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Macroinvertebrate redistribution of environmental pollution

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Chapter 8: Synthesis

The aim of this thesis was to elucidate the role of benthic macroinvertebrates in the redistribution of organic matter, nutrients and contaminants in WWTP associated systems. Departing from the mutual interactions between macroinvertebrates, algae and macrophytes and their surrounding environment in freshwater ecosystems (Fig. 8.1), the different roles of macroinvertebrates in organic matter degradation and transport, their contribution to nutrient cycling and their role in the fate of contaminants was studied, either in the presence or absence of macrophytes and algae. This chapter first highlights the main findings of the present thesis by refining and semi-quantifying the interaction scheme (Fig. 8.1) into a framework that shows the multiple roles of macroinvertebrates and their contribution to the pathways of organic matter, nutrients and contaminants in aquatic ecosystems impacted by WWTP discharges. Finally, the feasibility and challenges of large-scale implementation of a wastewater treatment step based on the processes elucidated in this thesis are discussed.

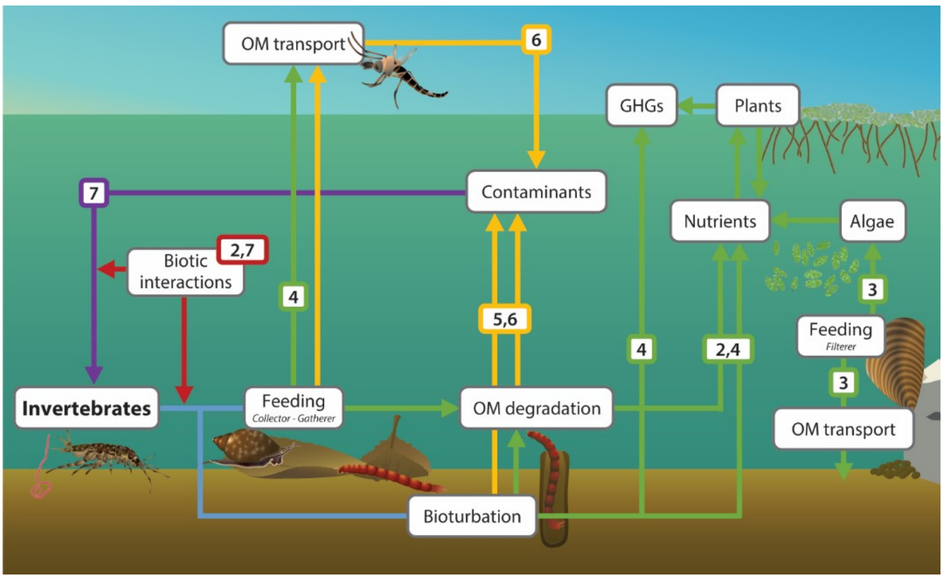

Figure 8.1: Schematic overview of interactions between organisms and their environment. Blue lines represent the main activities of macroinvertebrates studied in this thesis. Effects of organisms on nutrient dynamics (green arrows) and on contaminants (orange arrows), and effects of contaminants on organisms (purple arrow) are shown. Biotic interactions (red arrows) play a role in these organism-environment interactions. Numbers indicate in which chapters of this thesis these interactions have been investigated (Figure designed by L.A.M. Denkers).

The graphical representation of interactions between invertebrates and their environment, including algae and macrophytes, is grouped around three main pathways (Fig. 8.1):

I. Effects of organisms on nutrient dynamics in a contaminated environment (green arrows)
II. Effects of environmental contamination on organisms (purple arrows)
III. Effects of organisms on the fate of contaminants in their environment (orange arrows)

8.1 Invertebrate-Environment interactions

8.1.1 Macroinvertebrate interactions affect nutrient dynamics

In natural aquatic ecosystems biotic interactions affect nutrient dynamics and organic matter degradation. Biotic interactions between collector gatherers and shredders often occur when degrading heterogenous natural organic matter, such as leaf material, which may positively or negatively affect degradation rates (Little & Altermatt, 2018; Tonin et al., 2018). These interactions were also observed the present thesis (**Chapter 2, Chapter 7**), where they resulted in an increased sludge degradation. Yet, the species involved did not always benefit from these interactions, shown by lower growth and reproduction of tubificids in the presence of *Asellus aquaticus* (**Chapter 2**). Thus, we observed that a more diverse degrader community stimulated sludge degradation rates, but sometimes at the cost of the performance of individual species. Besides consumer-consumer interactions, macroinvertebrates also affect primary producers, either directly, through trophic interactions (Zhang et al., 2018), or indirectly, by changing nutrient availability (Schuijt et al., 2021). Filter feeders can enhance the flux of organic matter from the pelagic to the benthic environment, as they filter algal cells and other fine particulates from the water column (Basen et al., 2013; Vaughn et al., 2008; Wotton et al., 1998). Many filter feeders do not filter indiscriminately, but stop filtering if there are too many or too few particulates present, or if the particulates present are considered to be of poor quality. After initial ingestion, they may also choose to excrete the particulates as pseudofaeces, without passage through the gut system. Here we showed that filter feeders are also capable to remove the largest part of WWTP effluent cultured algae from the water column, indicating that they can limit the transport of algae-associated nutrients to locations downstream of WWTP discharges (**Chapter 3**). Hence, in a WWTP setting, nutrient retention would be the result of both the performance of the algae and the filtration rates and preferences of the filter feeders. Macroinvertebrates also affect the flux of nutrients from the benthic environment into the overlying water. Depending on the relative magnitude of organic matter degradation and bioturbation, macroinvertebrates can either stimulate of reduce this flux of nutrients. Whereas *A. aquaticus* activity increased the concentration of PO_4 in the overlying water (**Chapter 2**), the activity of *C. riparius* larvae reduced the aqueous PO_4 concentration during the degradation of WWTP activated sludge, likely due to the oxygenation of the sludge and the subsequent binding of phosphorus (**Chapter 4**). The oxygenation of the sludge also prevented the formation of gaseous CH_4, which is to our knowledge the first observation of GHG emission reduction by macroinvertebrates in a WWTP setting (**Chapter 4**). Furthermore, whereas the biomass of *A. aquaticus* remained in the aquatic environment, the emerged *C. riparius* adults transferred a part of the assimilated nutrients into the terrestrial environment. Thus, invertebrates may alter nutrient fluxes in a WWTP setting, either hampering or stimulating the growth of primary producers, depending on the traits of the invertebrate (community).

8.1.2 Effects of contaminants on macroinvertebrate mediated ecosystem processes

The amount of data on the effect of single contaminants on single macroinvertebrates is huge; for the 5 taxa studied in the present thesis 6577 ecotoxicity data are available for 520 different contaminants reported in 509 publications (EPA database; 23-05-2023). However, nor contaminants nor macroinvertebrates occur on their own in the multi-stress aquatic environment of today. Nonetheless, the number of hits on Web of Knowledge with the input "invertebrates" and "community" and "contaminants" is only 307 (30-05-2023). This huge discrepancy shows that the overwhelming amount of research has focused on traditional hazard assessment, with survival being the most frequently assessed endpoint. Traditional hazard assessment is however less useful to predict how environmental relevant concentrations of contaminants affect aquatic ecosystems in which multiple species occur, where non-lethal effects of contaminants also affect organism performance and thus ecosystem functioning (Burdon et al., 2023). The present thesis emphasized that macroinvertebrate mediated organic matter degradation is an important ecosystem function in both pristine and contaminated ecosystems, which proved to be more sensitive to contaminants than mortality (**Chapter 7**). Moreover, the presence of multiple collector gatherer macroinvertebrates modified each other's sensitivity to contaminants, for instance by dampening the adverse effects of contaminants on specific species through increased positive interactions under more stressful conditions (**Chapter 2**, **Chapter 7**), according to predictions of the Stress Gradient Hypothesis (Bertness & Callaway, 1994). Such biotic interactions may be used to create a WWTP sludge treatment step that is more resilient to contaminants than single species biobased solutions (**Chapter 7**). Hence, it is concluded that there is a strong bias towards assessing the lethal effect of compounds on organisms, ignoring sublethal effects and subtle biological interactions that may affect ecosystem functioning, as observed in the present thesis.

8.1.3 Macroinvertebrates affect contaminant distribution

The most strongly understudied effect of the mutual interaction between organisms and the environment concerns the influence of organisms on their surroundings, which may strongly affect the environmental distribution of contaminants (Gilroy et al., 2012). Bioturbation by invertebrates increases the oxygen concentration in sediments, which increases the bioavailability of metals. A novel insight generated in the present thesis was that invertebrates with a high bioturbation activity may increase their own exposure, leading to a higher observed sensitivity to sediment associated metals (**Chapter 5**). Due to their capacity to redistribute contaminants in the environment, macroinvertebrates can also affect the exposure of other organisms to these contaminants. A bioturbation mediated flux of contaminants from the sediment into the overlying water may thus result in increased exposure and effects on epibenthic or pelagic invertebrates (Pang et al., 2012), or decreased exposure and effects on invertebrates within the sediment (Colombo et al., 2016). Interestingly, in sediment with a high organic matter content, such as WWTP sludge, contaminant concentrations in both the sediment as well as in the overlying water may increase due to macroinvertebrate activity. This was shown in **Chapter 6**, where we observed that *C. riparius* larvae that bioturbate and feed on sludge organic matter promoted a flux of metals into the overlying water, but at the same time, they also reduced the amount of sludge, resulting in increased metal concentrations in the remaining sludge. The magnitude of metal redistribution due to bioturbation and consumption was even higher than due to aquatic-terrestrial transport by *C. riparius* adult biomass or by the uptake of metals by the larvae themselves (**Chapter 6**). Thus, macroinvertebrate activity and macroinvertebrate biotic interactions are highly important, both considering the effects of contaminants on individual species and on ecosystem functions, but also when considering the distribution and fate of contaminants in aquatic ecosystems, as emphasized in the present thesis.

The results obtained in the present thesis clearly showed that macroinvertebrates are not just a collection of single species affected by the contaminants present in wastewater. Macroinvertebrates are part of communities in which biotic interactions determine how (in)sensitive species are to contaminants, while their own and combined activity also shapes their environment, and thus the distribution and (trans)formation of nutrients, organic matter, and greenhouse gasses.

8.2 Towards a real-world functioning WWTP treatment cascade: Challenges and knowledge gaps

The motive of the scientific aim of this thesis rooted in a very practical question; "Can we improve the treatment of our wastewater using cascades of organisms?". In this thesis some of the fundamental processes and principles that form the basis of a potential wastewater treatment by macroinvertebrates have been addressed. Indeed, we showed that multiple macroinvertebrates and primary producers are capable to reduce the amount of sludge, nutrients and greenhouse gasses, and redistribute WWTP associated contaminants. Moreover, combinations of multiple macroinvertebrate species may enhance these processes, indicating that a multispecies setting does increase the efficiency and resilience of the processes that they mediate. However, these experiments have been performed in containers ranging from 0.025 to 50 L, for a duration of 7 to 28 days. While a controlled environment is essential to accurately measure biotic interactions and to elucidate the mechanisms underlying the studied processes, the size of these containers is dwarfed by the tens of thousands of m^3 of wastewater that an average WWTP treats every day. The scaling of the processes from experimental containers to a working treatment cascade is not linear, since increases in both space and time bring forth additional challenges and requirements. Furthermore, this thesis primarily assessed organic matter and nutrient dynamics, while the research on effects of contaminants was limited to a few metals and PAHs. The performance of a treatment cascade on the removal of other contaminants remains yet largely unknown. Therefore, before a functioning organism-based wastewater treatment cascade can be realized, the following challenges should be addressed:

8.2.1 Contaminants, Greenhouse Gasses and variable influent quality

As macroinvertebrates affect the distribution of contaminants, they may also cause the contaminants to be transported to unfavourable compartments, such as the effluent. In this thesis, we have shown that this occurred with four common metals (**Chapter 6**), but the effects on other (organic) contaminants remain unknown. Ideally, biodegradation processes should be optimized, meanwhile limiting the transport of contaminants into the overlying water. Non-biodegradable contaminants should preferably (hyper)accumulate either in macroinvertebrate biomass, or remain associated to the sludge. Although macrophytes and algae-mussel cascades might be able to remove some of the contaminants from the water, it would be more efficient to prevent an increased contaminant flux from the sludge into the water. To assess these macroinvertebrate-contaminant interactions, it would be beneficial to perform small scale experiments with (combinations of) macroinvertebrate species with different modes of bioturbation and a range of contaminants with different properties regarding, hydrophobicity, half-life and toxicity.

The same holds for the effects of macroinvertebrates on GHG emissions during sludge degradation. In Chapter 4 we showed that *C. riparius* larvae reduced CH_4 emissions, the first time that this was observed in a WWTP setting. Yet, GHG formation and emission depends on many more aspects, such as the microbial community, available nutrients, oxygen and temperature, and the effects of macroinvertebrates on GHG emissions in natural systems are poorly known, contradicting, and context dependent (Benelli & Bartoli, 2021; Kajan & Frenzel, 2006; Serrano et al., 2016).

Lastly, the effects of pulses of contaminant rich wastewater on macroinvertebrate mediated sludge degradation are also still unknown. Contaminants negatively impact organic matter and sludge degradation (**Chapter 7)**, and increased pulses of contaminants may further adversely affect these processes. Although macroinvertebrates are also able to cope with increased contaminant concentrations due to adaptation over multiple generations (Groenendijk et al., 1999), effects of sudden pulses and subsequent recoveries on macroinvertebrate performance and sludge degradation are unknown. The information that small scale experiments, like the ones performed in the present thesis, would provide on these topics would allow for preventive measures and informed responses to these real-world situations occurring at larger scales.

8.2.2 Modelling organic matter, nutrient and contaminant dynamics to provide a framework for monitoring the treatment efficiency of an organism-cascade

On an operational scale, the efficiency of the treatment cascade should be monitored regarding the removal of particulate organic matter, nutrients and contaminants. Although a simple comparison between the properties of the inflow and the outflow of the cascade may suffice for regulatory purposes, a more thorough grasp of the dynamics within and between each compartment of the treatment cascade would provide insights that may allow to construct a more flexible and robust system. As these treatment cascades will essentially simulate a flowing water body, the Spiralling Nutrient Dynamics model (Newbold et al., 1981) may be used to assess the flow and removal efficiency of particulate organic matter and nutrients throughout the cascade. Newbold et al. (1981) described how a dissolved nutrient atom moves a certain distance of stream length (S) either in the water (S_w), associated to particulate matter (S_p) or in macroinvertebrate consumers (S_c) before becoming dissolved again. Applying this model allowed to assess the efficiency of supplied nutrients utilization in a stream system, and may thus also serve to assess the efficiency of nutrient removal in a WWTP treatment cascade. As each compartment in the cascade host other species or communities with other rates of uptake, transformation and egestion, such a model could estimate the required relative size of each compartment to achieve optimal retention. Ideally, the degradation, transformation and distribution of contaminants should also be implemented in such a model, as these are tightly coupled to the nutrient dynamics the system (Tong et al., 2022), but also affect the performance of the biota within the system (Koelmans et al., 2001) (Fig. 8.1).

8.2.3 Scaling-up

When scaling up our mesocosm experiments in space and time towards a wastewater treatment plant, both populations of macroinvertebrates as well as a substantial degradation of sludge and removal of nutrients should be maintained. In large scale setups, the amount of sludge, overlying water, and container edges may differ from small scale experimental containers. Thus, making sure that the macroinvertebrates are provided with a suitable environment, which includes sufficient oxygen, food, physical space and structures, may require adjustments to the design of the cascade. Furthermore, the inter- and intraspecific macroinvertebrate performances and interaction effects may change over time and are not necessarily linear with increasing organism density (Little et al., 2020). In multi-species compartments, a competitive advantage of one species may only become prevalent after multiple generations due to intergenerational intraspecific facilitation (Costantini & Rossi, 1998), which may affect the overall sludge degradation. Ensuring a continuous growth of the invertebrate populations requires reliable production of new individuals. Whereas reproduction occurred almost always in the experimental setups (**Chapter 2,4,7**), this may not necessarily be the case in larger scale setups, due to possible limitations in available space to mate or to deposit eggs. Furthermore, at higher densities of invertebrates, intraspecific competition may result in a lower growth and possibly lower reproduction (Lamberti et al., 1987), resulting in less stable population sizes. In a large-scale culture of the black soldier fly (*Hermetia illucens*), this issue was solved by selecting a small part of the harvested pupae for raising these to adults under controlled conditions in brood chambers (Zurbrügg et al., 2018). The harvesting of macroinvertebrates from the treatment cascade also requires further development. Whereas in the present experimental setups, invertebrates were removed by hand from the remaining sludge, this would become completely unfeasible on a larger scale. Apart from sieving, it could be tried to coax invertebrates from the sludge by making the conditions in the sludge less favourable than outside of the sludge, for example by limiting aeration (Marian & Pandian, 1984), or by startling them using weak electrical pulses. Invertebrates with a terrestrial adult stage are therefore considered to have an advantage, as they leave the sludge themselves as part of their natural life cycle, strongly improving the ease of harvesting.

During the continuous culturing of organisms, environmental variability should be controlled within tolerable ranges, not only to ensure survival, growth and reproduction of the organisms, but also to maintain a sufficient rate of sludge degradation and nutrient removal. During colder periods in temperate climates, ambient temperatures may become so low that they prevent any activity of macroinvertebrates, while during summer times, excess temperatures may result in increased mortality (Spidle et al., 1995). The lower and upper limits of this temperature range for mortality and adequate performance differs between the species tested in this thesis. For example, whereas the bivalve *D. bugensis* remains active at temperatures as low as 8°C (Reeders & Bij De Vaate, 1990), the development time of *C. riparius* larvae until pupation increased from 6 to 14 days when temperature was decreased from 26.7 to 14 °C (Péry & Garric, 2006). Although aeration tank water and effluent have a more constant temperature than air and smaller water bodies, it may still be needed to ensure adequate temperatures, by using greenhouse setups or heating during colder periods, and cooling during warmer periods. Light may also be a limiting factor for primary producers in the treatment cascade, and artificial light may be required during times of the year with shorter days. Multiple invertebrate species on the other hand can complete their life cycle in the dark and are thus not reliant on any lighting.

8.2.4 Additional challenges developing a wastewater treatment cascade

Maintaining adequate oxygen concentrations, temperature ranges and light conditions requires energetic input. This increases the costs of the treatment cascade, but also increases the CO_2 emissions of WWTPs, which hampers to meet other Sustainable Development Goals. The use of solar and wind energy could minimize these disadvantages. Furthermore, the facilitations required for the maintenance of the treatment cascade will also require investments in additional construction materials, measuring equipment and harvesting equipment, although also conventional WWTPs have such needs.

Although the primary goal of the wastewater treatment cascade would be to improve the effluent quality, the costs of wastewater treatment may be suppressed if the harvested biomass could be marketed. Applications of WWTP harvested biomass are in development, including the use of chitin, fats and proteins from invertebrates, as well as the use of macrophytes as fertilizer and insulation material. Nonetheless, no readily available sales market is currently present for most of the biomass produced. A positive exception may be live chironomid larvae, to be sold as ornamental fish feed. Being for ornamental fish, contaminants in the larvae will not enter the food system, and moreover, the larvae currently sold for this purpose are often originating from contaminated environments as well (Sharifian Fard et al., 2014).

Currently, regulatory restrictions are in place for the rearing or transportation of invasive organisms, as well as the use of materials that are associated with sewage. Originally constructed to prevent health hazards from barely treated primary sewage, these regulations may not apply for the nowadays highly treated sewage. Nonetheless, to facilitate the large-scale use of WWTP associated materials and substances, regulatory changes are needed to provide a framework for this new reality. The same holds for regulations of non-native invasive species. The rules for rearing and transportation of these species are tight, and it is often completely forbidden. Multiple species that may fit well in treatment cascades are labelled as invasive species, although they sometimes already appear in the Netherlands for almost two centuries, like *D. polymorpha* (Waardenburg, 1827; named as *Mytilus lineatus*).

There are indeed some risks associated with the large-scale culturing of contaminated organisms, since large-scale escapes would result in the transport of contaminants into the environment, especially if the organisms are able to spread further than the contaminants would have been transported when discharged into the effluent. However, adequate measures can be taken to prevent these kinds of escapes, including the use of sand filters to prevent that aquatic organisms leave the system, or mesh nets to prevent terrestrial flying adults to escape. When using non-indigenous species, an additional risk may be that these could invest natural environments and disrupt local communities. However, non-indigenous species often cause the largest problems in ecosystems already under pressure from other stressors, including eutrophication, contaminants, and hydromorphological changes. Robust, healthy ecosystems are rarely greatly impacted by non-indigenous species. Hence, thriving non-indigenous species are a symptom of poor environmental quality. Moreover, the application of organisms that thrive in poor quality environments in a treatment cascade should increase the quality of the environment receiving WWTP discharges, thus actually increasing the robustness of these systems against the potential of non-indigenous species to become invasive.

Concluding remarks

Nowadays macroinvertebrates have to deal with multi-stress situations in nearly every aquatic environment in Europe, concerning nutrients, contaminants, temperatures and hydromorphological habitat degradation. Research on multi-stress becomes ever more integrated (Schäfer et al., 2023), and the effects of these stressors on aquatic ecosystems and the inhabiting macroinvertebrates are more and more understood. However, the important ecosystem functions that macroinvertebrates perform in these multi-stressed aquatic ecosystems remain a topic to be expanded. Understanding the negative impacts of these stressors on macroinvertebrates and taking adequate measures to restore their natural environment is essential, not only for their sake, but also for ours. Without macroinvertebrate and microbial activity, organic matter will accumulate, and benthic-pelagic coupling would come to a halt. The present thesis showed that their role may also become indispensable for the treatment of wastewater in the future, recognising how different macroinvertebrates, microbial communities and primary producers may be used to perform the degradation of contaminants and organic matter, as well as their redistribution to environmental sections that are easily removable during wastewater treatment, to prevent this pollution to enter the natural aquatic environment.

Wastewater treatment was founded over 4500 years ago by the people of Mohenjo-Daro, a simple yet ingenious settling tank, which required an understanding of solid settling rates and a recognition of the risks of wastewater for human health. The future of wastewater treatment is up to us, as we recognize the risks of wastewater for our aquatic environment. It again could involve simple yet ingenious techniques, this time requiring an understanding of ecological principles, ecosystem dynamics, and grasping the importance of macroinvertebrate redistribution of environmental pollution.