Variations in length, weight and length-weight relationships in the European minnow *Phoxinus phoxinus* (L.) due to fixation and preservation processes

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Variations in length, weight and length-weight relationships in the European minnow Phoxinus phoxinus *(L.) due to fixation and preservation processes.–* The present study attempts to clarify whether length parameters, total weight and their interrelationships on fixated and preserved fish samples are useful elements for fish biology research. Males European minnow *Phoxinus phoxinus* (L.) were captured, measured, weighed and then stored in 5% formalin. Four months later they were again measured and weighed. The fish were washed and preserved for another four months in 70% ethanol, measured and weighed again. There was no significant difference in length, weight, length and length-weight relationships nor in condition factor after formalin storage. A significant difference in fresh and formalin samples versus formalin-ethanol samples in lengths, weight and lengths and standard length-weight relationships was observed but differences in Fulton Condition Factor were not significant. In this species, preserving specimens in formalin for morphometric analysis may be valid. After formalin-ethanol storage samples are no longer valid other than for the condition factor.

Key words: Preservation distortion, Morphometry, Length-weight relationships, *Phoxinus phoxinus*.

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Introduction

Length and weight measurements in fish are a useful tool in several biological disciplines. In some studies, due to the large amount of samples or the revision of already collected specimen, these data have to be obtained from preserved specimens. In such cases, a subtle but systematic source of artifactual variation on resulting data is preservation distortion (PARKER, 1963). For instance, it has long been known that different tissues undergo different amounts and rates of shrinkage during fixation and preservation, and that such responses may be species-specific (PARKER, 1963; LOCKWOOD & DALY, 1975).

In addition, different preservation media, are likely to affect magnitudes and types of distortion (STRAUSS & BOND, 1990). However, some authors still continue to use morphometrics in preserved fish samples for species description (e.g. ELVIRA et al., 1990; KOCH & REIS, 1996), aquaculture management (e.g. DABROWSKY & POCZYCZYNSKI, 1988), aquatic ecology (e.g. ARCIFA et al., 1991; KJELLMAN et al., 1996), fish growth (e.g. PUIGCERVER, 1992; LEONARDOS et al., 1996), life history (e.g. URHO, 1996; VARGAS & SOSTOA, 1996), fisheries (e.g. Lough et al., 1980 in FOWLER & SMITH, 1983) without any corrections, providing misleading results and making correct extrapolation difficult.

The purpose of this study was to discuss the effect of fixation and preservation with the most common fixative and preservative used in fish biology studies, formalin and ethanol 70° (LOWE-MCCONNELL, 1971), on the length, weight, lengths and length-weight relationships of the European minnow Phoxinus phoxinus (L.). Ultimately, the aim was to provide a conversion factor that would permit an estimation of live lengths and weights of preserved specimens of this species.

Material and methods

P. phoxinus (Pisces, Cyprinidae) is a small freshwater fish distributed throughout Europe, and east and central Asia.

In July 1996, 34 samples of P. phoxinus (48-86 mm SL) from an introduced population in Sec Lake (Malniu Lakes, W Pyrenees, Spain) were collected using hand nets and transferred live to the laboratory within a few hours.

All fishes were anaesthetised with 2-Phenoxyethanol (Sigma) and measured as follows: length measures were taken from the left side of the fish (CHEN, 1996) by callipers to the nearest 0.1 mm, total length (L_t) was taken from the top of the snout to the tip of the tail with the lobes compressed so as to give the maximum possible measurement (HILE, 1948), and standard length (L_s) was taken as the distance from central hypural base to tip of snout. Both length measurements were taken because there is no agreement on a standardised way to measure fish length (BALON, 1974). Weight was taken to the nearest 0.001 g. The samples were blotted in damp towelling after the fish had been tilted head down to drain fluid from the body cavities (BILLY, 1982). All measurements were done twice.

Fixation was performed in buffered 5% (v/v) formalin (pH 7.4), being replaced within 48 hours by fresh 5% (v/v) formalin to ensure the maximum tissue quality through maintenance of formalin concentration.

The samples were stored for four months in the dark at room temperature (15-25°C) and then measured and weighed. They were then rinsed in water and preserved for another four months in 70% ethanol, measured and weighed again. This procedure covered a time span typical of routine laboratory processing of field samples. Such a long time should not be necessary as JENNINGS (1991) pointed out that shrinkage of juvenile bass either in formaldehyde or ethanol 70° occurs within the first six days. However, as KRUSE & DALLEY (1990) observed, there was a continuous shrinkage in capelin larvae over 24 weeks.

Total length-standard length, length-weight regression equations, L_s and L_t Fulton condition factors

$$K = 100 \times W / L^3$$

(BOLGER & CONNOLLY, 1989) were calculated.

The t-test was applied to analyse differences among single measurements and condition factor. Analysis covariance (ANCOVA) was performed for equation of regression. The level of significance was taken at 0.05.

Table 1. T-test applied within each variable treatments for *Phoxinus phoxinus:* fresh versus formalin (FR/FO), fresh versus formalin-ethanol (FR/FO-ET) and formalin versus formalin-ethanol (FO/FO-ET): L_S. Standard length; L_T. Total length; W. Total weight. (The length is given in mm, weight in g, representing mean ± standard error of the treatments; the difference between means is given in percentages.) (*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$)

Se aplica la t de Student para comparar los diferentes tratamientos en Phoxinus phoxinus: *fresco respecto formaldehido (FR/FO), fresco respecto formaldehido-etanol (FR/FO-ET) y formaldehido respecto formaldehido-etanol (FO/FO-ET): L_S. Longitud estándar; L_T. Longitud total; W. Peso total. (La longitud se expresa en mm y el peso en g, expresándose como media ± error estándar de cada tratamiento; la diferencia entre medias se expresa en porcentajes.) (*** $P \leq 0,001$; ** $P \leq 0,01$; * $P \leq 0,05$)*

	FR	FO	FO-ET	FR/FO	FR/FO-ET	FO/FO-ET
L_S	64.81 ± 1.3	63.89 ± 2.72	62.31 ± 2.57	(NS)1.5%	(***) 4%	(**) 2.5%
L_T	77.74 ± 1.4	76.73 ± 3.08	75.06 ± 2.75	(NS) 1%	(**) 3%	(NS) 2%
W	5.38 ± 0.27	5.63 ± 0.63	5.07 ± 0.57	(NS) 4%	(***) 6%	(**) 10%

Results

The season and type of fishing may have introduced gear-related sex selection bias into samples. Because few females were captured (n = 4), only males were processed. In this study, after four months of formalin storage, a decrease of 1 to 3.5% in length was seen.

Following four months in ethanol, shrinkage increased another 0.5 to 2.5 %. When comparing mean lengths and weight for the different treatments, FR samples were significantly different from FO-ET samples in the three parameters used, and FO samples versus FO-ET samples in L_S and weight (table 1). However, there were no significant differences in weight or in lengths between FR and FO samples. Both lengths and weight measures decreased significantly following FO-ET use.

The lengths and length-weight relationships are represented in table 2. The relationship between lengths and weight were double logarithmic. The equation follows the form

$$\log y = b \log x + a$$
$$y = ax^b$$

and the slope of the equations of regression was not significantly different from b = 3, as found in most fishes and other organisms with isometrical growth (SEBENS, 1987). The best approximation to length-weight cubic relationship was observed in the total length-weight relationship in FR samples. The better coefficient of determination (r^2) in FO samples was given by total length-weight relationship but by standard length-weight relationship in FO-ET and FR samples (table 2). Total length-weight relationship after FO-ET storage had the same metric equation as that after FO storage; but the former result was excluded from analysis due to poor coefficient of determination for $\log_{10}$-transformed weight length regression ($r^2 < 0.8$) (POPE et al., 1995). There was no significant difference between FR and FO samples in standard length-weight relationships nor in total length-weight relationships. However, there was a significant difference between FR and FO samples versus FO-ET samples in standard length-weight relationships.

The relationship between L_S and L_T was linear. The better coefficient of determination in lengths relationship was seen in FR samples. There were no significant differences between length relationships from FR

Table 2. Equation of regression and regression coefficient for the different treatments: L_S/L_T. Standard length and total length equation of regression; W/L_S. Predicting total weight from standard length; W/L_T. Predicting total weight from total length. ANCOVA was done to compare differences between the equations of regression after different treatments: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$ (W/L_T FO-ET has been excluded from analysis, see the text.)

*Ecuaciones y coeficientes de regresión para los diferentes tratamientos: L_S/L_T. Ecuación de regresión de la longitud estándar y la longitud total; W/L_S. Ecuación de regresión del peso y la longitud estándar; W/L_T. Ecuación de regresión del peso y la longitud total. Se aplicó una ANCOVA para comparar las diferencias entre las ecuaciones de regresión: *** $P \leq 0,001$; ** $P \leq 0,01$; * $P \leq 0,05$ (W/L_T FO-ET ha sido excluido de los análisis, ver el texto.)*

	L_S/L_T	W/L_S	W/L_T
FR	$L_S = 0.88\, L_T - 3.38$ $r^2 = 0.98$	$\log W = 2.81 \log L_S - 4.39$ $r^2 = 0.9$	$\log W = 2.98 \log L_T - 4.94$ $r^2 = 0.88$
FO	$L_S = 0.84\, L_T - 0.14$ $r^2 = 0.91$	$\log W = 2.9 \log L_S - 4.53$ $r^2 = 0.87$	$\log W = 3.05 \log L_T - 5.06$ $r^2 = 0.93$
FO-ET	$L_S = 0.86\, L_T - 1.76$ $r^2 = 0.9$	$\log W = 3.17 \log L_S - 5.04$ $r^2 = 0.95$	$\log W = 3.05 \log L_T - 5.06$ $r^2 = 0.71$
FR/FO	NS	NS	NS
FR/FO-ET	***	***	-
FO/FO-ET	*	***	-

versus FO samples; however, significant differences were observed between FR and FO versus FO-ET samples.

Fulton Condition Factor of *P. phoxinus* does not vary significantly in any of the treatments (table 3).

Discussion

Some authors consider L_S as the best length measurement (BALON, 1974; SNELSON, 1982) as samples sometimes have damaged fins. Uniform compression of the lobes could thus be a subjective action. In addition, L_T measure may be biased if there is allometric growth of the caudal fin. In this study, the best estimate of the length-weight relationship was given by the L_S in FR and FO-ET samples and by L_T in FO samples (table 2). Both lengths should therefore be measured and used differentially depending on whether FR, FO samples or FO-ET samples are used. Table 1 also indicates that the L_T was not significantly affected when comparing FO and FO-ET storage whereas L_S decreased significantly after both treatments. HILE (1936) and CLUTTER & WHITESEL (1956) reported weight loss after preserving fish in formalin. However, PARKER (1963), STOBO (1972) and BILLY (1982) reported a considerable increase in weight of fish preserved in formalin. It would likely seem that any material fixed in formalin should gain weight because of the chemical union of the formaldehyde molecule with the specimen proteins. In the present study, as reported by PARKER (1963), STOBO (1972) and BILLY (1982), weight increased after storage in formalin, but decreased below the fresh weight after storage in ethanol. A survey of studies reporting length changes associated with fixation and preservation shows that all authors except BILLY (1982) report a decrease in length. These authors

Table 3. Condition factor ($100 \times g/cm^3$) of fresh, fixed and preserved samples of *Phoxinus phoxinus* calculated from standard and total length ($\bar{x}\pm SD$). Comparisons after different treatments: FR/FO, FR/FO-ET and FO/FO-ET, are done with t-test: NS. Non significant.

Factor de condición ($100 \times g/cm^3$) de muestras frescas, fijadas y preservadas de Phoxinus phoxinus *calculados a partir de las longitudes total y estándar ($\bar{x}\pm DE$). Se efectúa una comparación de los diferentes tratamientos: FR/FO, FR/FO-ET y FO/FO-ET mediante una t de Student: NS. No significativo.*

	L_s condition	L_T condition
FR	1.750±0.131	1.033±0.071
FO	1.932±0.204	1.119±0.104
FO-ET	1.867±0.144	1.088±0.178
FR/FO	NS	NS
FR/FO-ET	NS	NS
FO/FO-ET	NS	NS

(CLUTTER & WHITESEL, 1956; PARKER, 1963; BALON, 1974; LESLIE & MOORE, 1986), showed an insignificant length shrinkage in several preservatives, attaining a maximum 96% of live length but usually not exceeding 1-2% shrinkage. Thus, total shrinkage oscillated between 3 to 6%. The authors referred above calculated the percentage of shrinkage only, and as it was less than 96% of live length, considered it was not significant. In the present study, significance (Paired t-test) was found at 2.5% shrinkage level. However, as stated above, when comparing FR versus FO samples, no significant difference was shown (table 1). Until now, no studies on the direct effects of preservatives on length-weight relationships are available. The differences in regression equations between fresh and formalin samples were not significant (ANCOVA F > 0.05). However, there were significant differences between regression equations of FR and FO versus FO-ET (table 2). The use of

FO preserved specimens did not seem to alter the regression equation in *P. phoxinus*, whereas FO-ET treatment did indicate some alteration. It was also observed that the condition factor was not significantly affected after either preservation treatment (table 3). The use of preserved specimens in formalin for morphometric analysis is therefore not always valid for single measurements but is valid for the relationship between measures, such as length-weight regression equation and condition factor. However, due to significant differences in length-weight regression observed on specimens FO-ET, its use by fish biologists should be avoided. Table 1 conversion factors should be used when necessary, i.e. in FO-ET samples of *P. phoxinus*. However, as no standard fish fixation and preservation technique has been agreed, they should be carefully used, since fixed and preserved samples undergo different rates of shrinkage depending upon original state and size of the specimens (STOBO, 1972; SCHNACK & ROSENTHAL, 1978; HAY, 1982), species and/or even populations involved, sample-preservative relationship (PARKER, 1963; LOCKWOOD & DALY, 1975; SAGNES, 1997) and the ionic concentration and storage time (SCHNACK & ROSENTHAL, 1978; HAY, 1982).

Resumen

Variación en la longitud, el peso y la relación longitud-peso en el foxino Phoxinus phoxinus *(L.) debidas a los procesos de fijación y preservación*

Este estudio trata de clarificar si los parámetros de longitud (L_T y L_s), peso total (W) y su interrelación (L_T / LS, L_s / W, L_T / W y factor de condición de Fulton) en ejemplares de peces fijados y preservados, son útiles para la investigación ictiológica. Se capturaron 44 individuos de foxino (*Phoxinus phoxinus* L.), que fueron medidos y pesados y posteriormente fijados en una solución de formaldehido 5% durante cuatro meses. Transcurrido este tiempo, fueron medidos y pesados de nuevo. Se lavaron y se preservaron en una solución de etanol 70% durante cuatro meses más, tras los cuales se repitió la operación de medida y pesaje. No hubo ninguna diferencia significativa en longitud,

peso ni sus interrelaciones tras la fijación en formaldehido 5% (tablas 1-3). En cambio, sí se encontró una diferencia significativa entre las muestras frescas (FR) y las conservadas en formaldehido 5% (FO) respecto de los ejemplares tras cuatro meses preservados en etanol 70° (FO-ET) en los siguientes parámetros: L_s, L_T, W y en las interrelaciones L_s / L_T y L_s / W. Sin embargo, la diferencia entre los factores de condición no fueron significativas (tabla 3). Se concluye que el uso para cálculos morfométricos de ejemplares de *P. phoxinus* conservados en FO es válido. Tras su conservación en FO-ET, estos ejemplares no son válidos para cálculos morfométricos excepto en el caso del factor de condición de Fulton.

Acknowledgements

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