Investigating the impact of the presence of water soldier (*Stratiotes aloides)* on water quality in a polder landscape with a high abundance of invasive crayfish

BSc Research Project – 24 EC

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1. Abstract

Water quality is experiencing a noticeable and concerning deterioration. In Europe, including the Netherlands, regulatory frameworks like the Water Framework Directive aim to achieve chemically and ecologically healthy water by 2027. Despite these efforts, Dutch agricultural ditches suffer from nutrient pollution, primarily from agricultural runoff, leading to eutrophication.

This study aims to investigate the impact of phytoremediation on water quality in a Dutch polder landscape, considering the presence of organisms that negatively affect water quality, by examining water soldier (*Stratiotes aloides*) as a potential nature-based solution for improving water quality in an area with a high abundance of invasive crayfish.

Water soldier has shown promise in reducing turbidity and nutrient levels to combat the effects of eutrophication. However, the presence of invasive crayfish complicates this dynamic by potentially altering nutrient cycling and aquatic macro-invertebrate community structure.

The study was conducted at Polderlab Vrouw Venne to assess both abiotic and biotic water quality in ditch tracks with and without water soldier. Abiotic parameters, including pH, turbidity, electrical conductivity, temperature, oxygen levels, and nutrient concentrations (specifically phosphate and nitrogen in the forms of nitrate and ammonia), were measured. Biotic assessments focused on aquatic macro-invertebrate richness, abundance, Shannon Diversity, taxa composition, and Belgian Biotic Index scores. Despite observable differences, only the Shannon Diversity Index varied significantly ($p=0.037$) between ditches with and without water soldier. Additionally, ditches with water soldier had slightly higher Belgian Biotic Index scores. When examining individual taxa, the abundances of *Baetidae* ($p=<0.001$), *Physidae* ($p=0.002$), and *Mysida* ($p=<0.001$) were higher in ditch tracks with water soldier.

The findings of this study underscore the complexity of managing water quality in agricultural landscapes and emphasize the need for nuanced approaches to effectively improve water quality.

2. Acknowledgements

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3. Introduction

Current state of bad water quality

Water is undeniably a fundamental element for life on Earth, ranking as the second most crucial resource after air. Its significance lies not only in sustaining human existence through drinking, but also in facilitating essential activities such as agriculture, manufacturing, and inland navigation (Zaman et al., 2017). However, despite its great importance, the quality of natural water bodies worldwide is experiencing a noticeable and concerning deterioration (Kılıç, 2021). This decline is due to pollutants ranging from urban stormwater runoff containing oil, lawn fertilizers, and other chemicals, to agricultural pollutants such as fertilizers and pesticides, as well as industrial waste chemicals, saltwater intrusion, and invasive species (Letchinger, 2000; Srivastav, 2020; Chowdhary et al., 2020; Johnson, 2007; McCormick et al., 2009). The urgency of addressing this issue is heightened by the combined effects of climate change and overpopulation (Gobler, 2020; Wurtsbaugh et al., 2019). Population growth increases pressure on industries, agriculture, and urbanization, which further strains water quality (Delpla et al., 2009). Climate change compounds these issues by altering weather patterns, leading to extreme conditions such as heavy rainfall and prolonged droughts, which can exacerbate water pollution. Flooding increases the transport of pollutants into water bodies, while droughts reduce water volumes, concentrating contaminants and further deteriorating water quality (Van Vliet et al., 2023).

The current state of water quality in Europe is concerning: only 40% of surface waters have good ecological quality, and only 33% have good chemical quality (Ecological Status of Surface Waters in Europe, 2021; European Environment Agency, n.d.). In the Netherlands, the situation is even worse, with only 1% of waters achieving both good ecological and chemical quality, placing the country at the bottom among all European Union (EU) member states (Didde, 2022). To address this issue, the Water Framework Directive (WFD) has been introduced, requiring EU member states to achieve and maintain chemically clean and ecologically healthy water by 2027 (Directive 2000/60/EC, 2014). The WFD relies on a river basin district strategy to ensure neighbouring countries collaborate in the management of shared rivers and other water bodies (European Commission, 2024). This is particularly important for a river delta like the Netherlands, where water and its pollutants from all over Europe converge in the rivers Meuse, Rhine, Scheldt, and Ems.

In addition to its major river systems, the Netherlands faces significant water quality challenges in its extensive network of agricultural ditches. These ditches span 300,000 kilometres across the country and are integral to agricultural water management, particularly in polders, which constitute about 60% of the Dutch landscape (Ligtvoet et al., 2008; Deltares, n.d.). Within these polders, the ditches are crucial for supplying water and draining excess from agricultural fields. Despite their importance to agriculture, these ditches have been overlooked in water quality regulations due to their limited size. Until 2018, they were not classified as water bodies under the Water Framework Directive (Compendium voor de Leefomgeving, 2021). However, recognizing their role in the broader hydrological system, the WFD was updated to include ditches also, reflecting their importance in achieving and maintaining water quality standards (Evers et al., 2018).

The main reason for inclusion of ditches in the WFD is the effect that nutrient pollution exerts, through these ditches, on the connected Dutch water system. Effective management of ditches is crucial for achieving overall water quality goals. A primary focus in these agricultural ditches is reducing nutrient levels, particularly nitrogen and phosphorus. These nutrients primarily originate from agricultural practices such as the application of artificial fertilizers and the use of animal manure (Schipper et al., 2022; CLO, 2022). Consequently, significant amounts of nitrogen and phosphorus can leach into ditches, especially during heavy rainfall or irrigation events (Vadas et al., 2005). When high concentrations of these nutrients enter water bodies, they contribute to a process known as eutrophication (Schindler, 1977; Khan & Ansari, 2005).

Eutrophication is characterized by several harmful effects on aquatic ecosystems. One of the most visible signs of eutrophication is the excessive growth of algae, often referred to as algal blooms (Anderson et al., 2002; Withers et al., 2014). These blooms can become so dense that they block sunlight from reaching deeper water layers, disrupting the photosynthesis of submerged plants. This reduction in sunlight and photosynthesis further diminishes oxygen levels in the water as the algae die off and decompose (El-Sheekh et al., 2021). As algae decay, bacterial activity increases because bacteria break down the organic material from the dead algae. This process, known as bacterial respiration, consumes oxygen (Diaz & Rosenberg, 2008). During the night, when submerged plants and algae do not perform photosynthesis, no new oxygen is added to the system. Consequently, the oxygen consumption by bacteria and other organisms can exceed the oxygen input, leading to significantly lower levels of dissolved oxygen in the water (Guo et al., 2018). This oxygen depletion can lead to anoxic conditions, where the oxygen levels are so low that fish and other aquatic organisms cannot survive, causing massive die-offs and a sharp decline in biodiversity (Prepas & Charette, 2005). Moreover, certain bacteria associated with eutrophication, such as cyanobacteria, can produce harmful toxins, posing potential hazards to both human and wildlife health (Osborne et al., 2001; Johnson et al., 2007). These combined effects severely disrupt the overall ecological balance of the aquatic environment, leading to long-term negative impacts on the health and sustainability of these ecosystems.

These algal blooms, along with the resulting oxygen depletion caused by eutrophication, severely threaten the biodiversity that thrives in these aquatic ecosystems. Protecting the water quality in these ditches against eutrophication and anoxia is especially essential, as they are significant reservoirs of biodiversity in the Netherlands. A study involving 279 standard pond net samples collected between 1980 and 2010 identified approximately one-third of the total Dutch freshwater fauna in these ditches. Moreover, this relatively high diversity was found in a sampling area representing only 0.0005% of the total ditch length in the Netherlands. This underscores the ecological importance of these water bodies (Verdonschot, 2012). Additional studies highlight the role of drainage ditches as crucial habitats for invertebrate biodiversity in agricultural landscapes (Painter, 1999; Armitage et al., 2003; Herzon & Helenius, 2008).

As eutrophication increasingly threatens the biodiversity found in Dutch ditches, impacting freshwater fauna populations and chemical water quality (Centraal Bureau voor de Statistiek, n.d.), there is growing concern that the Netherlands may not meet the Water Framework Directive's requirements within the next three years (Didde, 2022). This trend underscores the urgent need for effective action, as the inability to meet these requirements not only threatens the ecological integrity of Dutch water bodies but also carries significant social and economic implications. Poor water quality can affect human health through contaminated drinking water sources (Li & Wu, 2019) and recreational activities, such as swimming in cyanobacteria-infested waters caused by eutrophication (World Health Organization Regional Office for Europe & European Commission, 2002). It can also impact agriculture, as polluted water can affect crop irrigation and livestock health (Malakar et al., 2019; Umar et al., 2014). Furthermore, industries that rely on clean water, such as fisheries, could suffer significant economic losses (Parris, 2014; Mugidde et al., 2005). Therefore, the decline in water quality poses a multifaceted challenge that requires immediate and effective action. Investigating potential solutions to improve Dutch water quality is essential.

Traditional methods for managing water quality, such as circular agriculture or reducing nutrient runoff by installing infiltration ditches (Deltaplan Agrarisch Waterbeheer, 2022), are often expensive and inaccessible to many farmers due to their high costs (Maij et al., 2019). In contrast, nature-based solutions offer a more sustainable and cost-effective alternative by harnessing natural processes and ecosystems to address these challenges (O'Hogain et al., 2018). One promising nature-based solution approach is phytoremediation, where plants are used to improve water quality. Phytoremediation is not only cost-effective and non-invasive but also widely accepted as a means of mitigating environmental pollutants such as nutrients (Arthur et al., 2005). By utilizing the natural capabilities of plants, phytoremediation presents a practical and sustainable method for addressing water quality issues in polder landscapes.

Phytoremediation thus seems like a promising method for enhancing water quality in Dutch agricultural ditches. However, its success can be influenced by various interacting factors, including the presence of other organisms in the ecosystem (Jeong et al., 2015; Kumar et al., 2022). While phytoremediation can improve water quality, interactions with certain species, especially those that might negatively impact the process, are not fully understood. These negative interactions could potentially undermine the benefits of phytoremediation by disrupting nutrient balances, introducing toxins, or competing with the plants for resources (Kumar et al., 2022; Saladyga et al., 2023; Luo et al., 2017). Therefore, *the aim of this research is to examine the impact of phytoremediation on water quality in a Dutch polder landscape, considering the presence of organisms that negatively affect water quality.*

To investigate this, two model organisms have been selected based on their significant roles in the ecosystem and contrasting impacts on water quality: the phytoremediator water soldier (*Stratiotes aloides*), known for enhancing both biotic and abiotic water quality and historically abundant in Dutch shallow surface waters (Weeda et al., 1991; Brammer, 1979; Tarkowska-Kukuryk, 2006); and invasive crayfish, which may counteract this positive effect by degrading water quality and cutting up macrophytes like water soldier (Roessink et al., 2010; Gherardi & Acquistapace, 2007). Especially with their widespread infestation in significant portions of Dutch water bodies, this presents a considerable challenge to water quality management (NFDD & Anemoon, 2024). The following sections will discuss both selected model organisms in detail.

Water soldier as phytoremediator

Water soldier (*Stratiotes aloides*) is an aquatic plant and phytoremediator that exhibits a unique lifecycle, remaining submerged during autumn and winter and forming dense floating mats in spring and summer (Cook & Urmi-König, 1983). These dense mats result from the plant's ability to proliferate rapidly, aided by its high clonal growth rates. Within just a few growing seasons, water soldier can completely blanket surface waters (Cook & Urmi-König, 1983).

These high growth rates are especially helpful, given water soldier's potential to enhance water quality. This plant can improve abiotic water quality by preventing the re-suspension of sediment particles, thereby reducing water turbidity (Madsen et al., 2001). This reduction in turbidity has significant implications for abiotic water quality, as it enhances light penetration into the water column, promoting the growth of submerged aquatic vegetation and enhancing photosynthesis (Takamura et al., 2003). Additionally, water soldier can absorb excess nitrogen and phosphorus (Brammer, 1979) and limit ammonium conversion to nitrate by reducing oxygen levels in ditches through shading (Smolders et al., 2019). Kufel et al. (2010) hypothesized that during spring, water soldier primarily relies on nutrients from water in its submerged, rootless form, while in summer, its floating form with extensive roots can extract nutrients directly from bottom sediments, potentially reducing its dependence on dissolved nutrients in the water. By lowering nutrient levels in both the water column and sediments, water soldier competes with phytoplankton and filamentous algae, limiting their growth (Mulderij et al., 2007). Furthermore, it produces allelopathic compounds that inhibit phytoplankton growth, making it well-suited for meso- or eutrophic shallow stagnant waters (Strzałek & Koperski, 2009). Therefore, water soldier potentially serves as an ideal phytoremediator in agricultural ditches affected by eutrophication.

Beyond its impact on abiotic water quality, water soldier can also enhance biotic water quality by creating a favourable habitat for a wide array of aquatic organisms. The macrophyte's spiny leaves can provide habitat, breeding grounds, and food resources for aquatic macroinvertebrates (Obolewski & Strzelczak, 2009; Obolewski, 2005). Research conducted by Tarkowska-Kukuryk (2006) on the utilization of water soldier as a habitat for macroinvertebrates in a shallow eutrophic lake revealed that the fauna residing within the aquatic plants exhibited greater abundance and species diversity compared to those in the surrounding environment. Furthermore, there are species that specialize in water soldier, including the larvae of the endangered dragonfly green hawker (*Aeshna viridis*), which depend on water soldier for egg deposition (Rantala et al., 2004). Also, black terns (*Chlidonias niger*) are dependent on water soldier; they often construct their nests from fragments of leaves of this aquatic plant (Golawski et al., 2017).

Invasive crayfish as organism that negatively impacts water quality

Invasive crayfish are known to significantly influence nutrient concentrations (Angeler et al., 2001) in the water column through various mechanisms, potentially counteracting the benefits provided by water soldier. Firstly, their behaviours, including burrowing, walking, and tail flipping, cause bioturbation, which stirs up sediments and releases particle-bound nutrients into the water column. Additionally, crayfish feeding habits accelerate organic matter cycling, enriching the water with nutrients and altering the sedimentary nutrient pools (Angeler et al., 2001). Lastly, crayfish can release nutrients and negatively impact phytoremediation by cutting macrophytes like water soldier without consuming them, a behaviour known as non-consumptive clipping (Gherardi & Acquistapace, 2007). In a Dutch area called the Krimpenerwaard, the impact of crayfish on aquatic plant vegetation has even been linked to eutrophication and a blue-green algae bloom (Roessink & Ottburg, 2021).

Furthermore, crayfish have also been observed to adversely affect the biotic components of their environment. As omnivores, they have a very broad diet consisting of various aquatic invertebrates, (water) plants, detritus, fish, and amphibians (Roessink et al., 2010). Due to their ability to integrate into various levels of the food web and their reliance on the substantial energy reserves from detritus (Moyle & Light, 1996), crayfish have a significant impact on biotic water quality. Additionally, since crayfish cut up water plants and macrophytes, as mentioned above, the oxygen balance, clarity, and acidity of the water are influenced (Roessink et al., 2010). The change in turbidity is particularly important in this context, as crayfish may benefit from the turbid water by becoming less visible to potential predators like pike, which may find it challenging to thrive in such turbid conditions (Roessink et al., 2010).

In settings such as polder landscapes, where crayfish populations are often high and ditches already suffer from poor water quality due to eutrophication, crayfish can further degrade the water quality (Soes & Koese, 2010). Therefore, understanding the interaction between phytoremediation and organisms that can negatively affect water quality, using water soldier and invasive crayfish as model organisms, is crucial for effective water quality management. Regarding invasive crayfish, it is unclear how water soldier would impact them. On one hand, it could benefit them by providing breeding grounds, shelter for predators and the potentially heightened abundance of aquatic macro-invertebrate would provide food resources (Van Kleef et al., 2022; Correia, 2002; Alcorlo et al., 2005). On the other hand, the spiny leaves of water soldier might deter invasive crayfish from moving through these ditches, and the increased water clarity caused by water soldier can make them more vulnerable to their predators (Roessink et al., 2010).

This possible attraction or repulsion of water soldier on crayfish plays a crucial role in shaping water quality in Dutch agricultural ditches. If water soldier attracts crayfish, water quality might deteriorate. On the other hand, if invasive crayfishes would avoid water soldier, water quality might improve even further, in addition to the potential positive effects of water soldier on water quality. In Figure 1, these relationship between water soldier, invasive crayfish and water quality have been visualized. This study examines the direct impact of water soldier on water quality (green path Figure 1), while also taking the effects of a high invasive crayfish abundance (in this study defined as more than 100 crayfish per ditch) (blue path Figure 1) on water quality into account.

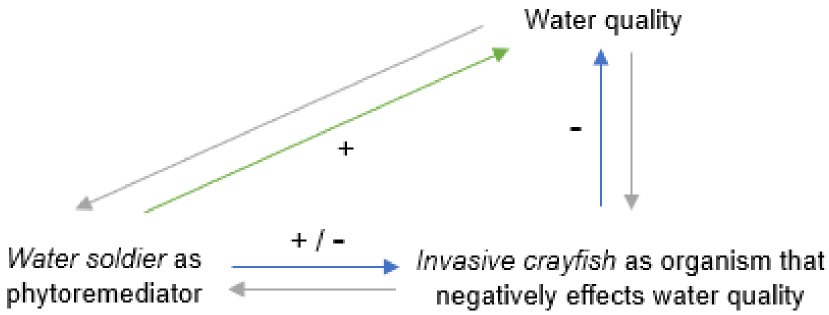


Figure 1: Overview of the relations between water soldier, invasive crayfish and water quality. *In this study, only the green and blue paths are examined. The grey arrows are present but have not been investigated.*

Objects and aims

Given the potential benefits of water soldier as a nature-based solution for improving water quality, it is crucial to determine its effectiveness in ditches with high invasive crayfish abundances. Therefore, the following research question has been formulated:

What impact does the presence of water soldier (*Stratiotes aloides*) have on water quality in ditches within a polder landscape with a high abundance of invasive crayfish?

In order to answer this question, the main research question has been split up into two sub-questions:

1. *What impact does the presence of water soldier have on abiotic water quality in ditches in a polder landscape with a high abundance of invasive crayfish?*
2. *What impact does the presence of water soldier have on biotic water quality in ditches in a polder landscape with a high abundance of invasive crayfish?*

By examining both abiotic and biotic aspects of water quality, a comprehensive understanding can be developed.

Approach

To investigate these questions, a study was conducted at Polderlab Vrouw Venne, a polder located in the Netherlands in a peat meadow area where it is investigated how sustainable agriculture can be applied in peatland areas (Polderlab, n.d.). The study area includes 10 ditches within an enclosed water system that features both tracks with and without water soldier presence, making it a suitable location to examine the impacts of water soldier on water quality. Additionally, the presence of at least two invasive crayfish species, the Red swamp crayfish (*Procambarus clarkii*) and the Spiny-cheeked crayfish (*Orconectes virilis*), is known in this polder, making it possible to study the impact of water soldier presence in combination with high abundances of invasive crayfish on water quality.

Abiotic water quality was assessed weekly from April through June by measuring pH, temperature, oxygen levels, electrical conductivity, turbidity, and nutrient levels of nitrate, ammonia, and phosphorus in ditch tracks with and without water soldier.
Biotic water quality was assessed once a month from March through May by comparing the ditch tracks with and without water soldier presence in terms of aquatic macro-invertebrate richness, abundance, Shannon Diversity, taxa composition and Belgian Biotic Index scores.
Crayfish abundances were measured once a month from April through May by counting the number of crayfish that were caught overnight in specialized crayfish traps in ditch tracks with and without water soldier.

Previous research in this area last year showed no significant differences between ditch tracks with and without water soldier in terms of abiotic and biotic water quality one year after the water soldier was planted (M. Kannekens, personal communication, March 6, 2024; Y. Hage, personal communication, March 6, 2024). However, both studies suggested that it would take a longer period of time to observe effects of water soldier on biotic and abiotic water quality parameters. Therefore, it is important to re-evaluate these effects now that the plants have been present for a longer period. Regarding invasive crayfish abundances, no comprehensive research on the number of crayfish has previously been conducted in Polderlab Vrouw Venne.

Expected outcomes

The impact of the presence of water soldier in ditch tracks with high invasive crayfish abundance is expected to result in measurable improvements in both abiotic and biotic water quality. Specifically, reductions in nutrient levels (phosphorus and nitrogen in the forms of nitrate and ammonia) are anticipated, alongside an increase in the diversity and abundance of macroinvertebrates. These outcomes underscore water soldier's potential as an effective tool for enhancing water quality in agricultural ditches, aligning with the objectives of the Water Framework Directive. Moreover, if water quality improves despite the presence of crayfish, these findings offer a dual benefit by addressing both water quality issues caused by eutrophication and invasive crayfish in the Netherlands. Ultimately, these research findings could inform water management practices and policies, promoting the integration of nature-based solutions in the maintenance and restoration of aquatic ecosystems in polder landscapes.

4. Methods

Study area

To assess the impact of water soldier presence on water quality, considering the high abundance of invasive crayfish, this study examined both biotic and abiotic components, including aquatic macroinvertebrates and various abiotic water variables. For this purpose, 10 ditches were selected in Polderlab Vrouw Venne, all of which are located within an enclosed water system (Figure 2).

The selected ditches were relatively equal in size and depth, with widths ranging from a minimum of 6 meters to a maximum of 14 meters and averaging 9 meters wide, and depths varying from a minimum of 40 cm to a maximum of 90 cm, averaging 60 cm deep (M. Schrama et al., personal communication, January 19, 2024). Among these ditches, water soldier has been present for varying durations. In ditch track 1, water soldier has been present the longest; it was planted here in November 2022. Ditch tracks 2 to 5 were planted twice, once in April 2023 and once in October 2023. Lastly, in ditch tracks 6 to 10 water soldier was introduced in November 2023 (K. Koot, personal communication, May 18, 2024). Only in ditch track 1 was water soldier present in high numbers; in the other ditches, water soldier was minimally present.

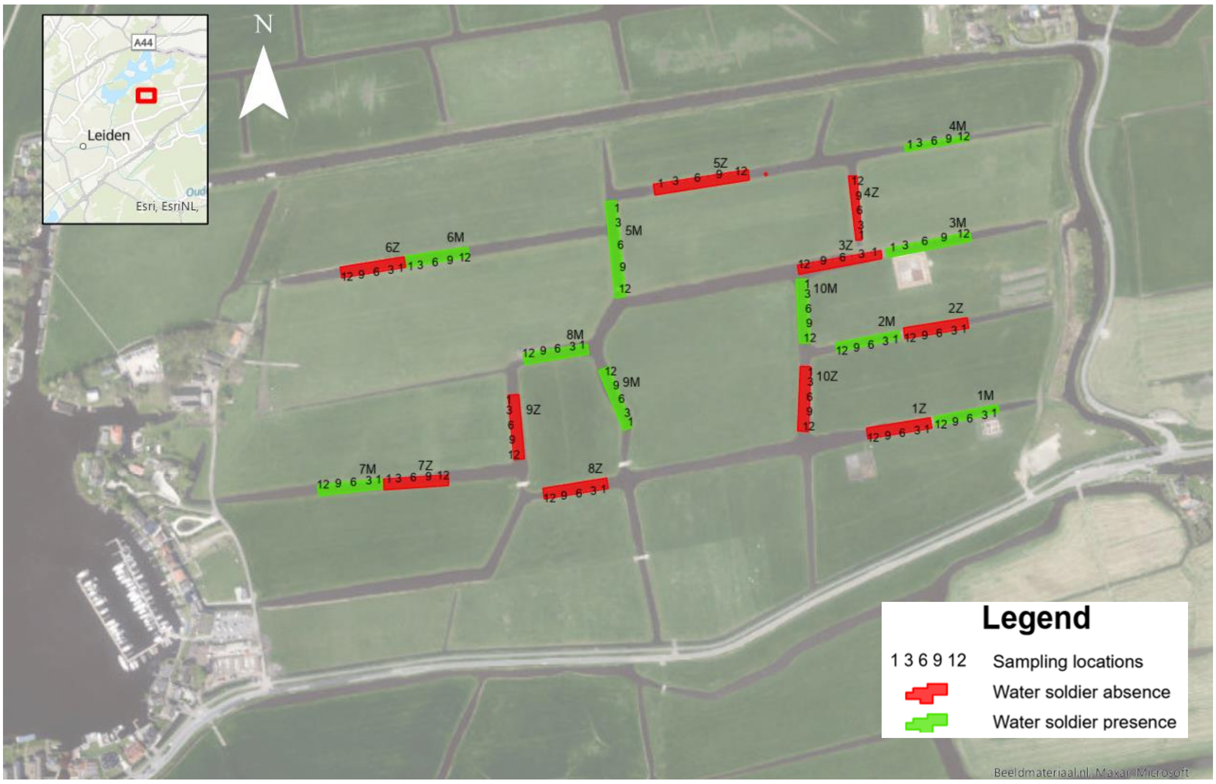


Figure 2: Study site in the Polderlab Vrouw Venne with ditch tracks featuring the presence or absence of water soldier
Not drawn to scale.

Experimental set up

As seen in Figure 2, the 10 selected ditches were divided into two tracks: one served as a treatment ditch track where water soldier was present, and the other served as a control ditch track where water soldier was absent. The barrier between these tracks consisted of branches in the water that kept the water soldier in place but did not prevent water flow or the migration of aquatic macro invertebrates between the treatments.

To maintain consistent sampling locations and order for every measurement week, a few measures were taken. Firstly, in order to keep the sampling locations the same in every sampling week, 12 flags were evenly distributed along one side of the ditch track (see Figure 2). The distance between flags was determined by the shortest side of the ditch track. Efforts were made to vary the placement between the north and south sides for some ditches and between the east and west sides for others. This distribution was as follows: 7 tracks on the north side, 7 on the south side, 5 on the east side, and 1 on the west side out of the total 20 ditch tracks (Figure 2).
Next, the sampling order was kept the same for both abiotic and biotic measurement weeks. Due to the large area of the Polderlab Vrouw Venne and long distances between ditches, the sampling order was based on the shortest walking route through the polder.
For crayfish abundance measurements, the sampling order was alternated each measurement week: it was reversed compared to the previous measurement week. This approach aimed to minimize walking time between ditches while still randomizing the sampling order across measurement weeks.
Lastly, to prevent interference between the measurements, biotic measurements were always conducted one week before the crayfish abundance measurements.

Data collection

Invasive crayfish abundance measurements

To assess whether invasive crayfish abundances influenced water soldier's capability to improve water quality, crayfish traps were utilized (Appendix A - Figure 1). A high crayfish abundance in this study was defined as more than 100 crayfish per ditch. This is considered as a high abundance because of their invasive nature, high reproduction rates and short generation time (Huner & Barr, 1991; GISB, 2011; Oficialdegui et at., 2020). Testing these effects at such high abundances increases the effect size, thereby potentially boosting statistical power.

Due to the limited number of crayfish traps available, only 2 or 3 ditches were sampled overnight over a one-week period. Each ditch track contained 12 traps, which were evenly distributed along one side of the bank (points 1 to 12, Figure 2). Each trap was baited with 3 halibut pellets and positioned at the bottom, parallel and as close to the ditch bank as possible, while still leaving enough room for air to prevent the drowning of bycatch (Figure 3). These traps were left overnight and emptied the following morning, where the number of crayfish caught in each trap, along with the time of placement and emptying were recorded. After recording, the crayfish were placed back in the ditch where they were caught. To minimize potential adverse effects, attempts were made to retrieve all bait used after sampling. This process was repeated until all ditches were sampled within the measurement week. Additionally, both tracks from the same ditch were ensured to be sampled on the same day. For statistical analysis, the total number of crayfish caught in each ditch track was aggregated.

A list of the materials used for the crayfish abundance measurements can be found in Appendix A (List 1).

Figure 3: Positioning of the used crayfish traps to measure invasive crayfish abundance

Abiotic water quality measurements

To assess the impact of the presence of water soldier on abiotic water quality in ditches with high crayfish abundances, nutrient concentrations and turbidity were primarily important, but for the sake of completeness, other water quality variables were also measured to determine the abiotic water quality. The following variables were measured in one day every week: pH, temperature, turbidity, oxygen levels, electric conductivity, and concentrations of nitrate, ammonia and phosphate. The same sampling order was always used to ensure consistent sampling of the same ditch track at the same time, thereby minimizing the influence of temperature changes due to the sun. The following order was used, based on the shortest walking route: 5M – 5Z – 4M – 4Z – 3Z – 3M – 10M – 2Z – 2M – 10Z – 1M – 1Z – 8Z – 9M – 8M – 6M – 6Z – 9Z – 7Z – 7M (Figure 2).

Measurement of pH, electric conductivity, dissolved oxygen levels and temperature

pH, electric conductivity, dissolved oxygen levels, and temperature were measured directly in the field at two locations in each ditch track (points 3 and 9 – Figure 2) and averaged out in the statistical analysis to reduce the likelihood of measuring errors. These variables were determined using a portable multimeter (*HACH model HQ4300*) with corresponding probes. Temperature was measured using the oxygen probe of the portable multimeter. The probes were rinsed three times with ditch water to ensure that they were clean before taking the next sample.

Measurement of turbidity

Turbidity was measured directly in the field at one location (point 6 – Figure 2) in every ditch track, using a Secchi transparency tube. This involved filling the tube completely with sample water from the surface, then slowly pouring it out until the distinction between the white and black quarters at the bottom of the tube became clear. Measurements were consistently conducted by the same person to prevent sampling bias, while facing the sun to ensure uniform lighting and minimize reflections from the sky inside the tube.

Measurement of nitrate, phosphate and ammonia levels

Nitrate, phosphate and ammonia levels were analysed inside a trailer present at Polderlab, in the same order as they were sampled. For this, a mixed sample containing 25 mL of water from point 3 and 25 mL of water from point 9 (Figure 2) was used. The time between sampling and analysis was always tried to be minimized and typically ranged between 3 to 5 hours. After sampling, nitrate concentration in the ditch water was determined using nitrate test packs (*Kyoritsu model WAK-NO3-S*). Phosphate levels were measured using a Checker photometer (*Hannah Instruments HI713 with reagent HI713-25*). Ammonia levels were also determined using a Checker photometer (*Hannah Instruments HI715 with reagents HI715A-0 and HI715B-0*).

A list of the materials used for the abiotic measurements can be found in Appendix A (List 2).

Biotic water quality measurements

To evaluate the impact of the presence of water soldier on biotic water quality in ditches with high crayfish abundances, aquatic macro invertebrates were used. These organisms are present in every water body and are rich in species, allowing for a broad spectrum of reactions to environmental conditions, making them valuable bioindicators for this study (Rosenberg, 1998). Monthly measurements were conducted within a designated week. The sampling order of ditch tracks was consistent and followed this sequence: 5M – 5Z – 4M – 4Z – 3M – 3Z – 2Z – 2M – 1M – 1Z – 10Z – 10M – 7M – 7Z – 9Z – 8Z – 9M – 8M – 6Z – 6M (Figure 2). It was ensured that both tracks of each ditch were always sampled on the same day.

The biotic measurements involved the use of an area kick net (*mesh size 2mm*) at three locations along the ditch track (between points 3-4, 6-7, and 9-10 - Figure 2). First, a fast and forceful sweep in the water was made (Figure 4 – 1), followed by three figure-eight movements while ensuring that each layer of the water column was sampled, without excessively scraping the bottom layer (Figure 4 – 2). Lastly, a sweep across the bottom layer was made to ensure that all macro invertebrates present were captured (Figure 4 – 3). These steps were carried out in one continuous take and as close as possible to the ditch bank.

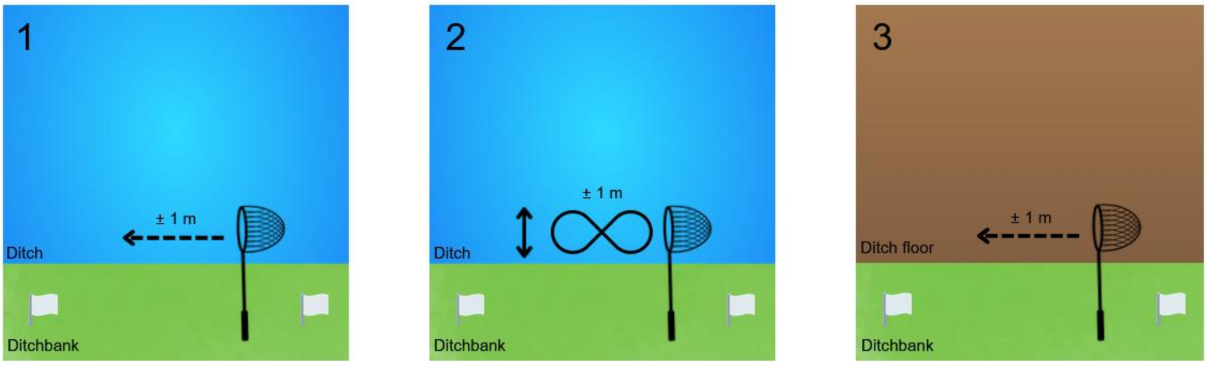


Figure 4: Abstract visualization of the sampling method used to assess biotic water quality.

The sampling was consistently conducted by the same person, ensuring a consistent speed and direction, to minimize sampling bias. The area sampled covered approximately 1 meter by 0.5 meters. Between sampling different points, each sample was initially washed, ensuring that the net was kept slightly above the water to prevent any new ditch water from contaminating the sample. After washing, all samples from the three locations were combined into a white container filled with a shallow layer of water for identification of the aquatic macro invertebrates.

For the identification, first, large crayfish were counted, recorded, and then returned to the ditch. The remaining individuals were transferred to cups containing a shallow layer of water, along with their corresponding order, and when possible, family. Identification was conducted using ObsIdentify (*version 3.15*) and identification keys (Bouchard, 2004; The Conchological Society of Great Britain and Ireland, n.d.; Drukker, 2017; Elliott et al., n.d.; UK Beetles, n.d.). In cases where the abundance of a specific taxon exceeded 100, it was recorded as 100+. Once it was visually estimated that 95% of the individuals were identified, the number of individuals of each taxon was noted down, and all macro invertebrates were released back into the ditch track from which they were collected.

With this data, taxa richness, abundance, Shannon Diversity, taxa composition and Belgian Biotic Index scores were calculated. Because there is not just one measure for biotic water quality, multiple ecological aspects were taken into account to provide a comprehensive view.
Firstly, taxa richness is the number of taxa present in a given area and is the simplest and most common measure of biodiversity (Magurran, 2003). It is crucial for determining environmental quality, as required by biological quality indices such as those used in the Water Framework Directive (Ramos-Merchante & Prenda, 2017).
Next, abundance is important because it provides specific insight into how water soldier and invasive crayfish affect individual taxa.
Thirdly, Shannon Diversity combines data on taxa richness with its corresponding abundance (Bartram & Ballance, 1996). If water soldier in crayfish-rich ditches improves water quality, higher taxonomic richness and increased abundance can be expected (Duran, 2006), resulting in a higher Shannon Diversity.
Furthermore, taxa composition was determined because shifts in composition can reflect alterations in habitat suitability caused by water soldier and invasive crayfish (Lalonde & Downing, 1992).
Lastly, the Belgian Biotic Index (BBI) was calculated. This index makes use of the number of taxa to indicate the biodiversity and the sensitivity of organisms to pollution (Vannevel et al., 2018). Since no specific biotic index developed for the Netherlands was available, the BBI was a suitable measure for assessing biotic water quality, as the majority of organisms found in this study were included in the BBI.

The BBI was determined using Table 1 and can be found in Appendix B (De Pauw & Vanhooren, 1983). Since some of the data were collected at the order level, and the Belgian Biotic Index sometimes includes only specific families within certain orders, this occasionally posed a challenge. For example, the Belgian Biotic Index only considers the family *Gammaridae* for the order *Amphipoda*. Since data for *Amphipoda* were available only at the order level in this study, it was assumed that all *Amphipoda* individuals found belonged to the family *Gammaridae*.

A list of the materials used for the biotic measurements can be found in Appendix A (List 3).

Data analysis

Only results with a probability of $\alpha \leq 0.05$ were assumed to be statistically significant.

Ditch 1, which was the only ditch with a thriving population of water soldier, was included in the statistical analysis because there were not enough replications to test it independently from the other ditches.

Invasive crayfish abundance

To assess the dependency between invasive crayfish abundance and the presence of water soldier, a test of dependence was conducted using a Welch T-test in *R (v. 4.4.0)*. This test resulted in a p-value of *0.026*, indicating that the presence or absence of water soldier and crayfish abundance were not independent variables; instead, they are interrelated. Therefore, in all statistical models used, only the presence or absence of water soldier is considered. This relationship is visualized as Model 2 in Figure 5, where invasive crayfish abundance and the presence of water soldier are shown to be interconnected. If the data for water soldier and invasive crayfish abundance were not dependent on each other, Model 1 (Figure 5) would have been used instead, where invasive crayfish serves as a mediator in affecting water quality through water soldier.

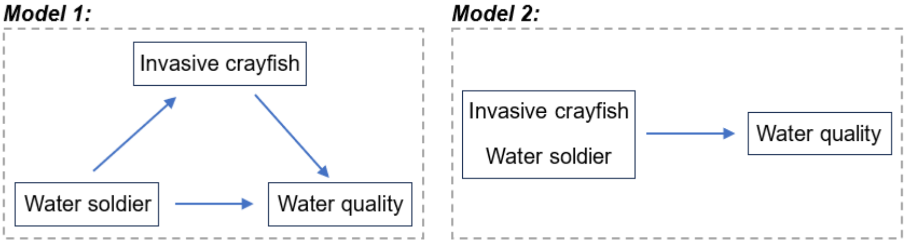


Figure 5: Statistical models for the relations between water soldier, invasive crayfish and water quality.

Model 1 shows the relationship between water soldier, invasive crayfish and water quality where the data of water soldier and invasive crayfish are not dependent on each other.

Model 2 shows the relationship between water soldier, invasive crayfish and water quality where the data of water soldier and invasive crayfish are dependent on each other.

Abiotic water quality

A linear mixed model (LMM) was constructed to test the impact of water soldier presence on abiotic water quality variables. Linear mixed models are frequently employed in phytoremediation studies because they can account for potential variations stemming from seasonal changes, differences in water temperature, or inherent differences across sampling locations (Balázs et al., 2018; Wang et al., 2021). For the variables 'electric conductivity' and 'temperature' a Generalized Linear Mixed Models using Template Model Builder (glmmTMB) was used, with respectively a negative binomial and gaussian distribution to better fit the data. This was determined by examining which model gave the best simulated residual plot using the *'DHARMa'* package available in *R*.

For every abiotic variable, two different models were made: one that included week as a random effect to capture any variation between the different measurement weeks, and one model that did not. The model with the lowest Akaike Information Criterion (AIC; Akaike, 1973) and Bayesian Information Criterion (BIC; Schwarz, 1978) values were chosen since they best explain the variation in the data. Also using the '*DHARMa'* package, a plot of the simulated residuals was created. In case the simulated residual plots showed different results for the best model than the AIC and BIC values, the model according to the best simulated residual plot were chosen. For most models this meant that presence/absence of water soldier and the week of sampling were taken into account as fixed effects, and that ditch number was taken into account as a random effect.

For abiotic water quality, the data from the weeks when crayfish abundance was measured was utilized. The abiotic data from the last crayfish measurement week (week 22) was missing, therefore the abiotic data from week 23 was included along with the data from weeks 15 and 18.

Biotic water quality

Since there is not a single method to quantify biotic water quality, a variety of statistical analyses were performed. First, the Shannon Diversity was calculated in *Excel* using the following formula, where S is the number of taxa and p_i the proportion of the total sample belonging to the *i*th taxa (Magurran, 2004):

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i)$$

Next, taxa richness, abundance and Shannon Diversity were analysed using linear mixed models (LMM). To determine if week should be included as a random effect, AIC-values, BIC-values and the simulated residual plots were checked. For all biotic variables, the presence or absence of water soldier was included as a fixed effect, and ditch number was considered as a random effect. For the abundance of *Physidae,* a Generalized Linear Mixed Models using Template Model Builder (glmmTMB) was used with a negative binomial distribution to better fit the data. This was determined by examining which model gave the best simulated residual plot.

Thirdly, differences in beta diversity were tested using a Bray-Curtis dissimilarity matrix and distance to centroid as a measure for differences in taxa composition. The difference in centroid values between the treatments of water soldier absence and presence was quantified and tested for significance using a PERMANOVA and post-hoc Tukey multiple comparison tests. By examining various biotic water quality variables with different statistical models, a comprehensive understanding of the identified biotic water quality could be obtained.

5. Results

Invasive crayfish abundance

To assess whether the invasive crayfish abundances in the Polderlab Vrouw Venne met the threshold for high abundance of 100 crayfish per ditch, Figure 1 in Appendix C was created. Over a 3-month period, an abundance of at least 127 crayfish per ditch was observed, indicating a high crayfish abundance during this study.

The impact on abiotic water quality

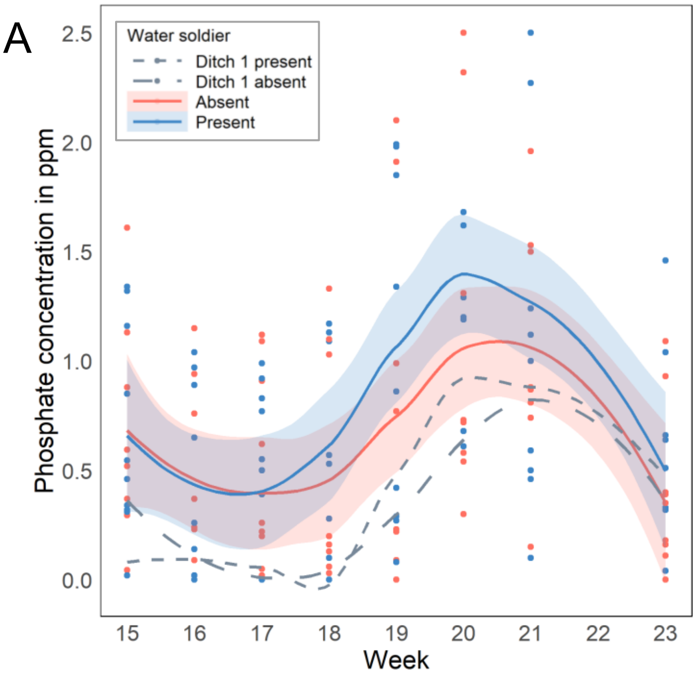
The impact of water soldier presence, while considering high crayfish abundances, on abiotic water quality was evaluated by examining the following water quality variables: turbidity, temperature, pH, electrical conductivity, oxygen levels, ammonia levels, phosphate levels, and nitrate levels. Each abiotic variable was measured in 20 ditch tracks over a 9/10-week period, resulting in 180 or 200 replications.

For the abiotic water quality variables tested, no significant differences were found between ditch tracks with or without water soldier. In table 1 the found means, standard deviations and p-values for every variable can be seen. Nitrate consistently measured at 0.2 ppm NO_3/N, so no statistical analysis could be performed on this variable.

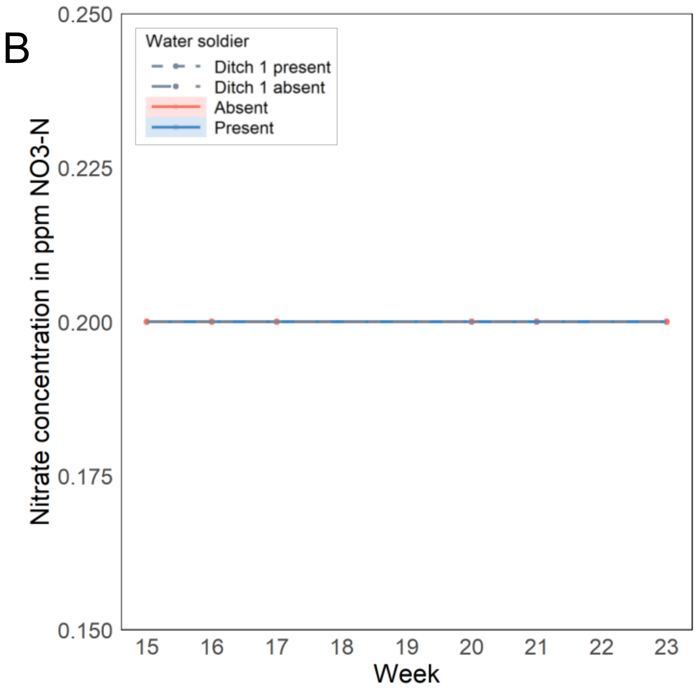
Table 1: The mean, standard deviation and p-value for all abiotic variables tested in ditches with water soldier presence and absence.
All nutrient concentrations and turbidity are given in ppm. Overall averages and standard deviations are given, while statistical tests were carried out using linear mixed models (LMM).

	Water soldier		
	Presence *Mean±SD*	Absence *Mean±SD*	*p-value*
Phosphate	0.76 ± 0.64	0.72 ± 0.55	0.696
Nitrate	0.2 ± 0.0	0.2 ± 0.0	-
Ammonia	0.89 ± 0.29	0.91 ± 0.33	0.78
Turbidity	33 ± 6	34 ± 6	0.707
Dissolved oxygen	9.83 ± 2.27	9.81 ± 2.19	0.971
Temperature	15.60 ± 2.84	15.51 ± 2.70	0.9
pH	7.79 ± 0.61	7.73 ± 0.65	0.183
Electric conductivity	538.82 ± 163.52	538.55 ± 156.42	0.935

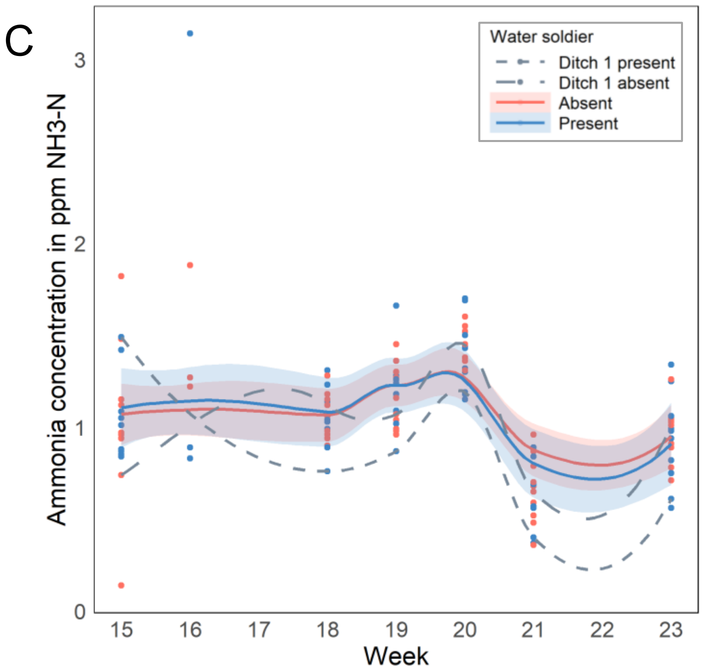
For visualizations in Figure 6A to 6H, which illustrate each abiotic water quality variable over a 9/10-week period, ditch 1 is shown separately. It can be observed that the measured values for phosphate and ammonia concentrations, electrical conductivity, and turbidity were lower in ditch track 1 where water soldier was present, while dissolved oxygen levels and pH were higher. The results for water temperature and nitrate concentrations in ditch track 1 with water soldier presence were inconclusive.



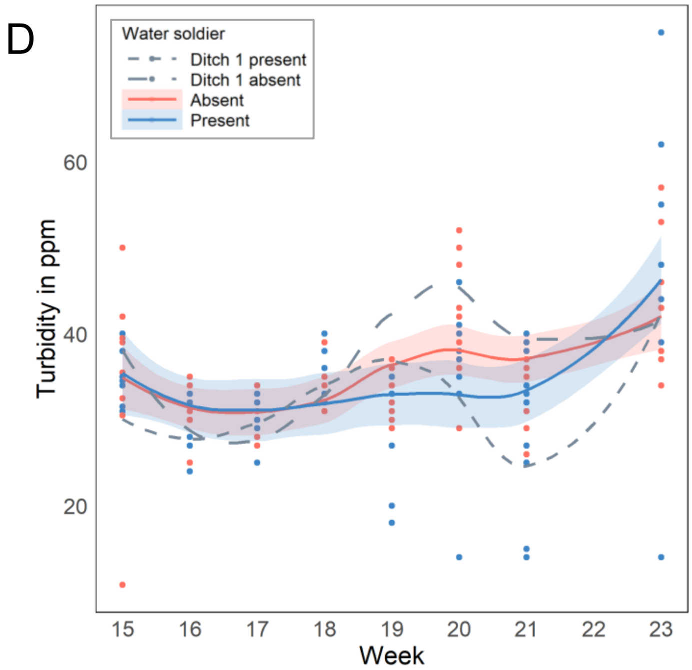
High phosphate levels are associated with bad water quality.



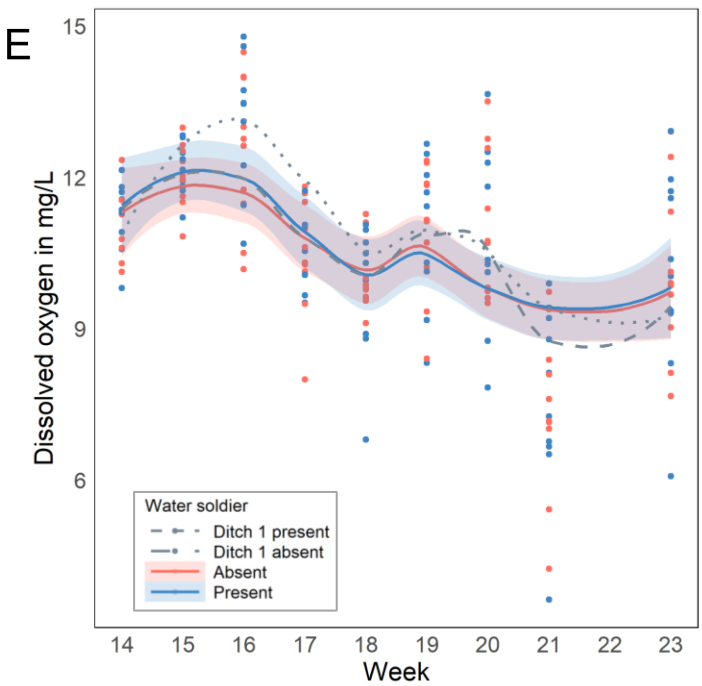
High nitrate levels are associated with bad water quality.



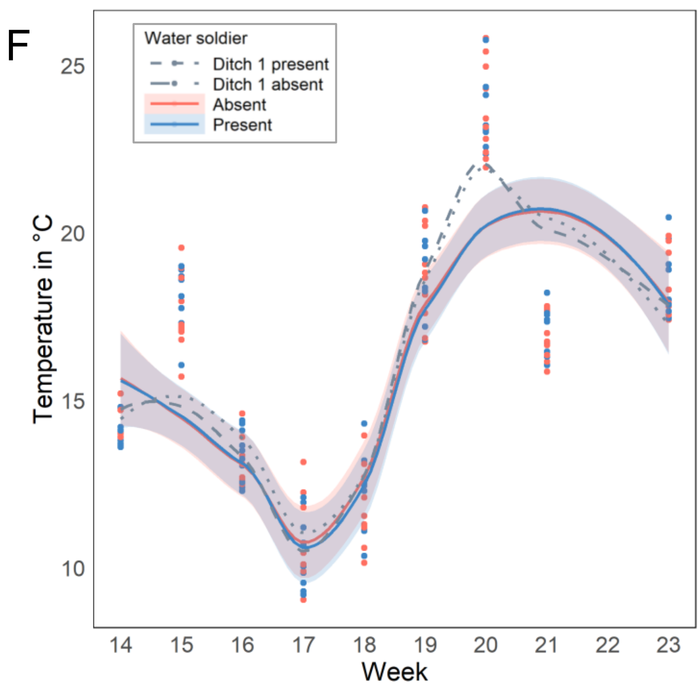
High ammonia levels are associated with bad water quality.

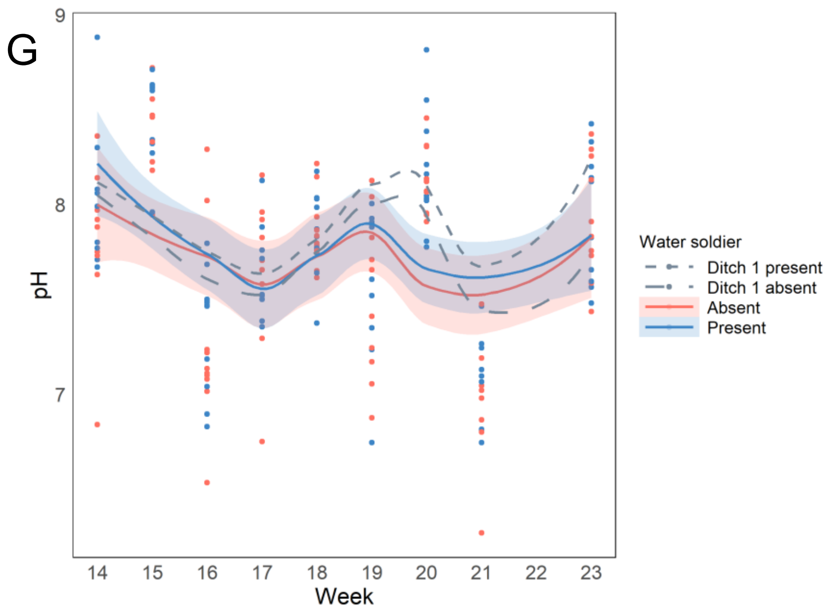


High turbidity levels are associated with bad water quality.

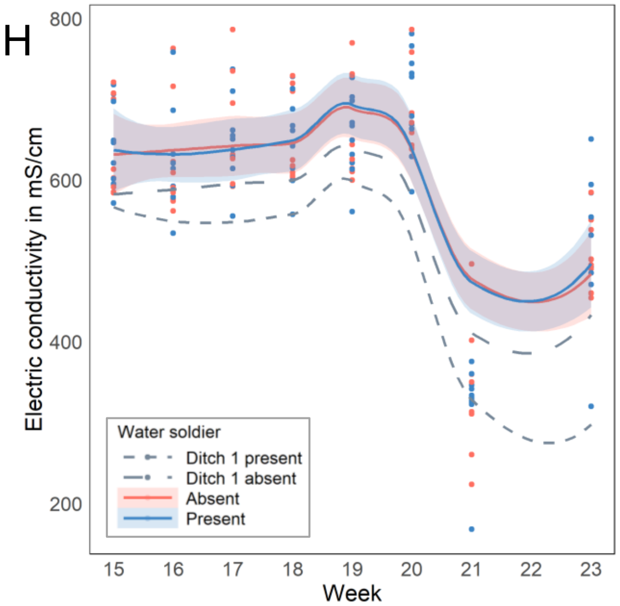


Low dissolved oxygen levels are associated with bad water quality.





Both high and low pH levels are associated with bad water quality



High electric conductivity levels are associated with bad water quality.

Figure 6: Abiotic water variables in ditch tracks with water soldier presence or absence
With A) Phosphate levels; B) Nitrate levels; C) Ammonia levels; D) Turbidity; E) Dissolved oxygen; F) Water temperature; G) pH and H) Electric conductivity.

The impact on biotic water quality

The impact of water soldier presence, while considering a high abundance of invasive crayfish, on biotic water quality is evaluated by examining taxa richness, abundance, Shannon diversity, taxa composition, and Belgian Biotic Index scores. Each biotic variable was measured 3 times in 20 ditch tracks, resulting in 60 replications. Each of these variables will be discussed in detail below.

Abundance of all taxa found

In Figure 7, an overview of all aquatic macro-invertebrate taxa found in ditch tracks with and without water soldier presence, along with their corresponding abundances, was created. For better visualization, taxa with very high abundances, specifically *Amphipoda*, *Diptera*, and *Corixidae*, were excluded from this figure. Substantial differences in the abundances of *Baetidae*, *Mysida*, *Physidae*, and *Zygoptera* between the treatments with and without water soldier presence can be observed, with a higher abundance in ditches with water soldier presence. In contrast shows *Unionidae* a higher abundance in ditches without water soldier. Only statistically significant differences were found for *Baetidae (p=<0.001)*, *Mysida (p=<0.001)* and *Physidae (p=0.002)* and can be seen in Table 2.

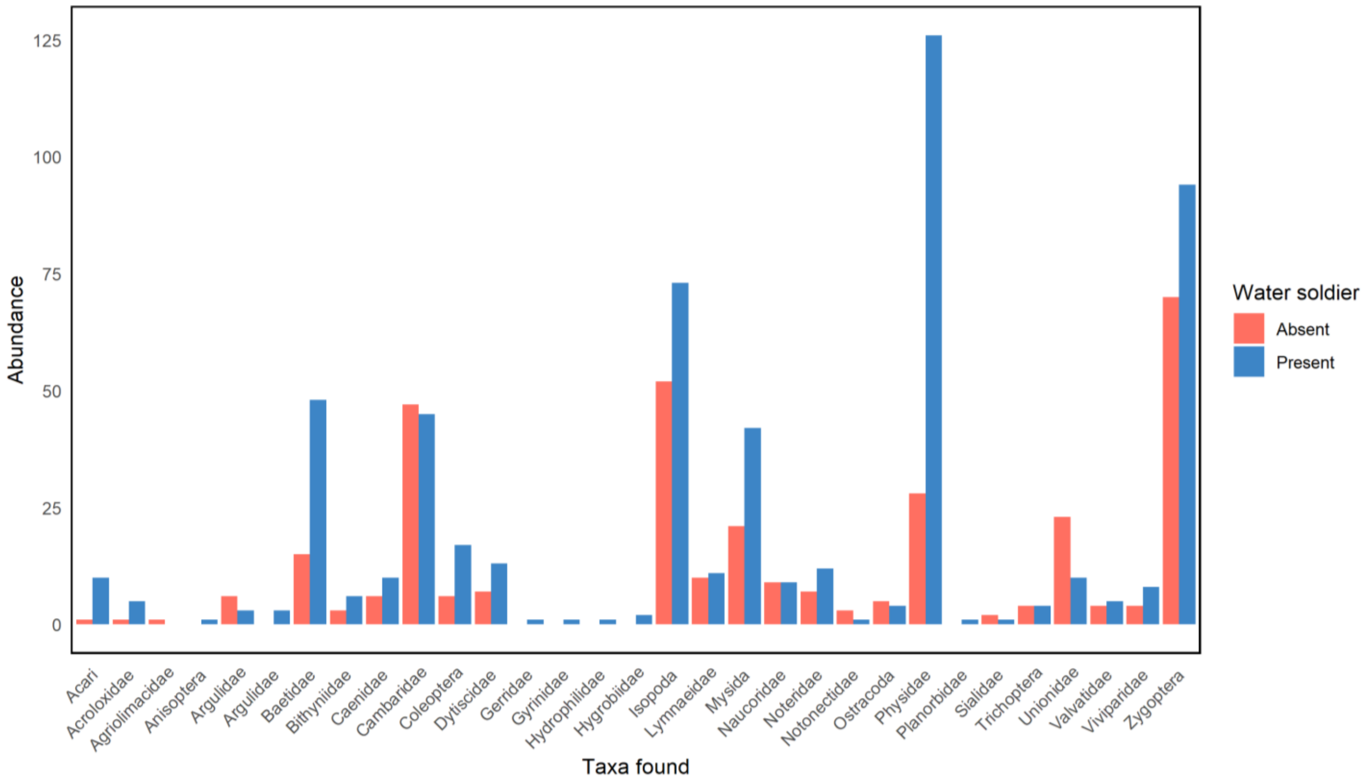


Figure 7: Selection of total abundances of taxa found in ditch tracks with water soldier presence or absence
For better visualisation, the taxa Amphipoda, Diptera and Corixidae with very high abundances were removed from this figure.

Table 2: The mean, standard deviation and p-value for the abundance of selected taxa in ditches with water soldier presence and absence.
Overall averages and standard deviations are given, while statistical tests were carried out using linear mixed models (LMM). Taxa with significant differences between treatments water soldier presence or absence are highlighted in bold.

	Water soldier			
	Presence *Mean±SD*	Absence *Mean±SD*	*p-value*	*Estimate*
Baetidae	**1.6 ± 3.9**	**0.5 ± 1.4**	**<0.001**	***1.16315***
Mysida	**1.4 ± 4.0**	**0.7 ± 1.29**	**<0.001**	**0.693117**
Physidae	**4.2 ± 9.6**	**0.9 ± 1.1**	**0.002**	**1.3352**
Unionidae	0.3 ± 0.6	0.8 ± 2.2	0.308	*-0.5603*
Zygoptera	3.1 ± 3.2	2.3 ± 3.1	0.314	0.3273

Taxon accumulation curve
Furthermore, a taxon accumulation curve was generated over time for all three measurement weeks (Appendix D - Figure 1) to evaluate the comprehensiveness of the search for aquatic macroinvertebrate richness in the ditches at the Polderlab Vrouw Venne. The curve indicates that the number of taxa found is approaching a plateau, suggesting that most of the taxa present have likely been identified.

Taxa richness

To examine the impact of water soldier presence on taxa richness, Figure 8 was created. Across all three measurement weeks, taxa richness of the aquatic macro-invertebrates was higher in ditch tracks with water soldier presence. However, Ditch 1 did not follow this pattern; in this case, ditch tracks without water soldier had a higher taxa richness. The taxa richness between the sample weeks remained relatively stable for both treatments. With mean ± SD values of taxa richness being 9 ± 3 for water soldier presence and 7 ± 3 for absence, the resulting p-value was 0.155 (estimate = 1.1333). This indicates that there is no significant effect of water soldier presence on taxa richness.

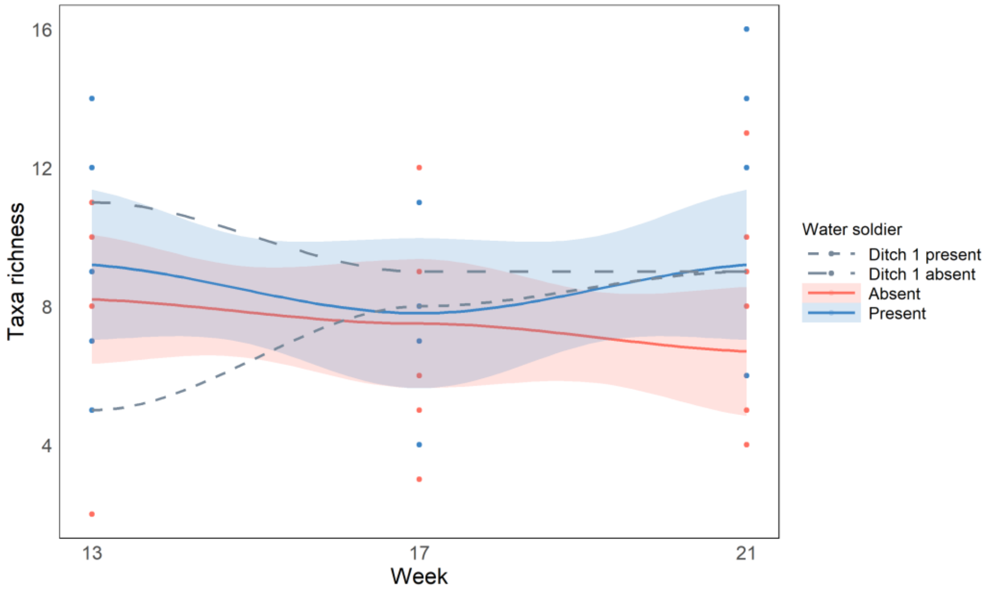


Figure 8: Taxa richness of aquatic macro-invertebrates in ditch tracks with water soldier presence/absence. *Ditch 1 is highlighted in grey, while in the categories 'Absent' and 'Present' all ditches are present.*

Abundance

Aquatic macro-invertebrate abundance is also a crucial factor in assessing biotic water quality, and is illustrated in Figure 9. Abundance is higher in ditch tracks without water soldier only in week 17; in weeks 13 and 21, abundance is higher in ditch tracks with water soldier. Ditch 1 follows the pattern observed in week 17, where abundance is greater in ditch tracks without water soldier than in those with water soldier. Overall, when considering all ditches, abundance increases over the different measurement weeks.

With mean ± SD values of abundance being 124 ± 71 for water soldier presence and 116 ± 55 for absence, the resulting p-value of 0.512 (estimate = 7.700) suggests that there is no significant difference in aquatic macro-invertebrate abundance related to the presence of water soldier.

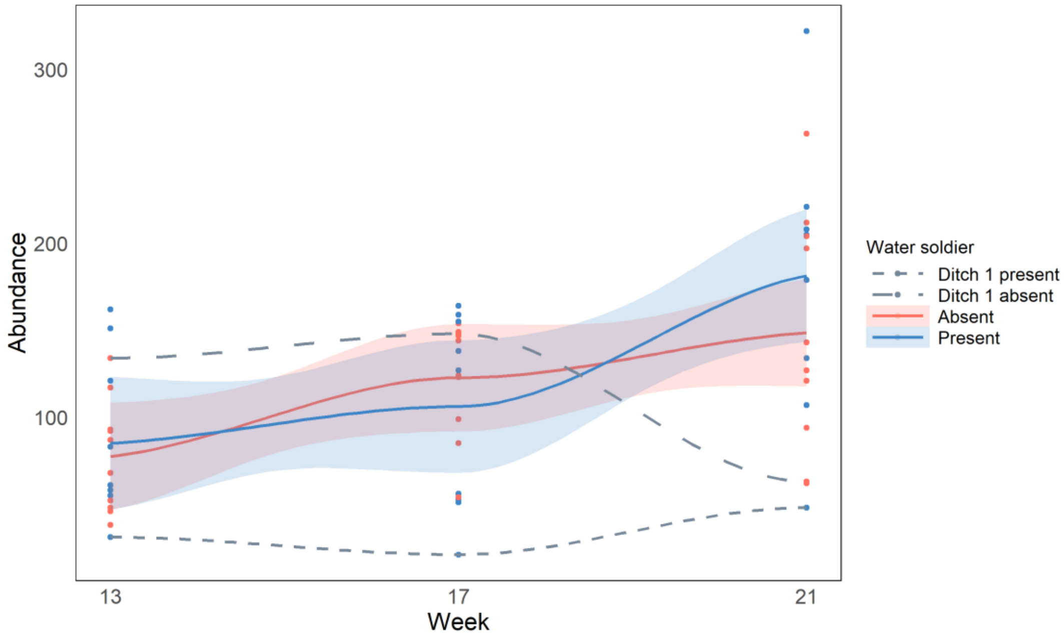


Figure 9: Total abundance of aquatic macro-invertebrates in ditch tracks with water soldier presence or absence
Ditch 1 is highlighted in grey, while in the categories 'Absent' and 'Present' all ditches are present.

Shannon Diversity
The Shannon Diversity, which combines both taxa richness (Figure 8) and abundance (Figure 9), is depicted in Figure 10. The Shannon Diversity is higher in ditch tracks with water soldier across all measurement weeks and shows a gradual decline over time. This trend is consistent in weeks 17 and 21, where the Shannon Diversity remains higher in ditch tracks with water soldier. However, in week 13, the Shannon Diversity is higher in ditch tracks without water soldier.
With mean ± SD values of Shannon Diversity being 1.32 ± 0.41 for water soldier presence and 1.12 ± 0.37 for absence, the resulting p-value of 0.037 (estimate = 0.20573) indicates that the presence of water soldier has a significant effect on Shannon Diversity, with slightly higher diversity observed in ditch tracks with water soldier presence.

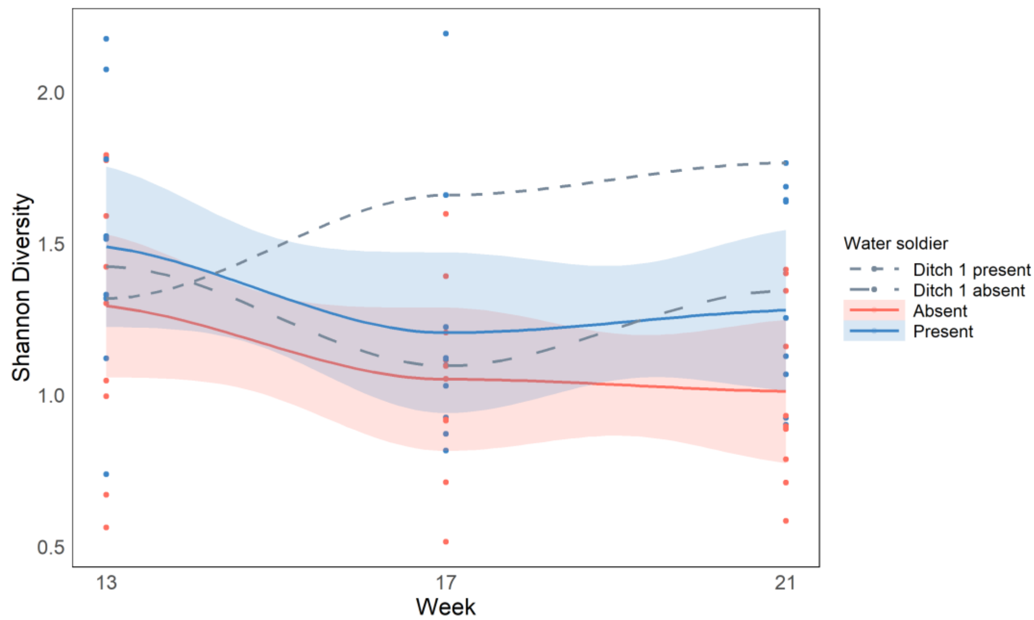


Figure 10: Shannon Diversity in ditch tracks with water soldier presence or absence
Ditch 1 is highlighted in grey, while in the categories 'Absent' and 'Present' all ditches are present.

Taxa composition

Taxa composition was analysed by creating a distance cloud for each treatment based on both taxon presence and coverage. Figure 11 visualizes the results of this analysis. The distance clouds for water soldier presence and absence show a significant overlap, indicating similar taxa composition between ditch tracks with and without water soldier. Additionally, the spread within each treatment is comparable, suggesting that the variation in taxa distribution is similar for both conditions. The resulting p-value of 0.499 indicates that there is no significant effect of water soldier presence on taxa composition.

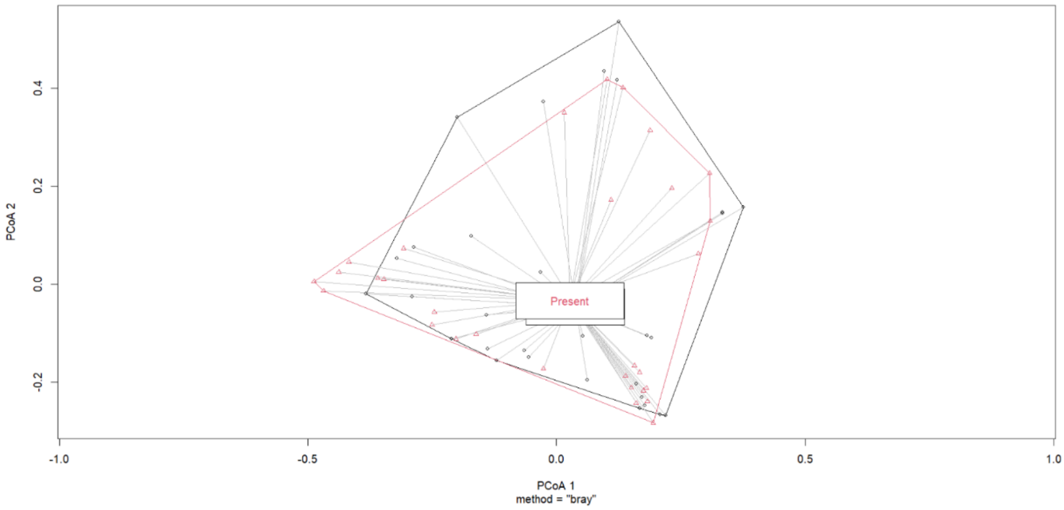


Figure 11: Presentation of the taxa composition within each treatment based on taxa presence and coverage, visualized in a distance cloud

Belgian Biotic Index

Lastly, the Belgian Biotic Index (BBI) was used as a measure for biotic water quality to compare ditch tracks with water soldier presence and absence in ditches with high crayfish abundance. This analysis yielded the following result: the average BBI score for ditch tracks with water soldier absence was 5.03, while the average BBI score for ditch tracks with water soldier presence was 5.2. A higher BBI score is associated with better water quality. According to the Belgian Biotic index classification, a score of 5-6 is classified as 'moderately polluted – critical situation' (De Pauw & Vanhooren, 1983).

6. Discussion

The purpose of this study was to examine the impact of the presence of water soldier on water quality in ditches within a polder landscape with a high abundance of invasive crayfish. For biotic water quality, only the Shannon Diversity was significantly impacted (*p=0.037*) by the presence of water soldier and a slightly higher Belgian Biotic Index score was observed in ditch tracks containing water soldier. Additionally, a significantly higher abundance of *Baetidae (p=<0.001)*, *Physidae (p=0.002)* and *Mysida (p=<0.001)* was found in ditches with water soldier. No differences in abiotic variables were detected between ditch tracks with water soldier absence or presence.

However, when considering only the visualizations of ditch track 1 with water soldier presence - the only ditch where water soldier was present in high abundance - large differences compared to ditch tracks without water soldier can be observed. Therefore, each variable will also briefly discuss ditch track 1 with water soldier presence, as it represents the most successful introduction of water soldier and the expected effects on water quality, providing insights into how the results of this study may appear in the future.

Limitations of study

For this study, there were a few limitations that should be taken into account.

Firstly, to address the research question - what is the impact of the presence of water soldier on water quality in ditches within a polder landscape with high invasive crayfish abundances - it would be valuable to examine both the direct effects of water soldier (green path - Figure 1) and the indirect effects of water soldier in combination with crayfish (blue path - Figure 1) separately. However, this was not feasible for two reasons:
Firstly, as mentioned earlier, in this study, the abundance of water soldier and invasive crayfish could not be treated as independent variables during the statistical analysis, making it impossible to isolate their effects on water quality.
Secondly, no statistical tests and models currently exists to evaluate the indirect effect of an explanatory variable (water soldier presence/absence) through a mediator (invasive crayfish abundance) on a response variable (water quality variables).
In future research, comparing both the direct and indirect effects would provide valuable insights into how both organisms affect water quality separately and combined when in each other's presence.

Moreover, there were also problems with the treatment "water soldier presence": to assess the impact of water soldier on water quality, 10 ditch tracks with either the presence or absence of water soldier were used. However, in reality, only Ditch 1 had a thriving population of water soldier. In the other ditches, water soldier was present in very low numbers, potentially due to the cutting of macrophytes by the invasive crayfish present (Gherardi & Acquistapace, 2007). Therefore, it is debatable whether these ditches could be considered as true 'treatment' ditches.
To address this issue, new water soldier was reintroduced into the ditches. Unfortunately, this reintroduction occurred after all measurements for this study were completed, so if this study is repeated next year, a larger difference in both abiotic and biotic factors associated with water soldier can be expected.

Furthermore, since all the studied ditches were interconnected through the polder landscape, this cannot be considered true replication as the ditches could influence one another. This means there was pseudoreplication in this study, but through the use of Linear Mixed Models (LMM) and Generalized Linear Mixed Models using Template Model Builder (glmmTMB) in the statistical analysis, this issue was accounted for.

Key findings

The impact on abiotic water quality

For abiotic water quality, a comprehensive analysis was conducted to determine if water soldier presence affected several key water quality parameters, including turbidity, temperature, pH, electrical conductivity, dissolved oxygen levels, ammonia, phosphate, and nitrate levels. The results indicate that water soldier did not significantly alter these abiotic conditions. It should be noted that possibly due to the direct connection between the ditch tracks with and without water soldier, only minimal differences may have been measured. Therefore, in future research, the measuring points that are furthest apart (e.g. points 12 & 12 ditch 6 - Figure 2) should be sampled to address this issue.

Nutrient levels of phosphate, ammonia and nitrate

The nutrient levels of phosphate and nitrogen, in the forms of ammonia and nitrate, did not significantly differ between ditch tracks with water soldier absence and presence, in combination with high abundances of invasive crayfish. Ditch track 1 with high water soldier presence, however, shows promising results where phosphorus and ammonia levels are lower compared to other ditches.

Nitrate concentrations were always at a low level of 0.2 ppm NO3-N, while phosphate levels were present at higher levels. This indicates that the water system in the Polderlab Vrouw Venne was nitrogen limited and not phosphorus limited. A possible explanation for this difference involves the chemical bonds in detritus between nitrogen and phosphorus (Vitousek & Howarth, 1991). In dead organic material, phosphorus is usually bound as esters, where various organisms produce enzymes that break down ester bonds in phosphorus, enabling them to utilize it. Additionally, nitrogen is typically bonded directly with carbon in detritus and is often found in complex forms. Releasing nitrogen from organic compounds requires the breakdown of the complex carbon-nitrogen structure, which involves multiple enzyme systems. This imbalance in the breakdown of chemical bonds is particularly noticeable in peat-rich systems (like Polderlab Vrouw Venne) where decomposition is slow. Here, nitrogen is released more gradually than phosphorus, potentially leading to lower nitrogen levels (Vitousek & Howarth, 1991).

The crayfish in the polder might also have contributed to this effect, as it has been shown that crayfish ingest higher amounts of detritus than other invertebrates (Rabeni et al., 1995). Being 'sloppy feeders', by breaking particles into smaller pieces without ingesting them, crayfish can transform large particles into smaller ones (Vanni, 2002). With the imbalance between nitrogen and phosphorus breakdown in these smaller particles, more phosphorus could be released then nitrogen.

The reason why no significant difference in nutrient concentration was found between ditch tracks with water soldier and those without water soldier might be that submerged plants primarily take up nutrients from the water, whereas floating plants predominantly absorb nutrients from the sediment through their roots, as hypothesized by Kufel et al. (2010). During this study, most of the plants that were present were floating from April and onwards. For future research, it would therefore be beneficial to also measure sediment nutrient levels to take this into account.

Electric conductivity

Electric conductivity did not significantly differ between ditch tracks with and without water soldier combined with high abundances of invasive crayfish. Electrical conductivity is a measure of the ability of water to pass an electrical current. Because dissolved salts and other inorganic chemicals conduct electrical current, conductivity increases as salinity increases, and can therefore be used as a measure of salt in the water (EPA, 2024).

Given the capability of water soldier to absorb pollutants and compounds from the water (Singh & Pant, 2023), it was expected that electric conductivity would be lower in ditch tracks with water soldier presence. This decrease is observed in Figure 6-H during week 21; however, it was not attributed to the water soldier. During week 21, very heavy rainfall diluted the inorganic chemicals, resulting in lower EC levels being measured.

Notably, Ditch 1, where water soldier has been present the longest, consistently shows lower electric conductivity compared to other ditches. This suggests that over a longer period, the presence of water soldier might contribute to a gradual decrease in electric conductivity levels in ditch water.

Turbidity

Turbidity did not significantly differ between ditch tracks with and without water soldier presence, when combined with high abundances of invasive crayfish. Turbidity is highly influenced by the presence of crayfish (Roessink et al., 2010), so it is possible that the negative effects of crayfish have overshadowed the potential positive effects of water soldier in this case. However, Ditch track 1 with water soldier presence, consistently showed lower turbidity from week 20 onwards compared to the other ditches, indicating that in a couple of years, turbidity in all ditch tracks with water soldier would be improved.

Turbidity measurements were conducted using a Secchi Transparency tube. However, reflected sunlight inside the tube caused large variations in measurements between sunny and cloudy days. For future research, it would be better to use a different, more accurate method for measuring turbidity. Additionally, taking chlorophyll A and B measurements into account would be beneficial, as they strongly influence turbidity and serve as good indicators of eutrophication (Fadel et al., 2016).

Dissolved oxygen

Dissolved oxygen levels did not significantly differ between ditch tracks with and without water soldier presence, in the presence of high abundances of invasive crayfish. The dissolved oxygen levels are remarkably similar between ditch tracks with and without water soldier. This is surprising, considering that the amount of oxygen produced by photosynthesis in the ditch tracks with water soldier should have been higher due to the greater number of plants. Crayfish may play a role in this phenomenon. Dorn and Wojdak (2004) observed that ponds with crayfish had lower dissolved oxygen levels. They suggested this could be due to reduced light penetration, the consumption of primary producers by crayfish, and/or increased respiration by decomposers.

Water temperature

Water temperature did not significantly differ between ditch tracks with and without water soldier presence, in the presence of high abundances of invasive crayfish. The water temperature is very similar between ditch tracks with and without water soldier. Because water temperature is influenced by multiple external factors like sunlight intensity, air temperature and vegetation cover (Pranoto et al., 2019; Kalny et al., 2017; Crisp & Howson, 1982), it might not the best measure for abiotic water quality. It is, however, taken into account because it can significantly influence biotic water quality: most aquatic organisms rely on the temperature of the water that surrounds them (Verma & Singh, 2013). Water soldier could have decreased water temperature by shading the ditch, but this did not happen.

pH

Water pH did not significantly differ between ditch tracks with and without water soldier presence, even in combination with high abundances of invasive crayfish. This finding aligns with a study by Efremov et al. (2019), which concluded that water soldier is indifferent to the pH value of water and, therefore, does not alter it.
However, this study found that pH levels were slightly higher in almost all weeks in ditches with water soldier presence and in Ditch 1, where water soldier has been present the longest. A possible explanation for this could be the photosynthetic activity of water soldier. During photosynthesis, water soldier reduces dissolved carbon dioxide levels by converting carbon dioxide into oxygen, which can lead to an increase in water pH (Ansari et al., 2020).

The impact on biotic water quality

For biotic water quality, a comprehensive analysis was conducted to determine if the presence of water soldier affected taxa richness, abundance, Shannon Diversity, taxa composition, and Belgian Biotic Index scores. The results indicate that water soldier did not significantly alter most aspects, except for Shannon Diversity, where a significant difference was found. Additionally, ditch tracks with water soldier had a slightly higher Belgian Biotic Index score and, when examining individual taxa, the abundances of *Physidae*, *Mysida*, and *Baetidae* were significantly higher in ditches with water soldier.
It should also be noted here that due to the direct connection between the ditch tracks with and without water soldier, migration of aquatic macro-invertebrates between the tracks was possible, which may have resulted in minimal differences being observed.

Overview of all taxa found

An overview of all macro invertebrates found shows significant differences for certain taxa, particularly *Baetidae*, *Mysida and Physidae*.
Baetidae, a family within the order Ephemeroptera (mayflies), were more abundant in ditch tracks where water soldier was present. This finding is consistent with several studies that have observed higher abundances of some *Baetis* species in the family *Baetidae* among aquatic macrophytes (Jenkins et al., 1984; Kubendran et al., 2017).
Similarly, there was a greater abundance of *Mysida* (opossum shrimps) in ditches with water soldier. Mysids are filter feeders, and the organic material trapped within the dense mats of water soldier may serve as a vital food source for them (Wittmann et al., 2014). Mysids play an essential role in aquatic food webs, acting as a crucial link (Mauchline, 1980; Mees & Jones, 1997). Therefore, their increased abundance in ditches with water soldier presence suggests a more developed and potentially more complex food web in these environments.
Lastly, *Physidae* (bladder snails) were more abundant in ditches where water soldier was present. A study by Son et al. (2021) confirmed these findings; they also observed a higher abundance of *Physidae* in ditches with submerged macrophytes like water soldier. This increase in abundance may be because *Physidae* feed on the water soldier and the organic material trapped among its leaves (Robert et al., 2024).

Although not significant, a noticeable result found is that the abundance of *Unionidae*, a family of freshwater mussels, was higher in ditch tracks without water soldier than with water soldier. However, this difference might not be related to water quality but rather to their habitat preference: mussels often require substrate or sediment to attach to or burrow into (Lewis & Riebel, 1984). In ditch tracks with water soldier, these may not be present in large quantities because the space is already occupied by these macrophytes. Therefore, the abundance of *Unionidae* may have decrease because they had little space to establish themselves.

Taxon accumulation curve

To check if a comprehensive picture of the taxa present in the study site, the Polderlab Vrouw Venne, a taxon accumulation curve has been made. The sampling method for assessing biotic water quality had several limitations that may have influenced this curve. Firstly, the mesh size of the area kick net used to collect the biotic samples was not fine enough to capture all individuals present in the ditches. For example, small organisms like water mites and water fleas were not included because they could pass through the net’s mesh. Secondly, in ditches that were very shallow or in ditch track 1 that had a high density of water soldier, the initial sweep - which is crucial for capturing fast-moving invertebrates - could not be performed as efficiently as in other ditches. This likely skewed the number and types of individuals collected. Lastly, the methods used introduced significant observation bias.

Certain orders, like Trichoptera (caddisflies), are not as easily spotted as others, making it likely that these organisms were sometimes overlooked.

Additionally to the sampling method, also the low sample size for biotic water quality measurements might have influenced this curve. This low sample size can reduce the statistical power of the study, which is the probability of detecting an effect if one truly exists. Statistical power depends on various factors, including sample size and effect size. In this study, there were only 60 replications for biotic water quality measurements. Given this small sample size, the effect size would have needed to be very large to detect a statistically significant effect. For further research, it would therefore be advisable to perform these measurements every week instead of every month, to increase the sample size and thereby the statistical power.

Although these limitations should be kept in mind, the taxon accumulation curve almost reaches a plateau, suggesting that additional sampling is unlikely to reveal more taxa since they have already been identified. This completeness in data collection is crucial for this study, as it indicates that the data gathered was useful for drawing conclusions.

Taxa richness

Taxa richness of aquatic macro invertebrates did not significantly differ between ditch tracks with and without the presence of water soldier in areas with high abundances of invasive crayfish. However, if only Figure 8 is considered, taxa richness was consistently higher in ditch tracks where water soldier was present. According to Higler (1977), the richness of invertebrates in water soldier stands does not depend on the specific structure of the plants or the chemical properties of the water. This study supports this observation: no significant differences in abiotic water quality variables were found between ditches with and without water soldier that could explain the observed higher taxa richness.
Additionally, in Ditch 1, where water soldier has been present the longest, the taxa richness is higher than in other ditches. This suggests that over time, as water soldier becomes established in all ditches, it may contribute to an increased taxa richness.

Abundance

Aquatic macro-invertebrate abundance did not significantly differ between ditch tracks with and without the presence of water soldier in areas with high abundances of invasive crayfish. Abundance increased over time during this study, likely due to temperature increases between measurement weeks over the three-month study period (Verma & Singh, 2013). In ditch track 1, where water soldier was present, the macro-invertebrate abundance was lower compared to the ditch tracks without water soldier. This can be attributed to the high density of water soldier in this ditch, which slowed down the sampling process, as previously mentioned.
Abundance estimates were often necessary due to the high numbers of individuals present in the samples. For example, there were often samples that contained only two taxa, but with many individuals from these taxa. Therefore, abundance might not be the most accurate estimate for biotic water quality in this study. It is, however, important for calculating the Shannon Diversity and taxa composition, so this biotic variable is still taken into account.

Shannon Diversity

Shannon Diversity significantly differed between ditch tracks and was slightly higher in ditch tracks with the presence of water soldier and invasive crayfish. Additionally, Shannon Diversity was higher in the last two measurement weeks in ditch track 1 with water soldier presence compared to the other ditches.

The Shannon Diversity Index is a widely used measure that considers both the abundance and evenness of species within a community (Farukuzzaman et al., 2023). This might explain why Shannon Diversity shows a significant difference, while abundance and taxa richness on their own did not. Shannon Diversity is more sensitive to the presence of rare taxa than simple abundance counts (Krebs, 1989). In contrast, richness only counts the number of taxa and not their abundance, which might overlook the presence of rare taxa.
This means that the Shannon Diversity is also more sensitive to taxa-specific effects. Both water soldier and invasive crayfish could disproportionately impact certain taxa, affecting their abundance and making the community structure more uneven. This unevenness might not significantly change taxa richness or the total number of individuals, but it will affect the Shannon Diversity due to changes in how these individuals are distributed among taxa.

Taxa composition
Taxa composition did not significantly differ between ditch sections with and without the presence of water soldier in areas with high abundances of invasive crayfish. This can be explained by the fact that water soldier did not affect abiotic water quality because the treatments were not separated from each other; therefore, the environmental conditions in both types of ditches were the same. A study by Obolewski et al. (2014) suggested that the differences in taxa composition found in their study were due to abiotic water quality variables. Thus, as water soldier becomes more established in all ditches over time and will influence abiotic water quality more, a more pronounced difference in taxa composition in the future can be expected.

Belgian Biotic Index (BBI)
The average BBI score for ditch tracks without water soldier was 5.03, while the average BBI score for sections with water soldier was 5.2. A higher BBI score is associated with better water quality (De Pauw & Vanhooren, 1983), indicating that ecological water quality, based on this test, was better in ditch sections with water soldier. However, the Belgian Biotic Index (BBI) has some limitations that might make it less suitable for measuring water quality in this study.
Firstly, some data were only available at the order level, whereas the BBI requires data at the family level. In these cases, it was assumed that all individuals from one order belonged to the family specified in the BBI. This assumption also posed a problem for estimating the number of systematic units, as this number is lower when only looking at order-level data. Thus, while keeping in mind that the BBI might not be the most suitable measure for water quality in this study, the results suggest that the presence of water soldier improve water quality.

Future research
As previously discussed, it is recommended to extend this study over the coming years to monitor how water quality changes between ditches with and without water soldier in areas with high crayfish abundance. Ditch track 1, where water soldier was present the longest and in the highest abundance, showed better water quality for some variables compared to ditches without water soldier. Further research is needed to determine if these benefits increase with longer presence.
Additionally, increasing the number of sampling rounds for biotic measurements would enhance the statistical power of the study. To minimize disturbances between these rounds, measurements should be conducted once a week.
Furthermore, in further research, sample locations for both abiotic and biotic measurements should be placed as far apart as possible to limit the effect of the direct connection between the ditch tracks.

To better understand the impact of water soldier and crayfish on water quality, an experimental setup where these factors are treated as independent variables would be valuable. Additionally, developing or using a statistical model that accounts for the effect of crayfish as a mediator in the effect of water soldier on water quality would allow for a more detailed analysis. This would help in distinguishing the specific influences of water soldier and crayfish on water quality.
Finally, it would be beneficial to include sediment nutrient levels, as well as chlorophyll A and B levels, in the analysis, in addition to the currently measured dissolved nutrient levels. This comprehensive approach would provide a more detailed understanding of water soldier's role as a nature base solution for improving water quality.

7. Conclusion

This study aimed to assess the impact of phytoremediation on water quality in a Dutch polder landscape, considering the presence of organisms that negatively affect water quality, by using water soldier (*Stratiotes aloides*) and invasive crayfish as model organisms. The findings reveal that water soldier had no significant impact on abiotic water quality parameters such as turbidity, temperature, pH, conductivity, dissolved oxygen, phosphate, ammonia, and nitrate levels. Regarding biotic water quality, only Shannon Diversity showed a slight increase in ditches with water soldier presence ($p=0.037$), suggesting subtle effects on community diversity. Also a slightly higher Belgian Biotic Index score was observed in ditch tracks with water soldier. However, overall taxa richness, abundance, and composition did not significantly differ between the treatments. Nonetheless, examining individual taxa revealed that the abundances of *Baetidae* ($p=<0.001$), *Physidae* ($p=0.002$), and *Mysida* ($p=<0.001$) were higher in ditch tracks containing water soldier.
Visualizations show that Ditch 1, where water soldier was present the longest, exhibited better water quality for some variables compared to ditches without water soldier. Therefore, further research is needed to determine if the impact of water soldier on water quality increases with longer presence. Additionally, future research should conduct controlled experiments to isolate the specific effects of water soldier and invasive crayfish on water quality parameters.
In conclusion, while the presence of water soldier did not substantially alter water quality parameters, except for Shannon Diversity, the Belgian Biotic Index and abundances of *Physidae*, *Mysida* and *Baetidae*, it underscores the complexity of managing water quality in agricultural landscapes. This emphasizes the need for nuanced approaches and highlights the potential role of nature-based solutions like water soldier in effectively improving water quality.

8. References

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. Pages 267-281 in B. N. Petrov and S. Caski, editors. Proceedings of the Second International Symposium on Information Theory. Akademiai Kaido, Budapest, Hungary.

Alcorlo, P., Geiger, W., & Otero, M. (2004). Feeding Preferences and Food Selection of the Red Swamp Crayfish, Procambarus clarkii, in Habitats Differing in Food Item Diversity. *Crustaceana*, *77*(4), 435–453.

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, *25*, 704-726.

Angeler, D. G., Sánchez-Carrillo, S., García, G., & Alvarez-Cobelas, M. (2001). The influence of Procambarus clarkii (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia*, *464*, 89-98.

Ansari, A. A., Naeem, M., Gill, S. S., & AlZuaibr, F. M. (2020). Phytoremediation of contaminated waters: An eco-friendly technology based on aquatic macrophytes application. *The Egyptian Journal of Aquatic Research*, *46*(4), 371-376.

Armitage, P. D., Szoszkiewicz, K., Blackburn, J. H., & Nesbitt, I. (2003). Ditch communities: a major contributor to floodplain biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *13*(2), 165-185.

Arthur, E. L., Rice, P. J., Rice, P. J., Anderson, T. A., Baladi, S. M., Henderson, K. L., & Coats, J. R. (2005). Phytoremediation—an overview. *Critical Reviews in Plant Sciences*, *24*(2), 109-122.

Balazs, H. E., Schmid, C. A., Feher, I., Podar, D., Szatmari, P. M., Marincaş, O., ... & Schröder, P. (2018). HCH phytoremediation potential of native plant species from a contaminated urban site in Turda, Romania. *Journal of environmental management*, *223*, 286-296.

Bartram, J., & Ballance, R. (1996). *Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programmes*. CRC press.

Brammer, E. S. (1979). Exclusion of phytoplankton in the proximity of dominant water-soldier (Stratiotes aloides). *Freshwater Biology*, *9*(3), 233–249.

Bouchard, R.W., Jr. (2004). Guide to aquatic macroinvertebrates of the Upper Midwest. Water Resources Center, University of Minnesota, St. Paul, MN. 208 pp.

Centraal Bureau voor de Statistiek. (n.d.). *SDG 6 Clean water and sanitation*. Centraal Bureau Voor De Statistiek. Retrieved April 7, 2024, from https://www.cbs.nl/en-gb/dossier/dossier-well-being-and-the-sustainable-development-goals/monitor-of-well-being-and-the-sustainable-development-goals-2023/the-sustainable-development-goals-in-the-monitor-of-well-being/sdg-s/sdg-6-clean-water-and-sanitation

Chowdhary, P., Bharagava, R. N., Mishra, S., & Khan, N. (2020). Role of industries in water scarcity and its adverse effects on environment and human health. *Environmental Concerns and Sustainable Development: Volume 1: Air, Water and Energy Resources*, 235-256.

CLO. (2022). Belasting van het oppervlaktewater met vermestende stoffen, 1990-2020 | Compendium voor de Leefomgeving [Webpagina]. Emissie Naar Lucht, Water En Bodem. https://www.clo.nl/indicatoren/nl019224-belasting-van-het-oppervlaktewater-met-vermestende-stoffen-1990-2021

Compendium voor de Leefomgeving. (2021). *Oppervlaktewater in Nederland*. Compendium Voor De Leefomgeving. Retrieved April 4, 2024, from

Cook, C. D., & Urmi-König, K. (1983). A revision of the genus Stratiotes (Hydrocharitaceae). *Aquatic botany*, *16*(3), 213-249.

Correia, A. M. (2003). Food choice by the introduced crayfish Procambarus clarkii. *Annales Zoologici Fennici*, *40*(6), 517–528.

Crisp, D. T., & Howson, G. (1982). Effect of air temperature upon mean water temperature in streams in the north Pennines and English Lake District. *Freshwater Biology*, *12*(4), 359-367.

De Pauw, N., & Vanhooren, G. (1983). Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*, *100*, 153-168.

Delpla, I., Jung, A. V., Baures, E., Clement, M., & Thomas, O. (2009). Impacts of climate change on surface water quality in relation to drinking water production. *Environment international*, *35*(8), 1225-1233.

Deltaplan Agrarisch Waterbeheer. (2022). BOOT lijst 2022. In *Deltaplan Agrarisch Waterbeheer*. Retrieved April 12, 2024, from https://agrarischwaterbeheer.nl/system/files/documenten/daw_overzicht_maatregelen_bootlijst_september_2022.pdf

Deltares. (n.d.). *Smart drainage of Dutch lowland*. Retrieved April 4, 2024, from https://www.deltares.nl/en/expertise/projects/smart-drainage-of-dutch-lowland

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *science*, *321*(5891), 926-929.

Didde, R. (2022). Wageningen World: Een dikke onvoldoende voor waterkwaliteit. *Wageningen University and Research*. Retrieved March 7, 2024, from https://www.wur.nl/nl/show-longread/een-dikke-onvoldoende-voor-waterkwaliteit.htm

Directive 2000/60/EC. (2014). *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy*. http://data.europa.eu/eli/dir/2000/60/2014-11-20

Dorn, N. J., & Wojdak, J. M. (2004). The role of omnivorous crayfish in littoral communities. *Oecologia*, *140*, 150-159.

Drukker, D. (2017). Soortzoeker haften van het laagland. Naturalis Biodiversity Center & EIS Kenniscentrum insecten en andere ongewervelden, Leiden.

Duran, M. (2006). Monitoring Water Quality Using Benthic Macroinvertebrates and Physicochemical Parameters of Behzat Stream in Turkey. *Polish Journal of Environmental Studies*, *15*(5).

Ecological status of surface waters in Europe. (2021). European Environment Agency. Retrieved March 19, 2024, from https://www.eea.europa.eu/en/analysis/indicators/ecological-status-of-surface-waters?activeAccordion=309c5ef9-de09-4759-bc02-802370dfa366

Efremov, A. N., Sviridenko, B. F., Toma, C., Mesterházy, A., & Murashko, Y. A. (2019). Ecology of Stratiotes aloides L.(Hydrocharitaceae) in Eurasia. *Flora*, *253*, 116-126.

Elliott, J. M., Humpesch, U. H., & Macan, T. T. (n.d.). *Key to larvae of mayflies (Ephemeroptera)*. https://dep.wv.gov/wwe/getinvolved/sos/documents/benthic/british_mayflykey.pdf

El-Sheekh, M., Abdel-Daim, M. M., Okba, M., Gharib, S., Soliman, A., & El-Kassas, H. (2021). Green technology for bioremediation of the eutrophication phenomenon in aquatic ecosystems: a review. *African Journal of Aquatic Science*, *46*(3), 274-292.

EPA. (2024). *Indicators: Conductivity*. Retrieved June 21, 2024, from https://www.epa.gov/national-aquatic-resource-surveys/indicators-conductivity

European Commission. (2024). *Water Framework Directive*. Retrieved April 2, 2024, from https://environment.ec.europa.eu/topics/water/water-framework-directive_en

European Environment Agency. (n.d.). *Chemicals in European surface water and groundwater bodies*. Retrieved March 19, 2024, from https://www.eea.europa.eu/en/european-zero-pollution-dashboards/indicators/chemicals-in-european-surface-water-and-groundwater-bodies

Evers, C. H. M., Van Den Broek, A. J. M., Buskens, R., Van Leerdam, A., Knoben, R. A. E., Van Herpen, F. C. J., & Pot, R. (2018). Omschrijving MEP en maatlatten voor sloten en kanalen voor de Kaderrichtlijn Water 2021-2027. In *STOWA* (No. 2018–50). Retrieved April 5, 2024, from https://www.stowa.nl/sites/default/files/assets/PUBLICATIES/Publicaties%202018/STOWA%202018-50%20Maatlatten%20defdef.pdf

Fadel, A., Faour, G., & Slim, K. (2016). Assessment of the trophic state and chlorophyll-a concentrations using Landsat OLI in Karaoun reservoir, Lebanon. *Leban. Sci. J*, *17*(2), 130-145.

Farukuzzaman, M., Sultana, T., Paray, B. A., Arai, T., & Hossain, M. B. (2023). Ecological habitat quality assessment of a highly urbanized estuary using macroinvertebrate community diversity and structure. *Regional Studies in Marine Science*, *66*, 103149.

Gherardi, F., & Acquistapace, P. (2007). Invasive crayfish in Europe: the impact of Procambarus clarkii on the littoral community of a Mediterranean lake. *Freshwater Biology*, *52*(7), 1249–1259.

GISB. (2011). *Procambarus clarkii*. Global Invasive Species Database. Retrieved July 3, 2024, from http://www.iucngisd.org/gisd/species.php?sc=608 on 05-07-2024.

Gobler, C. J. (2020). Climate change and harmful algal blooms: insights and perspective. *Harmful algae*, *91*, 101731.

Golawski, A., Kasprzykowski, Z., & Mroz, E. (2017). Wind differentiates reproduction in the non-expansive Black Tern Chlidonias niger and the expansive White-winged Tern Chlidonias leucopterus. *Aquatic Ecology*, *51*(2), 235-245

Guo, J., Zhang, C., Zheng, G., Xue, J., & Zhang, L. (2018). The establishment of season-specific eutrophication assessment standards for a water-supply reservoir located in Northeast China based on chlorophyll-a levels. *Ecological Indicators*, *85*, 11-20.

Herzon, I., & Helenius, J. (2008). Agricultural drainage ditches, their biological importance and functioning. *Biological conservation*, *141*(5), 1171-1183.

Higler, L. W. G. (1977). *Macrofauna-cenoses on Stratiotes plants in Dutch broads* (Doctoral dissertation, Rijksinstituut voor Natuurbeheer).

Huner, J. V., & Barr, J. E. (1991). Red swamp crawfish: biology and exploitation.

Jenkins, R. A., Wade, K. R., & Pugh, E. (1984). Macroinvertebrate–habitat relationships in the River Teifi catchment and the significance to conservation. *Freshwater Biology*, *14*(1), 23-42.

Jeong, S., Moon, H. S., & Nam, K. (2015). Increased ecological risk due to the hyperaccumulation of As in Pteris cretica during the phytoremediation of an As-contaminated site. *Chemosphere*, *122*, 1-7.

Johnson, P. T., Chase, J. M., Dosch, K. L., Hartson, R. B., Gross, J. A., Larson, D. J., ... & Carpenter, S. R. (2007). Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences*, *104*(40), 15781-15786.

Johnson, T. (2007). Battling seawater intrusion in the central & west coast basins. *WRD Technical Bulletin*, *13*.

Kalny, G., Laaha, G., Melcher, A., Trimmel, H., Weihs, P., & Rauch, H. P. (2017). The influence of riparian vegetation shading on water temperature during low flow conditions in a medium sized river. *Knowledge & Management of Aquatic Ecosystems*, (418), 5.

Khan, F. A., & Ansari, A. A. (2005). Eutrophication: an ecological vision. *The botanical review*, *71*(4), 449-482.

Kılıç, Z. (2021). Water pollution: causes, negative effects and prevention methods. *İstanbul Sabahattin Zaim Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *3*(2), 129-132.

Krebs, C. J. (1989). Ecological Methodology. Harper-Collins Publishers, New York. 654 pp.

Kubendran, T., Selvakumar, C., Sidhu, A. K., Krishnan, S. M., & Nair, A. (2017). Diversity and distribution of Baetidae (Insecta: Ephemeroptera) larvae of streams and Rivers of the southern Western Ghats, India. *Journal of Entomology and Zoology Studies*, *5*(3), 613-625.

Kufel, L., Strzałek, M., Konieczna, A., & Izdebska, K. (2010). The effect of Stratiotes aloides L. and nutrients on the growth rate of Lemna minor L. *Aquatic Botany*, *92*(3), 168-172.

Kumar, S., Pratap, B., Dubey, D., & Dutta, V. (2022). Interspecific competition and their impacts on the growth of macrophytes and pollutants removal within constructed wetland microcosms treating domestic wastewater. *International Journal of Phytoremediation*, *24*(1), 76-87.

Lalonde, S., & Downing, J. A. (1992). Phytofaun of eleven macrophyte beds of differing trophic status, depth, and composition. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(5), 992-1000.

Letchinger, M. (2000). Pollution and water quality, neighbourhood water quality assessment. *Project oceanography*

Lewis, J. B., & Riebel, P. N. (1984). The effect of substrate on burrowing in freshwater mussels (Unionidae). *Canadian Journal of Zoology*, *62*(10), 2023-2025.

Li, P., & Wu, J. (2019). Drinking water quality and public health. *Exposure and Health*, *11*(2), 73-79.

Ligtvoet, W., Beugelink, G. P., Franken, R., & Netherlands Environmental Assessment Agency. (2008). Evaluation of the Water Framework Directive in the Netherlands; costs and benefits. In *Netherlands Environmental Assessment Agency (PBL)*. Netherlands

Luo, J., Qi, S., Peng, L., & Xie, X. (2017). Enhanced phytoremediation capacity of a mixed-species plantation of Eucalyptus globulus and Chickpeas. *Journal of Geochemical Exploration*, *182*, 201-205.

Madsen, J. D., Chambers, P. A., James, W. F., Koch, E. W., & Westlake, D. F. (2001). The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, *444*, 71-84.

Magurran, A. (2003). *Measuring biological diversity*. John Wiley & Sons.

Magurran A. (2004). Measuring Biological Diversity. Blackwell Science Ltd, Oxford, United Kingdom.

Maij, H., Baarsma, B., Koen, C., van Dijk, G., Van Trijp, H., Volberda, H., ... & Thus, S. (2019). Goed boeren kunnen boeren niet alleen:" Je kunt niet groen doen als je rood staat" Verdienvermogen essentiële voorwaarde voor kringlooplandbouw.

Malakar, A., Snow, D. D., & Ray, C. (2019). Irrigation water quality—A contemporary perspective. *Water*, *11*(7), 1482.

Mauchline, J. (1980). The biology of mysids and euphausiids. *Adv. Mar. Biol.*, *18*, 1-677.

McCormick, F. H., Contreras, G. C., & Johnson, S. L. (2009). Effects of nonindigenous invasive species on water quality and quantity. *A dynamic invasive species research vision: opportunities and priorities*, *29*, 111-120.

Mees, J., & Jones, M. B. (1997). The hyperbenthos. *Oceanography and marine biology*, *35*, 212.

Moyle, P. B., & Light, T. (1996). Biological invasions of fresh water: empirical rules and assembly theory. *Biological conservation*, *78*(1-2), 149-161.

Mugidde, R., Gichuki, J., Rutagemwa, D., Ndawula, L., & Matovu, A. (2005). Status of Water Quality and its Implication on Fishery Production.

Mulderij, G., Mau, B., van Donk, E., & Gross, E. M. (2007). Allelopathic activity of Stratiotes aloides on phytoplankton—towards identification of allelopathic substances. In *Shallow Lakes in a Changing World: Proceedings of the 5th International Symposium on Shallow Lakes, held at Dalfsen, The Netherlands, 5–9 June 2005* (pp. 89-100). Springer Netherlands.

NFDD & Anemoon. (2024). *Verspreidingsatlas Rode Amerikaanse rivierkreeft*. Verspreidingsatlas. Retrieved May 20, 2024, from

Obolewski, K. (2005). Epiphytic macrofauna on water soldiers (Stratiotes aloides L.) in Słupia river oxbows. *Oceanol. Hydrobiol. Stud*, *34*(2), 37-54.

Obolewski, K., & Strzelczak, A. (2009). Epiphytic fauna inhabiting Stratiotes aloides in a new lake of the Słowiński National Park (Smołdzińskie lake, Poland). *Ecohydrology and Hydrobiology*, *9*(2–4), 257–267.

Obolewski, K., Strzelczak, A., & Glińska-Lewczuk, K. (2014). Does hydrological connectivity affect the composition of macroinvertebrates on Stratiotes aloides L. in oxbow lakes?. *Ecological engineering*, *66*, 72-81.‘

Oficialdegui, F. J., Sánchez, M. I., & Clavero, M. (2020). One century away from home: how the red swamp crayfish took over the world. *Reviews in Fish Biology and Fisheries*, *30*(1), 121-135.

O'Hogain, S., McCarton, L., O’Hogain, S., & McCarton, L. (2018). Nature-based solutions. *A Technology Portfolio of Nature Based Solutions: Innovations in Water Management*, 1-9.

Osborne, N. J., Webb, P. M., & Shaw, G. R. (2001). The toxins of Lyngbya majuscula and their human and ecological health effects. *Environment International*, *27*(5), 381-392.

Painter, D. (1999). Macroinvertebrate distributions and the conservation value of aquatic Coleoptera, Mollusca and Odonata in the ditches of traditionally managed and grazing fen at Wicken Fen, UK. *Journal of Applied Ecology*, *36*(1), 33-48.

Parris, K. (2014). Impact of agriculture on water pollution in OECD countries: recent trends and future prospects. *Water Quality Management*, 33-52.

Polderlab. (n.d.). *Hoe ziet een duurzame toekomst van de Hollandse polder eruit?* Retrieved May 28, 2024, from https://www.polderlab.org/

Pranoto, S., Pambudi, N. A., Wardani, N. S., Setyaji, A., Susanto, A., Setyawan, N. D., & Utomo, F. (2019). Parabolic trough collector's heat transfer analysis with changes of variation on the pipe absorber system. In *Journal of Physics: Conference Series* (Vol. 1153, No. 1, p. 012139). IOP Publishing.

Prepas, E. E., & Charette, T. (2005). Worldwide Eutrophication of. *Environmental Geochemistry*, *9*, 311.

Rabeni, C. F., Gossett, M., & McClendon, D. D. (1995). Contribution of crayfish to benthic invertebrate production and trophic ecology of an Ozark stream. *Freshwater crayfish*, *10*(1), 163-173.

Ramos-Merchante, A., & Prenda, J. (2017). Macroinvertebrate taxa richness uncertainty and kick sampling in the establishment of Mediterranean rivers ecological status. *Ecological Indicators*, *72*, 1-12.

Rantala, M. J., Ilmonen, J., Koskimäki, J., Suhonen, J., & Tynkkynen, K. (2004). The macrophyte, Stratiotes aloides, protects larvae of dragonfly Aeshna viridis against fish predation. *Aquatic Ecology*, *38*(1), 77–82.

Robert, A., Pinel-Alloul, B., Taranu, Z. E., & Harvey, E. (2024). Green landscape and macrophyte cover influence macroinvertebrate taxonomic and functional composition in urban waterbodies at multiple spatial scales.

Roessink, I., & Ottburg, F. G. W. A. (2021). Rivierkreeften. In *STOWA*. Retrieved May 21, 2024, from https://www.stowa.nl/sites/default/files/assets/DELTAFACTS/Deltafacts%20NL%20PDF%20nieuw%20format/Rivierkreeften%20v1-1-1%2020210819.pdf

Roessink, I., van Giels, J., Boerkamp, A., & Ottburg, F. G. W. A. (2010). *Effecten van rode-en geknobbelde Amerikaanse rivierkreeften op waterplanten en waterkwaliteit* (No. 2052). Alterra.

Rosenberg, D. M. (1998). A national aquatic ecosystem health program for Canada: We should go against the flow. *Bull. Entomol. Soc. Can*, *30*(4), 144-152.

Saładyga, M., Kucała, M., Adamski, M., Selvaraj, S., & Kaminski, A. (2023). Phytoremediation of a mixture of toxic cyanobacteria. Does phytoplankton composition affect the amount of toxins removed?. *Journal of Environmental Chemical Engineering*, *11*(3), 110158.

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. *Science*, *195*(4275), 260-262.

Schipper, P., Van Loon, A., Rozemeijer, J., Groenendijk, P., & Lukacs, S. (2022). Nutriënten en het ecologisch functioneren van oppervlaktewateren. STOWA.

Schwarz, G. (1978). Estimating the dimension of a model. *The annals of statistics*, 461-464.

Singh, H., & Pant, G. (2023). Phytoremediation: Low input-based ecological approach for sustainable environment. *Applied Water Science*, *13*(3), 85.

Smolders, F., Lucassen, E., Harpenslager, S., Van Schaijk, F., Lamers, L., & Roelofs, J. (2019). Kansen voor krabbenscheer in voedselrijke sloten van het veenweidegebied. *De Levende Natuur*. Retrieved May 5, 2024, from https://natuurtijdschriften.nl/pub/1010671/DLN201912000107.pdf

Soes, D. M., & Koese, B. (2010). Invasive crayfish in the Netherlands: a preliminary risk analysis.

Son, S. H., Kwon, S. J., Im, J. H., Kim, S. K., Kong, D., & Choi, J. Y. (2021). Aquatic macrophytes determine the spatial distribution of invertebrates in a shallow reservoir. *Water*, *13*(11), 1455.

Srivastav, A. L. (2020). Chemical fertilizers and pesticides: role in groundwater contamination. In *Agrochemicals detection, treatment and remediation* (pp. 143-159). Butterworth-Heinemann.

Strzałek, M., & Koperski, P. (2009). The Stratiotes aloides L. stand as a habitat in oxbow lake Bużysko. *Aquatic Botany*, *90*(1), 1-6.

Takamura, N., Kadono, Y., Fukushima, M., Nakagawa, M., & Kim, B. H. (2003). Effects of aquatic macrophytes on water quality and phytoplankton communities in shallow lakes. *Ecological research*, *18*, 381-395.

Tarkowska-Kukuryk, M. (2006). Water soldier Stratiotes aloides L.(Hydrocharitaceae) as a substratum for macroinvertebrates in a shallow eutrophic lake. *Polish Journal of Ecology*, *54*(3), 441-451.

The Conchological Society of Great Britain and Ireland. (n.d.). *Identifying British freshwater snails: shape of shell*. Retrieved March 3, 2024, from https://conchsoc.org/node/5300

UK Beetles. (n.d.). *Guide to families*. Retrieved March 3, 2024, from https://www.ukbeetles.co.uk/family-guide

Umar, S., Munir, M. T., Azeem, T., Ali, S., Umar, W., Rehman, A., & Shah, M. A. (2014). Effects of water quality on productivity and performance of livestock: A mini review. *Veterinaria*, *2*(2), 11-15.

Vadas, P. A., Kleinman, P. J. A., Sharpley, A. N., & Turner, B. L. (2005). Relating soil phosphorus to dissolved phosphorus in runoff: A single extraction coefficient for water quality modeling. *Journal of environmental quality*, *34*(2), 572-580.

Van Kleef, H., Kanters, S., Kampen, J., Lemmers, P., Koese, B., Schep, S., & Rip, W. (2022). Uitwerking ecosysteemaanpak beheersen rivierkreeften Molenpolder. In *Stichting-bargerveen*. Stichting Bargerveen.

Van Vliet, M. T., Thorslund, J., Strokal, M., Hofstra, N., Flörke, M., Ehalt Macedo, H., ... & Mosley, L. M. (2023). Global river water quality under climate change and hydroclimatic extremes. *Nature Reviews Earth & Environment*, *4*(10), 687-702.

Vannevel, R., Brosens, D., De Cooman, W., Gabriels, W., Lavens, F., Mertens, J., & Vervaeke, B. (2018). The inland water macro-invertebrate occurrences in Flanders, Belgium. *ZooKeys*, (759), 117.

Vanni, M. J., Flecker, A. S., Hood, J. M., & Headworth, J. L. (2002). Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters*, *5*(2), 285-293.

Verdonschot, R. (2012). *Drainage ditches, biodiversity hotspots for aquatic invertebrates: defining and assessing the ecological status of a man-made ecosystem based on macroinvertebrates*. Wageningen University and Research.

Verma, A. K., & Singh, T. N. (2013). Prediction of water quality from simple field parameters. *Environmental earth sciences*, *69*, 821-829.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur?. *Biogeochemistry*, *13*, 87-115.

Wang, G., Zhang, Q., Du, W., Ai, F., Yin, Y., Ji, R., & Guo, H. (2021). Microbial communities in the rhizosphere of different willow genotypes affect phytoremediation potential in Cd contaminated soil. *Science of the Total Environment*, *769*, 145224.

Weeda, E., R. Westra, C. Westra & T. Westra, (1991). Nederlandse Oecologische Flora, Wilde Planten en hun Relaties, deel 4. IVNVARA and VEWIN: 317 pp

Withers, P. J., Neal, C., Jarvie, H. P., & Doody, D. G. (2014). Agriculture and eutrophication: where do we go from here?. *Sustainability*, *6*(9), 5853-5875.

Wittmann, K. J., Ariani, A. P., & Lagardère, J. P. (2014). orders lophogastrida Boas, 1883, Stygiomysida tchindonova, 1981, and mysida Boas, 1883 (also known collectively as mysidacea). In *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Crustacea, Volume 4 Part B* (pp. 189-396). Brill.

World Health Organization Regional Office for Europe & European Commission. (2002). *Eutrophication and health*. Retrieved May 20, 2024, from https://ypen.gov.gr/wp-content/uploads/legacy/Files/Ydatikoi%20Poroi/Nitrorypansi/eutrophication%20and%20Health.pdf

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *Wiley Interdisciplinary Reviews: Water*, *6*(5), e1373.

Zaman, M. S., & Sizemore, R. C. (2017). Freshwater resources could become the Most critical factor in the future of the earth. *Volume 62 October 2017 Number 4*, *62*(4), 348.

9. Appendices

Appendix A: Materials used to assess invasive crayfish abundances and (a)biotic water quality

List 1: Materials used to assess the invasive crayfish abundance
- 10 ditch tracks with water soldier
- 10 ditch tracks without water soldier
- 72 Finnish crayfish traps (Figure 1)
- Rope to tie the crayfish traps
- 10 kg of 14 mm Halibut Pellets Coppens (*from Tijgernoten.nl*)

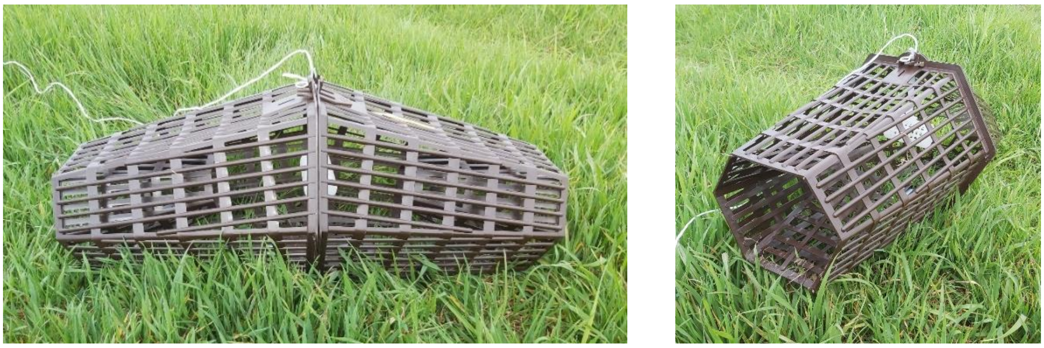


Figure 1 – Front and side view of finish crayfish traps that were used to assess invasive crayfish abundance

List 2: Materials used to assess the abiotic water quality
- 10 ditch tracks with water soldier
- 10 ditch tracks without water soldier
- Secchi tube
- Checker photometer for phosphate (*Hannah Instruments HI713*)
    - 180 packets of phosphate reagent (*Hannah Instruments HI713-25*)
- Checker photometer for ammonia (*Hannah Instruments HI715*)
    - 8 bottles (7 mL) of ammonia mid-range A (*Hannah Instruments HI715A-0*)
    - 8 bottles (7 mL) of Nessler reagent (*Hannah Instruments HI715B-0*)
- Portable multimeter (*HACH HQ4300*)
    - pH probe for Portable multimeter (*HACH HQ4300*)
        - pH buffer 4.01
        - pH buffer 7.00
        - pH buffer 10.01
    - Dissolved oxygen probe for Portable multimeter (*HACH HQ4300*)
    - Electric conductivity probe for Portable multimeter (*HACH HQ4300*)
    - Demiwater
- 180 nitrate pack tests (*Kyoritsu WAK-NO3-S*)

List 3: Materials used to assess the biotic water quality
- 10 ditch tracks with water soldier
- 10 ditch tracks without water soldier
- Area kick net (*mesh size 0.2 mm*)
- White container
- Cups to sort the aquatic macro-invertebrates in
- Tweezers
- Pipet

Appendix B: Table used to determine the Belgian Biotic Index

Table 1: Standard table to determine the Belgian Biotic Index (De Pauw & Vanhooren, 1983)

Table 1 displays characteristic aquatic macro invertebrates, each representing a specific tolerance class. This class indicates their sensitivity to pollution, ranging from hardly sensitive at the bottom to very sensitive at the top. For each sample, the organism with the highest tolerance class is identified, and the number of other taxa present in that same sample is determined to quantify the systemic units. For example, if the taxa Trichoptera is found, along with 15 other taxa, it results in a score of 8.

I Faunistic groups	II		III Total numbers of systematic units present				
			0–1	2–5	6–10	11–15	16 and more
			Biotic index				
1. Plecoptera or Ecdyonuridae (= Heptageniidae)	1	several S.U.*	–	7	8	9	10
	2	only 1 S.U.	5	6	7	8	9
2. Cased Trichoptera	1	several S.U.	–	6	7	8	9
	2	only 1 S.U.	5	5	6	7	8
3. Ancylidae or Ephemeroptera except Ecdyonuridae	1	more than 2 S.U.	–	5	6	7	8
	2	2 or < 2 S.U.	3	4	5	6	7
4. Aphelocheirus or Odonata or Gammaridae or Mollusca (except Sphaeridae)	0	all S.U.M mentioned above are absent	3	4	5	6	7
5. Asellus or Hirudinea or Sphaeridae or Hemiptera (except Aphelocheirus)	0	all S.U. mentioned above are absent	2	3	4	5	–
6. Tubificidae or Chironomidae of the *thummi-plumosus* group	0	all S.U. mentioned above are absent	1	2	3	–	–
7. Eristalinae (= Syrphidae)	0	all S.U. mentioned above are absent	0	1	1	–	–

* S.U.: number of systematic units observed of this faunistic group.

Appendix C: Abundance of invasive crayfish in Polderlab Vrouw Venne

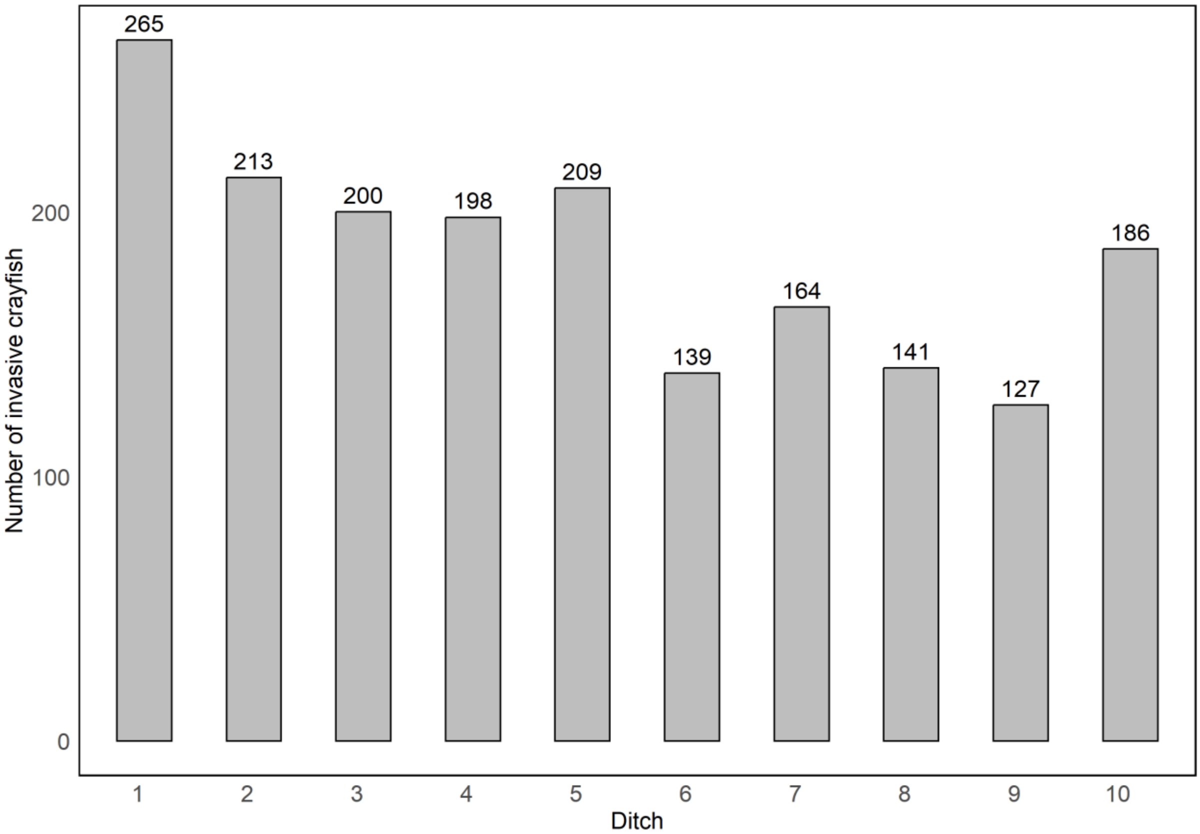


Figure 1: Abundance of invasive crayfish in the Polderlab Vrouw Venne
This histogram is based on the data of all crayfish from all measurement weeks in ditch tracks with water soldier presence and absence combined.

Appendix D : Taxon accumulation curve

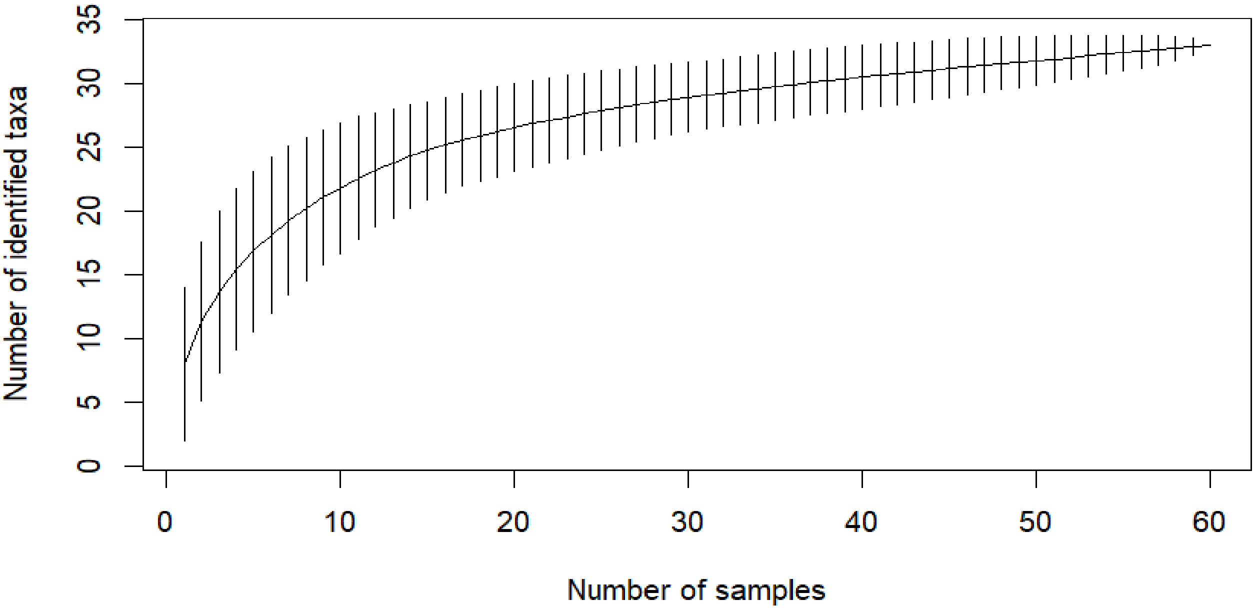


Figure 1: Taxon accumulation curve for treatments water soldier presence and absence combined
This curve is based on all biotic data gathered over a 3-month period. Samples 1-20 were taken in week 13, samples 21-40 were taken in week 17 and samples 41-60 were taken in week 21.