# Water quality affects the Condition of Barbus sclateri Günther, 1868 (Pisces, Cyprinidae) in semi-arid reservoirs from the Iberian Peninsula 

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#### Abstract

Resumen La calidad del agua afecta a la condición de Barbus sclateri Günther, 1868 (Pisces, Cyprinidae) en embalses de una zona semiárida de la Península lbérica.

El objetivo del presente trabajo fue analizar la relación entre la condición de los individuos y las variables ambientales en poblaciones de Barbus sclateri de embalses localizados en una zona semiárida del sureste de la Península Ibérica. Estos ecosistemas artificiales están caracterizados por una alta salinidad de sus aguas. Las relaciones Peso-Longitud de los individuos fueron utilizadas para la determinación de diferencias en la condición entre 6 poblaciones de Barbus sclateri de embalses con diferentes parámetros ambientales. Fueron obtenidas diferencias significativas en la condición entre las poblaciones estudiadas (ANCOVA, p<0,05). La condición de las poblaciones de Barbus sclateri fue dependiente de variables ecológicas relacionadas principalmente con la calidad de las aguas y, consecuentemente, con el sustrato y usos predominantes de la Cuenca Hidrográfica. La conductividad del agua fue la variable que presentó mayor efecto significativo sobre la condición de los peces. En conclusión, la salinización y la elevada conductividad del agua de los embalses podrían presentar un efecto negativo sobre la condición de los peces. Estos efectos deberían ser considerados cuando dichas poblaciones estén sujetas a la gestión para la pesca deportiva, o inmersas en programas de recuperación o cualquier otro plan de manejo.


Palabras clave: Barbus sclateri, Endemismo, Condición, Análisis ambiental, Hábitat.


#### Abstract

The objetive of this study was to investigate the relationships between fish condition and environmental variables in populations of Sclater's barbel (Barbus sclateri) from semi-arid reservoirs in the south-eastern Iberian Peninsula. These artificial ecosystems are characterised by waters of greater salinity. Weight-length relationship was used to test differences in fish condition between 6 populations of Sclater's barbel from reservoirs with different environmental parameters. Significant differences in fish condition among populations studied (ANCOVA, p < 0.05 ) were found. The condition of Sclater's barbel was dependent on ecological variables related mainly to water quality and, consequently, with the substrate and management of the basin. Water conductivity was the variable which affected in the most significant way the fish


condition. In conclusion, salinization and high water conductivity of reservoirs could have a negative effect on fish condition. These effects should be considered when such populations are subjected to sports fishing regulations, recovery programmes or any other management programme.

Key words: Barbus sclateri, Endemic fish, Body condition, Environmental assessment, Habitat differences.

## Introduction

The semi-arid regions of the Mediterranean basin have specific climatic conditions which have turned them into zones where irrigation-dependent agriculture is widely developed. The term «semi-arid» is applied to regions where the balance of water is negative, creating an environmental stress which, unlike those occurring in arid lands or deserts, is neither permanent nor predictible (Vidal-Abarca et al. 1992). Therefore and associated to such development, surface hydrology is highly modified by the engineering measures (Esteve et al. 1995). The high number of reservoirs in the Spanish Mediterranean area (Armengol \& García 1998) and concretely in the Murcian region (Vidal-Abarca et al. 1995) are clear examples of this.

Although reservoirs are considered as one of the main negative factors affecting Spanish fish fauna (Elvira 1996, 1998), their direct impact of those engineering works has not been analysed sufficiently (García de Jalón et al. 1992). Moreover, an ecological study of the ichthyofauna confined in the reservoirs has a interesting scientific value because it constitutes a natural reference for ascertaining the adaptations developed by fish species, once they colonize these artificial ecosystems (Granado-Lorencio et al. 1998).

Analysis of fish condition has become standard practice in the management of fish populations, as a measure of both individual and cohort (e.g., age or size group) fitness (Fisher et al. 1996). Measures of condition are generally intended to act an indicator of tissue energy reserves and may characterize components of the environment in which the fish exists (e.g., habitat, prey availability, competition). In this way, measures or indices of fish condition are of value to fishery managers who must assess population status, the impact of management actions and anthropogenic influences on the resource they are managing (Brown \& Austin 1996).

The present study analyses the relationship between several environmental variables, mainly related to water quality, and fish condition deduced from
weight-length relationship of six populations of Barbus sclateri Günther, 1868, confined in reservoirs from the Segura river basin, the most semi-arid zone of the Iberian Peninsula (Vidal-Abarca et al. 1987) and, probably, of Europe (Geiger 1973).

There is a widespread need to increase knowledge of the life history characteristics of fishes under threat as a necessary tool for management and conservation programmes (Wootton et al. 2000). In addition, a knowledge of which environmental conditions affect an endemic stream cyprinid in reservoirs, which represent new ecosystems for it, may be important because of the high rate of destruction of their natural habitats (Crivelli \& Maitland 1995).

## Material and Methods

Sclater's barbel is one of the most abundant endemic fish of the mid-south of the Iberian Peninsula, which includes the Guadiana, Guadalquivir and Segura river basins (Elvira 1995, Doadrio 2001). This benthic species reproduces between May and July (Herrera \& Fernández-Delgado 1992, Rodríguez-Ruiz \& Gra-nado-Lorencio 1992, Torralva et al. 1997, Soriguer et al. 2000). The populations studied inhabited six reservoirs located on Sector III (Mas, 1986) of the Segura river basin, which covers a drainage area of about $14,432 \mathrm{Km}^{2}$ in the most semi-arid region of the Iberian Peninsula (Fig. 1).

The sampling reservoirs are included in Group IV of the ecological classification made by Margalef (1976) in the Iberian Peninsula, which are characterized by waters of greater salinity, mainly due to high concentrations of chlorides.

A total of 751 Sclater's barbels from 6 sampling reservoirs were analysed (Fig. 1). Individuals were captured by passive fishing methods in November 1998. Limiting the sampling to this period prevented the capture of prespawning and spawning fish in this area (Torralva et al. 1997), and ensured that any variation in body condition was not affected by gonad development (Herrera \& Fernández-Delgado 1994, Encina \& Granado-Lorencio 1997a, 1997b).

| Sampling <br> reservoir | Volume <br> ( $\mathrm{Hm}^{3}$ ) | Sample <br> size | Water conductivity ( $\mu \mathrm{scm} \mathrm{cm}^{-1}$ ) |  | Water temperature <br> ( $\left.{ }^{\circ} \mathrm{C}\right)$ | pH | Water turbidity (cm) | $\mathrm{CPUE}_{\text {total }}$ | $\mathrm{CPUE}_{\text {R.sclese }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Argos | 10 | 27 | 1,650-1,750 | 8.20-9.22 | 17.2-17.4 | 7.22-7.28 | 31-44 | 1.25 | 0.29 |
| Alfonso XIII | 12 | 176 | 3,550-3,650 | 9.62-11.05 | 17.1-17.2 | 7.49-7.59 | 48-56 | 1.64 | 1.43 |
| Ojos | 1 | 205 | 400-600 | 7.72-9.38 | 16.5-16.7 | 8.66-8.76 | 28-42 | 1.86 | 1.81 |
| Mayes | 2 | 49 | 600-800 | 10.06-10.78 | 17.5-18.0 | 8.62-8.85 | 103-125 | 0.38 | 0.35 |
| Pliego | 10 | 173 | 1,800-2,000 | 6.96-7.01 | 17.0-17.1 | 8.00-8.10 | 60-61 | 1.68 | 1.22 |
| La Cierva | 7 | 121 | 550-650 | 6.54-8.38 | 16.1-16.2 | 8.00-8.20 | 93-101 | 1.24 | 1.21 |

Table 1. Environmental differences between sampling reservoirs. The mean value of environmental variables in each reservoir is presented. Water turbidity (Secchi transparence in cm ); CPUE of the whole of fish community ( $\mathrm{CPUE}_{\text {тотад }}$ ) and CPUE of B. sclateri (CPUE B.scla )


Figure 1. Sampling reservoirs from the Segura river basin, south-east of the Iberian Peninsula. Argos: 1; Alfonso XIII: 2; Ojos: 3; Mayes: 4; Pliego: 5; and La Cierva: 6.

Fish were caught with different types of sampling gear: simple monofilament nylon trawl-nets ( 12.5 m long, 1.80 m high) in the surface running transversely from the reservoir edge and in the reservoir centre, and pike-nets (prawn nets, 2 m length, 0.1 m entrance diameter) and minnow-traps (Harrison et al. 1986) ( 0.5 m length, 0.03 m entrance diameter) in the upwater shallows. All of these passive fishing methods were set for roughly 24 h . Further details of these catching methods may be seen in Miñano et al. (2002).

After capture, fish were anaesthetized with benzocaine and standard length ( $\pm 1 \mathrm{~mm}$ ) and total weigth $( \pm 0.1 \mathrm{~g})$ were obtained in situ for each individual. Fish smaller than $75 \mathrm{~mm}[<2+$ age clase, scale analysis in García de Jalón et al. (1999)] were excluded from the analysis to avoid possible differences in body shape between juveniles and adults (Murphy et al. 1990) and to minimize measurement errors associated with weighing small fish in the field (Vila-Gispert \& Moreno-Amich 2001). Fish killed as a result of being trapped in the nets for a long time (about $5 \%$ of captures, depending on the reservoir) were also excluded.

Each reservoir was characterised by eight environmental variables related to the morphology of the reservoir, water quality and possible intra or interspecific interactions: reservoir volume, conductivity, oxygen concentration, water temperature, pH , water turbidity, and relative fish abundance (Table 1). Water quality variables were obtained at several surface points ( 1 m depth) in each reservoir. Water turbidity was measured by Secchi transparence (cm) and relative abundance of species were analysed by catches per unit of effort (CPUE: fish number / net area $\left(\mathrm{m}^{3}\right)$ / 24 h ). More detailed information about the sampling reservoirs are given in García de Jalón et al. (1999) and Miñano et al. (2002).

The statistical analysis used to compare fish condition followed that used in two studies of Barbus meridionalis Risso, 1826 (Vila-Gispert et al. 2000, Vila-Gispert \& Moreno-Amich 2001) and proposed by García-Berthou \& Moreno-Amich (1993), which is based on the application of univariate analysis of covariance (ANCOVA) using total weight as the dependent variable and standard length as the covariate. The relationship between total weight and length was clearly non-linear, but was linear after log-transformation (ln). We therefore used the $\ln$-transformation of total weight as dependent variable and ln-transformation of standard length as the covariate. The homogeneity of the regression coefficients (slopes) of dependent-covariate relationships was tested with an ANCOVA design that analysed the pooled cova-
riate-by-factor interaction. If the covariate-by-factor interaction (homogeneity of slopes) was not significant ( $\mathrm{p}>0.05$ ), standard ANCOVA was applied to test significant differences in parameter «a» (the yintercept) between populations.

A stepwise multiple regression analysis was performed to determine the amount of variation in the $y$-intercept associated with environmental variables. Bivariate relationships between environmental variables were also analysed using Pearson's correlations for quantitative variables and Spearman's correlations for ordinal variables.

Statistical analyses were preformed with SPSS® software package and a significant level of 0.05 was accepted.

## Results

Sclater's barbel was the dominant fish species (80$100 \%$ ) in the majority of sampling reservoirs, coexisting with Chondrostoma polylepis Steindachner, Cyprinus carpio L., 1758, Carassius auratus (L., 1758), Micropterus salmoides (Lacépède, 1802) and Gambusia holbrooki (Agassiz, 1859), depending on the reservoir. Sclater's barbel has been shown to be dominant in the communities of the Segura river ba$\sin$ (García de Jalón et al. 1999, Miñano et al. 2002).

Parameters of the weight-length relationship in each population studied are presented in Table 2 and the results of the ANCOVA are shown in Table 3.

In the analysis involving all the sampling reservoirs (Analysis 1, Table 3), there were no significant homogeneity ( $\mathrm{p}=0.010$ ) among sampling sites on slopes of the relationship between total weight and standard length. However, because Mayes reservoir showed substantial daily variation in its water level due to the specific regulation it undergoes and which could affect the weight-length relationship of fish differently (Table 2), this reservoir was excluded from the analysis. In fact, in the analysis which excluded Mayes reservoir (Analysis 2, Table 3), there was a significant homogeneity of slopes ( $p=0.397$ ) among sampling reservoirs, while the y -intercept varied significantly ( $\mathrm{p}<0.0005$ ) among reservoirs.

The highest fish condition values were obtained in Ojos and La Cierva reservoirs, while Alfonso XIII reservoir showed the lowest value ( y -intercept higher and lower respectively, Table 2). As a result, sampling reservoirs (excluding Mayes) could be differentiated according to differences in parameter «a» (y-intercept) of the weight-length relationship.

Correlations between parameter «a» of the weig-ht-length relationship and environmental variables,

| Sampling <br> reservoirs | $\mathbf{n}$ | $\mathbf{b}$ (slope) | $\mathbf{a}$ (the y-intercept) | $\mathbf{r}$ | Mean $\pm \mathbf{C L}$ <br> of standard length (mm) |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Argos | 27 | 2.95 | -11.07 | 0.998 | $197.9 \pm 25.0$ |
| Alfonso XIII | 176 | 2.99 | -11.19 | 0.993 | $266.5 \pm 10.3$ |
| Ojos | 205 | 2.94 | -10.82 | 0.991 | $281.1 \pm 7.1$ |
| Mayes | 49 | 2.76 | -9.94 | 0.987 | $314.6 \pm 15.5$ |
| Pliego | 173 | 2.96 | -11.01 | 0.987 | $234.1 \pm 4.5$ |
| La Cierva | 121 | 2.93 | -10.92 | 0.979 | $249.4 \pm 4.5$ |

Table 2. Regression ( $\mathrm{a}, \mathrm{b}$ ) and correlation coefficients ( r ) of the ln-transformed weight-length relationship in each population studied.

| Source of variation | F | df | $\mathbf{p}$ |
| :--- | :---: | :---: | :---: |
| Analyse 1. Total sampling reservoirs |  |  |  |
| Preliminary design (test for interaction) | 18969.87 | 1,926 | $<0.0005$ |
| Length | 3.02 | 5,926 | 0.010 |
| Sampling site | 3.04 | 5,926 | 0.010 |
| Length $\times$ Sampling site |  |  |  |
| Analyse 2. Without Mayes reservoir | 22297.30 | 1.02 | 4,701 |
| Preliminary design (test for interaction) | 0.77 | 4,701 | 0.0005 |
| Length |  |  | 0.397 |
| Sampling site | 41928.45 | 1,701 | $<0.0005$ |
| Length $\times$ Sampling site | 100.72 | 4,701 | $<0.0005$ |
|  |  |  |  |
| Final design (no interaction) |  |  |  |
| Length |  |  |  |
| Sampling site |  |  |  |

Table 3. ANCOVA analysis of the weight-length relationship in Barbus sclateri: F-statistics, degrees of freedom (df) and palues. All variables (dependent and covariate) were $\ln$-transformed. Standard length is the covariate.

| Environmental <br> variables | $\mathbf{a}$ <br> (y-intercept) | Volume | Water <br> conductivity | Oxygen <br> concentration | Water <br> temperature | $\mathbf{p H}$ | Water <br> turbidity | CPUE $_{\text {B.sclateri }}$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Volume | $-0.93^{*}$ |  |  |  |  |  |  |  |
| Water conductivity | $-0.94^{*}$ | $0.81^{*}$ |  |  |  |  |  |  |
| Oxygen concentration | 0.57 | 0.24 | 0.65 |  |  |  |  |  |
| Water temperature | 0.74 | 0.64 | 0.73 | 0.46 |  |  |  |  |
| pH | $0.86^{*}$ | $-0.86^{*}$ | -0.65 | -0.40 | -0.71 |  |  |  |
| Water turbidity $^{0.09}$ | 0.15 | -0.21 | -0.47 | -0.65 | 0.08 |  |  |  |
| CPUE $_{\text {B.sclateri }}$ | 0.40 | -0.53 | -0.11 | 0.10 | -0.50 | 0.80 | 0.05 |  |
| CPUE $_{\text {ToтaL }}$ | 0.21 | -0.39 | -0.12 | 0.16 | 0.06 | 0.60 | -0.50 | 0.79 |

[^0]| Environmental variables | Partial correlation | Regression <br> equations | $\begin{gathered} \text { Adjusted } \\ \mathbf{r}^{2} \end{gathered}$ | F | df | $\begin{gathered} \mathrm{p} \\ \text { values } \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Model 1 <br> Water conductivity CPUE $_{\text {TOTAL }}$ | 0.980 | $\begin{aligned} & a=-10.821-0.00108 \text { (Water conductivity) } \\ & a=-11.076-0.00113 \text { (Water conductivity) }+ \\ & 0.171\left(\text { CPUE }_{\text {TOTAL }}\right) \end{aligned}$ | $\begin{aligned} & 0.889 \\ & 0.996 \end{aligned}$ | $\begin{array}{r} 24.073 \\ 221.885 \end{array}$ | $\begin{aligned} & (1,3) \\ & (2,2) \end{aligned}$ | $\begin{aligned} & 0.016 \\ & 0.004 \end{aligned}$ |
| Model 2 <br> (Without CPUE total ) <br> Water conductivity pH <br> Water turibidity | $\begin{aligned} & 0.962 \\ & -1.000 \end{aligned}$ | $\begin{aligned} & \mathrm{a}=-10.821-0.00108 \text { (Water conductivity) } \\ & \mathrm{a}=-11.677-0.00077 \text { (Water conductivity) }+ \\ & 0.108(\mathrm{pH}) \\ & \mathrm{a}=-11.731-0.00080 \text { (Water conductivity) }+ \\ & 0.106(\mathrm{pH})-0.00054 \text { (Water turbidity) } \end{aligned}$ | $\begin{aligned} & 0.889 \\ & 0.992 \\ & 1.000 \end{aligned}$ | $\begin{aligned} & 24.073 \\ & 121.013 \\ & 1398614 \end{aligned}$ | $\begin{aligned} & (1,3) \\ & (2,2) \\ & (3,1) \end{aligned}$ | $\begin{aligned} & 0.016 \\ & 0.008 \\ & 0.001 \end{aligned}$ |

Table 5. Stepwise multiple regression models used to predict the parameter «a» of the weight-length relationships from environmental variables in the reservoirs studied.
and intercorrelations between environmental variables are presented in Table 4. This first approach shows that reservoir volume, water conductivity, and pH present significant correlations with parameter «a».

The first stepwise multiple regression model showed that water conductivity and the CPUE of the whole fish community accounted for most of the variation (99.6\%) in parameter «a» of the weightlength relationship (Table 5). However, a second multiple regression model not involving intra and interspecific interaction variables (CPUEs) indicated that water conductivity, pH and water turbidity are the most important variables in explaining the variation in parameter «a» between sampling sites (Table 5). It should be mentioned that the high values obtained for the parameter $r^{2}$ adjusted are probably due to the relatively few sampling localities. Nevertheless, this does not affect the significant character of the analysis.

## Discussion

A critical factor when interpreting fish condition data in a way which may be both useful and applicable is to use the correct statistical methodologies when collecting and analyzing the data (Pope com.pers.). To investigate inter-population variations in fish condition, analysis of the weight-length relationships provided a good alternative method to the relative weight indices (ratio-related techniques), assuming that the genetically determined slope of the weight-length relationship does not vary between these populations
and that the slope is homogeneous on a local level (Sutton et al. 2000). Relative weight indices do not normally fulfil these underlying assumptions and have been criticized on statistical grounds (De Vlaming et al. 1985, Bolger \& Connolly 1989, Patterson 1992, García-Berthou \& Moreno-Amich 1993, Jakob et al. 1996). In fact, the adjustment of size variation in the data by regression-related techniques has recently been used with ANCOVA approach as a successful method with valid results (Vila-Gispert et al. 2000, Vila-Gispert \& Moreno-Amich 2001).

The results of the present study showed that condition of Sclater's barbel differed among several reservoirs. Differences in parameter «a» of the weig-ht-length relationship were probably caused by differences in environmental conditions of these artificial ecosystems. The fact that the sampling period was short and in November prevented capture of prespawning and spawning fish and thus ensured that differences in parameter «a» were not due to any seasonal variation in the condition of species, an aspect confirmed by several studies in the south of the Iberian Peninsula (Herrera \& Fernández-Delgado 1992, Rodríguez-Ruiz \& Granado-Lorencio 1992, Torralva et al. 1997). In addition, the absence of differences in the slope of the weight-length relationships between populations indicated that condition is independent of the fish body form (Winters \& Wheeler 1994).

An important constraint on a fish's capacity to regulate its allocation of energy to maintenance, activity and feeding is the effect of changes in the abiotic
environment (Wootton 1998). In the present study, the ecological variables that were correlated to fish condition are directly related to water quality (conductivity, pH and water turbidity). More specifically, water conductivity, the most significant variable in the multiple regression models (Table 5), was the water quality parameter that most clearly affected the condition of the Sclater's barbel. Fish condition was higher in reservoirs of low water conductivity (Ojos and La Cierva). On the other hand, Argos, Alfonso XIII and Pliego reservoirs showed lower fish condition values. These reservoirs are receivers in each one of their sub-basins, which are located in an important irrigated agriculture area. Consequently, these water bodies receive agrochemicals and an nutrient inputs from irrigated fields (via run-off), which, added to the saline character of the substrate of the reservoirs, causes high values of water conductivity. High water conductivity could act as a masking factor (sensu Wootton 1998) for fish growth in reservoir of higher values in this parameter. The effect on growth in fish that experience fluctuating salinities and conductivities between habitats has been reported, and it has been attributed to that the energy cost of osmotic ionic regulation will mean that less energy can be allocated to growth (Brett 1979). Salinization and high conductivity of surface waters resulting from the reintroduction of drainage and run-off water from irrigated agricultural fields is a common problem in semiarid zones (Petr \& Mitrofanov 1995), and, due to the general saline character of the Segura river basin (Vidal-Abarca et al. 1995), probably an important problem which has not been studied in depth in this river basin.

Our first model (Table 5), also showed a positive relationship between fish condition and the CPUE of the whole fish community. Because a high relative abundance (CPUE) is interpreted as increasing intra or interspecific competition and social stress, a relationship contrary to the one obtained it might be expected (Pottinger \& Pickering 1992, Sloman et al. 2001). However, because this relationship shows great complexity as regards ecological interactions (e.g. a non-linear realtionship between these variables), more investigation is needed before any conclusions can be reached.

Previous studies in running waters with the same species (Oliva-Paterna et al. 2003) and with Barbus meridionalis (Vila-Gispert et al. 2000, Vila-Gispert \& Moreno-Amich 2001) showed that ecological variables related to water flow and consequently to the physical structure of the streams were mainly responsible for the great variation in fish condition between populations. In the present study, reservoir volume
was correlated with fish condition but did not enter in our regression models, probably, because of the high correlation between reservoir volume and water conductivity (Table 4).

River-reservoir connectivity could affect the results obtained in the present study due to reproductive migration that Sclater's barbel shows in the south of Spain (Rodriguez-Ruiz \& Granado-Lorencio 1992). Because of the anthropogenic water regulation for agricultural purposes, the Segura river presents a modified flow dynamic with a continuous water flow, large well-developed pool refugia and lower temporal variations. In contrast, its tributaries show an intermittent flow regime, contain small pool refugia and exhibit substantial temporal variations in their physi-cal-chemical characteristics (Vidal-Abarca et al. 1992). Both Torralva et al. (1997) and Oliva-Paterna et al. (2003) concluded that Sclater's barbel condition was better in streams with a continous water flow. In this respect, our results show that the best fish condition was to be found in the Ojos reservoir, which is the only sampling reservoir located in the Segura river itself, the rest being located in tributaries. However, we need more data about river-reservoir connectivity and fish condition relationships to make any firm conclusions.

It is known that artificial river regulation, among other factors, has a strong influence on fish recruitment (Copp et al. 1991, Jurajda 1995), fish growth (Torralva et al. 1997), fish production (Almodóvar \& Nicola 1999) and on the whole fish community (Meador \& Matthews 1991, Penáz et al. 1995). In this study, as a first approach, it has also been shown that condition of Sclater's barbel populations confined in reservoirs is also affected. In particular, salinization and high water conductivity of reservoirs could have a negative effect on fish condition. These effects should be considered when such populations are subjected to sports fishing regulations, recovery programmes or any other management programme.

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[^0]:    Table 4. Correlation matrix of y-intercept of the weight-length relationships and environmental variables (Pearson's correlation coefficients for sampling sites. $\left(^{*}\right)$ Significant level $p<0.05$.

