

Original Research

**ENVIRONMENTAL VARIABLES AFFECT DISTRIBUTIONAL PATTERNS OF
THE RIFFLE BEETLE (Coleoptera: Elmidae) SPECIES IN THE SERBIAN
STREAMS AND RIVERS**

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Abstract

This study examined the impact of environmental variables on distributional patterns of 12 riffle beetle species (Coleoptera: Elmidae) in the Serbian streams and rivers. During six-year period (2019–2024), a total of 68 waterbodies of the Serbian streams and rivers from that riffle beetles were included (of total 226 running waterbodies covered by water status monitoring). During Regular National Annual Water Monitoring Programmes of the SEPA (Serbian Environmental Protection Agency), riffle beetle sampling was carried out simultaneously with aquatic macroinvertebrate samples in line with the AQEM protocol (multi-habitat sampling). Among all environmental variables analyzed, a total of 16 variables most important for aquatic macroinvertebrate community in general, and species' stream/river longitudinal distribution, were selected. Canonical Correspondence Analysis (CCA) revealed that the most significant parameters for each riffle beetle species depending on the species-specific ecological preference, and on the other hand, with regard to aquatic beetle species longitudinal distribution, the species' distributional patterns only corresponded with the RCC (River Continuum Concept) principle in larger Serbian rivers. It is concluded that limited riffle beetle species distribution in small and medium submountain and mountain streams/rivers is primarily affected by local water quality conditions and species-specific microhabitat preference rather the principle proposed by the RCC.

Keywords: riffle beetle, abiotic factors, distributional pattern, streams, rivers, Serbia.

INTRODUCTION

The longitudinal distribution of aquatic beetles in streams and rivers is mainly affected by environmental gradients that change from upstream to downstream. Principally, important variables include water temperature, flow velocity, dissolved oxygen, substrate type, vegetation cover, nutrient levels, and stream size. Upstream sections are typically cold, fast-flowing, and oxygen-rich, supporting specialized rheophilic species, while downstream

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reaches are warmer, slower, and richer in fine sediments and vegetation, favoring lentic-associated beetles. Human impacts such as pollution, dams, and land-use change can also modify these natural distribution patterns.

The River Continuum Concept (RCC) was first introduced by [1]. The concept asserts that the biological community in a stream system is largely regulated by geomorphological factors, and it was unique in that it proposed that streams were continua of both geomorphologic and biological factors: a new idea in ecosystemology at that time. The concept divides a river system into three major groups based on stream position order and comprising headwater streams, medium-sized streams, and large rivers. The transport, use, and storage of organic matter along the continuum take place in a symbiotic relationship with the distribution of macroconsumer groups. Also, this concept asserts that the macroinvertebrate community will exist in a fashion that optimally exploits its nutritive source of organic matter [2]. The present study also analyze did the riffle beetle species' distributional patterns correlated with the River Continuum Concept (RCC) principle.

Riffle beetles (order Coleoptera, family Elmidae), both larvae and adults, do not come to the surface to breathe. Instead, they rely on a specialized physical gill called a plastron – a dense, water-repellent layer of fine hairs that traps a thin film of air against their bodies. The longitudinal distribution of riffle beetles is strongly influenced by changes in water chemistry along the river continuum. Upstream reaches usually have cold, well-oxygenated water, low conductivity, low nutrient concentrations, and neutral to slightly acidic pH, which favor sensitive riffle beetle species adapted to fast-flowing habitats. Midstream sections often contain moderate nutrient and organic matter levels that can support higher species diversity. Downstream reaches generally show warmer water, higher conductivity, increased nutrients, and lower dissolved oxygen, conditions that reduce the abundance of many riffle beetles because most species require clean, oxygen-rich water. Plastron, permanent air film functions as a physical gill, allowing oxygen to diffuse from the surrounding water into the plastron while carbon dioxide diffuses out. Because of this mechanism, riffle beetles can remain submerged for long periods without surfacing. Also, riffle beetles provide excellent indicators of water quality and perhaps also climate change [3].

The main goal of this study is to provide the data on significance of different environmental variables in a six-year period (2019–2024) to riffle beetle species' distribution in the Serbian streams and rivers.

MATERIALS AND METHODS

Riffle beetle samples were taken using a hand net (25x25 cm, 500 µm mesh size) or specimens were manually collected using a tweezer. The samples were collected in the period 2019–2024 (six years, July–November). The majority of aquatic beetle specimens were collected with other aquatic macroinvertebrate samples during the Regular Annual Water Monitoring programmes conducted by the Serbian Environmental Protection Agency (SEPA). The multi-habitat sampling procedure [4] and AQEM protocol [5] were applied. The samples were preserved using 70–90% ethyl-alcohol. Containers were labelled with all necessary information, and transported to the laboratory for sorting, counting, and identification. For

identification, the stereomicroscope Carl Zeiss StereoDiscovery V.8 with Axio Cam ICc5 and ZEN2 imaging software and appropriate taxonomic aquatic beetle keys were used.

A total of 12 riffle beetle species, sampled in the Serbian streams and rivers in a six-year period (2019–2024) period at 68 watercourses (of total 226 running waterbodies covered by water status monitoring), were studied with regard to the selected environmental variables: *Elmis cf. aenea*, *Elmis cf. maugetii*, *Elmis cf. rioloides*, *Limnius volckmari*, *Limnius opacus*, *Limnius intermedius*, *Oulimnius tuberculatus*, *Riolus cupreus*, *Macronychus quadrituberculatus*, *Potamophilus acuminatus*, *Esolus angustatus* and *Esolus parallelepipedus*. Among all riffle beetle species recorded, some are common (e.g. *Elmis cf. maugetii*, *Limnius opacus*), but some, such as *Macronychus quadrituberculatus* and *Potamophilus acuminatus*, are scarcely found in Serbia. Regarding species' abundance per sampling site, a total abundance of beetle specimens is included. Due to well-known larval identification problem for this family, only imago specimens were considered, except three species for which larval stages were identified: *Riolus cupreus*, *Macronychus quadrituberculatus* and *Potamophilus acuminatus*.

Among all environmental variables measured, a total of 16 variables most important for aquatic macroinvertebrate assemblages in general, and species' distribution along stream/river, were selected, based on general ecological requirements of aquatic macroinvertebrate community – Physico–chemical parameters: pH, water temperature, water conductivity, dissolved oxygen (DO), oxygen saturation (%), orthophosphate (PO_4^{3-}), total phosphorus (TP), ammonium (NH_4^+), nitrates (NO_3^-), total nitrogen (TN), total organic carbon (TOC); Abiotic parameters: air temperature, water flow, sampling site elevation, canopy and riparian vegetation coverage. Water chemistry parameters were analyzed according to standardized analytical methods proposed for each parameter measured for the purposes of Regular Annual Monitoring Programmes conducted by the Serbian Environmental Protection Agency (SEPA), and the mean annual values of each parameter were included with regard to significance to riffle beetle species distribution. Also some additional parameters, not measured in regular water monitoring programmes, and found to be as important for benthic invertebrate fauna, were measured in the field, such as: sampling site elevation, water flow (preliminary rapid field estimation), and canopy and riparian vegetation coverage at sampling site (visual observation).

To get more precise data on effect of measured environmental variables to riffle beetle assemblages as well as species' distribution in the investigated Serbian streams and rivers (2019–2024 period), as well as correlations referred to composition of populations and environmental variables, Canonical Correspondence Analysis (CCA) was used [6–8]. Length of the vector represents its significance in the mean gradient. The analysis of the relationship between the structure of populations of elmids species and environmental variables was carried out on the same selected set of samples within all 226 sampling stations (2019–2024 period). The selection of the most significant influencing factors was conducted out using the previous (*forward*) selection, using the Pearson correlation test within the CCA framework analysis. Applying prior selection, based on Pearson's correlation test ($p \leq 0.05$), the gradient impact of environmental variables on each riffle beetle species populations was determined, and the most indicative parameters for riffle beetles were selected. Monte Carlo permutation test

(1000 permutations, $p \leq 0.05$) was used for confirming a statistical significance of the sum of all eigenvalues of individual factors. Canonical Correspondence Analysis (CCA) was performed in the statistical software Flora [8].

RESULTS AND DISCUSSION

Based on the Canonical Correspondence Analysis (Figure 1), by observing the relationship between parameters and riffle beetle species in the middle gradients, it was determined that there is a separation by the studied riffle beetle species, and also by the measured environmental variables. By using Monte Carlo permutation test (1000 permutations), a statistical significance of the sum of all individual eigenvalues was proven ($p \leq 0.05$).

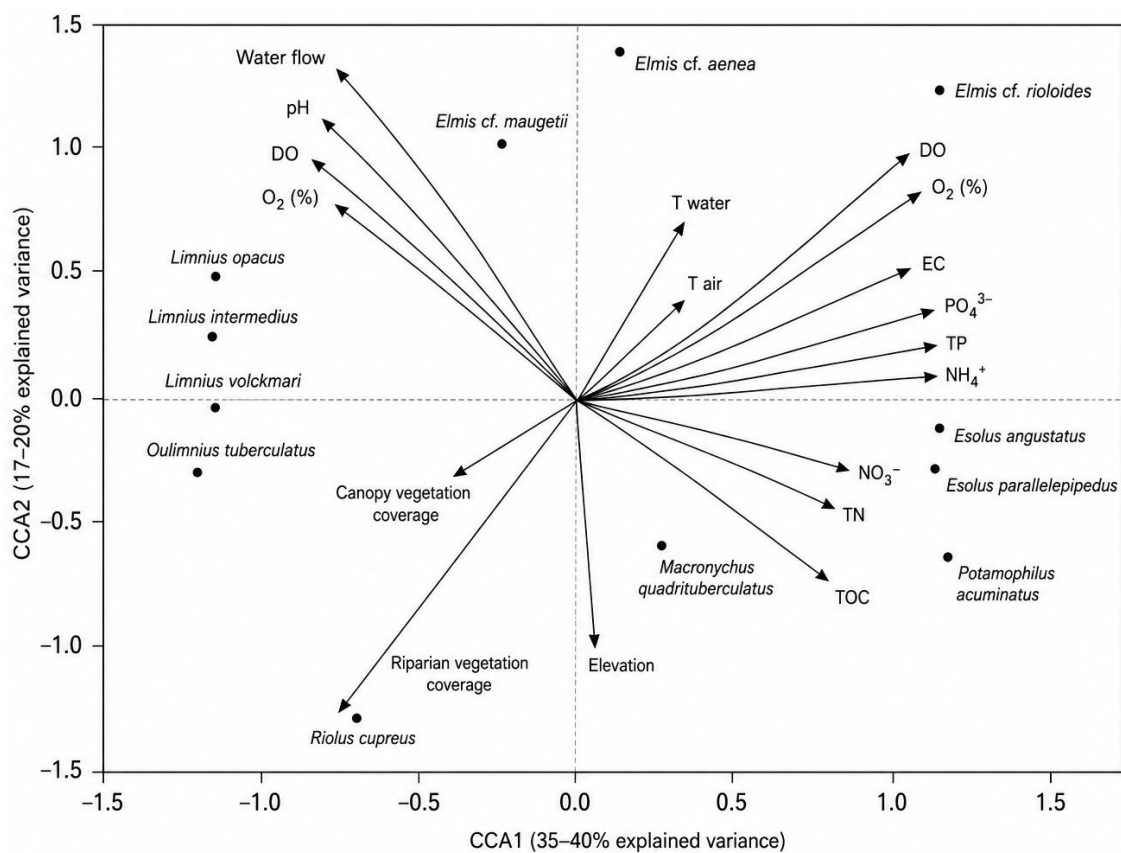


Figure 1 Canonical Correspondence Analysis (CCA) – Riffle beetle species and environmental variables

Canonical Correspondence Analysis (CCA) explained the relationship between species distribution and environmental variables across the studied stream/rivers in Serbia. The first axis, CCA1 (35–40% explained variance), mainly represents a gradient from clean, fast-flowing, oxygen-rich habitats on the negative side to warmer, nutrient-enriched, and more disturbed habitats on the positive side. The second axis, CCA2 (17–20% explained variance), reflects variation associated with temperature, elevation, and vegetation coverage. Species positioned close to environmental vectors are positively associated with those variables, while species located opposite to the vectors are negatively associated.

Species such as *Limnius opacus*, *Limnius intermedius*, *Limnius volckmari*, and *Oulimnius tuberculatus* are distributed on the negative side of CCA1, indicating strong associations with high water flow, elevated dissolved oxygen (DO), higher oxygen saturation (%), and relatively high pH. These species are therefore characteristic of clean, well-oxygenated, and fast-flowing stream environments with low nutrient concentrations. Among them, *Limnius opacus* and *Limnius intermedius* appear particularly sensitive to pollution and nutrient enrichment, making them good indicators of healthy stream ecosystems. *Limnius volckmari* occupies a more central position, suggesting moderate ecological tolerance, whereas *Oulimnius tuberculatus* is additionally associated with canopy and riparian vegetation, indicating the species' preference for shaded habitats.

Riolus cupreus is strongly associated with riparian vegetation coverage, canopy vegetation, and higher elevation, while being negatively related to nutrient variables. Its CCA position suggests that this species prefers cold, forested, minimally disturbed submountain and mountain streams with dense vegetation cover and low anthropogenic influence.

Elmis cf. maugetii and *Elmis cf. aenea* CCA positions indicated relationship with oxygen-rich and flowing water conditions. *Elmis cf. maugetii* is closely associated with water flow and high oxygen availability, reflecting preference for relatively clean running waters. *Elmis cf. aenea* shows a stronger association with the upper part of CCA2, suggesting tolerance for slightly warmer waters while still requiring good oxygen conditions.

Elmis cf. rioloides is positioned on the positive side of both axes and is positively associated with dissolved oxygen, oxygen saturation, conductivity (EC), and orthophosphate (PO_4^{3-}). This indicates that the species can tolerate moderate nutrient enrichment while still favoring oxygenated habitats. Its ecological position suggests adaptability to intermediate environmental conditions.

Species located on the positive side of CCA1, including *Esolus angustatus*, *Esolus parallelepipedus*, *Macronychus quadrituberculatus* and *Potamophilus acuminatus* are associated with higher nutrient concentrations, warmer temperatures, increased conductivity, and increased organic matter. *Esolus angustatus* and *Esolus parallelepipedus* are closely related to nitrate (NO_3^-), ammonium (NH_4^+), and electrical conductivity (EC), indicating tolerance to nutrient-enriched and potentially disturbed habitats. *Macronychus quadrituberculatus* is associated with total organic carbon (TOC) and total nitrogen (TN), suggesting preference for organic-rich stream environments. *Potamophilus acuminatus*, positioned furthest toward the enriched conditions, is strongly associated with TOC, TN, nitrate (NO_3^-), and higher water temperatures, indicating the highest tolerance to organic pollution and eutrophication among the recorded taxa.

Overall, the CCA showed a distinct ecological separation between sensitive riffle beetle species inhabiting clean, oxygen-rich, forested streams, and on the other hand, low-tolerant riffle beetle species adapted to nutrient-enriched, warmer, and more disturbed aquatic environments.

CONCLUSION

Riffle beetles are strongly associated with clean, fast-flowing freshwater habitats, and species distribution is mainly affected by environmental conditions related to water chemistry and habitat structure. Dissolved oxygen is one of the most important factors for riffle beetles in general, due to both larvae and adults depend on highly oxygenated water for respiration. Current velocity also plays a major role, as riffle beetles are specially adapted to live in riffles and flowing sections of streams and rivers. The efficiency of the plastron depends on high dissolved oxygen concentrations in the water. In polluted rivers, especially where organic pollution and eutrophication occur, dissolved oxygen levels decrease due to increased microbial decomposition and nutrient enrichment. Fine sediments and contaminants can also interfere with the plastron surface and reduce gas exchange efficiency. Therefore, many riffle beetle species are highly sensitive to pollution and are usually absent from degraded or oxygen-poor habitats, which is corroborated by the data obtained from the present study.

Water temperature influences species composition, with most riffle beetles preferring cool and stable thermal conditions commonly found in upstream and midstream stretches of stream/rivers. Substrate type is another key variable, since coarse materials such as gravel, cobbles, and stones provide surfaces for attachment, feeding, and shelter, but not considered in our study due to complexity. Measured water chemistry variable values, including low conductivity, low nutrient concentrations, and neutral to slightly acidic pH, are generally associated with higher riffle beetle diversity and abundance.

Organic pollution, eutrophication, and excessive sedimentation are negatively affect riffle beetle communities by reducing oxygen availability and covering coarse substrates with fine sediments.

The distribution of riffle beetle species in running waters of Serbia could not be explained by the River Continuum Concept (RCC), except in case of larger rivers. According to the RCC, upstream and midstream river stretches are characterized by low temperatures, fast currents, coarse substrates, and high dissolved oxygen concentrations. These conditions favor riffle beetles because their plastron respiration requires oxygen-rich flowing water. In these sections, riffle beetles can attach to stones and gravel and efficiently exchange gases through the plastron.

This River Continuum Concept (RCC) principle stated that physical and chemical conditions change gradually from upstream to downstream, leading to predictable changes in aquatic macroinvertebrate assemblages. In larger rivers downstream stretches are characterized by warmer water, slower, and richer in fine sediments and organic matter, while dissolved oxygen often decreases. Based on our results, longitudinal distribution of riffle beetles follows the environmental gradients predicted by the RCC only in case of larger Serbian rivers where riffle beetle species are distributed, but not supported the RCC principle for small and medium submountain and mountain streams/rivers, where local water microhabitat conditions and species-specific microhabitat preference shaped riffle beetle species distributional patterns.

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