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# Long-term trends of biometric indices in the large-lip killifish, *Tlaloc labialis* (Actinopterygii, Cyprinodontiformes, Profundulidae)

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## Abstract

Biometric indices have been widely used in studies of fisheries management, conservation, and biomonitoring of environmental stressors impacting fish health. Commonly used methods include somatic indices, such as the condition factor (*K*), and length–weight relationships (LWRs). The aim of this study was to explore the effects of long-term preservation on the LWRs and condition factors in *Tlaloc labialis* (Günther, 1866), a freshwater fish species endemic to southern Mexico and Guatemala. Fifty specimens were collected from the Grijalva River basin in southeastern México. Morphometric data were recorded at different stages of preservation: freshly collected, fixed in 10% formalin (72 h), and preserved in 70% ethanol for 10 years (2015–2025). We observed initial reductions in weight and condition factor after formalin fixation, followed by a substantial cumulative loss in weight (−33.92%) after one year of ethanol preservation. The *b*-value of the LWR for *T. labialis* increased markedly in specimens preserved in ethanol compared with fresh and formalin-fixed specimens. These results provide a quantitative basis for adjusting historical datasets, specifically by allowing the approximate reconstruction of original wet mass and body condition indices from long-preserved individuals, ensuring reliable biomonitoring and conservation assessments for this endemic species based on specimens from museum collections.

## Keywords

condition factor, endemic species, freshwater fish, Grijalva River, length–weight relationship, profundulids

## Introduction

Biometric indices have been used widely for fisheries management, species conservation, and fish-based biomonitoring of environmental stressors (e.g., Le Cren 1951; Froese 2006). Commonly used methods include somatic indices, such as the condition factor, and length–weight relationships (LWRs; Froese 2006; Montenegro and González 2012). The LWR constitutes a fundamental tool in fisheries and natural resource management that provides essential data for biomass estimation, growth trajectories, assessment of fish stock condition, and conservation studies of fishes (Froese 2006; Anzueto-Calvo et al. 2022). The LWR can be used to estimate fish biomass at a given length and to develop behavioral or predictive models at both the population and community levels (e.g., McCallum 2000; Anzueto-Calvo et al. 2017). Fulton's condition factor (*K*) provides a proxy of fish

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health and condition (e.g., plumpness, well-being) and is used to assess the condition of an individual fish or a population. It assumes that fish weighing more at a given length are in better condition than those weighing less at the same length (Fulton 1904; Froese 2006).

Several ecological factors affect LWRs and *K*, including biological attributes (fish sex, maturity, and health), environmental variables (habitat, hydrological parameters, and seasonality) and sampling conditions and possible sampling artifacts including sample size, length ranges, and preservation methods (Tesch 1971; Wootton 1998; Andreu-Soler et al. 2006; Hay et al. 2020). The technique and time of preservation can affect the values of biometric indices; however, this topic has arguably been less explored in ichthyological studies than LWRs themselves (e.g., Ajah and Nunoo 2003; Anzueto-Calvo et al. 2022). Recently, Hay et al. (2020) demonstrated that preserved museum specimens can effectively fill data gaps in LWRs for rare or non-commercial fish species by assuming isometric growth when sample sizes are small.

One species with prior research on the effects of preservation on biometric indices is the large-lip killifish, *Tlaloc labialis* (Günther, 1866), a small cyprinodontiform fish endemic to streams of southern Mexico and Guatemala (Miller et al. 2005; Domínguez-Cisneros et al. 2023). Prior research established initial baselines for LWRs in this species: Velázquez-Velázquez et al. (2015) provided the first LWR parameter estimates ($b$ = 3.003) for *T. labialis* from the Grijalva River basin, and Anzueto-Calvo et al. (2017) demonstrated that chemical preservation severely alters this species' biometric indices, artificially increasing the allometric coefficient. Despite the many insights from these previous studies, knowledge gaps remain regarding the long-term effects of preservation on biometric indices in *T. labialis*, including the effects of longer time periods. Therefore, in the present study, we aimed to evaluate the effects of long-term preservation on the LWRs and *K*-values for *T. labialis*. Our results provide a quantitative basis for adjusting historical datasets, specifically by allowing the approximate reconstruction of original wet mass and body condition indices from long-preserved individuals of *T. labialis*.

## Materials and methods

A total of 50 specimens of *T. labialis* were collected during 2015 from tributaries of the Grijalva River basin in Chiapas, Mexico using a seine net (3 × 2 m, 0.5 cm mesh size). After the catch, the fish were transferred to the laboratory. For fresh specimens, excess moisture was gently blotted using absorbent paper, and individuals were carefully straightened, if necessary, before being measured and weighed (cf. Hay et al. 2020). Subsequently, the specimens were fixed in 10% formalin and stored for 72 h at room temperature and then transferred to a 70% ethanol solution for final preservation and accessioning (Anzueto-Calvo et al. 2017) in the Fish Collection of the Zoology Museum of the University of Sciences and Arts of Chiapas (MZ-UNICACH 07813, 25; MZ-UNICACH 07814, 25). Based on the size at first sexual maturity (4.10 cm) reported by Anzueto-Calvo et al. (2024), fish were classified as mature ($n$ = 13) and immature ($n$ = 37). Overall specimen standard length ranged from 3.14 to 8.20 cm. Following a longitudinal, repeated-measures design, the exact same 50 specimens were monitored throughout the study. Standard length (SL) [cm] and total weight ($W$) [g] were recorded at four stages:

- when freshly collected,
- 72 h post-fixation in 10% formalin,
- one month after transfer to 70% ethanol, and
- after 1, 2, 5, 7, 9, and 10 years in 70% ethanol.

Before measuring and weighing, excess moisture was removed from each preserved specimen by blotting with absorbent paper.

The fish were euthanized with an overdose of MS-222 immediately after capture. This is consistent with the guidelines for the use of fishes in research published by the American Fisheries Society (AFS 2014). All field activities were authorized by the Mexican Ministry of Environment and Natural Resources (SEMARNAT Permit #SGPA/DGVS/02208/13).

The LWR for *T. labialis* was determined using the allometric equation (Ricker 1973)

$$W = aL^b$$

where $W$ is the weight of the fish in grams, $L$ is the standard length [cm], and $a$ and $b$ are the two model parameters (Froese 2006). The statistical significance level of the coefficient of determination ($r^2$) and 95% confidence intervals (CIs) of $a$ and $b$ in the LWR were also estimated (Anzueto-Calvo et al. 2022). The condition factor, $K$, was calculated using the equation (Heincke 1908)

$$K = \left(W \cdot L^{-3}\right) \times 100$$

To evaluate changes in fish condition and growth patterns over time, we tested the hypothesis that both the LWR parameters and the $K$-values vary significantly among the different measurement periods (i.e., freshly collected, 72 h post-fixation in 10% formalin, one month, one year, two, five, seven, nine, and ten years after transfer to 70% ethanol). Since our data did not comply with the assumptions of standard parametric statistical tests, and to account for the non-independent nature of the data, the non-parametric Friedman test was used to evaluate changes in body weight and Fulton's condition factor ($K$). To identify specific periods of change for both parameters, a post hoc Wilcoxon signed-rank test for paired samples was performed, applying the Bonferroni correction (Anzueto-Calvo et al. 2017). Additionally, to handle

repeated-measures dependency, a Linear Mixed Model (LMM) was applied to compare weight percentage changes between mature and immature individuals. The maturity stage (mature and immature), measurement, and their two-way interaction (maturity × measurement) were used as fixed effects, and individual fish identity (ID) as a random effect. All analyses were performed using the R statistical computing environment v4.4.3 (R Core Team 2025), including the car R package (Fox and Weisberg 2011), the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) packages.

## Results

An initial weight loss of −5.78% [standard deviation (SD) = 3.01] was recorded among the *T. labialis* specimens after 72 h following formalin fixation. The observed pattern of weight loss accelerated during the first month of ethanol preservation, reaching −29.94% (SD = 5.35) and then stabilizing at −33.92% change after one year. Statistically significant differences were found in weight loss between different preservation stages (Friedman $\chi^2(8) = 311.85$, $P < 0.001$). The post hoc test revealed that the most pronounced changes occurred during the initial stages of fixation and preservation ($P < 0.001$), and the same pattern was observed for length. The weight loss of the sampled fish stabilized after one year, with no further significant losses observed through the end of the ten-year study period (Fig. 1). However, the pattern of mass reduction was differential when separating specimens by maturity stage, i.e., comparing immature and mature specimens. Although both groups followed the same overall temporal trend of weight loss, a distinct and constant gap was observed: smaller, immature individuals proved to be more susceptible to preservation-induced shrinkage, stabilizing at roughly 35% weight loss, while more mature specimens maintained a significantly lower weight loss of around 26% across the 10-year monitoring period (Fig. 1). The linear mixed-model test confirmed that these observed differences between maturity stages were statistically significant (LMM $F(1, 48) = 44.74$; $P < 0.001$), as was the maturity × measurement interaction (LMM $F(7, 336) = 7.74$; $P < 0.001$).

In contrast to the pronounced weight loss, the reduction in standard length followed a similar trend but on a significantly smaller scale. During the initial fixation, specimens experienced a linear shrinkage of −1.19%, which increased about 2.1-fold after the first month in alcohol (to −2.53%). Subsequently, longitudinal shrinkage advanced gradually and at a slower rate, reaching −3.04% at one year of preservation. Length stabilized thereafter, with no further significant shrinkage observed until the end of the ten-year period.

The estimates of the parameters $a$ and $b$ for the LWRs, the coefficient of determination of $r^2$, and the 95% confidence intervals for $a$ and $b$ are presented in Table 1. The LWR for *T. labialis* along the period of time assessed showed evidence of positive allometric growth ($b > 3$) and ranged from 3.26 (95% CIs = 3.184–3.336) in fresh specimens to 3.574 (95% CIs = 3.469–3.678) at two years; after 10 years $b = 3.457$ (95% CIs = 3.344–3.571) (Fig. 2). The 95% confidence interval values for $b$ in fresh and fixed specimens do not overlap with those of the other periods except for fresh specimens vs. one year in ethanol, as detailed in Table 1. The increase in the $b$-values was highly pronounced from fresh specimens to those after one month in ethanol. Our ten-year monitoring period allowed us to confirm that while the initial shift in $b$ was rapid, a final adjustment occurred between the first month and the first year of ethanol preservation. No further significant changes in the $b$-value occurred among the sampled specimens during the subsequent annual intervals up to ten years from the date of capture (Table 1, Fig. 2).

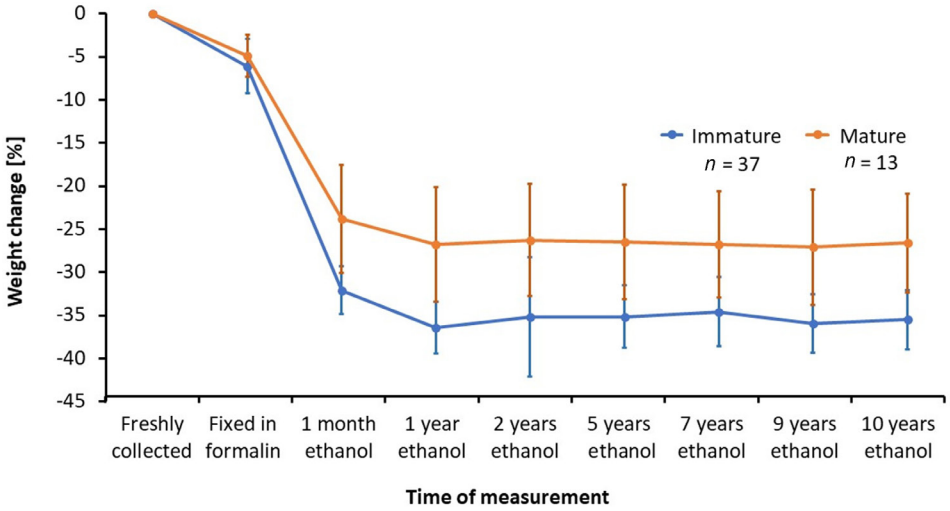


**Figure 1.** Temporal variation in the mean percentage of weight change of preserved *Tlaloc labialis* specimens over a decade of study, compared by maturity stages (immature versus mature). Error bars represent one standard deviation (±SD).

**Table 1.** Parameters of the length–weight relationship of preserved specimens of *Tlaloc labialis* during a 10-year study period.

| Time of measurement | *n* | SL [cm] | *W* [g] | *a* | *a* $CI_L$ | *a* $CI_U$ | *b* | *b* $CI_L$ | *b* $CI_U$ | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Freshly collected | 50 | 4.257 | 2.312 | 0.014 | 0.013 | 0.016 | 3.260 | 3.184 | 3.336 | 0.990 |
| Fixed in formalin | | | | | | | | | | |
| After 72 h | 50 | 4.207 | 2.182 | 0.014 | 0.013 | 0.016 | 3.260 | 3.191 | 3.329 | 0.990 |
| Preserved in ethanol | | | | | | | | | | |
| 1 month | 50 | 4.151 | 1.756 | 0.008 | 0.007 | 0.010 | 3.458 | 3.355 | 3.560 | 0.990 |
| 1 year | 50 | 4.128 | 1.682 | 0.007 | 0.006 | 0.009 | 3.489 | 3.335 | 3.642 | 0.980 |
| 2 years | 50 | 4.104 | 1.683 | 0.007 | 0.006 | 0.008 | 3.574 | 3.469 | 3.678 | 0.989 |
| 5 years | 50 | 4.139 | 1.696 | 0.008 | 0.007 | 0.009 | 3.487 | 3.377 | 3.597 | 0.988 |
| 7 years | 50 | 4.093 | 1.692 | 0.008 | 0.007 | 0.010 | 3.475 | 3.361 | 3.588 | 0.987 |
| 9 years | 50 | 4.101 | 1.680 | 0.008 | 0.006 | 0.009 | 3.512 | 3.400 | 3.624 | 0.988 |
| 10 years | 50 | 4.087 | 1.684 | 0.008 | 0.007 | 0.010 | 3.457 | 3.344 | 3.571 | 0.987 |

SL= mean standard length; *W* = actual mean weight of the fish from each measurement (the weight loss percentages mentioned in the text are the average of individual changes, whereas those reported in this table are based on average weights); *a* and *b* = parameters of $W = aL^b$ (log *a* is the intercept and *b* the slope of the log-transformed regression); $CI_L$ (*a*), $CI_U$ (*a*) = lower and upper 95% confidence intervals for *a*; $CI_L$ (*b*), $CI_U$ (*b*) = lower and upper 95% confidence intervals for *b*; $r^2$ = coefficient of determination (goodness-of-fit statistic).

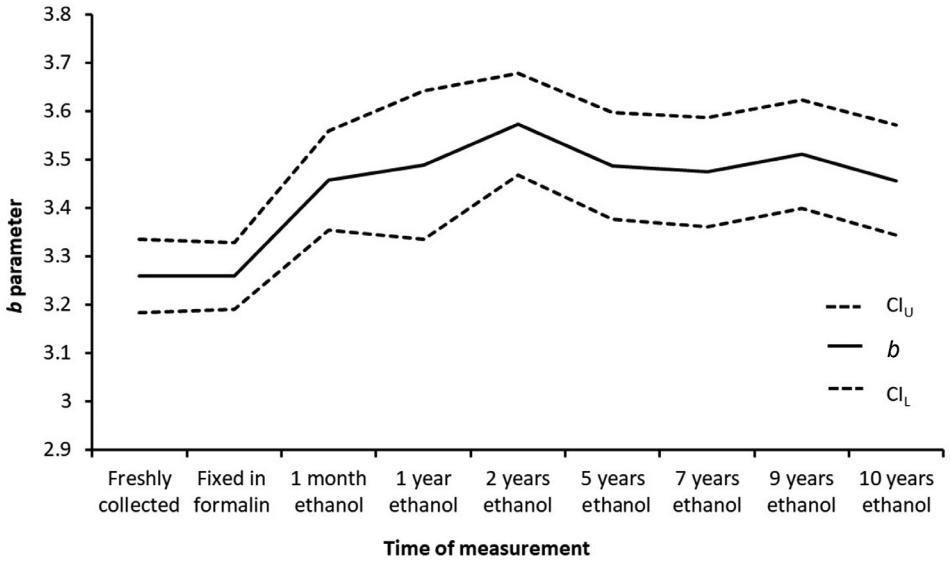


**Figure 2.** Temporal variation of *b*-values in the length–weight relationship of preserved *Tlaloc labialis* specimens during a decade of study. $CI_L$ (*b*), $CI_U$ (*b*) = lower and upper 95% confidence intervals for *b*, respectively.

The values of *K* decreased from 2.08 for freshly caught fish to 2.03 in the formalin-fixed specimens after 72 h of preservation, and this decrease continued to $K = 1.51$ after one year of preservation in ethanol (Fig. 3). After these changes, the *K*-values remained relatively constant during the study period. However, the mean *K*-values differed significantly among the nine time periods evaluated (Friedman $\chi^2(8) = 231.7$, $P < 0.001$).

## Discussion

The results of this study demonstrate that long-term preservation induces significant and permanent alterations in the biometric indices of large-lip killifish, *Tlaloc labialis*. We observed an initial reduction in weight of −5.78% after 72 h of formalin preservation, followed by a substantial cumulative loss reaching −33.92% after one year of preservation in ethanol. This phenomenon is fundamentally linked to the chemical properties of the preservatives used on fish specimens in natural history collections. While formalin acts as a fixative primarily due to its ability to provide excellent structural preservation through protein cross-linking (Shetty et al. 2020), ethanol serves as a potent dehydrating agent that replaces biological water within the tissues, leading to severe mass loss (Billy 1982). Our findings corroborate the claim that the transition from formalin fixation to alcohol storage is the most critical phase for morphological distortion in specimens for ichthyological studies (e.g., Anzueto-Calvo et al. 2017).

The increase in the allometric coefficient *b* from 3.26 in fresh specimens to 3.46–3.57 after ethanol preservation underscores that these artifacts are size-dependent. This shift is clearly demonstrated by the differential mass loss observed across life stages in our study,

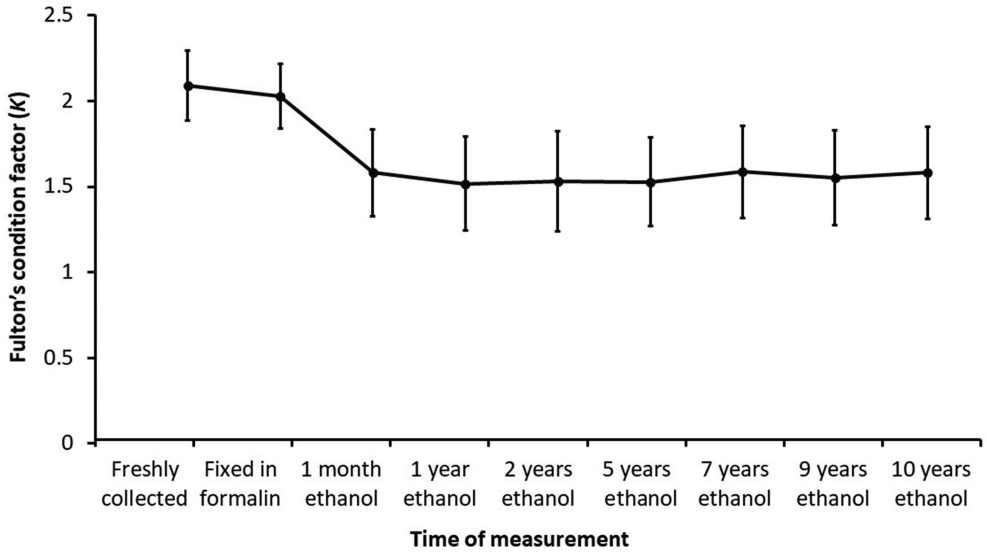


**Figure 3.** Changes in the mean Fulton's condition factor (*K*) of preserved *Tlaloc labialis* specimens over a decade of study. Dots indicate mean values and error bars represent one standard deviation (±SD).

where immature specimens lost 35% of their initial weight, whereas mature specimens experienced a lower reduction of 26%. This disproportionate desiccation in smaller individuals artificially steepens the slope of the LWR (Paradis et al. 2007). While size-dependent mass loss is a primary driver observed in *T. labialis*, this shift in the *b*-value is likely accelerated by complementary mechanisms, such as differential longitudinal shrinkage among size classes and the combined effects of tissue dehydration and overall body contraction (Shields and Carlson 1996). The value of *b* remained > 3 at all stages, including fresh specimens, indicating positive allometry in this population. This artificial inflation of the *b*-values could lead to erroneous biomass estimations if models derived from museum specimens were applied directly to live populations or used without correction. Similarly, the marked decline in Fulton's condition factor *K* from 2.08 to 1.51 in this study is interpreted as a direct consequence of mass loss and as a preservation artifact rather than a 'true' indication of poor biological health or the presence of environmental stressors affecting the sampled population.

Finally, while preserved specimens are crucial for long-term biological and ecological studies, the −33.92% weight loss experienced in *T. labialis* necessitates the use of correction factors. In contrast to Hay et al. (2020), who suggested that preservation-induced biases are negligible compared to natural seasonal oscillations in growth, our empirical data demonstrate that desiccation significantly alters both Fulton's condition factor, *K*, and the length–weight exponent, *b*. Nevertheless, we agree with Hay et al. (2020) that museum collections provide an invaluable framework for deriving key ecological parameters without the need for new field sampling. To enhance the practical value of historical collection data, we propose two specific correction equations to estimate the fresh weight ($W_F$) [g] of mature and immature specimens of *T. labialis* from museum specimens preserved for one year or more in alcohol ($W_P$, preserved weight), based on the stabilized overall mass loss of –35.5% for immature specimens and –26.8% for mature specimens, observed after one year, as follows:

- for mature specimens

$$W_F = W_P \cdot 0.732^{-1}$$

- for immature specimens

$$W_F = W_P \cdot 0.645^{-1}$$

These equations apply only to fish specimens preserved for ≥ 1 year in 70% ethanol following 72 hours of formalin fixation.

While these maturity-specific factors provide a solid basis for ecological analyses, researchers should note that biomass loss is size-dependent; in our study, immature specimens exhibited a higher mass loss than mature specimens. Nevertheless, these correction factors allow researchers to back-calculate the approximate original biomass of preserved specimens, overcoming a major limitation when utilizing historical museum series for ecological analyses.

These results provide a quantitative basis for adjusting historical data, ensuring accurate biomonitoring and conservation assessments for this endemic species in museum collections. Additionally, the contribution of this study to specimen-based research is significant because it indicates that, after the initial losses during the first year, the biometric indices of *T. labialis* specimens remain stable for at least a decade; this may also hold for closely related species.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

All field activities, specimen collection, and animal handling were authorized by the Mexican Ministry of Environment and Natural Resources (SEMARNAT, Permit #SGPA/DGVS/02208/13). Fish euthanasia was carried out immediately after capture using an overdose of MS-222, in accordance with the Guidelines for the Use of Fishes in Research published by the American Fisheries Society (AFS 2014).

### Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
Regarding the use of AI in the preparation of this manuscript, the authors declare the following: Gemini
Used for: Language, style and writing
The use of Gemini's AI was for revision and editing of English in the manuscript.

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### Author contributions

Conceptualization: EVV. Data curation: MJAC, BCM. Formal analysis: MJAC. Funding acquisition: EVV. Investigation: SEDC. Methodology: BCM. Supervision: SEDC. Writing - original draft: EVV. Writing - review and editing: SEDC.

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### Data availability

All of the data that support the findings of this study are available in the main text.