

AN ABSTRACT OF THE THESIS OF

Madeleine Demaries Weber for the degree of Master of Science in Fisheries Science. Presented on June 14, 2002. Title: A Morphometric Study of Growth and Condition in Juvenile English Sole (*Pleuronectes vetulus*) Relative to Environment.

Abstract approved:

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James H. Power

This thesis focuses upon whether stressful aspects of an organism's environment are reflected by that organism's shape. It presents an application of the powerful thin-plate spline and relative warp methods from morphometric analysis to demonstrate the overall utility of morphometrics in detecting environmental stress in an estuarine flatfish, the English sole or *Pleuronectes vetulus*.

Juvenile English sole were captured from the Yaquina Bay, Oregon, photographed using a digital camera, and then held without food in the laboratory for periods of 7 to 24 days. Landmarks on the outline of the ventral surface of the body were digitized from the images. The mean position of the landmarks for freshly caught sole was used to compute a reference specimen. The thin-plate spline method was then applied to quantify the intraindividual shape variation due to lab-induced environmental stressors for all fish. Relative warp analysis of the resulting landmark data yielded relative warp scores for each individual fish, and was analogous to a principal component analysis.

Analysis of covariance of the relative warp (principal component) scores showed that fish held without food acquire different shape characteristics in comparison with freshly caught fish, and that these shape differences reflect captivity and food deprivation effects. A discriminant function analysis using the data allows clear differentiation of stressed and non-stressed fish.

The underlying goal of this research was to examine the conceptual and methodological aspects of morphometrics relevant to its future potential use as a measure of developmental precision and environmental condition. The technique may have applicability for detecting environmental stress in natural populations of estuarine fish.

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**A Morphometric Study of Growth and Condition in Juvenile  
English Sole (*Pleuronectes vetulus*) Relative to Environment**

**by  
Madeleine Demaries Weber**

**A THESIS**

**submitted to**

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APPROVED:

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Madeleine Demaries Weber, Author

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## DEDICATIONS

To Mother Earth who in her graciousness provided me with the subjects without which this thesis could not have been written. To my mother, Kay Brackett Lowe, who set me upon the eternal and timeless path of wandering and wondering. To my father, Alan Andrew Begley, who gave me my mystical soul and lives within me still. To Alfred L. Lowe who opened his arms and made me his own. To Jerry Allen Weber whose support goes beyond the technical into the metaphysical. To Candace Begley whose mind and heart are always open to new knowledge and joy. To Cassandra Begley who reminds us all that we can strive to be better. To Alan Andrew Begley Jr. who inspires my spirit even now. To Z whose friendship and love causes me to walk in endless sunshine. And to Jessika, Sydney, and Nathan without whom this would all be meaningless.

# **A MORPHOMETRIC STUDY OF GROWTH AND CONDITION IN JUVENILE ENGLISH SOLE (*PLEURONECTES VETULUS*) RELATIVE TO ENVIRONMENT**

## **CHAPTER 1**

### **INTRODUCTION**

Recruitment success in fish populations is believed to be established during the larval and juvenile stages of development (Houde, 1987). During these stages intraindividual variations in overall growth and condition among members of a population can have important consequences for their continued survival and recruitment into a fishery. Consequently, indicators of physiological condition that can offer insight into the proximal growth and condition of juvenile fish have been extensively studied (Ferron & Leggett, 1994).

Mark-recapture methods are a classic method for evaluating fish growth, but are often impractical with juveniles that are not the target of a fishery. Observation of the progression of modal values in length-frequency distributions is another method that has also been used to evaluate how fish grow in relation to environmental conditions. This approach has coarse resolution and does not allow for spatial comparisons among individuals.

Other approaches to measuring fish growth and condition include histological assessments or evaluation of biochemical indicators. These indicators have an advantage in that they are not seriously affected by damage during net capture. The drawbacks to histological and biochemical indicators are that they are expensive, labor intensive, require special handling and facilities in the field, and may be biased by differential sample collection and processing.

Studies resulting from the above data collection methods have shown that fish larvae in good condition have increased foraging abilities, exhibit normal growth and development, are less susceptible to predation or disease, and are better able to combat environmental stressors. All of these factors may be beneficial for the survivorship of the organisms due to fact that they spend less time in the pre-reproductive phase (Suthers, 1992). Fish larvae in good condition grow and develop into juvenile and adult phases when they are able to obtain sufficient amounts of quality food resources. These fish do not have to divert energy towards coping with external stressors, and therefore grow and develop normally. Conversely, fish not locating sufficient food resources may not grow and develop normally, leading to an increase in intraindividual shape variations.

Recent studies have begun to focus upon the impact of environmental stressors on the benthic flora and fauna of estuaries, including its effects on fish condition (Kneib 1994; Brandt and Mason 1994; Deegan and Garritt 1997).

Estuaries collect the runoff from their associate watersheds, and are often associated with developed or urban areas. As a result, anthropogenic processes frequently alter the ecology of the estuarine ecosystem. Additionally, by their nature estuaries are locations where important physical, chemical, and biological parameters can be highly variable over both space and time.

One manifestation of environmental stress may be a more pronounced shape variation among individuals. There is ample evidence that organisms under stress do indeed suffer disruption of their biological growth mechanisms, which may then cause an increase in the random intraindividual variation of their shapes. An extreme example would be the numerous reports of malformed individuals occurring with greater frequency in proximity to aquatic stressors, such as pollutants. Intraindividual variations in shape are the result of both the organism's environment and its genetic composition. Such variations in shape arising from environmental sources may prove useful in detecting sublethal effects that might not otherwise be readily observed.

Until recently, our ability to discriminate such intraindividual shape variations among organisms had been based in part upon the subjective assessments. These subjective assessments limited our ability to detect any early environmental warning signs, if indeed they do exist. Recent advances now allow more quantitative approaches for measuring shape variation among organisms, and in turn relating these variations to the organism's environment.

These more recent quantitative approaches have been termed “geometric morphometrics”. These morphometric techniques have several advantages: they are relatively cheap, use less intensive laboratory techniques and facilities, and have been shown to be extremely sensitive to indicators of environmental stress (Clark, 1992).

This thesis focuses upon whether stressful aspects of an estuarine organism’s environment are reflected by that organism’s shape. The research reported here utilizes a comparatively new morphometric technique to quantify intraindividual shape variation due to environmental stressors observed in the estuarine flatfish known as the English sole or *Pleuronectes vetulus*. English sole are widely distributed throughout the lower Yaquina Bay estuary, and are readily captured at locations ranging from the dredged central channel to the mudflats that are exposed during low tides (Westrheim 1955; Toole 1980; Krygier and Pearcy 1986; De Ben et al. 1990). Adult English sole occupy coastal waters, but the larvae and juveniles utilize the Pacific coast estuaries as nursery grounds. English sole enter the Yaquina Bay as larvae beginning in late February, and benthic juveniles later emigrate from the estuary in the fall, with some portion over-wintering and departing by the following May (Boehlert and Mundy 1987; Gunderson et al. 1990; Chamberlain and Barnhart 1993). As a flatfish, English sole live in close association with bottom sediments and rely on infaunal organisms as the bulk of their diet (Hogue and Cary 1982; Becker and Chew 1987; Barry et al. 1996).

This research represents a pilot study to evaluate whether environmental stress, in the form of food deprivation, is detectable as a shape change utilizing current morphometric techniques. Future resource planners may potentially utilize the results of this study as a tool for evaluating morphometric changes in juvenile fish populations resulting from anthropogenic processes in estuarine environments.

## **CHAPTER 2**

### **METHODS**

#### **SAMPLING AND DIGITIZATION**

English Sole ranging in size from 30 to 70 mm were collected using a beach seine from a single location in Yaquina Bay estuary in Newport, Oregon during the summer of 1998. Beach seine nets were deployed by hand and used to enclose a specific area of the littoral zone. The catch was quickly recovered and live fish were transported to the laboratory for analysis.

Once in the laboratory, the fish were anesthetized using the agent MS-222 and photographed using a Kodak DCS 420D digital camera. The photographs were processed using image-processing software adjusted so that the fish body filled the image frame as much as possible and displayed at full resolution on a computer monitor. These digital images had a median resolution of 0.053 mm of fish body per image pixel.

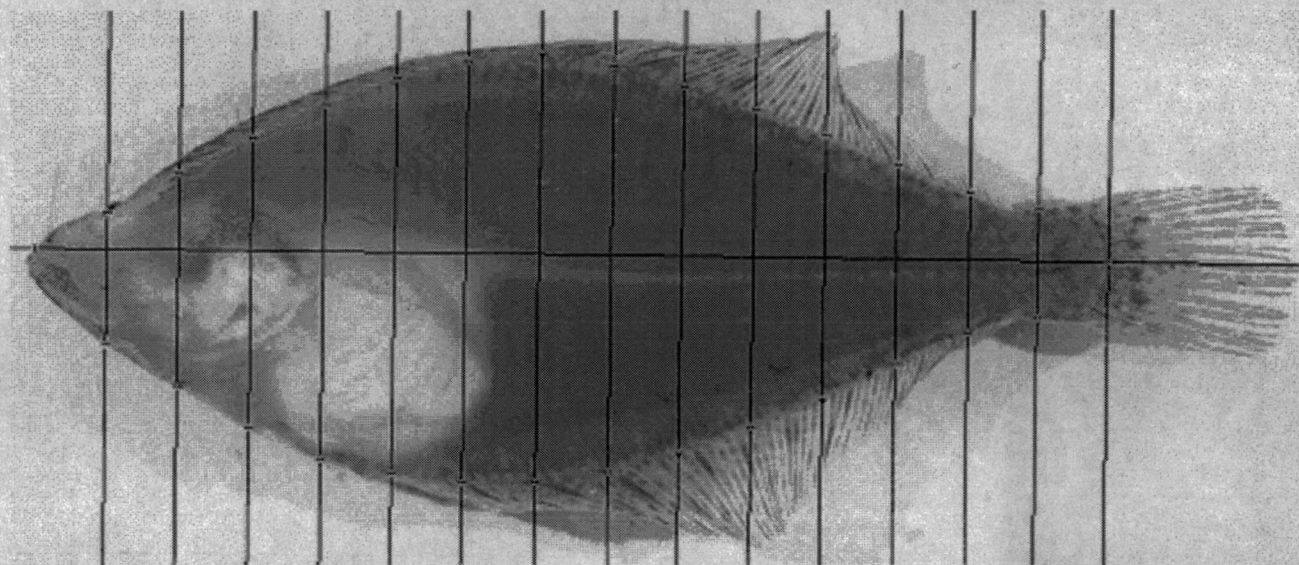
A subsample of fish were then held in laboratory tanks at ambient salinity and temperature, but without food, for periods ranging from 7 to 24 days. Three treatment classes were established under this analysis: freshly caught fish ( $n=65$ ), those fish held without food in the laboratory for 7-10 days ( $n=20$ ), and those fish starved for 22-24 days ( $n=34$ ).

After the lab-induced starvation periods, the fish constituting each treatment group were removed from the tank after the appropriate time had elapsed and were again anesthetized and photographed before being removed from the experiment. A total of 119 photographs ( $n= 119$ ) of *Pleuronectes vetulus* were digitized.

Digitization was accomplished by first locating the tip of the fish's snout and the posterior margin of the hypural plate on the digital image, and recording their coordinates. A baseline was then established by connecting these two points with a straight line. This baseline was partitioned along its length by 14 equispaced lines perpendicular to the baseline. The coordinates where these lines intersected the outer body margin were recorded as landmarks descriptive of the overall fish body shape. The image in Figure 1 represents a typical digital fish image.

## **THIN PLATE SPLINE MORPHOMETRIC ANALYSIS**

The analysis of intraindividual shape variations reported here used the morphometric techniques developed by Bookstein (1989, 1991) and Rohlf (1990). This technique, known as the thin-plate spline - relative warp analysis, was derived from an interpolation approach originally developed for computational surface theory and computer graphics. The thin-plate spline techniques are not widely known and so the analysis techniques are briefly summarized here and in more detail in the attached Appendix.



**Figure 1:** Photograph of ventral surface of English sole showing baseline and equispaced lines drawn perpendicular to baseline. Landmark coordinates were recorded where perpendicular lines intersected the fish body margin.

## **Landmark identification**

The landmark digitization resulted in  $p = 30$  landmarks corresponding to points on the margin of the body at the 14 intersecting lines. Figure 1 shows the positions of the landmarks that were identified for this study. Also shown are the positions of the 14 intersecting lines used to identify these landmarks. Analysis was done using the ventral (left) side of the fish.

## **Measuring**

The measuring and analysis of organism shape is confounded by variations in organism size – it is desirable to detect shape differences among organisms that are independent of differences in organism size. A measure of organism size that can be obtained from landmark data is centroid size. Centroid size is calculated as the square root of the sum of squared distances from each landmark to the centroid of all the landmark coordinates. Hence, the set of coordinates for a given organism were scaled by dividing by the organism's centroid size. This calculation removes size effects from the analysis of shape allowing shape to be examined independently.

The scaled landmarks were then converted to shape coordinates relative to the previously described baseline that extended from the snout to hypural plate. The tip of the fish snout was specified as  $(x,y)$  coordinates of  $(0,0)$ , while the posterior margin of the hypural plate was considered to have the coordinates of  $(1,0)$ .

The translation to shape coordinates ensures the landmark data are represented at a consistent orientation and a consistent scale.

The next step was the identification of a consensus, or reference organism. In this step only landmark coordinates from freshly caught fish were used, so that the reference specimen would represent the average shape of a fish prior to food deprivation. The landmark coordinates for the reference specimen were obtained by computing, for each landmark, the mean of the individual fish coordinates for that landmark. From this organism, a consensus or mean configuration was obtained and used as a reference to which all other observed specimens are compared. The consensus or reference organism was then used to obtain a set of vectors, called principal warps, that define the principal modes of variability possible in the observed specimens. It can be thought of a new, specialized coordinate system, based on the reference organism, within which the other specimens can be positioned. These principal warps also characteristically represent varying scales of shape variation, from broad changes over the entire organism to more localized differences. There are always 3 less principal warps than the number of landmarks recorded. The uniform (linear) components of shape change that span the space of affine differences such as translation, rotation, and uniform shape change among individuals have been removed from the analysis of shape by disregarding the last 3 eigenvectors in the bending energy matrix.

The reference organism's shape is now considered to lie at the point where all principal warp coordinate values are equal to zero (e.g. at the intersection of all the principal warp axes) in the principal warp vector space. All other specimens were then positioned in this principal warp coordinate system, so that the patterns and arrangement of those specimens relative to the reference organism can be examined for patterns.

The shape coordinates of each freshly caught fish were then positioned in the principal warp space, so that each fish would have a set of its coordinates on the 27 principal warps. These coordinates represent a point in multidimensional space corresponding to the shape of the individual fish. This is shown schematically in Figure 2 for only three dimensions, although this study had 27 principal warps, axes, or dimensions. Circles positioned in this space represented the shape of a specific fish. The reference specimen is at the origin of the 27 axes, or the intersection of the lines in Figure 2. The position of a fish in the principal warp space reflected how that fish's shape differed from that of the reference specimen.

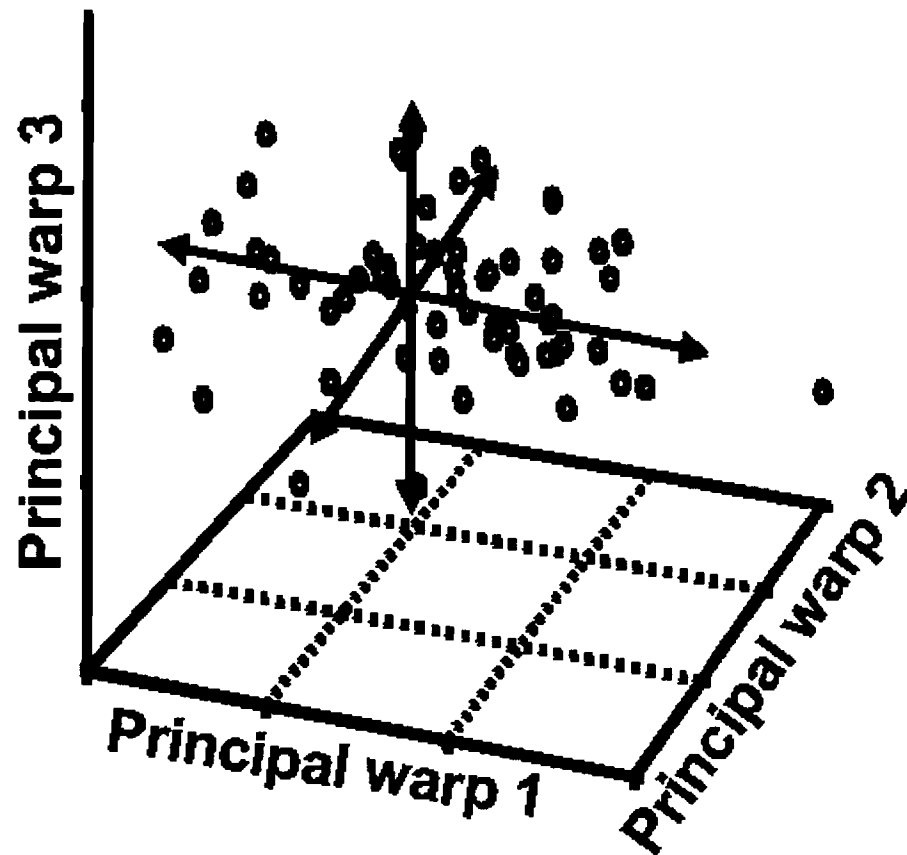


Figure 2: Example arrangement of individual specimen data on three principal warp axes. In this study there were 27 principal warp axes.

## Ordination

The next result was obtained by taking only the data (positions on principal warps) for freshly caught fish, and performing what is termed a relative warp analysis on this data to identify patterns within the cloud of points. The relative warp analysis is equivalent to a weighted principal component analysis, and indeed if the weighting parameter  $\alpha$  (see Appendix) is zero then the relative warp analysis is equivalent to principal component analysis. The terms relative warp and principal components may therefore be used interchangeably, and the relative warps are simply principal components vectors in this space and are used to describe the major trends in shape variation among specimens within a sample as deformations in shape. It was determined for this study that weighting was unnecessary, and a traditional principal component analysis was used to ordinate all the freshly caught fish in the principal warp space. Due to the fact that the analysis to this point only utilizes freshly caught fish, the variation or patterns (principal components = relative warps) in fish shapes can be explained by two sources: (1) natural shape variability do to environmental and genetic differences; (2) changes in shape due to development of the fish. Centroid size was used as a proxy way of expressing development.

The above processes allowed each specimen's geometric form to be archived and reconstructed as graphical representations of geometric shape differences among individuals. Each relative warp may be plotted as a deformation of the space of the reference configuration of landmarks.

This process can be shown by computing a thin-plate spline for each relative warp. Once the specimens were arranged in an appropriate coordinate system as defined by the reference organism, they were examined for any patterns evident in this arrangement. This gives the coefficients for a unit change in a relative warps score from that of the reference object. Deviations from the consensus configuration form the basis for characterizing the direction and magnitude of these differences.

### **Statistical analysis**

The scores of all fish on the principal component axes (relative warps) were then analyzed with respect to whether they were obtained from freshly caught or laboratory starved fish. The effect of size on shape had previously been removed, as described above, from the fish coordinate data. However, because the specimens were juvenile fish, their shape also varied due to developmental state as well as whether they represented freshly caught or laboratory-held fish. To accommodate for developmental shape differences, the fish centroid size values were considered to be a proxy value representing developmental state. The fish's principal component scores were then recombined with their associated centroid size values. A series of Analyses of Covariance (ANCOVA) were then performed with the data. Each ANCOVA was done using fish scores on a particular principal component axis as the dependent variate, with level of food deprivation as a treatment effect and centroid size as an independent variate to control for fish developmental state.

Finally, a discriminant function analysis was performed upon the resulting data to see if the lab-starved specimens could be reliably separated from the freshly caught fish.

## CHAPTER 3

### RESULTS

The reference organism coordinates (the mean landmark coordinates of the 65 freshly caught fish) are at the center of the blue crosses in Figure 3. The coordinates, or vectors, of a single fish are at the ends of the red lines. This example shows how a single fish's shape deviated from that of the reference specimen. In this case the individual fish's lower body margin extended further above or below that of the reference specimen. Recall that the fish's tip of snout is at coordinates  $(0,0)$  and the posterior margin of the hypural plate is at coordinates  $(1,0)$ . It is important to point out how even these small shape differences from a single specimen were detectable using morphometric techniques.

The reference specimen is used to define a multivariate space where each principal warp is represented as an axis in that space. Each particular principal warp axis further represents one kind of variation in shape that a fish would undergo through this investigation. There were 27 principal warps analyzed under this investigation. The modes of shape variation possible can range from broad scale, extending throughout the fish's body, to more localized variations.

Figure 4 shows how one particular principal warp's shape variations are expressed by shape variations as large scale, longitudinal shape changes extending over the entire fish's body. Figure 5 shows the shape variation patterns represented by another principal warp that are much more complex and localized.

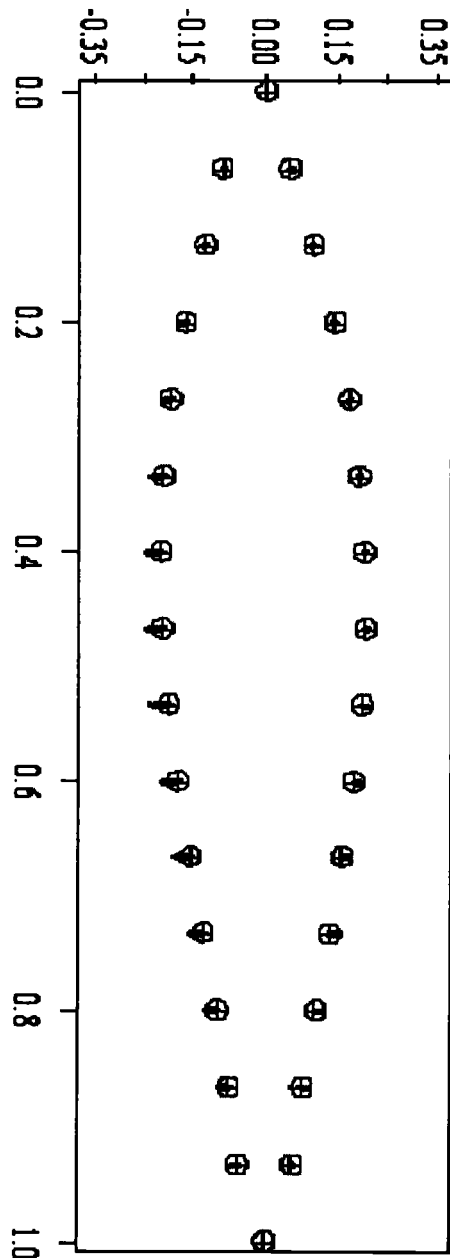


Figure 3: Example of observed shape variation in an individual fish. The reference organism coordinates are at the center of the blue crosses; the coordinates of the single fish are at the ends of the red lines.

pwarp=27

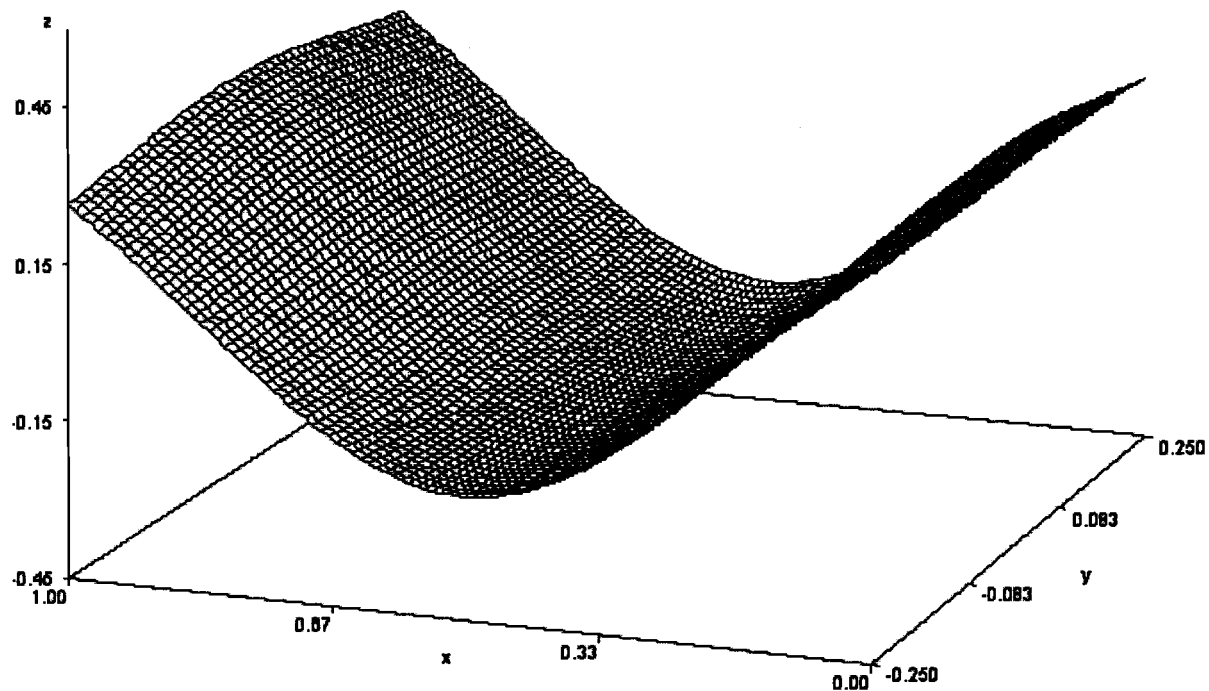


Figure 4: Plot of the relative effect of a single principal warp (vertical axis) on shape changes over a fish's body. The horizontal  $x$  and  $y$  axes correspond to the landmark coordinates on the fish. In this case the principal warp reflects broad scale longitudinal changes in fish shape along the  $x$  axis.

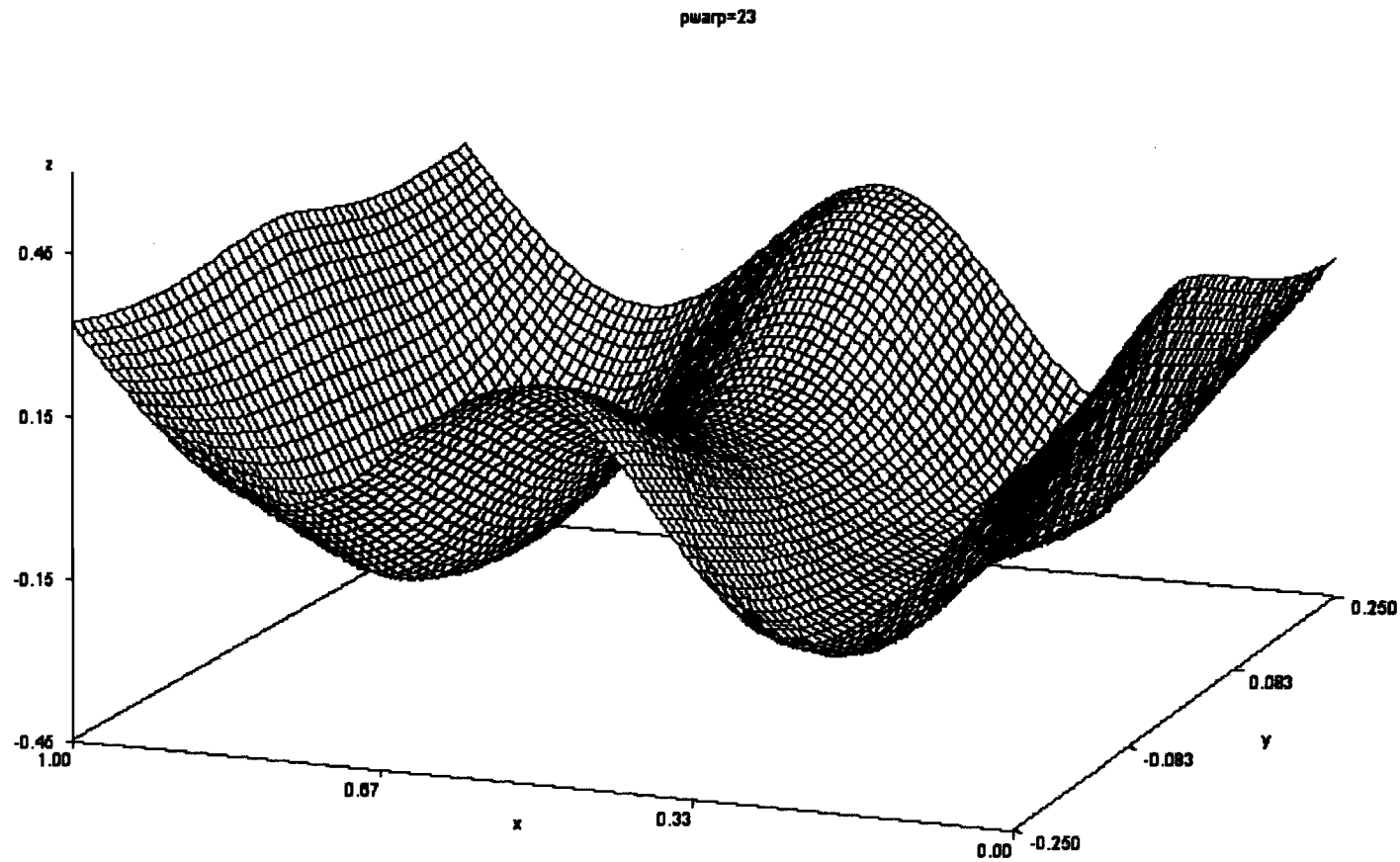


Figure 5: Plot of the relative effect of a single principal warp (vertical axis) on shape changes over a fish's body. The horizontal  $x$  and  $y$  axes correspond to the landmark coordinates on the fish. In this case the principal warp reflects complex localized shape changes in fish shape.

There were 54 relative warps or principal components that were computed using freshly-caught fish that had eigenvalues greater than 0. The last few relative warps represented highly localized, or smaller geometric, shape changes. The first few relative warps represented the largest geometric scale for a set of landmarks and typically exhibit the appearance of a growth or development gradient, or other systematic disproportion graded one-dimensionally across some transect of form (Rohlf and Splice 1990). I accounted for this gradient in the final analysis of treatment effects by developing a "model" of fish shape relative to growth and development only for freshly caught fish.

As Table 1 illustrates, the first three relative warps accounted for 66% of the variation in shapes among freshly caught fish. The first ten relative warps together explained approximately 92% of the shape variation observed in this investigation, and therefore only these first ten were examined in greater detail.

Relative Warps	Eigenvalues	Difference	Proportion	Cumulative Proportion
1	0.00014938	0.00002294	0.2825	0.2825
2	0.00012644	0.00004868	0.2391	0.5215
3	0.00007777	0.00003496	0.1470	0.6686
4	0.00004280	0.00001429	0.0809	0.7495
5	0.00002851	0.00001010	0.0539	0.8034
6	0.00001841	0.00000255	0.0348	0.8383
7	0.00001587	0.00000502	0.0300	0.8683
8	0.00001085	0.00000172	0.0205	0.8888
9	0.00000912	0.00000100	0.0172	0.9060
10	0.00000813	0.00000182	0.0154	0.9214

Table 1: Results of principal component (relative warp) analysis of the shapes data of freshly caught fish. Although 54 principle components were computed, the first ten accounted for 92% of the variation in the data.

The relative warps or principal components capture the shape variation in wild, freshly caught fish that arises from a combination of genetic, developmental, and environmental effects. These first ten relative warps were examined for patterns that describe the major trends in shape variation among specimens. Displacement of landmarks near the middle portion of the body (dorsal landmarks 6 through 9 and ventral landmarks 6 through 8) were found to be much less than at most of the other landmarks, especially those at the anterior and poster ends of the organism (ventral/dorsal landmarks 1 through 5 and ventral/dorsal landmarks 10 through 14). Relative warps 1, 4, 6, 7, 9, and 10 indicated expansion and compression of the landmarks located near the anterior and posterior regions. Relative warps 2, 3, 5, and 8 indicated markedly less compression and expansion among the landmarks, although the majority of displacements did fall at the anterior and posterior regions. These expansions and compressions were more pronounced among the ventral landmarks in relative warps 1 and 4. The remaining relative warps showed expansions and compressions distributed more evenly among the dorsal and ventral landmarks. Relative warps 3 and 5 showed compression and expansion along the middle portion of the body as well as at the anterior and posterior ends.

The principal component, or relative warp, analysis reported above was initially done only with fish that were freshly caught. To evaluate treatment effects and days in captivity, landmark data for fish that were held in the laboratory without food were plotted onto the previously generated principal components.

The prior principal component analysis was used to calculate what position starved fish would occupy on the principal component axes for freshly caught fish.

Analyses of covariance using these principal component scores data showed that the fish held in captivity without food did differ significantly in respect to treatment effects from the freshly caught fish. Clear differences based on starvation periods were observed. Of these first ten principal components, principal components 3 ( $p < 0.0001$ ), 5 ( $p < 0.0001$ ), and 7 ( $p = .0393$ ) were found to be statistically significant relative to treatment effects. These appear to be the smallest or best number of principal components necessary to discriminate days in captivity and treatment effects. The plots in Figures 6-15 are for the first 10 principal component score sets, and include the  $p$  values for the ANCOVAS. These plots showed that the fish scores on a given principal component axis have differing relationships to the duration of food deprivation and the magnitude of centroid size. For example, fish scores on principal component 2 are significantly related to centroid size, but not to the duration of food deprivation, while principal component 5 scores are related to starvation duration but not the centroid size of the fish.

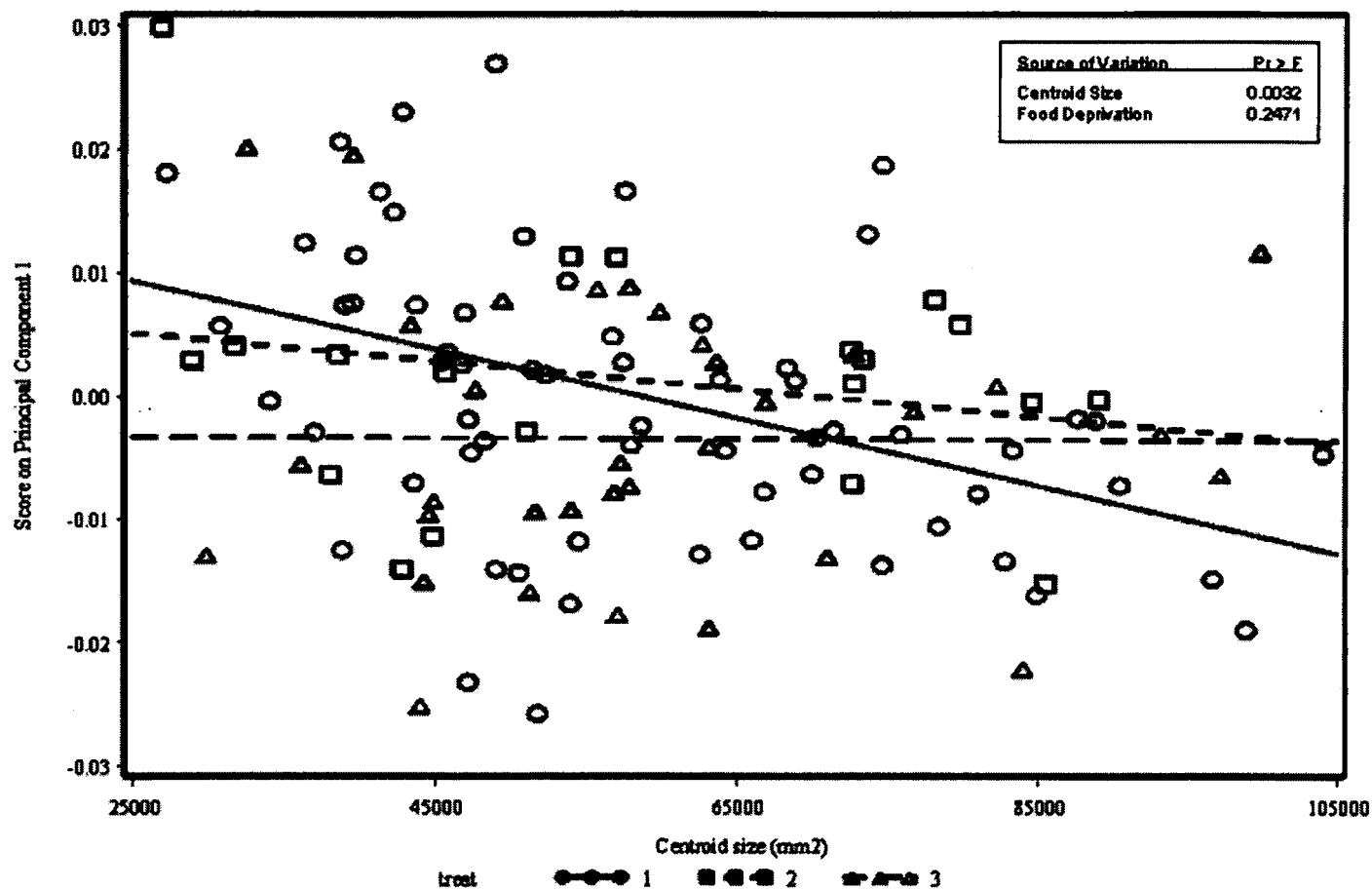


Figure 6: Plot of individual fish scores on principal component 1 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

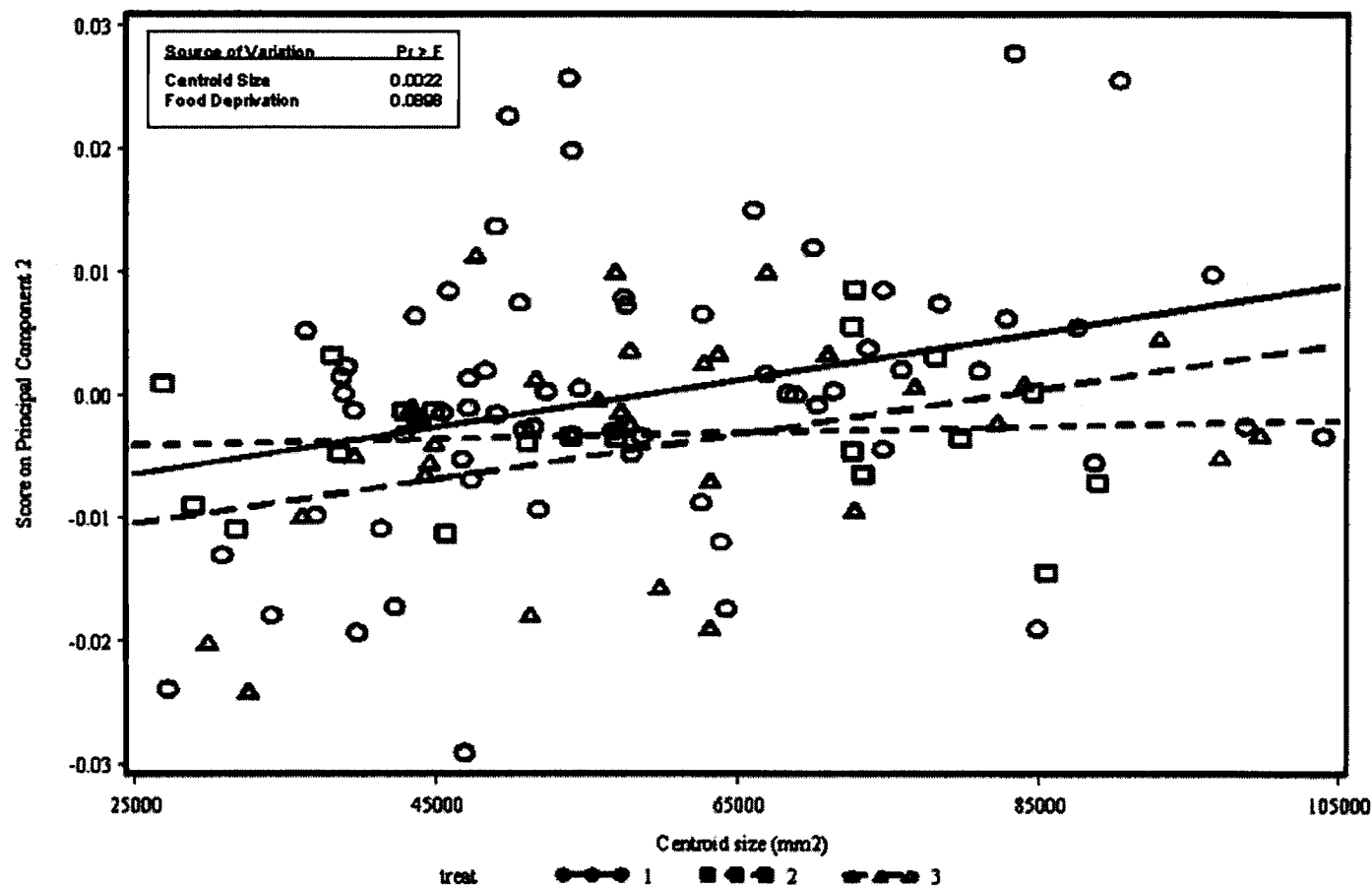


Figure 7: Plot of individual fish scores on principal component 2 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

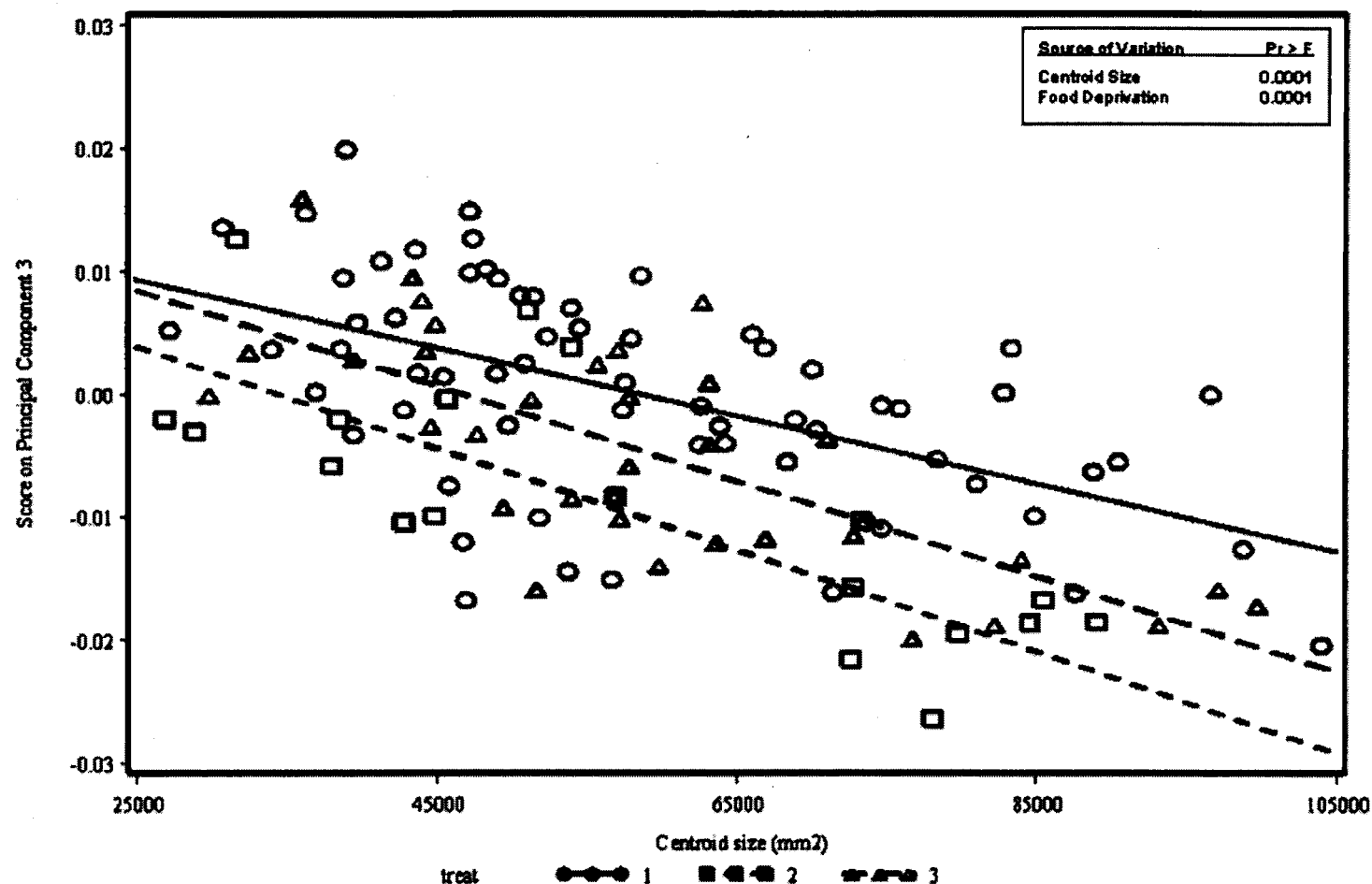


Figure 8: Plot of individual fish scores on principal component 3 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

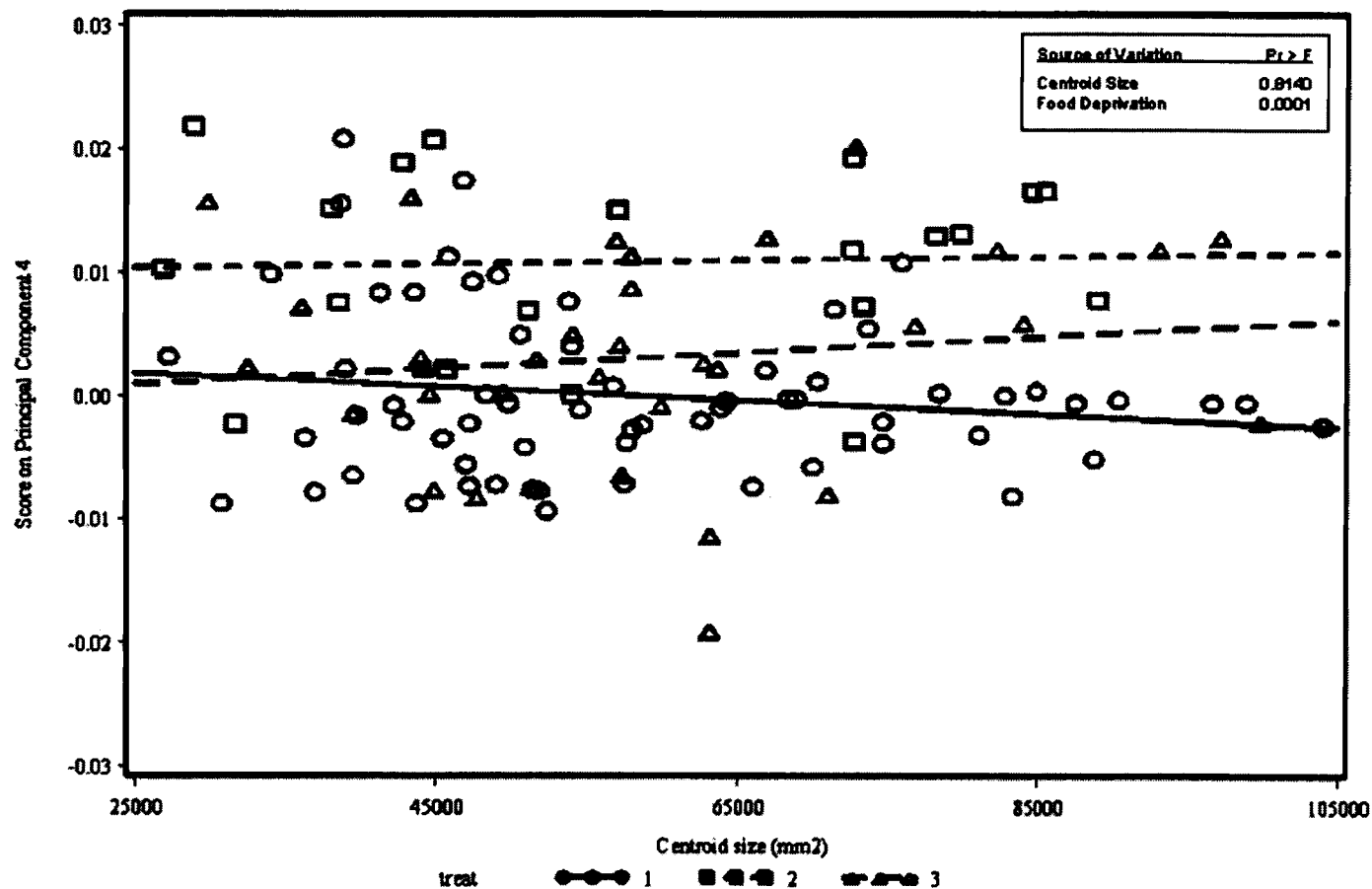


Figure 9: Plot of individual fish scores on principal component 4 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

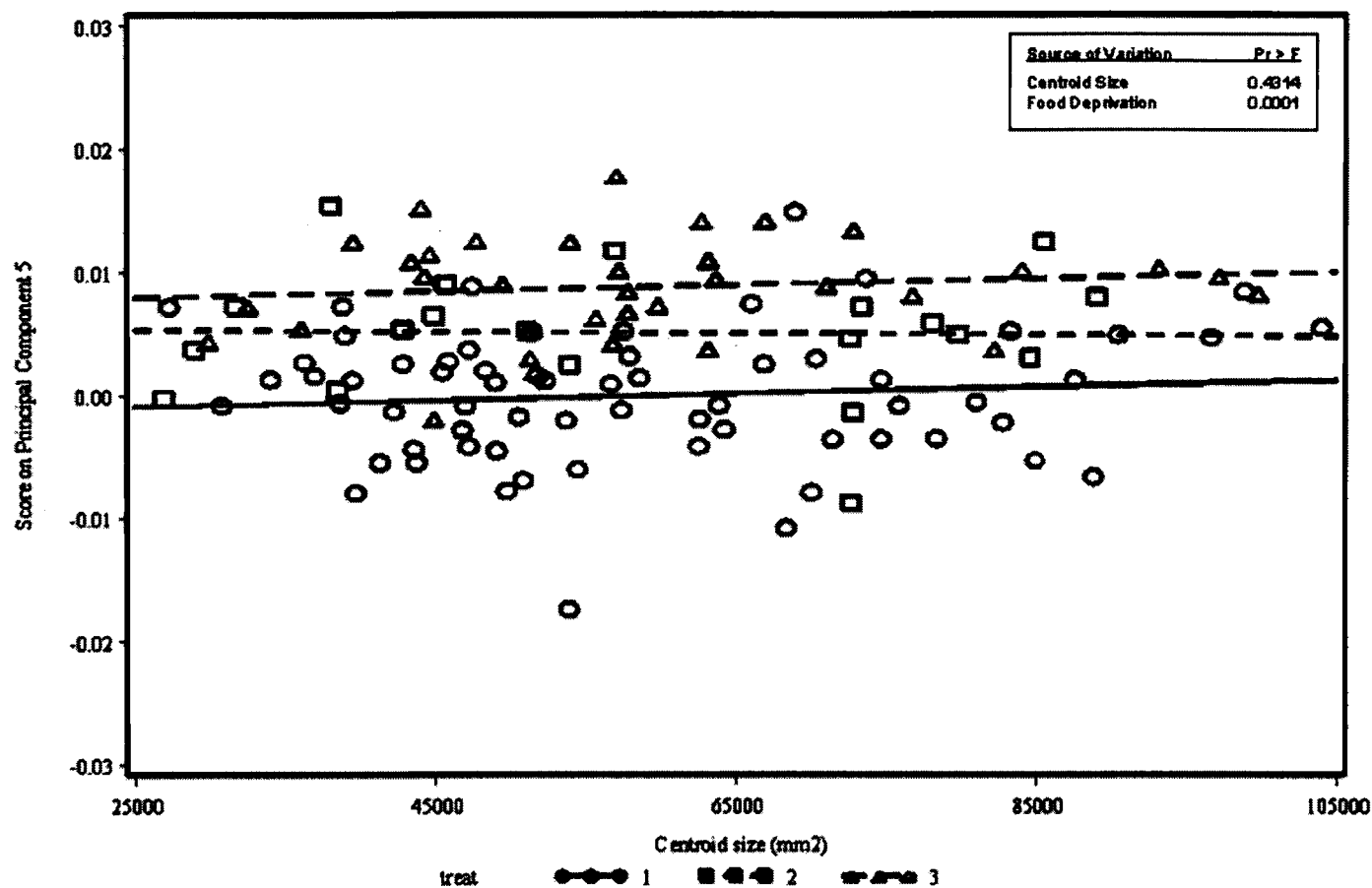


Figure 10: Plot of individual fish scores on principal component 5 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

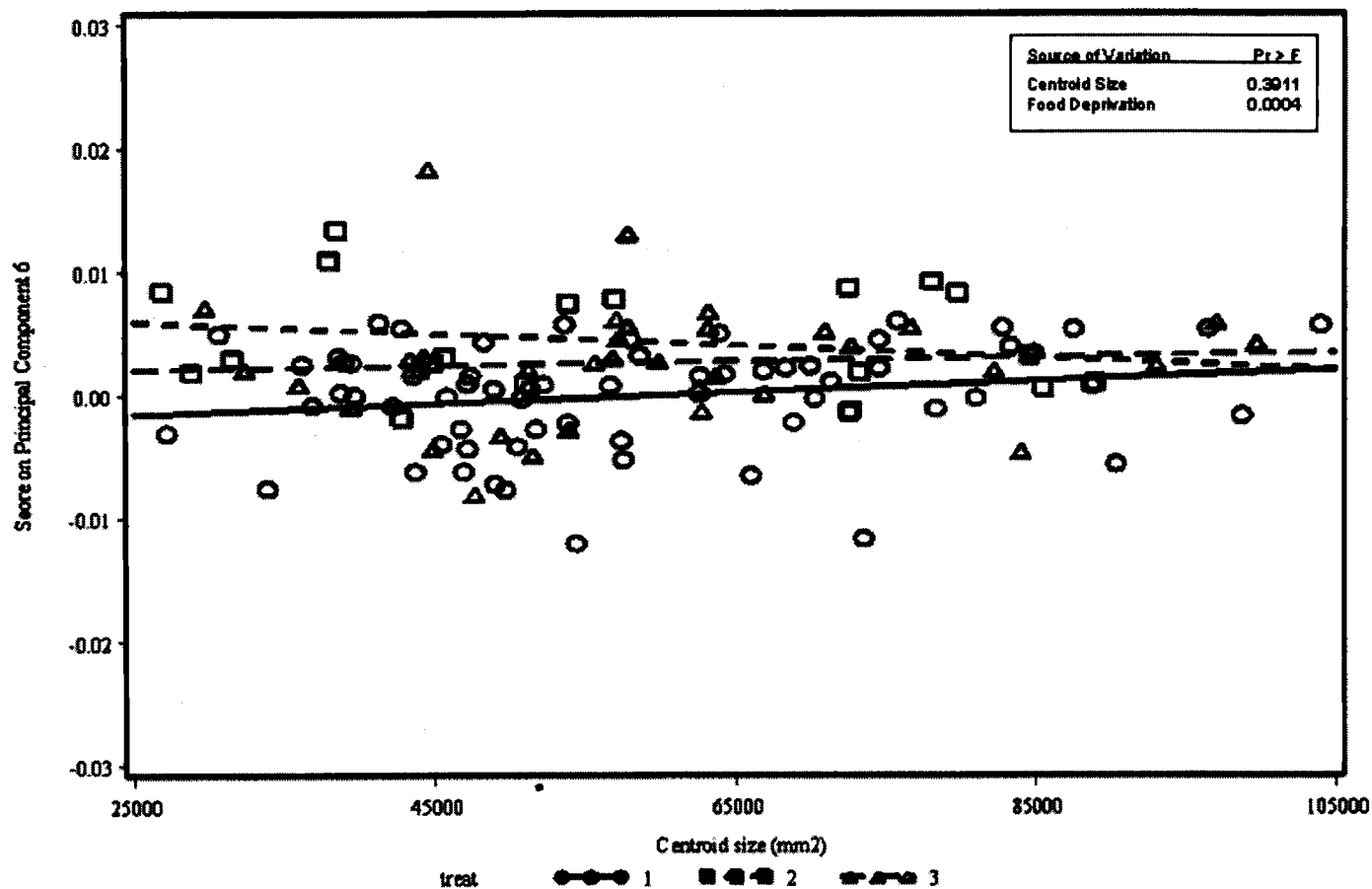


Figure 11: Plot of individual fish scores on principal component 6 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

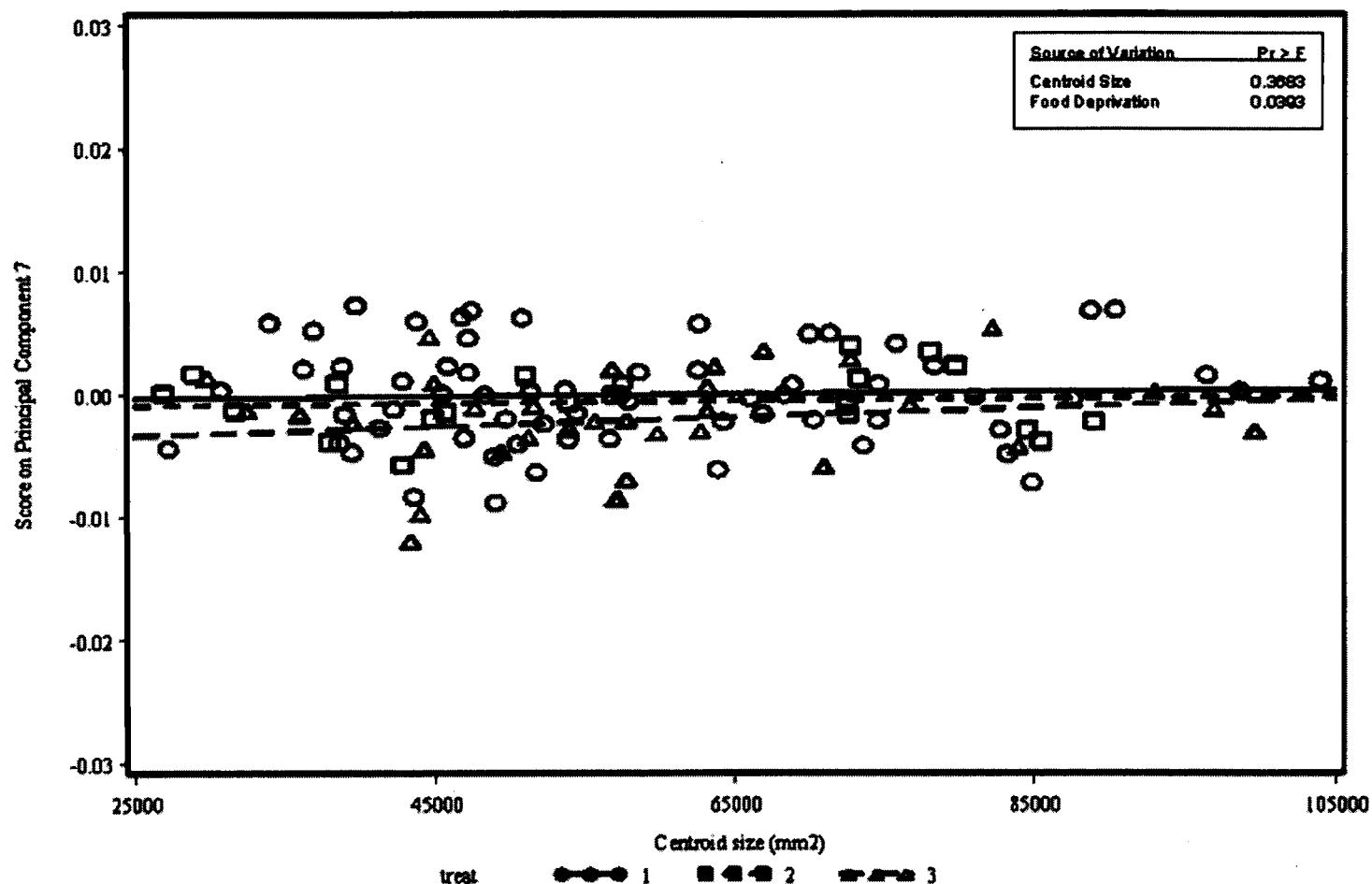


Figure 12: Plot of individual fish scores on principal component 7 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

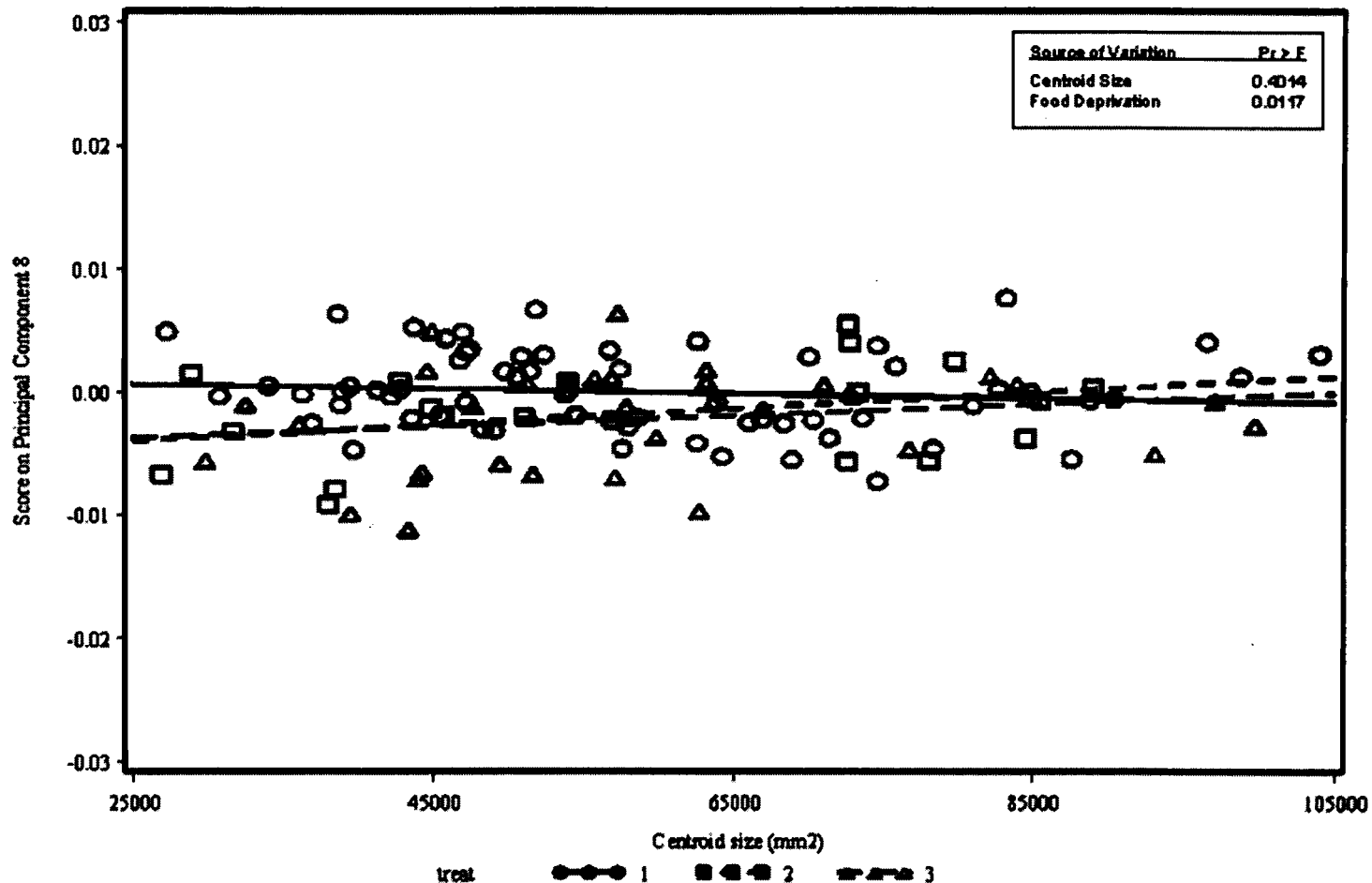


Figure 13: Plot of individual fish scores on principal component 8 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

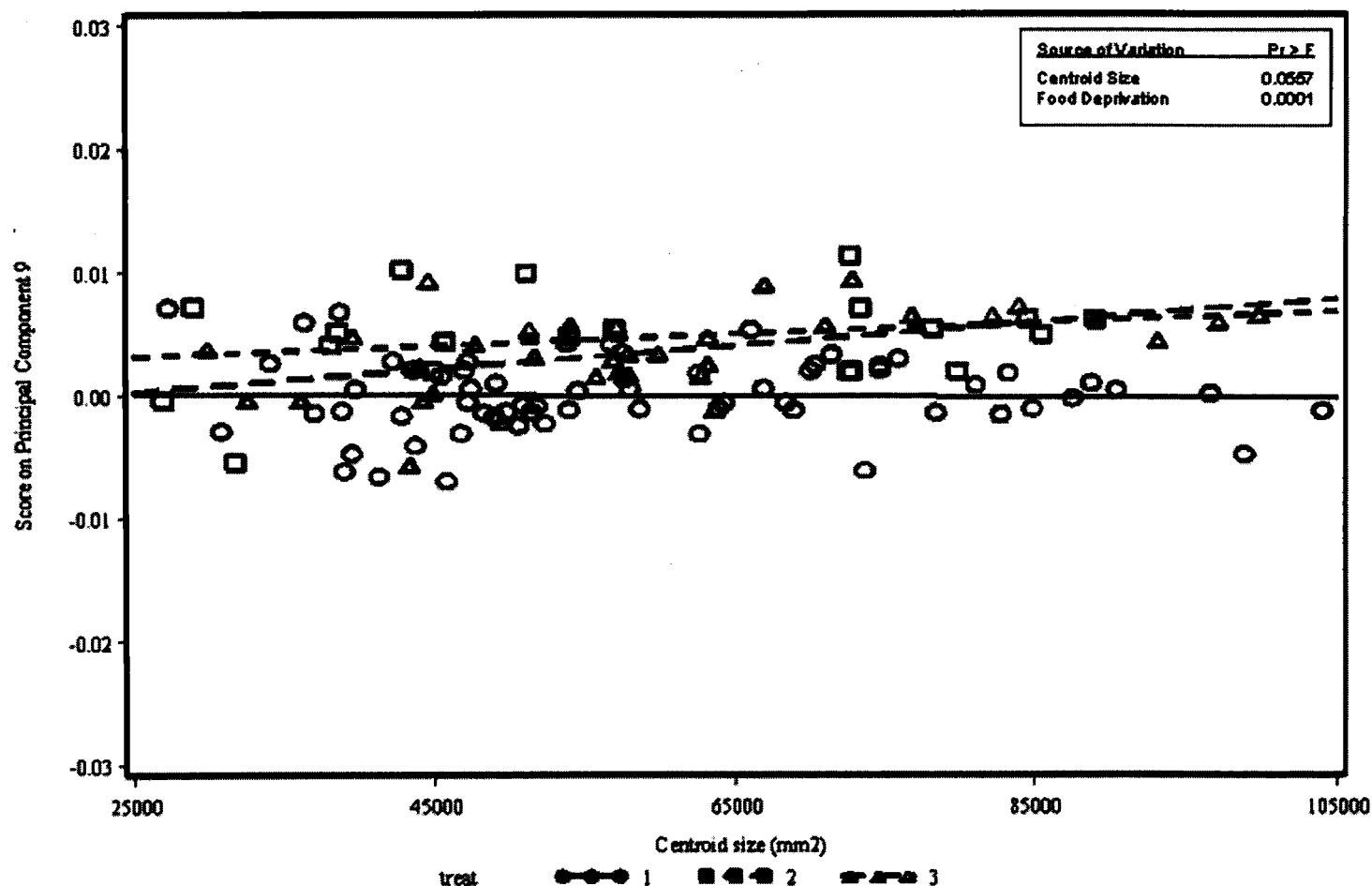


Figure 14: Plot of individual fish scores on principal component 9 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

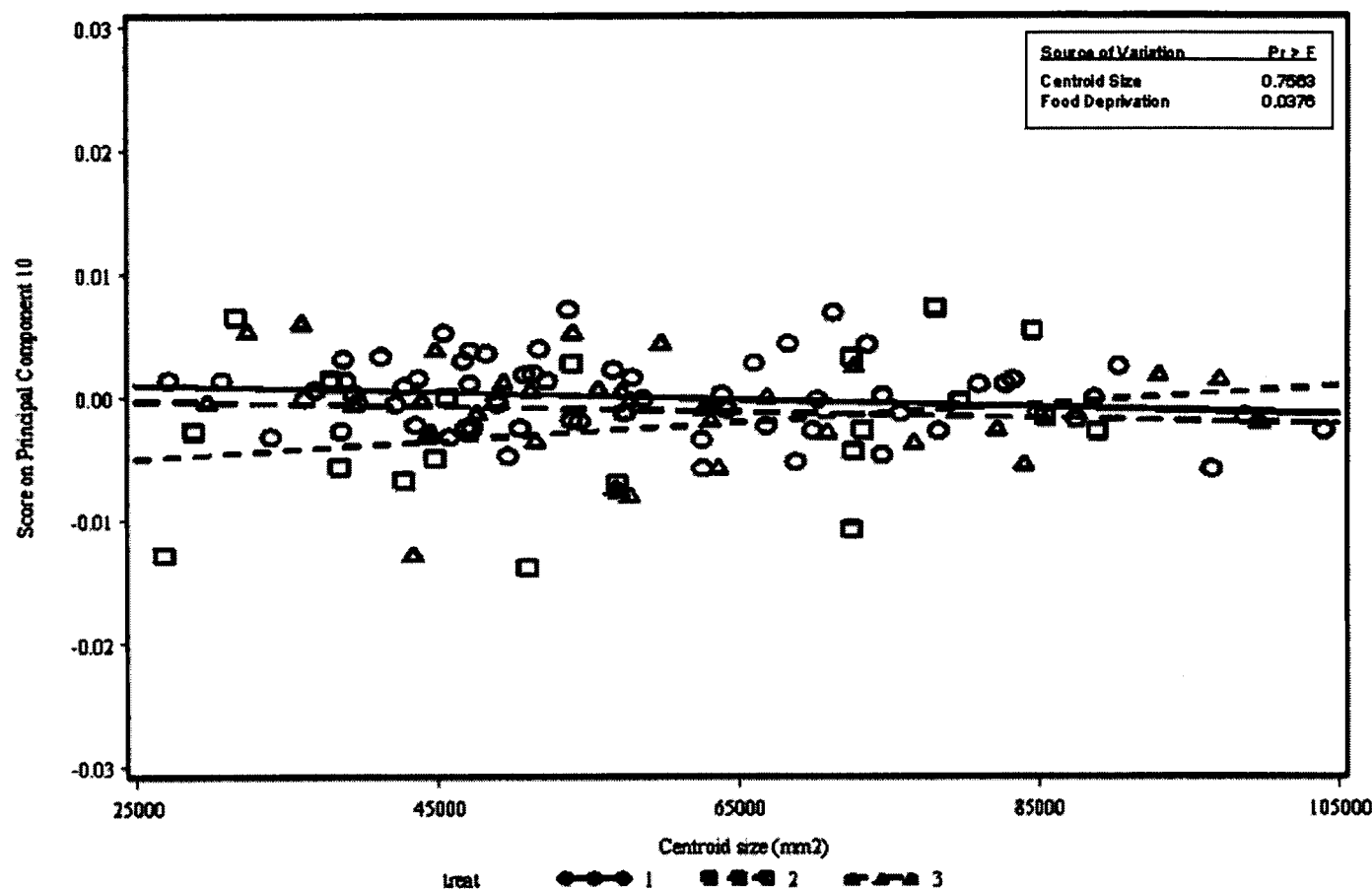


Figure 15: Plot of individual fish scores on principal component 10 (vertical axis) with respect to fish centroid size and treatment. Circles and solid line are freshly caught fish; squares and finely dashed line are fish held without food for 7 to 10 days, and triangles and coarsely dashed line are fish held without food for 22 to 24 days.

The attached figure 16 shows the effect of relative warp (principal component) 5 on fish shape change. It is similar to figure 3, except for the fact that the end of the red lines are where the landmark coordinates would be for a relative warp 5 score of 0.05. Relative warp 5 was chosen because that was the warp illustrated above (see figure 10) that showed the clearest indication of a food deprivation effect, but had the least influence of centroid size (development) on shape. A warp score of 0.05 was chosen because that was close to the range of the "worst" case scenario in food deprivation, but large enough to show a visible effect. It's very important to remember that figure 16 does not represent the shape of a fish with that warp score. The actual fish shape would be the summation of the fish's warp scores on all of the relative warps. This means that some of the shape changes visible in the figure would be canceled out by other relative warps, while some might also be augmented.

It does however seem clear that when the principal component scores on their respective axes are viewed collectively with respect to food deprivation it is evident that the effect of food deprivation on fish shape is readily discernible. To verify this, a discriminant function analysis was performed. The objective of the discriminant analysis was to determine the degree to which an individual fish's profile of scores on the independent variables corresponded to or resembled the typical profiles of each of a given set of discrete classes or treatment groups.

The discriminant analysis showed that the principal component scores of fish under the various treatments could indeed be used to differentiate fish on the basis of treatment effects.

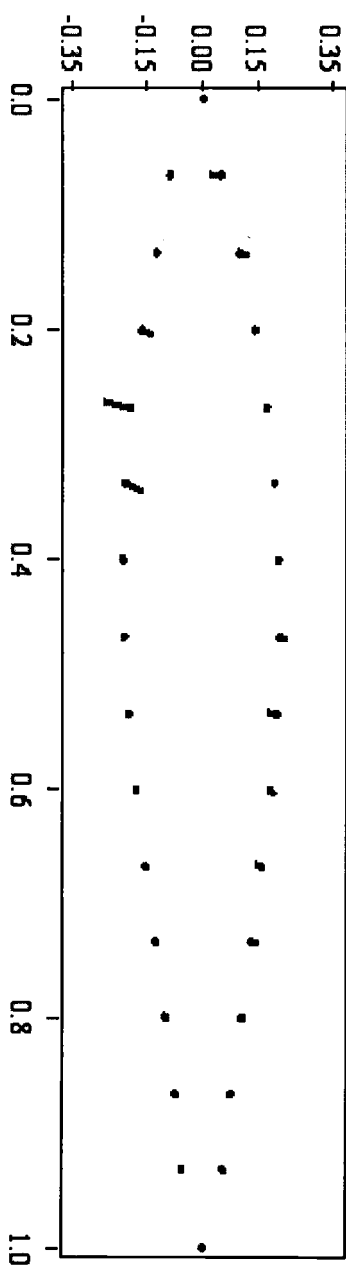


Figure 16:

This figure shows the effect of relative warp (principal component) 5 on fish shape change. The ends of the red lines are where the landmark coordinates would be for a relative warp 5 score of 0.05. This figure does not represent the shape of a fish with that warp score. The actual fish shape would be the summation of the fish's warp scores on all of the relative warps. Some of the shape changes visible on this figure might be canceled out by other relative warps, while other shape changes might also be augmented.

Table 2 lists the number of individual observations and percent classified into the various treatment groups. Ninety-two percent of the sixty-six individuals from treatment group one were correctly identified. Seventy percent of the twenty individuals from group two were correctly identified. Sixty-eight percent of the thirty-four individuals from group three were correctly identified. The discriminant analysis proved useful in identifying the extent to which specimens resembled each of several treatment groups to which they could be assigned.

	Discriminant Function				
		Freshly Caught	Without Food for 7-10 days	Without Food for 16-24 days	Total
Treatment	Freshly Caught	60 92%	2 3%	3 3%	65 100%
	Without Food for 7-10 days	3 13%	14 70%	3 13%	20 100%
	Without Food for 16-24 days	2 6%	9 26%	23 68%	34 100%
	Total	65 13%	25 21%	29 24%	119 100%

Table 2: Number of observations and percent classified into treatments using discriminant analysis function.

## CHAPTER 4

### CONCLUSIONS

This research documented the relative sensitivity of the English sole to lab simulated deficiencies in habitat quality. Although complete food deprivation is an artificial circumstance when compared to natural conditions, juvenile and adult fish can be quite resilient when faced with food deprivation. It was remarkable that food deprivation periods as short as one week resulted in detectable changes in fish shape that could be readily detected. These differences in fish shape were very subtle, and it is improbable that they could have been observed by a simple visual inspection. However, the morphometric procedures utilizing the thin-plate spline analysis that were employed readily captured even these subtle variations in shape.

The ultimate goal of this research was to utilize these new advances in morphometric techniques in order to evaluate whether differences in shape among individuals could first be detected and then related to the quality and condition of their environment. I was able to demonstrate through this investigation that stressful aspects of an organism's environment were reflected by that organism's shape. The geometric methods used to quantify these shape changes were based upon the analyses of body landmarks to characterize morphometric variations in wild and lab-starved English sole.

The morphometric techniques employed under this investigation are much less technically difficult when compared to the other commonly used methods for examining fish condition. The acquisition of data for the analysis is simple and, after an initial hardware and software investment, quite inexpensive in terms of cost and labor. Although, the thin-plate spline analysis is mathematically complex, its development in terms of software is also a one-time investment. The drawbacks to morphometric analysis are that it is nonspecific in that it shows how the whole animal is "different," but not specifically why, and there may also be a genetic component that clouds the overall picture of growth. The naturally occurring genetic variation that occurs among individuals is an important point that must be addressed. However, this genetic variation must be treated as a sort of "noise" component in the morphological evaluation of shape. Under this research the "noise" component was accounted for by evaluating the same individuals before and after the application of a lab induced environmental stressor. Despite the drawbacks, Morphometrics is considered one of the most useful tools in shape analysis to date and is also considered a promising tool in a multidisciplinary assessment of fish quality and condition (Bookstein, 1996).

The above research was performed both for its intrinsic scientific merit and because of the current scientific trend towards research that improves ecosystem risk assessment. This research examined the conceptual and methodological aspects of morphometrics relevant to its potential future use as a measure of developmental precision and environmental condition.

An explanation of data patterns based upon the covariances of landmark displacement locations could be phrased as an argument that particular anthropogenic processes deformed landmarks during ontogeny. Both the identification and assessment of intraindividual shape variations in fish may one day be used to characterize the risks associated with alterations to the estuary and its surrounding watershed.

Further work is needed to evaluate fish morphometric responses to other kinds of environmental stress under more natural field conditions. It is expected that the results of this project could contribute to a regional scale exposure assessment, whereby the consequences of natural or anthropogenic alterations to natural ecosystems may then be evaluated or even anticipated at the organism level. It is also my hope that management personnel responsible for decisions concerning sediment input and alterations in Pacific Northwest estuaries utilize the results of this research in the future.

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## **APPENDIX**

## APPENDIX

## THIN PLATE SPLINE MORPHOMETRIC ANALYSIS

## MEASURING

The analysis begins with the  $x$  and  $y$  coordinates of the  $p$  landmarks were determined from a consensus or reference organism.  $\mathbf{X}_c$  is the  $(2 \times p)$  matrix of coordinates of the consensus object, with the first row of  $\mathbf{X}_c$  being the  $x$  coordinates and the second row of  $\mathbf{X}_c$  being the  $y$  coordinates of the landmarks. The matrix  $\mathbf{Q}$  is a  $p \times 3$  matrix formed from  $\mathbf{X}_c$ , with the first column all 1's, second is the  $X$  coordinate of reference configuration and the third is  $Y$  coordinate of reference configuration.

A second symmetric ( $p \times p$ ) matrix,  $\mathbf{P}$  is formed using values of the function  $U(r)$  which is defined as:

$$U(r_{ij}) = r_{ij}^2 \ln(r_{ij}^2)$$

In this function  $r_{ij}$  is the distance between the  $i$ th and  $j$ th landmarks on the reference specimen.

A  $((p+3) \times (p+3))$  matrix  $\mathbf{L}$  is then formed out of submatrices  $\mathbf{P}$  and  $\mathbf{Q}$ . These submatrices were combined to form  $\mathbf{L}$  as follows:

$$L = \begin{bmatrix} P & Q \\ Q^T & 0 \end{bmatrix}$$

where the matrix **0** is a (3x3) matrix of zeroes.

The resulting elements of **L** are:

$$L = \begin{bmatrix} 0 & U(r_{12}) & U(r_{13}) & K & U(r_{1p}) & 1 & X_1 & Y_1 \\ U(r_{21}) & 0 & U(r_{23}) & K & U(r_{2p}) & 1 & X_2 & Y_2 \\ U(r_{31}) & U(r_{32}) & 0 & K & U(r_{3p}) & 1 & X_3 & Y_3 \\ M & M & M & M & M & M & M & M \\ U(r_{p1}) & U(r_{p2}) & U(r_{p3}) & K & 0 & 1 & X_p & Y_p \\ 1 & 1 & 1 & K & 1 & 0 & 0 & 0 \\ X_1 & X_2 & X_3 & K & X_p & 0 & 0 & 0 \\ Y_1 & Y_2 & Y_3 & K & Y_p & 0 & 0 & 0 \end{bmatrix}$$

It is important to remember that **L** is derived solely from the consensus organism. The inverse of **L** is found as  $L^{-1}$ , and a submatrix  $L_p^{-1}$  is then formed by taking the first  $p$  rows and columns of  $L^{-1}$ . This matrix (referred to as the bending energy matrix) is symmetric, and is decomposed to find its eigenvalues and eigenvectors, i.e.  $L_p^{-1} = E^t \Lambda E$ , where  $\Lambda$  is a ( $p \times p$ ) diagonal matrix of eigenvalues and **E** is a ( $p \times p$ ) matrix of eigenvectors whose columns are the eigenvectors of  $L_p^{-1}$ . These eigenvectors are the principal warps that express the modes of shape variation possible in the specimens.

Three of the eigenvalues in  $\Lambda$  are zero, so were dropped from both  $\Lambda$  and  $\mathbf{E}$  so that  $\Lambda$  became a  $((p-3) \times (p-3))$  matrix and  $\mathbf{E}$  became  $(p \times (p-3))$  matrix.

The next step is to subtract the x coordinates of the consensus organism from the corresponding X landmark coordinates of the specimens. This transforms each X coordinate in  $\mathbf{X}_x$  from its observed value to make it a deviation from the coordinate in the reference object. This is written in matrix notation as:

$$\mathbf{V}_x = \mathbf{X}_x - \mathbf{1}_n \otimes ([1 \ 0] \mathbf{X}_c) \quad (\text{Rohlf eqn. 8})$$

Breaking this expression down, the portion in parentheses in the above expression,  $(\mathbf{1}_n \otimes [1 \ 0])$ , says that by taking a vector (one column) that has n rows, with the value “1” in each row, and then taking the direct product of it with a matrix having one row and two columns, with a “1” in column 1 and a “0” in column 2. This forms an  $(n \times 2)$  matrix with all ones in the first column and all zeros in the second. Multiplying this matrix by the  $\mathbf{X}_c$  matrix yields an  $(n \times p)$  matrix, with each column containing the consensus organism’s X coordinate for that corresponding landmark. Similarly, the deviations from the Y coordinates of the consensus object was constructed as:

$$\mathbf{V}_y = \mathbf{X}_y - \mathbf{1}_n \otimes ([0 \ 1] \mathbf{X}_c) \quad (\text{Rohlf eqn. 9})$$

The  $V_x$  and  $V_y$  matrices are concatenated horizontally to form  $V$ :

$$V = [ V_x | V_y ] \quad (\text{Rohlf eqn. 7})$$

The  $V$  matrix was composed of deviations of the  $x$  and  $y$  coordinates of each specimen from the reference specimen. Again, the first  $p$  columns of  $V$  contain the deviations of the specimens'  $X$  coordinate from that of the consensus organism, and the  $(p + 1)$  to  $2p$  contain the deviations from the  $Y$  coordinate. Each row of  $V$  corresponded to one specimen, so there were  $n$  rows in  $V$ .

The next step is to position the deviations of the individual specimens from the reference specimen in the special principal warp space previously defined as the matrix  $E$ . This, and several other steps were accomplished to form a matrix  $W$  of dimensions  $(n \times 2(p - 3))$  as:

$$W = \left( \frac{1}{\sqrt{n}} V \right) (I_2 \otimes (E \Lambda^{-\alpha/2}))$$

Bookstein (1991) suggested the introduction of the parameter  $\alpha$  in the exponent of  $\Lambda$  in computing the weight matrix. The matrix  $\Lambda^{-\alpha/2}$  is used to scale the eigenvectors (= principal warps)  $E$  by their eigenvalues raised to some power that depends on the value  $\alpha$ .

The value of  $\alpha$  is chosen depending on what the researcher feels is important, and has choices ranging from giving all the principal warps an equal weight to differentially weighting them with respect to the associated bending energy. If  $\alpha$  is set to zero, then there is equal weighting because each element of  $\Lambda$  is raised to the zero power.  $\Lambda^{0/2}$  forms an identity matrix in which each principal warp (eigenvector) is multiplied by this identity matrix and so remains unchanged. The other often-used choice for  $\alpha$  is 1, so that each eigenvector is weighted by the inverse square root of its associated eigenvalue (bending energy).

The metric value of  $\alpha = 0$  was chosen for this investigation, as suggested by Bookstein (1991, p. 368). This value is of particular interest for exploratory studies because it gives all of the principal warps the same weight. Thus the analysis is not relative to bending energy, even though the principal warps are used as the basis vectors for the space. In this space the Cartesian distance between specimens was the affine-free Procrustes distance between specimens. In terms of the original coordinate data, it corresponded to the Cartesian distance between the x and y coordinates of a pair of specimens after differences explainable by affine transformations such as translation, rotation, and uniform stretching had been removed (Rohlf and Slice, 1990).

The specimen's X and Y deviations were separately positioned from the reference organism in the principal warp coordinate system.

This is why the direct product of an  $(2 \times 2)$  identity matrix (represented in the above equations as  $I_2$ ) and  $E\Lambda^{\alpha/2}$  was taken.  $E$  was a  $(p \times (p - 3))$  matrix, and  $\Lambda$  was a  $((p - 3) \times (p - 3))$  matrix, so their product had dimensions  $(p \times (p - 3))$ . Taking the direct product of the  $(2 \times 2)$  identity matrix and the product  $E\Lambda^{\alpha/2}$  was the same as forming a matrix from four  $((p - 3) \times p)$  submatrices as follows:

$$\begin{bmatrix} E\Lambda^{\alpha/2} & 0 \\ 0 & E\Lambda^{\alpha/2} \end{bmatrix}$$

The first  $p$  columns of  $V$ , corresponding to the  $X$  coordinates are transformed by  $E\Lambda^{\alpha/2}$  (the upper left submatrix), and the second  $p$  columns of  $V$ , corresponding to the landmark  $Y$  coordinates, are in turn transformed by  $E\Lambda^{\alpha/2}$  (the lower right matrix).

Divide by the square root of  $n$ , because the resulting matrix was a sum of squares and cross products matrix.

The end result was an  $(n \times 2(p - 3))$  weight matrix ( $W$ ) that was a horizontal concatenation of the weights for the  $X$  and  $Y$  coordinates, respectively:

$$W = [W_x | W_y].$$

Plotting these points (rows = specimens) represented in **W** could be viewed as a plot of specimens in the vector space that was defined by the reference specimen. **W** is therefore the data set representing the varying organism shapes that can be analyzed further.

## ORDINATION

One method to evaluate patterns of shape variation among specimens is to compute a singular value decomposition (SVD) of the **W** matrix. The SVD is defined as a decomposition of any rectangular matrix (with more rows than columns) into three matrices, e.g. decompose the matrix **W** to be **SDR<sub>T</sub>**, where **D** is a diagonal matrix holding the singular values, **R** was a matrix holding the relative warp vectors, and **S** held the scores that transformed the deviations to their positions on the relative warp vectors (principal components). A singular value decomposition of the matrix **W** was used to form three matrices:

$$\mathbf{W} = \mathbf{S} \mathbf{D} \mathbf{R}_T$$

This was why the factor  $1/\sqrt{n}$  was originally included in the formation of **W** above; it was the equivalent of the degrees of freedom ( $v$ ) in the principal component analysis. **S** had dimensions  $(n \times \min(n - 1, 2(p - 3)))$ . Its rows corresponded to the  $n$  specimens, and each column corresponded to a relative warp with singular value  $> 0$ .

**S** was a matrix of scores for each specimen on the associated relative warp axis. **D** represents a diagonal matrix of singular values and had dimensions  $[\min(n - 1, 2(p - 3)) \times \min(n - 1, 2(p - 3))]$ . It scales the relative warp axes. **R** had dimensions  $[\min(n - 1, 2(p - 3)) \times \min(n - 1, 2(p - 3))]$ . The rows of **R<sub>t</sub>** corresponded to relative warps (so the columns of **R** also corresponded to the relative warps). The columns of **R<sub>t</sub>** corresponded to principal warps (so the rows of **R** also correspond to the principal warps). The relative warps are therefore functionally equivalent to principal components and could then be examined for patterns within the dataset. If  $\alpha$  is chosen to be zero, as discussed above, then the above analysis is equivalent to a traditional principal component analysis.