Translocation of water soldier (*Stratiotes aloides*) impacts invasive crayfish density distributions in a Dutch polder landscape

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Preface

Before you lies the bachelor thesis ‘’*Translocation of water soldier (Stratiotes aloides) impacts invasive crayfish density distributions in a Dutch polder landscape*’’. This research project was done as the final assignment to graduate for the Bachelor Biology at the University of Leiden, the Netherlands.

During my previous years of study, I felt a void in actively conducting impactful research that generates novel knowledge applicable to societal issues. This internship precisely addressed this concern, especially when considering how invasive crayfish are a major problem in the Netherlands and across the world, affecting both nature and human matters. Furthermore, by engaging with multiple stakeholders, I gained insights into how organizations perceive research differently and acquired skills in integrating these diverse perspectives into a research plan.

Beyond this learning opportunity, I also witnessed the extensive collaboration involved in research like this. I want to express my gratitude to everyone who contributed. First and foremost, I would like to thank my internship partner, Anouk Hoogeveen. We have been through some tough times in the field, working long hours and concurring the elements of nature. However, thanks to your determination, patience and most importantly positive attitude we have pulled through. Without your company, this project would surely have been much tougher and far less fun. Next, I would like to thank both of my supervisors Fleur van Duin, MSc and Dr. Maarten Schrama. At first because of the opportunity and trust in me when you asked me to do this unique internship position on a relevant societal issue with involvement of multiple parties. Besides that, you have provided excellent guidance in order to complete the research project and produce relevant knowledge. Another person to thank because of his guidance is Bram Koese from EIS knowledge centre for insects and other invertebrates. Your knowledge and advise was of great importance and help during both the preparation and practical phase of the research. Furthermore I want to thank Martin Goosens and Robbert Poldermans from the waterboard Rijnland. Without your cooperation and involvement this research was not possible, since you provided the traps used in this project. The CML provided essential materials as well, which I want to thank Bo Bode for. At last I would like to thank Land van Ons, the non-profit organization which made research at the unique site possible in the first place.

I hope you will find useful information in this thesis.

Rick van Duijn
04-07-2024

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1 Abstract

Invasive alien crayfish (IAC) form a group of worldwide high-impact freshwater invasives which are associated with negative effects on water quality. In the Netherlands only one percent of the surface water is deemed to have good water quality, mainly due to the effects of eutrophication. For this reason the native macrophyte water soldier (*S. aloides*) is opted as a nature-based solution for enhancing water quality as it holds capabilities for counteracting eutrophication processes. IAC might interact with this plant and its effects, hence this study investigates whether water soldier deployment is compatible with IAC presence. To assess this, density distributions of IAC and several eutrophication related abiotic variables were investigated in water soldier-translocated tracks within a Dutch polder landscape. Density data were acquired by monthly sampling of ten water soldier-translocated and control tracks for three months in Spring (April-June). Abiotics were measured weekly. Results show how IAC densities were generally higher in the translocated tracks, however this difference faded over the sampling period. Furthermore, few water soldier individuals were observed after translocation indicating mortality. One track, which had experienced two growth seasons compared to zero or one in the other tracks, forms the exception. Given that abiotic conditions are therefore considered sufficient, the decline of the water soldier is likely due to biotic factors. The high density track showed a high crayfish density as well. This suggests that IAC possibly negatively impact small, weak water soldier populations while stable, high-density populations resist this effect, indicating density-dependency. More research, however, is needed to confirm this theory. Higher phosphate concentrations were found in water soldier tracks, possibly originating from dead water soldier material. Employment of water soldier in IAC invested waters is deemed possible, although introduction at high densities may be necessary to ensure the survival and growth of water soldier populations.

2 Introduction

Invasive alien species form a well-known phenomenon of current society where their negative impacts are often highlighted. Recently the Intergovernmental Platform on Biodiversity and Ecosystem Services published a major report considering a global assessment of invasive alien species. They reported how invasive alien species form a major threat for both nature and humans, whilst the number of alien species is still increasing (IPBES, 2023). Invasive alien species, for example, contribute alongside other drivers to 60% of global animals and plant extinctions and alter the environment, thereby changing the biodiversity and functioning of ecosystems. Additionally, the impacts of invasives are experienced by humans through changes in ecosystem services and quality of life, resulting in estimated annual global costs of > $423 billion (IPBES, 2023).

In freshwater ecosystems, invasives are recognised as a major driver of change and a threat for biodiversity (Carpenter et al., 2011; Reid et al., 2018; Sala et al., 2000). Freshwater change in its turn is considered as one of the planetary boundaries, which already crossed the border of earth's safe operating space (Richardson et al., 2023). A review on the consequences of invasive species in freshwater ecosystems lists the following three major consistent impacts: (I) strong negative influences on abundances of native macrophytes, zooplankton and fish, (II) increased nutrient and organic matter loading in the water possibly leading to eutrophication and (III) based on trophic position invasives have both positive but mostly negative impacts on the local native food web (Gallardo et al., 2015). Depending on species traits, invasive species have the potential to impact both the biotic and abiotic quality of a freshwater ecosystem, altering ecosystem functioning with repercussions for both humans and nature. Furthermore, through such effects homogenization of freshwater biota is at risk (Rahel, 2002).

2.1 Invasive alien freshwater crayfish

A group of species that embodies these impacts are invasive alien crayfish (IAC). They form a worldwide dispersed and established group of invasive species, only being absent at Antarctica (Lodge et al., 2012). This global spread has been facilitated by anthropogenic actions like aquaculture or pet trade, as has been shown for the red swamp crayfish (*Procambarus clarkii*) (Oficialdegui et al., 2019a; Oficialdegui et al., 2020). IAC posses a set of characteristic which make them a potential high-impact invasive alien species. At first, crayfish belong to the largest omnivorous invertebrates present in freshwater systems, occupying a central role in the food web (Geiger et al., 2005). Additionally, IAC are considered ecosystem engineers, altering the (a)biotic components of their environment which indirectly affects the biota again (Crooks, 2002; Reynolds et al., 2013). Furthermore, IAC are R-strategists by nature which provides them with the capability to disperse and increase in numbers at great rates, especially when considering their flexibility towards food and tolerance of environmental factors (Gherardi, 2006). A high egg count and reproductive speed have been found most important factors contributing to their success as invaders (Van Kuijk et al., 2021). Due to these characteristics, crayfish are likely to form self-sustainable populations when introduced outside of their home-range and become invasive (Gherardi, 2010). Once moved to a novel environment, IAC are linked to general negative ecological impacts on the native ecosystem (Souty-Grosset et al., 2016; Twardochleb et al. 2013). These negative impacts can be divided into three major categories: (I) effects on biota, (II) the role of IAC as vectors for disease (discussed in Box 1) and (III) alterations to the abiotic components of the environment.

BOX 1 Review of IAC impacts on various freshwater biotic components with a focus on Europe

IAC directly impact various species groups on different trophic levels through consumption due to their omnivorous diet. IAC mainly consume aquatic macroinvertebrates, macrophytes and detritus material, where macro-invertebrates are preferred when trophic availability is higher (Correia, 2002; Correia, 2003; Geiger et al., 2004; Gutiérrez-Yurrita et al., 1998; Ilhéu & Bernardo, 1993). Juvenile IAC primarily feed on macroinvertebrates, which continue to be a main part of their adult diet, yet a shift from predation in juveniles to herbivory and detritivory is apparent in adults (Alcorlo et al., 2005; Correia, 2002; Correia, 2003; Gutiérrez-Yurrita et al., 1998).

Primary producers such as macrophytes and microalgae are susceptible to the impacts of IAC. IAC have been found to reduce biomass, abundance and species richness of macrophytes, shown in both enclosure experiments and natural environments (Carreira et al., 2013; Gherardi and Acquistapace, 2007; Nyström et al., 1996; Nyström et al., 2001; Rodríguez-Pérez et al., 2015; Roessink et al., 2017; Van Der Wal et al., 2013). In the case of macrophytes, not only consumption but non-consumptive cutting of macrophytes leads to this decrease, one of the ecosystem engineer activities of IAC (Gherardi & Acquistapace, 2007). Furthermore, IAC show preferences for certain macrophytes, thereby changing the macrophyte community in both abundance and species composition accordingly (Carreira et al., 2013; Cronin et al., 2002). Other primary producers, such as microalgae, are also affected by IAC, both in terms of their abundance and composition (Gherardi & Lazzara, 2006).

Moving to a higher trophic level, IAC are linked to decreases in macroinvertebrate abundance and diversity, particularly impacting snails and diptera (Chucholl, 2013; Correia et al., 2005; Correia and Anastácio, 2007; Galib et al., 2022; Gherardi and Acquistapace, 2007; Herrmann et al., 2022; Nyström et al., 1996; Nyström et al., 2001). Furthermore, the effects vary across habitats and differ from Europe's native noble crayfish (Ercoli et al., 2015; Ruokonen et al., 2013). These effects are possibly facilitated by the fast learning curve for detecting and capturing new macroinvertebrate prey, which again shows a component of the invasive properties of IAC (Ramalho & Anastácio, 2010).

IAC impact not only the lower trophic levels of freshwater ecosystems, but also higher components of the native food web, including vertebrates. IAC affect amphibian embryo and larvae survival negatively through predation and injury, in some cases their influence was found to be stronger than that of natural predators and environmental characteristics (Cruz et al., 2006b; Falaschi et al., 2022; Ficetola et al., 2011; Ficetola et al., 2012; Gherardi et al., 2001; Grillas et al., 2018; Nunes et al., 2010; Nyström et al., 2001; Rebelo and Cruz, 2005). Cruz et al. (2008), for example, described the collapse of amphibian populations following the introduction of *P. clarkii*, recording a decline in abundance and the local extinction of seven species. Additionally, IAC limit dispersal of amphibians by excluding them from breeding sites (Cruz et al., 2006a; Cruz et al., 2006b; Ficetola et al., 2011). An alike trend was found where IAC presence affected dispersal on the spatially structured population level of amphibians (Bélouard et al., 2019; Dalpasso et al., 2022; Falaschi et al., 2021; Manenti et al., 2019). Apart from amphibians, IAC engage in direct interactions with fish, involving competition for food and shelter, with certain fish species predating on crayfish while crayfish feed on fish eggs (Mohammed et al., 2023; Reynolds, 2011; Setzer et al, 2011).

Besides the direct effects, IAC have been found to be vectors for various diseases and parasites as well. In Europe the invasion of IAC has led to a decrease and local extinctions of native crayfish due to the introduction of the crayfish plague in combination with direct competition (Bonelli et al., 2017; Brady et al., 2024; Capinha et al., 2013; Chucholl and Schrimpf, 2015; Holdich et al., 2009; Jussila et al., 2015; Martín-Torrijos et al., 2019; Tilmans et al., 2014). The crayfish plague is considered to be the most important biotic factor in interspecific competition between crayfish (Wiśniewski et al., 2020). This statement is supported by the finding of co-existence of native and non-native crayfish species in absence of the plague (Schrimpf et al., 2013). Besides the crayfish plague, other pathogens are transmitted through IAC that affect fish and amphibians as well (Dragičević et al., 2020; Imhoff et al., 2012; Martín-Torrijos et al.,2018; Oficialdegui et al., 2019b).

Aside from the impacts on the biota discussed in Box 1, crayfish influence the abiotic components of a freshwater ecosystem as well. Roessink et al. (2017) showed how IAC can have a significant effect on various water quality parameters after a certain, but realistic, density threshold has been reached. Additionally, IAC have been shown to impact the nutrient dynamics and increase the turbidity of the system (Angeler et al., 2001; Carvalho et al., 2016; Matsuzaki et al., 2008). IAC have been observed to affect nutrient concentrations in the water column in three ways, thereby potentially contributing to the eutrophication process (Angeler et al., 2001). Firstly, (I) they engage in bioturbation through their burrowing, walking and tail flipping behaviour which contribute to sediment resuspension and particle bound nutrient release in the water column, thereby increasing turbidity. Additionally, (II) their feeding behaviour plays an important role in organic matter cycling, where they enrich nutrients in the water column and modify the sedimentary nutrient pools. As an example, IAC increase rates of decomposition by directly consuming leaf litter or detritus materials (Chucholl, 2013; Jackson et al., 2014). Lastly, (III) another method by which crayfish might release nutrients into the system is through the non-consumptive cutting of macrophytes (Gherardi & Acquistapace, 2007). When comparing the different effects, Gao et al. (2023) found how sediment disturbance is a greater driver for increased turbidity than plant cutting/decrease. These ecosystem engineering activities have indirect effects on the biota as the available niches are being modified. Besides, the alterations in abiotic components can, in combination with the former named biotic impacts, lead to a switch in alternative stable states of freshwater systems (Matsuzaki et al., 2008).

It is important to emphasize that the effects are separately touched upon, but happen simultaneously and can interact with each other leading to cascading effects (Ficetola et al., 2012; Geiger et al., 2005; Gherardi and Acquistapace, 2007). A case study illustrates how a shift occurred from a clear, macrophyte-dominated lake to a turbid state following the introduction of an invasive crayfish. This transition was accompanied by eutrophication processes where a decrease in macrophytes and an increase in nutrients was recorded (Rodríguez et al., 2003). This in turn resulted in a decrease of richness and abundance of organism groups such as amphibians, macro-invertebrates and even birds, highlighting the negative cascading effects of an IAC (Rodríguez et al., 2005). IAC thus directly impact multiple organism groups belonging to various trophic levels through predation and competition, which in combination with its ecosystem engineer activities can lead to changes in (a)biotic water quality and freshwater ecosystem states.

2.2 The current state of water quality

In Europe, 40% of surface waters meet ecological quality standards, and 33% meet the chemical quality standards (Ecological Status of Surface Waters in Europe, 2021; European Environment Agency, n.d.). The Netherlands faces a particularly critical situation, with only 1% of the surface waters holding sufficient ecological and chemical water quality (Didde, 2022). To address this issue, the Water Framework Directive (WFD) has been put in act, requiring EU-member states to realize and maintain chemically clean and ecologically healthy water by 2027 (Directive 2000/60/EC, 2014). The prospects are that the Netherlands is unlikely to meet these objectives within the remaining three-year period as of writing this report (Didde, 2022). The Netherlands has a rich history of excessive nitrogen and phosphorus loads in its surface waters (CLO, 2022). Excessive nutrients form a driver of ecosystem change as they act as a bottom-up effect, triggering food-web changes that accelerate eutrophication, shifting clear macrophyte dominated systems to turbid macrophyte devoid systems, two recognized stable states of lentic water bodies (Gulati and Van Donk, 2002; Scheffer et al., 1993). Furthermore, eutrophication promotes algal blooms which have the potential to form dense surface mats which shade the water body and can lead to anaerobic conditions due to outcompeting of macrophytes and decomposition of the abundant dead algal material (Smith et al., 2006). For these reasons, reducing nutrient concentrations is one of the primary focal points for improving water quality in the Netherlands to combat eutrophication and its consequences. (Schipper et al., 2022).

2.3 Water soldier (*Stratiotes aloides*) as a nature-based solution

A potential nature-based solution for eutrophication is by planting water soldier (*Stratiotes aloides*), a species of submerged/emergent macrophyte native to Europe. *S. aloides* has experienced a decline throughout Europe and is now listed as a sensitive species on the Dutch Red List (Ministerie van Landbouw, Natuur en Voedselkwaliteit, n.d.; Efremov et al., 2019).

S. aloides possesses a set of relevant characteristics that makes it an interesting option for a nature-based solution. *S. aloides* follows a seasonal cycle of floating (spring–summer) and submerged (winter) phases which, in combination with turions (specialized buds) enables it to survive hard winter conditions and facilitates a wide distribution range (Efremov and Sviridenko, 2008; Efremov & Sviridenko, 2012; Efremov et al., 2019). The plant can persist the winter as long as the basal rosette or the water column does not fully freeze (Erixon, 1979). Furthermore, the plant shows morphological plasticity based on environmental conditions (Toma, 2019). *S. aloides* thus demonstrates a high degree of adaptability and tolerance to the environment, which makes for versatile deployment options. *S. aloides* typically thrives in phosphate poor, shallow, mesotrophic, slightly alkaline and sediment thick/detritus rich slow flowing/lentic water systems (Nielsen and Borum, 2008; Kłosowski et al., 2011). Outside from its broad home range, *S. aloides* shows high occupying capabilities during the floating phase as reproduction can happen at fast rates since the plant predominately reproduces vegetatively, even if sexual reproduction is possible (Smolders et al., 1995; Toma, 2012). During a growth season, individual numbers can increase by up to 70% (Renman, 1989), indicating *S. aloides* deployment may lead to fast outcomes with minimal human effort if the expected water quality improvements hold true.

Besides the suitability based on life history traits, *S. aloides* possesses several characteristics that directly mitigate the eutrophication process and its associated consequences previously mentioned. Firstly, *S. aloides* is associated with lower nutrient levels by absorbing excess nitrogen and phosphorus (Brammer, 1979; Strzałek et al., 2019), which can happen at great rates when considering the high reproduction and growth abilities. Secondly, submerged macrophytes decrease water flow velocities which promotes sedimentation and reduces turbidity (Madsen et al., 2001). Third, *S. aloides* inhibits free floating phytoplankton growth through allelopathic and shading effects, even when sufficient amount of nutrients for phytoplanktonic growth is present, thereby counteracting algal blooms (Brammer, 1979; Mulderij et al., 2005; Mulderij et al., 2006; Mulderij et al., 2007; Mulderij et al., 2008). Through this combined effects, *S. aloides* can enhance the abiotic water quality of a freshwater system and mitigate eutrophication processes.

At last, *S. aloides* holds restorative value since it is part of the Natura 2000 habitat type H3150 and is considered to be an important factor in restoring fen ponds (Arts et al., n.d.; Sarneel et al., 2011). This is especially relevant when considering the decline of this species and its associated fauna. *S. aloides* has been shown to household relatively high abundances epiphytic algae and is correlated with relatively high species richness of surrounding aquatic vegetation (Sugier et al., 2010; Toporowska et al., 2008). Furthermore, the spiny leaves of *S. aloides*, for example, provide habitat and breeding grounds for aquatic macroinvertebrates (Obolewski, 2005; Obolewski, 2009; Obolewski & Strzelczak, 2009; Suutari et al., 2008; Tarkowska-Kukuryk, 2006). Additionally, *S. aloides* provides habitat for the endangered dragonfly green hawker (*Aeshna viridis*) and black tern (*Chlidonias niger*), both of which are reliant on this plant for reproduction habitat (Beintema, 1997; Beintema et al., 2010; Rantala et al., 2004; Suutari et al., 2004). *S. aloides* thus enhances both abiotic and biotic components of the ecosystem, extending its impact even to the trophic level of birds.

However, there are some implications on using *S. aloides* as a nature-based solution. As stated by Harpenslager et al. (2016), successful restoration with *S. aloides* leading to a sustainable and self-facilitating population is density-dependent. This density-dependency overrules high stress environmental factors such as excessive concentrations of inorganic nitrogen which negatively impact *S. aloides* under normal conditions (Abeli et al., 2014). This tolerance shows yet another reason which makes *S. aloides* useful in restoration project in the eutrophic surface waters of the Netherlands.

2.4 Combatting invasive alien crayfish with water soldier

Because of the former stated impacts of IAC (see 2.1), especially concerning the effects related to eutrophication processes, IAC might contribute to declining water quality. In the Netherlands, six IAC species have established populations and it is estimated that IAC could have a substantial impact on the WFD scores (Boerkamp et al., 2011; De Vries, 2021;van der Meulen et al., 2009). The considerable impacts of IAC has led to most IAC species being included in the list of Invasive Alien Species of Union concern under the Invasive Alien Species Regulation of the EU (Regulation 1143/2014; Regulation (EU) 2016/1141). Consequently, countries are obliged to actively combat these species in order to conserve their native nature. This raises the question of how to effectively mitigate the impact of IAC, where no answer is simply given as a lack of knowledge is present on the efficiency of possible mitigating or eradicating strategies (De Hoop et al., 2016; Gherardi et al., 2011). These studies deem well known approaches such as intensive trapping as costly and non-sustainable for the long term. In the case of the Netherlands, De Hoop et al. (2016) considered eradication of IAC close to impossible and controlling populations most successful when different measures are combined. For example, Roessink et al. (2009) judges the intense trapping combined with the introduction of natural predators to be a promising strategy, still no research in the natural environment supports this statement.

Unsurprisingly, the Dutch government acknowledges that there is still no clear, effective strategy for controlling or eliminating alien crayfish species (Rijksoverheid, 2022). An strategy which is often opted, is creating a more robust system by altering environmental factors to combat crayfish, such as decreasing nutrient levels and therefore improving water quality, as this is considered to be a sustainable way to manage invasive crayfish populations (Rijksoverheid, 2022; Van Kleef et al., 2022). It is hypothesized that oligotrophic environments are more robust towards invasion of crayfish (Boerkamp et al., 2011; Lemmers et al., 2018; Koese & Evers, 2011; Soes & Koese, 2010). Lowering the phosphate load has been recognized as a mitigating strategy against IAC (Cusell et al., 2020). As discussed, IAC thrive in a self created eutrophic, turbid macrophyte-devoid habitat (see 2.1). Reducing the eutrophication processes through *S. aloides* implementation and thereby potentially IAC numbers is thus a promising strategy to restore freshwater systems. As stated by Scheffer et al. (1993), a combination of nutrient reduction and changes in the foodweb is needed in order to restore a stable clear state. Important to acknowledge is how macrophyte development, depend on turbidity of water caused by fish presence and impacts of grazers (Van De Haterd & Ter Heerdt, 2007; Veen et al., 2013). IAC in particular are considered as a potential bottleneck for shallow water body restauration in Europe with the use of submerged macrophytes (Van Der Wal et al., 2013). This prompts the question if *S. aloides* deployment is fundamentally compatible with IAC presence.

2.5 Possible outcomes of IAC and water soldier interactions

When considering all the earlier stated effects of *S. aloides* and IAC, several interaction pathways can be hypothesized. In the preferable situation, overall improvements in water quality induced by *S. aloides* creates a ripple effect that could lead to a decrease in the population of crayfish, as they typically thrive in possibly self-created nutrient rich environments. However, the exact relationship between both *S. aloides* and crayfish populations as water quality drivers is not yet fully understood. Moreover, *S. aloides* presence could indirectly affect crayfish densities negatively by providing habitat for predatory fish such as pike (Van Kleef et al., 2022).

Even though it is hypothesized that improving water quality has a negative impact on crayfish's niche, this effect might be negligible when considering the tolerance of IAC towards environmental variables. Furthermore, an opposite effect may arise where *S. aloides* provides a favourable habitat for crayfish. The expected water quality enhancement in combination with the habitat provisioning, could lead to heightened aquatic macroinvertebrate taxonomic richness and abundance (Duran, 2006). As aquatic macro-invertebrates are a significant part of crayfish diets, especially in juvenile stages a potential favourable nursery habitat could be created. Furthermore, *S. aloides* could present itself as a food source for IAC, however it is worth noting that *S. aloides* is a relatively tough plant, whereas crayfish tend to prefer more delicate macrophytes for feeding (Cronin et al., 2002). At last, structural properties of *S. aloides* could attract crayfish, for example because of shelter possibilities. These possible interactions lead to a contextual framework shown in figure 1.

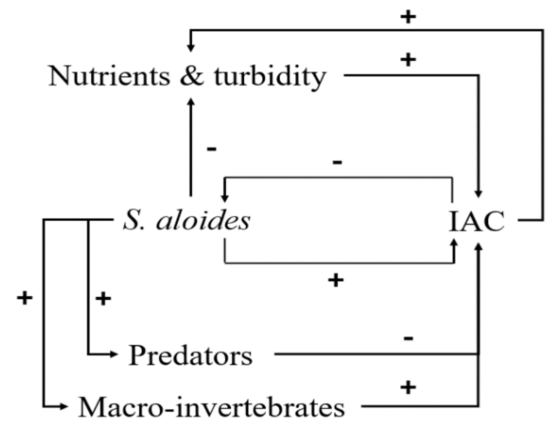


Figure 1: Simplified contextual framework of hypothesized pathways due to effects of *S. aloides* and IAC.

The aim of this study was to evaluate the compatibility of *S. aloides* with IAC presence. The main objectives of this study were to asses (I) how *S. aloides* translocations impact crayfish densities and (II) how differences in eutrophication related variables, if any, correlate with both *S. aloides* and IAC. IAC presence was studied by investigating crayfish densities in *S. aloides*-translocated and control ditches within a Dutch IAC invested polder landscape. Furthermore, abiotic variables such as nutrient levels and turbidity were measured.

3 Materials and Methods

3.1 Research site

This study has been conducted in Polderlab Vrouw Venne, a polder located in a peat meadow area in the Netherlands. This polder has a rich, intensive agricultural history with high levels of nitrogen and phosphorus and is experiencing the presence of two invasive crayfish species: Red swamp crayfish (*Procambarus clarkii*) and Spiny-cheeked crayfish (*Orconectes limosus*). The waterbodies were connected within an enclosed water system, although water flow in and out the system was allowed to regulate the water table. In advance of this study 10 ditches were selected within the research site suitable for *S. aloides*, see figure 2. These ditches were relatively wide (minimum 6 meters, maximum 14 meters, average 9 meters wide) and quite shallow (minimum 40 cm, maximum 90 cm, average 60 cm deep). The *S. aloides* translocations have been performed in different time frames: in ditch 1 water soldier has been placed in March 2022; ditch 2, 3, 4 & 5 in both April and October 2023; ditch 6, 7, 8, 9 & 10 in October 2023. In each ditch two ditch tracks were thus characterized: the translocated track and the control track. *S. aloides* was contained within the treatment tracks by a wall of sticks across the ditch, which prevented the plants from floating away. Furthermore, treatment sites located at the end of ditches were alternated between the two treatment states and intersections of ditches were evaded for sampling to prevent any bias.

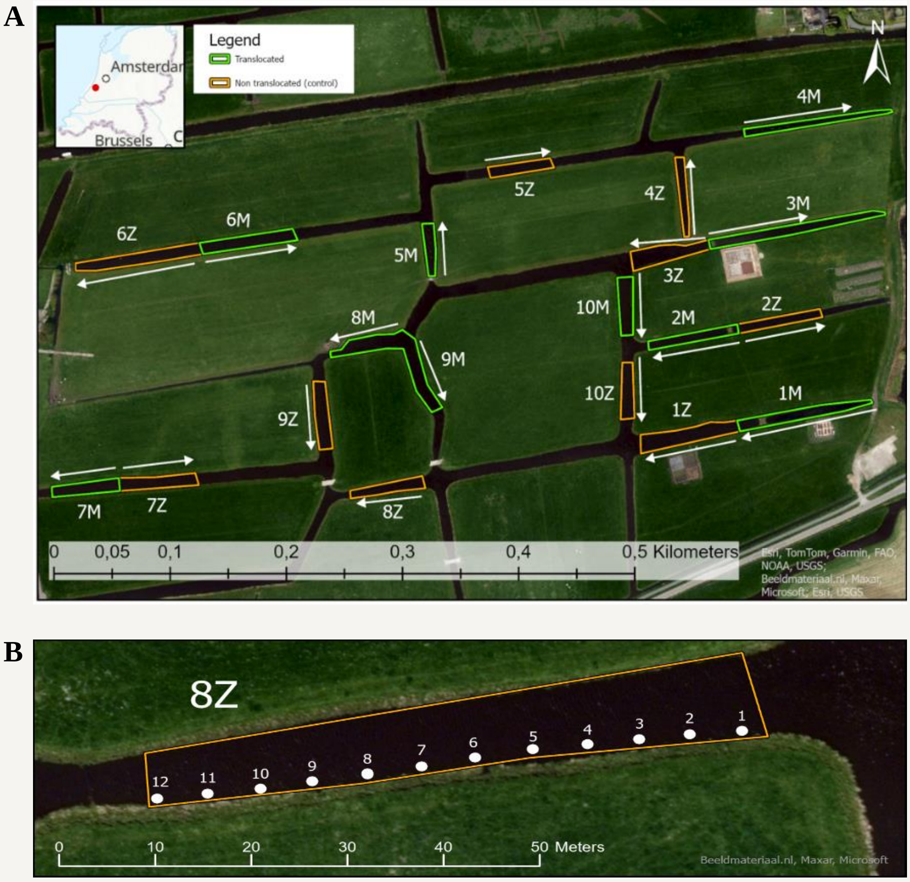


Figure 2: Maps showing the research area Polderlab Vrouwe Venne Polder. The placement of the ditch tracks are depicted through characters such as ‘’6M’’ where M depicts the *S. aloides* translocated tracks and Z the control tracks, coloured green and orange respectively (A). The white arrows indicate the placement of the landmarks in relation to two specifications: which bank side was sampled and the orientation of the landmarks 1–12. An detailed example of the landmark placement is visualised for track 8Z (B).

3.2 Crayfish sampling methods

To assess the density distributions of invasive crayfish in the *S. aloides* and control tracks, baited funnel traps were utilized to catch crayfish, see figure 3. In each ditch track 12 traps were evenly positioned along one bank, as this number is the standard for crayfish assessment. For this purpose, the shortest track of each ditch was measured in length and landmarks were then placed evenly along that length, serving as both placement indicators and anchors to tie the traps onto. The same positioning was then copied to the counterpart track, thereby eliminating possible effects due to difference in total sampling area. The landmarks were numbered on each track from 1–12, see figure 2. As bait, three 14 mm halibut pellets were used which were placed inside a cage within the trap to prevent feeding.

The placement of the traps followed specific steps. First, since crayfish are bottom dwellers, ground contact was always ensured. Second, whenever possible while maintaining bottom contact, the traps were not fully submerged to leave breathing room for bycatches such as frogs. Third, in accordance with the first two directions, the traps were placed as close to the ditch bank as possible. At last, following the results of a pilot study (see appendix A), it was decided to position the traps with the openings parallel to the ditch bank, see figure 3. Placement time of the traps was variable and dependent on the structure of the day, with traps typically placed after being emptied in the morning. Traps were left overnight to be emptied early the next morning, starting around 09:00. This approach was taken as a measure of standardisation and to reduce potential escapes since crayfish have been shown to enter and leave this type of trap over night (Lewis, 1997). Time of placement and emptying were noted.

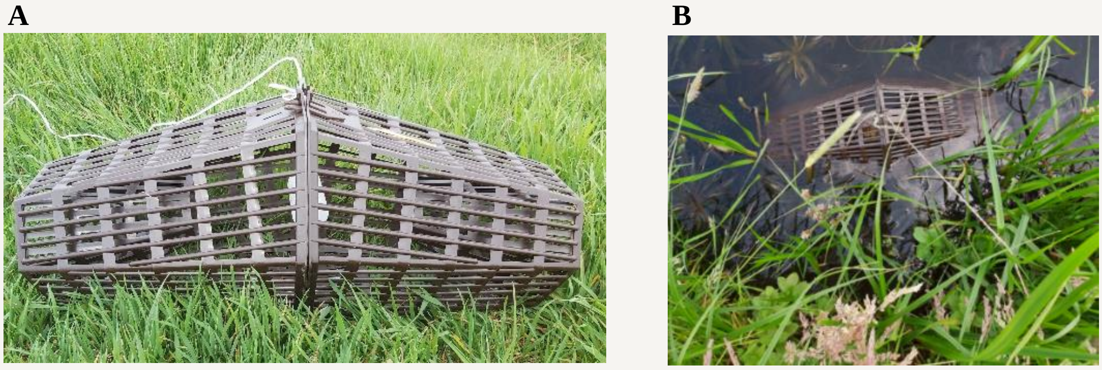


Figure 3: (A) Used baited funnel traps (610 x 315 x 250 mm), sometimes referred to as the ’’Finnish model’’ and (B) the placement and use of this trap.

Caught crayfish were counted, measured for length (from rostrum to telson rounding to 0.5 cm), sexed and identified on species level per trap before being released back into the water at the sampling site. The crayfish were measured by one person over the course of the whole study to reduce observer bias. Furthermore, missing claws were recorded as a measure of aggressiveness and bycatch was noted. At last, all used bait was recovered after sampling to prevent potential side effects.

This crayfish assessment was performed once a month over a three month period in spring (April-June). Each sampling round was completed within a one-week timeframe (four nights) as only six tracks could be sampled at once due to the available amount of traps. Furthermore, the sampling order was reversed every sampling round to introduce random sampling whilst accounting for the walking distances between sampling sites which made true randomisation not feasible. Additionally, counterpart ditch tracks were always sampled simultaneously.

3.3 Sampling methods for environmental and abiotic variables

To prevent confounding effects in treatment results, additional environmental variables were quantified to ensure observed trends are not influenced by these factors. Depth and emergent reed presence are deemed relevant since these represent parameters for ‘’nature friendly banks (NFBs)’’. NFBs are hypothesized to repel IAC due to abundant reeds and a gentle slope which hinders crayfish burrowing activity and attracts predators of IAC (Lemmers et al., 2019; Cusell et al., 2020; De Jong et al., 2019). At first depth of placement sites of the traps and the bank depth profile (0, 0.5, 1.0, 1.5, 2.0 and 2.5 meter from ditch bank) was measured at three evenly spaced locations per ditch track. The depth measurements were conducted with the use of a fishing pole (with reel) rigged with a light weight, 30 grams, in order to be able to measure first contact point with the thick sludge layer present in the ditches. This was done since this is most representative for the crayfish assessment since the traps were not pushed far into this layer. The distance from the ditch bank for the measurements was determined by a floating pole with marks at the former named distances. Furthermore the coverage of emergent reeds of the sampled bank length within each ditch track were quantified by visual estimation. These measurements were performed once in calendar week 24.

For this study, eutrophication related variables were of special interest for evaluating water quality effects induced by *S. aloides* and IAC. Therefore turbidity and the concentrations of the following nutrient indices were measured: phosphate, ammonia and nitrate. Phosphate and ammonia levels were quantified using Checker photometers where protocols of the included guides were followed. Due to the nature of these protocols processing showed difficulty in the field. Therefore, mixture samples were taken at each ditch track to be processed later in a more controlled environment. These consisted of 25 ml ditch water sampled at both landmark 3 and 9, totalling a mixture sample of 50 ml per track which accounted for possible differences within the track. Nitrate concentrations were measured from these samples as well with the KYORITSU PACKTEST Nitrate. To counteract any bias because of the time between sampling and processing, the same sampling order (5M, 5Z, 4M, 4Z, 3M, 3Z, 10M, 2M, 2Z, 1M, 1Z, 8Z, 9M, 8M, 6M, 6Z, 9Z, 7M, 7Z) was followed for each sampling day which consistently started at 09:00. Additionally, the first collected samples were also the first to be processed. Turbidity was measured once at each ditch track at landmark 6 using a Secchi tube. These variables were measured over the course of one day every week during the sampling period.

For a list of all used materials with specifications see appendix B.

3.4 Statistical analysis

Statistics were performed in RStudio, 2024.04.1 Build 748 (R 4.1.1). Before performing any statistics, the raw data needed to be prepared. For each sampling round the total haul of crayfish was calculated per ditch track, which forms the main response variable relevant to this research. Furthermore, to standardize the data for comparison with other studies, the catch per unit effort (CPUE) was determined. This was done by dividing the haul per ditch track in each sampling round by 12, giving the average amount of caught crayfish per trap, per track in a given sampling round. Additionally the sex ratio (F/M) was generated as a measure for including male and female counts and the average length of caught crayfish per ditch track was computed. Since the abiotic measurements were performed in a weekly manner the values corresponding to the crayfish sampling week and the week before and after this week were averaged. This approach provided a value for each track in each sampling round, capturing seasonal changes with a one-week buffer and accounting for missing data in various weeks.

Although CPUE and haul show the same distribution, the haul was used for statistical analyses since these hold the data structure of the raw count data, the terms might be used interchangeably forward. Before making of any models, data were inspected, visualised for initial visual correlations, and was tested for collinearity between the explanatory variables. Because of the interconnected treatment sites (nested experimental design) and replication over time, a correction for pseudo-replication was needed in the statistical models. This has been achieved by the use generalized linear mixed-effect models since these can account for the poisson distribution associated with count data as well (function lmer, package lme4 version 1.1-34). Furthermore, assumptions were checked using various diagnostic plots (package DHARMa version 0.4.6). When overdispersion was present in the model, a negative binomial distribution, log link, was specified in the model instead of a poisson distribution to prevent overestimations of p-values (function glmmTMB, package glmmTMB version 1.1.9). Within the models, ‘’Ditch’’ was specified as the random effect to account for both the spatial and temporal the pseudo-replication. All of the other explanatory variables, such as *S. aloides* treatment and depth, were included as random effects, leading to the most complex model. During model selection the principle of parsimony, the AIC and BIC scores and anova tests were used to find the best fit model containing only the most important predictors. In the case of the abiotic measurements, the crayfish haul became a predictor variable and the abiotic measurement the response variable, models were constructed in the same manner as described before. Additionally ANOVA’s were performed for basic comparisons.

A summary of the statistic models, including outputs, can be found in appendix C.

4 Results

Before describing results of the crayfish and abiotic assessments, the *S. aloides* treatment needs to be evaluated as some unexpected changes occurred during the sampling period. The *S. aloides* introductions are deemed unsuccessful as almost no individuals have been sighted floating up to calendar week 24. This indicated that the plants might not had survived the winter period as their typical cycle was being discontinued, see table 1. Only ditch track 1M contained vast amounts of visible *S. aloides*, which led to two different *S. aloides* treatment within the research site: translocation and presence of *S. aloides*. Since the translocation treatment contained nine of the ten replications, the main focus shifted to the effects of translocation of *S. aloides*. For this reason, ditch 1 was excluded from the statistical analysis to separate the effects of the presence and translocations of *S. aloides*. For the sake of consistency with this development translocated (TL) and non-translocated (NTL) will be used forward. If any data of ditch 1 were included in any way for a analysis, then this will be specified.

Ditch track	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M
Visible floating *S. Aloides*	INF	0	2	7	0	0	100	0	0	33

Table 1: Table showing the amount of visible floating *S. aloides* individuals counted in calendar week 24. In ditch track 1M such high amounts were present that counting them was impossible, hence the reason to name this infinite (INF) when considering the low amounts found in other tracks.

From march to June 2024, three sampling rounds for crayfish assessment were performed in calendar weeks 14, 18 and 22 where baited traps were used to catch crayfish in the treatment sites. During the whole of the sampling period, a total of 1842 IAC were caught, comprising two species: the red swamp crayfish (*Procambarus clarkii*) and the spiny cheeked crayfish (*Orconectes limosus*). The majority of the captured IAC were *P. clarkii* (~ 98%), while *O. limous* accounted for only a small fraction (~2%). Hence the reason to only include observations of *P. clarkii* for the statistical analyses.

Other creatures than crayfish were caught as well, these bycatches are visualized in figure 4. For all tracks at least one bycatch was noted during the sampling period. Tubenose gobies (*Proterorhinus semilunaris*) accounted for the largest portion of the bycatch, followed by bitterlings (*Rhodeus amarus*) and frogs (*Pelophylax sp.*). The bitterling counts are possibly underestimated as these fish slipped through the traps mazes when lifting it out of the water, but these fish are effectively caged in the water as they only fit through the mazes sideways.

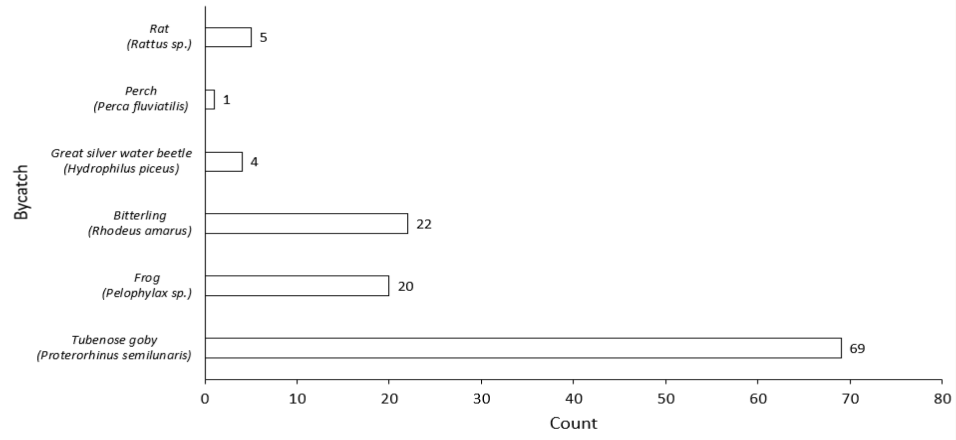


Figure 4: Bar graph showing the total amount and sort of bycatches caught during the whole sampling period.

4.1 Crayfish haul higher in translocated water soldier tracks

Overall, a significant difference was found between TL and NTL ditches ($p = 0.0123$). The average CPUEs, 2.27 and 2.87 respectively, was ~26% greater in TL than NTL tracks, see figure 5.

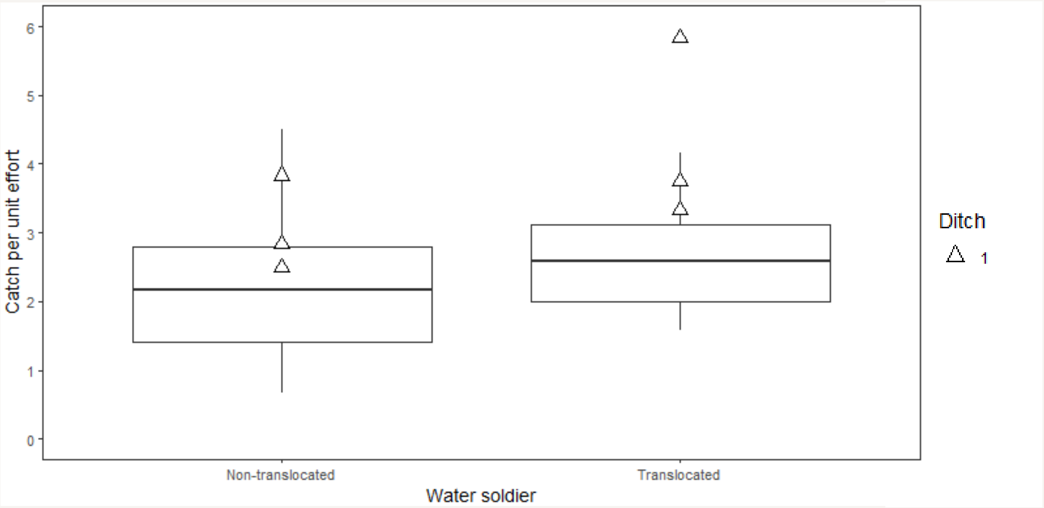


Figure 5: Boxplot showing the distribution of CPUE per track, categorized by *S. aloides* treatment, aggregating data across all sampling rounds. Data from ditch 1 are excluded from the boxes but are visualized separately as triangles.

Figure 5 shows the combined data of three sampling rounds, which were spread over three months, potentially missing seasonal changes. When a post-hoc analysis was performed, an almost significant difference ($p = 0.0857$) in CPUE between TL and NTL ditch tracks during the first sampling round was revealed, see figure 6. However, in the second and third sampling rounds, the differences between TL and NTL tracks balanced out. Furthermore, an increase of overall CPUE was found where the CPUE only differed between sampling round 1 and 3 ($p = 0.0235$). On average and regardless of water soldier treatment, more crayfish were caught per track during sampling round 3 compared to round 1 (excluding ditch 1).

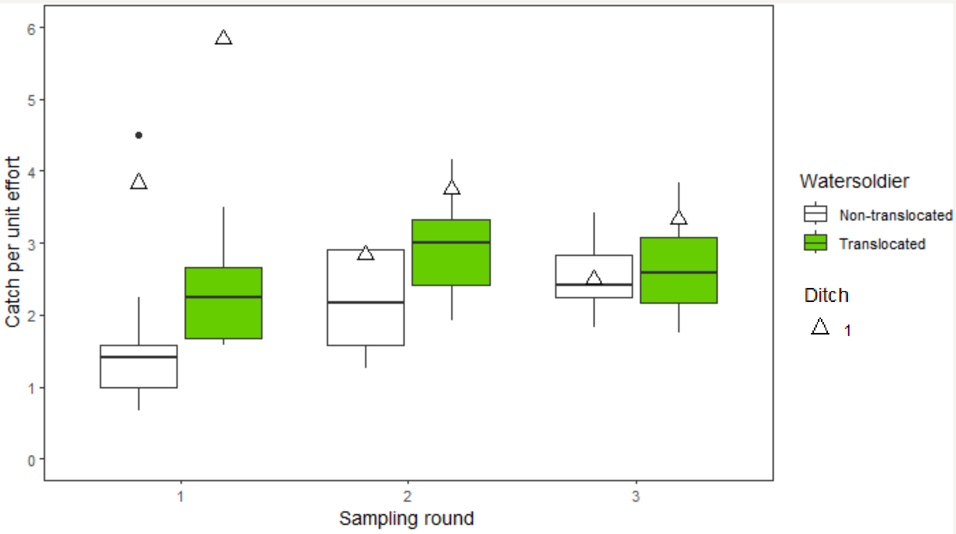


Figure 6: Boxplot showing the distribution of CPUE per track, categorized by sampling round and sorted by water soldier treatment. Data of ditch 1 are excluded from the boxes but are visualized separately as triangles.

Additionally, CPUE was found to vary with NTL and TL treatment-specific average length of the caught crayfish (*p* = 0.0263) while length itself showed no significance. In the TL tracks the CPUE decreased with increased averaged length while the NTL tracks showed the opposite trend, see figure 7. In figure 7 two influential datapoints are marked. These two points were part of the data originating from ditch tracks 4M and 5Z, excluding these points led to the loss of this relationship. Further investigation of these tracks showed interesting characteristics as 4M (TL) contained relatively high amounts of other macrophytes (personal observation) and ditch 5Z (NTL) has one of the highest placement depths and the lowest amount of reeds. Furthermore, no differences in average crayfish lengths between TL and NTL tracks were found, see figure 7.

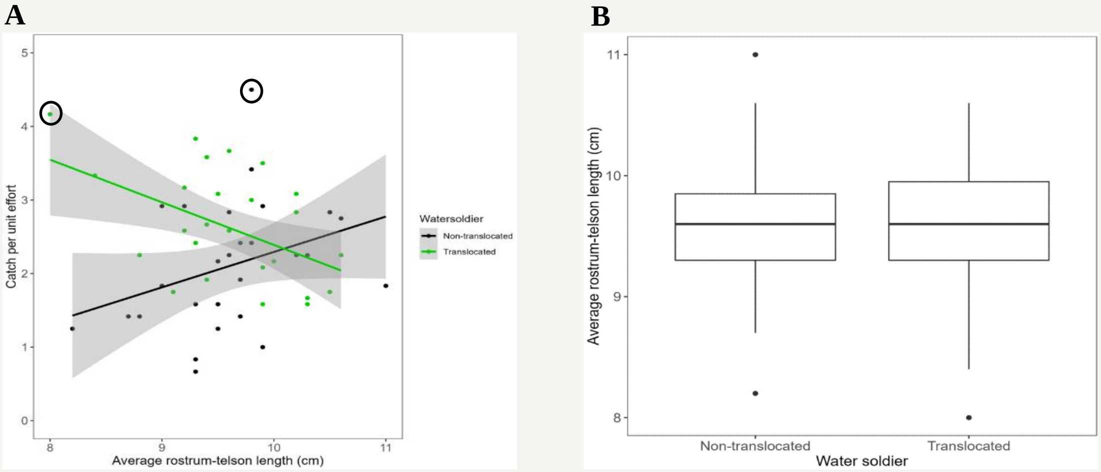


Figure 7: Graphs showing (A) the correlation between the average rostrum-telson length and the catch per unit effort per track of all sampling round in relation to TL and NTL *S. aloides* treatments and (B) a boxplot visualizing the average lengths found per track categorized by water soldier treatment. Influential data points have been marked with a circle (A) and no data of ditch 1 are included in both plots.

4.2 Higher phosphate concentration in water soldier tracks

When looking at the eutrophication related variables, only phosphate was found to correlate with water soldier translocation treatment (*p* = 0.0446). Phosphate concentrations were found to be higher in TL tracks and were not correlated with crayfish densities in any way, see figure 8.

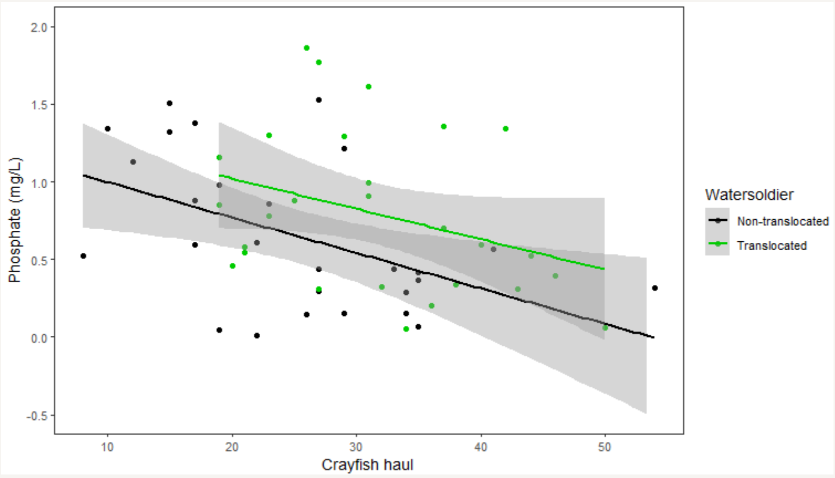


Figure 8: Graph showing the relationships between phosphate concentrations in mg/L and the crayfish haul per track sorted by water soldier treatment. Influential points are marked with circles and no data from ditch 1 are included. Note how the y-axis falls below zero, this is because of the computed confidence interval, logically no negative values of phosphate are possible.

4.3 Water soldier independent associations with crayfish haul

Besides water soldier treatment and its interaction, other variables have shown significant explanatory capabilities for the CPUE. Since the following relations were not associated to *S. aloides* treatments, data of ditch 1 were used for computing results. At first depth of trap placement was found to be the most significant variable within the model (*p* = 8e-08). As can be seen in figure 9, a positive relationship between CPUE and depth of placement site was present. Additionally, depth does not show interaction with any of the other variables and depth stayed consistent over the width of the ditch. Secondly, sex interacts with sampling round 3 (*p* = 0.003443) where a greater sex ratio, which means more females, correlates with an increase in CPUE in sampling round 3, see figure 9.

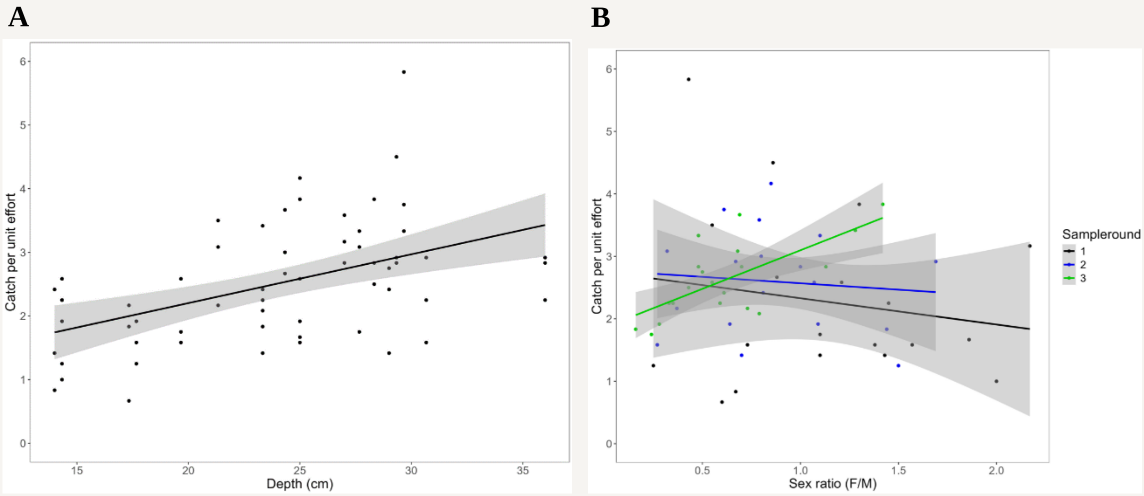


Figure 9: Graphs showing (A) the relationship between catch per unit effort and depth of trap placement per track of all sampling rounds combined and (B) the correlation between sex ratio and catch per unit effort per track sorted by sampling round. Data of ditch 1 are included.

Furthermore an interaction between sex and length is correlated with the CPUE (*p* = 0.022254), see figure 10. This interactions showed how the CPUE decreases with a lower sex ratio and a greater average length. Sex ratio was also found to be significant on its own, this was however only true when the interaction term was included in the model.

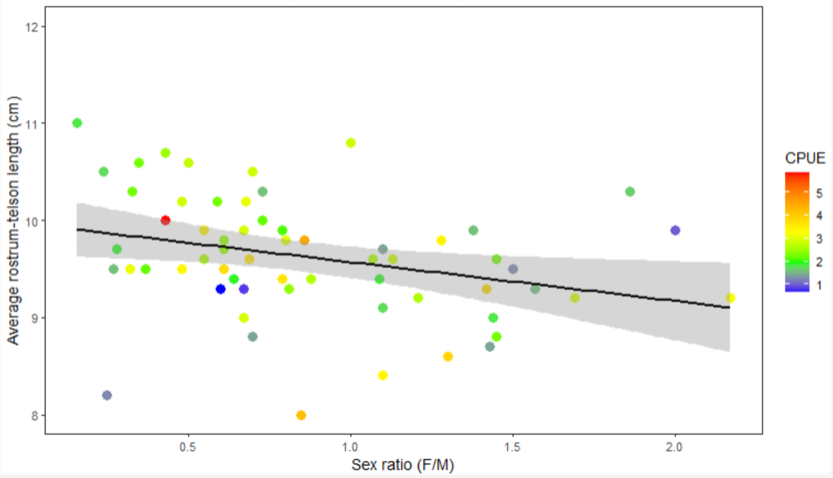


Figure 10: Graph showing the relationship between the sex ratio and average rostrum to telson length per track of all sampling rounds combined. Catch per unit effort is plotted as colored dots indicating its value. Data form ditch 1 are included.

4.4 Predominance of larger and male crayfish captures

Given that both sex and length have been identified as predictors of the haul, these variables were further investigated. Analysis of sex distributions revealed variations across the different sampling periods, as illustrated in figure 11. A difference in overall sex counts was found ($F(1) = 9.228$, $p = 0.00296$), where a Tukey post-hoc test confirmed higher male counts in sampling round three. This trend was visible in the sex ratios per track as well where an increase in frequency of lower ratios was found, see figure 11.

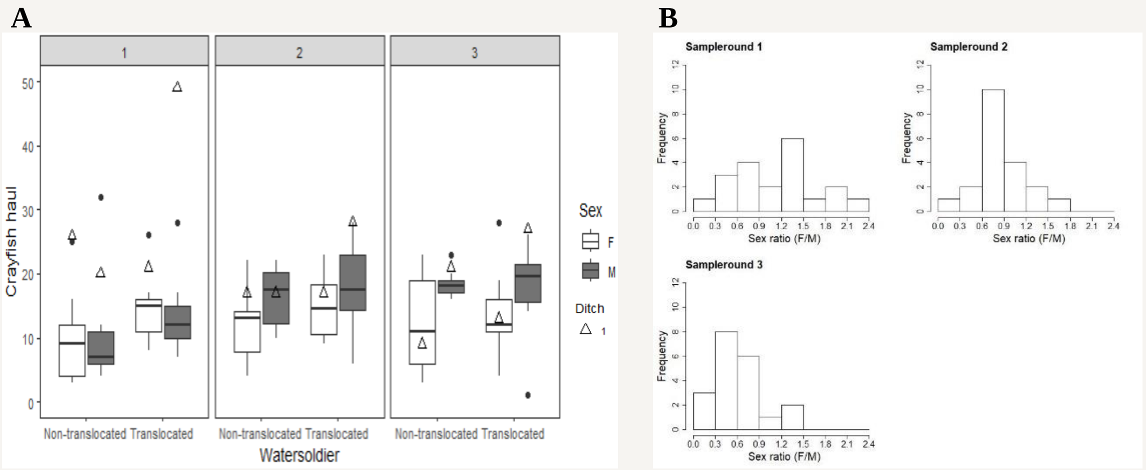


Figure 11: Graphs showing the distribution of male and female captures per sampling round per treatment (A) and the frequency of the sex ratios (F/M) per sampling round (B). The boxplots do not contain the data of ditch 1, these are plotted separately and visualized as triangles, the ratio plots do contain ditch 1 data.

During the sampling period crayfish with a telson-thorax length from 4.5 up to 14.5 cm were captured. As can be seen in figure 12, crayfish with a length of 8–11 centimeters were most frequently caught. Furthermore, the length distributions show no differences between treatments and sex, but larger crayfish were generally caught in sampling round three, see Figure 12.

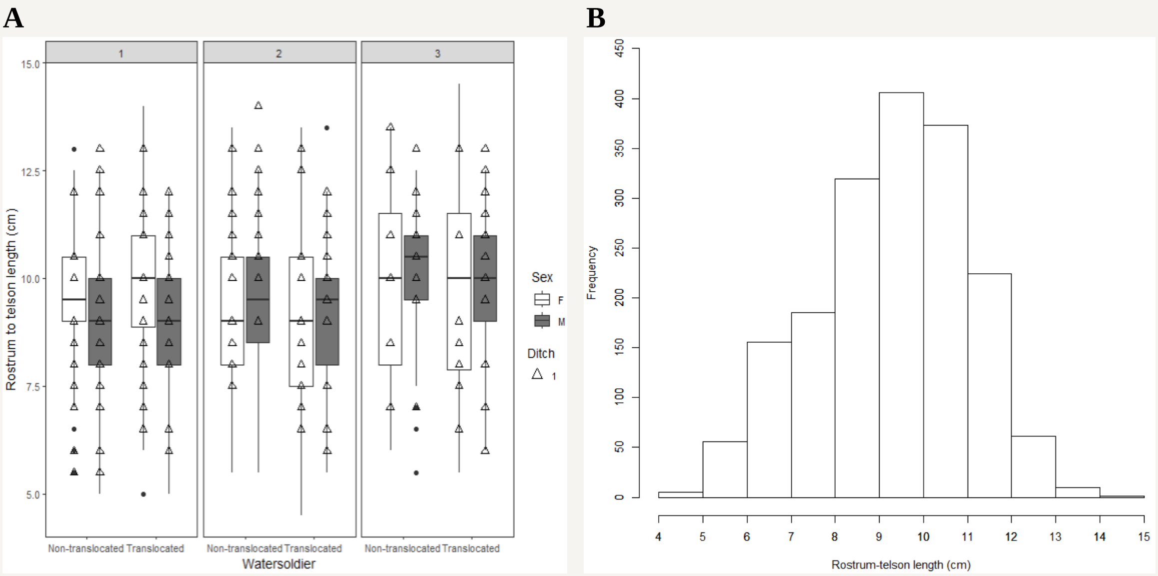


Figure 12: Graphs visualizing (A) the lengths of individual caught crayfish per treatment per sampling round and sorted by sex and (B) the frequency of lengths of all caught specimens, including ditch 1. Data from ditch 1 are excluded from the boxplots but are plotted separately as triangles.

5 Discussion

Water soldier (*S. aloides*) is considered to be a nature-based solution for improving water quality, particularly by combating eutrophication processes. Invasive alien crayfish (IAC), however, might interact with *S. aloides* and its effects. Therefore, the aim of this study was to investigate whether *S. aloides* deployment is compatible with IAC presence. The main objectives of this study were how IAC densities were affected by *S. aloides* translocations and how eutrophication related variables were correlated with both *S. aloides* presence and IAC densities. In nine of the ten sampled *S. aloides* tracks hardly any individual plants were observed after translocation. Nevertheless, the results show how overall IAC densities were 26% higher in *S. aloides* translocated tracks. However, when looking at the monthly sampling rounds, the first sampling round displayed the greatest difference, which faded over the subsequent rounds, indicating a seasonal trend. In case of the eutrophication related variables, only phosphate showed higher concentrations in *S. aloides* tracks and no association to crayfish densities. At last, greater ditch depth was associated with higher crayfish densities while depth showed no relationship with *S. aloides* treatments.

S. aloides was planted prior to this study, which took place from April to June 2024, at least before winter 2023. During the sampling period, however, hardly any individuals of *S. aloides* were observed floating in almost all of the sampled tracks. Due to this unexpected development, essentially two treatments were present in the research site: *S. aloides* presence (n = 1) and *S. aloides* translocation(n = 9). Since translocations comprised nine of the ten replications and to separate the effects of these two treatments, ditch 1 was excluded from the main analysis. When referring to translocated (TL) or non-translocated (NTL/control) tracks, ditch 1 is being excluded. If any data of ditch 1 were used for computing results and interpretation, then this will be specified accordingly as non-water soldier treatment related trends could still be of interest.

Only in ditch track 1M, a vast abundance of *S. aloides* was observed. This track formed a more stable population, as this track had experienced two growing seasons during 2022 and 2023. The other tracks with little to none observed *S aloides* were only transplanted in 2023. Records from Finland show how *S. aloides* can stay submerged over the whole year and do not float to the surface, which depends on the abiotic site conditions (Erixon, 1979; Harpenslager et al., 2015; Nielsen and Borum, 2008; Renman, 1989). However, given its presence in 1M, it is unlikely that abiotic conditions pose a bottleneck for settling and growth, particularly considering its tolerance to environmental variables (Efremov et al., 2019). This suggests that *S. aloides* was affected by biotic factors in other tracks during its submerged dormant phase in the winter, resulting in increased mortality. Logically the question arises if the biotic factor responsible for this hypothesized mortality are IAC.

Overall more crayfish were found in the *S. aloides* TL ditches, but a seasonal shift was revealed when looking at the individual sampling rounds. A higher catch per unit effort (CPUE) was recorded in the TL tracks for the first sampling round in April, this effect however evened out during the three-month sampling period. Since hardly any *S. aloides* were observed floating, with the exception of ditch 1, impacts on IAC due to their (a)biotic quality improvements are unlikely as these mainly depend on the emergent phase of the plant where most growth, occupancy and reproduction occur (Harpenslager et al., 2015; Smolders et al., 1995; Toma, 2012; Renman, 1989). This does not directly infer that the translocations of *S. aloides* had no impact. Although no causal relations can be manifested, a hypothesized plausible pathway leading up to the *S. aloides* decline and the found crayfish density distribution can be thought of. When considering the feeding behaviour of crayfish, the former mentioned seasonal shift could be due to trophic food availability and competition behaviour for resources. *S. aloides* partly decays in the winter period during its submerged phase (Efremov and Sviridenko, 2008), thereby providing a considerable amount of plant/detritus material in the generally macrophyte devoid studied polder landscape. When considering how the hard spiny leaves become more feedable for IAC as they loose their rigid structure when separated from the plant (Cronin et al., 2002), the TL tracks offer themselves as a food hotspot during winter. Especially given that IAC are invertebrates, their feeding activity typically reduces with temperature (Valido et al., 2020). This could lead to a shift towards non-moving prey as plant/detritus material thereby affecting the vulnerable *S. aloides* populations which were recently planted, thereby explaining the initial found differences. A potential explanation for the fading of this trend over the sampling rounds follows from macro-invertebrate abundance. In the research site an *S. aloides* independent increase of macro-invertebrate abundance over the three sampling rounds was found (unpublished data). A trend which might be due to higher growth and reproduction rates of macro-invertebrates with increasing temperature (Bonacina et al., 2022) and mirrors findings of Hill et al. (2016) who found increasing macro-invertebrate abundances in British agricultural landscapes from spring to summer. As a consequence of this increase in trophic availability of macro-invertebrates and a increase in crayfish activity with higher temperatures, a dietary shift towards macro-invertebrates can be expected (Correia, 2002; Alcorlo et al., 2004; Gutiérrez-Yurrita et al., 1998). In combination with competition behavior, this could lead to a more even distribution of crayfish as food is more abundant throughout the whole research site.

When considering this hypothesized interaction between IAC and TL tracks in relation to the data from ditch 1, an interesting theory arises. Ditch 1 had the greatest IAC density during the whole study where the water soldier-present track consistently showed a higher density throughout all sampling rounds. Together with the decline of the other younger less populated translocated ditch tracks, the simultaneous presence of high numbers of both IAC and *S. aloides* suggests that *S. aloides* can withstand IAC influences at high densities. This theory matches the findings of Harpenslager et al. (2016) which states how successful restoration with *S. aloides* leading to a sustainable and self-facilitating population is density-dependent and overrules stress factors if sufficient density is reached.

The eutrophication related variables showed little differences between *S. aloides* translocations and no association with IAC densities. Phosphate was the only variable found to be correlated with the translocation treatment. However, instead of the expected phosphate uptake by *S. aloides* (Brammer, 1979), the phosphate concentrations were higher in in TL tracks. This difference could be caused by the effects discussed in previous paragraphs as the hypothesized decline and decomposition of *S. aloides* plants could have led to a increase in phosphate concentration. As previously mentioned, nutrients uptakes are expected during a growth season (spring–summer), which was not experienced by most TL tracks, especially when considering the low amount of *S. aloides* individuals.

Outside from the impacts of water soldier solely on IAC densities, a positive correlation was found between the average length and CPUE from TL tracks while NTL tracks showed the opposite. However, when looking at the spread of the data points two specific tracks, 4M (high CPUE, small length) and 5Z (high CPUE, moderate length), seemed to contribute most to these trends. This was supported by (I) the finding of the loss of this relationship when removing these points from the analysis and (II) corresponding length distributions between *S. aloides* and control treatment. Further investigation of these tracks showed differences from other tracks since 4M contained relatively vast amounts of macrophytes and 5Z was a deep ditch with low amounts of reeds. Although these data are not seen as outliers, these specific habitat conditions are considered to be the driving factor for this trend rather than water soldier translocations, as will be shown shortly for depth. In the case of the abundant macrophytes, they could function as a nursey habitat due to shelter and food availability, hence the greater amount of smaller crayfish.

Besides the results of the CPUE in relation to *S. aloides*, other variables were found to influence the CPUE independently of *S. aloides* treatments, hence data of ditch 1 were included in these results. Sex and length were found to have a negative interaction on the CPUE when data of ditch 1 were included. Unlike sex, length was found to correlate with TL and NTL tracks, which makes interpretation of this trend difficult as this conflicts with the two water soldier treatments. However following the reasoning of the previous paragraph, the interaction between sex and length is still considered to be true for all ditches. As a result of this interaction sex was found to be a significant predicter as well. This means that on average a higher CPUE is expected with a higher F/M ratio, which could be due to intraspecific aggression behaviour of males (Oyama et al., 2020). The interaction adds that the increase in CPUE because of having a higher F/M sex ratio, is less pronounced for bigger individuals. In other words when the average length of caught crayfish is greater, the positive effect of more female individuals is being reduced. These results match the well recognised bias associated with this type of trap towards the higher catching probability of big and/or male crayfish due to territorial and aggressive behaviour (Hilber et al., 2020; Dorn et al., 2005; Price & Welch, 2009; Chucholl, 2011).

Because of the effects of lengths and sex on crayfish captures, one must recognize how only part of the IAC population was effectively sampled, which is supported by the found length and sex distributions. Parallel to the change in captures, a seasonal trend for sex where more male captures were recorded over the sampling period. Barbaresi et al. (2004) found that the sexes of *P. clarkii* show different movement behaviours as females tend to be more nomadic in their reproduction phase and move less with increasing temperatures. This corresponds with personal observations, since egg/larvae carrying females were only caught during the first sampling round and a rise in average temperature was true as the sampling period comprised April–June. The increase in male captures is likely an effect of the former stated sex-length dependant bias and a decrease in movement of female crayfish. When looking at the distributions of the thorax-telson length of caught crayfish, values center around 10 centimetres with a minimum of 4.5 cm and a maximum of 14.5 cm. This shows a bias for larger crayfish, again aligning with the previously mentioned bias.

At last depth of trap placement site was found to be the strongest predictor of the CPUE which increased with increasing depth, depth showed no relationship with *S. aloides* treatment. As part of the depth measurements, the overall ditch depth profile was measured. These showed little fluctuation which indicates that the depth of the placement sites can be viewed as an estimate for the bank slope. Consequently, a lower slope is associated with lower crayfish densities. This matches the hypothesised repellent capabilities nature friendly banks based on hindrance of burrowing activity due to a gentle slope (Lemmers et al., 2019; Cusell et al., 2020; De Jong et al., 2019).

Although one of the initial focuses of this study was to investigate the impact of water soldier translocations on crayfish densities, an hypothesized opposite impact was revealed as well. It is important to recognize that the proposed idea of density-dependant resistant capabilities of water soldier towards IAC is based on one high-density *S. aloides* track. Above that IAC are not the only biotic factor possibly affecting water soldier growth and settling, fish and birds have also been found to effect development and growth macrophytes (Van De Haterd & Ter Heerdt, 2007; Veen et al., 2013). Furthermore, only part of the total IAC population was effectively sampled due to catching biases for male and larger crayfish, thereby missing distributions juvenile IAC. At last no measurements were performed before the translocations, therefore possible preexisting differences in IAC densities and abiotic variables could not be accounted for and could have influenced the results, depth functions as an example for this. However, pre-existing differences are estimated to have low impact, as treatment sites were located closely together, often adjacent, and thus experienced similar environmental conditions. In future studies, a BACI approach is preferred for *in situ* studies whenever possible in order to identify possible pre-existing trends in the variables of interest within the research site. Furthermore, isolation experiments, for example by the use of mesocosms, could be conducted to better understand the interactions between IAC and water soldier. It would be preferable to conduct investigations at various densities for both IAC and *S. aloides*, while covering different stages of the *S. aloides* life cycle. In this way potential density thresholds can be identified for IAC impacts on *S. aloides* and its abiotic water quality improvement.

6 Conclusion

The results show how higher IAC densities and phosphate concentrations were found in *S. aloides* translocated tracks. Furthermore, newly planted *S. aloides* tracks (0/1 experienced summers) faded while successful growth and settlement was observed in the only longer existing *S. aloides* track (two experienced summers). This indicates that (I) abiotic factors are not limiting and (II) biotic factors are likely driving this mortality. The high crayfish densities in both *S. aloides* devoid and dense tracks suggests possible impacts of IAC on water soldier settlement and growth when water soldier populations are weak and densities are low. *S. aloides* may experience density-dependent resistant capabilities against the hypothesized impacts induced by IAC. More research, however, is needed to support this statement. Little associations between abiotic components were found, probably owing to the lack of *S. aloides* individuals due to the former named decline. The phosphate increase, could have been caused by increased nutrients from dead *S. aloides* material. Based on the results, water soldier employment is thought to be compatible with crayfish invested freshwater systems, however, introduction at high densities may be necessary to ensure the survival and growth of water soldier populations. Therefore, future research should focus on density dependent relationships between IAC and *S. aloides* and their effects on water quality.

References

Abeli, T., Rossi, G., Smolders, A. J. P., & Orsenigo, S. (2014). Nitrogen pollution negatively affects Stratiotes aloides in Central-Eastern Europe. Implications for translocation actions. *Aquatic Conservation*, *24*(5), 724–729. https://doi.org/10.1002/aqc.2497

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. https://doi.org/10.1163/1568540041643283

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*(1/3), 89–98. https://doi.org/10.1023/a:1013950129616

Arts, G. H. P., Brouwer, E., & Smits, N. A. C. (n.d.). Deel II - 381 Herstelstrategie H3150: Meren met krabbenscheer en fonteinkruiden. In *natura2000.nl*. Ministerie van Lanbouw, natuur en voedselkwaliteit. Retrieved June 16, 2024, from https://www.natura2000.nl/sites/default/files/PAS/Herstelstrategieen/Deel%20II-1/H3150V1_19-4-2021.pdf

Beintema, A. J. (1997). European Black Terns (Chlidonias niger) in Trouble: Examples of Dietary Problems. *Colonial Waterbirds*, *20*(3), 558-565. https://doi.org/10.2307/1521611

Beintema, A. J., Van Der Winden, J., Baarspul, T., De Krijger, J. P., Van Oers, K., & Keller, M. (2010). Black TernsChlidonias nigerand Their Dietary Problems in Dutch Wetlands. *Ardea*, *98*(3), 365–372. https://doi.org/10.5253/078.098.0310

Bélouard, N., Petit, E. J., & Paillisson, J. (2019). Variable effects of an invasive species on the reproduction and distribution of native species in pond networks. *Freshwater Biology*, *64*(3), 544–554. https://doi.org/10.1111/fwb.13241

Boerkamp, A, I. Roessink, J. van Giels en F.G.W.A. Ottburg (2011). Onderzoek naar de effectiviteit van de combinatie van twee beheermaatregelen voor rode Amerikaanse rivierkreeften. Wageningen, Alterra, Alterra-rapport 2244. https://edepot.wur.nl/200044

Bonacina, L., Fasano, F., Mezzanotte, V., & Fornaroli, R. (2022). Effects of water temperature on freshwater macroinvertebrates: a systematic review. Biological Reviews/Biological Reviews of the Cambridge Philosophical Society, 98(1), 191–221. https://doi.org/10.1111/brv.12903

Bonelli, M., Manenti, R., & Scaccini, D. (2017). Mountain protected areas as refuges for threatened freshwater species: the detrimental effect of the direct introduction of alien species. *Eco.mont*, *9*(2), 23–29. https://doi.org/10.1553/eco.mont-9-2s23

Brady, D. J., Meade, R., Reynolds, J. D., Vilcinskas, A., & Theissinger, K. (2024). The Crayfish Plague Pathogen Aphanomyces astaci in Ireland. *Microorganisms*, *12*(1), 102. https://doi.org/10.3390/microorganisms12010102

Brammer, E. S. (1979). Exclusion of phytoplankton in the proximity of dominant water-soldier (Stratiotes aloides). *Freshwater Biology*, *9*(3), 233–249. https://doi.org/10.1111/j.1365-2427.1979.tb01506.x

Capinha, C., Larson, E. R., Tricarico, E., Olden, J. D., & Gherardi, F. (2013). Effects of climate change, invasive species, and disease on the distribution of native European crayfishes. *Conservation Biology*, *27*(4), 731–740. https://doi.org/10.1111/cobi.12043

Carpenter, S. R., Stanley, E. H., & Zanden, M. J. V. (2011). State of the world's freshwater ecosystems: physical, chemical, and biological changes. *Annual Review of Environment and Resources*, *36*(1), 75–99. https://doi.org/10.1146/annurev-environ-021810-094524

Carreira, B. M., Dias, M. P., & Rebelo, R. (2013). How consumption and fragmentation of macrophytes by the invasive crayfish Procambarus clarkii shape the macrophyte communities of temporary ponds. *Hydrobiologia*, *721*(1), 89–98. https://doi.org/10.1007/s10750-013-1651-1

Carvalho, F., Pascoal, C., Cássio, F., & Sousa, R. (2016). Direct and indirect effects of an invasive omnivore crayfish on leaf litter decomposition. *Science of the Total Environment*, *541*, 714–720. https://doi.org/10.1016/j.scitotenv.2015.09.125

Chucholl, C. (2013). Feeding ecology and ecological impact of an alien 'warm-water' omnivore in cold lakes. *Limnologica*, *43*(4), 219–229. https://doi.org/10.1016/j.limno.2012.10.001

Chucholl, C., & Schrimpf, A. (2015). The decline of endangered stone crayfish (Austropotamobius torrentium) in southern Germany is related to the spread of invasive alien species and land-use change. *Aquatic Conservation*, *26*(1), 44–56. https://doi.org/10.1002/aqc.2568

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/nl0192-belasting-van-oppervlaktewater-met-vermestende-stoffen

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1141. (2016). *Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council*. http://data.europa.eu/eli/reg_impl/2016/1141/2022-08-02

Correia, A. M. (2002). Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (Procambarus clarkii) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). *Acta Oecologica*, *23*(6), 421–429. https://doi.org/10.1016/s1146-609x(02)01166-9

Correia, A. M. (2003). Food choice by the introduced crayfish Procambarus clarkii. *Annales Zoologici Fennici*, *40*(6), 517–528. http://www.jstor.org/stable/23736508

Correia, A. M., Bandeira, N., & Anastácio, P. M. (2005). Predator–prey interactions of Procambarus clarkii with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica*, *28*(3), 337–343. https://doi.org/10.1016/j.actao.2005.06.002

Correia, A. M., & Anastácio, P. M. (2007). Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecological Research*, *23*(4), 729–734. https://doi.org/10.1007/s11284-007-0433-5

Cronin, G. M., Horvath, T., Lindquist, N., Miller, M. A., Wahl, M., Hill, A. M., Bolser, R. C., Hay, M. E., & Lodge, D. M. (2002). Crayfish feeding preferences for freshwater macrophytes: the influence of plant structure and chemistry. *Journal of Crustacean Biology*, *22*(4), 708–718. https://doi.org/10.1163/20021975-99990285

Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, *97*(2), 153–166. https://doi.org/10.1034/j.1600-0706.2002.970201.x

Cruz, M. J., Pascoal, S., Tejedo, M., & Rebelo, R. (2006a). Predation by an Exotic Crayfish, Procambarus Clarkii, on Natterjack Toad, Bufo Calamita, Embryos: Its Role on the Exclusion of this Amphibian from its Breeding Ponds. *Copeia*, *2006*(2), 274–280. https://doi.org/10.1643/0045-8511(2006)6[274:PBAECP]2.0.CO;2

Cruz, M. J., Rebelo, R., & Crespo, E. G. (2006b). Effects of an introduced crayfish, Procambarus clarkii, on the distribution of south-western Iberian amphibians in their breeding habitats. *Ecography*, *29*(3), 329–338. https://doi.org/10.1111/j.2006.0906-7590.04333.x

Cruz, M. J., Segurado, P., Sousa, M., & Rebelo, R. (2008). Collapse of the amphibian community of the Paul do Boquilobo Natural Reserve (central Portugal) after the arrival of the exotic American crayfish Procambarus clarkii. *HERPETOLOGICAL JOURNAL*, *18*(4), 197–204. https://www.thebhs.org/publications/the-herpetological-journal/volume-18-number-4-october-2008/532-03-collapse-of-the-amphibian-community-of-the-paul-do-boquilobo-natural-reserve-central-portugal-after-the-arrival-of-the-exotic-american-crayfish-i-procambarus-clarkii-i

Cusell, C., Brederveld, B., Doef, L., Jnas, M., Lammers, D., Tangerman, M., ... & van de Craats, A. (2020). Rode Amerikaanse rivierkreeften in Nederland: relaties met milieu-en omgevingsfactoren. Stichting Toegepast Onderzoek Waterbeheer (STOWA). https://www.stowa.nl/sites/default/files/assets/PUBLICATIES/Publicaties%202020/STOWA%202020-08%20rivierkreeften%20highres.pdf

Dalpasso, A., Ficetola, G. F., Giachello, S., Lo Parrino, E., Manenti, R., Muraro, M., & Falaschi, M. (2022). Similar species, different fates: Abundance dynamics in spatially structured populations of common and threatened frogs. *Diversity and Distributions*, *28*(4), 770–781. https://doi.org/10.1111/ddi.13483

De Hoop, L., Van Der Loop, J., Van Kleef, H. H., De Hullu, E., & Leuven, R. S. E. W. (2016). Maatregelen voor het elimineren en beheersen van invasieve exoten van EU- belang in Nederland. In *Rivierkreeft.nl* (Be 00235). Radboud Universiteit. Retrieved June 16, 2024, from https://rivierkreeft.nl/wp-content/Informatiebronnen/Hoop%20et%20al_2016_Maatregelen%20exoten_Radboud%20Univ.pdf

De Vries, R. (2021). Rivierkreeften in Nederland. *Dierplagen Informatie*, *4*, 4–7. https://edepot.wur.nl/577954

Didde, R. (2022, January 2). 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

Dragičević, P., Bielen, A., Petrić, I., & Hudina, S. (2020). Microbial pathogens of freshwater crayfish: A critical review and systematization of the existing data with directions for future research. *Journal of Fish Diseases*, *44*(3), 221–247. https://doi.org/10.1111/jfd.13314

Duran, M. (2006). Monitoring Water Quality Using Benthic Macroinvertebrates and Physicochemical Parameters of Behzat Stream in Turkey. *Polish Journal of Environmental Studies*, *15*(5), 709-717. https://www.pjoes.com/Monitoring-Water-Quality-Using-Benthic-Macroinvertebrates-and-Physicochemical-Parameters,87923,0,2.html

Ecological status of surface waters in Europe. (2021, November 18). 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. (2008). The ecobiomorph of water soldier Stratiotes aloides L. (Hydrocharitaceae) in the west Siberian part of its range. *Inland Water Biology*, *1*(3), 225–230. https://doi.org/10.1134/s199508290803005x

Efremov, A. N., & Sviridenko, B. F. (2012). Seasonal and spatial dynamics of Stratiotes aloides (Hydrocharitaceae) plants. *Botanica Serbica, 36*(1), 59–62. https://botanicaserbica.bio.bg.ac.rs/arhiva/pdf/2012_36_1_555_full.pdf

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. https://doi.org/10.1016/j.flora.2019.03.009

Ercoli, F., Ruokonen, T. J., Koistinen, S., Jones, R. I., & Hämäläinen, H. (2015). The introduced signal crayfish and native noble crayfish have different effects on sublittoral macroinvertebrate assemblages in boreal lakes. *Freshwater Biology*, *60*(8), 1688–1698. https://doi.org/10.1111/fwb.12601

Erixon, G. (1979). Population ecology of a Stratiotes aloides l. stand in a riverside lagoon in N. Sweden. *Hydrobiologia*, *67*(3), 215–221. https://doi.org/10.1007/bf00023178

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

Falaschi, M., Giachello, S., Lo Parrino, E., Muraro, M., Manenti, R., & Ficetola, G. F. (2021). Long-term drivers of persistence and colonization dynamics in spatially structured amphibian populations. *Conservation Biology*, *35*(5), 1530–1539. https://doi.org/10.1111/cobi.13686

Falaschi, M., Muraro, M., Gibertini, C., Monache, D. D., Lo Parrino, E., Faraci, F., Belluardo, F., Di Nicola, M. R., Manenti, R., & Ficetola, G. F. (2022). Explaining declines of newt abundance in northern Italy. *Freshwater Biology*, *67*(7), 1174–1187. https://doi.org/10.1111/fwb.13909

Ficetola, G. F., Siesa, M. E., De Bernardi, F., & Padoa-Schioppa, E. (2012). Complex impact of an invasive crayfish on freshwater food webs. *Biodiversity and Conservation*, *21*(10), 2641–2651. https://doi.org/10.1007/s10531-012-0323-1

Ficetola, G. F., Siesa, M. E., Manenti, R., Bottoni, L., De Bernardi, F., & Padoa-Schioppa, E. (2011). Early assessment of the impact of alien species: differential consequences of an invasive crayfish on adult and larval amphibians. *Diversity and Distributions*, *17*(6), 1141–1151. https://doi.org/10.1111/j.1472-4642.2011.00797.x

Galib, S. M., Sun, J., Gröcke, D. R., & Lucas, M. C. (2022). Ecosystem effects of invasive crayfish increase with crayfish density. *Freshwater Biology*, *67*(6), 1005–1019. https://doi.org/10.1111/fwb.13897

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2015). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151–163. https://doi.org/10.1111/gcb.13004

Gao, J., Yuan, H., Zhang, H., Liao, M., Wang, H., Kang, Y., Liu, Z., & Jeppesen, E. (2023). Effects of the invasive red swamp crayfish (Procambarus clarkii) on eelgrass-dominated clearwater lakes: the role of bioturbation. *Hydrobiologia*, *851*(8), 1993–2005. https://doi.org/10.1007/s10750-023-05437-7

Geiger, W., Alcorlo, P., Baltanás, A., & Montes, C. (2005). Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions*, *7*(1), 49–73. https://doi.org/10.1007/s10530-004-9635-8

Geiger, W., Otero, M., & Alcorlo, P. (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. https://doi.org/10.1163/1568540041643283

Gherardi, F., Renai, B., & Corti, C. (2001). CRAYFISH PREDATION ON TADPOLES : a COMPARISON BETWEEN a NATIVE (AUSTROPOTAMOBIUS PALLIPES) AND AN ALIEN SPECIES (PROCAMBARUS CLARKII). *Bulletin FrançAis De La PêChe Et De La Pisciculture*, *361*, 659–668. https://doi.org/10.1051/kmae:2001011

Gherardi, F., Tricarico, E., & Ilhéu, M. (2002). Movement patterns of an invasive crayfish,Procambarus clarkii, in a temporary stream of southern Portugal. Ethology Ecology & Evolution, 14(3), 183–197. https://doi.org/10.1080/08927014.2002.9522739

Gherardi, F. (2006a). Crayfish invading Europe: the case study ofProcambarus clarkii. *Marine and Freshwater Behaviour & Physiology/Marine and Freshwater Behaviour and Physiology*, *39*(3), 175–191. https://doi.org/10.1080/10236240600869702

Gherardi, F., & Lazzara, L. (2006b). Effects of the density of an invasive crayfish (Procambarus clarkii) on pelagic and surface microalgae in a Mediterranean wetland. *Archiv Für Hydrobiologie*, *165*(3), 401–414. https://doi.org/10.1127/0003-9136/2006/0165-0401

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. https://doi.org/10.1111/j.1365-2427.2007.01760.x

Gherardi, F. (2010). Invasive crayfish and freshwater fishes of the world. *Revista Científica Y Técnica - Oficina Internacional De Epizootias/Scientific and Technical Review - International Office of Epizootics/Revue Scientifique Et Technique - Office International Des Épizooties*, *29*(2), 241–254. https://doi.org/10.20506/rst.29.2.1973

Gherardi, F., Aquiloni, L., Diéguez-Uribeondo, J., & Tricarico, E. (2011). Managing invasive crayfish: is there a hope? *Aquatic Sciences*, *73*(2), 185–200. https://doi.org/10.1007/s00027-011-0181-z

Grillas, Célia & Ugnon-Coussioz, Eliot & Priol, Pauline & Renet, Julien. (2018). Low occurrence of the great crested newt Triturus cristatus at the limits of its range: An alarming preliminary study. Herpetological Bulletin. 145. 14-18. https://www.thebhs.org/publications/the-herpetological-bulletin/issue-number-145-autumn-2018/1859-03-low-occurrence-of-the-great-crested-newt-i-triturus-cristatus-i-at-the-limits-of-its-range-an-alarming-preliminary-study

Gulati, R. D., & Van Donk, E. (2002). Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review*. *Hydrobiologia*, *478*(1/3), 73–106. https://doi.org/10.1023/a:1021092427559

Gutiérrez-Yurrita, P. J., Sancho, G., Bravo, M. Á., Baltanás, Á., & Montes, C. (1998). Diet of the Red Swamp Crayfish Procambarus Clarkii in Natural Ecosystems of the Doñana National Park Temporary Fresh-water Marsh (Spain). *Journal of Crustacean Biology*, *18*(1), 120–127. https://doi.org/10.1163/193724098x00124

Harpenslager, S. F., Smolders, A. J. P., Kieskamp, A. a. M., Roelofs, J. G. M., & Lamers, L. P. M. (2015). To Float or Not to Float: How Interactions between Light and Dissolved Inorganic Carbon Species Determine the Buoyancy of Stratiotes aloides. *PloS One*, *10*(4), e0124026. https://doi.org/10.1371/journal.pone.0124026

Harpenslager, S. F., Lamers, L. P., Van Der Heide, T., Roelofs, J. G., & Smolders, A. J. (2016). Harnessing facilitation: Why successful re-introduction of Stratiotes aloides requires high densities under high nitrogen loading. *Biological Conservation*, *195*, 17–23. https://doi.org/10.1016/j.biocon.2015.12.031

Herrmann, A., Grabow, K., & Martens, A. (2022). The invasive crayfish Faxonius immunis causes the collapse of macroinvertebrate communities in Central European ponds. *Aquatic Ecology*, *56*(3), 741–750. https://doi.org/10.1007/s10452-021-09935-5

Hilber, T., Oehm, J., Effenberger, M., & Maier, G. (2020). Evaluating the efficiency of three methods for monitoring of native crayfish in Germany. Limnologica, 85, 125821. https://doi.org/10.1016/j.limno.2020.125821

Hill, M. J., Sayer, C. D., & Wood, P. J. (2016). When is the best time to sample aquatic macroinvertebrates in ponds for biodiversity assessment? Environmental Monitoring and Assessment, 188(3). https://doi.org/10.1007/s10661-016-5178-6

Holdich, D., Reynolds, J., Souty-Grosset, C., & Sibley, P. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, *394–395*, 11. https://doi.org/10.1051/kmae/2009025

Ilhéu, M., & Bernardo, J.M. (1993). Aspects of trophic ecology of red swamp crayfish (Procambarus clarkii Girard) in Alentejo, south of Portugal. *Limnética/LimnéTica*, *6*(1), 417–423. https://doi.org/10.23818/actas.06.52

Imhoff, E. M., Mortimer, R. J. G., Christmas, M., & Dunn, A. M. (2012). Horizontal transmission of Thelohania contejeani in the endangered white-clawed (Austropotamobius pallipes) and the invasive signal crayfish (Pacifastacus leniusculus). *Parasitology*, *139*(11), 1471–1477. https://doi.org/10.1017/s0031182012000777

IPBES (2023). Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Roy, H. E., Pauchard, A., Stoett, P., Renard Truong, T., Bacher, S., Galil, B. S., Hulme, P. E., Ikeda, T., Sankaran, K. V., McGeoch, M. A., Meyerson, L. A., Nuñez, M. A., Ordonez, A., Rahlao, S. J., Schwindt, E., Seebens, H., Sheppard, A. W., and Vandvik, V. (eds.). IPBES secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.7430692

Jackson, M. C., Jones, T., Milligan, M., Sheath, D., Taylor, J., Ellis, A., England, J., & Grey, J. (2014). Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology*, *59*(6), 1123–1135. https://doi.org/10.1111/fwb.12333

Jong, de, B., L. Bovend'aerde, J. Mandemakers, R. van de Haterd, J. Kampen & C. Cusell (2019). Bureauonderzoek naar het effect van uitheemse rivierkreeften, andere grazers en biobouwers op de ontwikkeling van jonge verlanding met een doorkijk naar potentiële maatregelen. Tussenrapportage BN228-LZ. VBNE, Driebergen. https://www.natuurkennis.nl/Uploaded_files/Publicaties/bureaustudie-naar-effect-uitheemse-rivierkreeften-op-jonge-verlanding.f86158.pdf

Jussila, J., Vrezec, A., Makkonen, J., Kortet, R., & Kokko, H. (2015). 8. Invasive Crayfish and Their Invasive Diseases in Europe with the Focus on the Virulence Evolution of the Crayfish Plague. In *Biological Invasions in Changing Ecosystems* (pp. 183–211). De Gruyter Open Poland. https://doi.org/10.1515/9783110438666-013

Kłosowski, S., Jabłońska, E., & Szańkowski, M. (2011). Aquatic vegetation as an indicator of littoral habitats and various stages of lake aging in north-eastern Poland. *Annales De Limnologie*, *47*(3), 281–295. https://doi.org/10.1051/limn/2011008

Koese, B. & Evers, N. (2011). A national inventory of invasive freshwater crayfish in the Netherlands in 2010. Reportnr. 2011-03, EIS-Nederland, Leiden. http://dx.doi.org/10.13140/RG.2.2.16971.00804

Lemmers, P., B.H.J.M. Crombaghs & R.S.E.W. Leuven (2018) Invasieve exotische kreeften in het beheergebied van waterschap Rivierenland. Verkenning van effecten, risico's en mogelijke aanpak. Natuurbalans - Limes Divergens BV, Radboud Universiteit & Nederlands Expertise Centrum Exoten, Nijmegen. http://dx.doi.org/10.13140/RG.2.2.14508.92809

Lemmers, P., Verhees, J., van der Kroon, R., & Leuven, R. S. E. W. (2019). Minder uitheemse rivierkreeften in natuurvriendelijke oevers. H2O Online, 23, 1-5. https://www.h2owaternetwerk.nl/images/2019/December/H2O-Online_191223_Rivierkreeften.pdf

Lewis, S. D. (1997). Life History, Population Dynamics, and Management of Signal crayfishin Lake Billy Chinook, Oregon. [Master Thesis, Oregon State University]. https://ir.library.oregonstate.edu/concern/graduate_thesis_or_dissertations/9s161822p

Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C., Arcella, T., Baldridge, A. K., Barnes, M. A., Chadderton, W. L., Feder, J. L., Gantz, C. A., Howard, G. W., Jerde, C. L., Peters, B. W., Peters, J. A., Sargent, L. W., Turner, C. R., Wittmann, M. E., & Zeng, Y. (2012). Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on ecosystem Services. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 449–472. https://doi.org/10.1146/annurev-ecolsys-111511-103919

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*(1/3), 71–84. https://doi.org/10.1023/a:1017520800568

Manenti, R., Falaschi, M., Monache, D. D., Marta, S., & Ficetola, G. F. (2019). Network-scale effects of invasive species on spatially-structured amphibian populations. *Ecography*, *43*(1), 119–127. https://doi.org/10.1111/ecog.04571

Martín-Torrijos, L., Gelder, S. R., & Diéguez-Uribeondo, J. (2018). A new kid in town: First case of an alien worm, Xironogiton victoriensis (Annelida: Clitellata) on a native European freshwater crayfish. *Aquaculture*, *496*, 39–42. https://doi.org/10.1016/j.aquaculture.2018.06.086

Martín-Torrijos, L., Kokko, H., Makkonen, J., Jussila, J., & Diéguez-Uribeondo, J. (2019). Mapping 15 years of crayfish plague in the Iberian Peninsula: The impact of two invasive species on the endangered native crayfish. *PloS One*, *14*(8), e0219223. https://doi.org/10.1371/journal.pone.0219223

Matsuzaki, S. S., Usio, N., Takamura, N., & Washitani, I. (2008). Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia*, *158*(4), 673–686. https://doi.org/10.1007/s00442-008-1180-1

Ministerie van Landbouw, Natuur en Voedselkwaliteit. (n.d.). *Krabbenscheer (Stratiotes aloides)*. Beschermde Natuur in Nederland: Soorten En Gebieden in Wetgeving En Beleid. Retrieved March 21, 2024, from https://minlnv.nederlandsesoorten.nl/content/krabbenscheer-stratiotes-aloides

Mohammed, E., Amen, R., Abdelwahab, H. M., & Winkelmann, C. (2023). Potential impacts of invasive crayfish on native benthic fish: shelter use and agonistic behaviour. *NeoBiota*, *83*, 131–153. https://doi.org/10.3897/neobiota.83.102975

Mulderij, G., Mooij, W., Smolders, A., & Van Donk, E. (2005). Allelopathic inhibition of phytoplankton by exudates from Stratiotes aloides. *Aquatic Botany*, *82*(4), 284–296. https://doi.org/10.1016/j.aquabot.2005.04.001

Mulderij, G., Smolders, A. J. P., & Van Donk, E. (2006). Allelopathic effect of the aquatic macrophyte, Stratiotes aloides, on natural phytoplankton. *Freshwater Biology*, *51*(3), 554–561. https://doi.org/10.1111/j.1365-2427.2006.01510.x

Mulderij, G., Mau, B., Van Donk, E., & Gross, E. M. (2007). Allelopathic activity of Stratiotes aloides on phytoplankton—towards identification of allelopathic substances. *Hydrobiologia*, *584*(1), 89–100. https://doi.org/10.1007/s10750-007-0602-0

Mulderij, G., Mau, B., De Senerpont Domis, L. N., Smolders, A. J. P., & Van Donk, E. (2008). Interaction between the macrophyte Stratiotes aloides and filamentous algae: does it indicate allelopathy? *Aquatic Ecology*, *43*(2), 305–312. https://doi.org/10.1007/s10452-008-9194-7

Nielsen, L. T., & Borum, J. (2008). Why the free floating macrophyte Stratiotes aloides mainly grows in highly CO2-supersaturated waters. *Aquatic Botany*, *89*(4), 379–384. https://doi.org/10.1016/j.aquabot.2008.04.008

Nunes, A. L., Cruz, M. J., Tejedo, M., Laurila, A., & Rebelo, R. (2010). Nonlethal injury caused by an invasive alien predator and its consequences for an anuran tadpole. *Basic and Applied Ecology*, *11*(7), 645–654. https://doi.org/10.1016/j.baae.2010.09.003

Nyström, P., Brönmark, C., & Granéli, W. (1996). Patterns in benthic food webs: a role for omnivorous crayfish? *Freshwater Biology*, *36*(3), 631–646. https://doi.org/10.1046/j.1365-2427.1996.d01-528.x

Nyström, P., Svensson, O., Lardner, B., Brönmark, C., & Granéli, W. (2001). THE INFLUENCE OF MULTIPLE INTRODUCED PREDATORS ON a LITTORAL POND COMMUNITY. *Ecology*, *82*(4), 1023–1039. https://doi.org/10.1890/0012-9658(2001)082[1023:TIOMIP]2.0.CO;2

Obolewski, K. (2005). Epiphytic macrofauna on water soldiers (Stratiotes aloides L.) in Słupia river oxbows. *Oceanol. Hydrobiol. Stud*, *34*(2), 37-54. https://www.researchgate.net/publication/287499781_Epiphytic_macrofauna_on_water_soldiers_Stratiotes_aloides_L_in_Slupia_River_oxbows

Obolewski K., Glińska-Lewczuk K., Kobus S. (2009). An attempt at evaluating the influence of water quality on the qualitative and quantitative structure of epiphytic fauna dwelling on Stratiotes aloides L., a case study on an oxbow lake of the Łyna river. *J. Elem. 14(1)*, 119 – 134. http://dx.doi.org/10.5601/jelem.2009.14.1.13

Obolewski, K., & Strzelczak, A. (2009). Epiphytic fauna inhabiting Stratiotes aloides in a new lake of the Słowiński National Park (Smołdzińskie lake, Poland). *Ecohydrology & Hydrobiology*, *9*(2–4), 257–267. https://doi.org/10.2478/v10104-010-0003-2

Oficialdegui, F. J., Clavero, M., Sánchez, M. I., Green, A. J., Boyero, L., Michot, T. C., Klose, K., Kawai, T., & Lejeusne, C. (2019a). Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (Procambarus clarkii). *Freshwater Biology*, *64*(8), 1382–1400. https://doi.org/10.1111/fwb.13312

Oficialdegui, F. J., Sánchez, M. I., Monsalve-Carcaño, C., Boyero, L., & Bosch, J. (2019b). The invasive red swamp crayfish (Procambarus clarkii) increases infection of the amphibian chytrid fungus (Batrachochytrium dendrobatidis). *Biological Invasions*, *21*(11), 3221–3231. https://doi.org/10.1007/s10530-019-02041-6

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. https://doi.org/10.1007/s11160-020-09594-z

Oyama, T., Momohara, Y., Yano, H., Kamio, M., Fujiyama, N., & Nagayama, T. (2020). Sex recognition and agonistic strategies of male and female crayfish. *Behaviour, 157(6)*, 575–596. https://doi.org/10.1163/1568539x-bja10014

Rahel, F. J. (2002). Homogenization of freshwater faunas. *Annual Review of Ecology and Systematics*, *33*(1), 291–315. https://doi.org/10.1146/annurev.ecolsys.33.010802.150429

Ramalho, R. O. & Anastácio, P. M. (2010). Crayfish learning abilities: how does familiarization period affect the capture rate of a new prey item? *Ecological Research*, *26*(1), 53–58. https://doi.org/10.1007/s11284-010-0754-7

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. https://doi.org/10.1023/b:aeco.0000021005.22624.16

Rebelo, R., & Cruz, M. J. (2005). Vulnerability of Southwest Iberian amphibians to an introduced crayfish, Procambarus clarkii. *Amphibia-Reptilia/Amphibia-reptilia*, *26*(3), 293–303. https://doi.org/10.1163/156853805774408577

Regulation 1143/2014. (2019). *Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species*. http://data.europa.eu/eli/reg/2014/1143/2019-12-14

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2018). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews/Biological Reviews of the Cambridge Philosophical Society*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Renman, G. (1989). Life histories of two clonal populations of Stratiotes aloides L. *Hydrobiologia*, *185*(3), 211–222. https://doi.org/10.1007/bf00036609

Reynolds, J. (2011). A review of ecological interactions between crayfish and fish, indigenous and introduced. *Knowledge and Management of Aquatic Ecosystems*, *401*, 10. https://doi.org/10.1051/kmae/2011024

Reynolds J, Souty-Grosset C and Richardson A (2013). Ecological Roles of Crayfish in Freshwater and Terrestrial Habitats. *Freshwater Crayfish 19(2)*. 197-218. https://hdl.handle.net/102.100.100/577501

Richardson, K., Steffen, W., Lucht, W., Bendtsen, J., Cornell, S. E., Donges, J. F., Drüke, M., Fetzer, I., Bala, G., Von Bloh, W., Feulner, G., Fiedler, S., Gerten, D., Gleeson, T., Hofmann, M., Huiskamp, W., Kummu, M., Mohan, C., Nogués-Bravo, D., . . . Rockström, J. (2023). Earth beyond six of nine planetary boundaries. *Science Advances*, *9*(37). https://doi.org/10.1126/sciadv.adh2458

Rijksoverheid. (2022). Ontwikkeling Beheersingsaanpak Uitheemse Rivierkreeft. https://open.overheid.nl/documenten/458ddf7b-9579-4937-abba-f17f89d0df29/file

Rodríguez, C. F., Bécares, E., & Fernández-Aláez, M. (2003). Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (Procambarus clarkii). *Hydrobiologia*, *506–509*(1–3), 421–426. https://doi.org/10.1023/b:hydr.0000008626.07042.87

Rodríguez, C., BÉcares, E., Fernández-Aláez, M., & Fernández-Aláez, C. (2005). Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions*, *7*(1), 75–85. https://doi.org/10.1007/s10530-004-9636-7

Rodríguez-Pérez, H., Hilaire, S., & Mesléard, F. (2015). Temporary pond ecosystem functioning shifts mediated by the exotic red swamp crayfish (Procambarus clarkii): a mesocosm study. *Hydrobiologia*, *767*(1), 333–345. https://doi.org/10.1007/s10750-015-2523-7

Roessink, I., Gylstra, R., Heuts, P., Specken, B., & Ottburg, F. (2017). Impact of invasive crayfish on water quality and aquatic macrophytes in the Netherlands. *Aquatic Invasions*, *12*(3), 397–404. https://doi.org/10.3391/ai.2017.12.3.12

Roessink, I., S. Hudina & F.G.W.A. Ottburg, 2009. Literatuurstudie naar de biologie, impact en mogelijke bestrijding van twee invasieve soorten: de rode Amerikaanse rivierkreeft (Procambarus clarkii) en de geknobbelde Amerikaanse rivierkreeft (Orconectes virilis). Wageningen, Alterra, Alterra-rapport 1923. 62 blz. 54 fig.; 10 tab.; 132 ref. https://edepot.wur.nl/51003

Ruokonen, T. J., Karjalainen, J., & Hämäläinen, H. (2013). Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific. *Freshwater Biology*, *59*(1), 12–25. https://doi.org/10.1111/fwb.12242

Sala, O. E., Chapin, F. S., Iii, N., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M., & Wall, D. H. (2000). Global Biodiversity Scenarios for the year 2100. *Science*, *287*(5459), 1770–1774. https://doi.org/10.1126/science.287.5459.1770

Sarneel, J. M., Soons, M. B., Geurts, J. J., Beltman, B., & Verhoeven, J. T. (2011). Multiple effects of land-use changes impede the colonization of open water in fen ponds. *Journal of Vegetation Science*, *22*(3), 551–563. https://doi.org/10.1111/j.1654-1103.2011.01281.x

Scheffer, M., Hosper, S., Meijer, M., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*, *8*(8), 275–279. https://doi.org/10.1016/0169-5347(93)90254-m

Schipper, P., Van Loon, A., Rozemeijer, J., Groenendijk, P., & Lukacs, S. (2022). Nutriënten en het ecologisch functioneren van oppervlaktewateren. STOWA. https://www.stowa.nl/deltafacts/waterkwaliteit/kennisimpuls-waterkwaliteit/nutrienten-en-het-ecologisch-functioneren-van

Schrimpf, A., Maiwald, T., Vrålstad, T., Schulz, H. K., ŚMIETaNA, P., & Schulz, R. (2013). Absence of the crayfish plague pathogen (Aphanomyces astaci) facilitates coexistence of European and American crayfish in central Europe. *Freshwater Biology*, *58*(6), 1116–1125. https://doi.org/10.1111/fwb.12112

Setzer, M., Norrgård, J. R., & Jonsson, T. (2011). An invasive crayfish affects egg survival and the potential recovery of an endangered population of Arctic charr. *Freshwater Biology*, *56*(12), 2543–2553. https://doi.org/10.1111/j.1365-2427.2011.02679.x

Smith, V. H., Joye, S. B., & Howarth, R. W. (2006). Eutrophication of freshwater and marine ecosystems. *Limnology and Oceanography*, *51*(1part2), 351–355. https://doi.org/10.4319/lo.2006.51.1_part_2.0351

Smolders, A., Hartog, C. D., & Roelofs, J. (1995). Observations on fruiting and seed-set of Stratiotes aloides L. in the Netherlands. *Aquatic Botany*, *51*(3–4), 259–268. https://doi.org/10.1016/0304-3770(95)00463-a

Soes, D. M., & Koese, B. (2010). *Invasive crayfish in the Netherlands: a preliminary risk analysis.* EIS-Nederland, Leiden. https://www.researchgate.net/publication/254892939_Invasive_crayfish_in_the_Netherlands_a_preliminary_risk_analysis

Souty-Grosset, C., Anastácio, P. M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., & Tricarico, E. (2016). The red swamp crayfish Procambarus clarkii in Europe: Impacts on aquatic ecosystems and human well-being. *Limnologica*, *58*, 78–93. https://doi.org/10.1016/j.limno.2016.03.003

Strzałek, M., Kufel, L., & Wysokińska, U. (2019). How does Stratiotes aloides L. affect the growth and turion formation of Spirodela polyrhiza (L.) Schleiden? *Aquatic Botany*, *154*, 45–52. https://doi.org/10.1016/j.aquabot.2019.01.001

Sugier, P., Lorens, B., Chmiel, S., & Turczyński, M. (2010). The influence of Ceratophyllum demersum L. and Stratiotes aloides L. on richness and diversity of aquatic vegetation in the lakes of mid-eastern Poland. *Hydrobiologia*, *656*(1), 43–53. https://doi.org/10.1007/s10750-010-0433-2

Suutari, E., Suhonen, J., Rantala, M. J., & Salmela, J. (2004). Intraguild predation and interference competition on the endangered dragonfly Aeshna viridis. *Oecologia*, *140*(1), 135–139. https://doi.org/10.1007/s00442-004-1559-6

Suutari, E., Salmela, J., Paasivirta, L., Rantala, M. J., Tynkkynen, K., Luojumäki, M., & Suhonen, J. (2008). Macroarthropod species richness and conservation priorities in Stratiotes aloides (L.) lakes. *Journal of Insect Conservation*, *13*(4), 413–419. https://doi.org/10.1007/s10841-008-9188-8

Tarkowska-Kukuryk, M. (2006b). Water soldier Stratiotes aloides L. (Hydrocharitaceae) as a substratum for macroinvertebrates in a shallow eutrophic lake. Polish Journal of Ecology, 54(3), 441–451. https://www.infona.pl/resource/bwmeta1.element.baztech-article-BGPK-1469-5873

Tilmans, M., Mrugała, A., Svoboda, J., Engelsma, M., Petie, M., Soes, D., Nutbeam-Tuffs, S., Oidtmann, B., Roessink, I., & Petrusek, A. (2014). Survey of the crayfish plague pathogen presence in the Netherlands reveals a new Aphanomyces astaci carrier. *Journal of Invertebrate Pathology*, *120*, 74–79. https://doi.org/10.1016/j.jip.2014.06.002

Toma, C. (2012). Reproduction of Stratiotes aloides in Ilmoilanselka Lake (Finland). *Baltic Coastal Zone. Journal of Ecology and Protection of the Coastline*, *16, 103-111.* https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-d5e3ce1f-1311-4cfe-bf71-0c8f5a9d6e78

Toma, C. (2019). Phenotypic plasticity of Stratiotes aloides L. (Hydrocharitaceae). *Botanica Pacifica*, *8*(2). https://doi.org/10.17581/bp.2019.08213

Toporowska, M., Pawlik-Skowrońska, B., & Wojtal, A. (2008). Epiphytic algae on Stratiotes aloides L., Potamogeton lucens L., Ceratophyllum demersum L. and Chara spp. in a macrophyte-dominated lake. *Oceanological and Hydrobiological Studies*, *37*(2), 51–63. https://doi.org/10.2478/v10009-007-0048-8

Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*(4), 1367–1382. https://doi.org/10.1899/12-203.1

Van De Haterd, R. J. W., & Ter Heerdt, G. N. J. (2007). Potential for the development of submerged macrophytes in eutrophicated shallow peaty lakes after restoration measures. In *Springer eBooks* (pp. 277–290). https://doi.org/10.1007/978-1-4020-6399-2_25

Van der Meulen, M., Vos, J., Verweij, W., & Kraak, M. (2009). Effecten van exotische rivierkreeften op de KRW-maatlatscores. H2O,42(14/15), 41-43. https://issuu.com/h2o-magazine/docs/20090710085647

Van Der Wal, J. E. M., Dorenbosch, M., Immers, A. K., Forteza, C. V., Geurts, J. J. M., Peeters, E. T. H. M., Koese, B., & Bakker, E. S. (2013). Invasive crayfish threaten the development of submerged macrophytes in lake restoration. *PloS One*, *8*(10), e78579. https://doi.org/10.1371/journal.pone.0078579

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. https://stichting-bargerveen.nl/wp-content/uploads/2022/12/Rapportage-Ontwikkeling-systeemaanpak-rivierkreeften_def.pdf

Van Kuijk, T., Biesmeijer, J. C., Van Der Hoorn, B. B., & Verdonschot, P. F. M. (2021). Functional traits explain crayfish invasive success in the Netherlands. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-82302-4

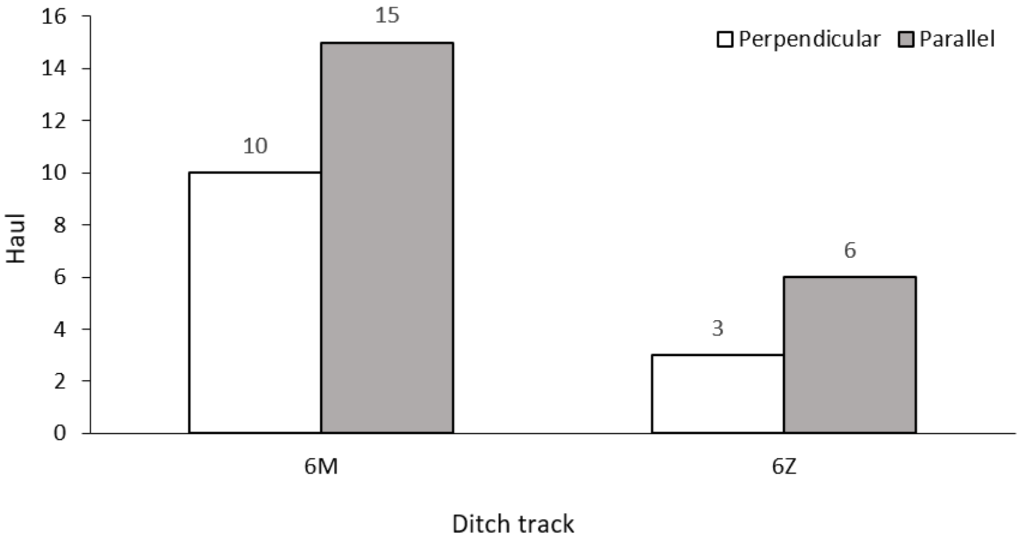
Veen, G. F., Sarneel, J. M., Ravensbergen, L., Huig, N., Van Paassen, J., Rip, W., & Bakker, E. S. (2013). Aquatic grazers reduce the establishment and growth of riparian plants along an environmental gradient. *Freshwater Biology*, *58*(9), 1794–1803. https://doi.org/10.1111/fwb.12168

Wiśniewski, K., Szarmach, D., & Poznańska-Kakareko, M. (2020). The role of abiotic and biotic factors in interspecific competition of Polish crayfish – comprehensive literature review. *Oceanological and Hydrobiological Studies*, *49*(4), 428–441. https://doi.org/10.1515/ohs-2020-0038

Appendix A Pilot study

In advance of the sampling period (27-03-2024), a pilot study was performed to go through the planned workflow and identify the placement of the traps. For this purpose, one ditch (6M – *S. aloides* present, 6Z *S. aloides* absent) was sampled in the manner as proposed at the time. This includes the placement of twelve traps in each track, landmarks were already placed when the pilot was performed.

The results of the pilot study are shown in the figure below. Parallel placement of the traps, which means that the openings of the funnel traps are parallel to the ditch bank, resulted in higher hauls. Based on the strong differences, it was determined to employ the parallel placement of the traps, although this was only tested for once.



Appendix B Materials

Below follows a list of all the materials with specifications used for this study:

- 72 baited funnel traps – ‘’Finnish model’’
- Bait – (14 mm) halibut pellets
- Rope
- Bamboo sticks and duct-tape (landmarks)
- Proper measurement device for crayfish length
- Container for collecting crayfish from traps
- Rod with reel rigged with 30 grams for depth assessment
    - Alternatively, in this specific research site, a stick with 60 centimeters of line at the end with distance indications can be used as this already exceeds the maximum found depth.
- Floating stick with distance measurements for ditch depth profile assessment
- KYORITSU PACKTEST Nitrate (WAK-NO3)
- HANNA INSTUMENTS Ammonia LR Checker, 0.00-3.00 ppm (HI700)
    - With associated reagents
- HANNA INSTUMENTS Phosphate low range Checker colorimeter: Range 0.00 to 2.50 ppm (mg/l) (HI713)
    - With associated reagents
- Sechi tube
- 50 ml tubes

Appendix C Statistic models summaries

Model haul excluding ditch 1

Used model:

```
glmmTMB(Haul ~ Sampleround + Watersoldier +`Sexratio(F/M)`+ Depth + Length +
                    Sampleround:Watersoldier + Sampleround:`Sexratio(F/M)` + Length:Watersoldier + (1|Ditch),
                        family = nbinom1(link = "log"),
                        data = subset(ALL_VARIABLES, Ditch %in% 2:10))
```

Output model:

```
     AIC      BIC   logLik deviance df.resid 
   383.7    411.6   -177.9    355.7       40 

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev. 
 Ditch  (Intercept) 5.927e-11 7.699e-06
Number of obs: 54, groups:  Ditch, 9

Dispersion parameter for nbinom1 family (): 0.656 

Conditional model:
                                     Estimate Std. Error z value Pr(>|z|)    
(Intercept)                          1.061671   1.162774   0.913   0.3612    
Sampleround2                         0.263137   0.241268   1.091   0.2754    
Sampleround3                        -0.044465   0.279604  -0.159   0.8736    
WatersoldierTranslocated             3.544315   1.415584   2.504   0.0123 *  
`Sexratio(F/M)`                     -0.155528   0.136011  -1.143   0.2528    
Depth                                0.026306   0.006224   4.226 2.38e-05 ***
Length                               0.153828   0.127237   1.209   0.2267    
Sampleround2:WatersoldierTranslocated -0.159728   0.173766  -0.919   0.3580    
Sampleround3:WatersoldierTranslocated -0.111694   0.196962  -0.567   0.5707    
Sampleround2:`Sexratio(F/M)`        -0.004364   0.206675  -0.021   0.9832    
Sampleround3:`Sexratio(F/M)`         0.438874   0.220833   1.987   0.0469 *  
WatersoldierTranslocated:Length     -0.332747   0.149787  -2.221   0.0263 *  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Output post hoc (emmeans):

```
$emmeans
 Watersoldier     Sampleround emmean     SE  df asymp.LCL asymp.UCL
 Non-translocated 1             3.01 0.1097 Inf      2.80      3.23
 Translocated     1             3.36 0.0953 Inf      3.18      3.55
 Non-translocated 2             3.27 0.0892 Inf      3.10      3.45
 Translocated     2             3.46 0.0848 Inf      3.30      3.63
 Non-translocated 3             3.36 0.1058 Inf      3.15      3.57
 Translocated     3             3.60 0.0830 Inf      3.44      3.76

Results are given on the log (not the response) scale. 
Confidence level used: 0.95 

$contrasts
 contrast                                                         estimate    SE  df z.ratio p.value
 (Non-translocated Sampleround1) - Translocated Sampleround1      -0.35118 0.133 Inf  -2.650  0.0857
 (Non-translocated Sampleround1) - (Non-translocated Sampleround2) -0.25924 0.133 Inf  -1.947  0.3735
 (Non-translocated Sampleround1) - Translocated Sampleround2      -0.45070 0.138 Inf  -3.263  0.0140
 (Non-translocated Sampleround1) - (Non-translocated Sampleround3) -0.34695 0.167 Inf  -2.079  0.2981
 (Non-translocated Sampleround1) - Translocated Sampleround3      -0.58643 0.135 Inf  -4.346  0.0002
 Translocated Sampleround1 - (Non-translocated Sampleround2)       0.09194 0.129 Inf   0.712  0.9805
 Translocated Sampleround1 - Translocated Sampleround2            -0.09952 0.126 Inf  -0.788  0.9696
 Translocated Sampleround1 - (Non-translocated Sampleround3)       0.00423 0.147 Inf   0.029  1.0000
 Translocated Sampleround1 - Translocated Sampleround3            -0.23525 0.127 Inf  -1.852  0.4320
 (Non-translocated Sampleround2) - Translocated Sampleround2      -0.19145 0.121 Inf  -1.579  0.6124
 (Non-translocated Sampleround2) - (Non-translocated Sampleround3) -0.08770 0.148 Inf  -0.591  0.9917
 (Non-translocated Sampleround2) - Translocated Sampleround3      -0.32719 0.119 Inf  -2.748  0.0662
 Translocated Sampleround2 - (Non-translocated Sampleround3)       0.10375 0.135 Inf   0.770  0.9725
 Translocated Sampleround2 - Translocated Sampleround3            -0.13573 0.126 Inf  -1.080  0.8895
 (Non-translocated Sampleround3) - Translocated Sampleround3      -0.23949 0.131 Inf  -1.828  0.4473

Results are given on the log (not the response) scale. 
P value adjustment: tukey method for comparing a family of 6 estimates 
```

Model haul including ditch 1

Used model:

```
glmmTMB(Haul ~ Sampleround + Watersoldier +`Sexratio(F/M)` + Depth + Length +
                    Sampleround:`Sexratio(F/M)` +  Sexratio(F/M)`:Length  +  (1|Ditch),
                    family = nbinom1(link = "log"),
                    data = subset(ALL_VARIABLES, Ditch %in% 1:10))
```

Output model:

```
AIC      BIC   logLik deviance df.resid
   437.4    462.5   -206.7    413.4       48

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 Ditch  (Intercept) 1.745e-09 4.177e-05
Number of obs: 60, groups:  Ditch, 10

Dispersion parameter for nbinom1 family (): 1.08

Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                  -0.322116   1.505950  -0.214 0.830628
Sampleround2                  0.072307   0.214595   0.337 0.736157
Sampleround3                 -0.475864   0.236887  -2.009 0.044556 *
WatersoldierTranslocated      0.268806   0.069398   3.873 0.000107 ***
`Sexratio(F/M)`               3.256429   1.540361   2.114 0.034509 *
Depth                         0.034542   0.006436   5.367    8e-08 ***
Length                        0.308055   0.158120   1.948 0.051387 .
Sampleround2:`Sexratio(F/M)`  0.017208   0.206582   0.083 0.933612
Sampleround3:`Sexratio(F/M)`  0.695799   0.237865   2.925 0.003443 **
`Sexratio(F/M)`:Length       -0.372556   0.162973  -2.286 0.022254 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Model Phosphate excluding ditch 1

Used model:

```
lmer(Phosphate ~ Haul + Watersoldier + (1|Ditch), data = subset(ALL_VARIABLES, Ditch %in% 2:10))
```

Output model:

```
REML criterion at convergence: 65.8

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.99083 -0.57151  0.04341  0.40664  2.17868

Random effects:
 Groups   Name        Variance Std.Dev.
 Ditch    (Intercept) 0.1172   0.3423
 Residual             0.1171   0.3423
Number of obs: 54, groups:  Ditch, 9

Fixed effects:
                          Estimate Std. Error        df t value Pr(>|t|)
(Intercept)               0.890116   0.192312 31.215684   4.629 6.13e-05 ***
Haul                     -0.009513   0.005547 46.670368  -1.715   0.0929 .
WatersoldierTranslocated  0.205825   0.099481 43.181435   2.069   0.0446 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Haul
Haul        -0.729
WtrsldrTrns  0.029 -0.351
```

Model sex count comparison

Used model:

```
aov(Haul ~ Sex*Samplingground, data = Sexcounts_total)
```

Output model:

```
                   Df Sum Sq Mean Sq F value  Pr(>F)   
Sex                 1    445   444.7   9.228 0.00296 **
Samplingground      2    127    63.7   1.322 0.27070   
Sex:Samplingground   2    186    93.1   1.932 0.14958   
Residuals         114   5494    48.2                   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Post-hoc (Tukey):

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Haul ~ Sex * Samplingground, data = Sexcounts_total)

$Sex
    diff      lwr      upr     p adj
M-F 3.85 1.339269 6.360731 0.0029558

$Samplingground
    diff       lwr      upr     p adj
2-1 2.05 -1.636164 5.736164 0.3866777
3-1 2.30 -1.386164 5.986164 0.3034824
3-2 0.25 -3.436164 3.936164 0.9858034

$`Sex:Samplingground`
         diff         lwr       upr     p adj
M:1-F:1  0.75  -5.6134529  7.113453 0.9993705
F:2-F:1  0.45  -5.9134529  6.813453 0.9999489
M:2-F:1  4.40  -1.9634529 10.763453 0.3463475
F:3-F:1 -0.75  -7.1134529  5.613453 0.9993705
M:3-F:1  6.10  -0.2634529 12.463453 0.0682907
F:2-M:1 -0.30  -6.6634529  6.063453 0.9999932
M:2-M:1  3.65  -2.7134529 10.013453 0.5591149
F:3-M:1 -1.50  -7.8634529  4.863453 0.9834778
M:3-M:1  5.35  -1.0134529 11.713453 0.1524164
M:2-F:2  3.95  -2.4134529 10.313453 0.4702913
F:3-F:2 -1.20  -7.5634529  5.163453 0.9940501
M:3-F:2  5.65  -0.7134529 12.013453 0.1122816
F:3-M:2 -5.15 -11.5134529  1.213453 0.1846307
M:3-M:2  1.70  -4.6634529  8.063453 0.9713006
M:3-F:3  6.85   0.4865471 13.213453 0.0270691
```