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Variability in Population Traits of a Sentinel Iberian Fish in a Highly Modified Mediterranean-Type River

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Abstract: Human pressures on water resources have been suggested as a driver of biological traits that induce changes in native fish populations. This study highlighted the interplay between environmental stress factors, mostly related to flow regulation, and the longitudinal river gradient in biological traits such as the growth, size structure and somatic condition of a sentinel fish, *Luciobarbus sclateri*. We found an increase in size-related metrics and somatic condition at population levels associated with downstream reaches, although fragmentation and habitat alteration, flow regime alteration and the abundance of non-native fish were also significantly involved in their variability. Age-related parameters and growth were only explained by flow regime alterations and the abundance of non-native fish species. The high plasticity observed in *L. sclateri* population traits suggests that this is a key factor in the species adaptability to resist in a strongly altered Mediterranean river basin. However, the interplay of multiple stressors plays an important role in fish population dynamics and could induce complex responses that may be essential for long-term monitoring in sentinel species.

Keywords: sentinel species; longitudinal gradient; human impacts; flow regime alteration; non-native fish; fragmentation; habitat alteration; Mediterranean rivers; Segura River basin



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1. Introduction

Freshwater ecosystems are considered among the most altered as a consequence of the historical pressure of human activities [1,2]. Hydraulic management to take advantage of water resources and the effects of climate change are inducing quantitative and qualitative changes in river systems [3,4]. These changes imply hydro-morphological, chemical and biological alterations which affect the freshwater fauna [2,5]. Hydraulic management is especially intense in Mediterranean regions where water resources are scarce [6]. Rivers in semi-arid regions, such as the Iberian Peninsula, are heavily impacted by the construction of a large number of dams and weirs [7,8].

Mediterranean regions are characterized by marked seasonality and inter-annual variability with severe periods of floods and droughts [9,10]. The native freshwater fauna is adapted to such natural variability and displays great resistance and resilience [11–13]; however, it is considered especially sensitive to human impacts [14,15]. Human pressures are particularly severe in Mediterranean regions and they coincide with high natural variability, causing severe alterations to fluvial ecosystems [8,16,17]. Despite the high adaptability of freshwater fauna, the expected increase in human impacts under future scenarios of global climate change could increase its vulnerability to such pressure, especially in Mediterranean regions [11,18,19].

The flow regime is considered one of the main driving forces of freshwater ecosystems, determining the structure and ecological dynamic of rivers [19,20]. In the Iberian Peninsula, the alteration of the natural flow regime by dam regulation is one of the most important

stress factors that negatively affects the native fish fauna [21–23]. The effects of flow regime alteration have been widely documented in Mediterranean fish populations [24–27]. In addition, flow management infrastructures cause loss of connectivity and habitat alterations related to the fragmentation process (e.g., increase in lentic habitats, changes in water quality), which could facilitate the establishment and spread of non-native fish species, altering the composition of the community [28,29]. However, the effects of these stress factors have been poorly studied in Mediterranean rivers [30–32].

The Segura River basin is located in the southeast of the Iberian Peninsula and is characterized by a marked environmental variability and displays a wide variety of human impacts along its longitudinal gradient [33,34]. Furthermore, this basin shows a well-documented range of impacts on the natural flow regime [35,36] and is considered one of the most regulated Mediterranean river systems [37]. Therefore, the Segura River offers an opportunity to study the effect of multiple human-induced stressors along an intensively altered basin. The fish assemblage in the Segura River basin is characterized by a low number of species where *Luciobarbus sclateri* (Günther, 1868) appears to be dominant [38]. This native species is an endemic potamodromous fish considered a sentinel species in the southern Iberian Peninsula [12], and its biology and ecology have been well documented [39–41], being the unique native fish widely distributed in the Segura River basin [42]. Therefore, *L. sclateri* populations in the Segura River could be considered a useful tool through which to assess the intra-specific variability along a wide longitudinal gradient strongly affected by environmental alterations mainly related to flow regulation. However, few authors have described the effects of multiple stressors on its populations, and nothing is known about its intra-specific variability along longitudinal gradients [43–45].

The main objective of the present study was to assess the variability of *L. sclateri* population traits in relation to different environmental stressors along the longitudinal gradient of a highly regulated river system. Our hypothesis was that *L. sclateri* would exhibit high phenotypic plasticity that enables populations to survive along the longitudinal gradient of a highly impacted river basin. *L. sclateri* has been evaluated as near threatened (NT) on a regional scale due to the intensification of human pressures which caused a decrease in habitat quality (mainly pollution and flow regime alteration) and an increase in the establishment and spread of several non-native fish species [46]. Furthermore, in recent years, a severe decline in *L. sclateri* populations has been documented in the Segura River basin [38,47]. The use of different biological traits provides ecological insights into how populations respond to multiple stress factors, allowing us to understand how species' traits could predispose species to local extinctions [48] and to establish more successful management and recovery programs [8,49].

2. Materials and Methods

2.1. Study Area and Sampling Design

This study was conducted in the Segura River basin, situated in the semi-arid Mediterranean climatic zone in the southeast of the Iberian Peninsula (Figure 1). The river basin drains an area of 18,870 km² and is characterized by a strong climatic and altitudinal gradient, and significant annual and inter-annual natural variation in the flow regime [6]. Multiple human activities—primarily related to agriculture, but also to electricity generation and human supply—have developed in the study area [33,34,37]. Irrigation accounts for 90% of the water demand and is considered the main pressure on the water resources. A total of 33 dams (>10 m height and >1 hm³ of reservoir) and 170 smaller obstacles exist along the longitudinal gradient of the river with a capacity of regulation of approximately 1200 hm³. Since 1979, this basin has received an external water transfer from the Tajo River with an average of 350 hm³year^{−1}, so the storage capacity increased by around 140% of the natural input (871 hm³year^{−1}) [37,50]. Furthermore, local agricultural practices add an artificial source of pollutant discharge (mainly phosphates and nitrates) into the river [51,52]. As a result, the fluvial and riparian habitats of the Segura River basin have been severely altered [33,53], in addition to strong modifications in the natural flow regime [54].

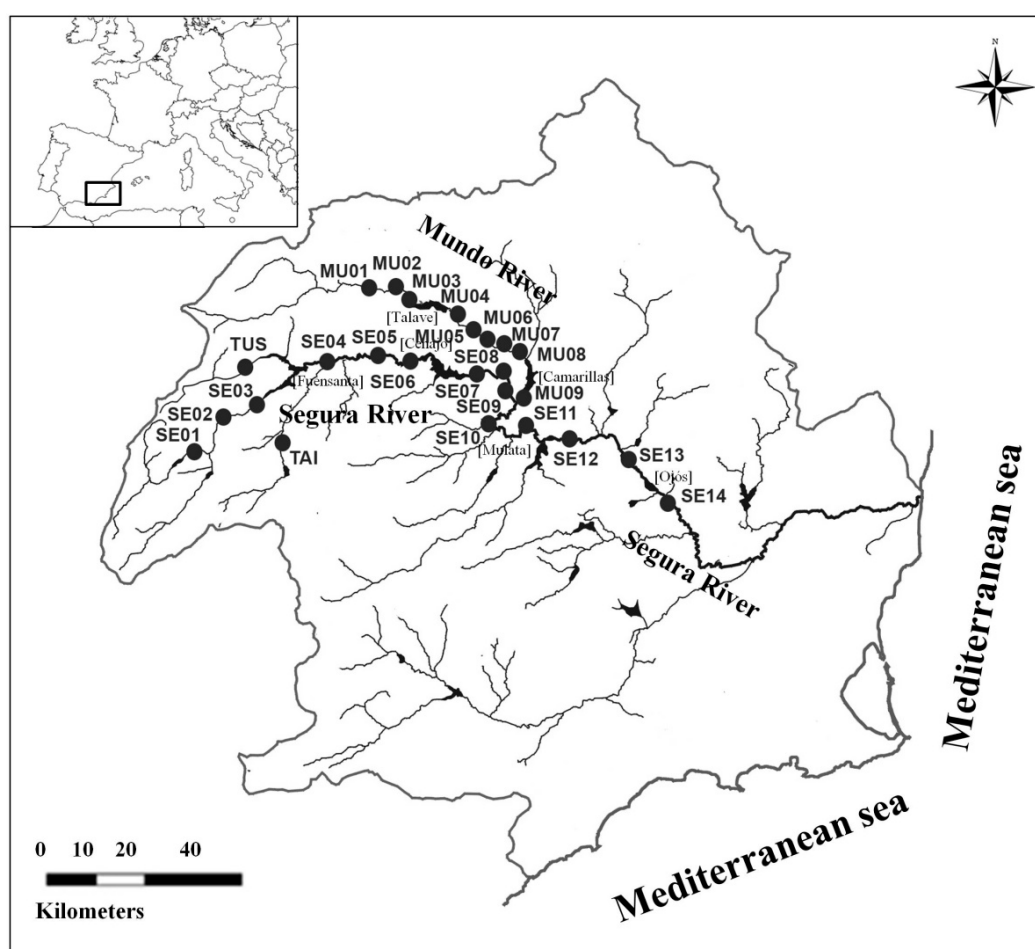


Figure 1. Study area. Location of the Segura River basin in the southeast of the Iberian Peninsula. Sampling sites are marked (●): TAI (Taibilla River), TUS (Tus River), MU (Mundo River) and SE (Segura River). Names of large reservoirs are between parentheses [].

We sampled a total of 25 sites in fluvial reaches distributed along upstream–downstream gradients from the upper Segura main stem (SE) to the middle Segura (195 km), from the upper Mundo River (MU) to the Mundo–Segura confluence (51 km) and in two upper tributaries (Tus and Taibilla) (Figure 1). The range in altitude is 112–809 m.a.s.l. in the study area; the water conductivity ranged from 316 to 1303 $\mu\text{S cm}^{-1}$ and the range of the mean annual water temperature was 13.9–16.9 °C during the study period. The distribution of sampling sites reflects the different hydrological flow regimes present in the study area. Sampling sites placed in areas with the most natural flow regime (e.g., MU01 and SE01; Figure 1) were characterized by a strong seasonal variation, alternating summer droughts and spring/autumn short-time flow peaks (Figure 2a). An impact gradient on the natural flow regime in the study area was described by Amat-Trigo [35] and Amat-Trigo et al. [36]. Impacted areas exhibit a seasonal inversion of the natural flow pattern (high flow levels in spring and summer, low flow levels in autumn and winter) due to the water demand for agricultural practices. Furthermore, impacted areas can be characterized by two extreme flow impacts: reaches downstream of the Cenajo reservoir (sampling sites SE06, SE07 and SE09; Figure 1) showed a high level of contingency and low variability, but also low predictability (Impact 1, Figure 2b), while other reaches (e.g., sampling sites MU09, SE12 and SE13; Figure 1) were characterized by more stable and high levels of base flow throughout the year, high values of temperature and spell peaks, in addition to the inversion in flow seasonality (Impact 2, Figure 2c).

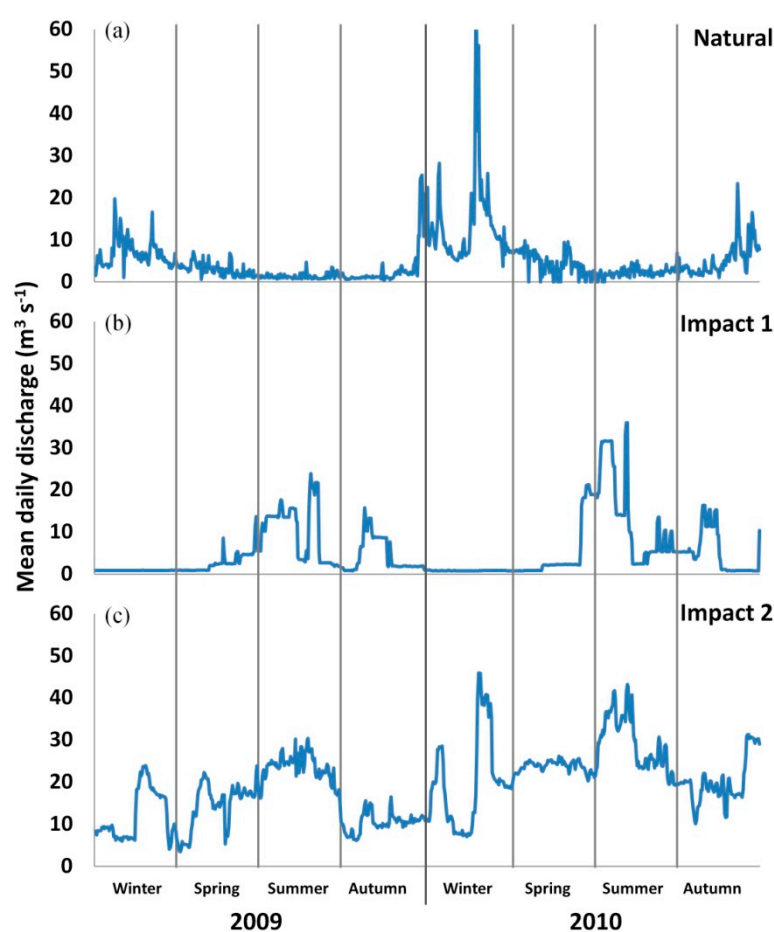


Figure 2. Flow variation (mean daily discharge; $\text{m}^3 \text{s}^{-1}$) at three representative fluvial sectors in the study area. Representative flow regimes were measured at three gauging stations: (a) Natural flow regime at the upper part of the Mundo River, (b) Impact 1 just downstream of the Cenajo reservoir, and (c) Impact 2 downstream of the La Mulata dam (see location in Figure 1). Flow discharge data were obtained from the Segura Hydrographic Confederation.

The fish assemblage in the study area is composed of both native and non-native species [38]. Non-native species are dominant (90% of total species richness) and *L. sclateri* is the only widely distributed native species in the study area. The most abundant species are cyprinids: the native *L. sclateri* and the non-native species *Pseudochondrostoma polylepis* (Steindachner, 1864), *Gobio lozanoi* (Doadrio and Madeira, 2004) and *Alburnus alburnus* (Linnaeus, 1758). The non-natives *Cyprinus carpio* (Linnaeus, 1758) and *Lepomis gibbosus* (Linnaeus, 1758) are locally abundant. Furthermore, the natives *Squalius pyrenaicus* (Günther, 1868) and *Salmo trutta* (Linnaeus, 1758), as well as the non-natives *Gambusia holbrooki* Girard 1859, *Oncomorhynchus mykiss* (Walbaum, 1792), *Micropterus salmoides* (Lacépède, 1802), *Sander lucioperca* (Linnaeus, 1758) and *Esox lucius* (Linnaeus, 1758), are present in the study area.

2.2. Environmental Variables

We described a total of ten environmental variables and gradient descriptors in the study area (Table 1). Water conductivity was measured in situ using a multi-parameter (340i WTW), and ecological status was assessed according to the EU Water Framework Directive, Fluvial Habitat Index (IHF) [55], Riparian Quality Index (RQI) [56] and altitude [57]. The Longitudinal Connectivity Index (ICL) [58] and free reach (length of reach available for free movement) were calculated using databases available online from the official monitoring service of the Segura Hydrographic Confederation (CHS) (<https://www.chsegura.es/chs/cuenca/restauracionderios/obstaculos/visorjs.html>; accessed on 12 September 2018).

Water temperature and daily river discharge data were also obtained from the CHS online databases with data gathered by gauging stations distributed in the study area (<https://www.chsegura.es/chs/cuenca/redesdecontrol/estadisticashidrologicas/>; accessed on 12 September 2018). Mean monthly values of water temperature for 2009 and 2010 were calculated. Mean daily discharge ($\text{m}^3 \text{s}^{-1}$) over a 16-year period (1994–2010) was used to calculate the mean daily base flow (MDBF) as the total base flow component of the hydrograph divided by the number of recording days, and flow variability as the daily range between $Q_{10\%}$ and $Q_{90\%}$ discharge divided by the median value. These two flow metrics (MDBF and flow variability) were calculated using time series analysis (TSA) of the River Analysis Package (RAP version 3.0.7) [59].

Table 1. Environmental variables and gradient descriptors measured or calculated at sampling sites. Altitude (meters above sea level), ecological status (categorized as: 1 = high; 2 = good; 3 = moderate; 4 = poor), Fluvial Habitat Index (IHF) and Riparian Quality Index (RQI) (%), conductivity ($\mu\text{S cm}^{-1}$), free reach (km), Longitudinal Connectivity Index (ICL), water temperature ($^{\circ}\text{C}$), mean daily base flow (MDBF) ($\text{m}^3 \text{s}^{-1}$) and flow variability ($(Q_{10\%} - Q_{90\%})/\text{median}$).

Sampling Site	Altitude	Ecological Status	IHF	RQI	Conductivity	Water Temperature	Free Reach	ICL	MDBF	Flow Variability
MU01	560	2	72	55	602	14.7	4.13	255	1.59	−2.44
MU02	540	2	81	85	619	14.7	2.43	255	1.59	−2.44
MU03	520	2	81	85	619	14.7	5.39	255	1.59	−2.44
MU04	480	3	69	43	639	14.7	3.25	205	8.58	−1.71
MU05	460	3	75	53	674	14.7	2.02	205	8.58	−1.71
MU06	430	3	70	64	688	14.7	3.67	205	8.58	−1.71
MU07	396	3	72	46	724	14.7	3.79	205	8.58	−1.71
MU08	354	3	70	34	1303	14.7	2.29	205	8.58	−1.71
MU09	350	4	67	39	1109	15.4	3.54	138	14.04	−1.62
SE01	860	3	78	94	364	14.3	25.88	84	2.91	−3.14
SE02	685	1	61	98	387	14.3	32.33	84	2.91	−3.14
SE03	491	1	63	87	390	14.3	12.58	84	2.91	−3.14
SE04	470	1	67	78	340	13.8	30.02	0	4.14	−2.58
SE05	452	1	68	80	401	13.8	30.02	0	4.14	−2.58
SE06	432	1	78	98	401	13.8	30.02	0	4.14	−2.58
SE07	363	2	67	77	447	14.1	7.73	126	1.77	−13.10
SE08	325	2	73	66	492	14.1	5.96	126	1.77	−13.10
SE09	306	2	58	69	579	14.1	1.7	126	1.77	−13.10
SE10	290	2	62	32	756	15.9	7.18	126	14.04	−1.62
SE11	260	3	57	44	803	15.9	10.64	90	14.04	−1.62
SE12	200	2	66	70	861	16.4	4.98	106	14.22	−1.55
SE13	148	2	58	45	1139	16.4	6.88	365	15.14	−1.47
SE14	112	4	64	36	1157	16.8	4.36	365	2.46	−3.31
TAI	640	2	61	43	566	14.7	15.67	58	-	-
TUS	809	1	84	65	432	14.5	12.81	76	1.59	−2.44

2.3. Fish Sampling and Population Traits

Sites were sampled using electrofishing (working voltage between 200 and 350 V, 2–3 A), following the CEN standard protocol [60]. Each sampling site was considered an independent population, taking into account the distance between sampling sites, the presence of non-passable barriers (dams and weirs) and the biological characteristics of *L. sclateri*. Fish were collected in 100-m-long wadable sections blocked by nets that acted as barriers. Fish sampling sessions were carried out in 30–45 min. Sampling was conducted during October–November 2009 to prevent the capture of spawning fish [45] and to avoid variation in body condition due to gonad development [39,43,61]. Fish manipulation was carried out following the European Union Directive 2010/63/UE on the protection of animals used for scientific purposes and it was not necessary to obtain authorization from the research ethics commission. In concordance with administrative permits, a total of 1529 specimens of *L. sclateri* were caught, processed in the field and returned to their habitat. Fork length ($\text{FL} \pm 0.1 \text{ cm}$) and weight ($\text{TW} \pm 0.1 \text{ g}$) were measured. A subsample of scales (611 specimens) was taken and cleaned later in a laboratory to determine the age according to Herrera et al. [40] and Torralva et al. [45].

L. sclateri populations were evaluated at sampling sites to assess intra-specific variability. The fish population traits we studied were relative abundance, size-related parameters, age-related parameters, relative growth rates and somatic condition. Relative abundance was measured as the number of *L. sclateri* individuals caught per hour (catch per unit effort, CPUE) in a standardized sample area without significant differences in habitat complexity and, thus, assuming that catch efficiency remains constant. Size-related parameters included mean, maximum and range of fork length (FL), and a size diversity index calculated as a Shannon-Wiener modification using the number of size classes grouped in 2 cm length ranges. Age-related parameters included mean, maximum and range of age, determined for a subsample of *L. sclateri* scales. Back-calculated lengths were estimated by the Fraser-Lee equation following the methodology used by Torralva et al. [45] and Miñano et al. [62] based on the counting and measuring of scales' annuli, and checked according to Musk et al. [63]. Proportions (%) of back-calculated lengths for each age class were obtained from the Walford method [64] and used to calculate the mean individual growth index (GI). Relative growth rates at the site level were estimated from the mean individual growth index (GI). The methodology used to calculate the growth index (GI) is detailed in Masó et al. [65] and Amat-Trigo et al. [66], which followed the Hickley and Dexter procedure [67]. We used the mean values of GI at age 1 year, age 2 years and maturity (individuals older than 2 years) according to the age of maturity previously established for the species in the Segura River basin [43,45,61]. Somatic condition was expressed as predicted values of log-transformed weight (mean value at sampling site) obtained from the application of univariate analysis of covariance (ANCOVA) using total weight (WT) as the dependent variable and fork length (FL) as the covariate; differences in variation were tested by ANOVA and Tukey's HSD post hoc tests [68]. Individuals with an FL less than 75 mm were considered juveniles [43]. Bivariate relationships between population traits were analyzed using Spearman's rank correlations. Statistical analysis was performed with the SPSS software package v. 24.

2.4. Effect of Environmental Stress Factors on Population Traits

We conducted a model selection analysis based on the Akaike Information Criterion with a correction for small samples sizes (AICc) [69] to determine the stressors associated with the variability in *L. sclateri* population traits. To establish model ranking, the MuMIn (Multi-Model Inference) R Package was used [70]. Stress factors were obtained by the reduction in transformed environmental variables and gradient descriptors (log for numeric variables, arcsine square root for proportions) using principal component analysis (PCA) with varimax rotation [71] using R package *psych*. Spearman's rank correlation analysis was used to test the redundancy between the variables. Principal component axes scores were used as stressors. The ratio of the abundance of non-native fish species (abundance of non-native species/total abundance) was also considered a stressor. General linear model results (GLMs) for the best models ($\Delta\text{AICc} < 2.0$) were used to describe the response of *L. sclateri* biological traits to the stress factors. These analyses were performed in the R statistical environment (Version 1.40.4).

3. Results

3.1. Relative Abundance and Population Traits

The abundance of *L. sclateri* showed high spatial variation, ranging from 13.33 to 150.00 catches per unit of effort (CPUE, Supplementary Table S1). The highest value was found in the Taibilla tributary (sampling site TAI) and the lowest was found at SE04, downstream of the Fuensanta reservoir (Figure 1).

The mean size fork length in the whole study area was 18.3 ± 6.55 cm FL, with the maximum value detected at MU03 (58.2 cm). The maximum validated age was 15+ years, with individuals this old detected at sampling sites SE04, SE07 and SE09; however, the mean age at the population level was 4.9 ± 1.43 years. The population size/structure differed among sampling sites, although a polymodal distribution pattern was evident

in most sampling sites along the longitudinal gradient (Figure 3). Both size and age parameters displayed lower values in headwaters (Figure 3 and Table S1), with maximum fish sizes below 30 cm (FL), lower size ranges and lower size diversity index values (Figure 3a,b,c). The other sampling sites had specimens longer than 40 cm (FL), with higher values at sampling sites just downstream of non-passable obstacles (e.g., SE07, Figure 3) and upstream of large reservoirs (e.g., MU03, Figure 3).

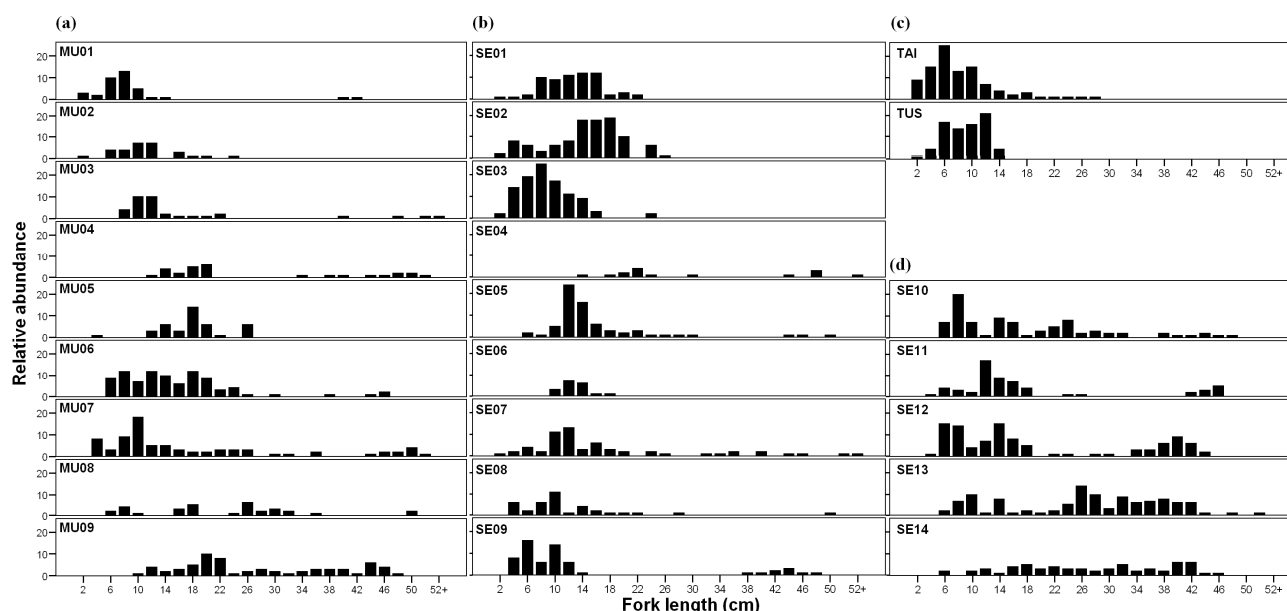


Figure 3. Size distribution (2 cm fork length (FL) groups) of *L. sclateri* at sampling site. Plot distribution: (a) Mundo River (MU) sampling sites, (b) sampling sites placed from Segura (SE) riverbed to Mundo River confluence, (c) sampling sites at tributaries Tus and Taibilla (TAI) Rivers and (d) sampling sites from Segura–Mundo confluence to the latest downstream sampling site (SE14).

The growth index (GI) displayed high variability for individuals of each age class between sampling sites. The mean GI value at age 1 year across the study area was 66.74 ± 1.28 , with a maximum value of 82.47 detected at sampling site SE01, while the mean value of GI at age 2 years was 39.34 ± 1.13 , with a maximum of 53.52 at sampling site MU09. For mature fish, the mean GI value in the study area was 17.69 ± 1.57 , with a maximum of 32.53 detected at site MU05. The lowest GI values were detected in the TUS tributary (age 1 year = 45.72; age 2 years = 19.26; mature = 0.03).

Somatic condition was higher in mature individuals and also showed greater variability among sampling sites. The mean of predicted values of log-transformed weight for mature individuals was 1.87 ± 0.03 , with a maximum value of 2.43 at sampling site MU04, whereas the mean value for immature individuals was 0.63 ± 0.03 , with a maximum of 0.90 at sampling site SE10. Generally, lower values of somatic condition were detected in headwaters for both mature (TUS = 1.28) and immature (SE01 = 0.44) individuals. Population traits at the site level are shown in Table S1 (Supplementary Material). The relationships among population traits are presented in Table S2 (Supplementary Material).

3.2. Environmental Factors

The first three PC axes obtained from the dimension reduction of environmental variables and gradient descriptors explained 79.7% of the total variance (Table 2). PC1 was associated with habitat alteration and fragmentation, which were directly related to poor ecological status and low RQI, high values of conductivity and water temperature and low connectivity (high values for ICL and low values of free reach). PC2 was associated with the longitudinal gradient, which was directly related to high altitude, IHF and low water

temperatures. PC3 was associated with flow regime alteration, which was directly related to flow variability and MDBF.

Table 2. Loadings and proportions of variance extracted by PCA of environmental variables with varimax rotation. Loadings > 0.50 are marked in bold.

Env. Variables	PC1	PC2	PC3
Ecological status	0.817	−0.150	0.202
Fluvial Habitat Index (IHF)	0.081	0.882	0.036
Riparian Quality Index (RQI)	− 0.648	0.427	−0.286
Altitude	−0.339	0.832	−0.071
Free reach	− 0.909	0.052	0.087
Longitudinal connectivity (ICL)	0.869	0.021	−0.068
Conductivity	0.750	−0.443	0.393
Water temperature	0.520	− 0.534	0.466
Mean daily base flow (MDBF)	0.170	−0.473	0.700
Flow variability	−0.038	0.164	0.950
Proportion of variance	0.366	0.241	0.191

3.3. Effects of Environmental Factors on Population Traits

Table 3 displays the best models obtained from the model selection analysis based on AICc. The longitudinal gradient (PC2) was significantly linked with size-related parameters and somatic condition metrics. The gradient from the upper sampling sites to the downstream sites was associated with increment in population traits including the size range, mean and maximum size, size diversity index and somatic condition metric (longitudinal gradient axis, Figure 4). The longitudinal gradient interplayed with other stressors such as the non-native fish species, showing a significant effect on size range and maximum size (size-related parameters, Table 3). In addition, this environmental factor interplayed with the flow regime alteration (PC3), displaying a significant effect on mean size (size-related parameters, Table 3) and somatic condition for mature individuals (somatic condition, Table 3). Finally, the longitudinal gradient also interplayed with habitat alteration and fragmentation (PC1), displaying a significant effect on the size diversity index (size-related parameters, Table 3) and somatic condition in immature individuals (somatic condition, Table 3).

Table 3. General linear models (GLM) results for the best models obtained by model selection analysis based on the Akaike Information Criterion with a correction for small samples sizes (AICc). GLM analysis was conducted for each population trait: CPUE, size- and age-related parameters, growth and somatic condition. (● $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

SIZE-RELATED PARAMETERS					
Size range	Longitudinal gradient + Non-native species				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	26.264	5.239	5.013	5.81×10^{-5}	***
Longitudinal gradient	−4.660	1.921	−2.426	0.024	*
Non-native species	18.278	8.792	2.079	0.050	●
Mean size	Longitudinal gradient + Flow regime alteration				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	18.420	1.114	16.537	1.62×10^{-13}	***
Longitudinal gradient	−3.097	1.119	−2.768	0.0115	*
Flow regime alteration	2.578	1.117	2.307	0.0313	*

Table 3. Cont.

Maximum size	Longitudinal gradient + Non-native species				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	29.456	5.913	4.981	6.27×10^{-5}	***
Longitudinal gradient	−4.812	2.168	−2.220	0.038	*
Non-native species	23.839	9.924	2.402	0.026	*
Size diversity index	Longitudinal gradient + Habitat alteration and fragmentation				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	2.231	0.051	43.977	$<2 \times 10^{-16}$	***
Longitudinal gradient	−0.023	0.051	−4.434	0.0002	***
Habitat alteration and fragmentation	0.117	0.056	2.098	0.048	*
ABUNDANCE (CPUE)					
CPUE	Null				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	61.525	7.786	7.902	5.3×10^{-8}	***
AGE-RELATED PARAMETERS					
Age range	Flow regime alteration				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	8.765	0.519	16.900	4.36×10^{-14}	***
Flow regime alteration	−1.586	0.520	−3.048	0.006	**
Mean age	Non-native species				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	3.237	0.749	4.324	2.73×10^{-4}	***
Non-native species	2.985	1.245	2.397	0.025	*
Maximum age	Non-native species				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	7.206	1.658	4.345	2.59×10^{-4}	***
Non-native species	5.060	2.759	1.834	0.080	●
SOMATIC CONDITION					
Immature somatic condition	Longitudinal gradient * Habitat alteration and fragmentation				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	0.642	0.023	28.162	9.97×10^{-14}	***
Longitudinal gradient	−0.119	0.028	−4.291	0.0007	***
Habitat alteration and fragmentation	−0.015	0.028	−0.552	0.589	
L. gradient: H. alter. and fragment.	0.110	0.043	2.578	0.022	*
Mature somatic condition	Longitudinal gradient + Flow regime alteration				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	1.869	0.058	32.211	$<2 \times 10^{-16}$	***
Longitudinal gradient	−0.188	0.058	−3.228	0.004	**
Flow regime alteration	0.107	0.058	1.844	0.080	●

Table 3. Cont.

GROWTH					
Growth index (GI) at age 1	Null				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	66.676	2.387	27.930	$<2 \times 10^{-16}$	***
Growth index (GI) at age 2	Flow regime alteration				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	40.724	1.723	23.639	$<2 \times 10^{-16}$	***
Flow regime alteration	3.607	1.728	2.087	0.049	*
Growth index (GI) for mature	Flow regime alteration				
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	18.165	2.042	8.894	9.73×10^{-9}	***
Flow regime alteration	5.614	2.049	2.740	0.012	*

In addition to the interplay with the longitudinal gradient, the flow regime alteration (PC3) was linked to variability in the age range (age-related parameters, Table 3) and the growth index (GI) for mature fish and fish aged 2 years (growth, Table 3). An increase in the PC3 axis implied high flow variability and base flow (Impact 2, Figure 2c) and was associated with increased mean size and somatic condition of mature individuals, and GI at age 2 years and for mature individuals (flow regime axis, Figure 4). In contrast, a decrease in the PC3 axis implied low base flow and variability (Impact 1, Figure 2b) and was associated with a decreased age range (flow regime alteration axis, Figure 4).

In addition to the interplay with the longitudinal gradient, non-native fish species were linked to the variability in the mean and maximum age (age-related parameters, Table 3). An increased relative abundance of non-native fish species was associated with an increase in these two age-related parameters (non-native fish species axis; Figure 4).

Finally, habitat alteration and fragmentation (PC1) were linked to the size diversity index (size-related parameters, Table 3) and somatic condition of immature individuals (somatic condition, Table 3) and always exhibited an interplay with the longitudinal gradient. An increase in habitat alteration and fragmentation was associated with increased size diversity index (habitat alteration and fragmentation axis, Figure 4). Furthermore, the only interaction found in this study occurred between this stressor and the longitudinal gradient, which was associated with increased somatic condition for immature individuals (longitudinal gradient axis, Figure 4). Finally, the model selection analysis did not find any stress factors among those considered in this study to explain the variability in the growth index (GI) of fish at age 1 year or abundance (CPUE).

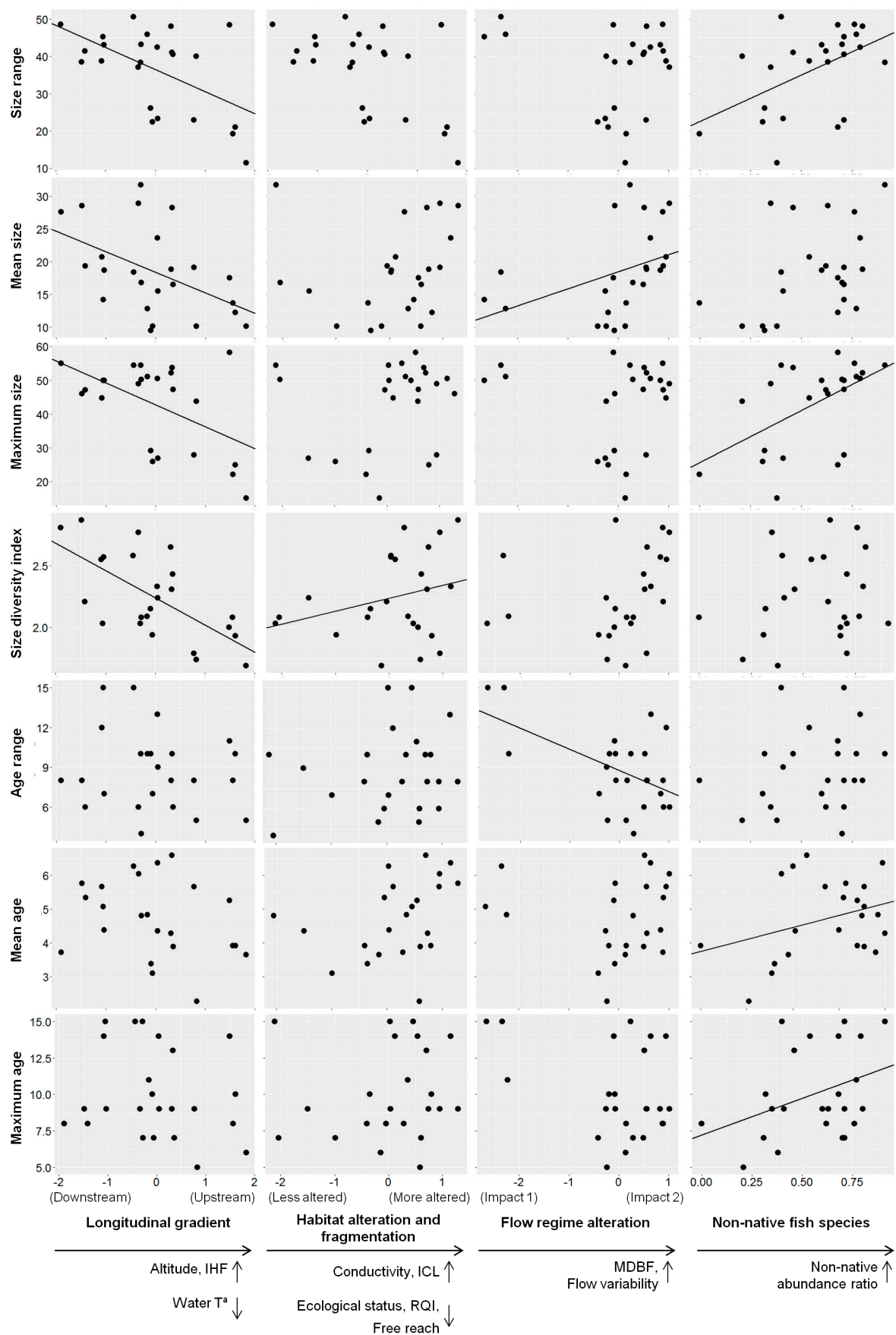


Figure 4. Cont.

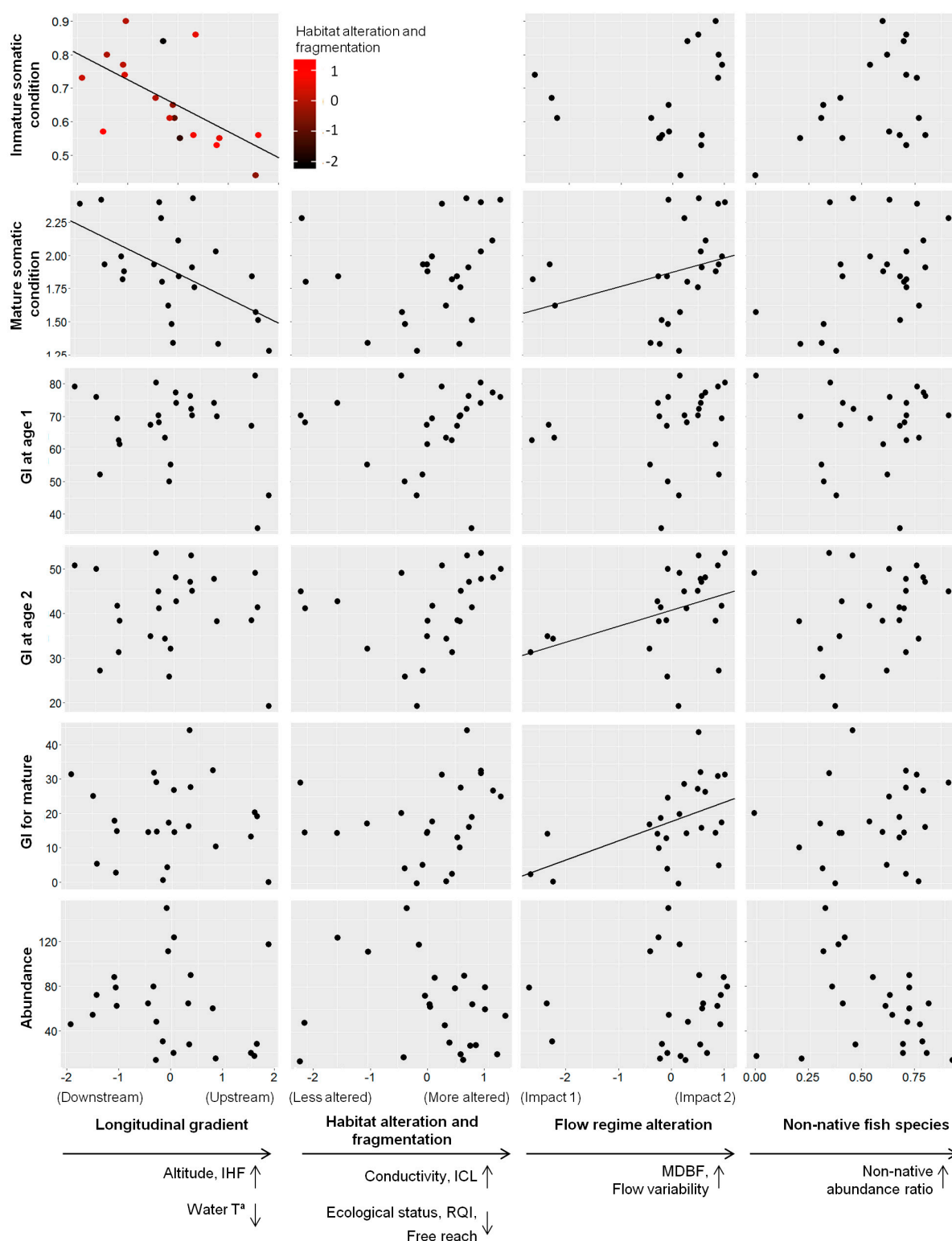


Figure 4. Relationship between environmental factors (longitudinal gradient, habitat alteration and fragmentation, flow regime alteration and non-native fish species) and *L. sclateri* population traits. Significant results (GLM p -value < 0.05) are represented with marked trend line on the plot. In x-axes, arrows pointing down indicate decrease in environmental variables (i.e., water temperature), and arrows pointing up indicate increase in environmental variables (i.e., altitude) and also increase in non-native abundance ratio.

4. Discussion

In this study, we analyzed the variation in population traits of *L. sclateri* in response to environmental factors in the fluvial reaches of the Segura River basin. Our results confirm significant variability in population traits along the longitudinal gradient due to the effect of human impacts, mostly those related to flow regulation such as fragmentation and habitat alteration, flow regime alteration and the relative abundance of non-native fish species. The use of biological traits related to size and age, relative abundance, growth and somatic condition allowed us to identify the population-level responses of this sentinel Iberian fish to different stressors and to verify the complex effect of multiple stressors in a heavily modified Mediterranean-type river.

The longitudinal gradient is a key factor in the structure and dynamic of freshwater ecosystems, so it is essential to consider its effects in the assessment of multiple stressors in river systems [72–75]. Environmental conditions along the longitudinal gradient determine the availability of resources such as food, refuges and breeding areas [34,76]. Consequently, a marked effect of the spatial variation on fish populations was expected, especially in Mediterranean-type rivers which exhibit a strong climatic and altitudinal gradient [10,77,78]. The increment of resources downstream along natural river systems promotes large sizes and a wider range of size classes in the fish fauna [73,76,79]. We found significant variability in the size parameters and somatic condition of *L. sclateri* populations associated with spatial variation, with an increase in these biological traits along the longitudinal gradient. The size population structure displayed a polymodal pattern, with high variability among sampling sites. The size distribution results highlighted a lack of some size groups, and this was more evident in sampling sites downstream of reservoirs. Small and medium-sized individuals were scarce downstream of operational dams (i.e., at sites MU09, SE04, SE09 and SE11). The GLM results (Table 3) showed that size and somatic condition parameters were significantly associated with the longitudinal gradient. In fact, habitat alteration and fragmentation, flow regime alteration and relative abundance of non-native fish species were all related to the variability of population parameters, reflecting the severe alterations that have affected the Segura River basin [34,37].

Human impacts increase along the longitudinal gradient as a consequence of the greater accessibility to water resources [77]. As a result, the interplay among spatial variation and human stress factors shaped the environmental conditions that act as a “filter” of biological traits [80]. The selected traits determine the biological responses of freshwater fauna to cope with altered conditions [18,75,81]. The Segura River basin shows a strong influence of human alterations mainly related to agricultural supply [33,37]. Agricultural practices involve strong hydraulic management that results in a loss of connectivity (habitat fragmentation), flow regime alteration and water quality and habitat degradation and encourages the establishment of non-native fish species [18,32,82,83]. Our results show the interplay between environmental stress factors, mostly those related to flow regulation, and the longitudinal gradient in some biological traits such as size and somatic condition. In addition, we found that age and growth variations were significantly associated with the isolated effect of the flow regime alteration and the relative abundance of non-native fish species. This finding suggests that the magnitude of human impacts in the study area could be masking some ecological responses to longitudinal gradients [84,85].

The flow regime is considered the main driver of freshwater ecosystems, defining the structure, function and dynamic of rivers, and affecting the individual fitness and growth rate of fish populations [80,86,87]. We found significant relationships between flow regime alteration and some population traits of *L. sclateri* including mean size, age range, somatic condition for mature individuals and growth variability. Our results show an increase in these population traits associated with fluvial sectors that had a high level of base flow throughout the year (reflected as Impact 2 in Figure 2) and a flow regime pattern that reduced the strong seasonal variability of Mediterranean-type rivers. Although these fluvial sectors display an inversion in flow seasonality related to agricultural water demands, they also provide an increased availability of water, refuge and food resources [25,76,88].

Furthermore, high flow levels promote changes in body shape and muscle development, which induce better swimming performance and increased somatic condition [89,90], a finding that was previously documented in the Segura River basin [43]. In contrast, we observed a decrease in population traits (mean size, age range, somatic condition for matures and growth variability) associated with strong inversion in flow seasonality and base flow reduction (Impact 1 in Figure 2). We found this flow pattern in sampling sites downstream of the Cenajo reservoir, a consequence of the operating characteristics of its dam [34,35,54]. The extreme hydrological conditions caused by this type of water regulation result in a poor-quality habitat, especially for adult barbels [91], and are considered a limiting factor for the growth of barbel species in the Iberian Peninsula [25,92]. These two contrasting results in the response of fish populations under different hydrological flow patterns have been described in other Iberian rivers as well [25,93].

Non-native fish species tend to be dominant in human-altered ecosystems, such as the Mediterranean rivers, where more stable environmental conditions that result from flow regulation measures encourage their establishment and spread [82,83,94]. Iberian fish communities have exhibited significant changes over recent decades as a consequence of the introduction of a wide range of non-native fish species [95–97], and these changes are especially evident in the Segura River basin [38]. The negative responses of native fish populations associated with the presence and abundance of non-native species in the fish assemblage are well documented [75,98,99]. Our results show an increase in the maximum size and size range, and the mean and maximum age of *L. sclateri* populations associated with a higher ratio of non-native fish abundance. The proliferation of non-native species is generally a result of changes in the environmental conditions caused by flow regulation [4,8]. The lack of small size classes of fish at sampling sites placed downstream of reservoirs where the presence of non-natives is favored (i.e., SE04 in Figure 3) suggests an effect of the fish assemblage composition on the structure of *L. sclateri* populations. In general, predation by non-native fish could affect the population structures and dynamics of native fish species in the Iberian Peninsula [95]. Some studies from other Iberian rivers confirmed the inclusion of different barbel species in the diet of top predator fish such as *E. lucius* [100,101] and *S. lucioperca* [102]. Predatory fish (*E. lucius*, *S. lucioperca* and *M. salmoides*) showed a higher occurrence in the lower reaches of the study area. They could be inducing higher predation pressure on certain size classes of *L. sclateri* and therefore affecting the population size structure as Bravo et al. [103] showed in the Palancar River, where *M. salmoides* predation was directly related to the lack of 0+ individuals of dominant species such as *L. sclateri*.

The results of this study highlight relevant associations between human impacts, most of which were related to flow regulation, and the population traits of *L. sclateri* along a longitudinal gradient, providing insights into the population-level responses of this sentinel Iberian fish to environmental conditions at the site level. The key role of the longitudinal gradient in driving the increase in human impacts as a result of greater accessibility related to the lower reaches of rivers is evident, since most of the stressors were related to the spatial variation, so this dependence makes it difficult to interpret the effect of isolated stressors. In addition, there is a wide variety of human impacts present in the study area that were not considered in this study. For example, pollution could be driving the response of *L. sclateri* to environmental conditions [32], or predatory mammals (*Lutra lutra*), whose predation on *L. sclateri* in the Segura River basin was recently confirmed [104].

The ability of this species to adapt to changes in local conditions has been shown by the variation in population size/structure along the longitudinal gradient. Although human impacts exerted significant effects on the biological traits we evaluated, our findings also suggest that the wide inter-population plasticity displayed by *L. sclateri* may be a mechanism for this species to successfully inhabit a highly modified Mediterranean-type river. Cyprinids in general show great adaptability to environmental alterations [12,25,92,105], and *L. sclateri* showed a tolerance to the effects of flow regulation previously studied in the same river basin [45,61].

In recent decades, declines in fish populations have been documented for several Iberian fish species and there have been drastic reductions in fish species that were previously widely distributed in the study area. Native fish species are vulnerable to the rapid increase in human pressure on the water resource; this is especially so in Mediterranean areas where an increase in the magnitude of extreme weather events is expected under climate change scenarios [106]. Therefore, the use of well-known and widely distributed sentinel species, such as *L. sclateri*, may prove a useful tool to increase the knowledge of the adaptability and population responses to gradients of single and multiple stressors, which is essential to establish and improve management actions to protect native fish species.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4441/13/6/747/s1>, Table S1: Population traits of *L. sclateri* at sampling site. Table S2. Coefficients of Spearman rank correlation between population traits.

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