ORIGINAL ARTICLE

Longitudinal recovery gradient of macroinvertebrates during different hydrological scenarios in a downstream river reach

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ABSTRACT

Macroinvertebrate community composition in regulated rivers is highly dependent on high and low flow events. Significant reduction or augmentation of the flow downstream from a dam can lead to a decrease in the variety of the sensitive macrozoobenthic taxa. Such decreases may lead to a degraded ecological status. In this research, the macrozoobenthic community was investigated in a river section downstream of a multipurpose dam with strategic significance for northeast Bulgaria. Samples were collected for five years from the Golyama Kamchia River, in close proximity to the Ticha dam. Three sampling sites were established in a longitudinal gradient, from 75 to 1770 meters from the dam. Sixteen invertebrate samples were taken between 2017 and 2021. Double sampling (spring and autumn) was conducted in 2019. The goal was to find how macroinvertebrate communities are influenced by the dam-induced modified environmental conditions, from the management of the dam, and which of the parameters is considered to have the strongest effect. Likewise for the study was important to understand longitudinal recovery gradients from the application of the present "minimum acceptable flow – compensation flow" in hydrologically differing years. The main physicochemical parameters were measured alongside the velocity of the water at each sampling site. Additionally, the maintained hydrological regime was explored for a relationship by several hydrological indices with the macroinvertebrate community composition. The analysis of the biological data through 7 biological indices showed that hydrologically sensitive taxa (Ephemeroptera, Plecoptera and Trichoptera - EPT) were very good indicators for damming impact, detecting disturbances not so well differentiated by other type-specific indices and the resulting ecological status. The order Trichoptera was the most influenced taxa group during the study. In 2020 the base flow released from the dam dropped to its lowest level (0.095 $m^3 s^{-1}$ from 0.552 $m^3 s^{-1}$) for an explored 10-year period of hydrology. This event continued for more than a year and led to a rupture of the previously observed recovery gradient. Downstream of the second sampling site, at about 800 m below the dam wall, a decrease in the number of trichopteran families was observed and at the third site, at 1.8 km from the dam they were significantly reduced.

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Key words: dams; rivers; downstream reaches; longitudinal; recovery gradient; macroinvertebrates.

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INTRODUCTION

Free-flowing rivers are one of the most dynamic ecosystems in the world (Grill *et al.*, 2019; Cooke *et al.*, 2021). Hydro-morphological alterations on the river continuum carried out in the Anthropocene by dam building seriously affect lotic water bodies, constraining about 17% of the river flow worldwide (Cooke *et al.*, 2021). Such a remarkable water volume retained or abstracted from the streams undoubtedly changes the hydrological regime at a global scale, which influences the protection of species and habitats' diversity (Ashton, 2012; Wang *et al.*, 2020). Following that, insufficient regulation of discharge into downstream river reaches from dams is a significant impediment to the presence of diverse freshwater biota (Voelz and Ward, 1991), because the organisms have evolved developing life strategies coupled with natural flow regimes (Bunn and Arthington, 2002; Rivas and Koleva-Lizama, 2017; Carlisle *et al.*, 2019). Therefore, the natural variability of stream flows, the so-called "natural flow regimes" (NFR), is crucial to sustain populations of different freshwater organisms, along with ecosystem health, function, and services (Poff *et al.*, 2007; Poff *et al.*, 2010; European Commission, 2015; Wegscheider *et al.*, 2023).

Modifications of the hydrological regime also led to changes in the physical habitats, which in turn strongly determines community composition and supports the production and sustainability of aquatic ecosystems (European Commission, 2015; Wegscheider *et al.*, 2023). The effect of these hydrological alterations and rate of recovery can be detected by one of the most sensitive river communities, whose composition and structure reflect the overall degradation in rivers - macrozoobenthic organisms (Doichev *et al.*, 2020; Wang *et al.*, 2020; Dusable *et al.*, 2022). In this "biological quality element" (BQE), different taxa are specifically affected by contamination or change in the main food resources (Dolédec *et al.*, 2021). Modification towards homogenization of flow, for example, troubles the development of macroinvertebrates and other aquatic organisms. The constancy of the discharge from a dam brings to society a multitude of benefits such as eradicated fluctuations of water velocity and volume, which can assure the lack of inundation, but at the cost of creating favourable conditions for alien and invasive species (Meißner *et al.*, 2018; Zeiringer *et al.*, 2018). That type of management of reservoirs modulates the hydrological regime by changing one or more of the specific hydrological parameters in downstream reaches, which are: magnitude, duration, frequency, annual variability, and daily fluctuation of the discharge (Agostinho, 2008; Carlisle *et al.*, 2019).

The European legislation and in particular the Directive 2000/60/EC (Water Frame Directive, WFD) admits the role of hydrologic alteration, acknowledging changes in the specific hydrologic parameters (magnitude, duration, frequency, annual variability, and daily flow fluctuation) as a primary factor in retaining the quality of river ecosystems and the achievement of a "good ecological status" as defined by the different BQEs and indicators (European Commission, 2015). Hydrological variables and the relevant flow regime are part of the "hydro-morphological quality elements" (European Communities, 2000), supporting BQEs and the consequent induced or inhibited longitudinal trends of improvement. Hydro-morphological conditions are also responsible for altering the values of some basic parameters as water temperature (T), dissolved oxygen (DO), total suspended solids (Meißner *et al.*, 2018; Casserly *et al.*, 2021, Dolédec *et al.*, 2021) and the concentration of nutrients (Dusabe *et al.*, 2022).

The aim of the present study is to evaluate and analyse the specific longitudinal gradient of macroinvertebrates downstream of a multipurpose dam with great regional importance. This is based on finding the most influential environmental variables (physico-chemical parameters or/and hydrological indices) for the computed biological indices and the resulting ecological status (ES). The research is also seeking to create an opportunity for assessing the type of environmental flow which will be best suited for the downstream river reach. This was founded on presently applied "minimum acceptable flow - compensation flow" and the impact from it on the achievement and sustainment of the ecological minimum, set by WFD - "good ecological status", during a timespan with diverse hydrological conditions.

METHODS

Study area

According to the regional River Basin Management Plan (RBMP) (2016), the Kamchia River is the biggest intra-territorial river in Bulgaria (length: 245 km), which flows directly into the Black Sea. It is formed by the Golyama Kamchia and the Luda Kamchia rivers. The Golyama Kamchia River is considered its headwaters. The watershed area of the whole basin is 5358 km^2 (Marinov, 1957), from which 61.7% is used for agriculture, 37.7% is forested and a very small fraction (0.6%) is occupied by urban areas (Skoulikidis *et al.*, 2022).

The studied Golyama Kamchia reach is located in the canyon of Preslavska Planina mountain. The geological structure here consists of an alternation of carbonated and non-carbonated rocks, which are distributed at about 40% of the Kamchia River catchment. Those rocks are composed of slates and sandy limestones, clayey marls and limestones, clayey-sandy slates (Nikolova, 2010). This stretch of the river is located in the WFD water body BG2KA900R1020, which spans from the Ticha dam (the height of the dam wall is 54.5 m) to the end of the gouge, near the town of Veliki Preslav. It has been characterised as a "heavily modified water body" and is protected by the Habitats Directive, having 4 protected areas of Natura 2000, classified as "site of community interest – SCI". The Bulgarian typology classifies the lotic ecosystem here as a "semi-mountain river – R4", in ecoregion 12 – Pontic province (RBMP, 2016).

The Ticha Dam was built in 1971-1972 on the Golyama Kamchia River. The reservoir is with a watershed of about 1000 km^2 (Davidova *et al.,* 2008). Four main rivers are draining their waters into the multipurpose dam (RBMP, 2016). The perimeter of the reservoir is about 100 km, with a maximum water storage capacity of 311.800.000 m^3 (Davidova *et al.,* 2008). This stagnant water body must meet domestic and industrial needs, irrigation, and hydropower production and it should maintain the riverine ecosystem downstream from the dam wall (Kolcheva and Ilcheva, 2016).

According to the regional RBMP (2016), the dam is classified as a "big and deep reservoir – L11" and it is part of a "special protected area – SPA" with the name "Kotlenska Planina" BG0002029. The hydropower station is built on the dam wall, and when possible, provides the main part of the environmental flow, working on two modes discharging 5 or 0.5 $m^3 s^{-1}$. The rest of the flow comes from the drain valve, which is never completely closed, or, in extreme cases, from the spillway as well (RBMP, 2016).

We monitored three sampling sites longitudinally located from 75 m to 1770 m from the dam wall (Fig. 1). This distance was chosen because Voelz and Ward (1991) reported dynamic and very significant augmentation in macroinvertebrate taxa (from 15 to 40 taxa) for a relatively short distance (1.4 km). All sites are located entirely within the protected area "Golyama Kamchia" BG0000501. The plant habitat communities of alluvial forests – 91E0*, are the only ones forming the riverine galleries here (http://natura2000.moew.government.bg).

The first sampling site (GK1) is located immediately downstream of the dam wall at about 75 m from its base, at 149 m above sea level in an area where the river channel is straightened with dikes constructed from rock material. Tree vegetation is not present on the dikes, and as a consequence the site is totally exposed to direct sunlight. The bottom substrate consists primarily of macrolithal (20–40 cm, about 75%), megalithal (above 40 cm, about 5%) on which representatives of Dreissenidae family develop very well. The accumulation of silt occurs in maximum 15% of the overall microhabitats. The remaining 5% of microhabitat types are represented by mesolithal (6–20 cm), microlithal (2–6 cm) and akal (0.2–2 cm), depending on the discharge. Woody debris is absent. The width of the water current of GK1 is between 5 and 9 m. The depth also varies, depending on the river flow but is between 23 and 165 cm. The most dominant macrophyte species at GK1 are *Schoenoplectus lacustris* and *Potomogeton nodosus.*

The second site (GK2), located 830 m below the dam at 138 m above sea level, has dikes as well, but they are not immediately close to the water table. Tree vegetation is present on the riverbanks and on the dikes. The arboreal species are typical for the riverine galleries in habitat 91E0* and predominantly consist of *Salicion albae* with *Alnus glutinosa* and *Fraxinus excelsior.* This assures a shadiness between 60 and 70% of the river channel. The bottom substrate on GK2 consists predominantly of mesolithal (40%) and macrolithal (30%). The remaining 30% of microhabitats are represented by microlithal (20%), akal (5%) and psammal (5%). Woody debris is available in the water column. Several logs are submerged in the periphery of the water table. The width of the water surface varied from 6 to 12 m during the study. This site is shallower in comparison to GK1 with a minimum registered depth of 15 cm and a maximum of 70 cm. Macrophytes are found only at the lenthic part of the water table and are dominated by genus *Carex*.

The third site (GK3), located 1770 m from the dam, has no constructed dikes. Trees are present on the banks, again with species characterizing habitat 91E0*. The specific characteristic here is the presence of channel "islands" with terrestrial grasses and some single trees (mostly willows). About 85% of the water table is shaded. The benthic substrate of GK3 is more diverse than GK1 and GK2. Microlithal (50%) dominates the site. Akal (10%), psammal (10%) and psamopellal (10%) are representing 30% more of the mineral microhabitats while mesolithal (15%) and rarely macrolithal (5%) dominate on riffles. Woody debris is present and includes whole logs fallen into the water current. The width of the water table was from 15 to 19 m and the registered average depth was between 10 and 60 cm. *Schoenoplectus lacustris* are the present dominant macrophyte.

Collection and determination of macroinvertebrates

In total, 18 samples were taken during the five-year period, from 2017 to 2021. Macrozoobenthic organisms were collected in accordance with the multihabitat method described by Cheshmedjiev *et al.* (2011). Standardized hand net or the so-called kick-net with 500 µm mesh size, with projection of the frame 25 x 25 cm was used for kick-sampling. One "kick" or sub-sample covered 0.125 m^2. Ten sub-samples were taken (covering 1.25 m^2) at every site (30 m reach) proportionally to the microhabitat distribution within the riverbed and if needed wash sampling was conducted as well. (Cheshmedjiev *et al.*, 2011).

The sampling events took place in June 2017, October 2018, June 2019, October 2019, June 2020, and July 2021. Subsequently, samples were washed and cleaned of mechanical impurities. Determinations to family level and in some taxa to genus (except for Tricladida and Oligohaeta, identified as such) were made with a 20-80x magnification stereomicroscope (BRESSER, Researcher ICD LED).

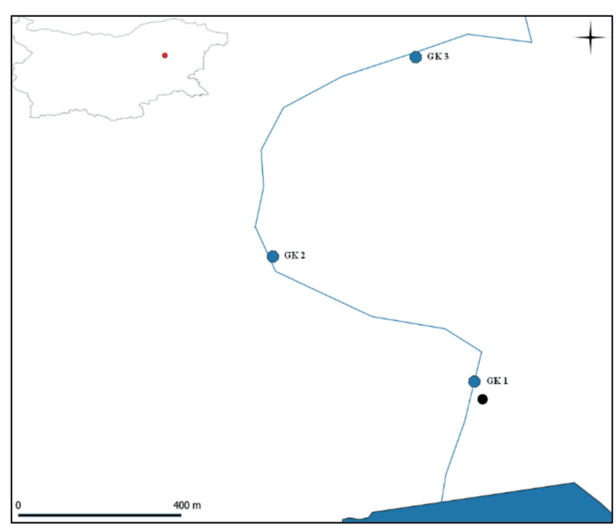

Fig. 1. Map of the sampling sites. GK1, first sampling site; GK2, second sampling site; GK3, third sampling site; Black point, gauging station; Blue points, sampling sites.

Characterizing the flow conditions

The data about the discharge of water for environmental flow downstream of the Ticha dam was collected from the managing organisation – Irrigation systems "Shumen". They provided information about the average daily flow (Q) values. Flow time series 1 year prior to every sampling event were used for the calculation of hydrological indices. In order to use a small number of variables for further analysis, only several indices were chosen. They were related solely to the magnitude and rate of change of the hydrological regime due to the findings of White *et al.*, (2017). According to their results, those two types of hydrological indices have the strongest influence on macroinvertebrates in regulated streams from spring and autumn samplings. The calculated indices are mean average discharge (QMEAN), median discharge (Q50), coefficient of variation of discharge (QCVANN), number of positive changes in flow conditions (QPORR), and number of negative changes in flow conditions (QNERR).

In addition, we made measurements of the water velocity 18 times in total for the studied river reach, or six times for every sampling site. Values of maximum (V_{MAX}) and average (V_{AV}) water velocity were obtained, by a winch in open channels – FP 201 Nibco – NSF – PW. The instructions of the device require repeated smooth immersion from the water surface to the sediment. Thus, the propeller calculates both parameters for the whole water column (Global Water Instrumentation Inc., - a Xylem brand, Golden River, California, USA). Each measurement of V_{MAX} and V_{AV} consists of three sub measurements, which were done for each sampling event at each site. One at the centre of the water table representing purely lotic conditions, one near the shore, and one in between. Afterwards, the results of the velocities were averaged and given as final values for the sampling event.

Physicochemical parameters

In situ measurements of the physicochemical parameters include water temperature (T), pH, dissolved oxygen (DO) and electric conductivity (CD). T and CD were measured by a portable conductometer (WTW 196 LF, WTW Gmbh, Valheim, Germany). The values of DO and pH were obtained by a multiparametric probe (Senso Direct 150 Lovibond; Tintometer GmbH, Dortmund, Germany). Water samples were taken during spring in 2017, 2019, and 2020, as well as during the autumn of 2018 and 2019, according to Bulgarian State Standard EN ISO 5667-6:2016. Those measurements of the water were matching the biological sampling events.

The parameters phosphates (PO_4), nitrites (NO_2), nitrates (NO_3), and ammonia (NH_3) were determined using the multiparametric photometer Hanna HI 83200 (Hanna Instruments, Smithfield, RI, USA) in laboratory conditions. After tempering the water to 25 °C, four different reagents were applied for the measures of nutrient concentrations – phosphate low range reagent HI 93713-0, with accuracy ± 0.04 mg L^{-1}; nitrite low range reagent HI 93707-0, with accuracy ± 0.06 mg L^{-1}; nitrate HI 93728-0, with accuracy ± 0.5 mg L^{-1}; ammonia medium range reagents HI 93715A-0, HI93715B-0, with accuracy ± 0.05 mg L^{-1}. Measurements were carried out until three of the results are close in value and the eventual differences are with smaller margins from the specified accuracy. Then, the results were averaged.

Ecological status assessment

Determining the taxonomic composition of macrozoobenthic communities to family and on some occasions to genus levels is relatively fast and allows the determination of several indices for the assessment of the ES. Methods in the Ordinance H-4/2012, classify surface waters in Bulgaria according to their BQE values in high, good, moderate, poor and bad ecological status, in compliance with the EU WFD.

In situ measured physicochemical parameters and the chemical laboratory-analysed parameters are the so-called accompanying information which can serve as additional information for defining the ES (European Commission, 2005).

The indices listed below were calculated for macroinvertebrate communities:

- The abundance (A) was calculated from the composite sample for a surface of 1 m^2.
- Total number of taxa (S) is a type-specific simplified metric reflecting community changes and is a good additional parameter to serve as a support for major indices (Cheshmedjiev and Varadinova, 2013). We reported even the families with single member representative for calculation of this index in accordance with the findings of Guareshi *et al.* (2017).
- Ephemeroptera, Plecoptera and Trichoptera richness (EPT) is a metric which exhibits great stability in reference conditions and it is suitable for semi-mountainous river sections (Cheshmedjiev and Varadinova, 2013) and at the same time is sensitive to hydrological alteration, especially in downstream from dams river sections (Carlisle *et al.*, 2012, Meißner *et al.*, 2018; Mellado-Díaz *et al.*, 2019).
- Relative abundance of EPT (%EPT) is a parameter that does not have a scale which can be directly correlated with the ES.
- Adapted biotic index (BI) is a type-specific index used in Bulgaria for more than 23 years in the state monitoring of surface waters (Cheshmedjiev and Varadinova, 2013).
- Biological monitoring working party (BMWP) is a biotic index of global use, with several regional modifi-

cations. It is based on the presence/absence of invertebrate families differently sensitive to organic pollution, without considering their abundance (Pineda-Pineda *et al.*, 2018). The interpretation of the results was done in accordance with Kucuk and Alpbaz (2008).

- Average score per taxon (ASPT, that is the BMWP value divided by the number of scoring families), is also based on the sensitivity of invertebrate families to organic charge and has proved to be sensitive to other alterations such as sealing, clogging, habitat degradation, discharge of toxic mixtures (Šidagytė *et al.*, 2013) and hydrological modification (Mellado-Díaz *et al.*, 2019). The interpretation of the results was done in accordance with Hieu *et al.*, (2008).
- Dominance index (DOMN) (Simpson, 1949) demonstrates the ability of more tolerant and resilient taxa to increase their numbers at the expense of other members of the community while facing adverse conditions and environmental degradation.

Data analysis

Principal component analysis (PCA) was used to extract the main information from the three groups of parameters (biological, hydrological, and physicochemical) in order to reduce the dimensionality of multivariate data and to visualize better the results. The analysis was performed in R environment (R 4.2.2) using “factoextra” and “FactoMineR” packages. The physicochemical parameters which had gaps in the data were filled by averaging results from all conducted measurements at the sampling site. Afterwards, some of the variables from every group were chosen for further analysis, based on the PCA test for adequacy - Kaiser-Meyer-Olkin (KMO), performed by the add-in “Real Statistics” using Excel. After minimizing the redundancy by PCA and identifying the main variation for every data set, multiple factor analysis (MFA) was conducted in R (R 4.2.2) by using variables with the highest KMO. MFA is multivariate data analysis which summarises complex data. This analysis is very well suited for ecological research where several samplings are done on different dates. This is due to MFA capability of simultaneous consideration of multiple data series while balancing the influence of each set of variables (Pagès, 2002).

RESULTS

Macroinvertebrate responses

Taxonomically, the poorest site was GK1. The richness reached once 18 taxa, “accomplished” high ecological status once and good status twice (Fig. 2). The first three sampling events reached only the moderate range of the index. BI and EPT never reached values compatible with good ecological status. They were at the range of moderate and poor status. BMWP registered values predominantly into the moderate condition range. This index reached the highest score and good status in October 2019 and was determined as poor for the first two sampling events. ASPT varied in scoring between 3.66 and 5 points and reached good status only in 2018 when the families were the lowest in numbers. For the rest of the period ASPT stayed at moderate ecological status (Fig. 2).

The abundance varies from about 600 ind m^{-2} to 1207 ind m^{-2} for the whole period of sampling (Tab. S1 and Fig. 2). The minimum values were registered in October 2018 and the maximum ones a year later in October 2019 (Tab. S1). The quantity of macroinvertebrates from the insect orders Ephemeroptera, Plecoptera and Trichoptera (%EPT) relative to the abundance, were between 0.11 and 2.65 %, excluding 2020. In the year just referred %EPT reached 53.92%, which is the highest score for this metric at all sites. This was due to the dominance of Baetidae and corresponds to the lowest-ever value of DOMN at GK1 and the other two sampling sites (Fig. 2). In the rest of the study period DOMN values were between 0.61 and 0.88, always due to the non-insect domination of Gammaridae.

At GK2 considerable improvement was observed both in taxonomic richness and ecological status in comparison to GK1. S fluctuated between 13 and 26 families (Fig. 2). The lowest value was registered in 2017 and the highest one in October 2019. BI reached high ecological status (Government of Bulgaria, 2012) three times and registered moderate and good status as well. The EPT reached a greater number of families and evaluated GK2 as good ecological status for the period from 2019 to 2021. This was an effect of Trichoptera richness improvement (Tab. S1) because plecopterans were not present and ephemeropteran added only 1 or 2 taxa between GK1 and GK2. The number of insect and non-insect taxa, giving the value of S doubled GK1 values and families from order Trichoptera were more than doubled as well (Tab. S1). BMWP enters within moderate, good, and high status proportionally dividing the results between those three classes from 64 to 122 points. The lowest scores were from 2017 and 2018 and the highest ones from October 2019 and July 2021. ASPT did not show significant improvement longitudinally compared to the other biological indices (Fig. 2) for the distance from GK1 to GK2. This metric registered here good status in 2017 and 2020.

At the second sampling site mean abundance of invertebrates increased more than two-fold and kept increasing for every sampling event in comparison to the first site. In June 2019 the quantity of organisms were almost four times more than GK1. Orders Ephemeroptera, Plecoptera and Trichoptera kept augmenting longitudinally their percentage of the whole abundance, excluding 2020 sampling collection. The mean %EPT (without 2020) at GK2 were 4.16 in comparison to 0.92 at GK1. In 2020 a decrease was observed. DOMN varies in the range from 0.60 (2020) to 0.90 (2017) because of Gammaridae dominance (Fig. 2). GK3 was characterized by an almost permanent high ecological status, according to BI (4) and S (above 16). An exception was observed in the autumn of 2018, when the river's ecological status was evaluated as moderate. EPT also improved from GK2 to GK3 for most of the sampling occasions (Fig. 2). Only 2020 demonstrated a decrease in the number of families from GK2 to GK3 from the sensitive insect taxa. EPT is one of the few indices that registered improvement between the second and the third site in 2018. BMWP registered values characterizing the site as having high status during 2017, October 2019 and 2021. As moderate ecological status in 2018 and as good in the spring of 2019 and 2020. The sampling events from 2018 and 2020 were related to a decrease in the values for BMWP from GK2 to GK3 and in the rest of the occasions with improvement. ASPT reached good status three times in 2017, October 2019 and 2021, which coincided with the good status for BMWP at the third site. For the rest of the time, ASPT was with values beneath the threshold for a good status of 4.9 points.

The mean abundance at GK3 (1455) was lower than

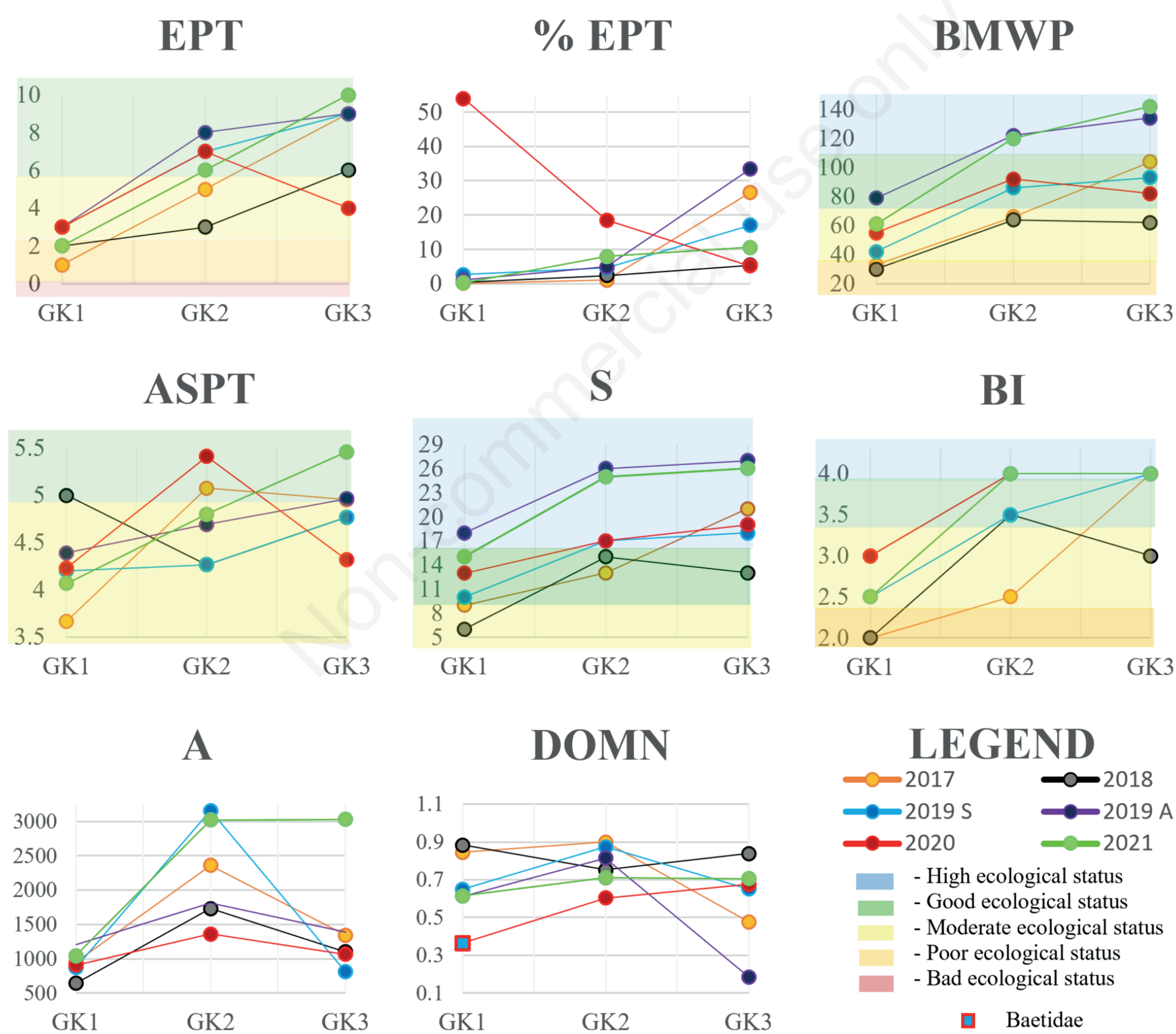

Fig. 2. Dynamics of the biological indices. EPT, number of families from the orders Ephemeroptera, Plecoptera, Trichoptera, %EPT, Ratio between the abundance of EPT individuals and total invertebrate abundance; BMWP, Biological monitoring working party index; ASPT, average score per taxon (= the BMWP value divided by the number of scoring families); S, total number of taxa; BI, adapted biotic index; A, abundance (calculated from the composite sample for a surface of 1 m^2); DOMN, dominance index.

GK2 (2240) and higher than GK1 (936). The number of individuals increased only in 2021, and on other occasions decreased. The quantity of the insect orders structuring the EPT index always increased from GK2 to GK3. An exclusion was in 2020 when the decrease from the first site continued and the proportion of sensitive insects was only 5.16%. The mean value of %EPT was 18.58%, which was a considerable augmentation from the second sampling site. The highest value was registered in October 2019 when %EPT was 33.38%. This sampling event at the third site was related to the lowest value of DOMN (0.18) for the whole studied river reach. The worst or the highest result was registered in 2018.

All eight biological indices were subjected to PCA. The results show that the indices with the greatest contribution are BMWP, S, EPT and BI (Fig. 3). All are located near the axis of dimension 1 (PC1). This dimension explains 56.3% of the variation within the biological data. DOMN, %EPT and A contribute greatly to PC2 (Tab. S2), which explains 23% of the variation in the "biological" dimension (Fig. 3). The strongest KMO value is for BI (0.926) followed by EPT (0.601) and S with BMWP having the same value (0.548) (Tab. S2).

Characterizing the flow conditions

Measurements of V_{MAX} and V_{AV} were done at all sampling sites, to distinguish localities in their specific hydromorphological characteristics. The results are presented in Tab. S3. During the period of samplings, peaks in the daily flow and overall higher water tables were registered only from January to March in 2017 and 2018 (Fig. 4). For the rest of the study period (2019 – 2021), Q remained almost stationary but showed two greatly differing magnitudes that were with values of about 0.6 m^3 s^{-1} (2019)

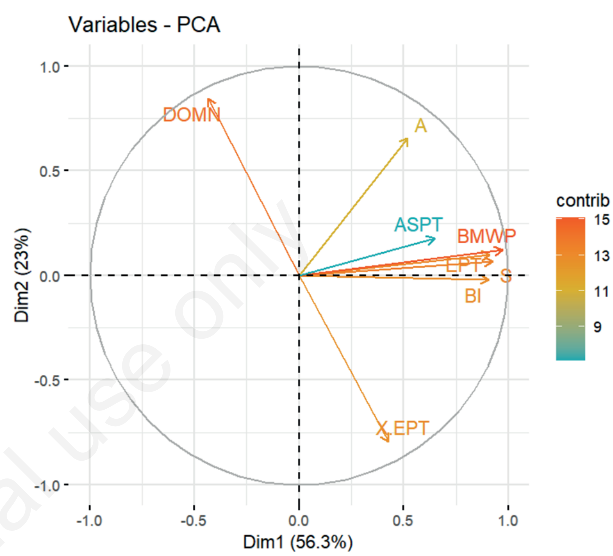

Fig. 3. PCA biplot of the biological indices.

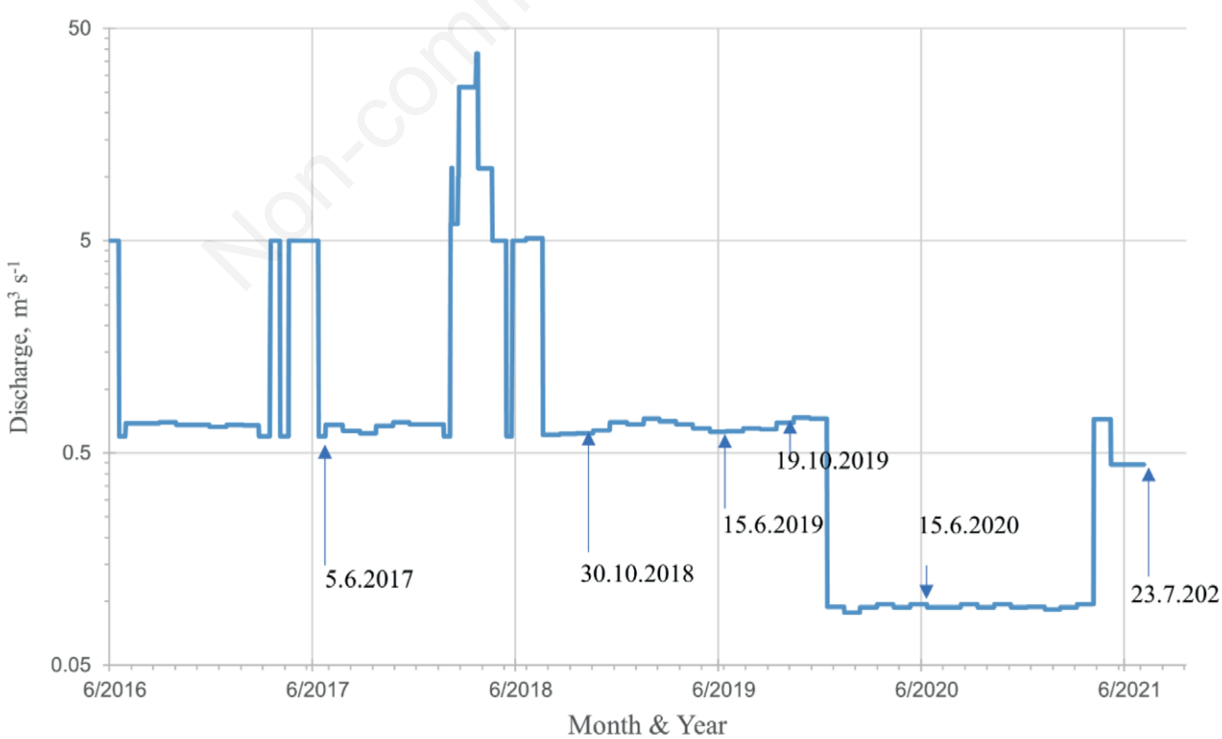

Fig. 4. Hydrological regime for the studied period. Including the data 1 year prior to the first sampling event. The arrows point out the dates of samplings

and 0.09 m^3 s^{-1} (2020) (Fig. 4). Based on the daily flow time series (Tab. S4) QMEAN, Q50, QCVANN, QPORR and QNERR were calculated (Tab. S3). Afterwards, PCA considering V_{AV}, V_{MAX}, Q and the hydrological indices was conducted. The results show that the major contribution is due to the indices QMEAN, QPORR, QNERR and Q50 (Fig. 5). All are closely located to PC1 axis and are dominating factors in this dimension (Tab. S5), which is responsible for 54.7% of the variation. V_{AV}, V_{MAX}, and Q are dominating factors in PC2, responsible for 29.9% of the variation from the flow characteristics. The strongest KMO value is for QNERR (0.375) followed by QMEAN (0.371), QPORR (0.360) and Q50 with the lowest result (0.288) (Tab. S5).

Physicochemical parameters

Results from the measured *in situ* parameters, classified using the threshold values of Ordinance H-4/2012, are shown and marked with colours (Tab. S3). The measurements of the T did not register an amplitude between GK1 to GK2 or GK3 of more than 1°C. The other *in situ* parameters had results within the range of good and high ecological status (Tab. S3).

Measurements in laboratory conditions showed that NO_3 reduced its mean values from GK1 to GK3 with about 1.5 mg l^{-1}. The lowest results were always at GK3 for this parameter. For the others was not always like this, but the tendency of reduced mean concentrations remained for NO_2 and NH_3 (Tab. S3).

All eight physicochemical parameters were subjected to PCA (Fig. 6). The results show 6 variables with great contribution - CD, T, pH, NH_3, NO_2 and PO_4 (Tab S6). All, excluding phosphates are located near the axis of dimension 1 (PC1) and are dominating factors there. Only phosphates dominate in PC2. The second dimension explained 26.1% and the first one 46.3% of the variation. DO and NO_3 were not considered for further MFA due to low KMO values (Tab. S6). The strongest KMO value is for NO_2 (0.861) followed by CD (0.767), NH_3 (0.719), T (0.696), PO_4 (0.677) and pH (0.657) (Tab. S6).

Multidimension relationships

MFA shows that dimension 1 is responsible for 49.49% of the variation and dimension 2 for 33.41%. In the first axis, the greatest contribution is divided between 4 biological indices and the flow characteristics expressed by 4 hydrological indices (Fig. 7). The biological component formed 44.70%, the hydrological 50.45% and the chemical 4.84% for the construction of the axis 1 (Tab. S7). Within dimension 2 the domination is for the physicochemical variables with 88.19%, followed by the biological component with 11.28% and 0.53% for the flow characteristics (Fig. S1).

The correlation circle illustrates very well the clear negative correlation between all biological indices and flow characteristics, which are far from the origin and close to axis 1. The quality of S and BMWP is the highest in the factor map from the biotic component (Fig. 7).

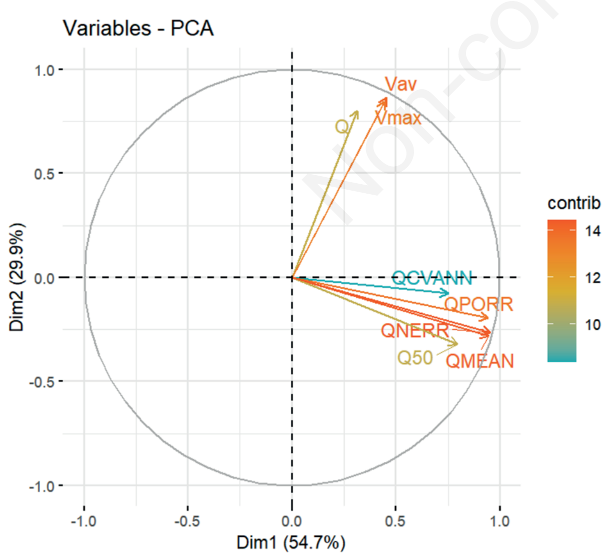

Fig. 5. PCA biplot of hydrological indices. Q, mean daily flow; Q50, median discharge; QMEAN, mean average discharge; QCVANN, coefficient of variation of discharge; QPORR, number of positive changes in flow conditions; QNERR, number of negative changes in flow conditions; V_{MAX}, maximum water velocity; V_{AV}, average water velocity.

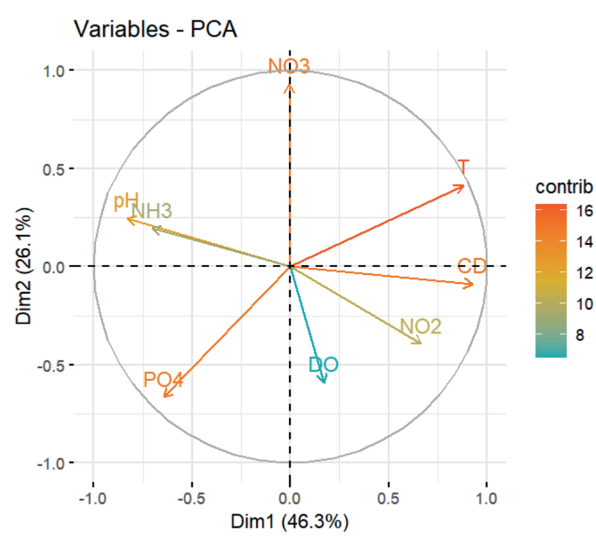

Fig. 6. PCA biplot of the physicochemical parameters. T, water temperature; pH, active reaction; DO, dissolved oxygen; CD, electric conductivity; PO_4, phosphates; NO_2, nitrites; NO_3, nitrates; NH_3, ammonia.

Those two indices have almost identical contributions due to their close location. The third place of importance is BI, followed by EPT. All flow characteristics are very closely organized in the factor map and differ by very little space. The most distant one from the origin is QNERR, closely followed by QMEAN and QPORR. Only Q50 shows a visibly weaker contribution.

The most important parameter for dimension 2 is CD. The second most contributing one is pH, which correlates negatively with T (the third most important) and CD. The fourth (NO_2) and the fifth (PO_4) are visible with less contribution, due to the close proximity to the centre of the factor map and are negatively correlated with each other.

DISCUSSION

The dams are a significant anthropogenic pressure for lotic ecosystems with severe global influence (Krajenbrink *et al.*, 2019; Wang *et al.*, 2020). In spite of the fact that Dusabe *et al.*, (2022) reported only weak dam-induced effect on low-diversity downstream river reaches, all aspects of NFR are still changed (Krajenbrink *et al.*, 2019). The consequence from this influence is a significant reduction of macroinvertebrate richness (Trottier *et al.*, 2022) which is better detected from the metrics reflecting the state of compositional changes than the diversity indices (Wegscheider *et al.*, 2023). Those organisms are important for the overall vitality of a riverine ecosystem because they impact the biotic component through their trophic function into the instream food web and impact the abiotic component through biogeochemistry processes (Wang *et al.*, 2020; Wang *et al.*, 2021). The effect can continue for a long distance from the dam wall (Mellado-Diaz *et al.*, 2019) or to be with short-distance influence as reported by Voelz and Ward (1991) and to reach some phase of equilibrium conditions after about 3 km.

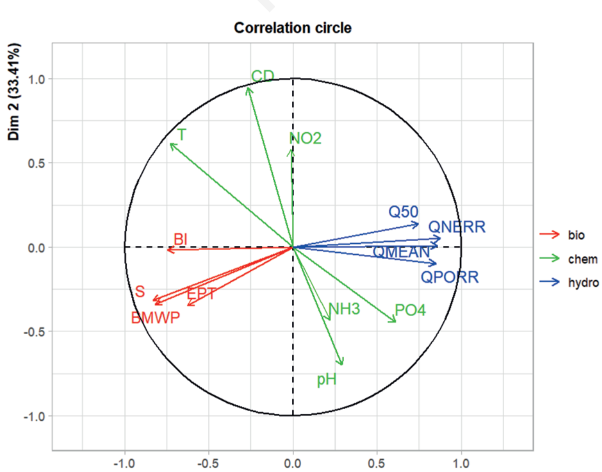

Fig. 7. MFA correlation circle of all significant variables.

It is not only macroinvertebrates that suffer negative effects from the numerous dams induced alterations on the ecosystems, but terrestrial organisms as well (Wang *et al.*, 2020). Another example is fish diversity, which is also globally affected by specific latitudinal gradients demonstrating a serious decrease in diversity in tropical regions, a less pronounced one in temperate regions and a weak impact on boreal biomes (Trottier *et al.*, 2022). Fish beta diversity can be affected too, which in turn can influence the phytoplankton diversity (Wang *et al.*, 2021).

Most studies connected to regulated rivers are planned and executed short term (Krajenbrink *et al.*, 2019) although they should be designed in a manner that must allow observations during diverse flow and water level scenarios. This is needed to obtain samples from extreme high and low flow events, which are crucial in structuring the biodiversity in rivers (Wegscheider *et al.*, 2023). Although this is an assumption made by Wegscheider *et al.* (2023) about large rivers, it seems that the same principles are valid for lotic water bodies of smaller size, too (White *et al.*, 2017).

This is consistent with our study, because in 2018 the overall richness of macroinvertebrates was improved for half of the studied longitudinal distance (800 m), followed by a decline. That deterioration of the ecological status registered from indices S, BI and BMWP, observed from the sampling event at GK3 (Fig. 2) was due to a reduction in the results from GK2 during the autumn of 2018. According to the conducted MFA, those indices are negatively correlated to flow characteristics related to the mean annual discharge and the rate of change (Fig. 7). A possible reason for that is the drift of the macrozoobenthic organism, due to the abrupt change of the outflow or retention of high-water levels inappropriate for the zoobenthic fauna during the year (Fig. 4). In our case such a statement arises from the fact, that 1 year prior to the sampling, the daily flow was retained at higher levels for prolonged periods (Fig. 4) and registered the greatest number of positive (QPORR) and negative changes (QNERR) in the flow magnitude (Tab. S3). Furthermore, in this year, the runoff reached its highest peak, during our monitoring and the second largest one, for the period from 2011 to 2021. Orders Ephemeroptera, Plecoptera and Trichoptera which are sensitive to hydrological alteration (Carlisle *et al.*, 2012; Meißner *et al.*, 2018; Mellado-Díaz *et al.*, 2019), also reacted to this perturbation. EPT normally registered from 9 to 10 families on GK3, but in 2018 stayed at the lower limit for good ecological status. The abundance of EPT taxa was about 5% which is atypically low for the site and is often a response within downstream river reaches (Wang *et al.*, 2021) although Krajenbrink *et al.*, (2019) reported the opposite. In the autumn of 2018, the recovery gradient for Trichoptera and EPT was not interrupted but appeared to be less pronounced (Fig. 2).

An additional reason to emphasize the significance of EPT is the research of Moskova *et al.* (2008), who reported that the evaluation of the sapro-biological condition in the Kamchia River watershed is well reflected by this metric. This shows that orders Ephemeroptera, Plecoptera and Trichoptera can be used for ecological evaluation in the Golyama Kamchia River. Our results confirm this statement, because the two type-specific indices (BI and S) did not detect the disturbance from the 2020 flow drop and evaluated the river ecological status as high (Fig. 2) despite the extremely sharp decline of the daily flow (Fig. 4). In contrast to S and BI, EPT and %EPT reacted to that shortage of water intendent for "minimum acceptable flow" and registered their lowest values on GK3 (Fig. 2) with score within the range of moderate status (Cheshmedjiev and Varadinova, 2013).

Thereby, in 2020 the deterioration of the longitudinal gradient is visible from the drop of the EPT, from GK2 to GK3 (Fig. 2). This index is dependent mainly on Trichoptera, which for most of the sampling events increased their richness by about 1.7 km with 3 to 6 taxa (Tab. S1). Plecopterans were not present probably because of higher winter and lower summer water temperatures (Elis and Jones, 2016). Ephemeropteran added only 1 or 2 taxa for 1.7 km. They tended to become more abundant as a group in a downstream direction (Tab. S1). Their abundance always increased excluding 2020 when they decreased from GK1 to GK3. This is visible from the metric %EPT which had high results because it was constructed predominantly of Baetidae, the dominant family at GK1 in 2020 (Fig. 2). In general, Ephemeroptera is not heavily impacted in downstream river reaches (Wang *et al.*, 2020) and the Golyama Kamchia River is not an exception.

Therefore, during years of atypically low flow events the overall benthic assemblages in the Golyama Kamchia River seemed to be less affected and kept improving with the distance but those consisting of the EPT were unable to sustain the recovery gradient for more than about 800 m, because afterwards a significant reduction of the Trichoptera families occurred. Furthermore, as the results show the sampling event from October 2019 was with the best scores of almost every used index (Fig. 2). As for example lowest DOMN, highest %EPT at GK3, highest S score for every site, overall high results for BMWP, *etcetera*. MFA negatively correlated these metrics to a flow characteristic as QPORR, QNERR and QMEAN. The daily flow stayed constant with 0 changes in a positive or negative manner and a mean value of 0.67 $m^3 s^{-1}$. It seems that these flow conditions, a combination of climatic conditions, water chemistry and others, positively influenced the macroinvertebrates and their community. As a result, they succeeded in accomplishing high ecological status on GK2 and even raised the scores at GK3 (Fig. 2), for the biological indices mostly contributing to axis 1 (Fig. 7). This longitudinal trend of recovery was similarly good for 2021. The MFA individual factor map for the sampling sites visualized very well the similarities and distributed the points of GK2 and GK3 for the autumn of 2019 and 2021 closely in the dimension of the bottom left quadrant (Fig. S2)

Those stationary flow conditions in the downstream heavily modified WFD water body significantly differed from the other years of the conducted monitoring (2017, 2018, 2020) (Fig. 4), from the whole period from 2011 and from the pre-dam period (1937 – 1953). Before the building of the dam, the flow was characterised by two peaks. A primary maximum in the spring (April-May), a secondary maximum in November and overall high-water levels from January to June. From June to the end of September or the beginning of October water tables were low (Marinov, 1957). These fluctuations are specific to the nivo-pluvial river type (Skoulikidis *et al.*, 2022).

Further modified study with more sampling sites and more frequent biotic investigation is needed to determine what kind of environmental flow is most adequately suited to sustain at least good ecological status of the overall WFD water body. For now, it seems that more stagnant water tables are better for this biological indicator, but we cannot be sure whether or not periphyton growth will negatively influence the river system and reduce the quantity and quality of benthic habitats. This in turn may lead to the need for pulse flow application (Flinders and Hart, 2009). Naturally, the intensity of this process depends on varieties of factors such as nutrients and not only on flow characteristics (Kilroy *et al.*, 2020). This is why the trophic state of the downstream river reach cannot be overlooked due to the possibility of impairment of the macroinvertebrate biological indices and community structure (Aspin *et al.*, 2020). For the observed period, Golyama Kamchia River is not an exception because physico-chemical variables had a 4.84% contribution to dimension 1 and an 88.19% contribution to dimension 2 of the MFA (Tab. S7). In this data analysis dimension 2 is responsible for 33.41% of the variation (Fig. 7).

Our results confirm the ecological responses of macroinvertebrate communities to hydrological alterations from numerous studied rivers globally (Carlisle *et al.*, 2012, Elis and Jones, 2016; Schneider *et al.*, 2018, Meißner *et al.*, 2018; Mellado-diaz *et al.*, 2019). Furthermore, we found out that in the studied river stretch, families from order Trichoptera were more than doubled in a downstream direction, illustrating very well the longitudinal recovery gradient of regulated streams, described by Voelz and Ward (1991). Trichoptera deserves special attention not only because of their local diversity richness, but also because they represent the most complicated trophic relationships of aquatic insects and together with this, the numerous functional traits they show, makes them critical for the functionality of freshwater ecosystems (Morse *et al.*, 2019).

CONCLUSIONS

This is the first study in Bulgaria which gathered information about the longitudinal recovery gradient of macroinvertebrates downstream of a multipurpose reservoir. Additionally, is the first one to collect data for this particular river reach. The conducted research described the environmental context in order to properly assess the longitudinal recovery gradient of macroinvertebrates in 1770 m from the dam, but the climatic conditions and quantity of the rainfall were not included. They have a significant connection to streamflow variability (Malede *et al.*, 2022) and can influence the annual flow variability not only of free-flowing rivers but also at regulated streams such as the studied Golyama Kamchia River reach. The years significantly differing in their hydrological conditions need to be taken into great consideration by managing organizations responsible for dam management.

According to the results of the study in years of high flow events (2018) overall longitudinal recovery gradient occurred at a greater distance for EPT. The overall richness considering insect and non-insect taxa improved for half of the studied longitudinal distance, followed by degradation. For events of opposite significance or periods of atypically low flow conditions, the overall benthic assemblages seemed to be less affected and kept improving with the distance but those consisting of the EPT were unable to sustain the recovery gradient for more than about 800 m, because afterwards significant reduction occurred.

A possible conclusion from that mode of recovery gradient, in order to better evaluate the ecology status of a river site is that different indices could be preferred to others, depending on the hydrology 1 year prior to the sampling collection. This could be of assistance when a decision should be reached as to which results to neglect and which to be considered as more relevant. As a result, it can be hypothesized that the type-specific indices such as BI and S, also BMWP are more stringent and relevant than EPT for annual hydrological regimes which are characterized by high flow events for different periods. *Vice-versa*, EPT was more stringent when low flows took place, because then the degradation was on insect taxa predominantly. This comes to show that the longitudinal recovery gradient of macroinvertebrates for extreme hydrological conditions does exist, but it is shortened to about 800 meters (2018).

Another possible conclusion can be extracted from the years with more stable flow conditions and more average and permanent flow when no fluctuation occurred, but a certain level of the daily flow magnitude was kept. This led to registering the highest scores for the majority of indices and to the emergence of a very balanced and diverse community of macroinvertebrates at 1.77 km from the dam. On this occasion, the improvement of the ecological status was longitudinally consistent, and it was not interrupted or slowed down (October 2019, 2021).

At unpolluted river stretches, like this one, where the hydrologic connectivity is not possible to be restored because of the great importance of the multipurpose dam, an environmental flow should be considered and an adequate hydrological regime retained. This is needed because the appropriate flow characteristics could be a prerequisite for maintaining and restoring the ecological functions of the Golyama Kamchia River.

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Online supplementary material:

Tab. S1. Taxonomic distribution at GK1, GK2 and GK3 for every sampling event, indicated by year.

Tab. S2. Results from PCA on biological indicators.

Tab. S3. Environmental variables indicated chronologically by month and year.

Tab. S4. Daily flow time series.

Tab. S5. Results from PCA on hydraulics and hydrology indices.

Tab. S6. Results from PCA on physicochemical parameters.

Tab. S7. Results from MFA on biological, physicochemical and hydrological variables.

Fig. S1. MFA_Complex_Full_Partial_axes.

Fig. S2. MFA_Complex_Full_IND.