

## **Methods for shape analysis of landmark data from articulated structures**

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### **ABSTRACT**

Landmark-based geometric morphometric methods are powerful tools in the study of size and shape. These methods allow one to describe the shape of rigid structures using a set of variables that can be used for statistical hypothesis testing, and to generate graphical representations of shape differences as deformations. However, when the landmarks chosen for an analysis span multiple rigid structures that articulate, variation describing the position of landmarks on one structure relative to those on another is also present in the data. In this paper, I develop three novel methods to remove the effects of arbitrary positioning of articulated structures. The separate subset method constructs shape variables for each subset of landmarks separately, then combines the resulting information. The fixed angle method rotates one subset of landmarks so the angle between subsets is invariant among specimens, and then treats them as a rigid structure for the shape analyses. The orthogonal projection method estimates the distortion due to the effects of articulation motion, approximates it with a vector, and then removes this dimension from the shape data before statistical analysis. I describe each of these methods in detail, and demonstrate their use on a data set containing landmarks from skulls and lower jaws from several populations of the threespine stickleback, *Gasterosteus aculeatus*. The results using all methods are compared to previous findings and to each other, and the implications for studies of functional morphology are discussed.

*Keywords:* articulation, Burnaby's size correction, morphometrics, thin-plate spline.

### **INTRODUCTION**

Morphometrics, the study of shape variation and its covariation with other variables, is an integral part of organismal biology. By quantifying morphological variation, it is easier to identify the relationship between morphology and ecology (e.g. Losos, 1990; Ricklefs and Miles, 1994) and thus make more informed inferences on the evolution of organisms. The field of morphometrics has recently undergone a revolution, in which traditional approaches based on sets of distance measurements have given way to geometric methods (Rohlf and Marcus, 1993). These new methods capture shape using the Cartesian coordinates of homologous landmarks after non-shape variation (specimen location, orientation

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and size) have been removed from the data. These methods are quite powerful, and generate a set of shape variables that can be used for statistical hypothesis testing, and provide a way of describing visually patterns of shape differences in the data.

Although geometric morphometric methods have been used to address many types of questions, their use in functional morphology has been surprisingly limited (but see Shaefer and Lauder, 1996). Current geometric methods can be used to analyse landmark configurations from single rigid structures (e.g. skull), but sets of landmarks that span multiple parts of an articulated structure (e.g. skull and mandible) cannot be analysed (Gillham and Claridge, 1994; Rosenberg, 1997). Because many functional morphological studies are concerned with articulated structures, there is an obvious need for landmark-based methods to analyse such data. Bookstein (1991, p. 313) outlined an approach that expresses the rigid motion of one subset of landmarks relative to another by defining a baseline and describing the displacements of landmarks relative to it using shape coordinates. His method is general, and can describe all types of rigid motion and relative displacements of one subset of landmarks. My aim here is to present several simpler approaches to study the shape of articulated structures, after the effects of arbitrary rotations have been removed. First, I review current geometric morphometric methods and discuss the problem of articulated structures. I then describe three methods to remove variation due to the effects of articulation-generated rotation from landmark data, and compare their results using an example data set. Finally, the advantages and disadvantages of the methods are discussed.

## GEOMETRIC MORPHOMETRICS AND ARTICULATED STRUCTURES

Geometric morphometric methods usually begin with the collection of two- or three-dimensional Cartesian coordinates of homologous landmarks. However, direct analysis of these data is not useful, since various types of non-shape variation (such as position, orientation and size) are present in these values. Therefore, a generalized least-squares superimposition is performed to remove this variation, and to align the specimens to a common coordinate system (Rohlf and Slice, 1990). The aligned specimens identify points in a non-Euclidean space (similar to that in Kendall, 1984), which is approximated by a Euclidean tangent space for standard multivariate statistical analyses (Rohlf, *in press*). The thin-plate spline can be interpreted as one method of generating a coordinate system for this tangent space.

The thin-plate spline is a global interpolating function that maps (transforms) perfectly the landmark coordinates of one specimen to the coordinates of the landmarks in another specimen, thus allowing the differences between the two specimens to be described in a manner similar to D'Arcy Thompson's transformation grids (Thompson, 1917). The parameters for this mapping can be decomposed into uniform and non-uniform components, which, when taken together, describe perfectly the shape of one specimen as deformations of another. The non-uniform component can be further decomposed into geometrically orthogonal elements called 'partial warps' (Bookstein, 1991). The partial warp scores, plus the uniform shape components, can then be treated as a set of shape variables for statistical comparisons of within- and between-group variability (e.g. Adams and Funk, 1997; Caldecutt and Adams, 1998).

The difficulty of applying current geometric methods to data from articulated structures becomes clear with an example. Suppose there is an organism on which seven landmarks span two structures that articulate at a common point (Fig. 1a). Because the two structures

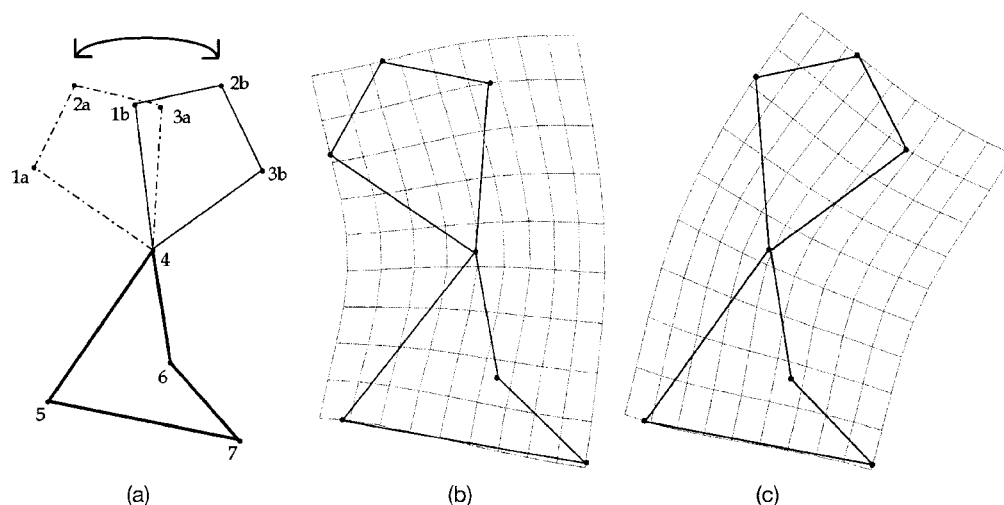
articulate, one subset of landmarks may be arbitrarily rotated relative to the other. Recording the  $x,y$  coordinates of these landmarks for different rotations yields different values for landmarks 1–3. If these two rotations were then treated as separate specimens, superimposed using generalized least-squares, and shape variables were obtained with the thin-plate spline, the results would differ. Thus, although the two configurations of landmarks differ merely by an arbitrary rotation of the upper subset of landmarks relative to the lower set (Fig. 1b,c), they would be erroneously interpreted as having different shapes.

### ACCOUNTING FOR BIOLOGICAL ARTICULATIONS

The example in Fig. 1 demonstrates that the relative position of two subsets of landmarks can affect the shape variables of a specimen. If this effect is systematically biased in a data set (e.g. males are preserved with their jaws closed, while females are preserved with their jaws open), any shape differences found among groups may be an artifact. It is therefore desirable to remove this effect, so that shape variables adjusted for the effects of articulation angle (hereafter called ‘rotation-free shape’) may be obtained and used in statistical analyses. I describe three alternative methods for removing articulation motion below.

#### Separate subset method

One obvious way to generate rotation-free shape is to analyse each subset of landmarks separately, and combine the results from the two analyses. In this *separate subset method*, the landmarks are separated into two groups, each containing the articulation point. Each subset is superimposed using generalized least-squares, and shape variables are generated using the thin-plate spline. The overall shape of each specimen is then determined by



**Fig. 1.** (a) Two landmark configurations differing by a 50° rotation of the upper subset of landmarks. (b and c) The same landmark configurations represented as thin-plate spline transformation grids.

combining the two sets of shape variables (one for each subset), and an additional variable which is the ratio of the size of the two subsets (centroid size is used: Bookstein, 1991). These variables are then treated as rotation-free shape variables, and used in all subsequent statistical analyses.

### Fixed angle method

A second method is to standardize all specimens so that the articulation angle is invariant among specimens. For this *fixed angle method*, the Cartesian coordinates of  $p$  landmarks from  $N$  specimens are recorded, and an articulation angle is calculated for each specimen. The articulation angle is defined as the angle between the articulation point and the centroid of each subset of landmarks. For each specimen, one subset of landmarks is rotated so that the articulation angle is set at some fixed angle  $\theta_1$  for all specimens. The specimens are then treated as rigid structures, superimposed using generalized least-squares, and shape variables are generated using the thin-plate spline.

### Orthogonal projection method

A third possible way to remove the effects of small variation in articulation position is to estimate directly the distortion due to the effects of articulation motion and remove it from the data set. A simple way to implement this method is to use orthogonal projections. Burnaby (1966) described a method to remove the effect of one or more extraneous variables from a data set by projecting into a space orthogonal to these variables. His original formulation was designed to remove general size from a set of linear distance measurements so that the remaining variation could be considered size-independent descriptors of shape. Using Burnaby's (1966) method, a subspace complementary to the size vector is generated, and the specimens are projected into this subspace. The size vector is then considered to be held constant with respect to variation in the resulting variables (Rohlf and Bookstein, 1987).

Generation of this subspace is easily performed through matrix algebra. For  $N$  specimens and  $q$  variables, one defines the extraneous variable as a  $q \times 1$  column vector,  $\mathbf{f}_1$ . The matrix  $\mathbf{L}$  projects the data onto the space orthogonal to  $\mathbf{f}_1$  and is defined as:

$$\mathbf{L} = \mathbf{I}_q - \mathbf{f}_1(\mathbf{f}_1'\mathbf{f}_1)^{-1}\mathbf{f}_1'$$

where  $\mathbf{I}$  is a  $q \times q$  identity matrix, and  $\mathbf{L}$  is a  $q \times q$  matrix of rank  $q - 1$  (Rohlf and Bookstein, 1987). One then multiplies the data matrix by  $\mathbf{L}$  to generate a set of adjusted specimens. Matrix  $\mathbf{L}$  is of rank  $q - 1$ , and the adjusted specimens contain variation only in the hyperplane orthogonal to the original vector  $\mathbf{f}_1$ .

Burnaby's (1966) method can be applied here by defining  $\mathbf{f}_1$  as the vector that approximates the distortion due to changes in articulation angle. The approach is analogous to Bookstein's (1991) method of estimating rigid motions by exact infinitesimal linearization, but uses a simple linear approximation. For this *orthogonal projection method*, the Cartesian coordinates of  $p$  landmarks from  $N$  specimens are recorded, and an articulation angle (as defined above) is calculated for each specimen. Specimens are superimposed using generalized least-squares and shape variables are calculated using the thin-plate spline. This is the set of shape variables for the unadjusted specimens, and variation due to the rotation of articulated structures will be removed from these variables.

The one-dimensional articulation vector,  $\mathbf{f}_1$ , is calculated by first rotating one subset of landmarks on each specimen so that the articulation angle  $\theta_1$  is the same for all specimens. These specimens are retained as data set 1. The same subset of landmarks is then rotated to another angle  $\theta_2$ , where  $\theta_2 > \theta_1$  (note: values  $\theta_2 < \theta_1$  can also be used). These new specimens are retained as data set 2. Data sets 1 and 2 are combined, the specimens are superimposed using generalized least-squares, and shape variables are calculated using the thin-plate spline. Because the only difference between shape variables at  $\theta_1$  and  $\theta_2$  for each specimen is the relative position of the two subsets of landmarks, the difference between these shape variables can be considered an approximation of the effect of arbitrary articulation positions on shape. Thus, the 'articulation vector' for each specimen is defined as the difference between its shape variables from rotations  $\theta_1$  and  $\theta_2$ . The average articulation vector is calculated, treated as  $\mathbf{f}_1$ , and the shape variables from the unadjusted specimens are projected onto a subspace orthogonal to this vector to yield rotation-free shape variables.

The rotation-free shape variables obtained from the above procedure can be used for descriptions of shape and comparisons of shape among groups. However, because any variation along one dimension of the data set has been held constant, the covariance matrix is singular, and any statistical analyses that use the inverse of the covariance matrix of these variables (e.g. multivariate analysis of variance, canonical variates analysis, etc.) cannot be performed directly. Therefore, one should use a generalized inverse of the shape variables, or a two-step canonical variates analysis (CVA) (see Campbell and Atchley, 1981) to eliminate the singularity. I used the two-step CVA before all group comparisons of shape.

### AN EXAMPLE

To demonstrate the use of the three methods described above, I analysed a data set containing landmarks from skulls and lower jaws of stickleback fish (*Gasterosteus aculeatus*). The specimens were part of a larger study comparing the trophic osteology of four stickleback populations in Alaska (Caldecutt and Adams, 1998). Caldecutt and Adams found differences in head shape between populations and between sexes, but were unable to include landmarks from the lower jaws of the specimens. I included several landmarks from the lower jaw and re-analysed their data, first to determine whether statistical conclusions from methods taking articulation into account were similar to those from standard techniques and, second, to determine if additional information could be obtained by the inclusion of landmarks from both parts of articulated structures.

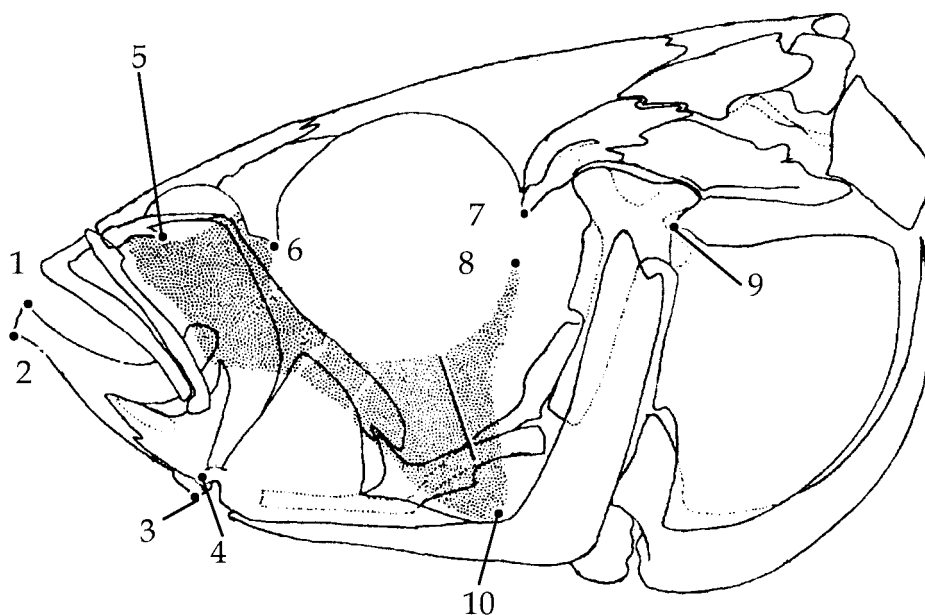
Caldecutt and Adams (1998) analysed 50 males and 50 females from each of four populations to compare head shape among populations and between the sexes. They used 23 landmarks to represent the shape of the skull, but no landmarks from the lower jaw were used, because they were unable to control its position relative to the rest of the skull. They found significant differences among populations, which were interpreted in terms of the feeding regimes of those populations (i.e. benthic feeders vs limnetic feeders). Trophic habitat is statistically associated with other morphological characters in stickleback (see McPhail, 1994, and references therein), and may also be associated with head shape. Caldecutt and Adams (1998) also found significant sexual dimorphism in their data set, although multiple comparisons revealed that this dimorphism only existed in one population (Meadow Creek). In addition, using a relative warp analysis (a principal components analysis of shape variables; Rohlf, 1993), they described the major trend in shape variation in the data set. They found that the first relative warp described variation between fish with

deepened skulls, relatively smaller orbits and an enlarged suspensorium, and those with shallow heads, relatively larger orbits and a smaller suspensorium – a contrast similar to the benthic–limnetic dichotomy.

I selected 200 specimens from Caldecutt and Adams' original sample (25 males and 25 females per population) and re-digitized a subset of their landmarks (Table 1, Fig. 2). Landmark #4, the quadrate-articular joint, was treated as the articulation point for the lower jaw. In addition, I digitized three landmarks on the lower jaw of each specimen,

**Table 1.** Morphological landmarks used in this study (the last column denotes the landmark numbers from Caldecutt and Adams, 1998)

| Landmark | Description                                | Subset     | Prev. # |
|----------|--------------------------------------------|------------|---------|
| 1        | Anterodorsal tip of dentary                | Jaw        | —       |
| 2        | Anteroventral tip of dentary               | Jaw        | —       |
| 3        | Angular process                            | Jaw        | —       |
| 4        | Quadrate-articular joint                   | Art. point | 22      |
| 5        | Palatine-lachrymal at nasal capsule        | Head       | 3       |
| 6        | Lachrymal-lateral ethmoid contact at orbit | Head       | 7       |
| 7        | Anteroventral extent of sphenotic at orbit | Head       | 11      |
| 8        | Posterodorsal extent of third suborbital   | Head       | 10      |
| 9        | Operculum-hyomandibular joint              | Head       | 14      |
| 10       | Posteroventral extent of third suborbital  | Head       | 20      |



**Fig. 2.** Positions of landmarks used in this study. For all analyses, landmark #4, the quadrate-articular joint, is considered the articulation point of the lower jaw. Modified from Caldecutt and Adams (1998), after Bowne (1994) (see Table 1 for descriptions).

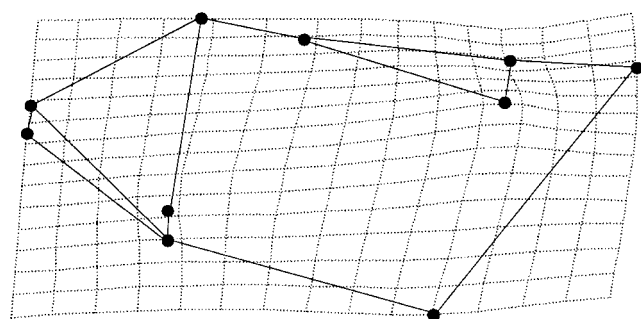
knowing that variation in the position of the lower jaw relative to the rest of the skull was present in the data. I generated rotation-free shape variables using all three methods, and compared the results to those of Caldecutt and Adams (1998), and to each other.

Using rotation-free shape, I found significant differences between populations, as well as between the sexes, confirming Caldecutt and Adams' (1998) findings (Table 2). As in their study, differences between populations were greatest, followed by differences between the sexes; the interaction between population and sex had the smallest effect. This pattern was always found, regardless of which method was used to generate rotation-free shape variables. Multiple comparison tests revealed that within-population sexual dimorphism was not significant, but the comparisons of males and females from the Meadow Creek population were nearly significant. This trend is similar to the findings of Caldecutt and Adams (1998), but their larger sample size allowed for greater statistical power to identify sexual dimorphism in the Meadow Creek population.

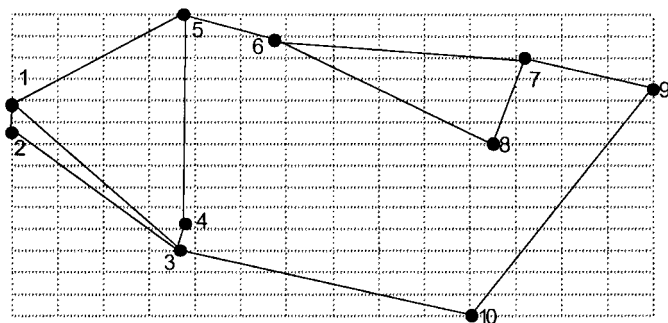
I described the major trends in shape variation in the data by visualizing specimens along the first relative warp axis using the fixed angle data set. For the separate subset and orthogonal projection data sets, I approximated the relative warps procedure by first computing the principal component (PC) scores for each specimen using the rotation-free shape variables, and then regressed the shape variables of the unadjusted specimens onto the first PC scores to obtain visualizations along this axis. Results from all three 'relative warps' analyses were similar, so only the results from the fixed angle data are presented here. With each of these data sets, I found that the major trend in shape variation was very similar to that found by Caldecutt and Adams (1998). Extremes along the first relative warp contrasted specimens with a deeper skull (landmarks 8 and 10) and a relatively smaller orbit (landmarks 6, 7 and 8) to those with a shallow skull and relatively larger orbit (Fig. 3). Furthermore, positive deviations along the first relative warp described specimens with a relatively longer lower jaw (as seen by the posterior movement of the angular process), whereas negative deviations described specimens with a relatively shorter jaw. Thus this axis

**Table 2.** Results of two-way MANOVA of shape variables from each of the three articulation methods

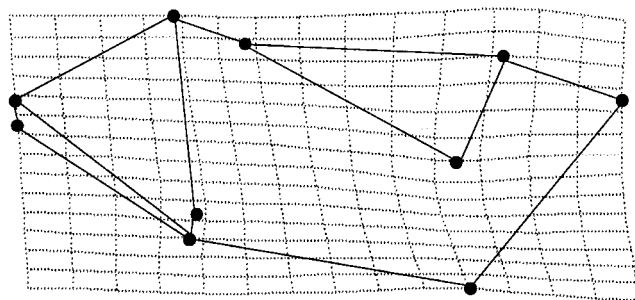
| Source                              | Wilks' $\lambda$ | $F$   | d.f.   | $P$     |
|-------------------------------------|------------------|-------|--------|---------|
| <b>Separate subset method</b>       |                  |       |        |         |
| Population                          | 0.0183           | 33.47 | 45,530 | <0.0001 |
| Sex                                 | 0.6587           | 6.14  | 15,178 | <0.0001 |
| Population $\times$ sex             | 0.6000           | 2.21  | 45,530 | <0.0001 |
| <b>Fixed angle method</b>           |                  |       |        |         |
| Population                          | 0.0176           | 31.69 | 48,527 | <0.0001 |
| Sex                                 | 0.6519           | 5.91  | 16,177 | <0.0001 |
| Population $\times$ sex             | 0.5533           | 2.42  | 48,527 | <0.0001 |
| <b>Orthogonal projection method</b> |                  |       |        |         |
| Population                          | 0.0163           | 35.21 | 45,530 | <0.0001 |
| Sex                                 | 0.6027           | 7.82  | 15,178 | <0.0001 |
| Population $\times$ sex             | 0.5395           | 2.72  | 45,530 | <0.0001 |



Positive Deviation



Mean Shape



Negative Deviation

**Fig. 3.** Visualizations of the major trend in shape variation, as found from a relative warp analysis of the fixed angle data set. Positive deformations along this first RW axis describe specimens with deeper heads, relatively smaller orbits and longer lower jaws, whereas negative deformations along this axis describe specimens with shallower heads, relatively larger orbits and shorter jaws.



seemed to differentiate fish with respect to jaw length, a result that Caldecutt and Adams (1998) did not find. Walker (1997) found that fish in lakes with high relative littoral area had longer jaws, which is consistent with my findings. This result also implies that the information gained from including landmarks from the lower jaw revealed additional contrasts between specimens along RW1, and possibly between benthic and limnetic feeders.

Finally, I compared the results from each of the three rotation-free methods to results from a 'naïve' analysis, in which the 10 unadjusted landmarks were treated as if they were from a single rigid structure. Again I found significant differences between populations and between the sexes, with a significant interaction term between population and sex. However, when I compared the probability values from the naïve analysis to those from the three rotation-free methods, I found that, on average, the probabilities for the rotation-free methods were 30 times smaller, indicating that they had higher power. Thus, by not accounting for biological variation in the relative position of the lower jaw, the naïve analysis contained random variation in the resulting shape variables that reduced the statistical power of the subsequent analyses.

## DISCUSSION

The relationship between form and function is an integral part of ecological and evolutionary studies (Ricklefs and Miles, 1994). The link between the two is often difficult to make, however, in part because of a lack of appropriate methods for quantifying morphology. Geometric morphometric methods have the advantage that they provide a consistent set of shape variables for hypothesis testing, which can also be used to generate graphical representations (deformation grids) of morphology. These deformation grids are particularly useful to describe the shape differences between groups and how shape changes with continuous variables (e.g. size). A disadvantage of current techniques, however, is that they cannot be used to analyse articulated structures, which are often of greatest interest in studies of functional morphology.

In this paper, I have described three new morphometric methods to adjust landmark data for articulation motion before the generation of shape variables. Using an example data set of landmarks from skulls and lower jaws from stickleback fish (Caldecutt and Adams, 1998), I found that all three methods gave similar statistical results, which were consistent with results from previous analyses using only the skull. Thus the analysis of landmarks from both parts of the articulated structure did not alter previous conclusions from analyses of just one subset of landmarks. By including landmarks from the lower jaw, I was able to identify additional components of variation that Caldecutt and Adams (1998) did not observe. I found that specimens with deep skulls and small orbits had longer jaws, whereas specimens with shallow skulls and large orbits had shorter jaws. Only by including landmarks from both parts of an articulated structure could such an association be found. Finally, when these results were compared to those from a naïve shape analysis, I found that the probability values from the rotation-free methods were roughly 30 times smaller, implying higher statistical power. Thus, variation due to the relative position of the lower jaw was present in the original data set, and needed to be accounted for before statistical hypothesis testing.

Although it is clear that articulation variation must be accounted for before statistical analysis, how to choose between the rotation-free methods is not so obvious. One option is not to choose, but rather to compare the results from all three methods. Although no major

differences were found for the data set presented here, it is possible that each method may detect subtle differences in other data sets. Because the methods seem to yield consistent results, one may decide to choose on the basis of computational simplicity. Here the separate subset method has the advantage over the other methods, as one merely generates two landmark data sets from the original set of specimens and combines the resulting shape variables. However, since the two subsets of landmarks are biologically coupled as an articulated structure, dissociating the two for statistical analysis may lead to a loss of some biomechanical information. The fixed angle method is also relatively simple to implement. Once the articulation angle has been standardized, the specimens are analysed as if they were rigid structures. A disadvantage with this method is that the value of the articulation angle to be standardized must be decided by the researcher (e.g.  $50^\circ$ ). Because this choice is arbitrary, standardizing different articulation angles may yield slightly different results. There is also a problem of how to define the articulation angle. Here I defined it as the angle between the articulation point and the centroid of each subset of landmarks, but the most biologically meaningful angle may be between the articulation point and two landmarks on the articulation surface (e.g. teeth) that touch when the articulation is 'closed'.

Of the three methods presented, the orthogonal projection method requires the most computational steps. This method also requires one to choose two angles to standardize, and may suffer from the same difficulties as the fixed angle method. In addition, the method removes mathematically the average articulation vector from all specimens, rather than adjusting each specimen individually for their own articulation vector. However, there are several reasons for doing this. First, if one were to remove the individual articulation vector for each specimen separately, a slightly different dimension would be eliminated from the shape variables for each specimen. Thus, the resulting rotation-free shape variables would not be comparable. Use of the average vector assumes that articulation vectors for all specimens are relatively homogeneous (i.e. they are oriented in approximately the same direction), although this generally seems to be the case. For the stickleback data set, the pairwise angles between articulation vectors ranged from  $0.7^\circ$  to  $12.9^\circ$ , with an average of  $4.1^\circ$ . This implies that the articulation vectors for all specimens were pointed in the same general direction. Therefore, use of the average articulation vector seems warranted for this data set, but other data may not be as well behaved.

One reason for choosing the orthogonal projection method over the other methods is that one could analyse both rotation-free shape and the articulation vectors for the same data set. Because the articulation vector for each specimen is calculated before its elimination from the shape variables, the vector itself may be used as data for comparison of groups of specimens. Although further study is needed, analysis of the articulation vectors could potentially identify *modes* of articulation motion, and may provide evidence of functional differences between groups.

Although the results presented here were similar for all methods, the ultimate reason for choosing between them should be statistical in nature. Computer simulations, under a variety of known conditions, should be conducted to determine the type I error rates and statistical power of each method. Using this procedure, it would be possible to determine when each method performs well and when each performs poorly. Thus, the decision for choosing one method over another for a particular data set would be based on which method has the most acceptable type I error rate, or which has the greatest statistical power.

The field of geometric morphometrics is a rapidly evolving area of research, and extensions of current methods for new problems are constantly being investigated (e.g. Fink and Zelditch, 1995). While some of these new applications are controversial (see discussions in Adams and Rosenberg, 1998; Rohlf, 1998), the tools of geometric morphometrics are a great aid in the description and understanding of morphological variation. The ability to account for variation in landmark data spanning articulated structures further expands the realm of geometric morphometrics, by allowing the exploration of shape variation that, until now, could not be investigated. These rotation-free methods broaden the scope of geometric morphometrics.

### ACKNOWLEDGEMENTS

I thank E. Abouheif, W. AlGharaibeh, M. Bell, F. Bookstein, C.P. Klingenberg, F.J. Rohlf and D. Slice for comments on earlier versions of the manuscript, and W. Caldecutt for digitizing additional landmarks for the data example. Specimens were collected on NSF grant DEB-9317572 (to M.A. Bell). This work was sponsored in part by NSF grants IBN-9800636 (D.C.A.) and DEB-9728160 (to F.J. Rohlf), and a Sigma Xi Grant-in-aid of Research (D.C.A.), and is contribution number 1047 from the program in Ecology and Evolution at the State University of New York at Stony Brook.

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