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The effects of freezing on the morphometrics of sardine *Sardinops sagax* (Jenyns, 1842)

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ABSTRACT

The effects of freezing on the morphometrics of sardine *Sardinops sagax* were studied. Results were variable depending on the length of the freezing period, but all fish showed significant ($p < 0.001$) decreases in both caudal length and wet body weight compared to initial measurements taken from unfrozen fish. The absolute weight loss of sardine ranged between 0.256 and 0.868 g, which corresponded to a relative weight loss between 0.86 and 2.49%. The absolute loss in caudal length ranged between 0.281 and 0.458 cm, whereas the relative loss in caudal length ranged between 1.57 and 2.54%. The effect of freezing duration on morphometric measurements was variable, and whereas shrinkage was most common, no effect was observed in a number of cases. Most of the morphometric measurements showed small changes and, if they decreased, the decrease was gradual. Only one morphometric measurement (LM 12, the distance between the snout and the top of the head) showed a significant increase in half of the eight freezing periods. The relevance of these results to studies of morphological variability in fish is discussed.

1. Introduction

Fisheries research often requires the preservation of samples collected in the field, generally because of time and/or equipment constraints, with the most widely used preservation methods being freezing or fixation (usually in formalin or alcohol). The preservation of samples generally results in a loss of body weight and body shrinkage in both freshwater and marine fish (Ajah and Nunoo, 2003; Buchheister and Wilson, 2005; Florin and Lingman, 2008). If preservation of samples also affects and alters the morphological characteristics of fish, it will likely affect measurements of these morphometric variables, which will bias interpretations of these results. Such bias could be important when comparing the morphometric measurements of fish from different localities and/or periods, for example in studies where morphometric variation may be used to assess the existence of discrete stocks of a particular species. This could be particularly important in cases where samples are processed (*i.e.* morphometric measurements are taken) after different freezing periods and then compared.

Effects of preservation are species specific (Florin and Lingman, 2008). The population structure of sardine *Sardinops sagax* in the Benguela current system is currently under investigation (de Moor and Butterworth, 2009), with spatial variability in sardine morphometrics being one of several methods used to assess stock structure (van der Lingen et al., 2009). At present, there is little information concerning the effect of freezing duration on sardine. The purpose of this study was to investigate the effect of freezing, and the effect of different freezing periods, on caudal length, weight and various morphometric measurements of sardine *S. sagax*. This was done to develop a standardized protocol for processing frozen sardine samples for morphological analysis.

2. Materials and methods

Adult sardine *S. sagax* (Jenyns, 1842) were collected from commercial catches taken by purse-seine nets in March 2009, with samples of 120 fish taken from a landing at Gans Bay (34°35.56′S, 19°20.17′E) and 120 fish from a landing in Saldanha Bay (32°29.55′S, 17°57.71′E), South Africa. After collection the fish were immediately transported to the laboratory for processing, where each fish was weighed to the nearest 0.001 g, and then placed on a photographic stage and photographed with its left side facing upward. Photographs were taken with a Canon 400D digital SLR camera (image resolution: 10 megapixels) with a fixed focal length of 24 mm to avoid image distortion. In total 11 landmarks were defined (Fig. 1 and Table 1) along the contours of the fish's body, with ten of these landmarks (1–10) used to calculate the morpho-

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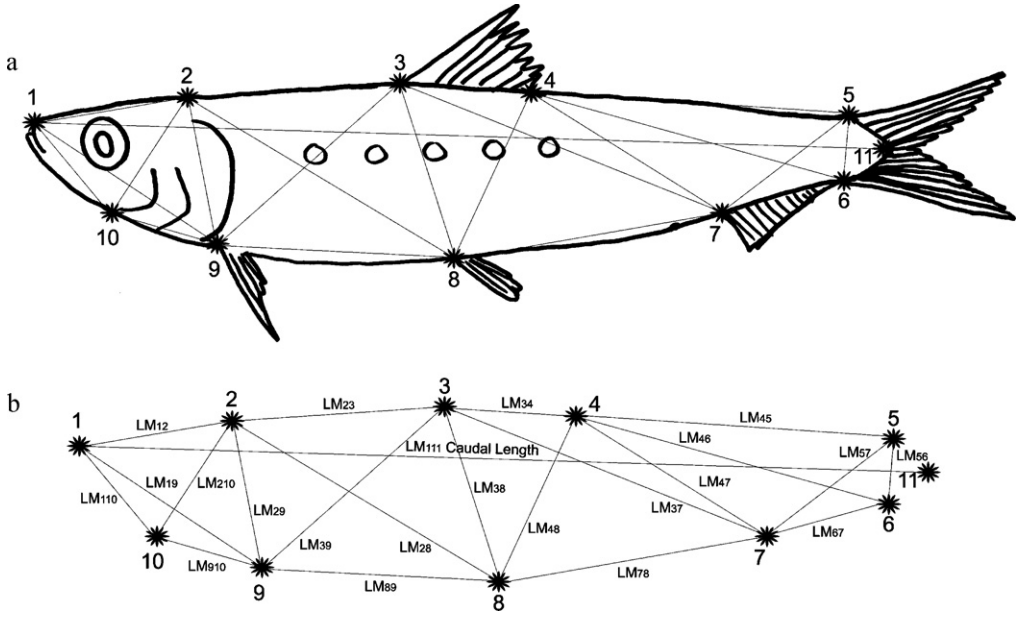

Fig. 1. A box-truss network of linear distances among morphometric landmarks for sardine *Sardinops sagax*. (a) The locations of the 11 landmarks on the body of the fish and (b) the box-truss network linking landmarks.

metric measurements that make up the segments of a box truss network (Strauss and Bookstein, 1982; Silva, 2003; De La Cruz-Agüero and García-Rodríguez, 2004).

Landmarks were marked with pins before the photographs were taken to ensure that they were easy to locate with the imaging software. The fish was then photographed next to a waterproof label (containing all the relevant information) and a graduated rule (used to calibrate distances in the photographs). Once photographed, each fish was placed in an individual plastic bag containing a waterproof label (for later identification). The sardine were then randomly divided into eight groups of 30 fish each, placed in a −70 °C freezer, and frozen. Changes in caudal length, weight and morphometric measurements of all fish within a single group were then assessed for different freezing periods, with groups examined after being frozen for one, two, three, four, six, eight, 12 or 17 weeks.

After each freezing period the fish were thawed and each individual was again weighed to the nearest 0.001 g and photographed using the above technique. Photographs were analysed using freeware software tpsDig2 (Rohlf, 2005). The landmarks were marked on each photograph, the scale determined using the graduated rule and the distances between the landmarks (Fig. 1b) measured to the nearest 0.01 cm and recorded.

Table 1
Description of each of the 11 morphometric landmarks defining the truss network. The landmark positions are illustrated in Fig. 1.

Description	Landmark number
The tip of the snout	1
The occipital ridge – first line on top of the head above the gill plate	2
The front insertion point of the dorsal fin	3
The back insertion point of the dorsal fin	4
The upper insertion point of the caudal fin	5
The lower insertion point of the caudal fin	6
The front insertion point of the anal fin	7
The front insertion point of the pelvic fin	8
The front insertion point of the pectoral fin	9
The hinge of the jaw, aligned with the first line on the gill plate	10
The beginning of the caudal fin in the middle of the body or where the expanded bones at the end of the backbone start that support the caudal fin	11

Effect of freezing on sardine caudal length and weight were assessed using paired-sample *t*-tests between measurements taken at the start and end of each freezing period (Underwood, 2007). The mean absolute change (fresh minus thawed) in caudal length and weight was calculated, as were the mean percentage changes (relative to fresh size). Positive values for these variables indicate shrinkage. To correct for any change, predictive regression equations were derived to allow fish caudal length and weight before freezing to be calculated from the measurements after freezing for different periods of time. Analysis of covariance was used to check for the influence of fish size within samples and to compare the regressions from different freezing periods.

Each morphometric distance measured was corrected to remove size-effects and possible allometric relationships between variables for each of the eight freezing periods (Strauss, 1985). To standardise all variables to the mean length of all sampled individuals, the equation (Lleonart et al., 2000; Murta et al., 2008):

$$LM_s = LM \times \left(\frac{CL_m}{CL}\right)^b \quad (1)$$

was adopted. LM_s is the standardised morphometric measurement for an individual fish, LM is the morphometric measurement of that fish before correction, CL_m is the mean caudal length of all sampled individuals, CL is the measured caudal length of the individual fish, and b is the slope of the geometric mean regression (Ricker, 1973) calculated with the log-transformed variables CL and LM for all samples. Once the size effect was removed, the effect of freezing on the landmark measurements for different freezing periods was examined using paired-sample *t*-tests to establish which morphometric measurements changed significantly due to freezing duration (Underwood, 2007). The results were then subjected to a sequential Bonferroni procedure to adjust significance levels to control for Type I errors in multiple testing situations (Quinn and Keough, 2004). This procedure can increase the chance of Type II errors (Moran, 2003; Nakagawa, 2004) but the more conservative approach was preferred. The percentage change was calculated for each morphometric measurement for each freezing period. These percentage changes can be applied to standardised morphometric data to correct for the effect of freezing for different periods when comparing fresh and frozen fish.