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Deliverable D3.2: Manuscripts on experimental results (BOKU, AU)

RIVERS – Lead beneficiary: BOKU

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LAKES – Lead beneficiary: AU

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Executive summary- Rivers

This deliverable contains abstracts of published and submitted manuscripts on experimental results within WP 3.2 (rivers). **Manuscript 1** investigates the effects of a two months experimentally induced extreme low-flow scenario on the physical, biological, and functional characteristics in a macrophyte-rich lowland stream. A significant decline in the stream wetted habitat area, an increase in water temperature, and an increase in the accumulation of fine organic matter with reduced flow, but no significant changes in dissolved oxygen or benthic chlorophyll a concentrations was identified. For **Manuscript 2** an experiment in large outdoor flumes was conducted to assess the effects of low flow, fine sedimentation, and nutrient enrichment on the structure of the benthic macroinvertebrate community in riffle and run habitats of lowland streams. For most taxa, a negative effect of low flow on abundance in the riffle habitat was found, whereas the effect was partly mitigated by fine sedimentation and by nutrient enrichment. In contrast, fine sediment in combination with low flow rapidly affected macroinvertebrate composition in the run habitat, with decreasing abundances of many species. **Manuscript 3** describes the response of benthic algae species composition, traits, biovolume and Chl-a concentration to low flow in combination with nutrient enrichment and fine sedimentation. Experiments were conducted in large outdoor stream flumes. Strong responses in the benthic algae community to sudden changes in low flow and fine sedimentation were observed, mediating rapid species turnover with a decreased algal biovolume and increased abundance of large, motile species. The objective of the study presented in **Manuscript 4** was to assess the single and combined effects of hydropeaking and cold thermopeaking on the drift of aquatic macroinvertebrates in experimental flumes. It was found that hydropeaking induced significantly higher drift rates of most macroinvertebrate taxa, whereas combined hydropeaking and cold thermopeaking revealed reduced total drift rates. **Manuscript 5** presents results on the interactive effects of higher flow velocity and nutrient enrichment for an oligotrophic stream periphyton community. The results showed a significant lower biomass development in the hydropeaking treatment, compared to the no-hydropeaking treatment. Nutrient subsidy effects were not observed, because the biomass development of periphyton was highly diminished through the pulsed flow velocity increase. Fish passage performance was investigated in a study presented in **Manuscript 6** where a full-scale experimental vertical slot fishway under two different slot configurations (C1 & C2) was used. Results show that the chub performed a higher number of upstream movements in C2, while for the barbel, a large-bodied potamodromous bottom-oriented fish, the performance was similar in both configurations. The study presented in **Manuscript 7** analysed the responses of potamodromous fish facing combinations of a primary stressor (two levels of connectivity reduction due to water scarcity) and a secondary stressor (three levels of oxygen depletion due to increase organic load of anthropogenic nature). Results show that at the unconnected level the primary stressor (lack of connectivity) overrode the effect of the secondary stressor (oxygen depletion), but when connectivity existed oxygen depletion caused a reduction of fish movements with decreasing oxygen

concentrations. **Manuscript 8** focused on the response of macroinvertebrate drift to single and combined effects of water scarcity and dissolved oxygen (DO) depletion over two seasons (winter and spring). Results showed that both stressors individually and together had a significant effect on macroinvertebrate drift ratio for both seasons. Single stressor effects showed that macroinvertebrate drift decreased with flow velocity reduction and increased with DO depletion, in both winter and spring experiments. Combined stressors interaction induced a positive synergistic drift effect for both seasons.

Four more manuscripts were submitted presenting experimental studies on fish movement and on effects of flow events and nutrient addition on stream periphyton and macroinvertebrates. **Several further manuscripts are in preparation** and are listed in this deliverable.

Executive summary- Lakes

This deliverable contains abstracts of published and submitted manuscripts on experimental results within WP 3.2 (lakes) conducted in Denmark, UK and Germany. The experiments included studies of effects of a heatwave and pulsed N-dosing (Denmark), combined impact of nutrient addition, warming and extreme rainfall events (UK) and extreme effects on mixing and of browning (Germany). The experiment in Germany was started one year later than planned as there were problems with permissions. The results from this experiment are therefore online in the pipeline (see planned papers at page 27-28).

Heat wave experiment in experimental ponds in Denmark: A one-month heating experiment (+5^{o}C) was conducted in mid-summer in existing experimental ponds (since August 2003) having two nutrient levels crossed with three temperatures and four replicates of each. **Manuscript 1** focuses on effects on the fluxes of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). It was published online in *Freshwater Biology* in April 2017. **Manuscript 2** focuses on effects on growth of two species of submerged macrophytes, *Elodea canadensis* and *Potamogeton crispus*, pre-adapted to different temperature. The paper was published in *Climate Research* in December 2015.

Pulse-dosing of Nitrogen in experimental ponds in Denmark: The effects of a N pulse on N processing and storage in shallow lake ecosystems based on a $K^{15}NO_3$ pulse addition (increased in NO_3-concentration from c. 0.1 to 2 mg N/L) were studied in twelve mesocosms with relatively low nutrient levels (same systems as mentioned above). In **Manuscript 3** the dosed NO_3 was followed for five months in the primary and secondary producers and turnover and loss rates were calculated. It was published online in *Freshwater Biology* in May 2017. **Manuscript 4** focuses on how periphyton (on inert substrata), epiphyton and epipelon biomass responded to a nitrogen (N) pulse, an approximately tenfold enrichment of the NO_3-pool, under three contrasting warming scenarios. It was published online in *Hydrobiologia* in March 2017.

Multiple stress experiment in UK mesocosms: investigating the combined impact of nutrient addition, warming and extreme rainfall events on shallow lake environments. The first two papers are on biological responses, specific metrics of water quality (chlorophyll *a* and cyanobacteria) and fish. The third paper uses outcomes from the experiment for methodological advancement in UV spectroscopic analysis for estimating freshwater DOM. **Manuscript 5** focuses on the response of phytoplankton and cyanobacteria, both metrics of water quality, to the combined effect of the three stressors, asking whether warming and nutrient enrichment exacerbate the effect of one another (synergistic) as predicted, and whether this interaction may be altered by significant biomass loss as a result of extreme rainfall events. The paper aims to provide mechanistic insight of the process underlying these responses. This manuscript will be submitted to *Global Change Biology* by August 2017. **Manuscript 6** tests the short term effect of extreme rainfall events on fish stress levels and whether responses to environmental perturbations were modified by conditions within the mesocoms, warming and nutrient enrichment, alone and in combination. This manuscript is due for submission by the end of June 2017. **Manuscript 7** uses the response of algae to future environmental scenarios in lakes to estimate extinction coefficients in the UV range of algae derived DOM. These estimates will be used to update current models based on UV spectroscopic analysis for estimating freshwater DOM. This manuscript is currently under review in *Inland Waters*, submitted March 2017.

RIVERS

Paper 1: Experimental drought changes ecosystem structure and function in a macrophyte-rich stream

Published in Aquatic Sciences in May 2017 with DOI: 10.1007/s00027-017-0536-1

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Abstract: Water abstraction continues to increase worldwide, causing periods with extreme low-flow in many streams, which will likely intensify in the future due to climate change. Extreme low-flow may have major effects on in-stream habitats, organisms, and consequently ecosystem functions. We investigated the effects of a 2 months experimentally induced extreme low-flow scenario on the physical, biological, and functional characteristics in a macrophyte-rich lowland stream using a before-after, control-impact (BACI) approach. We quantified nutrient dynamics, including inorganic nitrogen and phosphorus concentrations, ammonium uptake, and whole-stream metabolism. We found a significant decline in the stream wetted habitat area, an increase in water temperature, and an increase in the accumulation of fine organic matter with reduced flow, but no significant changes in dissolved oxygen or benthic chlorophyll a concentrations. Furthermore, the relative demand and overall uptake of ammonium was lower in the low-flow reach relative to the control reach, whereas the relative demand and uptake of phosphate were higher at low-flow. Our results demonstrate that low-flow conditions cause resource limitation in stream biota most likely due to increased thickness of the diffusive boundary layers and an enhanced heterotrophic activity in the accumulated fine organic matter. Our results imply that the basal resources for productivity shift from autotrophic towards more heterotrophic resources causing a shift at higher trophic levels towards more detritivore based and less herbivore based food webs with implications for the invertebrate community composition and the distribution of functional feeding groups. Based on the strong links observed between low-flow and nutrient uptake, we suggest that functional metrics are suitable to assess the effects of low-flow conditions in small streams.

Paper 2: Multiple stress response of lowland stream benthic macroinvertebrates depends on habitat type

Published in Science of the Total Environment in May 2017 with DOI: 10.1016/j.scitotenv.2017.05.102

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Abstract: Worldwide, lowland stream ecosystems are exposed to multiple anthropogenic stress due to the combination of water scarcity, eutrophication, and fine sedimentation. The understanding of the effects of such multiple stress on stream benthic macroinvertebrates has been growing in recent years. However, the interdependence of multiple stress and stream habitat characteristics has received little attention, although single stressor studies indicate that habitat characteristics may be decisive in shaping the macroinvertebrate response. We conducted an experiment in large outdoor flumes to assess the effects of low flow, fine sedimentation, and nutrient enrichment on the structure of the benthic macroinvertebrate community in riffle and run habitats of lowland streams. For most taxa, we found a negative effect of low flow on macroinvertebrate abundance in the riffle habitat, an effect which was mitigated by fine sedimentation for overall community composition and the dominant shredder species (*Gammarus pulex*) and by nutrient enrichment for the dominant grazer species (*Baetis rhodani*). In contrast, fine sediment in combination with low flow rapidly affected macroinvertebrate composition in the run habitat, with decreasing abundances of many species. We conclude that the effects of typical multiple stressor scenarios on lowland stream benthic macroinvertebrates are highly dependent on habitat conditions and that high habitat diversity needs to be given priority by stream managers to maximize the resilience of stream macroinvertebrate communities to multiple stress.

Paper 3: Responses of benthic algal communities and their traits to experimental changes in fine sediments, nutrients, and flow

Accepted in Freshwater Biology in May 2017

Authors: Érika Maria Neif[a], Daniel Graeber[b*], Liliana Rodrigues[a], Simon Rosenhøj-Leth[b], Tinna M. Jensen[b], Peter Wiberg-Larsen[b], Frank Landkildehus[b], Tenna Riis[b], Annette Baattrup-Pedersen[b]

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Abstract: 1- Lowland stream ecosystems are subjected to multiple anthropogenic stressors, usually nutrient enrichment in combination with sedimentation of fine particles and low flow periods in summer. Here, we investigated the temporal development of the benthic algae community in response to these three stressors and linkages to the trait characteristics of the community to explore the mechanisms responsible for stress-induced community changes.

2- We investigated the response of benthic algae species composition, traits (life forms, cell size categories), biovolume and Chl-a concentration to low flow in combination with nutrient enrichment and fine sedimentation in twelve large outdoor stream flumes (12 m long) resembling small streams in size and habitat characteristics. The experiment consisted of two phases: a normal-flow phase followed by a low-flow phase (90% current velocity reduction), each spanning four weeks. We applied a eutrophication scenario (mean increases of 1.14-5.48 mg N L-1 and 0.01-0.06 mg P L-1 in the flumes for dissolved inorganic nitrogen and phosphate, respectively) throughout the experiment. Under low flow, we supplemented this with a fine sedimentation scenario (> 90% stream bed cover). We took samples once in the normal-flow phase and every week during the low-flow phase.

3- We observed strong responses in the benthic algae community to sudden changes in low flow and fine sedimentation, mediating rapid species turnover with a decreased algal biovolume and increased abundance of large, motile species. However, we did not observe any pronounced responses to nutrient enrichment. In contrast to the observations for other variables, we found a continuous increase in Chl-a concentration during low flow. This was likely due to continuous fine sedimentation during this phase, reducing light availability which probably resulted in an increase of cell-level Chl-a concentration in response to light limitation and lower rates of light-induced Chl-a degradation.

4- The rapid response of the benthic algal community to the applied stressors suggests that even short periods of major stressor exposure may significantly affect benthic algae in lowland systems. We suggest that short-term stress events may have cascading effects on several important ecosystem processes given the importance of benthic algae for the productivity of these systems.

Paper 4: Effects of hydro- and thermopeaking on benthic macroinvertebrate drift

Published in Science of the Total Environment in December 2016 with DOI: 10.1016/j.scitotenv.2016.08.022

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Abstract: The operation of storage hydropower plants is commonly linked to frequent fluctuations in discharge and water level (hydropeaking) of downstream river stretches and is often accompanied by cooling or warming of the water body downstream (cold or warm thermopeaking, respectively). The objective of this study is to assess the single and combined effects of hydropeaking and cold thermopeaking on the drift of selected aquatic macroinvertebrates in experimental flumes. The study specifically aims to (1) investigate the macroinvertebrate drift induced by hydropeaking, (2) identify taxon-specific drift patterns following combined hydropeaking and cold thermopeaking and (3) quantify diurnal drift differences under both impact types. Overall, hydropeaking induced significantly higher drift rates of most macroinvertebrate taxa. Combined hydropeaking and cold thermopeaking, however, revealed reduced total drift rates, however with strong taxon-specific response patterns. Hydropeaking during night led to significantly higher drift rates than during daytime, while in combination with thermopeaking the same trend was observable, although insignificant. Taxon-specific analysis revealed lower drift rates following hydropeaking for rheophilic and interstitial taxa (e.g. Leuctra sp., Hydropsyche sp.), whereas many limnophilic taxa adapted to low current showed markedly increased drift (e.g. *Lepidostoma hirtum* and Leptoceridae). In line with previous studies, our results confirm a significant loss of limnophilic macroinvertebrate taxa following hydraulic stress. The mitigating effect of cold thermopeaking might be explained by behavioural patterns, but requires further investigation to clarify if macroinvertebrates actively avoid drift and intrude into the interstitial, when cold water is discharged. Our results imply that river restoration projects must address the hydrological regime and, if necessary need to include suitable management schemes for hydropower plants. Besides operative management measures, the construction of reservoirs to buffer hydropeaks or the diversion of hydropeaks into larger water bodies could mitigate hydropeaking effects and foster biological recovery including limnophilic taxa.

Paper 5: Antagonistic and synergistic effects on a stream periphyton community under the influence of pulsed flow velocity increase and nutrient enrichment

Published in Science of the Total Environment in August 2016 with DOI: 10.1016/j.scitotenv.2016.08.158

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Abstract: Aquatic ecosystems are generally affected by multiple stressors, and therefore, analysing single stressor responses is not appropriate to evaluate the whole range of effects on these ecosystems. We assessed the interaction effects of two strong stressors (higher flow velocity due to e.g. daily hydropeaking) and nutrient enrichment for an oligotrophic stream periphyton community. As periphyton has a rapid reproduction rate and very short life cycles, it can therefore be expected to reflect short-term impacts and sudden changes/disturbances in the environment. We measured biomass development, algal group distribution and photosynthesis efficiency during a time period of 33days in an experimental flume setting in Lunz am See (Austria). We conducted the experiment with two treatments (no hydropeaking and hydropeaking) and three nutrient enrichments (nitrate, phosphate and nitrate+phosphate enrichment) and control (no nutrient addition). The results showed a significant lower biomass development in the hydropeaking treatment (HP), compared to the no-hydropeaking treatment (NHP) in a later successional stage (day 33). Nutrient subsidy effects were not observed, because the biomass development (chlorophyll-a) of periphyton was highly diminished through the pulsed flow velocity increase. Also a negative synergistic interaction (more negative than predicted additively) was observed. Our study confirmed for periphyton communities that for different algal groups and functional guilds the same multiple stressor combination can be detrimental for one species group (e.g. Chlorophyta) while beneficial for another (e.g. diatoms). We conclude for multiple stressor studies to consider the successional stage and community composition, when estimating the interaction effects of these stressors.

Paper 6: Passage performance of two cyprinids with different ecological traits in a fishway with distinct vertical slot configurations

Accepted in Ecological Engineering in April 2017 with DOI: 10.1016/j.ecoleng.2017.04.031

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Abstract: Adequately designed vertical slot fishways (VSF) mitigate the impact of anthropogenic obstructions on fish migrations. Until now, most studies on VSF were conducted focusing on high priority species, particularly salmonids, while other species, such as cyprinids, have received less attention. In Mediterranean rivers, where water availability is a problem, effective low discharge fishways are desirable. Attempting to contribute towards filling this gap, the present study focuses on the behaviour and passage performance of two Iberian cyprinids with different ecological traits, the Iberian barbel (*Luciobarbus bocagei*, Steindachner, 1864) and the Southern Iberian chub (*Squalius pyrenaicus*, Günther, 1868). Fish passage performance was investigated in a full-scale experimental VSF under two different slot configurations (C1 and C2), which require different discharges for equal mean water depths in the pools (Q = 110 L s−1, for C1, and Q = 81 L s−1, for C2). Results show that the chub, a small-bodied fish that utilises the upper portion of the water column, performed a higher number of upstream movements in C2, while for the barbel, a large-bodied potamodromous bottom-oriented fish, the performance was similar in both configurations. With similar overall passage success, slot configuration C2 requires a lower discharge to operate, making it the more cost-effective geometry, especially in regions affected by water scarcity.

Paper 7: Potamodromous fish movements under multiple stressors: Connectivity reduction and oxygen depletion

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Abstract: Rivers are impacted by multiple stressors that can interact to create synergistic, additive or antagonistic effects, but experimental studies on fish encompassing more than one stressor are seldom found. Thus, there is the need to study stressors through multifactorial approaches that analyse the impact of fish exposure to multiple stressors and evaluate fish sensitivity to stressor combinations. Some of the most common impacts to Mediterranean rivers are of two natures: i) water abstraction and ii) diffuse pollution. Therefore, the present study aims at studying the responses of potamodromous fish facing combinations of: 1) a primary stressor (two levels of connectivity reduction due to water scarcity), and 2) a secondary stressor (three levels of oxygen depletion due to increase organic load - of anthropogenic nature). Schools of five wild fish from a cyprinid species (Luciobarbus bocagei) were placed in a flume, equipped with see-through sidewalls to allow for behavioural analysis, and subjected to different combinations of the stressors. Results show that at the unconnected level the primary stressor (lack of connectivity) overrode the effect of the secondary stressor (oxygen depletion), but when connectivity existed oxygen depletion caused a reduction of fish movements with decreasing oxygen concentrations. This multifactorial study contributes to improved prediction of fish responses upon actual or projected pressure scenarios.

Paper 8: Macroinvertebrate short-term responses to flow variation and oxygen depletion: A mesocosm approach

Published in Science of the Total Environment in May 2017 with DOI:

10.1016/j.scitotenv.2017.05.056

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Abstract: In Mediterranean rivers, water scarcity is a key stressor with direct and indirect effects on other stressors, such as water quality decline and inherent oxygen depletion associated with pollutants inputs. Yet, predicting the responses of macroinvertebrates to these stressors combination is quite challenging due to the reduced available information, especially if biotic and abiotic seasonal variations are taken under consideration. This study focused on the response of macroinvertebrates by drift to single and combined effects of water scarcity and dissolved oxygen (DO) depletion over two seasons (winter and spring). A factorial design of two flow velocity levels – regular and low (vL) - with three levels of oxygen depletion - normoxia, medium depletion (dM) and higher depletion (dH) - was carried out in a 5-artificial channels system, in short-term experiments. Results showed that both stressors individually and together had a significant effect on macroinvertebrate drift ratio for both seasons. Single stressor effects showed that macroinvertebrate drift decreased with flow velocity reduction and increased with DO depletion, in both winter and spring experiments. Despite single stressors opposing effects in drift ratio, combined stressors interaction (vL × dM and vL × dH) induced a positive synergistic drift effect for both seasons, but only in winter the drift ratio was different between the levels of DO depletion. Stressors interaction in winter seemed to intensify drift response when reached lower oxygen saturation. Also, drift patterns were different between seasons for all treatments, which may depend on individual's life stage and seasonal behaviour. Water scarcity seems to exacerbate the oxygen depletion conditions resulting into a greater drifting of invertebrates. The potential effects of oxygen depletion should be evaluated when addressing the impacts of water scarcity on river ecosystems, since flow reductions will likely contribute to a higher oxygen deficit, particularly in Mediterranean rivers.

Paper 9: Using river microalgae as indicators for freshwater biomonitoring: Review of published research and future directions

Accepted in Ecological Indicators in June 2017

Authors: Naicheng Wu, Xuhui Donga, Yang Liud, Chao Wang, Annette Baattrup-Pedersen, Tenna Riis

Trait-based approaches may give insights into underlying mechanisms of relationships between biological communities and environmental stressors, and are increasingly used in ecological studies, but are only very recently considered for freshwater riverine microalgae. Here, we i) review the research trend in riverine microalgae during the past 26 years in order to conduct a quantitative and qualitative analysis for global trends in the research field, ii) summarize the use of algae traits in riverine biomonitoring and iii) propose future research perspectives. The bibliometric analysis showed that the annual number of publications on microalgae increased significantly from 1991 to 2016, although their proportions to total numbers of scientific articles remained steady. The studies have become increasingly concerned on issues arisen from global environmental changes such as "eutrophication", "pollution", "land use", "biomonitoring", "biodiversity", "functional group", etc. The use of algae traits in biomonitoring has become popular and includes e.g. functional diversity, cell size, guild, life form, eco-morphology, spore formation as well as algal quality. Here we collate all relevant algal traits, their different categories and propose their responses to resource supply and disturbance frequency in a conceptual model, which should be validated in future studies. In order to expand the knowledge and future use of microalgae in biomonitoring research efforts should also include: i) description of relationships between algal traits and ecosystem functions (e.g., nutrient uptake, metabolism, energy transfer across the food web) and underlying mechanisms; ii) selection of robust traits reflecting and disentangling the effects of multiple stressors; iii) water resource management in an interdisciplinary manner linking risk assessment and management scenarios by an integrated modelling system using microalgae.

Submitted Manuscript 1: Effects of flow events and nutrient addition on stream periphyton and macroinvertebrates: an experimental study using flumes

Submitted to Knowledge and Management of Aquatic Ecosystems

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Abstract: We used flume experiments to study the effects of a temporary increase in stream flow on macroinvertebrates, leaf litter breakdown and soft-bodied benthic algae, both as a single stressor and eventually in combination with an increase in nutrient supply. We compared species composition of macroinvertebrates and benthic algae between the flumes and the nearby stream from which the flumes were supplied with water, in order to understand how well the flume experiments reflected the processes in the associated stream ecosystem. As single stressors, nutrient addition and an increased flow velocity from 1.3 to 2.8 cm/sec lead to an increase in the biomass of benthic algae, likely reflecting an improved transfer of nutrients into algal patches. However, the combined effect of flow and nutrient addition was smaller than the sum of both individual effects, likely because an increased biomass also was more susceptible to scouring. We found differences in macroinvertebrate and benthic algal taxon identity and abundance between stream and flumes. Since biodiversity is assumed to stabilize ecological functioning in response to disturbances and variation, we conclude that care should be taken in applying results from small scale experiments to stream ecosystems.

Submitted Manuscript 2: Does season matter for potamodromous fish movements? An experimental approach in a vertical slot fishway

Submitted to Aquatic Sciences

Authors: Romão, F.A.[a], Santos, J.M.[b], Katopodis, C.[c] , Pinheiro, A.N.[a] & Branco, P.[a,b]

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Abstract: The majority of fishway studies are conducted during the reproductive period, when fish migrate upstream to spawning grounds; yet uncertainty remains on whether results may be biased if the same studies were performed outside of the migration period. To understand how season affects fish motivation, as characterized by a number of entry and passage related metrics, the present study assessed the performance of the Iberian barbel (*Luciobarbus bocagei*, Steindachner, 1864) in an experimental full-scale vertical slot fishway during spring (the reproductive season) and early-autumn (non-reproductive season). Results revealed that, no significant differences were detected on passage performance between the two seasons, with the exception of entry efficiency which was higher in early-autumn. However, differences among seasons were noted in the plasma lactate concentration, used as a proxy for muscular fatigue after the fishway negotiation. Lactate concentrations were significantly higher in early-autumn than in the spring. This suggests that, for potamodromous cyprinids, the evaluation of fish passage performance in fishways does not need to be restricted to the reproductive migratory season and may be extended to early-autumn, when movements associated with shifts in home range may occur. Furthermore, lactate differences detected between seasons imply that fish may incur physiological adjustments at different times of the year. The increased effort during the non-reproductive period suggests that adapting the operating regime of fishways, at different biologically meaningful seasons in a year, should be assessed by considering the physiological state of the target species.

Submitted Manuscript 3: To swim or to jump? Passage behaviour of a potamodromous cyprinid over an experimental broad-crested weir

Submitted to River Research and Applications

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Abstract: Physical stressors, such as man-made obstacles, are considered one of the main causes that negatively affect freshwater fish. Even small weirs may impact fish populations, particularly potamodromous cyprinids, by partially or totally blocking upstream migratory movements. Recent studies have addressed the effect of key hydraulic parameters on upstream movements past small weirs, but little is known on how these parameters interact to induce a swimming or a jumping behaviour in negotiating such obstacles. This study aims to evaluate the passage behaviour (swimming vs. jumping) of Iberian barbel (*Luciobarbus bocagei*), a potamodromous species, over an experimental small broad-crested weir, considering the effect of different plunge pool depths (D), waterfall heights (H), and flow discharges (Q). Results showed that passage behaviour was highly dependent from the combinations of plunge pool depths and waterfall heights tested (Freeman–Halton test; $P < 0.0001$). Barbel negotiated most configurations by swimming (overall negotiations: 81.5% swimming vs. 18.5% jumping), except the ones with the higher H (25 cm). Therefore, higher waterfalls proved to be preponderant in the switching of passage behaviour from swimming to jumping. Contrarily, there was no evidence that passage behaviour was discharge related. These results are useful to identify potential migration obstacles, and should be taken into consideration when designing fishways for potamodromous cyprinids.

Submitted Manuscript 4: Structural microhabitat use of endemic cyprinids as a tool to inform restoration practices in Mediterranean rivers

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Abstract: 1. Endemic freshwater fish from the Mediterranean region are amongst the most threatened species in the world due to increasing river degradation caused by anthropogenic stressors. Because of such threats, the number of river restoration projects has greatly increased. However, they are seldom planned with inputs from the species' life history, often resulting in erroneous practices that compromise their success.

2. This study assessed the seasonal and size-related microhabitat use of three endemic cyprinids (Iberian barbel, Luciobarbus bocagei; Iberian straight-mouth nase, *Pseudochondrostoma polylepis*; and calandino, *Squalius alburnoides*) in a Mediterranean river by employing a multivariate approach to analyse both structural resource use and availability data.

3. All species showed evidence of non-random microhabitat use. The barbel and nase shifted to faster-flowing positions (>25 cm s-1) with a coarser substratum (>150 mm particle size) during spring and to sheltered positions (50–100% in-stream cover) during autumn. Calandino selected more covered areas in autumn (>60% cover) and shifted to shallower positions from this season (>40 cm) to summer (<35 cm).

4. Significant size-related differences in microhabitat use were consistent for the three species, with smaller individuals occupying on average shallower microhabitats. Significant resource-use overlap between species size classes increased from autumn to summer, occurring mainly between juvenile and small adult barbel and calandino.

5. The results can be used to inform river restoration practices in Mediterranean-type rivers. Cover was found to be critical for all species, in particular for calandino, which could be classified as a shelter-oriented eurytopic species. Restoration scenarios should therefore consider maintaining cover habitats during unfavourable flow conditions for this species through the control of mosaics of submerged aquatic macrophytes (SAM). Lithophilic spawners, which were highly responsive to velocity and substratum, could particularly benefit from the creation of gravel bed-forms and artificial riffles to enhance depth and flow variability.

Further Manuscripts in preparation

- Schülting, L., Feld, C. K., Hudek, H., Zeiringer, B., Graf, W. (in prep.). Macroinvertebrate drift patterns during hydropeaking simulations with varying ramping velocities.
- Bondar-Kunze, Kasper V., & Hein, T.. Responses of periphyton communities in a pool riffle sequence to abrupt changes of temperature and flow velocity in flume experiments
- Auer, S., Zeiringer, B., Kaiser L., Schmutz S. (in prep.). Thermopeaking effects on early live stages of European graylings (*Thymallus thymallus* L.)
- NIVA (in prep.). Effects of a flow event and nutrients on stream periphyton and macroinvertebrates: an experimental study using flumes.
- AU: 2 further manuscripts in prep.
- ULisboa: 1 further manuscripts in prep.

LAKES

Paper 1: Heat-wave effects on greenhouse gas emissions from shallow lake mesocosms

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Abstract: 1. Shallow lakes are a key component of the global carbon cycle. It is, therefore, important to know how shallow lake ecosystems will respond to the current climate change. Global warming affects not only average temperatures, but also the frequency of heat waves (HW). The impact of extreme events on ecosystems processes, particularly greenhouse gas (GHG) emissions, is uncertain.

2. Using the world's longest-running shallow lake experiment, we studied the effects of a simulated summer HW on the fluxes of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). The experimental mesocosms had been exposed to different temperature treatments and nutrient loading for 11 years prior to the artificial HW.

3. In general, there was an increase in total GHG emissions during the 1-month artificial HW, with a significant increase in CO_2, CH_4 and N_2O being observed in the shallow lake mesocosms. No significant effect of the HW on CO_2 emissions could be traced, though, in the mesocosms with high nutrient levels. Furthermore, the data suggested that in addition to the direct effect of increased temperature on metabolic processes during the HW, biotic interactions exerted a significant control of GHG emissions. For example, at low nutrient levels, increased CO_2 emissions were associated with low macrophyte abundance, whereas at high nutrient levels, decreased phytoplankton abundance was linked to increased emissions of CO_2 and CH_4.

4. In contrast to the observable heat-wave effect, no clear general effect of the long-term temperature treatments could be discerned over the summer, likely because the potential effects of the moderate temperature increase, applied as a press disturbance, were overridden by biotic interactions. This study demonstrates that the role of biotic interactions needs to be considered within the context of global warming on ecosystem processes.

Paper 2: Heat wave effects on biomass and vegetative growth of macrophytes after long-term adaptation to different temperatures: a mesocosm study

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Abstract: Elevated temperatures and extreme climatic events, such as heat waves, can negatively affect submerged macrophytes. Here, we investigated how submerged macrophytes adapted to 3 different temperatures (ambient, ca. +3°C and ca. +4.5°C) responded to a heat wave. After 10 yr of adaptation, the shoots of 2 species of submerged macrophytes, *Elodea Canadensis* and *Potamogeton crispus*, were collected from each of the 3 temperature treatments and transferred to 2 heated treatments for 1 mo. The 2 heated treatments were then exposed to a 1 mo heat wave with an additional 5°C temperature increase. For P. crispus, total biomass did not differ among the plants adapted to the different temperatures or between the 2 heated treatments for the duration of the experiment. Plants adapted to the highest temperatures, however, produced a larger number of smaller turions before the heat wave and allocated less biomass to elongation before and after the heat wave. For *E. canadensis*, the plants adapted to higher temperatures had higher total biomass before and during the heat wave and allocated more biomass to roots and leaves during the heat wave. Most indicators (e.g. length and biomass) of macrophyte performance measured during the experiment did not differ between the 2 heated treatments. In summary, after the 10 yr adaptation to higher temperatures, the submerged macrophytes showed adaptive changes in growth and asexual reproduction and responded in a complex way to the heat wave depending on species, growth status and adaptation temperature.

Paper 3: Effect of a nitrogen pulse on ecosystem N processing at different temperatures: A mesocosm experiment with $^{15}NO_3$ addition

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Abstract: 1. Shallow lakes may play an important role for the nitrogen (N) balance in drainage basins by processing, transferring and retaining N inputs. An increase in the frequency of storm-induced short-term N pulses and increased water temperatures are both likely outcomes of climate change, potentially affecting the N processing in lakes.

2. An experiment with a $K^{15}NO_3$ -pulse addition (increase in NO_3 concentration from c. 0.1 to 2 mg/L) was carried out in 12 mesocosms with relatively low (applies to Danish lakes) total N (TN) and total phosphorus (TP) concentrations (c. 0.3 mg N/ L and 0.04 mg P/ L) to assess the effects of an N pulse on N processing and storage in shallow lake ecosystems. The mesocosms have a hydraulic retention time of approximately two and a half months, and at the time of the experiment, they had

been adapted to contrasting temperatures for a period of 10 years: ambient, T3 (heating according to the Intergovernmental Panel on Climate Change 2007 A2 scenario, +3.7–4.5°C, depending on season) and T5 (heating with A2 + 50%, +4.9–6.6°C).

3. Macrophytes and filamentous algae retained up to 40% and 30% of the added ^{15}N, respectively, reflecting their high biomass in the mesocosms. Macrophytes and filamentous algae constituted between 70% and 80% of the biomass of all primary producers during the experiment in the T3 and ambient treatments and between 20% and 40% in T5. By comparison, less than 1% of the added ^{15}N diffused to the sediment and less than 5% was lost to the atmosphere as N_2 gas. Snails represented the long-term storage of ^{15}N, retaining up to 6% of the tracer and with detectable enrichment 100 days after tracer addition.

4. We found no significant differences among the temperature treatments in the ^{15}N turnover after pulse dosing. However, a larger percentage of ^{15}N was stored in macrophytes in the ambient and T3 mesocosms, reflecting higher biomasses than in T5 where filamentous algae were more abundant. Macrophytes and filamentous algae rather than temperature were therefore key controllers of N processing during the summer N pulse in these shallow, relatively low TP lakes.

Paper 4: Temperature effects on periphyton, epiphyton and epipelon under a nitrogen pulse in low-nutrient experimental freshwater lakes

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Abstract: The ongoing global climate change involves not only increased temperatures but may also produce more frequent extreme events, such as severe rainfall that could trigger a pulse of nutrients to lakes. In shallow lakes, this may affect primary producers through a number of direct and indirect mechanisms. We conducted a six-month mesocosm experiment to elucidate how periphyton (on inert substrata), epiphyton and epipelon biomass responded to a nitrogen (N) pulse, an approximately tenfold enrichment of the NO3-pool, under three contrasting warming scenarios: ambient temperature and ca. ?3_C and ca. ?4.5_C elevated temperatures (hereafter T1, T2 and T3). After the N pulse, we found a higher periphyton biomass at elevated than at ambient temperatures but no change in epiphyton biomass. Epipelon biomass was lower in T3 than in T1.

Both periphyton and epiphyton biomasses correlated negatively with snail biomass, while periphyton biomass correlated positively with light. Different responses to higher temperatures under short-term extreme nutrient loading conditions may be attributed to differences in the access to nutrient sources and light. Our data suggest that the biomass of periphyton in oligotrophic clear water lakes will increase significantly under conditions exhibiting short-term extreme nutrient loading in a warmer climate.

Paper 5: Do extreme weather events change the response of cyanobacteria to the combined effects of global warming and nutrient enrichment?

To be submitted to Global Change Biology – September 2017.

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Abstract: There is great concern that harmful algal blooms (HABs) of cyanobacteria in nutrient impacted lakes will be exacerbated by global warming, further threatening water and ecological quality. Here we report results from a lake mesocosm experiment that indicates that the effect of warming depends upon the nutrient gradient experienced. In ambient mesotrophic mesocosms, warming increased nutrient release resulting in eutrophic conditions and an increase in phytoplankton abundance. Some additional (direct) warming effects were seen for cyanobacteria with increases in buoyancy-regulating and often toxic genera that pose the greatest risk – *Microcystis* spp. and *Dolichospermum* spp. In mesocosms with additional nutrient enrichment (N and P) warming further increased internal nutrient loading resulting in hypertrophic conditions. In these conditions cyanobacteria and phytoplankton abundance did not increase, but slightly decreased, highlighting an antagonistic interaction. This antagonism is contrary to expectations and suggests the combination of hypertrophic conditions and warming introduces other factors that limits the response of phytoplankton and cyanobacteria. This unexpected result highlights the importance of environmental context in predicting the response of cyanobacteria to anthropogenic stressors. The experiment also tested how the response to warming or nutrient addition alone, and in combination, could be altered by significant biomass loss as a result of extreme rainfall events, which are predicted to increase as a result of climate change. We found no significant effect of a summer flushing event suggesting that, one-off events like this are unlikely to help reduce the symptoms of enhanced algal blooms and HABs. Our results indicate that, in shallow lakes, the effects of warming on internal nutrient cycling will make remediation from eutrophication more challenging.

Paper 6: The short-term stress response of three-spined sticklebacks to climate-related stressors in shallow lakes: a mesocosm study.

In preparation to be submitted early June 2017.

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Abstract: As part of a mesocosm-based study on the impacts of climate change stressors on shallow lakes the short-term effect of extreme rainfall events on fish was investigated. Replicate 3000 L mesocosms were established using lake water, lake sediment mixed with washed sand, and an inoculation of phytoplankton, zooplankton and macroinvertebrates. Water temperature was elevated by within-mesocosm heating (ambient and ambient +4^{o}C), nutrient loading was increased by the addition of nitrate and phosphate, and intermittent extreme rainfall events were simulated, to mimic the changes predicted as being likely to occur due to climate change. A range of physicochemical parameters was measured at intervals throughout the study. Three-spined sticklebacks (*Gasterosteus aculeatus* L.) were sampled from enclosures within the experimental mesocosms on two occasions (November 2014, May 2015) at intervals of 1 h and 24 h following a stimulated rainfall event. Cortisol levels were determined in gill tissue homogenates to evaluate whether the rainfall event was detected by the fish, and if so, whether the response of the fish was modified by conditions within the mesocosms. During November, the rainfall event resulted in elevated cortisol levels in fish from all treatment groups. However, in May, an increase in cortisol levels after exposure to the rainfall event was detected only in fish from nutrient-enriched mesocosms, and from both heated and unheated treatment groups. This outcome suggested that the physicochemical conditions within the mesocosms, in particular high pH and algal abundance, exacerbated the impact of an additional stressor upon the fish. The findings support concerns that climate related alterations in the aquatic environment may render ecosystems less able to cope with increasingly frequent environmental perturbations.

Paper 7: The contribution of algae to freshwater dissolved organic matter: implications for UV spectroscopic analysis.

Submitted to Inland Waters. Under review.

Authors: Jessica L. Adams*, Edward Tipping, Heidrun Feuchtmayr, Heather Carter, Patrick Keenan

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Abstract: Dissolved organic matter (DOM) is an important constituent of freshwater. It controls aquatic ecological and biochemical cycling, and can be problematic in the water treatment process. Thus, the demand for rapid and reliable monitoring is growing, and spectroscopic methods are potentially useful. A model with three components, two absorbing in the ultraviolet (UV) range and one non-absorbing, present at a constant concentration, was previously found to give good predictions of dissolved organic carbon concentration, [DOC], across 1700 freshwaters (R^2=0.98). However, the model underestimated [DOC] in shallow, eutrophic lakes in the Yangtze Basin, China, raising the possibility that DOM derived from algae might be poorly estimated, and this is supported by new data for eutrophic British lakes. We estimated the extinction coefficients in the UV range of algae-derived DOM, from published data on algal cultures and new data from outdoor mesocosm experiments, in which high concentrations of DOC were generated under conditions similar to those in natural waters. The results demonstrate the weak UV absorbance of DOM from algae compared to DOM from terrestrial sources. Introduction of the new extinction coefficients into the three-component model allowed the contribution of algae-derived DOM to DOC in surface water samples to be estimated. Concentrations of DOC due to algae ranged from zero to 8.3 mg DOC L^{-1}, and the fraction of algae-derived DOC ranged from zero to 97%. Further work is required, using data from a wide range of contrasting waters, to determine whether a generally-applicable model can be established to predict [DOC] from spectroscopic data.

Papers in the pipeline - Drafts - Danish experiments

Paper 1: Heat-wave effects on microbial loop in shallow lake mesocosms

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Paper 2: Impact of Heat Waves on Zooplankton Community Structure in Heated and Nutrient Enriched Mesocosms

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Abstract: Shallow lakes, besides being the most widespread inland water bodies in the world are very sensitive to external perturbations, including land use and climate change. Today, unprecedented rates of warming threaten the functioning of lakes, especially when combined with additional multiple stressors especially eutrophication. Lately, extreme events such as heat waves complicate the impacts of stressors on ecosystem structure and functions. To elucidate the effects of heat waves on zooplankton community structure and functioning 24 flow-through mesocosms (1.9 m in diameter, 1.5 m in total depth, imitating a shallow lake) were used, located in Lemming, Denmark. The mesocosms simulate two nutrient levels combined with three different temperature scenarios (unheated, A2 scenario and A2+50% scenario). A 5°C heat wave was applied during a full month (1st July- 1st August 2014). Samplings for physical-chemical variables and zooplankton were performed from 19th June to 9th October. During the heat wave application, sampling frequency increased. To investigate the the key effects on zooplankton community structure nutrient enrichment, heat scenarios and heatwave simulation used as a factor in linear mixed model. Heat wave had decreasing effects on taxonomic diversity, size diversity and community evenness. However, zooplankton abundance (including biomass of Cladocera, Copepoda and Rotifera) showed resistance to the extreme event, thus no strong adverse effects of heat wave were observed. Adverse effects were also observed in high nutrient mesocosms, along with the low nutrient mesocosms heated with A2+50% scenario. This pattern indicates zooplankton community in oligotrophic shallow lakes might be under threat of extreme events especially with increasing global temperatures in the future as well as eutrophic ones.

Paper 3: Effect of a short term heat wave on phytoplankton community under different temperatures and nutrient concentrations: a mesocosm experiment

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Abstract: Phytoplankton are short-lived organisms responding fast and directly to environmental fluctuations which make them useful indicators of deterioration in lake ecosystems to stressors. Due to climate change, extreme events including heat waves will be more common in the future and such changes also augment eutrophication, a problem lakes are already facing today. To understand the effects of temperature and nutrient stressors on phytoplankton taxonomic groups and size diversity we used a mesocosm experiment in Silkeborg, Denmark, a system which has been already continuously operated for 11 years. There are 24 mesocosms (1.9 m in diameter, 1.5 m in total depth, imitating a shallow lake) simulating two nutrient levels (un-enriched and enriched with additional nitrogen and phosphorus) and three different temperature scenarios (ambient, IPCC A2 and A2+%50) with 4 replicates. Heat wave was imitated for 1 month by rising the temperature 5°C (from 1st July till 1st August 2014). Samplings for physical-chemical variables and phytoplankton were performed from 19th June to 9th October. During the heat wave application, sampling frequency increased. To investigate the key effects on phytoplankton community structure nutrient enrichment, heat scenarios and heatwave simulation used as a factor in linear mixed model. In low nutrient mesocosms heat wave increased total phytoplankton biomass with A2+50% scenario. The effects in high nutrient mesocosms observed mostly after heat wave as a decrease of total phytoplankton biomass. While heat wave decreased species richness with heat scenarios, it didn't affect the size diversity in both low and high nutrient mesocosms. Our results indicate that heat wave may support the mineralisation in oligotrophic systems and increase the primary production. Nutrients are likely to be the stronger factor for eutrophic systems than heat stressors.

Papers in the pipeline – German experiments

Paper 1: Impacts of browning on phytoplankton communities exposed to nutrient enrichment

Authors (changes possible): Birger Skjelbred[1], Ute Mischke[2], Stella A. Berger[3], Jens C. Nejstgaard[3], Darren P. Giling[3,6], Erik Sperfeld[3], Susanne Stephan[3], Tim Walles[3], Jeremy Fonvielle[3], Andreas Ballot[1], Sigrid Haande[1], Alexis Guislain[2], Hans-Peter Grossart[3,4], Mark O. Gessner[3,5], Anne Lyche Solheim[1]

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Abstract: Nutrient loading and browning by humic substances are two important and co-occurring stressors affecting lakes in Northern Europe. The combined impact of browning and nutrient enrichment on phytoplankton was assessed in a large-scale enclosure experiment, conducted in Lake Stechlin, north-eastern Germany, during summer 2015. The phytoplankton in the enclosures was initially dominated by a filamentous cyanobacterium, *Dolichospermum zinserlingii* (biomass 1±0.4 mg l-1), which collapsed after two weeks. Microcystin also occurred in all enclosures during the first two weeks of the experiment. Preliminary results indicate that browning reduced the total phytoplankton biovolume and -diversity, apparently as a result of light limitation. Although cyanobacteria generally tolerate low light intensities, the browning reduced their proportion, particularly that of *Planktothrix*. The strong absorption of blue light by the added humic substances is likely the most important mechanism accounting for this decline, because cyanobacteria require blue light to maintain their competitive advantage when growing at low light intensities. Also the chlorophytes decreased their proportion in the brown vs. clear enclosures, whereas cryptophytes increased possibly because of their mixotrophic life-style. These changes indicate a shift from an autotrophic to a heterotrophic food web. Nutrient effects were less clear, probably because of top-down control by significant zooplankton populations developing in the absence of fish predation. In the brown enclosures, the unclear nutrient effects can also be related to less nutrient limitation, due to mixotrophs grazing on bacteria. However, the role of nutrients and zooplankton in explaining the observed shift from cyanobacteria to cryptophytes will require further exploration.

Paper 2: Testing the effects of experimental lake browning and nutrient enrichment on primary production in large-scale enclosures

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Abstract: Increasing levels of humic substances and nutrients resulting from climate and land-use change are widespread. Both stressors threaten fresh waters individually and may also act in concert, but the interactive effects are not well known. To unravel how the biomass (chlorophyll *a*) and primary production (PP, ^{14}C method) of three major size classes of phytoplankton respond to these stressors, we performed a seven-week experiment with natural lake plankton in a large enclosure facility (www.lake-lab.de). A nutrient gradient, corresponding to seven phosphorus levels ranging from oligo-mesotrophic to eutrophic conditions, was crossed with three levels of humic substances. In enclosures without humic substances added, rates of PP increased at increased nutrient levels, whereas chlorophyll *a* did not clearly respond to nutrients. In enclosures receiving humic substances, phytoplankton biomass and PP went through distinct phases during the experiment, mostly driven by light availability. PP sharply declined immediately after humic substances were added, whereas phytoplankton biomass declined later. The euphotic was greatly reduced directly after the addition of humic substances and only rose again after one month of bleaching. This gradually improved light availability in the enclosures. Picoplankton biomass started to increase concomitantly, presumably because the larger size fractions were heavily grazed by zooplankton. Overall, our results demonstrate that lake browning is a dominant stressor of planktonic primary producers, switching nutrient to light limitation even in oligo-mesotrophic conditions.

Paper 3: Alkaline phosphatase activity in lake plankton under global change – a large-scale enclosure experiment on browning and nutrient enrichment effects

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Abstract: Climate and land-use changes have increased the leaching of humic substances and nutrients from catchments into fresh waters, thus causing browning by coloured dissolved organic matter and eutrophication. We investigated the effects of these two stressors on the activity of the exoenzyme alkaline phosphatase (AP). The aim was to inform about nutrient conditions in lake plankton in the future, given that AP produced by algae and heterotrophic microbes provides bioavailable (inorganic) phosphorus for growth. We conducted an experiment in 21 large enclosures deployed in a deep clear-water lake in north-eastern Germany (http://www.lake-lab.de) by crossing three levels of browning (clear, meso-, polyhumic) with seven levels of nutrient supply corresponding to a gradient from oligo- to eutrophic conditions. We determined AP Michaelis-Menten kinetics at least weekly over the following 40 days by incubating plankton samples with a fluorogenic model substrate. Both stressors independently reduced AP activity and also affected AP kinetics interactively. Specifically, the observed reduction of AP activity with increasing nutrient supply was weakened at higher browning levels. Furthermore, the AP kinetics indicate that browning led to non-competitive inhibition, whereas elevated nutrient supply caused competitive inhibition of the enzyme. These results imply a reduction in the inorganic phosphorus provision from organic matter by both stressors, but different mechanisms mediating the effects. Overall, our results suggest that browning and nutrient enrichment can notably influence AP kinetics and thus affect phosphorus cycling in lake plankton under future global change.

Paper 4: Changes in competition between periphyton and macrophytes induced by brownification: A large-scale enclosure experiment

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Abstract: Inputs of humic substances from terrestrial into aquatic ecosystems have been increasing during the past decades in many regions. This brownification of inland waters can provide additional resources and reduce light availability, thus affecting primary producers. However, effects on interactions between periphyton and macrophytes, which compete for light and nutrients, are still unknown. We tested the impacts of humic substances on submerged macrophytes and periphyton in a large-scale enclosure experiment at different levels of nutrient supply. A total of 21 fishless enclosures received single pulses of humic substances at three levels, crossed with seven levels of phosphorus and nitrogen. Submerged macrophytes (*Stuckenia pectinata*) and artificial substrates for periphyton growth were exposed for four weeks at five different water depths. Brownification (highest level) resulted in a significant decline of both, macrophyte and periphyton biomass in 1.5, 3 and 6 m depth. However, the relative response of periphyton biomass to brownification in 1.5 and 3 m depth was stronger than that of macrophytes relative to controls (macrophytes 53 and 60% decline versus periphyton 91 and 96%, respectively). As a consequence, shading of periphyton on macrophytes in shallower water was reduced and macrophytes gained in importance relative to periphyton. Overall, this lower shading by periphyton did not compensate the effects of reduced light supply in browner water and total (theoretical) benthic primary producer biomass decreased by about 50%. A brownification-induced shift from periphyton to macrophyte in shallow waters has potential implications for several ecosystem functions such as carbon and nutrient storage.

Further papers in preparation

- Skjelbred, Mischke, Lyche-Solheim, Ballot, Haande, Berger, et al. optional, if too much for the other papers. Cyanobacteria responses, details of the different taxa, toxins, relationship with light etc.
- Lyche-Solheim, A., Mischke, U., Skjelbred, B., et al. Impacts on ecological status based on German and Norwegian classification system: WFD metrics and nEQRs Norwegian and German system, implications for managers.
- Berger, S., Gessner, M., Lyche-Solheim, A., Nejstgaard, J., et al. Lake Stechlin MARS experiment synthesis
- Nejstgaard, J., Berger, S., Gessner, M., Lyche-Solheim, A. et al. Large mesocosms operations for the MARS experiment
- additional papers on zooplankton responses, microbial loop etc. are in early stages of preparation