Assessment of the biotic & abiotic drivers of *Stratiotes aloides* establishment in a polder landscape

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Name:	Laura Sassen (3693694)
Daily supervisor:	F.C.M. van Duin, MSc
Supervisor:	Dr. M.J.J. Schrama
Internship partner:	Kasper Nagels (3708500)
Internship location:	Institute of Environmental Sciences (CML) Einsteinweg 2, 2333 CC, Leiden, The Netherlands
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Contact for raw data and lab journal:	F.C.M. van Duin – fleur.vanduin@naturalis.nl

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Table of Contents

Abstract

In the Netherlands, just 1% of its surface waters meet both the ecological and the chemical water quality standards because of eutrophication. A potential nature-based solution to this problem is the macrophyte *Stratiotes aloides,* a native plant that faces decline in populations. To reintroduce the macrophyte in water bodies in the Netherlands, defining clear environmental parameters for establishment is vital for a practical implementation. Therefore, in this study the aim is to assess the different drivers of the establishment of *S. aloides* in agricultural ditches at Polderlab Vrouw Venne. This includes assessment of invasive crayfish densities and the amount of coverage of *S. aloides,* as well as potential abiotic drivers including oxygen levels, pH, temperature, electrical conductivity, turbidity, ammonia and phosphate. The results indicate that the (a)biotic factors ammonia, phosphate, turbidity, temperature and dissolved oxygen and invasive crayfish did not have a significant effect on the establishment of *S. aloides*. These variables did not significantly correlate to the amount of coverage of *S. aloides* as well. Visualisations do indicate some trends (even though not significant) and with the knowledge that the data analysis for the coverage of *S. aloides* was depended on a single-year observation, future research should gather data over multiple years to see if the indicated potential trends still apply. While the (a)biotic factors did not contribute significantly to the establishment of *S. aloides,* it highlights the difficulty of defining clear environmental parameters for the establishment of *S. aloides,* which is vital for its practical implementation to use it as a nature-based solution.

Introduction

The environmental state of water bodies

To quantify the earth's capacity to handle environmental pollution without losing planetary stability, nine planetary boundaries have been chosen, including freshwater change. As it stands now, we are outside the threshold a safe operating space on this planet (Richardson et al., 2023). The quality of water bodies across the globe is undergoing a rapid decline (Hasan et al., 2023). Due to several causes, the water quality does not currently meet the standards that have been set by the governmental bodies like the European union (Voulvoulis et al., 2017). About 40% of the surface waters in Europe complies with the ecological quality benchmarks, while about 30% fulfils the chemical quality criteria for a good status (European Commission, 2025). The Water Framework Directive (WFD) was implemented to tackle this challenge, obligating the EU member states to achieve and preserve both chemically clean and ecologically sound water bodies by the year 2027 (*Directive 2000/60/EC*, 2014). A change like this is needed in the Netherlands, as a lot of water containing contaminants flow together through the rivers like the Rhine and Meuse (Winkels & Diem, 1991) .

A large factor that contributes to the current state of the aquatic ecosystems is the nutrient runoff from the agricultural land. Stretching over 300,000 kilometres throughout the Netherlands, agricultural drainage ditches play a vital role in managing the agricultural water within the polders (Highler, 1989). The extensive use of manure to fertilize the fields results in huge amounts of nitrogen and phosphorus leaking into the ground and surface water, especially in the Netherlands (Chardon & Schoumans, 2007; de Boer et al., 1997). Elevated levels of these nutrients in aquatic systems could drive ecosystem change, as these changes can initiate bottom-up processes that alter food webs, as well as increased eutrophication processes, which can lead to fewer macrophytes (Gulati & van Donk, 2002; Phillips et al., 2016; Singh & Yadav, 2025). The nutrient pollution transmitted through these ditches significantly impacts the connected Dutch water system as only 1% of surface water in the Netherlands meets the water quality standards (R. Didde, 2023). Therefore, finding a way to manage the ditches is essential for meeting broader water quality objectives. Especially reducing nitrogen and phosphorus concentrations is very important to achieve, because they mainly come from the use of fertilizers and animal manure during farming activities (Allen & Mallarino, 2008; Schoumans et al., 2014; Smith et al., 2007).

Aquatic ecosystems experience a lot of negative impacts because of eutrophication. For example, eutrophication processes can lead to algal blooms which can block sunlight from getting to the deeper water layers. This can reduce photosynthesis in the submerged plants, which in turn could lead to further loss of oxygen as the algae eventually die and decompose. When the algae decompose, bacteria can break down the organic matter, which can increase respiration of the bacteria which uses up oxygen

(Boyd, 2020; Diaz & Rosenberg, 2008; Dorgham, 2014; El-Sheekh et al., 2021; Mulukutla et al., 2025). This could result in too much oxygen being used by bacteria and other organisms for the amount that can be returned, leading to a decline in the dissolved oxygen levels (Diaz & Rosenberg, 2008; Guo et al., 2018). These low oxygen levels are known as hypoxia, which can create negative conditions for fish and many aquatic species that are often causing widespread mortality (Diaz & Rosenberg, 2008; Mulukutla et al., 2025). On top of that, there are certain cyanobacteria that occur when eutrophication processes are taking place. These cyanobacteria can form toxins, which could be dangerous to the many organisms that live in and around water bodies (Igwaran et al., 2024). When taking all these negative effects in consideration, it is crucial to employ regulations to reduce the risk of harmful consequences.

Because research showed that excessive amounts of nitrogen and phosphorus continue to leak into surface water and the ground, the regulations aimed at limiting manure use were not effective (Schipper et al., 2022). This shows that investigating possible ways to improve the Dutch water quality is needed, as well as a focus on implementing stricter legislations or other environmental measures to reduce nutrient levels. However, addressing the nutrient pollution alone is not enough, as biological pressures from invasive species such as crayfish also play a critical role in shaping the ecological state of freshwater systems (Reynolds et al., 2013).

Invasive crayfish as agents of ecological disruption in freshwater systems

There are about seven different crayfish species classified as invasive in the Netherlands, of which *Procambarus clarkii, Faxonius virilis, Faxonius limosus* and *Procambarus acutus* are classified as highly invasive species (Roessink & Ottburg, 2025). Their rapid growth, early maturity, high fecundity and ability to survive in extreme environmental conditions enable them to thrive. This leads to rising invasive crayfish populations, deteriorating water quality, and increasing damage to the dikes and banks (Lemmers et al., 2022; Nunes et al., 2017). The impacts for both the biotic and abiotic components will be discussed next.

Impact of the North American crayfish species on biota

Invasive species have a negative impact on the biotic water quality in aquatic ecosystems; studies have shown that North American crayfish species have nearly led to the complete disappearing of floating and submerged aquatic macrophytes, and have led to a decline in abundance of aquatic communities and of native aquatic populations in the area close to the infested rivers (Foster & Harper, 2006a, 2006b; Harper et al., 2002; Johnson et al., 2009; Nunes et al., 2017). The red swamp crayfish (*P. clarkii)* has been shown to consume the roots of water plants as well as young, small fish and their eggs (Farrag et al., 2021; Visser, 2023). Invasive species can be a cause for the loss of native species, which could lead to changes in the ecosystems functionality which in turn could lead to unwanted consequences for communities and ecosystems (Mollot et al., 2017). It has been found that the presence of invasive crayfish species can lead to a decline in the richness and abundance of various organisms (Gherardi, 2007).

Impact of the North American crayfish species on abiotic components of freshwater

Apart from the impacts on the biotic components of freshwater ecosystems, it was also concluded that habitats invaded by these species show an increase in water turbidity, as well as nitrogen and organic matter concentrations (Gao et al., 2024; Palmas et al., 2019), which eventually could lead to eutrophication (Angeler et al., 2001). This comes from activities like their burrowing, tail flipping and walking (Angeler et al., 2001; Harvey et al., 2014; Statzner & Sagnes, 2008). Additionally, the invasive crayfish construct burrows in dikes and banks, which weaken them. One consequence of this is an increased risk of dike and dam failure. Another consequence is the damage done to water drainage systems, leading to a mobilisation of soil nutrients, which deteriorates water quality (Angeler et al., 2001; Faller et al., 2016; Gylstra et al., 2016; Haubrock et al., 2019; Koese & Vos, 2013; Lemmers et al., 2018, 2022). Furthermore, the feeding of invasive crayfish can influence the cycling of organic matter: studies have shown that macrophytes are being cut by invasive crayfish species and not always consumed (Crehuet et al., 2007; Gherardi & Acquistapace, 2007; Nyström & Strand, 1996), leading to a decline of these macrophytes and possibly adding nutrients into the water column (Alcorlo et al., 2004; Gherardi & Acquistapace, 2007; Nyström et al., 2001; Rodríguez et al., 2003; Wal et al., 2013).

All of the above could interact and strengthen the total impact; invasive crayfish species can prey on local macroinvertebrate populations which can change resources for other species (Covich et al., 1999; Feminella & Resh, 1989; Gamradt et al., 1997; Klose & Cooper, 2012; Lodge et al., 2000; Stelzer & Lamberti, 1999), as well as the impact of invasive crayfish species on abiotic factors which can result in a switch from a stable state, to a non-stable state of freshwater ecosystems (Matsuzaki et al., 2008). All these listed effects show that a durable solution must be found to tackle these problems. There is no robust strategy for reducing and controlling the amount of invasive crayfish species, also due to the interconnected water bodies (Gherardi et al., 2011; Lemmers et al., 2021; Ministerie van Landbouw, 2022; Souty-Grosset et al., 2016). Intensive trapping is too expensive and not feasible for the long term (de Hoop et al., 2016; Gherardi et al., 2011; Lemmers et al., 2022). Therefore it is crucial to gain knowledge on how to manage the negative effects of invasive crayfish, but until now, there is a knowledge gap for combatting invasive crayfish (de Hoop et al., 2016; Gherardi et al., 2011). The elimination of invasive crayfish in the Netherlands is deemed nearly impossible, with population control being the most effective when multiple strategies are integrated (De Hoop et al., 2016). Possible nature-based solution should be investigated and if successful, implemented to tackle the water quality problem. *Stratiotes aloides* is an example of a possible candidate for the use as a nature-based solution.

Stratiotes aloides and its environmental properties in the context of nature-based solutions

S. aloides (also known as water soldier) is an example of an aquatic macrophyte native to the Netherlands and is found in Eurasia and parts of North America (A. N. Efremov et al., 2019). It is part of the Natura 2000 habitat type H3150 (Arts, z.d.; Cusell et al., 2013) and would be a logical choice to implement as a nature-based solution because of its contribution as a keystone species in the process of restoring fen ponds (Sarneel et al., 2011). The macrophyte is declining in the Netherlands as well as that it is considered to be a sensitive species (Smolders et al., 2003a). Given its preference for lentic environments (Erixon, 1979; Kłosowski et al., 2011; Nielsen & Borum, 2008), *S. aloides* aligns well with the potential restoration efforts aimed at improving water quality in agricultural drainage ditches. It spends a significant part of the year floating: after winter, it rises to the surface in spring and remains there until autumn (A. N. Efremov et al., 2019). A previous study found that, under in situ conditions, the plant numbers increased by up to 70% during the growing season, with no observed mortality (Renman, 1989). The plant reproduces in a vegetative manner during the floating phase, although sexual reproduction is also an option (Cook & Urmi-König, 1983; Smolders et al., 1995; Toma, 2012). It survives winter through a few properties; first of all through its specialized buds which are also called turions, that make it possible for the macrophyte to occur in different sorts of habitats (A. N. Efremov et al., 2019; A. N. Efremov & Sviridenko, 2008; A. Efremov & Sviridenko, 2012). Secondly, it survives in the winter if the water column or basal rosette do not get frozen entirely (Erixon, 1979).

Moreover, *S. aloides* may rapidly enhance water quality when present in agricultural drainage ditches, but only when it can establish its populations, which requires specific environmental conditions: this macrophyte flourishes in slow flowing, slightly alkaline, phosphate-poor, shallow, mesotrophic waters rich in detritus (Kłosowski et al., 2011; Nielsen & Borum, 2008). *S. aloides* displays a continuous morphological plasticity, forming individuals with varying numbers of emergent and submerged leaves which are influenced by environmental conditions (Department of Carpology, Kazimierz Wielki University & Toma, 2019).

S. aloides is known to absorb nitrogen and phosphorus when high levels of these nutrients are present (Brammer, 1979; Strzałek et al., 2019). It can prevent the conversion of ammonium to nitrate to some extent as well, because the coverage *S. aloides* provides on the water surface leads to less oxygen, which is needed during this process. This is beneficial as high levels of ammonium are toxic to the macrophyte (Harpenslager et al., 2016; Smolders et al., 2019). Additionally, this plant positively influences the process of sedimentation because when it is submerged, it reduces water flow. This as well results in a lower turbidity (Madsen et al., 2001). The macrophyte limits the growth of phytoplankton and filamentous algae because it reduces the nutrient levels which they need for their survival (Mulderij et al., 2007). It is hypothesised that *S. aloides* takes up nutrients from the water in the spring when it is submerged, but during the summer, nutrient uptake is performed via its roots that facilitate nutrient uptake from the sediment (Kufel et al., 2010). Because of all the described benefits, *S. aloides* could be a suitable phytoremediator to restore water quality in the eutrophicated agricultural ditches.

Introduction of *S. aloides* could also have a positive effect on the aquatic macroinvertebrate populations (and therefore possibly water quality) by providing habitats with their spiny leaves (Obolewski, 2005; Obolewski et al., 2009; Obolewski & Strzelczak, 2009; Suutari et al., 2009; Tarkowska-Kukuryk, 2006). Reintroduction into these systems could hugely benefit the aquatic life and species abundance as *S. aloides* harbours larger epifauna abundance in standing water environments (Obolewski et al., 2014; Tarkowska-Kukuryk, 2006). Taking all these properties into account, *S. aloides* can thus be a potential candidate for phytoremediaton in the Netherlands for agricultural drainage ditches. This is because they represent a typical lentic freshwater system where these restoration measures could be tested and implemented effectively. As it is known that *S. aloides* is listed as a sensitive species and is experiencing decline (A. N. Efremov et al., 2019; Ministerie van Landbouw, 2022), research is needed to see if reintroduction for implementation of this plant as a phytoremediator can be achieved.

Objects and aims

It is known that the successful reintroduction of *S. aloides* resulting in a stable and self-sustaining population is influenced by population density, as high-density populations flourish, even when abiotic conditions are not optimal, while establishment at low densities under the same conditions seem impossible (Harpenslager et al., 2016). Therefore, widespread implementation of *S. aloides* as a nature-based solution will require knowledge of factors influencing establishment of *S. aloides* in an ecosystem. Previous research has pointed towards a negative relationship between *S.aloides* and sulphate and high levels of nitrogen (Abeli et al., 2014; Smolders et al., 2003b), but van Duijn (2024) demonstrated that *S. aloides* was able to establish in agricultural drainage ditches with relatively poor water quality. Defining clear environmental parameters for establishment is vital for a practical implementation of *S. aloides*. However, the drivers that are required for the successful establishment of *S. aloides* in polder landscapes are unclear, which highlights the need to perform research on this topic. Therefore, the aim of this study was to assess the factors that affect establishment, by investigating the following research question: “To what extent do (a)biotic factors influence the establishment of *S. aloides* in a Dutch polder landscape?”

To answer the question: “To what extent do (a)biotic factors influence the establishment of *Stratiotes aloides* in a Dutch polder landscape?” these following components will be analysed in the statistical analysis:

1.1: What impact do abiotic variables have on the establishment of *S. aloides* in a polder landscape?

1.2: What impact do invasive crayfish densities have on the establishment of *S. aloides* in a polder landscape?

2.1: What impact do invasive crayfish densities have on the amount of coverage of *S. aloides* in a polder landscape?

2.2: What impact do abiotic variables have on the amount of coverage of *S. aloides* in a polder landscape?

Expected outcomes

It is expected that the invasive crayfish will contribute significantly to the establishment of *S. aloides* because there are a number of (in)direct effects of invasive crayfish on macrophytes. *S. aloides* could be experiencing grazing of the crayfish, however, the leaves of *S. aloides* are strong (Cronin et al., 2002) so if the effects of grazing crayfish occurred, it would likely be on their roots, as that is when they are in the submerged phase (A. N. Efremov et al., 2019). It is hypothesised that this could make it more resistant to underwater grazing, which is what makes it a suitable candidate for this study. Additionally, the cutting of the plant without consuming it could damage the plant (Gherardi & Acquistapace, 2007), affecting its growth and coverage. Crayfish could be attracted to *S. aloides* due to feeding opportunities and the hypothesized reasoning that crayfish can find shelter in dense mats of *S. aloides*. Additionally, the burrowing of the crayfish in the sides of the ditch bank could disturb the macrophyte from taking up nutrients from the sediment, which is where they mainly obtain their nutrients when they are in the floating phase of their life-cycle (Kufel et al., 2010). For example, the burrowing activity that disturbs the sediment and releases nutrients can increase turbidity (Angeler et al., 2001; Carvalho et al., 2016; Matsuzaki et al., 2008) and thereby possibly eutrophication which can affect photosynthesis by *S. aloides* and limit establishment.

Previous findings of a study performed in a Dutch polder landscape showed that the crayfish haul was higher in the translocated water soldier tracks (van Duijn, 2024). Additionally, the data of the crayfish densities per ditch showed a seasonal shift, which may have something to do with the seasonal cycle of *S. aloides* (van Duijn, 2024). In the past, records from Finland have shown that abiotic conditions on site can influence *S. aloides* to stay submerged all year (Erixon, 1979; Harpenslager et al., 2016; Nielsen & Borum, 2008; Renman, 1989). However, in one particular ditch track, a successful population of *S. aloides* was observed (van Duijn, 2024). Given that the abiotic conditions within the polder did not show a significant difference (Hoogeveen, 2024), it is not very likely that the abiotic conditions at the research site are significantly affecting the establishment of *S. aloides* for this particular location. Another reason to suspect that the abiotic factors do not have a significant effect on the establishment is that *S. aloides* can survive in a broad range of abiotic variables (A. N. Efremov et al., 2019). Therefore, it is hypothesised that the observed differences in the establishment of *S. aloides* within this polder, are likely caused by crayfish, or that the crayfish act as a mediator which can lead to the unsuccessful establishment of *S. aloides* for some ditches.

Materials and methods

Polderlab experimental setup

To investigate the effects of abiotic variables and crayfish on the establishment of *S. aloides* in agricultural ditches, this study was set in the Dutch polder landscape Vrouw Venne (The Netherlands, 52.191612, 4.552275), where large numbers of *S. aloides* were introduced. The coverage of *S. aloides* was recorded, crayfish numbers were monitored and abiotic variables were measured in 10 ditches (see Figure 1). Each ditch consisted of a track with *S. aloides* and one control track without *S. aloides*. For this study it is important to note that the data-analysis only covers the ditches with *S. aloides* as the establishment of this plant is the subject of interest, therefore excluding the ditches without the *S. aloides* treatment. *S. aloides* has been introduced in different amounts over different years for all treatment ditches (Appendix A). The ditches are interconnected and range from ~38 metres in length to a maximum of ~138 metres. The width of the ditches has a range of ~4 to ~9 metres.

All data was collected between week 15 and week 24 in 2025. Crayfish and abiotic variables were monitored simultaneously over three sampling rounds during April and May. The sampling order of ditch tracks was randomised for each sampling week. The abiotic samples of the turbidity, phosphate and ammonium were measured once per sampling round for each ditch track, all on one day. For the fieldwork schedule, see Appendix B. For the randomized sampling schedule, see Appendix C. For a full list of materials, see Appendix D.

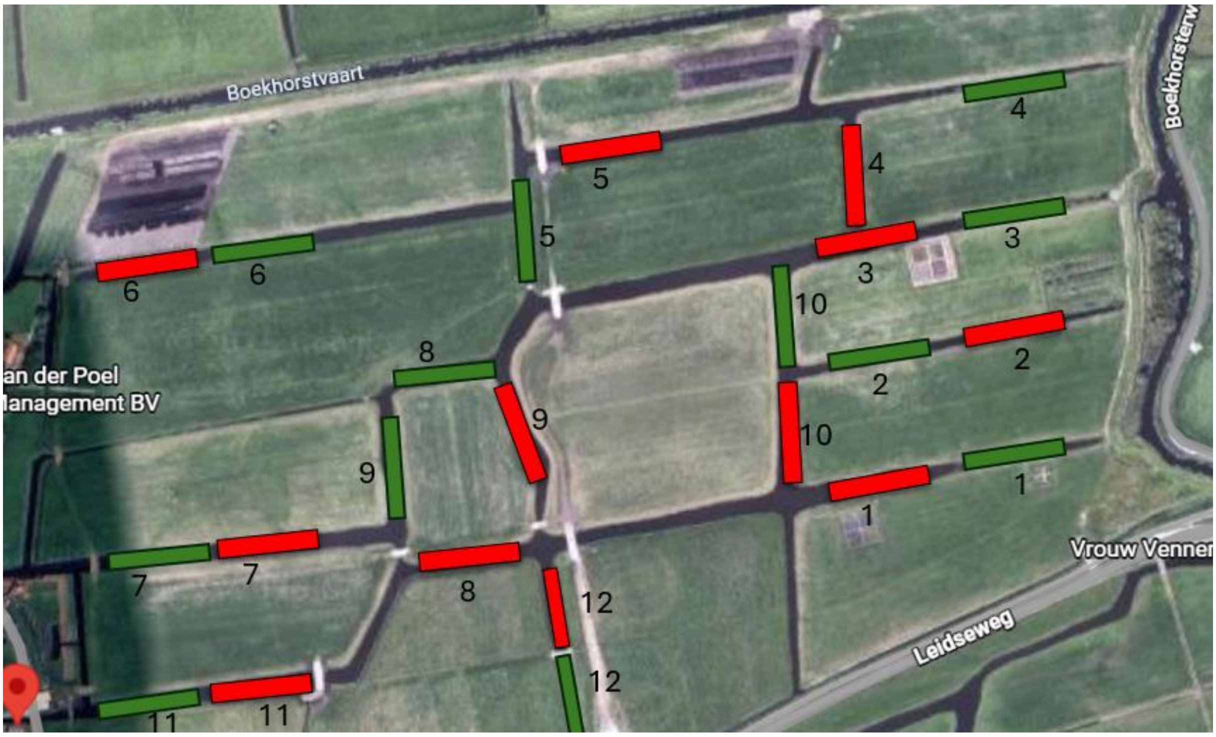

Figure 1*: Research site in the Polderlab Vrouw Venne (Google Maps) with drawn ditch tracks, coloured green for presence of S. aloides and red for the control/absence of S. aloides***. *Not drawn to scale. Ditches 8 & 9 are present on the map but not included in the study.*

Crayfish sampling methods

To assess the density distributions of the invasive crayfish in the ditches, funnel traps were used, see Figure 2. The bait used included three 14 mm halibut pellets in a cage within the trap (which prevented feeding). In each ditch track, 12 traps were placed with an even distance between them (+-4 metres). This kind of method is used in research on invasive crayfish (Lewis, 1997).

The steps of placing the traps included: Firstly, ground contact should be ensured (since crayfish are bottom feeders). Then, the traps should leave breathing room for bycatch (e.g. frogs) at the top if possible, however bottom contact should always be the priority, so the crayfish can always get into the traps. After this, the traps should be located to the ditch bank as close as possibly can. The openings should be positioned parallel to the ditch bank (Figure 2).

Figure 2: *Funnel trap placement (also called "Finnish model" (610 x 315 x 250 mm)).*

The time of placement for each ditch depended on the randomized schedule for each day (Appendix C). Every trap was left overnight. Traps were emptied in the morning, which started around 9:00. After emptying the traps into a bucket, the crayfish were counted. After this the crayfish were directly released into the water at the same sampling site. Missing claws were noted, as well as bycatch. The crayfish sampling occurred over three sampling rounds during April, May and June. Each crayfish round was performed within one week over four nights, due to the amount of traps that were available. For the preparation of the raw data, see "Statistical analysis".

Abiotic variables sampling methods

Measurement methods for pH, electric conductivity, dissolved oxygen and temperature

The pH, electric conductivity, dissolved oxygen levels and temperature were measured during the crayfish sampling process, so at the same time the traps were emptied for a track. The device used was a portable multimeter (HACH model HQ4300) with accessory probes. The measurements were performed three times for each track, at the beginning, the middle, and the end of where the traps were located. The temperature was always measured with the oxygen probe.

Measurement methods for ammonia and phosphate levels

Ammonia and phosphate samples were taken in the field, and the samples were directly analysed in the field afterwards in the same order as they were sampled. Per track, a mixture of 25 mL water at the point of the first crayfish traps and 25 mL water of the last crayfish trap was used. Phosphate levels were measured with a Checker photometer (Hannah Instruments HI713 with reagent HI713-25). Ammonia levels were measured using a Checker photometer (Hannah Instruments HI715 with reagents HI715A-0 and HI715B-0).

Measurement of turbidity

The turbidity was measured in the field at one location per track, at the middle of where the crayfish traps were located. For this a Secchi transparency tube was used. We ensured to be facing the sun, so there was uniform light which minimised reflections inside the tube. Measurements were performed by filling the tube all the way to the top with sample water from the surface. After this, the water was poured out until we could distinguish between the black and white quarters at the bottom of the tube.

Methods for determining the degree of coverage of *S. aloides*

To determine the establishment of *S. aloides* for each treatment ditch, the coverage *of S. aloides* was measured in one designated week (calendar week 23), as this was a week in which most of the *S. aloides* plants were visible at the surface. This was done by the use of a (handmade) floating bamboo quadrat of 1m^2 which was placed in the water multiple times per ditch along the whole length of it. To account for differences in the total measured surface area between ditches (because of differences in length and width between ditches), calculations were made for each ditch to determine the number of sampling points for the width prior to sampling (Table 1). A visual estimation of the percentage of floating plants within the square was made by one person for all samples. To estimate *S. aloides* coverage for the entire ditch track, the total of samples per ditch was averaged.

Table 1: *Ditch width categories and sampling methods. The distance in ditch length between measurements always remained constant. The amount of width measurements per 5 metres depended on the width category of the ditch. This resulted in a certain amount of measurements per ditch, which resulted in a relatively similar percentage of measured surface per ditch.*

Category	Width Range (m)	Ditches	Width Sampling Method	Length per ditch	Total amount of measurements	% of measured surface
Narrow	< 5 m	Ditch 4	2 width measurements every 5 metres; at the wider end: 3 width measurements done twice	Ditch 4: ~115 m	Ditch 4: 51	Ditch 4: ~10%
Medium	5 – 5.4 m	Ditches 1, 3	3 width measurements every 5 metres	Ditch 1: ~138 m Ditch 3: ~138 m	Ditch 1: 54 Ditch 3: 76	Ditch 1: ~11% Ditch 3: ~11%
Wide	> 5.4 m	Ditches 2, 5, 6, 7, 10, 11, 12	4 width measurements every 5 metres	Ditch 2: ~75 m Ditch 5: ~38 m Ditch 6: ~50 m Ditch 7: ~50 m Ditch 10: ~62 m Ditch 11: ~38 m Ditch 12: ~54 m	Ditch 2: 60 Ditch 5: 40 Ditch 6: 40 Ditch 7: 51 Ditch 10: 40 Ditch 11: 19 Ditch 12: 57	Ditch 2: ~11% Ditch 5: ~11% Ditch 6: ~11% Ditch 7: ~11% Ditch 10: ~12% Ditch 11: ~11% Ditch 12: ~11%

Statistical analysis

The statistical analyses were performed in RStudio, "R version 4.4.2 (2024-10-31 ucrt)". Before performing the statistical analyses, the raw data has been prepared and for every sub question, an analysis was performed. This will be described per question. Furthermore, before the models were made, the data was inspected and visualised, as well as the collinearity between variables (package corrplot version 0.95). Assumptions per model have been checked with diagnostic plots (package car version 3.1-3). The PCA plot and the boxplots were made by using the package ggplot2, version 3.5.1. During the process of choosing a model, the concept of parsimony, along with AIC scores was employed to determine the most suitable model that retained only the most relevant explanatory variables. Because of this, you will notice that pH is not included in either model. But firstly, before describing the statistical method per question, please note the following:

Preparation of the response variables ‘Established’ and ‘Coverage’:

Since *S. aloides* coverage could only be recorded once (when plants were visible at the water surface), for every round and each individual ditch, the same corresponding average coverage of *S. aloides* was used. This is a relatively good approach because within the sampling period, no big changes in the amount of plants occurred. Because of the interconnection between ditches (called a nested experimental design) and the temporal replication, there needed to be a correction for the pseudo-replication. When performing the statistical analysis, it was statistically difficult to perform any tests because of the repeating percentage of average coverage for each ditch per round and additionally, a linear mixed-effect model was not possible. To be able to still include an alternative to the linear mixed-effect model, it was decided to average all explanatory variables over the three rounds in order to be able to use the percentage of average coverage as a response variable and ultimately derive the labels ‘established’ or ‘non-established’ from this. This as well acted as a replacement for the linear mixed-effect model that could not be used. The percentage of average coverage determined whether a ditch could be labelled as ‘established’ or not. The cutoff value for a ditch to be established was determined to be 25%, see the discussion for the reasoning why this cutoff value was determined. To provide more insight, the model for the coverage was employed as well.

The statistical methods will be described per sub question:

1.1: What impact do abiotic variables have on the establishment of *S. aloides* in a polder landscape?

1.2: What impact do invasive crayfish densities have on the establishment of *S. aloides* in a polder landscape?

2.1: What impact do invasive crayfish densities have on the amount of coverage of *S. aloides* in a polder landscape?

2.2: What impact do abiotic variables have on the amount of coverage of *S. aloides* in a polder landscape?

For Q 1.1 & Q 2.2: The three abiotic measurements per ditch per round that were performed with the HACH, including pH, EC, dissolved oxygen and temperature were averaged by dividing the total per abiotic measurement by three. This resulted in a value for each track per sampling round. The other abiotic measurements including turbidity and pH already had one 'averaged' value per ditch track per round, due to the sampling method as described above.

For Q 1.2 & Q 2.1: The calculation of the total crayfish haul was performed, from which the catch per unit effort (CPUE) was calculated. This has been done by dividing the total haul per ditch track per sampling round by 12 (the amount of traps per track), which results in the average caught crayfish per trap, per track in a sampling round. With the resulting data for the variable CPUE, statistical analyses were performed

Per question, the CPUE and abiotic variables were incorporated into the model like this:

Q1.1, Q1.2: LMestablished <- lm(Established ~ Temperature + Oxygen + EC + Turbidity + Phosphate + Ammonia + CPUE)

Q2.1, Q 2.2: LMcoverage <- lm(Coverage ~ Temperature + Oxygen + EC + Turbidity + Phosphate + Ammonia + CPUE)

Results

From April to June 2025, three rounds for crayfish estimation measurements were performed in weeks 15, 19 and 20 for all ditches, including the control ditches without water soldier. During the three sampling rounds, a total of 1268 crayfish were caught. Which is on average for all rounds approximately 63 individuals per ditch track for both the treatment and control ditches. The raw data showed some varying patterns for relations between the different (abiotic) variables and the *S. aloides* coverage (fig. 5-11).

The amount of coverage of *S. aloides* for each ditch

Overall, *S. aloides* was present in most of the studied ditch tracks, except for ditch 5 and 10 (Table 2). Ditch 1 was the only ditch that displayed a very high amount of coverage by *S. aloides*, in dense mats where almost only *S. aloides* was visible on the surface. The other ditches that were marked as 'established' did have dense mats of floating *S. aloides* as well, but in patches and located more at the sides of the ditch. The other ditches displayed some floating *S. aloides,* mostly at the end of the ditches and sporadically though some parts of the ditches.

Ditch track	1M	2M	3M	4M	5M	6M	7M	10M	11M	12M
Average coverage (%)	84,6	12,2	15,4	23,3	0	6,9	25,3	0	25,3	6,9

Table 2: *Table showing the amount of coverage (averaged) in % for each ditch in calendar week 23. Ditches in which S. aloides had an average coverage below 25% were considered non-established. The established ditches are highlighted in the table.*

Q1.1 The influence of abiotic variables on the establishment of S. *aloides*

For the abiotic variables, no significant differences were found for all tested variables between ditches that were marked as 'established' (meaning >25% coverage of *S. aloides* on the water surface) and ditches that were marked as 'non-established'. In Table 3 the means, standard deviations and p-values for every variable can be found (pH was excluded from the model as this variable displayed multicollinearity).

The differences in the means (Table 3, Figure 4) for the abiotic variables suggest that ditches labelled as 'established' may be associated with slightly higher oxygen levels, EC, turbidity and ammonia. Ditches that were labelled as 'non-established' show higher temperature and phosphate concentrations. Although these differences are not statistically significant, they may indicate small differences between established and non-established ditches.

Q1.2 The influence of invasive crayfish densities on the establishment of S. *aloides*

The influence of invasive crayfish densities on the establishment of *S. aloides* were included in the model as well, which resulted in a non-significant p-value of 0.478 (table 3). Even though the p-value is not significant, the mean of the CPUE is slightly higher for the established ditches, suggesting that there are more crayfish with substantial populations of *S. aloides,* or that *S. aloides* is established better in ditches with more crayfish.

Table 3: *The mean, standard deviation and p-value for all (a)biotic variables in "non-established" vs. "established" ditches. All nutrient concentrations are given in ppm.*

Variable	Not Established (Mean ± SD)	Established (Mean ± SD)	p-value
Temperature	16.82 ± 1.57	15.76 ± 0.65	0.184
Dissolved oxygen	6.58 ± 1.13	7.23 ± 1.07	0.702
Electrical conductivity	899.22 ± 55.94	930.02 ± 65.50	0.175
Turbidity	48.56 ± 42.87	58.90 ± 31.03	0.174
Phosphate	1.32 ± 0.74	1.05 ± 0.31	0.506
Ammonia	0.75 ± 0.48	0.97 ± 0.61	0.534
CPUE	1.57 ± 0.54	1.83 ± 0.52	0.487

The results for the measured variables were inconclusive, therefore a PCA biplot was created. This was done to provide additional insight into potential underlying patterns and correlations between environmental variables and establishment status even though the statistics were inconclusive. In the PCA plot in Figure 3, dissolved oxygen and phosphate point in the same direction (positive PC2), which indicated a positive correlation between these variables along this axis. Temperature, ammonia and turbidity showed a strong influence on PC1, which indicated a possible positive correlation between these variables. The CPUE had a strong influence on PC1 and pointed in the opposite direction to pH, EC, oxygen and phosphate. Non-established sites appeared to be more spread across both axes, while established sites were more clustered to the top left and right. In Figure 4 an overview for each of the variables and their 'established' status was created. It displays the differences between the established and non-established ditches, even though no significant differences were found.

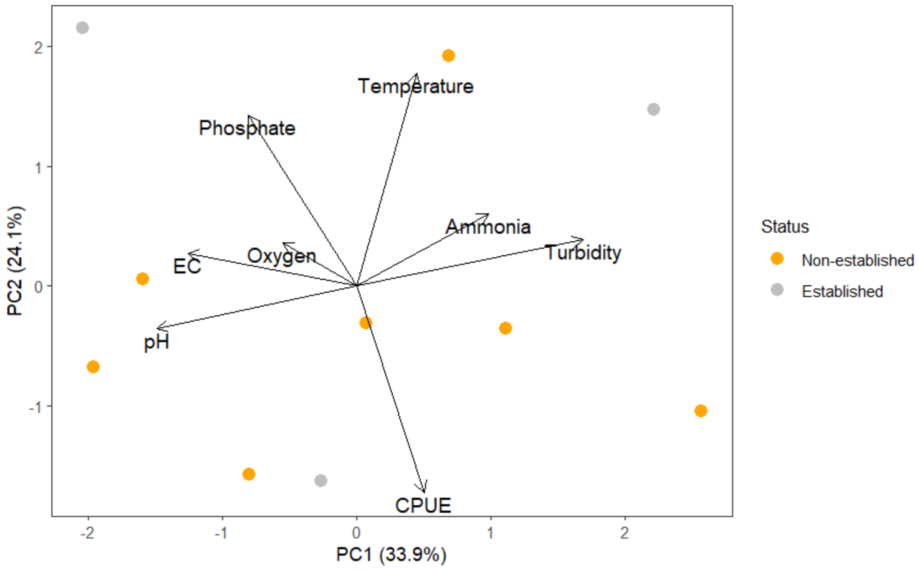

Figure 3: PCA biplot for all variables, based on the establishment status. It shows the relationship between variables based on whether they are labelled as established or not established. Each point represents a ditch, where orange points are labelled as non-stablished and grey points are established ditches.

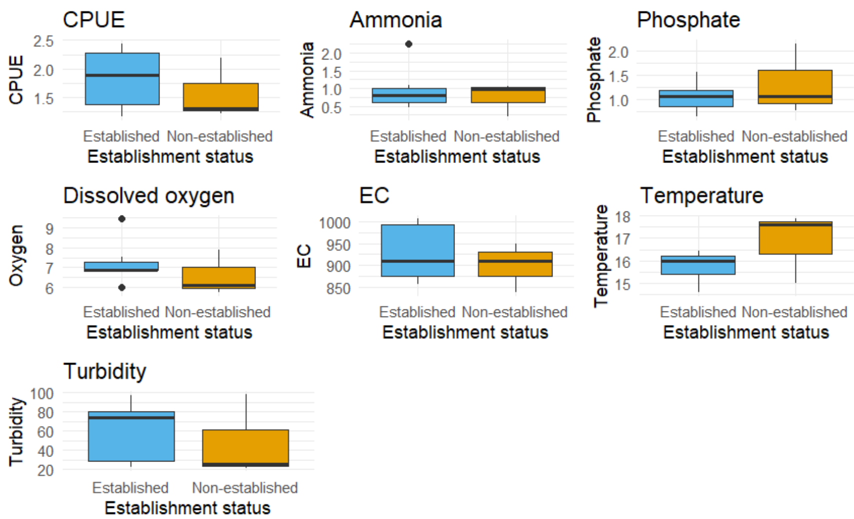

Figure 4: Boxplots per variable, for established and non-established ditches.

Key observations from the fieldwork

Please note that the p-values are derived from the statistical model. These values are modelled with the averaged dataset over the three rounds, as described in the methods for the statistical analysis. However, be aware that the described observations from the different plots are explorative from the raw data.

Q2.1 The influence of invasive crayfish densities on the coverage of *S. aloides*

Invasive crayfish and S. aloides coverage

Figure 5 shows a scatterplot of the CPUE against the *S. aloides* coverage (%) across the three sampling rounds, displaying the distribution and potential patterns in the raw data per ditch over time. Ditch 1 displays a high CPUE for round 1, but for the other rounds in this ditch the CPUE was not the highest. Round 3 data showed a relatively consistent CPUE range (1.5-2.5) which seemed to result in a varying amount of coverage. The p-value shows a non-significant correlation for this variable, p=0.8629 (Table 4).

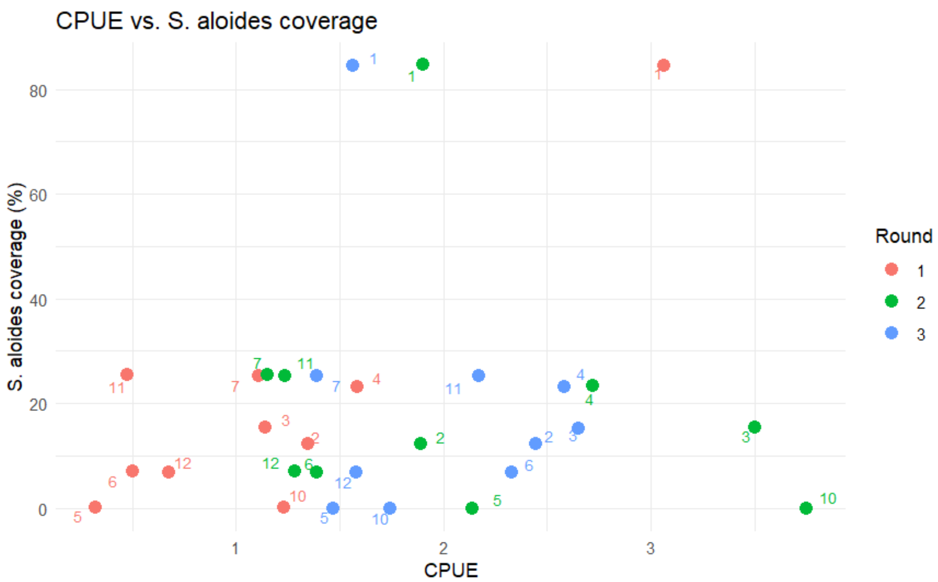

Figure 5: *Scatterplot of the CPUE against S. aloides coverage (%) across the 3 sampling rounds.*

Q2.2 The influence of abiotic variables on the coverage of *S. aloides*

The influence of the abiotic variables on the amount of coverage of *S. aloides* were assessed by measuring: oxygen levels, temperature, ammonia levels, phosphate levels, turbidity and electrical conductivity.

For the abiotic variables, no significant correlations were found between the coverage of *S. aloides* and the abiotic variables. In Table 4 the means, standard deviations and p-values for every abiotic variable can be found.

Dissolved oxygen and S. aloides coverage

For the dissolved oxygen and *S. aloides* coverage plot (Figure 6) at 0-30% of coverage, dissolved oxygen (DO) levels are widely scattered, which did not indicate a trend. Round 3 had lower DO values overall. High *S. aloides* coverage (i.e. ditch 1) did not correlate with high DO levels. Dissolved oxygen has a non-significant p-value of 0.2220 in Table 4, indicating no significant correlation between DO and *S. aloides* coverage.

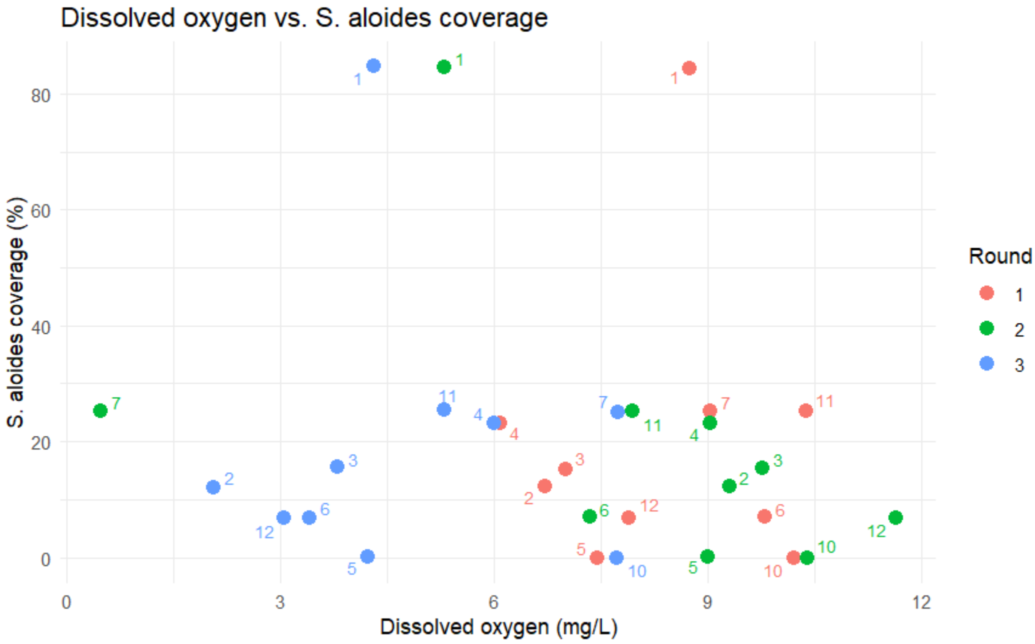

Figure 6: *Scatterplot of the dissolved oxygen (mg/L) against S. aloides coverage (%) across the 3 sampling rounds.*

Electrical conductivity vs. S. aloides coverage

In Figure 7 a plot of the electrical conductivity and *S. aloides* coverage shows no clear pattern for the EC in relation to the amount of coverage. EC shows a non-significant correlation with a p-value of 0.1138 (Table 4).

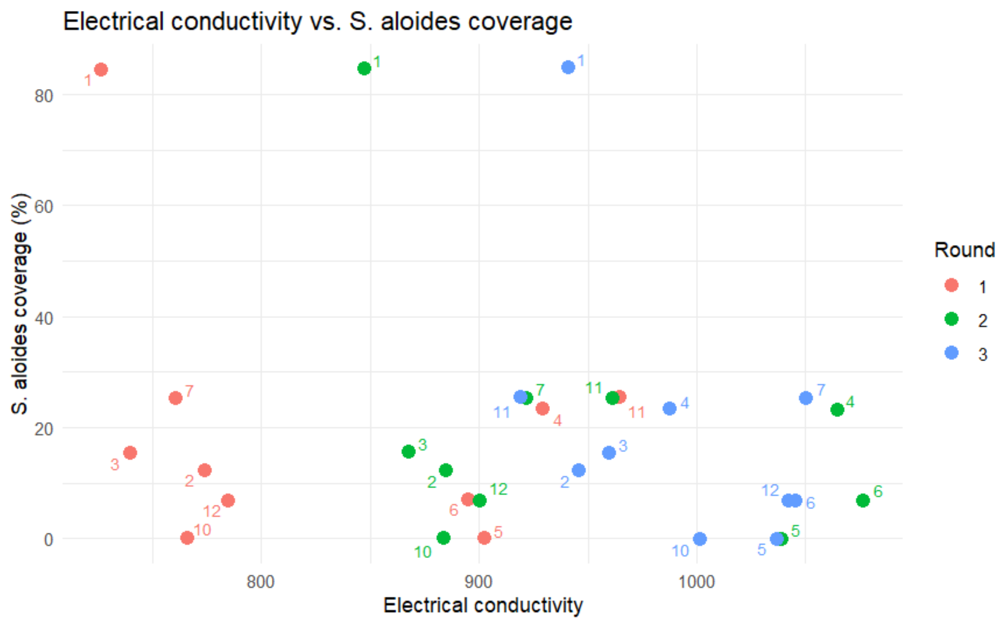

Figure 7: *Scatterplot of the electrical conductivity against S. aloides coverage (%) across the 3 sampling rounds.*

Phosphate and S. aloides coverage

In Figure 8 it can be seen that lower to medium range phosphate concentrations seemed to result in a higher coverage of *S. aloides* for ditch 1. Phosphate shows a non-significant p-value of 0.4594 (Table 4).

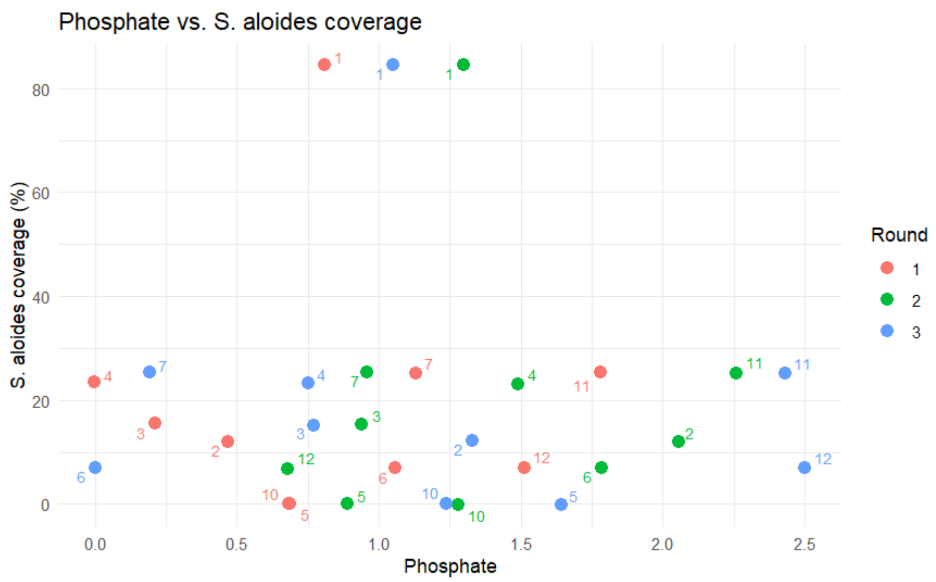

Figure 8: *Scatterplot of phosphate concentrations against S. aloides coverage (%) across the 3 sampling rounds.*

Ammonia and S. aloides coverage

In Figure 9 the plot for the ammonia concentrations and coverage showed no patterns as the datapoints were scattered. Ditch 12 is an outlier. In Table 4, ammonia shows a non-significant p-value of 0.5106.

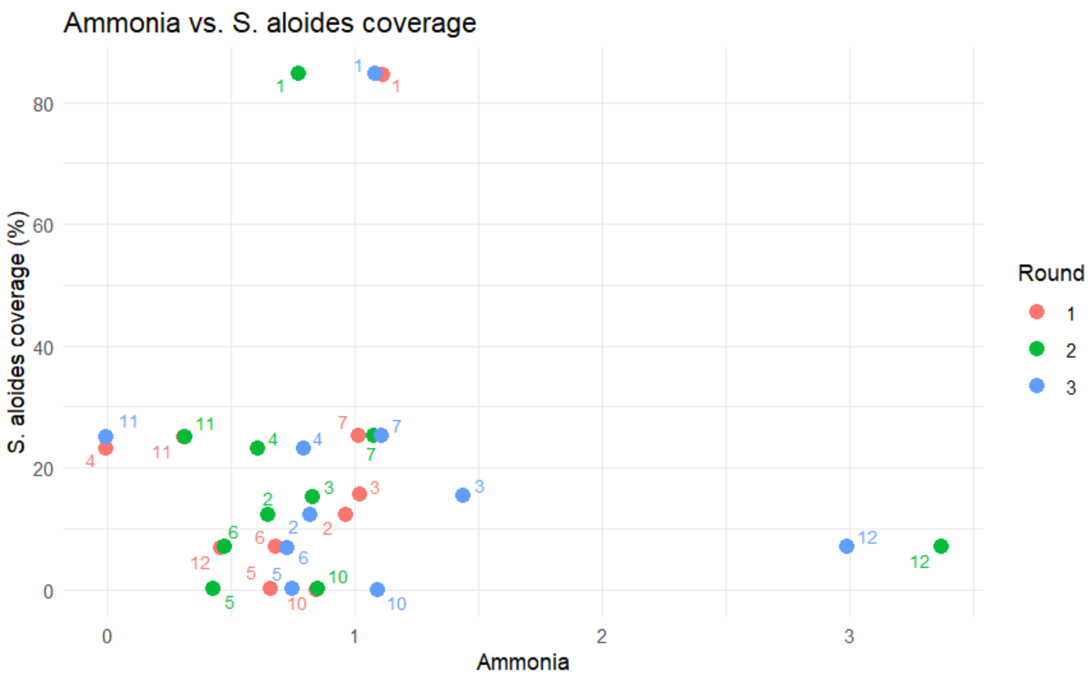

Figure 9: *Scatterplot of ammonia concentrations against S. aloides coverage (%) across the 3 sampling rounds.*

Turbidity and S. aloides coverage

The plot for turbidity vs. *S aloides* coverage in Figure 10 showed no clear patterns regarding the amount of coverage as datapoints were scattered. Round 3 generally had low turbidity, round 3 had high turbidity and round one was slightly higher than round 3 for most ditches. Turbidity shows a non-significant p-value of 0.0546 (Table 4).

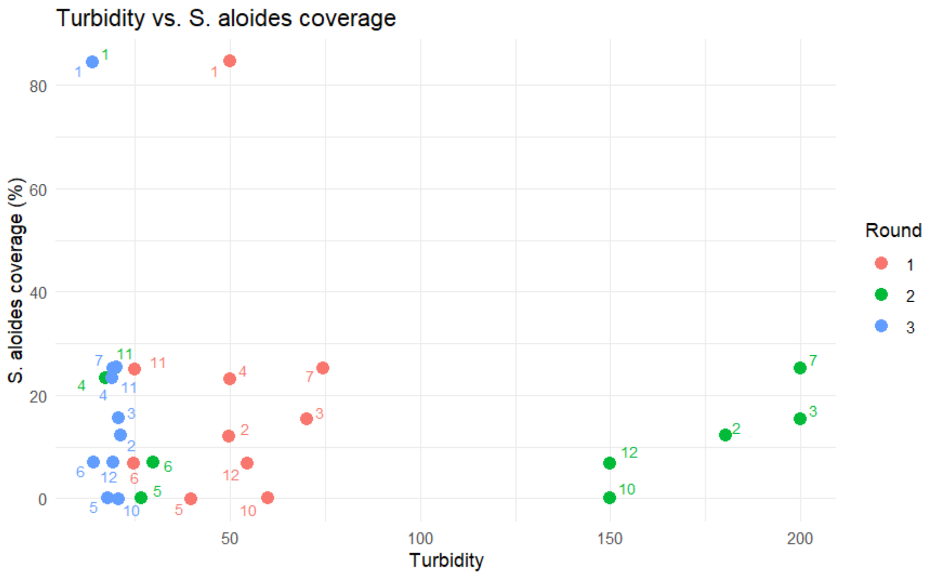

Figure 10: *Scatterplot of the turbidity against S. aloides coverage (%) across the 3 sampling rounds.*

pH and S. aloides coverage

The plot in figure 11 displays how the pH varied per round and results in a varying coverage per ditch. This was left out of the model due to the method described in 'statistical analysis'.

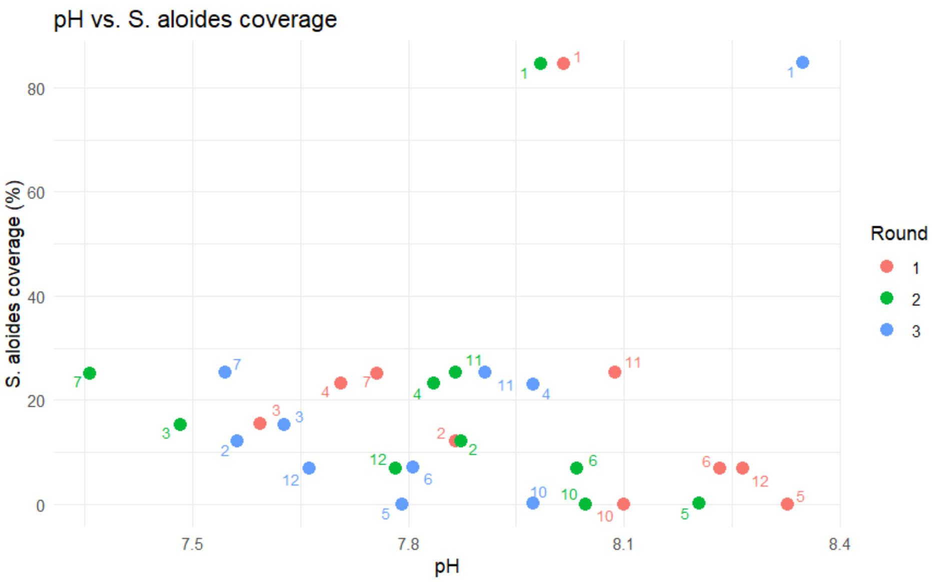

Figure 11: *Scatterplot of the pH against S. aloides coverage (%) across the 3 sampling rounds*

Table 4: *The mean, standard deviation and p-value for all (a)biotic variables tested for a correlation between the coverage of S.aloides in all ditches. All nutrient concentrations are given in ppm.*

Variable	Coverage (%) (Estimate ± SE)	p-value
Temperature	11.62 ± 5.75	0.1808
Dissolved oxygen	-9.68 ± 5.52	0.2220
Electrical conductivity	-0.39 ± 0.14	0.1138
Turbidity	-0.95 ± 0.23	0.0546
Phosphate	-15.64 ± 17.21	0.4594
Ammonia	8.96 ± 11.28	0.5106
CPUE	8.82 ± 18.65	0.6829

Discussion

The aim of this study was to investigate the effect of abiotic variables and invasive crayfish on the amount of coverage and the establishment of *S. aloides* within a polder landscape. No significant effect of abiotic variables on the establishment of *S. aloides* were discovered. However, as noted in the results, some observations across different ditches with varying coverage reveal differences or suggest certain trends, which will be discussed here as well.

Limitations of the study

Invasive crayfish and interactions

Given that this study explores the influence of abiotic variables on the establishment of *S. aloides* in a polder landscape with a high abundance of invasive crayfish, it would have been useful to separately assess both the direct effects of abiotic factors on *S. aloides* establishment and their indirect effects mediated through interactions with invasive crayfish. This was not feasible because the abiotic factors could not statistically be analysed as non-dependent variables because of the reasons described above, where it was mentioned that a linear-mixed model was not possible with this kind of data. Nevertheless, there is no statistical approach available to model the indirect effects of abiotic factors (explanatory variables) on the establishment of *S. aloides* (response variable) mediated by invasive crayfish occurrence. Future research should aim to distinguish and compare both direct and indirect effects to provide more insight as to how each variable individually, as well as in combination, influences establishment of *S. aloides*.

Temporal element

First of all, as seen in the methods for the statistical analysis, the data assembled over the three rounds was averaged. This was due to the fact that there was only one measurement round performed for the coverage of *S. aloides*, and the rest of the data was assembled in a temporal manner over three rounds from April-June. This resulted in a statistical analysis on a small dataset, with small of degrees of freedom. This was not optimal for the analysis, because a mixed-effect model was preferred to account for the interconnected ditches as well as the repeated collection of data for all other variables on the same site. A suggestion for further research on the establishment of *S. aloides* with a setup like this would be to include temporal data, so more measurements of the coverage of *S. aloides* over the years for this same location. This would fix the temporal issue to a certain extent. Apart from the temporal component is the amount of replications for this research question that should be addressed; no significant differences were found meaning no effects of the (a)biotic variables on the establishment were found, but this is solely based on an analysis for three rounds or replications of data.

Small differences between ditches

Because all ditches at the research site were interconnected, replication could not fully be achieved. The potential influence between ditches containing *S. aloides* introduced pseudo-replication. As previously noted, the use of a linear mixed model was not feasible; instead, averaging the data across the three sampling rounds served as a pragmatic alternative. Nonetheless, minor variations between ditches and within measured variables likely persist as a result of this limitation.

***When is S. aloides established*?**

As described in the statistical analysis section, for this study, ditches with >25% *S. aloides* coverage were classified as 'established.' However, in Harpenslager et al. (2016), *S. aloides* populations are referred to as "dense mats of floating vegetation" or "high-density stands." In their study, low-density populations were quantified as 83.4 ± 2.3 g DW m^{-2} (5 plants m^{-2}), while high-density populations were measured at 253.2 ± 1.0 g DW m^{-2} (16.7 plants m^{-2}).

This highlights a challenge: how can such density-based classifications be accurately quantified in an experimental setup like the present study, where observations and measurements using a quadrat were non-invasive and based on visual coverage estimates? From my own observations, the ditches in this study did have one example of an established ditch according to the descriptions above (ditch 1). However, I have seen more ditches with patches of dense mats of floating *S. aloides*. The question is where to draw the line for this study with this experimental setup. The 25% cutoff value seemed appropriate for this study as the ditches that have a coverage of >25% do have dense mats of floating vegetation, but not for the entire ditch. *S. aloides* has been introduced and translocated at the research site multiple times over several years from 2022-2025 (Appendix A). The numbers of (individual) *S. aloides* plants were not monitored in between, until last year for the first time. Last year, the introduction of *S. aloides* seemed to have failed for most ditches except for ditch 1, which contained vast amounts of *S. aloides*. This year, the amount of *S. aloides* has improved, and for the ditches of >25% coverage the term "established" seems to be right as they are growing in populations and have at least that amount of coverage. For future studies (at this research site), the coverage of *S. aloides* should be monitored regularly every year to gain a more complete understanding of the establishment for this particular research site.

Key observations

The impact of abiotic variables on the establishment and amount of coverage of S. aloides

An analysis of several abiotic variables including phosphate, ammonia, dissolved oxygen levels, pH, electrical conductivity, temperature and turbidity within ditches in a polder landscape were performed. The results show that the abiotic factors did not have a significant effect on the establishment of *S. aloides* and on the amount of coverage of *S. aloides* per ditch. As described above, it should be taken into account that, perhaps due to the interconnected ditches (and ditches with *S. aloides* that are directly connected to ditches without *S. aloides*), minimal differences may have been measured due to dilution or movement of water. Because there are no baseline measurements for all ditches, it is hard to say if the measured variables influence *S. aloides* in its establishment, or if it has already occurred the other way around. Furthermore, results that were expected to not indicate a significant difference are listed first. After that, the results that were unexpected will be evaluated.

Electrical conductivity

A plot of the different ditches with their coverage over the three rounds for the EC (Figure 7) showed no pattern. The EC gives an indication of water's ability to conduct electricity, thus how many ionised particles the water contains (Walton, 1989). Factors such as (in)organic compounds or microorganisms can influence the EC but it is found that a higher EC (meaning more ionised particles in the water) indicated bad water quality (Verma & Mehta, 2025). It is believed that *S. aloides* can absorb nutrients (Kufel et al., 2010) which could potentially reduce the concentration of dissolved ions in the water, leading to a decline in electrical conductivity. This implies there could be an effect of *S. aloides* on the EC, but not the other way around.

Phosphate

Although not significant, lower phosphate concentrations seemed to result in a higher coverage of *S. aloides* (Figure 8). But it is important to notice that this effect might be the other way around: *S. aloides* is known for taking up phosphate (Brammer, 1979). A possibility why there is no significant correlation between phosphate and the amount of coverage could be that submerged plants mainly obtain nutrients directly from the surrounding water, while floating plants typically absorb nutrients from the sediment via their root systems (Kufel et al., 2010). As this study was performed while most of the plants that were present were floating as this is part of the lifecycle of *S. aloides* during spring and summer (A. Efremov & Sviridenko, 2012), it could be the case that phosphate was obtained mainly from the sediment instead of the water, resulting in no significant differences. So, in future studies, sediment nutrient levels should be studied as well.

Turbidity

Although not significant, a higher turbidity tends to result in a lower coverage of *S. aloides* (Figure 10). This suggests that more turbid water results in less plant coverage. However, this might be the other way around as *S. aloides* is known to reduce water flow, resulting in less turbid water (Madsen et al., 2001). Biotic factors such as crayfish, who can influence turbidity of water (Roessink et al., 2010) and other factors such as algae, organic matter, and sediment particles, may have confounded potential patterns (Stroud Water Research Center, 2022; Velthuis et al., 2023). The higher turbidity for round 2 compared to round 1 could have been caused by the rise of the temperature, as it is known that with higher temperatures, crayfish activity goes up (Rodríguez Valido et al., 2021) and therefore the sediment could be more disturbed, which could have led to this increase in turbidity (Gao et al., 2024; Palmas et al., 2019). The lower turbidity in round 3 may have resulted from increased nutrient uptake and plant growth due to warmer temperatures (Costa et al., 2023; Zhang et al., 2021).

Water temperature

Water temperature was similar between ditches and did not have a significant effect on the establishment or coverage. This was probably due to the influence of several factors that were alike for all ditches in this study site like sunlight, temperature of the air, and plants that were present in ditches that might provide shade (Rutherford et al., 1997; Stefan & Preud'homme, 1993). Therefore, this variable might not be the best measure.

pH

pH (acidity) was left out of the analysis as described in the statistical methods. It could be that *S. aloides* exhibits a general tolerance to varying pH levels (A. N. Efremov et al., 2019), which may explain why pH would have minimal impact on the overall coverage of *S. aloides*.

Dissolved oxygen

For the dissolved oxygen and *S. aloides* coverage plot (Figure 6), dissolved oxygen (DO) levels are widely scattered and seem to result in a low to moderate plant coverage (0-30%), which did not indicate a trend. Round 3 had lower DO values overall, which was probably due to the fact that temperatures were higher in the last round compared to the other rounds and water with a higher temperature holds less oxygen (US EPA, 2015).

Overall, at low DO levels, a high coverage was observed. This is against the expectations as it is known that aquatic species like *S. aloides* typically enhance oxygen levels within aquatic environments (Ansari et al., 2020). However, in a previous study it was found that a high density of *S. aloides* can lower oxygen levels (Harpenslager et al., 2016), so the effect is probably density-dependent for a high coverage. Observations from earlier research showed that DO-levels were lower in ponds containing crayfish, and argued it could be because of factors such as diminished light availability, crayfish feeding on plants and heightened decomposer respiration (Dorn & Wojdak, 2004), but this is likely not the main cause as high crayfish densities were found in all ditches.

Ammonia

Ammonia levels did not significantly differ between ditches that were labelled as 'established' or 'non-established'. It did not show a significant correlation on the amount of coverage of *S. aloides* either. In Figure 9 the ammonia per round was displayed for each ditch with a different coverage. The ammonium concentrations measured in this study (0–3.3 ppm) indicate low to moderately elevated nitrogen loads in the investigated systems, with some locations approaching high N-loads. Although increased $NH4^+$ availability has been linked to the decline and failed reintroduction of *S. aloides* in the literature (Abeli et al., 2014; Harpenslager et al., 2016; Zantout et al., 2011), this study did not find a significant effect of $NH4^+$ on the establishment of *S*. aloides. This may suggest that most concentrations were below a critical threshold, or that other environmental factors were more limiting for *S. aloides* establishment in these systems than ammonium availability.

The impact of invasive crayfish densities on the amount of coverage and establishment of *S. aloides*

The invasive crayfish, measured in CPUE, did not have a significant effect on the establishment of *S. aloides* or showed a significantly correlation with the coverage, which is against the expectations formulated in the introduction. Round 3 data showed a relatively consistent CPUE range (1.5-2.5) regardless of the amount of coverage (Figure 5). With these results we can't confirm that there is an effect of the crayfish densities, but last year at this research site, results showed two things:

First of all, that higher crayfish densities were present in ditches containing *S. aloides* compared to ditches were no plants were introduced (van Duijn, 2024). This could possibly explain why some ditches containing low coverage of *S. aloides* have trouble to establish due to impacts of the invasive crayfish. It is known that a sustainable and self-facilitating population is density-dependent and that high-densities of *S. aloides* thrive under pressure, while low-densities can't withstand under similar conditions (Harpenslager et al., 2016). The high-density resistance to pressure of factors like invasive crayfish was seen last year as well, as crayfish densities were high in ditch 1 (van Duijn, 2024) but it remained established to this year. As well as the opposite effect where the high densities of invasive crayfish in all ditches (van Duijn, 2024) might have led to *S. aloides'* unsuccessful establishment. It would be unlikely that there is no effect of the invasive crayfish densities on this macrophyte, when we know that these effects could be present.

It is likely that the experimental setup of this research has led to the detection of minimal effects of the CPUE on the establishment of *S. aloides*. This is due to the fact that crayfish could have an effect on *S. aloides* and vice versa. Additionally, the interconnected ditches can influence each other, as well as the complex interactions as seen in the introduction that the statistics could not cover entirely.

Future research

As mentioned before, monitoring the coverage of *S. aloides* over multiple years is important for developing a more robust statistical model and for understanding how various factors influence its coverage over time, rather than relying on a single-year observation. As the study last year at this research site did not observe many *S. aloides* plants in all ditches except for ditch 1 (Appendix A) (van Duijn, 2024), the measured variables in these non-established ditches could potentially function as baseline data when the coverage of *S. aloides* is monitored in future studies at this research site. In this study, ditches 1, 7, and 11 were classified as established, as their coverage exceeded the 25% threshold - a cutoff determined based on the rationale outlined in the opening paragraph of the discussion. In the future, alternative ways to determine a cutoff value for the establishment of *S. aloides* should be considered.

Moreover, conducting research for over the years would improve the study's statistical robustness. In the future, it would be valuable information to focus on separating both direct and indirect effects of abiotic variables and invasive crayfish on the establishment of *S. aloides*. Using a statistical model that incorporates the role of crayfish as a mediating factor in the relationship between water soldier and water quality could provide a more specific understanding of their interactions. Lastly, taking sediment nutrient concentrations into account could add valuable insight to the analysis as it would result in a more thorough perception of the role of *S. aloides* in nutrient uptake and the different drivers of its establishment.

A probable limiting factor for the establishment of *S. aloides* could be ammonia, which was highlighted to be a primary obstacle at elevated concentrations for the establishment of *S. aloides* at low densities of the macrophyte (Abeli et al., 2014; Smolders et al., 2019; Zantout et al., 2011). In this year's study, the effect of ammonia was not found in ditches with elevated ammonia concentrations. It could be that ammonia concentrations were not the only variable playing a role in influencing successful establishment as a limiting factor. Therefore, it is still likely that the invasive crayfish could play a role in this. The crayfish densities which were not found to have a significant effect, are expected to do so in a different experimental setup. Even if abiotic conditions were suitable for *S. aloides* to establish, crayfish may mediate the response of *S. aloides* particularly in low-density populations that lack resilience as opposed to those that are established (Abeli et al., 2014; Harpenslager et al., 2016; Zantout et al., 2011). Apart from that, other biotic pressures could be of influence. Outside of crayfish, studies have shown that fish and birds can influence growth and establishment of macrophytes (van de Haterd & Ter Heerdt, 2007; Veen et al., 2013). For a successful reintroduction of *S. aloides,* an approach that addresses all measured (a)biotic factors and long-term monitoring of the coverage of *S. aloides* is necessary. This study highlights the difficulty of defining clear environmental parameters for the establishment of *S. aloides,* which is vital for its practical implementation to use it as a nature-based solution.

Conclusion

The aim of this study was to investigate the different drivers on the amount of coverage and establishment of *S. aloides* in a Dutch polder landscape. The results indicate that the (a)biotic factors ammonia, phosphate, turbidity, pH, temperature, dissolved oxygen and invasive crayfish did not have a significant effect on the establishment of *S. aloides*. These variables did not significantly correlate to the amount of coverage of *S. aloides* as well. Even though it can't be said that the variables significantly correlate to a higher coverage or may result in the (non-)establishment of *S. aloides,* the findings offer insight as to how the successful reintroduction of the macrophyte can be studied in the future. The results for abiotic variables show no significant effect on the establishment or coverage, but for some of them a hypothesised opposite effect came up, for example for EC, phosphate and turbidity. The results that showed no effect of these variables on established and non-established ditches could indicate that they are not limiting the establishment. However, the surprising result for ammonia showed that there might be an additional biotic limiting factor, e.g. crayfish, contributing to the decline of *S. aloides* when exposed to non-favourable conditions at low coverage. However, both of these variables did not have a significant effect on the establishment or coverage by itself. Therefore, future studies should focus on monitoring the amount of coverage and establishment of *S. aloides* over multiple years in combination with a statistical method or experimental setup in which the interactions between variables can be studied.

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Appendix

Appendix A – *S. aloides* introductions per track and year

Year	Season / Date	Ditch Numbers	No. of Plants Introduced	Observed Before Introduction	Remarks
2022	—	1	>1500	0	-
2023	Spring	2, 4, 6, 5	150–200	0	-
2023	Autumn	6, 7, 8, 9, 2, 3, 4, 5	ca. 1200	0 / unknown	-
2024	12 June	2–9	60–910 (varies by track)	0–100	-
2025	15 & 16 June	2, 3, 4	730–780	0	Partly harvested from ditch 1

Appendix B - Fieldwork schedule

	April			May					June	
	week 15	week 16	week 17	week 18	week 19	week 20	week 21	week 22	week 23	week 24
Abiotics		■				■				■
Degree of coverage *S. aloides*									■	
Crayfish assessment	■				■				■	
HACH abiotics	■				■					■

Appendix C – Crayfish density assessment & HACH measurements randomised schedule

ROUND 1, week 15

1. 11 7 6

2. 12 5

3. 3 4 10

4. 1 2

ROUND 2, week 19

1. 5 4 10

2. 1 2 12

3. 7 11

4. 6 3

ROUND 3*, week 23

1. 12 1 3

2. 4 2 10

3. 6 11

4. 5 7

* For round 3, HACH measurements were performed in week 24 on one day (13-6-2025)

Appendix D – Materials

List 1: Sampling materials for assessing invasive crayfish abundance

- 72 Finnish crayfish traps

- Rope
- 10 kg of 14 mm Halibut Pellets Coppens (from Tijgernoten.nl)
- 10 ditch tracks with water soldier

List 2: Sampling materials used to measure the abiotic water quality

- Portable multimeter (HACH HQ4300)
- pH probe for Portable multimeter (HACH HQ4300)
- Dissolved oxygen probe for Portable multimeter (HACH HQ4300)
- Electric conductivity probe for Portable multimeter (HACH HQ4300)
- pH buffer 4.01
- pH buffer 7.00
- pH buffer 10.01
- Secchi tube
- Checker photometer for phosphate (Hannah Instruments HI713)
- +- 90 packets of phosphate reagent (Hannah Instruments HI713-25)
- Checker photometer for ammonia (Hannah Instruments HI715)
- 2 bottles (7 mL) of ammonia mid-range A (Hannah Instruments HI715A-0)
- 2 bottles (7 mL) of Nessler reagent (Hannah Instruments HI715B-0)
- Demi water
- 10 ditch tracks with water soldier

List 3: Sampling materials for assessing the amount of coverage of S. aloides

- 1m^2 quadrat (handmade, bamboo)
- Rope