4 The effect of alcohol and freezing preservation on carapace size and shape in *Liocarcinus depurator* (Crustacea, Brachyura)

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4.1 Abstract

Morphometric and shape analysis are usually performed on preserved specimens. The current paper examines the effect of two common preservation methods, freezing and alcohol, on the shape and magnitude of crab's carapace. The carapace widths and images of the carapace of two batches of the swimming crab, *Liocarcinus depurator* were taken before and after preservation. The carapace width was measured by two operatives and discrepancy between the two was analysed. The carapace images were analysed using geometric morphometric analysis. The carapace widths decreased significantly, though minimally, after preservation. Geometric morphometric indicated significant differences after preservation in the uniform shape components only indicating global differences rather than localised differentials.

Keywords: *Liocarcinus depurator*, alcohol, freezing, preservation, shape differences, geometric morphometry, crab.

4.2 Introduction

Since crustaceans have hard exoskeletons and potentially numerous "landmarks" they should constitute an ideal group for the application of geometric morphometric methods. Few such studies on Crustacea can, however, be found. Cadrin (1995) applied box-truss methods to discriminate between sexes and potential fishery stocks of the American lobster, *Homarus americanus* (Milne Edwards). Rosenberg's (1997) work on the shape difference between major and minor chelipeds of the fiddler crab *Uca pugnax*, (Smith) probably pioneered the use of landmark-based morphometric analysis in extant crustaceans (see Reyment for works on fossil Crustacea). The differences observed suggested that the major claw could produce more crushing power, and that selection for "fight effectiveness" may have played an important role in the evolution of the cheliped shape. Rufino et al. (2004) used geometrical morphometric techniques to elucidate subtle differences in the carapace shape of male and female *Liocarcinus depurator*.

Recently, reviews of the techniques applied to crustaceans can be found in, Rosenberg (2002, claw shape variation across the genus *Uca*), Cadrin and Friedland (1999, lobster stock identification) and Cadrin (2000, fisheries stock identification).

In many field studies, individuals are not measured immediately after capture, but are preserved for later measurement. The effect of the preservation on the size of the individuals is often ignored, however significant. The ultimate effect of preservation distortion may largely depend on the degree of accuracy needed for each specific study. The effect is often quite variable, for example, many fish species, both adults and larvae, either shrink or enlarge after preservation with alcohol, formaldehyde or freezing (e.g. *Sprattus sprattus*, *Encheylopus cimbrius* and *Pomatoschistus minutus* (Fey 1999); *Clupea harengus* and *Osmerus eperlanus* (Fey 2002); *Mullus barbatus* and *M. surmuletus* (Al-Hassan et al. 2000)). The few studies carried out on crustaceans include that of Melville-Smith (2003) who showed that the carapace of the rock lobster (*Panulirus cygnus*) shrank significantly after cooking and freezing, although the shrinkage was minimal. No study has been conducted on the effect of preservation procedure on the over-all shape of the individuals and whether preservation acts in a differential manner on different parts of the body.

Clearly, the interpretation of significant differences in morphometry between species is difficult if the preservation method causes a differential effect between species. Such differential effects of preservatives on different parts of an organism will cause differences in shape, which will be more readily appreciated with geometric morphometry than with any other technique.

The present study examines the effect of different preservation methods on size and shape in the portunid crab *Liocarcinus depurator* (Linnaeus). It is the dominant brachyuran by-catch (untargeted species) in Mediterranean demersal fisheries and shows a wide bathymetric range throughout the continental shelf and upper slope (Abelló et al. 1988; Abelló et al. 2002; Rufino et al. submitted). *L. depurator* inhabits several types of substrata, although it is most commonly found on mud (Minervini et al. 1982; Rufino et al. submitted). A wide-ranging species, *L. depurator* has been reported from Mauritania and the Canary Islands to Norway in the eastern North Atlantic and throughout the Mediterranean Sea (d'Udekem d'Acoz 1999).

4.3 Materials and methods

Individuals of *Liocarcinus depurator* were collected by trawling off Barcelona (western Mediterranean). The carapace widths (CW) of 120 fresh individuals (60 males and 60 females) were measured by two operatives using the same digital calipers with a resolution of 0.01 mm. The upper view of the carapace was also scanned into a digital image using a calibrated HP Precisionscan 3.1. Thirty male and 30 female crabs, randomly-selected, were frozen (~ –20ºC) and the remaining 30 males and females were stored in 70% ethanol. Three weeks later, the frozen crabs were defrosted and the measurements repeated on both groups of crabs.

Landmarks (see Fig. 1) were used to quantify carapace shape and "Centroid" magnitude (defined as the squared root of the summed, squared distance of all landmarks in relation to the geometric centroid [calculated using tpsRel (Rohlf 2003b)]) was used as a measure of crab size in addition to CW.

Figure 1 shows the locations of the 15 landmarks identified for the geometrical morphometric analysis. The first landmark was located centrally on the posterior margin of the carapace. The second landmark was the point of maximum curvature of the posterior carapace margin. Landmarks 3 to 11 represent the tips of and anterior notch formed by the four anterolateral teeth. Landmarks 12 to 14 represent the tips of and the notch between the two anterior teeth. Landmark 15 along with landmark 3 delineated the maximum carapace width. Coordinates of the landmarks were digitised using tpsDig (Rohlf 2003a).

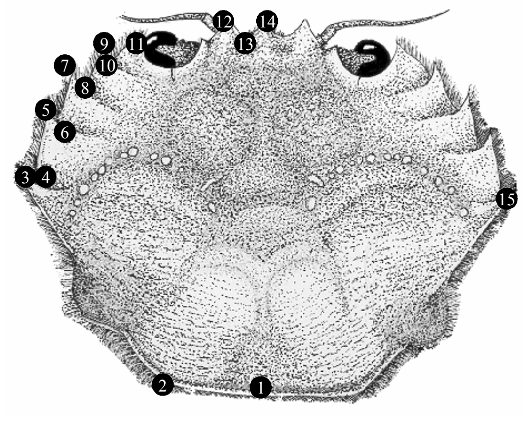

Fig. 1. Landmarks selected on the *Liocarcinus depurator* carapace

Details on the procedures for geometrical morphometric analysis can be found in Adams et al. (2003), nevertheless only a brief description is given here. After digitising, landmark maps were rotated, scaled (to unit centroid size) and translated through a Generalised Least squares Superimposition (GLS) procedure (generalised procrustes) to eliminate scale and orientation distorsions ('*tpsRel*' (Rohlf 2003b)). A thin-splate spline procedure was used to fit an interpolated function to an average map (consensus configuration) of the carapace shape and

derive the uniform and non-uniform (partial warps) components of shape variation. The two uniform components describe differences that affect all parts of the carapace equally (global differences). The magnitude of the first of these indicates the degree of stretching along the x-axis relative to the average carapace map. Whereas the magnitude of the second indicates compressions or dilatations along the y-axis (Cavalcanti et al. 1999). The non-uniform shape components (partial warps) describe localised departures from the average carapace map.
The approach followed in the data treatment was to calculate the difference between the operative's measurements and between before/after conservation, in order to obtain independent samples. A two-sample Wilcoxon test was used to test differences between genders and the non-parametric one-sample Wilcoxon test corrected for tied observations (exactRankTests package in R-project (Ihaka and Gentleman 1996)) was used to test if the CW change due to the conservation or different author measurements, was significantly different from zero (which would correspond to absence of a significant change). Ninety-five percent confidence intervals were estimated using one-sample Wilcoxon test corrected for tied observations (exactRankTests package in R-project (Ihaka and Gentleman 1996)).

4.4 Results

Carapace width (CW) measurements were not normally distributed and the variances between treatments were not approximately equal. Therefore, differences between operators' measurements and before and after preservation were analysed separately.

Within the size ranges measured, no significant difference ($p>0.05$) between measurements on males or on females was found (Wilcoxon test corrected for tied observations: $W = 7622$, p-value = 0.4332) so, in the remaining analyses, both sexes were pooled.

There were no significant differences between median operator differences across the groups of male and female crabs before or after preservation (Mood's median Test Chisquared = 9.54, df = 7, $p = 0.216$). Fig 2 shows boxplots of the difference data for each group showing how one or two outliers are evident but that the bulk of the differences were very small. There are no discernible patterns between genders or preservation methods nor between before and after preservation. The median differences between the measurements made by the two operators was 0.01mm and proved highly significant (Wilcoxon 1-sample test $W=7484.5$, $p<0.001$, $N=238$).

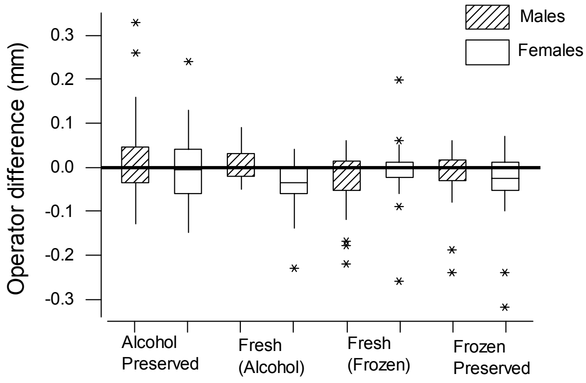

Fig. 2. Box plots of the differences in measurements on individual *L.depurator* carried out by two different operators

Since the average carapace width being measured was 39.4 mm to find a systematic error of 0.025% between operatives is remarkably good, despite the "significance". With such large numbers of observations even tiny and essentially trivial differences tend to become "statistically significant". There was no significant correlation ($r = -0.074$, $df = 236$, $p = 0.256$) between operatives' and the size of the crabs, confirming a standard systematic error between operatives which did not vary with crab dimension or applied treatment.

From the geometric morphometry, Centroid size was significantly correlated with CW ($r = 0.992$, $df = 118$, $p < 0.001$ see Fig.3).

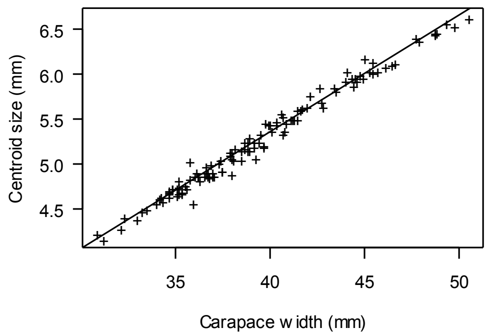

Fig. 3. Relationship between carapace width (measured with a digital caliper) and the centroid size (based on 15 landmarks on the crab's carapace) of 120 *Liocarcinus depurator* (60 males and 60 females). centroid = 0.183 + 0.129*CW

Carapace width and centroid magnitude, showed different results in relation to the effect of preservation technique. Carapace width decreased (median difference = 0.08 mm) after the animals were preserved in alcohol (Table 1), while the centroid magnitude only decreased by 0.01mm (median difference not significant, Table 1). The effect of freezing was smaller. Centroid magnitude actually increased (median difference = -0.01, but not significantly, Table 1). Carapace width, on the other hand decreased significantly (median difference = 0.07-0.05 mm, Table 1) after freezing. However, the variability associated with centroid magnitude differences after freezing was far greater than any other treatment

For both the difference in carapace width and in centroid magnitude, with preservation technique there was no significant correlation with the carapace width (i.e. size of the individual), showing no differential effect of preservation technique with crab size.

Table 1. Median difference of the carapace width (mm) and centroid size of *Liocarcinus depurator*, between before and after preservation (alcohol or frozen), and respective results of the one-sample Wilcoxon test (V: statistic and p: pvalue)

					Wilcox	
Cons	Measure	Author	Estimated median.	Median	V	p-value
Alc	Centroid		0.02	0.01	990	0.065
	CW	A	0.09	0.08	1441	0.000
		B	0.1	0.08	1709	0.000
Fro	Centroid		-0.01	-0.02	806	0.701
	CW	A	0.07	0.06	1423	0.000
		B	0.05	0.04	1619	0.000

Cons.:conservation; Alc:Alcohol; Fro: Frozen

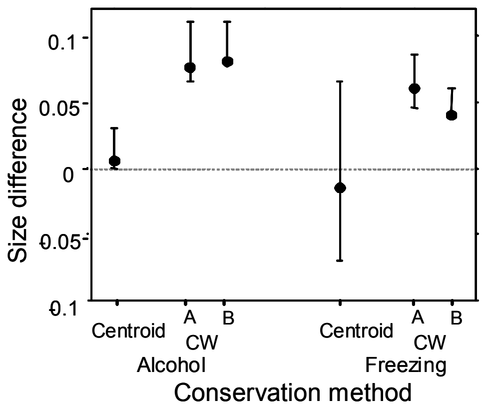

Fig. 4. Median and 95% CI (Wilcoxon), of the difference between conservation methods, on the centroid magnitude and on carapace width of *Liocarcinus depurator* for the two operatives measurements (A and B)

Analysis of the uniform components for crabs preserved in alcohol (Repeated measures MANOVA) indicated a significant effect of the preservation on the shape (Wilks = 0.743, F = 10.016, $p < 0.001$). The resultant discriminant function was not particularly powerful (63% success for pre-preservation crabs and 65% success for post-preservation crabs). Freezing also showed a significant multivariate effect (Wilks = 0.792, F = 7.627, p = 0.001), providing an even less powerful discriminant (58% success for pre-preservation crabs and 60% for post-preservation specimens). Despite determining significant effects of preservation on the global shape parameters, the multivariate analysis could not discriminate well between preserved and fresh crabs simply on the basis of their shape.

The non-uniform shape components exhibited no significant effects of preservation for both alcohol (Manova:- Wilks = 0.177; F = 1.415; p = 0.244) and freezing (Manova:- Wilks = 0.128; F = 2.082; p = 0.068). Thus, although an over all shrinkage (global change) was evident in the preserved carapaces such was not reflected in local effects at particular landmark locations.

4.5 Discussion

Differences in carapace width measurements taken by the two operatives were always smaller than the differences measured between preserved and fresh specimens. The effect of operative was shown to be truly systematic and did not vary with treatment or crab size. The precision of each operative was very similar, again emphasising the systematic nature of the difference.

Both alcohol and freezing preservation methods caused significant shrinking of *L. depurator*'s carapace. Shrinking by alcohol preservation was slightly greater than that caused by freezing. 70% ethanol is well known for removing water from immersed specimens and presumably does so quicker than exposure to -20ºC which can also create dehydration but usually only under a vacuum.

The individuals of *Liocarcinus depurator* studied ranged from 30.69 mm to 50.53 mm CW. A maximal shrinkage of a maximum of 100µm, represents a mere 0.33 to 0.20% decrease, which in practice is relatively trivial. Melville-Smith (2003) also found shrinkage of the carapace after freezing in the rock lobster *Panulirus cygnus.* Remarkably, given the severity of the treatment, Ibbot (2001) found no effect of boiling and subsequent freezing on the carapace length of the southern rock lobster (*Jasus edwardsii*).

Centroid magnitude showed no significant differences due to the different preservation methods, and seemed the most variable of the two measures, particularly for crabs that had been frozen. The inability of centroid magnitude to reflect the obvious differences in CW measured indicates its limitations as a general indicator of 'size'. It must ultimately be more variable than a single easily identifiable measure since it is composed of many, often imprecisely located, measures. In the present instance, the variability associated with centroid magnitude hid the significance of the obvious shrinkage and even resulted in the opposite effect being identified (although not significant).

Geometrical morphometric analysis indicated that the effect of preservation on crab shape was significant only in the uniform shape component. All differences in shape were global. Despite the significance, the discriminating power of the derived function was low. The potential for the technique to address tiny differences like those inflicted on the crab carapace by preservation appears limited at present. The errors in digitising, establishing landmark locations and then interpolating the shape functions are clearly greater than the difference revealed by precise measurements of a single, but easily identifiable dimension. Increasing the precision of the geometrical morphometric techniques to rival simple measures of 'size' in discriminating power must be an urgent aim. The power to analyse shape as an overall phenomenon with the precision of simple measurements will make geometric morphometry extremely valuable.

4.6 Acknowledgements

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References

Abelló P, Valladares FJ, Castellón A (1988) Analysis of the structure of decapod crustacean assemblages off the Catalan coast (North-West Mediterranean). Mar Biol 98: 39-49

Abelló P, Carbonell A, Torres P (2002) Biogeography of epibenthic crustaceans on the shelf slope of the Iberian Peninsula Mediterranean coasts: implications for the establishment of natural management areas. Sci Mar 66 (Suppl.2): 183-198

Adams DC, Rohlf FJ, Slice D (in press) Geometric Morphometrics: Ten Years of Progress Following the 'Revolution'. Ital J Zool. Proceedings of the Rome Geometric Morphometrics Workshop

Al-Hassan LAJ, Bujawari JA, El-Silini OA (2000) The effect of some preservatives and freezing on certain body dimensions of two species of the family Mullidae collected from Benghazi waters, Libya. Acta Ichthyol Piscatoria 30: 127-136

Cadrin SX (1995) Discrimination of the American lobster (*Homarus americanus*) stocks off southern New England on the basis of secondary sexual character allometry. Can J Fish Aquat Sci 52: 2712-2723

Cadrin SX (2000) Advances in morphometric identification of fishery stocks. Rev Fish Biol Fish 10: 91-112

Cadrin SX, Friedland KD (1999) The utility of image processing techniques for morphometric analysis and stock identification. Fish Res

Cavalcanti MJ, Monteiro LR, Lopes PRD (1999) Landmark based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). Zool Stud 38: 287-294

d'Udekem d'Acoz C (1999) Inventaire et distribution des crustacés décapodes de l'Atlantique nort-oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25ºN. Patrimoines Naturels (M.N.H.N./S.P.N.) 40: 1-383

Fey DP (1999) Effects of preservation technique on the length of larval fish: Methods of correcting estimates and their implication for studying growth rates. Arch Fish Mar Res/Arch Fisch Meeresforsch 47: 17-29

Fey DP (2002) Length correction of larval and early-juvenile herring (*Clupea harengus* L.) and smelt (*Osmerus eperlanus* L.) after preservation in formalin and alcohol. Bull Sea Fish Inst Gdynia 155: 47-51

Ibbot S, Gardner C, Frusher S (2001) The effect of cooking on carapace length of southern rock losbter, *Jasus edwardsii* (Hutton 1875) (Decapoda, Palinuridae). Crustaceana 74: 221-224

Melville-Smith R, Thomson AW (2003) The effect of cooking and freezing on the carapace measurements of western rock lobster, *Panulirus cygnus* George 1962. Crustaceana 76: 605-609

Minervini R, Giannotta M, Falciai L (1982) A preliminary report on the decapod crustaceans in the estuarine area of the Tiber. Quad Lab Tecnol Pesca 3: 305-318

Rohlf FJ (2003a) TpsDig, version 1.39, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook

Rohlf FJ (2003b) TpsRel, version 1.29, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook

Rosenberg MS (1997) Evolution of shape differences between the major and minor chelipeds of *Uca pugnax* (Decapoda: Ocypodidae). J Crustacean Biol 17: 52-59

Rosenberg MS (2002) Fiddler crab claw shape variation: a geometric morphometric analysis across the genus *Uca* (Crustacea : Brachyura : Ocypodidae). Biol J Linn Soc 75: 147-162

Rufino MM, Abelló P, Jones DA (in press) An application of geometric morphometrics to crustaceans: male and female carapace shape differences in *Liocarcinus depurator* (Decapoda: Brachyura). Ital J Zool. Proceedings of the Rome Geometric Morphometrics Workshop

Rufino MM, Abelló P, Maynou F (in press) *Liocarcinus depurator*: Review of current knowledge on life history and fishery data. In: Tully O (ed) The assessment and management of European crustacean fisheries: EDFAM Concerted Action