pattern. To begin with, the condition number of the **P** matrix was 3.9, suggesting that correlations between the variables would not cause problems with collinearity. We therefore proceeded to calculate the selection gradients using our estimates of selection differentials (**S**) and the variance–covariance matrix (**P**):

$$\boldsymbol{\beta} = \mathbf{P}^{-1}\mathbf{S} = \begin{bmatrix} 1 & -0.59 \\ -0.59 & 1 \end{bmatrix}^{-1} \begin{bmatrix} 1.11 \\ -0.42 \end{bmatrix} = \begin{bmatrix} 1.32 \\ -0.36 \end{bmatrix} \quad (10)$$

Note that the selection gradients confirm that greater PLD is associated with greater survival (β_{PLD} ± SE = 1.32 ± 0.215), but that after accounting for the correlated effects of PLD, the direct relationship between survival and larval growth is actually positive (β_{growth} = 0.36 ± 0.208). These results illustrate that because selection differentials are the sum of both indirect and direct selection (i.e. $S_{growth} = P_{2,1}\beta_{PLD} + P_{2,2}\beta_{growth} = 1.32 \times -0.59 + 1 \times 0.36 = -0.42$), they cannot reveal the direct effects of selection. When correlations among traits are large, selection differentials may even be somewhat misleading. In this case a substantial, negative correlation between PLD and larval growth combined with strong selection for larvae with longer PLDs actually caused a reversal in the apparent direction of selection on larval growth. In contrast, selection gradients provide direct measurements of selection and can be much more useful in analyzing selection on multiple traits. Fig. 2 contrasts nonparametric visualizations of the

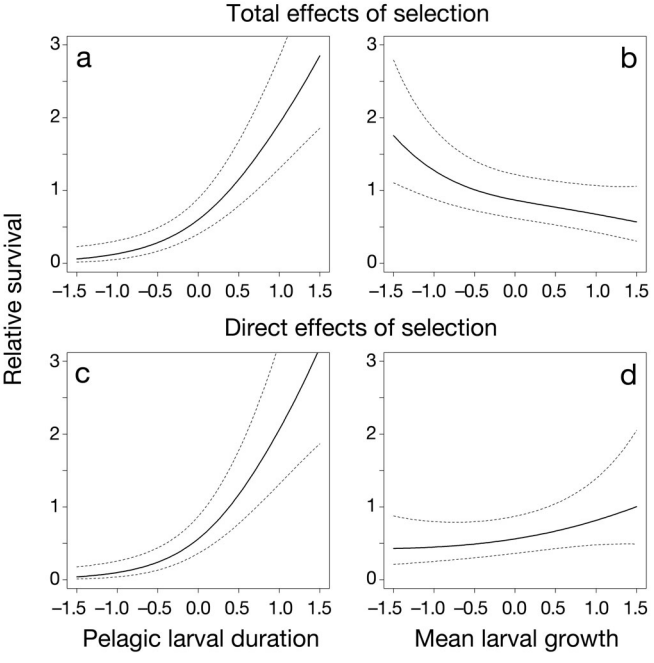

Fig. 2. *Stegastes partitus*. Fitness functions relating relative survival to phenotypic trait values (expressed as SD away from the mean). (a, b) Univariate relationships between trait values and survival representing both direct and indirect effects of selection. In contrast, relationships in (c, d) were calculated when both variables were included in the analysis and therefore illustrate the direct effects of selection (i.e. 'partial' relationships between traits and relative survival, after statistically accounting for trait correlations). Dashed lines: expected value of survival ± 2 SE

total effects of selection (which include direct and correlated effects and are analogous to selection differentials) with the direct effects of selection (analogous to selection gradients). Again, note the reversal in the apparent direction of selection on larval growth rate.

When comparing values of selection differentials and gradients within a large database of selection estimates, Kingsolver et al. (2001) found that, on average, values of selection differentials and gradients tended to be similar, although there were many individual exceptions. Whether differentials and gradients are similar depends on both the degree of correlation between traits studied and the magnitude of selection on the correlated traits (Fig. 1). Because many of the traits that can be measured via otolith proxies are likely to be correlated, we emphasize the importance of studying selection within a multivariate framework.

Example 2. Measuring selection on trait combinations

Our second example illustrates how relative survival may be associated with certain combinations of traits (often referred to as 'correlational selection'), and how a multivariate approach can be used to measure these effects. The data come from a study of selective mortality on early life history traits in a reef-associated wrasse, *Thalassoma bifasciatum* (Grorud-Colvert & Sponaugle 2011). Larvae of this species settle and bury into sand and rubble habitat for 3 to 5 d while they undergo metamorphosis. The before-selection sample represents fish that were captured immediately after emergence (i.e. fish aged 0 to 4 d post emergence). The after-selection sample represents fish of the same cohort that were captured as surviving juveniles (ages >9 d post emergence). For this example, we analyzed selective mortality on size at settlement and width of the metamorphic band (an indicator of settlement condition). Again, these are 2 correlated traits that were inferred from otolith records (r = −0.47). We used data from cohort 3 in the original study, in which 27 fish were sampled before and 48 after selection (Grorud-Colvert & Sponaugle 2011).

We calculated the standardized selection differentials (Eq. 1) to be −0.124 (± 0.248 SE) for size at settlement and 0.597 ± 0.261 for metamorphic band. These values suggest that fish with wider metamorphic bands survived better as juveniles (p = 0.022; assuming *Z*-scores for differentials are normally distributed). The selection differential for size at settlement is small relative to its SE, suggesting no strong evidence for selection on this trait (p = 0.617). Here we illustrate that calculating selection gradients provides measures of direct selection (using Eq. 3) and permits measurement of nonlinear selection (Eqs. 4 & 5), particularly correlational selection. The condition number of the **P** matrix was 2.79, suggesting that correlations between the variables would not cause problems with collinearity. Directional selection gradients ($\boldsymbol{\beta}$) were estimated to be 0.204 ± 0.347) for size at settlement and 0.693 ± 0.322 for metamorphic band width. Nonlinear selection gradients were obtained by first calculating the matrix of nonlinear selection differentials (C):

$$\mathbf{C} = \mathbf{P}^{\star} - \mathbf{P} + \mathbf{S}\mathbf{S}^{\mathrm{T}} = \begin{bmatrix} 1.13 & -0.19 \\ -0.19 & 0.80 \end{bmatrix} - \begin{bmatrix} 1 & -0.47 \\ -0.47 & 1 \end{bmatrix} + \begin{bmatrix} -0.12 \\ 0.60 \end{bmatrix} \begin{bmatrix} -0.12 & 0.60 \end{bmatrix} \quad (11)$$

$$\mathbf{C} = \begin{bmatrix} 0.13 & 0.28 \\ 0.28 & -0.20 \end{bmatrix} + \begin{bmatrix} 0.02 & -0.07 \\ -0.07 & 0.36 \end{bmatrix} = \begin{bmatrix} 0.15 & 0.21 \\ 0.21 & 0.16 \end{bmatrix}$$

The nonlinear differentials were then used to estimate nonlinear gradients:

$$\boldsymbol{\gamma} = \mathbf{P}^{-1}\mathbf{C}\mathbf{P}^{-1} = \begin{bmatrix} 1 & -0.47 \\ -0.47 & 1 \end{bmatrix}^{-1} \begin{bmatrix} 0.15 & 0.21 \\ 0.21 & 0.16 \end{bmatrix} \begin{bmatrix} 1 & -0.47 \\ -0.47 & 1 \end{bmatrix}^{-1} = \begin{bmatrix} 0.63 & 0.67 \\ 0.67 & 0.65 \end{bmatrix} \quad (12)$$

The nonlinear selection gradients revealed marginal evidence for nonlinear, quadratic selection on settlement size (γ_{size} = 0.63 ± 0.33, p = 0.056) but little evidence of nonlinear, quadratic selection directly on metamorphic band (γ_{band} = 0.65 ± 0.54, p = 0.23). Importantly, evidence for nonlinear selection on the combination of settlement size and metamorphic band was strong ($\gamma_{size \times band}$ = 0.67 ± 0.31, p = 0.03), indicating that fish that were large at settlement and had wide metamorphic bands survived even better than would have been predicted from the additive effects of these traits. These patterns were corroborated by a non-parametric analysis of relative survival (Eqs. 6 & 8) and a subsequent visualization of the bivariate, individual selection surface. Fig. 3 indicates that expected values of relative survival strongly increase in the upper-right quadrant, regions of space associated with both large metamorphic bands and large size at settlement. Collectively, these analyses revealed that while fish in good condition were expected to survive better on average, fish that were both large at settle-

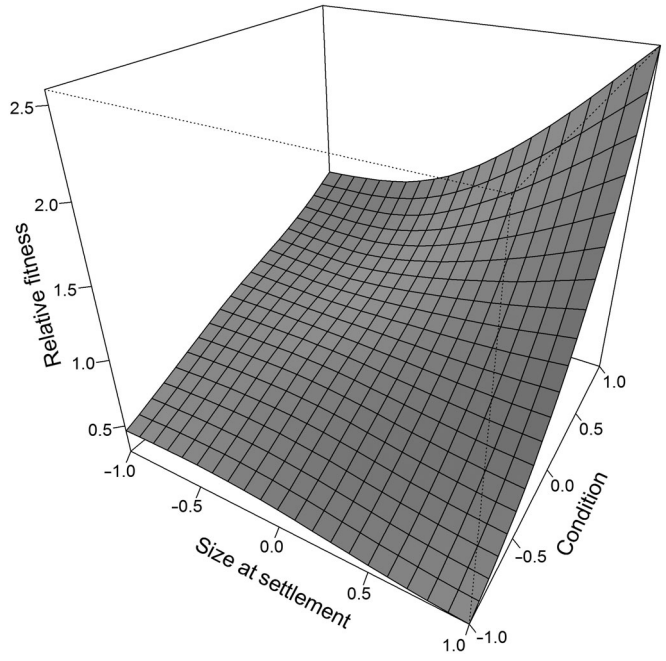

Fig. 3. *Thalassoma bifasciatum*. Bivariate fitness surface illustrating the joint effects of size at settlement and condition on relative values of post-settlement survival. Positive correlational selection is evidenced by an interaction between size and condition whereby individuals with high values of both traits experienced greater survival than would be expected from the additive effects of these 2 traits

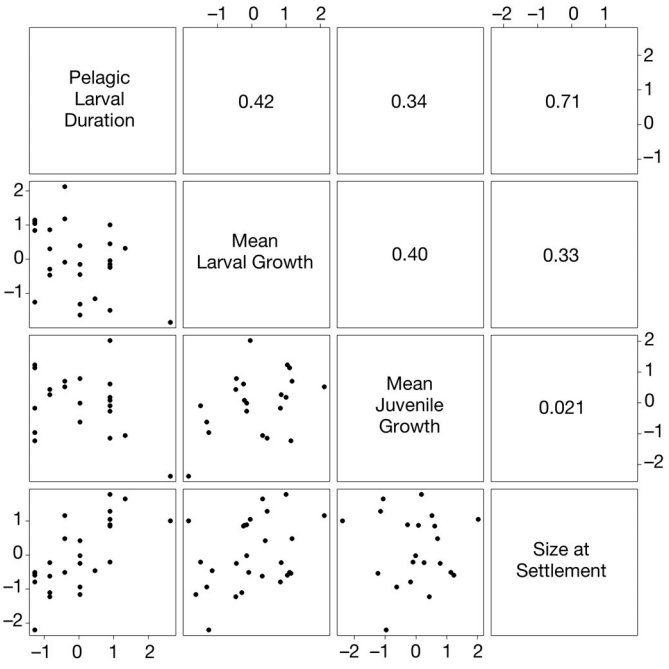

Fig. 4. *Stegastes partitus*. Relationships among early life history traits in the population before selection. Data are scaled as SD from the mean. Pairwise correlations are provided in the upper panels, but we note that pairwise correlations alone are often insufficient to detect problems with collinearity. See the Methods for a detailed assessment of collinearity

ment and in good condition survived exceptionally well (Fig. 3). These results suggest complex links between physiological condition, body size, behavior, and predation risk and point toward new avenues of research. Importantly, these synergistic effects would not have been detected by examining a series of selection differentials.

Example 3. Analyzing selection on three or more traits

Our final example describes an approach used to measure and visualize selection on 3 or more traits. Again, the data come from Rankin & Sponaugle's (2011) study of early life history traits and selective mortality in the coral reef damselfish *Stegastes partitus*. Here we set out to measure selection on all 4 of the traits in the original study: PLD, mean larval growth (MLG), mean juvenile growth (MJG), and size at settlement (SAS). We used data from cohort 'C' in the original study, in which 27 fish were sampled before selection and 38 were sampled after selection. The before-selection sample consisted of fish of age 1 to 7 d post settlement, and the after-selection sample consisted of fish that had survived 15 to 21 d post settlement (Rankin & Sponaugle 2011).

Initial examination of the **P** matrix indicated that the condition number was exceedingly high ($\kappa = 1032.7$), indicating severe problems with multicollinearity. These results suggested that any subsequent calculations of selection gradients on the full suite of traits would be unreliable. Plotting the relationships among variables provided some insight into the problem (Fig. 4). Both PLD and SAS were substantially correlated with 2 or more of the other variables. Although this provides some information (e.g. note the strong relationship between PLD and SAS), a better assessment of potential problems with collinearity is provided by the VIFs, which were 195.8, 89.2, 1.8, and 160.4 for PLD, MLG, MJG, and SAS, respectively. The extreme values measured for PLD and SAS (and to a lesser extent MLG) indicate that these variables could be removed from the data set with very little loss of information. Although VIF values identified PLD, SAS, and MLG as potential candidates for removal, in this study there was a clear, *a priori* reason for removing SAS. SAS is derived directly from larval growth and larval duration, and exploratory removal of this variable was sufficient to reduce collinearity. We therefore dropped

this derived trait from our subsequent selection analyses and concentrated on measuring selection on the 3 primary traits (PLD, MLG, and MJG).

The **P** matrix for the reduced set of traits indicated no problems with collinearity (κ = 3.18). Similarly, VIF values were 1.3, 1.4, and 1.2 for PLD, MLG, and MJG, suggesting that estimates of selection gradients would be reliable. Results are summarized in Table 1. Note that because measurements of juvenile growth were unavailable for several individuals, our resampling procedure was restricted to those individuals for which measurements of all traits were available. Selection gradient analyses revealed significant selection for fish that grew faster as larvae (β_{MLG} = 0.733, p = 0.035) but slower as juveniles (β_{MJG} = −0.785, p = 0.027). Because these 2 traits are moderately correlated (r = 0.398, Fig. 4), the opposing selection resulted in selection differentials being weaker than gradients (and non-significant; Table 1). Again, selection gradient analysis provided a clearer description of the direct effects of selection. Slow juvenile growth has also been directly associated with greater survival in other populations of this species, and may be related to decreased risk-taking associated with slow growth during the juvenile phase (Johnson & Hixon 2010). Evidence for nonlinear selection was weak, in part because of missing data and relatively low sample sizes. None of the nonlinear, quadratic differentials or gradients were significant at the α = 0.05 level, although the point estimates for correlational selection on the MJG× MLG and the MJG×PLD terms were moderately strong (Table 1). These patterns were corroborated by piecewise visualizations of the multivariate selection surface. When present, selection on each of the traits was more or less directional, with the exception of slight correlational selection for a combination of fast larval growth and slow juvenile growth (Fig. 5).

General recommendations

Analyzing selection within a multivariate framework can be very revealing, and the procedure for calculating selection gradients is only slightly more detailed than traditional analyses of selection differentials. As an informal guide, we include a flow chart outlining the steps that can be involved in calculating selection gradients (Fig. 6). Note that the R scripts provided in the online supplements follow this outline and include options for visualizing selection surfaces.

Selection gradient analysis enables the estimation of the actual, direct effects of selection by accounting for indirect effects. It also allows the joint effects of traits to be explicitly considered, and provides the capability to resolve many details of the individual selection surface in multiple dimensions. Selection gradient analysis therefore provides a powerful tool for understanding selective mortality in natural populations. However, employing these tools is only part of the way forward. A challenge for empirical studies will be to ensure that sample sizes are large enough to provide adequate statistical power to estimate some of the more subtle features of multivariate selection. In our experience, samples of hundreds of individuals may be required to characterize certain features of selection (e.g. nonlinear selection) with much certainty. We recommend sample sizes of >75 in each of the before- and after-selection samples, noting that in some cases (e.g. when using passive collectors such as light traps), sample sizes will be limited. Although the data-collecting effort required to describe multivariate selection is substantial, we believe that the advances in understanding that result from a multivariate approach will be well worth the effort.

Table 1. *Stegastes partitus.* Summary of selection coefficients for Example 3. PLD: pelagic larval duration; MLG: mean larval growth; MJG: mean juvenile growth; **S**: standardized selection differential. **Bold** values highlight estimates that are significantly different than zero at the alpha = 0.05 level. **γ**: nonlinear selection gradients; **β**: linear selection gradients; **C**: nonlinear selection differential

	Linear selection coefficients						Nonlinear selection coefficients					
	Differentials			Gradients			Differentials			Gradients		
Trait	**S**	SE	p	**β**	SE	p	**C**	SE	p	**γ**	SE	p
PLD	0.284	0.303	0.349	0.298	0.309	0.335	0.198	0.49	0.686	−0.164	2.31	0.943
MLG	0.285	0.313	0.363	**0.733**	**0.347**	**0.035**	0.333	0.568	0.558	0.632	2.307	0.784
MJG	−0.595	0.326	0.068	**−0.785**	**0.356**	**0.027**	0.108	0.746	0.885	0.192	3.52	0.957
PLD×MLG							−0.244	0.436	0.576	−0.101	1.76	0.954
PLD×MJG							−0.323	0.591	0.585	−0.463	2.66	0.862
MLG×MJG							−0.142	0.442	0.748	−0.666	2.26	0.768

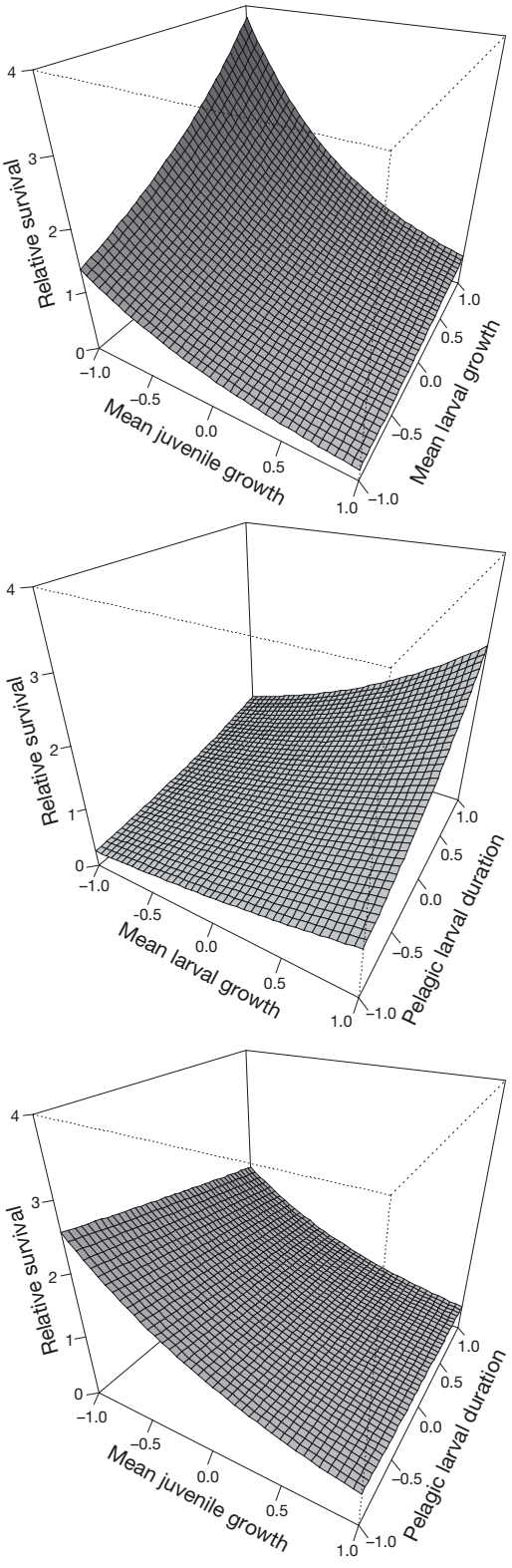

Fig. 5. *Stegastes partitus*. Pairwise representations of the multivariate fitness surface. Each panel displays the relationship between standardized trait values and expected values of relative fitness, holding the third, omitted trait at its average value

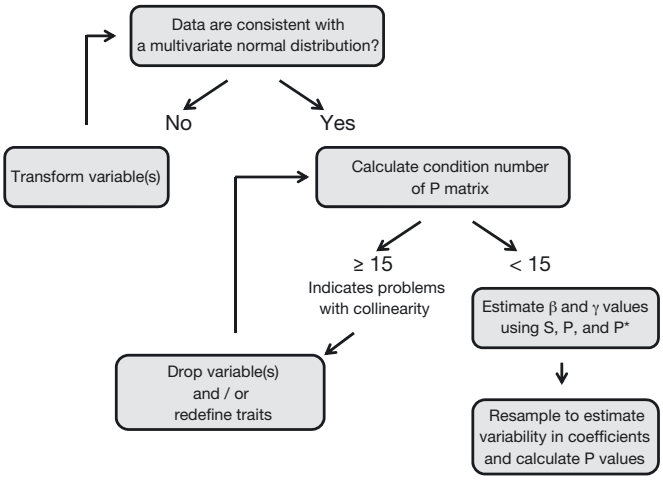

Fig. 6. Basic procedures for calculating selection gradients from cross-sectional data. Of particular importance are the assumptions that the before-selection sample is consistent with a multivariate normal distribution and that multicollinearity among the phenotypic traits will not appreciably affect the calculations. See main text for detailed recommendations on how to ensure these assumptions are met

CONCLUSIONS

Selection is a multivariate phenomenon. In nature, it is extremely unlikely that an individual's chance of survival (or reproduction) is influenced by only a single phenotypic trait. Rather, probability of survival is often influenced by a suite of traits, sometimes independently, sometimes in combination. In addition, traits that can confer a survival advantage (e.g. size- and growth-related traits) are often correlated to some degree. For all of these reasons, selection should be studied in a multivariate framework.

Our hope is that more cross-sectional studies of selective mortality adopt a multivariate approach and calculate selection gradients as well as differentials. Although selection differentials can be informative, there is a pressing need for a more accurate description of natural selection in marine environments. Indeed, studies based on single-trait measures of selection have revealed that selective mortality is widespread, often very strong, and particularly important during the early life stages of marine species (reviewed by Sogard 1997, Pechenik 2006, Perez & Munch 2010). More recent research has suggested that patterns of selection may vary across time and space and may change in response to several factors, including ontogenetic niche shifts (Gagliano et al. 2007), composition of local predators and competitors (Holmes & McCormick 2006, McCormick & Meekan 2007), distribution of trait values (Johnson et al.

2010), or changes in abiotic factors such as water temperature (Grorud-Colvert & Sponaugle 2011, Rankin & Sponaugle 2011). In addition, fishing mortality is a potentially powerful selective force (reviewed by Law 2000, 2007). Measures of selection (e.g. selection differentials) associated with different intensities and types of fishing are critical to quantifying or predicting evolutionary responses to fisheries-induced selection (e.g. Rijnsdorp 1993, Swain et al. 2007). However, patterns of fishing selectivity may be complex (Kuparinen et al. 2009), and fishing mortality may select on a variety of correlated traits (e.g. size at various ages, age at maturity, boldness), affecting both the interpretation of selection differentials and predicted evolutionary responses. In both basic and applied studies of selective mortality, a thorough, multivariate consideration of selection will lead to a more rigorous evaluation of how and why selection varies in marine systems and will undoubtedly lead to new and exciting progress in this field of study.

Acknowledgements. This work was conducted while D.W.J. was a Postdoctoral Associate at the National Center for Ecological Analysis and Synthesis, a center funded by the National Science Foundation (grant no. EF-0553768), the University of California, Santa Barbara, and the State of California. Empirical data were collected under NSF grants OCE- 9986359 and OCE 0550732 to S.S. We thank S. Cappell, C. Cooper, E. D'Alessandro, K. Denit, A. Exum, J. Fortuna, L. Gundlach, C. Guigand, K. Huebert, J. Llopiz, A. Mass, M. Paddack, C. Paris, D. Pinkard, D. Richardson, and M. Sullivan for assistance in the data collection. R. R. Warner and 3 anonymous reviewers provided helpful comments on an earlier version of the manuscript.

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Editorial responsibility: Stylianos Somarakis, Heraklion, Greece

Submitted: June 20, 2012; Accepted: August 28, 2012
Proofs received from author(s): November 30, 2012