Response to Reviewer 1:

Thank you very much for valuable comments, significantly improving the quality of presented paper. Below we tried to include the improvements and detailed comments.

General Comments:

The authors test the influence of river incision on environmental flow estimation based on the macroinvertebrate index using data from 12 waterbodies in Poland. The topic is interesting and is one of the emerging trends in environmental and ecological studies. The introductory remarks justify the theme and are well presented.

I suggest to add few sentences to better introduce environmental/minimum flow estimation.

We added more information in the Introduction: "Environmental Flow is an amount of water required to maintain biological diversity in the river ecosystem. This definition requires to quantify ecological response of aquatic elements to flow alteration, which data are rather scare in the literature (Poff and Zimmerman, 2010). Therefore, it appears crucial to define estimate empirical ranges of environmental flows that ensure optimal habitat conditions for living organisms (Bunn and Arthington, 2002; Acreman et al., 2014)."

The other background information are up to date and provide sufficient information to put this work in the international context. The sampling campaign is well described in the methods and is suitable for the aim of the study. The statistical analyses should be described in detail to be possible to evaluate their robustness, I would expect a substantial revised paragraph in the next version.

The paragraph about statistical analysis was improved according to your suggestion.

Discussion and conclusion sections are generally well written, supported by the results but
Should be compared with the current literature and putted in the international context.

We added current literature to the discussion:

Shields, F.D., Knight, S.S. & Cooper, C.M. Effects of channel incision on base flow stream habitats and fishes. Environmental Management 18, 43–57 (1994). https://doi.org/10.1007/BF02393749.

Fornaroli, R., Cabrini, R., Sartori, L. et al. Predicting the constraint effect of environmental characteristics on macroinvertebrate density and diversity using quantile regression mixed model. Hydrobiologia 742, 153–167 (2015). https://doi.org/10.1007/s10750-014-1974-6.

The new version of discussion:

4. Discussion

The present study showed that river bed transformation, disturbing sedimentation processes and increasing the incision of the river bed vastly increases the environmental flow values for macroinvertebrates habitat suitability. This is important because incision processes are common in most European rivers (Gore, 1996). Channel incision decreases the area of suitable habitat for macroinvertebrates and increases the potential environmental flow to an extremely high level to obtain the minimum beneficial habitat capacity for macroinvertebrates (Bravard et al., 1997; Skalski et al., 2020). In incised channels, the degree of lateral connectivity between the river and floodplain is reduced, and the degree of modification of the substrate material is higher (Wyżga et al., 2012). As a consequence of channelization and incision, the continuity of the floodplain and shelf zone along the river is disrupted (Walther and Whiles, 2008; Kędzior et al., 2016; Anim et al., 2018; dos Reis Oliveira et al., 2019). Moreover, incision results in a concomitant decrease in sediment supply to the channels, reducing the microhabitat diversity and the quality of macroinvertebrate habitats (Wyżga, 2007; McKenzie et al., 2020). During the incision process, morphological changes in the channel, especially in the case of highly incised rivers, decrease the area of shelf habitat, and fluvial deposits are drastically reduced. Thus, to keep areas wet, flow requirements must be much higher than the mean annual flow and associated with inundation hazards.

Linkage between mean annual flow and environmental flow estimation has been the subject of consideration for many years (Tennant, 1976), based on the assumption that to obtain good stream environment conditions, some percentage of the average flow is required (Richter et al., 2012; van Niekerk et al., 2019). According to Tennant (1976), 10% of the average flow is the minimum flow recommended to sustain short-term survival habitat for most aquatic life forms. Thirty percent was recommended as a base flow to sustain good survival biota conditions. Sixty percent provides excellent to outstanding habitat for most aquatic life forms during their primary periods of growth and for most recreational uses. However, what about strongly channelized and incised rivers, which are the most common channel types in Europe? Our survey indicated that to obtain macroinvertebrate diversity, we need a much higher volume of water than 10% of MAF. In the case of incision, a high volume of water is needed to cover the shelves and sediment storage, which are the principal elements of macroinvertebrate habitats and refuges in a dynamic river system (Duan et al., 2009; Anim et al., 2018).

It is obvious that macroinvertebrates are closely linked to the substrate, which is highly variable in terms of particle size (Bravard et al., 1997; Merz and Ochikubo Chan, 2005; Duan et al., 2009). Alluvial processes are strongly disturbed in an incised river, leading to deepening of the channel and bed degradation (Wyżga, 2007). The areas shown in Fig. 7, which are 100% suitable for macroinvertebrates, are extremely low in incised rivers throughout the spring and autumn. In most rivers with an augmented bed, the sedimentation process is disturbed, and thus only habitats located closer to the surface, where lateral erosion occurs, provide a suitable habitat for macroinvertebrates. Modern restoration efforts often involve the artificial addition of sediments to sand (dos Reis, Oliveira et al., 2019) or modification of channel morphology to restore the sedimentation process (Violin et al., 2011; Anim et al., 2018).

The biotic integrity of rivers is primarily restricted by downstream transport of sediments controlling the integrity of fluvial ecosystems (Katano et al., 2009; White et al., 2016). Substrate characteristics such as size, stability, compactness, quality, and dynamics are a key parameter determining the occurrence and variation in macroinvertebrate communities. High substrate stability, substrate heterogeneity, and low compactness determine high macroinvertebrate diversity (Beisel et al., 2000; Duan et al., 2009). On the other hand, fine sediments can be regarded as a potential stressor for macroinvertebrates (Meißner et al., 2019). In highly incised sectors of the river, a deficiency of sediment and its compactness as well as a lack of food sources (Shields et al., 1994; Jowett, 2003) lead to impoverishment of the taxonomic composition of macroinvertebrates and favour taxa adapted to high flow only (Wyżga et al., 2013). Our results indicates that prevention of optimal conditions requires more volume of water which exceeds the mean annual flow. This conclusion seems paradoxical and rather dangerous, because increased discharge augments incision processes. We can thus fall into a kind of ecological trap. A solution may be to pay careful attention to the bed morphology, especially in the case of incised channels. There is still a problem to gather information on flow- ecological response of any organisms and extend the survey in international context should be done (Poff, and Zimmermann, 2010; Fornaroli et al., 2015). We then have two options to preserve the high biodiversity of invertebrates according to the EU water directive: to vastly increase the water volume or to restore sedimentation processes to obtain a hydrodynamic balance. As a consequence, suitable habitats for invertebrates and fish will be enlarged. The second option seems much more realistic. Only then we will be able to successfully maintain the diversity of aquatic biota.

Detailed comments:

Lines 15-28: the abstract is revised and improved:

Prediction of the environmental flow allowing to keep biological diversity and river health developed as a response to the degradation of aquatic ecosystems. Meanwhile the incision and channelization is one of the global common threads, there is still gap in the linking channel incision and environmental flow modelling. The goal of the study was to test the influence of river incision on environmental flow estimation based on the macroinvertebrate communities. The 240 macroinvertebrate assemblages (151 466 macroinvertebrates belonging to 92 families) of 12 waterbodies differing in the bed substrate, amplitude of discharge were surveyed in southern Poland. Generalized additive models supported by nonmetric multidimensional scaling revealed the optimal river parameters (depth and velocity) for the highest values of the biological monitoring working party macroinvertebrate index reflecting the habitat suitability. Using the hydraulic method (rating curve) and 2D modelling the optimal environmental flows for incised and redeposited rivers were estimated. The river incision significantly increased the values of e-flow calculations in relation to redeposited channels. The area of habitat suitability decreased with the bed incision intensity. In incised channels where sedimentation processes are altered by dam reservoirs or bedrock downcutting, the areas of suitable habitat vanish. We conclude that to obtain more suitable conditions covering the shallow zones of the incised river, the higher values of discharge should be applied, up to enormous levels. In habitat modelling, careful attention should be paid to the morphology of the modelled river, its geometry, and the fluvial processes in the active channel.

Line 41: The subject is missing.

Improved

Line 43-45: Substitute "this parameter" with "flow".

It was improved in the manuscript.

Line 46: Substitute "demand" with "abstraction".

It was improved in the manuscript.

Line 50: Its determination.

It was improved in the manuscript.

Line 52: Delete "it".

It was improved in the manuscript.

Line 56: This method was introduced much earlier.

We added the reference of: Gippel C.J., Stewardson M.J. 1998. Use of wetted perimeter in defining minimum environmental flows. Regulated Rivers: Res. Manage. 14, 53-67.

Line 56: Define "Q" the first time.

Improved

Rewritten the sentences to: Determination of discharge (Q) for environmental flow involves defining the breaking point of the hydraulic variable discharge curves as the e-flow.

Line 64: Recently the functional flows concept was introduced and should be mentioned here.

Yes you are right, we added the reference: Yarnell, S. M.; Petts, G. E.; Schmidt, J. C.; Whipple, A. A.; Beller, E. E.; Dahm, C. N.; Goodwin, P.; Viers, J. H. Functional Flows in Modified Riverscapes: Hydrographs, Habitats and Opportunities. Bioscience 2015, 65 (10), 963–972. https://doi.org/10.1093/biosci/biv102.

Rewritten the sentences to: In this case, environmental flow is defined in the category of deviation from the natural flow regime (Yarnell et al., 2015).

Line 65-66: Most of those methods were not specifically developed for macroinvertebrates but, the ones the focus at biological responses were frequently developed for fish. I suggest to mention the most of this methods were developed for fish.

Improved

Rewritten the sentences to: The methods presented above focus on the fish distribution and rarely on diversity and availability of habitats for freshwater macroinvertebrates, which is the most important and sensitive indicator of the ecological state of the ecosystem (Jowett et al., 2008; Birk et al., 2012).

Line 74: Substitute "array of" with "available in".

It was improved in the manuscript.

Line 75: Substitute "vastly" with "greatly", it is not clear which of the previous mentioned methods highlight this need.

We have delated this sentence because it is highlighted in our results.

Line 97: Substitute "silica" with "silicate".
It was improved in the manuscript.

Line 113: I suggest to include the area of subsamples and the total area sampled for clarity.
Improved
Rewritten the sentences to: We collected 20 subsamples (1 m^2 each subsamples) from each low-flow channel along a representative 100 m section of each river according to a sampling procedure for the BMWP_PL index (Bis and Mikulec, 2013).

Line 122: This choice is not adequately supported, most details should be added.
Improved.
We added sentence: The BMWP index was originally developed to represent water quality, but subsequent studies showed that it reflects ecological quality of the waterbodies and can be also related to hydromorphological impoverishment such like channel incision or straightening (Wyżga et al., 2013; Mutz et al., 2013; Mikuś et al., 2021).

Line 125-126: This index was developed for water quality, not for e-flow evaluation, this should be stated somewhere as different index (e.g. LIFE) were specifically developed.
There are two reasons why we have chosen the BMWP_PL index. First of all, in the beginning, when the index was developed it was oriented on pollution biotic index, however in the literature there are information about using BMWP_PL index for the ecological state and habitat quality of the rivers such a depth, velocity, bankfull channel (Wyżga et al. 2013, Mikuś et al. 2021). Mutz et al 2013 also confirmed that Biological Monitoring Working Party score system, used in a gravel bed river responds more to the hydromorphological channel characteristics (either channelized or not) than to water quality.
The second reason is related to invertebrate representation in the river. LIFE index is not developed for Poland especially for upland and mountainous gravel bed rivers. The existing in the literature family evaluation LIFE index is not relevant for families in given region. We have collected 128 macroinvertebrate families, and the LIFE scores were proposed only form 75% of the data. As a consequence many sampling scores in spite of high macroinvertebrates diversity had no any LIFE scores. BMWPPL index which is a part of the ecological integrity national monitoring fully covered the 240 assemblages giving more valuable information about diversity loss linked to the e-flow development. Our data confirmed that distribution of BMWPPL had similar pattern as LIFE index (Mantel-Cochran Haenszel test: $chi^2MH=21.4$. $p<0.0001$).

Line 164: Those sites are probably "location" within the same "site", please clarify.

Improved

Rewritten the sentences to: For each research section, we choose 20 points at each subsampled area differing in water velocity and water depth as the main environmental variables creating habitat heterogeneity for macroinvertebrates.

Line 168: "highest" should be better defined here.

Improved

Rewritten the sentences to: we constructed a GAM model as the best fitted method to mark out the range of hydromorphological attributes (where the BMWP_PL suitability index is the highest obtained from the GAM model curve).

Line 171: Delete "were".

It was improved in the manuscript.

Line 181-182: Probably also the sediment load downstream of the reservoir changed substantially unless proper mitigation measures were putted in place. This need to be clearly stated.

Improved.

Rewritten sentence to: The Dobczyce retention reservoir, which influences the hydrology and morphology of the river, is located upstream of the examined sector of the river (12 km). Constructing of the retention reservoir in 1986 led to a significant decline in average annual flow values (MAF values varied from 12.22 m3/s; in 1951-1985 to 10.57 m3/s in 1986-2015, $F = 49.90$, $p < 0.0001$) and broke the continuity of the sediment transport. The reduction in flow, blockade of sediment supply and longitudinal training work of the Raba led to incision of the riverbed, change the natural sediment load and permanent compactness of the bed material.

Line 185: "WUA" should be defined and is still not clear how it was calculated.

Explained in the text.

Rewritten the sentences to: We also wanted to estimate minimum flow values for two rivers which were modelled using CCHE2D. The highest values of depth and velocity obtained from the GAM model (Figure 5) for each type of river and season were plotted against the number of pixels having values resembling suitability model. Giving those calculations we were able to obtain the Weighted Usable Area (WUA) showing the most suitable habitat parameters (GAM depth plus GAM velocity). Each pixel covered 0.25 m^2 of total river area, so the number of counted calculated cells were the given values of velocity and depth of each type of river were summarized and multiplied by the surface area. Based on those calculations using CCHE2D model we were able to find the relationship between usable area and *flow values. To calculate the optimal environmental flow values the curve between flow and suitable area was created. The optimum of environmental flow was estimated as 50% of WUA values for CCHE2D modelled rivers below which the habitat retention level will have negative effect on populations conditions (Jowett et al., 2008).*

Line 196: These categories should be clearly reported.

Improved.

Rewritten the sentences to: Descriptive physical properties (water depth and velocity) were classified into two or three categories: Low, Medium and High. We used minimum and maximum values of depth and velocity range in each river group and divided them into 33 percentile ranges of the total value variability. In the case when the ranges were less than 0.5 m depth we have chosen two groups of 50 percentiles of the depth ranges.

Line 201-202: The description of the GAM models should be substantially improved as the details provided do not allows for a proper evaluation.

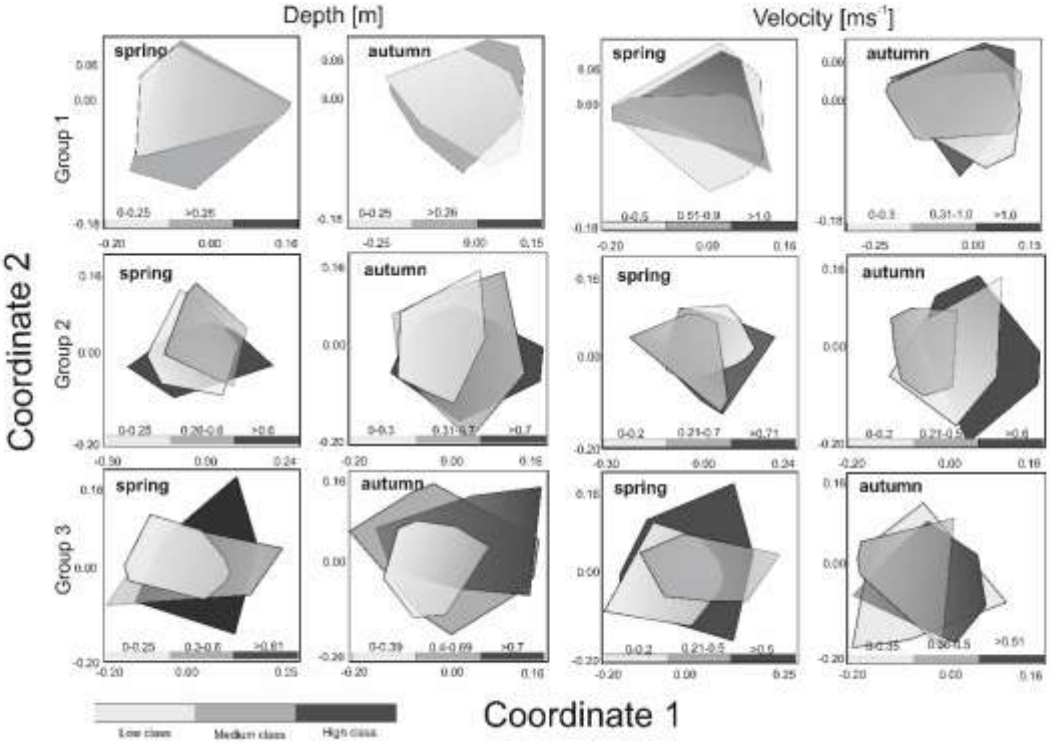
Line 202-204: As for GAM, GLM should be defined and properly described.

We improved the description of the GAM and GLM as well as we improved all Data Analysis as below: "ANOVA was used to verify the statistical significance of the differences in environmental data between the three river types groups (Statsoft, 2013). Non-metric multidimensional scaling (NMDS) was used to test the relationship between the macroinvertebrate taxonomic composition of the assemblages of the 12 rivers assigned to three types groups (Group 1, Group 2 and Group 3) and hydromorphological variables (water velocity and depth) during the spring and autumn. Descriptive physical properties (water depth and velocity) were classified into two or three categories: Low, Medium and High. We used minimum and maximum values of depth and velocity range in each river group and divided them into 33 percentile ranges of the total value variability. In the case when the ranges were less than 0.5 m depth we have chosen two groups of 50 percentiles of the depth ranges. The significance of differences between depth and velocity classes was tested by ANOSIM on the Bray-Curtis dissimilarity matrix with 499 permutations of the data. PAST software (version 3.13) was used to analyze NMDS and ANOSIM (Hammer et al., 2001).

To develop habitat suitability functions of macroinvertebrates, reflecting the optimal conditions in the river, generalized additive models (GAMs) procedures were chosen. The advantage of the method described by Jovett and Davey (2007), is that it calculates the probability of relations between dependent biotic variables and independent flow parameters. To choose the best-fitting model, we have ranked the available models according to Akaike information criteria procedure and ΔAICc values, which reflects the difference of AIC between a given model and the lowest AIC. The best fitting model, describing the relationship between independent variables (depth and velocity and its two-way interaction between *them) and macroinvertebrate BMWP_Pl index, was generalized additive model with Poisson error distribution and log link function. We have also measured the accuracy of the GAM procedures (Shearer et al., 2015). The total deviance explained calculated as the relative difference between the residual and the null deviances of the model ([null deviance-residual deviance]/null deviance) was adopted. The course of the regression line of the BMWP-PL and depth and velocity for each group of the bed material rivers was obtained using CurveExpert software, where the best fitted line for the set of nonlinear curves was applied and ranked. The BMWP_PL curve maximum values were regarded as the most suitable for invertebrates and the most preferred. We were interested in calculation of optimal condition for depth and velocity separately to obtain the optimal conditions allowing to calculate the discharge which are needed for hydraulic and 2CEDD modelling. The preferred depths and velocities for each season and river bed material groups were used to calculate the hydraulic discharges which are the most suitable for BMWP_Pl variables and recognized as environmental flow."*

Description of mixed models in results:
There were significant effects of depth and velocity and its combination on variation of BMWP_PL index. Generally, the percentage of the total deviance was the highest for the combination of both hydrological parameters, however depth parameter alone described similar level of the total deviance. Velocity explained 38.1 and 44.5% of the total deviance of BMWP_PL variation in the mountain rivers (Group 1) for spring and autumn respectively. In other river groups the total deviance described for velocity varied between 6-29%. Bringing into consideration that both hydrological parameters alone described more of the total deviance, we regarded them in further analysis separately.

Line 217: I suggest to use transparency as it is difficult to understand how much the polygons are overlapping.
Improved.
Revised Fig. 4 is below.



Line 234: Please, use the same y scale for the different plots.

We would like to stay on the scales which were defined for each river group range. When we expand the ranges, the models extrapolate the curves to the given extensions. So we would like to avoid the extrapolation of the data.

Line 238-239: This is not necessarily true, the use of univariate models (there are not enouth details in the methods to evaluate this point) do not account for both velocity and depth at the same time. In this case the author can expect "highest values at high water velocity OR in shallower zones".
See Fornaroli, R.; Cabrini, R.; Sartori, L.; Marazzi, F.; Canobbio, S.; Mezzanotte, V. Optimal Flow for Brown Trout: Habitat – Prey Optimization. Sci. Total Environ. 2016, 566–567, 1568–1578. for an example of multivariate regression.
It was done purposely to reach the values of depth and velocity separately to include them later in the hydrological and CCHE2D modelling. We agree that much better will be to obtained both factors together in the mix model. But it was 100% methodological purpose.

Line 242: This point should be clarified.
Improved.
In the first version of the paper, we used several hydraulic methods, the final version of the manuscript was based only on the hydraulics of flows in the rating curve, excluding the wetted periemter method.

At the stage of editing, the description of this method was not removed. The text was clarified both in the results and in the methodology.
Rewritten sentence to: "Using the optimal depth characteristics reflecting the habitat suitability (Fig. 5), the environmental flow based on rating curve was defined. The results are shown in Table 4."

Line 251: The table must be read without the text, there are too many acronyms that make difficult to get the content of the table.
We would like to leave the acronyms of flow type in the table, but we added acronyms explanation under the table for better understanding the meaning of the table 4.

Line 271: Delete space.
It was improved in the manuscript.

Line 276: The lowest flow.
Improved

Line 276-278: A relevant citation should be inserted here.
This sentence was deleted and developed in the discussion chapter.

Line 282-283: This is unclear, please reword.
Improved

Line 285: Substitute "would have to " with "should".
It was improved in the manuscript.

Line 301: Delete one dot.
It was improved in the manuscript.

Line 304: Delete the first dot.
It was improved in the manuscript.

Line 304: Substitute "connectivity of" with "connectivity between".
It was improved in the manuscript.

Line 323: Substitute "varied" with "variable".
It was improved in the manuscript.

Line 325-326: This sentence need to be reworded.

Improved