Organisms make ecosystems function

Identifying functional indicators of anthropogenic stress in aquatic ecosystems

Gea H. van der Lee

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Gea Heleen van der Lee

This research was conducted at the Institute for Biodiversity and Ecosystem Dynamics (IBED) through the department of Freshwater and Marine Ecology (FAME) at the Universiteit van Amsterdam (UvA) in The Netherlands under the auspices of Graduate School for Production Ecology & Resource Conservation (PE&RC).

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No man ever steps in the same river twice,
for it is not the same river and he is not the same man.

--- Heraclitus---

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CHAPTER 1

GENERAL INTRODUCTION

[I]t is not the organism but human societies which can serve as an adequate model when it comes to describing functions (roles) within an ecological system and the functioning of the whole system. ... In contrast to parts of an organism, a particular species has no clearly defined role within an ecosystem ... That is, like a person within a human society, who may be teacher, spouse, child, politician etc., either at the same time or at different times, it can have several roles. Roles can change and the same person as well as the same species can even take opposing roles in time. ''The'' one and only role of a species does not exist. Roles are strongly context-dependent. Also, species (…) can live in different ecological systems (…) like persons can change the society in which they live, e.g. by emigrating. ... It also can easily be decided if an organism is alive or dead, while this is enormously difficult to decide for a human society (…) or an ecological system (…).

— Jax (2005) —

DEFINING ECOSYSTEM STRUCTURE AND FUNCTIONING

The above analogy by Jax (2005) illustrates the complexity of describing functions within an ecological system and the functioning of the whole system. Moreover, it exemplifies that the word 'function' can have different meanings (see Box 1). To avoid misunderstandings and to apply the notion of ecological functions in practice, a clear definition of this term is thus warranted.

Here, we define an ecological system or ecosystem according to Odum (1971), as any discrete unit in nature that includes all living organisms occurring in a given area and their non-living environment with which they interact. This definition implies that an ecosystem has spatial boundaries. In some cases these boundaries seem fairly defined, like a lake (Odum, 1971). However, in reality the lake shore may transition gradually from an aquatic to a terrestrial environment, making it ambiguous what the exact position of the boundary is. Moreover, the water level of a lake can change, altering the position of the boundary over time (Lévêque, 2003). Ecosystem boundaries are thus virtually absent in nature and must therefore be delimited based on the specific perspectives and objectives of the observer (Odum, 1971; Jax, 2005). These arbitrary boundaries are permeable and subject to the input of matter (e.g. nutrients) and energy (e.g. light) from the surrounding environment (Wiens et al., 1985).

An ecosystem can be described by its structure and its functioning. The structure of an ecosystem refers to the organisms present (biotic structure), the non-living, physical or chemical features of the environment (abiotic structure), and their interactions (Blair et al., 2000). I use the terms 'ecosystem function' and 'ecosystem functioning' interchangeably to describe different environmental (e.g. flow regime and sediment flux) and ecological

processes (e.g. metabolism, decomposition, nutrient cycling and trophic transfer) that sustain an ecosystem (Box 1 definition 2; Jax, 2005; Palmer & Febria, 2012). So, while the structure commonly reflects the status of an ecosystem at a given time, the functioning eflects the dynamic processes taking place in an ecosystem through time (Palmer & Febria, 2012).

The contribution of organisms to the realization and maintenance of ecological processes can be studied by ascribing functional roles to organisms (Box 1 definition 3; Box 2; Jax, 2005). In other words, functional roles indicate what organisms are doing in an ecosystem in a similar way as we speak of 'jobs' and 'professions' fulfilled by persons in human society, according to the analogy at the start of the introduction (Jax, 2005; Dussault, 2019). It is, however, important to emphasize that *'The one and only role of a species does not exist'* (Jax, 2005). Rather, the functional roles that organisms fulfil are context-based, including the actual and potential interactions with other organisms and their environment, meaning that roles can change over space and time (Dussault, 2019). For example, some macroinvertebrates that usually eat plant material can also eat certain dead organisms, but they only fulfil the 'detritivore' role if these dead organisms are actually present. In a similar way, the role of a person depends on the interactions with other persons within society (Jax, 2005).

Under unimpacted conditions, the structure and functioning of ecosystems changes over time due to the natural variability in environmental processes (Lévêque, 2003). This natural variability is driven in the short-term by diurnal and seasonal changes and in the long-term by climatic, geologic or geomorphologic events (Landres, 1992; Lévêque, 2003). When events, i.e. external forces, factors or stimuli, lead to the (temporary) restructuring of an ecosystem, they are defined as a disturbance (Stanley et al., 2010). Although the word disturbance has a negative connotation in common language, it is recognized that natural disturbances are essential for many ecosystems in order to maintain their heterogeneity (Landres, 1992; Rapport & Whitford, 1999; Lévêque, 2003). For example, a natural flood event may open up resources that were unavailable in the pre-existing state of the ecosystem, which can subsequently be colonized by individuals of the same or different species (Lévêque, 2003).

Disturbances can be characterized by their intensity, frequency, predictability and duration (Lake, 2000; Lévêque, 2003). Although disturbances have traditionally been viewed as pulses (i.e. rapid and discrete events, such as floods), it is now well established that they can also occur as press (i.e. disturbances with a sharp rise that are sustained at a constant level, such as sediment input from a landslide) or ramp (disturbances that increase intensity over time, such as prolonged droughts) (Lake, 2000; Stanley et al., 2010). Beside natural variability, human activities or by-products from human activities may superimpose additional external forces, factors or stimuli on ecosystems (Odum et al., 1979; Stanley et.

Box 1. Function and functioning in ecology

Four different meanings of function in ecology - The word 'function' is commonly used in ecology, although with several different meanings. In order to resolve this issue, Jax (2005) identified four important meanings of the word 'function' in ecology:

1. Individual-level processes, described as state changes in time and causal relations that give rise to them (e.g. a nutrient is assimilated by a plant using solar energy).
2. Systemic processes that together sustain an ecosystem, relating to the movement or storage of energy and matter (e.g. nutrient cycling).
3. Individual roles of organisms within an ecosystem (e.g. primary producer).
4. The services an ecosystem provides for human beings (e.g. a stream can eliminate sewage pollution through self-purification).

This is further summarized by Farnsworth et al. (2017), stating that an organism has certain capabilities (3) that are realized in practice (1), which can be described as ecological processes in a more complex context of the larger systems of which they are part (2). When these ecological processes are interpreted from a human perspective, depending on their practical use, they are called a service (4).

A philosophical debate - The use of the word 'function' has raised a philosophical debate whether we can assign purpose in ecology (see Jax, 2005; DeLaplante & Picasso, 2011; Toepfer, 2011). The critique arises that by asking the question 'what for?', one would assign teleological properties to organisms and ecosystems, i.e. state that something is created and used for a conscious end or purpose. This would require an intelligent design by an external agent like God or an immanent nature of the cosmos (DeLaplante & Picasso, 2011). Teleological questions have, therefore, often been considered methodologically unscientific (Toepfer, 2012).

Evolutionary theory offered a popular strategy to naturalize teleological reasoning by replacing the role of the designer with mechanisms of natural selection, i.e. by stating that organisms are adapted to their environments and their parts are adapted to the functions they serve (DeLaplante & Picasso, 2011). Ascribing functional roles to organisms within ecosystems would, however, imply that some traits of the organism have been shaped by ecosystem-level selection and

this would conflict with natural selection theory, which assumes individuals within populations are the unit at which natural selection operates (Dussault, 2019).

Dussault (2019) proposed three arguments why the ascription of functional roles diverges from the natural selection theory: 1) the functional roles of organisms are perceived as context-based properties of organisms and do not entail claims about selective history, 2) the aim is to explain ecological processes rather than the presence of ecosystems, and 3) organisms are not 'designed' to fulfill the role, but the organisms are adapted on organism-level to function in interaction with other organisms and their environment to their own benefit. The total sum of individual-level processes fulfilled by each organism form the ecological processes within the ecosystem.

Although there seems no consensus on the interpretation of the word 'function' in ecology, there is general agreement that it will unlikely be eliminated and can be useful in describing and explaining biological objects without presupposing an intelligent design in nature (Jax, 2005; DeLaplante & Picasso, 2011; Toepfer, 2012).

Box 2. Not all traits are functional traits

Traits are often defined as any morphological, physiological or life-history attribute inherent to an organism (Violle et al., 2007). All these traits are 'biological', as they are crucial for the fitness and performance of organisms. It is, however, important to recognize that not all traits are 'functional', i.e. significantly modulating ecosystem processes (Mlambo, 2014). Functional trait groupings can be made on basis of similar responses to environmental changes or on basis of similar functional roles in ecological processes (Dussault, 2019). In this thesis, my main focus is on the groupings based on functional roles. Contrasting with many traditional trait-based studies, the functional roles in ecological processes are concerned with 'what' organisms do (e.g. remove particles), regardless of 'how' they do it (e.g. scrape or excavate) (Bellwood et al., 2018). A classic grouping of functional roles in an ecosystem is based on similarities in resource use, encompassing for example producers, consumers and decomposers (Cummins, 1974).

al., 2010). Anthropogenic disturbances may be categorized into five major groups, i.e. overexploitation, flow modification, habitat degradation and destruction, water pollution and the introduction of exotic species (Rapport et al., 1985; Dudgeon et al., 2006).

To complicate matters, an ecosystem that has been modified, degraded or otherwise changed by human activities is often referred to as disturbed (Stanley et al., 2010). Moreover, the term 'stress' has often been used interchangeably with the term 'disturbance' (Borics et al., 2013). Although it has been attempted to discriminate between disturbance and stress based on cause and effect relationships (Rykiel, 1985), positive or debilitating effects on the ecosystem (Rapport & Whitford, 1999) or based on the frequency of the event (Borics et al., 2013), in this thesis I do not differentiate between both terms. Rather, I only focus on additional human-induced external forces, factors or stimuli applied to ecosystems and use the term anthropogenic stress to describe this.

Now that I have defined the concepts of ecosystem structure and functioning under anthropogenic stress, I will elaborate on the need of science and management to: 1) define the nominal state of an ecosystem, and 2) search for suitable ecosystem indicators to accurately discern the effects of anthropogenic stress on ecosystems.

CHALLENGES IN SPECIFYING THE NOMINAL STATE OF ECOSYSTEMS

A challenge in making statements about changes in the structure and functioning of ecosystems is that it requires the observer to define the nominal state of an ecosystem (Landres, 1992; Jax, 2005). As mentioned by Jax (2005), *'[it] can easily be decided if an organism is alive or dead, while this is enormously difficult to decide for a human society (…) or an ecological system (…)'.* Specifically, while the addition or lack of certain organs could be devastating to an organism, the addition or lack of certain species is often within the boundaries of natural variability of most ecosystems (Landres, 1992). There is thus need to define the nominal state by the limits of allowable change, i.e. the normal operating range (Kersting, 1984; Landres, 1992).

The nominal state of an ecosystem is often determined based on a set of areas of the same ecosystem type that are least impacted by human activities, i.e. spatial reference conditions (Stoddard et al., 2006). Even if near-natural ecosystems still exist, finding suitable spatial reference conditions is difficult, as there is substantial natural variability within an ecosystem type due differences in factors like climate, geology, geomorphology and historic events (Landres, 1992; Johnson et al., 2010). Alternatively, the nominal state can be derived from the prevailing conditions in the past, i.e. historic reference conditions (Stoddard et al., 2006). However, as ecosystems naturally shift over time the standard of comparison also changes (Landres, 1992; Johnson et al., 2010).

As alternative, or when the purpose is not to determine to what degree an ecosystem is degraded, but to understand the cause-effect relationships between anthropogenic stress and ecosystem structure and functioning, it has been argued that we rather need to find control conditions instead of reference conditions (Downes, 2010). Control conditions should closely match the impacted ecosystems, except for the human impact(s) of interest (Downes, 2010). Thus in contrast to reference conditions, control conditions may be impacted by a suite of comparable anthropogenic stressors at both locations and should only differ in the studied stressor(s). In this thesis, I used such control sites and compared them along specific gradients of anthropogenic stress to improve our understanding of ecosystem change.

SEARCHING FOR SUITABLE ECOSYSTEM INDICATORS

To gain insight into how ecosystems change under anthropogenic stress, we need suitable ecosystem indicators (Dale & Beyeler 2001). Yet, finding suitable indicators is challenging, considering the numerous organisms present and their countless interactions that sustain complex processes (Landres, 1992). Traditionally, most biomonitoring schemes have relied on structural indicators based on single point-in-time taxonomic inventories of species groups that comprise the ecosystem, such as fish, macrophytes, algae and macro-invertebrates (Cummins 1974; Landres, 1992; Boulton, 1999; Dale & Beyeler 2001). Especially macroinvertebrates are commonly used, as they are a diverse and abundant group with a wide range of sensitivities to anthropogenic stress (Rosenberg & Resh, 1993). Structural indicators of the status or condition of an ecosystem include, for example, species diversity (i.e. richness, evenness and composition) and the presence of indicator species (e.g. Rosenberg and Resh 1993).

It is often assumed that these structural attributes are representative of the functioning of an ecosystem and that direct measurements of functional attributes are thus irrelevant (Lecerf et al., 2006; Friberg et al., 2011). However, anthropogenic stress may impact species composition without affecting ecological processes and vice versa ecological processes may alter in the absence of a change in the species composition (Cairns & Pratt, 1985; Sandin & Solimini, 2009). Therefore, it has been argued that direct measurements of ecosystem functioning and the underlying roles that organisms play in these processes are necessary to gain insight into how ecosystems change under anthropogenic stress (Karr, 1999; Young et al., 2008; Friberg et al., 2011; Palmer and Febria, 2012).

Functional roles may, for example, be quantified as the number and biomass of organisms fulfilling specific roles in ecosystem functioning (Box 2; Landres et al., 1992). Although there is information for a variety of traits and taxonomic groups in online databases (Culp et al., 2011), these traits are often selected based on ease of measurement and not on functional value (Bellwood et al. 2019). Also, these trait-databases mostly

include static broad categories, while the realized functional role is adaptive and depends on the context (Jax, 2005; Bellwood et al. 2019). The challenge is thus to find indicators that have causal links with ecosystem functions (Bellwood et al. 2019). Measurements of the natural abundance of stable isotopes may, for example, be used as integrated measure of the realized trophic positon of species (Dawson et al., 2002).

Direct measurements of ecosystem processes rely on repeated measurements through time in order to obtain process rates (Palmer & Febria, 2012). Environmental processes may, for example, be measured with temperature- and water level loggers to obtain information on the thermal and flow regimes respectively. The ideal way to measure ecological processes would be to directly measure the flow of energy and cycling of matter through an ecosystem, but this is difficult to achieve (Odum, 1971; Jørgensen, 2009). Instead, measurements of ecological processes are based on indirect changes in quantities through time, such as the amount of organic matter that is produced or decomposed (e.g. by using mass loss of leaves to estimate decomposition rates) or the by-product that is released during this process (e.g. by using changes in dissolved oxygen concentrations to estimate ecosystem metabolism; Odum, 1971; Jørgensen, 2009).

As these measurements are indirect, they may reflect different aspects of the complex ecological processes that they are supposed to describe (Odum, 1971). As such, there are seemingly endless number of variables which may be measured to describe ecosystem functioning (Jax, 2005; Canning & Death, 2018). Several methods which can potentially be used to measure ecological processes are described in Box 3. There is a need to explore to what extent these different attributes can help us to understand how ecosystems function.

AIM, OBJECTIVES AND OUTLINE OF THIS THESIS

From a scientific, but also from a management perspective there is a need to find suitable indicators to gain insight into how ecosystems function, and to detect, monitor, assess and diagnose deviations from control conditions. This resulted in the formulation of the following aim of this thesis:

To identify functional indicators of anthropogenic stress in aquatic ecosystems using ecosystem processes and functional roles of organisms, and to explore their potential use in biomonitoring schemes.

The focus of this thesis is on linear shaped small (width < 10 m) and shallow (depth < 1 m) permanent freshwater ecosystems in lowland areas, including lowland streams and drainage ditches. These two water types can be distinguished based on flow. Ditches are characterized by a current velocity of less than 5 cm/s in either direction, whilst in lowland

Box 3. Potential measurements of ecological processes in freshwater ecosystems

Ecological processes that may be used as indicators of ecosystem functioning, include metabolism, decomposition rates, nutrient cycling and trophic transfers. Potential methods to measure these ecological processes in freshwater ecosystems are described below:

1. Ecosystem metabolism encompasses the rate of production (i.e. photosynthetic fixation of inorganic to organic carbon by algae, bryophytes and aquatic macrophytes) and respiration (i.e. loss or mineralization of organic to inorganic carbon by all organisms) of organic matter (Odum, 1956; Woodwell and Whittaker, 1968). A common method to estimate ecosystem metabolism incudes the measurement of dissolved oxygen (DO) concentrations either in the open channel or in air-tight chambers enclosing part of the water body (Tank et al., 2010; Staehr et al., 2012a).
2. Decomposition describes the rate of detrital mass loss through leaching, microbial and invertebrate activities and abrasion (Webster and Benfield, 1986). Decomposition releases energy into the food web and forms an important step in nutrient cycling (Friberg et al., 2011). Decomposition rates are commonly estimated by measuring the mass loss of leaves or artificial substrates incubated in the water body over a certain period of time (Meyer, 1980; Boulton and Boon, 1991; Young et al., 2008).
3. Nutrient cycling involves the sequence of uptake of mostly inorganic elements by biota, the transfer of these elements to higher trophic levels and the release back into the environment (Mulholland & Webster, 2010). Methods used to assess aspects of nutrient cycling include, for example, the measurement of nutrient transformation processes like denitrification under controlled conditions (Udy et al., 2006).
4. Trophic transfer involves the feeding of one organism on another or on dead organic matter (Odum, 1971). Trophic transfers may be demonstrated by enriching organic matter with labeled stable isotopes and subsequently measuring the uptake in organisms (De Goeij et al., 2008; Mulholland & Webster, 2010).

streams flow is unidirectional and reaches current velocities over 20 cm/s (Peters et al., 1988). Furthermore, ditches are man-made, excavated to drain wetlands, as opposed to streams which are natural. Normalized and channelized streams fall in between these two water types, as their longitudinal and cross profile is altered or even completely excavated by humans, and although flow is unidirectional, current velocity is generally lower than in natural streams (Peters et al., 1988).

The positioning of these waters in densely populated lowland areas exposes them to a combination of stressors, including hydro-morphological degradation and pollution (Paul and Meyer, 2001; Riis and Sand-Jensen 2001; Schinegger et al. 2012; Bracewell et al. 2019). Nutrient enrichment, in particular, is considered to be one of the predominant water quality issues in surface waters (Janse & Van Puijenbroek, 1998; Smith et al., 1999; Smith and Schindler, 2009) and therefore has a key focus in this thesis, although other stressors were studied as well, amongst others, alteration of discharge dynamics, low dissolved oxygen concentrations, increased water temperatures and contamination from pesticides, pharmaceuticals and personal care products.

In this thesis, we conduct multiple field studies (**Chapter 2-7**) and a literature study (**Chapter 8**) to meet the following objectives (Figure 1):

1. Measure abiotic features over time to obtain information on environmental processes

To achieve the first objective, we measure two key environmental processes over time, including discharge dynamics (**Chapter 2**) and dissolved oxygen dynamics (**Chapter 3**). In **Chapter 2**, we record the timing of extreme peak discharges in four streams throughout two years using water level loggers. We relate these discharge peaks, as functional indicator, to the population dynamics of the caddisfly *Agapetus fuscipes*, which is a good indicator of undisturbed streams. To gain more insight into the impact of eutrophication on the functioning of drainage ditch ecosystems, we identify daily and seasonal temporal patterns of dissolved oxygen dynamics in drainage ditches along an eutrophication gradient in **Chapter 3**.

2. Measure the realized functional roles of organisms in resource use

To achieve the second objective, we study the functional detritivore role of macroinvertebrates using trait-databases and relate this to the particulate organic matter decomposition rates (**Chapter 4**). We explore how the role that macroinvertebrates and microbes play in particulate organic matter decomposition changes under low dissolved oxygen stress. In **Chapter 5**, we assess the plasticity of the role that primary and secondary macroinvertebrate consumers fulfill along a eutrophication gradient. Specifically, we use stoichiometry and stable isotope analysis to assess if nutrient enrichment causes a shift in the trophic position of the macroinvertebrate consumers.

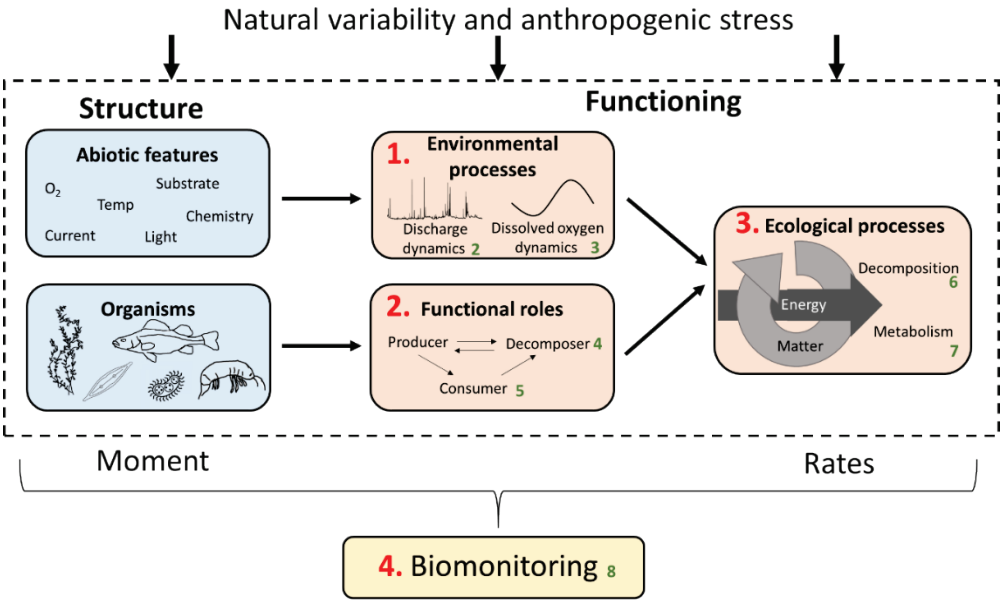


***Figure 1**: Schematic overview of the structure and functioning of aquatic ecosystems in relation to the four objectives (large red numbers) and the main focus of the chapters (small green numbers) of this thesis. First, abiotic features are measured over time to obtain information on environmental processes. Second, organisms are studied through their functional roles in resource use. The environmental context determines the contribution of organisms to the realization and maintenance of ecological processes Third, we evaluate direct measurements of these ecological processes. Fourth, the use of both structural and functional indicators are compared and ideas are explored to improve understanding of ecosystem functioning and its associated biomonitoring.*

3. Evaluate direct measurements quantifying ecological processes

To achieve the third objective, we focus on measurements related to decomposition (i.e. mass loss of artificial substrates; **Chapter 6**) and ecosystem metabolism (i.e. open channel dissolved oxygen; **Chapter 7**), as these have been advocated previously as suitable functional indicators by Young et al. (2008). First, we evaluate the use of organic matter decomposition as functional indicator to diagnose the impact of various stressors originating from agricultural activities and WWTP discharges (**Chapter 6**). The estimation of ecosystem metabolism from diel dissolved oxygen curves involves several challenges, like the difficulty to split up productivity and respiration rates, the estimation of re-aeration rates and the lack of anaerobic respiration rates. In **Chapter 7**, we explore whether passive acoustics monitoring may potentially overcome these challenges.

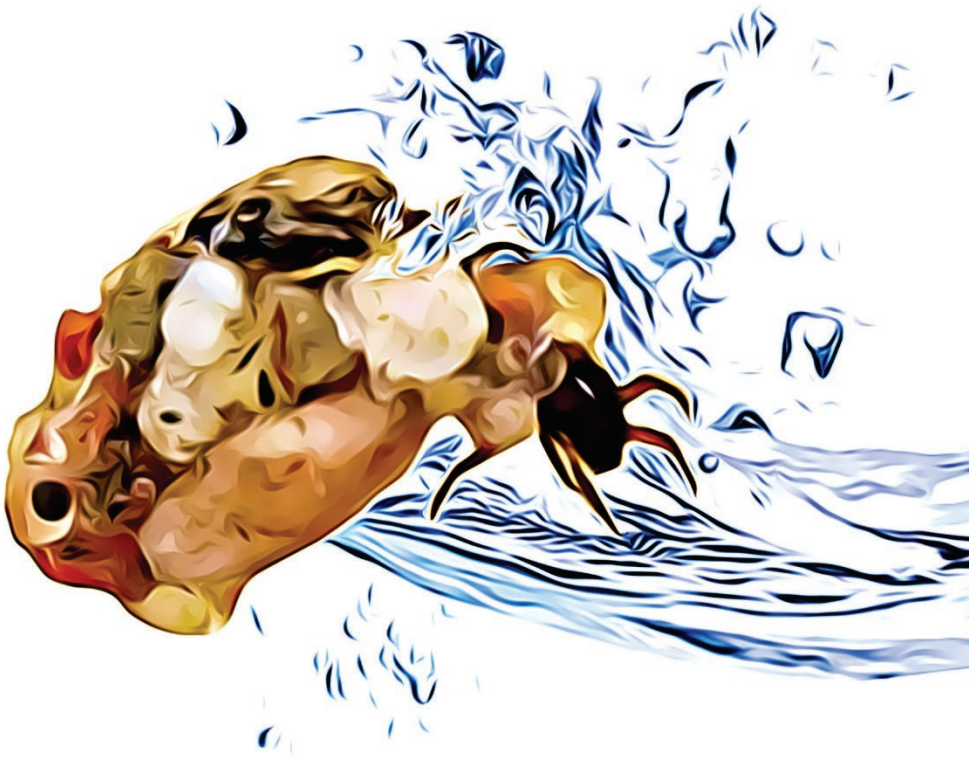
4. Explore ideas to improve our understanding of ecosystem functioning

To achieve the fourth objective, we perform a literature study (**Chapter 8**) in which we evaluate the use of functional indicators (i.e. ecosystem metabolism and decomposition) compared to structural indicators (e.g. taxonomic inventories of the community composition) in the understanding and biomonitoring the impact of multiple anthropogenic stressors on running waters. We explore ideas on how knowledge on suites of interacting traits that evolved under local abiotic and biotic conditions of all organism groups can help to better comprehend multiple stressor effects on ecosystem structure and function.

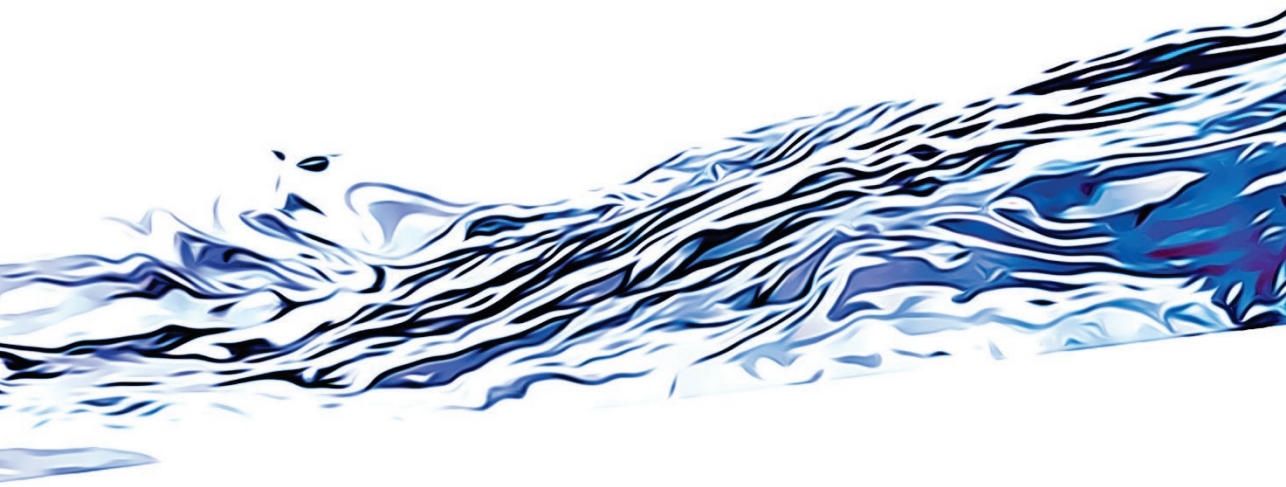
Finally, I provide a synthesis that describes a framework on how abiotic and biotic hierarchical filters control the functioning of ecosystems (**Chapter 9**). I propose how studying the appropriate temporal scales can improve our comprehension of how organisms respond to anthropogenic stress and how studying the known unknowns of functional roles of these organisms can improve our understanding of ecosystem functioning. Finally, I suggest possible steps on how to monitor and predict ecosystem functioning in practice.


1

CHAPTER 2



PERSIST OR PERISH: CRITICAL LIFE STAGES DETERMINE THE SENSITIVITY OF INVERTEBRATES TO DISTURBANCES



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Gea H. van der Lee, Michiel H.S. Kraak, Ralf C.M. Verdonschot &
Piet F.M. Verdonschot

ABSTRACT

A large proportion of studies assessing the impact of disturbances on the invertebrate community composition focus on a single life stage, assuming that those are an adequate indicator of environmental conditions. The effect of a specific disturbance may, however, depend on the life stage of the exposed organism. Therefore, we focused on the effect of spates on the caddisfly *Agapetus fuscipes* CURTIS (Trichoptera: Glossosomatidae) during different larval stages. A two year field study was performed in which we measured the discharge dynamics and population development of *A. fuscipes* in four lowland streams in The Netherlands. A stage-structured population model (i.e. StagePop) was used to test the impact of peak discharge on the different life stages, as larval instars 1 - 4 were not effectively sampled in the field. Four different mortality rates in response to spates were simulated, including a constant low, a constant high, a decreasing and an increasing impact per larval stage. This way, we were able to show a potential association between spates and population declines, where the stage-population model including decreasing impact by spates with increasing larval life stage most accurately described the population development of the larval instars 5 - 8. Focusing only on late instars could thus potentially result in underestimation of the effects of spates on this species. In conclusion, determination of responses of critical life stages to specific disturbances may help to identify the causes of the presence and absence of species, and thereby aid more effective management and restoration of degraded aquatic systems.

INTRODUCTION

Natural flow variations, including spates and droughts, largely determine the spatial and temporal dynamics of invertebrate populations in running waters, as species have evolved traits that enable them to survive, exploit and even depend on these flow regimes (Resh et al., 1988; Poff et al., 1997; Lytle & Poff, 2004). Disturbances outside the predictable flow regime to which stream organisms were originally adapted can, however, reduce population densities, and these adverse effects increase with the frequency, intensity and severity of the disturbance (Poff, 1992; Lytle & Poff, 2004). A specific disturbance may, however, lead to very different ecological responses depending on the (ontogenetic) life stage of the exposed organism, i.e. eggs, different larval stages, pupae and adults each have different sensitivities (Lancaster & Downes, 2010). This was shown for chemical pollution to which early instars of different insect species were commonly more sensitive than later larval stages (e.g. McCahon & Pascoe, 1988; Stuijfzand et al., 2000; Pineda et al., 2012). For hydrological disturbance it has been postulated that invertebrate responses depend on the timing of the event relative to the life history of the constituent invertebrate species (Boulton, 2003; Lytle & Poff, 2004; Nijboer, 2004). Hence, the timing of harmful events in relation to the critical periods in the life cycle of the exposed species may be important in determining changes in the population structure after a disturbance (Lancaster & Downes, 2010; Miller et al., 2012; Wesner, 2019).

Studies assessing the impact of disturbances on invertebrate population dynamics that take the organism's entire life cycle into consideration are, however, rare (but see Kohler & Hoiland, 2001; Elliot, 2006; Elliot, 2013, Pandori & Sorte, 2018). Most studies focus on a single life stage, generally late instars or aquatic adults (Lancaster & Downes, 2010). This is partly due to the practical limitations involved in the sampling of early instars, since the mesh size of sample nets is commonly too large to retain these small individuals (Cummins & Wilzbach, 1988). Alternatively, stage-structured population models (e.g. StagePop) may be used to simulate the impact of a disturbance on life stages that are difficult to sample in the field (Kettle & Nutter, 2015). Hereby, the un-impacted population dynamics are modelled by using previously required information on reproduction, natural death rates and stage durations. Different scenarios can then be simulated in which the environmental variables or disturbances have a different effect on the death rates during each life stage, subsequently affecting the population dynamics during later stages (Kettle & Nutter, 2015). Such stage-structured population models have previously been used to evaluate the effectiveness of pest control during different life stages of an invasive culicid mosquito species (Wieser et al. 2019). Stage-structured population models were further successfully applied to assess the effects of invasive species and drought on crayfish population dynamics during different life stages (Yarra & Magoulick, 2019). Hence, these stage-population models may be a promising tool to simulate the effect of hydrologic

disturbances on invertebrate population dynamics during different life stages, including those life stages that are difficult to collect.

The present study applied this approach to the caddisfly *Agapetus fuscipes* CURTIS (Trichoptera: Glossosomatidae), as there is adequate information available on the population dynamics of *A. fuscipes* in unimpacted upper courses of European streams where the species can locally reach high densities (e.g. Nielsen, 1942; Castro, 1975; Becker 1990; Sangpradub et al., 1999; Becker, 2005). *A. fuscipes* is a case-building species with a univoltine life cycle consisting of an egg stage, eight larval instars, a pupae stage and a terrestrial flying adult (Castro, 1975). Several life stages of *A. fuscipes* are generally simultaneously present in the stream (Becker, 2005). Some knowledge on the stress responses for different life stages of *A. fuscipes* is already available, showing that unpredictable drops in stream water levels may result in the desiccation and subsequent loss of pupae above the water line (Nielsen, 1942; Marchant & Hehir, 1999). A laboratory experiment showed that late instar larvae endure more respiratory difficulties than early instar larvae when material is deposited upon them (Majecki et al., 1997). Moreover, terrestrial adults may be affected by disturbance of the riparian vegetation, with attendant impacts on larval population densities (Harrison et al., 2000). The literature is, however, ambiguous concerning the sensitivity of *A. fuscipes* to spates. Some studies reported that *A. fuscipes* populations were very susceptible to spates (Jones et al., 1977), while others reported that larvae were relatively unaffected (Giller et al., 1991). The discrepancy between these studies may be related to the timing of the peak discharge in relation to its life cycle, as argued above, but this explanation lacks verification.

Therefore, we aimed to gain a better understanding of the effect of spates on the population dynamics of *A. fuscipes* during different larval stages. We hypothesized that first instar larvae are more sensitive to spates than final instar larvae, i.e. high currents may cause dislodgment of first instar larvae as they attach themselves poorly to the gravel (Jones et al., 1977; Nijboer, 2004). To test this hypothesis, we performed a two year field study in which we measured discharge dynamics and the population development of *A. fuscipes* in four lowland streams in The Netherlands and tested the larval instar specific mortality rates in response to spates using a stage-structured population model. Normally, these four streams have a relatively stable discharge pattern. However, in the first year of the field study several severe spates occurred, providing a 'natural experiment' to evaluate the effect of these spates on the population development of *A. fuscipes* compared to the more stable second year.

MATERIALS AND METHODS

Study area

The field study was performed in two regions in The Netherlands, one region in Zuid-Limburg (region I: 50°54'N; 5°48'E), and one region in the Veluwe (region II: 52°04'N; 5°52'E) (Figure 1a). In each region, two headwater streams were selected; region I Bunderbosbeek (BU) and Strabekervloedgraaf (ST: Figure 1b), region II Seelbeek (SE) and Oude beek (OB).

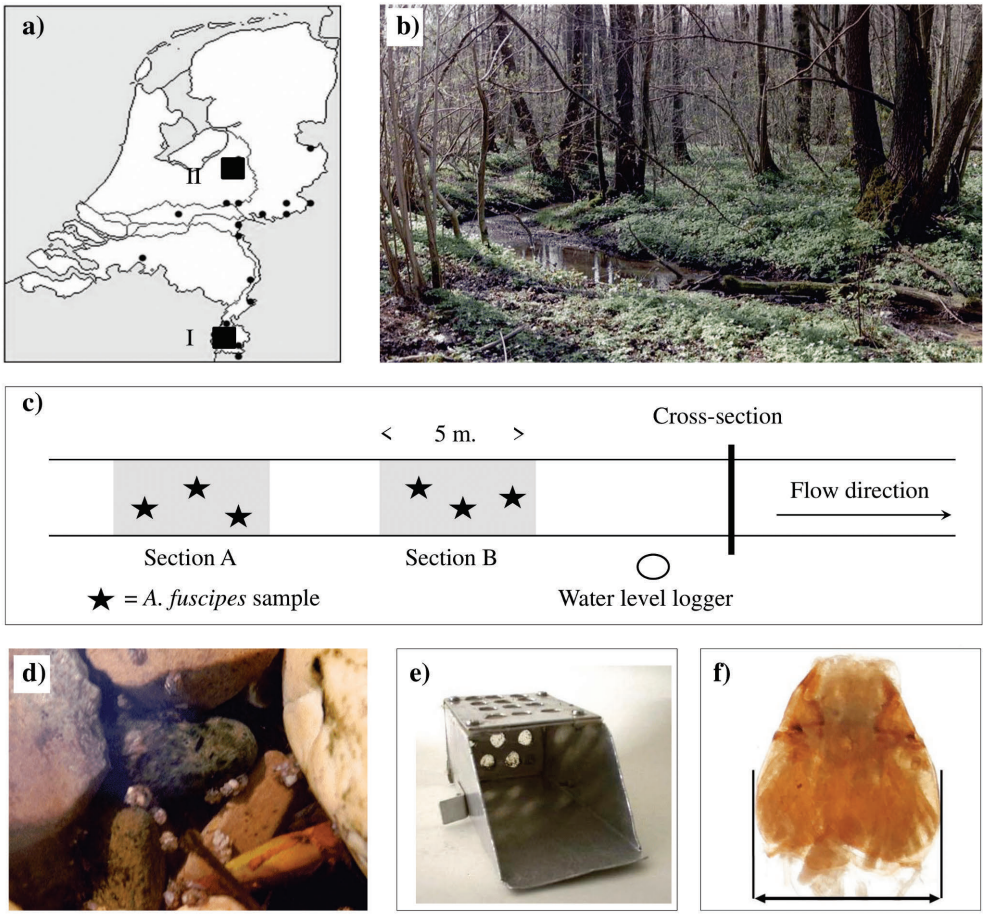

Figure 1*: a) Occurrence of A. fuscipes (dots) and location of study regions (squares) in The Netherlands: I Zuid-Limburg and II Veluwe, b) Picture of the Strabekervloedgraaf (ST stream), c) Schematic overview of the field set-up, d)* A. fuscipes *larvae on gravel bed, e) shovel used for sampling, f) measured head capsule width of* A. fuscipes*.*

The water chemistry differed between the two regions, as the soil in Zuid-Limburg is more calcareous than in the Veluwe, leading to a higher mean pH (BU = 7.2 ± 0.1 and ST = 7.3 ± 0.2 vs SE = 6.9 ± 0.2 and OB = 7.0 ± 0.1), higher mean electrical conductivity (BU = 702 ± 108 and ST = 558 ± 90 vs SE = 342 ± 48 and OB = 193 ± 11 µS/cm) and higher concentration of some micro-ions in the stream water (Supplementary material 1, Table S1). In spring and autumn, mean daily water temperatures were similar in the four streams (Supplementary material 1, Table S1 and Figure S1a). In summer, the water temperature was higher in the ST stream than in the other streams, while in winter the water temperature was lower in the ST and SE streams than in the BU and OB streams. In each stream, two sections of 5 m length were selected, which were up to 10 cm deep and up to 2 m wide (Figure 1c). Coarse gravel was the most frequently observed substrate category in each section, i.e. 59 % coverage in the SE stream, 61 % in the OB stream, 74 % in the BU stream and 83 % in the ST stream (Supplementary material 1, Figure S2). Larvae generally inhabit these gravel beds, feeding on biofilms growing on hard substrates (Figure 1d; Castro, 1975; Becker, 1990).

Data collection

Discharge dynamics - The water level (m) of each stream was measured every 15 minutes for two years from April 2002 until April 2004 with a mini-Troll model ssp-100 (In-Situ inc, Ft. Collins, CO, USA) installed in a monitoring well (Figure 1c). To be able to translate the water level measurements into discharge, a cross-section profile of the stream was measured every two weeks at 10 cm intervals across the channel. Discharge (Q) was calculated in m3/s from the corrected water level and cross-section data using the slope-area method (Boiten, 2000; details in Nijboer et al., 2003). Discharge data were normalized to the median flow (Q_{50} or base flow) to enable comparison of the streams with different flow magnitude (Riis et al., 2008).

Agapetus fuscipes - Population density and head capsule width were measured every two weeks during the first year. In the second year sampling was continued every three months, which was considered sufficient to follow general trends in population dynamics based on the results from the first year. In each 5 m stream section, three random samples were taken from the gravel beds (Figure 1c; N = 6 per stream). For each sample, the top gravel layer was collected with a shovel from a surface area of 45 cm^2 and placed into plastic buckets (Figure 1e). A total area of 270 cm^2 was sampled per stream. This sampling surface was considered sufficient as the density of *A. fuscipes* was very high on these gravel beds, and could reach up to 388 larvae/270 cm^2 (25th percentile = 9; median = 64; 75th percentile = 103 larvae/270 cm^2). The samples were stored for one night aerated at 5 °C, sieved through a 0.16 mm mesh sieve and sorted. All larvae and pupae of *A. fuscipes* were preserved in 70 % ethanol. The head capsule width was measured under 50x

magnification with a microscope equipped with a horizontal micrometer scale, to the nearest 0.025 mm (Figure 1f). The *A. fuscipes* larvae were assigned to eight larval instars, based on the head width classes defined by Castro (1975). Based on the entire sampling collection over two years, the number of specimens increased from the first larval instar to the fifth (Figure 2), suggesting that early instars 1 - 4 were not collected effectively from the field.

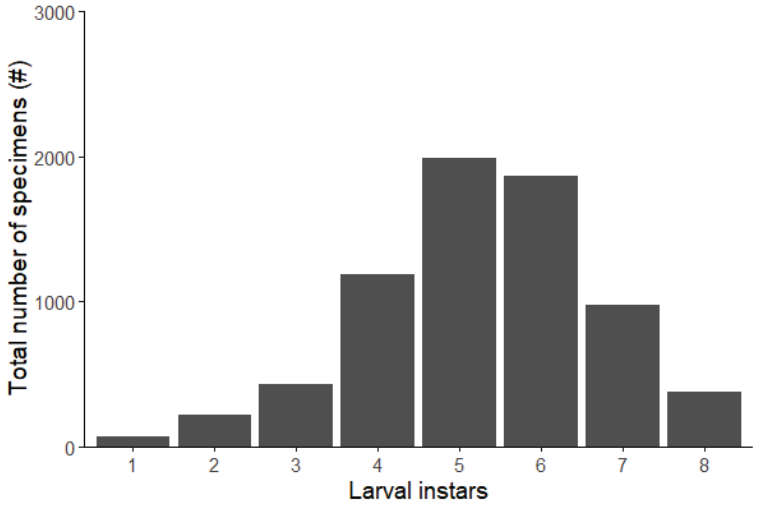

***Figure 2**: Number of specimen per larval instar collected during the entire sample collection period of two years.*

Stage-structured population model

As larval instars 1 - 4 were not collected effectively from the field, we used a stage-structured population model to analyze the effect of peak discharges on the development of the *A. fuscipes* population density. The natural life cycle of the population was modeled using the delay- differential equation formulation by Nisbet & Gurney (1983). This formulation assumes that once an individual is born it passes through different life stages, unless it dies. The stage durations and background or natural death rates were based on parameters measured previously in unimpacted streams (see model parameters). Discharge peaks were then used as input to the model to alter the mortality rates of each individual in the population based on its life stage and the intensity of the spate. This way we could assess the effect of discharge during all stages, i.e. also those that were not sampled effectively, on the population development during later stages. We simulated four scenarios with different sensitivity (mortality rates) to spates and tested which scenario corresponded best with the measured *A. fuscipes* population density of larval instars 5 - 8 (see model testing).

Model parameters

Parameter values were obtained from Castro (1975), who extensively monitored *A. fuscipes* in the Breitenbach, an unimpacted headwater stream in Central Germany with dimensions and temperature regimes similar to our studied streams (Supplementary material 1, Figure S1b). The stage-structured population model comprised the egg stage, the eight larval instars, the pupal and adult stage. The time for one larval instar to develop into the next instar depends on the cohort and water temperature (Castro, 1975). After each moult, the larvae leave their old case and build a new one from sand grains (Hanna, 1961). The mean durations of these stages over all cohorts were 33, 25, 34, 44, 42, 45, 50, 40, 27, 29, and 4 days, respectively (Castro, 1975). The sex-ratio was 2 : 1 (♂ : ♀), with a female laying 200 eggs on average during her 4 day life as an adult, so we simplified the reproduction rate to 200/4 = 50 eggs per female per day (Castro, 1975). Background mortality rates were estimated from the data on population density of larval instars 4 to 8 of Castro (1975), by dividing the area under the density curve by the duration of each larval instar (Southwood, 1978). The other stages were either not sampled or not sampled effectively, so we extrapolated the results assuming a linear trend. This resulted in slightly higher background mortality rates of the early life stages than those of the later life stages, decreasing from 0.016 to 0.006 day^{-1}.

Model testing

The simulation was initiated with the immigration of 1 adult per day over 120 days, as most adults emerge over a four month period, resulting in the presence of several developmental stages present at the same time (Becker, 2005; Nijboer, 2004). For each measurement year the model was run separately, as *A. fuscipes* populations may recover quickly after stress (Nijboer, 2004). A 'spin up' time was applied to get all stages established, with 394 days for the BU stream, SE stream and OB stream and 424 days for the ST stream to match the respective pupation period in each stream. To determine the effect of spates we selected discharge peaks exceeding thresholds relevant for invertebrate communities in lowland streams, including small spates of 2 - 4 times the Q_{50}, medium spates of 4 - 8 times the Q_{50} and high spates of > 8 times the Q_{50} (Verdonschot & van den Hoorn, 2010). Mortality rates were set to test four different scenarios (i.e. responses) to spates: 1) constant low, no impact by spates, 2) constant high, all larval stages are highly sensitive to spates, 3) decrease with instar stage, the sensitivity of the larvae to spates decreases exponentially with increasing larval stage, and 4) increase with instar stage, the sensitivity of larvae to spates increases exponentially with increasing larval stage (Figure 3). We assumed a density loss of 80 % for high spates, 40 % for medium spates, and 20 % for small spates (Bond & Downes, 2003; Death, 2008; Supplementary material 2).

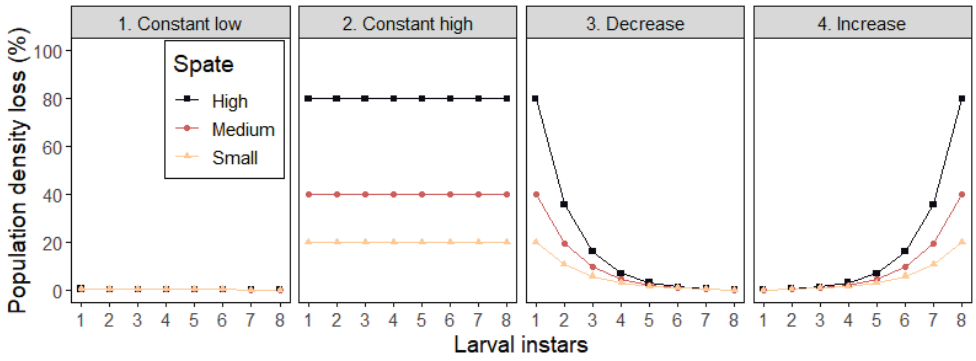

***Figure 3**: Four potential scenarios for the effects of spates on* A. fuscipes *population density losses per larval instar: 1) constant low, no impact by spates, 2) constant high, all larval stages are highly sensitive to spates, 3) decrease with instar stage, the sensitivity of the larvae to spates decreases with increasing larval stage, and 4) increase with instar stage, the sensitivity of larvae to spates increases with increasing larval stage (corresponding mortality rates for population density loss in each model in Supplementary material 2).*

The root mean square error (RMSE) was then used to test which scenario corresponded best with the measured *A. fuscipes* population density development of larval instars 5 - 8. The RMSE estimates the standard deviation of the model, so smaller values indicate a better fit. The unit is the same as the unit of the dependent variable, i.e. number of specimen/270 cm^2. The analysis was performed in R version 3.4.1, using r package 'StagePop' to construct the stage-structured population models (Kettle & Nutter, 2015), 'PBSddesolve' to solve the delay- differential equations (Schnute et al., 2003) and 'Metrics' to calculate the RMSE values (Hamner et al., 2018).

RESULTS

Discharge dynamics

The base flow (Q_{50}) was higher in the OB stream (0.014 m^3/s) than in the other three streams (0.004 m^3/s). The flow duration curves showed that in the BU stream more peak discharges occurred in the first measurement year than in the second year (Supplementary material 3). All peak discharges took place from May to October 2002 with one high spate (> 8 times the Q_{50}) on 13 July 2002 (Figure 4a). Discharge peaks occurred during both years in the ST stream, although they were higher in the first year. High spates took place on 13 July, 20 and 21 August, and 3 November 2002 (Figure 4b). Discharge in the SE stream was rather stable during both years, as only some small spates occurred between April and May 2002 and one in November 2002 (Figure 4c). In the OB stream, more peak discharges occurred in the first year than in the second year, with one high spate on 27 October 2002 (Figure 4d).

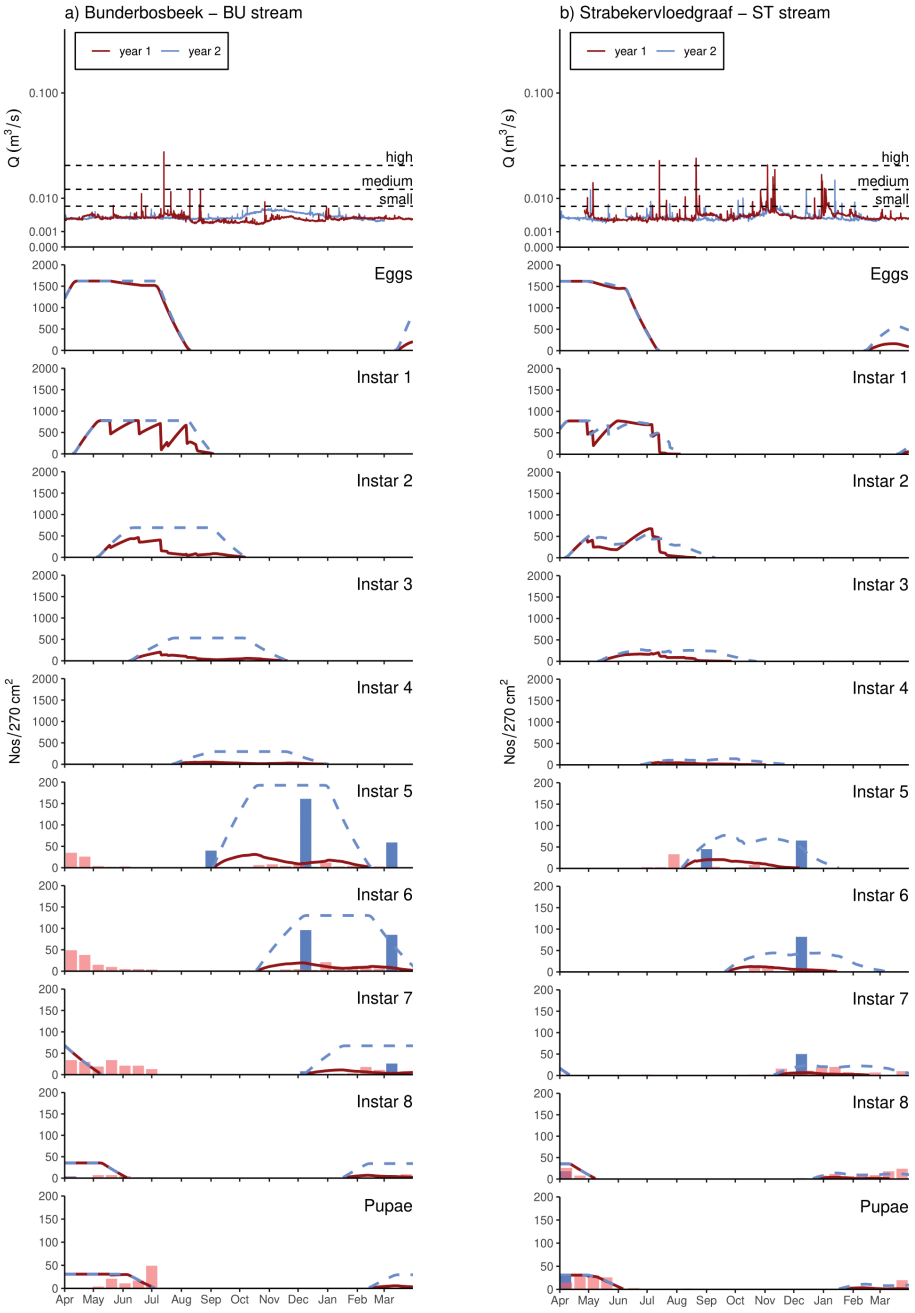

***Figure 4**: Impact of discharge dynamics (Q) on* A. fuscipes *population densities during different life stages from April 2002 - April 2004 in four streams a) Bunderbosbeek (BU stream), b) Strabekervloedgraaf (ST stream), c) Seelbeek (SE stream), d) Oude beek (OB stream). Measurements of population densities of larval instars 5 - 8 and pupae from year 1 are shown in red bars (sampled onthly) and from year 2 in blue bars (sampled every 3 months). Legend continued on the next page…*

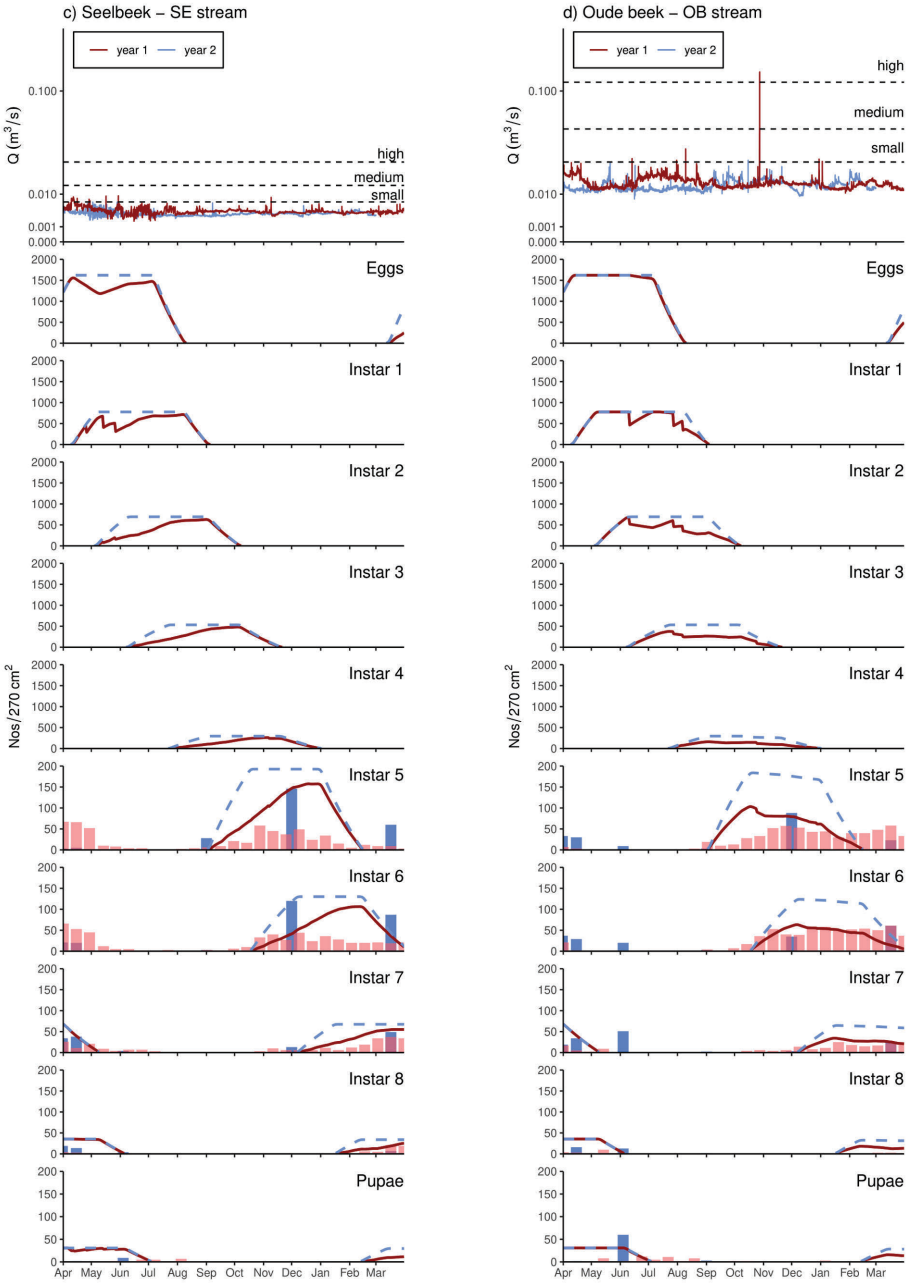

***Figure 4 (legend continued)**: The best fitting stage-structured population model, which assumes that the sensitivity of larvae to spates decreases with increasing larval stage, is shown for each aquatic life stage by a red solid line for year 1 and a blue dashed line for year 2. Note the discharge is in sqrt-scale and y-axis for stages egg - instar 4 is 10 times larger than instar 5 – pupae.*

Agapetus fuscipes

Pupae started to appear from the end of March in the ST stream and the end of April in the BU, SE and OB stream (Figure 4). In all streams, the majority of the individuals had pupated by September. Matching the start of the pupation period of the model to the data on *A. fuscipes* resulted in comparable timing between the model and the data for the larval instars 5 - 8. In all streams, the population density of larval instars 5 - 8 was lower in the first measurement year than in the second year, except for the OB stream where the population density was similar during both years.

In the BU stream, a high spate and several smaller spates occurred in the first year when primarily larvae of instar stages 1 - 3 were present (Figure 4a). Here, the models including either a constant high impact by spates or a decreasing impact by spates were better able to represent the population development in larval instars 5 - 8 than the models including either a constant low impact by spates or an increasing impact by spates (Table 1). In the ST stream, spates of various intensities occurred during different life stages (Figure 4b). The model including a decreasing impact by spates most accurately described the population development in larval instars 5 - 8 in this stream (Table 1). In the SE stream, several small spates occurred between instar stage 7 to instar stage 1 (Figure 4c). The model including a constant high impact by spates most accurately described the population development in larval instars 5 - 8 (Table 1). In the OB stream, the population density of larval instars 5 - 8 was similar during both years (Figure 4d). The high spate during instar stages 4 - 6 did not seem to be associated with a decrease in population density. The model including decreasing impact by spates most accurately described the population development in larval instars 5 - 8 in this stream (Table 1).

Table 1: Comparison of different model scenarios for the population density of larval instars 5 - 8 over two measurement years using the root mean square error (N = 32). Δ_i represents distance from the model with the lowest RMSE, and thus best fit.

	Bunderbosbeek BU stream		**Strabekervloed-graaf ST stream**		**Seelbeek SE stream**		**Oude beek OB stream**	
	RMSE	**Δ_i**	**RMSE**	**Δ_i**	**RMSE**	**Δ_i**	**RMSE**	**Δ_i**
Constant low	160	133	162	137	130	80	117	68
Constant high	27	0	39	14	49	0	74	25
Decrease with stage	28	1	25	0	72	23	49	0
Increase with stage	134	107	66	41	64	15	92	43

DISCUSSION

For three of the four studied streams the stage-population model including a decreasing impact by spates with larval life stage most accurately described the *A. fuscipes* population development of larval instars 5 - 8, supporting our hypothesis. It must be stressed that the study was based on a 'natural experiment' where several high spates occurred during early stages, while there was only one data point for a high spate during later periods in the life cycle. In the fourth stream, the population density of larval instars 5 - 8 was most accurately described by the model including constant high impact by spates. In that stream only several small spates occurred between instar stage 7 to instar stage 1 in the first year. The lowered population density of larval instars 5 - 8 in that year may not have been directly related to the flow, but instead to the large amount of deposited sand, silt and detritus on the gravel beds during this period (extra observations in Supplementary material 1, Figure S3). *A. fuscipes* larvae may endure respiratory difficulties when material is deposited upon them, in particular during later stages (Majecki et al., 1997). The effects of flow and sediment transport are, however, difficult to separate as both factors interact (Hynes, 1970a). Controlled experiments are needed to understand the mechanistic effects of flow and sediment transport on invertebrate species during a spate (e.g. Bond & Downes, 2003; Gibbins et al., 2007), but to our knowledge such experiments have not been combined with testing for the effect on specific life stages.

Comparable to our study, Elliot (2006) assessed the effects of a severe spate on different life stages of four Elmid beetles species in a 'natural experiment'. The effects of the spate were negligible for the larvae as they were buried in the gravel, which served as a refuge from the spate. In contrast, all adult densities were negatively affected by the spate, but the magnitude varied between species, presumably related to species specific habitat requirements (Elliot 2006). The same spate had limited effect on a Baetid mayfly, as the specimens present during the spate were in larval stages 2 - 3, and probably small enough to burrow between small stones in the substratum to avoid effects of the spate (Elliot, 2013). In agreement with these studies, Sagnes et al. (2008) observed that the aquatic insect larvae can make use of different hydraulic habitats while growing, i.e. dependent on the species they may prefer higher or lower shear velocity conditions with increasing larval stage. Beside the timing of spates during the invertebrate life cycle, Lancaster (1992) concluded that the time of day at which a disturbance takes place should be taken into account when interpreting the effect of peak discharges on invertebrates, as the density of Baetis nymphs in her study was reduced significantly by the spate created after sunset, but not at dawn or mid-afternoon.

Similar to other aquatic ecological studies, the early life stages of *A. fuscipes* (larvae instar 1 - 4) were not sampled effectively in this study. The smallest larvae may have been present in a different (micro) habitat than the larger larvae, like the sand under the stones,

as only the top layer of gravel was collected. Alternatively, they may have been mechanically damaged in the buckets during transport or passed through the sieve when sorting the samples, as the mesh of the sieve was larger than the head width of larval instars 1 - 4. To compensate for the ineffective sampling, we applied the stage-structured population model (i.e. StagePop), which proved to be a valuable tool to obtain an indication of the duration, timing and mortality patterns of the early life stages of *A. fuscipes* for which sampling was ineffective.

The natural life cycle of the population in the model was based on previously obtained parameter values, such as stage durations, of an un-impacted headwater stream in Central Germany. However, actual stage durations are dependent on water temperature and cohort (Castro, 1975). This temperature dependency may have caused slight differences between the timing and duration of each life stage, potentially causing uncertainty in the sensitivity of the population model compared to the field situation. In future studies, the model could thus be improved by making each stage duration temperature dependent (Kettle & Nutter, 2015). Additionally, in some streams (e.g. BU and SE stream) that were disturbed by peak discharges during the first year, the *A. fuscipes* population grew fast and recovered during the following stable year. Similar to previous studies, we observed a simultaneous presence of different life stages of *A. fuscipes* in each stream, which may spread the risk of high mortality to discharge peaks (Becker, 2005). Nijboer (2004) proposed that after a reduction in the population density by hydrological disturbances, the remaining females may be able to lay more eggs than normal, as there is less competition for food. Such density-dependent processes would need to be studied further in experiments, and could be included in the model to gain a better understanding the influence of spates on the local extinction of populations. Despite these uncertainties in the stage-population model, we were able to show that population declines may have been associated with the timing of spates, coinciding with the presence of early life stages.

Our study supports previous findings that floods can result in severe declines in stream invertebrate densities (see studies in Lake, 2000 and Death, 2008). Although recovery from floods by invertebrates is typically high, some previous studies observed changes in species composition following repeated, severe and/or unpredictable flooding (e.g. Giller et al., 1991; Scrimgeour et al., 1988; Robinson et al., 2003). It is generally accepted that the effects of spates depend greatly on the taxon, as taxa have different resistance (ability to tolerate disturbance) and resilience to flow (ability to recover after a disturbance) (Death, 2008; De Brouwer et al., 2017). This study provided initial indication that the resistance to peak discharge of invertebrates not only depends on the taxon, but also varies between life stages. This may have implications for management and restoration of freshwater ecosystems, as the current single-life-stage-based assessments with a strong focus on late instar or aquatic adult life stages may not elucidate which stressors or

disturbances actually constrain invertebrate population densities during their entire life cycle, which may lead to unsuccessful management efforts. Restoration measures might aim at environmental factors relevant for late instars or aquatic adult life stages, which may not be the limiting life stage for that species (Bond & Lake, 2003; Lancaster & Downes, 2010). The assessment of the critical life stages of a specific species to specific disturbances may help to identify the actual cause for the presence and absence of species and thereby aid more effective management and restoration of degraded aquatic systems.

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SUPPLEMENTARY MATERIAL 1

Water chemistry

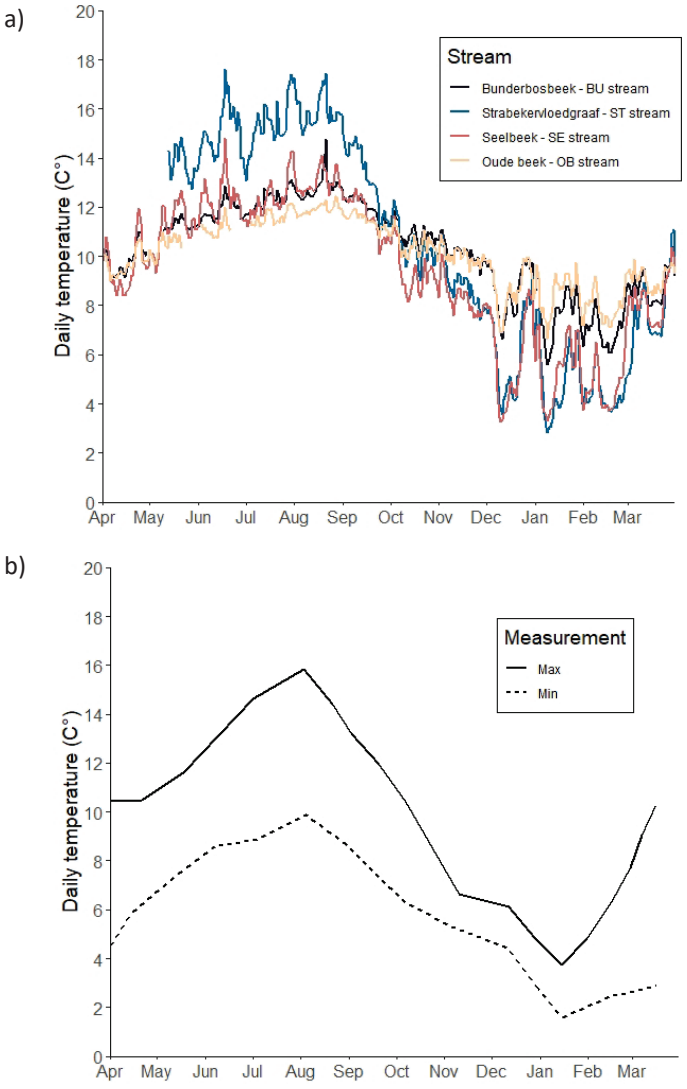


Figure S1*: Daily water temperature for a) mean temperature for the four studied streams, based on measurements taken every 15 minutes from April 2002 until April 2003 with an OTD-diver (Van Essen instruments, Delft, The Netherlands) and b) minimum and maximum temperature in the un-impacted stream Breitenbach, based on measurements from July 1971 to June 1972 (Castro, 1975).*

Table S1: Water chemistry of the streams sampled monthly from February 2002 until April 2003, and analyzed according to NEN-EN-ISO/IEC 17025 (N = 17). Mean daily water temperature per northern meteorological season based on 15 minute measurements from April 2002 until April 2003 with an OTD-diver (Van Essen instruments, Delft, The Netherlands).

	Bunderbosbeek BU stream				Strabekervloedgraaf ST stream				Seelbeek SE stream				Oude beek OB stream			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
pH	7.2	0.1	7.0	7.4	7.3	0.2	7.0	7.5	6.9	0.2	6.7	7.5	7.0	0.1	6.8	7.3
EC (µS/cm)	702	108	522	896	558	90	437	735	342	48	226	438	193	11	168	209
Ca (mg/l)	98.0	8.5	83.2	112.0	77.9	6.3	64.8	90.0	33.0	2.5	30.0	38.7	18.0	2.7	8.4	21.1
Cl (mg/l)	51.0	3.2	42.5	55.8	26.2	2.6	18.3	29.4	25.5	2.6	16.4	27.4	14.2	2.7	3.9	15.6
Fe (mg/l)	<0.005	<0.005	<0.005	0.029	0.008	0.003	0.006	0.013	0.007	0.001	0.006	0.007	0.013	0.005	0.008	0.022
HCO_3 (mg/l)	95.4	17.2	64.0	129.0	116.8	18.7	84.0	152.0	30.6	2.8	26.0	38.0	26.3	3.0	19.0	34.0
K (mg/l)	1.4	0.3	1.1	2.0	1.5	0.3	0.9	2.3	1.5	0.2	1.1	1.8	2.5	0.5	0.6	3.3
Mg (mg/l)	12.0	2.5	2.9	13.8	12.2	1.1	8.8	13.6	5.0	0.4	3.6	5.5	2.8	0.5	1.0	3.1
Na (mg/l)	13.1	1.2	10.1	15.0	12.4	1.1	9.0	13.3	16.9	1.6	11.3	18.3	10.1	3.8	2.4	19.7
N Kjeld. (mg N/l)	<0.2	<0.2	<0.2	0.9	0.4	0.2	0.3	0.9	0.5	0.2	0.3	1.0	0.5	0.3	0.2	0.9
NH_4 (mg N/l)	<0.03	<0.03	<0.03	0.11	0.06	0.02	0.03	0.10	0.05	0.02	0.03	0.11	0.04	0.01	0.03	0.04
NO_3 (mg N/l)	18.3	1.3	16.0	20.3	17.9	2.2	13.2	22.0	11.1	1.5	7.3	13.6	6.6	3.0	1.5	17.6
P total (mg P/l)	0.05	0.02	0.02	0.06	0.05	0.02	0.03	0.09	0.04	0.03	0.02	0.09	0.27	0.64	0.02	1.84
PO_4 (mg P/l)	0.029	0.015	0.012	0.083	0.009	0.003	0.006	0.014	0.009	0.004	0.006	0.018	0.010	0.005	0.006	0.023
SO_4 (mg/l)	118.9	5.6	110.0	133.0	75.1	3.5	68.0	81.1	40.0	1.4	36.8	42.0	14.6	2.0	6.7	15.8
Temp (°C)																
Spring	9.9	1.1	8.0	11.7	10.1	3.1	5.2	14.8	9.7	1.5	7.1	12.7	9.7	0.7	8.4	11.0
Summer	12.3	0.6	11.2	14.8	15.5	1.0	13.1	17.6	12.6	0.8	11.2	14.8	11.6	0.4	10.7	12.4
Autumn	11.0	0.9	9.2	12.6	10.9	2.1	7.4	15.0	9.8	1.6	7.5	12.8	10.7	0.7	9.4	12.0
Winter	7.8	1.1	5.6	9.9	5.2	1.6	2.8	9.3	5.3	1.5	3.3	8.7	8.4	0.8	6.7	10.0

Substrate coverage

In each 5 m stream section, the areal coverage of each substrate category was mapped every two weeks during the first year using the grid method sectioned in 10 cm squares (Gordon et al.. 1992). The substrate categories included silt and fine detritus (8 - 4 ϕ); coarse detritus, leaves and branches (< 4 ϕ); sand (4 – -1 ϕ); fine gravel (-1 – -3 ϕ); coarse gravel (-3 – -6 ϕ); and gravel (fine or coarse) covered by one of the other categories (Wentworth. 1922). Cobbles (-6 – -8 ϕ) and boulders (< −8 ϕ) were not present in the studied stream sections. Areal substrate coverage maps were digitalized in ArcInfo, and per 10 cm squares the most prevailing substrate type was determined in ArcGrid (Figure S2).

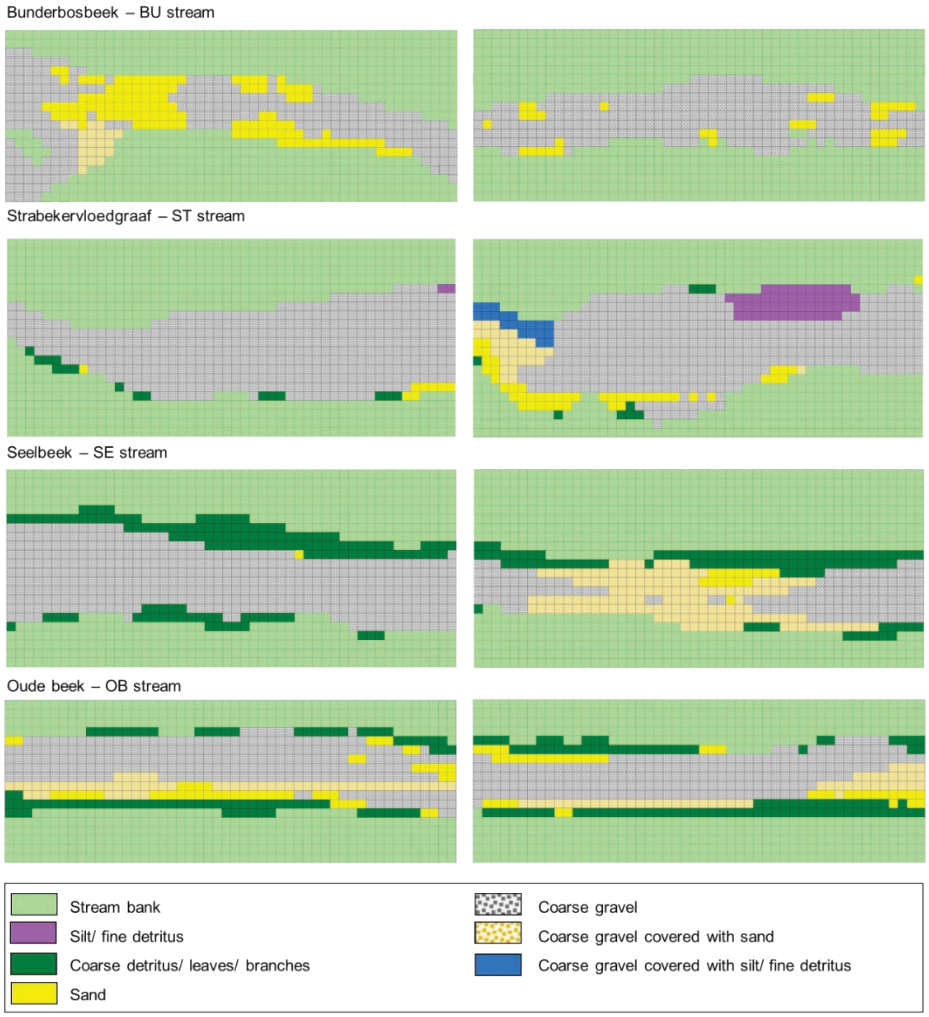


***Figure S2**: Most frequently observed substrate category in the two 5 meter sections per stream from April 2002 - April 2003. Each square on the map measures 10 by 10 cm in reality.*

We categorized the substrate into suitable, i.e. fine and coarse gravel inhabited by *A. fuscipes* (gravel), and unsuitable, i.e. fine and coarse gravel covered by one of the other categories (covered gravel). Gravel bed coverage followed a seasonal pattern in the BU stream and SE stream, with a high percentage of gravel covered by sand, silt or detritus during autumn (Figure S3a), and autumn and spring respectively (Figure S3c). The gravel bed coverage changed in a flashy pattern in the ST stream (Figure S3b) and OB stream (Figure S3d).

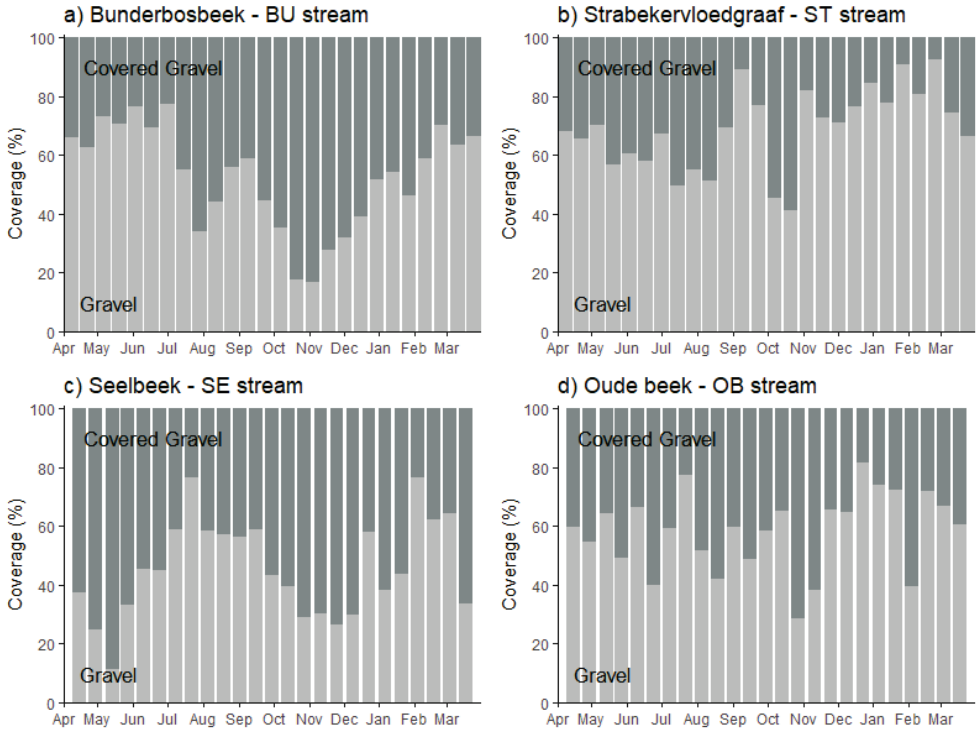


***Figure S3**: Gravel coverage from March 2002 until March 2003 in four streams a) Bunderbosbeek (BU stream), b) Strabekervloedgraaf (ST stream), c) Seelbeek (SE stream), d) Oude beek (OB stream).*

SUPPLEMENTARY MATERIAL 2

Table S1: Death rates set for each model to ensure approximate population density loss given the model assumes exponential decay.

Spate intensity	Larvae instar	Model							
		Constant low		Constant high		Decrease		Increase	
		Density loss (%)	Death rate (day^{-1})	Density loss (%)	Death rate (day^{-1})	Density loss (%)	Death rate (day^{-1})	Density loss (%)	Death rate (day^{-1})
High	1	0.6	0.015	80.0	2.175	80.0	2.175	0.3	0.008
High	2	0.5	0.014	80.0	2.175	35.9	0.976	0.7	0.018
High	3	0.5	0.013	80.0	2.175	16.1	0.438	1.5	0.04
High	4	0.4	0.012	80.0	2.175	7.2	0.197	3.2	0.088
High	5	0.4	0.011	80.0	2.175	3.2	0.088	7.2	0.197
High	6	0.4	0.010	80.0	2.175	1.5	0.040	16.1	0.438
High	7	0.3	0.009	80.0	2.175	0.7	0.018	35.9	0.976
High	8	0.3	0.008	80.0	2.175	0.3	0.008	80.0	2.175
Medium	1	0.6	0.015	40.0	1.086	40.0	1.086	0.3	0.008
Medium	2	0.5	0.014	40.0	1.086	19.8	0.538	0.6	0.016
Medium	3	0.5	0.013	40.0	1.086	9.8	0.267	1.2	0.032
Medium	4	0.4	0.012	40.0	1.086	4.9	0.132	2.4	0.065
Medium	5	0.4	0.011	40.0	1.086	2.4	0.065	4.9	0.132
Medium	6	0.4	0.010	40.0	1.086	1.2	0.032	9.8	0.267
Medium	7	0.3	0.009	40.0	1.086	0.6	0.016	19.8	0.538
Medium	8	0.3	0.008	40.0	1.086	0.3	0.008	40.0	1.086
Small	1	0.6	0.015	20.0	0.545	20.0	0.545	0.3	0.008
Small	2	0.5	0.014	20.0	0.545	11.0	0.298	0.5	0.015
Small	3	0.5	0.013	20.0	0.545	6.0	0.163	1.0	0.027
Small	4	0.4	0.012	20.0	0.545	3.3	0.089	1.8	0.049
Small	5	0.4	0.011	20.0	0.545	1.8	0.049	3.3	0.089
Small	6	0.4	0.010	20.0	0.545	1.0	0.027	6.0	0.163
Small	7	0.3	0.009	20.0	0.545	0.5	0.015	11.0	0.298
Small	8	0.3	0.008	20.0	0.545	0.3	0.008	20.0	0.545

SUPPLEMENTARY MATERIAL 3

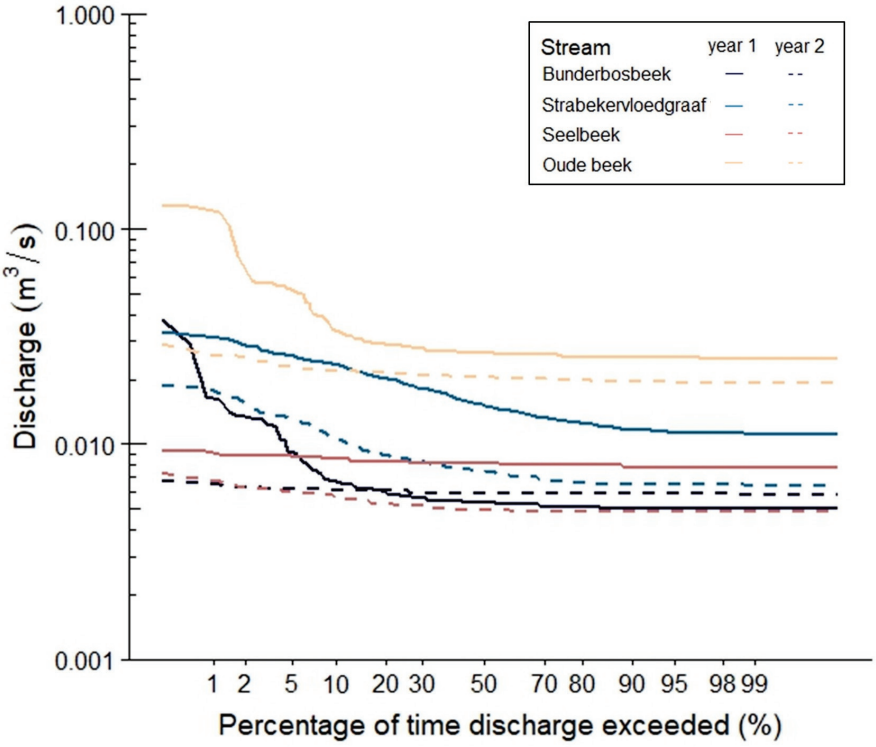
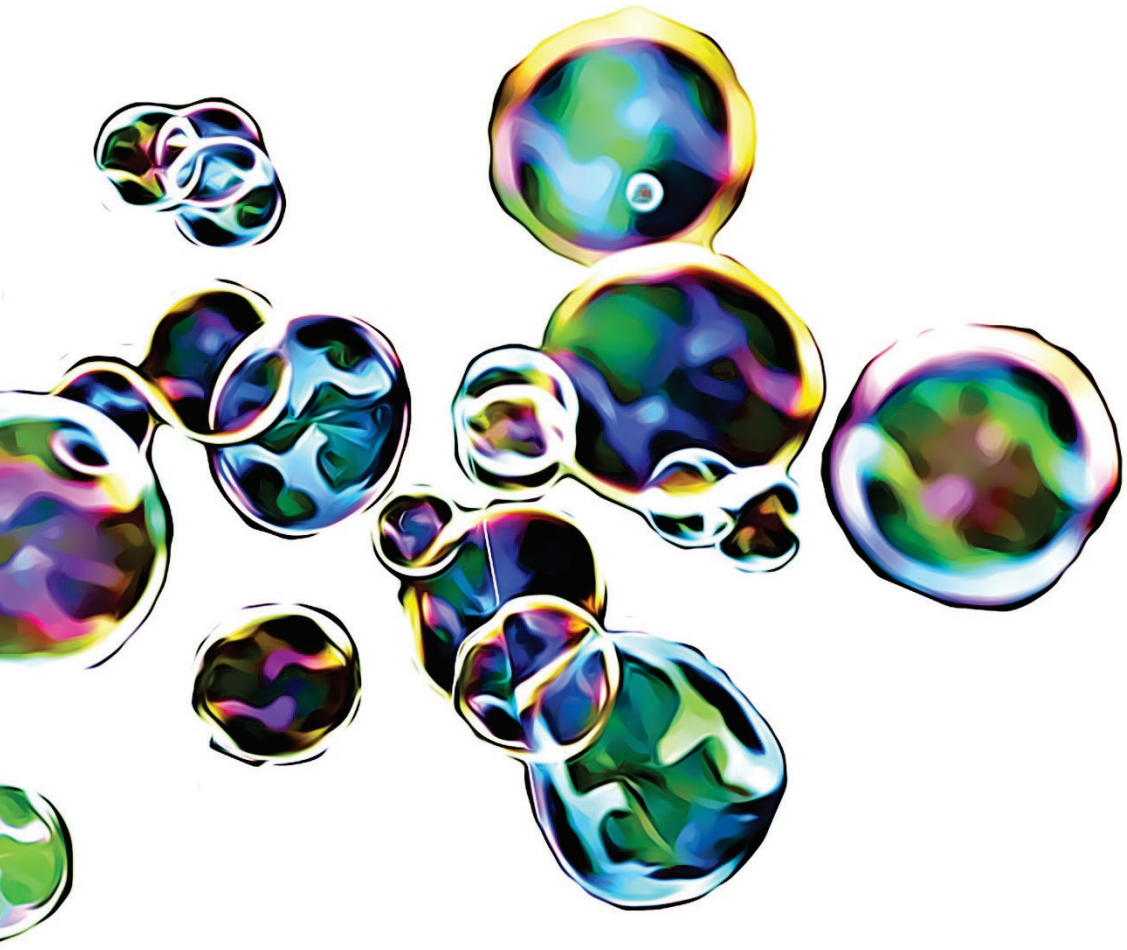


***Figure S1**: Flow duration curves for the four streams during year 1 (April 2002 - April 2003) and year 2 (April 2003 - April 2004). Based on 15 minute data.*

CHAPTER 3

DISSOLVED OXYGEN DYNAMICS IN DRAINAGE DITCHES ALONG A EUTROPHICATION GRADIENT



This chapter is based on the paper in *Limnologica* 72: 28-31

Gea H. van der Lee, Ralf C.M. Verdonschot, Michiel H.S. Kraak &
Piet F.M. Verdonschot

ABSTRACT

The impact of eutrophication on the functioning of drainage ditch ecosystems is understudied. Therefore, we performed a field study to quantify the dissolved oxygen dynamics of ditches at different depths and seasons along a eutrophication gradient. During summer, a clear distinction in daily variation in dissolved oxygen saturation of the top water layer was observed between the trophic states. We recommend including dissolved oxygen dynamics as a functional parameter in drainage ditch monitoring programmes.

INTRODUCTION

Eutrophication, the enrichment of surface waters with nutrients from agricultural and urban sources, is one of the key human induced stressors in freshwater ecosystems (Schindler, 2006; Smith & Schindler, 2009). In unshaded waters, eutrophication results in excessive plant and algal growth. The senescence and subsequent decay of this biomass can lead to prolonged periods of oxygen depletion, negatively impacting ecosystem structure and functioning (Smith & Schindler, 2009). Accordingly, the EU Water Framework Directive 2000/60/EC prescribes including assessment of both ecosystem structure and functioning in monitoring strategies. However, in practice the monitoring of surface waters often only encompasses structural measurements related to physico-chemical parameters or biological community composition (Bunn, 1995). Likewise, the impact of eutrophication is assessed by analyzing nutrient concentrations in the water column, and by determining the presence and absence of algal and macrophyte species.

These point-in-time measurements of structure, however, do not reflect the dynamic processes in ecosystems, and how they are affected by eutrophication (Palmer & Febria, 2012). Monitoring of freshwater ecosystems should thus also include functional measurements, such as primary production and community respiration (Palmer & Febria, 2012). To this purpose measuring the dynamics of dissolved oxygen (DO) concentrations in time and space is highly relevant, as changes in DO are the result of whole-system primary production and community respiration (Odum, 1956; Young et al., 2008). Additionally, DO is essential for the survival of aquatic organisms (Fox & Taylor, 1995). This, in combination with the latest advances in optical DO sensors and data logging technology that allow for relatively inexpensive and easy to execute DO measurements, has proliferated research on DO dynamics and metabolism in streams and lakes (reviewed in Hoellein et al., 2013 & Staehr et al., 2012a). Yet, only few studies focused on the spatial and temporal dynamics of DO in agricultural drainage ditches (Kersting & Kouwenhoven 1989; Veeningen, 1982; Verdonschot, 2012).

Drainage ditches are linear water bodies which can harbour a high invertebrate biodiversity (Herzon & Helenius, 2008; Verdonschot, 2012). These waters are present in most lowland areas of the temperate and boreal zones of the Northern Hemisphere. For example, in The Netherlands alone, total ditch length is estimated to be around 300,000 km (Higler, 1989). Water movement in drainage ditches in The Netherlands is negligible (<5 cm/s), and they are commonly surrounded by agricultural areas. Hence, drainage ditches may be particularly prone to eutrophication (Janse & Van Puijenbroek, 1998). However, to our knowledge, no previous studies have focussed on the impact of eutrophication on the DO dynamics in drainage ditches. The aim of this study was therefore to quantify DO dynamics in drainage ditches along a eutrophication gradient. To this purpose, we

performed a field study in which we performed continuous measurements of dissolved oxygen at different depths and seasons in drainage ditches that differed in trophic status.

MATERIALS AND METHODS

The field study was performed from 2016 to 2017 in two comparable regions in The Netherlands; the sampling sites were located near the village of Tienhoven (Region A; 52°10'N; 5°06'E) and near the village 't Doosje (Region B; 52°41'N; 6°07'E) (Figure 1a). In each region, three ditches were selected based on similar width (3.9 ± 1.1 m) and depth (0.4 ± 0.1 m), and their typical plant community structure during summer, each representing a trophic state, respectively (Figure 1b): 1) mesotrophic, characterized by a species rich plant community with numerous growth forms and many open water spaces between individual plants, 2) eutrophic, with a high biomass of submerged vegetation and dense layers of filamentous algae, filling the entire water column, and 3) hypertrophic, with a duckweed and frogbit floating vegetation layer with some submerged vegetation in the water column (Portielje & Roijackers, 1995; Janse & Van Puijenbroek, 1998). This vegetation is naturally occurring in these systems, however, to retain the drainage function of ditches regular maintenance (e.g. mowing and dredging) is carried out, which can alter the vegetation composition (Verdonschot, 2012). For each ditch, nutrient concentrations and macrophyte community cover-abundance were recorded during summer (Supplementary material 1).

Dissolved oxygen concentrations (mg/L) and temperature (°C) were measured with optical HOBO® Dissolved Oxygen Loggers U26-001, protected by the antifouling protective guard U26-GUARD-2 (Onset Computer Corporation, Bourne, MA, USA). The DO loggers were placed in the centre of each ditch from 10 cm under the water surface to 5 cm above the bottom, with approximately 10 cm steps in depth (Figure 1c). Water DO concentration and temperature were measured every 10 minutes for five consecutive days during each meteorological season. The measurements were taken simultaneously per region to minimize the variation in weather conditions between measurements. Percent DO saturation (%) was calculated from the DO concentrations and temperature, assuming 0 ‰ salinity and 1 atm. barometric pressure, using DOTABLES developed by the U.S. Geological Survey (2011). Vertical variations in DO saturation were observed during summer, therefore data from the loggers placed 10 cm under the water surface were used for further analysis.

We performed a linear mixed effects analysis for repeated measures of the relationship between dissolved oxygen saturation and trophic state during different seasons. We fitted a model for the extreme low (5^{th} percentile), mean, and extreme high (95^{th} percentile) dissolved oxygen saturation in the ditches using maximum likelihood (ML). As fixed effects, we entered region (without interaction term), trophic state and season (with interaction term) into the model. As random effects, we selected intercepts for ditches. Shapiro-Wilk normality test and the Levene's test revealed no deviations from

normality in the residuals and homogeneity of variance for each model. P-values of the fixed effects were estimated by Satterthwaite's method of approximation, and $p < 0.05$ was considered significant. Data analyses were performed in R version 3.4.1 using packages 'lme4' and 'lmerTest'.

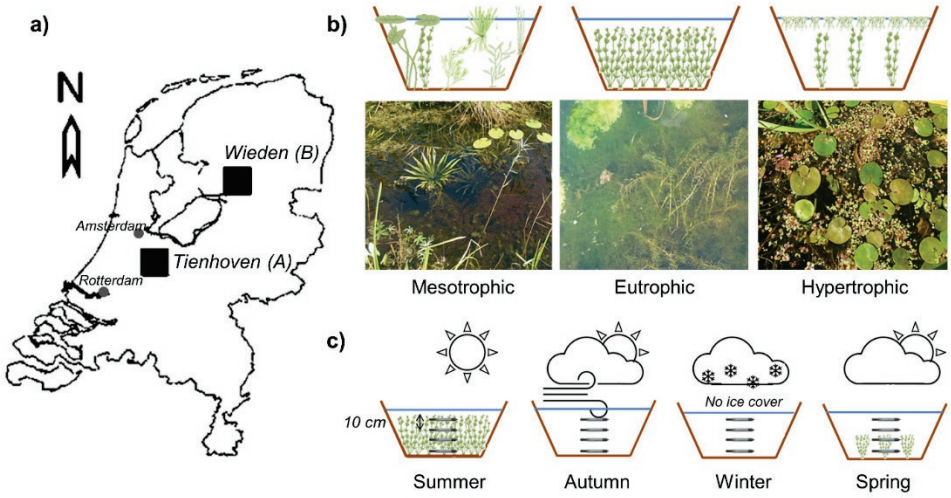


***Figure 1**: a) Map of the The Netherlands showing the study areas. b) Macrophyte community structure in the drainage ditches reflecting the three trophic states (pictures taken in area B Wieden). c) Field set-up for measuring vertical profiles of dissolved oxygen concentrations in drainage ditches over five days during different seasons.*

RESULTS

Summer was the only season when vertical variation in DO saturation was observed. During this period, the DO saturation could decrease to 0 % at water depths larger than 40 cm beneath the water surface, in relation to thermal stratification. An example of vertical stratification in DO saturation and temperature in region A Tienhoven in the mesotrophic and eutrophic ditch is shown in Figure 2. The models of the extreme low (5th percentile), mean, and extreme high (95th percentile) DO saturation measured 10 cm under the water surface showed that the effect of region, season, and trophic state separately were not significant, while the interaction between trophic state and season showed some significant effects (Supplementary material 2; Figure 3). Specifically, during summer the mean DO saturation in ditches of the hypertrophic state was significantly lower (mean ± sd; Tienhoven = 48 ± 16 %; Wieden = 39 ± 30 %; t-value = -2.26; *p*-value = 0.033) than in the eutrophic

(Tienhoven = 113 ± 75 %; Wieden = 86 ± 48 %) and mesotrophic state (Tienhoven = 111 ± 37 %; Wieden = 80 ± 18 %). Additionally, the 5th percentile DO saturation was significantly lower in the eutrophic (Tienhoven = 10 %; Wieden = 31 %; t-value = -2.19; *p*-value = 0.042) and hypertrophic state (Tienhoven = 23 %; Wieden = 6 %; t-value = -2.63; *p*-value = 0.017) than in the mesotrophic state (Tienhoven = 58 %; Wieden = 58 %). Large daily fluctuations in DO saturation in the eutrophic state were recorded compared to the other states, i.e. minimum values from 0 % in the early morning up to 300 % in the late afternoon (Figure 2 over time in Tienhoven; Figure 3). During autumn and winter, the mean DO saturation in all trophic states ranged between 46 and 100 %. During spring, the 95th percentile DO saturation was nearly significantly higher in the eutrophic state (Tienhoven = 141 %; Wieden = 325 %; t-value = 1.90; *p*-value = 0.070), and significantly higher in the hypertrophic state (Tienhoven = 170 %; Wieden = 294 %; t-value = 2.24; *p*-value = 0.034) than in the mesotrophic state (Tienhoven = 106 %; Wieden = 97 %). In region B Wieden, maximum values up to 392 % were reached in the eutrophic and hypertrophic state (Figure 3).

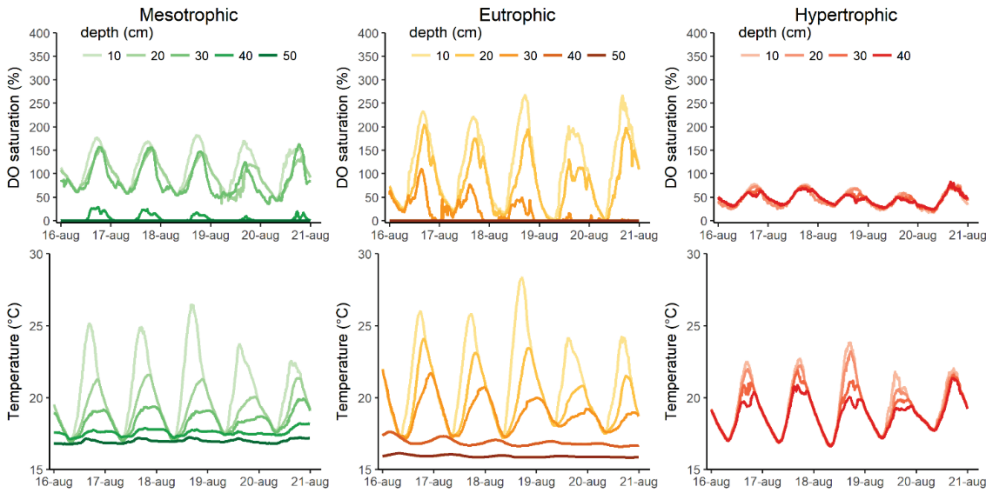


***Figure 2**: Vertical variation in dissolved oxygen (DO) saturation (%) and temperature (°C) dynamics measured in drainage ditches with a different trophic state during 5 days in summer (region A Tienhoven as example).*

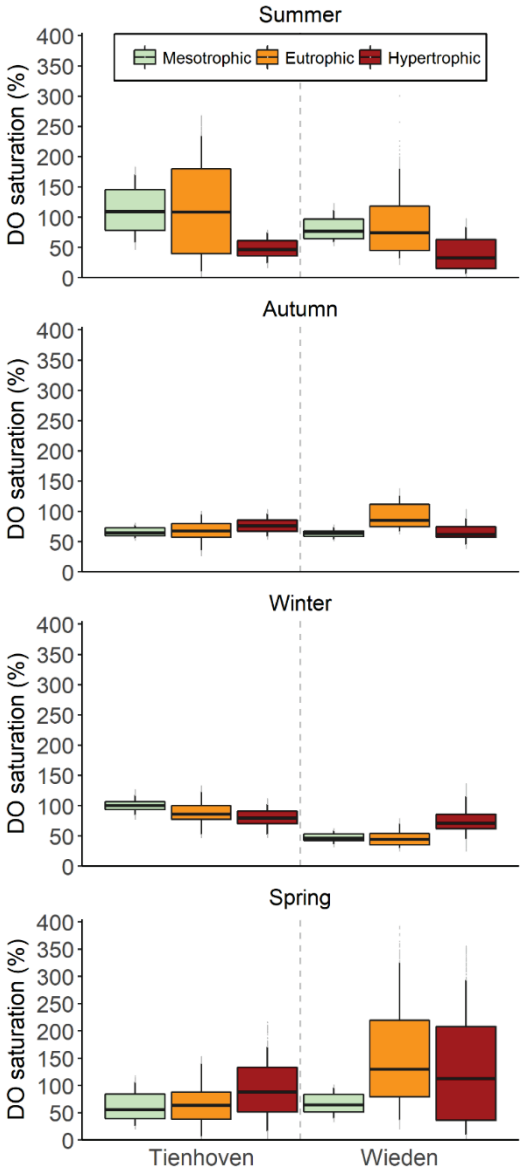


***Figure 3**: Boxplot of dissolved oxygen (DO) saturation dynamics in drainage ditches of different trophic states. Boxes are inter-quartile ranges (25th percentile to 75th percentile); whiskers extend to 5th and 95th percentiles; dots are outliers. Data includes measurements taken 10 cm under the water surface in each ditch every 10 minutes during 5 days in each season (N = 720).*

DISCUSSION

The present study gauged the impact of trophic state on dissolved oxygen dynamics in drainage ditches by conducting simultaneous high frequency DO measurements using multiple sensors. The results showed that the DO dynamics in the top water layer (10 cm under water surface) of the mesotrophic ditches were comparable during all seasons. The DO dynamics of the eutrophic and hypertrophic ditches differed from the mesotrophic ditches during spring and summer. During summer, the eutrophic ditches were characterized by high daily fluctuations in DO saturation, while the hypertrophic ditches had low daily minimum and mean DO saturation. During spring, both eutrophic and hypertrophic ditches measured high daily maximum DO saturation. Vertical variations in DO saturation were only observed during summer when DO saturation could decrease to 0 % in bottom water layers, independent of the trophic state.

The large impact of eutrophication on the DO dynamics in drainage ditches during spring and summer is presumably related to the small dimensions of ditches. Specifically, the large bottom surface-to-water volume leads to a dominant role of macrophytes and algae in DO production. During spring, phytoplankton blooms often occur in eutrophied ditches, which explain the extremely high DO saturation levels in the late afternoon due to primary production (Janse & Van Puijenbroek, 1998; Smith & Schindler, 2009). During summer, the moderate enrichment in eutrophic ditches causes an excess growth in submerged macrophyte biomass, so high DO production continues (Portielje & Roijackers, 1995; Janse & Van Puijenbroek, 1998). In contrast, at very high nutrient loadings a dense floating layer of e.g. duckweed and frogbit forms in the hypertrophic ditches (Portielje & Roijackers, 1995; Janse & Van Puijenbroek, 1998), which may have halted oxygen production by reducing light availability, and may have decreased the exchange of oxygen between water and atmosphere (Kersting & Kouwenhoven, 1989; Verdonschot, 2012).

The large bottom surface-to-water volume further results in a substantial influence of organic matter decomposition on DO consumption (Verdonschot, 2012). The increased primary production due to eutrophication increases DO consumption by microbes and invertebrates that decompose the excess dead organic matter, which explains the anoxic conditions in the early morning due to continued DO consumption by respiration with no DO production due to photosynthesis (Kersting & Kouwenhoven, 1989). Similar to lakes, DO consumption due to decomposition in relation to thermal stratification can also result in anoxia in the bottom water layers of drainage ditches during summer (Coloso et al., 2008. Staehr et al., 2012b). Stratification with anoxic conditions at the bottom impede the estimation of primary production and ecosystem respiration from dissolved oxygen concentrations (Hoellein et al., 2013), as oxygen produced by epipelon and epiphyton may be directly consumed by the microbial community.

In conclusion, continuous measurements of dissolved oxygen have been proposed to monitor and assess the functioning of freshwater ecosystems, such as lakes and rivers (Palmer & Febria, 2012; Young et al., 2008). The present study demonstrated that single, intermittent measurements of DO concentration, currently included in monitoring programmes, are indeed not relevant for drainage ditches due to large daily variations in DO. Rather, continuous measurements of DO are necessary to show daily variation in minimum and maximum DO saturation, which occur outside the working hours when incidental measurements are taken. Here, we demonstrated the impact of eutrophication on DO dynamics of drainage ditches during spring and summer, with a clear distinction in daily fluctuation in DO saturation between the different trophic states during summer. Therefore, we recommend the inclusion of continuous measurement of DO in the top water layers during summer as a functional parameter in monitoring programmes of drainage ditches. These measurements will help to distinguish trophic states of drainage ditches, therefore indicating why certain species may be present or absent, as DO is essential for the survival of many aquatic organisms. This will provide presently missing information that will enable effective management and restoration of degraded drainage ditch systems.

ACKNOWLEDGEMENTS

We would like to thank Mariska Beekman and Isabel Trujillo Rocha for their help in the field, Dick Brus for statistical advice, Erik de Haan and Rosalie Martens (Natuurmonumenten) and Bert van der Tol (Streekmuseum VredeGoed) for providing access to the study sites. Two anonymous reviewers provided valuable suggestions to improve the manuscript.

SUPPLEMENTARY MATERIAL 1

Table S1: Macrophyte species recorded in ditches of different trophic state (mesotrophic, eutrophic, hypertrophic) in each region during summer 2017; r = 1 individual; + = 2 - 5 individuals; 1 = 6 – 50 individuals; 2m = < 5 %; 2a = 5 – 12 %; 2b = 13 – 25 %; 3 = 26 – 50 %; 4 = 51 – 75 %; 5 = 76 – 100 % (Braun-Blanquet, 1932).

Latin name	region A Tienhoven			region B Wieden		
	Meso	Eutro	Hyper	Meso	Eutro	Hyper
Acorus calamus		r	+		+	
Agrostis stolonifera	+				1	
Alisma plantago-aquatica				+	r	+
Berula erecta	+	+		2m	+	1
Bidens sp.		r				
Butomus umbellatus			2a			r
carex sp.	+				+	+
Ceratophyllum demersum					2a	3
Cicuta virosa				1		
Eleocharis palustris	2m	1			2a	1
Elodea nuttallii	2b	5		2b	3	2a
Equisetum fluviatile	2m	+		r		
Galium palustre	+			+		+
Glyceria fluitans					1	1
Glyceria maxima			2m		1	2a
Hottonia palustris				1		
Hydrocharis morsus-ranae	+			2m	+	3
Hypericum maculatum				+		
Iris pseudacorus	1	1		+	2m	1
Juncus effuses		1	r		+	
Juncus subnodulosus	1			2a		
Lemna minor			2m			3
Lemna trisulca				2a		1
Lotus uliginosus	+			+		
Lycopus europaeus				+		
Lysimachia thyrsiflora	+					
Lysimachia vulgaris				r		

Table S1 (continued)

Latin name	region A Tienhoven			region B Wieden		
	Meso	Eutro	Hyper	Meso	Eutro	Hyper
Lythrum salicaria					+	+
Mentha aquatic	1	1		+		
Myosotis laxa/ scorpioides	+	+	1	+	+	+
Myriophyllum spicatum				+		
Nuphar lutea	1	+				
Nymphaea alba				1		
Oenanthe aquatic				1		
Oenanthe fistulosa	+					
Persicaria amphibia	r			+		
Phragmites australis		+		2a	1	+
Potamogeton compressus	1					
Potamogeton crispus						1
Potamogeton lucens	1			1		r
Potamogeton natans				1	r	
Potamogeton obtusifolius				2m		
Potamogeton pectinatus						1
Potamogeton trichoides					+	
Rorippa microphylla		+				
Rumex hydrolapathum	1	+		+	+	+
Sagittaria sagittifolia	1		+	1	r	+
Scutellaria galericulata						r
Sium latifolium	2m	+				
Sparganium emersum					+	
Sparganium erectum	2b	1	2b	2b	1	1
Spirodela polyrhiza			2m			2m
Stachys palustris	+					
Stratiotes aloides				1		
Typha angustifolia				2a		
Chara sp.				2b		
Filamentous algae				+	5	

Table S2: Nutrient concentrations in grab water samples taken on one point-in-time during summer 2017, analysed for total nitrogen (TN; Hach LCK 138) and total phosphorous (TP; Hach LCK 349) (N = 3). Nutrients concentrations can, however, vary greatly over time in these systems, e.g. concentrations are often low during summer, as most nutrients are directly taken up by primary producers with temporary increases in concentrations due to agricultural run-off (Veeningen, 1982; Verdonschot, 2012).

Area	**Eutrophiction state**	**TN (mg/L)**		**TP (mg/L)**	
		Mean	**SD**	**Mean**	**SD**
Region A Tienhoven	Mesotrophic	0.85	0.07	0.04	0.00
	Eutrophic	1.60	0.09	0.18	0.03
	Hypertrophic	2.54	0.52	0.10	0.01
Region B Wieden	Mesotrophic	1.52	0.19	0.03	0.00
	Eutrophic	2.21	0.11	0.06	0.02
	Hypertrophic	2.03	0.03	0.06	0.01

Table S1: Linear mixed effect models fitted for the 5th percentile, mean, and 95th percentile dissolved oxygen saturation in the ditches using maximum likelihood (N = 24). Fixed effects were region (without interaction term), trophic state and season (with interaction term), and random effects were intercepts for ditches. Reported for the fixed parts are estimate (B), standard error (SE), degrees of freedom (df), t-value and *p*-value estimated by Satterthwaite's method of approximation. Reported for the random parts are variance and standard deviation (SD). Reported for the model summary are akaike information criterion (AIC), bayesian information criterion (BIC), deviance (Dev.), and the degrees of freedom of the residuals (df_{res}).

		model 1: 5th percentile DO saturation					model 2: Mean DO saturation					model 3: 95th percentile DO saturation				
Fixed parts		**B**	**SE**	**df**	**t**	***p***	**B**	**SE**	**df**	**t**	***p***	**B**	**SE**	**df**	**t**	***p***
(Intercept)		55.4	8.2	23.1	6.77	**<0.01**	65.8	13.5	24.0	4.88	**<0.01**	71.2	26.5	24.0	2.69	**0.01**
Region	Wieden	-1.8	4.7	6.0	-0.38	0.72	-2.1	7.5	24.0	-0.28	0.79	9.5	14.7	24.0	0.65	0.52
Trophic state	Eutrophic	-3.5	11.1	23.9	-0.31	0.76	14.9	18.3	24.0	0.81	0.42	35.0	35.9	24.0	0.97	0.34
	Hypertrophic	-2.9	11.1	23.9	-0.26	0.80	6.2	18.3	24.0	0.34	0.74	16.5	35.9	24.0	0.46	0.65
Season	Winter	5.8	10.9	18.0	0.54	0.60	8.9	18.3	24.0	0.49	0.63	12.6	35.9	24.0	0.35	0.73
	Spring	-22.1	10.9	18.0	-2.03	0.06	-1.1	18.3	24.0	-0.06	0.95	25.5	35.9	24.0	0.71	0.49
	Summer	3.7	10.9	18.0	0.34	0.74	31.1	18.3	24.0	1.70	0.10	65.2	35.9	24.0	1.81	0.08
Trophic state : Season	Eutrophic : Winter	-16.2	15.4	18.0	-1.05	0.31	-22.1	25.9	24.0	-0.85	0.40	-26.5	50.8	24.0	-0.52	0.61
	Hypertrophic : Winter	-8.9	15.4	18.0	-0.58	0.57	-2.4	25.9	24.0	-0.09	0.93	3.9	50.8	24.0	0.08	0.94
	Eutrophic : Spring	-8.0	15.4	18.0	-0.52	0.61	31.4	25.9	24.0	1.21	0.24	96.4	50.8	24.0	1.90	0.07
	Hypertrophic : Spring	-17.0	15.4	18.0	-1.10	0.29	40.4	25.9	24.0	1.56	0.13	114.0	50.8	24.0	2.24	**0.03**
	Eutrophic : Summer	-33.7	15.4	18.0	-2.19	**0.04**	-11.5	25.9	24.0	-0.44	0.66	30.9	50.8	24.0	0.61	0.55
	Hypertrophic : Summer	-40.5	15.4	18.0	-2.63	**0.02**	-58.5	25.9	24.0	-2.26	**0.03**	-78.5	50.8	24.0	-1.54	0.14
Random parts		**Variance**			**SD**		**Variance**			**SD**		**Variance**			**SD**	
	Ditch	3.9			2.0		0.0			0.0		0.0			0.0	
	Residual	118.7			10.9		335.7			18.32		1292.0			35.9	
Model summary		**AIC**	**BIC**	**Dev.**	**df_{res}**		**AIC**	**BIC**	**Dev.**	**df_{res}**		**AIC**	**BIC**	**Dev.**	**df_{res}**	
		213.5	231.2	183.5	9		237.7	255.4	207.7	9		270	287.7	240.0	9	

CHAPTER 4

OXYGEN DRIVES BENTHIC-PELAGIC DECOMPOSITION PATHWAYS IN SHALLOW WETLANDS

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ABSTRACT

Oxygen availability is perceived as an important environmental factor limiting POM decomposition. In shallow wetlands, however, the impact of commonly observed anoxic conditions in the benthic layer on the relative contribution of microbes and invertebrates to POM decomposition remains largely unknown. Therefore, the aim of this study was to determine if dissolved oxygen drives benthic-pelagic decomposition pathways in shallow wetlands. Dissolved oxygen concentration, invertebrate community composition, microbial decomposition and invertebrate consumption were measured in the benthic and pelagic layer of 15 permanent drainage ditches. We showed that an increased duration of anoxic conditions in the benthic layer of the ditches was related to increased microbial decomposition in this layer, while invertebrate consumption decreased in the benthic layer and increased in the pelagic layer. The increased invertebrate consumption in the pelagic layer was related to the presence of amphipods. We concluded that anoxic conditions in the benthic layer of shallow wetlands relate to an increase in microbial decomposition and a decrease in invertebrate consumption, as detritivorous invertebrates move to the pelagic layer to consume particulate organic matter. This illustrates that environmental conditions, such as dissolved oxygen, may drive the relative importance of aquatic organisms to ecosystem functioning.

INTRODUCTION

Dead particulate organic matter (POM) fuels shallow wetland food webs by serving as a food source for interacting microbes and invertebrates (Moore et al., 2004; Whatley et al., 2014). The rate of POM decomposition is therefore influenced by microbial and invertebrate community composition, but also by POM quality, and by the physicochemical environment (Webster & Benfeld, 1986; Tank et al., 2010; Handa et al., 2014). Especially, oxygen availability has been proposed an important environmental factor limiting POM decomposition (Brinson et al., 1981; Webster & Benfeld, 1986). In shallow wetlands, considerable differences in daily and seasonal dissolved oxygen concentrations have been measured along depth gradients in the water column (Clare & Edwards, 1983; Kersting & Kouwenhoven, 1989; Sharitz & Batzer, 1999; Verdonschot, 2012). This may alter the relative importance of microbes and invertebrates for POM decomposition in each layer, as they are differently adapted to function under low dissolved oxygen conditions (Webster & Benfeld, 1986; Covich et al., 1999).

Dissolved oxygen concentrations are the result of whole-system primary production (release of O_2) and respiration (uptake of O_2) (Odum, 1956). In shallow wetlands, anoxic conditions typically occur in the benthic layer from late spring through summer, as the oxygen demand is high at the bottom due to POM processing, in combination with stratification of the water column due to high water temperatures and decreased water movements (Kersting & Kouwenhoven, 1989; Longhi et al., 2016). Despite the differences in oxygen conditions between the benthic and pelagic layer, POM decomposition in shallow wetlands is pre-dominantly studied in the benthic layer (Hunting et al., 2016; Longhi et al., 2016; Vonk et al., 2016). However, a large amount of plant biomass already decomposes in a standing-dead position in the pelagic layer before the shoot material collapses to the benthic layer (Kuehn & Suberkropp, 1998). Furthermore, algal litter in the pelagic layer can also form an important food source in eutrophic shallow wetland food webs (Campeau et al., 1994). Hence, in shallow wetlands POM decomposition takes place in both the benthic and pelagic layer, yet under varying oxygen conditions.

It is generally believed that anoxic conditions slow down invertebrate consumption rates by lowering invertebrate densities (Brinson et al., 1981; Webster & Benfeld, 1986). Some aquatic invertebrate species are killed or suffer from sub-lethal effects under anoxic conditions (Fox & Taylor, 1955; Davis, 1975), while mobile organisms may move to the pelagic layer (Kolar & Rahel, 1993). Furthermore, it has been suggested that anoxia slows down microbial decomposition rates, as oxygen is energetically the most favourable electron acceptor (Sørensen et al., 1979). Data from microbial litter breakdown studies do, however, not always support the assumption that decomposition proceeds more rapidly under aerobic than anoxic conditions (Nichols & Keeney, 1973; Cole & Pace, 1995). A diverse assemblage of microbial functional groups coexists in freshwater sediments and water, and

depending on the prevailing conditions one or the other functional group may become active and dominant (Gons, 1982; Palmer et al., 2000). Thus, the relative contribution of the interacting microbes and invertebrates to POM decomposition and consumption in the benthic and pelagic layer of shallow wetlands remains largely unknown.

The aim of this study was therefore to determine if dissolved oxygen drives benthic-pelagic POM decomposition pathways in shallow wetlands. We hypothesized that low dissolved oxygen concentrations in the benthic layer 1) do not change microbial decomposition rates, as other functional groups adapted to low oxygen become active and dominant (Palmer et al., 2000), and 2) lead to lower invertebrate consumption, as they die (Fox & Taylor, 1955; Davis, 1975) or move to the pelagic layer(Kolar & Rahel, 1993). To test these hypotheses, we performed a field study in which we continuously monitored dissolved oxygen concentrations, determined invertebrate community composition, and quantified microbial decomposition and invertebrate consumption in the benthic and pelagic layer of 15 permanent peat drainage ditches in The Netherlands.

MATERIALS AND METHODS

Study area

The present field study was performed in 15 permanent peat drainage ditches located in an extensive agricultural area near Tienhoven, The Netherlands (52°09'N - 52°10'N; 5°05'E - 5°06'E). Drainage ditches are linear water bodies with negligible water movement (0-5 cm/s). The ditches were selected based on similar width (3.7 ± 1.0 m) and depth (0.6 ± 0.1 m). Physicochemical characteristics of the water column and sediment from the ditches were determined three times between May and July 2016 (methods in Supplementary material 1; results in Table 1). Water temperature, dissolved oxygen and decomposition were measured over 55 days between 26 May and 20 June 2016. Each measurement was performed in the benthic layer (<10 cm above the sediment) and in the pelagic layer (<10 cm below water surface).

Table 1: Overview of the physicochemical characteristics of the water column and sediment in the ditches (N = 15).

Water column	Mean	SD	Sediment	Mean	SD
Water temp (°C)	8.0	0.6	C:N ratio	14.5	1.1
Conductivity (µS/cm)	291	72	Tot P (mg/g)	0.6	0.3
Tot C (mg/L)	44.6	9.7	Organic matter (%)	17.2	12.8
DOC (mg/L)	20.9	9.3			
Tot N (mg/L)	1.3	0.6			
PO_4 (mg/L)	0.06	0.02			
SO_4 (mg/L)	6.7	4.7			
Cl (mg/L)	16.9	10.4			

Dissolved oxygen conditions

In each ditch, dissolved oxygen concentration and water temperature were measured every ten minutes during 55 days with HOBO® Dissolved oxygen loggers U26-001 (Onset Computer Corporation). Loggers were placed 10 cm (pelagic layer) under the water surface and 4 cm above the bottom sediments (benthic layer). A reading of 0.2 mg/L or lower was considered to be anoxic, a reading between 0.2 and 2.0 mg/L hypoxic, and a reading of 2.0 mg/L and higher as oxic (Diaz, 2000). For each ditch the percent time anoxic, hypoxic and anoxic was calculated for the benthic and pelagic layer.

Invertebrate community composition

Invertebrate community composition was determined using activity traps (Verdonschot, 2010). The traps were deployed 10 cm under the water surface (N = 5 per ditch) and on the bottom substrate (N = 5 per ditch) for one week, both at the start and at the end of the field experiment. The traps were carefully retrieved from the water, the contents poured through a sieve (250 µm mesh), and thereafter washed into a bottle with 70% ethanol. In the laboratory, the collected invertebrates were identified to the lowest practical taxonomic level, except for Tricladida, Hirudinea, and Oligochaeta which were not identified further. Bias caused by differences in taxonomic resolution (family, genus, and species) was reduced by applying a conservative adjustment procedure (Schmidt-Kloiber & Nijboer, 2004). We defined taxa as detritivore based on a combination of the primary functional feeding group (i.e. collector gatherer, shredder or filter feeder) and food type (i.e. detritus or dead plants) based on the trait-database by Tachet et al. (2002) supplemented with additional literature (Moller Pilot, 2009 & 2013) (complete list of detritivores is provided in Supplementary material 2). The invertebrate data of the traps deployed at the start and the end of the experiment were aggregated per ditch (total N = 10 per ditch) before further analyses were conducted.

Microbial decomposition and invertebrate consumption

Decomposition rates were measured using standard substrates, the Decomposition and Consumption Tablets (DECOTABs) (www.ibed.uva.nl/decotab; Kampfraath et al., 2012). The DECOTABs were prepared by boiling 20 g/L of purified agar dissolved in deionized water for 3 minutes. The mixture was cooled down under continuous stirring to 60 °C at which point 60 g/L of powdered cellulose and 60 µmol/L ascorbic acid as were added. The mixture was then poured into polycarbonate moulds (15 mm diameter; 884 mm^3 volume), and after cooling the DECOTABs were removed from the moulds and stored at 7 °C. Initial DECOTAB dry mass (70 °C, 2 days) was determined from a subset of 70 DECOTABs.

To facilitate retrieval from the field, we deployed cages (height 2 cm; diameter 10 cm) containing three DECOTABs closed off by either fine mesh (width 51 µm) to quantify decomposition by microcrobes, or coarse mesh (width 4 mm) to quantify the joint microbial

decomposition and invertebrate consumption. Mass loss by leaching of compounds from the DECOTABs under controlled conditions was negligible (Vonk et al., 2016). The cages were deployed 10 cm under the water surface (fine cages N = 5 per ditch; coarse cages N = 5 per ditch) and on the bottom substrate (fine cages N = 5 per ditch; coarse cages N = 5 per ditch). After 55 days of exposure, the cages were retrieved, DECOTABs were rinsed, dried in a stove (70 °C, 2 days), and weighed. Mass loss was calculated as mean initial weight minus individual weight after exposure in the field. Microbial decomposition was defined as the mass loss of DECOTAB in the fine cages. Consumption by invertebrates was calculated by subtracting mass loss of DECOTAB in fine mesh cages from mass loss of DECOTAB in the coarse mesh cages. The decomposition rates of the five replicates per ditch were averaged for further analysis, excluding outliers according to the Dixon's Q test (Dean & Dixon, 1951).

Statistical analysis

Significant differences among oxygen conditions (percent of time anoxic, hypoxic, and oxic) in the benthic and pelagic layer were analysed using Mann-Whitney pairwise comparisons, because of deviations from homogeneity and normality of variances. To exclude the effect of water temperature as confounding factor in our experiment, water temperature was related to the mean oxygen concentration in each layer using a linear regression. To meet assumptions of normality, invertebrate data were $\log_{10}(x + 1)$-transformed. Differences in the invertebrate community composition between the benthic and pelagic layer were compared using a paired sampled t-test. Microbial decomposition (i.e. mass loss in fine mesh cages) and invertebrate consumption (i.e. mass loss in coarse cages minus microbial decomposition) in the benthic and pelagic layer were compared using Kruskal-Wallis tests, because of deviations from homogeneity of variances. Post hoc testing was performed using Mann-Whitney pairwise comparisons (Bonferroni corrected: 0.05/3, a = 0.017) to compare the four groups. To assess the effect of stratification of the water column on decomposition pathways, microbial decomposition and invertebrate consumption were related to the percent time the benthic layer was anoxic using linear regression analysis. A linear regression analysis was further used to relate the dominant detritivore invertebrate groups to invertebrate decomposition. Data analyses were performed in R version 3.1.0.

RESULTS

Dissolved oxygen conditions

The duration of anoxic, hypoxic, and oxic conditions in the 15 ditches during the 55 days of measurements differed significantly (all $p < 0.01$) between the benthic and pelagic layer (Figure 1). The pelagic layer was almost always oxic in all ditches (mean percent of time ± SD = 93 ± 11 %), and anoxia and hypoxia were rarely observed. The benthic layer showed large variations in dissolved oxygen conditions with ranges between the ditches

from 0 to 91 % of the time anoxic, 4 to 22 % of the time hypoxic, and 4 to 95 % of the time oxic. The percent of time that the hypoxic and anoxic conditions occurred in the benthic layer was significantly higher than in the pelagic layer ($p = 0.004$ and $p = 2.3 * 10^{-5}$, respectively). Dissolved oxygen concentrations did not relate significantly to water temperature in the benthic layer ($R^2 = 0.08$, $p = 0.30$), or the pelagic layer ($R^2 = 0.05$, $p = 0.44$).

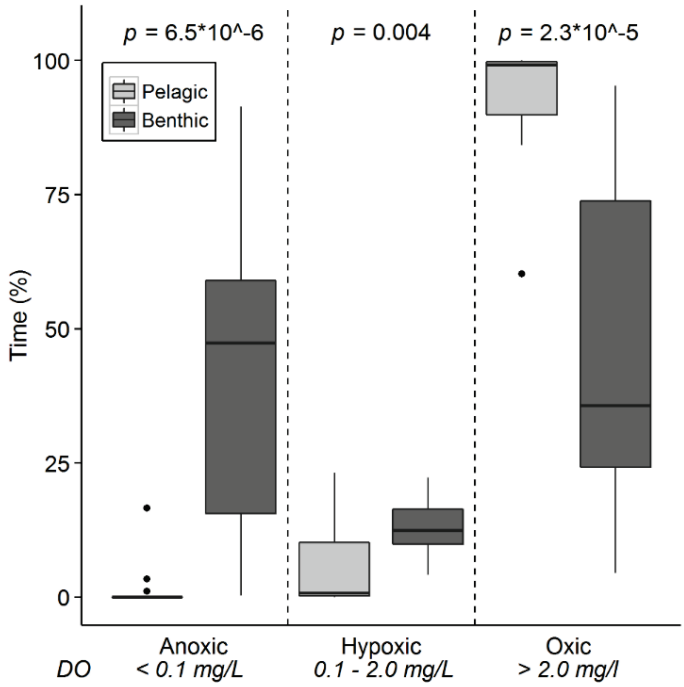

***Figure 1**: Boxplot of dissolved oxygen (DO) conditions during 55 days of measurements in the pelagic and benthic layer. Boxes are inter-quartile ranges (25th percentile to 75th percentile); whiskers extend to 1.5 * IQR; dots are outliers.* p-*values indicate statistical difference between the benthic and pelagic layer are for percent time anoxic, hypoxic and oxic (Mann-Whitney pairwise comparisons; N = 15).*

Invertebrate community composition

A total of 2857 individuals belonging to 79 taxa were caught in the activity traps. The detritivores comprised 63.9 % of the total invertebrate abundance. The four most dominant (>10 % of the detritivore abundance) detritivore taxonomic groups were corixids, amphipods, oligochaetes, and isopods, which constituted 30.0 %, 29.4 %, 13.9 %, and 11.2 % to the total detritivore abundance, respectively.

Significant differences were observed between the overall invertebrate richness and abundance in the benthic and pelagic layer (all $p < 0.05$; Table 2). There was, however, no significant difference in detritivore richness between the layers ($p = 0.06$). Detritivores

were significantly more abundant in the benthic layer (mean individuals ± SD = 72 ± 42) than in the pelagic layer (mean individuals ± SD = 50 ± 31, $p = 7.72 * 10^{-3}$), which was reflected by significantly higher number of corixids and isopods in the benthic layer. Abundance of amphipods and oligochaetes did not differ significantly between the benthic and pelagic layer.

Table 2: Richness and abundance of invertebrates caught in the benthic and pelagic layer. Statistical difference in richness (number of taxa) and abundance (number of individuals) between the benthic and pelagic layer are presented (paired sampled t-test; N = 15).

Invertebrate metric		**Benthic layer**		**Pelagic layer**		***p***
		Mean	**SD**	**Mean**	**SD**	
Richness	Overall	19.3	7.1	15.7	3.9	0.03
(number of taxa)	Detritivores	9.5	3.2	7.8	1.9	0.06
Abundance	Overall	109.3	62.6	81.2	31.0	0.02
(number of individuals)	Detritivores	72.1	42.1	49.5	31.2	$7.72*10^{-3}$
	Corixids	31.1	28.8	8.1	7.2	$1.74*10^{-5}$
	Amphipods	14.7	12.4	21.1	24.1	0.12
	Oligochaetes	5.1	7.7	9.0	10.7	0.06
	Isopods	12.4	15.2	3.7	5.3	$1.35*10^{-3}$

Microbial decomposition and invertebrate consumption

The contribution of microbial decomposition and invertebrate consumption to DECOTAB mass loss was significantly different between the benthic and pelagic layer during the 55 days of the measurements ($p = 3.5 * 10^{-7}$; Figure 2). In the benthic layer the microbial decomposition (mean mass loss ± SD = 42 ± 13 mg) was higher than invertebrate consumption (mean mass loss ± SD = 14 ± 11 mg), while the opposite was observed in the pelagic layer where invertebrate consumption (mean mass loss ± SD = 31 ± 13 mg) was higher than microbial decomposition (mean mass loss ± SD= 14 ± 4 mg).

Effect of anoxic conditions in the benthic layer

An increase in anoxic periods in the benthic layer related to a significant increase in microbial decomposition in the benthic layer ($R^2 = 0.49$, $p = 0.004$), but showed no relation to the microbial decomposition in the pelagic layer (Figure 3a). Invertebrate consumption significantly decreased in the benthic layer ($R^2 = 0.42$, $p = 0.009$), and significantly increased in the pelagic layer ($R^2 = 0.29$, $p = 0.04$) in relation to increasing time that the benthic layer was anoxic (Figure 3b). Ditches that were not or for limited time anoxic in the benthic layer showed similar microbial decomposition and invertebrate consumption in the benthic and pelagic layer (compare Figure 3a and 3b).

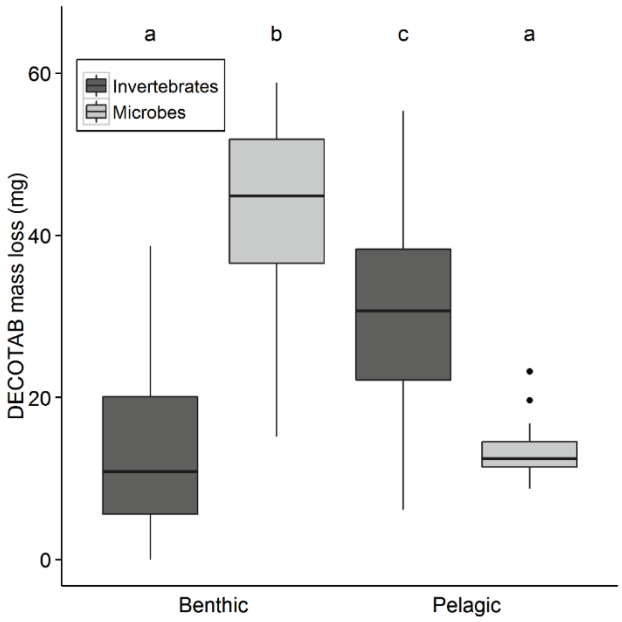

***Figure 2**: Boxplot of loss of DECOTAB mass (mg) after 55 days of exposure in the benthic and pelagic layer, expressed as invertebrate consumption and microbial decomposition. Boxes are inter-quartile ranges (25th percentile to 75th percentile); whiskers extend to 1.5 * IQR; dots are outliers. Different letters indicate statistical difference between DECOTAB mass loss (Kruskal-Wallis test and post-hoc Mann-Whitney pairwise comparisons; N = 15).*

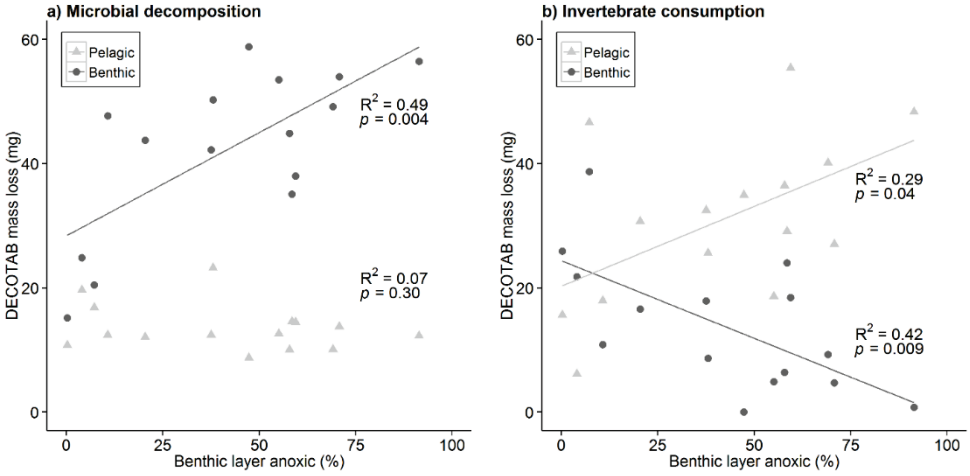

***Figure 3**: Relation between anoxic conditions (DO < 0.2 mg/L) in the benthic layer (% of time) and loss of DECOTAB mass (mg) after 55 days of exposure in the benthic and pelagic layer for A) microbial decomposition, and B) invertebrate consumption (linear regression; N = 15).*

Detritivorous invertebrates and consumption

The number of amphipods (97% *Gammarus pulex* and 3% *Crangonyx pseudogracilis*) caught in the traps related significantly to the invertebrate consumption of the DECOTABs in the pelagic layer ($R^2 = 0.41$, $p = 0.01$), but not in the benthic layer ($R^2 = 0.05$, $p = 0.40$; Figure 4). No significant relation was observed between invertebrate consumption and the number of corixids (pelagic $R^2 = 0.01$, $p = 0.71$; benthic $R^2 = 0.01$, $p = 0.68$), number of oligochaetes (pelagic $R^2 = 0.00$, $p = 0.86$; benthic $R^2 = 0.07$, $p = 0.33$), or the number of isopods caught in the traps of each layer (pelagic $R^2 = 0.08$, $p = 0.32$; benthic $R^2 = 0.00$, $p = 0.82$).

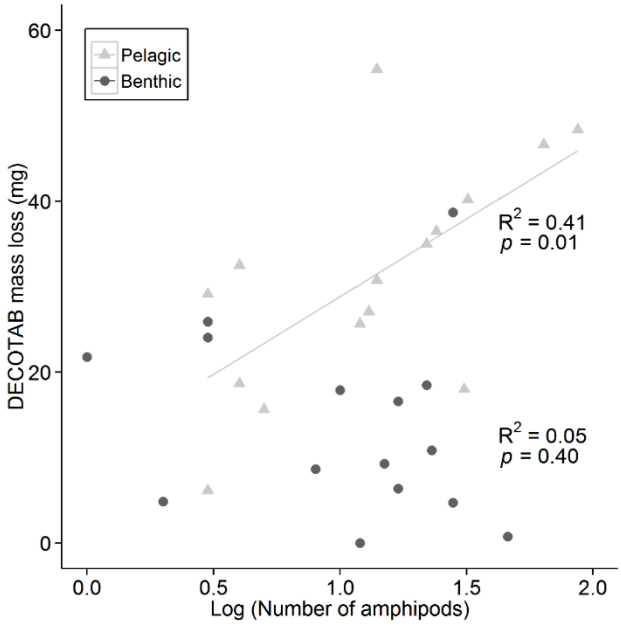

***Figure 4**: Relation between number of amphipods ($log_{10}(x+1)$) and loss of DECOTAB mass (mg) after 55 days of exposure in the benthic and pelagic layer (linear regression; N = 15).*

DISCUSSION

We determined microbial decomposition and invertebrate consumption in permanent drainage ditches, characterized by different dissolved oxygen conditions in the benthic layer compared to the pelagic layer. The pelagic layer was almost always oxic in all drainage ditches, while the benthic layer covered the entire oxic-anoxic range. Similar dissolved oxygen patterns have been observed in shallow wetland environments other than drainage ditches (Ritter & Montagna, 1999; Sharitz & Batzer, 1999). Our results showed that an increased duration of anoxic conditions in the benthic layer related to increased microbial

decomposition (rejecting our first hypothesis), while invertebrate consumption simultaneously decreased in this layer (accepting our second hypothesis).

Longer time periods of anoxic conditions in the benthic layer related to higher microbial decomposition rates. Faster POM breakdown at low oxygen concentrations has also been reported for lakes (Nichols & Keeney, 1973; Cole & Pace, 1995). It was, however, not possible to determine whether low dissolved oxygen concentrations were the cause or the consequence of the high microbial activities in the benthic layer, as under anoxic conditions oxygen production and consumption rates cannot be directly derived from dissolved oxygen concentrations. Primary production rates of epipelon and epiphyton can be high in the benthic layer of shallow wetlands (Gons, 1982), but this oxygen may be immediately used by the microbial community, still leading to anoxic conditions. Under complete oxic conditions in the benthic and pelagic layer decomposition rates were similar, so we assume that under anoxic conditions in the benthic layer additional functional groups of bacteria have become metabolically active, including denitrifiers, manganese-iron reducers, sulphate reducers, or fermenters (Palmer et al., 2000). Adaptation of these microbial functional groups to daily and seasonal anoxic conditions, in combination with excess availability of an alternative electron acceptor (e.g. nitrate), may thereby enhance microbial decomposition in shallow wetlands (Longhi et al., 2016). Such changes in the microbial community can occur abruptly between oxic and anoxic states (Bush et al., 2017). However, other processes that influence microbial decomposition rates may also have changed under anoxic conditions in the benthic layer, such as an increased release of compounds from the sediments needed for decomposition (Skoog et al., 2009), or differences in numbers and diversity of organisms that graze upon microbes and periphytic algae (Lee, 1992), which may have stimulated microbial decomposers in breaking down POM (Kuehn et al., 2014). No effect of anoxic conditions in the benthic layer on the microbial decomposition in the pelagic layer was observed, which confirms the idea that microbial functional activity corresponds to small-scale variations in chemical conditions (Palmer et al., 2000), in this case presumably the widely varying oxygen conditions over a water column height of less than one meter.

Invertebrate consumption decreased in the benthic layer and increased in the pelagic layer as the benthic layer was anoxic for a longer time period. This suggests that the invertebrates moved higher up in the water column to avoid prolonged anoxic conditions. Yet, we observed that the detritivores remained present in the benthic layer under low oxygen conditions, which implies that they were present, but did not consume POM. Kolar & Rahel showed that in the absence of predators invertebrates moved to the pelagic layer as benthic dissolved oxygen concentrations declined, but in the presence of fish most taxa remained in the benthic layer and slowed down their activity (Kolar & Rahel, 1993). Further, larvae of four species of caddisfly (Bjelke, 2005) and the amphipod *Gammarus*

pulex (Maltby et al., 1990) reduced or even stopped POM consumption under low oxygen conditions. These findings are coherent with the conclusion by Verdonschot & Verdonschot (2014) that most invertebrate taxa are capable to survive a certain period of anoxia in drainage ditches, but that such events can have negative impact on their functioning (e.g. emergence and recruitment). The increase in invertebrate consumption in the pelagic layer only related to increased activity of amphipods (mainly *Gammarus pulex*) in this layer. Although functional feeding groups are often treated as one guild, sharing specific traits (Blonder, 2003), in reality detritivorous invertebrates have different abilities to consume POM and this capacity may alter under anoxic stress (Bjelke, 2005). Similar to our study, Tiegs et al. (2008) showed that the extremely mobile and very effective leave shredder *Gammarus fossarum* was a key player in the decomposition processes in their leaf litter bag experiment in streams. Gammarids were 100 to 200 times more abundant on leaf litter packs than in areas adjacent to these packs (Haeckel et al., 1973). We thus suggest that mobile invertebrates, such as gammarids, take refuge in the benthic layer from predators while reducing their detritivorous activity due to low oxygen concentrations, and that they migrate to the pelagic layer to consume POM under oxic conditions.

To conclude, anoxic conditions in the benthic layer of shallow wetlands relate to an increase in microbial decomposition, and a decrease in invertebrate consumption in this layer as detritivorous invertebrates move to the pelagic layer to consume particulate organic matter. Oxygen may thus drive benthic-pelagic decomposition pathways in shallow wetlands, which illustrates that environmental conditions determine the relative importance of groups of aquatic organisms to ecosystem functioning.

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SUPPLEMENTARY MATERIAL 1

Water temperature was measured every ten minutes during 55 days between May and July 2016 with HOBO® Dissolved oxygen loggers U26-001. Data for each ditch was averaged for one loggers placed 10 cm under the water surface and one logger placed 4 cm above the bottom sediments. Additionally, chemical characteristics of the water and sediment in the ditches were determined on three moments in time. Conductivity was measured using the TDS & EC meter hold (HQ EZ1). Surface water samples were taken, filtered with 0.2µm Whatman GF/F glass fiber filters, and analyzed for total carbon, dissolved organic carbon, and total nitrogen on the elemental analyzer (Elementar Vario EL), and for orthophosphate, sulphate and chloride on the continuous flow analyzer (Skalar SAN++ system). The top 2 cm of four sediment cores were pooled, sieved through 500 µm, stored in a freezer at -22 °C, and milled. The organic matter content was determined after heating dry sediment samples at 550 °C for 4 h. Homogenized portions of 200 mg dry sediment were digested with 4 mL HNO3 (65 %) and 1 mL H2O2 (30 %), using the microwave. Digestates were diluted and phosphorus concentrations were determined by ICP (Perkin Elmer ICP-OES 8000). A homogenized p ortion of dry sediment was used to determine carbon and nitrogen content, using the elemental analyzer as described above.

Table S1: Overview of the physicochemical characteristics of the water column and sediment in each ditch (water temperature N = 17280 readings, other parameters N = 3 samples).

Oxygen	**Water**																**Sediment**					
Benthic layer anoxic	Water temp (°C)		Conductivity (µS/cm)		Tot C (mg/L)		DOC (mg/L)		Tot N (mg/L)		PO_4 (mg/L)		SO_4 (mg/L)		Cl (mg/L)		C:N ratio		Tot P (mg/g)		Organic matter (%)	
(%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.3	19.7	2.2	372	131	45.6	1.5	20.6	7.9	1.4	0.3	0.05	0.03	14.7	2.8	28.6	13.6	12.4	6.3	0.3	0.1	5.4	1.7
4.0	19.2	2.2	404	76	44.2	6.3	18.1	5.0	1.3	0.1	0.03	0.01	14.4	6.4	34.7	7.8	13.0	5.5	0.2	0.1	4.3	1.0
7.2	18.3	2.1	262	31	34.5	8.3	10.5	1.6	0.9	0.4	0.05	0.03	1.6	0.3	12.3	0.6	15.3	1.1	0.4	0.3	10.7	6.9
10.7	19.0	2.3	204	33	31.6	4.7	12.6	1.0	0.8	0.1	0.03	0.01	1.2	0.3	11.7	0.7	15.4	1.1	0.3	0.3	7.8	7.7
20.4	18.6	2.1	226	6	45.9	5.2	22.8	7.5	1.5	0.2	0.07	0.00	4.5	1.7	7.7	1.0	15.3	3.7	1.1	0.3	26.3	2.5
37.5	19.0	2.2	365	43	58.6	29.5	33.1	27.6	2.4	1.2	0.07	0.04	10.0	3.9	28.5	14.8	14.5	4.9	0.6	0.1	13.7	0.6
38.0	19.1	1.9	395	70	48.6	4.7	19.3	3.5	1.3	0.1	0.07	0.09	8.9	4.7	31.5	7.0	14.8	5.2	1.1	0.4	20.5	8.5
47.3	18.9	2.3	269	37	65.8	12.1	43.8	8.0	2.6	0.3	0.09	0.05	8.3	4.3	9.0	1.9	14.9	4.4	0.9	0.5	25.3	22
55.1	18.4	2.4	223	10	45.7	2.1	25.4	1.0	1.5	0.0	0.07	0.09	7.2	1.2	7.7	0.2	14.6	3.8	0.8	0.1	26.1	1.4
57.8	17.8	2.1	273	39	37.3	7.1	11.5	1.7	0.9	0.3	0.08	0.01	4.5	0.9	10.0	0.6	13.3	4.0	0.4	0.2	7.1	4.0
58.5	19.5	2.1	386	104	56.1	11.2	30.1	12.8	2.0	0.7	0.05	0.05	13.2	10.4	29.2	7.5	13.6	7.5	0.6	0.1	11.2	0.5
59.4	18.5	1.9	248	28	43.7	10.3	18.4	6.2	1.1	0.2	0.04	0.05	2.6	1.5	5.2	1.3	14.5	4.2	0.9	0.9	22.2	23.8
69.1	17.7	1.9	226	12	33.0	2.5	10.2	2.3	0.8	0.1	0.09	0.03	1.5	0.2	7.8	0.1	13.9	1.1	0.4	0.3	7.3	5.0
70.7	19.4	2.3	239	44	39.3	5.3	21.1	6.5	1.3	0.3	0.04	0.05	3.3	4.0	16.2	3.7	15.4	4.6	0.5	0.2	16.8	15.7
91.4	19.2	2.3	276	50	39.1	9.2	15.5	5.2	0.9	0.3	0.04	0.04	4.1	2.3	13.0	1.9	16.6	4.8	1.1	0.1	53.9	4.3

SUPPLEMENTARY MATERIAL 2

Table S1: List of detritivores based on combination of functional feeding group (CG = collector gatherer, SH = shredder, FI = filter feeder) and food (DET = detritus or dead plant). Information based on Tachet (2010). 1) Additional information Chironomidae based on Moller Pilot (2009, 2013). 2) Coleoptera larvae (lv) were assessed separate from adults for feeding groups (not for richness).

Taxa	Family	Functional feeding group			Food	Detri-tivore
		CG	SH	FI	DET	
Crangonyx pseudogracilis	Amphipoda	0	1	0	1	1
Gammarus pulex	Amphipoda	1	1	0	1	1
Argyroneta aquatica	Aranea	0	0	0	0	0
Sphaeridae	Bivalvia	0	0	1	1	1
Odontomyia sp.	Brachycera	1	1	0	1	1
Ablabesmyia sp.	Chironomidae[1]	0	0	0	0	0
Chironomus sp.	Chironomidae[1]	1	0	1	1	1
Cladopelma gr. *lateralis*	Chironomidae[1]	1	0	0	1	1
Clinotanypus nervosus	Chironomidae[1]	0	0	0	0	0
Cricotopus sp.	Chironomidae[1]	1	0	0	1	1
Endochironomus sp.	Chironomidae[1]	0	0	1	1	1
Microtendipes sp.	Chironomidae[1]	1	0	0	1	1
Polypedilum sp.	Chironomidae[1]	1	0	1	1	1
Procladius sp.	Chironomidae[1]	0	0	0	0	0
Psectrocladius gr. *limbatellus/sordidellus*	Chironomidae[1]	1	0	0	1	1
Psectrocladius gr. *platypus/obvius*	Chironomidae[1]	1	0	0	0	0
psectrotanypus varius	Chironomidae[1]	0	0	0	0	0
Tanypus kraatzi	Chironomidae[1]	1	0	0	1	1
Zavrelia marmorata	Chironomidae[1]	1	0	0	1	1
Agabus bipustulatus	Coleoptera	0	1	0	0	0
Graphoderus cinereus	Coleoptera	0	0	0	0	0
Haliplus flavicollis	Coleoptera	0	1	0	0	0
Hydaticus seminiger	Coleoptera	0	0	0	0	0
Hydaticus transversalis	Coleoptera	0	0	0	0	0
Hydrochara caraboides	Coleoptera	0	1	0	1	1
Hyphydrus ovatus	Coleoptera	0	0	0	0	0
Laccobius minutus	Coleoptera	1	1	0	1	1
laccophilus hyalinus	Coleoptera	1	1	0	1	1
Laccophilus minutus	Coleoptera	1	1	0	1	1
Noterus clavicornis	Coleoptera	0	0	0	0	0
Noterus crassicornis	Coleoptera	0	0	0	0	0
Rhantus exoletus	Coleoptera	0	0	0	0	0
Agabus sp.	Coleoptera lv.[2]	0	0	0	0	0
Cybister sp.	Coleoptera lv.[2]	0	0	0	0	0
Graptodytes sp.	Coleoptera lv.[2]	0	0	0	0	0
Haliplus sp.	Coleoptera lv.[2]	0	1	0	0	0
hydrophilus sp.	Coleoptera lv.[2]	0	0	0	0	0
Hyphydrus sp.	Coleoptera lv.[2]	0	0	0	0	0
Laccophilus sp.	Coleoptera lv.[2]	0	0	0	0	0
Astacidea	Decapoda	0	1	0	1	1
Caenis horaria	Ephemeroptera	1	0	0	1	1
Caenis robusta	Ephemeroptera	1	0	0	1	1
Cloeon dipterum	Ephemeroptera	1	0	0	1	1

Table S1 (continued)

Taxa	Family	Functional feeding group			Food	Detri-
		CG	SH	FI	DET	tivore
Anisus vortex	Gastropoda	0	0	0	1	0
Bithynia leachii	Gastropoda	0	0	1	1	1
Bithynia tentaculata	Gastropoda	0	0	1	1	1
Gyraulus albus	Gastropoda	0	0	0	0	0
Gyraulus crista	Gastropoda	0	0	0	0	0
Lymnaea stagnalis	Gastropoda	0	0	0	1	0
Physa fontinalis	Gastropoda	0	0	0	1	0
Physella acuta	Gastropoda	0	0	0	1	0
Planorbis carinatus	Gastropoda	0	0	0	1	0
Radix labiata/balthica	Gastropoda	0	0	0	1	0
Valvata piscinalis	Gastropoda	0	0	0	1	0
Corixa punctata	Heteroptera	1	0	0	1	1
Corixidae	Heteroptera	1	0	0	1	1
Cymatia coleoptrata	Heteroptera	0	0	0	0	0
Ilyocoris cimicoides	Heteroptera	0	0	0	0	0
Notonecta sp.	Heteroptera	0	0	0	0	0
Plea minutissima	Heteroptera	0	0	0	0	0
Ranatra linearis	Heteroptera	0	0	0	0	0
Sigara distincta	Heteroptera	1	0	0	1	1
Sigara falleni	Heteroptera	1	0	0	1	1
Sigara fossarum	Heteroptera	1	0	0	1	1
Sigara semistriata	Heteroptera	1	0	0	1	1
Sigara striata	Heteroptera	1	0	0	1	1
Erpobdella octoculata	Hirudinea	0	0	0	0	0
Hydracarina	Hydracarina	0	0	0	0	0
Asellus aquaticus	Isopoda	1	1	0	1	1
Proasellus meridianus	Isopoda	1	1	0	1	1
Sialis lutaria	Megaloptera	0	0	0	0	0
Anophelinae	Nematocera	1	0	0	1	1
Ceratopogonidae	Nematocera	0	0	0	0	0
Chaoborus sp.	Nematocera	0	0	0	0	0
Dixella sp.	Nematocera	1	0	1	1	1
Aeschnidae	Odonata	0	0	0	0	0
Coenagrionidae	Odonata	0	0	0	0	0
Oligochaeta	Oligochaeta	1	0	0	1	1
Tricladida	Platyhelminthes	0	0	0	0	0
Agrypnia pagetana	Trichoptera	0	1	0	0	0
Athripsodes aterrimus	Trichoptera	0	1	0	0	0
Holocentropus picicornis	Trichoptera	0	0	0	0	0
Oecetis struckii	Trichoptera	0	1	0	0	0
Triaenodes bicolor	Trichoptera	0	1	0	1	1
Tricholeiochiton fagesi	Trichoptera	0	0	0	0	0


4

CHAPTER 5

EUTROPHICATION INDUCES SHIFTS IN THE TROPHIC POSITION OF INVERTEBRATES IN AQUATIC FOOD WEBS

This chapter is based on the manuscript under review by *Ecology*

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ABSTRACT

Changes in the ecological stoichiometry of primary producers may have important implications for energy and matter transfer in food webs. We hypothesized that nutrient enrichment alters the trophic position of many aquatic invertebrates, as the nutritional quality of primary producers increases. This hypothesis was tested by analysing the ecological stoichiometry and stable isotope signature of a wide range of aquatic taxa including primary producers, primary and secondary consumers along a eutrophication gradient in an agricultural landscape. Our results show (1) that carbon:nutrient ratios of primary producers decreased along the eutrophication gradient, while the elemental composition of consumers remained homeostatic, and (2) that the trophic position of secondary consumers shifted towards herbivory, while the trophic position of primary consumers remained constant. These findings show that eutrophication can alter the structure of aquatic food webs by shifting the diet of aquatic invertebrates from animal to plant material.

INTRODUCTION

Agricultural expansion and intensification are among the most predominant human drivers of global environmental change (Tilman et al., 2001). Excessive application of nitrogen (N) and phosphorus (P) fertilizers in agriculture diminishes nutrient limitations in the surrounding water bodies, altering ecosystem structure and functioning (Conley et al., 2009; Guignard et al., 2017). Enrichment of surface waters through runoff and leaching of nutrients from agricultural lands is therefore considered to be one of the primary water quality issues globally (Smith et al., 1999; Smith & Schindler, 2009).

The increased nutrient supply changes the abundance and species composition of algae and macrophytes in lakes, rivers and streams (Hough et al., 1989; Hilton et al., 2006; Phillips et al., 2016). Besides the commonly perceived increase in algal and macrophyte biomass (Smith et al., 1999; Huisman et al., 2018), nutrient enrichment also alters the ecological stoichiometry of primary producers (Sterner & Elser, 2002; Burson et al., 2016). Since primary producers have a high degree of flexibility in their elemental composition, increased nutrient loadings result in lower carbon (C): nutrient ratios in their tissues (Sterner & Elser, 2002; Garbey et al., 2004; Finlay & Kendall, 2007; Person et al., 2010). This change in elemental composition may improve the nutritional quality of primary producers as food for potential consumers, with important implications for energy and matter transfer to higher trophic levels in food webs (Elser et al., 2001; Woodward, 2009).

In contrast to primary producers, most consumer species tend to maintain a relatively constant (homeostatic) elemental composition of their tissues, even if the nutritional quality of their food changes (Sterner & Elser, 2002; Frost et al., 2003; Evans-White et al., 2005). Increased nutrient loading thus decreases the elemental imbalance between primary producers and consumers, and thereby reduces stoichiometric constraints on the metabolism and growth of consumers, i.e. consumer organisms feeding on more nutritious plants or algae can employ energetically less costly mechanisms to meet their nutrient uptake, assimilation and retention (Sterner & Hessen, 1994; Frost et al., 2003; Sardans et al., 2012 Schoo et al., 2012; Teurlincx et al., 2017). Compared to primary consumers, secondary consumers are less likely to encounter stoichiometric constraints, since their bodies have a comparable elemental composition as their homeostatic food sources, i.e. the primary consumers (Frost et al., 2003).

The lower C: nutrient ratios of primary producers in response to increased nutrient loading may cause changes in trophic transfer. In particular, omnivorous consumers may decrease their relative consumption of animal material when simultaneously offered high quality plant material, as shown in laboratory choice experiments (e.g. Eubanks & Denno, 2000; Janssen et al., 2003; Zhang et al., 2018). Although these laboratory experiments are extremely valuable, field studies are needed to assess if these changes in trophic transfer also occur under natural conditions (Lancaster et al., 2005).

Trophic interactions of consumers can be studied in the field using stable isotope analysis of C and N (Woodward & Hildrew, 2002). Stable carbon isotope signatures ($\delta^{13}C$) can be used to quantify the contribution of specific food sources to the diet of consumers (Finlay & Kendall, 2007; Parnell et al., 2010). Enrichment of the stable nitrogen isotope signature ($\delta^{15}N$) provides insight into the trophic position of organisms (Cabana & Rasmussen, 1996) and sources and transformations of nitrogen (Diebel & Van der Zanden, 2009). Studies on the trophic positions of different taxa suggested that aquatic invertebrates may commonly feed on both plant and animal food (Lancaster et al. 2005) and that the trophic position of these omnivorous invertebrates may vary substantially across sites (Anderson & Cabana, 2007).

Although a few studies assessed how trophic interactions were impacted by increased nutrient loading (Singer & Battin, 2007; Bergfur et al., 2009; Baumgartner & Robinson, 2017), inherent differences in community composition between field sites with low and high nutrient loadings have limited the assessment of potential shifts in trophic position of specific taxa. In particular, a comparison across different locations makes it difficult to disentangle the extent to which shifts in the trophic structure of food webs should be attributed to variation in community composition or to variation in the nutritional quality of primary producers. To assess potential shifts in trophic positions across a wide range of taxa while controlling for variation in community composition, sampling of the same taxa along the eutrophication gradient would be required.

The aim of our study was to assess if nutrient enrichment drives changes in trophic transfer in aquatic communities. We hypothesized that with increased nutrient loading many aquatic invertebrates shift their trophic position from consuming animal material towards increased consumption of primary producers, as the C: nutrient ratios of primary producers decrease. To test this hypothesis, we analysed the ecological stoichiometry (C:N, C:P, N:P) and stable isotope signature ($\delta^{13}C$ and $\delta^{15}N$) of primary producers, primary consumers and secondary consumers along a eutrophication gradient in a 675 m permanent drainage ditch. Due to the ditch' unique position in the landscape, draining a nature reserve into an agricultural area, this ditch enabled sampling of the same set of taxa along a strong gradient in nutrient loading. First, we investigated the common expectation of ecological stoichiometry (Sterner and Elser, 2002) that nutrient enrichment resulted in lower C: nutrient ratios of primary producers, while primary and secondary consumers maintain a constant elemental composition. Then, we used the $\delta^{15}N$ stable isotope signatures to determine whether consumers shifted their trophic positions along the eutrophication gradient.

MATERIALS AND METHODS

Study site

The permanent drainage ditch (1 - 3 m wide, < 1 m deep, 0 - 5 cm/s water flow) is located in a drainage-ditch-rich peatland area in the north of the Netherlands (Figure 1). These drainage ditches were originally dug to drain excess water from the surrounding fields. At the beginning of the studied section (52°44'14.7"N 6°06'49.0"E), the ditch is positioned adjacent to a nature reserve containing oligo- to mesotrophic fen meadows, whilst towards the end of the ditch section (52°44'35.0"N 6°06'33.7"E) land use consists of intensively farmed agricultural fields grazed by cattle (up to 1.5 large sized livestock/ha) and fertilized with 10 - 15 metric ton stable manure per hectare per year. The macrophyte community in the ditch changed along the nutrient enrichment gradient from a species rich wetland-plant community with numerous growth-forms and many open water areas, via dense beds of submerged vegetation and filamentous algae filling the water column, to open water with some emergent vegetation and duckweed.

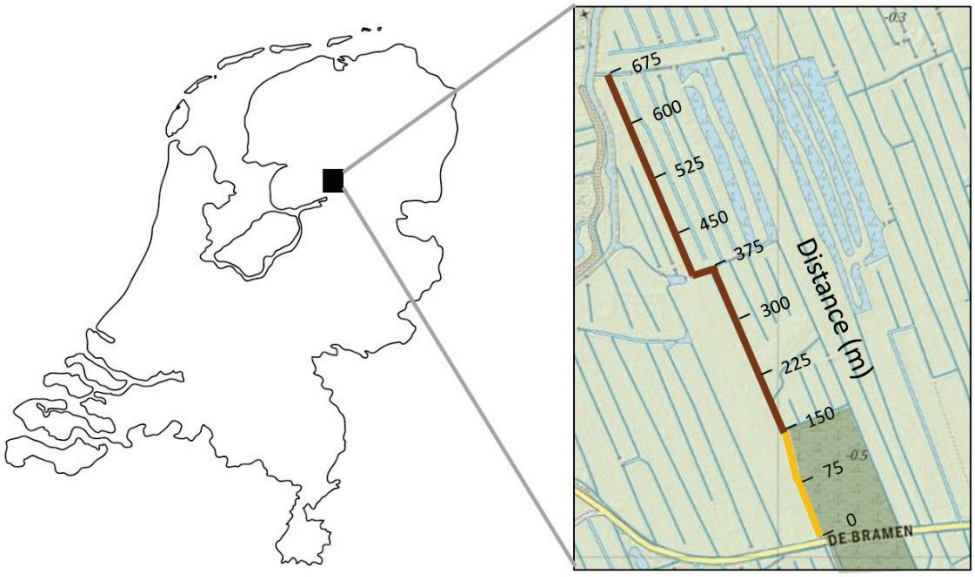

***Figure 1**: Location of the drainage ditch and division of the ditch into nine 75m sub-sections. The dark green area indicates the nature reserve containing oligo- to mesotrophic fen meadows. The yellow line indicates the ditch sections next to the nature reserve and the brown line indicates the ditch sections next to agricultural lands.*

Sampling

The 675 m long ditch was first divided into nine 75-m subsections. In the middle of each of subsection, we collected sediment from the bottom of the ditch, total suspended matter, graminoid vegetation from the banks, epiphyton, four species of macrophytes and eighteen invertebrate taxa in late October of 2017 and 2018. For the sediment, the top 2-cm of the bottom substrate of the ditch was collected in 50 mL tubes (N = 3). For the total suspended matter, 1 L of water was collected in plastic bottles, which were allowed to settle overnight at 4 °C before carefully drawing up 100 mL from the bottom of the bottle using a syringe (N = 3). All other samples were collected in singular from each subsection. Graminoid vegetation was collected from the banks next to the ditch. Epiphyton (i.e. biofilms attached to submerged plants) was scraped from several new shoots of *Phragmites australis* stems using a blade. Macrophyte taxa were selected based on their growth form and occurrence throughout the entire ditch and consisted of summer-floating *Stratiotes aloides*, submerged *Elodea nuttallii* and *Lemna trisulca* and emergent *P. australis*. Aboveground parts of all macrophytes were collected by hand and washed to remove any attached material and invertebrates.

The consumer community of aquatic invertebrates was collected by sweeping a 0.5-mm mesh hand-net through the submerged vegetation and over the top of the sediment of the ditch. The animals were transported to the laboratory on the same day and were kept one to two nights at 4 °C under aerated conditions to allow for gut clearance (Evans-White *et al.*, 2005). Eighteen invertebrate taxa were selected based on their occurrence throughout the entire 675 m ditch section. Primary consumers included molluscs (Bivalvia: Sphaeriidae; Gastropoda: *Bithynia* spp., *Lymnaea* sp., *Planorbarius* sp., *Planorbis* spp., *Valvata* spp.), insects (Trichoptera: Phryganeidae; Ephemeroptera: *Cloeon* spp.; Diptera: Chironomidae) and crustaceans (Isopoda: Asellidae; Amphipoda: Crangonyctidae/ Gammaridae). Secondary consumers included insects (Coleoptera: *Noterus* spp.; Odonata: Anisoptera and Zygoptera; Heteroptera: Corixinae, *Ilyocoris* sp. and *Notonecta* spp.) and annelid worms (Hirudinea: *Erpobdella* spp.). For large taxa at least two or three individuals were collected and for small taxa approximately 15-30 individuals (Evans-White et al., 2005; Bergfur et al., 2009). Mollusc shells and caddisfly cases were removed, while whole organisms were used for all other taxa. Samples were stored at -20 °C.

Elemental and stable isotope analysis

Prior to analysis all samples were freeze-dried. The sediment was dry sieved (2 mm mesh) to remove mollusc shell fragments and dead plants. Thereafter, the samples were grounded to fine powder using a ball-mill for 5 min at 400 rpm for the sediment, a herb grinder for the plants, and a mortar and pestle for the invertebrates. For total C, total N, $\delta^{13}C$ and $\delta^{15}N$, 5 - 20 mg freeze-dried material was weighted to the nearest 0.01 mg in tin capsules and

analysed using a Vario Isotope elemental analyser (Elementar Analysesysteme GmbH, Langenselbold, Germany) in conjunction with an Bio Vision isotope ratio mass spectrometer (Elementar UK, Manchester, UK). For total P, 1-20 mg sample was digested using 250 µL HNO_3 (65%) and 125 µL H_2O_2 (30%) in a microwave assisted system (Multiwave 3000, rotor 64MG5, Anton Paar GmbH) operated at 350 W for 20 min with a 10 min ramp and 450 W for 30 min with a 5 min ramp (Cedergreen & Marcussen, 2013), diluted to 4.6 mL and analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer Optima 8300, Waltham, MA, USA). Some low mass samples were not analysed for total P. The precision (mean ± SD) of our standards were $\delta^{13}C$: -30.36 ± 0.03 ‰, $\delta^{15}N$: 0.69 ± 0.1 ‰, C: 72.07 ± 0.32 % and N: 10.9 ± 0.06 % for acetanilide 99% (Sigma-Aldrich, St. Louis, Missouri) and P: 0.11 ± 0.01 % for Granodiorite (Silver Plume, Colorado, GSP-2).

Isotope ratios were expressed as delta (δ) values, in parts per mil (‰), according to the equation

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} is the stable isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) between the heavy and light isotope in the sample and $R_{standard}$ is the stable isotope ratio of the standard reference material (Peedee Belemnite carbonate for $\delta^{13}C$; atmospheric N_2 for $\delta^{15}N$). A higher delta value indicates that the sample is more enriched in the heavy isotope (Fry, 2006). Additionally, we calculated the trophic position (TP) of each consumer following the simplest model (Post, 2002):

$$TP = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base}) / \Delta_n$$

where λ is the trophic position of the organisms at the baseline, $\delta^{15}N_{base}$ and $\delta^{15}N_{consumer}$ are the $\delta^{15}N$ values of the organisms at the baseline and of the consumer, respectively, and Δ_n is the expected enrichment in $\delta^{15}N$ per trophic level. We used the mean $\delta^{15}N$ of the primary producers (λ = 1) to establish the $\delta^{15}N$ baseline at each ditch section and used Δ_n = 2.55 based on the compilation of three meta-analyses by Matthews & Mazumder (2008).

Statistical analysis

To establish the presence of a eutrophication gradient, linear regressions were performed on the ecological stoichiometry (C:N, C:P and N:P ratios) and isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) of the ditch sediment, total suspended matter and graminoid vegetation along the length of the ditch. Thereafter, linear regression analyses were repeated for the primary producers, primary consumers and secondary consumers to assess if they followed a similar trend in ecological stoichiometry and isotope signature. For the consumers, we also tested

for changes in their trophic position along the length of the ditch. As we conducted analyses on six independent variables, Bonferroni correction was applied to correct for multiple hypothesis testing (significance level of 0.05/6 = 0.008). All analysis were performed in R version 3.6.0. (R Core Team, 2019) using the *lm* function in the *stats* package.

RESULTS

C:N and C:P ratios of the sediments of the ditch and C:P ratios of the graminoid vegetation on the banks decreased significantly along the length of the ditch, from the nature reserve into the agricultural area (Figure 2a and b). N:P ratios of the ditch sediment and graminoid vegetation also decreased significantly, indicating a larger P than N enrichment in the agricultural area (Figure 2c). The $\delta^{13}C$ signature of the graminoid vegetation and ditch sediment did not change significantly along the length of the ditch (Figure 2d). In contrast, the $\delta^{15}N$ signature of the ditch sediment increased significantly along the ditch. The $\delta^{15}N$ signature of the graminoid vegetation on the banks of the ditch was lower in the nature reserve (first 150 m) than in the agricultural area, but this trend was not significant (Figure 2e). For suspended matter, no significant changes were detected in elemental stoichiometry (mean ± SD of C:N = 32.6 ± 3.3, C:P = 1298 ± 521, N:P = 39.2 ± 12.8) and stable isotope signatures (mean ± SD of $\delta^{13}C$ = -32.0 ± 1.2, $\delta^{15}N$ = -0.3 ± 3.6) (see Table S1 and Table S2 in Supplementary material 1).

The C: nutrient ratios of all primary producers combined decreased significantly along the ditch (Figure 3a and b; Table 1). The decline in C:N ratio was strongest in the two submerged macrophytes *Elodea nuttallii* and *Lemna trisulca*, while the decline in C:P ratio was most pronounced in the epiphyton (details individual taxa, see Figure S1 and S2; Table S1). The N:P ratio of the primary producers did not change significantly along the ditch, although the variance was large adjacent to the nature reserve due to high N:P ratios of the epiphyton (Figure 3c; Table 1; see also Figure S3). The elemental stoichiometry of the primary and secondary consumers did not change significantly along the ditch and the variance in their stoichiometry was small, indicating that these invertebrates were homeostatic (Figure 3a, b and c; Table 1; details individual taxa, see Figure S1 - S3; Table S1).

The $\delta^{13}C$ signature of the primary producers and consumers was constant along the ditch (Figure 3d). Moreover, the $\delta^{13}C$ signatures of the different taxa within the primary and secondary consumers (mean ± SD: 33.1 ± 2.3 ‰) were very similar to the $\delta^{13}C$ signatures of the primary producer taxa with the exception of *Stratiotes aloides* (see Table S2). Hence, the contribution of specific primary producer taxa to the diet of the consumers could not be quantified based on their $\delta^{13}C$ signature. In contrast, the $\delta^{15}N$ signature of the primary producers, primary consumers and secondary consumers increased significantly along the ditch (Figure 3e).

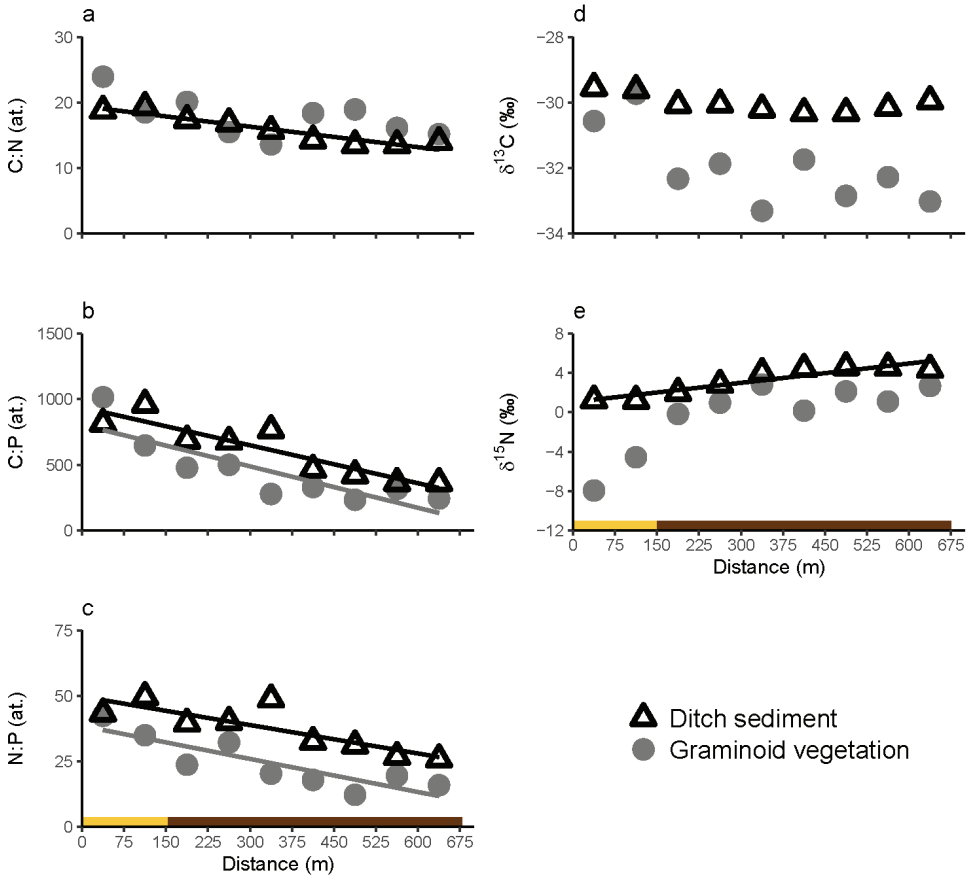

***Figure 2**: Elemental stoichiometry and stable isotope signature of graminoid vegetation and ditch sediment along the length of the ditch. (a) C:N ratio (graminoids: $R^2 = 0.37$, $p = 0.08$; sediment: $R^2 = 0.90$, $p < 0.001$), (b) C:P ratio (graminoids: $R^2 = 0.73$, $p = 0.003$; sediment: $R^2 = 0.83$, $p = 0.001$), (c) N:P ratio (graminoids: $R^2 = 0.75$, $p = 0.003$; sediment: $R^2 = 0.70$, $p = 0.005$), (d) $\delta^{13}C$ (graminoids: $R^2 = 0.51$, $p = 0.03$; sediment: $R^2 = 0.43$, $p = 0.06$) and (e) $\delta^{15}N$ (graminoids: $R^2 = 0.64$, $p = 0.01$; sediment: $R^2 = 0.86$, $p < 0.001$). Lines indicate significant linear regressions (using a Bonferroni corrected significance level of $p < 0.008$; $N = 9$ in all graphs). The colored bar below the graphs indicates whether the ditch sections were located adjacent to the nature reserve (yellow bar) or agricultural lands (brown bar).*

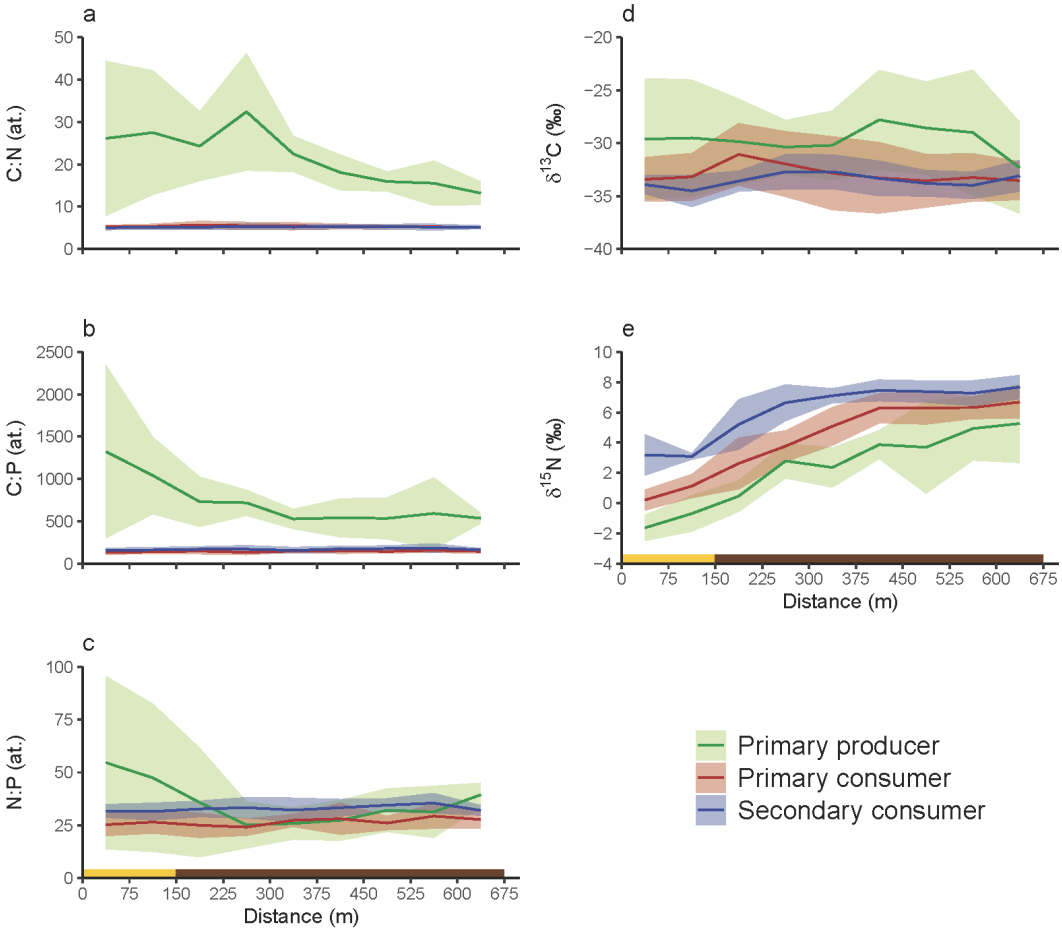

***Figure 3**: Stoichiometry and stable isotope signature of primary producers, primary consumers and secondary consumers along the length of the ditch. (a) C:N ratio, (b) C:P ratio, (c) N:P ratio, (d) $\delta^{13}C$ and (e) $\delta^{15}N$. The mean values (line) ± SD (ribbon) are shown, based on N = 5 taxa of primary producers, N = 11 taxa of primary consumers and N = 7 taxa of secondary consumers. The colored bar below the graphs indicates whether the ditch sections were located adjacent to the nature reserve (yellow bar) or agricultural lands (brown bar). Details of individual taxa are shown in Figure S1 - S5.*

Table 1: Elemental stoichiometry, stable isotope signature and trophic position of primary producers, primary consumers and secondary consumers. Reported are the mean ± SD over the length of the ditch, and the R^2 and *p* values resulting from linear regression along the length of the ditch. Significant regressions are presented in bold (Bonferroni corrected, $p < 0.008$). n/a = not applicable. For details of the individual taxa, see supplementary material Table S1 and S2.

	Primary producers				**Primary consumers**				**Secondary consumers**			
	Mean ± SD	R^2	*p*	N	Mean ± SD	R^2	*p*	N	Mean ± SD	R^2	*p*	N
C:N (at.)	22.1 ± 10.9	**0.20**	**0.002**	43	5.3 ± 0.6	0.01	0.32	91	5.2 ± 0.6	0.01	0.60	55
C:P (at.)	771 ± 503	**0.22**	**0.004**	36	142 ± 27	0.04	0.11	68	168 ± 35	0.04	0.12	45
N:P (at.)	36.2 ± 23.6	0.07	0.11	36	26.4 ± 4.9	0.04	0.12	67	33.1 ± 3.9	0.05	0.13	45
$\delta^{13}C$ (‰)	-29.6 ± 4.3	0.00	0.98	43	-32.9 ± 2.7	0.01	0.31	92	-33.5 ± 1.4	0.01	0.46	55
$\delta^{15}N$ (‰)	2.2 ± 2.8	**0.65**	**<0.001**	43	4.3 ± 2.5	**0.76**	**<0.001**	92	6.1 ± 2.0	**0.61**	**<0.001**	55
Trophic position	1	n/a	n/a	n/a	1.8 ± 0.5	0.00	0.94	92	2.5 ± 0.5	**0.31**	**<0.001**	55

The trophic positions of the consumer taxa were calculated by comparing their $\delta^{15}N$ signature against the $\delta^{15}N$ signature of the primary producers. The trophic position of the primary producers was set at 1, and in theory the trophic position of primary consumers should be 2 and that of secondary consumers should be 3. In line with expectation, the trophic positions of taxa pre-assigned as secondary consumer were higher than those of taxa pre-assigned as primary consumer, except for the gastropod *Valvata* spp. (Figure 4a). The trophic position of the primary consumers was on average 1.8 ± 0.5 (mean ± SD) and did not display a significant trend along the ditch (Figure 4b; Table 1). In contrast, the trophic position of the secondary consumers decreased significantly from 2.9 ± 0.5 adjacent to the nature reserve to only 1.9 ± 0.3 at the end of the ditch in the agricultural fields (Figure 4b; Table 1).

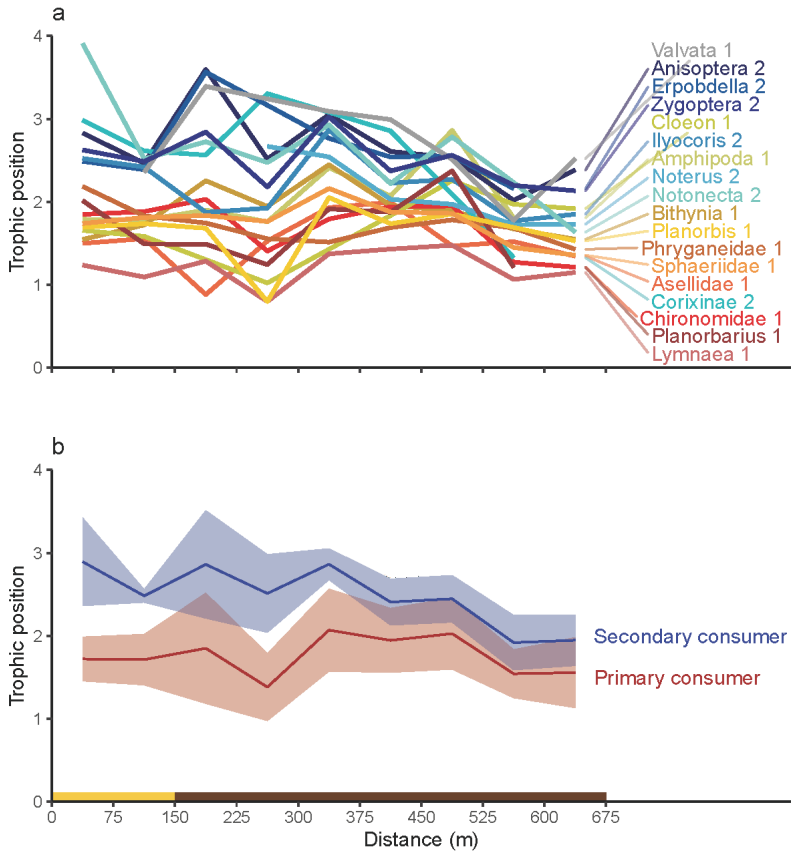

***Figure 4**: Trophic position of primary and secondary consumers along the length of the ditch. (a) Trophic positions of individual taxa pre-assigned as primary consumer (1) or secondary consumer (2). (b) The mean trophic position (line) ± SD (ribbon) of the primary consumers (N = 11 taxa) and secondary consumers (N = 7 taxa). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

DISCUSSION

Our results show a downward shift in the trophic position of secondary consumers along the eutrophication gradient, while the trophic position of the primary consumers remained constant. Furthermore, in line with expectation, the C:N and C:P ratios of the primary producers decreased along the eutrophication gradient, while the elemental composition of the consumers remained homeostatic, in agreement with many other studies in the field of ecological stoichiometry (Sterner & Elser, 2002). This suggests that the secondary consumers may have adjusted their diet from consumption of primary consumers to increased consumption of primary producers. In terrestrial systems, many invertebrates have physiological, morphological and behavioural adaptations, allowing them to forage and process both plant and animal material (see review by Coll & Guershon, 2002). There is increasing evidence that omnivory is also common amongst aquatic invertebrates (France 1997; Lancaster et al., 2005; Figueroa et al., 2019) and our results provide strong field support for the idea that these omnivores may change their trophic position depending on the resources available along productivity gradients (Wootton, 2017).

One of the reasons why omnivores may shift their trophic position from carnivory towards herbivory with increased nutrient loading is that the C: nutrient ratio of primary producers decreases and thus becomes more comparable to the body stoichiometry of the consumers (Figure 3a-c). Likewise, a laboratory food choice experiment showed that the aquatic snail *Lymnaea stagnalis* increased the relative consumption of plant material compared to animal material when plant C: nutrient ratios were lower (Zhang et al., 2018). Similar findings were also made in a field study on two *Macrobrachium* shrimp taxa across tropical streams with different dissolved P concentrations (Snyder et al., 2015). The trophic positions of the shrimps, measured by $\delta^{15}N$, were lower relative to their potential food sources (i.e. leaf litter, periphyton and insects) in streams with high dissolved P concentrations, similar to the secondary consumers in our study. Since the P content of the shrimps remained homeostatic and there were no differences in P excretion rates, Snyder et al. (2015) suggested that the shrimps shifted their diet from resources with high P contents (e.g. insects) in P-limited streams to resources low in P (leaf litter) in streams with high dissolved P concentrations.

Nutrient enrichment can also lead to changes in the abundance of prey, providing another explanation for the shifts in trophic position with different productivity levels (Lancaster et al., 2005).For example, Fox et al. (2009) observed a shift in the diet of decapods from feeding mainly as predators in an oligotrophic estuary to feeding mainly as herbivores in a eutrophic estuary, where invertebrate prey were scarce and macroalgae abundant. The ability of aquatic invertebrate omnivores to change their trophic position in response to variation in the abundance and nutritional quality of their potential resources may have important implications for our understanding of species distributions in aquatic

ecosystems. In particular, it has been argued that by changing their feeding habits in response to resource availability and/or quality, omnivores are able to exploit a wide range of environments (Lancaster et al., 2005; Wootton, 2017). This view is supported by our findings, where all investigated aquatic invertebrates were present across the entire eutrophication gradient.

Our results rely on the use of the $\delta^{15}N$ signature of primary producers and consumers to determine the trophic positions of consumer species. Although this method is commonly used (e.g. Post, 2002; Middelburg, 2014), it faces two major uncertainties. First, the $\delta^{15}N$ of the primary producers at the baseline can vary both spatially and temporally, e.g. due to agricultural activities (Figure 3e; see also Boon & Bunn, 1994; Cabana & Rasmussen, 1996; Peipoch et al., 2012). Motile consumers may integrate $\delta^{15}N$ over larger spatial and temporal scales than the primary producers (Post, 2002). Moreover, the baseline was based on bulk samples of primary producers, whereas some taxa may selectively consume specific plant parts or additional food sources, for example bacteria or detritus (Cross et al., 2005; Peipoch et al., 2012). Therefore, we used multiple species of primary producers and consumers to add robustness to our results, and we sampled all organisms at the end of the growing season to gain a seasonally integrated measure of the stable isotope signature.

Second, our approach assumed a fixed ^{15}N enrichment of 2.55 ‰ per trophic level, however, the ^{15}N trophic fractionation may typically range between 2 - 4 ‰, depending on the studied taxa (Matthews & Mazumder, 2008; Middelburg, 2014) and the C: N ratio of the consumed food (Adams & Sterner, 2000). An important advance of the present study was the use of the same set of taxa along the entire eutrophication gradient, thereby minimizing effects of variation in species composition on trophic fractionation. It seems unlikely that changes in the C:N ratio of the consumed food had a major effect on the ^{15}N fractionation per trophic level, as we observed a shift in the trophic position of the secondary consumers, but not of the primary consumers. Species-specific variation in the baseline and trophic fractionation may have resulted in uncertainty in establishing the precise trophic position of each consumer taxon in our study. Nevertheless, the relative change in trophic position of the different consumer taxa along the eutrophication gradient is evident.

In conclusion, we observed a trophic shift in the diet of secondary consumers along the eutrophication gradient, from consumption of other animals near the nature reserve towards increased consumption of plant material in the agricultural influenced areas. This shift is presumably related to enhanced nutrient availability and the increased nutritional quality of primary producers in more eutrophic parts of the landscape. Hence, increasing eutrophication due to the intensification of agriculture and urban expansion may alter the food web structure of aquatic ecosystems, as aquatic invertebrate omnivores adjust their trophic position to the prevailing nutrient conditions.

ACKNOWLEDGEMENTS

We thank Jochem Baan and Mariska Beekman for assisting the fieldwork, Titus Rombouts for filtering the water samples, Jorien Schoorl, Rutger van Hall and Brechtje Hilbers for stable isotopes analysis and nutrient analysis, and Rosalie Martens from Natuurmonumenten for access to their property.

SUPPLEMENTARY MATERIAL 1

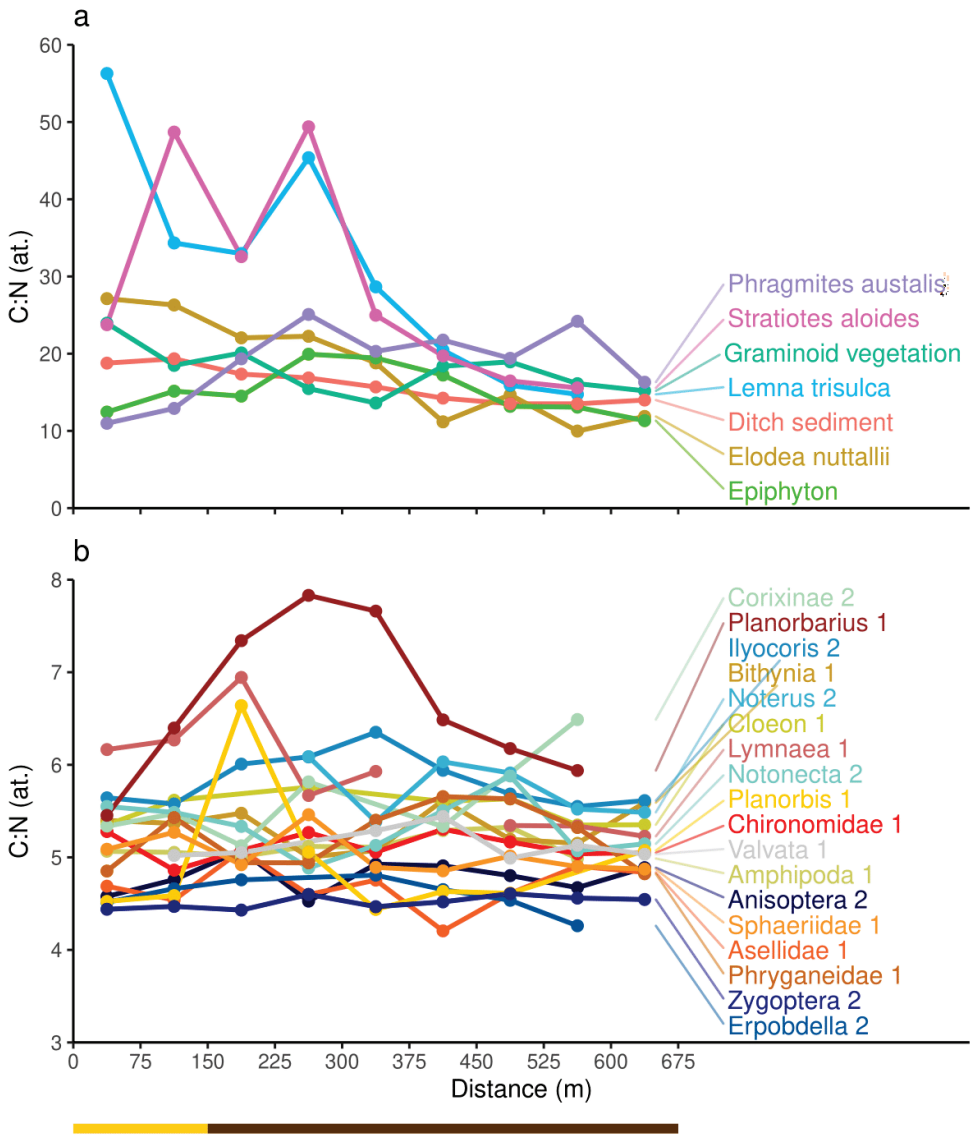

***Figure S1**: C:N ratios of individual taxa along the length of the ditch. (a) Aquatic primary producers, ditch sediment, suspended matter and graminoid vegetation on the banks. (b) Primary consumers (1) and secondary consumers (2). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

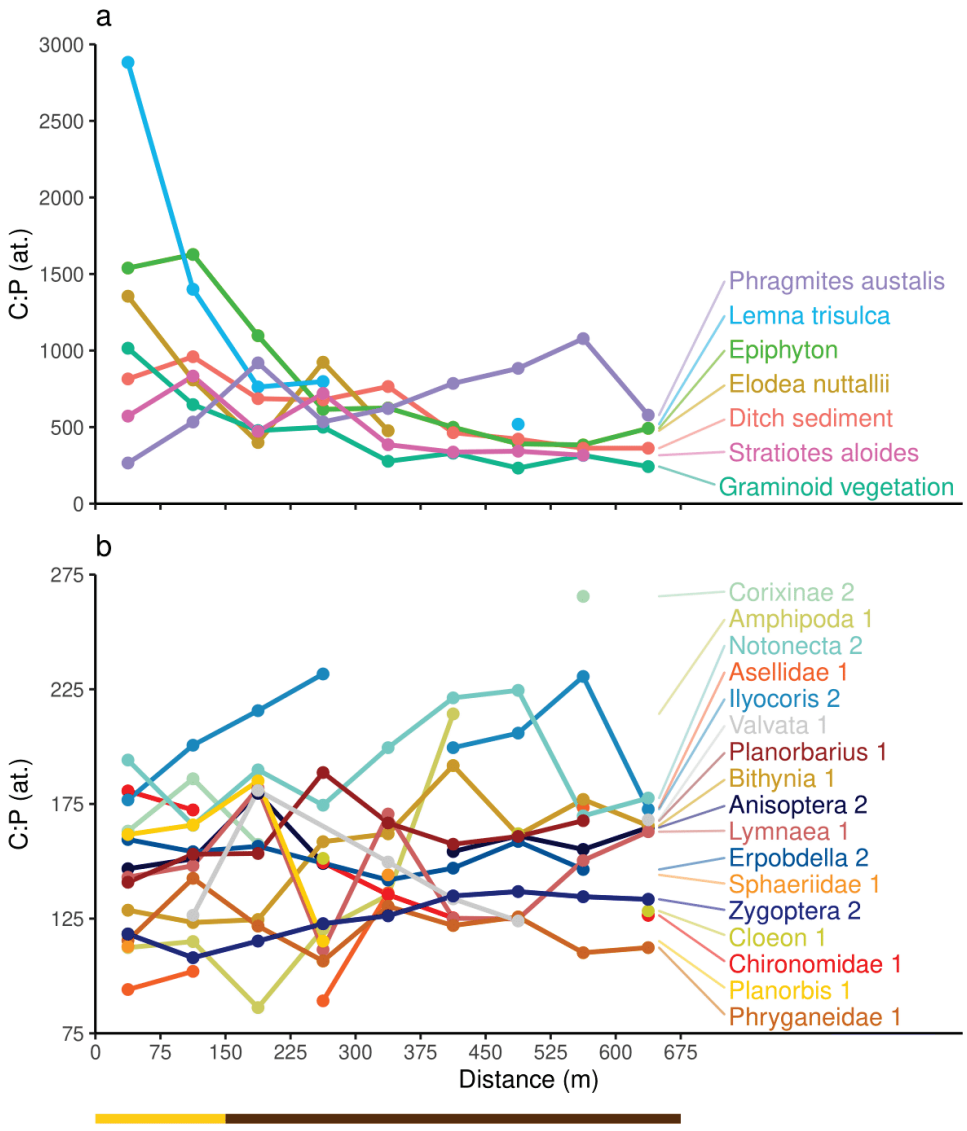

***Figure S2**: C:P ratios of individual taxa along the length of the ditch. (a) Aquatic primary producers, ditch sediment, suspended matter and graminoid vegetation on the banks. (b) Primary consumers (1) and secondary consumers (2). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

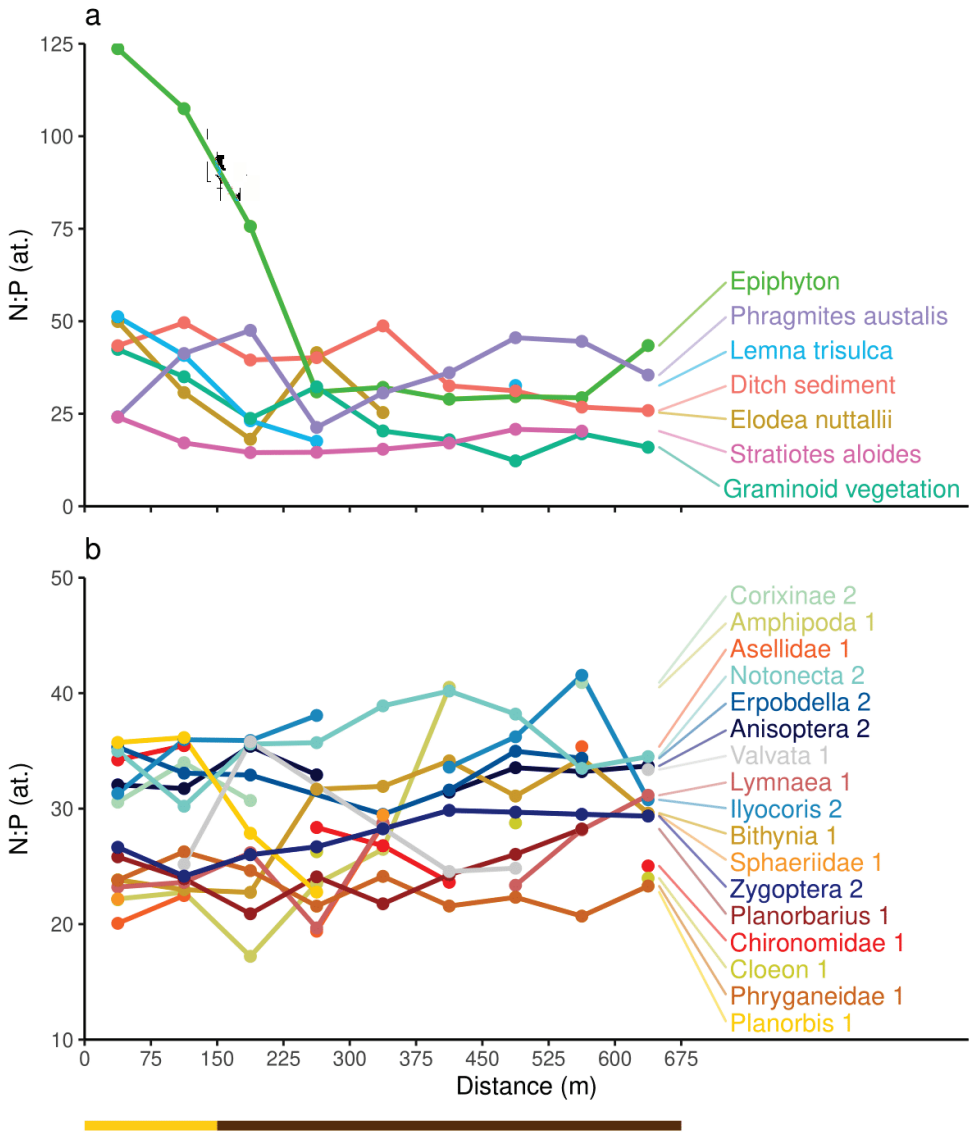

***Figure S3**: N:P ratios of individual taxa along the length of the ditch split. (a) Aquatic primary producers, ditch sediment, suspended matter and graminoid vegetation on the banks. (b) Primary consumers (1) and secondary consumers (2). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

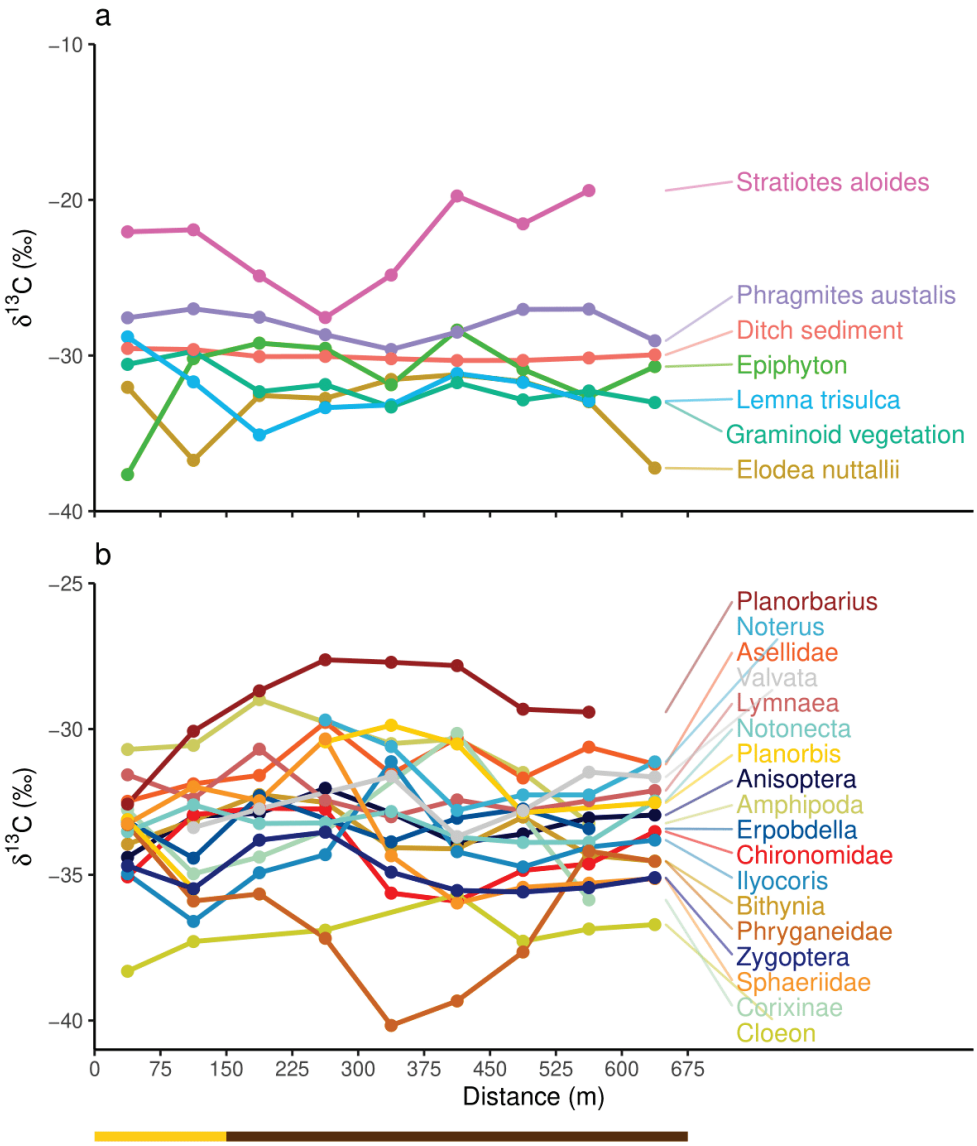

***Figure S4**: Carbon isotope signature ($\delta^{13}C$) of individual taxa along the length of the ditch. (a) Aquatic primary producers, ditch sediment, suspended matter and graminoid vegetation on the banks. (b) Primary consumers (1) and secondary consumers (2). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

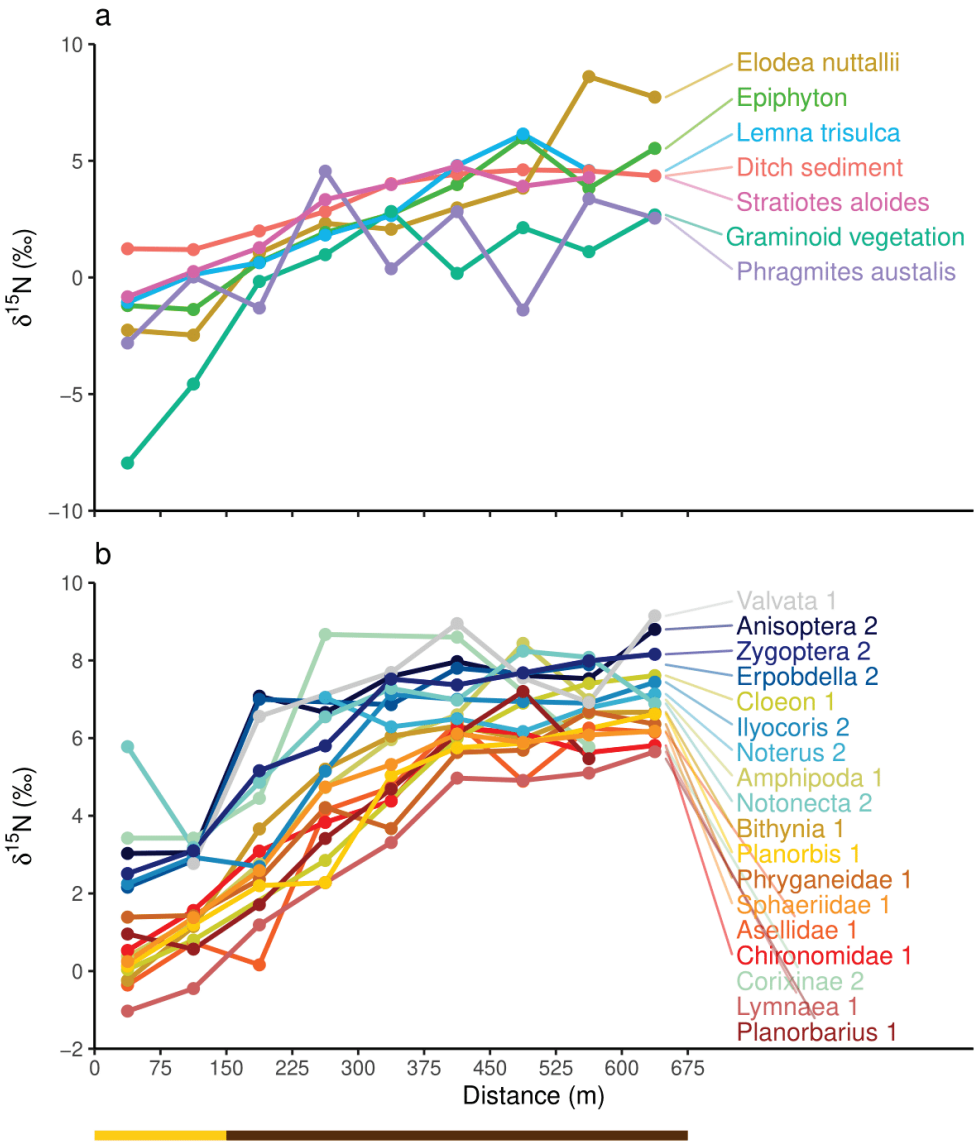

***Figure S5**: Nitrogen isotope signature ($\delta^{15}N$) of individual taxa along the length of the ditch. (a) Aquatic primary producers, ditch sediment, suspended matter and graminoid vegetation on the banks. (b) Primary consumers (1) and secondary consumers (2). The colored bar below the graphs indicates whether the ditch sections were located next to the nature reserve (yellow bar) or agricultural lands (brown bar).*

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Table S1: Elemental stoichiometry of environmental parameters, primary producers, primary consumers and secondary consumers. Reported are the mean and standard deviation (SD) over the length of the ditch, and the R2 and p values resulting from linear regressions along the length of the ditch. Significant regressions are presented in bold (Bonferroni corrected, $p < 0.008$). For individual taxa the linear regression was performed only if $N > 4$. n/a = not applicable.

		C: N (at.)					C: P (at.)					N: P (at.)				
		Mean	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**
Environment	Graminoid vegetation	17.8	3.1	0.37	0.08	9	448	254	**0.73**	**0.003**	9	24.4	10.0	**0.75**	**0.003**	9
	Ditch sediment	15.9	2.3	**0.90**	**<0.001**	9	612	218	**0.83**	**0.001**	9	37.5	8.9	**0.70**	**0.005**	9
	Suspended matter	32.6	3.3	0.04	0.62	9	1298	521	0.02	0.71	9	39.2	12.8	0.08	0.47	9
Primary producers	TOTAL	22.1	10.9	**0.20**	**0.002**	43	771	503	**0.22**	**0.004**	36	36.2	23.6	0.07	0.11	36
	Elodea nuttallii	18.3	6.6	**0.89**	**<0.001**	9	792	384	0.46	0.21	5	33.1	12.7	0.23	0.42	5
	Epiphyton	15.1	3.1	0.06	0.54	9	808	489	**0.78**	**0.002**	9	55.7	37.3	0.63	0.01	9
	Lemna trisulca	31.1	14.5	**0.77**	**0.004**	8	1272	957	0.60	0.12	5	33.1	13.5	0.24	0.40	5
	Phragmites australis	18.9	4.8	0.25	0.17	9	689	249	0.32	0.12	9	36.3	9.4	0.10	0.40	9
	Stratiotes aloides	28.9	13.5	0.37	0.11	8	497	194	0.56	0.03	8	18.0	3.4	0.00	0.99	8
Primary consumers	TOTAL	5.3	0.6	0.01	0.32	91	142	27	0.04	0.11	68	26.4	4.9	0.04	0.12	67
	Amphipoda	5.1	0.1	0.15	0.34	8	131	44	0.54	0.10	6	25.4	8.0	0.54	0.10	6
	Asellidae	4.7	0.2	0.00	0.86	9	119	36	0.78	0.05	5	25.2	6.8	0.77	0.05	5
	Bithynia spp.	5.3	0.2	0.00	0.93	9	155	24	0.62	0.01	9	29.2	4.7	0.55	0.02	9
	Chironomidae	5.1	0.1	0.00	0.90	9	148	24	0.84	0.01	6	28.9	4.9	0.76	0.02	6
	Cloeon spp.	5.5	0.2	0.06	0.61	7	140	20	n/a	n/a	4	25.3	2.9	n/a	n/a	4
	Lymnaea spp.	5.9	0.6	0.64	0.02	8	147	23	0.00	0.94	9	25.5	3.7	0.36	0.12	8
	Phryganeidae	5.2	0.3	0.05	0.56	9	121	11	0.12	0.35	9	23.1	1.8	0.36	0.09	9
	Planorbarius spp.	6.7	0.9	0.00	0.97	8	161	14	0.20	0.26	8	24.4	2.4	0.19	0.28	8
	Planorbis spp.	4.9	0.7	0.01	0.83	8	157	30	n/a	n/a	4	30.6	6.5	n/a	n/a	4
	Sphaeriidae	5.0	0.2	0.27	0.15	9	128	22	n/a	n/a	2	25.8	5.1	n/a	n/a	2
	Valvata spp.	5.1	0.2	0.00	0.93	7	147	23	0.00	0.91	6	28.7	4.8	0.00	0.92	6

Table S1 (continued)

		C: N (at.)					C: P (at.)					N: P (at.)				
		Mean	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**
Secondary consumers	TOTAL	5.2	0.6	0.01	0.60	55	168	35	0.04	0.12	45	33.1	3.9	0.05	0.13	45
	Anisoptera	4.8	0.2	0.04	0.59	9	158	11	0.06	0.57	8	33.0	1.3	0.05	0.60	8
	Corixinae	5.6	0.5	0.35	0.21	6	193	50	n/a	n/a	4	34.0	4.8	n/a	n/a	4
	Erpobdella spp.	4.6	0.2	0.19	0.33	7	152	7	0.25	0.26	7	33.1	2.1	0.01	0.88	7
	Ilyocoris spp.	5.8	0.3	0.03	0.68	9	204	22	0.00	0.91	8	35.4	3.5	0.02	0.73	8
	Noterus spp.	5.7	0.3	0.22	0.35	6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0
	Notonecta spp.	5.3	0.3	0.03	0.66	9	191	21	0.02	0.73	9	35.8	3.0	0.07	0.49	9
	Zygoptera	4.5	0.1	0.44	0.05	9	126	10	**0.76**	**0.002**	9	27.8	2.0	**0.72**	**0.004**	9

Table S2: Stable isotope signature of environmental parameters, primary producers, primary consumers and secondary consumers. Reported are the mean and standard deviation (SD) over the length of the ditch, and the R2 and p values resulting from linear regressions along the length of the ditch. Significant regressions are presented in bold (Bonferroni corrected, $p < 0.008$). For individual taxa the linear regression was performed only if $N > 4$. n/a = not applicable.

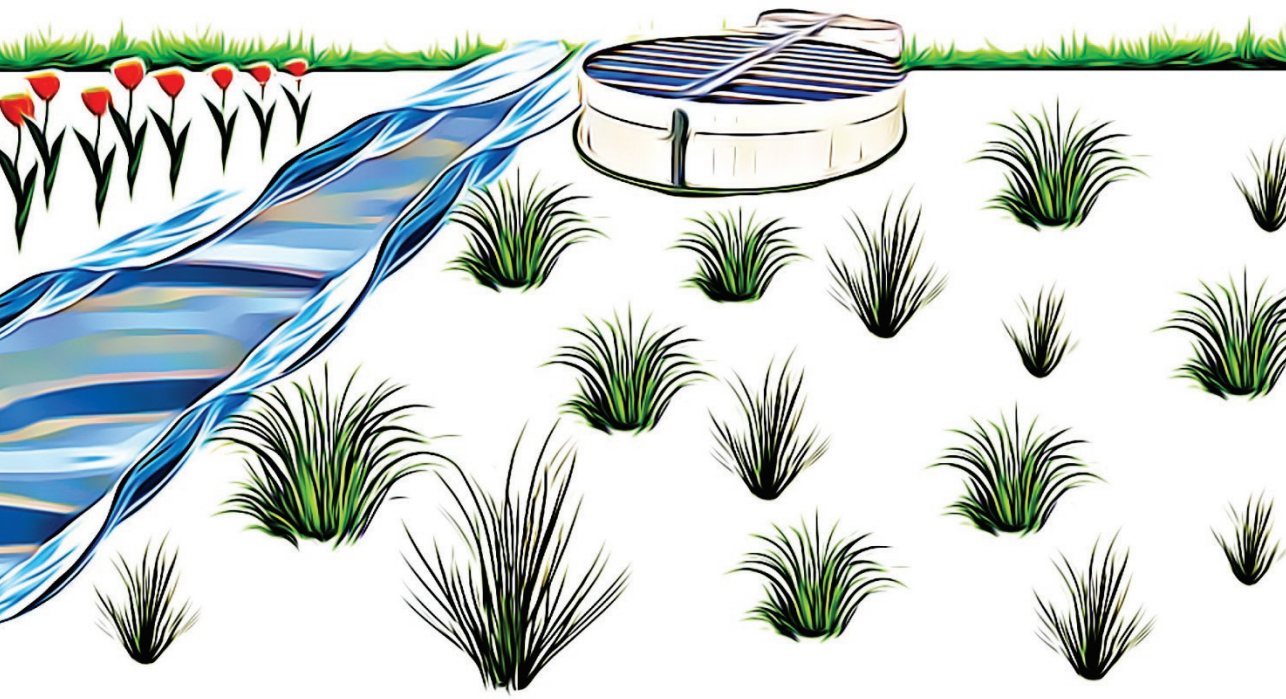
		$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)					Trophic position				
		Mean	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**
Environment	Graminoid vegetation	-32.0	1.2	0.51	0.03	9	-0.3	3.6	0.64	0.01	9	n/a	n/a	n/a	n/a	n/a
	Ditch sediment	-30.0	0.3	0.43	0.06	9	3.2	1.5	**0.86**	**<0.001**	9	n/a	n/a	n/a	n/a	n/a
	Suspended matter	-13.7	1.6	0.62	0.01	9	3.0	0.9	0.62	0.01	9	n/a	n/a	n/a	n/a	n/a
Primary producers	TOTAL	-29.6	4.3	0.00	0.98	43	2.2	2.8	**0.65**	**<0.001**	43	n/a	n/a	n/a	n/a	n/a
	Elodea nuttallii	-33.2	2.2	0.02	0.75	9	2.7	3.8	**0.90**	**<0.001**	9	n/a	n/a	n/a	n/a	n/a
	Epiphyton	-31.2	2.7	0.09	0.43	9	2.4	2.7	**0.88**	**<0.001**	9	n/a	n/a	n/a	n/a	n/a
	Lemna trisulca	-32.2	1.9	0.07	0.52	8	2.5	2.5	**0.91**	**<0.001**	8	n/a	n/a	n/a	n/a	n/a
	Phragmites australis	-28.0	1.0	0.05	0.56	9	0.9	2.5	0.29	0.14	9	n/a	n/a	n/a	n/a	n/a
	Stratiotes aloides	-22.7	2.8	0.16	0.32	8	2.6	2.1	**0.82**	**0.002**	8	n/a	n/a	n/a	n/a	n/a
Primary consumers	TOTAL	-32.9	2.7	0.01	0.31	92	4.3	2.5	**0.76**	**<0.001**	92	1.8	0.5	0.00	0.94	92
	Amphipoda	-30.7	1.3	0.40	0.09	8	4.6	2.9	**0.91**	**<0.001**	8	2.0	0.4	0.24	0.22	8
	Asellidae	-31.2	0.8	0.19	0.24	9	3.7	2.7	**0.82**	**0.001**	9	1.5	0.3	0.02	0.74	9
	Bithynia spp.	-33.5	0.8	0.25	0.17	9	4.6	2.5	**0.80**	**0.001**	9	1.9	0.3	0.02	0.75	9
	Chironomidae	-34.2	1.3	0.05	0.55	9	4.1	2.1	**0.85**	**<0.001**	9	1.7	0.3	0.33	0.10	9
	Cloeon spp.	-37.0	0.8	0.34	0.17	7	4.5	3.2	**0.97**	**<0.001**	7	1.7	0.4	0.32	0.18	7
	Lymnaea spp.	-32.2	0.7	0.18	0.25	9	2.9	2.5	**0.94**	**<0.001**	9	1.2	0.2	0.02	0.75	9
	Phryganeidae	-36.4	2.3	0.01	0.76	9	4.2	2.1	**0.93**	**<0.001**	9	1.7	0.2	0.45	0.05	9
	Planorbarius spp.	-29.2	1.6	0.25	0.21	8	3.8	2.5	**0.86**	**0.001**	8	1.7	0.4	0.00	0.92	8
	Planorbis spp.	-31.2	3.1	0.00	0.97	8	3.6	2.5	**0.92**	**<0.001**	8	1.6	0.4	0.00	0.91	8
	Sphaeriidae	-33.8	1.9	0.48	0.04	9	4.3	2.3	**0.84**	**0.001**	9	1.8	0.2	0.22	0.20	9
	Valvata spp.	-32.5	0.9	0.31	0.20	7	7.1	2.1	0.56	0.05	7	2.7	0.5	0.20	0.31	7

Table S2 (continued)

		$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)					Trophic position				
		Mean	**SD**	**R^2**	***P***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**	**Mean**	**SD**	**R^2**	***p***	**N**
Secondary consumers	TOTAL	-33.5	1.4	0.01	0.46	55	6.1	2.0	**0.61**	**<0.001**	55	2.5	0.5	**0.31**	**<0.001**	55
	Anisoptera	-33.2	0.7	0.02	0.69	9	6.6	2.1	**0.72**	**0.004**	9	2.7	0.5	0.27	0.15	9
	Corixinae	-33.6	2.0	0.00	0.94	6	5.7	2.4	0.35	0.21	6	2.6	0.7	0.41	0.17	6
	Erpobdella spp.	-33.3	0.7	0.03	0.72	7	6.0	2.4	0.76	0.01	7	2.6	0.4	0.10	0.50	7
	Ilyocoris spp.	-34.3	1.4	0.16	0.28	9	5.4	2.2	**0.82**	**0.001**	9	2.2	0.4	0.19	0.24	9
	Noterus spp.	-31.5	1.2	0.27	0.29	6	6.7	0.4	0.04	0.69	6	2.1	0.4	**0.91**	**0.003**	6
	Notonecta spp.	-33.3	0.5	0.01	0.76	9	6.4	1.6	0.56	0.02	9	2.6	0.6	0.55	0.02	9
	Zygoptera	-34.9	0.8	0.18	0.26	9	6.1	2.1	**0.87**	**<0.001**	9	2.5	0.3	0.23	0.20	9

CHAPTER 6

STRUCTURAL AND FUNCTIONAL ASSESSMENT OF MULTI-STRESSED LOWLAND WATERS



This chapter is based on the manuscript accepted by *Freshwater Science*

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ABSTRACT

Water bodies in densely populated lowland areas are commonly impacted by pollution from agricultural activities and wastewater treatment plant (WWTP) discharges. Yet, the selection of appropriate water quality assessment methods for these water bodies is under debate. Therefore, we aimed to compare the use of structural and functional metrics in their ability to 1) detect adverse effects from anthropogenic stress on aquatic ecosystems, and 2) diagnose the potential causes of the observed adverse effects. For this purpose, we compared the responses of several structural (both taxonomic and trait-based) and functional (i.e. ecological process) metrics to a series of stressors in 20 lowland water bodies impacted to a varying degree by agricultural activities and WWTP discharges. The measured stressors included nutrients, dissolved oxygen saturation, water temperature and proxies for pesticides, pharmaceuticals and personal care products. The results showed a significant negative relation between the combined proxy for organic toxicants and the taxonomic-based evenness metric, as well as the trait-based $SPEAR_{organic}$ and $SPEAR_{pesticides}$ metrics. The microbial decomposition and invertebrate consumption showed an opposite and more complex relation to the stressors. These findings suggest that process-based metrics may be able to detect patterns of anthropogenic stress that were not evident from the structural metrics alone, and thereby provide complementary information to aid water quality assessment. In terms of diagnostic value, the results indicated that both SPEAR metrics may be used to diagnose the combined presence of organic toxicants from agricultural activities and WWTPs discharges. It is concluded that taxonomic, trait-based and functional metrics provide complementary information that, when integrated, allow for more thorough water quality assessment strategies.

INTRODUCTION

Freshwater ecosystems in densely populated lowland areas are generally impacted by a combination of hydro-morphological degradation and pollution, resulting in poor water quality (Paul & Meyer, 2001; Riis & Sand-Jensen, 2001; Needelman et al., 2007; Schinegger et al., 2012). The mixtures of pollutants entering these water bodies can largely be attributed to agricultural and urban land use (Allan, 2004; Burdon et al., 2019). Pesticides and excess nutrients may enter the water via run-off and spray drift from agricultural activities (Schulz, 2004; De Zwart, 2005; Bracewell et al., 2019). Urbanization can lead to an increase in almost all types of pollutants originating from non-point source run-off and municipal wastewater treatment plant (WWTP) discharges (Paul & Meyer, 2001). Depending on the particular processes used to treat influent wastewater, WWTPs may represent a major source of input of nutrients, pharmaceuticals and personal care products (PPCPs), pesticides and a suite of generally unknown contaminants (Carey & Migliaccio, 2009; Rosi-Marshall & Royer, 2012; Petrie et al., 2015; Munz et al., 2017). The input of excessive nutrients by agricultural land use and WWTPs can result in eutrophication, thereby indirectly altering dissolved oxygen regimes (Paul & Meyer, 2001; Van der Lee et al., 2018). Moreover, water temperatures tend to be higher due to increased solar radiation resulting from riparian vegetation removal and the warming influence of wastewater inputs into receiving water bodies (Allan, 2004; Kinouchi et al., 2007). Although several studies have demonstrated the negative effects of agricultural activities and WWTP discharges on the water quality, the selection of the appropriate assessment methods to aid effective management in multi-stressed water bodies is still under debate (Pascoal et al., 2003; Bonada et al., 2006; Hagen et al., 2006; Schäfer et al., 2007; Friberg et al., 2011; Peters et al., 2013).

Traditionally, most biomonitoring schemes have relied on structural metrics based on taxonomic inventories of various organism groups, with invertebrates being the most widely used (Boulton, 1999; Bonada et al., 2006; Resh, 2008). Species diversity, i.e. species richness, evenness and composition, is commonly used in water quality assessment, as the detrimental effects of human disturbances on species diversity are generally well established (Metcalfe, 1989; Norris & Thoms, 1999; Cao & Hawkins, 2019). However, most of the metrics calculated directly from taxonomic data respond to general degradation, and thus have limited value in identifying the impact of specific stressors on the ecological status of freshwater ecosystems (Clews & Ormerod, 2009; Friberg et al., 2011; Gieswein et al., 2017; Lemm et al. 2019). Metrics based on species traits may provide the specificity that is lacking in taxonomic metrics (Statzner & Bêche, 2010). A promising trait-based method is the SPEcies At Risk (SPEAR) approach, designed to distinguish between the impacts of different types of stressors on freshwater invertebrates (Liess & Von der Ohe, 2005). The $SPEAR_{organic}$ is based on taxon-specific sensitivities to organic contaminants (i.e. carbon-

based synthetic chemicals) gleaned from databases of ecotoxicity studies providing lethal effect concentrations (LC_{50}) (Liess & Von der Ohe, 2005; Beketov & Liess, 2008). The $SPEAR_{pesticides}$ combines the $SPEAR_{organic}$ with life history traits that differentiate a taxon's ability to recover from pulses of contamination typical of pesticide exposure, such as generation time, migration ability, and timing and duration of life stages (Liess & Von der Ohe, 2005; Knillmann et al., 2018). Several studies reported significant correlations between the $SPEAR_{pesticides}$ and the presence of elevated pesticide concentrations in streams (Liess & Von der Ohe, 2005; Schäfer et al., 2007; Beketov et al., 2009; Schäfer et al., 2012), and between $SPEAR_{organic}$ and concentrations of petrochemicals and surfactants (Beketov & Liess, 2008; Kuzmanović et al., 2016). An increased understanding of the diagnostic value of this approach could thus aid water quality assessment in multi-stressed lowland water bodies.

In addition to improving diagnostic value of water quality assessment approaches, there is also a recognized need to adopt indicators of ecosystem function, as anthropogenic stress may impact ecosystem structure and function differently (Bunn, 1995; Gessner & Chauvet, 2002; Young et al., 2008). Specifically, species composition may change without affecting ecological processes, i.e. the loss of a species is compensated for by a species with a functionally similar role within the ecosystem (Jackson et al., 2016). Alternatively, ecological processes may change in absence of a change in species composition, e.g. an increase in growth rate may enhance productivity, but does not change species composition (Bunn, 1995; Sandin & Solimini, 2009). To include functional indicators in assessment approaches, several direct measures of ecosystem processes have been suggested (e.g. metabolism and nutrient cycling), and particularly organic matter breakdown has been advocated as a sensitive parameter to assess the impact of human-induced stress (Gessner & Chauvet, 2002; Young et al., 2008). For example, organic matter breakdown may be enhanced by nutrient enrichment (Ferreira et al., 2015) and higher water temperatures (Ferreira & Chauvet, 2006), but could be inhibited by toxic contaminant concentrations (Schäfer et al., 2007; Young et al., 2008). Despite the importance of including both structural and functional metrics in water quality assessments, few studies have actually done so and usually only a single stressor is considered (but see Schäfer et al., 2011; Matthaei et al., 2010, Piggott et al. 2012).

Because the goal of many water-quality assessments is to detect whether multiple anthropogenic stressors have changed ecosystem structure and functioning, there is a legitimate question as to the sufficiency of the currently used metrics. Hence, our objective was to determine whether functional metrics provide added value in the detection of anthropogenic stress. We evaluated this by comparing responses of several structural (both taxonomic and trait-based) and functional (i.e. based on ecological process) metrics to a series of stressors. We hypothesized that functional metrics can reveal patterns that would

not be evident from structural metrics alone. As the goal of water-quality assessments is also to diagnose the potential causes of the observed adverse effects, we aimed to determine whether the trait-based SPEAR-approach offers value in distinguishing toxic pollution from agricultural activities and WWTPs discharges from other stressors in multi-stressed lowland water bodies.

MATERIALS AND METHODS

Study sites

The present field study was performed in 20 permanent lowland water bodies throughout the Netherlands. The selected water bodies were small (mean ± SD = 4.5 ± 1.8 m) and shallow (0.8 ± 0.2 m) with a minimal flow (5.3 ± 6.8 cm/s) and often with a channelized geomorphology (i.e. including drainage ditches). To comprise a wide range of stress from nutrients, low dissolved oxygen concentrations, water temperature, pesticides and PPCPs, sites were selected which were impacted to varying degrees by agricultural activities and WWTP effluent (Table 1; details in Supplementary material 1). Four sites were chosen that were primarily surrounded by nature in the riparian zone. In addition, 12 sites were selected that were surrounded to varying degree by agricultural land use in the riparian zone of which five sites were surrounded by intensive horticulture. Four other sites were chosen that directly received WWTP effluent. Since water bodies in the Netherlands are generally strongly connected, we used a measure of the anthropogenic sources of gadolinium (used in medical procedures) as an index of the degree to which each study site was influenced by effluent input from WWTPs (details in Supplementary material 1). A natural gadolinium anomaly (Gd*) without any WWTP impact is around 1.3 (Rabiet et al., 2005; Petelet-Giraud et al., 2009). Hence, the Gd* values reported in Table 1 indicate that WWTP effluent also reached several sites that did not directly receive WWTP discharge. Sampling was conducted from the 20th of August (week 34) until the 23rd of October (week 43) 2018 (Table 2).

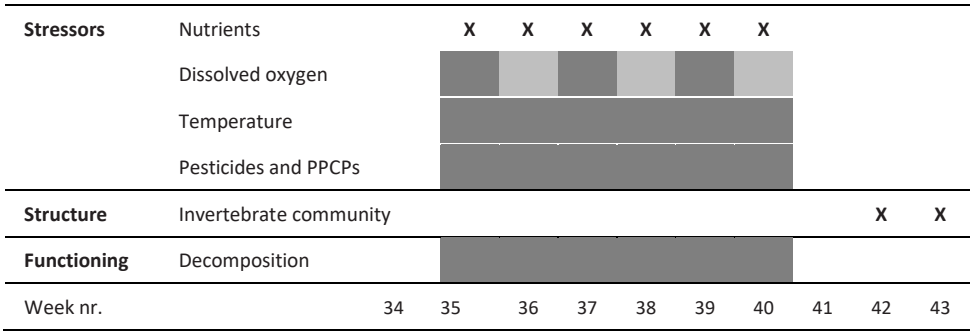
Stressors

Nutrients – One surface water grab sample was collected weekly at each site for six weeks, filtered over a 1.2 µm filter and analyzed for total dissolved nitrogen (TDN) and orthophosphate (PO_4-P) on a continuous flow analyzer (SAN++ system, Skalar Analytical B.V., Breda, The Netherlands). The mean nutrient concentrations over the six weeks were calculated for further analysis.

Table 1: Overview of study sites: coordinates, water body dimensions, land use of the riparian zone (150m on both sides along 600m), and a proxy for the degree of WWTP effluent influence indicated by the gadolinium anomaly. Natural Gd* values in water bodies without any WWTP impact are around 1.3 (Rabiet et al. 2005, Petelet-Giraud et al. 2009). Details of methods in supplementary material 1.

Coordinates (WGS 84)		Water body dimensions			Land use riparian zone			Effluent influence
Latitude	Longitude	Depth (m)	Width (m)	Flow velocity (cm/s)	Agri-culture (%)	Nature (%)	Urban (%)	Gadolinium anomaly (Gd*)
52°49'22.7"N	5°54'26.5"E	1.0	6.0	0.9	2	98	0	4.3
53°00'22.3"N	5°48'43.4"E	0.7	2.5	2.0	4	96	0	5.3
52°53'29.0"N	4°49'34.8"E	0.6	4.5	2.2	93	0	7	6.8
52°45'51.4"N	4°40'52.0"E	1.2	6.0	11.6	88	0	12	4.5
52°40'33.0"N	4°50'02.3"E	0.8	6.0	2.6	0	0	100	4.2
52°17'07.2"N	4°32'34.6"E	0.8	8.0	2.3	100	0	0	13.8
52°17'23.2"N	4°30'37.7"E	0.6	4.0	1.3	89	0	11	4.4
52°17'05.3"N	4°29'54.7"E	1.2	5.5	1.0	99	0	1	5.6
52°08'08.2"N	4°48'37.6"E	1.0	2.8	1.4	26	73	1	1.5
52°12'43.4"N	4°53'10.6"E	0.8	1.5	1.5	16	0	84	26.7
52°15'20.5"N	5°05'15.2"E	1.0	5.0	1.3	78	0	22	1.2
51°25'40.9"N	4°46'46.8"E	1.0	3.0	1.3	92	0	8	1.6
51°30'46.1"N	4°50'57.2"E	0.4	1.5	7.3	71	0	29	30.3
51°33'54.2"N	4°59'13.1"E	0.6	4.5	3.4	78	21	1	4.8
51°36'08.3"N	5°04'32.9"E	0.4	5.0	25.5	43	17	41	50.3
51°30'15.0"N	5°10'19.9"E	0.4	4.0	14.7	64	0	36	25.6
51°24'27.2"N	5°41'37.0"E	0.8	4.0	4.7	79	15	6	1.1
51°17'52.0"N	5°36'18.8"E	0.8	5.5	1.4	84	16	0	1.1
51°13'50.3"N	5°37'31.4"E	0.6	3.0	2.2	11	82	7	2.5
51°18'09.7"N	5°29'09.6"E	0.6	8.0	17.7	81	18	1	9.2

Table 2: Sampling scheme per week in 2018. A cross indicates a grab sample and a filled bar indicates a time-integrated sample. The dissolved oxygen measurements and invertebrates sampling was split between different weeks for the different sites, indicated by dark and light grey bars and crosses.



Dissolved oxygen and temperature – Dissolved oxygen (DO) concentrations (mg/L) were measured with optical HOBO® Dissolved Oxygen loggers U26-001, protected by the antifouling protective guard U26-GUARD-2 (Onset Computer Corporation, Bourne, MA, USA). Water temperature (°C) was measured with HOBO® Temperature/Light loggers UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). Both Dissolved Oxygen and Temperature/Light loggers were placed mid-channel, 15 cm under the water surface (Van der Lee et al., 2018). Measurements were taken every ten minutes. Temperature/Light loggers were placed at each site for six weeks continuously, while the Dissolved Oxygen loggers were placed three times during the six week period for six consecutive days, rotating weekly between the sites (Table 2). Percent DO saturation was calculated from the DO concentrations and temperature, assuming 0 ‰ salinity and 1 atm barometric pressure, using DOTABLES developed by the U.S. Geological Survey (2011). Thereafter, the percent time that the dissolved oxygen levels were below 10 % saturation (DO < 10) was calculated, as many invertebrate taxa do not tolerate these low oxygen levels (Connolly et al., 2004).

Pesticides and PPCPs – Polar organic chemical integrative samplers (POCIS) containing 200 mg of Oasis hydrophilic-lipophilic balance sorbent (Waters, MA, USA) were applied for the sampling of polar compounds from the surface water (Alvarez et al., 2004; details in Supplementary material 2). At each site, four POCIS retained in stainless steel cages were deployed for six weeks in the middle of the water column. After field exposure, the POCIS were cleaned in the field with local water and a scrubbing sponge to remove any biofouling that had accumulated on the polyether sulfone membranes and stored at -20 °C. Frozen POCIS were freeze-dried overnight at −53 °C in a Scanvac CoolSafe freeze-dryer. Dry sorbent of the four POCIS per site was pooled and eluted three times with 3 mL LC grade acetonitrile under vacuum in a glass solid phase extraction column. Finally, the extracts were topped up to 10 mL with acetonitrile by weight and stored at -20°C until analysis.

POCIS acetonitrile extracts were subjected to three *in vitro* chemical activated luciferase gene expression (CALUX®) bioassays at the BioDetection Systems laboratories (Amsterdam, The Netherlands). Extracts were converted to dimethylsulphoxide before exposure in the bioassays. Estrogen receptor (ERα), androgen receptor antagonism (anti-AR) and progesterone receptor antagonism (anti-PR) CALUX assays were performed according to previously described protocols (Hamers et al., 2006; Sonneveld et al., 2004; Van der Linden et al., 2008). The activities of the extracts were expressed as bioanalytical equivalents of the corresponding reference compounds. Subsequently, the bioanalytical activities were divided by the effect-based trigger (EBT) value of each assay to obtain a measure of the ecotoxicological risk caused by the bioactive compounds present at the study sites (Brion et al., 2019; Escher et al. 2018; supplementary material 2 Table S1). ERα risk was considered to be a proxy for the presence of pharmaceuticals and personal care products (PPCPs) (Välitalo et al., 2016) and the mean of anti-AR and anti-PR risks was

considered to be a proxy for the presence of pesticides in the surface waters (Pieterse et al., 2015). In addition, we calculated a combined proxy for presence of organic toxicants using the mean of the proxy for PPCPs and pesticides. Although these proxies do not provide information on the concentrations of individual chemical compounds, they do provide a promising tool to interpret the harmful effects of groups of often (un)known, unregulated and unmonitored compounds present in surface waters (De Baat et al., 2019).

Invertebrate community composition

Three invertebrates samples were collected at each site on one occasion by sweeping a pond net (1mm mesh size, 25 cm width) three times over a length of 0.5 m of submerged vegetation (surface per sample = 0.125 m^2). The samples were stored overnight at 4°C with oxygen supply, washed over 1 mm and 250 µm sieves, sorted alive and preserved in 70% ethanol until identification. Invertebrates were identified to the genus level with a few exceptions, specifically Oligochaeta (order), Hydracarina (order) and Diptera (family). A total of 14968 individuals belonging to 94 invertebrate taxa were collected.

Numerous mathematical functions have been proposed for measuring diversity (Ludwig et al., 1988; Beisel et al., 2003), although few metrics have been specifically developed for small lowland water bodies with limited flow (Verdonschot et al., 2012). Here, we calculated richness in the simplest way as the total number of taxa in the sample (Ludwig et al., 1988). Evenness was estimated using the Smith & Wilson evenness (Evar) index, which describes the species abundance distributions using statistics to avoid dependence on species richness (Smith & Wilson, 1996; Heip et al., 1998). A commonly used water quality metric based on community composition in streams is the number or relative abundance of Ephemeroptera, Plecoptera and Trichoptera taxa, as these orders are considered sensitive to many pollutants in streams (Wallace et al., 1996; Norris & Thoms, 1999). However, these EPT metrics were not considered to be suitable for application in our drainage ditches, as Plecoptera are mostly absent from these types of water bodies and Ephemeroptera are represented by a few abundant insensitive species (Verdonschot et al., 2012). Hence, as alternative to the EPT-index for drainage ditches we used the number of Trichoptera families as taxonomic metric based on community composition, which was as recommended by Verdonschot et al. (2012).

As trait-based approach, we calculated the $SPEAR_{pesticides}$ index using the SPEAR calculator 2019.10 (Version 1.1.1) as implemented in www.systemecology.eu/indicate (Liess & Von der Ohe, 2005, recently revised by Knillmann et al., 2018). The $SPEAR_{organic}$ was calculated using the same calculator, but excluding the life history traits, i.e. manually setting all traits except for the taxon-specific sensitivities to organic contaminants to sensitive. The mean of each metric was calculated over the replicate samples per site for further analysis.

Microbial decomposition and invertebrate consumption

Decomposition was measured using standard substrates, the DEcomposition and COnsumption TABlets (DECOTABs) (Kampfraath et al., 2012). The DECOTABs were prepared by boiling 20 g/L of purified agar dissolved in deionized water for 3 minutes. The mixture was cooled down under continuous stirring to 60 °C at which point 60 g/L of powdered cellulose and 60 µmol/L ascorbic acid were added. The mixture was then poured into polycarbonate moulds (35 mm diameter, 6.7 cm^3 volume) and after cooling the DECOTABs were removed from the moulds and stored at 7 °C. The DECOTABs were deployed in cages (height 2 cm, diameter 10 cm) placed 15 cm under the water surface. To quantify decomposition by microbes the cages were closed off with fine mesh (width 51 µm) on both sides and to quantify the joint microbial decomposition and invertebrate consumption the cages were closed off at the top with coarse mesh (width 4 mm) and at the bottom with fine mesh to collect incompletely decayed DECOTAB fragments mobilized by decomposer activity (Brinson et al., 1981). At each site, five fine and five coarse mesh cages were deployed, each containing two DECOTABs. The DECOTAB cages were lost at one site. For the other sites, the cages were retrieved after 42 days of field exposure. In the laboratory, the DECOTABs were rinsed, dried in a stove (70 °C, 2 days) and weighed. Mass loss was calculated as mean initial DECOTAB weight (subset of 40 DECOTABs not deployed in the field) minus the individual DECOTAB weight after exposure in the field. Microbial decomposition was defined as the mass loss of the DECOTABs in the fine mesh cages. Invertebrate consumption was calculated by subtracting the mean mass loss of the DECOTABs in fine mesh cages from the mass loss of the DECOTABs in the coarse mesh cages. The mean mass loss was then calculated over the replicate DECOTAB cages per site for further analysis.

Statistical analyses

To test for collinearity between the measured stressors (i.e. co-occurrence of stressors at each sampling site) a Pearson correlation analysis was performed. To meet the assumption of normal distribution for this analysis, all stressor variables were $\log_{10}(x+1)$–transformed, except for the DO < 10 % which was logit transformed. Then, the relation between the different structural and functional end-points and stressors was analyzed using single- and two-variable regression analysis. For the single-variable models, we related each end-point and stressor using linear and unimodal (i.e. univariate quadratic function) relations. Unimodal relations were considered, as moderate amounts of stress may increase diversity and organic matter breakdown, while they may be suppressed under influence of high stress levels (Niyogi et al., 2002, Woodward et al., 2010). For the two-variable models, we related each end-point to each pair of stressors. The fits of the single (linear and unimodal) and two-variable models to the data were compared by using the small-sample version of

the Akaike Information Criterion (AICc). When the difference between the fits was relatively small (ΔAICc < 4) the simplest (i.e. linear) model was selected (Burnham & Anderson, 2004). For the two-variable models the variance inflation factor (VIF) was computed to estimate how much of the variance of a regression coefficient is inflated due to multicollinearity in the model, with VIF > 4 indicative of multicollinearity (Miles & Shevlin, 2001). The proxy for organic toxicants was only considered in the single-variable models, as it is a composite measure of the other measured toxic stressors. For the regression analysis the data were not transformed, as the residuals were approximately normally distributed (visual inspection of quantile-quantile plot). As we conducted analyses on seven independent variables, Bonferroni correction was applied to correct for multiple hypothesis testing (significance level of 0.05/7 = 0.007). All analysis were performed in R version 3.6.3 (R Core Team, 2019) using the 'codyn' package to calculate the Evar evenness index, the 'Hmisc' package to compute the Pearson correlation coefficients, the 'stats' package to fit the regression models, the 'AICcmodavg' package to compute the AICc, the 'cars' package to compute the VIFs.

RESULTS

Several stressors were significantly positively correlated to each other (Table 3). The strongest correlation was between the proxy for pesticides and the PO_4-P concentrations ($r = 0.90$, $p = 0.001$). TDN concentrations were significantly correlated to all other stressors, except for the percent of time that the dissolved oxygen levels were below 10 % saturation. Moreover, water temperature was significantly correlated to the PO_4-P concentrations and the proxy for PPCPs.

For all metrics, the models fitted with a single stressor using the linear function performed better than the models using the unimodal function (Table 4) and the models including two stressors (Supplementary material 3 Table S1), except for the relation between microbial decomposition and the proxy for PPCPs, where the model using the unimodal function performed best (ΔAICc = 7.8). The structural metrics based on the taxa richness and the number of Trichoptera families did not relate to any of the stressors (Table 4, Figure 1). The evenness metric related significantly to the combined proxy for organic toxicants, i.e. the mean of the proxy for pesticides and the proxy for PPCPs. The variation in eveness values from the regression line reduced with higher values of organic toxicants. Both the $SPEAR_{pesticides}$ and $SPEAR_{organic}$ were also significantly related to the combined proxy for organic toxicants, although a higher proportion of variability was explained for the $SPEAR_{organic}$ ($R^2 = 0.57$, $p < 0.001$) than the $SPEAR_{pesticides}$ ($R^2 = 0.39$, $p = 0.002$). Moreover, the $SPEAR_{organic}$ also showed a negative relation to the proxy for PPCPs (Table 4, Figure 1).

The microbial decomposition related significantly to several stressors (Table 4, Figure 1), although the strongest fit was with the TDN concentrations using a positive linear

function (R^2 = 0.88, $p < 0.001$), followed by the more complex fit with the proxy for PPCPs using a unimodal function (R^2 = 0.69, $p < 0.001$). The invertebrate consumption showed a significant positive relation to the combined proxy for organic toxicants (R^2 = 0.60, $p < 0.001$) and the proxy for PPCPs (R^2 = 0.44, $p < 0.001$), as well as with TDN concentrations and water temperature when considering $p < 0.05$.

Table 3: Collinearity between stressors measured at 20 drainage ditches and lowland streams. Reported are the Pearson correlation coefficients with significant values in bold ($p < 0.05$). Stressors include total dissolved nitrogen (TDN) concentrations, orthophosphate (PO_4-P) concentrations, the percent time that the dissolved oxygen saturation is below 10 % (DO < 10), mean water temperature (temperature), the proxy for the presence of pesticides (pesticides) and the proxy the presence of pharmaceuticals and personal care products (PPCPs).

	TDN	PO_4-P	DO < 10	Temperature	PPCPs	Pesticides
TDN	1	**0.60**	0.41	**0.58**	**0.60**	**0.54**
PO_4-P	**0.60**	1	0.37	**0.45**	0.13	**0.90**
DO < 10	0.41	0.37	1	0.04	0.13	0.37
Temperature	**0.58**	**0.45**	0.04	1	**0.56**	0.35
PPCPs	**0.60**	0.13	0.13	**0.56**	1	0.09
Pesticides	**0.54**	**0.90**	0.37	0.35	0.09	1

DISCUSSION

The present study compared the use of several structural (both taxonomic and trait-based) and functional metrics in water quality assessment of multi-stressed lowland water bodies. The sites were surrounded to a varying degree by agricultural land use and some sites directly received WWTP effluent. Yet, the gadolinium anomaly showed that several sites that did not directly receive WWTP discharge were also impacted by effluent to some extent through a WWTP higher upstream, sewage overflow, or the inlet of river water which is a common practice during dry summer months to retain a constant water level in The Netherlands (Verdonschot et al., 2012). These anthropogenic activities resulted in the combined presence of multiple stressors, including nutrient enrichment, increased water temperature and elevated proxies of pesticides and PPCPs. Due to the correlation and co-presence of these stressors, their individual contribution to changes in ecosystem structure and functioning could not be unraveled and neither could potential interactive effects between different stressors be determined (Ferreira et al., 2015; Piggott et al., 2015). For example, water temperature can affect the bioavailability of toxicants and thereby alter their subsequent effects on ecosystem structure and function (Peters et al., 2013).

Table 4: Regression statistics for single variable models using linear and univariate quadratic (Unim.) functions between each structural and functional metric and each stressor. When the difference between both fits was relatively small (ΔAICc < 4) the simplest (i.e. linear) model was selected (bold). Adjusted R^2 and *p*-value of selected best fitting models are presented (N = 20, except for microbial decomposition and invertebrate consumption N = 19). Significant regressions are presented in bold (Bonferroni corrected, p < 0.007). Stressors include total dissolved nitrogen (TDN) concentrations, orthophosphate (PO4-P) concentrations, the percent time that the dissolved oxygen saturation is below 10 % (DO < 10), mean water temperature (temperature), the proxy for the presence of pesticides (pesticides) and the proxy the presence of pharmaceuticals and personal care products (PPCPs), and proxy for combined presence of organic toxicants (Toxic).

	Taxonomic														
	Richness (#)					**Evenness (-)**					**Trichoptera fam. (#)**				
	AICc			**Best model**		**AICc**			**Best model**		**AICc**			**Best model**	
	Linear	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***	**Linear**	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***	**Linear**	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***
TDN (mg/l)	**114.5**	114.4	0.1	0.04	0.20	**-22.8**	-20.3	-2.5	0.11	0.08	**47.6**	50.6	-3.0	0.07	0.13
PO_4-P (mg/l)	**113.7**	116.9	-3.2	0.07	0.13	**-20.0**	-17.7	-2.3	-0.02	0.47	**49.8**	50.6	-0.8	-0.04	0.59
DO < 10 (%)	**116.3**	117.8	-1.5	-0.05	0.80	**-19.6**	-16.5	-3.1	-0.05	0.69	**49.6**	52.6	-3.0	-0.03	0.50
Temperature (°C)	**115.7**	114.1	1.6	-0.02	0.43	**-23.5**	-21.2	-2.3	0.14	0.06	**49.4**	51.6	-2.2	-0.02	0.43
PPCPs (%)	**114.5**	115.8	-1.3	0.04	0.21	**-27.4**	-25.2	-2.2	0.29	0.01	**46.7**	48.1	-1.4	0.11	0.08
Pesticides (%)	**113.3**	115.5	-2.3	0.10	0.10	**-20.0**	-16.8	-3.2	-0.03	0.48	**49.8**	52.7	-2.9	-0.04	0.56
Toxic (%)	**116.2**	119.3	-3.1	-0.05	0.69	**-29.8**	-27.0	-2.8	**0.37**	**0.003**	**46.6**	48.3	-2.7	0.16	0.05

	Trait-based										**Ecological process**									
	$SPEAR_{pesticides}$ (-)					**$SPEAR_{organic}$ (-)**					**Microbial decomposition (%)**					**Invertebrate consumption (%)**				
	AICc			**Best model**		**AICc**			**Best model**		**AICc**			**Best model**		**AICc**			**Best model**	
	Linear	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***	**Linear**	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***	**Linear**	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***	**Linear**	**Unim.**	**Δ**	**$R^2_{adj.}$**	***p***
TDN (mg/l)	**24.5**	21.1	3.4	-0.05	0.69	**29.5**	28.7	0.8	-0.02	0.46	**135.3**	137.9	-2.6	**0.88**	**<0.001**	**160.7**	162.2	-1.5	0.17	0.04
PO_4-P (mg/l)	**22.9**	26.1	-3.2	0.03	0.22	**28.9**	31.9	-3.0	0.00	0.31	**174.5**	171.2	3.3	0.03	0.23	**163.0**	165.2	-2.2	0.07	0.15
DO < 10 (%)	**21.4**	20.9	0.5	0.10	0.09	**28.5**	30.2	-1.7	0.03	0.24	**171.4**	173.8	-2.4	0.17	0.04	**163.0**	162.0	1.0	0.06	0.15
Temperature (°C)	**24.6**	26.9	-2.3	-0.05	0.79	**27.6**	29.5	-2.1	0.07	0.14	**168.7**	171.9	-3.2	0.28	0.01	**160.4**	160.6	-0.2	0.18	0.04
PPCPs (%)	**19.1**	22.1	-3.0	0.20	0.03	**17.7**	19.9	-2.2	**0.43**	**0.001**	162.8	**154.9**	7.9	**0.69**	**<0.001**	**153.4**	156.4	-3.0	**0.44**	**<0.001**
Pesticides (%)	**22.1**	24.7	-2.6	0.07	0.13	**29.0**	31.0	-2.0	0.00	0.32	**175.9**	178.4	-2.5	-0.04	0.62	**164.0**	167.1	-3.1	0.01	0.28
Toxic (%)	**13.7**	16.7	-3.0	**0.39**	**0.002**	**12.0**	12.2	-0.2	**0.57**	**<0.001**	**160.7**	159.6	1.2	**0.52**	**<0.001**	**146.9**	149.9	-3.0	**0.60**	**<0.001**

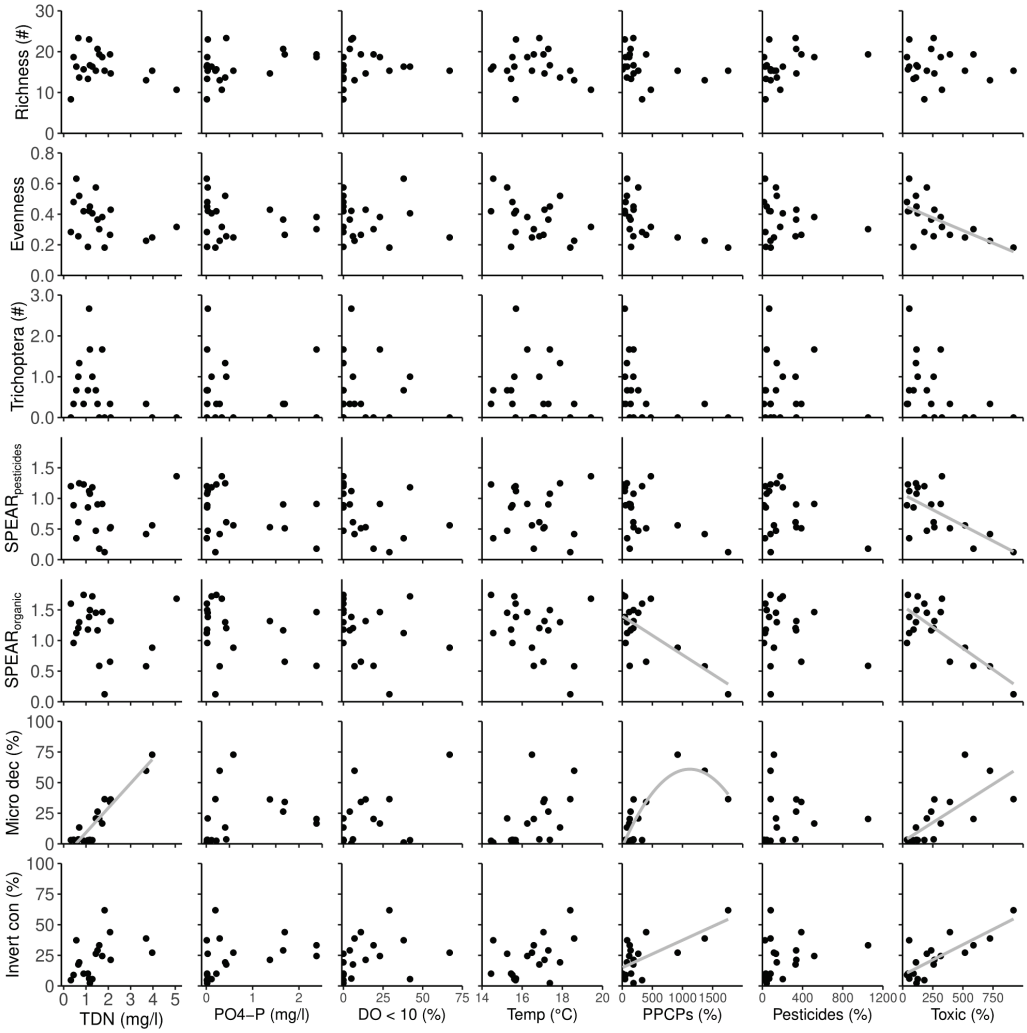


***Figure 1**: Relationships between structural and functional metrics and different stressors. Lines indicate significant regressions using best fitting linear or univariate quadratic function (Bonferroni corrected significance level of p < 0.007; N = 20, except for microbial decomposition and invertebrate consumption N = 19). See Table 4 for the details on regression statistics and abbreviations of labels*

To disentangle individual and combined effects of specific stressors on ecosystem structure and functioning other approaches may be needed, like mesocosm experiments (e.g. Townsend et al., 2008; Pigott et al., 2012). Since the combined presence of multiple stressors is common in water bodies in densely populated areas (Allan, 2004; Ormerod et al., 2010), the design of the present study did allow us to compare the ability of structural and functional metrics to 1) detect adverse effects from anthropogenic stress on aquatic ecosystems, and 2) diagnose the potential causes of the observed adverse effects in water quality assessment under realistic field conditions.

In line with our hypothesis, the process-based metrics detected patterns of anthropogenic stress that were not evident from the structural metrics alone. Specifically, the microbial decomposition and invertebrate consumption showed an opposite and more complex relation to the stressors than the evenness metric, which was the only structural metric that showed a significant response in our study. Similar to previous studies, the microbial decomposition was stimulated by an increase in dissolved inorganic nutrient availability (see review by Ferreira et al., 2015). The DECOTABs used in this study, consisting only of cellulose, may be particularly sensitive to nutrient gradients in the water column, as microbes need to assimilate nutrients from the water column when decomposing low-nutrient substrates (Gulis et al., 2006; Schäfer et al., 2012). The microbial decomposition showed a unimodal relation to the proxy for PPCPs. The negative effects of toxicants on microbial decomposition may be limited by the replacement of sensitive species with tolerant ones, maintaining their function as decomposers (Blanck, 2002). Supporting this line of reasoning, no negative effects on microbial organic matter breakdown rates were observed in the laboratory when mixtures of pharmaceuticals (Hughes et al., 2016) and pesticides (Feckler et al., 2018) were added to microbial communities from disturbed sites. The invertebrate consumption was positively related to the proxy for organic toxicants and to a lesser extent TDN concentrations and water temperature. This finding is in contrast with other studies that have frequently observed impaired invertebrate consumption rates in relation to stress from agricultural activities (e.g. Lecerf et al., 2006; Schäfer et al., 2007; Piscart et al., 2009; Schäfer et al., 2012) and WWTP discharges (e.g. Englert et al., 2013, Münze et al., 2017). Possible explanations for the difference in functional response may relate to the characteristics of the receiving water bodies, variation in the composition of the stressors and the sensitivity of the dominant decomposer invertebrate species (Dangles & Malmqvist, 2004, Hagen et al. 2006, Solagaistua et al., 2018). The impact of anthropogenic stress on ecosystem processes thus appears to be context-dependent, meaning that the relation between taxonomic-based and functional metrics is not always straightforward. Therefore, functional metrics may provide additional information on degradation of the water bodies compared to only using structural metrics.

In terms of diagnostic value, the results of this study indicated that the $SPEAR_{pesticides}$ and $SPEAR_{organic}$ can both potentially be used to diagnose the combined presence of organic toxicants from agricultural activities and WWTPs discharges, however, they may have limited application in differentiating between both sources of pollution. Various studies have suggested that the $SPEAR_{pesticides}$ relates specifically to pesticide exposure, as the calculation includes life history traits that differentiate a taxon's ability to recover from pulses of contamination typical of pesticide exposure (e.g. Schäfer et al. 2007, Beketov et al., 2019, Knillmann et al. 2018). However, the results from the present study suggest that the $SPEAR_{pesticides}$ may also relate to other organic toxicants in WWTPs discharges (e.g. PPCPs), although we cannot exclude that the invertebrates at these sites did not integrate effects of pesticide peaks in WWTP discharges prior to the sampling period. Munz et al. (2017), for example, reported a high number of pesticide peaks in WWTP effluent in May and July, and considered these pesticide peaks the main explanatory factor for lowered $SPEAR_{pesticide}$ values. Various studies used the $SPEAR_{pesticides}$ to detect impact of WWTP discharges in flowing water, more or less depreciating the $SPEAR_{organic}$ (Burdon et al. 2016, Burdon et al. 2019). However, our results showed that a higher proportion of the proxy of organic toxicants was explained by the $SPEAR_{organic}$ than by the $SPEAR_{pesticides}$. One explanation could be that the reduced flow and hydro-morphological habitat degradation of lowland water bodies, including channelization, dredging and riparian vegetation removal and control, may have hampered the colonization of the sensitive $SPEAR_{pesticide}$ taxa, irrespective of the presence of organic toxicants (Rasmussen et al. 2012a). To distinguish between different sources of pollution, the SPEAR approach may need to be combined with other methods, like the quantification of sources of stress based on land use maps (De Vries et al. 2019) and using proxies for WWTP discharge such as the gadolinium anomaly used in this study. Moreover, the taxonomic-based evenness metric showed a wider variation in values at the sites with a low toxic pollution than at high toxic pollution, indicating that there may have been other (unmeasured) stressors that impacted the invertebrates. Therefore, it would be a valuable addition to future research efforts to also test the applicability of SPEAR metrics designed for other types of stressors in multi-stressed lowland water bodies, such as heavy metal pollution (Malaj et al. 2012) and salinity (Schäfer et al. 2011).

The selection of metrics should depend on the goals of the water quality assessment approach. If the goal is to detect adverse effects from anthropogenic stress on both ecosystem structure and functioning, we recommend to include process-based metrics besides taxonomic-based metrics, as their relation with anthropogenic stress may be opposite to the taxonomic-metrics and more complex. If the goal is to diagnose the potential causes of the observed adverse effects, the application of the $SPEAR_{organic}$ and $SPEAR_{pesticides}$ seems promising in distinguishing elevated levels of toxic pollution, although

this trait-based approach should still be verified for other types of stressors in multi-stressed lowland waters. If an additional goal is to identify sources of stress (i.e. determine whether the toxic pollution derived from agricultural land use or WWTP discharges), the SPEAR approach may need to be combined with other methods, extracting information from land use maps and proxies such as the gadolinium anomaly. In conlcusion, the taxonomic, trait-based and functional metrics may provide complementary information that, when integrated, allow for more thorough water quality assessment strategies.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL 1

Water body dimensions

Water body size (width and depth) and flow velocity were measured on a single occasion. Flow velocity was measured in the middle of the water body with an electromagnetic current meter (Valeport model 802, Devon, UK).

Land use of the riparian zone

The land use of the riparian zone (i.e. nature including forest, agriculture, or urban) was taken from the LGN5 map, providing land use types in 2003 and 2004 using the clipping function in ArcMap (Hazeu, 2005). The riparian zone was delineated as a zone of 150 m on both sides of the sampling site along a length of 600 m upstream in lotic waters and from the middle in lentic waters.

Wastewater treatment plant effluent influence

A proxy for the degree of direct and indirect effluent input from wastewater treatment plants (WWTPs) was quantified using the gadolinium anomaly (Gd*) in the water. Gadolinium (Gd) is a rare earth element (REE) that is used as a paramagnetic contrast agent in the form of stable Gd complexes in medical magnetic-resonance imaging (MRI). REEs have a specific geochemical behavior and occur in strict ratios with very little naturally occurring variation (Kulaksız & Bau, 2011). Gd complexes are excreted by people within hours after a MRI scan, and pass through the WWTP almost unchanged, thus providing a good tracer for WWTP effluent in surface waters (Rabiet et al., 2005; Petelet-Giraud et al., 2009).

One surface water grab sample was collected each week for six weeks, and 8 mL of each of the weekly samples were combined to obtain a composite sample per site. These composite samples were filtered over 0.45 µm filters, after which the filtrate was acidified with 0.334 mL 65% HNO_3 (Suprapur®) and stored at 4 °C until analysis on a Thermo Scientific i-Cap ICP-MS, with an APEX ESI (Omaha, US) sample introduction system. The geogenic background concentration of Gd (Gd_{geo}) was calculated for each sample individually, based on the presence of at least five other REEs in the sample which are naturally occurring and unaffected by anthropogenic activities. The gadolinium anomaly, i.e. Gd enrichment compared to Gd_{geo}, was calculated as follows: $Gd^* = Gd_{measured} / Gd_{geo}$ (Kulaksız & Bau, 2011). Natural Gd* values in water bodies without any WWTP impact are around 1.3 (Rabiet et al., 2005; Petelet-Giraud et al., 2009).

SUPPLEMENTARY MATERIAL 2

POCIS were constructed using two stainless steel rings, with an inner diameter of 5.4 cm, to retain sorbent between two membranes, leaving approximately 46 cm^2 of surface area exposed to the surrounding water. Stainless steel rings (Exposmeter, Sweden), nuts and bolts, as well as all used tools were cleaned in acetone before assembly of the samplers. Polyether sulfone (PES) diffusion limiting membrane filters (Pall Corporation, NY, USA, 0.1 µm pore size, 90 mm diameter) were used to enclose the sorbent, and were cleaned before POCIS assembly in HPLC grade methanol:ultra-pure water (50:50, v:v) followed by rinsing in ultra-pure water. As a receiving phase, 200 mg of Oasis hydrophilic-lipophilic balance (HLB) sorbent (Waters, MA, USA) was enclosed between the PES membranes. The HLB was conditioned in its original column by sequentially eluting with 40 mL acetone, 40 mL dichloromethane and 40 mL methanol (Biosolve, The Netherlands, all chromatography grade) and dried under vacuum, followed by final assembly of the POCIS. POCIS were stored at 4 °C in food-grade Mylar zip lock bags until deployment.

At each site, four POCIS retained in stainless steel cages were deployed for six weeks in the middle of the water column. After field exposure, the POCIS were cleaned in the field with local water and a scrubbing sponge to remove any biofouling that had accumulated on the polyether sulfone membranes, and stored at -20 °C. Frozen POCIS were freeze-dried overnight at −53 °C in a Scanvac CoolSafe freeze-dryer. Dry sorbent of the four POCIS per site was pooled and eluted three times with 3 mL LC grade acetonitrile under vacuum in a glass solid phase extraction column. Finally, the extracts were topped up to 10 mL with acetonitrile by weight, and stored at -20°C until analyses.

POCIS acetonitrile extracts were subjected to three *in vitro* chemical activated luciferase gene expression (CALUX®) bioassays at the BioDetection Systems laboratories (Amsterdam, The Netherlands). Extracts were converted to dimethylsulphoxide before exposure in the bioassays. Estrogen receptor (ERα), androgen receptor antagonism (anti-AR) and progesterone receptor antagonism (anti-PR) CALUX assays were performed according to previously described protocols (Hamers et al., 2006; Sonneveld et al., 2004; Van der Linden et al., 2008). The activities of the extracts were expressed as bioanalytical equivalents of the corresponding reference compounds. Subsequently, the bioanalytical activities were divided by the effect-based trigger (EBT) value of each assay to obtain a measure of the ecotoxicological risk caused by bioactive compounds present at the study sites (Brion et al., 2019; Escher et al., 2018; Table S1).

ERα risk was considered a proxy for the presence of pharmaceuticals and personal care products (Välitalo et al., 2016), and the mean of anti-AR and anti-PR risks was considered a proxy for the presence of pesticides in the surface waters (Pieterse et al., 2015). In addition, we calculated a combined proxy for toxic pollution using the mean of the proxy for PPCPs and pesticides. Although these proxies do not provide information on the

concentrations of individual chemical compounds, they do provide a promising tool to interpret the harmful effects of groups of often (un)known, unregulated and unmonitored compounds present in surface waters (De Baat et al. 2019).

Table S1: Overview of CALUX assays performed on POCIS extracts. Effect-based trigger (EBT) values were previously defined by Brion et al. 2019 (ERα) and Escher et al. 2018 (anti-AR and anti-PR).

CALUX assay	Endpoint	Reference compound	EBT	Unit
ERα	Estrogenic activity	17β-estradiol	0.28	ng EEQ/L
anti-AR	Antiandrogenic activity	flutamide	14.4	µg FEQ/L
anti-PR	Antiprogestogenic activity	Ru486	0.013	µg REQ/ml

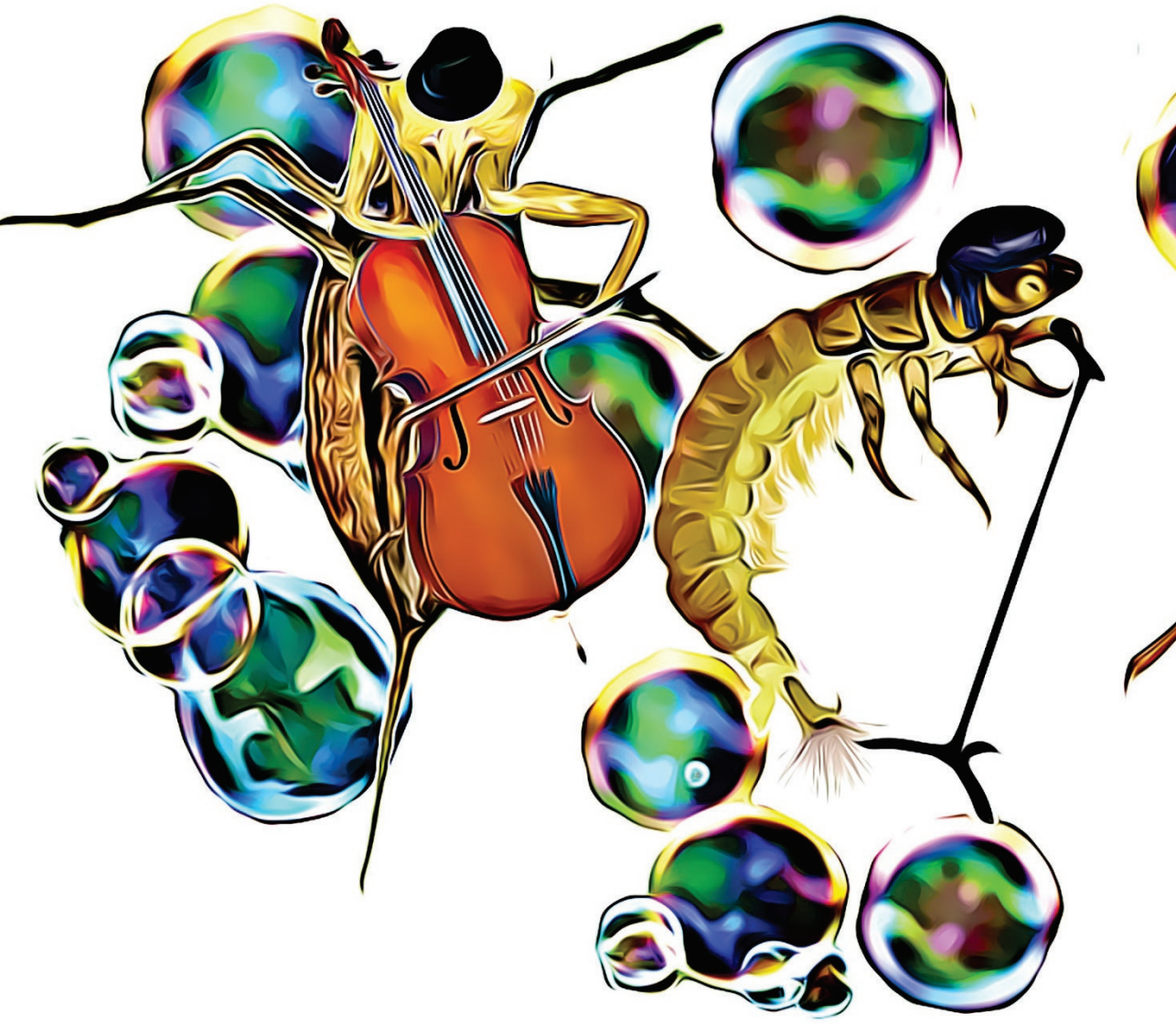
SUPPLEMENTARY MATERIAL 3

Table S1: Regression statistics for two- variable models using linear functions between each structural and functional metric and each pair of stressors. A variance inflation factor (VIF) > 4 is indicative of multicollinearity. The ΔAICc was calculated against the best fitting single variable model (see Table 4, main text). Stressors include total dissolved nitrogen (TDN) concentrations, orthophosphate (PO4-P) concentrations, the percent time that the dissolved oxygen saturation is below 10 % (DO < 10), mean water temperature (temperature), the proxy for the presence of pesticides (pesticides) and the proxy the presence of pharmaceuticals and personal care products (PPCPs).

		VIF	ΔAICc						
			Richness (#)	Evenness (-)	Trichoptera (#)	$SPEAR_{pesticides}$ (-)	$SPEAR_{organic}$ (-)	Microbial decomposition (%)	Invertebrate consumption (%)
TDN	PO4-P	1.03	-0.5	-9.8	-4.3	-12.3	-19.6	-3.1	-15.8
TDN	DO < 10	1.08	-3.9	-10.1	-4.3	-10.9	-19.4	-3.3	-16.7
TDN	Temperature	1.70	-4.4	-8.9	-4.4	-13.9	-18.6	-0.2	-14.9
TDN	PPCPs	1.39	-3.8	-5.2	-2.9	-7.5	-7.0	0.4	-9.7
TDN	Pesticides	1.01	-0.5	-9.7	-4.2	-11.4	-19.6	-3.3	-16.0
PO4-P	DO < 10	1.02	-3.6	-12.8	-6.3	-9.5	-18.7	-38.0	-17.3
PO4-P	Temperature	1.04	-2.1	-9.3	-6.1	-12.4	-18.0	-36.0	-15.5
PO4-P	PPCPs	1.02	-2.1	-3.7	-2.8	-4.9	-4.4	-24.2	-1.6
PO4-P	Pesticides	4.17	-3.1	-12.9	-6.6	-11.5	-20.0	-40.9	-19.2
DO < 10	Temperature	1.01	-5.5	-9.0	-5.6	-10.7	-16.3	-28.5	-13.4
DO < 10	PPCPs	1.10	-3.9	-5.4	-3.5	-6.8	-8.7	-28.0	-9.2
DO < 10	Pesticides	1.01	-3.2	-12.9	-6.2	-8.5	-18.7	-39.2	-18.3
Temperature	PPCPs	1.46	-4.4	-5.1	-3.5	-6.6	-8.8	-29.6	-9.6
Temperature	Pesticides	1.02	-1.8	-9.2	-6.0	-11.5	-17.9	-36.6	-16.0
PPCPs	Pesticides	1.04	-1.9	-3.1	-2.5	-2.4	-19.6	-28.0	-3.2


6

CHAPTER 7



FRESHWATER ECOACOUSTICS: LISTENING TO THE ECOLOGICAL STATUS OF MULTI-STRESSED LOWLAND WATERS

This chapter is based on the paper in *Ecological Indicators* 113: 106252

Gea H. van der Lee*, Camille Desjonquères*, Jérôme Sueur, Michiel H.S. Kraak, & Piet F.M. Verdonschot (*equal contribution)

ABSTRACT

A major challenge in water quality assessment is to identify suitable indicators to monitor and assess the effects of anthropogenic stressors on the ecological status of freshwater ecosystems. Passive acoustic monitoring is a novel approach that could potentially be used to detect invertebrate species and ecological processes such as dissolved oxygen dynamics in freshwater environments. The aim of the present study was to evaluate to what extent sounds can be used for water quality assessment. We performed a field study to relate acoustic indices to the intensity of several stressors, the invertebrate community composition and the dissolved oxygen dynamics in 20 temperate lowland streams and drainage ditches impacted to a varying degree by agricultural activities and discharges from waste water treatment plants. Our results showed that the recorded acoustic patterns were primarily associated with the fluctuation in dissolved oxygen saturation, while specific frequency bands could be related to the sound-producing invertebrate community. We observed that acoustic indices do not allow to detect the adverse effects of anthropogenic stressors on the invertebrate community composition, presumably due to the prevalence of Heteroptera which are relatively insensitive to stressors, but make a lot of sounds. A strong relation between acoustic indices and oxygen fluctuation indicate that passive acoustic monitoring may be used to estimate metabolism in water bodies. We suggest that the next step in freshwater ecoacoustics will be to precisely characterise each source of sound emitted during the processes of primary production, respiration and re-aeration, in order to distinguish these parameters. This may overcome some of the challenges encountered in the estimation of metabolism from diel dissolved oxygen curves.

INTRODUCTION

Freshwater ecosystems are commonly impacted by various anthropogenic activities, including agricultural activities and municipal wastewater treatment plant (WWTP) discharges (Burdon et al., 2019; Ormerod et al., 2010). These activities can result in a combination of stressors, such as excess nutrients, increased water temperatures, pesticides, pharmaceuticals, personal care products and a suite of other contaminants, which can cause substantial changes in the community composition and ecological processes of the impacted water bodies (Allan, 2004; Karr, 1999; Palmer & Febria, 2012). A major challenge in protecting and restoring these ecosystems is to identify cost-effective indicators to monitor, assess and evaluate the effects of these stressors on the ecological status of freshwater ecosystems (Bonada et al., 2006; Friberg et al., 2011).

The majority of ecological indicators used in water quality assessments are based on point-in-time measurements of the community structure of various organism groups (Boulton, 1999; Resh, 2008). Communities, especially of macroinvertebrates, are relatively diverse and can therefore be used to assess a variety of anthropogenic stressors (e.g. Clapcott et al., 2012). These structural-based indicators, however, do not capture the dynamic properties of freshwater ecosystems (Palmer & Febria, 2012). It has been argued that repeated measurements of ecological processes could be helpful to capture system dynamics employing functional-based indicators (Bunn, 1995). To this purpose, diel change in dissolved oxygen (DO) concentrations have been measured with loggers in the open channel to calculate ecosystem metabolism, where DO concentrations are associated with photosynthesis during the day and respiration at night (Odum, 1956; Young et al., 2008). Yet, these structural and functional based indicators require different sampling techniques. Thus the development of a sampling method that can monitor and assess structural and functional indicators with a single technique could improve the efficiency of water quality assessments.

A potential approach to combine structural and functional water quality assessment is to use passive acoustic monitoring, a method proposed by ecoacoustics that samples ambient sounds to tackle ecological questions (Farina & Gage, 2017; Sueur & Farina, 2015). This non-invasive and continuous monitoring method is emerging to address ecological questions in many ecosystems (Desjonquères et al., 2020b; Gibb et al., 2019; Linke et al., 2018; Sugai et al., 2019a, 2019b). Some terrestrial studies have already managed to link acoustic patterns to the degrees of human influence (Burivalova et al., 2018; Buxton et al., 2018a), however, only a limited number of studies have applied ecoacoustic monitoring in freshwater environments (but see Desjonquères et al., 2015; Karaconstantis et al., 2020; Linke et al., 2020).

Environmental sounds can emanate not only from sound producing species, whose presence and activity depends on local ecological conditions, but also from ecological

processes and anthropogenic activities (Linke et al., 2018). In freshwater environments, there are four main taxa known for producing sounds: amphibians, crustaceans, fish and insects (Desjonquères et al., 2020a). In temperate zones, most of the sounds recorded in freshwater environments appear to be linked to insects belonging to the insect orders Heteroptera, Coleoptera, Trichoptera and Odonata, Heteroptera and more specifically the family Corixidae include the majority of soniferous species (Aiken, 1985; Desjonquères, 2016; Desjonquères et al., 2018). Sounds linked to ecological processes are likely due to a combination of primary production through plant photosynthesis as well as the decomposition of organic matter by microbial activity (Linke et al., 2018). Although all these species and processes can potentially be detected and monitored in freshwater environments, the knowledge on species- and process-specific sounds is still emerging, making it unclear to what extent sounds can be used as a monitoring tool in water quality assessment strategies.

One of the main challenges of acoustic monitoring is to extract ecologically meaningful attributes of sounds and to link these sounds to ecosystem variables, such as community composition and ecological processes (Linke et al., 2018; Sueur & Farina, 2015). To address this challenge, acoustic indices, which are the equivalent of ecological diversity indices based on sound rather than on the number of species sampled, have been developed (Sueur et al., 2014). Such acoustic indices compute specific features of the sound spectrum (frequency representation) or waveform (temporal representation), which are thought to represent meaningful information about the ecosystem (Gage et al., 2017).

The aim of the present study was, therefore, to evaluate the potential of passive acoustic monitoring in freshwater environments by relating acoustic patterns to ecosystem structure and function in water bodies along a gradient of anthropogenic stress. We hypothesized that different acoustic indices relate to different structural and functional aspects of freshwater ecosystems. To test this hypothesis, we conducted a field study in 20 temperate lowland streams and drainage ditches throughout the Netherlands. First, we tested to what extent the invertebrate community reflected the intensity of the measured stressors including dissolved oxygen dynamics to confirm their use in water quality assessment. Then, we assessed if the sound-producing invertebrate community also reflected the same relationship to these stressors and dissolved oxygen dynamics. Finally, to evaluate the potential of passive acoustic monitoring in freshwater ecosystems, we correlated the acoustic indices to the measured stressors, the invertebrate community composition, the sound-producing invertebrate community composition and the dissolved oxygen dynamics.

MATERIALS AND METHODS

Study outline

The 20 study sites were surrounded by varying degrees of agricultural land use in the riparian zone and WWTP effluent (Supplementary material 1). The sites had a comparable width (mean ± SD = 4.5 ± 1.8 m), depth (0.8 ± 0.2 m) and flow velocity (5.3 ± 6.8 cm/s). At each site, we measured nutrient concentrations, water temperature and proxies for pesticides, pharmaceuticals and personal care products to estimate the intensity of the stress originating from the agricultural activities and the WWTP discharges. As structural and functional indicators, we determined invertebrate community composition and dissolved oxygen dynamics, respectively. At all study sites, we recorded the underwater sounds from which we calculated acoustic indices. The study was conducted between August 20th and October 23th 2018. We chose this period, as the dissolved oxygen dynamics are most distinct during this period (Van der Lee et al., 2018) and it is likely to be within the reproductive period of several aquatic insect species (Jansson, 1974). All of this is expected to result in the most distinct sound patterns between sites. Stressors, dissolved oxygen dynamics and acoustics data was collected during the first six weeks of the sampling period, while invertebrate samples were collected during the last two weeks of the sampling period to avoid disturbing the acoustic sampling.

Stressors

Nutrient concentrations were measured by collecting a weekly surface water grab sample at each site for six weeks. The samples were filtered over a 1.2 µm filter and analysed for total dissolved nitrogen (TDN) and orthophosphate (PO_4-P) on a continuous flow analyser (SAN++ system, Skalar Analytical B.V., Breda, The Netherlands). The mean nutrient concentrations over the six weeks were calculated for further analysis.

Water temperature (°C) was measured every ten minutes for six weeks with HOBO® Temperature/Light loggers UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). At each study site, the loggers were placed in the mid-channel, 15 cm under the water surface. The mean water temperature over the six weeks was calculated for further analysis.

Proxies for pesticides, pharmaceuticals and personal care products were derived from bioassays subjected to passive sampler extracts. To this end, polar organic chemical integrative samplers (POCIS) containing 200 mg of Oasis hydrophilic-lipophilic balance sorbent were used (Waters, MA, USA; Alvarez et al., 2004). At each site, four POCIS were deployed for six weeks in the middle of the water column to absorb polar compounds from the surface water. After field exposure, the POCIS extracts were prepared and pooled for further analysis (details in Chapter 6). POCIS extracts were subjected to three in vitro chemical activated luciferase gene expression (CALUX®) bioassays at the BioDetection Systems laboratories (Amsterdam, the Netherlands), including the Estrogen receptor (ERα),

the androgen receptor antagonism (anti-AR) and the progesterone receptor antagonism (anti-PR) CALUX assays. The activity of the extracts were expressed as bio-analytical equivalents of the corresponding reference compounds and divided by the effect-based trigger (EBT) value of each assay to obtain a measure of the ecotoxicological risk caused by the bioactive compounds present at each site (Brion et al., 2019; Escher et al., 2018). We considered ERα risk as a proxy for the presence of pharmaceuticals and personal care products and the mean of anti-AR and the anti-PR risks as a proxy for the presence of pesticides in the surface waters (Pieterse et al., 2015; Välitalo et al., 2016). Each stressor variable was scaled to a standard deviation of one and centred at its mean for further analysis.

Invertebrate community composition

Six invertebrate samples were collected at each site on a single occasion. Three subsamples were taken with a pond net (1mm mesh size, 25 cm width) that was swept over a length of 0.5 m of submerged vegetation, while the other three subsamples were taken with the same net swept over the top layer of the sediment. The samples were stored overnight at 4 °C with oxygen supply, washed over 1 mm and 250 µm sieves, sorted alive and preserved in 70% ethanol until identification. Overall, a total of 33298 individuals belonging to 106 invertebrate taxa were collected. Invertebrates were identified to the genus level with a few exceptions, specifically Oligochaeta (order), Hydracarina (order) and Diptera (family). Corixidae were, if possible, further identified to the species level, as they are the family with the highest number of sound-producing species (Aiken, 1985). In total, 26 of the identified taxa are known for producing sounds (Table 1). The sum of taxon abundance for the six replicate invertebrate samples per site was $\log_{10}(x+1)$ transformed before further analysis to minimize the effect of high density taxa.

Table 1: Invertebrate genera collected in this study that produce sound. Sound production in freshwater invertebrates was reviewed by Desjonquères (2016).

Order	Family	Genus
Trichoptera	Hydropsychidae	*Hydropsyche*
Coleoptera	Dytiscidae	*Cybister, Dytiscus*
	Haliplidae	*Haliplus*
	Hydrophilidae	*Anacaena, Enochrus, Helophorus*
Heteroptera	Corixidae	*Callicorixa, Corixa, Cymatia, Hesperocorixa, Micronecta, Paracorixa, Sigara*
	Nepidae	*Nepa, Ranatra*
	Pleidae	*Plea*
	Naucoridae	*Ilyocoris*
Decapoda	-	-

Dissolved oxygen dynamics

Dissolved oxygen (DO) concentrations (mg/L) were measured with optical HOBO® Dissolved Oxygen loggers U26-001, protected by the antifouling protective guard U26-GUARD-2 (Onset Computer Corporation, Bourne, MA, USA). Following Van der Lee et al., (2018) measurements were taken every ten minutes for six consecutive days in the mid-channel, 15 cm under the water surface. This was repeated three times at each site during the six week period, rotating weekly between the sites. Percent DO saturation was calculated from the DO concentrations and temperature, assuming 0 ‰ salinity and 1 atm barometric pressure, using DOTABLES developed by the U.S. Geological Survey (2011) for further analysis (Van der Lee et al., 2018). The mean DO saturation was calculated per 10 minute time step over the 18 measurement days. The DO dynamics were represented by the mean DO saturation, while the fluctuation was calculated as the maximum values minus the minimum values.

Acoustic sampling

The underwater sounds were monitored with nine autonomous recording platforms consisting of a HTI-96 hydrophone (flat frequency response between 20 Hz and 40 kHz, High Tech Inc., Long Beach, MS, USA) connected with a 20 m cable to one channel of an autonomous SM2 audio recorder (Wildlife Acoustics, Maynard, MA, USA). A single SM2 recorder connected to a hydrophone was set up at each site to record uncompressed audio files (in the wav format) at a 44.1 kHz sampling frequency and a 16 bit digitization depth. The hydrophones were placed next to the dissolved oxygen sensors 15 cm below the water surface, with their piezoelectric element directed downward toward the sediment. The recording schedule was set to one minute every ten minutes, 24 hours a day for six consecutive days. This was repeated two times at each site during the six week period, rotating between the sites. There was a two to three week interval between the first and second recording week.

To ensure the quality of the recordings used for the analysis, two randomly selected recordings per day were systematically examined by listening to the recording and inspecting the spectrogram. Due to one malfunctioning hydrophone, the data obtained with this hydrophone (5652 recordings) was excluded from our analyses. Recordings with high levels of anthropogenic noise were identified in one site and this data was also excluded (1792 recordings). We were not able to identify and remove occasional anthropogenic noise, however we expect it to have a negligible effect on our results. As rain can introduce unwanted noise and bias acoustic analyses, we also removed recordings collected when it was raining. Rain periods were assessed using the closest Royal Netherlands Meteorological Institute (KNMI) meteorological station (Supplementary material 1), resulting in the removal of 2879 recordings. This way, we obtained a final dataset of 26989 recordings with

an average of 1350 ± 575 (mean ± SD) one-minute recordings for each site (min: 209; max: 2095).

Acoustic analyses

The systematic examination of the sound recordings also allowed us to identify the specific frequency bands in which the main acoustic patterns occurred. This way, we identified three frequency bands which appeared to delimit most types of sounds: 0-2 kHz, 2-7 kHz and 7-22.05 kHz (Figure 1). For each of these three bands, we computed three acoustic indices: the Acoustic Complexity Index (*ACI),* the Spectral Entropy (H_f), and the amplitude measured as the sum of raw amplitude from the spectrogram (*Amp)*. These three indices were chosen because they are indicative of different aspects of the soundscape (Buxton et al., 2018b; Towsey et al., 2018) and they have been used previously in some of the first ecoacoustic studies in freshwater environments (Desjonquères et al., 2015; Karaconstantis et al., 2020; Linke & Deretic, 2020). *ACI* is a measure that calculates the average difference of spectral amplitude between time windows (Pieretti et al., 2011). *Hf* is analogous to the Shannon entropy index from community ecology. Instead of species probability of presence, *Hf* uses the amplitude of each frequency bin in the mean spectrum (Sueur et al., 2008). This index thus yields a measure of the evenness of the probability mass function.

As sound is variable and dynamic over time it was recorded over multiple full days, which raises the issue of temporal autocorrelation and pseudo-replication (Desjonquères et al., 2020a). So far most research in ecoacoustics did not take into account such considerations, even though it may impact the statistics and subsequent interpretation of the results. In this study, we proposed a solution to this problem by employing Functional Data Analysis (FDA), which allows to represent a set of temporal data points as a single continuous mathematical function (Ramsay et al., 2009). Here, we used this method to describe and model the daily variations in each acoustic index at each site. To represent the smooth variation of the acoustic indices over a day, we calculated the mean values per ten minute time step resulting in 144 mean values per index per site. To obtain a representation of the daily variation for each site, we used 140 spline bases of order four and a smoothing parameter of 10^4. This specific smoothing parameter was chosen to optimise the trade-off between degrees of freedom and generalised cross validation (Ramsay et al., 2009). The modes of variation between the sites were then displayed in a Functional Principal Component Analysis (FPCA) for each acoustic index (Ramsay et al., 2009). This way, we obtained the coordinates of each site in a functional FPCA space. In such a FPCA, each axis represents the maximum variance between the sites in the shape of the splines. The scores for the first FPCA axis (FPC1) explained more than 67% of all acoustic indices and was used in subsequence analyses.

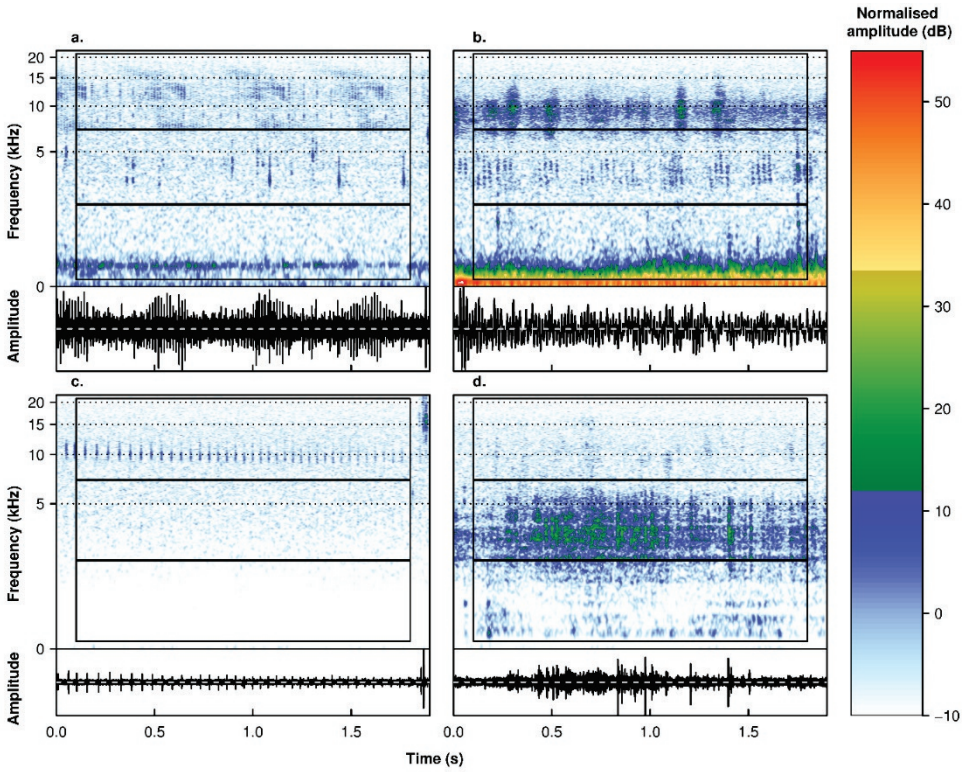


***Figure 1**: Example of the main acoustic patterns in the recordings with the three frequency bands indicated that delimit most types of sounds: 0-2 kHz, 2-7 kHz and 7-22.05 kHz. (a) Recording from site 2 at 1:50 pm on August 25th showing the sounds of gusts of wind and continuous low frequency sounds potentially corresponding to photosynthesis (0-2 kHz), ticking and bubble sounds (2-7 kHz and 7-22.05 kHz); (b) Recording from site 8 at 6:00 am on August 24th showing the sounds of gusts of wind and continuous low frequency sounds potentially corresponding to photosynthesis (0-2 kHz), Corixidae sounds (2-7 kHz) and Micronecta sounds (7-22.05 kHz); (c) Recording from site 12 at 5:40 pm on August 28th showing ticking sounds (7-22.05 kHz); (d) Recording from site 8 at 14:40 pm on September 16th showing anthropogenic noise (2-7 kHz). Information on the sites in Supplementary material 1. The selected sound recordings can be found in Supplementary material 2-5.*

Statistical analysis

To assess whether the sound-producing invertebrate community represents the same indication of the water quality as the entire community, we tested to what extent the separate unconstrained ordination (PCA) of the invertebrate taxa, both of the entire community and of only the sound-producing community, related to the stressors and dissolved oxygen dynamics. Then, to evaluate the potential of passive acoustic monitoring, we correlated the FPC1 of each acoustic index to a separate unconstrained ordination (PCA) of the measured stressors, the entire invertebrate community composition, the sound-

producing invertebrate community composition and the dissolved oxygen dynamics. Significant relations between each acoustic index and each PCA was tested using a 999 permutation process. Significant vectors ($p<0.05$) for acoustic indices were plotted on the ordination to show the correlation with the ordination configuration.

All analyses were performed in R (R Core Team, 2019; v. 3.6.0) using the *seewave* package to compute the acoustic indices (Sueur et al., 2018; v. 2.1.3), the *fda* package to compute the FPCA analysis (Ramsay et al., 2014; v. 2.4.8) and the package *vegan* to compute the PCA and to fit the acoustics indices on the ordinations (Oksanen et al., 2013; v. 2.5-6).

RESULTS

The acoustic recordings contained various sound types associated with different processes including sound production by invertebrates, ticking and bubble sounds, wind and anthropogenic noise (Figure 1, Supplementary Material 2-5). These sound types were in most cases confined to specific frequency bands (0-2, 2-7 and 7-22.05 kHz). The daily variation of acoustic indices depended on the index, the site and the frequency band considered (Figure 2). Some of the indices, such as Hf_{0-2} and Amp_{0-2}, had similar temporal patterns with a maximum or minimum in the afternoon (12:00-18:00), but showed strong differences in the magnitude of the daily peaks. While others, like Amp_{2-7}, showed less differences in the magnitude of daily variation but higher differences in temporal patterns. Finally other indices, such as Hf_{2-7}, Hf_{7-22} or Amp_{2-7} differed both in magnitude of daily variations and in temporal patterns, with in some sites a secondary peak of activity at night.

In terms of the stressors, the entire invertebrate community showed significant correlations with orthophosphate concentrations, the proxies for pesticides and pharmaceuticals and the fluctuation in dissolved oxygen saturation (Figure 3a, Table 2). The sound-producing invertebrate community only correlated significantly to the mean water temperature (Figure 3b, Table 2).

In terms of the acoustic indices, the intensity of the stressors only correlated significantly to Hf_{0-2} (Figure 4a, Table 3). Similarly to the stressors, the entire invertebrate community also correlated significantly with this acoustic index, as well as to Amp_{0-2} (Figure 4b, Table 3). The sound-producing invertebrate community composition correlated significantly with *ACI* and *Hf* in the frequency band 2-7 kHz as well as $Amp_{7-22.05}$ and ACI_{0-2}. The loading scores for $Amp_{7-22.05}$ and ACI_{0-2} were in the same direction as the loading score of *Micronecta* on PC2 (Figure 4c, Table 3). The dissolved oxygen saturation correlated significantly with all acoustic indices, except for ACI_{0-2} and Amp_{2-7} (Figure 4d, Table 3). All arrows were mainly associated with the y-axis, representing fluctuations in dissolved oxygen. The strongest correlation was found with Amp_{0-2} ($R^2 = 0.52$, $p = 0.001$).

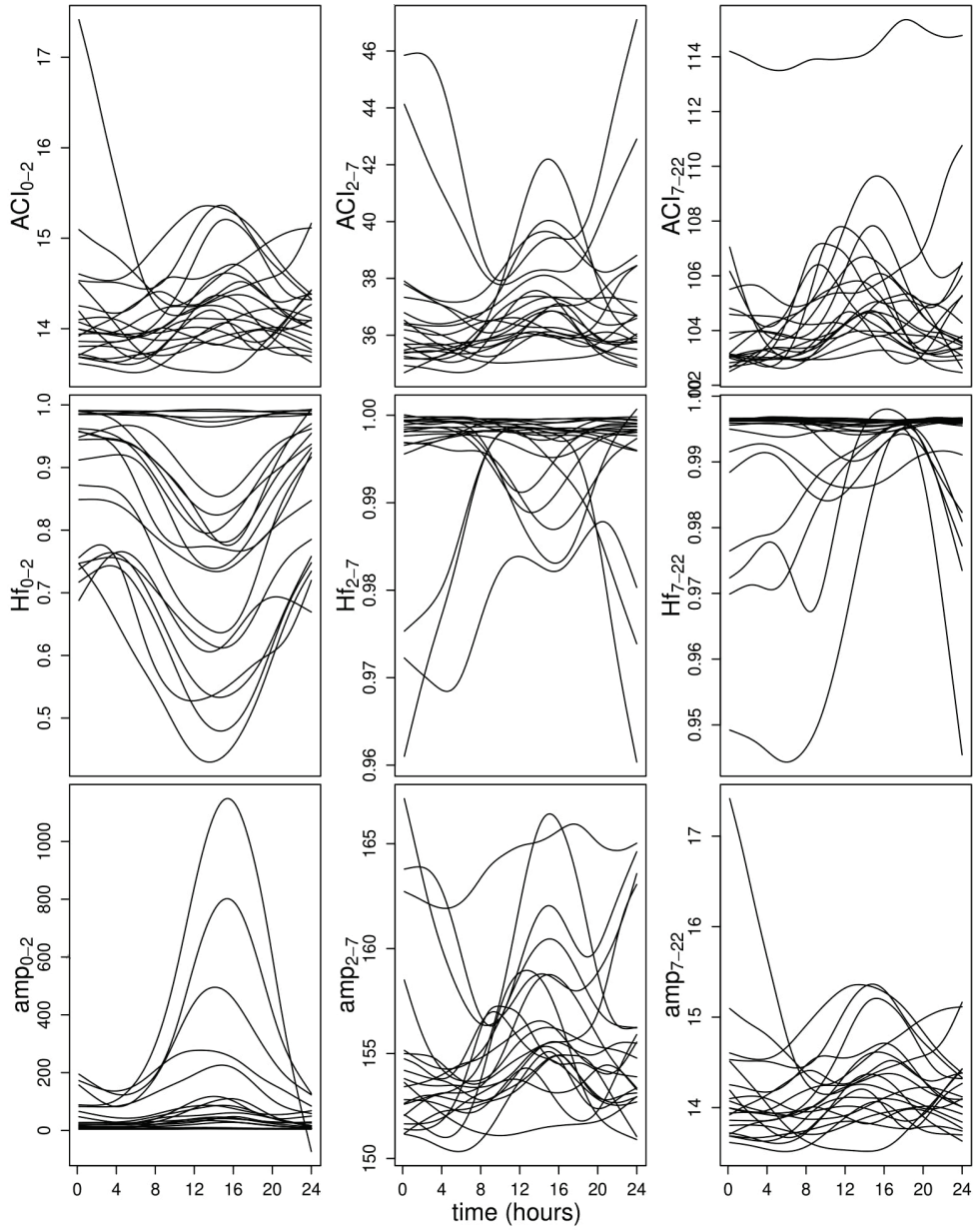


***Figure 2**: FDA representation of the smooth variation of the acoustic indices over a single day. Each curve shows the FDA representation for a frequency band of an acoustic index in a site. The curves were obtained using a spline basis and a smoothing parameter. Notation: $ACI_{0\text{-}2}$ corresponds to the ACI index over the 0-2 kHz frequency band.*

Table 2: Relations between the stressors and dissolved oxygen dynamics and the ordination of the invertebrate communities (all taxa and only sound-producing taxa), as squared correlation coefficient (R^2) and significance *(p)* permutation-tested using 999 randomizations (N = 20).

	All invertebrate taxa		Sound-producing invertebrate taxa	
	R^2	p	R^2	p
Total dissolved nitrogen	0.08	0.541	0.204	0.148
Orthophosphate	0.31	0.038	0.003	0.982
Temperature	0.11	0.403	0.431	0.006
Proxy for pesticides	0.29	0.049	0.025	0.782
Proxy for pharmaceuticals	0.46	0.010	0.132	0.260
Mean dissolved oxygen	0.07	0.551	0.010	0.938
Fluctuation dissolved oxygen	0.40	0.011	0.087	0.482

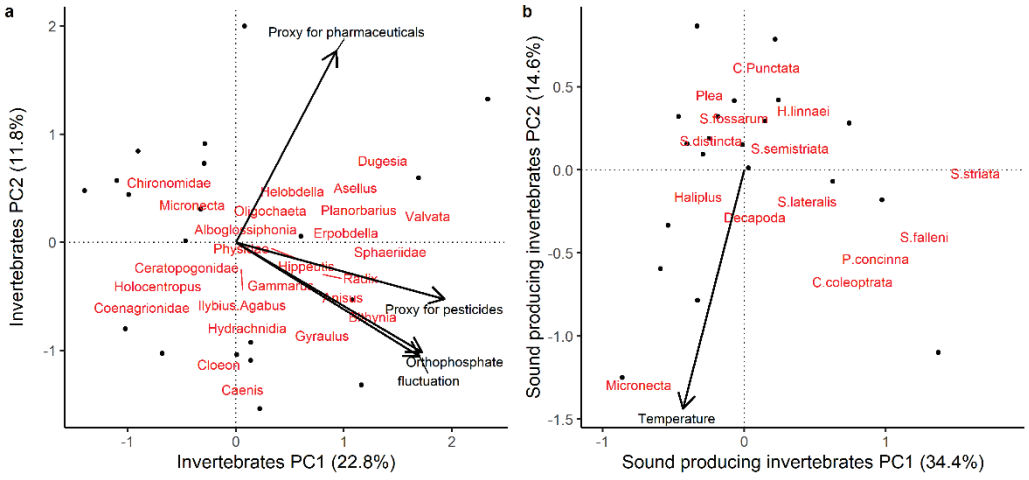


***Figure 3**: Relations between the stressors, dissolved oxygen dynamics and the ordination of a) entire invertebrate community composition and b) sound-producing invertebrate community composition. Only significant vectors (p < 0.05) are plotted (details in Table 2). Taxa shown are prioritized on abundance.*

Table 3: Relations between the acoustic indices and the ordination of different environmental variables as squared correlation coefficient (R^2) and significance *(p)* permutation-tested using 999 randomizations (N = 20).

		Stressors		All invertebrate taxa		Sound-producing invertebrate taxa		Dissolved oxygen saturation	
		R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*
ACI	0-2	0.08	0.509	0.07	0.558	0.35	0.025	0.31	0.044
	2-7	0.11	0.346	0.20	0.143	0.41	0.031	0.37	0.017
	7-22.05	0.05	0.588	0.07	0.532	0.00	0.969	0.06	0.656
Hf	0-2	0.30	0.048	0.54	0.004	0.15	0.247	0.47	0.009
	2-7	0.11	0.326	0.16	0.236	0.38	0.019	0.44	0.006
	7-22.05	0.18	0.164	0.06	0.557	0.24	0.096	0.46	0.004
Amp	0-2	0.21	0.108	0.40	0.015	0.13	0.279	0.52	0.001
	2-7	0.02	0.815	0.21	0.127	0.09	0.425	0.20	0.149
	7-22.05	0.08	0.509	0.07	0.558	0.35	0.025	0.31	0.044

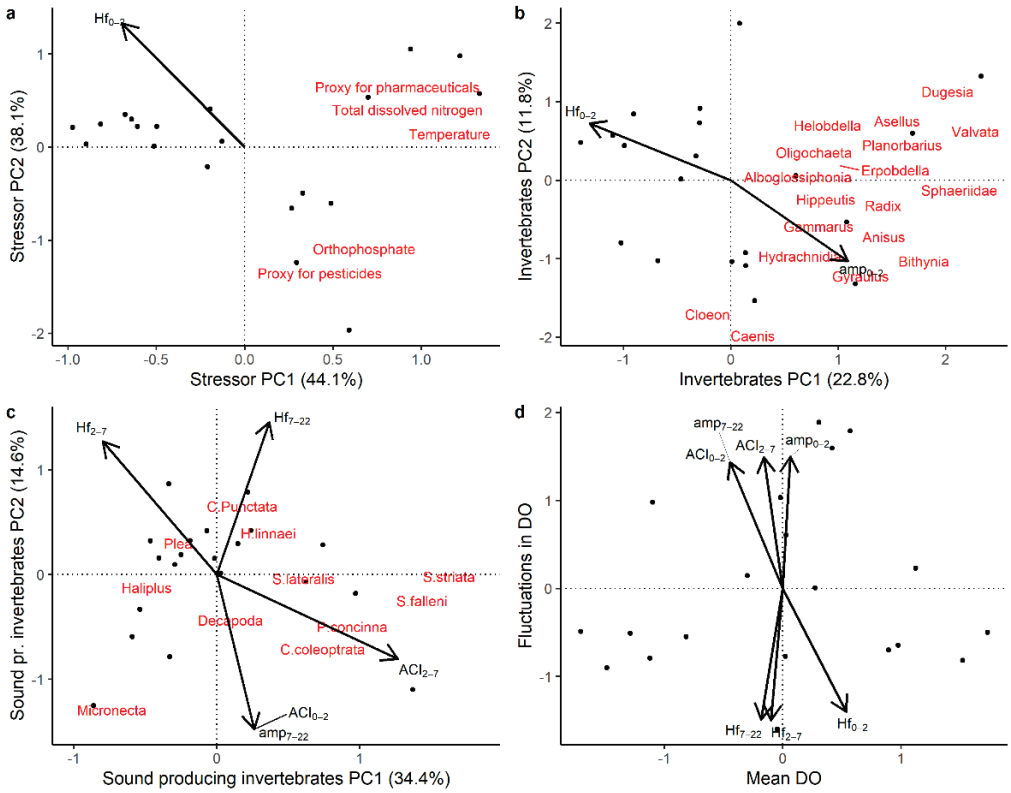


***Figure 4**: Relations between the acoustic indices and the ordination of different environmental variables, including a) stressors, b), entire invertebrate community composition c) sound-producing invertebrate community composition and d) dissolved oxygen dynamics. Only significant vectors (p < 0.05) are plotted (details in Table 3). Taxa shown are prioritized on abundance.*

DISCUSSION

The present study tested the relationship between acoustic patterns and the intensity of various anthropogenic stressors, invertebrate community composition and dissolved oxygen dynamics in temperate lowland streams and drainage ditches under a gradient of anthropogenic stress from agricultural activities and WWTP discharges. Our results showed that the acoustic patterns were primarily associated with the composition of the sound-producing invertebrate community and the fluctuation in dissolved oxygen saturation. Below we discuss to what extent these results corroborate the utility of passive acoustic monitoring in water quality assessment.

The sound-producing invertebrate community composition correlated to two acoustic indices in the frequency band 2-7 kHz (*ACI* and *Hf*), while the presence of *Micronecta* appeared to be associated with the high frequency band ($Amp_{7\text{-}22.05}$). This is coherent with the findings from previous studies showing that sounds produced by soniferous aquatic insects are concentrated within the 5-6.5 kHz range (Aiken, 1982), while *Micronecta* is known to generate a high-pitch sound with a dominant frequency in the 7-12 kHz band (Desjonquères et al., 2020b; Sueur et al., 2011). Indeed a wide diversity of invertebrates produce underwater sounds (Aiken, 1985; Desjonquères, 2016; Desjonquères et al., 2020a) and the family Corixidae is considered to be one of the main emitters of sounds in (shallow) freshwater environments (Desjonquères et al., 2018). Moreover, the daily pattern of these indices showed a distinct second peak at night, corroborating previous findings that Heteroptera chorus peak at 2 a.m. (Linke et al., 2020).

The acoustic indices were, however, not related to the intensity of the measured stressors. While the entire invertebrate community composition indicated several of the anthropogenic stressors, including nutrients, pollutants and dissolved oxygen fluctuation, the sound-producing taxa only related significantly to temperature. This corroborates the common use of invertebrate community composition in water quality assessment, as they are a diverse group reflecting a wide range of sensitivity to anthropogenic stressors (Rosenberg & Resh, 1993). The lack of a relationship between the measured stressors and the acoustic indices may be due to the fact that the majority of sound-producing invertebrate taxa occurring in these lowland waters belong to the order of Heteroptera, which are generally considered moderately tolerant to chemical stressors, such as nutrient loading and organic toxicants (Lock et al., 2013; Von der Ohe & Liess, 2004). Previous studies rather related their presence to the habitat structure of water bodies, such as vegetation coverage (Dias-Silva et al., 2010; Olosutean & Ilie, 2013), which was not included in our study. This was confirmed by Desjonquères et al., (2018) who showed that when vegetation density was included as part of the studied gradient, the acoustic community could indeed be related to the entire invertebrate community. So, even though the acoustic indices could represent the sound-producing invertebrate community, they may have limited value as

indicators in water quality assessment, as they did not represent the impact of different anthropogenic stressors.

Almost all acoustic indices (7 out of 9) were associated with the daily fluctuation in dissolved oxygen saturation and many of them showed a peak in the afternoon, which is a pattern typically observed for dissolved oxygen saturation in these water bodies. Similarly, Felisberto et al. (2015) observed that acoustic patterns in low (0.4-0.8 kHz) and medium (1.5-3.5 kHz) frequency bands followed the same diel cycle as measured by dissolved oxygen loggers in a marine environment. Interestingly, these frequency bands are similar to the ones for which high correlations values were observed in this study. Dissolved oxygen saturation in the water column is affected by 1) the release of oxygen by photosynthetic primary producers during the day, 2) the uptake of oxygen through respiration by all organisms and 3) the exchange of oxygen with the air (i.e. re-aeration) (Odum, 1956). Previous studies have shown that each of these processes produces sounds. Specifically, Kratochvil & Pollirer (2017) reported that an aquatic plant, *Elodea canadensis*, emits short sound pulses with a wide frequency band as it produces and releases oxygen bubbles in the water. Freeman et al. (2018) observed similar results for marine macroalgae and argued that these sounds may thus be used as an indicator for photosynthetic activity. The respiration by microorganisms decomposing organic matter (both aerobically and anaerobically) is also suspected to produce ticking sounds, as gas bubbles are formed and expelled (Felisberto et al., 2015; Linke et al., 2018). Lastly, Morse et al. (2007) were able to relate sounds at the water-air interface to re-aeration rates.

Our findings, along with these studies, indicate that acoustic indices could be used to estimate metabolism in water bodies, which may subsequently be used in water quality assessment. Passive acoustic monitoring may even overcome certain challenges encountered in the estimation of metabolism from diel dissolved oxygen curves, such as the possibility to split up different components if different processes emit different acoustic patterns, the ability to estimate re-aeration rates and the inclusion of anaerobic respiration (Staehr et al., 2012a). Future research should focus on the selection of suitable frequency bands or the detection of specific acoustic patterns that are not sensitive to confounding factors, affecting the acoustic patterns emitted by metabolism-related processes, such as the sounds of invertebrates, surface agitation due to wind and the influence of water movement on bubble formation and retention (Felisberto et al., 2015; Freeman et al., 2018). This may be achieved with a combination of laboratory and field studies identifying the acoustic patterns emitted by specific sources of underwater sounds.

In conclusion, the presently employed acoustics indices allowed the detection of sound-producing invertebrate taxa as well as the fluctuation in dissolved oxygen saturation. In terms of water quality assessment, the acoustic indices poorly indicated the intensity of the anthropogenic stressors compared to the traditional method which samples

invertebrate communities with a net, presumably due to the dominance of the relatively insensitive Heteroptera in the sound-producing community. In contrast, the strong relation between acoustic indices and oxygen fluctuation indicated that passive acoustic monitoring may be used to estimate metabolism in these water bodies. The knowledge of these sounds is still emerging, we therefore suggest that the next step in freshwater ecoacoustics is to precisely characterise the sounds individually emitted by photosynthesis, respiration and re-aeration, so these processes can be distinguished. This would greatly enhance the potential of ecoacoustics as a monitoring tool in freshwater environments.

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SUPPLEMENTARY MATERIAL 1

Table 1: Overview of study sites: coordinates, water body dimensions and closest Royal Netherlands Meteorological Institute (KNMI) meteorological station.

	Coordinates (WGS 84)		Water body dimensions			Closest meteorological station	
Nr	Latitude	Longitude	Depth (m)	Width (m)	Flow velocity (cm/s)	name	Proximity (km)
1	52°49'22.7"N	5°54'26.5"E	1.0	6.0	0.9	Marknesse	15
2	53°00'22.3"N	5°48'43.4"E	0.7	2.5	2.0	Leeuwarden	25
3	52°53'29.0"N	4°49'34.8"E	0.6	4.5	2.2	De Kooy	5
4	52°45'51.4"N	4°40'52.0"E	1.2	6.0	11.6	De Kooy	20
5	52°40'33.0"N	4°50'02.3"E	0.8	6.0	2.6	Berkhout	10
6	52°17'07.2"N	4°32'34.6"E	0.8	8.0	2.3	Amsterdam/Schiphol	20
7	52°17'23.2"N	4°30'37.7"E	0.6	4.0	1.3	Amsterdam/Schiphol	20
8	52°17'05.3"N	4°29'54.7"E	1.2	5.5	1.0	Amsterdam/Schiphol	20
9	52°08'08.2"N	4°48'37.6"E	1.0	2.8	1.4	Amsterdam/Schiphol	20
10	52°12'43.4"N	4°53'10.6"E	0.8	1.5	1.5	Amsterdam/Schiphol	10
11	52°15'20.5"N	5°05'15.2"E	1.0	5.0	1.3	De Bilt	15
12	51°25'40.9"N	4°46'46.8"E	1.0	3.0	1.3	Gilze-Rijen	20
13	51°30'46.1"N	4°50'57.2"E	0.4	1.5	7.3	Gilze-Rijen	10
14	51°33'54.2"N	4°59'13.1"E	0.6	4.5	3.4	Gilze-Rijen	5
15	51°36'08.3"N	5°04'32.9"E	0.4	5.0	25.5	Gilze-Rijen	10
16	51°30'15.0"N	5°10'19.9"E	0.4	4.0	14.7	Eindhoven	15
17	51°24'27.2"N	5°41'37.0"E	0.8	4.0	4.7	Eindhoven	20
18	51°17'52.0"N	5°36'18.8"E	0.8	5.5	1.4	Ell	15
19	51°13'50.3"N	5°37'31.4"E	0.6	3.0	2.2	Ell	10
20	51°18'09.7"N	5°29'09.6"E	0.6	8.0	17.7	Eindhoven	15

SUPPLEMENTARY MATERIAL 2-5

Acoustic recordings presented in Figure 1. Available online.

CHAPTER 8

PERSPECTIVES ON FUNCTIONAL ASSESSMENT OF ANTHROPOGENIC STRESSORS ON STREAM ECOSYSTEMS

This chapter is based on the manuscript under review by *Freshwater Science*

Piet F.M. Verdonschot & Gea H. van der Lee

ABSTRACT

Both ecosystem structure and function have been given attention in studies assessing the effect of anthropogenic stressors on running water ecosystems. However, few studies have examined to what extent functional assessment can aid surface water quality assessment compared to more traditional measures of ecosystem structure. This study examined how the functional parameters metabolism and decomposition are affected by different anthropogenic stressors. Although some general trends were identified, the overall impact of anthropogenic stressors on ecosystem functioning was complex, as the processes are largely context dependent and there is usually a combination of multiple stressors involved. We provide perspectives on how to deal with these issues in the future. Next, studies that combined structural and functional measures under anthropogenic stressors both from a mechanistic and assessment-based approach were addressed. It became evident from studies combining structural and functional measures, primarily associated with shredders and decomposition rates, that knowledge on the functional roles of individual species is necessary to understand the relation between structure and function. Based on this notion, we explored ideas on how mechanistic understanding of structure and related processes can improve assessment of stream ecosystems in the future. A way forward would be 1) to increase understanding of the role of individual species in the functioning of stream ecosystems, and 2) to quantify the response of individual species to stressors and combinations thereof. We propose that knowledge on suites of interacting traits that evolved under local environmental abiotic and biotic conditions of all organism groups could help to better comprehend multiple stressor effects on ecosystem structure and function, and thereby aid water management in taking more accurate measures.

INTRODUCTION

Over the last decades, both ecosystem structure and function have increasingly been given attention in studies assessing the effect of anthropogenic stressors on running water ecosystems (Dale & Beyeler, 2001; Giller et al., 2004; Young et al., 2008; Von Schiller et al., 2017). Already in 1960, Hynes & Pentelow illustrated patterns in structural and functional parameters after a point discharge of organic waste in a river, using several biological groups and biological oxygen demand as parameters (Hynes & Pentelow, 1960). Structural parameters have since then gained a proven record in water quality assessment, as they are sensitive to a variety of anthropogenic stressors. Communities, especially of macroinvertebrates, are relatively diverse and can thus reflect small changes in species losses (e.g. Clapcott et al., 2012). Moreover, single species trait-based information can provide a causal diagnosis of different anthropogenic stressors (Culp et al., 2011). A large body of evidence is, however, showing that aquatic ecologists should not only study the organisms present within the environment, but also their roles in ecosystem processes or functioning, as changes in species compositions, especially species losses, can alter ecosystem function (Boulton, 1999; Karr, 1999; Loreau et al., 2002; Gücker et al., 2006; Bergfur et al., 2007; Palmer & Febria, 2012). Nevertheless, water quality assessment continued to rely mostly on structural parameters like indicator species, species diversity and species composition (e.g. Rosenberg & Resh, 1993; Bailey et al., 2004). Hereby, a positive relation between structure and function in running water ecosystems is often assumed (Lecerf, 2006).

Studies have considered function from a structural perspective, using food webs (Hladyz et al., 2011a), functional feeding groups (Cummins & Klug, 1979), or other functional traits to better understand and explain ecosystem processes (Dolédec et al., 1999; McGill et al., 2006; Bergfur et al., 2007; Poff et al., 2010; Frainer & McKie, 2015; Raffard et al., 2017; Truchy et al., 2019). However, 'true' ecosystem processes refer to system dynamics, like leaf litter decomposition (Gessner & Chauvet, 2002), ecosystem metabolism (Fellows et al. 2006), primary and secondary production (Wallace et al., 1996; Udy et al., 2006) and the mechanisms behind these processes, like nutrient uptake (Niyogi et al., 2004; Bukaveckas, 2007), carbon flux into the food web (Marcarelli et al., 2011) and oxygen regimes (Cox, 2003). Measurements of functional parameters that provide insights into these "true" ecosystem processes, are relatively inexpensive, straightforward to deploy and in case of metabolism amenable to automation (Collier et al., 2013).

Functional measures inform about how ecosystems operate, while structural measures inform about the condition of the ecosystem (Dale & Beyeler, 2001; Tilman, 2001). A combination of ecosystem structural and functional measures could thus potentially strengthen tools to assess ecosystem integrity and help water management in taking accurate measures (Young et al., 2008; Feio et al., 2010). Although several studies

have attempted to quantify the response of different stressors on ecosystem processes (e.g. Sponseller & Benfield, 2001; Bergfur et al., 2007; Young et al., 2008; McKie & Malmqvist, 2009; Frainer & McKie, 2015; Bruder et al., 2016; Clapcott et al., 2016; Von Schiller et al., 2017; Frainer et al., 2018), few examined to what extent functional assessment can aid surface water quality or status assessment compared to more traditional measures of ecosystem structure (but see Pascoal et al., 2001; Hagen et al., 2006; Death et al., 2009). Therefore, the purpose of this paper is to provide more insight into the use of ecosystem function in relation to structure in the assessment of anthropogenic stressors on running waters. Hereby, we focused on two main questions: 1) Do functional parameters add to the understanding of the effects of anthropogenic stressors on streams and rivers?, and 2) Do structural and functional parameters provide complementary information on the effects of anthropogenic stressors on streams and rivers?.

We focused on stressors that are commonly caused by human activities and that show general effects on ecosystem structure and functioning, including warming, light increase, nutrient and organic loading, hydro-morphological modification and chemical stress (Birk et al., 2012; Hering et al., 2015). Stress from invasive species was excluded, as their impact on the ecosystem is dependent on the ecology of each species and therefore difficult to generalize (Von Schiller et al., 2017). As functional parameters we included ecosystem metabolism (primary production and respiration) and decomposition of particulate organic matter, which have previously been suggested to be suited for water quality assessment, as they are sensitive to anthropogenic stressors and relatively easy and inexpensive to measure (Young et al., 2008). As structural measures we focused on the most commonly included biological groups, i.e. bacteria, fungi, algae, macrophytes and macroinvertebrates. We did not include all papers published on this topic, but rather attempted to have looked at a relevant set to provide insight and perspectives on whether functional assessment can assist water management in making more effective decisions, building on three major review papers in the field (Webster & Benfield, 1986; Young et al., 2008; Tank et al., 2010).

We start by examining how ecosystem processes (i.e. metabolism and decomposition) are affected by different anthropogenic stressors. Next, we discuss that this relation is context dependent and usually involves a combination of multiple stressors. We provide perspectives on how to deal with these topics in the future. Then we address studies that combined structural and functional measures under anthropogenic stress both from a mechanistic and assessment-based approach. Lastly, we explore ideas on how mechanistic understanding of structure and related processes can improve assessment of stream ecosystems in the future.

METABOLISM UNDER ANTHROPOGENIC STRESSORS

Metabolism is the flux of matter and energy within a stream, encompassing photosynthetic fixation of inorganic to organic carbon, and respiratory loss or mineralization of organic to inorganic carbon (Odum, 1956; Woodwell & Whittaker, 1968; Chapin et al., 2006). Primary production in streams is performed by algae, bryophytes and aquatic macrophytes, while all life forms respire to carry out metabolic activity (Figure 1; Likens, 1975; Brown et al., 2004). Metabolism is usually estimated from measurements of the diel change in dissolved oxygen (DO) concentration in the open channel or by enclosing part of the stream in an air-tight chamber and measuring DO changes in the chamber (Tank et al., 2010; Staehr et al., 2012a). Both techniques estimate metabolism with inherent uncertainty. For the chamber methods containment artifacts and scaling issues are problematic, while for the open-system methods uncertainty is associated with the estimates of DO diffusing between the air and water (reaeration rates). These technical issues are further discussed elsewhere, e.g. Staehr et al. (2012a) and Demars et al. (2015).

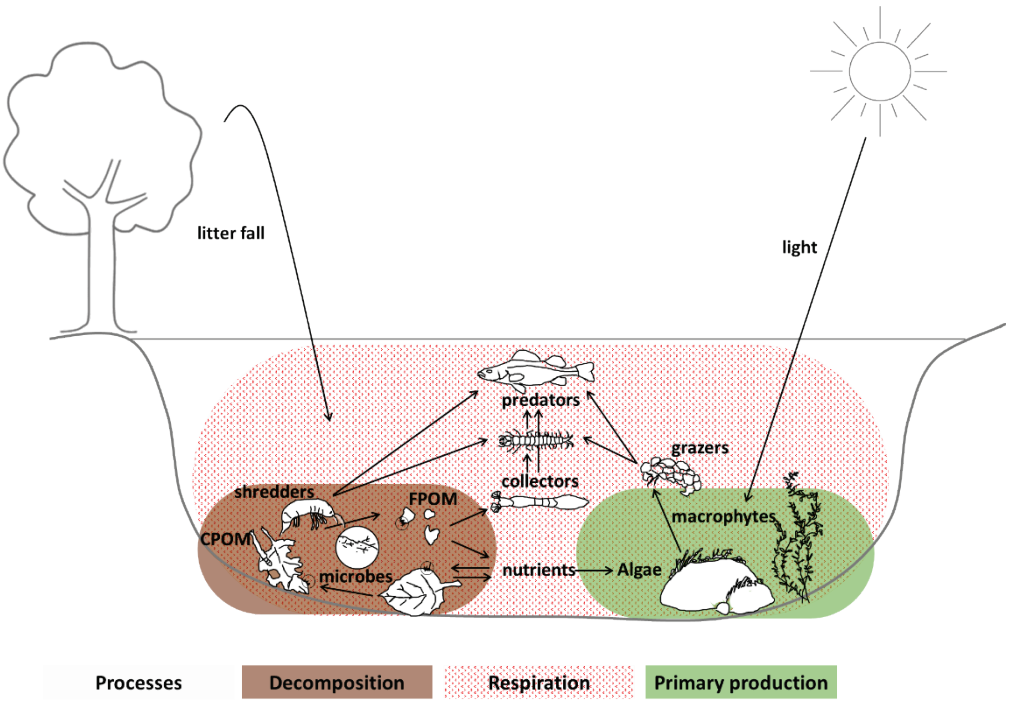

***Figure 1**: Aquatic food web structure in relation to ecosystem processes (primary production, respiration and decomposition).*

Light and nutrients are required for photosynthesis and generally lead to higher primary production (e.g. Phinney & McIntire, 1965; Steinman & McIntire, 1987; Bott et al., 2006; Dodds, 2006; Bernot et al., 2010). An increase in these parameters is termed stressors when acting as a debilitating agent or i.e. setting ecosystems in a self-reinforcing motion that creates further degradation (Odum et al., 1979). A positive link is also frequently observed between primary production and warming of stream water. Warming may increase primary production through increased temperature-dependent physiological rates and changes in the trophic structure (Phinney & McIntire, 1965; Petchey et al., 1999; Rasmussen et al., 2011), however, this positive relation is presumably also mediated through increased light availability (Young et al., 2008). The impact of organic pollution is ambiguous as some studies observed an increase (Quinn & McFarlane, 1989; Paul & Meyer, 2001; Bott et al., 2006; Gücker et al., 2006, Aristi et al., 2015) and others a decrease in primary production (Meyer & Edwards, 1990; Minshall et al., 1992; McTammany et al., 2003; Rodríguez-Castillo et al., 2017). Aristi et al. (2015) argued that the specific impact of organic pollution is related to whether the increased nutrient supply promoted activity of primary producers or whether the reduced light availability suppressed the activity of primary producers. In terms of hydrological stress, studies commonly observed a decrease in primary production under high flows and floods (e.g. Uehlinger et al., 2003; Roberts et al., 2007; Val et al., 2016), while the opposite was the case for low flows and droughts (e.g. Marcarelli et al., 2010; Acuña et al., 2010; Val et al., 2016). High flows and floods generally related to decreased substrate stability and the resulting increase in turbidity may reduce light conditions (Uehlinger, 2000; Morgan et al., 2006; Leggieri et al., 2013). Furthermore, scouring may reduce the biomass of epiphytic and benthic algae and abundance and diversity of macrophytes (Riis & Biggs, 2003; Vilches & Giorgi, 2010) and as such reduce primary production. Other authors have, however, reported that after a flood epiphyton was more productive because of the greater availability of nutrients (Stevenson, 1990). Low flows and droughts may enhance primary production through increased substrate stability, reduced shear stress on periphyton, enhanced macrophyte establishment, increased light availability and increased nutrient concentrations due to decreased dilution (Marcarelli et al., 2010; Val et al., 2016). Morphological alterations of natural streams are diverse, ranging from the removal of large instream wood to the construction of new artificial channels (Elosegi & Sabater, 2013). The change in primary production due to morphological alterations is, therefore, dependent on whether the channel cross-sectional area is increased or decreased, resulting in different flow velocity patterns, shear stress and sediment transport (Gücker et al., 2009). Lastly, toxicants and other forms of chemical pollution may inhibit primary production if the present algae and macrophytes are sensitive to the particular substance (Peters et al., 2013), however, this effect may be offset by

nutrients in toxic discharge (Young et al., 2008) or by a reduction in grazers (Niyogi et al., 2002).

Respiration is primarily driven by the availability of labile organic matter and temperature, and to a lesser extent nutrients (Bernot et al., 2010). Both organic pollution and increased nutrient loading form a subsidy for microbial processes and thus generally result in increased respiration (e.g. Mulholland et al., 2001; Roberts et al., 2007; Aristi et al., 2015). Warming is also often related to enhanced respiration as temperature is an important regulator of metabolic activity (Phinney & McIntire, 1965; Sinsabaugh, 1997; Acuña et al., 2008; Rasmussen et al., 2011). The sensitivity of respiration to temperature may, however, be obscured by the quantity and quality of organic matter in the stream (Mulholland et al., 2001; Acuña et al., 2004; Jankowski et al., 2014). For example, in the study of Roberts et al. (2007) high temperatures coincided with low organic matter availability during summer and thus resulted in reduced respiration. No impact of light availability on respiration is expected, as processes that contribute to respiration are not necessarily dependent on light (McTammany et al., 2007; Young et al., 2008). Light availability could, however, be related to respiration if algae are responsible for a large portion of respiration in the stream (e.g. Benson et al., 2013). Little is known to what extent hydro-morphological changes impact ecosystem respiration (Elosegi & Sabater, 2013). Increased flow may enhance respiration through the input of organic matter, while the transport capacity of organic matter decreases during low flow events (e.g. Roberts et al., 2007; Val et al., 2016). In other streams, a small reduction in respiration was observed after floods and an increase after droughts (Acuña et al., 2004; Uehlinger et al., 2003; Uehlinger, 2006). This difference is presumably related to the differences in stability of the bed sediment between streams (Uehlinger & Naegeli, 1998). The effects on respiration from scouring during flooding may be restricted, as microbes are protected from abrasion in the hyporheic zone (Uehlinger & Naegeli, 1998; Chester & Norris, 2006; Benson et al., 2013). Like primary production, the effect of toxicants and other chemical pollution on respiration is dependent on whether a shift in community can compensate for toxicant induced species losses or whether other stressors cancel out the effect of toxicants (Schäfer et al., 2012a).

DECOMPOSITION UNDER ANTHROPOGENIC STRESS

Decomposition, the loss of detrital mass over time, takes place through several interacting processes, including leaching, microbial (fungal and bacterial) and macroinvertebrate activities and abrasion (Figure 1; Webster & Benfield, 1986; Graça, 2001). These processes are subject to the chemical and physical properties of organic matter, the microbial and macroinvertebrate community present and the environmental factors (Webster & Benfield, 1986). Estimates of decomposition rates are mostly based on the measurement of mass loss of leaves incubated over a certain period in the stream (Meyer, 1980; Boulton & Boon,

1991). Decomposition in coarse-mesh bags is affected both by physical fragmentation, invertebrate and microbial activity, while in fine mesh bags it is mainly affected by microbial activity (Gessner & Chauvet, 2002). These litter-bag studies may, however, overestimate the importance of macroinvertebrates to decomposition rates as losses of incompletely decayed fragments from physical and biological fragmentation are included in the estimates (Brinson et al., 1981; Heard et al., 1999; Lepori et al., 2005; Friberg et al., 2009) and shredders may aggregate on experimental leaf packs when litter resources in streams are rare (Tiegs et al., 2008). Other factors causing variation in the results are, for example, the intra- and interspecific variability in quality (e.g. nutrients ratios) of the incubated leaves (Gessner & Chauvet, 1994; Suberkropp & Chauvet, 1995; Lecerf & Chauvet, 2008), incubation time (Bergfur et al., 2007) and natural variation in external factors, like seasonality (Frainer et al., 2014; Frainer & McKie, 2015) and inter-annual variability (Yeung et al., 2018).

A systematic quantitative assessment of litter decomposition across a gradient of nutrient enrichment at the continental scale by Woodward et al. (2012) showed that breakdown rates are low at both ends of the nutrient gradient. Microorganisms can assimilate nutrients from the water column, resulting in higher rates of decomposition when there is external nutrient loading, with the largest effects when background nutrient concentrations are low (Suberkropp & Chauvet, 1995; Woodward et al., 2012; Ferreira et al., 2015; Jabiol et al., 2019). This positive effect on decomposition rates by microbes may, however, be counteracted by a reduction in shredder abundance when elevated nutrient concentrations result in low dissolved oxygen levels (Hagen et al., 2006) or other stressors deteriorating the environmental conditions (Woodward et al., 2012). Similarly, organic pollution can lead to a rapid decline of dissolved oxygen, which frequently coincided with slower decomposition rates (Chauvet, 1997; Pascoal & Cássio, 2004). Both field and laboratory studies demonstrated that warming results in faster breakdown rates, particularly related to microbial processes (see review by Webster & Benfield, 1986). High flows are generally associated with increased decomposition rates, which may relate to enhanced physical fragmentation (Heard et al., 1999; Lepori et al., 2005; Paul et al., 2006) and changes in the consumer community (Gaudes et al., 2009). Especially in autumn, enhanced decomposition rates may be related to increased sediment transport and high current velocities (Ferreira et al., 2006b). Low flows, droughts and morphological alterations generally resulted in lower decomposition rates, because conditions became unfavorable for key shredders species (Gelroth & Marzolf, 1978; Schlief & Mutz, 2009; Mendoza-Lera et al., 2012; González et al., 2013) and fungal performance decreased or ceased (Bruder et al., 2011). The sensitivity of macroinvertebrates to reduced flow depends on the species and the amount of flow reduction (Dewson et al., 2007a) and in some cases no change in macroinvertebrate composition and decomposition was observed after flow reduction (e.g.

Dewson et al., 2007b). Furthermore, decomposition of organic substrates in intermittent and ephemeral streams is affected by drying-rewetting cycles (Fierer & Schimel, 2002) resulting in higher leaching effects (Shumilova et al., 2019). Decomposition was usually slower in streams impacted by toxic pollution and other chemical stressors, such as metal pollution (Carlisle & Clements, 2005; Chaffin et al., 2005), pesticides (Schäfer et al., 2007), acidification (Dangles et al., 2004) and salinity (Schäfer et al., 2012b). Meta-analyses by Peters et al. (2013) and Ferreira et al. (2016) showed that the effect of pesticides and metals was more evident in the presence of macroinvertebrate decomposers than when only microbes were involved in decomposition. Tolerant microorganisms may be able to replace sensitive species, maintaining their function (Blanck, 2002; Feckler et al., 2018).

CONTEXT DEPENDENCE OF FUNCTIONAL PARAMETERS

Although some general trends of anthropogenic stressors on ecosystem functioning were identified, the overall response is complex as the processes largely depend on site-specific factors (Tank et al., 2010). First, site-specific abiotic factors may impact how anthropogenic stressors affect ecosystem processes, e.g. temperature and pH can strongly affect toxicant bioavailability and subsequently cause high variability in the effect on ecosystem functioning (Peters et al., 2013; Ferreira et al., 2016). These abiotic factors may also vary in time and space. For example, Frainer & McKie (2015) found that the changes in decomposition along a land-use gradient with increasing nutrients became less obvious due to the loss of detritivore functional diversity along the gradient in autumn and effects were completely offset by a shift in functional trait composition in spring. Beside temporal and spatial scale, habitat heterogeneity can lead to different functional responses (Collier et al., 2013). Second, the presence of dominant taxa can influence the effect of a stressor on process rates (Dangles & Malmqvist, 2004). This is particular evident in decomposition studies where the impact of stressors on specific species of amphipods (Dangles & Guerold, 2001; Dangles et al., 2004; Lecerf et al., 2006; Piscart et al., 2009; Rasmussen et al., 2012b), isopods (Bergfur et al., 2007), caddisflies (Robinson et al., 1998; McKie et al., 2006) and stoneflies (Carlisle & Clements, 2005) caused significant differences in decomposition rates. Furthermore, dominant functional feeding groups may change along a stressor gradient, as was shown for an acidification gradient by Layer et al. (2013). The authors showed that the resistance of a dominant generalist herbivore-detritivore delayed the recovery of specialist grazers along the acidification gradient, and thereby may delay functional recovery. Third, the impact on functioning depends on the length and position of the studied stressor gradient. In moderately stressed systems process rates may increase, while process rates may be suppressed under influence of high stress, which can lead to a non-monotonic response (Niyogi et al., 2002; Hagen et al., 2006; Young & Collier, 2009; Clapcott et al., 2010; Woodward et al., 2010). Studies comparing process rates in reference streams to one level

of alteration of the stream or a short section of the gradient may lead to different results (Woodward et al., 2012; Feld et al., 2016).

It is thus important to consider the impact of abiotic factors in time and space, the presence of dominant taxa and the length and position of the stressor gradient when studying the impact of anthropogenic stressors on ecosystem functioning. A histogram can, for example, be used to check the range and distribution of stressor values, which can be compared to values reported previously in the targeted ecosystem (Feld et al., 2016). Moreover, Truchy et al. (2019) provided a valuable approach to partition the influence of context dependence, in their case spatial structuring and community composition, from the effect of environmental variables on ecosystem functioning using variance partitioning analysis. Such an approach can potentially increase the explanatory power in studies on ecosystem processes (Truchy et al., 2019).

MULTIPLE STRESSOR EFFECTS ON FUNCTIONAL PARAMETERS

Anthropogenic activities commonly result in changes in multiple stressors (Ormerod et al., 2010), e.g. agricultural activities can result in increased nutrient loading, decreased oxygen concentration, increased concentration of pesticides, sedimentation and removal of riparian vegetation. However, frequently studies focus on the effect of a single stressor in isolation, even though this stressor may not be limiting or influencing the functional response (Clapcott et al., 2010). For instance, the removal of riparian vegetation results in more available light for which one would expect an increase in primary production, but removal of vegetation can also increase turbidity through sediment runoff decreasing primary production (Young & Huryn, 1999; Frankforter et al., 2010). The removal of riparian vegetation may also limit the distribution of shredders, e.g. directly by removal of egg deposition structures or indirectly by altering the quality of allochthonous inputs and thus influence leaf breakdown rates (Sponseller & Benfield, 2001). Further, interacting stressors may lessen (antagonism) or amplify (synergism) the effects of each individual stressor (Piggott et al., 2015). Synergism was, for example, observed by Matthaei et al. (2010) when the negative effects of fine sediment inputs on algal production were stronger at reduced flow. In the study of Bruder et al. (2016) stressor interactions associated with agricultural land use were less common than additive effects, but in some cases the synergistic interactive effects on decomposition were of the same magnitude as the main stressor effects. Gücker et al. (2009) showed two cases of antagonism in agricultural streams, namely physical stress counteracted the effects of eutrophication diminishing respiration, whereas eutrophication counteracted the effects of physical stress enhancing primary production.

Quantification of multiple stressor effects need thus be accounted for when assessing anthropogenic impacts on ecosystem functioning. Potential approaches include

an experimental design incorporating different sequences of naturally occurring stressors with several sampling dates: before application of the stressors, after the first application of stressors (e.g. treatment: A, B, A, B and control), after the second application of stressors (e.g. treatment: B, A, A, B and control) and on additional occasions after the second application to follow recovery of the system (see Giller et al., 2004). The effect of multiple stressors on ecosystem functioning may also be disentangled using extensive statistical analysis on large datasets (see protocol presented by Feld et al., 2016). Another promising perspective was provided by Bruder et al. (2019) who advocated ecological network theory as a way forward. Ecological networks include both biotic (trophic and non-trophic) and environmental interactions and can show the direct and indirect impacts of multiple stressors on organisms, communities and ecosystem processes. Beside the need to include multiple stressors, it is recommended to use similar methods to consider multiple processes simultaneously, as different processes can interact or respond in opposite direction and thereby influence the overall functioning of ecosystems (Gilling et al., 2018).

MECHANISMS BETWEEN STRUCTURAL AND FUNCTIONAL RESPONSES

A selection of studies that combined structural and functional measures when studying the effect of anthropogenic stressors are listed in Table 1. Several studies focused on the combination of decomposition as functional measure and diversity and/or abundance of macroinvertebrates and/or microbes as structural measures, yielding various outcomes. For example, the loss of fungal diversity in eutrophied streams reported by Lecerf & Chauvet (2008) contrasted with the results of other eutrophication experiments showing either a positive effect on both fungi and decomposition (Gulis & Suberkropp, 2003) or no effect at all (Ferreira et al., 2006a). Lecerf & Chauvet (2008) suggested that oxygen depletion on the surface of leaves, caused by intense deposition of fine sediments and oxidation of organic matter by other microorganisms, was detrimental to aquatic hyphomycetes. Despite the increase of fungal spores and mycelial biomass due to nutrient enrichment, the oxygen regime could strongly determine the observed effect on decomposition (see also Bruder et al., 2016). The relation between microbial and invertebrate structure and decomposition may also change with environmental conditions, like temperature oscillations related to, amongst others, climate or season (Dang et al., 2009) or site-specific differences in habitat (Robinson et al., 1998). Even the plant species used in litter decomposition studies may affect the relation observed between structure and function, e.g. in the study by Bruder et al. (2016) fungi appeared important for birch litter decomposition but played a minor role for mahoe litter decomposition.

Apart from environmental conditions, the functional roles of individual species are important in affecting the relation between ecosystem structure and function (Dangles & Guerold, 2001), as the effects of anthropogenic stressors on ecosystem processes is often

mediated through effects on the community composition (Truchy et al., 2019). Currently this knowledge is mainly associated with the role shredders play in decomposition under different types of stressors. Shredder species can differ substantially in the degree that they decompose litter (Dangles & Guerold, 2001). Dangles & Guerold (2000) demonstrated that acidification changed the shredder community structure which in turn changed the litter breakdown process and impact upland stream functioning. Specifically, the difference in acid-tolerance between *Protonemura* sp. (Plecoptera: Nemouridae) and *Gammarus fossarum* determined the breakdown rate (Dangles & Guerold, 2001). Also liming caused negative effects on both macroinvertebrate assemblages, in terms of evenness and abundance of shredders and litter decomposition due to a change in relative occurrence of limnephilid caddisflies versus stoneflies (Mckie et al., 2006). Contrary, Jonsson & Malmqvist (2003) in experiments showed that increase in shredder richness enhanced leaf breakdown rates.

Even minor changes in community composition, such as the loss of a single species, can thus lead to disproportionate changes in decomposition (e.g. Carlisle & Clements, 2005; Lecerf et al., 2006; Bergfur et al., 2007). Others have, however, observed that taxonomic richness of shredders has greater effect on litter processing than shredder biomass (e.g. Huryn et al., 2002; Jonsonn et al., 2002). Frainer et al. (2014) observed a variation in detritivore functional diversity (i.e. a combination of feeding strategy, biomass, emergence period and substrate and current velocity preference) between seasons in stream pools and riffles, indicating the importance of fluctuations in the relative abundances not of species, but of species functional roles for ecosystem process rates. Furthermore, the functional roles appear not only to be driven by environmental conditions but also by geographical position which suggests that spatial structuring in ecosystem functioning beyond that attributable to species sorting along environmental gradients can be important (Truchy et al., 2019). Species richness could enhance decomposition rates in experiments, even when all species belonged to the same guild (Jonsson & Malmqvist, 2000). Three potential mechanisms could explain the increase in decomposition rates with increasing species richness, namely 1) facilitation between species (i.e. niche partitioning), 2) less negative interactions (i.e. behavioral interactions might be less in diverse communities, so more time can be spent on feeding) (Jonsson & Malmqvist, 2000), and 3) the insurance effect (i.e. diverse communities are more likely to include tolerant species, which are able to compensate for those negatively affected by a given stressor) (Loreau et al., 2002; McKie et al., 2009). In general, environmental conditions can thus moderate structure - function relationships either by suppressing the role of a dominant taxon or by degrading diversity (Cardinale & Palmer, 2002), which underlies the importance of understanding species roles in ecosystem processes when assessing ecological consequences of anthropogenic stressors (Carlisle & Clements, 2005).

Table 1: A selection of studies providing mechanistic (structure-based) explanations of the functional response to anthropogenic stressors.

Structural measure	FuncFtional measure		
	Production	**Respiration**	**Decomposition**
Bacteria	Corcoll et al. 2015	Masseret et al. 1998, Carlisle and Clements 2005	Pascoal et al 2001, Lecerf et al. 2006, Hladyz et al. 2011b
Fungi			Suberkropp and Chauvet 1995, Gulis and Suberkropp 2003, Bärlocher and Corkum 2003, Gulis et al. 2006, Ferreira et al. 2006b, Bergfur et al. 2007, Castela et al. 2008, Lecerf and Chauvet 2008, Bruder et al. 2016
Algae	Crossey and La Point 1988, Masseret et al. 1998, Cardinale and Palmer 2002, Uehlinger et al. 2003, Death et al. 2009, Hladyz et al. 2011b, Corcoll et al. 2015, Truchy et al. 2019	Crossey and La Point 1988, Corcoll et al. 2015	Smeti et al. 2019
Macrophytes			Gücker et al. 2006
Invertebrates (general)	Clapcott et al. 2010, Savoy et al. 2019	Clapcott et al. 2010	Robinson et al. 1998, Jonsson and Malmqvist 2000, Loreau et al., 2002, Woodcock and Huryn 2004, Acuña et al. 2005, Hagen et al. 2006, Gücker et al. 2006, Bergfur et al. 2007, McKie et al. 2009, Dang et al. 2009, Death et al. 2009, Gücker et al. 2009, Clapcott et al. 2010, Hladyz et al. 2011b, Riipinen et al. 2009, Schäfer et al. 2012b, Collier et al. 2013, Smeti et al. 2019, Truchy et al. 2019
Shredders			Brown et al. 1983, Dangles and Guerold 2001, Pascoal et al. 2001, Huryn et al. 2002, Jonsson et al. 2002, Jonsson and Malmqvist 2003, Woodcock and Huryn 2004, Dangles et al. 2004, Carlisle and Clements 2005, Gulis et al. 2006, McKie et al. 2006, Lecerf et al. 2006, Bergfur et al. 2007, McKie and Malmqvist 2009, Riipinen et al. 2009, Frainer et al. 2014
Grazers	Hill et al. 2001, Friberg et al. 2009, Hladyz et al. 2011b		
Filter-feeders	Cardinale and Palmer 2002, Friberg et al. 2009		Cardinale and Palmer 2002, Cardinale et al. 2002

Although most studies focused on explaining the mechanisms between structural parameters and decomposition, some examples exist of studies that focused on the relation between structural parameters and metabolism (Table 1). For example, Uehlinger et al. (2003) showed that floods temporarily reduced periphyton biomass and composition, as well as ecosystem metabolism, although metabolism recovered relatively fast while a persistent shift in the periphyton structure was observed with high inter-annual variation. Other studies reported a lowering of periphyton species richness and diversity, because sensitive species were replaced by resistant species (e.g. green algae and heterotrophs), in combination with increased community metabolism due to heavy metals (Crossey & La Point, 1988), treated waste water (Masseret et al., 1998) and pharmaceuticals (Corcoll et al., 2015). Invertebrate community changes were also shown to affect metabolism, e.g. an increase in grazing snails resulted in an increase in productivity of their food resource (periphyton) (Friberg et al., 2009). Moreover, it has recently been proposed that characterizing productivity regimes can also provide information on the structure of aquatic communities, as the magnitude and timing of primary production can affect primary and secondary consumers (Savoy et al., 2019).

STRUCTURE AND FUNCTION IN ASSESSMENT OF ANTHROPOGENIC STRESS

Several studies in Table 1 stated specifically that structural and functional measures were complementary and should be used in concert to reflect an integrated assessment of the stream ecosystem. It was proposed that in combination, they capture a broader range of potential effects and provide insights into the functional consequences of changes in community structure (e.g. Crossey & La Point, 1988; Pascoal et al., 2001; Friberg et al., 2009; Collier et al., 2013). For example, in the study by Pascoal et al. (2001) macroinvertebrates increased in density but decreased in taxon richness in relation to an increase in nutrient concentrations from waste water effluent, while leaf breakdown rates were significantly stimulated. That both structural and functional attributes can give complementary information on changes was also shown by Friberg et al. (2009) studying a temperature gradient. Higher temperature related to an increase in density and decrease in diversity of invertebrates, as well as an increase in decomposition rates. While some other structural (i.e. macrophytes and fish community composition) and functional (i.e. algal productivity) measures did not respond to the temperature gradient (Friberg et al., 2009). The difference in response of structural and functional measures to reach-scale pressures and local habitat conditions may strengthen their complementarity (Collier et al., 2013).

Other studies argued that functional measures were even more sensitive than structural measures (e.g. Dangles et al., 2004; Gulis et al., 2006; Riipinen et al., 2009). Gulis et al. (2006) showed that decomposition responded to low levels of eutrophication, while all of the streams were classified as having ‘very good’ ecological conditions according to

the used macroinvertebrate index. Measurements of leaf litter breakdown also demonstrated a stronger difference in response to acidification in comparison to structural measures, like total abundance, biomass and species richness of detritivores (Dangles et al., 2004; Riipinen et al., 2009). Moreover, it has been argued that functional parameters have added value in assessment because they vary less than structural parameters among bioregions (e.g. Young et al., 2008). In a study including three bioregions of New Zealand, four out of five functional indicators exhibited predictable relationships with land use independent of bioregion (Clapcott et al., 2010).

However, others found high variability in functional measures (see context dependence of functional parameters) and argued that structural measures give a better indication of anthropogenic stressors (e.g. Crossey & La Point, 1988; Hagen et al., 2006; Death et al., 2009). The results of the study of Crossey & La Point (1988) on the effectiveness of community production and respiration measurements as monitoring tools for environmental impact evaluations indicated that inherent variability may limit the use of these community functional measures in routine environmental monitoring. Hagen et al. (2006) concluded that leaf breakdown rates may not be a useful indicator of stream integrity because of the complex effects that agricultural land use had on breakdown rates, while the land use gradient was reflected consistently in the structural invertebrate measures. Similarly, Bergfur et al. (2007) and Death et al. (2009) concluded that macroinvertebrate metrics performed much better than leaf litter breakdown rates along an enrichment and water abstraction gradient, respectively. The functional responses, or lack thereof, to water abstraction could only be explained by more thorough investigation of the individual responses of each of the New Zealand streams, which would not make it a more efficient or complementary than structural measures in assessment (Death et al., 2009).

Taking all these studies into consideration, it may be concluded that data on community composition together with functional measures, such as decomposition rates, greatly contributed to the interpretation of stressor effects and plea for a combined approach (Huryn et al., 2002; McKie et al., 2006; McKie & Malmqvist, 2009).

FUTURE PERSPECTIVES ON STREAM ASSESSMENT

Good water management requires action and prediction based on diagnostic information. Mechanistic understanding of the structure and related processes can support more accurate management (Elosegi et al., 2017). Therefore, a way forward could be 1) to increase understanding of the role of individual species in the functioning of stream ecosystems, and 2) to quantify the response of individual species to stressors and combinations thereof. To understand the role of individual species in the functioning of stream ecosystems single trait approaches have been advocated in the past, but appeared

unsuccessful. Potentially, the use of the evolutionary perspective of how functional traits have coevolved in response to natural selection could improve this knowledge (Southwood, 1977; Grime, 1979; Winemiller, 1992). Selection pressures do not act independently on single traits but rather on species with a suite of multiple interacting traits (Pilière et al., 2016). The adaptive value of a particular trait may differ within and across species, depending on the life stage, other traits possessed by the species and the prevailing environmental conditions (Statzner & Bĕche, 2010; Rubach et al., 2011; Wilkes et al., 2017). Species plasticity in resource requirement enlarges its fundamental niche and plasticity in the proportional resource uptake results in expansion of the realized niche (Berg & Ellers, 2010). Both enlarge the adaptive value of a trait and thereby strengthen the functional role of a species in a community. Local environmental (abiotic and biotic) conditions thus determine the structure and function of a local community (Leibold et al., 2004). Knowledge on suites of interacting traits that evolved under local environmental abiotic and biotic conditions (including multiple stressor effects) would help to better understand ecosystem structure and function (Leibold et al., 2004; Hamilton et al., 2019). Furthermore, the tolerance of species traits to one environmental factor (or stressor) can affect the response of communities and functional groups to other stressors. An environmental factor (or stressor) induced shift in a certain trait adaptation by natural selection will strengthening positive co-tolerance among taxa, but a lack of such exposure and adaptation can be expected to decrease co-tolerance and reduce resistance (Vinebrooke et al., 2004). This reasoning does not only account for macroinvertebrates but can be projected on many organism groups, including microbes (e.g. McGee, 2011; Winnemiller et al., 2015). Development of molecular technology that allows for identification and characterization of the functional traits of microbial communities may thereby add to a better understanding of ecosystem functioning (Sims et al., 2013). However, most response and effect traits are based on several interacting genes that are difficult to understand in combination. Moreover, many genes indicative of a trait might not be expressed and therefore be irrelevant for measurable traits but picked up by DNA-mining approaches.

To quantify the response of individual species to stressors and combinations thereof, the development of multiple trait-based species or species assemblage quantified sensitivities to specific stressors could offer potential. Such approach requires a more targeted selection of physiological, behavioural or life-cycle traits, i.e., ones that have a clear mechanistic relationship to single stressors (fundamental determinants of intrinsic sensitivity) and the processes induced by the stressor (e.g. Rubach et al., 2010; Ippolito et al., 2012). Several recent studies have addressed multiple important traits (e.g. Poff & Allan, 1995; Lamouroux et al., 2004). But there has been rather little distinction made between the traits or trait combinations that are truly functional in terms of ecosystem functioning and those that portray other life history characteristics. Yet, a direct emphasis on the

(quantified) relationships between traits, groups of traits or interconnected trait types (functional types) and ecosystem functioning might further increase our understanding of ecosystem functioning (e.g. Harvey et al., 2017; Seibold et al., 2018; Delmas et al., 2019). Knowledge on functional types of all members of a species assemblage (functional categorization) and their interspecies connections could portray local environmental characteristics and constraints set by habitat templates (Goedkoop & Johnson, 1996; Heino, 2005). Changes in species assemblages, such as the disappearance of a single species due to an environmental change, could imply a loss of a functional type which would cause a change in ecosystem functioning or could change the functioning of other species in the same assemblage. Knowledge on the functional roles of dominant, foundation or key species would strongly strengthen the understanding of ecosystem functioning. Knowledge on the effects of losses of individual species or the introduction of novel species whom possess different functional characters can, for example, be extracted from studies on alien and invasive species (e.g. Carlsson et al., 2004; Anderson & Rosemond, 2007; Gutiérrez et al., 2014). Changes of species composition that change the relative share of functional characters would make it possible to study, assess and predict single stressor and multiple stressors effects on ecological assemblages.

ACKNOWLEDGEMENTS

We would like to thank Ralf Verdonschot and two anonymous reviewers for their helpful comments and suggestions.

CHAPTER 9

SYNTHESIS

The aim of this thesis was to identify functional indicators of anthropogenic stress in aquatic ecosystems using ecosystem processes and functional roles of organisms, and to explore their potential use in biomonitoring schemes. For discharge dynamics (**Chapter 2**) and oxygen dynamics (**Chapter 3**), I showed that detailed quantification of these environmental processes is necessary to comprehend the temporal patterns in abiotic features leading to biotic responses. For example, timing of extreme disturbances, like peak discharges, showed to be important in explaining changes in caddisfly population dynamics (**Chapter 2**). Low dissolved oxygen concentrations, which may impact the survival of many aquatic organisms, were measured outside the working hours when incidental measurements are usually taken (**Chapter 3**). Both studies emphasized the need to consider temporal scales better when trying to understand how ecosystems change under anthropogenic stress and how organisms respond to these changes.

Indeed, initial evidence was provided that environmental processes are an important factor in setting the context in which organisms fulfill their functional roles in resource use, and therewith regulate ecological processes (**Chapter 4, 5**). Specifically, low dissolved oxygen saturation determined to what extent microbial and macroinvertebrate detritivores fulfilled their role in organic matter decomposition (**Chapter 4**), while eutrophication induced a shift in the diet of secondary consumers towards increased herbivory, as the carbon: nutrient ratios of primary producers decreased (**Chapter 5**).

The impact of anthropogenic stressors on ecological processes is complex, as there is usually a combination of multiple stressors involved (**Chapter 6, 8**). For example, organic matter decomposition was higher at sites with higher dissolved nitrogen concentrations, masking the potential effects from toxic pollution (**Chapter 6**). Advancements in measurement techniques may provide more information on specific ecological processes, e.g. passive acoustic monitoring may be used to characterize photosynthesis, respiration and re-aeration in more detail than ecosystem metabolism estimated from measurements of dissolved oxygen concentrations (**Chapter 7**).

In order to increase our understanding of how ecosystems change under anthropogenic stress at a specific site, we need to recognize that the functioning of ecosystems is shaped by a set of hierarchically arranged abiotic and biotic filters changing over time. Specifically, it was postulated in **Chapter 8** that we need to increase our knowledge of 1) the response of species to natural variability in abiotic features and the superimposed stress caused by anthropogenic activities, and 2) the functional role these species play in the functioning of ecosystems in the context of the local dynamics in environmental processes.

In this synthesis, I first provide a framework exploring how the abiotic and biotic hierarchical filters control the functioning of ecosystems. Then, I explore how studying the appropriate temporal scales can improve our comprehension of how organisms respond to

anthropogenic stress. Next, I propose future directions on how to study the known unknowns of organisms' functional roles in ecosystem functioning. Lastly, I suggest possible steps on how to monitor and predict ecosystem functioning in practice.

HIERARCHICAL FILTERS CONTROLLING THE FUNCTIONING OF ECOSYSTEMS

If we wish to understand how ecosystems function, it must be recognized that the functioning of ecosystems is shaped by a set of hierarchically arranged abiotic and biotic filters (Figure 1; Lévêque, 2003; Tockner et al., 2010). The geographic position of an ecosystem controls the regional climate, geology and geomorphology (Lévêque, 2003). These drivers can generally not be shaped by local management (Verdonschot, 1998), although human activities do impact ecosystems at this level through, for example, anthropogenic climate change (e.g. Dhungel et al., 2016). The environmental processes in an ecosystem are a result of the interaction between climate, geology and geomorphology, which in freshwater bodies primarily comprise the temporal variability in hydrology, morphology and chemistry (Verdonschot, 1998; Tockner et al., 2010).

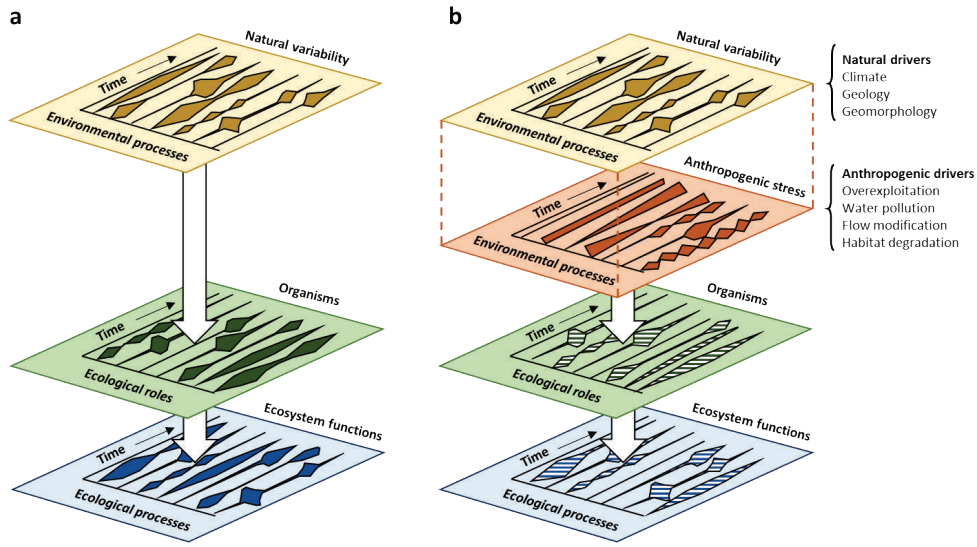

***Figure 1**: a) Schematic representation of how the natural variability in the environment acts as a filter on the presence of organisms, which in turn contribute to the realization and maintenance of ecological processes in an ecosystem over time, and b) the same situation under anthropogenic stress. Figure adapted from Tockner et al. (2010).*

Hydrological processes relate to changes in water quantity over time, such as discharge dynamics (**Chapter 2**). Morphological processes include changes in longitudinal and lateral channel patterns and substrate composition, like channel migration and sediment dynamics. Chemical processes relate to changes in water and sediment quality over time, including the dynamics of substances like dissolved oxygen (**Chapter 3**), nutrients, organic matter and major ions. These environmental processes strongly interact, e.g. the morphology is shaped by the hydrological processes in a stream (Verdonschot, 1998). Under unimpacted conditions, the natural variability in environmental processes act as an abiotic filter for the presence and abundance of organisms in an ecosystem, as their suite of multiple interacting traits allow them to exploit the available resources (Figure 1a; Lévêque, 2003; Tockner et al., 2010). These organisms interact with other organisms and their environment and thereby form a biotic filter that determines the realization and maintenance of ecological processes in an ecosystem (Jax, 2005). Although the filters are drawn linear and sequential, they may also be iterative (Hamilton et al., 2019), e.g. ecological processes like primary production and respiration alter the dissolved oxygen dynamics, which may in turn impact the organisms present (**Chapter 3**).

Anthropogenic stress from overexploitation, flow modification, habitat degradation and destruction and water pollution may superimpose an additional filter, interfering with the natural variability in local environmental processes (Figure 1b; Dudgeon et al., 2006; Tockner et al., 2010). For example, channelization may alter hydrological processes by increasing the number and intensity of discharge peaks (**Chapter 2**). Agricultural activities and WWTP discharges may alter chemical processes by increasing nutrient and contaminant concentrations in an ecosystem (**Chapters 5, 6**), as well as changing dissolved oxygen dynamics (**Chapter 3**). Hence, the combination of natural variability and anthropogenic stress impacts the presence of organisms and subsequently ecological processes (Tockner et al., 2010). Each of these hierarchical filters acts over time and considering the appropriate temporal scale is, therefore, an important step to understand how organisms respond to anthropogenic stress.

IT IS TIME TO STUDY THE APPROPRIATE TEMPORAL SCALES

A large proportion of studies assessing the impact of anthropogenic stress on organisms focussed on a single life stage, assuming that those are an adequate indicator of environmental conditions (Lancaster & Downes, 2010). To improve our understanding of how organisms respond to disturbances, it is thus necessary to select the appropriate temporal scale (**Chapter 2**). The temporal scale for an ecologist typically ranges from a day to a century (Lévêque, 2003). Selection of the appropriate temporal scale depends on the chosen ecological phenomena and the organism of interest (Figure 2a; Lancaster, 2008). Here, we were interested in changes in populations and communities, which should be

studied at a temporal scale of at least one generation. Generation time refers to the time it takes for an individual to produce its first offspring, which differs between organisms (Lancaster, 2008).

An approximate indication of generation times for different organism groups is presented in Figure 2b. The generation time between organism groups typically ranges from a day for bacteria (Jannasch, 1969) to several years for fish (Nie, 1996; Mims et al., 2010), however, even within organism groups variation can be large. For example, the generation time of macroinvertebrates ranges from several weeks (e.g. some Naididae, Culicidae and Chironomidae) to multiple years (e.g. certain species of Elmidae, Odonata and Hirudinea) and in some cases even decades (e.g. Unionidae), although an annual generation time is most common in northern temperate zones (Hynes, 1970b; Tachet et al., 2000). Within species, generation times may vary depending on local conditions, like temperature and resource availability (Hynes. 1970b; Lancaster, 2008; e.g. *Ageputus fuscipes* in **Chapter 2**). Once the appropriate temporal scale has been selected, one should determine whether and how environmental processes within that temporal scale might influence ecological phenomena (Lancaster, 2008).

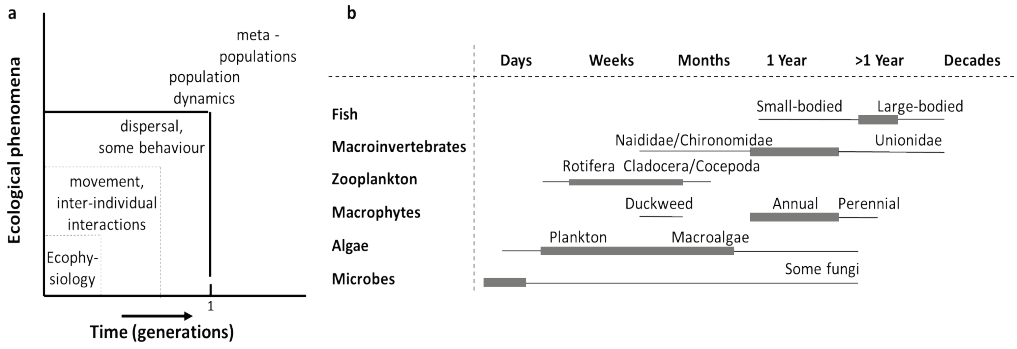

***Figure 2**: a) Different ecological phenomena approximately scaled to the generation time of an organism (adapted from Lancaster, 2008), and b) approximate indication of the time for different organism groups to produce one generation in northern temperate zones (based on fish: Nie, 1996; Mims et al., 2010; macroinvertebrates: Hynes, 1970; Tachet et al., 2000; Thorp & Covich, 2009; zooplankton: Allan, 1976; Sigee, 2005; Thorp & Covich, 2009; macrophytes: Barrat-Segretain, 1996; Philbrick & Les, 1996; Packer et al., 2017; algae: Hassall, 1845; Transeau, 1916; Azim et al., 2005; microbes: Jannasch, 1969; Wurzbacher et al., 2011).*

To determine whether and how environmental processes impact organism populations within the selected time scale, we also need to consider the temporal patterns of disturbances (Underwood, 1994). Broadly, three types of temporal patterns of disturbances can be distinguished, namely pulse, press and ramp (Figure 3; Lake, 2000). The classification of disturbances into these categories depends on the generation time of the

organism concerned, i.e. a disturbance lasting two months would be deemed a ramp or press for an organism with a generation time of a few weeks, but would be considered a pulse for organism with a generation time of one year (Underwood, 1994). Although there are some difficulties involved in describing disturbance in three broad categories (see for example Box 1), it is nevertheless useful to think in different types of disturbance (Underwood, 1994). Press and ramp disturbances have a rather constant long-term impact on populations, whereas for pulse disturbances it is relevant to consider the population dynamics in relation to the temporal patterns of the disturbance (**Chapter 2**; Underwood, 1994). The intensity, frequency, predictability and duration of pulses has already been extensively documented for discharge dynamics (**Chapter 2**; Downes, 2010; Stanley et al., 2010), and advances in measurement technologies hold promise to increase our underdestanding of temporal patterns of other environmental processes as well (Ensign et al., 2017).

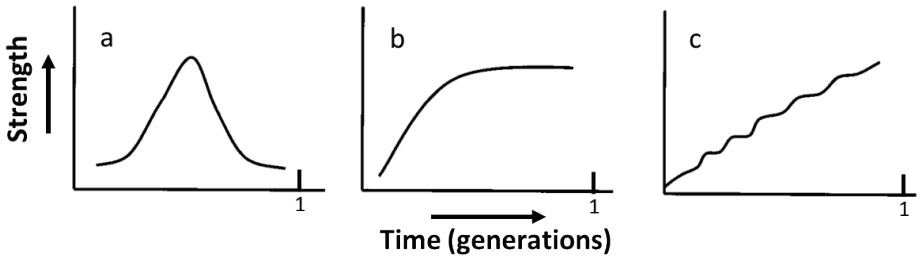

***Figure 3**: Three types of stress distinguished by temporal trends in the strength of the disturbing force in relation to the generation time of an organism: a) pulse, b) press and c) ramp. Figure adapted from Lake (2000).*

Box 1. Challenges in classifying temporal patterns of disturbances

There are some challenges in classifying different types of disturbances, for example, when a pulse disturbance has long-term press effects (Underwood, 1994). For instance, many pesticides are sprayed at particular times of the year (e.g. pre-emergent herbicides), while others are sprayed multiple times throughout the growing season (e.g. most insecticides), producing multiple pulses of pesticide inputs in surface waters over a year (Relyea & Hoverman, 2006). Many of these pesticides stick to the sediment, making sediments a sink for pesticides when accumulation is higher than degredation of the compound (Konvang et al., 2003). In the sediment, these pesticides form a press or ramp disturbance to most sediment-dwelling organisms, as they are exposed by direct inadvertent ingestion of sediments and by uptake from pore water and overlaying water where contaminants are released back through diffusive fluxes (Simpson & Batley, 2016). Physical disturbance of sediment may also result in a pulse back into the surface water with a different timing than the original pulses (Simpson & Batley, 2016), further complicating the classification of the temporal pattern of the disturbance.

In this thesis, I quantified the temporal patterns of dissolved oxygen saturation using sensors with datalogging capabilities (**Chapter 3**). These measurements revealed diurnal cycles in dissolved oxygen saturation originating from day-night patterns in production and respiration (Figure 4a). The minimum and maximum values occured outside the working hours when incidental measurements are usually taken (**Chapter 3**). Eutrophication altered the intensity of these diurnal cycles during spring and especially during summer (Figure 4b). Likewise, advances in measurement technologies hold promise to detect temporal variability in other chemical processes as well, that have been tedious to detect up to now using manual grab sampling (Ensign et al., 2017). For example, developments in high frequency loggers of some nutrients (PO_4, NH_4 and NO_3) could enhance our understanding of patterns and controls of nutrient dynamics (Mulholland et al., 2010; Pellerin et al., 2016). Also morphological processes, like sediment fluxes and streambed mobility, may now be measured at high frequency, employing dataloggers like the passive acoustics methods used in **Chapter 7** or by using automated imagery.

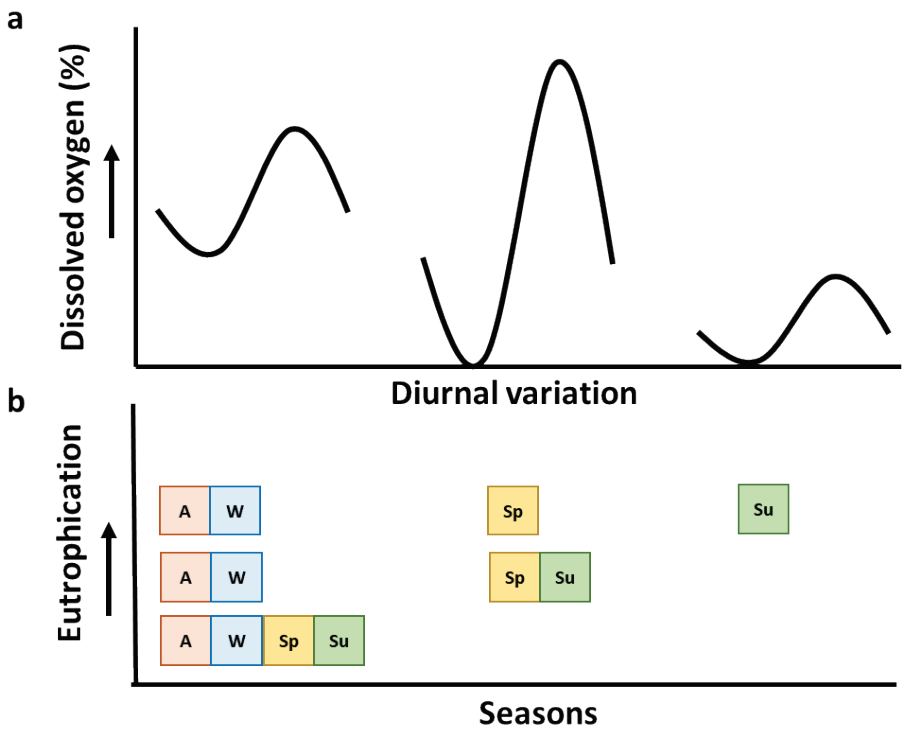

***Figure 4**: Dissolved oxygen dynamics in drainage ditches with a) diurnal variation in dissolved oxygen saturation and b) the occurrence of these diurnal patterns over different seasons (A= autumn, W = winter, Sp = spring, Su = summer) dependent on eutrophication state ranging from meso- to hypertrophic (adapted from Chapter 3).*

Whether population dynamics are actually impacted by a pulsed disturbance depends mainly on the intensity, frequency, predictability and duration of the pulses in relation to the timing of critical periods in the life cycle of the exposed species (**Chapter 2**; Lancaster and Downes 2010). Various species have evolved traits to avoid unfavourable conditions by, for example, going into a resting state (Transeau, 1916; Hynes, 1970). While certain other life history traits, such as generation time, migration ability, and timing and duration of life stages may increase a species ability to recover after a disturbance (which is included in the trait-based SPEAR metrics used in **Chapter 6**). In northern temperate zones, a regular succession of life stages of different species occurs throughout the year in relation to seasonal variations in climatic factors. Nonetheless, the exact timing of the consecutive life stages depends on local conditions and may vary slightly between years (Figure 5; **Chapter 2**; Transeau, 1916; Hynes 1970; Lévêque, 2003). Many species within the same genus coexist together through differences in timing of life history (Figure 6; Hynes 1970), stressing the importance of studying the succession of life stages on species level.

The exact number of generations that needs to be studied to understand the relation between environmental processes and population dynamics depends on the natural variability in the population dynamics, but Lancaster (2008) argues that useful time-series may encompass at least 20 generations. For organism groups with longer generation times, long-term datasets of the environmental processes and population dynamics would be required, however, collecting this information is logistically challenging (Lancaster, 2008). To overcome this challenge, development and parametrization of numerical population models including abiotic and biotic interactions may provide potential to extrapolate between temporal scales and thereby facilitate our understanding on how environmental processes impact population dynamics over longer temporal scales (Relyea & Hoverman, 2006; Lancaster, 2008). An example of such numerical model was used in **Chapter 2** to simulate the population dynamics of a caddisfly species. Although the stage-population model could still be improved by, for example, making each stage duration dependent on temperature and resource availability and by including biotic interactions like competition, it did seem a promising tool to simulate the invertebrate population dynamics over time (**Chapter 2**).

Based on the above information, I suggest that to determine how anthropogenic stress drives population dynamics we need to 1) select the appropriate temporal scales based on the generation time of the studied organism, 2) quantify the temporal patterns of environmental processes within this unit of time using high frequency measurements, 3) link the intensity, frequency, predictability and duration of pulse disturbances to the timing of critical periods in the life cycle of the exposed species, potentially using population models.

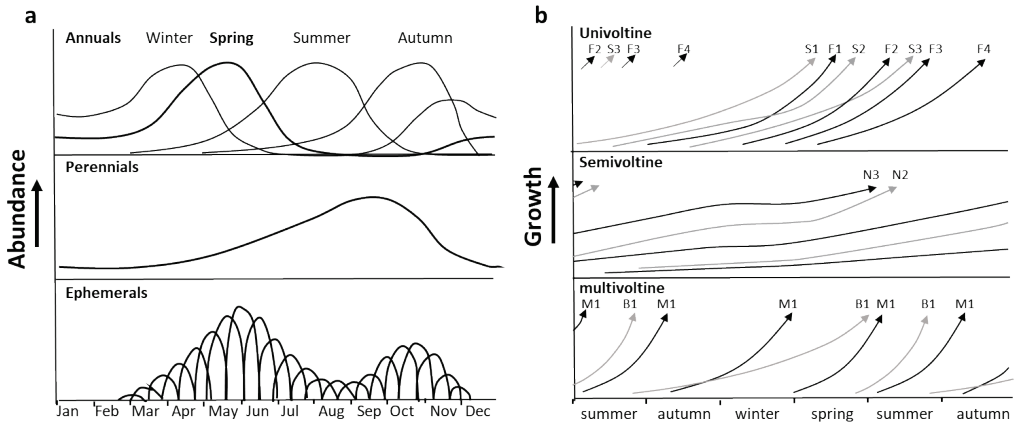

***Figure 5**: Typical periodicity of three ecological groups based on generation time of a) abundance of algae in ponds and streams (adapted from Transeau, 1916), including annuals (with spring annuals being the largest seasonal group), perrenials and ephimerals and b) growth of aquatic insects in streams, with arrows indicating emergence, including univoltine ($F_{1,2,3}$ = fast seasonal cycle and $S_{1,2,3}$ = slow seasonal cycle; adapted from Hynes, 1970), semivoltine (N_2 = 2 year cycle and N_3 = 3 year cycle; adapted from Hynes, 1970) and multivoltine (B_1 = 2 generations per year and M_1 = 3 generations per year; based on data by Wagner et al., 2011). Both panels depict a schematic representation of typical periodicity. Note the differences in axes scales.*

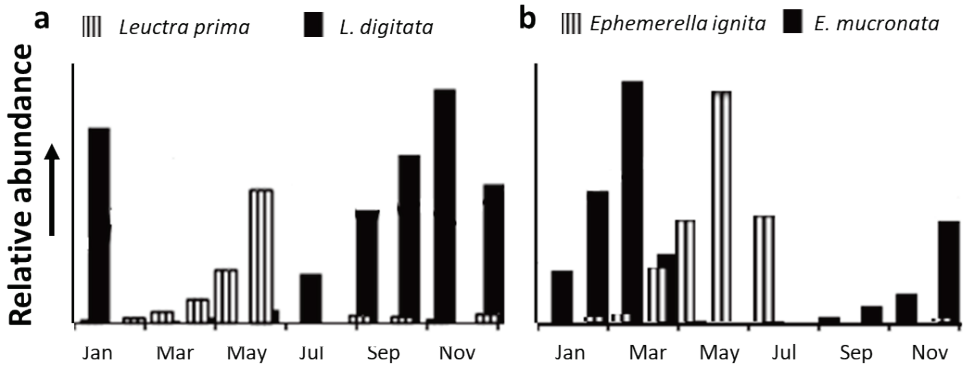

***Figure 6**: Temporal variability in the relative abundance of two species of the same genus in a) stoneflies (Plecoptera:* Leuctra*) and b) mayflies (Ephemeroptera:* Ephemerella*). Adapted from data of the Breitenbach stream measured in 1987-1988 by Wagner et al. (2011).*

THE KNOWN UNKNOWNS OF ORGANISMS' FUNCTIONAL ROLES IN ECOSYSTEM FUNCTIONING

In the previous section, I focused on how environmental processes impact population dynamics, typically studied by population ecologists. Measurements of direct ecological processes which are of interest to ecosystem ecologists, such as decomposition rates, were presented in several chapters of this thesis (see **Chapters 4, 6, 8**). Although some steps were made to link population and ecosystem ecology, such as using the natural abundance of stable isotopes to determine the functional roles of organisms rather than using static trait-databases (**Chapter 5**), it still remains a challenge to identify to what extent different species present in an ecosystem realize and maintain ecological processes over time (**Chapter 8**). The main question we need to ask to link these two disciplines is *'what do species do in ecosystems?*' (Lawton, 1994). The obvious answer is '*many things'*, considering that ecosystems would not exist without species. However, the actual links between functional roles and ecosystem functioning remain largely unknown (Lawton, 1994; Lévêque, 2003; Bellwood et al., 2018). In this section, I propose some future directions on how to study these known unknowns of functional roles.

Before identifying functional roles, it is important to select the ecosystem function(s) of interest, as one organism may fulfill many different roles (Bellwood et al., 2018). Then, we need to identify those species that have a fundamental ecological niche able to deliver the specific ecosystem function(s). The fundamental ecological niche of an organism includes the sum of all adaptations crucial for the fitness and performance of organisms and may be characterized using three principal axes, describing where an organism lives (habitat) and what it does (functional role) over time (Figure 7; Lévêque, 2003; Pianka, 2011). This fundamental ecological niche is, however, a hypothetical, idealized niche in which the environmental conditions are optimal and there are no negative interactions with other organisms, like competition and predation (Hutchinson, 1957; Pianka, 2011).

In reality, the fundamental ecological niche is seldom fully utilized at a certain time and location. The context, i.e. the actual set of environmental conditions and positive and negative interactions with other organisms, determine which part of the organisms' fundamental ecological niche is realized at a certain time and location (Figure 7; **Chapters 4, 5, 8**; Hutchinson, 1957; Lévêque, 2003; Pianka, 2011; Rodriguez-Cabal et al., 2012). For example, in **Chapter 5** we showed that secondary macroinvertebrate consumers adjusted their diet along a eutrophication gradient, i.e. they presumably shifted their realized niche based on the available resources. This finding indicates that the functional role of organisms cannot be inferred from static, broad trait databases, but needs to be derived from the local temporal context (Bellwood et al., 2018). There is thus need to measure to what extent

functional roles are performed by the organisms within a specific ecosystem. This would be a laborious task and perhaps impossible to perform considering the number of species present within an ecosystem. Therefore, I recommend focusing on the species that are presumed to play a key role in the selected function(s), i.e. the most dominant or keystone species for a particular function under specific contexts. Examples of such key species include the gammarid shredders assessed in the decomposition study of **Chapter 4** or the generalist consumers assessed in the trophic transfer study of **Chapter 5**.

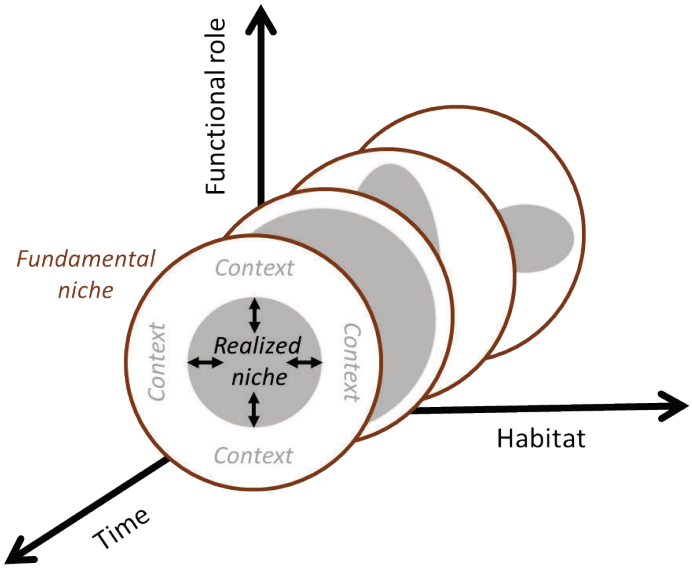

***Figure 7**: The ecological niche of an organism in terms of where it lives (habitat) and what it does (functional role) over time. The context, i.e. the environment and interactions with other organisms, determines which part of the fundamental niche (brown line) is actually realized at a certain time and location (grey shape). Based on Lévêque, 2003; Pianka, 2011; Rodriguez-Cabal et al., 2012.*

In this thesis, I only focused on functional roles of organisms based on trophic interactions. There are, however, also functional roles of organisms that do not directly involve trophic interactions between species, but that may have played an important (unknown) role in ecosystem functioning (Jones et al., 1994; Wright & Jones, 2006; Kéfi et al., 2012; Dussault, 2019). One important non-trophic role in ecosystem functioning is fulfilled by ecosystem engineers, i.e. organisms that directly or indirectly physically modulate the availability of resources to other species and thereby alter environmental and ecological processes (Figure 8; Jones et al., 1994; Wright & Jones, 2006; Jones et al., 2010). Organisms may modify the environment with their own living or non-living structure,

termed autogenous engineers, e.g. macrophytes in water bodies create physical habitat with their tissue, impact light, oxygen and temperature regimes and alter sedimentation rates. Or organisms may change the environment by transforming living and non-living matter from one physical state to another, termed allogenous engineers, e.g. beavers that cut down trees to construct dams and burrowing organisms that actively rework soils (Jones et al., 1994). We know in theory that these ecosystem engineers are present in most ecosystems. However, their role in ecosystem functioning has received limited attention in empirical studies as opposed to trophic interactions, except for spectacular examples such as beaver activities (Lévêque, 2003; Wright & Jones, 2006; Borst et al., 2018). Understanding the functional roles of organisms in ecosystem functioning may thus be further improved by more explicitly including these non-trophic interactions in future studies.

Based on the above information, I propse that one way forward to improve our understanding of how organisms contribute individually and collectively to the functioning of ecosystems is to 1) select the ecosystem function(s) of interest, 2) quantify to what extent different species fulfill the roles in that specific function(s) within the local temporal context, 3) extend the study on functional roles by including non-trophic interactions, like the role of ecosystem engineers.

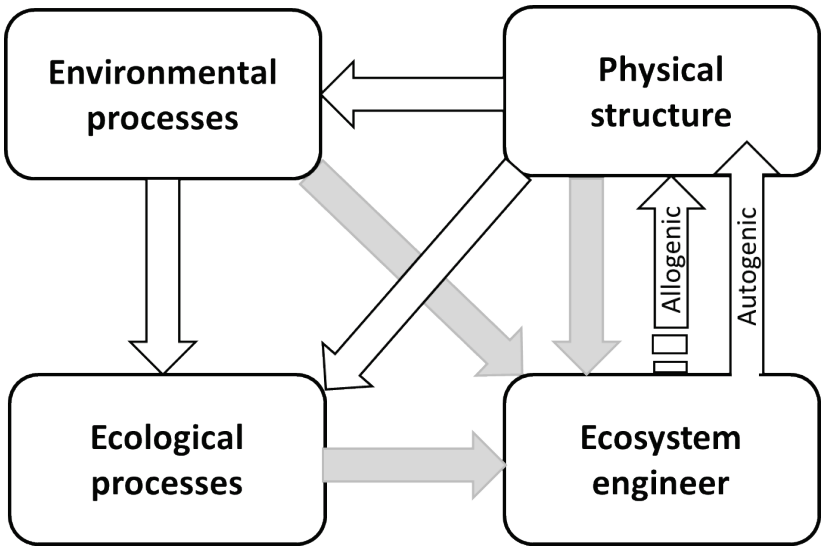

***Figure 8**: Pathways in which ecosystem engineers may impact ecosystem functioning through non-trophic interactions. The white arrows indicate cause-effect relationships in an engineered ecosystem and the grey arrows indicate feedback mechanisms back on the ecosystem engineer. The solid white arrow for autogeneous engineers represents the physical structure inserted in in its environment. The striped white arrow for allogenous engineers represents the action of the engineer on other living or non-living structures. Adapted from Jones et al., 2010.*

MONITORING AND PREDICTING ECOSYSTEM FUNCTIONING IN PRACTICE

In this synthesis, I argued that to improve our understanding of ecosystem functioning under anthropogenic stress, we need to study ecosystem functions within a framework of hierarchically arranged abiotic and biotic filters changing over time. I propose that if we aim to *monitor* ecosystem functioning in practice, we need to address these abiotic and biotic filters in reverse order (Figure 9). First, we need to select the ecosystem function(s) of interest, as the importance of organisms in fulfilling a functional role depends on the selected function(s). Then, we need to quantify to what extent different species fulfill a role in the specified function(s) within the local temporal context. In future studies, I recommend focusing on the species that are presumed to play a key role in the selected function(s) based on both trophic and non-trophic interactions. To understand how the population dynamics of these species change under anthropogenic stress, we need to select the appropriate temporal scale based on the generation time of the studied organism. Within this unit of time, we need to link the temporal patterns of the environmental processes to the timing of critical periods in the life cycle of the exposed species.

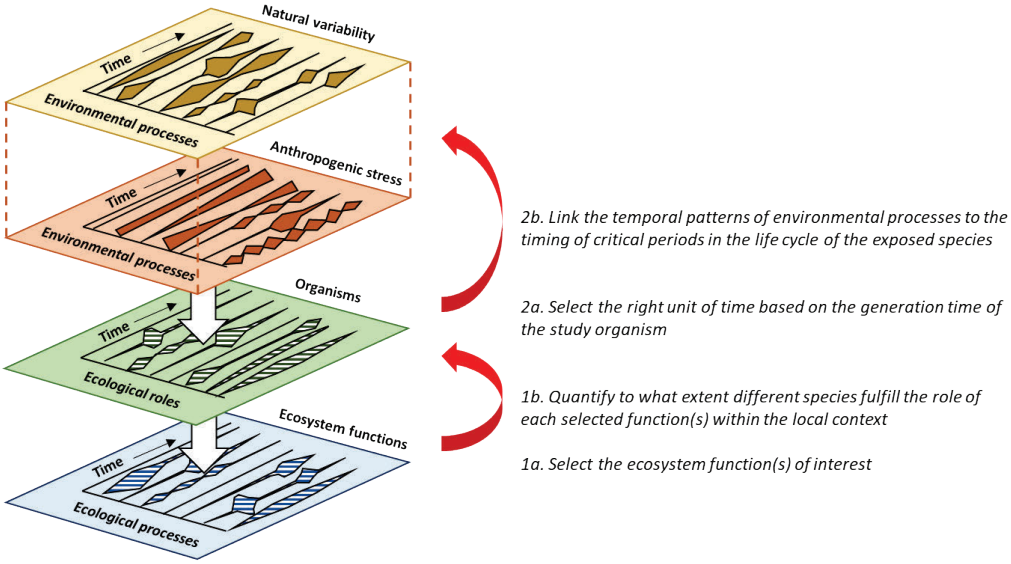

***Figure 9**: Perspectives on how to monitor changes in ecosystem functioning under anthropogenic stress in reversed order of the set of hierarchially aranged abiotic and biotic filters.*

If we want to take this framework one step further and would aim to *predict* how ecosystem functioning changes under anthropogenic stress within the local temporal context, we also need to know which species may be able to take over a specific functional role when the organism(s) previously playing the key role in the selected function(s)

disappear. In **Chapter 8**, we described potential factors that may impact the degree that organisms may fulfill functional roles under anthropogenic stress, based mainly on knowledge associated with the role shredders play in decomposition. In short, some species present in low numbers, or newly colonizing species may be able to replace the key organism(s) previously fulfilling the functional role, and then fulfill this role within the new context (Lévêque, 2003). In some cases, species loss may occur without detectable loss in ecosystem function, because of the density-dependent compensation by other species (Figure 10; Schultze & Mooney, 1994). However, species may substantially differ in the degree that they fulfill a functional role, which could result in either an increase or decrease in a specific function, or alternatively there may be no species present to fulfill the functional role (Figure 10; Vinebrook et al., 2004). Currently, we know too little about the functional roles of most species to make such predictions (**Chapter 8**). Therefore, I argue that we first need to increase our knowledge on which potential trophic and non-trophic functional roles different species may fulfill.

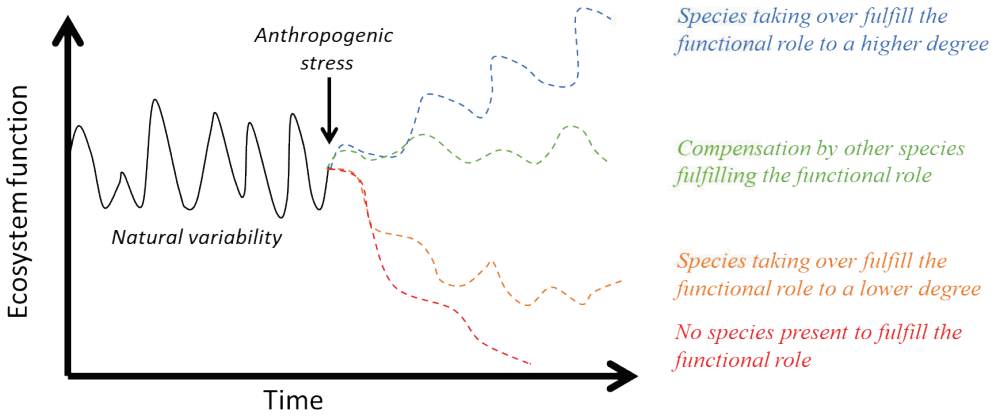

***Figure 10**: Hypothetical scenarios describing the potential impact of anthropogenic stress on the functional role fulfilled by organisms in an ecosystem over time.*

To conclude, Oscar Wilde is said to once have stated *"Society exists only as a mental concept: in the real world there are only individuals"* (Elliot & Turner, 2012). Similarly, ecosystem boundaries are virtually absent in nature, but are delimited based on the specific perspectives and objectives of the observer (Jax, 2005). In the real world, there are organisms that perform processes and it is the total sum of these individual-level processes fulfilled by each organism that make ecosystems function. From this thesis, it can be concluded that the functional roles fulfilled by organisms can varry due to changes in

context, resulting in complex patterns in ecosystem processes. Therefore, I recommend that future research on ecosystem functioning should focus on the potential trophic and non-trophic functional roles fulfilled by organisms within different contexts changing over time.

--- Ultimately, organisms make ecosystems function ---

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SUMMARY

Organisms make ecosystems function

Identifying functional indicators of anthropogenic stress in aquatic ecosystems

Ecosystems are discrete units that include all living organisms occurring in a given area and their non-living environment with which they interact. They can be described by their structure and functioning. The structure commonly reflects the abiotic and biotic status of an ecosystem at a given time, while its functioning reflects the dynamic environmental and ecological processes taking place through time. The contribution of the biota to the realization and maintenance of ecological processes can be studied by ascribing functional roles to organisms. Under unimpacted conditions, the structure and functioning of ecosystems changes over time due to the natural variability in the environmental processes. Human activities or by-products from human activities may superimpose additional external forces, factors or stimuli on ecosystems, which may alter the structure and functioning of ecosystems outside the boundaries of the natural variability.

To gain insight into ecosystem changes under anthropogenic stress, we need suitable ecosystem indicators. Traditionally, most biomonitoring schemes have relied on structural indicators based on taxonomic inventories of species groups that comprise the ecosystem, assuming these are representative of the functioning of an ecosystem. However, anthropogenic stress may impact ecosystem structure and functioning differently. It has thus been argued that direct measurements of ecosystem functioning are necessary, including measurements of functional roles of organisms and of environmental and ecological processes. Therefore, the aim of this thesis was to identify functional indicators of anthropogenic stress in aquatic ecosystems using ecosystem processes and functional roles of organisms, and to explore their potential use in biomonitoring schemes. To this purpose multiple field studies and a literature study were conducted in linear shaped, small, shallow permanent freshwater ecosystems in lowland areas, including streams and drainage ditches.

To obtain information on environmental processes as indicator of ecosystem functioning under anthropogenic stress, we measured discharge dynamics (**Chapter 2**) and dissolved oxygen dynamics (**Chapter 3**) over time. The aim of **Chapter 2** was to gain a better understanding of the effect of extreme disturbances on the population dynamics of organisms during different life stages, focusing on the effect of peak discharges on different larval stages of the caddisfly *Agapetus fuscipes*. We measured discharge continuously with water level loggers in four streams throughout two years and related the timing of extreme

peak discharges to the population dynamics of *A. fuscipes* using a stage-population model. This way, we were able to show a potential association between extreme peak discharges and population declines when the hydrologic disturbances occurred during their sensitive early life stages. In **Chapter 3**, we quantified the dissolved oxygen dynamics of drainage ditches at different water depths and seasons along a eutrophication gradient. Diurnal cycles in dissolved oxygen saturation were observed, originating from day-night patterns in production and respiration. Eutrophication altered the intensity of these diurnal cycles during spring and especially during summer. Both studies emphasized that the quantification of environmental processes is necessary to comprehend temporal patterns of abiotic features to which biota respond.

The response of organisms to changing environmental processes was studied by quantifying their realized niche in resource use under different contexts. The aim of **Chapter 4** was to determine if dissolved oxygen drives the role that invertebrates and microbes fulfil in particulate organic matter decomposition in drainage ditches. We showed that an increased duration of anoxic conditions in the benthic layer of the ditches was related to increased microbial decomposition, while simultaneously invertebrate consumption decreased in the benthic layer and increased in the pelagic layer. In **Chapter 5**, we assessed if nutrient enrichment drives changes in trophic transfer in aquatic communities. Using stoichiometry and stable isotope analysis, we were able to show that nutrient enrichment shifted the diet of secondary consumers towards increased herbivory. Both chapters provided initial evidence that environmental processes are an important factor in setting the context in which organisms fulfill their functional role in resource use, and therewith alter ecosystem functioning.

Next, we evaluated direct quantifications of ecological processes, focusing on decomposition (i.e. mass loss of artificial substrates; **Chapter 6**) and ecosystem metabolism (i.e. open channel dissolved oxygen dynamics; **Chapter 7**). In **Chapter 6**, we assessed the use of organic matter decomposition as functional indicator to diagnose the impact of various stressors originating from agricultural activities and WWTP discharges. We showed that the impact of these anthropogenic stressors on ecological processes was complex, as a combination of multiple stressors were involved. In **Chapter 7**, we evaluated to what extent passive acoustic monitoring may be used to quantify ecological processes in freshwater environments. Our results showed that the recorded acoustic patterns were primarily associated with the fluctuation in dissolved oxygen saturation, indicating that this novel approach may be further developed to estimate metabolism in water bodies. We suggested that passive acoustic monitoring may even overcome certain challenges encountered by the estimation of metabolism from diel dissolved oxygen curves, such as the possibility to split up different components of ecological processes that emit different acoustic patterns, the ability to estimate re-aeration rates and the inclusion of anaerobic respiration.

In the literature study of **Chapter 8**, we compared the use of functional indicators (i.e. ecosystem metabolism and decomposition) to that of structural indicators (e.g. taxonomic inventories of the community composition) to assess the impact of multiple anthropogenic stressors on running waters. We argued that that we need to increase our knowledge of 1) the response of species to natural variability in abiotic features and the superimposed stress caused by anthropogenic activities, and 2) the functional role these species play in the functioning of ecosystems in the context of the local dynamics in environmental processes.

In the synthesis, I proposed that if we aim to understand how ecosystem functions change under anthropogenic stress, it must be recognized that the functioning of ecosystems is shaped by a set of hierarchically arranged abiotic and biotic filters. Specifically, temporal variation in environmental processes (i.e. hydrology, morphology and chemistry) influenced by anthropogenic stress act as an abiotic filter for the presence and abundance of organisms in an ecosystem, as their suite of multiple interacting traits allows them to exploit the available resources. Considering the appropriate temporal scale is an important step to improve our comprehension of how these organisms respond to anthropogenic stress. This includes the need to 1) select the appropriate temporal scales based on the generation time of the studied organism, 2) quantify the temporal patterns of environmental processes within this unit of time using high frequency measurements, 3) link the intensity, frequency, predictability and duration of pulse disturbances to the timing of critical periods in the life cycle of the exposed species, potentially using population models. Next, the trophic and non-trophic interactions between the organisms present and their environment results in a biotic filter that determines the realization and maintenance of ecosystem functioning. However, the links between functional roles and ecosystem functioning remain largely unknown. To study these known unknown functional roles, there is need to 1) select the ecosystem function(s) of interest, 2) quantify to what extent different species fulfill the roles in that specific function(s) within the local temporal context 3) extend the study on functional roles by including non-trophic interactions, like the role of ecosystem engineers.

I proposed that to *monitor* how ecosystem functioning changes under anthropogenic stress within the local context, we need to address the relevant filters in reverse order. To be able to do this we need to know which potential trophic and non-trophic functional roles different species may fulfill and the sensitivity of these species to different disturbances over their life cycle. If we would aim to *predict* how ecosystem functioning changes under anthropogenic stress within the local context, we also need to know which species may be able to take over a functional role when key organisms in the selected function(s) disappear.

It can be concluded from this thesis that the functional roles fulfilled by organisms can vary due to changes in context, resulting in complex patterns in ecosystem processes. Therefore, I recommended that future research on ecosystem functioning should focus on the potential trophic and non-trophic functional roles fulfilled by organisms within different contexts changing over time. Ultimately, organisms make ecosystems function.

SAMENVATTING

Organismen laten ecosystemen functioneren

Functionele indicatoren van antropogene stress in aquatische ecosystemen

Een ecosysteem omvat alle levende organismen binnen een bepaald gebied en de wisselwerking die zij met elkaar en het omringende milieu hebben. Een ecosysteem kan gekarakteriseerd worden door de structuur die het heeft en de manier waarop het functioneert. De structuur wordt gedefinieerd als de niet-levende (abiotische) en levende (biotische) toestand van een ecosysteem op een bepaald moment, terwijl het functioneren alle, meestal dynamische, processen omvat die plaatsvinden door de tijd. Ecosysteemprocessen zijn gerelateerd aan veranderingen in het milieu, zoals bijvoorbeeld de afvoerdynamiek van een beek en de kringloop van stoffen. De kringloop van stoffen vindt plaats doordat energie en materie van het ene organisme aan het andere worden doorgegeven, waardoor de één brandstof levert aan de ander. Zo zetten primaire producenten, zoals planten, zonlicht om in hun eigen biomassa. Consumenten eten deze planten en worden zelf weer gegeten door andere organismen. Niet alle planten en dieren worden echter gegeten. Ze kunnen ook dood gaan. Dit dode organische materiaal wordt vervolgens afgebroken door reducenten, zoals schimmels en bacteriën. Organismen spelen dus elk hun eigen rol in het functioneren van een ecosysteem.

Zonder menselijke invloed worden de structuur en het functioneren van een ecosysteem bepaald door de natuurlijke variatie van het milieu. Menselijke activiteiten kunnen deze natuurlijke variatie verstoren, waardoor de structuur en het functioneren van een ecosysteem kunnen worden aangetast. Om de effecten van menselijke activiteiten op de structuur en het functioneren van ecosystemen vast te stellen zijn indicatoren nodig. In de huidige monitoringsprogramma's wordt meestal gebruik gemaakt van indicatoren die gebaseerd zijn op de structuurkenmerken van een ecosysteem. Er wordt bepaald welke soorten in een ecosysteem aanwezig zijn, waarbij ervan uit wordt gegaan dat indicatoren op basis van de soortensamenstelling ook representatief zijn voor het functioneren van een ecosysteem. Menselijke activiteiten kunnen de structuur en het functioneren van een ecosysteem echter op verschillende wijze veranderen. Het is daarom belangrijk om ook het functioneren van een ecosysteem zelf direct te meten. Directe metingen van bijvoorbeeld functionele rollen van soorten, maar ook van milieu- en ecologische processen, zouden de basis moeten vormen voor dergelijke functionele indicatoren. Het doel van dit proefschrift was dan ook om geschikte functionele indicatoren van menselijke invloed op aquatische ecosystemen te identificeren en om de potentiële meerwaarde van deze indicatoren in

monitoringsprogramma's te evalueren. Om dit doel te bereiken werden een aantal veldstudies en een literatuurstudie uitgevoerd. Deze studies richtten zich specifiek op rechte, smalle en ondiepe beken en sloten in laaglandgebieden.

Allereerst hebben we onderzocht hoe de dynamiek van waterafvoer in beken (**Hoofdstuk 2**) en de zuurstofhuishouding in sloten (**Hoofdstuk 3**) veranderen onder menselijke invloed. Het doel van **Hoofdstuk 2** was om vast te stellen wat het gevolg is van extreme verstoringen in het milieu op de populatiedynamiek van organismen gedurende verschillende levensfases. Het onderzoek was specifiek gericht op het effect van afvoerpieken op de populatiedynamiek van de kokerjuffer *Agapetus fuscipes* gedurende verschillende larvale stadia. In deze studie hebben we twee jaar lang de waterafvoer van vier laaglandbeken gemeten. Vervolgens hebben we een populatiemodel gebruikt om de populatiedynamiek van *A. fuscipes* aan de afvoerpieken te koppelen. Met deze gegevens hebben we kunnen aantonen dat verstoringen in de waterafvoer van een beek kunnen leiden tot een afname van de populatiegrootte van *A. fuscipes*, vooral als de extreme afvoerpieken plaatsvinden tijdens de gevoelige eerste levensfases van de kokerjuffer. **Hoofdstuk 3** richtte zich op de effecten van eutrofiëring op de zuurstofhuishouding in sloten. In alle sloten werd een dag-nacht cyclus in zuurstofconcentraties waargenomen. De eutrofiëring veroorzaakte in eerste instantie een veel groter verschil tussen de pieken en dalen en bij een verder toenemende mate van eutrofiëring tot lagere gemiddelde zuurstofconcentraties. Het verschil in zuurstofhuishouding tussen de sloten langs een eutrofiëringsgradient was al zichtbaar in de lente, maar werd versterkt gedurende de zomer. Beide studies toonden aan dat de kwantificering van processen nodig is om verstoringen in het milieu waarop organismen reageren beter te begrijpen.

Hierop aansluitend hebben we de effecten van veranderingen in het milieu op de rol die organismen in ecologische processen spelen bestudeerd. Het doel van **Hoofdstuk 4** was om het effect van lage zuurstofconcentraties op de rol van macrofauna en micro-organismen in de afbraak van organisch materiaal in sloten te bepalen. We hebben laten zien dat zuurstoflоosheid net boven de waterbodem een verhoogde afbraak van organisch materiaal door micro-organismen kan veroorzaken. Tegelijkertijd nam de afbraak van dood organisch materiaal door ongewervelde zoetwaterdieren in deze zuurstofloze laag af, doordat de dieren naar de zuurstofrijke waterkolom migreerden. In **Hoofdstuk 5** hebben we onderzocht wat het effect is van eutrofiëring op de positie van macrofauna in de voedselketen. Door middel van een analyse van de voedselkwaliteit en posities in het voedselweb hebben we aangetoond dat eutrofiëring in een agrarisch gebied kan leiden tot verandering in het dieet van secundaire consumenten. Bij verrijking van de planten met voedingsstoffen veranderde de macrofaune hun consumptie van dierlijk naar plantaardig materiaal. Beide studies toonden aan dat het milieu een belangrijke factor is die de context

waarin organismen hun rol in ecologische processen vervullen bepaalt en daarmee ook het functioneren van een ecosysteem.

Vervolgens hebben we enkele directe metingen van ecologische processen geëvalueerd. Dit onderzoek richtte zich op het meten van de afbraak van organisch materiaal, het meten van massaverlies met behulp van kunstmatige substraten (**Hoofdstuk 6**) en het meten van zuurstofconcentraties in het water om respiratie en primaire productie te bepalen (ecosysteem metabolisme; **Hoofdstuk 7**). In **Hoofdstuk 6** hebben we getoetst wat de toegevoegde waarde is van het meten van de afbraak van organisch materiaal als functionele indicator ten opzichte van de meer gangbare structuurindicatoren in monitoringsprogramma's. Hiertoe hebben we onderzoek gedaan in twintig sloten en langzaam stromende beken verspreid over Nederland, die in verschillende mate verstoord waren door landbouwactiviteiten en lozingen van rioolwaterzuiveringen. We lieten zien dat het effect van deze menselijke activiteiten op het functioneren van ecosystemen niet rechtstreeks gerelateerd was aan de structuurindicatoren. Dit kwam doordat er vaak meerdere stressoren aanwezig waren, die gecombineerd een verschillend effect hadden op de structuur en het functioneren van het ecosysteem. In dezelfde sloten en beken hebben we in **Hoofdstuk 7** onderzocht of geluidsopnames onderwater gebruikt kunnen worden om ecologische processen te kwantificeren. Onze resultaten lieten zien dat de patronen in het geluid overeenkomen met de metingen van de zuurstofhuishouding. Dit gaf een eerste indicatie dat deze nieuwe methode gebruikt kan worden om ecosysteemmetabolisme te bepalen. Wellicht zouden onderwater geluidsopnames zelfs enkele problemen kunnen ondervangen die zich voordoen bij het bepalen van ecosysteemmetabolisme met behulp van zuurstofmetingen. Zo zou deze methode de mogelijkheid kunnen bieden om de bijdrage van verschillende deelprocessen (productie, respiratie en rearatie) te onderscheiden op basis van de verschillende type geluiden die erbij worden geproduceerd. Ook zou het wellicht mogelijk zijn om onderdelen van ecosysteemmetabolisme waar te nemen die niet gerelateerd zijn aan de zuurstofhuishouding, zoals bijvoorbeeld anaerobe respiratie.

In **Hoofdstuk 8** hebben we het gebruik van functionele indicatoren ecosysteemmetabolisme en afbraak van organisch materiaal en structuurindicatoren in de kwaliteitsbeoordeling van stromende wateren middels een literatuuronderzoek vergeleken. Op basis hiervan beargumenteerden we dat er een beter begrip nodig is van de samenhang tussen de structuur en het functioneren van een ecosysteem en hoe deze samenhang beïnvloed wordt door menselijke activiteiten. Hiervoor is meer kennis nodig over 1) hoe het milieu de soortensamenstelling in een ecosysteem stuurt en 2) welke functionele rollen de soorten in een ecosysteem onder de lokale omstandigheden waarbij ze voorkomen spelen.

In de synthese stelde ik voor om het functioneren van een ecosysteem te benaderen op basis van hiërarchisch gerangschikte abiotische en biotische filters. De

temporele variatie in het milieu, zoals de hydrologische, morfologische en chemische processen, vormen de abiotische filter. Deze filter bepaalt welke soorten in een ecosysteem kunnen voorkomen, omdat organismen aan specifieke omstandigheden over de tijd zijn aangepast. Menselijke activiteiten kunnen er voor zorgen dat deze filter anders gaat fungeren, waardoor de soortensamenstelling kan veranderen. Het is dus van belang om de juiste tijdschaal in acht te nemen om beter te begrijpen wat de effecten van menselijke activiteiten zijn op het voorkomen van soorten. Specifiek houdt dit in dat 1) de juiste tijdschaal moet worden geselecteerd op basis van de generatietijd van de organismen die onderzocht worden, 2) binnen deze tijdschaal de temporele patronen in milieuvariabelen moeten worden gekwantificeerd door middel van hoogfrequente metingen, en 3) de intensiteit, frequentie, voorspelbaarheid en duur van verstoringen moeten worden gerelateerd aan de gevoelige perioden van de levenscyclus van de onderzochte organismen. De wisselwerking tussen organismen en het omringende milieu vormt de biotische filter. Deze filter bepaalt hoe het ecosysteem functioneert. Organismen spelen dus een belangrijke rol in het functioneren van ecosystemen, maar er is nog weinig inzicht in de precieze functionele rollen die organismen hierin vervullen. Om een beter idee te krijgen van deze bekende onbekende functionele rollen is het van belang om 1) specifieke ecosysteem functie(s) te selecteren, 2) te kwantificeren welke soorten deze functie(s) vervullen onder de lokale omstandigheden, en 3) hierbij niet alleen de functionele rollen mee te nemen die direct betrekking hebben op de voedselverwerving van organismen, maar ook op de niet-voedsel gerelateerde rollen, zoals die van ecosysteemingenieurs of biobouwers. Dit zijn organismen die een leefgebied creëren, wijzigen, vernietigen of onderhouden, zoals bijvoorbeeld planten die habitat leveren aan andere organismen, maar ook de licht-, zuurstof-, temperatuurhuishouding in het water beinvloeden.

In dit proefschrift werd geconcludeerd dat de functionele rollen die organismen spelen afhangen van de lokale omstandigheden. Hierdoor valt het effect van menselijke activiteiten op het ecosysteem functioneren vaak niet rechtstreeks te herleiden uit de structuurindicatoren die worden gebruikt in de huidige monitoringsprogramma's. Daarom raadde ik aan dat het toekomstig onderzoek naar het functioneren van ecosystemen zich zal moeten richten op de trofische en niet-trofische rollen die soorten kunnen vervullen onder lokale omstandigheden en hoe de vervulling van deze functionele rollen verandert over tijd. Het zijn tenslotte de organismen die ecosystemen laten functioneren.

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Good company in a journey makes the way seem shorter
--- Izaak Walton ---

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AUTHOR CONTRIBUTIONS

Chapter 2: G.H. van der Lee, M.H.S. Kraak, R.C.M. Verdonschot & P.F.M. Verdonschot

PFMV contributed to the study conception and design; PFMV, RCMV and others (see acknowledgements) collected and prepared the data; GHvdL performed the data analysis; GHvdL wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript.

Chapter 3: G.H. van der Lee, R.C.M. Verdonschot, M.H.S. Kraak & P.F.M. Verdonschot

GHvdL was involved in the study design, acquired and analyzed the data, and wrote the main manuscript text; MHSK, RCMV and PFMV were involved in the study design and critically revised the manuscript.

Chapter 4: G.H. van der Lee, M.H.S. Kraak, R.C.M. Verdonschot, J.A. Vonk & P.F.M. Verdonschot

GHvdL was involved in the study design, acquired and analyzed the data, and wrote the main manuscript text; MHSK, RCMV, JAV and PFMV were involved in the study design and contributed to writing the manuscript.

Chapter 5: G.H. van der Lee, J.A. Vonk, R.C.M. Verdonschot, M.H.S. Kraak, P.F.M. Verdonschot & J. Huisman

GHvdL, JAV and PFMV designed the study; GHvdL conducted the field work with participation of JAV and RCMV; GHvdL analyzed the data with input from JH, JAV and PFMV; GHvdL wrote the first draft of the manuscript and all authors contributed substantially to revisions.

Chapter 6: G.H. van der Lee, M.L. de Baat, N. Wieringa, M.H.S. Kraak, R.C.M. Verdonschot & P.F.M. Verdonschot

GHvdL was involved in the study design, acquired and analyzed the data, and wrote the main manuscript text; NW and MLdB were involved in the study design, acquired the data, and critically revised the manuscript; MHSK, RCMV and PFMV were involved in the study design and critically revised the manuscript.

Chapter 7: G.H. van der Lee*, C. Desjonquères*, J. Sueur, M.H.S. Kraak, & P.F.M. Verdonschot (*equal contribution)

GHvdL, CD and PFMV conceived the ideas and designed methodology; GHvdL conducted the field work, collecting the data and identifying invertebrate samples; CD and PFMV participated in the field work; CD and GHvdL analysed the data and led the redaction of the manuscript and all authors contributed critically to the drafts.

Chapter 8: P.F.M. Verdonschot & G.H. van der Lee

PFMV and GHvdL contributed equally to the manuscript.

ABOUT THE AUTHOR

Gea H. van der Lee was born on the 10th of February 1992 in Delft, the Netherlands, where she attended primary and secondary school. She always loved to be out in nature and enjoyed learning new things. This led her to move to Utrecht in 2010 for her bachelor studies in Environmental Sciences with a specialization in 'Water and Nature'. She spend one semester abroad at the School of Geography at Leeds University, UK. For her bachelor thesis she did a four month internship at the water board Hoogheemraadschap De Stichtse Rijnlanden, where she conducted research on the spatial patterns of aquatic macrophytes in Dutch polders in relation to the water management. Her work was highly commended by the Undergraduate Awards and she presented the results at their Global Summit in Dublin, Ireland. After finishing her bachelor degree *Cum Laude*, she continued with a master degree in Sustainable Development at the University of Utrecht which she also finished *Cum Laude*. She specialized in 'Environmental Change and Ecosystems' with all research projects focussed on aquatic ecology. In her first research project she collaborated with Warsaw University of Life Sciences to study nutrient retention in the relatively undisturbed Biebrza floodplain in Poland. Her master thesis was in collaboration with the Norwegian Institute for Water Research NIVA and focussed on invertebrate community change in acidified waters in Norway. She performed statistical analysis of long-term data sets to analyze temporal changes in the invertebrate community in relation to changes in environmental parameters.

Gea's fascination for aquatic ecology paved the way for her to start a PhD at the department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam. This trajectory (February 2016 – February 2020) led to the thesis you hold in your hands. Gea now works as a Junior Researcher Aquatic Ecology in the team Freshwater Ecology at Wageningen Environmental Research. In this position she contributes to the Water Quality Knowledge Impulse (KIWK), a project that aims to combine existing and new knowledge on factors that influence water quality, and thereby strengthen the basis for water management authorities to take measures to improve water quality and biodiversity in the Netherlands.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

Perspectives on functional assessment of anthropogenic stress on stream ecosystems

Writing of project proposal (2 ECTS)

KNAW Fund: using stable isotopes to reveal the impacts of eutrophication on aquatic food web structure

Post-graduate courses (3.6 ECTS)

Stable isotope applications in microbiology and environmental studies; SENSE (2017)
Aquatic ecology; PE&RC (2018)
Generalized linear models; PE&RC (2019)

Competence strengthening / skills courses (1.5 ECTS)

Teaching & supervision; UvA (2016)
Individual supervision; UvA (2017)
Presentation course for PhDs; UvA (2019)
IBED Career events; UvA (2019)

Scientific integrity / ethics in science activity (0.3 ECTS)

Academic integrity; UvA (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.9 ECTS)

NAEM (2017, 2018)

Discussion groups / local seminars / other scientific meetings (6.6 ECTS)

Stream ecology group meeting (2016-2018)
IBED Seminars (2016-2019)

International symposia, workshops and conferences (8.8 ECTS)

SFS; Raleigh (2017), Detroit (2018), Salt Lake City (2019)
SEFS; Zagreb (2019)

Societally relevant exposure (0.3 ECTS)

Landschap: adaptief monitoren (2018)

Lecturing / supervision of practicals / tutorials (3 ECTS)

Introduction to aquatic sciences (2016-2018)
Ecosysteem dynamica (2017)

Supervision of students (3 ECTS)

5 MSc research projects; 2 MSc literature projects, 1 BSc research project


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