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Big fish, small fish: still the same species. Lack of morphometric evidence of the existence of two sturgeon species in the Guadalquivir river

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Abstract I have analyzed a published set of morphometric data of sturgeons (Acipenser spp.) caught in Iberian rivers. These data claimed to prove the presence of both A. naccarii and A. sturio in the Iberian Peninsula. However, graphical representation of the scores of individual specimens on the principal components (PC) extracted by a principal-components analysis (PCA) performed on the covariance matrix offered no suggestion of the existence of two morphologically dissimilar groups in the sample. PC extraction from the covariance matrix of the original variables preserves allometries and does not distort the geometric space. However, I also carried out a PCA using the correlation matrix of the morphometric variables after the application of a commonly employed univariate size-adjustment technique because, in the particular study under consideration, ordination of specimens was of interest. Again, this analysis provided no evidence of the presence of two distinct groups within the sample. Neither did bivariate log-log scatterplots of the morphological measurements against total length. Furthermore, a multivariate analysis of variance (MANOVA) of the same size-adjusted variables found no significant differences between the two groups identified by the investigators in their sample. Examination of the two composite variables that these authors used to separate those groups showed them to be badly affected by ontogenetic allometry, thereby leading to the ascription of large and small specimens to different groups. I conclude that there is no morphological evidence to support the claim of the presence of A. naccarii in the Iberian Peninsula.

Introduction

Reliable estimates of biological diversity (both extant and historical) are obviously a necessary prerequisite for the implementation of management and recovery programmes for threatened species. Indeed, this reliability can prove critical to the success of subsequent conservation strategies (Meffe and Carroll 1994). Therefore, analyses providing biodiversity estimates must be as rigorous and detailed as possible (Mayden 1992; Mayden and Kuhajda 1996). Sturgeons (family Acipenseridae) are one group of fishes that contains many imperilled species, some critically so or even extinct (Birstein 1993). Furthermore, in the past they have been relatively neglected by systematists and taxonomists, and now the estimates of their diversity so urgently necessary to current preservation efforts have become arduous because of the present sheer rarity of the intended study objects (e.g. Mayden and Kuhajda 1996). Despite this, different authors are undertaking research that attempts to remedy this situation (Elvira et al. 1991; Mayden and Kuhajda 1996; Garrido-Ramos et al. 1997; Birstein and DeSalle 1998; Birstein et al. 1998; Ludwig and Kirschbaum 1998; Elvira and Almodóvar 1999).

Traditionally, the European Atlantic sturgeon Acipenser sturio L. 1758 has been considered the only sturgeon species native to the Iberian Peninsula (Classen 1944; Almaça 1988; Doadrio et al. 1991; Elvira et al. 1991; Elvira and Almodóvar 1993; Pereira 1995). Recently, however, Garrido-Ramos et al. (1997) stated that two sturgeon species, A. naccarii Bonaparte 1836, a species generally regarded as restricted to the Adriatic Sea (particularly its northern part: Holcik 1989) and A. sturio are present among specimens collected from the Guadalquivir river from the end of the last century until the early 1980s. They based their claim on morphometric and genetic analyses. Other authors have been unable to replicate their genetic findings (Doukakis et al. 2000) and, as I will show later in this paper, the conclusions Garrido-Ramos et al. based on their study of
morphology are compromised by their application of an inadequate methodology that is unable to separate ontogenetic and evolutionary allometry (Cock 1966; Klingenberg 1996). Failure to distinguish between and to account for allometry arising from growth processes within a taxon from that originated by phylogenetic variation between taxa confounds the identification of shape dissimilarities, and urgently calls for a more detailed examination of the morphological data that supposedly provide the basis for the claim of native status for A. naccarii in Iberian rivers. The apparent existence of plans for the release of specimens of A. naccarii into the Guadalquivir River (Ruiz-Rejo´n et al. 1998) makes a more thorough appraisal of that morphometric evidence urgently warranted.

In this paper, I undertake such a task by applying common multivariate and univariate techniques for allometric correction (Humphries et al. 1981; Bookstein et al. 1985; Reist 1985, 1986; Klingenberg 1996) to the raw morphometric measurements employed by Garrido-Ramos et al. (1997; their Table 1). I also critically re-examine their morphometric methodology, evaluate its true ability (or lack of it) to identify and correct allometric growth, and discuss how methodological shortcomings have affected their conclusions.

Materials and methods

Data

Table 1 of Garrido-Ramos et al. (1997) is the source of all morphometric measurements of Acipenser spp. used in my analyses. Garrido-Ramos et al. reported the values of the following six morphometric variables: total length (TL, cm), distance from the tip of the snout to the base of the barbels (A, cm), distance from the base of the barbels to the cartilaginous arch of the mouth (C, cm), distance from the tip of the snout to the cartilaginous arch of the mouth (F, cm), width of the snout at the point of barbel insertion (B, cm) and distance from the tip of the snout to the frenulum (LFR, cm). From these measurements they also derived the subtraction C-A and the ratio F:B (Garrido-Ramos et al. 1997: their Table 1). I also critically re-examine their morphometric methodology, evaluate its true ability (or lack of it) to identify and correct allometric growth, and discuss how methodological shortcomings have affected their conclusions.

The measurements originated from specimens that had been treated quite differently: live or freshly caught, stuffed and preserved in ethanol, and with times since capture ranging from over a century to perhaps 15 to 20 yr, an aspect that could affect the current state and integrity of specimens. However, there is no mention by Garrido-Ramos et al. of any possible influence of this factor on their findings, so I assumed they were tacitly considered to be negligible. Therefore, to ensure comparability, morphometric data have been entered in subsequent calculations as they appear in Table 1 of Garrido-Ramos et al. (1997) and have not been altered save for the correction of obvious transcription or typographical error: e.g. the published measurements for Specimen EBD-8174 are A = 5.20, B = 11.40, C = 12.60, F = 12.60, C-A = 2.20 and F:B = 1.14. I inferred that the correct value for C should be 7.40, and used this in further analyses.

Garrido-Ramos et al. provided no information on their measuring protocols, but from their Fig. 2A and B it is clear that F should be equal to A+C. Indeed, this was always the case in the specimens measured by Classen (1944) and in most of the remaining specimens. However, in other specimens (MUC1, PSN-1), F was slightly smaller than A+C (e.g. MUC1, F = 10.60 cm, A + C = 11.00 cm) while in others again F was larger than A+C (e.g. SE-1, SE-3, F = 4.60, 4.30, 3.20 cm; A + C = 3.90, 4.54, 2.56 cm, respectively). The discrepancy between A + C and F is as high as 0.85 and 0.64 cm for reported values of F of 4.30 and 3.20 cm, respectively, i.e. 20% of the reported measurements. While this may certainly point to a preservation problem (the major discrepancies correspond to stuffed individuals about one century old), it also leads to speculation as to whether the same reference system (e.g. projections along the longitudinal axis of the body) was used for all measurements in all specimens. Again, to maintain comparability and given that the reported values of F are not a linear combination of reported values of C and A, I have retained F in further analyses.

The cases of the subtraction C-A and the ratio F:B are, however, more complicated, and deserve more detailed comment as these two calculations were the main basis of Garrido-Ramos et al.’s claims. I contend that their use instead of the original measurements, and particularly their use as substitutes of other, commonly used, techniques to deal with the effects of size and allometric growth, is mistaken, as I will show below.

Preliminary examination of the data in Table 1 of Garrido-Ramos et al. and their specific ascriptions revealed that their putative Acipenser naccarii were considerably larger and comprised a much smaller size range (TL range = 117 to 203 cm; mean TL ± SE = 158.95 ± 7.1 cm) than the specimens they identified as A. sturio (TL range = 20 to 175 cm; mean TL ± SE = 59.79 ± 12.3 cm). This is a situation where a clear distinction between ontogenetic and evolutionary allometry (Cock 1966; Klingenberg 1996) is critically relevant, and the problem of allometric growth clearly requires some consideration.

The study of allometry has a long tradition (Huxley 1932; Huxley and Teissier 1936), and has produced a truly substantial body of literature (e.g. Cock 1966; Gould 1966; Pimentel 1979; Cheverud 1982; Reist 1985, 1986; Reiss 1989; Bookstein 1993; Marcus 1993; Klingenberg 1996; and references therein). Within this literature, the specific problem of separating size-related variation within groups from between-group differences has also received ample attention (Burnaby 1966; Pimentel 1979; Thorpe 1983; Kohli and Bookstein 1987; Marcus 1990; Klingenberg 1996).

Since the study of Huxley (1932), the formula for simple allometry between two morphological trait measurements x and y (see also Klingenberg 1996) has been:

\[ y = b \cdot x^a \]

very often linearized through log-transformation as:

\[ \log y = \log b + z \cdot \log x \]

where \( z \) and \( b \) are constants. The constant \( z \) is frequently called allometric coefficient.

Eq. (1) shows that for the subtraction of two morphometric measurements (as when generating C-A), to render a truly

\[ \text{Variables} \quad R^2 \quad F \quad (df) \quad x \quad \text{Student’s } t \quad p \quad (df) \]

<table>
<thead>
<tr>
<th>Variables</th>
<th>R²</th>
<th>F (df)</th>
<th>( x )</th>
<th>Student’s t</th>
<th>p (df)</th>
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<td>89.19 (1, 23)</td>
<td>0.7847</td>
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<td>123.74 (1, 23)</td>
<td>0.8664</td>
<td>11.123 (23)</td>
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<td>108.25 (1, 23)</td>
<td>0.6154</td>
<td>10.404 (23)</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td>log LFR</td>
<td>0.913</td>
<td>231.81 (1, 22)</td>
<td>0.7342</td>
<td>15.225 (23)</td>
<td>&lt;0.0000</td>
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