

## RESEARCH ARTICLE

WILEY

# Influence of the *Salmo (trutta) trutta* on the population structure, the growth, and the habitat preference of a *Cottus gobio* population

Luca Bonacina<sup>1</sup>  | Sergio Canobbio<sup>2</sup> | Riccardo Fornaroli<sup>1</sup> 

<sup>1</sup>Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Milan, Italy

<sup>2</sup>Bioworks Professional Practice, Guanzate, CO, Italy

## Correspondence

Luca Bonacina, Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy.

Email: [l.bonacina9@campus.unimib.it](mailto:l.bonacina9@campus.unimib.it), [luca@bonacina.eu](mailto:luca@bonacina.eu)

## Funding information

Uniacque Spa; Università degli Studi di Milano-Bicocca

## Abstract

The bullhead *Cottus gobio* is a small-sized fish whose range extends over most of the European continent, and it is listed in Annex II of EU “Habitat” Directive for its great conservation interest. In the last decades, bullhead populations suffered local decline. Among the factors that negatively affect bullhead populations, major threats are pollution, habitat deterioration, and the massive introduction of salmonids. This study aims to better understand in which way the presence of the *Salmo (trutta) trutta* affects *Cottus gobio* populations. The investigation was carried out in two stretches of a stream located in the Orobic Alps (Italy). The downstream stretch hosts a fish assemblage constituted of both bullhead and brown trout, while in the upstream stretch only bullhead is present. An insurmountable barrier isolates the upstream population of *C. gobio* from trout, while the environmental conditions of the two stretches proved to be fully comparable. We evaluated population structure, habitat preference, and body shape of bullhead populations in both stretches: the results indicate that the presence of trout decreases the number of bullhead adults, reduces the average adult body size, and induces a bullhead suboptimal habitat occupation. However, both populations of *C. gobio* showed a well-structured population and good performance indexes, so trout do not seem to be a threat to the population survival.

## KEYWORDS

bullhead, conservation, endangered species, habitat suitability, interspecific interaction, population demography, salmonids

## 1 | INTRODUCTION

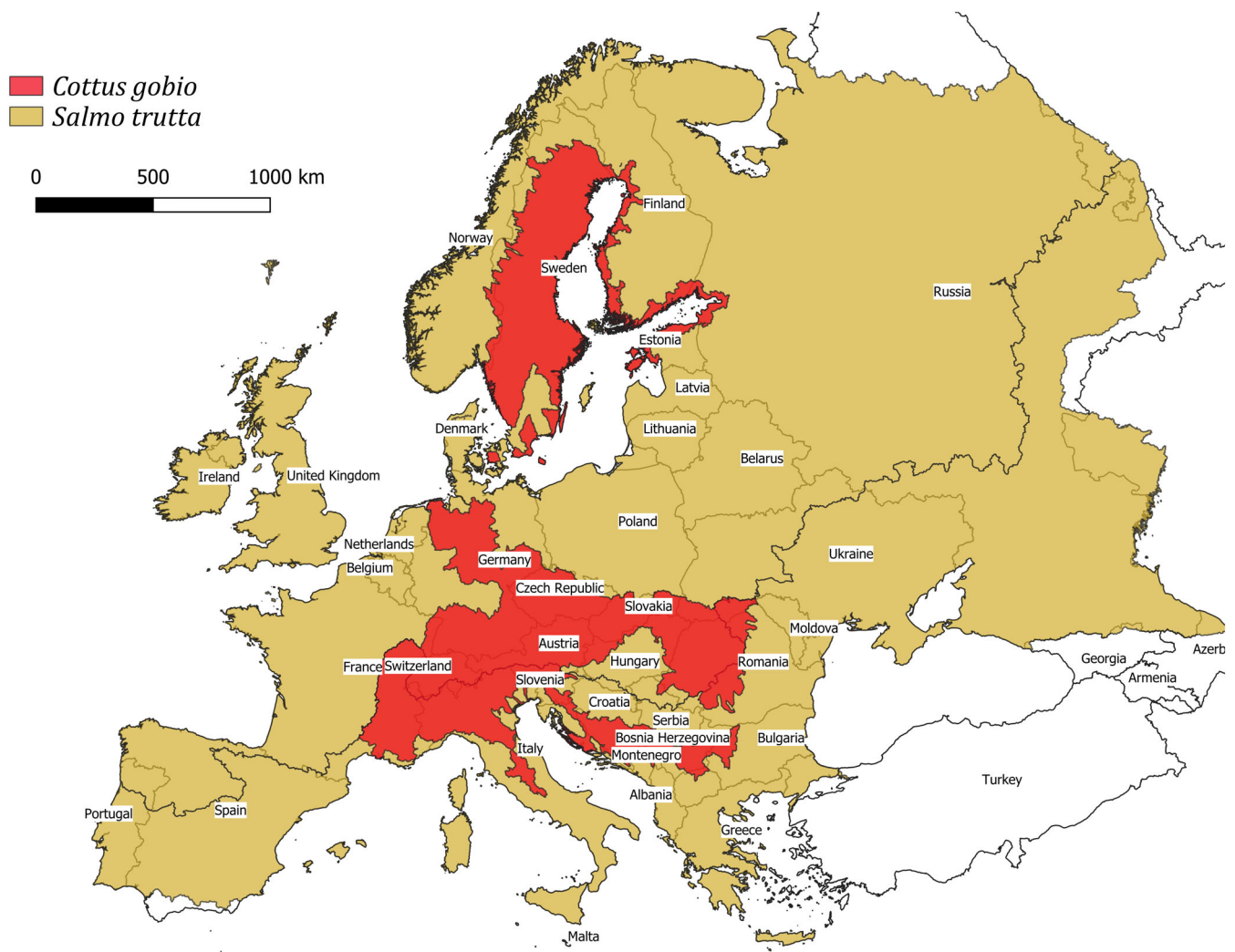
The bullhead *Cottus gobio*, Linnaeus, 1758, is a small-sized, bottom-dwelling freshwater fish species belonging to the Cottidae family. *Cottus gobio* is widely distributed throughout the European continent, from Sweden to Italy and from France to Estonia (Figure 1) even if some populations attributable to the species *C. gobio* have been

observed also in Spain, the United Kingdom, Belgium, Norway, Siberia, and Greenland (Elliott, 2006; Frilund, Koksvik, Rikstad, & Berger, 2009; Holmen, Olsen, & Vøllestad, 2003; Koli, 1969; Mills & Mann, 1983; Van Liefveringe, Seeuws, Meire, & Verheyen, 2005).

In the last decades, however, local extinction phenomena occurred, and the species is currently fragmented in different populations whose grade of isolation is growing (Legalle, Santoul, Figuerola,

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *River Research and Applications* published by John Wiley & Sons Ltd.



**FIGURE 1** Geographical range of *Cottus gobio* and *Salmo trutta* (data provided by IUCN Red list) [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Mastrorillo, & Céréghino, 2005; Van Liefferinge et al., 2005). In Italy, the species is widespread throughout streams and rivers in the Alps (up to 800–1,200 m a.s.l.) and in the springs of the pre-alpine region, while in central Italy the range distribution has undergone a contraction (Franchi, Pompei, & Barbaresi, 2012; Gandolfi, Zerunian, Torricelli, & Marconato, 1991) so that the presence of the bullhead is now supposed to be restricted to a few watercourses of the Tiber River basin, the Tuscan-Emilian Apennines and the Marche region (Freyhof & Kottelat, 2011; Lorenzoni, Ghetti, Carosi, & Dolciami, 2010). The factors that mostly constitute a threat to the species are water pollution, habitat deterioration, predation, and competition with alien species.

Bullhead generally prefers fast-flowing riffle habitats (Carter, Copp, & Szomlai, 2004; Mann, 1971; Roussel & Bardonnnet, 1996), stony substrates such as gravel, cobble, pebble, and boulder (Tomlinson & Perrow, 2003), cold, well-oxygenated, and clean waters (Jermacz, Kobak, Dzierżyńska, & Kakareko, 2015). Dams and weirs can reduce the availability of suitable reproductive habitats due to

fragmentation and the increase of fine sediment deposition (Fischer & Kummer, 2000). Riverbed modifications as channeling, widening, and deepening can negatively affect bullhead populations by reducing suitable substrates and increasing the exposure to predators (Perrow, Punchard, & Jowitt, 1997).

The brown trout (*Salmo (trutta) trutta*) is a European species of salmonid fish, native to northern and central Europe widespread from Ural Mountains to Iberian Peninsula (Figure 1). Over time the brown trout has been widely introduced worldwide in North and South America, Asia, and Australia (MacCrimmon & Marshall, 1968). It is a very adaptable species, which can colonize a wide variety of environments, and has high economic value for food and angling purposes. In Italy the Atlantic haplotype *Salmo trutta* was probably introduced in the XIX century from North European fish hatcheries (Bettoni, 1895) and at present, it is extremely common and widespread throughout the peninsula, in Sicily and Sardinia, so much that it threatens the Italian indigenous trouts (Splendiani, Palmas, Sabatini, & Caputo Barucchi, 2019; Zerunian, 2002): the Adriatic trout (*Salmo ghigii*), the marble

(*Salmo marmoratus*) and the Mediterranean trouts (*Salmo macrostigma*) as well as some very local endemisms (*carpio*, *Salmo cenerinus*, *Salmo cettii*, and *Salmo fibreni*) of which the systematic status is still today very debated (Splendiani et al., 2019). Since the middle of 19th century massive stocking activities with the Atlantic strain of *S. trutta* have been carried out to improve angling opportunities and human consumption so that this species is now considered as one of the world's 100 most invasive species (Jonsson & Jonsson, 2012) imperiling native species by predation, habitat and food competition, and hybridization (Budy et al., 2013).

Indeed, the massive release of brown trout in rivers and streams for angling purposes greatly increases the predation pressure causing a decline in bullhead populations through the predation of juveniles (Lorenzoni et al., 2018; Marconato, 1986; Simon & Townsend, 2003; Zerunian, 2002). Furthermore, the similar ecological preferences and the similar feeding habits between brown trout and bullhead can also lead to interspecific competition phenomena (Elliott, 2006; Holmen et al., 2003; Louhi, Mäki-Petäys, Huusko, & Muotka, 2014). For its great conservation interest, the bullhead is listed under the Annex II of the Council Directive 92/43/EEC (European Commission, 1992) and in the general action plan for Italian freshwater fish conservation (Zerunian, 2003). Thus, while it is classified as a “Least concern” species in the IUCN Vertebrates Red List (Rondinini, Battistoni, Peronace, & Teofili, 2013), the occurring fragmentation of populations and the reduction of the distribution range raise conservation issues in Italy.

The autoecology of the bullhead, such as poor vagility and the requirement of good environmental quality, together with the fact that it is not a species subject to manipulation (i.e., breeding and release for angling purposes, or relocation), could make the bullhead occurrence a useful indicator for evaluating the integrity and the conservation status of freshwater ecosystems (Charles, Subtil, Kielbassa, & Pont, 2008; Tomlinson & Perrow, 2003; Utzinger, Roth, & Peter, 1998), but more knowledge is needed about the causal links among the different stressors and their effects on populations. Thus, based on the hypothesis that the introduction of brown trout can negatively affect bullhead populations due to the increased predation and/or interspecific competition, the aims of the present research were:

- i. to present a case study of coexisting and long-term non-coexisting populations of bullhead with introduced trout in the same stream of the Orobian Alps (northern Italy);
- ii. to compare the population status of bullhead with the presence and the absence of trout, to understand in which way, *S. trutta* affects *C. gobio* populations in terms of density, growth performance, population structure, and habitat preference.

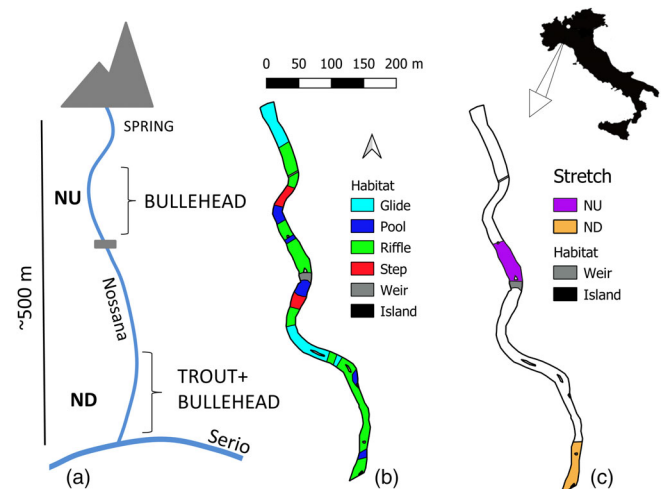
## 2 | MATERIAL AND METHODS

### 2.1 | Study area

The study site is represented by Nossana stream, a tributary of Serio River located in the Orobian Alps (Province of Bergamo, northern Italy)

that flows on the surface for 500 m. The Nossana watershed covers an extension of about 80 km<sup>2</sup> and is constituted by a predominant drainage system towards the subsoil that feeds a spring (Vigna & Banzato, 2015), characterized by a high permeability due to a fracture system within two geological formations, limestone and dolomite (Ferlinghetti, Arzuffi, & Beretta, 2011). Nossana spring ensures the drinking water service to the city of Bergamo and the surrounding municipalities. Two stretches of the stream have been selected: Nossana upstream (NU) and Nossana downstream (ND). The first is located close to the spring: it is characterized by the presence of a bullhead population because previously released trout have been removed since the early 2000s to avoid the potential proliferation of pathogens in a fish hatchery located downstream. The second stretch is located before the confluence with the Serio River and is characterized by the presence of both bullhead and trout emisppecies populations. Brown trout are periodically released from the hatchery or can go upstream from the Serio River. The two reaches are divided by an insurmountable barrier, constituted by a 7 m high artificial weir, and have the same environmental conditions in terms of width, water depth, channel substrates and habitat availability (Figure 2).

Nossana stream has a torrential water regime, with a flow rate that can vary between 0.5 and 15 m<sup>3</sup>/s (flow data is assessed by the managing company of the drinking water service): however, a minimum flow of about 0.5 m<sup>3</sup>/s is guaranteed by law due to the presence of the spring water collection activity. The selected stream represents a suitable model to study the *S. trutta* and *C. gobio* interaction due to: (i) an intact and morphologically diversified environment that can sustain bullhead populations, (ii) the long-term absence of brown trout in the upper stretch and the absence of other fish species in the whole stream, (iii) the similar geomorphological and environmental conditions between the two stretches.



**FIGURE 2** (a) Schematic representation of the study area, (b) maps of the superficial stream reach, and (c) location of the sampling stretches [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## 2.2 | Data collection

Fish data were collected eight times from September 2016 to September 2020 in the two stretches. Each sampling was carried out by two-pass electrofishing using the removal method (Moran, 1951; Zippin, 1958). Fish were captured during low flow periods using electric current, and applying a similar fishing effort in NU and ND (Seber & Le Cren, 1982). For all the captured fish, the species was identified, and individuals were counted to determine the species abundance, in terms of density (number of individuals/m<sup>2</sup>) and standing crop (g/m<sup>2</sup>). The total length (TL) was measured to the nearest 0.1 cm for all specimens while the body weight (W) was measured to the nearest 0.1 g. All the fish were released back into their natural environment at the end of the fieldwork. In autumn 2020 we also monitored the habitat occupied by every captured bullhead, both in NU and ND stretches.

To assess the environmental quality and characteristics of the stretches, water temperature was measured in continuous by iButton devices (range -5 to 26°C, resolution: ±0.0625°C, measurement interval: 10 min) fixed in the riverbed of the two stretches from Autumn 2018 to after the end of the fieldwork. Physico-chemical parameters (i.e., electric conductivity, dissolved oxygen, and oxygen saturation) were measured in the field using a probe HACH-HQ40d while pH, nitric nitrogen, ammonia nitrogen, and phosphate were determined in the laboratory according to Standard Methods (APHA/AWWA/WEF, 2012). Macroinvertebrate samplings were also performed on the occasion of each fish sampling with a Surber net (0.10 m<sup>2</sup>, 500 µm mesh) following a standardized multi-habitat sampling procedure (Barbour, Gerritsen, Blaine, & Stribling, 1999). Ten replicates for each stretch were collected then merged in the field and preserved with 96% ethanol. In the laboratory, taxa were identified at the family level according to standard keys. Water quality was evaluated using the LIMeco index for water quality assessment (European Community, 2000; Italian Ministry of the Environment, 2010) while the ecological status was evaluated through the STAR ICMi indicator as reported in Fornaroli, Calabrese, Marazzi, Zaupa, and Mezzanotte (2019). A riverbed habitat map was created in QGIS based on hydraulic and geomorphological data collected for both reaches during the surveys. Water velocity, water depth and size of substrates were monitored at 1 m steps along transects perpendicular to the flow, placed every 5 m. Those data were firstly interpolated to produce thematic maps for each variable (i.e., water velocity, water depth, substrate composition) and then reclassified to create the habitat classification map as it is described in Fornaroli et al. (2016).

## 2.3 | Age and growth

The age of bullhead was determined throughout the analysis of the length-frequency distribution (Bagenal, 1978; Harvey & Cowx, 2000) using the “ELEFAN” function of the *TropFishR* package (Mildenberger, Taylor, & Wolff, 2017) within R project software (R Core Team, 2020)

since that for bullhead is not possible the age determination using direct monitoring as the skin scales.

To estimate the infinite length ( $L_{inf}$ ) and the growth coefficient ( $K$ ) we performed the moving average over 5 length classes (5 mm each); we identified the optimal  $L_{inf}$  value among 30 steps in the range 12–14.5 and, similarly, among 20 steps in the range 0.3–1.5 for the optimal  $K$ . We specified a maximum age of the bullhead of 4 years.

The theoretical growth was estimated separately for the two bullhead populations, using the von Bertalanffy growth curve model (Von Bertalanffy, 1938) (Equation (1)) and the mean length of the different age classes was estimated and compared between the two bullhead populations.

$$TL_t = L_{inf} * (1 - e^{K(t-t_0)}) \quad (1)$$

where  $TL_t$  is the total length of the fish at time  $t$ ,  $L_{inf}$  is the theoretical maximum length (cm),  $K$  is the rate of approach to  $L_{inf}$ , and  $t_0$  is the theoretical age at which  $TL_t = 0$ .

The total Length-Weight Relationship (LWR) (Froese, 2006; Le Cren, 1951) was estimated for the upstream and downstream populations of bullhead by the least-squares method (Ricker, 1975) based on Equation (2) to compare the growth between the two bullhead populations.

$$W(g) = a * L^b (cm) \quad (2)$$

where  $W$  = weight,  $L$  = length,  $a$  and  $b$  constants.

Furthermore, the index of growth performance was calculated by the equation of Pauly and Munro (1984) (Equation (3)) for both populations:

$$\Phi = \log_{10}K + 2\log_{10}L_{inf} \quad (3)$$

where  $K$  and  $L_{inf}$  are the growth parameters of the Von Bertalanffy model.

Multiple working hypotheses were evaluated requiring tests between groups of data classified based on the presence or absence of trout. Because normality and homogeneity of variance could not be achieved in most log-transformed data, differences in population characteristics were tested using nonparametric procedures. We used Wilcoxon Signed Rank test for paired data and Wilcoxon Rank Sum test ( $\alpha < .05$ ) to evaluate differences in population traits between treatments. We used the two stretches (NU and ND) as treatments in terms of (i) density of individuals, (ii) standing crop, (iii) body length of each age class, (iv) length-weight curves, and (v) the frequency of individual of each length class. We used Spearman  $\rho$  statistic to estimate a rank-based measure of association among density of individuals of fish species and macroinvertebrate families over time. These comparisons allowed us to identify differences in the structure of the two populations of bullhead and understand the ecological implications of interspecific interaction.

## 2.4 | Habitat preference

Habitat use of bullhead was defined by measuring current velocity, water depth, and substrate type at each fish location. To identify the fish position we used backpack electrofishing, as direct observation via snorkeling was difficult because of water turbulence, and stream bank direct observation was also difficult because of the lack of suitable observation points (i.e., bridges) and small sizes of the fishes (Vismara, Azzellino, Bosi, Crosa, & Gentili, 2001).

Habitat availability in the two study stretches was derived from thematic maps produced for the whole stream with a cell dimension of 1 m<sup>2</sup>. Maps of water velocity were reclassified in three classes: <0.1 m/s, from 0.1 m/s to 0.3 m/s and >0.3 m/s; similarly maps of water depth were reclassified in three classes: <0.1 m, from 0.1 m to 0.3 m and >0.3 m.

Univariate suitability curves were defined, according to the procedure outlined by Boove (1986): (i) each variable was divided into classes, and frequencies of utilization and availability were computed; (ii) preferences for each class interval of the measured variable were computed from relative frequencies of utilization and availability as follows:

$$P_i = \frac{U_i}{A_i} \quad (4)$$

where  $P_i$ : relative preference value of a target species for a specific interval of the measured variable,  $U_i$ : % of utilization of a specific interval of the measured variable,  $A_i$ : % of availability of a specific interval of the measured variable in the studied river sector at the time the organisms were sampled; (iii) preferences were then normalized to a maximum value of 1.

Univariate suitability curves for adult brown trout were not evaluated in this work due to the very low number of individuals but were available for the mainstem of Serio River, for stretches similar to the Nossana stream in terms of size, flow and morphology (Viganò et al., 2015).

## 3 | RESULTS

### 3.1 | Environmental characterization and ecological status of the stretches

Water temperature was constant the whole year ( $8.3 \pm 0.2^\circ\text{C}$ ) both in NU and ND, due to the presence and proximity of the spring.

**TABLE 1** Water quality, ecological status, and macroinvertebrate densities expressed as mean of the samplings of each year

Year	Water quality (LIMeco)		Ecological status (STAR_ICMi)		Macroinvertebrate density (n° ind/m <sup>2</sup> )	
	NU	ND	NU	ND	NU	ND
2016	0.854 ± 0.036	0.875 ± 0.000	0.78 ± 0.01	0.75 ± 0.07	679.3 ± 439.0	655.7 ± 142.7
2017	0.860 ± 0.031	0.860 ± 0.031	0.84 ± 0.03	0.75 ± 0.08	875.0 ± 228.0	869.5 ± 336.8
2018	0.850 ± 0.056	0.863 ± 0.028	0.76 ± 0.10	0.76 ± 0.01	810.6 ± 470.8	601.0 ± 252.0
2019	0.854 ± 0.036	0.854 ± 0.036	0.77 ± 0.03	0.77 ± 0.06	608.0 ± 159.5	860.3 ± 295.6
2020	0.875 ± 0.000	0.875 ± 0.000	0.78 ± 0.04	0.74 ± 0.03	654.0 ± 235.9	774.0 ± 232.5
Quinquennium	0.859 ± 0.034	0.866 ± 0.023	0.79 ± 0.06	0.75 ± 0.05	734.3 ± 316.1	745.1 ± 239.8

Note: Blue and green indicate “excellent” and “good” status, respectively.

Dissolved oxygen ranged from 10.50 to 12 mg/L, close to saturation (>95%). Electrical Conductivity was about  $240 \pm 40$  mS/cm and pH  $7.9 \pm 0.3$ . The ecological status of both stretches resulted constantly high since LIMeco and STAR\_ICMi indicators resulted to be always “elevated” and “good” respectively (LIMeco =  $0.859 \pm 0.034$  in NU and  $0.866 \pm 0.023$  in ND while STAR\_ICMi =  $0.79 \pm 0.06$  in NU and  $0.75 \pm 0.05$  in ND for the quinquennium) as reported in Table 1.

### 3.2 | Density and standing crop

*Salmo (trutta) trutta* (ND) and *Cottus gobio* (ND, NU) were the only fish species inhabiting the two investigated stretches. A total of 570 bullhead specimens were collected. The size of the sampled bullhead ranged from 3.7 to 14.2 cm in the upper stretch while from 1.6 to 13.2 in the lower one (Figure A1, Appendix A). A total of 111 trout individuals were collected in the lower stretch with a total length ranging between 7 and 44 cm. The density and the standing crop of both bullhead and trout have been reduced considerably in the first 2 years (2016–2018) while have been kept constant later. The change of density over time was similar for the two bullhead populations and the brown trout inhabiting the lower stretch as confirmed by the Spearman correlation statistic ( $\rho = .82$ ,  $p < .05$  between d\_C.gobio\_NU and d\_C.gobio\_ND;  $\rho = .79$ ,  $p < .05$  d\_C.gobio\_ND and d\_S.trutta\_ND; Table A1). The change of standing crop of the two bullhead populations over time was similar ( $\rho = .86$ ,  $p < .05$ , Table A1) while the standing crop of brown trout showed a different pattern ( $\rho = .68$ ,  $p = \text{n.s}$  with sc\_C.gobio\_NU;  $\rho = .32$ ,  $p = \text{n.s}$  with sc\_C.gobio\_ND; Table A1), highly influenced by the presence of few large individuals. Wilcoxon Test showed statistically significant differences between the two bullhead populations (density:  $V = 28$ ,  $p = .016$ ; standing crop:  $V = 28$ ,  $p = .016$ ): density was 50% higher and standing crop was 20% higher in the NU population of bullhead compared to the ND one (Figure 3a).

### 3.3 | Demographic characteristics

Five age classes (0p–4p) of bullhead were identified by the analysis of the length-frequency distribution and the Von Bertalanffy growth curve equations were extrapolated (Figure 3b):

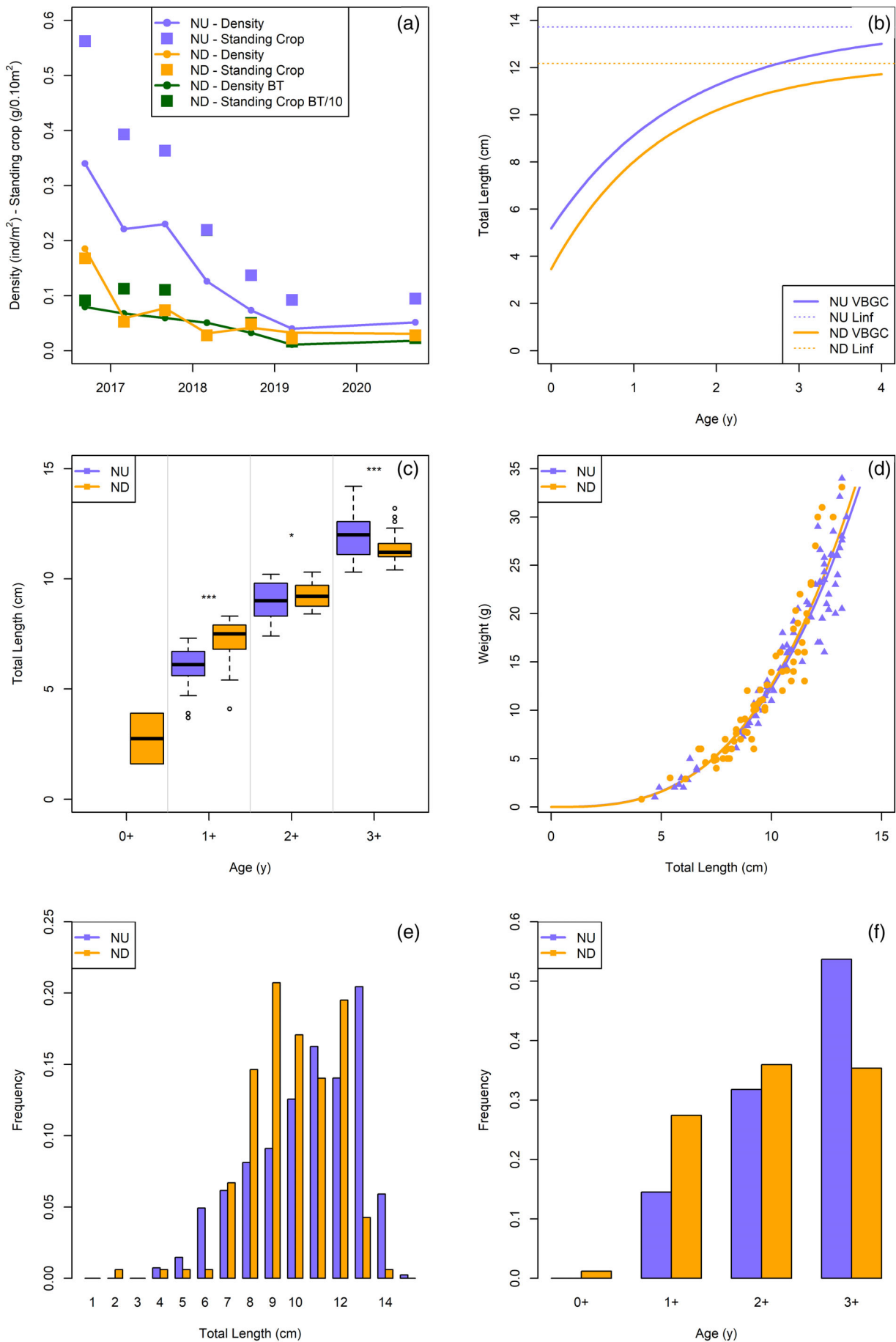
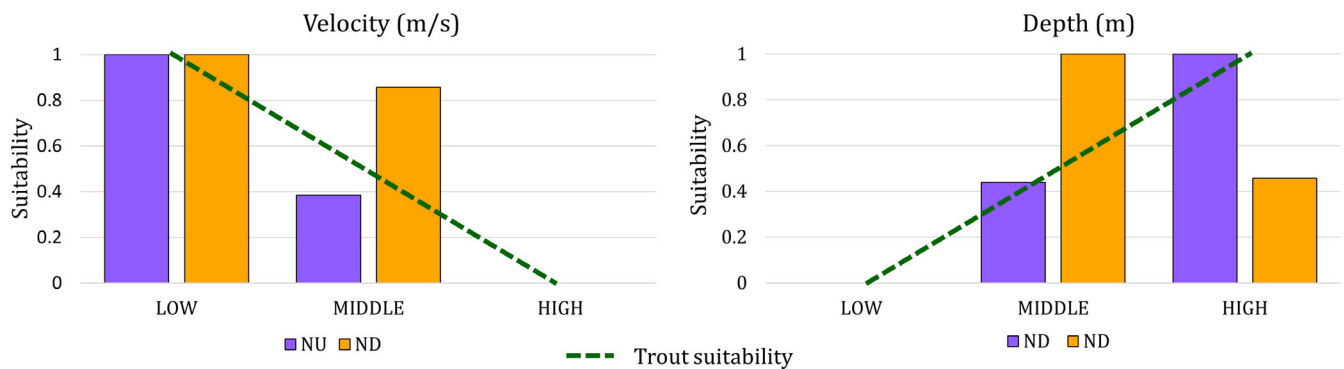


FIGURE 3 Legend on next page.



**FIGURE 4** Habitat suitability of bullhead populations in the two stretches. Trout suitability (green dot line) is shown only for qualitative comparison [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

$$NU: TLt = 13.72 * \left(1 - e^{-0.62 * (t + 0.764)}\right)$$

$$ND: TLt = 12.17 * \left(1 - e^{-0.74 * (t + 0.452)}\right)$$

The theoretical maximum length was lower in the downstream bullhead population (12.17 vs. 13.72 cm in NU and ND, respectively). The size of each age class was compared between the populations, as shown in Figure 3c. No 0+ individuals were caught in NU while they were present in ND (only 2); 1+ individuals were on average longer in ND than in NU ( $W = 329, p < .001$ ); 2+ individuals were only slightly longer in ND if compared with NU ( $W = 3,124, p < .05$ ) while 3+ individuals were on average shorter in ND than in NU ( $W = 8,668, p < .001$ ). The index of growth performance was calculated separately for upstream and downstream populations:  $\Phi$  was 2.07 and 2.03 respectively indicating very well growth performance in both stretches ( $\Phi > 1.8$ ) (Pauly, 1979).

### 3.4 | Growth

LWR equations estimated separately for the upstream and downstream populations were:

$$\begin{aligned} NU: W &= 0.0143 (0.0105 - 0.1952) * TL^{2.933 (2.802 - 3.064)} \left( n = 79, R^2 = .96 \right) \\ ND: W &= 0.0126 (0.0079 - 0.0204) * TL^{2.998 (2.785 - 3.211)} \left( n = 66, R^2 = .93 \right) \end{aligned}$$

showing similar trend (Figure 3d). 95% confidence intervals are reported in brackets for each coefficient.  $n$  indicates the number of individuals. Both  $b$  coefficients (NU = 2.933,  $t(77) = -1.019$ ;  $p = .312$ ; ND = 2.998,  $t(64) = -0.018$ ;  $p = .986$ ) indicated an isometric growth rate ( $b \sim 3$ ).

### 3.5 | Population structure

The population structure was studied investigating the frequency of each dimensional class estimated both based on the length data collected in the field (Figure 3e) and on the age classes obtained by the analysis of the length-frequency distribution (Figure 3f). Both populations presented a good structure even if the ND population had a smaller proportion of 3+ adults (35% vs. 54%) and a higher proportion of 1+ young (27% vs. 15%) as underlined also by the age-length analysis (Figure 3c).

### 3.6 | Habitat suitability

The bullhead is a benthic species that prefers cold, clear, fast-flowing small streams and middle-sized rivers. According to the meso-habitat survey, the habitat preference of bullhead was different between the two stretches. In ND, the bullhead preference for habitats of middle velocity (0.1–0.3 m/s) and middle depth (0.1–0.3 m) increased (from 0.38 to 0.85 and from 0.43 to 1, respectively) while the preference for higher depths ( $\geq 0.3$  m) decreased (from 1 to 0.45) compared to NU (Figure 4).

## 4 | DISCUSSION

The environmental characterization confirmed that Nossana stream represents an optimal environment for bullhead, especially for the presence of stable and diversified stony substrates, high concentration of oxygen and low water temperature. Many studies showed that springs and riverine reaches close to the source are among the habitats preferred by bullhead (Lorenzoni et al., 2018; Tomlinson & Perrow, 2003).

**FIGURE 3** Comparison between bullhead populations in terms of: density and standing crop trends (a), theoretical growth curves (b), the total length distribution of the age classes among stretches (c) (levels of statistical significance for Wilcoxon Rank Sum test between stretches are as follows: \*\*\* $p < .001$ ; \*\* $p < .01$ ; \* $p < .05$ ; ns,  $p > .05$ ); length-weight curves for bullhead populations (d), frequencies of the length classes (e), and frequencies of the age classes (f). Trout standing crop in the plot A is expressed in  $g/0.01 \text{ m}^2$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

The five-year survey revealed that the biomass and the density of bullhead were significantly lower in the downstream stretch, showing that the presence of the *S. trutta* was influencing it. The densities of both the bullhead populations in NU and ND in the years 2016–2018 exceeded the 0.2 individuals/m<sup>2</sup>, while in the following period (2018–2020) they decreased below 0.2 individuals/m<sup>2</sup>. This led the populations below the low-density threshold of 0.15 individuals/m<sup>2</sup> for headwater bullhead populations (Perrow et al., 1997). These values are comparable to other Alpine bullhead population densities, such as those defined for Valgrande streams, that ranged between 0.001 and 0.21 ind/m<sup>2</sup> (Foglini, Sala, Zellino, & Volta, 2018). The bullhead density decrease that occurred in Nossana stream can be ascribed to an exceptional flood event that occurred in the autumn of 2018, in which the mean daily flow passed 15 m<sup>3</sup>/s. The event negatively also affected the densities of the more skilled swimmer *S. trutta*.

The parameters of the theoretical growth curves calculated separately for the two bullhead populations showed that bullhead in the absence of trout had a greater maximum theoretical length than bullhead in the presence of trout even if both showed similar maximum length compared to other Italian populations of bullhead (Table 2).

Nevertheless, the change rate of body shape was about three (2.93 and 2.99 in NU and ND), indicating an isometric growth (Ricker, 1975). This slope was different from those recorded in central Italy (Lorenzoni et al., 2018) and in the Verbano Cusio Ossola Province (Foglini et al., 2018) that showed negative allometric growth. Conversely, the Tiber basin populations showed a positively allometric growth with a slope value greater than 3 (Lorenzoni et al., 2018) (Table 2). These deviations could be related to the differences in size structure and contribution of age classes among the compared bullhead populations due to different environmental conditions and predation pressure. For example, the non-optimal corpulence indicator of the Valgrande bullhead (2.05) was ascribed to a low productive environment and high predatory pressure (Foglini et al., 2018). The other Italian populations, included those of Nossana stream, showed a good body shape having a corpulence indicator included in the optimal range (2.6–3.4).

The analysis of the population structure, together with the analysis of length-frequency distribution, showed that, in presence of trout, 3+ bullhead specimens were shorter while 1+ bullhead ones were

longer (Figure 3a). In addition, at the same time, 3+ downstream adults were a smaller percentage of the total population while 1+ were a larger one (Figure 3e,f) compared to the upstream population. This suggests that the trout, besides decreasing the number of bullhead, could influence the population structure by lowering the life expectancy and promoting faster growth in younger individuals. Indeed, rapid development can occur in populations exposed to strong predator selection to maximize fitness (Melotto, Manenti, & Ficetola, 2020).

The age classes analysis revealed that in Nossana stream bullhead did not have a long life expectancy: this is in agreement with the observations of Zerunian (2002), according to which in water resurgences of the pre-Alpine region the maximum age reaches 4–5 years (and the growth is fast) while mountain habitats are marked by longer life cycles and slower growth (9–10 years, with a length of 15–16 cm). Also, lithology can affect longevity since in soft waters the life expectancy can get to 10 years while in hard waters, such as the one of Nossana, the longevity reaches 4 years (Mills & Mann, 1983).

In Italy, where bullhead is often syntopic with brown trout (Carosi, Ghetti, Forconi, & Lorenzoni, 2015; Franchi et al., 2012; Lorenzoni et al., 2010), as also reported for many European countries, including the United Kingdom (Elliott, 2006; Mann, 1971), Belgium (Van Liefferinge et al., 2005) and Norway (Holmen et al., 2003), the two species can compete for food and/or habitats since they have similar ecological preferences and feeding habits (Lorenzoni et al., 2018; Marconato, 1986; Simon & Townsend, 2003).

As shown through the habitat suitability analysis, the presence of trout induced a bullhead suboptimal habitat occupation: *C. gobio* preferences shifted toward faster flow velocities and shallower depths. This probably occurs to avoid the salmonid predators, that are more skilled in deeper and slower water, and whose larger specimens tend to occupy those habitats. The two species may compete also for food availability (Irons, Sass, McClelland, & Stafford, 2007) and some diversifications in their diet and microhabitat preferences have been already reported in other studies (Elliott, 2006; Franchi et al., 2012). To precisely ascribe the observed bullhead habitat shift to trout predation, instead of food competition, an investigation about the diet of bullhead populations (upstream and downstream) and trout should be

Stream	Region	Study	$L_{inf}$ (cm)	$b$	$L_{max}$ (cm)
Vallaccia creek	Umbria	Froese and Pauly, 2016	—	3.30	12.0
Bagni creek	Umbria	Reported in Lorenzoni, 2018	—	3.23	12.8
Topino river	Umbria	Reported in Lorenzoni, 2018	—	3.16	14.2
Toce stream	Piemonte	Foglini, 2018	15.5	2.81	14.6
S.Giovanni stream	Piemonte	Foglini, 2018	13.5	2.72	12.5
Valgrande stream	Piemonte	Foglini, 2018	12.8	2.05	12.0
Gorga creek	Marche	Lorenzoni, 2018	13.1	2.63	11.8
Cesano creek	Marche	Lorenzoni, 2018	12.7	2.71	10.9
Nossana upstream	Lombardia	Present study	13.72	2.93	14.2
Nossana downstream	Lombardia	Present study	12.17	2.99	13.2

**TABLE 2** Parameters of bullhead populations from different Italian streams



carried out. However, according to macroinvertebrate densities, calculated from the samples collected in the survey period (2016–2020), Nossana stream is a highly productive environment, and the two stretches have comparable macroinvertebrate densities (annual mean of  $734.3 \pm 316.1$  specimens/m<sup>2</sup> and  $745.1 \pm 239.8$  specimens/m<sup>2</sup> in NU and ND, respectively) (Table 1). Nevertheless, the correlation matrix between macroinvertebrate abundances and bullhead densities showed that temporal changes in bullhead density in ND were significantly correlated with temporal changes in Baetidae (negatively) and Chironomidae (positively) abundances ( $\rho = -.79$ ,  $p < .05$  and  $\rho = .80$ ,  $p < .05$ , respectively, Table A1).

The results suggest that interspecific competition phenomena occurred between brown trout and bullhead in the downstream stretch, as observed also in other countries by Elliott (2006), Holmen et al. (2003) and by Franchi et al. (2012) in Central Italy. However, the competition in Nossana stream does not seem particularly strong since the bullhead population keeps good demographic and structural population indexes.

## 5 | CONCLUSION

The present case study showed a marked interspecific competition between brown trout and bullhead populations. This hypothesis is supported by: (i) the significantly lower density and standing crop of bullhead sympatric with brown trout and (ii) the changes in size and age structure of bullhead population between the two stretches, with a reduction in the proportion of adult specimens and an increase in the proportion of younger specimens proportions when trout are present. Moreover, (iii) the disproportionate length between young and adult specimens and (iv) the suboptimal habitat preference of downstream bullhead also supports the hypothesis.

The pressure induced by the presence of brown trout, however, did not seem to be an existential threat to the studied bullhead population, because a good population structure and good growth performance indices were found. However, further analysis on diet is needed to understand if food competition occurs and how direct predation influences the trout–bullhead coexistence. The information collected in the present study showed the effective impact of trout presence on bullhead populations and confirmed that management and regulation of salmonid introductions in streams is needed to protect the native species *C. gobio*.

### ACKNOWLEDGMENTS

This work was funded by Uniacque S.p.a. The authors wish to thank all the people who joined the field activities, Vanessa De Santis for useful advice, and an anonymous reviewer that helped to improve the manuscript. Open Access Funding provided by Università degli Studi di Milano-Bicocca within the CRUI-CARE Agreement.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### ORCID

Luca Bonacina  <https://orcid.org/0000-0002-4695-5932>

Riccardo Fornaroli  <https://orcid.org/0000-0001-6326-5653>

### REFERENCES

- Bagenal, T. B. (1978). *Methods for assessment of fish production in freshwaters*. London: B. S. Publication.
- Barbour, M. T., Gerritsen, J., Blaine, S. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and Wadeable Rivers: Periphyton, benthic macroinvertebrates, and fish. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency Policy, 339. Washington, D.C: Office of Water. Retrieved from. <http://www.epa.gov/OWOW/monitoring/techmon.html>
- Bettoni, Eugenio. (1895). In M. Hoepli (Ed.), *Piscicoltura d'acqua dolce*. Milano: Eugenio Bettoni.
- Boove, K. D. (1986). *Development and evaluation of habitat suitability criteria for use in the instream flow incremental methodology*. Washington, D. C: Ken D. Bovee.
- Budy, P., Thiede, G. P., Lobon-Cervia, J., Fernandez, G. G., McHugh, P., McIntosh, A., ... Jellyman, P. (2013). Limitation and facilitation of one of the world's most invasive fish: An intercontinental comparison. *Ecology*, *94*(2), 356–367.
- Carosi, A., Ghetti, L., Forconi, A., & Lorenzoni, M. (2015). Fish community of the river Tiber basin (Umbria-Italy): Temporal changes and possible threats to native biodiversity. *Knowledge and Management of Aquatic Ecosystems*, *416*, 22. <https://doi.org/10.1051/kmae/2015018>
- Carter, M. G., Copp, G. H., & Szomlai, V. (2004). Seasonal abundance and microhabitat use of bullhead *Cottus gobio* and accompanying fish species in the River Avon (Hampshire), and implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *14*(4), 395–412. <https://doi.org/10.1002/aqc.617>
- Charles, S., Subtil, F., Kielbassa, J., & Pont, D. (2008). An individual-based model to describe a bullhead population dynamics including temperature variations. *Ecological Modelling*, *215*(4), 377–392. <https://doi.org/10.1016/j.ecolmodel.2008.04.005>
- Elliott, J. M. (2006). Periodic habitat loss alters the competitive coexistence between brown trout and bullheads in a small stream over 34 years. *Journal of Animal Ecology*, *75*(1), 54–63. <https://doi.org/10.1111/j.1365-2656.2005.01022.x>
- European Commission. (1992). Council directive 92/43/ECC. *Official Journal of the European Union*, *94*(1259), 40–52.
- European Community. (2000). Directive 2000/60/EC of the European Parliament. *Official Journal of the European Communities*, *43*, 1–84.
- Ferlinghetti, R., Arzuffi, A., & Beretta, L. (2011). Piano di gestione del S.I.C. IT2060009 “Val Nossana - Cima di Grem”. <https://doi.org/10.9790/1684-1302022628>
- Fischer, S., & Kummer, H. (2000). Effects of residual flow and habitat fragmentation on distribution and movement of bullhead (*Cottus gobio* L.) in an alpine stream. *Hydrobiologia*, *422–423*, 305–317. [https://doi.org/10.1007/978-94-011-4164-2\\_25](https://doi.org/10.1007/978-94-011-4164-2_25)
- Foglini, C., Sala, P., Zellino, C., & Volta, P. (2018). Autoecology of the bullhead *Cottus gobio* in the Province of Verbano Cusio Ossola. *IdroLIFE Project*, *1*. <https://idrolife.eu/wp-content/uploads/2021/01/A2-Autoecology-of-Cottus-gobio.pdf>
- Fornaroli, R., Cabrini, R., Sartori, L., Marazzi, F., Canobbio, S., & Mezzanotte, V. (2016). Optimal flow for brown trout: Habitat – Prey optimization. *Science of the Total Environment*, *566–567*, 1568–1578. <https://doi.org/10.1016/j.scitotenv.2016.06.047>
- Fornaroli, R., Calabrese, S., Marazzi, F., Zaupa, S., & Mezzanotte, V. (2019). The influence of multiple controls on structural and functional characteristics of macroinvertebrate community in a regulated Alpine river. *Ecology*, *12*, 1–20.
- Franchi, E., Pompei, L., & Barbaresi, R. (2012). Distribution and abundance of European bullhead *Cottus gobio* in the watercourses of Pesaro-

- Urbino Province (Marche, central Italy). *Italian Journal of Freshwater Ichthyology*, 2, 48–56.
- Freyhof, J., & Kottelat, M. (2011). *Cottus gobio*. The IUCN red list of threatened species. Version 2021-3. Retrieved from <http://www.iucnredlist.org/details/5445/0>
- Frilund, G. E., Koksvisk, J., Rikstad, A., & Berger, H. M. (2009). *Cottus gobio* (Linnaeus, 1758), a new fish-species in Nord-Trøndelag. *Fauna Norvegica*, 29, 55–60.
- Froese, R. (2006). Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. *Journal of Applied Ichthyology*, 22(4), 241–253. <https://doi.org/10.1111/j.1439-0426.2006.00805.x>
- Gandolfi, G., Zerunian, S., Torricelli, P., & Marconato, A. (1991). *I pesci delle acque interne italiane*. Roma: Istituto Poligrafico e Zecca dello Stato.
- Harvey, I., & Cowx, J. (2000). Monitoring the bullhead. *Conserving Natura 2000 Rivers*, 4, 1–30.
- Holmen, J., Olsen, E. M., & Vøllestad, L. A. (2003). Interspecific competition between stream-dwelling brown trout and Alpine bullhead. *Journal of Fish Biology*, 62(6), 1312–1325. <https://doi.org/10.1046/j.1095-8649.2003.00112.x>
- Irons, K. S., Sass, G. G., McClelland, M. A., & Stafford, J. D. (2007). Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? *Journal of Fish Biology*, 71(Suppl. D), 258–273. <https://doi.org/10.1111/j.1095-8649.2007.01670.x>
- Italian Ministry of the Environment. (2010). Decreto 8 November 2010, n. 260 Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali, per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152.
- Jermacz, L., Kobak, J., Dzierżyńska, A., & Kakareko, T. (2015). The effect of flow on the competition between the alien racer goby and native European bullhead. *Ecology of Freshwater Fish*, 24(3), 467–477. <https://doi.org/10.1111/eff.12162>
- Jonsson, B., & Jonsson, N. (2012). Ecology of Atlantic Salmon and Brown Trout – Habitat as a template for life histories. *Springer Fish & Fisheries Series*, 33, 708. <https://doi.org/10.1111/j.1467-2979.2012.00472.x>
- Koli, L. (1969). Geographical variation of *Cottus gobio* L. (Pisces, Cottidae) in Northern Europe. *Annales Zoologici Fennici*, 6(4), 353–390.
- Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the length-weight relationship and seasonal cycle in gonad weight and condition in the perch. *British Ecological Society*, 20(2), 201–219.
- Legalle, M., Santoul, F., Figuerola, J., Mastrorillo, S., & Céréghino, R. (2005). Factors influencing the spatial distribution patterns of the bullhead (*Cottus gobio* L., Teleostei Cottidae): A multi-scale study. *Biodiversity and Conservation*, 14(6), 1319–1334. <https://doi.org/10.1007/s10531-004-9673-7>
- Lorenzoni, M., Carosi, A., Giovannotti, M., Porta, G. L., Splendiani, A., & Barucchi, V. C. (2018). Population status of the native *Cottus gobio* after removal of the alien *Salmo trutta*: A case-study in two Mediterranean streams (Italy). *Knowledge and Management of Aquatic Ecosystems*, 419, 22. <https://doi.org/10.1051/kmae/2018006>
- Lorenzoni, M., Ghetti, L., Carosi, A., & Dolcianni, R. (2010). La fauna ittica ei corsi d'acqua dell'Umbria. Sintesi delle Carte Ittiche regionali dal 1986 al 2009, Petrucci Editore.
- Louhi, P., Mäki-Petäys, A., Huusko, A., & Muotka, T. (2014). Resource use by juvenile brown trout and Alpine bullhead: Influence of interspecific versus intraspecific competition. *Ecology of Freshwater Fish*, 23(2), 234–243. <https://doi.org/10.1111/eff.12072>
- MacCrimmon, H. R., & Marshall, T. L. (1968). World distribution of Brown Trout, *Salmo trutta*. *Journal of the Fisheries Research Board of Canada*, 25(12), 2527–2548. <https://doi.org/10.1139/f68-225>
- Mann, R. H. K. (1971). The populations, growth and production of fish in four small streams in Southern England. *British Ecological Society*, 40(1), 155–190.
- Marconato, A. (1986). Distribuzione dell'ittiofauna e gestione della pesca. *Quaderni ETP*, 14, 139–149.
- Melotto, A., Manenti, R., & Ficetola, G. F. (2020). Rapid adaptation to invasive predators overwhelms natural gradients of intraspecific variation. *Nature Communications*, 11(1), 3608. <https://doi.org/10.1038/s41467-020-17406-y>
- Mildenberger, T. K., Taylor, M. H., & Wolff, M. (2017). TropFishR: An R package for fisheries analysis with length-frequency data. *Methods in Ecology and Evolution*, 8(11), 1520–1527. <https://doi.org/10.1111/2041-210X.12791>
- Mills, C. A., & Mann, R. H. K. (1983). The bullhead *Cottus gobio*, a versatile and successful fish. *Annual Report of the Freshwater Biological Association*, 51, 76–88. Retrieved from. <https://agris.fao.org/agris-search/search.do?recordID=AV2012055893>
- Moran, P. A. P. (1951). A mathematical theory of animal trapping. *Biometrics*, 38, 307–311.
- Pauly, D. (1979). Gill size and temperature as governing factors in fish growth: a generalization of Von Bertalanffy's growth formula. *Bel'Inst. Meereskd. Vniv. Kiel*, 63, 156.
- Pauly, D., & Munro, J. L. (1984). One more on the comparison of growth in fish and invertebrates. *Fishbyte*, 2(1), 21.
- Perrow, M. R., Punchard, N. T., & Jowitt, A. J. D. (1997). *The habitat requirements of bullhead (Cottus gobio), and brown trout (Salmo trutta) in the headwaters of selected Norfolk rivers: Implications for conservation and fisheries*. Eastern Area: Ecological Consultancy & Environment Agency.
- R Core Team. (2020). *R: A language and environment for statistical computing*. Retrieved from [www.R-project.org](http://www.R-project.org)
- Ricker, W. (1975). Computation and of interpretation biological statistics of fish populations. *Journal of Fisheries Research Board of Canada*, 191, 1–382. <https://doi.org/10.1038/108070b0>
- Rondinini, C., Battistoni, A., Peronace, V., & Teofili, C. (2013). Lista rossa dei vertebrati italiani, 56 WWF Italia. Settore Diversità Biologica. Retrieved from [www.iucn.it](http://www.iucn.it)
- Roussel, J. M., & Bardonnnet, A. (1996). Differences in habitat use by day and night for brown trout (*Salmo trutta*) and sculpin (*Cottus gobio*) in a natural brook. Multivariate and multi-scale analyses. *International Journal of Ichthyology*, 20, 43–53.
- Seber, G. A. F., & Le Cren, E. D. (1982). Estimating population parameters from catches large relative to the population. *Journal of Animal Ecology*, 36(3), 631–643.
- Simon, K. S., & Townsend, C. R. (2003). Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. *Freshwater Biology*, 48(6), 982–994. <https://doi.org/10.1046/j.1365-2427.2003.01069.x>
- Splendiani, A., Palmas, F., Sabatini, A., & Caputo Barucchi, V. (2019). The name of the trout: Considerations on the taxonomic status of the *Salmo trutta* L., 1758 complex (Osteichthyes: Salmonidae) in Italy. *European Zoological Journal*, 86(1), 432–442. <https://doi.org/10.1080/24750263.2019.1686544>
- Tomlinson, M. L., & Perrow, M. R. (2003). Ecology of the bullhead. *Conserving Natura 2000 Rivers*, 4(4), 1–19.
- Utzinger, J., Roth, C., & Peter, A. (1998). Effects of environmental parameters on the distribution of bullhead *Cottus gobio* with particular consideration of the effects of obstructions. *Journal of Applied Ecology*, 35(6), 882–892. <https://doi.org/10.1111/j.1365-2664.1998.tb00006.x>
- Van Liefvering, C., Seeuws, P., Meire, P., & Verheyen, R. F. (2005). Micro-habitat use and preferences of the endangered *Cottus gobio* in the River Voer, Belgium. *Journal of Fish Biology*, 67(4), 897–909. <https://doi.org/10.1111/j.0022-1112.2005.00782.x>
- Viganò, G., Confortola, G., Fornaroli, R., Cabrini, R., Canobbio, S., Mezzanotte, V., & Bocchiola, D. (2015). Effects of future climate change on a river habitat in an Italian Alpine catchment. *Journal of*

*Hydrologic Engineering*, 21. [https://doi.org/10.1061/\(ASCE\)HE.1943-5584.0001293](https://doi.org/10.1061/(ASCE)HE.1943-5584.0001293)

Vigna, B., & Banzato, C. (2015). The hydrology of high-mountain carbonate areas: An example of some Alpine systems in southern Piedmont (Italy). *Environmental Earth Sciences*, 74, 267–280.

Vismara, R., Azzellino, A., Bosi, R., Crosa, G., & Gentili, G. (2001). Habitat suitability curves for brown trout (*Salmo trutta fario* L.) in the river Adda, Northern Italy: Comparing univariate and multivariate approaches. *Regulated Rivers: Research & Management*, 50, 37–50.

Von Bertalaffy, L. (1938). A quantitative theory of organic growth. *Human Biology*, 10(1), 181–213. <https://doi.org/10.1016/j.solener.2019.02.027%0A>

Zerunian, S. (2002). In Edagricole (Ed.), *Condannati all'estinzione? Biodiversità, biologia, minacce e strategie di conservazione dei pesci d'acqua dolce indigeni in Italia*. Bologna: Edagricole; pp. X + 220.

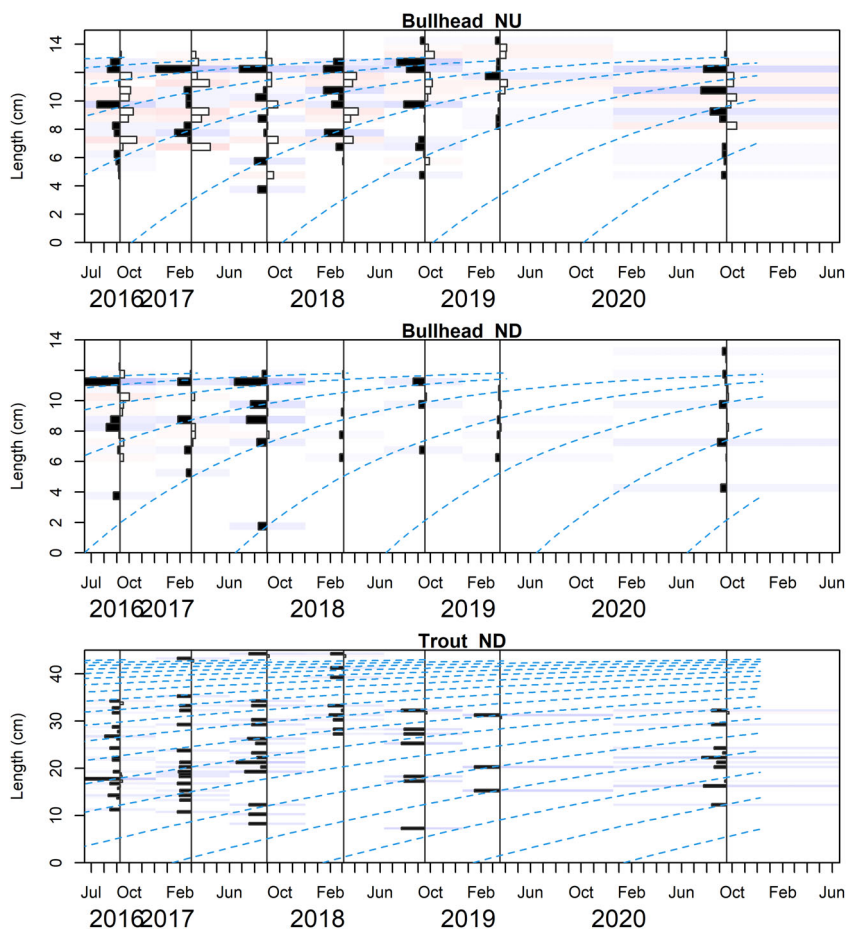
Zerunian, S. (2003). Piano d'azione generale per la conservazione dei Pesci d'acqua dolce italiani. Ministero dell'Am- biente e Istituto Nazionale Fauna Selvatica. *Quaderni Conservazione Natura*, 17, 123.

Zippin, C. (1958). The removal method of population estimation. *Journal of Wildlife Management*, 22(1), 82–90.

**How to cite this article:** Bonacina, L., Canobbio, S., & Fornaroli, R. (2022). Influence of the *Salmo (trutta) trutta* on the population structure, the growth, and the habitat preference of a *Cottus gobio* population. *River Research and Applications*, 1–16. <https://doi.org/10.1002/rra.4018>

## APPENDIX A

Figure A1



**FIGURE A1** Output of the analysis of the length-frequency distribution. Positive and negative bins are represented with black and white bars [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



TABLE A1 (Continued)

P		$\rho$																	
		Leucotruidae_NU	Perlo didae_NU	Nemo uridae_NU	Baetidae_NU	Hepta geniidae_NU	Brachy centridae_NU	Glossomatidae_NU	Hydro psychidae_NU	Limne philidae_NU	Philotamidae_NU	Rhyacophilidae_NU	Elmidae_NU	Hydracnidae_NU	Blephariceridae_NU	Chironomidae_NU			
Elmidae_ND	n.s	<.01	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	n.s	n.s	n.s	n.s			
Athericidae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Blephariceridae_ND	n.s	<.01	<.05	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	<.05	<.01	n.s	n.s	n.s			
Chironomidae_ND	n.s	n.s	<.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Limoniidae_ND	n.s	<.01	<.05	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	<.05	n.s	n.s	n.s	n.s			
Psychodidae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Simuliidae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Gammaridae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Lumbriculidae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Lumbricidae_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
Tubificidae_ND	n.s	<.05	<.05	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	<.05	<.05	n.s	n.s	n.s			
d_C.gobio_NU	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
d_C.gobio_ND	n.s	n.s	<.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<.05			
d_S.trutta_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
sc_C.gobio_NU	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
sc_C.gobio_ND	n.s	n.s	<.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s			
sc_S.trutta_ND	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s			
P		$\rho$																	
		Limonidae_NU	Simuliidae_NU	Ancyloides_NU	Dugesidae_NU	Leucotruidae_NU	Perlo didae_NU	Nemo uridae_NU	Baetidae_NU	Hepta geniidae_NU	Brachy centridae_NU	Limne philidae_NU	Rhyacophilidae_NU	Elmidae_NU	Athericidae_NU	Blephariceridae_NU	Chironomidae_NU	Limonidae_NU	Psychodidae_NU
.29	.22	-.43	.64	.28	.82	.14	-.22	-.58	.32	.76	-.22	.17	.22	.12	.58	.22	.32	.62	.62
-.49	-.81	.41	.62	.30	.37	.44	-.74	-.36	-.62	-.56	.25	-.94	.12	.93	.58	-.90	-.62	-.20	-.20
-.57	-.90	.20	.41	.02	.63	.61	-.89	-.39	-.20	-.48	-.02	-.71	-.07	.85	.80	-.78	-.20	-.20	-.20
.20	.54	-.41	-.61	.27	-.07	.16	.57	.07	-.20	.04	.46	.50	.09	-.58	-.04	.30	-.20	-.20	-.20
.33	.18	-.41	.41	.41	-.82	.47	-.14	-.68	.00	.70	.09	.21	.37	.15	.09	.00	.00	.00	.00
.45	.34	-.17	-.17	-.41	-.21	-.31	.00	.20	1.00	.64	-.52	.61	.00	-.44	-.25	.68	1.00	1.00	1.00
.45	.34	-.17	-.17	-.41	-.21	-.31	.00	.20	1.00	.64	-.52	.61	.00	-.44	-.25	.68	1.00	1.00	1.00
.23	-.34	1.00	-.17	-.10	.42	-.31	-.20	.61	-.17	-.42	.31	-.41	.43	.22	-.25	-.34	-.17	-.17	-.17
.37	.90	-.61	-.41	.09	-.56	-.29	.82	.11	.20	.48	.02	.79	-.07	-.85	-.45	.78	.20	.20	.20
.37	.37	-.25	-.25	.67	-.14	.40	.09	-.13	-.25	.09	.76	.22	.56	-.24	.28	.07	-.25	-.25	-.25

(Continues)



TABLE A1 (Continued)

$\rho$	Simuliidae_NU	Ancyloides_NU	Dugesia_NU	Leucotrachea_NU	Perloides_NU	Nemouridae_NU	Baetidae_NU	Heptageniidae_NU	Brachycentrines_NU	Limnephilidae_NU	Rhyacophilidae_NU	Elmidae_NU	Athericidae_NU	Blephariceridae_NU	Chironomidae_NU	Limonidae_NU	Psychodidae_NU	
	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	n.s	n.s	n.s	n.s	n.s	n.s	<.01	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<.05	n.s	n.s	n.s
$\rho$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Simuliidae_ND	Gammaidae_ND	Lumbricidae_ND	Lumbriculidae_ND	Lumbricidae_ND	Tubificidae_ND	Heptageniidae_ND	Brachycentrines_ND	Limnephilidae_ND	Rhyacophilidae_ND	Elmidae_ND	Athericidae_ND	Blephariceridae_ND	Chironomidae_ND	Limonidae_ND	Psychodidae_ND			
-.09	.25	-.37	-.36	.64	.15	-.87	.36	.02	.30	.30	.30	.17	.69					
.66	-.44	.36	.31	.62	-.87	.36	.31	.38	.09	.09	.09	.49	-.18					
.36	-.45	.18	.61	.41	-.87	.47	.61	.71	.50	.50	.50	.86	-.14					
-.58	.11	-.36	-.07	-.61	.43	.00	-.07	-.29	.00	.00	.00	-.36	.11					
-.31	.43	-.22	.36	.41	.00	.47	.36	-.18	.29	.29	.29	.11	.71					
-.51	.32	-.25	.20	-.17	.47	.47	.20	.20	.41	.41	.41	.20	.41					
-.51	.32	-.25	.20	-.17	.47	.47	.20	.20	.41	.41	.41	.20	.41					
.21	.00	.76	-.20	-.17	-.35	.87	-.20	.00	-.20	-.20	-.20	.00	-.20					
-.50	.30	-.53	-.32	-.41	.87	.47	-.32	-.50	-.21	-.21	-.21	-.64	.21					
-.58	.00	-.39	.40	-.25	.18	.47	.40	.04	.40	.40	.40	.04	.53					
-.21	.22	-.11	-.45	-.31	.81	.47	-.45	-.27	-.29	-.29	-.29	-.53	.09					
.28	.01	.18	.50	.62	-.80	.47	.50	.34	.38	.38	.38	.70	.05					
.00	.64	.51	-.41	-.17	-.35	.47	-.41	-.61	-.41	-.41	-.41	-.20	-.41					
-.08	-.08	-.39	-.47	-.26	.73	.47	-.47	-.47	-.47	-.47	-.47	-.79	.00					
.13	-.59	.02	.60	.10	-.44	.47	.60	.87	.60	.60	.60	.76	.00					
-.70	.50	-.02	.04	-.34	.56	.47	.04	-.22	.18	.18	.18	-.22	.71					
-.51	.22	-.52	-.24	-.34	.97	.47	-.24	-.36	-.12	-.12	-.12	-.60	.42					
.21	.00	.76	-.20	-.17	-.35	.47	-.20	.00	-.20	-.20	-.20	.00	-.20					
.62	-.43	-.25	.41	1.00	-.35	.47	.41	.41	.20	.20	.20	.41	.20					
.04	-.47	-.49	.47	.41	.07	.47	.47	.31	.32	.32	.32	.13	.54					
.30	-.58	.23	.11	-.21	-.45	.47	.11	.52	.11	.11	.11	.37	-.56					
-.14	.07	.00	.58	.21	-.66	.47	.58	.23	.49	.49	.49	.59	.16					
-.25	.37	-.04	-.79	-.61	.72	.47	-.79	-.79	-.68	-.68	-.68	-.93	-.25					
-.13	-.02	.27	-.46	-.61	.43	.47	-.46	-.14	-.32	-.32	-.32	-.43	-.21					
-.51	.32	-.25	.20	-.17	.47	.47	.20	.20	.41	.41	.41	.20	.41					

(Continues)

