ISSN 0032-9452, Journal of Ichthyology, 2023, Vol. 63, No. 5, pp. 930–936. © Pleiades Publishing, Ltd., 2023.
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Length–Weight Relationships and Body Condition Indices of a South American Bioindicator, the Native Neotropical Fish Species, *Cnesterodon decemmaculatus* (Poeciliidae)

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Received January 9, 2023; revised April 20, 2023; accepted April 21, 2023

Abstract—The main goal of our study was to determine the length-weight relationships of *Cnesterodon decemmaculatus*, as well as to estimate its general fitness through Fulton condition factor (*K*), relative condition factor (K_n) and the residuals of length–weight relationships. To determine the best index to explain the growth pattern of *C. decemmaculatus*, a total of 472 adult females were collected from Yuspe River in Córdoba (Argentina), between 2012 and 2019. The length–weight relationships between body weight (*W*) and standard length (*SL*) was established by the equation: $W = 0.16 \times SL^{3.14}$, showing a positive allometric growth. Meanwhile, somatic indices presented similar variation patterns, increasing their values during the breeding season (between October and February) since the individuals gain weight due to gonadal maturation and embryonic growth, and decreasing between March and September. We found that *K* was significantly associated with fish length, therefore, it would not be appropriate to use it to assess the general body condition of *C. decemmaculatus* females. According to our results we highly recommend establishing the length–weight relationships first and then selecting the somatic index according to it. We strongly suggest that for future investigation with *C. decemmaculatus* as a bioindicator, the analyses of residuals of the length–weight relationship is the best method to determine variation patterns of general fish conditions at both field and laboratory studies.

Keywords: fish growth, body condition, South American freshwater systems, *Cnesterodon decemmaculatus*

DOI: 10.1134/S0032945223050156

INTRODUCTION

Fish growth is influenced by both internal and external factors such as sex, age, food, population density or changes in environmental conditions (Weatherley and Gill, 1987; Imsland and Jonassen, 2003). Integrative information about the influences of the aquatic environment could be obtained through indicators of fish growth and general health conditions (Searcy et al., 2007; Zapfe and Rakocinski, 2008). These indices have been used primarily in population dynamics studies and species management (Imai et al., 2002; Ortiz de Zárate and Babcock, 2016) to compare different habitat qualities and to assess the responses of fish to pollution (Amara et al., 2007; Vasconcelos et al., 2009; Piazza and La Peyre, 2010; Brázová et al., 2021). In this sense, the increasing environmental degradation of freshwater systems worldwide highlights the need for studies that attempt to address the effects of anthropogenic activities on the normal fish growth and health of natural populations.

When growth is analyzed as a length function, relative growth is obtained (Huxley, 1993; Volpedo et al., 2015). The fish length–weight relation (LWR) is often used to convert lengths into biomass, compare fish growth between different areas, study ontogenetic allometric changes and determine the condition of individuals (Teixeira-de Mello et al., 2009; Froese et al., 2011). On the other hand, to determine the general health condition, different morphometric indices have been developed based on the LWRs, with the premise that the heaviest individuals of a given length have the best health condition (Sutton et al., 2000). Among these indices, the most widely used is the Fulton condition factor (Fulton, 1904), which estimates the fish condition under the assumption of isometric growth. However, this assumption is not fulfilled in all fish populations (Rennie and Verdon, 2008). As an alternative when isometric growth does not occur, other indices, such as the relative condition factor (K_n) (Le Cren, 1951) or the residuals of LWR (Sutton et al., 2000), could be used to estimate the relative fish condition of a population.

Fish have been widely used in environmental water quality assessment since they offer an integrated response from factors that directly and indirectly stress the entire ecosystem and manifest the ecological relevance of disturbances. Recently, a species that has gained importance as a bioindicator in the Neotropical region is *Cnesterodon decemmaculatus* (Jenyns, 1842) (Poeciliidae, Cyprinidontiformes). This small viviparous fish is widely distributed across three South American countries, Argentina, Uruguay and Brazil (Lucinda, 2005). They present sexual dimorphism where males are smaller than females. Male growth rate decelerates at maturity while female growth continues to follow a normal, decelerating growth trajectory (Reznick et al., 2021). It inhabits both pristine and degraded environments with different degrees of alteration. As a tolerant fish species, its population size increases in polluted freshwater systems (Hued and Bistoni, 2005; Vera-Candioti et al., 2014; Zambrano et al., 2018). Furthermore, since it has been described as a species of easy handling and acclimatization to laboratory conditions, it has been used as a model organism in toxicity bioassays (Vera-Candioti et al., 2014; Bonifacio et al., 2016, 2020). Although, in recent years, there has been a notable increase in knowledge about the biology of this species (Zambrano et al., 2018; Rautenberg et al., 2022), the study of its growth patterns has not yet been addressed, and the only morphometric index used to assess the health status of individuals has been Fulton's condition factor (Menéndez-Helman et al., 2012; Ossana et al., 2019; Bonifacio et al., 2020; Young et al., 2020).

The main goal of our study was to determine the length–weight relationships of *C. decemmaculatus*, as well as the general health condition of fish through the application/estimation of three different condition indices in order to determine the best index that shows the growth pattern of this species. Our results will allow us to obtain appropriate tools for field and laboratory assessments using *C. decemmaculatus* as a fish bioindicator for ecotoxicological studies.

MATERIALS AND METHODS

Adult females of *C. decemmaculatus* were collected from the Yuspe River (the Suquía River tributary is a medium-size hydrological system located in the semiarid region of the central area of Argentina, in Córdoba Province) around two to four times per year, from 2012 to 2019. This region is characterized by a wet season from October to March (with a historical record of an average monthly rainfall of 100–120 mm), and a dry season from April to September, with an average rainfall of 10 mm (Pasquini et al., 2012). The site of sampling (64°32′ W, 31°17′ S) was selected as a reference site for fish capture due to the high quality of its waters determined in different studies (Hued and Bistoni, 2005; Zambrano et al., 2018; Rautenberg et al., 2022). Downstream the river channel crosses Córdoba city, the most populated urban center of the Province (1.5 million inhabitants according to Instituto Nacional de Estadisticas y Censos, 2010: https://www.indec.gob.ar/indec/web/Nivel4-Censo-Provincia-999-999-14-000-2010).

Fish were caught using hand fishing nets with a 2 mm mesh and then transported alive to the laboratory, where they were sacrificed by an overdose of tricaine methanesulfonate (MS-222, Sigma-Aldrich). All procedures comply with the Guide for Care and Use of Laboratory Animals (National Institutes of Health, 2011). All individuals were weighed and measured to explore the LWR and evaluate their general condition.

In fish, weight (W) is exponentially related to its length (L), according to the potential model: $W = aL^b$, where a is the y-intercept and b is the logarithmically transformed slope of the relation (Le Cren, 1951; Froese, 2006). Based on the slope b of this relationship, it can be determined if the growth of an individual is isometric (b = 3, all dimensions of the fish grow at the same rate), negative allometric (b < 3, the increase in weight is less than that predicted by the increase in length, being more elongated as it grows) or positive allometric (b > 3, the fish increases its weight more than expected based on the increase in length, that is, it becomes more rounded as it grows) (Froese et al., 2011). According to this, LWR was estimated through the following equations: $W = a \times SL^b$ and $\log W = \log a + b\log SL$, were W is body weight (g) and SL is standard length (cm). To ensure the significant difference in fish growth from isometric growth, the b value was calculated using a t-test at 0.05 significance.

Different studies have shown that high fish condition indices values indicate a good general fish health, which reflects a high food availability and/or environmental quality. In this sense we applied the following indices: first, the Fulton condition factor was calculated (Ricker, 1975), which relates the weight and length of the individuals through the following equation: $K = W/SL^3 \times 100000$, and assumes isometric growth pattern of fish. It is also possible that in some species or populations, the isometry assumption is not fulfilled (Le Cren, 1951), for which the Le Cren relative condition index was also calculated. It relates the accurate weight with an average weight (W') calculated for the population: $K_n = W/W'$.

Although K_n does not depend on the isometry assumption, it assumes that the growth slope is the same between samples. Rennie and Verdon (2008) pointed out that slopes of length–weight regressions could differ among fish populations, due to variation in ecological niches between populations rather than energetic status specifically. Therefore the K_n estimation is appropriate to describe single populations only and not useful to compare between populations. Third, the residuals of LWR were calculated, where

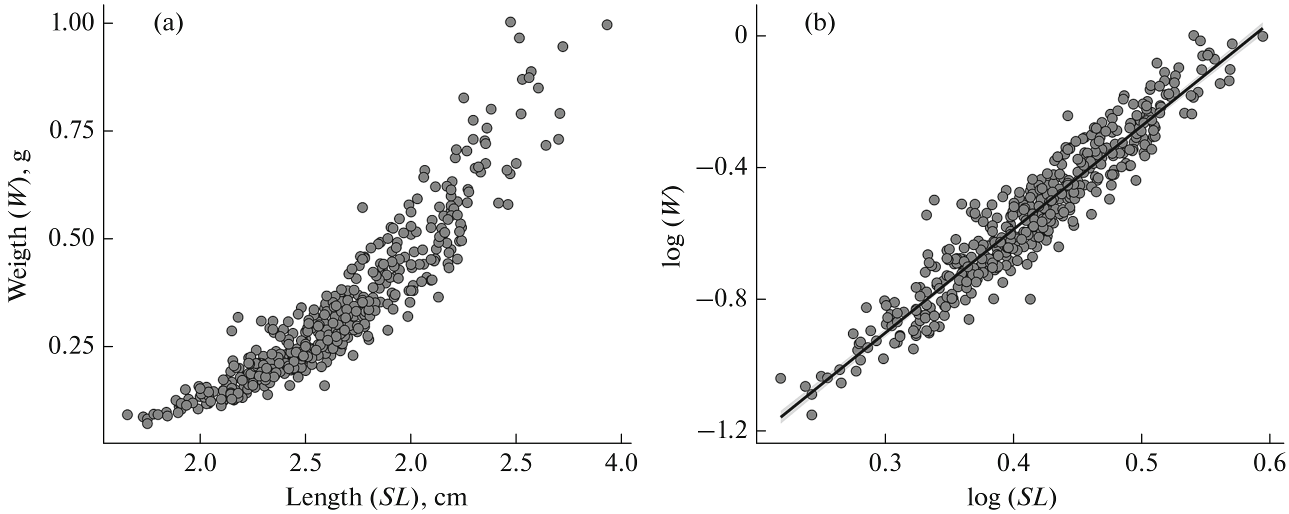

Fig. 1. Length–weight data for adult females of *Cnesterodon decemmaculatus* (a: $W = 0.16 \times SL^{3.14}$, $n = 472$, $R^2 = 0.9$) and double-logarithmic plot of the data (b: $\log(W) = \log(-1.85) + 3.14\log(SL)$).

the residual of the i_{th} fish (e_i) is the difference between the logarithm of the observed weight and the logarithm of the predicted weight that arises from the substitution of the logarithm of the observed length for the fish in the logarithm length–weight regression (Ogle, 2018), as follows: $e_i = \log(W_i) - (\log(a) + b\log(SL_i))$. This index has the advantage of not having any of the previous restrictions, so it is widely applicable (Sutton et al., 2000).

Furthermore, to evaluate whether the condition indices presented biases related to length, a linear regression was performed between each index and the *SL* (Rennie and Verdon, 2008). Each index was estimated monthly from 2012 to 2019 to determine the mean annual variation. All the analyses were performed using R v4.2.1 software (R Core Team, 2022).

RESULTS

A total of 472 adult females of *C. decemmaculatus* were examined to determine LWR and to estimate and compare the condition factor results. The length of the individuals ranged from 1.66 to 3.93 cm, with an average of 2.63 ± 0.40 cm, whereas weight varied from 0.07 to 1.0 g, with an average of 0.33 ± 0.17 g. Fish LWR was described through the equation: $W = 0.16 \times SL^{3.14}$ (Fig. 1). The exponential value *b* is significantly greater than 3 (*p*-value = 0.003, 95% confidence interval (CI) = 3.05–3.24); therefore, *C. decemmaculatus* females have positive allometric growth.

Results of the three condition indices calculated *K*, K_n, and residuals of LWR are shown in Table 1. The mean *K* values varied between 1.50 and 1.89 with an average of 1.66 ± 0.28. K_n presented mean annual values between 0.92 and 1.14, with an average of 1.01 ± 0.17. It was observed for the residuals of LWR, mean annual values ranging from –0.04 to 0.05 and an average value of $-1.2e - 18 \pm 0.07$.

When analyzing the values obtained for the three indices for each sampling year, it was observed that the lowest and highest values correspond to 2016 and 2014, respectively. On the other hand, throughout the year, somatic indices showed similar variation patterns (Fig. 2), increasing the average general condition of *C. decemmaculatus* females from October to February and then decreasing between March and September.

Finally, the linear regression between *K* and *SL* was significantly positive ($p = 0.004$), while K_n and residuals of LWR were not significantly related to fish length ($p = 0.97$ and 0.86, respectively).

DISCUSSION

The present study highlights the importance of having information from the LWR and the condition indices that best reveal the general fish health. These biological parameters are useful tools to be applied in field studies that attempt to determine both the natural variation of populations and changes related to environmental quality where the species inhabits.

Through the analysis of the LWR, we registered that *C. decemmaculatus* females present a positive allometric growth ($b = 3.14$). This result is strengthened by fish samplings carried out during eight consecutive years (2012–2019), and supported by the results obtained by Teixeira-de Mello et al. (2009), who also reported a positive allometric growth but based on a single year of sampling for females ($b = 3.29$) of *C. decemmaculatus* in Santa Lucía River (Uruguay). Our work was carried out at a site located on a mountain stream with *quasi* pristine conditions whereas Teixeira-de Mello et al. (2009) made their study on a

Table 1. Somatic parameters for adult females of *Cnesterodon decemmaculatus* for each year of study

Year	n	Standard length, cm		Wet weight, g		K	K_n	Residuals of LWR
		mean ± *SD*	min–max	mean ± *SD*	min–max	mean ± *SD*		
2012	16	2.98 ± 0.22	2.58–3.33	0.44 ± 0.12	0.25–0.66	1.63 ± 0.19	0.98 ± 0.12	–0.0100 ± 0.05
2013	45	2.52 ± 0.46	1.84–3.93	0.28 ± 0.17	0.10–0.99	1.62 ± 0.33	0.99 ± 0.21	–0.0100 ± 0.08
2014	28	2.85 ± 0.30	2.30–3.38	0.46 ± 0.19	0.18–0.83	1.89 ± 0.31	1.14 ± 0.18	0.0500 ± 0.07
2015	70	2.76 ± 0.46	2.03–3.72	0.39 ± 0.23	0.12–1.00	1.67 ± 0.28	1.01 ± 0.17	0.0006 ± 0.07
2016	35	2.62 ± 0.22	2.26–3.11	0.27 ± 0.07	0.20–0.47	1.50 ± 0.17	0.92 ± 0.11	–0.0400 ± 0.05
2017	104	2.72 ± 0.33	1.91–3.70	0.36 ± 0.12	0.10–0.73	1.77 ± 0.28	1.08 ± 0.18	0.0300 ± 0.07
2018	154	2.51 ± 0.40	1.91–3.70	0.27 ± 0.16	0.08–0.89	1.59 ± 0.23	0.98 ± 0.14	–0.0100 ± 0.06
2019	20	2.43 ± 0.40	1.75–3.06	0.24 ± 0.12	0.07–0.44	1.53 ± 0.21	0.95 ± 0.13	–0.0300 ± 0.07
All	472	2.63 ± 0.40	1.66–3.93	0.33 ± 0.17	0.07–1.00	1.66 ± 0.27	1.01 ± 0.17	–1.2e – 18 ± 0.07

n, the number of specimens; *SD*, standard deviation; *K*, Fulton's condition factor; K_n, relative condition factor; LWR, length–weight relation.

plain river system with a history of significant pollution and eutrophication (Teixeira-de Mello et al., 2012; Alvareda et al., 2020).

The differences between *b* values could be due to the fish health status. Individuals from polluted sites could present liver hypertrophy due to its detoxifying activity increase at polluted sites and consequently increasing its total weight. Also food availability varies between rivers and streams that run across plains, where conditions are more stable and benign, and those located in mountain regions where the environmental conditions are more unstable and stressful due to the stronger summer floods because of the high steep slopes and water velocities. These main differences could cause less food availability which is reflected in a slightly lower *b* value of *C. decemmaculatus* females from the studied populations at Yuspe River compared with individuals from Santa Lucia basin.

In female fish, fecundity is directly related to body size, as has been indicated by Jonsson et al. (1997). For instance, in the viviparous poeciliid fish, *Phalloceros caudimaculatus*, a positive correlation between female

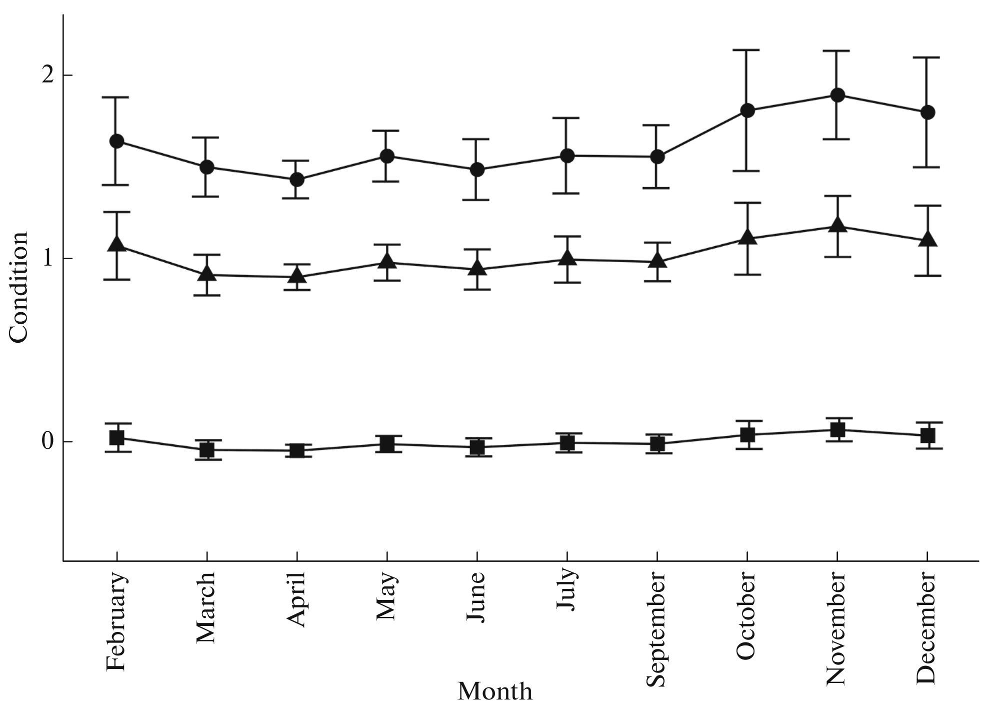

Fig. 2. Mean values of Fulton's condition factor *K* (●), relative condition factor K_n (▲), and residuals of the length–weight relationship (■) for females of *Cnesterodon decemmaculatus* in each month, considering all years.

size and offspring number has been recorded (Machado et al., 2002) supporting the idea that a greater size of females implies a bigger body cavity to accommodate more oocytes and, consequently, a greater number of embryos in viviparous fishes. This pattern agrees with the positive allometric growth we registered for *C. decemmaculatus* and has also been recorded for other poeciliids such as *Gambusia holbrooki*, *Poecilia reticulata* and *P. vivipara* (Montag et al., 2011; Franco et al., 2014).

Condition indices based on the LWR could indicate the general health condition or reproductive status. Similar variation patterns were registered for all the condition indices where the highest and the lowest values were registered in 2014 and 2016, respectively. According to what is mentioned in the literature as normal index values that indicate a fish optimal health condition, all the averages estimated for each year are within a range of normal variation expected for the population, including the highest and the lowest values. However, the unstable conditions due to periods of drought and floods that characterize these mountain rivers could be the reason for the little differences observed among years. This river dynamic could provoke differences in food availability in the environment (Froese, 2006; Ogle, 2018).

The positive allometric growth we registered in *C. decemmaculatus* females (Fig. 1) reveals that Fulton condition factor K was a function of the SL (p = 0.004). Since K becomes a function of fish length when the assumption of isometric growth is violated (Sutton et al., 2000) changes in the general fish health condition within or between natural populations could be a simple artifact of the average size variation of individuals in the populations (Rennie and Verdon, 2008). Most of the studies that relate the variation patterns of condition indices use the Fulton's condition factor without previously determining if the population presents an isometric growth. Thus, the estimations of the general fish health condition of these individuals are probably length-biased (Muzzalifah et al., 2015; Stavrescu-Bedivan et al., 2017; Famoofo and Abdul, 2020).

On the other hand, the relative condition index K_n and the residuals of LWR were not significantly related to fish length; therefore, they would be more appropriate to use to assess the general health condition of *C. decemmaculatus*. However, a disadvantage of K_n is that to estimate its value, the length–weight equations of the different populations of the same fish species are needed, making comparisons among different geographic regions or water bodies difficult (Lloret et al., 2013). In this sense, since the analysis of the residuals of LWR is free from these assumptions, it becomes an excellent index to compare the general fish condition among different populations (Sutton et al., 2000).

Several factors could affect the condition of individuals, such as seasons, food availability, population density, and environmental and habitat conditions, among others (Olurin and Aderibigbe, 2006), leading to annual and seasonal variations as we observed through the somatic indices calculated in our work. In particular, it can be seen that the estimated values of *C. decemmaculatus* population increased between October and February, which coincides with the reproductive activities of the species (Lorier and Berois, 1995) during the more benign environmental conditions in the wet season (spring and summer). As a consequence, individuals gain weight because of gonadal maturation and embryonic growth. This pattern has been observed in other females of the Poeciliidae family, showing increased gonad size, greater reproductive investment, and high fecundity in the wet season (Vargas and de Sostoa, 1996; Rautenberg et al., 2015; Huang et al., 2019).

In summary, our results will allow us to have suitable tools to study the biology of *C. decemmaculatus* and determine how growth and general condition of this neotropical native fish species could change with environmental natural variations. Future studies should contemplate comparisons of growth patterns and fish health conditions among different water bodies across the Sudamerican distribution of *C. decemmaculatus* considering also the evaluation of these parameters in males. Knowing the natural variations of the studied parameters will also allow their use as indicators of the environmental quality alterations. Furthermore, we suggest that before applying condition indices, it is necessary to establish the LWR of the species under study to properly decide which index has to be used and thus avoid reaching erroneous conclusions. Finally, we also emphasize their use not only at field work but also in laboratory studies that mainly include long-term trials.

CONCLUSIONS

When studies are carried out to determine the environmental/pollution impact on fish, the most used index is the condition factor. This index assumes an isometric growth of individuals, while actually this is not necessarily the case. The present study highlights the importance of having information about the species-specific LWR and the selection of a condition indices that best reveal the general fish health, to be applied in field studies that attempt to determine either the natural variation or population changes related to environmental quality where the species inhabits. In this sense, we suggest using the residuals of the LWR to estimate the *C. decemmaculatus* condition. Knowing the variation pattern of the parameters used, deviations from it could be estimated in future works for other sections of the basin that are severely impacted by pollution.

FUNDING

This work was supported by grants from the National Research Council (CONICET, PIP 112-201101-01084) and the Secretariat of Science and Technology of the National University of Córdoba (SECyT, 2014–2015.Res no: 203/14).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors state no conflict of interest with respect to the research, authorship, and/or publication of this article.

Statement on the welfare of humans or animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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