Analysis of the ontogenetic variation in body and beak shape of the *Illex argentinus* inner shelf spawning groups by geometric morphometrics

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In this paper we analysed size and shape differences of body (mantle and fins) and inferior beak of the squid Illex argentinus *from two Patagonian inner-shelf spawning groups. A new method was developed to reconstruct three-dimensional coordinates based on the 2-D projection of the beak on a plane. Shape of the beak did not vary between groups, sexes and maturity condition of individuals. Also, no beak shape changes were observed through the ontogeny. In contrast, as larger and heavier squids were considered in shape analysis, body widened near the fin insertion, and fin area increased. Our results suggest that shape adaptations of* I. argentinus *through the ontogeny reflect modifications to optimize swimming performance rather than to increase gamete holding capacity.*

INTRODUCTION

Morphometric analysis has been widely used to study shape variations through the ontogeny of cephalopods (e.g. paralarvae, juveniles and adults) and to distinguish between specimens belonging to different taxa (e.g. species and subspecies) or population units (e.g. fishery stocks) (Haefner, 1964; Vidal, 1994; Zecchini *et al.*, 1996; Carvalho & Nigmatullin, 1998; Hernández-García & Castro, 1998; Barón & Ré, 2002; Shea & Vecchione, 2002; Vega *et al.*, 2002). Classical methods of morphometric analysis include both uni and multivariate statistics (e.g. simple and multiple regression, covariance, principal component and discriminant analysis) based on linear measurements of body dimensions (Cadrin, 2000). These approaches present several limitations, including loss of information and risk of selecting dimensions that do not fully represent shape variation (Bookstein *et al.*, 1985; Adams *et al.*, 2004; Zelditch *et al.*, 2004). Geometric morphometrics were developed to solve these difficulties, showing a very rapid progress over the last few years in many areas of biology (Rohlf, 1998; Richtsmeier *et al.*, 2002; Adams *et al.*, 2004). However, the use of this methodology in the study of cephalopods has been limited so far, being exclusively employed to discriminate the morphology of statoliths from a few species (Lombarte *et al.*, 2006; Neige, 2006).

Environmental variables are known to cause inter and intra specific morphometric variations in soft and hard structures of organisms, especially in species with a wide range of distribution (Darwin, 1859). Being a neritic–oceanic species distributed over the South American shelf from southern Brazil (22°S) to the Malvinas Islands (52°S) (Haimovici *et al.*, 1998), the Argentinean short-fin squid *Illex argentinus* (Castellanos, 1960) provides a good example of such condition. Several stocks have been described over its geographical range of distribution based on the asynchrony of spawning and the adaptation of early life-stages to take advantages of particular spatiotemporal cycles of primary productivity (Haimovici *et al.*, 1998; Silva, 2003). As a consequence of their adaptation, body, beak and statolith shape of individuals from some stocks present differences that some authors have described using traditional morphometric methods (Brunetti & Ivanovic, 1991; Arkhipkin, 1993; Arkhipkin & Laptikhovsky, 1994; Martinez *et al.*, 2002). In spite of these advances, the use of morphometrics to help interpret the population structure of *I. argentinus* is far from being exhausted, especially considering that new analytical methods have been developed and that morphometric dissimilarities have been compared only between samples from some stocks (Brunetti & Ivanovic, 1991; Martinez *et al.*, 2002). Studies analysing shape variation throughout the ontogeny of ommatrephids have been conducted for a few species (Brunetti & Ivanovic, 1991; Shea & Vecchione, 2002), and part of them is only focused on early live stages (Vidal, 1994). The only antecedent for *I. argentinus* is the description of morphometric relationships of the beak (Ivanovic & Brunetti, 1997).

In waters from San Matías Gulf (Northern Patagonia, 41°–42°S 63°–65°W) two main spawning groups (summer and spring spawners) of *I. argentinus* have been clearly identified based on maturation chronology and size-structure of

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mature individuals (Morsan & González, 1996; Crespi-Abril *et al.*, 2008). The objectives of this paper are to analyse size and shape differences of body (mantle and fins) and beak of squids from both groups and to provide a description of shape variation with size and weight.

MATERIALS AND METHODS

Squid samples were obtained in four surveys conducted between July 2005 and February 2006 in waters of San Matías Gulf on board commercial bottom trawlers operating 120 mm mesh-size nets. Samples were preserved in sealed plastic bags refrigerated with ice until they were processed in the laboratory. Specimens were classified as belonging to the spring (SpSG) or summer spawning (SSG) groups based on the criteria (seasonality of mating and gonad maturation, size-structure and size at maturity) used by Crespi-Abril *et al.* (2008). Differences in body shape were tested using 330 individuals: 107 females (60 immature and 47 mature) and 122 males (43 immature and 79 mature) from the SpSG; 36 females (26 immature and 10 mature) and 65 males (38 immature and 27 mature) from the SSG. Individuals were selected to include the widest rage of size and maturity conditions as well as the best possible state of preservation. Beak shape differences between both groups were analysed in a subset of 138 individuals: 18 females and 33 males from the SpSG; 51 males and 36 females from the SSG. Photographs were taken on each specimen with a digital camera Sony DSC-W70 (7.2 megapixels) mounted on a table top to ensure parallelism between the focal plane of the camera and frontal plane of the squid. Animals were spread ventral side facing up to avoid fins from hanging from the lateral sides of the mantle. Each photograph included a scale to standardize the individual sizes. Six landmarks arranged in two dimensions were used to characterize body shape (Table 1). Landmark coordinates were obtained using the software TPSDig version 2 (Rohlf, 2001). Four of the landmarks selected (landmarks 1–4) were of type I (points where at least two distinct structures meet; i.e. the posterior tip of the body) and two (landmarks 5 and 6) of type II (points that are supported by geometric criteria; i.e. projection of the meeting point of fin and mantle margins on the longitudinal axis of the body) (Bookstein, 1991). All landmarks were distributed assuming axial symmetry on individuals and reducing the degrees of freedom in the analyses (Figure 1).

Lower beaks were cleaned using fresh water to remove rest of tissues and photographed at once from five different angles using a reflecting device assembled with four mirrors inclined at 45° angles relative to the focal plane (Figure 2) and a digital camera mounted on a table top. Based on each image, a truss network based on 58 inter-landmark distances was constructed to estimate a three-dimension Cartesian coordinate of 13 landmarks using the simplified multidimensional scaling approach (Carpenter *et al.*, 1996). Coordinates of landmarks (Table 1) were estimated using the software Morpheus *et al.* (Slice, 1998).

Data analysis

In the analysis of body shape, the command 'Unbend specimens' developed on the software TpsUtil Version 1.37 (Rohlf, 2001) was used to correct the bending that individuals could present. To make this correction, landmarks 1, 5 and 6

Table 1. Description of the location and type of each landmark in the body and the lower beak.

Anatomic structure	Landmark	Type (Bookstein, 1991)	Description
Body	1	I	Posterior end point of the mantle
Body	2	I	Lateral end of the fin
Body	3	I	Meeting point of the fin and the mantle
Body	4	I	Lateral point of the anterior part of the mantle
Body	5	II	Projection of landmark 4 on the longitudinal axis
Body	6	II	Projection of landmark 3 on the longitudinal axis
Beak	1	I	Anterior extreme of the hood
Beak	2	I	Right knob of the wind
Beak	3	I	Extreme of the right wind
Beak	4	I	Extreme of the left wind
Beak	5	I	Left knob of the wind
Beak	6	I	Posterior extreme of the crest
Beak	7	I	Posterior extreme of the hood
Beak	8	I	Posterior extreme of the right wall
Beak	9	I	Posterior extreme of the left wall
Beak	10	I	Ventral intersection of the right knob with the wind
Beak	11	I	Dorsal intersection of the right knob with the wind
Beak	12	I	Dorsal intersection of the left knob with the wind
Beak	13	I	Ventral intersection of the left knob with the wind

were forced to be in the main longitudinal axis (Figure 1). TpsRelw software Version 1.44 (Rohlf, 2001) was used to translate, rotate, and scale the landmark configurations for all individuals by using the generalized least squares (GLS) superimposition method. Translation and rotation is achieved by superimposing the landmark configurations and adjusting their individual inclination and relative position by minimizing the square root of the sum of squared differences between corresponding landmarks (Rohlf, 1999). Scaling is performed by correcting the landmark configurations in such way that all present the same centroid size. The program was also used to calculate the average individual map (consensus configuration), to derive the uniform (that affecting to the same extent all of the landmarks of the form under study) and non-uniform (all other landmark local differences) components of variation, and to estimate size of individuals as the centroid size (the square root of the sum of the squared deviations of
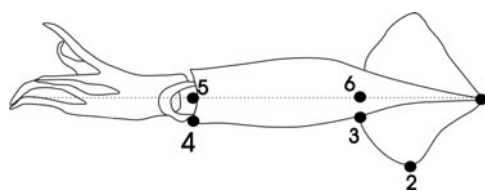

Fig. 1. Landmark configuration on the body of the *Illex argentinus*. Landmarks 1–4 belong to the type I and landmarks 5–6 are of the type II (Bookstein, 1991). Dashed line represents the longitudinal axis of the body.
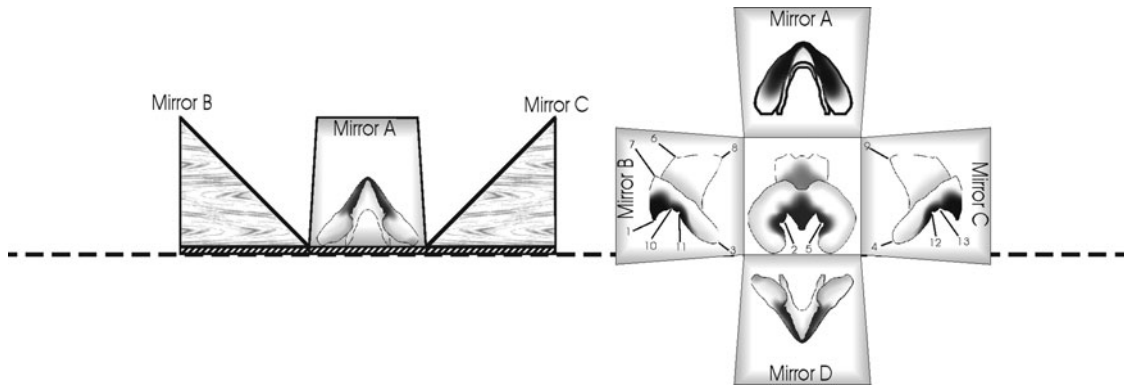
Fig. 2. Schematic view of the reflecting device used in this study, showing the four inclined mirrors and the position of the beak in the centre. Thirteen landmarks are identified with numbers in the right part of the figure.

landmarks from a centroid point) (Rohlf, 1998; Richtsmeier *et al.*, 2002). For the analysis of beak morphometry, superimposition method, components of variations (uniform and non-uniform) and size were derived using the Morphologika2 package developed to process three-dimensional data. The main shape changes in body and beak were visualized through relative warp (RW) analysis, which is a principal component analysis performed over the uniform and non-uniform components of variation, where RW are the principal component orthogonal axis used to describe the main tendencies in shape variation between specimens within a sample (Bookstein, 1998).

Once photographed, individuals were measured (dorsal mantle length in cm), weighed (in grams), dissected and classified as immature (stages I–III) or mature (stages VI–VII) following the scale of maturity developed by Nigmatullin (1989). Since *I. argentinus*, as most Ommastrephid squids, is a semelparous species dying shortly after spawning (Haimovici *et al.*, 1998), these two broad stages of maturity were used as phases of an ontogenetic scale to analyse changes in shape through the ontogeny. In order to do so, the following relationships were examined for beak and body shape: (1) RW1 on size (centroid size); and (2) RW1 on weight (in grams). Given that squids from the SSG attain maturity at lower sizes than those from the SpSG (Crespi-Abril *et al.*, 2008), and considering that fully mature gonads may occupy a large portion of the mantle that could cause a deformation of the body outline,

we expected to be able to distinguish body shapes of mature SSG individuals from those of immature SpSG specimens of similar size and weight. Therefore, we analysed the relationship of RW1 on an index that could reflect the degree of occupation of the mantle cavity by the gonads: ovary weight on total weight (OvW/TW).

Considering that previous studies on the morphology of squids have been conducted using classic morphometrics based on linear measurements, changes of squids' body shape were analysed using this methodological approach to facilitate comparisons with other studies and to complement the results obtained by geometric morphometrics. For this, the relationships between selected linear dimensions of the body parts that changed the most through the first RW (principal axis of shape variation) and the standard measure of size in squids (dorsal mantle length, DML) were studied using bivariate regression analysis, along with isometry and ANCOVA tests.

RESULTS

Relative warp analysis performed on the beak and on the body of *I. argentinus* showed that the first and second relative warps explained more than 75% of the total shape variation in both cases (RW1 61% and RW2 15.3% for beak; RW1 52.45% and RW2 22.86% for body). Lower beak and body shapes were similar for both sexes of the species (Figure 3).
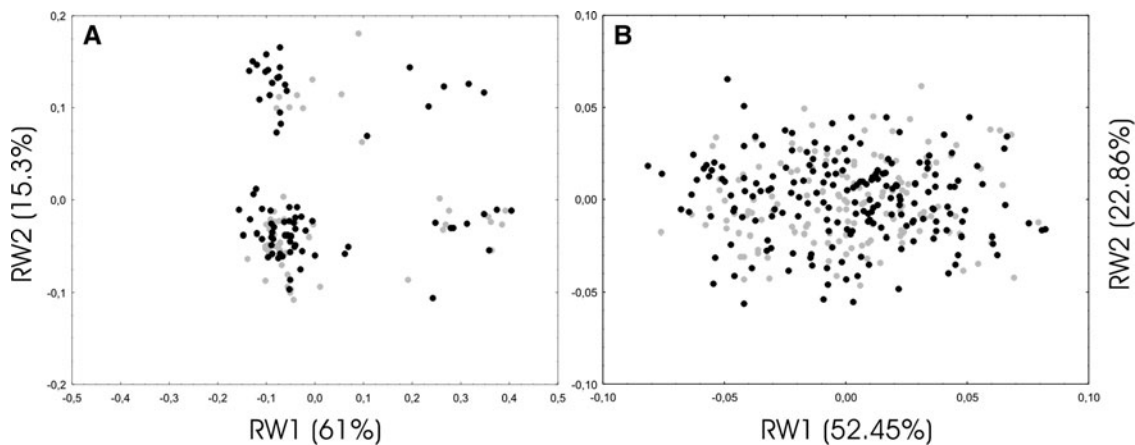

Fig. 3. Scatterplots of the first and second components of the relative warp analysis using beak landmarks (A) and body landmarks (B). Black dots: males; grey dots: females.
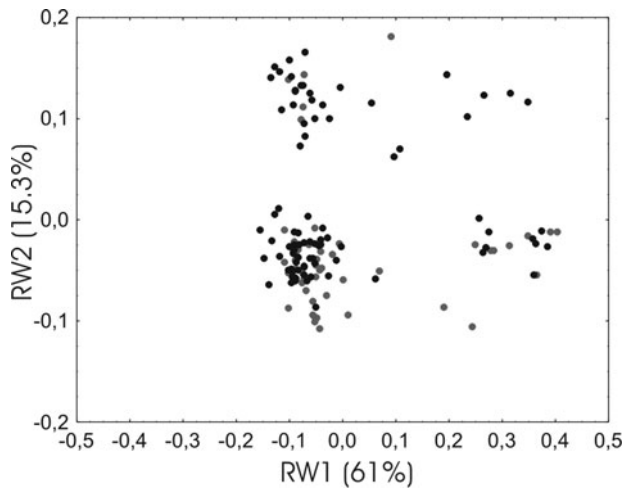
Fig. 4. Scatterplots of the first and second components of the relative warp analysis using beak landmarks. Black dots: summer spawning group (SSG); grey dots: spring spawning group (SpSG).

In the case of the lower beak, RW1 and RW2 did not allow separating individuals from SSG and SpSG (Figure 4). In contrast, body shape presented some degree of separation between individuals of both spawning groups along RW1 (Figure 5). Similarly, some separation was observed along RW1 for mature and immature individuals (Figure 5). Overall, body shape changed gradually from immature SSG (positive values) to mature SpSG individuals (negative values) (Figure 5). In comparison with positive values, negative magnitudes of RW1 were associated to wider mantle, fins occupying higher proportion of the mantle, and wider fins with the lateral ends slightly displaced towards the anterior part of the body (Figure 5).

Beak shape of *I. argentinus* did not change with increasing centroid size and weight (Figure 6). On the contrary, body shape changed as these variables increased (Figure 7). Both spawning groups presented a similar trend in shape change: smaller and lighter individuals were characterized by a more constricted mantle and smaller fin than larger and heavier individuals (Figure 7). In the particular case of SpSG, the rate of shape variation decreased when individuals reached an approximate weight and centroid size of 200 g and 20 (21 cm in DML) respectively (Figure 7).

Geometric morphometrics revealed that fin was the body part that changed the most through the ontogeny. Therefore, relationship of fin length (FL) (Euclidean distance between the landmarks 1 and 2; Figure 1) on DML was analysed. FL increased allometrically relative to DML according to the function $FL = a*DML^b$, with 'b' significantly higher than 1 (95% CI: 1.098–1.166). Maturity condition did not affect the rates of change of log FL relative to log DML, both in females (ANCOVA, $P > 0.92$, df: 1) and males (ANCOVA, $P > 0.64$, df: 1).

Mature females of both spawning groups showed some degree of separation in body shape (RW1) for individuals with a similar proportion of the ovary weight (OvW) relative to total weight (W) (Figure 8).

DISCUSSION

Our geometric morphometry analysis shows that shape of the lower beak of *I. argentinus* is similar when compared between mature and immature specimens, both sexes, and both main spawning groups (SpSG and SSG). Also, it evidenced that beak shape remains constant throughout the ontogeny. This result differs from that obtained by Ivanovic & Brunetti (1997) who reported that the relationships rostral length on
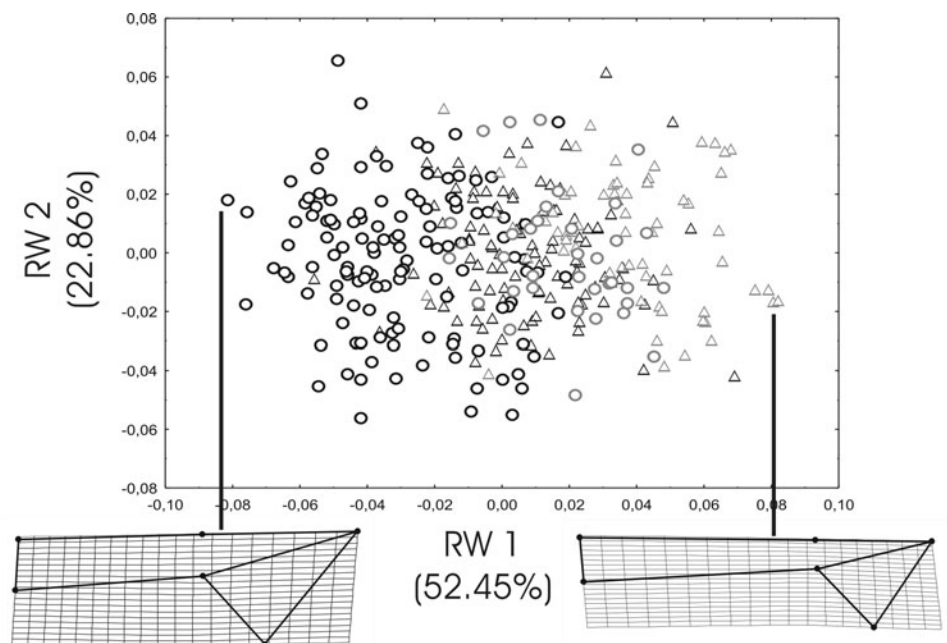

Fig. 5. Scatterplot of the first and second components of the relative warp analysis using body landmarks. Triangles: summer spawning group (SSG); circles: spring spawning group (SpSG); grey symbols: immature specimens; black symbols: mature specimens. The grids represent the shape of individuals at the extremes of RW1.
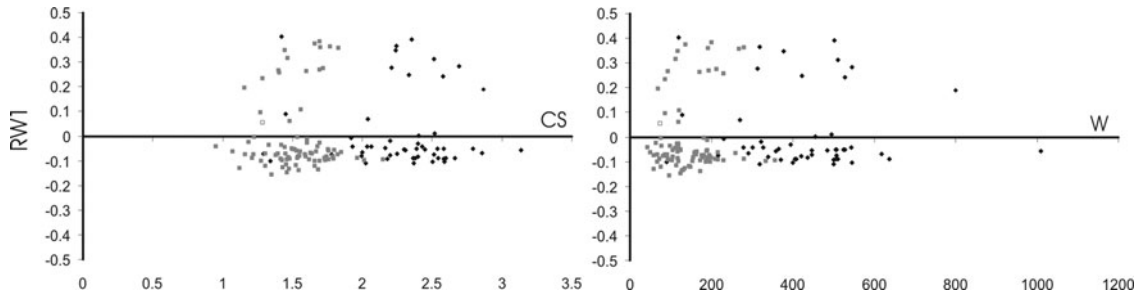
Fig. 6. Scatterplot of the relationship RW1 on centroid size (CS) (left) and RW1 on weight (W, in grams) (right) for the lower beak of *Illex argentinus*. Grey dots: summer spawning group (SSG); black dots: spring spawning group (SpSG).
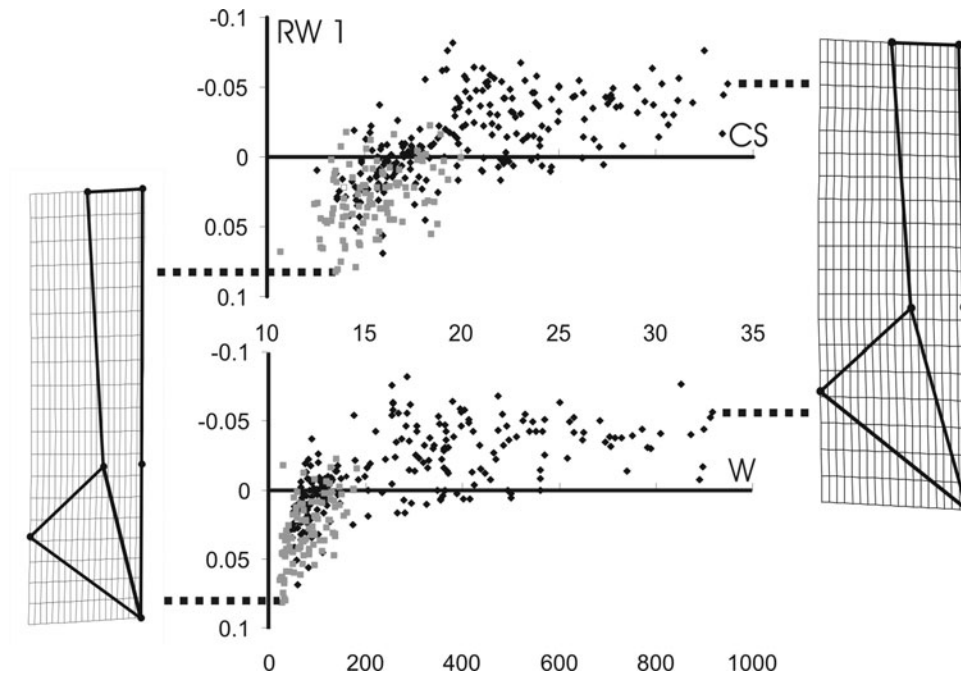

Fig. 7. Scatterplots of the relationship RW1 on centroid size (CS) (up) and RW1 on weight (W, in grams) (down). Grey dots: summer spawning group (SSG); black dots: spring spawning group (SpSG). Grids correspond to the shapes of the smallest/lightest and largest/heaviest individuals.

hood length and crestal length on rostral length of the lower beak were allometric. It is difficult to elucidate the reasons for this difference, but since those authors based their analysis on a set of individuals from mid-shelf and slope spawning groups, altogether and with no distinction of sex, allometry could have been an artefact from pooling the data. On the other hand, our analysis of the variation of shape (RW1)

Fig. 8. Scatterplot of the relationship RW1 on OvW/W (proportion of ovary weight relative to total weight). Grey dots: summer spawning group (SSG); black dots: spring spawning group (SpSG).

with size and weight may have not detected a change in the relationship between some particular dimensions of the beak. One example of this circumstance was given by Rufino *et al.* (2006), who could not discriminate the shapes of male and female carapaces of the crab *Liocarcinus depurator* (Linnaeus, 1758) using landmark-based geometric morphometrics, but with further analysis using classic bivariate morphometry found differences in the relationship of two particular dimensions that allowed the identification of sexes.

As reported by Crespi-Abril *et al.* (2008), SSG individuals attain smaller maximum sizes and sizes at maturity (ML50%) than those from the SpSG. This difference was attributed to the distinct environmental conditions (i.e. primary productivity and temperature) that each particular spawning group may experience through its ontogeny (Crespi-Abril *et al.*, 2008), a phenomenon observed when comparing groups of many other squid species (Forsythe, 1993; Hatfield, 2000; Forsythe *et al.*, 2001; Jackson & Domeir, 2003). In this study, we compared body and beak shapes between two spawning groups of *I. argentinus* to elucidate if these were distinguishable one from the other. The results demonstrated that body shape of squids presented
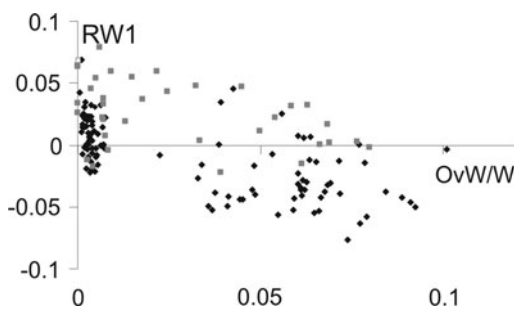
a higher variability than beak shape, even though shape was similar between sexes. Both spawning groups of *I. argentinus* were not strictly separated based on body shape, but there was a gradual change from immature individuals of the SSG to mature individuals of the SpSG (Figure 5). However, we found that as squids grow (in size and weight) body widens near the fin insertion and the fin area increases (Figure 7). Therefore, body shape differences between individuals from the two spawning groups are probably due to uneven size composition in the samples compared rather than to intrinsic shape differences between groups.

Below centroid sizes of 20 (approximately 21 cm in ML) and weights of 200 g, body shape differences among individuals of different sizes and weights are pronounced, while over these thresholds the principal component of shape (RW1) stabilizes (Figure 7). Only squids belonging to SpSG attain centroid sizes and weights higher than 20 and 200 g respectively, and therefore SSG specimens change their shape steadily throughout their whole ontogeny. The analysis performed using the classical morphometrics approach also detected a positive allometric growth of a fin linear dimension (FL) relative to DML independently of maturity condition, which agrees with the findings obtained with geometric morphometrics. Allometric growth of mantle and fins has been observed in several species of squids in particular and in the group as a whole (Haefner, 1964; O'Dor & Hoar, 2000; Barón & Ré, 2002; Zeidberg, 2004), and this pattern has been explained as an adaptation to a more efficient swimming capacity of adults compared to early life stages (O'Dor & Hoar, 2000; Thompson & Kier, 2004; Bartol *et al.*, 2008).

RW1 values were similar in mature SSG individuals and immature SpSG specimens of similar size and weight (Figure 7). From our analysis it became apparent that mature females from both spawning groups reach similar OvW/TW values, and that for similar OvW/TW, smaller SSG females display higher RW1 values (Figure 8). In other words, we did not distinguish body shapes of females belonging to different spawning groups if comparing individuals of the same size, but we did find differences between females of similar OvW/TW (and different size) when comparing different spawning groups. Therefore, we conclude that in specimens of *I. argentinus* from the SSG and SpSG, size related shape adaptation to optimize swimming capacity is more relevant than alteration of body shape to accommodate the gametes as individuals grow and mature.

To our knowledge, this paper is the first application of landmark-based geometric morphometrics to the study of body and beak shape in cephalopods. Although in this work allometric growth of fin was detected using both geometric and classical approaches, these methodologies are not equivalent. The first one presents several advantages compared to classical methods: (1) shape can be reconstructed and visualized to facilitate comparisons and analysis of its variations; (2) the technique isolates the effect of size in the analysis of shape in contrast to classical morphometrics; and (3) as mentioned previously, there is less loss of information and lower risk of selecting dimensions that are uninformative in relation to shape variation. Although the resolution of both methodological approaches depends on the number and adequacy of dimensions or landmarks employed, the previously mentioned advantages of geometric morphometrics may make the method capable to detect changes in shape that could be masked using the classical approach.

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