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An unpolluted regulated stream and its recovery gradient dependency from environmental variables

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ABSTRACT

An unpolluted regulated stream and its recovery gradient dependency from environmental variables.

Periods of thermal stratification in deep dams can result in increased liberation of ammonia and phosphates at the sediment-water interface, which can cause nutrient enrichment in the downstream river section. We studied the recovery gradient of a thermally altered, regulated, unpolluted stream and its dependency on the main environmental variables. Macroinvertebrate assemblages were assessed in the spring-summer and autumn periods, at three sampling sites in a 1.25 km river reach, downstream from the dam, for 4 years (2019-2022). Water samples were collected from all riverine sites and one site in the dam. Nutrients were measured and hydrological alteration indices were calculated for the longitudinal gradient survey. We aimed to unravel the main environmental variables linked to the dam-induced hydro-morphological pressure affecting macroinvertebrate communities. Complex interactions between nutrients and the main physical parameters were identified as responsible for the observed trends. The analysis revealed natural-born hypolimnetic ammonia as the most contributing parameter to the biomonitoring metrics reflecting the status of macroinvertebrates. Ammonia enters the river system mainly during the dam thermal stratification periods (spring-summer) and as a result, macroinvertebrate communities had the highest recovery rates in the autumn. In this season the stream can achieve good ecological status even as near as 25 m below the dam wall if ammonia concentrations are low. Therefore, seasonal fluctuations of parameters such as dissolved oxygen and temperature in the hypolimnion are affecting invertebrate communities.

KEY WORDS: hypolimnion, ammonia, regulated streams, recovery gradient, macroinvertebrates, nutrients.

RESUMEN

Una corriente regulada no contaminada y su dependencia del gradiente de recuperación de variables ambientaless.

Los períodos de estratificación térmica en presas profundas pueden provocar una mayor liberación de amoníaco y fosfatos en la interfaz sedimento-agua, lo que puede provocar un enriquecimiento de nutrientes en el tramo río abajo. Se estudió el gradiente de recuperación de un pequeño río no contaminado, regulado y alterado térmicamente y su dependencia de las principales variables ambientales. Se evaluaron las comunidades de macroinvertebrados en los períodos primavera-verano y otoño, en tres sitios de muestreo, en un tramo de río de 1.25 km aguas abajo de la presa, durante un período de 4 años (2019-2022). Se recolectaron muestras de agua de todos los sitios en el tramo del río y de un sitio en la presa. Los nutrientes y parámetros ambientales fueron medidos y se calcularon índices de alteración hidrológica a lo largo del gradiente longitudinal. Nuestro objetivo era desentrañar las principales variables ambientales vinculadas a la presión hidro-morfológica inducida por la presa que afecta a las comunidades de macroinvertebrados. Se identificaron interacciones complejas entre los nutrientes y los principales parámetros físicos como responsables de las tendencias observadas. El análisis reveló que el amoníaco hipolimnético natural es el parámetro que más contribuye a las métricas de biomonitoreo que reflejan el estado de los macroinvertebrados. El amoníaco ingresa al sistema fluvial principalmente durante los períodos de estratificación térmica de la presa (primavera-

verano) y, como resultado, las comunidades de macroinvertebrados tuvieron las mayores tasas de recuperación en el otoño. En esta estación del año, el río puede alcanzar un buen estado ecológico incluso a una distancia de hasta 25 m por debajo de la pared de la presa si las concentraciones de amoníaco son bajas. Por lo tanto, las fluctuaciones estacionales de parámetros como el oxígeno disuelto y la temperatura en el hipolimnio están afectando a las comunidades de invertebrados.

PALABRAS CLAVE: *hipolimnio, amoníaco, ríos regulados, gradiente de recuperación, macroinvertebrados, nutrientes.*

INTRODUCTION

The thermal regime of unregulated river reaches depends principally on atmospheric temperature, hydro-morphological settings, topography and degree of shadiness. (Halaj et al., 2015; Salmaso et al., 2016). Regulated rivers downstream from dams normally maintain higher winter and lower summer water temperatures (T), as compared to free-flowing streams (Salmaso et al., 2016; Meißner et al., 2018). This can be a consequence of the temperature buffer capacity of the reservoir, the slower temperature exchange of the water masses or the depth of the reservoir discharge channel (epi- vs hypolimnetic release). As a result, a reduction in annual and daily temperature fluctuations occurs (Olden & Naiman, 2010). Thus, T in regulated rivers show a weaker correlation with air temperature (Webb et al., 2008), since qualitative and quantitative discharge fluctuations unite their significance with climatic conditions and hyporheic exchange. (Webb et al., 2008; Salmaso et al., 2016).

The natural temperature regime maintenance is essential because it influences physicochemical parameters such as dissolved oxygen (DO) and suspended solids (Webb et al., 2008) by influencing the solubility of gasses and the intensity of chemical reactions within the water column (Erickson & Stefan, 2000). In addition, temperature is one of the most significant parameters determining the intensity of biological processes occurring in lotic ecosystems (Caissie et al., 2007; Webb et al., 2008). Specifically, in stream macroinvertebrates, temperature exerts a strong influence on metabolism, growth, feeding, fecundity, mating emergence or survival rates (Dallas & River-Moore, 2012). Seasonal alterations of such rates can result in disruption of the complete life cycle due to failure to find mates or food (Olden & Naiman, 2010).

One of the many activities causing thermal pressures on freshwater ecosystems is river regulation and water abstraction (Dallas, 2008). Physicochemical alterations as a result of temperature regime change are not very well studied, nor widely recognized as a serious impact. For example, at the EU level, only 13 countries have considered this anthropogenic influence as an ecologically important impact derived from river regulation by dams (Kampa et al., 2017).

In the Dalgachka River, Ovcharovo Dam was designed for irrigation purposes and has a 29 m height wall. It has a hypolimnetic release, so it tends to warm up the river down from the dam during cold months and cool it down in the spring/summer period, as usual (Meißner et al., 2018). In addition, thermally stratified lentic water bodies, such as the studied one, can accumulate ammonia (NH_3) near the bottom due to the decomposition of organic matter, nitrogen fixation processes and/or the excretion of nitrogenous wastes from animals (Environmental Protection Agency, 2013). This accumulation of inorganic nitrogen can be further enhanced by a release from the sediments due to reduced oxygen concentrations (Mermillod-Blondin et al., 2024). The depletion of oxygen quantity could be exacerbated by thermal stratification (Rodal-Morales et al., 2024) which can become stronger with rising temperatures because of climate change. In the case of the studied water objects the consequence could be further dam eutrophication and nutrient enrichment of the downstream river section.

As the management of water bodies and their quality gains greater importance (Rodal-Morales et al., 2024), and considering the short period (till 2027) for achieving good ecological status for all surface water bodies in the EU (European Commission, 2019), gaining a better understanding of the interactive effect from hydro-morphological pressure becomes more important.

The subsequent thermal, oxygen and nutrient regime alterations are essential and require a long-term monitoring program within a water body that is not affected by additional anthropogenic pressure. Dalgachka River is such an example because: (1) it is free from intensive aquaculture and therefore organic pollution within the lentic ecosystem is lacking; (2) its environmental flow program maintains a constant runoff for ecological purposes of about 20 l/s (although the minimum acceptable flow should not be less than 40 l/s); (3) agricultural practices around the stream are conducted in a heterogeneous landscape with an extensive natural riparian habitat (Fig. 1); (4) the river reach is part of the European network NATURA 2000 and is protected by the Birds Directive, bordering protected sites by the Habitats Directive (Fig. 2). Considering all pointed facts, the goal of the study emerged.

The main objective was to evaluate the environmental parameters with the greatest significance for the longitudinal recovery gradient of macroinvertebrate communities in the river reach. In addition, the study aims to elucidate the influence of processes related to water quality parameters within the dam and their role in the ecological status assessment in the riverine sampling sites, using multivariate analyses and the use of biomonitoring metrics. The study hypothesized that thermal stratification in the dam can lead to internal loading and to increased concentrations of ammonia and phosphates at the bottom layer, which can cause nutrient enrichment in the downstream river section. Especially, considering that anaerobic conditions augment the content of dissolved inorganic nitrogen and phosphorus in the water column (Mermillod-Blondin et al., 2024).

MATERIALS AND METHODS

Study area

Dalgachka River is a small stream (14 km long) springing from the northern slopes of Preslavska Planina mountains, near the town of Targovishte, North-East Bulgaria. The main lithology of the slopes consists of consolidated non-carbonate rocks: sandy limestones, marls and clayey marls with sandstone interbed and orbitoline sandstones (Nikolova, 2010). It includes several small dams and is part of the Kamchia River watershed. The national typology classifies the studied stream as a “semi-mountain river – R4”, in ecoregion 12 (Pontic Province) and the dam built in the riverbed as a “small and semi-mountain lake type – L12” (RBMP, 2016). The “broad river type”, according to Lyche Solheim et al. (2019) is “R-09: mid-altitude, siliceous, very small-small”.

The Ovcharovo dam was constructed in 1977 for irrigation purposes, about 4 km downstream from the spring. It has a perimeter of about 2.5 km. The dam wall is 29 m high and its uppermost part is located at 306 m above the sea level. The maximum storage capacity is about 3 x 10^6 m^3 but the maintained volume was less than 2 x 10^6 m^3. A “minimum acceptable flow” (Government of Bulgaria, 2012) or environmental flow of 0.019 m^3/s is released from the hypolimnion as an average from June 2018 till November 2022.

One sampling site was established in Ovcharovo Dam (OV) for water quality samplings and analysis from the epilimnion. Measurements *in situ* and collection of water samples for water quality parameters were carried out at about 3 m depth.

Three sampling sites were monitored in the Dalgachka River. They are longitudinally distributed within a 1255 m reach downstream from the dam, at an average elevation of 262 m a.s.l (Fig. 1 and Fig. 2). The distance between sampling sites from the spillway was established considering the stream width/length ratio and the territory of the protected area (Fig. 2). Outside of NATURA 2000 and further downstream, the pressure from agriculture intensifies and the management of the dam is no longer with great significance for macroinvertebrates.

The first stream sampling site (Dalgachka 1 – D1) is located about 25 m downstream of the dam wall (Fig. 1 and Fig. 2). The average width of the stream is 0.85 m and the average depth is 0.08 m. The stream reach is moderately shaded with *Salix fragilis* and *Salix purpurea* as dominant tree and shrub species, respectively. Helophytes are represented by 4 species (Table S1, supplementary information, available at https://www.limnetica.net/en/limnetica) and cover 5% of the stream banks and channel. The riverbed substrate consists pri-

Figure 1. Picture of the sampling sites. *Imagen de las localidades de muestreo*.

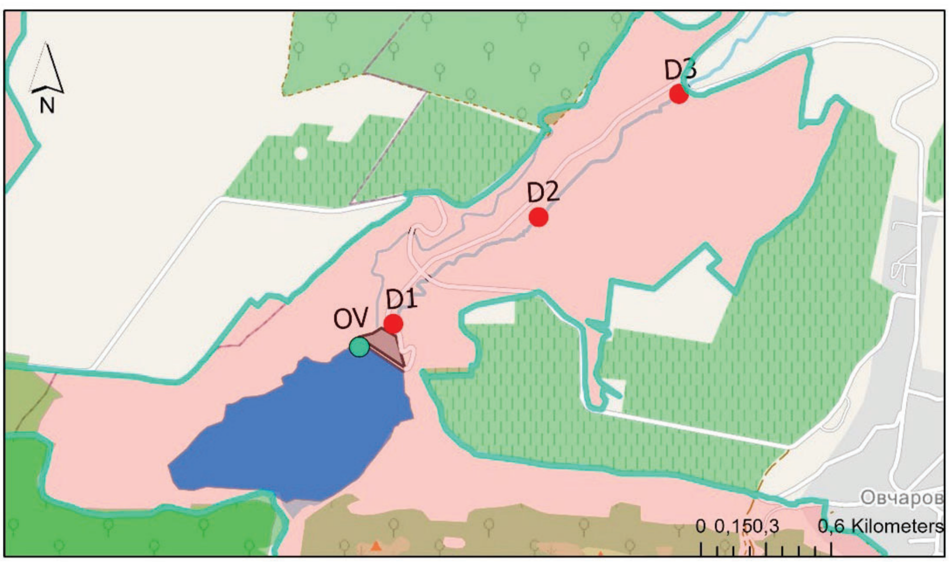

Figure 2. Map of the sampling sites. OV - sampling site at the dam. D1 - first sampling site at the river. D2 - second sampling site at the river. D3 - third sampling site at the river. The area in pink color is protected area by the Birds Directive and in dark green is protected area by the Habitats Directive. *Mapa de las localidades de muestreo. OV - localidad de muestreo en la presa. D1 - primera localidad de muestreo en el río. D2 - segunda localidad de muestreo en el río. D3 - tercera localidad de muestreo en el río. En color rosa es área protegida por la Directiva de Aves y en verde oscuro es área protegida por la Directiva de Hábitats.* Coordinates of the sampling sites: OV coordinates - 43.1879°N 26.6282°E, D1 coordinates - 43.188810°N, 26.629713°E, D2 coordinates - 43.191573°N, 26.635145°E, D3 coordinates - 43.194996°N, 26.640548°E

marily of macrolithal (20–40 cm diameter; 40%). Mesolithal (6-20 cm), microlithal (2-6 cm), akal (0.2–2 cm diameter) and empty dreissenid shells are evenly distributed at the site with about 15% for every sediment type.

The second stream sampling site (Dalgachka 2 - D2) is located 675 m downstream from the reservoir. The average width of D2 is 1.05 m and the average depth is 0.06 m. The sampling site is completely shaded by broadleaved trees (*Salix fragilis*). Helophytes are not present in D2 (Table S1). The substrate is dominated by mesolithal

(45%) and macrolithal (25%). Microlithal (10%), akal (10%), psammal (5%) and agrillal (<0.02 cm diameter; 5%) compose the rest of the size classes.

The third sampling site in Dalgachka River (D3) is located 1.25 km from the dam. D3 is 1.15 m wide on average with a depth of 0.06 m. The site is almost totally shaded by the tree canopy (*Salix fragilis*). Shrubs and helophytes are present as well (Table S1). Mesolithal (50%) dominates the bottom substrate. Microlithal represents 20%, followed by macrolithal, akal and psammal (10% each one).

In order to address the possible influence of the riparian habitat on invertebrate communities the QBR index (Munné et al., 2003) was calculated. This index evaluates both the riparian forest quality (cover, composition, structure) and the main hydromorphological alterations.

Collection and determination of macroinvertebrates

A total of 17 macroinvertebrate samples were collected from D1, D2 and D3 during the four years (2019-2022). The sampling events took place in June 2019, October 2019, June 2020, June 2021, June 2022, and November 2022. In June 2019 it was not possible to take samples at D2 due to the lack of surface flow. This was a consequence of the very small volume of the runoff and its infiltration in the substrate.

Macroinvertebrates were sampled in accordance with Cheshmedjiev et al. (2011). Standardized kick-net (500 µm mesh size, 25 x 25 cm frame) was used for kick sampling. One "kick" covered 0.125 m^2 and corresponded to one sub-sample. Ten sub-samples were taken (1.25 m^2) at every site (30 m length) proportionally to the microhabitat distribution.

Laboratory identifications were made using a 20-80x magnification stereomicroscope (BRESSER, Researcher ICD LED). The general taxonomic level was family, except for Tricladida and Oligohaeta, identified as such. Some taxa (*Dugesia* sp., *Sphaerium* sp., *Astacus astacus*, *Ephemera* sp., *Chironomus* sp., *Rheotanytarsus* sp., *Nepa cinerea* and *Ranatra linearis*) were identified to genus and species level.

Environmental variables

Physicochemical parameters

In situ measurements included T, pH, DO and conductivity (CD). T and CD were measured with a portable conductometer WTW 196 LF (WTW Gmbh, Valheim, Germany). DO and pH were measured with a multiprobe Senso Direct 150 Lovibond (Tintometer GmbH, Dortmund, Germany).

Collection of water samples for standard nutrient analyses was taken in every sampling event, according to Bulgarian State Standard EN ISO 5667-6:2016. The parameters analyzed in the laboratory were phosphates (PO_4), nitrites (NO_2), nitrates (NO_3) and ammonia (NH_3). Those nutrients were analyzed using standard colorimetric analysis, with references to the methods found in the guidance for photometer Hanna HI 83200 (Hanna Instruments, Rhode Island, USA).

Hydrological and hydraulic parameters

The environmental flow discharge data was collected from the dam management office. They provided information about the average runoff on ten-day periods. Based on their data, a graphic of the hydrological regime was created for the studied period (Fig. 3). Flow time series 1 year prior to every sampling event were used for the calculation of hydrological indices. Six indices related to the magnitude and rate of change of the environmental flow were chosen, following the findings of White et al. (2017). The indices are: (1) mean annual average discharge (QMEAN); (2) median annual discharge (Q50); (3) coefficient of variation of the annual discharge (QCVANN); (4) number of positive changes in flow conditions for 1 year, of more than 0.004 m^3/s (QPORR); (5) number of negative changes in flow conditions for 1 year of more than 0.004 m^3/s (QNERR).

In addition, we included one more hydrologic parameter related to the environmental flow and two related to the reservoir water volume. The parameters are: (1) total volume of water intended for environmental flow 1 year prior to sampling events (V); (2) water volume in the dam during sampling events (VD); (3) average water volume

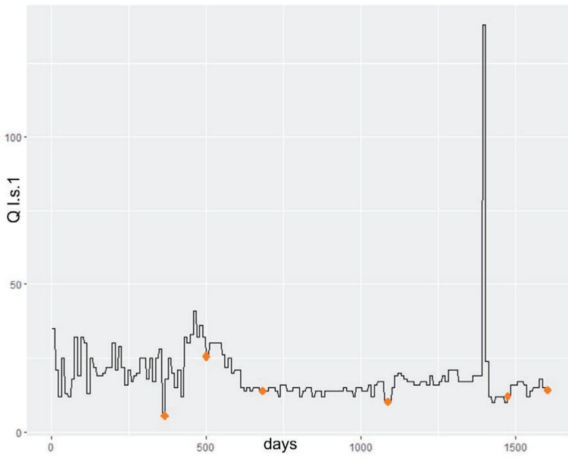

Figure 3. Hydrological regime from June 2018 to November 2022. Points show sampling events. *Régimen hidrológico de junio de 2018 a noviembre de 2022. Los puntos muestran eventos de muestreo.*

in the dam for 1 year prior to sampling events (VD_{AV}).

During fieldwork we made *in situ* measurements of the average water velocity (Vel) by the propeller of Global Water Flow Probe FP 201 Nibco – NSF – PW (Global Water Instrumentation Inc., - a Xylem brand, Golden River, California, USA). Based on this information and the width and depth of the channel, the instantaneous discharge (Q) in the river was calculated too.

Biomonitoring metrics and Ecological Status (ES) assessment

Several biological indices for the determination of the ES were used. According to the Water Framework Directive (WFD) (European Commission, 2000) and the Bulgarian Ordinance H-4/2012, the ES is a result calculated from indices using biological quality elements (BQEs). The mentioned ordinance, in compliance with the WFD, classifies surface waters in Bulgaria according to their BQEs, in high, good, moderate, poor and bad ecological status.

In situ and laboratory-analyzed physicochemical parameters, or the so-called physicochemical quality elements (PCQEs) according to the WFD, are classified as accompanying information which serves as supporting data for the ES assessment. Results from PCQEs classify surface waters in high, good or moderate status (European Commission, 2005; Government of Bulgaria, 2012).

The following metrics were calculated using macroinvertebrate communities:

- Total number of taxa, or richness (S), as a simple biodiversity metric reflecting changes at a certain taxonomic level.
- Ephemeroptera, Plecoptera and Trichoptera richness (EPT) is a widely used index which exhibits stability in unpolluted sites, is suitable for semi-mountain river sections (Cheshmedjiev & Varadinova, 2013), sensitive to hydrological alterations (Carlisle et al., 2012, Meißner et al., 2018, Mellado-Díaz et al., 2019) and more rigorous than other indices in years with atypically low flow conditions (Doychev, 2023).
- Relative abundance of EPT taxa (%EPT) to the total abundance of macroinvertebrates.
- Adapted Biotic Index (BI) is an index which has been developed and widely used in Bulgaria for more than 24 years (Cheshmedjiev & Varadinova, 2013) mainly concerning the state monitoring program and WFD obligations (Government of Bulgaria, 2012).
- Biological Monitoring Working Party (BMWP) is an almost globally applied index, developed for UK streams (Hawkes, 1998), with several regional modifications. It is based on the sensitivity of invertebrate families to organic pollution.
- Average score per taxon (ASPT) is a derivative of the BMWP (ASPT=BMWP / no. of families), thus equally based on the sensitivity to organic pollution but less influenced by the number of taxa. Its sensitivity to other anthropogenic impacts, such as hydrological alterations, has been previously noted (Šidagytė et al., 2013; Mellado-Díaz et al., 2019).
- The dominance index (DOMN) (Simpson, 1949) demonstrates the ability of more resistant and resilient taxa to increase their relative abundance in adverse conditions.

Data analysis

Principal component analysis (PCA) was conducted to extract the main information from the sets of environmental variables in order to reduce the dimensionality of multivariate data and visualize better the results. PCA was applied separately on hydrological indices and physicochemical parameters in the R environment (R 4.2.3, R Core Team, 2023), using "factoextra" and "FactoMineR" packages. The significance ($P<0.05$) of the environmental variables for principal components 1 (Dim 1) and 2 (Dim 2) were determined using the "dimdesc" function in "FactoMineR". The contribution for each of the explaining variables in Dim 1 and Dim 2 simultaneously, was visualized by a bar plot and only those which were statistically significant and above the average contribution were selected for further analysis (Kassambara, 2017).

Simple linear regression analysis was chosen as a final step because of its reliability and simplicity (Philips et al., 2024). The "important" environmental variables, defined from the PCA, were used for linear regression analysis in XLSTAT (Lumivero, 2023) to seek firm interactions between them and with biomonitoring metrics. Only statistically significant models with $R^2 > 0.36$ are represented since this value is good enough for making predictions (Phillips et al., 2018).

RESULTS

Environmental variables

Physicochemical parameters

Ovcharovo Dam registered T of the epilimnion during sampling events between 14.2 and 26.3 ºC. DO was in the interval between 6.93 and 10 mg/l. The highest DOs was from autumn samplings and the lowest were from June 2019 and 2022. CD varied in the interval from 226 and 290 µS/cm² and pH was between 8 and 8.33 (Table S2, supplementary information, available at https://www.limnetica.net/en/limnetica).

NH_3 and NO_2 from the reservoir showed slight fluctuations and registered values from 0 to 0.22 mg/l and from 0.02 to 0.09 mg/l respectively. The lowest and the highest results for NH_3 were from the autumn. NO_3 at OV demonstrated clear seasonal differences with mean values for autumn

a

b

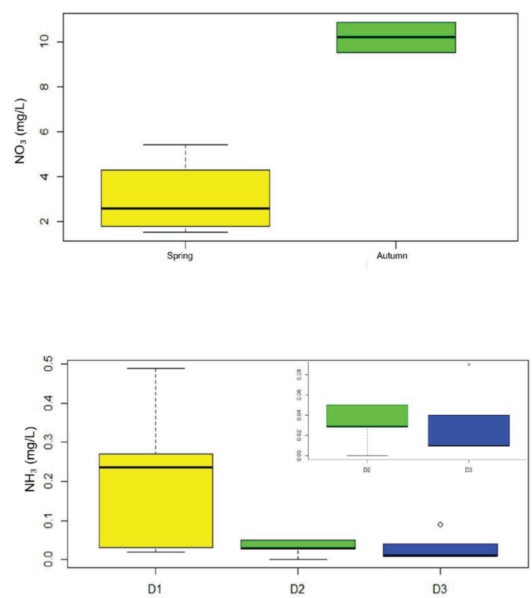

Figure 4. a) Boxplots for nitrate concentrations from the dam by seasons b) Boxplots for ammonia concentrations from the river by sampling sites. *a) Diagramas de caja con concentraciones estacionales de nitrato de la presa por estaciones. b) Diagramas de caja para las concentraciones de amoníaco del río por localidades de muestreo.*

samplings of 10.2 mg/l and spring-summer ones of 2.22 mg/l (Fig. 4a). PO_4 fluctuated from 0 to 0.2 mg/l without any visible seasonal pattern (Table S2).

Stream water quality measurements showed that the average T at D2 was with 2ºC higher than D1 and 1ºC higher than D3. DO was with the lowest average values on D1 (6.34 mg/l) and longitudinally increased with the distance from the dam, reaching 7.5 mg/l at D2 and 7.9 mg/l at D3. The pH was in the spectra between 7.69 and 7.98 at all stream sites. The lowest was registered at D1 and the highest at D2. The average value of CD was between 370 µS/cm² at D2 and 504 µS/cm² at D3. D1 was with very close results to D2 (Table S2).

A clear longitudinal reduction of average NH_3 concentrations was observed, from a maximum of 0.214 mg/l at D1 to the minimum values at D3 of 0.016 mg/l (Fig. 4b, Table S2). Nitrites also demonstrated a similar pattern and in a downstream direction were reducing their concentrations but only till D2, where the lowest mean val-

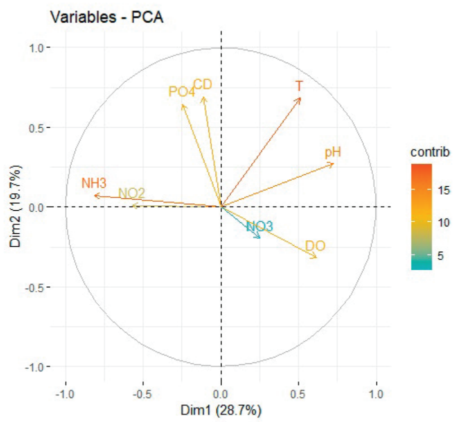

Figure 5. PCA correlation circle with physicochemical parameters from all stream sites. The contribution of every variable is colored – "contrib". *Círculo de correlación de la PCA con los parámetros fisicoquímicos de todas las localidades de muestreo del río. La contribución de cada variable está coloreada – "contrib".*

ue is registered. Measurements of NO_3 and PO_4 did not detect a clear distinction between sampling sites longitudinally (Table S2).

Physicochemical variables resulting from all stream sites were subjected to PCA. Dim 1 and Dim 2 respectively, explained 28.7% and 19.7% of the variation (Fig. 5). The first three parameters with the greatest contribution for Dim 1 are NH_3, pH and DO. For Dim 2 those parameters are CD, T and PO_4 (Table S3, supplementary information, available at https://www.limnetica.net/en/limnetica). Only nitrates are not statistically significant (Table S4, supplementary information, available at https://www.limnetica.net/en/limnetica) and because of that, we have chosen for testing with linear regression models only variables with contribution close to the average one (Fig. S1, supplementary information, available at https://www.limnetica.net/en/limnetica) for both principal components – T, NH_3, pH, CD, DO and PO_4.

Simple linear regression models were built from measurements in OV and D1 to analyze how physicochemical conditions in the dam could influence water quality in the river. Two of the models were statistically significant (Fig. 6, Table S5). It is visible that higher concentrations of dissolved oxygen from the dam (DOd) are related to less NH_3 and less PO_4 at D1 in 85% and 78% of the cases, respectively (Fig. 6, Table S5 (supplementary information, available at https://www.limnetica.net/en/limnetica)).

Hydrological and hydraulic parameters

Considering water volumes in the dam, during the samplings, is clear that June 2022 coincides with the highest water volume. The lowest VD was from June 2020. VD_{AV} shows completely different results, registering a maximum in November 2022 and a minimum in June 2021 (Table S6, supplementary information, available at https://www.limnetica.net/en/limnetica).

The data gathered by water velocity measurements, show that Vel is in the range between 0.25 and 0.35 m/s at all sampling sites. D1 had higher

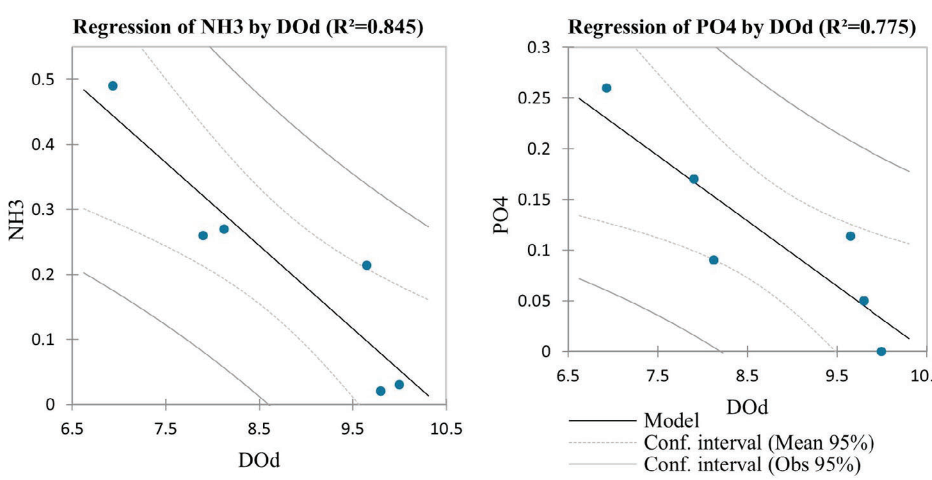

Figure 6. Linear regression analysis between oxygen concentrations in the dam (DOd) and nutrients from D1. *Análisis de regresión lineal entre las concentraciones de oxígeno en la presa (DOd) y nutrientes del D1.*

velocities due to the smaller width of the channel and D2 and D3 registered similar values from every measurement, excluding June 2019, when surface flow at D2 was not registered. The daily flow during sampling collections was between 0.005 and 0.025 m^3/s (Table S6).

From the results considering V, is visible that the period before the sampling event in June 2021 corresponds to the smaller environmental flow. October 2019 was with the highest results. The difference between both periods was more than 300 000 m^3. The autumn of 2019 registered the highest Q50 and QMEAN. Those values where slightly higher than the results of June 2019 and 2020. The lowest Q50 and QMEAN were connected to June 2021 (Table S6).

The first year of the monitoring – 2019, considering both sampling events is specific in comparison to the rest because of the larger number of positive and negative changes in the daily flow discharge – QPORR and QNERR. This variability is almost absent considering the period connected to the samplings from June 2021 when only 2 changes of more than 4 l/s occurred and the environmental flow was almost static (Fig. 3 and Table S6).

PCA considering hydrological indices, hydraulics and water volume data shows that 51.5% of the variance was explained by Dim 1 and 26.5% by Dim 2 (Fig. 7). The variables with the greatest contribution in Dim 1 are Q50, QMEAN and QNERR. In Dim 2 the longest vectors are for QCVANN, VD_{AV} and V (Table S7, supplementary information, available at https://www.limnetica.net/en/limnetica). Only Q was not statistically significant (Table S8, supplementary information, available at https://www.limnetica.net/en/limnetica). Because of that, we decided to use for further data analysis hydrological indices with contributions greater than the average one for both principal components – Q50, QNERR, V, QMEAN, QCVANN, VD and QPORR (Fig. S2, supplementary information, available at https://www.limnetica.net/en/limnetica).

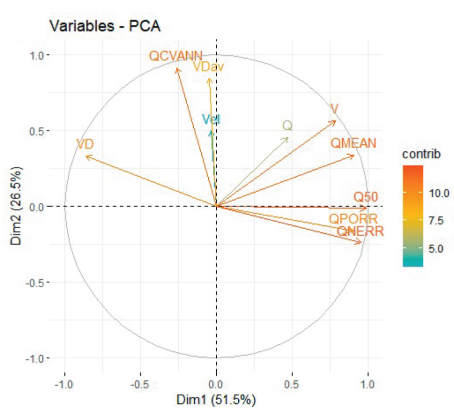

Figure 7. PCA correlation circle with hydraulic parameters, hydrological indices and dam water volumes. The contribution of every variable is colored – "contrib". *Círculo de correlación de la PCA con parámetros hidráulicos, índices hidrológicos y volúmenes de agua de la presa. La contribución de cada variable está coloreada – "contrib".*

PCA demonstrates very well the strong positive correlation between most of the hydrological indices related to the magnitude of the discharge and its negative and positive changes. Those are the most contributing variables in Dim 1, which correlate negatively with the water volume of the dam, during samplings (Fig. 7, Table S8). This means that during the studied period augmenting the water volume of the dam is related to reduction and homogenization of the environmental flow and vice versa.

In Dim 2 QCVANN and VD_{AV} are positively correlated between each other and with Vel and Q (Fig. 7, Table S8). That principal component reveals that during those 4 years, retaining higher annual water volumes in the dam resulted in a higher coefficient of variation of the annual discharge.

Macroinvertebrate responses

Considering the total number of macroinvertebrate taxa is clear that D2 has the greatest number (45) and D1 and D3 are equivalent (38) (Table S9, supplementary information, available at https://www.limnetica.net/en/limnetica). Those results are not valid if we pay attention to the different sampling events and the scores from all used biological indices (Fig. 8).

BMWP dynamic demonstrates that results are registered at D1 within poor, moderate and good ES. Sampling events from the spring are predominantly giving "poor" results. The autumn collection of organisms shows quite high scores that reach good ecological status (GES). The sec-

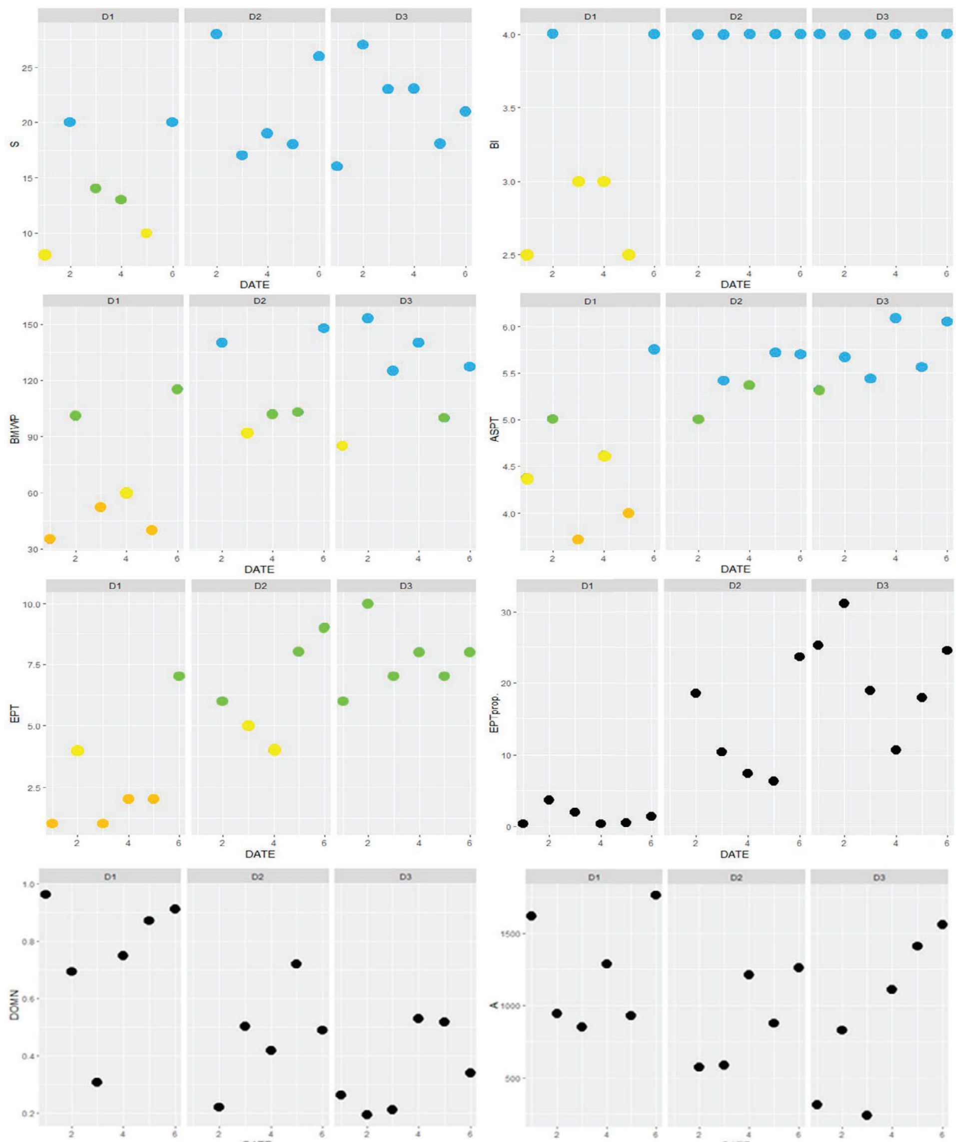

Figure 8. Temporal and spatial dynamics of the biological indices: BMWP, ASPT, EPT, EPTprop. - Ratio between the abundance of EPT individuals and total invertebrate abundance, BI, S, DOMN, and A. Color coding of the ecological status assessment in the figure: blue – high ecological status; green – good ecological status; yellow – moderate ecological status; orange - poor ecological status. *Dinámica espacial y temporal de los índices biológicos: BMWP, ASPT, EPT, EPTprop. - Ratio entre la abundancia de individuos del EPT y la abundancia total de invertebrados, BI, S, DOMN y A. Codificación de colores de la evaluación del estado ecológico en la figura: azul – buen estado ecológico; verde – buen estado ecológico; amarillo – estado ecológico moderado; naranja – deficiente estado ecológico.*

ond sampling site again showed maximum results in October 2019 and November 2022, but their values reached "high ecological status". Samplings from 2020, 2021 and June 2022 had values of about 100 points and stayed at moderate and good ES. D3 registered the higher results for BMWP and the biggest fraction of sampling dates with results better than the GES condition (Fig. 8). ASPT as a derivative of BMWP also divides the results into 4 ecological status classes. All of them are registered at D1. The highest result is from November 2022 and the worst one is from June 2019. For D2 scores are in the GES and better than the GES spectre. D3 shows the best ASPT values from all sites. High ecological status was achieved for all sampling events excluding June 2019. The best score is from June 2021 (Fig. 8).

EPT registered three ES classes at D1. GES was achieved once, at the last sampling event and once reached moderate status in October 2019. In the rest of the cases EPT stayed at "poor" ES (Fig. 8). Longitudinally, EPT improved its scores registering moderate and good status at D2, reaching the highest score for the site in November 2022. D3 never registered less than 6 families and was always at GES. The highest score was from October 2019, when 10 families were found.

% EPT does not have a scale for assessing the ES but clearly shows that the proportion of EPT representatives is growing downstream from the dam, within the protected site "Ovcharovo". Figure 8 illustrates the tendency and demonstrates that at D1 the abundance of Ephemeroptera, Plecoptera and Trichoptera is less than 5% even considering the highest result from October 2019. At D2 the ratio of those insects grew to almost 20 and 25% in the autumn samplings. In 2020, 2021 and June 2022 they were about 10 and less than 10%. D3 has the highest proportion of EPT taxa, reaching its maximum score of above 30% in October 2019 and the lowest proportion from June 2021 (Fig. 8).

According to figure 8 BI was not very sensitive to changes in the ecosystem conditions on a seasonal or interannual base because the index registered only moderate and high ES. At D1, BI was 4 times in moderate status and better than GES from autumn samplings. Further away from the impoundment BI was always with the same result – better than GES.

S was giving more detailed information than BI, registering 3 ES classes. As always, the first sampling site had the worst results from all, with values throughout all classes and equally good results from October 2019 and November 2022. At D2 results were in high status at every sampling event registering the top score for the whole studied river stretch of 28 families in the autumn of 2019. At D3 the maximal registered value was from the same sampling collection but with 27 families. The smallest number of registered families was from June 2019.

DOMN registered the highest scores at D1 with an average value of 0.75. The highest result here was from June 2019 and the lowest one from June 2020. The second most balanced community at D1 was from October 2019, which is the period with the lowest score for D2 (0.22) and D3 (0.19). D2 had mean value for this index of 0.47 and D3 with 0.34. A, as DOMN registered the greatest results at D1. The first site had mean abundance of 1232 individuals per 1.25 m^2 and with about 900 individuals per 1.25 m^2, for D2 and D3 (Fig. 8).

QBR results demonstrated the dominance of *Salix fragilis* L. within the riparian habitat at all sampling sites. In some areas of the river which are not described by QBR, the riparian zone is preserved as narrow stretches mainly dominated by young individuals of willow or small patches of poplar trees. According to the NATURA 2000 classification the habitat found near the riverbed corresponds to habitat 91E0* - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*.

QBR achieved GES at all riverine sites. D1 registered the worst score and D2 and D3 had equal results, reaching 90 points (Fig. S3, supplementary information, available at https://www.limnetica.net/en/limnetica).

Interrelationships

All biological indices were used for linear regression analysis with both types of environmental parameters. Additionally, interconnectedness was searched between hydrological indices and physicochemical parameters.

Regression of biological indices by environmental parameters

Statistically significant linear models are found between BI-NH_3, S-NH_3, BMWP-NH_3, EPT-NH_3, ASPT-NH_3 and S-PO_4. The highest R^2 is for BI-NH_3 ($P < 0.0001$) and the lowest for S-PO_4 ($P < 0.005$) (Fig. 9). Supplementary information about simple linear regression significance for every single model is provided in Table S10 (available at https://www.limnetica.net/en/limnetica). The best linear fitting for ammonia, considering "t" coefficients, is confirmed for the index adapted for Bulgaria and used in the state monitoring program – BI.

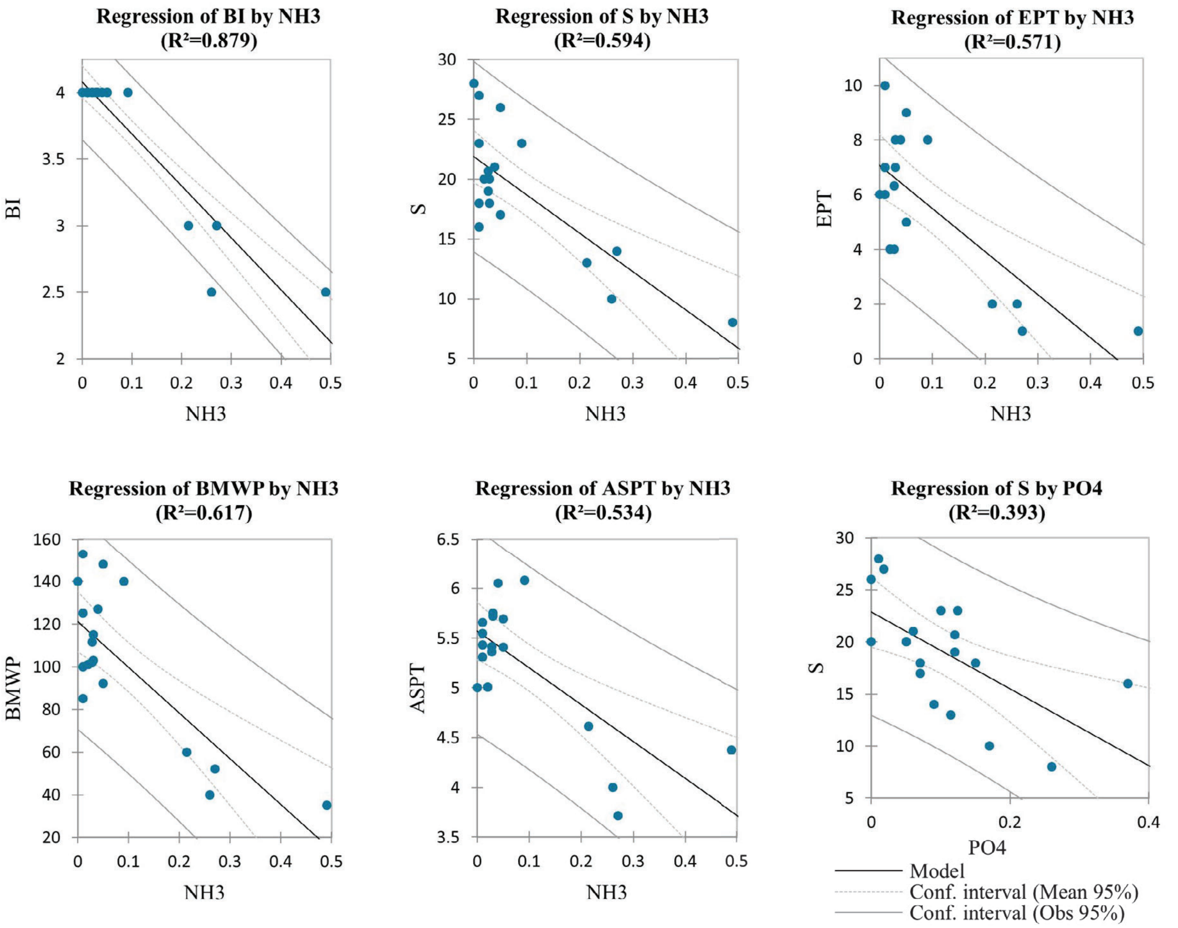

Figure 9. Linear regression analysis between physicochemical parameters from the river and biomonitoring metrics. *Análisis de regresión lineal entre parámetros fisicoquímicos del río y métricas de biomonitoreo.*

Hydrological indices calculated from the hydrological regime 1 year before the sampling event and VD (considering D1) did not create statistically significant models with the macrozoobenthic response, but only with physicochemical parameters.

Regression of physicochemical parameters by hydrological indices

The mean discharge and the total volume of the runoff are the only two hydrological indices that generate statistically significant linear models with conductivity (Fig. 10). V created models with slightly better standardized coefficients than QMEAN (Table S11, supplementary information, available at https://www.limnetica.net/en/limnetica) but augmenting them both result in CD reduction in more than 41% of the cases (Fig. 10).

DISCUSSION

The duration of the stratification and the mixing of thermally differentiated water layers play an important role in nutrient cycling and in the dissolved oxygen concentrations within the water column of lentic ecosystems (Dory et al., 2024). Unfortunately, in the context of the climate change processes, thermal stratification could make oxygen depletion in the sediment-water interface an even more serious problem leading to inorganic nitrogen enrichment predominantly in the form of

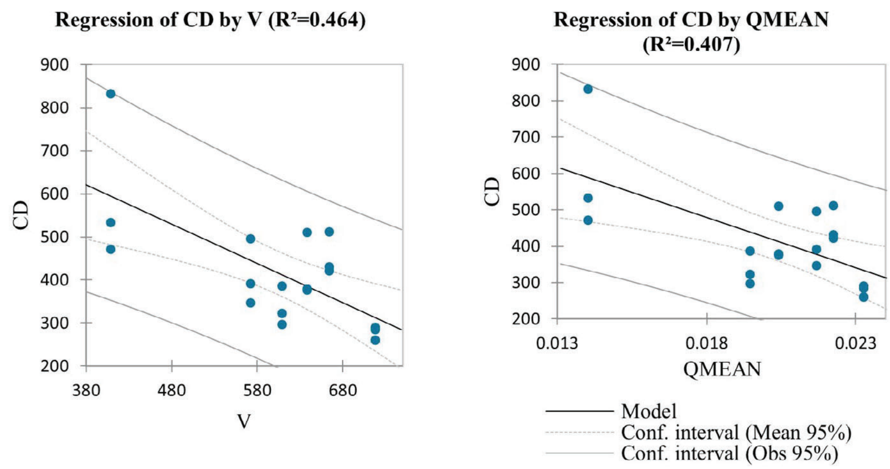

Figure 10. Linear regression analysis between hydrological indices and physicochemical parameters. *Análisis de regresión lineal entre índices hidrológicos y parámetros fisicoquímicos..*

ammonia (Rodal-Morales et al., 2024).

The limitations of our study related to the lack of information about the vertical profiles of temperature, oxygen and nutrients from Ovcharovo Dam do not allow firm conclusions related to laboratory-gathered data and analysis. We rely on results from the conducted statistical analysis based on a survey in the epilimnion and riverine sampling sites and on well-studied processes in dams and lakes (Beutel et al., 2008; Rodal-Morales et al., 2024) to confirm or reject the hypothesis.

The results confirm our hypothesis since the most influential environmental parameters for macroinvertebrate assemblages are exactly those related to natural internal loading processes, ammonia and phosphates. Considering the seasonality and that the worst ecological status in the stream is related to the thermal stratification of the dam in the spring-summer periods, our hypothesis is accepted. A management addressing the reduction of the ubiquitous biogenic element that acts as a pollutant in natural water bodies - NH_3 (Fan et al., 2021) and PO_4, from reservoirs in unpolluted regulated streams, could be a practical implication, assuring better recovery gradient for macroinvertebrates.

The studied dam-river system shows very well the same inverse proportionality from the upper reaches of the Yellow River in China (Zhao et al., 2020) in figure 6, where the linear regression analysis demonstrates the relationship between oxygen in the dam (DOd) and NH_3 at D1. In 85% of the cases augmenting the oxygen concentration at OV leads to NH_3 reduction at D1 (Fig. 6). This result is a consequence of the fact that when DOd is higher, during the autumn samplings, NH_3 in the first riverine sampling site is at its lowest values (Table S2). Considering this and the close values of T at OV and D1 during those samplings (Table S2) gives a good sign for the lack of stratification within the pelagic habitat in the dam and of possible oxygen replenishment in the sediment-water interface. This can be explained by the capability of oxygen to affect ammonia-oxidizing microorganism activity and to facilitate the transformation of ammonia (Zhao et al., 2020).

In addition, the relationship in figure 6, if explained by the well-established stratification processes, could be a consequence of the decomposition of organic matter which can lead to steady consumption of the available oxygen (Charlton, 1980). This is connected to the conversion of nitrogen to ammonia and ammonium through a process called ammonification (Environmental Protection Agency, 2013). In this way, the organic matter produced and incorporated into the water system from the epilimnion by photosynthesis (Charlton, 1980), could result in NH_3 accumulation, which can cause damage to *Nitrosomonas* spp. and *Nitrobacter* spp. bacteria and to inhibit the nitrification processes (Environmental Protection Agency, 2013).

Rising concentrations of ammonia in deeper parts of dams can be related not only to the incapability of biological nitrification but to reduced ammonia assimilation as well (Beutel, 2006). The latter is related to the slow growth of anaerobic bacteria (Beutel et al., 2008).

The impact of NH_3 in an ecosystem is likely to influence fish and invertebrate populations through chronic toxicity because of the reduced capability of growing and reproducing (Environmental Protection Agency, 2013). Considering the aquatic organisms most sensitive to ammonia group, i.e. macroinvertebrates, we should emphasize and the possibility of damaging their tissues by oxidative and physiological stress (Zhang et al., 2023). On the other hand, fish species have a similar response to ammonia as mammals and react to this toxicant with convulsion, coma or death, depending on their tolerance. Other effects could be the depolarization of neurons, excessive calcium ions influx and cell death within the central nervous system (Randall & Tsui, 2002).

These impacts can be minor and hard to notice for some distance in the downstream river reach. Longitudinally, those impacts can vary greatly within different hydrological scenarios, river flow rates, temperatures and pH amplitudes (Environmental Protection Agency, 2013). The assimilation rate for the riverine system and the uptake lengths for ammonia are variable and can range from less than 100 m to a few hundred meters in oligotrophic headwater streams (Zeiringer et al., 2018), as the studied reach of Dalgachka River.

Although ammonium concentrations in lakes are low and stable, because of its quick assimilation, this biogenic element could be responsible for 95% of the primary production in oligotrophic water bodies (Olofson et al., 2021), and in water bodies such as Ovcharovo Dam could be with great influence on the longitudinal recovery gradient. As a confirmation of this statement comes the fact that NH_3 is the most important environmental variable for the entire river reach, according to the PCA (Fig. 5 and Table S3), and the generated statistically significant linear regression models with 5 biological indices (Fig. 9). Therefore, it is directly influencing the ecological status assessment of Dalgachka River. BI was with the greatest significance, followed by BMWP, S, EPT and ASPT (Fig. 9, Table S10). Those biological indices were influenced by the ammonia from about 53% (ASPT) to 88% (BI) of the cases.

Phosphates are the other environmental parameter that generated a statistically significant model but only with S. This model was the least important since PO_4 has an impact on macroinvertebrate richness solely in 39% of the cases (Fig. 9, Table S10). Phosphates also seem to be influenced by the oxygen quantity in the dam in 78% of the cases (Fig. 6). But unlike ammonia, which has the greatest contribution in PCA on physicochemical parameters, PO_4 are just third in Dim 2 and sixth on the simultaneous contribution (Fig. 5, Table S3, Fig. S1). Therefore, they are of much less importance for the studied river reach.

Nevertheless, phosphates cannot be neglected because they did not show great importance for the studied period between 2019 and 2022 and should be considered by addressing management practices in dams. Especially, keeping in mind that, oxygen and nitrate concentrations could be important factors inhibiting internal loading with phosphorus compounds from the sediments in oligo-mesotrophic dams with a mean depth of 22 m (Beutel et al., 2008). Ovcharovo Dam has similar characteristics near the water tower, considering that the volume of the dam was never at its maximum and has a 29 m height wall and low overall concentrations of nutrients in the epilimnion (Table S2).

Nitrates in OV show very clear seasonal differences (Fig. 4a). Unfortunately, we did not have the opportunity to measure this nitrogen-containing parameter from the profundal, but we can suppose that the higher quantity of nitrates during the autumn samplings is a natural consequence of the mixing processes occurring in this period. Apparently, the water column is mixed if the temperature at OV and D1 is almost equal during autumn samplings (Table S2). Therefore, all the nutrients that accumulated during the spring-summer stratification are distributed throughout the dam, which can be an explanation for the higher concentration of nitrates at the epilimnion in October and November. We can suggest that nitrates were higher in deeper zones of the dam during warm months and because of a regression model (P<1, R^2=0.467) of phosphates from D1 by nitrates from OV. This linear regression analysis is not represented since it is not statistically significant, but still, those results can show a probable tendency which relates augmented nitrates of the epilimnion with reduced phosphates at D1.

The other physicochemical parameter that succeeds in building statistically significant regression models is the most widely used salinity parameter in Europe – CD (European Commission, 2024). Unlike ammonia, it built dependency with two hydrological indices, related to flow magnitude (Fig. 10, Table S11) but not to biomonitoring metrics.

According to the R^2 values in figure 9, in 46% of the cases augmentation of the total volume intended for environmental flow can lead to CD reduction. This is valid for QMEAN as well in 41% of the cases. Although CD did not influence directly biological indicators in Dalgachka River, Alkan et al. (2013) found a moderate correlation between ammonia and conductivity within several river reaches. This could be a possible interrelation in the Dalgachka River and an eventual negative influence if low flows are maintained in the future.

Protected sites like "Ovcharovo" usually are not influenced by severe anthropogenic pressure, although human activity is not prohibited. The lack of serious impact, different than agricultural activities near the riverbed (Fig. 1), maintaining GES in the QBR sense (Fig. S3), and NH_3 entering from the hypolimnion of the dam, additionally assures that no other variables are seriously influencing the score of the biological indices and the ecological status. This means that mitigation measures as the use of multiple intakes for the environmental flow (Kampa, 2017), could be of great benefit to the Dalgachka River. That will assure the achievement of the WFD goal at all sampling sites, and not only for the more distant from the dam where the influence from the hydro-morphological alteration is weak. Multiple intakes will discharge waters with more oxygen content, more seasonally balanced temperatures and as a consequence less NH_3.

In addition, our data can serve for the specification of the most appropriate annual timing for conducting monitoring on macroinvertebrates within thermally altered river stretches, because almost all indexes achieve the highest results during autumn samplings (Fig. 8). Therefore, to conduct a switch from the current mode of work related to the state monitoring agencies in Bulgaria, which normally monitor rivers in the spring-summer period (RBMP, 2016). This will permit the evaluation of the ecosystem during a period with the theoretically highest score and will allow the exclusion of seasonal samplings to assess the condition of the benthic community.

CONCLUSION

Our study is part of a wider research program that is focused on macroinvertebrate recovery gradients downstream from dam's river reaches with different technical specifications, diverse purposes and catchment areas. The previously published work (Doychev, 2023) was related to a bigger and strategically important dam for North-East Bulgaria with a great number of purposes. The results presented here demonstrate the significance of thermal stratification from small dams, with high walls and the influences of seasonal dynamics in the lentic ecosystem, with hypolimnetic release on stream without additional anthropogenic pressures.

We found that ammonia had the greatest significance for the ecological status assessment system in the studied unpolluted stream, related to BQE – macroinvertebrates. We can also suggest that ammonia is generated during the spring-summer thermal stratification of the dam from the sediment-water interface. Considering this, our study could be beneficial in achieving GES or better than GES condition by establishing measures that are able to mitigate the negative pressure of ammonia by improving the quality of the environmental flow.

Multiple intakes can be considered as an effective measure that will improve macroinvertebrate recovery and will assure the achievement of good ecological status in all seasons and at all sites, by balancing the temperature, oxygen and nutrient content. Those characteristics proved to be of great importance for the ecological flow - "a hydrological regime consistent with the achievement of the environmental objectives of WFD in natural surface water bodies" (European Union, 2015), if retained at about 20 l/s.

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