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Preliminary Study of the Relationship Between Caddisfly Larvae and Environmental Variables in Şimşir Stream (Karabük, TURKEY)

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ABSTRACT

Şimşir Stream is located in Yenice Forest, which has been selected as one of the 100 forest hotspots with high biodiversity and needs urgent protection, and the study area includes two protection zones. Determining the current situation in an area that has not yet been exposed to human influence helps to monitor the response of the rivers in cases that may arise later, such as climate change, pollution, land use, and morphological degradation. Trichoptera are one of the most important groups of macroinvertebrates used to assess such disturbances in streams. Trichoptera larvae were collected from 8 stations along the Şimşir Stream during 2019 and 2020 seasonally by kick-sampling. During this investigation, 17 genera belonging to 15 families were identified. Environmental variables were recorded to determine the relationship with larvae. Most of the stations, except the station located on the tributary, showed positive correlations with water velocity, dissolved oxygen, pH, electrical conductivity, and stream width. It was found that the rate of the boulder in the substrate was one of the most important variables affecting the distribution of larvae. The only station that was sampled in the tributary of the stream had different taxa from other stations, and this showed the contribution of the tributary to the fauna.

Key words: Yenice Forest, *Thremma anomalum*, *Helicopsyche bacescui*, forest hot spot, reference, condition.

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INTRODUCTION

Biological monitoring uses systematically the biological responses to determine environmental changes (Rosenberg & Resh, 1993). Benthic macroinvertebrates are commonly used as indicators in biomonitoring studies in running waters. This group is preferred because it consists of animals that tend to move less, have a wide species richness that can be affected to varying degrees by disturbances from different sources, and have a life cycle long enough to monitor (Reece & Richardson, 1999; Niemi & McDonald, 2004; Bae, Kil, & Bae, 2005). First of all, it is necessary to know the community structure and its relationship with undisturbed environmental variables in running waters where there is no human impact to carry out biological monitoring (Hodkinson & Jackson, 2005). This state, which is free from human influence, is known as the "reference condition". In the case of physical, chemical, or morphological degradation, the change in the habitat quality of the stream can be determined by understanding how much deviation is from this reference condition according to the presence, absence, or abundance data of macroinvertebrate (Council of European Communities, 2000). Trichoptera are one of the most important groups of macroinvertebrates used in biological monitoring studies to determine the effects of organic pollution (Dohet, 2002), climate change (Hering et al, 2009; Sáinz-Bariáin et al, 2016), metal pollution (Sola & Prat, 2006), morphological deterioration (Chakona, Phiri, & Day, 2009), and water velocity (de Brouwer, Besse-Lototskaya, ter Braak, Kraak, & Verdonschot, 2017) in aquatic ecosystems. They are found in almost all kinds of river habitats and the diversity of species on a wide scale for different river conditions has made the Trichoptera order a group that can be used in biological monitoring studies.

Yenice Forest was selected as one of the 100 forest hotspots with high biodiversity with urgently required protection by the World Wide Fund for Nature (WWF) in 1999, and it was determined as one of the Key Biodiversity Areas by the Doğa Derneği according to criteria set by the International Union for Conservation of Nature (IUCN) (Erciyas & Lise, 2006). It is shown as one of the 122 plant areas in Turkey that should be protected (Avcı, 2005). Yenice Forest, Turkey's largest uninterrupted forest (Erciyas & Lise, 2006), includes 3 different protection areas: Yenice Wildlife Development Area, Çitdere Nature Protection Area, and Kavaklı Nature Protection Area. The Şimşir Stream and Çitdere which are subjects of this project, are located in the first two protection areas.

Studies on benthic macroinvertebrates in the region are limited. Tanatmış (2004) conducted a study on the order Ephemeroptera found in the Filyos (Yenice) River and one of the 54 stations is located in the Şimşir Stream. Sipahiler (2014) collected adult Trichoptera specimens in Zonguldak and Karabük provinces, including Yenice Forest, and identified the species found in these regions. However, a study investigating the relationship of larvae with environmental variables has not been conducted in this region.

The goal of this paper was to determine the relationship between Trichoptera larvae and environmental variables in Şimşir Stream in Yenice Forest, which is currently far from human influence. By comparing the future studies in this region with the data obtained from these studies, it can be determined whether there is any deterioration in the stream and the degree of deterioration.

MATERIALS AND METHODS

Study area

Şimşir Stream, which takes its source from Hodulca Hill in the Western Black Sea Region, passes through Şeker Canyon and mixes with the Filyos River, and discharges into the Black Sea (Fig. 1). The rugged topography restricted human activities in Yenice Forest, thus ensuring that the biodiversity of the region remains intact (Erciyas & Lise, 2006; Öztekinci & Coşkun, 2021).

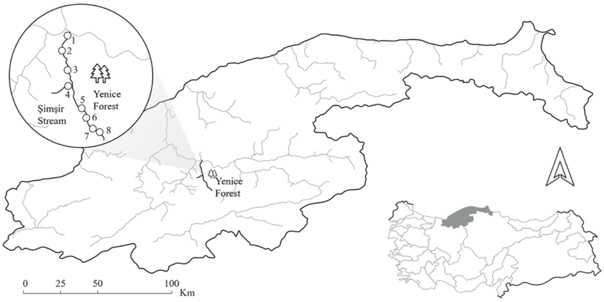

Fig. 1. Northwestern Anatolia and stations studied on Şimşir Stream.

Yenice Forest is Turkey's largest uninterrupted forest (Erciyas & Lise, 2006) and the most important reason for maintaining this integrity is its high average elevation and slope values; this slope rises up to 33% in Şimşir Stream (Öztekinci & Coşkun, 2021). In this forest, mixed natural old forests form the margins of the Şimşir Stream and Çitdere catchment areas (Erciyas & Lise, 2006). The basin where the Şimşir Stream is located has Cretaceous lithological units and contains limestone (Coşkun, 2020).

Sampling and identification

Benthic macroinvertebrate larvae were collected seasonally from 8 stations along the stream during 2019 (summer, autumn) and 2020 (spring, summer, and autumn) by kick-sampling (D-frame net with 500 µm mesh size). While the 7 stations were located on the main branch of Şimşir Stream, only one station (the 4th station) was located on the tributary of the stream. Other tributaries of the stream were also selected for sampling, but this could not be done because they were dry most of the year. Collected samples were passed through sieves with different mesh sizes to separate unwanted materials such as stones, leaves, and twigs, and they were taken to the laboratory by adding ethyl alcohol. Trichoptera larvae were separated and identified with the Leica EZ4 stereomicroscope to the lowest possible systematic level. In cases where no distinction could be made at the genus level, the two genera were given together (such as Ser/Sch). Due to the high endemism rate of Trichoptera species in Turkey, the fact that the diagnoses are mostly made on males, and the lack of association studies of larvae with adults, it is often not possible to diagnose larvae at the species level. Larvae can be described by methods such as metamorphotype, DNA barcoding, rearing of larvae, or raising eggs from adults, but these studies are very limited for this group in Turkey (Sipahiler, 2007, 2013a, 2013b; Ekingen & Kazancı, 2019).

Environmental data

Environmental variables such as pH, electrical conductivity, dissolved oxygen, and temperature were measured in situ using Hanna 98194, simultaneous with benthic invertebrate sampling. Water velocity was measured by Hydro-bios rod held current meter. Variables such as the riparian vegetation, the structure of the stream substrate, shading, and stream width were recorded visually. Cummins (1962) and Harrelson, Rawlins, and Potyondy (1994) were followed to determine the size of the substrate. The two categories given as “pebble” (16-64 mm) and “gravel” (2-26 mm) are combined and given as a single category (gravel) between 2-64 mm. The CANOCO program (Ter Braak &Smilauer, 2002) was used to determine the effects of these environmental variables on the distribution of Trichoptera larvae. Variables showing high correlation with each other were not included in the analysis.

RESULTS AND DISCUSSION

The sampled stations are located between 188 m a.s.l and 1001 m a.s.l (Fig. 2). The dissolved oxygen values were measured between 7.6 and 13.91 mg/l, whereas the water temperature ranged from 10.41 to 19.53 °C. The alkalinity of pH values ranging from 7.58 to 8.65 is related to the geological structure of the region containing limestone (Coşkun, 2020). Electrical conductivity values were measured between 76 and 385 μS/cm. The lowest electrical conductivity values were measured at the 4th station, which is a tributary of the stream, during all periods. The stream width at the sampled stations varies between 2 and 12 m. It was observed that the stream water velocity varied between 0.20 and 1.02 m/s. Since vegetation and shading around the stream were intense and similar at all stations, these environmental variables were not included in the analysis.

Sipahiler (2014) stated that 29 adult species belonging to 26 genera and 18 families inhabited Karabük province. During the study, 17 genera belonging to 15 families were found (Table 1). *Rhyacophila* Pictet, 1834 and *Hydropsyche* Pictet, 1834 larvae were found in most of the samples (94.4% and 91.6%, respectively), and in this respect, it is similar to a study conducted in rivers in the Eastern Black Sea Region (Ekingen & Kazanci, 2021). In the CCA analysis, the first and second axes show strong species-environment correlations (r: 0.95 and r: 0.70, respectively) (Table 2). While the first axis in the CCA ordination diagram represents 48% of the variations that can be explained by environmental variables, the two axes together represent 66% (Monte Carlo perm. Test, p-value: 0.020).

Table1. List of genera/species found in the stations in Şimşir Stream during the study.

Family	*Genus/Species*	S1	S2	S3	S4	S5	S6	S7	S8
Rhyacophilidae	*Rhyacophila*	*	*	*	*	*	*	*	*
Glossosomatidae	*Glossosoma*		*	*	*	*			
Philopotamidae	*Philopotamus*				*			*	*
	Wormaldia			*	*				
Polycentropodidae	*Plectrocnemia*			*					
Hydropsychidae	*Diplectrona*				*	*			
	Hydropsyche	*	*	*	*	*	*	*	*
Psychomyiidae	*Tinodes*	*						*	
Brachycentridae	*Micrasema*		*	*	*		*	*	*
Uenoidae	*Thremma anomalum*				*				
Goeridae	*Lithax/Silo*			*	*	*		*	
Lepidostomatidae	*Lepidostoma/Dinarthrum*					*		*	
Limnephilidae	*Chaetopteryx*								*
Sericostomatidae	*Oecismus*					*			
	Sericostama/Schizopelex			*	*	*	*	*	*
Beraidae	*Beraeamyia*	*			*	*			*
Helicopsychidae	*Helicopsyche bacescui*				*				

Table 2. Results of canonical correspondence analysis (CCA).

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.333	0.133	0.098	0.05	2.031
Species-environment correlations:	0.947	0.703	0.621	0.646	
Cumulative percentage variance					
of species data:	17	23.8	28.9	31.4	
of species-environment relation:	47.3	66.2	80.2	87.2	
Sum of all eigenvalues					1.955
Sum of all canonical eigenvalues					0.704

According to the CCA analysis (Fig. 3), the first three variables that best explain the change in the distribution of the larvae were stream width, altitude, and the percentage of the boulder on the stream substrate. It was seen that the stations were mostly ordered according to the altitude. (Fig. 3). Most of the stations showed positive correlations with water velocity, dissolved oxygen, pH, electrical conductivity, and stream width.

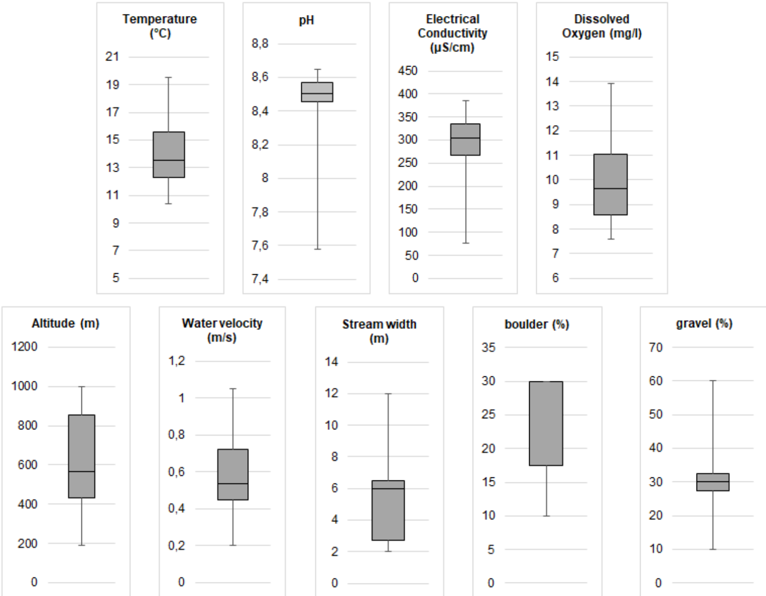

Fig. 2. Boxplot of nine environmental variables.

Station 4, unlike other stations, was not located on the main branch of Şimşir Stream, but it was sampled on a tributary at 567 m a.s.l. According to the CCA graph, Station 4 was located in a different place than the other stations (Fig. 3). The physicochemical variables that distinguished station 4 from most of the other stations were stream width, pH, electrical conductivity, and water velocity. This difference was also observed in the composition of Trichoptera in the station. In this study, *Thremma anomalum* McLachlan, 1876 and *Helicopsyche bacescui* Orghidan and Botosaneanu, 1953 were only found at this station. The habitats of the genera of the Uenoidae family, to which the genus *Thremma* belongs, are restricted to cold and small mountain streams (Vineyard & Wiggins, 1988). Although it was stated in the literature that *T. anomalum* was found only in cold waters near the headwaters (Graf, Murphy, Dahl, Zamora-Munoz, & Lopez-Rodriguez, 2008), Živić et al (2013) stated that the habitat of *T. anomalum* was not limited to cold waters only, so the classification as a cold stenotherm (<10°C) should be re-evaluated. In another study, it was observed that the water temperature varies between 5.2 and 16.8 °C in rivers where *T. anomalum* was found (Waringer, González, & Malicky, 2020). Likewise, *H. bacescui* is classified as a cold stenotherm (<10°C) in the literature (Graf et al, 2008), but the highest water temperature at the station (4th station) where these two species were found was 16.99°C in summer. According to the results, the presence of *T. anomalum* and *H. bacescui* are not limited to cold streams, but the reason why these species are not found in the main branch of the stream may be the high ratio of boulders and gravel in the substrate structure of the station where these species were found. In the literature (Vineyard & Wiggins, 1988; Živić, Markovic, Simic, & Kucinic, 2009; Živić et al, 2013) it has been stated that the regions where these species live are rivers with boulder substrate. On the other hand, Station 4, has a high level of gravel besides its boulder substrate.

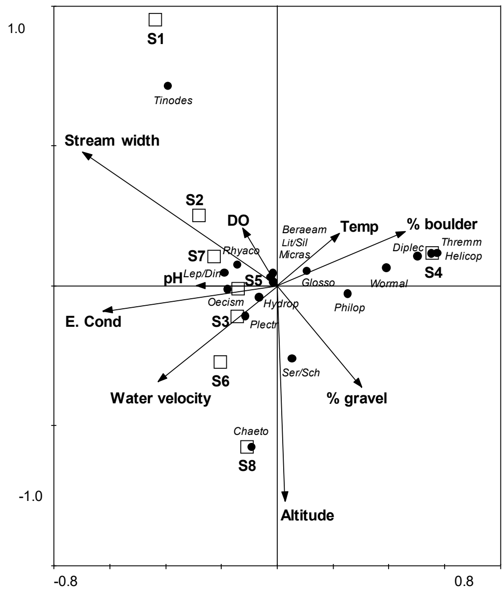

Fig. 3. CCA diagram showing the relationship between environmental variables and distribution of Trichoptera larvae. (Themma) *Thremma anomalum*, (Helicop) *Helicopsyche bacescui*, (Philop) *Philopotamus*, (Wormal) *Wormaldia*, (Diplec) *Diplectrona*, (Micras) *Micrasema*, (Lit/Sil) *Lithax*/*Silo*, (Beraeam) *Beraeamyia*, (Ser/Sch) *Sericostoma*/*Schizopelex*, (Glosso) Glossosoma, (Chaeto) *Chaetopteryx*, (Rhyaco) *Rhyacophila*, (Hydrop) *Hydropsyche*, (Plectr) *Plectrona*, (Oecism) Oecismus, (Lep/Din) *Lepidostoma*/*Dinarthrum*

A scale of the vulnerability of the species to climate change was created, and the species were matched with numbers between 0 and 6 on this scale: A value of 0 indicates that the effect is low, while a value of 6 indicates that the effect of climate change is high (Hershkovitz, Dahm, Lorenz, & Hering, 2015). *T. anomalum* and *H. bacescui* have a value of “2” in the literature (Graf et al, 2008). This means that they will not be affected much by climate change. However, in this study, these two species were found only at the station in the tributary of the stream (Station 4), and this station was one of the rare tributaries that did not dry out in all seasons. Although these species will not be affected or slightly affected by the increase in temperature that will occur with the effect of climate change, they may disappear locally due to the drying of the tributary of the stream. It has been reported that the drought caused by climate change will seriously affect the biota of the rivers, especially in the middle latitudes, and drought periods may be prolonged in temporary waters, and permanent waters may become temporary and even dry up completely (Fenoglio, Bo, Cucco, Mercalli, & Malacarne, 2010). Fenoglio, Bo, Cucco, & Malacarne (2007) observed that the abundance and taxonomic richness of macroinvertebrates in the hyporheic zone during dry periods in streams decreased as the drought period lengthened. Although it is stated in the literature that *H. bacescui* is frequently found in periodically drying streams (Živić et al, 2009) these adaptive abilities of the species may not work if the natural regime of the stream is disturbed.

Information about the pH value of the stream in which *T. anomalum* inhabits was found in only one publication and it was stated that it ranged from 7 to 7.7 (Živić et al, 2013). In this study, the values measured at Station 4, the only station where *T. anomalum* was found, were measured between 7.58 and 8.53 throughout the study, and the pH values at this station during the periods when *T. anomalum* was present varied between 8.40 and 8.48. According to the CCA graph, the 4th station has a low pH value and although the species found here seem to prefer low pH values, it is because the lowest pH value measured at all stations during the study belongs to this station at 7.58.

Two genera from the Hydropsychidae family were found in this study: *Diplectrona* Westwood, 1840 and *Hydropsyche*. *Diplectrona* is similar to *T. anomalum* and *H. bacescui* in terms of river region, altitude, substrate structure, water velocity, temperature preferences (cold stenotherm), and sensitivity to climate change (Graf et al, 2008). Only *D. atra* of the genus *Diplectrona*, which is represented by five species in Turkey, is found in the Western Black Sea region, and its adult was previously recorded by Sipahiler (2019) in Yenice Forest.

Hydropsyche is a genus represented by 75 species in Turkey (Darılmaz & Salur, 2015; Sipahiler, 2016a). Longitudinal studies of this genus in rivers have reported that there are different Hydropsychids species with different feeding habits at different altitudes (Bing, Müller, Glaser, Brandl, & Brändle, 2015). In this study, *Hydropsyche* appears to show a wide ecological preference by placing close to the middle in the CCA graph. Since this genus could not be identified at the species level using larvae, the different ecological demands of the species belonging to this genus could not be determined and therefore it was located in the middle of the graph. The positive relationship with the water velocity may be related to the dominant feeding habits of this genus by passive filtering. Looking at the longitudinal distribution of hydropsychids in uncontaminated streams of England, it was found that they were lined up as *Diplectrona felix*- *Hydropsyche* spp.- *Cheumatopsyche lepida* (Edington & Hildrew, 1995). In this study conducted in the Western Black Sea region, although *Hydropsyche* was found at all stations, *Diplectrona* was found at stations close to the source. In another study conducted in the Eastern Black Sea Region (Ekingen & Kazanci, 2021), *Hydropsyche* was found in most stations, while larvae belonging to the *Cheumatopsyche* genus were found in the lower parts of the stream. In this respect, the longitudinal distribution of Hydropsychids at the species level which Edington & Hildrew (1995) stated, seems to be valid at the genus level in the Black Sea Region of Turkey as well.

The ecological preferences of most of the genera found in the study could not be interpreted due to the fact that they were located near the middle of the CCA graph. The reasons for being in the middle may be that they have a wide ecological tolerance, or that there are different species with different ecological requirements in the genus.

With this study, it has been observed that the larvae found here can also be found in alkaline waters in general.

CONCLUSION

Yenice Forest is one of the least disturbed forest habitats in Turkey, but it is still known to be affected by forestry activities (Avcı, 2005; Öztekinci & Coşkun, 2021). The increase in road networks in the forest, the emphasis on the use of machinery in forest cutting, and illegal tree cutting are seen as threats that will affect Yenice Forest (Öztekinci & Coşkun, 2021). On the side of Şimşir Stream, there is a road opened for the transportation of forest products. It has been stated that no matter how small and sensitive forest activities are, they can cause physical deterioration that can harm aquatic ecosystems (Gökbulak, Serengil, Özhan, Özyuvacı, & Balcı, 2008) and that forestry activities will increase the influx of sediment into the stream and cause long-term damage to the invertebrates living here with the accumulation of sediment in the stream bed (Campbell & Doeg, 1989) but there is no study on effects of timber harvesting in this region.

The taxa, which are not found in other stations except for the tributary, show the contribution of the tributaries of the river to the fauna. Other tributaries were not sampled in this study as they were dry in some periods, but sampling them in the future will contribute to the determination of the fauna.

The fact that larval identifications cannot be made at the species level hinders the comparison and discussion of ecological information. The high level of endemism for Trichoptera in Turkish rivers makes the use of larval identification keys in other regions inconvenient for larval identification. For example, it is known that 50% of the *Tinodes* genus, which is represented by 32 species in Turkey, is endemic (Sipahiler, 2016b). The reason why some genera and variables could not be included in the discussion in this study is that ecological information at the species level is mostly not common at the genus level. Studies on the association of larvae and adults will not only enable the description of larvae but also increase the knowledge on the ecology of endemic species and make it possible to compare and use this information in future studies. Prioritizing the association studies between larvae and adults in later studies will be useful in determining the ecological preferences of the larvae and evaluating the river conditions.

Especially in biological monitoring studies, the determination of the changes in the rivers and taking measures accordingly requires the existence of reference data obtained from previous studies in the region. Since Yenice Forest has high biodiversity, is Turkey's largest uninterrupted forest, and is a region where human influence is low due to the topographic structure, the data to be obtained from the studies to be carried out in this region will be important in the evaluation of changing environmental conditions in the future.

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