Bioremediation potential of Constructed Floating Wetlands (CFWs) in the Limfjord, Denmark

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Abstract: Nutrient enrichment is one of the major global threats to the health of freshwater and coastal marine ecosystems due to the severe environmental effects of eutrophication. Danish estuaries and coastal water have suffered from a noticeable eutrophication impact since the 1950s. The Limfjord is the Danish estuary that receives the highest amount of nutrients with an approximate annual load of 11,000–17,000 t N and 220-400 t P. From the mid-1980s onwards Denmark has undertaken a thorough and efficient strategy to enhance the environmental state of groundwater and surface water by applying several instruments aimed at achieving the nutrient reduction targets. Constructed floating wetlands (CFWs) are a relatively recent bioremediation technology based on a floating vegetated system. This study investigates the bioremediation potential of a Constructed floating wetland with two native species of halophytes (*Aster tripolium L.* & *Salicornia europaea L.*) and two species of filter-feeding bivalves (*Mytilus edulis* & *Ostrea edulis*). Two CFWs were deployed at the Limfjord from 30/06/22 to 09/08/22 and the growth development of all the organisms was measured twice during the experimental period. The results showed that *Ostrea edulis* had a 0% survival success, that the two species of halophytes *Aster tripolium L.* and *Salicornia europaea L.* had a reduced growth rate due to the high salinity levels 29.14($\pm$1.29)ppt and that *Mytilus edulis* had a successful development with an increase in Dry weight and condition index. The findings in this study show that the deployment of CFWs is highly sensitive to salinity levels and that CFWs have the potential to be applied as bioremediation tools in lower salinity areas of the Limfjord.

Table of Contents

Acknowledgments

I would like to show my heartfelt appreciation to my supervisors, Majken Pagter & Niels Iversen at the Department of Chemistry and Bioscience at Aalborg University for their careful guidance and constructive advice during every phase of the thesis. A profound thanks is further directed to Helle Blendstrup her continuous laboratory assistance was greatly appreciated.

Diego Mayorga Rojas 19.08.2022

1 Introduction

Nutrient enrichment of aquatic ecosystems due to anthropogenic pressures like urban growth, industrial development, and intensified agriculture deteriorate the structure and function of ecosystems through eutrophication which is one of the major global threats to the health of freshwater and coastal marine ecosystems [Ansari and Gill, 2014]. From the Arctic to the Antarctic, ecosystems are affected by eutrophication's visible effects [Smith and Schindler, 2009].

The environmental effects of eutrophication include severe changes in pelagic and benthic food web structure, increased phytoplankton biomass, extensive macroalgal blooms, frequent hypoxia events, and alteration of biogeochemical processes [Maar et al., 2021; Riemann et al., 2016]. These effects are not restricted to the biological and ecological characteristics of the habitat as they also influence the ecosystem services and associated activities [Ansari and Gill, 2014].

During the 20th century, eutrophication had a noticeable impact on Danish estuaries and coastal waters, mainly driven by the rising nutrient inputs from agriculture, industry, and urban areas between 1950 and the mid-1980s [Riemann et al., 2016].After world war II synthetic-N fertilizer inputs increased from 15 kg N ha^{-1} in 1945 to 143 kg N ha^{-1} in 1983 at their highest point, with severe environmental issues as a result [Dalgaard et al., 2014].

The Danish estuary that receives the highest amount of nutrients is the Limfjorden, with an approximate annual load of 11,000–17,000 t N and 220-400 t P. The Limfjorden system as a whole is considered to be in "poor ecological condition," with large sections of the system experiencing periodic hypoxia throughout the year [Taylor et al., 2019].

From the mid-1980s onwards, Denmark has undertaken a thorough and efficient strategy to enhance the environmental state of groundwater and surface water [Dalgaard et al., 2014]. Following the adoption of the first Action Plan on the Aquatic Environment in 1987, subsequent Action Programmes have been created to ensure the reduction of nitrogen and phosphorus leaching into the aquatic environment [Danish Environmental Protection Agency, June 2017].

Between 1985 and 2003 the P supply decreased by 869 t $year^{-1}$ (≈69%) and the N supply decreased by 4000 t $year^{-1}$ (≈20%). However, this reduction has not reduced the total area affected by oxygen depletion events occurring in the Limfjorden [Riisgård et al., 2012a].

The first efforts to reduce nutrient loss to the aquatic environment focused mainly on improving wastewater facilities and regulating agricultural sources. After a markedly decrease, the N concentrations have remained fairly constant since 2003 with annual averages of 40-60 μg l^{-1}, and the P levels have been stable since 1998 as seen in Figure 1, because of this, several new instruments aimed at achieving the reduction targets have been adopted in the subsequent Action Programmes.The measures include, among others, stricter catch crops schemes, afforestation, and further re-establishments of wetlands [Danish Environmental Protection Agency, June 2017].

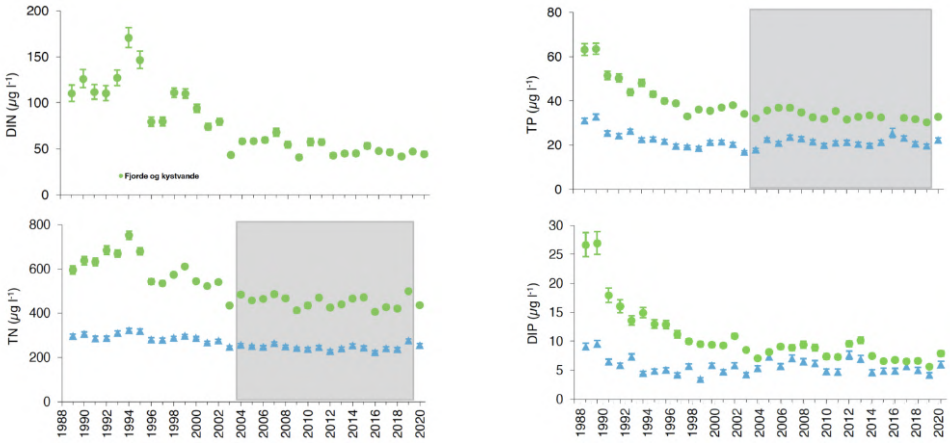


Figure 1. Annual average concentrations (±95 confidence limits)for DIN, DIP, TN and TP for fjords and coastal waters (0-10m)

Natural wetlands suffered from extensive drainage during the 1990s due to agricultural intensification.In Denmark, wetlands were drained by changing the natural course of rivers and channeling water from neighboring areas[Kandel et al., 2019].But, In the past few decades, considerable efforts have been made to restore these highly productive ecosystems.

Some of the ecosystem processes related to nutrient cycling that occur in natural wetlands can be imitated by employing constructed wetlands (CWs) and constructed floating wetlands (CFWs) [Vymazal, 2007].

Constructed floating wetlands (CFWs) are a relatively recent technology that began in the late 1970s to optimize the physical, chemical, and biological parameters of aquatic ecosystems [Shpigel et al., 2013], the technology is based on a floating vegetated system where plants are supported by a buoyant mat on the surface of water bodies [Liu et al., 2021], the roots and rhizomes of the plants develop below the floating device, extending down into the water column and acting as a host for biofilms, zooplankton, microalgae, and small vertebrates and invertebrates which promote the breakdown of pollutants and nutrients, decrease metabolic stress and improve plant health [Shahid et al., 2020].

Transport of total suspended solids (TSS), nutrient cycling and uptake by plants, animals, and microorganisms, nitrification/denitrification, decay, mineralization, immobilization, and degradation of organic pollutants are the main physical, chemical, and biological processes in CFWs [Shpigel et al., 2013].
Additionally, the plant and animal products can be processed into biogas, bio-fertilizers,

and biomaterials as well as being used for human or animal food.Some other advantages of CFWs include easy operation and maintenance, no land usage, economic construction, and floating mats that adjust to the water level.[Li et al., 2010]

While CFWs have been tested in a variety of geographical regions and systems, most of the studies have been carried out under controlled conditions in micro- or mesocosm trials. As a result, in situ applications of CFWs for nutrient removal are still uncommon, particularly in cold climate marine ecosystems[Choudhury et al., 2019; Shpigel et al., 2013]. Diverse strategies to tackle the environmental conditions, and achieve year-round steady operation of CFWs can be applied, such as selecting plants that can thrive in saline environments like halophytes [Webb et al., 2012], and the addition of filter-feeding bivalves [Li et al., 2010].

1.1 Problem statement

Since our understanding of the efficiency of Constructed floating wetlands (CFWs) applied in coastal estuary areas for brackish water purification is still limited, this thesis will experimentally address some of the existing knowledge gaps.

The main objective of this project is to:

Investigate the bioremediation capacity of a Constructed Floating Wetland (CFW) in a brackish water environment in the Limfjorden, Denmark.

The specific objectives include:

- Evaluation of the survival rate and growth characteristics of two native species of halophytes (*A.tripolium* & *S.europaea*) and bivalve filter-feeders (*M.edulis* & *O.edulis*) in a Constructed floating wetland (CFW).
- Assessment of the viability of constructed floating wetlands (CFWs) and its strengthening techniques for improving the environmental state in a brackish water system.

The objectives are based on the following hypothesis:

Constructed floating wetlands (CFW) can substantially decrease nutrient concentrations and improve the environmental state of the Limfjorden, Denmark.

2 Theory

2.1 Constructed floating wetlands (CFWs)

Constructed floating wetlands (CFWs) are an innovative ecological engineering tool that mimics some of the physical, chemical, and biological processes that take place in natural wetlands [Bi et al., 2019]. CFWs have been used to treat stormwater, natural water, agricultural runoff, secondary treated wastewater effluent, and high-concentration wastewater such as domestic sewage and industrial wastewater [Shen et al., 2021]. Studies dealing with industrial wastewater treatment include petrochemical refinery wastewater, mining effluent, meat processing effluent, manure wastewater, swine farm effluent, and aquaculture effluent[Pavlineri et al., 2017]. CFWs are frequently used due to their many advantages: Low investment, high efficiency, no additional land occupation, strong adaptability to water depth, flexible operation, simple maintenance, and green economy [Wang et al., 2020]. They also have profound and long-lasting bottom-up effects on the aquatic ecosystem by potentially enriching biological habitats and enhancing recreational and aesthetic values [Shen et al., 2021].

In CFWs aquatic or terrestrial plants are grown in a buoyant mat. Aerial organs of the plants develop and remain above the water, whereas plant roots are suspended beneath the floating bed and into the water column. The plants absorb nutrients directly from the water using a hanging network of roots and rhizomes covered with biofilms that act as a filter and provide a wide bioactive surface area for the biochemical transformation of pollutants (Figure 3 [Shen et al., 2021]. One of the first reports of their application as a purification tool is from Germany in 1979.

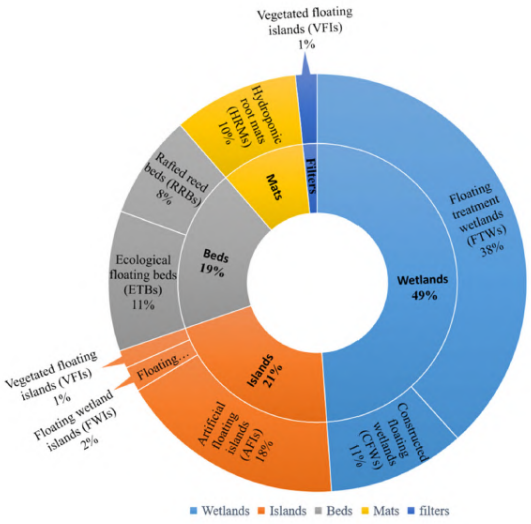


Figure 2. Different terms and their percentage of use by the studies focusing on floating treatment wetlands. Retrieved from [Shen et al., 2021]

This was followed by applications in ponds and reservoirs in the 1980s in other European countries, the US, and Japan [Bi et al., 2019]. Constructed floating wetlands (CFWs) are also known by many other names like floating treatment wetlands (FTWs), artificial floating islands (AFIs), and ecological floating beds (ETBs), see Figure 2.

2.2 CFW component elements and design parameters

The main components responsible for pollutant removal efficiency in conventional CFWs are macrophytes along with the biofilm attached to the roots, and other stable living and non-living surfaces [Bi et al., 2019]. Some of the most significant design parameters include the growth media, water depth, vegetation coverage ratio, and methods for achieving buoyancy. In addition, the purification performance can be enhanced by including additional components and combining other technologies, such as biofilm carriers, filter-feeding bivalves, immobilized microorganisms, and macroalgae [Colares et al., 2020].

Unfortunately, even after more than three decades of the use of CFWs for wastewater and stormwater runoff treatment, there are no fixed design parameters available, as [Colares et al., 2020] stated, the main objective when designing a CFW system is to maximize contact between the root-biofilm network and the polluted water going through the system. Thereby, designing CFWs should consider the diversity of factors affecting the overall performance, and select the optimal parameters based on the local environment and water quality characteristics.

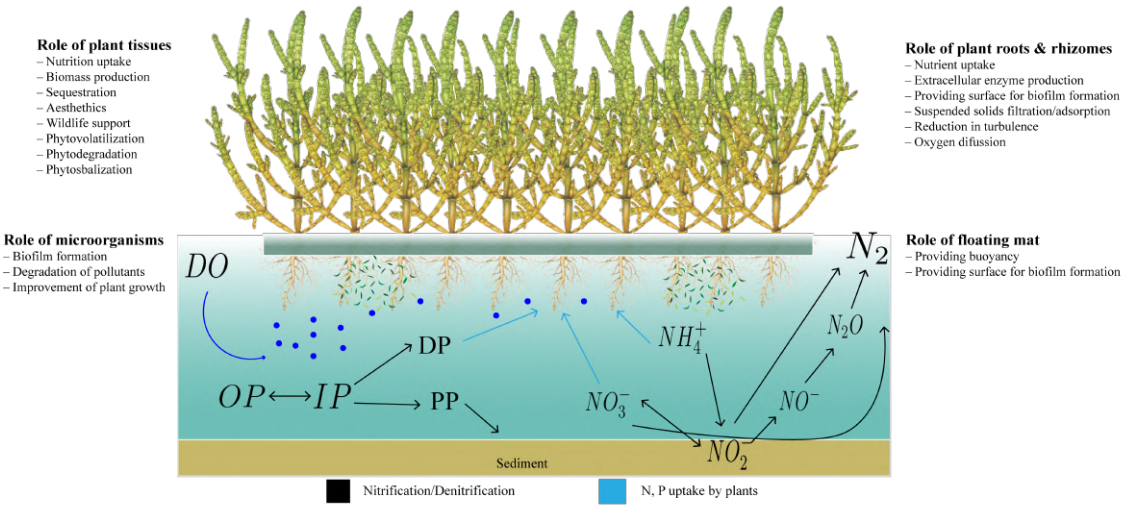


Figure 3. Nutrient removal pathways in CFWs, OP(organic phosphorus), IP(inorganic phosphorus), DP (dissolved phosphorus), PIP (particulate phosphorus), DO (dissolved oxygen).

2.2.1 Vegetation

Macrophytes are key components in CFWs. The primary removal mechanism for dissolved species of nitrogen, phosphorus, heavy metals, and dissolved organic matter is uptake by the roots, along with the translocation and accumulation of pollutants in the above-surface plant biomass. The roots secrete organic compounds and release oxygen, which promotes the breakdown of organic pollutants. Roots also act as a living surface for the development of biofilms and are effective at capturing and settling particle matters. Additionally, plants provide habitat and shelter for a variety of wildlife [Bi et al., 2019].

Macrophyte species selection is crucial, not only for the system performance but also for the local ecosystem integrity. To select the species [Pavlineri et al., 2017] established the following general criteria:

(1) Non-invasive and preferably native species.
(2) Preferably perennial species.
(3) Wetland plants or plants capable to adapt and develop in hydroponic conditions.
(4) Plants with an extensive root system and aerenchyma.
(5) Plants with high nutrient uptake.
(6) Plants adapted to local climatic conditions.

Macrophytes in CFWs uptake nutrients for their growth and reproduction, therefore, the harvesting of biomass can be used to increase N and P uptake and to avoid further reintroduction of nutrients back to the water through decomposition. The recovery of nutrients and the biomass generated from the CFW system can be used to aggregate value and increase revenue for the system. There are two popular types of vegetation harvesting: above-ground tissue harvesting and harvesting the whole plant. Removing the whole plant can overlook reproductive problems and nutrient allocation in vegetation and is more suitable for plants that are harder to harvest, it should be taken into account that harvesting is deeply dependent on the selected species [Colares et al., 2020].

Adding submerged plants can enhance nutrient removal by providing more surface area for the development of biofilms, offering habitat for zooplankton, increasing the dissolved oxygen concentration, and effectively preventing sediment resuspension, which decreases the turbidity and release of phosphate from sediments [Bi et al., 2019].

2.2.2 Biofilm

Biofilms generally begin with the adherence of bacterial cells to natural and man-made surfaces. Adhered bacterial cells produce an extracellular polymeric substance (EPS) matrix that modifies the surface physicochemical properties, and influences the adherence of successive colonizers such as algae, cyanobacteria, and protists. By being the early colonizers' bacteria play a crucial role in determining the structure and function of the mature biofilm. Along with diatoms and other microorganisms, bacteria are responsible for microfouling, allowing the posterior attachment of larger organisms such as algae, mussels, and barnacles which causes macrofouling [de Carvalho, 2018].

Bacteria play an essential role in marine environments, including driving biogeochemical cycles and providing materials and energy to higher trophic levels. Individual cells are shielded by biofilms from environmental stresses, such as desiccation, temperature and pH fluctuation, competition and predation, UV exposure, and depleted nutrient conditions [de Carvalho, 2018].

In CFWs, macrophytes are essential for the establishment, growth, and development of biofilms. The function and structure of microbial communities are modified by plant root exudates, such as organic acids, sugars, and other secondary metabolites.

Additionally, biofilm carriers can be included in CFWs to provide a bigger attachment surface for bacterial growth and propagation, increasing the amount of biomass and the proliferation of slow-growing denitrifying and nitrifying organisms [Nsenga Kumwimba et al., 2021]. Bacterial biofilms attached to biological surfaces have higher biodiversity than those growing on artificial biofilm carriers [Bi et al., 2019].

2.2.3 Strengthening techniques

Although CFWs can improve water quality and potentially enrich biological habitats, there are still many limitations in their practical application and research, particularly in marine ecosystems and locations with harsh cold seasons. Low microbial activity, low metabolic processes, and short-term plant growth period are some of the factors decreasing the efficacy of CFWs. To enhance the purification performance several components can be added to the system, this additional supplements can be aeration systems, artificial biofilm carriers, supplementation of the substrate, seasonal salt-tolerant plant species, shellfish, and seaweeds in marine environments[Nsenga Kumwimba et al., 2021]. A general overview of the biological processes that happen in a CFW when benthic organisms are added to the system can be seen in Figure 4.

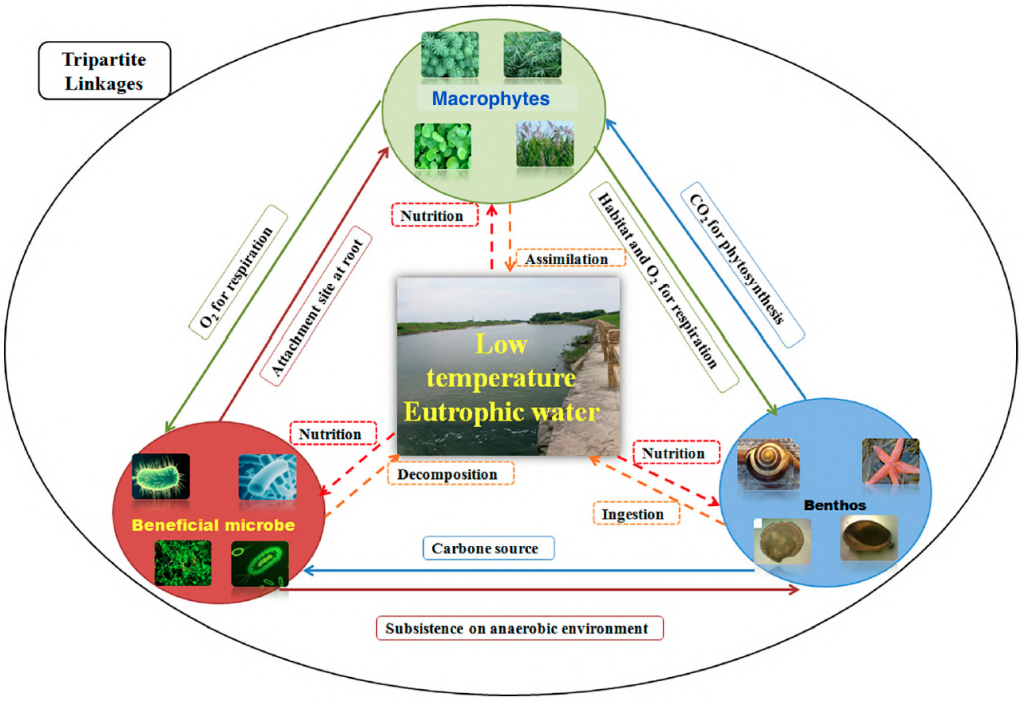


Figure 4. Overview of the synergistic processes between macrophytes, benthos and microorganisms when used as a strengthening technique in CFWs. Figure adapted from [Nsenga Kumwimba et al., 2021]

2.3 Filter-feeding bivalves

The class of Bivalves is the second most diverse among mollusks with more than 10,000 species. Bivalves have a shell excreted by the mantle that also contains the body, the valves from the shell are joined together by a hinge and they possess no head. Most of them are marine organisms, and the rest live in estuaries or freshwater ecosystems. Based on the mechanisms in which they obtain their food bivalves can be classified into four main categories: (1) suspension-feeders, (2) deposit-feeders, (3) carnivores, and (4) wood-consuming.
Suspension-feeder bivalves capture organic and inorganic particles from the water column. Particles enter through the inhalant aperture (siphon) and are captured by the ctenidial filaments (gills), after that a pair of labial palps transport, select, and reject the particles that will be ingested by the mouth and into the stomach, finally, the intestine moves trough the waste to the anus to be expelled as feces. The particles that are too big to be digested are also expelled as pseudofeces [Lobo-da Cunha, 2019].

2.3.1 Nutrient cycling

Bivalves remove and assimilate large quantities of suspended particles such as plankton, bacteria, and detritus from the water column, the waste generated from their digestion is deposited as feces and pseudofeces or excreted as dissolved nutrients where they can subsequently be utilized by the phytoplankton and microorganisms in the sediments [Dame, 2016]. Bivalves contribute to nutrient cycling through remineralization, translocation, and transformation processes, the specific pathways contributing either as a sink or source of nutrients depend heavily on the physical features of the ecosystem (Table 1).

Table 1. Nutrient source and sink processes in suspended mussels & oysters culture types in different water depth systems. Table adapted from [Smaal et al., 2018]

Depth system	Culture type	Regeneration (source)	Retention (sink)	Removal (sink)
Shallow	Suspended	*Pelagic* CO_2 (DIC), NH_4 & PO_4 excretion organisms		*Pelagic* — PON, PON, POP harvest mussel & oyster tissue
		Benthic — CO_2 (DIC), NH_4, PO_4 & Si biodeposit mineralization — NO_2/NO_3 nitrification from NH_4	*Benthic* — PO_4 binding to sediment — POC, PON, POP, POSi burial of biodeposits	*Benthic* — N_2 nitrification/ denitrification from NH_4
Deep	Suspended	*Pelagic* — CO_2 (DIC), NH_4 & PO_4 excretion organisms		*Pelagic* — PON, PON, POP harvest mussel & oyster tissue
			Benthic (deep fjord basin) — POC, PON, POP, POSi burial of biodeposits — CO_2 (DIC), NH_4 ,PO_4 & Si biodeposit mineralization	

Inorganic and organic carbon can be processed and stored by marine bivalves either as calcium carbonate crystals in their shells or from photosynthetically active organisms that they utilize as food. Nitrogen, present in several forms in the marine environment, is removed by bivalves through assimilation into their tissue and shell biomass, and by influencing the biogeochemical processes through their depositions (Figure 5). Assimilation and biodeposition rates depend on complex interactions between biological, geochemical, and physical variables [Kellogg et al., 2014].

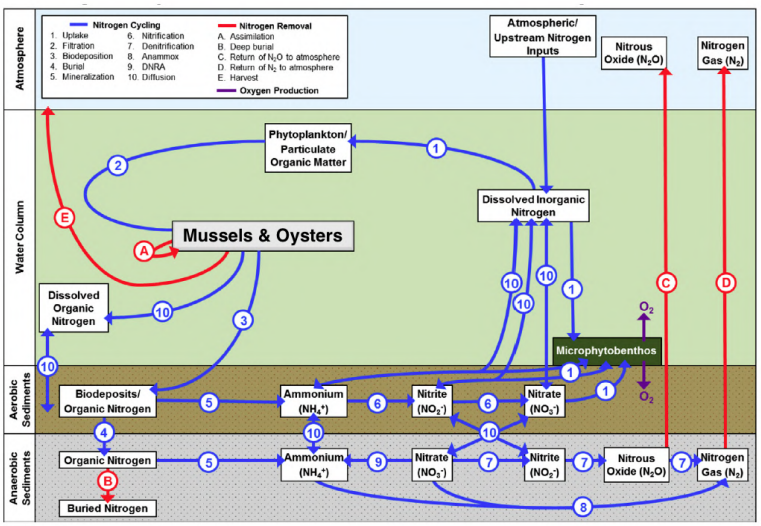
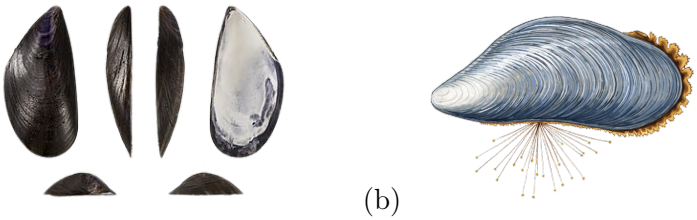


Figure 5. Nitrogen cycling and removal pathways of mussels & oysters. Figure adapted from [Kellogg et al., 2014]

2.3.2 *Mytilus edulis*

Mytilus edulis or Blue Mussels are filter-feeding bivalves. They have a pair of valves made of calcium carbonate and protein, joined together by ligaments and muscles, which they use to protect themselves against the surrounding environment. They secrete byssal threads that allow them to move and attach to almost any substrate. Two short siphons on the inside of their shells direct the water flow in and out [Tyler-Waters, 2008].



(a) (b)

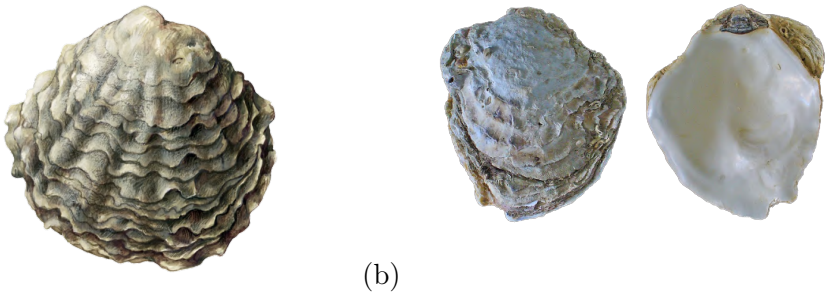
Figure 6. (a) Photos of *M. edulis* shells; (b) Illustration of *M. edulis* showing bysal threads

The shell length usually ranges between 5-10 cm, and the largest may reach 15-20 cm, the colour of the shells is usually blue or purple but sometimes can be brown. The difference between growth rates is mainly due to environmental conditions such as temperature, salinity, food availability, tidal exposure, competition for space and food, parasitism and pollution [Tyler-Waters, 2008].

Several factors contribute to mortality and dynamics of Blue mussels populations, desiccation, intra- and interspecific competition, and oxygen depletion are some of them, but predation is the most important. The vulnerability of mussels decreases as they grow past the preferred size by their predators. They may be preyed upon by starfish, crabs, fish and birds [Tyler-Waters, 2008].

2.3.3 *Ostrea edulis (Linnaeus 1758)*

Ostrea edulis L. or European flat oyster is a sessile bivalve that belongs to the Ostreidae family with a similar external appearance to many other Ostrea species. *O. edulis* is relatively oval in shape formed by two valves, a flat dorsal valve that sits within a concave central valve, joined together at the hinge. The dorsal valve is mainly brown, and the ventral valve exhibits a mix of colouration's that include brown, red, green, white, yellow, pink, purple and blue [Colsoul et al., 2021].



(a) (b)

Figure 7. (a) Illustration of *O. edulis* concave central valve; (b) *O. edulis* Photo of both valves

The internal anatomy of *O. edulis* consists of multiple organs, including a single adductor muscle firmly attached to both valves that controls the opening and closing movements that the organisms employs for protection against disturbances or for feeding purposes. The pseudofeces are regularly extruded by the closure of the two valves whilst the true feces are extruded trough the anus [Colsoul et al., 2021].

Juveniles of *O. edulis* normally develop as males until they are approximately two years of age, after which a gender alternation happens. The number of larvae produced by O. edulis has been reported to be proportional to the size (mean diameter). Their capability of producing off springs, usually happens between 2-3 years after settlement when they are sexually mature [Kamermans et al., 2020].

2.4 Halophytes

Halophytes are those plant species that can maintain their morphological, anatomical, and physiological characteristics and complete their life cycle even under high saline conditions, over 200 Mm NaCl. Halophytes are vastly diverse around the world, as they have developed their tolerance mechanisms according to the specific characteristic of the saline environment where they grow. Halophytes can be a good source of food, fuel, fiber, and compounds with medical importance, which makes them a good substitute for conventional crops.

The classification of halophytes is dependent on many factors, ecologically, they can be classified as (a) obligate, (b) facultative, and (c) habitat-indifferent [Hasanuzzaman et al., 2014].

Table 2. Ecological classification of halophytes, and their respective growing conditions.

Type of halophytes	Growing conditions
Obligate	Exclusively growing in saline environments. They show sufficient growth and development under high saline conditions.
Facultative	Capable of establishing themselves on salty soils. Their optimum development lies in a salt-free or low-salt habitat.
Habitat-indifferent	Able to cope with salty soils. They usually grow in salt-free habitats.

Salt tolerance mechanisms

Halophytes have evolved to tolerate high concentrations of salts through a diversity of strategic, adaptive, and fundamental defense mechanisms that work at the cellular level. Halophytes are capable of absorbing and storing ions such as Na^+,Cl^- and K^+ in the vacuole to avoid cytoplasmic toxicity, and in older leaves where leaf senescence appears first, they are also able to exclude the accumulation of Na^+ by decreasing the number of ions uptaken by cells in the root cortex, and by lowering the root permeability which actively helps in salt exclusion (Figure 8).
In addition, halophytes increase the activity, expression, and biosynthesis of enzymatic and non-enzymatic antioxidant compounds to protect the cells from oxidative stress induced by the formation of reactive oxygen species (ROS) under high salinity conditions [Hasanuzzaman, 2020].

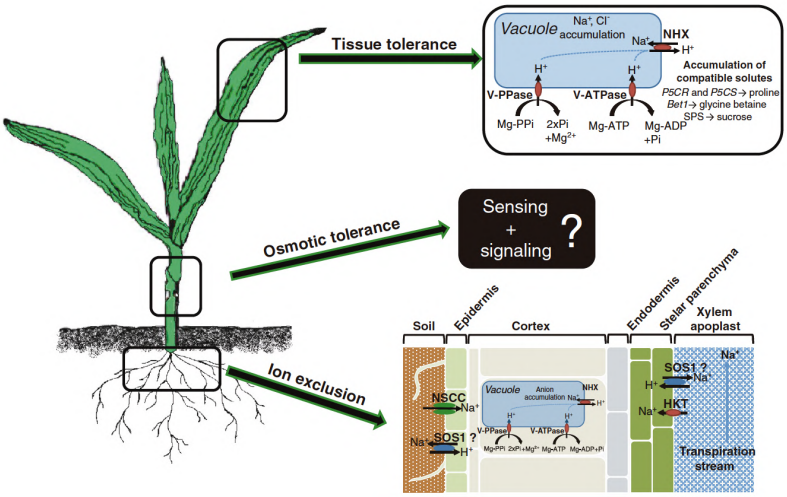


Figure 8. Main mechanisms of salinity tolerance in halophytes. Figure retrieved from [Roy et al., 2014]

Many halophytes develop roots, steam, and/or leaf adaptations to withstand stress conditions, the root vascular system is reduced, stems develop succulence, and leaves are generally small, and succulent. Their coping mechanisms make them optimal candidates for marine CFWs, and coastal environmental restorations [Ben Hamed et al., 2021].

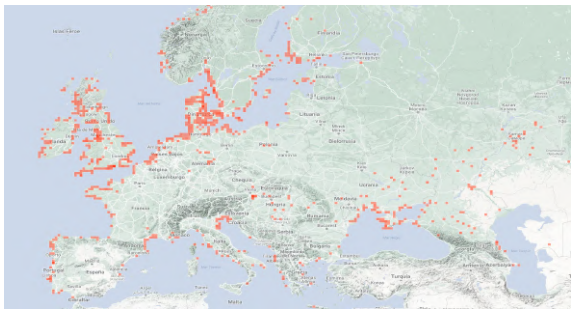
2.4.1 *Aster tripolium L.*

Aster tropolium L. is a common salt-marsh plant belonging to the Asteraceae family that grows in estuaries with frequent tidal inundations, but can also be found in non-saline soils. It commonly appears in areas dominated by *Salicornia*, *Spartina stricta* or *Suaeda maritima*, and may assume dominance or co-dominance with *Glyceria maritima* in their next stage of succession [Clapham et al., 1942]. *A. tripolium* has developed complex tolerance mechanisms that allow it to grow in highly saline soils, up to 300 mM NaCl [Ventura and Sagi, 2013]. It is a novel kind of food source for human and animal consumption with high levels of omega-3, and omega-6 [Duarte et al., 2017].
Additionally the phytoremediation efficiency of xenobiotics reported by [Turcios et al., 2021] has proven its potential as an halophyte that can be used in different saline environments for water treatment.

Table 3. Morphological and phenological characteristics of *A. tripolium*

Aster tripolium L., Tripolium pannonicum (Jacq) Dobrocz.; *Tripolium pannonicum subsp. tripolium* (L.) Greuter	
Stem	Erected to 30-60cm, glabrous, leafy, branched.
Leaves	Alternate, fleshy, three-nerved, entire or faintly denticulate.
Flowers	Disk florets yellow, ray florets right blue to lilac, occasionally white. Plants flower in their second year.
Root morphology	Swollen, subject rhizome only (1.3-3.8 cm) in length, numerous adventitious roots extending to a depth of (15-30 cm), the rhizome and roots eventually acquire a corky covering.
Longevity	Perennial, short lived. The leaves of next season remain above the surface during winter.
Phenology	Germinates from March inwards, shoots appear in April or May, flowers in August and September and fruits are dispersed from October onwards.

(a)



(b)

(c)

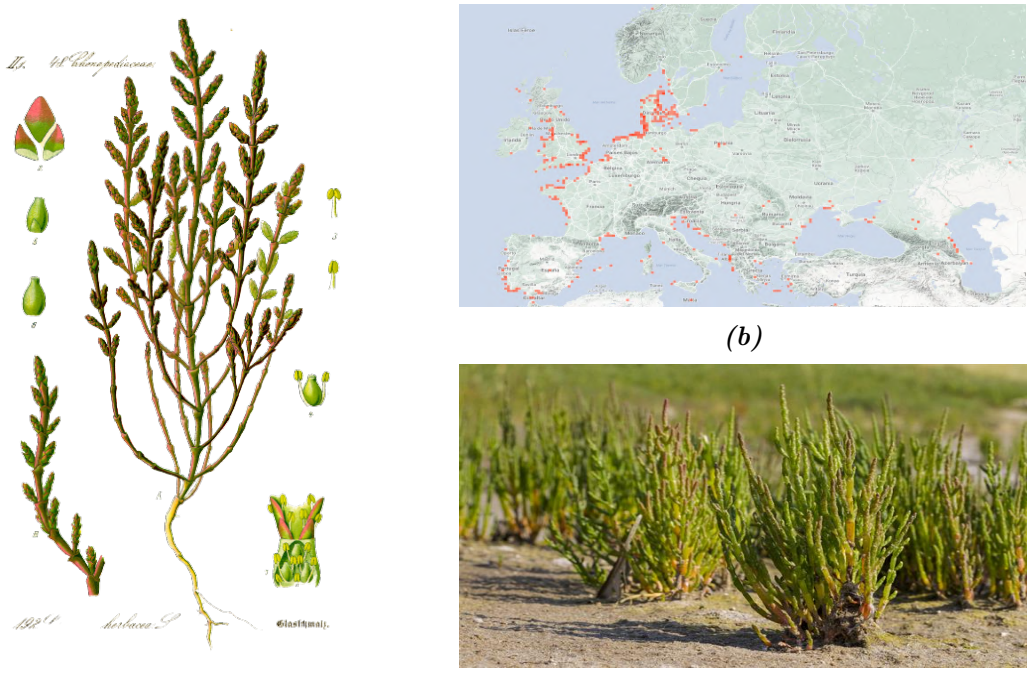
Figure 9. (a) *Aster tripholium L.* illustration from «Bilder ur Nordens Flora» Stockholm 1926, (b) *A.tripolium* distribution map based on reported sightings from (https://www.inaturalist.org), (c) Photo of *A. tripolium* during flowering stage

2.4.2 *Salicornia europaea L.*

Salicornia europaea L. belongs to the Amaranthaceae family, subfam. Salicornioideae, and consists of plants capable of grow in highly saline areas in temperate and subtropical regions around the world. *Salicornia spp.* have been reported to grow under hyper-saline drainage water concentrations, up to 500 mM NaCl by [Ventura and Sagi, 2013]. Many applications have been suggested for *Salicornia* species due to its richness in secondary metabolites, minerals, and vitamins. Additionally, *S. europaea*, and *A. tripolium* have been applied in CFW for the removal of nutrients from marine aquaculture, producing high-yields of valuable by-products [Cárdenas-Pérez et al., 2022].

Table 4. Morphological and phenological characteristics of *S. europaea*

Salicornia europaea L.	
Stem	Erect to 35cm, fairly richly branched, branches with a succulent covering. Dark green and ultimately flushed pink or red.
Leaves	Opposite, connate, highly reduced leaves generate from each stem node.
Flowers	Central flower and lateral flowers, deeply embedded in fleshy tissue. Flowers are usually arranged in triangles.
Root morphology	Root systems tend to be superficial, highly branched, and woody main roots. The upper part of the main root and lower regions of the stem are surrounded by a thin layer of cork.
Longevity	Summer annual that perennates as a seed bank in salt marshes.
Phenology	Germinates from early January to late April. Vegetative growth ends in late July or August. Flowers from mid-August to mid-September but flowers may be seen from late July. Seeds are fully mature from mid-September and get dispersed from the dead parent plant.



(a) ***(b)*** ***(c)***

Figure 10. (a) *Salicornia europaea L.* illustration from Flora von Deutschland, Österreich und der Schweiz 1885, (b) *S. europaea* distribution map based on reported sightings from (https://www.inaturalist.org), (c) Photo of *S. europaea* during vegetative stage

3 Materials and Methods

3.1 Study site

Figure 11. Geographical location of the study site in the Limfjord marked by a red shaded square with the specific location of the pilot experiments indicated with a blue square

The Limfjord is a eutrophic 1,500km^2 water system that connects the North Sea via Thyborøn Kanal in the west, with the Kattegat in the east. It has a mean annual water volume of 7.4km^3 with a freshwater inflow of 2.6km^3. It is a shallow water system with an average depth of 5m and a tidal amplitude of 10-20cm, and a salinity gradient that goes from west to east with brackish conditions in the inner areas.[Riisgård et al., 2012a].

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From 30/06/2022 to 09/08/22 two CFWs were deployed in the Limfjord next to Lindholm Strandpark ($57°03'36.6''N 9°54'35.4''E$).The CFWs were kept in place by using the rafts from Aalborg Fjordhaver as tying support. The placement site was 10 and 12 meters away from the shore for Platforms A and B, respectively, and the water depth was variable with a minimum of 2.5m. The exact location is shown in Figure 12.

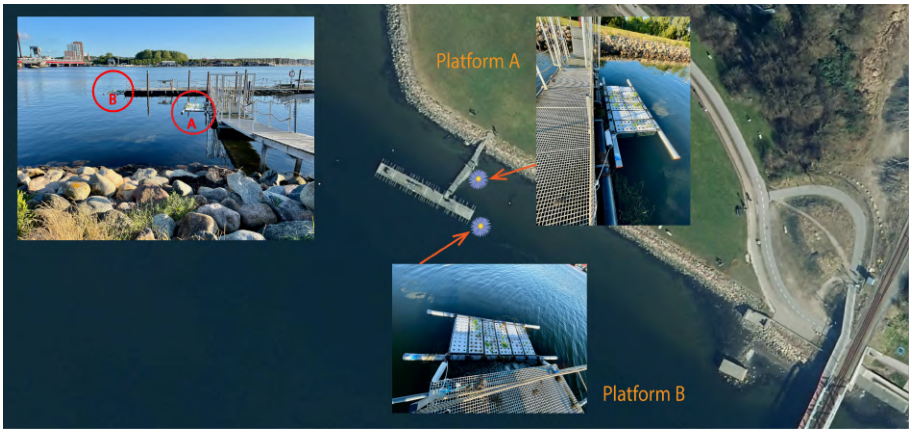


Figure 12. Satellite and photographic view of the experimental site showing the exact location of both CFWs (Platform A & Platform B)

3.2 Design & assemblage of the Constructed floating wetlands (CFWs)

Two constructed floating wetlands were built, each one of them was assembled with two PVC pipes (125mm Ø × 4m Length) joined together by 7 steel pipes (30mm Ø × 1.35m Length) fixed from both ends using saddle clamps, the structure provided strength and buoyancy to the system. The device was divided into two sections of 6 modules (Figure 13).

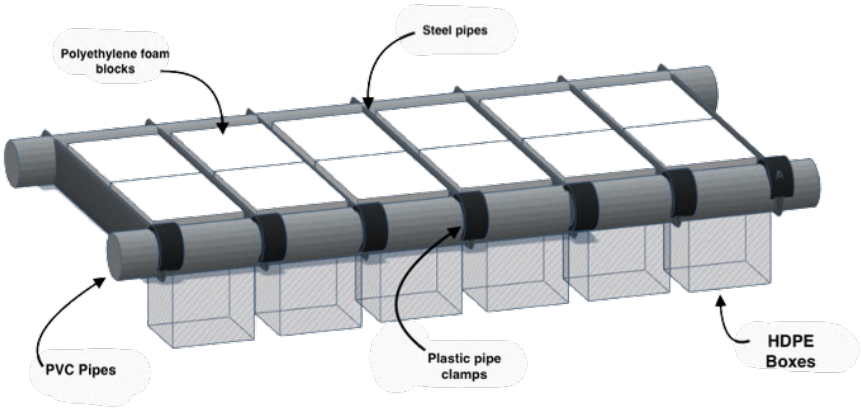
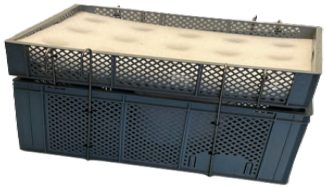


Figure 13. 3D-Model showing the structural and support components of each CFW

Each one of the modules was assembled with two perforated HDPE boxes (Dansk Transport Emballage A/S, Vojens, Denmark) of different sizes; (L×W×H, 600×400×274 mm) for the bottom box and (L×W×H, 600×400×121 mm) for the top box. On the upper box, a double-layered polyethylene foam block (RAJA PACK Ltd, UK) was perforated and held in place to provide support for the plants (Figure 14b,14c). The modular boxes were hanged securely to the system with steel hooks of two different sizes. (Figure 14a).

(a)

(b)



(c)

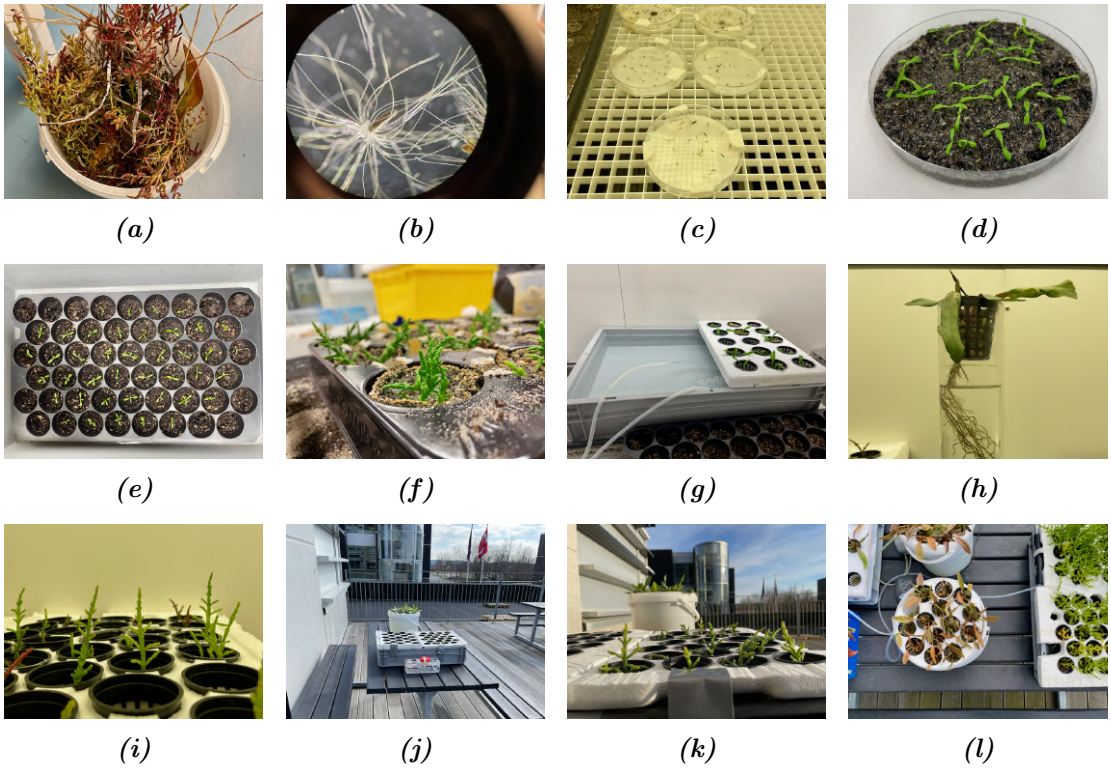
Figure 14. (a) Picture of the CFWs base structure, (b)Side view of the modular boxes showing both levels and the steel hooks, (c) Top view of the modular boxes with the perforated polyethylene foam blocks

3.3 Plant material

Salicornia europaea L. and *Aster tripolium L.* seeds were collected at Løgstør Bredning ($57°00'05.5''N 9°17'12.0''E$) the last week of October 2021 when the seeds of both species started to get dispersed (Figs 15a, 15b). Following collection, the seeds were stored in dry conditions at 5^{o}C for six weeks to simulate cold stratification conditions. A second plant material collection was done on the 10th of June 2022 in which young plants of ≈10cm tall of *A. tripolium* & *S. europaea* were collected at the same location that the seeds at Løgstør Bredning.

After the cold stratification period *A. tripolium* seeds were placed on moistened filter paper in Petri dishes (Fig. 15c) and *S. europaea* seeds were placed in Petri dishes with a mix of 50:50 sand:soil. The seeds were germinated in a temperature and relative humidity controlled growth chamber (Snijders Scientific, Jumo Imago F3000, Netherlands) with a 16-/8-h photoperiod, $20/18^{o}$C (day/night) air temperature, and $65 \pm 5\%$ relative humidity with a light irradiance of 25 μmol m^{-2} s^{-1}(Fig. 15d).

Germinated seedlings after a 3–4 week period were transplanted into seedling trays with a 50:50 sand:soil mixture (Fig. 15e), seedlings were irrigated with tap water as needed and twice a week half-strength Hoagland solution until they reached 1-2cm shoot length(Fig. 15f). The growth chamber was set to the same air temperature and (day/night) length conditions, with a higher light irradiance of 305 μmol m^{-2} s^{-1}.



(a) ***(b)*** ***(c)*** ***(d)*** ***(e)*** ***(f)*** ***(g)*** ***(h)*** ***(i)*** ***(j)*** ***(k)*** ***(l)***

Figure 15. Pictures illustrating the different stages of *A. tripolium* and *S. europaea* preparation, from seed collection until the final growing stages in the hydroponic systems.

When the plants reached a size of ≈5cm they were transferred to plastic hydroponic net pots (5.5cm Ø) with stone wool as a substrate (Grodan Vital, ROCKWOOL B.V.) and

placed in hydroponic systems (Fig. 15h). Polypropylene containers (L×W×H, 600×400×170 mm) (Dansk Transport Emballage A/S, Vojens, Denmark) with a capacity of 16 L were used as tanks for the hydroponic systems, tanks were filled with 14 L of solution and they were constantly aerated by small compressors (At-a9500, ATMAN)(Fig. 15g). During the first three weeks plants were grown using solution **A** (Table 5). The salinity of solution **A** was increased stepwise over two weeks by adding 5 g/L of NaCl until a 25 g/L NaCl final concentration was obtained to avoid osmotic impacts on the plants.

After reaching the desired salinity concentration, the solution from the hydroponic systems was changed to solution **B** (Table 5) prepared with artificial seawater (Tetra Marine SeaSalt, Hanover, Germany) with extra nutrients to prevent a shortage. The growth chamber was set to 20/18ºC (day/night) air temperature with an elongated day length of 18h to prevent flowering of the plants, and a light irradiance of 305 μmol m^{-2} s^{-1}(Fig. 15i).

Table 5. Formula for the hydroponic solutions modified from [Turcios et al., 2016] using $C_6H_8O_7$.Fe.H_4N instead of Fe−EDDHA

Solution A		**Solution B**	
Formula	Concentration [mg/l]	Formula	Concentration [mg/l]
KNO_3	303	Tetra Marine SeaSalt	25,000
$Ca(NO_3)_2.4H_2O$	472	$NaNO_3$	303
$NH_4H_2PO_4$	115	$H_2NaO_4P.H_2O$	44
$MgSO_4.7H_2O$	123	$C_6H_8O_7$.Fe.H_4N	0.28
KCl	1.865		
H_3BO_3	0.775		
$MnSO_4.H_2O$	0.17		
$ZnSO_4.7H_2O$	0.29		
$CuSO_4.5H_2O$	0.06		
$MoNa_2O_4.2H_2O$	0.06		
$C_6H_8O_7$.Fe.H_4N	0.28		

Four weeks before transferring the plants to their final location on the floating platforms, the hydroponic systems were moved from the growth chambers to an exterior location at the university building (Fredrik Bajers Vej 7H, 9220 Aalborg Øst, Danmark) to expose the plants to uncontrolled environmental conditions (temperature, light, humidity, precipitation & wind) similar to what they were going to experience once they were placed at the Limfjord (Figs 15j, 15l). The young plants collected in June were transferred to the same hydroponic systems at the exterior terrace of the university building, the salinity concentration of the water in which the young collected plants were grown was increased stepwise over a week until a 25 g/L NaCl final concentration was obtained(Fig. 15k).

Temperature and relative humidity were measured every hour during those four weeks with an RH/Temp Data Logger (EL-USB-2+, LASCAR electronics, UK) with minimum and maximum values of (-3ºC - 30ºC) and (22% - 96.5%) registered for temperature and humidity respectively.

3.4 Bivalves

Mytilus edulis of different sizes were collected in two different sites, the first sampling was at the end of June in the Limfjord ($57°03'49.93''N 9°53'41.71''E$) close to Lindholm Brygge 31, Aalborg. The second batch of collected organisms was obtained from a fishing vessel in Løgstør Kanalhavn ($56°58'02.7''N 9°14'42.9''E$) in the first week of July.
Ostrea edulis were obtained at Havnegade 20, 7790 Thyholm ($56°39'07.5"N 8°38'08.5"E$) from a fishing company (Jensen Fiske- & Muslingeexport AS, Denmark) in the first week of July.

To record the changes in the organisms during the experimental period, 450 *M. edulis* and 68 *O. edulis* were tagged, numerical tags were printed with a label printer and adhered to the organism with superglue (Loctite SuperGLue, Henkel, USA). The organisms were kept for 96h at 10 ºC in fish tanks with water collected from the Limfjord, supplied with constant oxygen using small air compressors (At-a9500, ATMAN).

Figure 16. *O. edulis* and *M. edulis* with the printed and adhered tags

3.5 Experiment

For the final experimental assembling of the Floating devices, the pots with the plants previously grown in the hydroponic system were inserted into the holes of the polyethylene foam blocks(Fig. 18b), and the bivalves were placed in the bottom boxes of the modules that were completely immersed in the water column (Fig. 18a). In each one of the platforms (A and B) 60 *A. tripolium*, 60 *S. europaea*, 400 *M. edulis* and 48 *O. edulis* were placed following the plan that can be seen in Figure 13 and Table 6 after being randomly selected.

Table 6. Distribution plan for the organisms in the CFWs divided by modules

Module	Organism(n)	Module	Organism(n)
A	*S. europaea (15)*	F	*M. edulis (100)* *A. tripolium (15)*
B	*A. tripolium (15)*	G	*O. edulis (12)* *A. tripolium (15)*
C	*M. edulis (100)* *S. europaea (15)*	H	*O. edulis (12)* *S. europaea (15)*
D	*M. edulis (100)* *S. europaea (7)* *A. tripolium (8)*	I	*O. edulis (12)*
E	*O. edulis (12)* *S. europaea (8)* *A. tripolium (7)*	J	*M. edulis (100)*

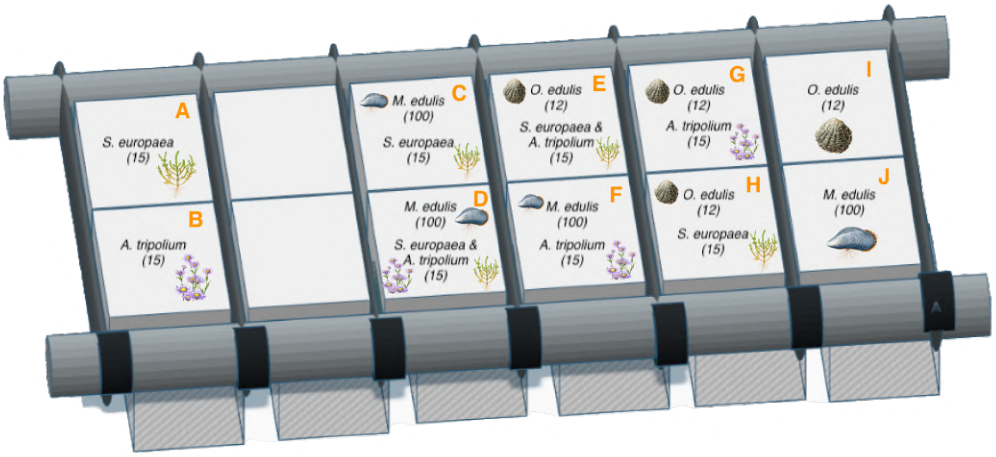


Figure 17. Distribution plan for the organism in the CFWs

The floating platforms were deployed at the Limfjord on the 30th of June (Figs 18c, 18d, 18e), an acclimatization period of 25 days was considered before the first sampling. Two sample collections were done (Halophytes & Filter-feeders), the first on the 26th of July and the last on the 9th of August. During the experimental period, a visual assessment of the organisms was done every 48h by registering the color of leaves and wilting symptoms of the plants as well as the survival rate.

Physicochemical parameters of the Limfjord water at the experimental location were measured with a Hydrolab DS5X Water Quality Multiprobe (Hach Environmental, Loveland, USA) deployed next to Platform B 1 m below the water surface. Temperature, salinity (HYDROLAB conductivity sensor,Hach Environmental) , dissolved oxygen (DO) (Hach LDO Sensor, Hach Environmental), and chlorophyll a (Chlorphyll a fluorometer sensor, Hach Environmental) were recorded hourly by the data logger. The logger was taken to the lab once during the experimental period for cleaning purposes.

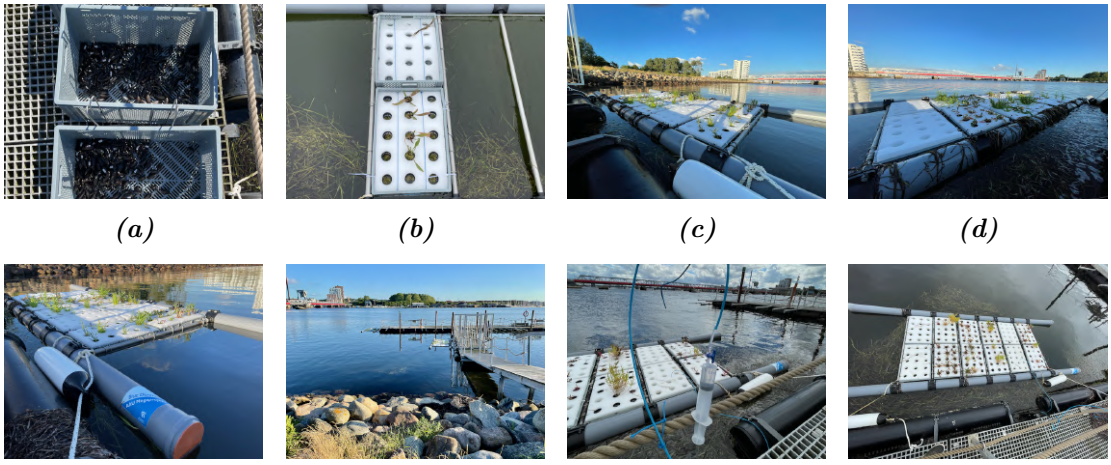


Figure 18. Pictures illustrating the CFWs setup at the study site

Meteorological data from Aalborg Kommune weather station (Temperature, humidity, rain, wind speed and solar radiation) based on 10km x 10km grid interpolated averages and the daily tidal amplitude of the Limfjord were retrieved from the Danish Meteorological Institute (DMI) website.

3.6 Collection & analysis of water samples

Unfiltered water samples were collected once a week in 5 L bottles for quantifying Chlorophyll a. Samples of 1.5-2 L were filtered through a Whatman GF/C filter and extracted in 96% ethanol for 20 hours, after the extraction period the samples were centrifuged for 10 minutes at 10,000 m/s^2 (2500rpm). The absorbance of the extracts was measured at 665 and 750 nm using a Genesys 20 spectrophotometer (Thermo Spectronic, USA), and the concentration of chlorophyll a was calculated according to equation 3.1

$$C_V = \frac{10^4 * V_e * A_{665K}}{83.4 * V_f * l} \tag{3.1}$$

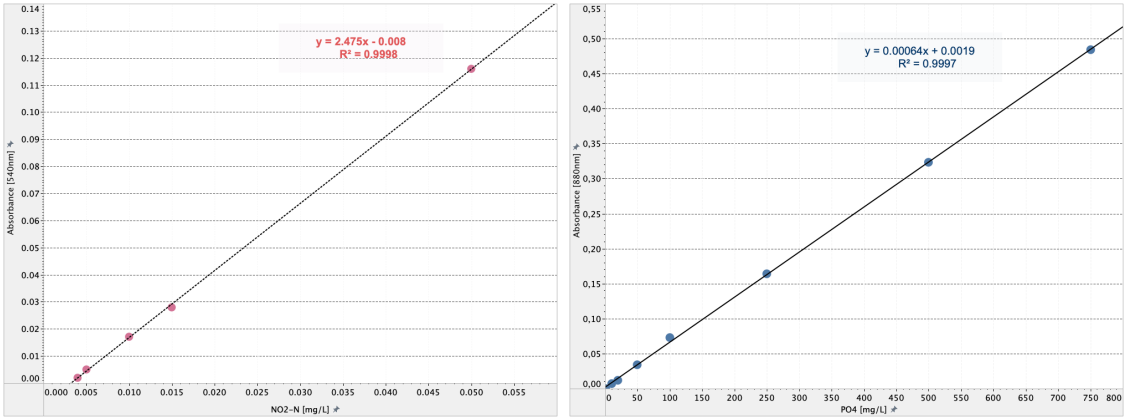
where:

C_V = Chlorophyll concentration of the water sample, μ g l^{-1}
V_e = Volume of ethanol extract, in ml
A_{665K} = A_{665nm} − A_{750nm}
83.4 = Absorption coefficient in 96% ethanol, lg^{-1} * cm^{-1}
V_f = Filetered water volume, in ml
l = Lenght of cuvette, in mm

Laboratory test results from chlorophyll a quantification were compared with the field readings from the Hydrolab DS5X data logger.

Water samples were also taken from inside both platforms and in a single location close to the experimental setup every 48h from 13:00-14:00. For each of the three sampling locations, 5 replicates of 15ml were collected and immediately filtered through a 0.45-μm cellulose acetate syringe filter (Whatman, Maidstone, United Kingdom), the samples were sealed and kept frozen at -18ºC for their later analysis (Figs 18g, 18h).

Using the filtrated water samples NH_4-N and NO_3-N were determined by a colorimetric reaction using a Technicon TrAAccs 800 (Technicon Industrial Systems, Tarrytown, NY). NO_2-N was determined by a spectrophotometric procedure according to DS 222:1975 in which by applying the Griess reaction sulfanilamide added to each sample forms a diazonium salt that reacts with N−(1-naphthyl)−ethylenediamine−dihydrochloride (NED dihydrochloride) forming a pink coloured dye that was measured at 540nm using a UV-Vis Genesys 20 spectrophotometer (Figure 19a).



(a) *(b)*

Figure 19. (a) Standard curve of know concentrations NO_2-N in (mg/L) vs. absorbance at 540nm, (b) Standard curve of known concentrations of $PO_4{}^{3-}$ in (mg/L) vs. absorbance at 880nm

Dissolved Orthophosphate ($PO_4{}^{3-}$) was determined by the ascorbic acid reduction method described by Murphy and Riley [1962], in which the existing phosphate of the samples is transformed into ammonium phosphomolybdate by adding a mixed solution of sulfuric acid, potassium antimonyl tartrate, and ammonium molybdate, that when reduced to ascorbic acid forms a blue dye that was then measured with a UV-Vis spectrophotometer at 880nm (Figure 19b).

3.7 Collection & analysis of organisms

Plants and bivalves were sampled twice, the first sampling (**S1**) was done the 26/07/2022, and the second sampling (**S2**) two weeks after on 09/08/2022. In each sampling 50% of the plants alive from each species were randomly harvested. Once at the laboratory, the collected plants were rinsed with distilled water and divided into shoots and roots. Organs were measured and weighed to obtain the root and shoot length, leaf area, leaf thickness, and fresh weight (FW) before being dried at 65ºC until constant weight for 48-72 h using a (Memmert GmbH, Germany) drying oven to estimate the dry weight (DW) values. The leaf thickness was measured from 50 randomly selected leaves with a digital caliper to the nearest 0.1mm, after the thickness was measured photos of each leaf were taken with a white background, and the photos were then processed with a mobile application (LeafByte, version 1.3.0) that uses background removal and scale identification to count the pixels and perform a calculation of the area in cm^2.

Number of leaves, nodes, stems and water content (WC) were also measured from each collected plant. The water content percentage (WC %) was calculated as follows:

$$WC\% = \left(\frac{FW - DW}{FW}\right) * 100$$

(a) ***(b)*** ***(c)*** ***(d)***

Figure 20. Pictures illustrating two of the initial steps in the measurement procedures for *A. tripolium* and *S. europaea*

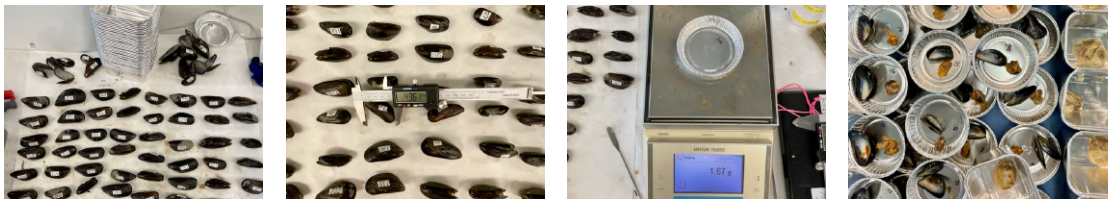
For Mussel and Oysters, on each sampling date, half of the tagged bivalves were collected and transported to the laboratory where they were cleaned thoroughly to remove as many fouling organisms as possible(Fig. 21a), once they were cleaned the individual wet weight was registered(Fig. 21c). Length and width were measured from each one of them with a digital caliper (±0.01 mm)(Fig. 21b), and finally, the mussels and oyster were opened and their tissues were separated from the shells to be weighed and dried at 65ºC until constant weight for 48-72 h to estimate the dry weight (DW) values (Fig. 21d).

For calculating the condition index (CI,$mg.cm^{-3}$) of *M. edulis* equation 3.2 from [Riisgård et al., 2012b] was applied.

$$CI = \frac{W}{L^3} \tag{3.2}$$

where:

W = Dry weight of soft tissue, in mg
L^3 = Shell length, in cm



(a) *(b)* *(c)* *(d)*

Figure 21. Pictures illustrating some of the laboratory measurement procedures for *M. edulis* and *O. edulis*

And for estimating growth rates, the equations from [Pleissner et al., 2012] were used. Actual growth rate (mg day^{-1}) was estimated with with equation 3.3 and the weight specific growth rate (% day^{-1}) with equation 3.4.

$$G_{act} = \frac{W - W_0}{\Delta t} \tag{3.3}$$

$$\mu = \frac{ln\left(\frac{W}{W_0}\right)}{\Delta t} * 100 \tag{3.4}$$

where:

W_0 = Dry weight of soft tissue at time 0, in mg
W = Dry weight of soft tissue at the end of experiment, in mg
Δt = Duration of the experiment, in days

The calculation of *Ostrea edulis* condition index (CI) was done using equation 3.5 as described by [Walne and Mann, 1975].

$$CI = \frac{(DW_T * 1000)}{DW_S} \tag{3.5}$$

where:

DW_T = Dry weight of soft tissue, in g
DW_S = Dry weight of the shell, in g

3.8 Statistical analysis

Normality assumptions were tested using the Shapiro-Wilk test and visually by using $Q-Q$ normal plots and histograms. Normally distributed data were analyzed using Fisher or Welch one-way ANOVA and non-parametric data was analyzed with the Kruskal-Wallis H test. Effect sizes were estimated with $Eta - (\eta)$ squared ω_p^2 for parametric data and non-parametric with rank epsilon squared (ϵ^2), and for the pairwise comparisons Games-Howell test or Students t-test for parametric data, and Dunn test for non-parametric data. All statistical analyses were performed using R Statistical Software (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria).

4 Results

4.1 Physicochemical parameters

Water parameters

The time-series graph and summary of the physicochemical parameters recorded during 50 days at the study location can be seen in Figure 22 and Table 7, respectively.

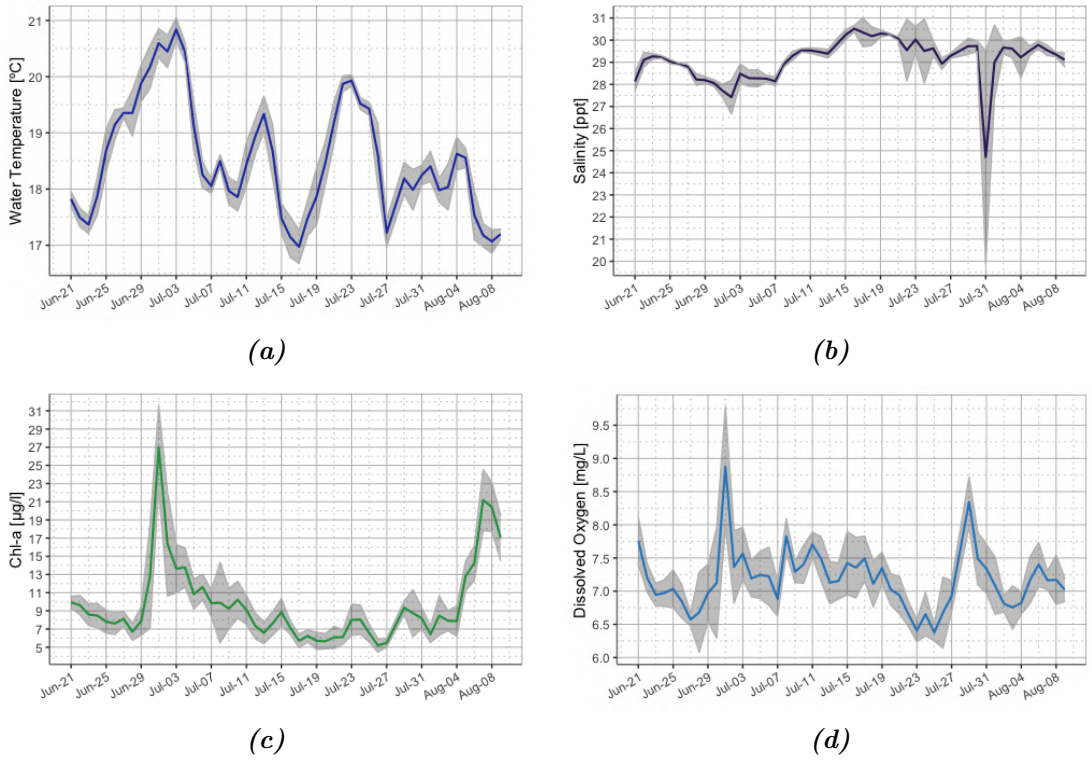


Figure 22. Time series of the mean daily values of **(a)** Water temperature (ºC), **(b)** Salinity (ppt), **(c)** Chl-a (μg/L), and **(d)** Dissolved oxygen (mg/L) measured with the DS5X-Hydrolab at the study location from 21/06/22 to 09/08/22.

Table 7. Minimum, maximum and mean values (±SD) of the water physicochemical parameters measured at the study location from 21/06/22 to 09/08/22

Parameter	Min	Max	Mean(±sd)
Water temperature (ºC)	16.48	21.37	18.59 (±1.07)
Salinity (ppt)	16.87	30.82	29.14 (±1.29)
Chl-a (μg/L)	3.81	35.73	9.74 (±4.89)
Dissolved Oxygen (mg/L)	5.83	10.34	7.14 (±0.55)

Weather parameters

The time-series graph and summary of the weather data retrieved from the DMI website during the same 50-day period can be observed in Figure 23 and Table 8, respectively.

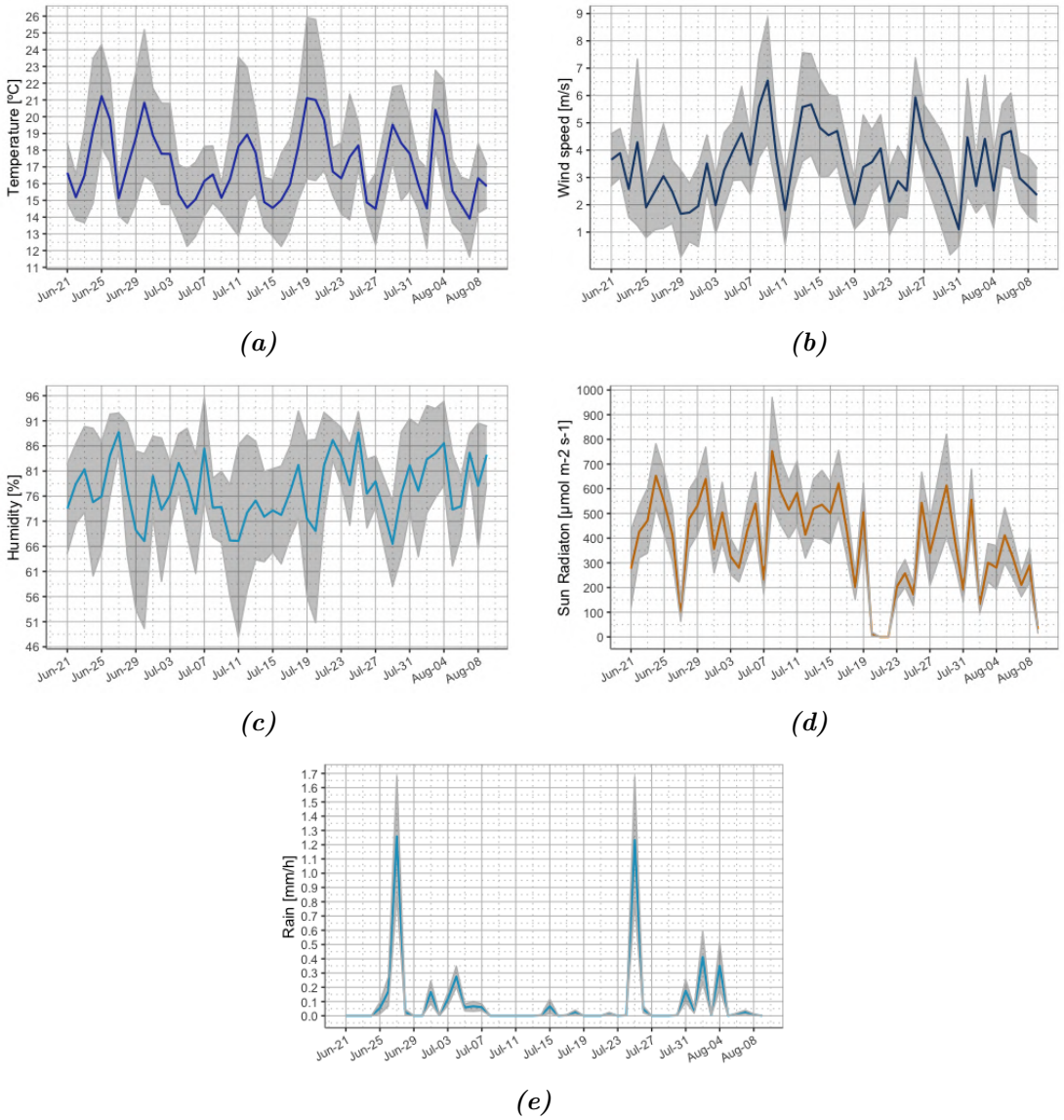


Figure 23. Time series of the mean daily values of **(a)** Air temperature (ºC), **(b)** Wind speed (m/s), **(c)** Humidity (%), **(d)** Sun Radiation (μmol $m^{-2}s^{-1}$), and **(e)** Rain (mm/h) retrieved from the DMI website.

Table 8. Minimum, maximum and mean values (±SD) of the weather parameters from the 21/06/22 to 09/08/22 that were retrieved from the DMI website

Parameter	Min	Max	Mean(±sd)
Temperature (ºC)	9.9	31.2	17.15 (±3.44)
Humidity (%)	36	96.7	77.51 (±12.03)
Windspeed (m/s)	0	12.5	3.42 (±1.92)
Rain (mm/h)	0	8.4	0.10 (±0.55)
Sun radiation (μmol $m^{-2}s^{-1}$)	0	1062	205.19 (±261.93)

Tidal range

The highest tide recorded during the sampling period was 17.3cm above the mean sea level and the lowest was -16.1cm below the mean sea level. The average value of the high tide was 6.96(±4.41)cm and the average value of the low tide -6.36(±3.71)cm. Tidal range data is illustrated on Figure 24.

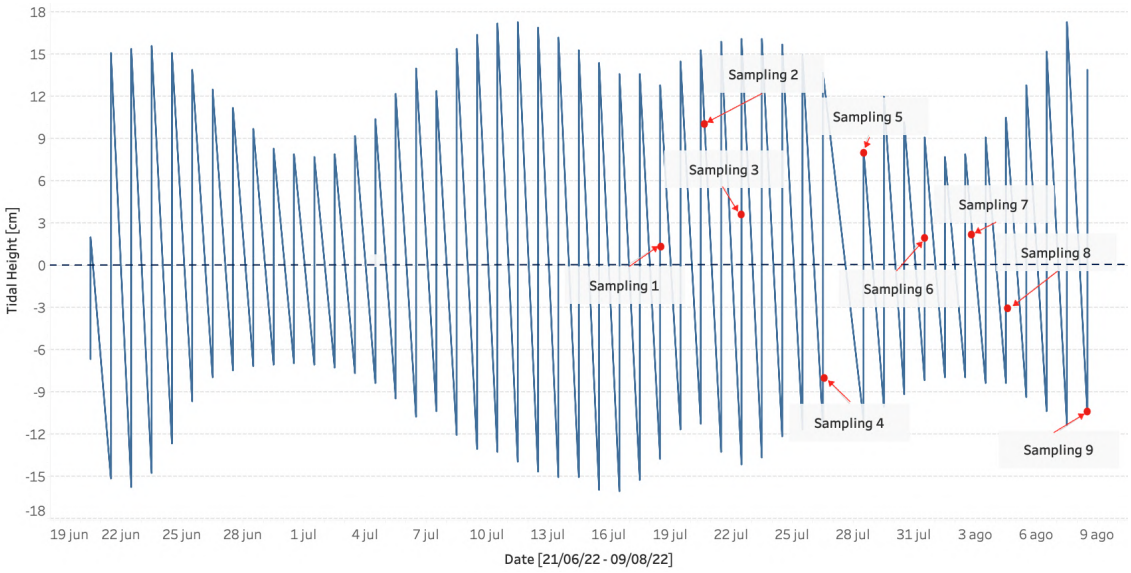


Figure 24. Tidal range from 21/06/22 until 09/08/22. The average sea level is indicated with a doted blue line, tags with red arrows and red dots are used to mark the tide level when the water samples were collected.

Water samples were collected 9 times during the study period. The date, hour, and tidal height at the time the samplings were made are shown in Table 9.

Table 9. Date, hour, and tidal height (cm) during the water collection dates

Sampling	Date	Hour	Tidal height (cm)
1	19/07/22	14:00	1.3
2	21/07/22	13:00	9.9
3	23/07/22	14:00	3.3
4	27/07/22	13:00	-7.8
5	29/07/22	14:00	8.2
6	01/08/22	13:00	2.2
7	03/08/22	14:00	4.2
8	05/08/22	13:00	-3.2
9	09/08/22	14:00	-10.5

Water nutrients

Water samples were collected and analyzed for quantifying the concentration of PO_4^{3-}, NO_2-N, NH_4-N, and NO_3-N. The results from these analyses were evaluated to detect discrepancies between the concentration of nutrients found inside the CFWs and in a near sampling location selected as a reference point for the mean local concentration of nutrients at the Limfjorden.

The different NH_4-N measured concentrations are illustrated in Figure 25, a Kruskal-Wallis non-parametric test was done to compare the median values between the different sampling locations, with no statistically significant difference found between them ($p = 0.51$). A summary of the NH_4-N measurements statistics is shown in Table 10.

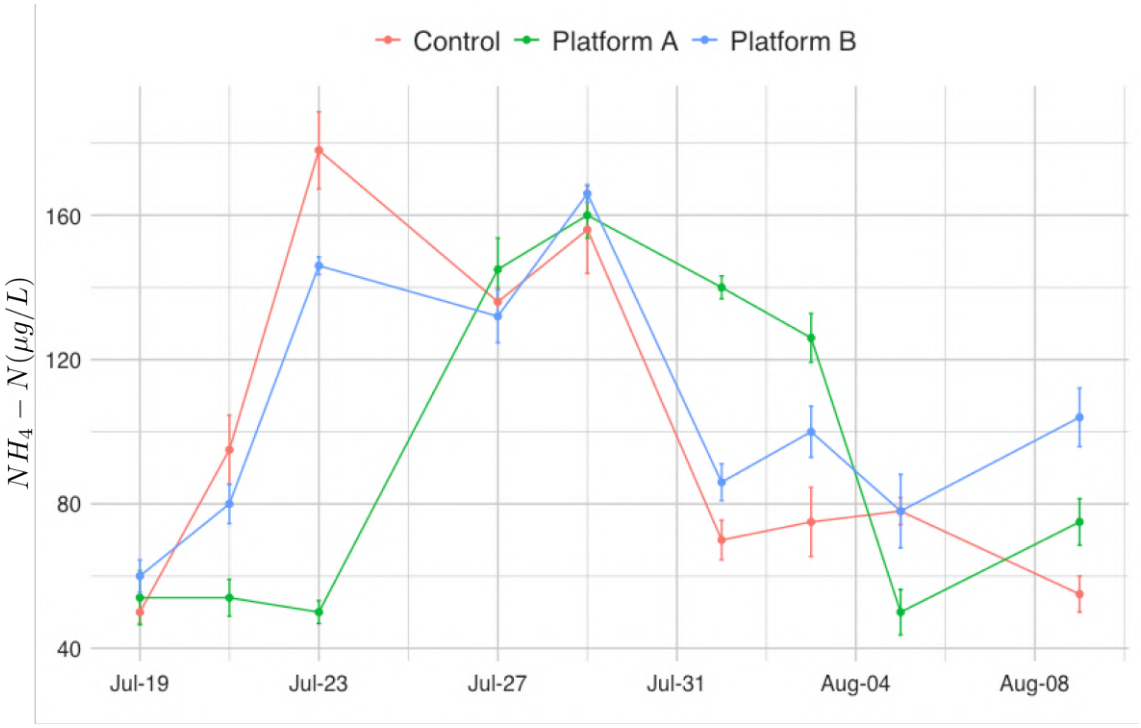


Figure 25. Average NH_4-N concentration values in each sampling date and location

Table 10. Summary of NH_4-N nutrient analysis results showing the sampling location, median, maximum and minimum values.

	Units	Sampling Location	Median	Max	Min
NH_4-N	$\mu g/l$	Control	78	178	46
		Platform A	75	160	50
		Platform B	100	166	60

NO_3-N calculated concentrations are illustrated in Figure 26, a Kruskal-Wallis non-parametric test was done to compare the median values between the different sampling locations, an unusually high value was measured on the 03/08/22 (189.4 μg/L) at the control location, but the statistical test showed no significant difference between them ($p = 0.27$). A summary of the NO_3-N measurements statistics is shown in Table 11.

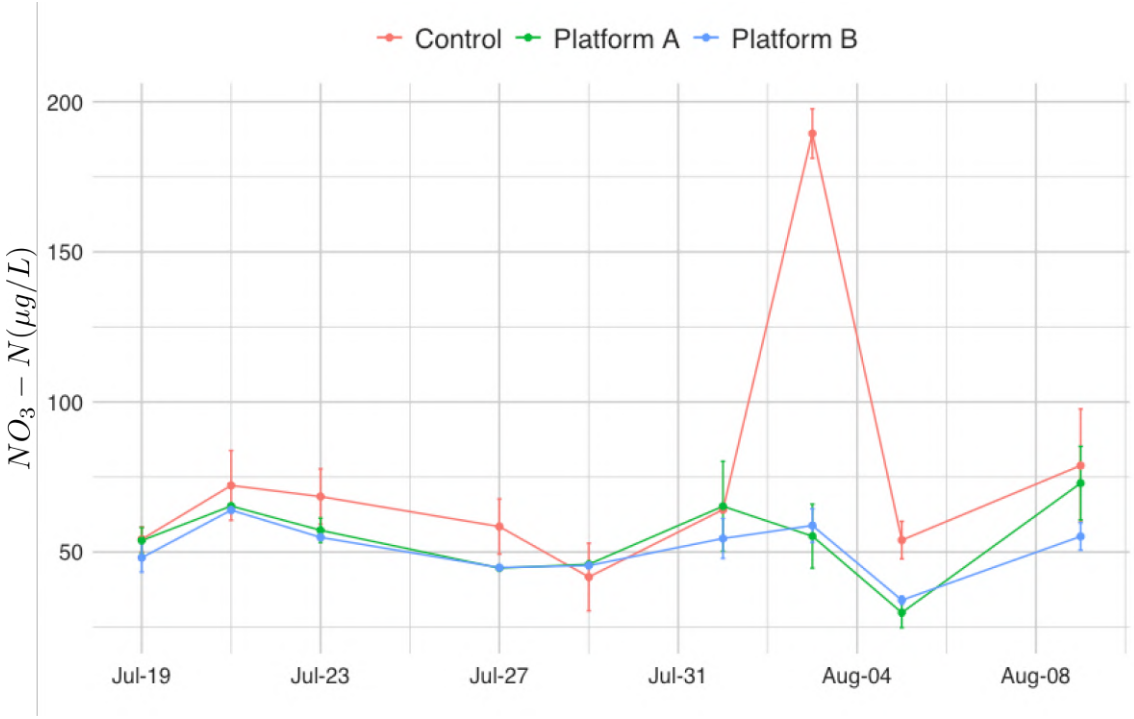


Figure 26. Average NO_3-N concentration values in each sampling date and location

Table 11. Summary of NO_3-N nutrient analysis results showing the sampling location, median, maximum and minimum values

	Units	Sampling Location	Median	Max	Min
NO_3-N	μg/l	Control	64.12	189.4	41.66
		Platform A	55.27	72.93	29.78
		Platform B	54.51	63.95	33.95

The different NO_2-N measured concentrations are illustrated in Figure 27. The concentrations were higher in the control water samples two in two sampling dates (21/07/22 and 03/08/22). A Kruskal-Wallis non-parametric test was done to compare the different median values between sampling locations with no statistically significant difference found ($p = 0.39$).

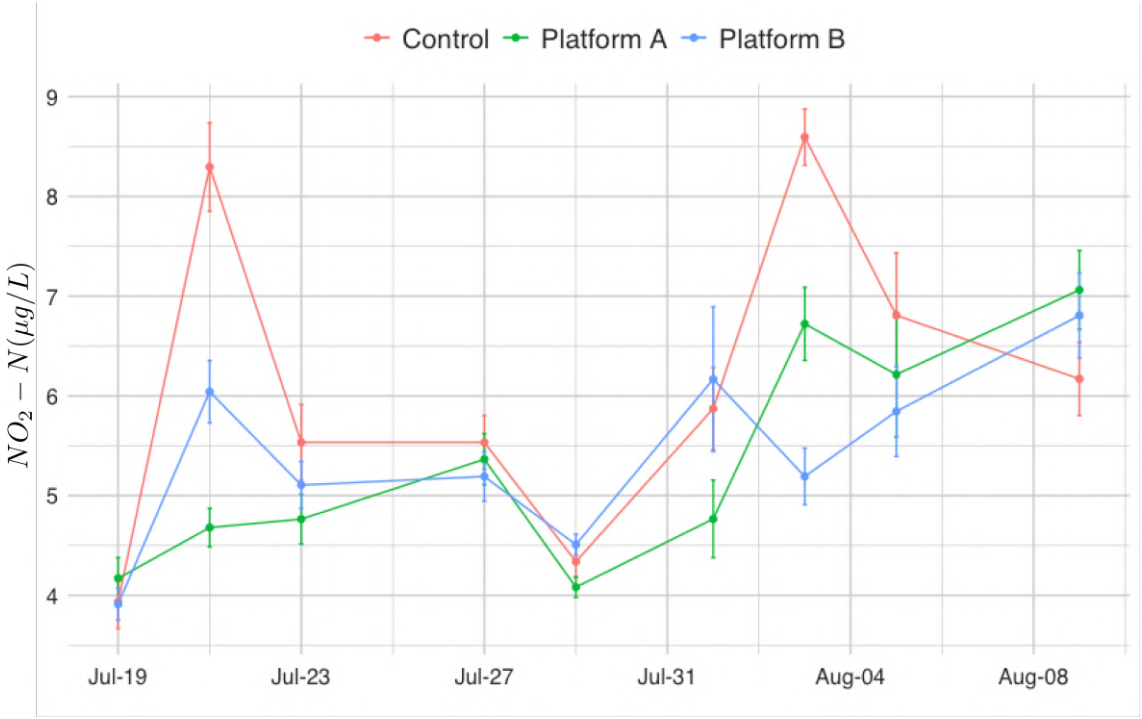


Figure 27. Average NO_2-N concentration values in each sampling date and location

Table 12. Summary of NO_2-N nutrient analysis results showing the sampling location, median, maximum and minimum values

	Units	Sampling Location	Median	Max	Min
NO_2-N	$\mu g/l$	Control	5.87	13.18	4.33
		Platform A	4.76	7	4.04
		Platform B	5.19	7.48	3.91

The different PO_4^{3-} measured concentrations are illustrated in Figure 28. High values were measured on both platform water samples the 03/08/22 but the Kruskal-Wallis test revealed that the difference between mean concentration values was not statistically significant ($p = 0.56$). A summary of the PO_4^{3-} measurements statistics is shown in Table 13.

Table 13. Summary of PO_4^{3-} nutrient analysis results showing the sampling location, median, maximum and minimum values

	Units	**Sampling Location**	Median	**Max**	**Min**
PO_4^{3-}	μg/l	Control	23	46.66	6.33
		Platform A	18.33	87.66	9
		Platform B	22	74.66	7.66

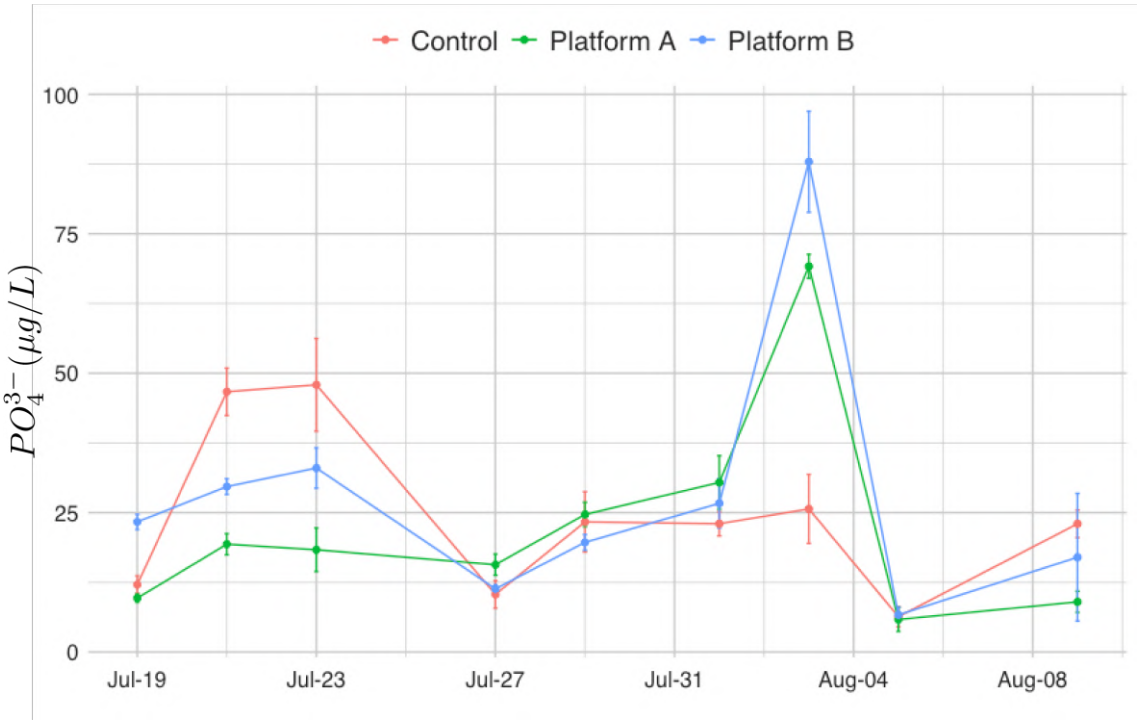


Figure 28. Average PO_4^{3-} concentration values in each sampling date and location

4.2 Growth and physiological characteristics of *Aster tripolium L.* & *Salicornia europaea L.*

The survival rate of *S. europaea* was generally lower than *A. tripolium* survival rate during the entire experimental period. After the first 25 days *A. tripolium* had an ≈12% higher survival rate than *S. europaea*, 30 days after both had decreased by 5%. The survival rate of both species kept decreasing as time passed, but *S. europaea* had higher mortality with a survival rate decrease of 15% after 35 days and 10% after 40 days which was higher compared to the 8% and 5% decrease after 35 and 40 days respectively of *A. tripolium* (Table 14).

Table 14. Surival rate of *A. tripolium* & *S. europaea* during the experimental period.

Initial number of organisms = 150		*A. tripolium*	*S. europaea*
Days after deployment	Date	Survival rate [%]	Survival rate [%]
25	30/06/22	75	63
30	05/07/22	60	58
35	10/07/22	52	43
40	15/07/22	47	33

The shoot height of *A. tripolium* decreased in both samplings, comparing the highest shoot measurements from the three groups it can be observed that the tallest shoots on the control sample were above 22cm, by Sampling 1 the tallest shoots were just above 17cm, and by the end of the experiment on Sampling 2, the tallest shoots were below 15cm 29. *S. europaea* shoots were similar between the control group and Sampling 1 by the 2nd sampling the height of the tallest shoots was different from the other 2 groups, with the tallest ones measuring less than 17cm.

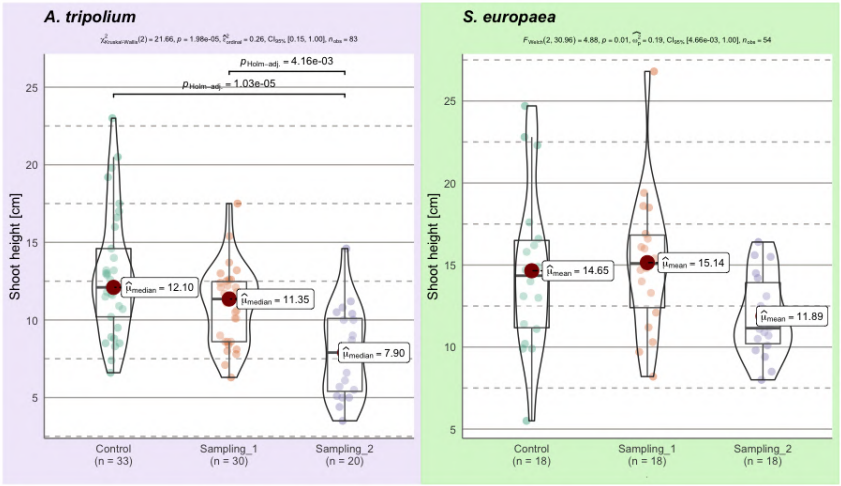


Figure 29. Shoot height of *A. tripolium* & *S. europaea*. A non-parametric Kruskal-Wallis test was done for A. tripolium and a parametric Welch's t-test for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within *A. tripolium* are shown by black lines at the top of the graph.

The root length as opposed to the shoot height decreased more than 35% from the control groups to Sampling 1 in both *A. tripolium* and *S. europaea*, after Sampling 1 it kept decreasing and the values were much less diversified with the highest values below 15cm for *A. tripolium* and 5cm for *S. europaea* in Sampling 2 (Fig. 30).

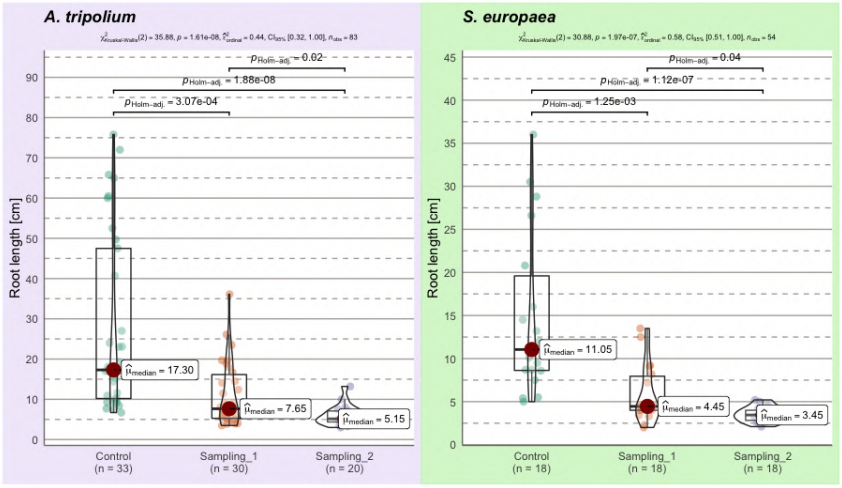


Figure 30. Root length of *A. tripolium* & *S. europaea*. A non-parametric Kruskal-Wallis test was done for A. tripolium and for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within each species are shown by black lines at the top of the graph.

The number of leaves of *A. tripolium* and stems of *S. europaea* increased slightly from the control to Sampling 1 but by the end of the experiment on Sampling 2, a decrease in both can be observed (Fig. 31). There was no statistically significant difference found in the change of the number of leaves/stems during the experimental period but in both *A. tripolium* and *S. europaea* significant differences were found in the decrease from Sampling 1 to Sampling 2.

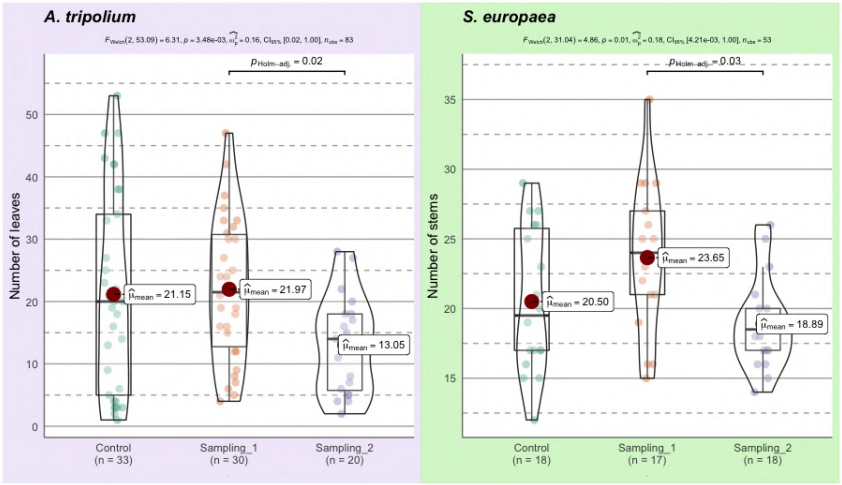


Figure 31. Number of leaves of *A. tripolium* and number of stems of *S. europaea*. A parametric Welch's t-test was done for A. tripolium and for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within each species are shown by black lines at the top of the graph.

The values from leaf area (31) and number of leaves (32) of *A. tripolium* show that the plants were shredding older leaves and growing new and smaller ones, at the end of the experimental period on Sampling 2 it can be seen that the density of leaves had decreased slightly but the change in the area between the three different groups was statistically significant. For *S. europaea* the leaf area (Fig. 32) had no statistically significant changes between the control of both samplings, which may show stagnation in the growth of younger stems.

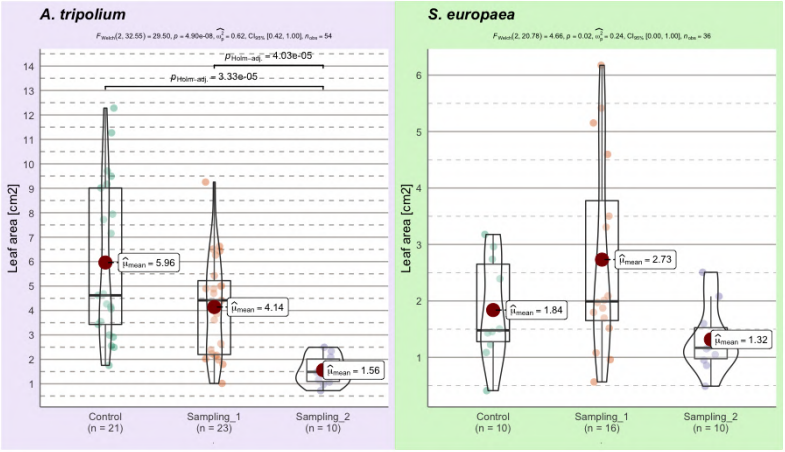


Figure 32. Leaf area of *A. tripolium* & *S. europaea*. A parametric Welch's t-test was done for A. tripolium and for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within *A. tripolium* are shown by black lines at the top of the graph.

The water content of *A. tripolium* showed no statistically significant difference between the three groups but as seen from (Fig. 33) it seems that it is slightly higher in the Sampling 2 group, the plants were smaller but had a higher content of water than the ones from Control and Sampling 1. *S. europaea* clearly shows a tendency in water increase over time as there were significant differences found between the three groups.

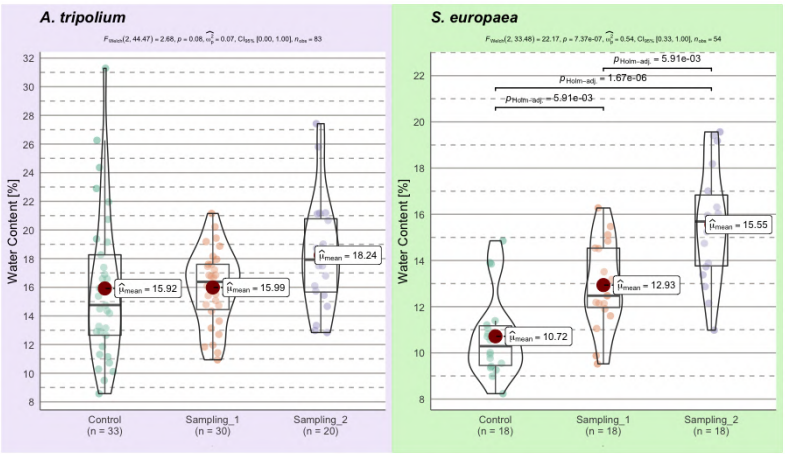


Figure 33. Water content of *A. tripolium* & *S. europaea*. A parametric Welch's t-test was done for A. tripolium with no statistically significant difference found ($p > 0.05$) and for *S. eruopaea* with statistically significant differences found ($p < .05$). Significant differences within *S. europaea* are shown by black lines at the top of the graph.

S. europaea dry weight values increased from Control to Sampling 1 and then decreased by Sampling 2, a statistically significant difference was found when comparing the group median values, the highest values increased from ≈2.2g to ≈3g from Control to Sampling 1 respectively, but by the 2nd Sampling dry weight values were lower but with just significant differences between Sampling 1 and Sampling 2. In the case of *A. tripolium* a small increase from Control to Sampling 1 followed by a decrease on Sampling 2 can be observed in (Fig. 34) even though statistically significant differences were not found.

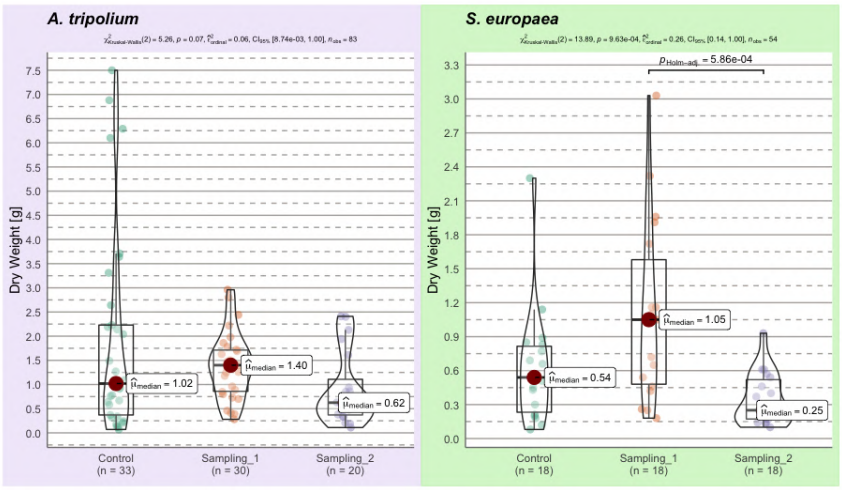


Figure 34. Dry weight of *A. tripolium* & *S. europaea*. A non-parametric Kruskal-Wallis was done for A. tripolium with no statistically significant difference found ($p > 0.05$) and for *S. eruopaea* with statistically significant differences found ($p < .05$). Significant differences within *S. europaea* are shown by black lines at the top of the graph.

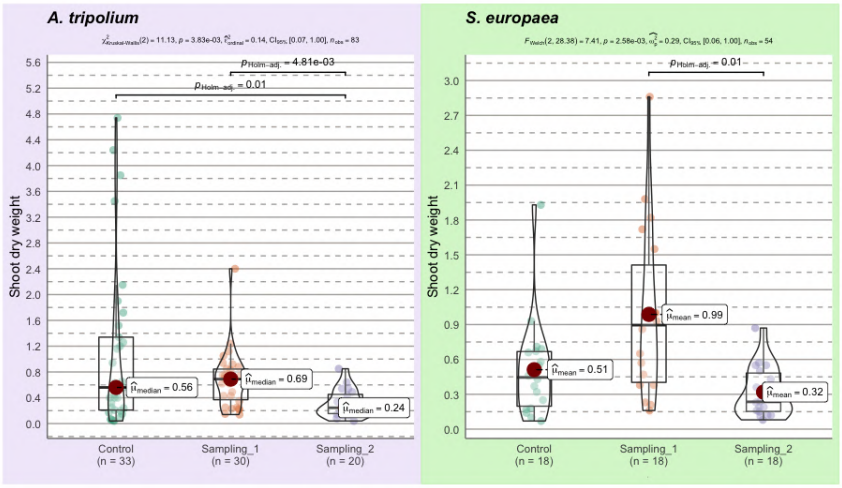


Figure 35. Shoot dry weight of *A. tripolium* & *S. europaea*. A non-parametric Kruskal Wallis test was done for A. tripolium and a parametric Welch's t-test for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within each species are shown by black lines at the top of the graph.

A. tripolium shoot dry weight had a significant decrease over time (Fig. 35) with significant differences between the three groups. The root dry weight remained stable with just a small decrease by Sampling 2 (Fig. 36) that was not statistically significant, but both graphs

show that the dry weight decrease over time was mainly due to a change in the biomass that was located above the water. For *S. europaea* statistically significant differences were found in both shoot dry weight (Fig. 35) and root dry weight (Fig. 36) in both cases there was a small increase from Control to Sampling 1 and a decrease with significant differences from Sampling 1 to Sampling 2.

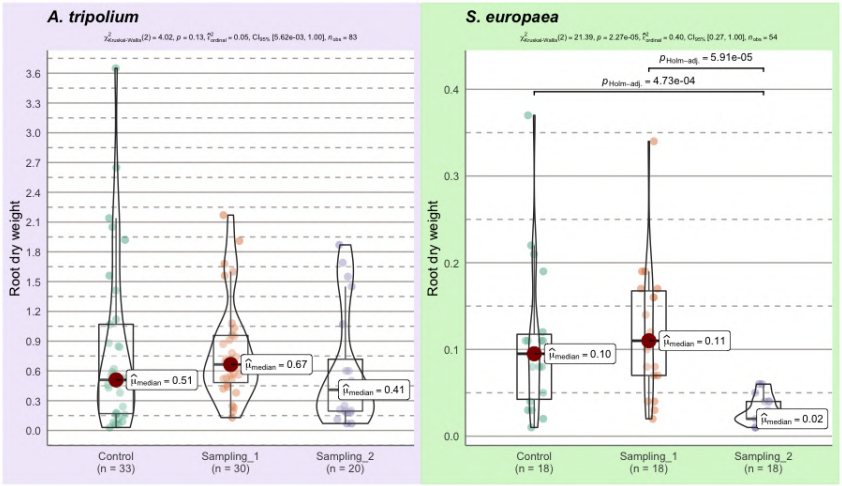


Figure 36. Root dry weight of *A. tripolium* & *S. europaea*. A non-parametric Kruskal-Wallis was done for A. tripolium with no statistically significant difference found ($p > 0.05$) and for *S. eruopaea* with statistically significant differences found ($p < .05$). Significant differences within *S. europaea* are shown by black lines at the top of the graph.

Root shoot dry weight shows for both *A. tripolium* and *S. europaea* what has been observed from the previous graphs (Figs 35, 36) R/S ratio increased trough time for *A. tripolium* and decresed for *S. europaea* in both cases significant differences where found between the Control and Sampling 1 and 2 (Fig. 37).

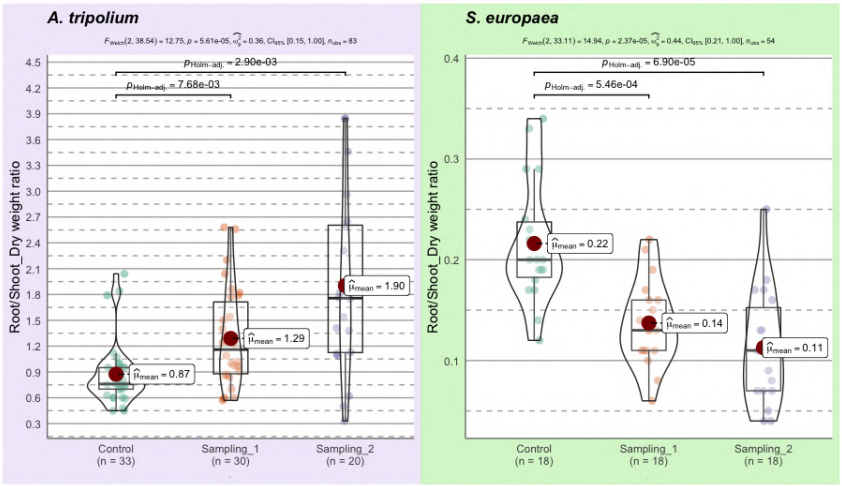


Figure 37. Root/Shoot dry weight ratio of *A. tripolium* & *S. europaea*. A parametric Welch's t-test was done for A. tripolium and for *S. eruopaea*, in both cases a statistically significant difference was found ($p < .05$). Significant differences within each species are shown by black lines at the top of the graph.

4.3 Growth and physiological characteristics of *Mytilus edulis* & *Ostrea edulis*

M. edulis had a good development during the experimental period, there was a daily increase in length of 0.16mm, an average soft tissue growth of 9.86mg every day, and the condition index increase as well from 2.18 to 3 which can be seen on (Table 15)

Table 15. *M. edulis.* Mean shell length (mean±SD)(n), rate of increase in shell length, mean dry weight of tissue (mean±SD), actual growth rate, condition index (mean±SD) and actual specific growth rate. Samples collected after 0 and 15 days.

L_0 (mm)	L_{15} (mm)	$\Delta L/\Delta t$ (mm day^{-1})	W_0 (mg)	W_{15} (mg)	$\Delta W/\Delta t = G_a$ (mg day^{-1})	CI_0 (mg cm^{-3})	CI_{15} (mg cm^{-3})	μ_a (%day^{-1})
48.01 ± 5.23 (48)	50.53 ± 4 (144)	0.16	242 ± 6.97 (48)	390 ± 10.51 (144)	9.86	2.18 ± 0.45 (48)	3 ± 0.58 (144)	9.92

M. edulis increased its size trough time in each one of the three different groups, there were significant differences found between the Control and Sampling 1 and 2 (Fig. 38) although there was no difference between the mussels length from the Control group and the ones from Sampling one the length values remained almost the same (Fig. 38). Regarding *O. edulis* there were no significant differences in length among the three different groups, the length values from the Control group are similar to the values from the 2nd Sampling.

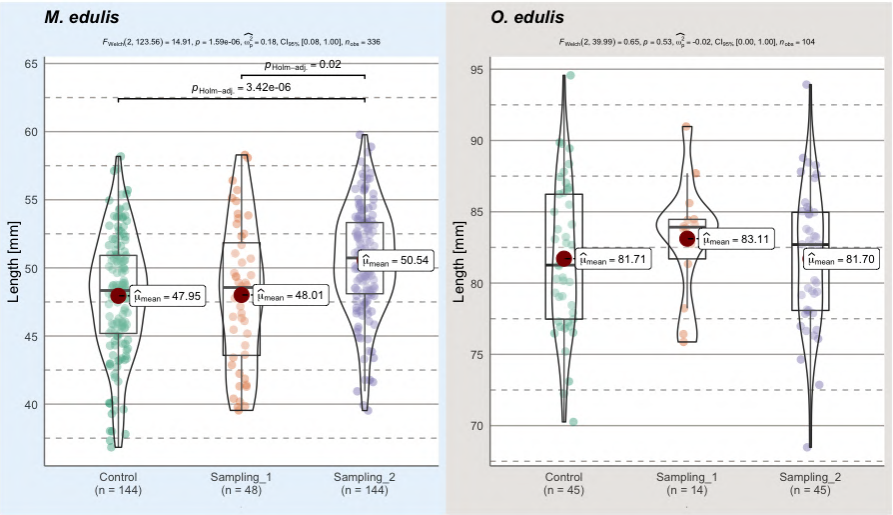


Figure 38. Length of *M. edulis* & *O. edulis*. A parametric Welch's t-test was done for M. edulis with statistically significant difference found ($p < 0.05$) and for *O. edulis* with no statistically significant differences found ($p > .05$). Significant differences within *M. edulis* are shown by black lines at the top of the graph.

Table 16. *O. edulis.* Survival rate (%) after 15 and 74 days, and condition index (mean±SD) after 0 and 15 days

Ostrea edulis			
Survival rate (15d)	Survival rate (74d)	CI_0	CI_{15}
62%	0%	32.94(±9.41)	32.27(±10.21)

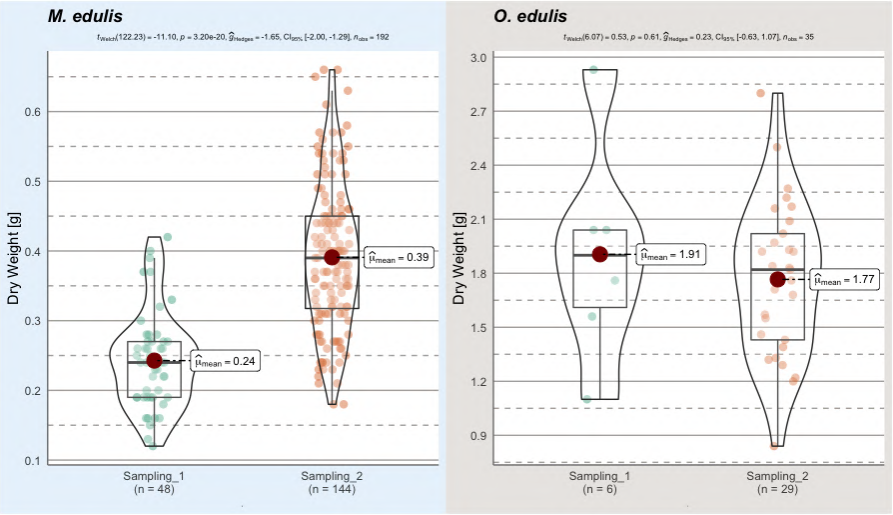


Figure 39. Dry weight of *M. edulis* & *O. edulis*. A parametric Welch's t-test was done for M. edulis with statistically significant difference found ($p < 0.05$) and for *O. edulis* with no statistically significant differences found ($p > .05$).

Dry weight and condition index of *M. edulis* increased over time from Sampling 1 to Sampling 2 (Figs 39, 40) in both cases there were statistically significant differences between the mean values. In contrast the dry weight (Fig. 39) and the condition index (Fig. 40) of *O. edulis* seems to have decreased from Sampling 1 to Sampling 2 although no statistically significant differences were found. The survival rate of *O. edulis* at the end of was of 62% and after 74 days it was 0%.

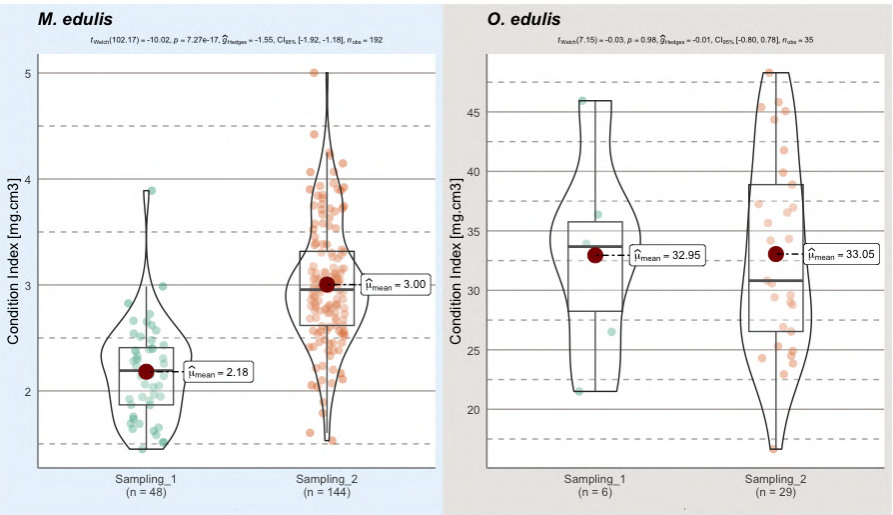


Figure 40. Condition index of *M. edulis* & *O. edulis*. A parametric Welch's t-test was done for M. edulis with statistically significant difference found ($p < 0.05$) and for *O. edulis* with no statistically significant differences found ($p > .05$).

4.4 Constructed Floating Wetlands

The Constructed floating wetlands (CFWs) were deployed at the Limfjord on the 30th of June. CFW A (Figs 41a, 41b) was closer to the shoreline ($\approx 10m$), and CFW B was deployed at $\approx 12m$ in one of the edges of the Fjordhaver rafts (Figs 41c, 41d). A high amount of seagrass trapped inside the CFWs was removed completely before the deployment but some of the remnants can be seen trapped between the Fjordhaver rafts and the PVC pipes from the CFWs (Figs. 41b, 41d).

(a) CFW A (30/06/22)

(b) Side view of CFW A (30/06/22)

(c) CFW B (30/06/22)

(d) Side view of CFW B (30/06/22)

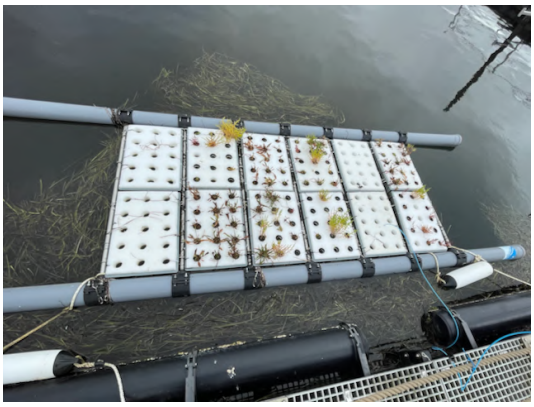
Figure 41. Pictures showing the upper view and side view of CFW A (Figs 41a, 41b) and CFW B (Figs 41c, 41d) the 30th of June a few minutes after being deployed at the Limfjord

On the 14th of July after 14 days of deployment CFW A plants (Fig. 42a) already showed a change in pigmentation, in the case of *S. europaea* it can be observed than many of the stems have changed from a green coloration to yellow, orange and red and *A. tripolium* also shows a change from green to red in some of the older leaves, the same patter in color change can be seen as well for CFW B (Fig. 42b).
The high amount of sea grass affected both CFWs unequally, in the case of CFW A (Fig. 42a) the rafts from the Fjordhaver acted as a physical barrier that decreased the amount of sea grass and allowed the currents to remove most of it. In contrast, CFW B (Fig. 42b) due to its location in one of the exterior edges of the fjordhaver rafts had to sustain the full waves strength which pushed a high amount of sea grass over the CFW and covered almost the half of it.

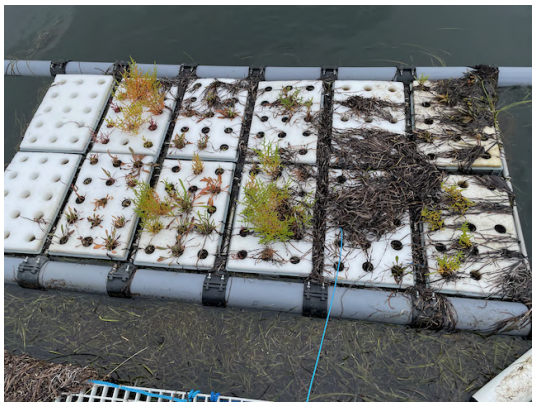
23 days after the deployment of the CFWs (Figs 42c, 42d) on the 23rd of July the change in pigmentation in both halophytic species appears to have slowed down as it looks similar to the change that had already happened by the 14th of July, from the sea grass accumulation around CFW A (Fig. 42c) it seems that the CFWs acted as a small barrier that decreased the currents strength and kept a high amount of seagrass near the PVC tubes. In the case of CFw B (Fig. 42d) the sea grass that was covering the CFW had already dried and was being removed slowly by the wind.

(a) CFW A (14/07/22)

(b) CFW B (14/07/22)



(c) CFW A (23/07/22)



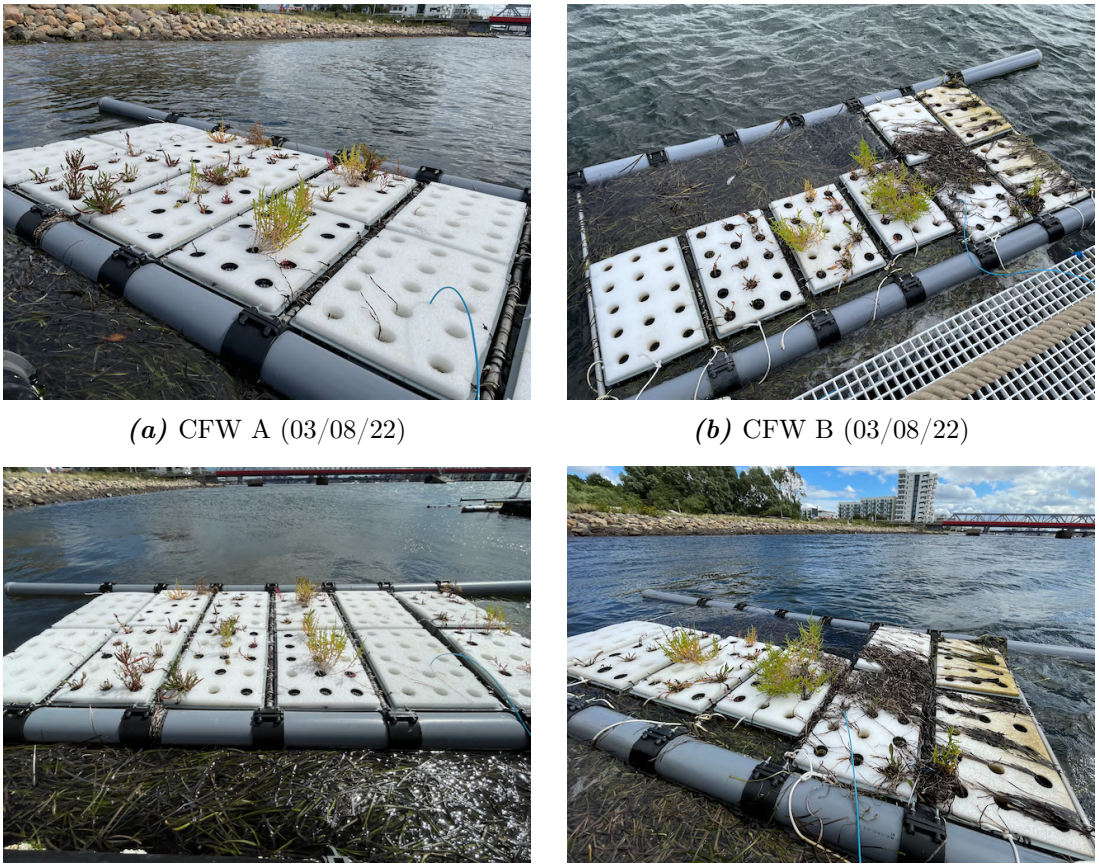
(d) CFW B (23/07/22)

Figure 42. Pictures showing the upper view of CFW A and CFW B after 14 days of deployment (Figs 42a, 42b) and 23 days after deployment (Figs 42c, 42d)

34 days after the CFWs was deployed the first sampling had already been done and it can be observed from CFW A (Fig. 43a) that *A. tripolium* was already losing some of the older leaves and that *S. europaea* was developing a corky main stem and was also losing some of the older secondary stems. CfW B (Fig. 43b) had lost 4 upper boxes with halophytes due to a summer storm that happened the day before the sampling, and the bottom boxes carrying the filter-feeders (*M. edulis* & *O. edulis*) remained in place but were filled with seagrass, the weight of the seagrass inside the boxes started sinking one of the PVC pipes from the CFW.

On the 5th of August, 36 days after deployment the halophytes were still shredding the older leaves and stems. The new *A. tripolium* leaves were greener and some of the plants were already showing signs of flower development, *S. europaea* in some of the plants had

changed to a deep red pigmentation with the development of small flowers (Figs 43c, 43d). It can be seen on the right side of CFW B and from the distinct coloration of the floating mats that the seagrass was constantly being pushed over them (Fig. 43d).



(a) CFW A (03/08/22)

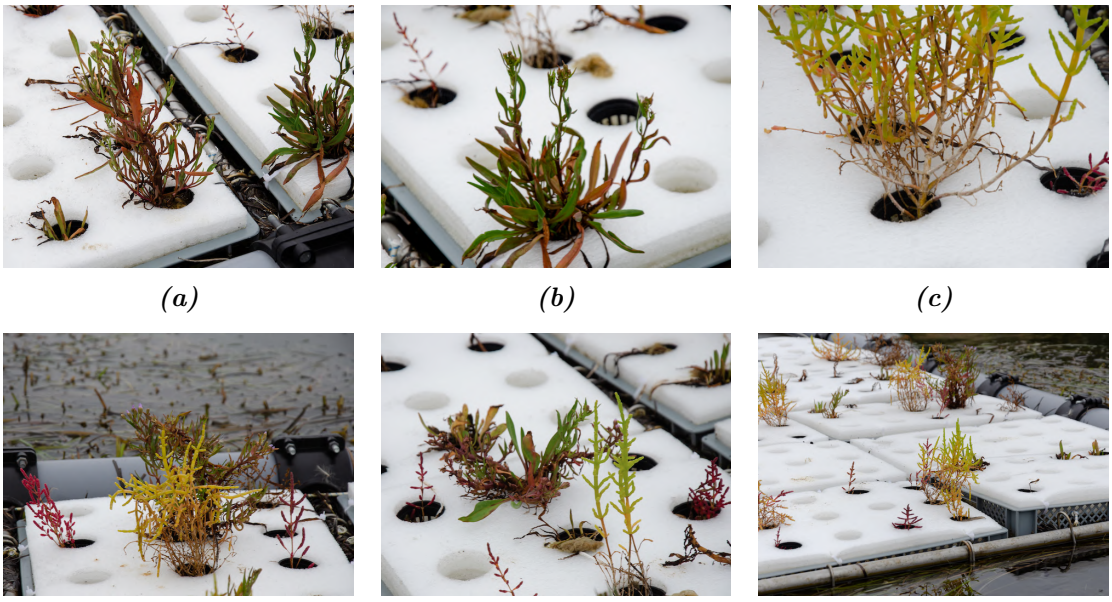
(b) CFW B (03/08/22)

(c) CFW A (05/08/22)

(d) CFW B (05/08/22)

Figure 43. Pictures showing the upper view of CFW A and CFW B after 34 days of deployment (Figs 43a, 43b) and 36 days after deployment (Figs 43c, 43d)

The last sampling was done on the 9th of August 40 days after the deployment of the CFWs. The halophytes had decreased in size and gone through many morphological changes (Fig. 44f). In the remaining *A. tripolium* the leaves that the plants had at the beginning of the experiment had been shredded after going through a pigmentation change. Based on the observations from the remaining plants some of the older leaves were also going trough a pigmentation change and the majority of the young leaves were greener, some of the surviving *A. tripolium* were already showing clear signs of flowering and in some cases, the flower buds were already opening (Figs 44a, 44b, 44d). The remaining *S. europaea* were either red and with flowering signs as in (Figs 44d, 44e) or yellow with corky stems extending from the base of the plants to half of the main stem height (Figs 44c, 44e).



(a) *(b)* *(c)*

(d) *(e)* *(f)*

Figure 44. Pictures from different perspectives of CFW A remaining halophytes before the last sampling on the 9th of August

CFW B was removed after 75 days due to buoyancy issues, as observed from (Figs 45a, 45b, 45c) the boxes were full of fouling organisms, which increased the weight of the system. Among the different species that adhered to the CFWs boxes the ascidian *Styela clava* can be observed at the edge of the box (Fig. 45a, *Ciona intestinalis* can be observed in (Fig. 45b) and the presence of barnacles and small blue mussels can be seen in the entire box (Fig. 45c).

(a) *(b)* *(c)*

Figure 45. Pictures from one of the bottom boxes from CFW B after 75 days, showing the entire base and most of the size area of the box covered by fouling organisms

5 Discussion

5.1 Evaluation of the growth characteristics and survival rate of *Aster tripolium L.* & *Salicornia europaea L.*

S. europaea and *A. tripolium* were selected due to their capability to survive in highly saline environments and their proven potential to adapt and develop in hydroponic conditions, as both species have been used before in multitrophic aquaculture systems (IMTA) to remove waste dissolved nitrogen (N) and phosphorus (P) [Waller et al., 2015; Quintã et al., 2015] and in constructed wetlands for the treatment of wastewater of marine aquaculture [Webb et al., 2013]. The survival rate of *A. tripolium* and *S. eruopaea* after 40 days of experimental period was 47% and 33% respectively (Table 14) .

Based on the observations from the CFWs (Fig. 42) some of the plants were expelled from the transplanting pots by waves and/or the wind, the plants that had a longer root system were able to endure the waves and the wind. *S. europaea* root was shorter (Figure. 30) and less developed (Figure. 36), similar to what [Davy et al., 2001] reported on the taxonomic description (Table. 4). In contrast *A. tripolium* root system was longer (Fig. 30) and more developed (Fig. 36) as described by [Clapham et al., 1942] (Table. 3) . This morphological difference between species could explain why *S. europaea* had a lower survival rate during the first 25 days (63%) than *A. tripolium* (75%).

The high salinity levels during the study period may be a reason for the low survival rate of the selected halophytic species. It was reported by [Geissler et al., 2009] that the survival rate of *A. tripolium* started decreasing after surpassing a 15 g/L salinity concentration and reached a minimum of 20% when the salinity levels reached 30 g/L. According to [Puccinelli et al., 2022] *S. europaea* is considerably more salt tolerant than *A. tripolium* as the optimal hydroponic salinity levels for *A. tripolium* are between 2.9 - 4.7 g/L and for *S. europaea* between 16.5 - 24.7 g/L. However, *S. europaea* has been shown to grow in conditions where the NaCl levels were as high as 35 g/L [Rozema and Schat, 2013] and with yields of 1.5 (kg $m^2 year^{-1}$) under 30 g/L NaCl hydroponic concentrations [Nazarian, 2016], and *A. tripolium* was grown successfully in a RAS system with salinity levels of 16 g/L by [Waller et al., 2015]. Interestingly, When comparing the survival rate of the two species in the present study it is clear that *A. tripolium* had a better performance than *S. europaea* contrary to what has been shown by the aforementioned literature.

Both *A. tripolium* and *S. europaea* had increased their dry weight and number of leaves or stems slightly after the first 25 days, but subsequently both decreased as evidenced by a lower dry weight and fewer leaves/stems by the end of the experimental period. *A. tripolium* increase in dry weight after the first 25 days can be explained by a higher number

of leaves, as the plants were growing new smaller, and thicker leaves. According to [**?**]*A. tripolium* subjected to higher mechanical stress like wave impact when they grow closer to the shore grow smaller and thicker leaves, and also reduce their height, which can be seen as well in the decreasing root length and shoot height (see Fig.30, Fig.29) and in the reduced leaf area (Fig. 32). The older leaves were not only lost as a way of dealing with mechanical stress caused by the impact of the waves but also as an adaptation to high salinities. It was reported by [Geissler et al., 2009] that *A. tripolium* translocates NaCl into the old leaves to reduce salt accumulation, the toxic effect of these ions can be seen in the signs that the old leaves show before dying off.

It has been reported by [Ramani et al., 2006; **?**; Shennan et al., 1987] that *A. tripolium* does not show differences in succulence among different levels of salinity, and the relative water content (RWC) remains stable among the different NaCl concentrations. However, [Geissler et al., 2009] reported that the leaf water content in *A. tripolium* under different salinity concentration treatments was the highest (89%) at a 25% seawater salinity concentration $\approx$ 9 g/L and then decreased as the salinity increased, similar to what [Wiszniewska et al., 2019] found, where *A. tripolium* root and shoot water content was highest at a $\approx$ 8.76 g/L salinity concentration. The water content in this study did not show a significant increase over time.

A change in tissue coloration was observed in *A. tripolium* as the leaves went from a deep green pigmentation to a yellowish one with orange spots by the end of the experimental period (Fig. 44). [Duarte et al., 2017] reported that salt stressed *A. tripolium* changed from a light-harvesting activity to photo-protection by increasing the proportion of carotenoids to chlorophyll, which would explain the leaves' change of coloration.

The root:shoot ratio R/S followed a different pattern in the two species as it decreased from 0.22 to 0.11 in *S. europaea*(Fig. 37) and increased from 0.87 to 1.90 in *A. tripolium* (Fig. 37). The water content had a significant increase in *S. europaea* (Fig.33) during the entire experiment. The presence of NaCl in the substrate produces succulence in *S. europaea*, an increase in water content in the plant organs that allows halophytes to cope with high levels of salinity by diluting salts and toxic ions accumulated in the plant cells, for this purpose *S. europaea* has a unique kind of spiral cells (tracheoidioblasts) that function as low-salt water reservoirs in highly-saline environments [Gri, 2017], and they also accumulate sodium ions, betaine, and proline which allows them to adjust the osmotic potential attracting water into the cells and maintaining turgor which helps them coping efficiently with high salinity [Cárdenas-Pérez et al., 2022; Moghaieb et al., 2004]. *S. europaea* shoots developed a corky stem during the experimental period, which is a morphological characteristic that the plants present as they grow [Davy et al., 2001] however according to [English and Colmer, 2013] species within the Salicornioideae store excess ions in older tissues within the shoot that will eventually be shed from the plant.

According to [Cárdenas-Pérez et al., 2022] the concentration of photosynthetic pigments in *S. europaea* is highest under non-saline conditions and decreases with increasing salinity levels until 400 mM NaCl. At higher NaCl concentrations the pigment concentration remains relatively stable showing that *S. europaea* is capable of protecting the photosynthetic system under extreme saline stress conditions. A change in tissue pigmentation was also observed in *S. europaea* as the branches went from green to yellow and finally to a red coloration by the end of the experimental period (data not shown). It was reported by [Ozturk et al., 2018] that *S. europaea* accumulates anthocyanin in the later vegetative stage and that anthocyanin is also related to salt tissue accumulation. In both of these mechanisms the lower pigment concentration and anthocyanin accumulation would explain the pigmentation change during this experiment.

The negative growth rate that *A. tripolium* and *S. europaea* had after the first sampling may be caused by two main factors, the high salinity levels (Table 7) and the low concentration of nutrients. High salinity levels induce several mechanisms in halophytes to help them cope with the higher amount of salt in the environment which increases the energy consumption and therefore reduces the size of the plants, high salinity levels can also cause growth reductions due to insufficient osmotic adjustment and damage from reactive oxygen species (ROS) [Flowers et al., 2015]. It was shown by [Cárdenas-Pérez et al., 2022] that very high salinity levels impose a growth reduction of *S. europaea* associated with reduced tugor and the high energy cost of salt secretion and osmoregulation. [Buhmann et al., 2015] found that *A. tripolium* growing hydroponically under high salinity conditions showed a growth reduction of 65% with a decline in nutrient uptake of $\geq$30%. Regarding the nutrient concentration [Buhmann et al., 2015] showed that a nitrate-N and phosphate concentration of at least 10 and 0.3 mg/L^{-1}, respectively, was necessary for reasonable biomass production.

5.2 Evaluation of the survival success and growth characteristics of *Mytilus edulis* & *Ostrea edulis*

M. edulis and *O. edulis* were selected for their capability of working as bio-engineering tools to control some of the effects of eutrophication and by that providing as well ecosystem scale services [Petersen et al., 2016; Kotta et al., 2020; Cranford et al., 2011]. During the experimental period, the environmental conditions were ideal for the growth of both species.

The ideal development conditions can be observed on the length increase (Fig. 38), higher condition index (CI) values (Fig, 40) and dry weight gain (Fig. 39) of *M. edulis*. It was shown by [Riisgård et al., 2012a] that blue mussels exposed to a salinity of 30 ppt had a faster growth rate (6.2% d^{-1}) than mussels growing under low salinity 10 ppt levels (1.6% d^{-1}), and there were no significant differences of growth with salinity levels between 15 to 25 ppt, during the experimental period the salinity concentration was of 29.14($\pm$1.29), a concentration that falls into the optimal salinity levels for the growth of the mussels. Based on the dry weight and length increase *M. edulis* with a density of 400 mussels per m^2 could remove by tissue growth 598.6$\pm$3.28 g C m^2 y^{-1}, 124$\pm$1.09 g N m^2 y^{-1} and 14.6$\pm$0.36 g P m^2 y^{-1}.

Chl-a concentration during the experimental period was of 9.74 ($\pm$4.89) $\mu g/L^{-1}$ in average, the concentration of chl-a may be close to the threshold algal concentration that lies between 6.3 and 10.0 $\mu g/L^{-1}$ at which the filtration rate of *M. edulis* decreases due to saturation, as found by [Riisgård et al., 2011]. The 9.92 (% day $^{-1}$) increase and 9.86 (mg day $^{-1}$) growth (Table 15) are similar at what [Riisgård et al., 2012a] found from smaller mussels (25 $\pm$0.6 mm) sampled at Løgstør Bredning, and Skive Fjord during 2009. The condition index (CI) after 15 days of the experimental period increased by 0.82 from 2.18 to 3 this is lower than the condition index increase reported by [Riisgård et al., 2012a] from three different class sizes of mussels during a 16-day experimental period. The daily shell length increase of 0.16 mm found in this study is lower than the increase of 0.18 and 0.23 mm that [Riisgård and Poulsen, 1981] found in mussels from two different locations in the Limfjord after 15 days of monitoring.

The water temperature during the experimental period was on average 18.59 ($\pm$1.07) ^{o}C with a minimum of 16.4 ^{o}C and a maximum of 21.3 ^{o}C, these temperature levels are found between the optimal thermal range for *M. edulis* and *O. edulis*. [Almada-Villela et al., 1982] reported that between 3 and 20 ^{o}C *M. edulis* growth increases logarithmically, above 20 ^{o}C the growth declines sharply, and between 3 and 5 ^{o}C growth rates are constant but low.

O. edulis length (Table 38), dry weight (Table 39) and condition index (CI) (Table 40) were lower with a high percentage of mortality (38%) by the end of the experimental period. *O. edulis* is a stenohaline species with a salinity tolerance between 25-37 ppt as reported by [Helmer, 2019], but the salinity levels were high enough to provide the needed conditions for its survival.

There were a small number of oyster spats adhered to the shells of the sampled *O. edulis* at the end of the experimental period (data not shown) this could explain the decrease in growth and condition index (CI) as variations of CI are associated with the use of energy for the reproductive period as shown by [Austin et al., 1993]. The oysters sampled had a high amount of silt adhered to the shells, high quantities of sediment in the water can block the digestive and respiratory tracts, which causes high stress to the oysters and death after a certain time. In a evaluation of *O. edulis* offshore farming in 2004 [Pogoda et al., 2011] registered a mortality rate of 40% in one of their study locations due to a high sediment load in the water column, and [Colden et al., 2017] after 2 years of monitoring different oyster reefs designs found that the reefs with a high sediment load experienced in some cases a 100% mortality. During the experimental period, a high amount of seagrass was trapped inside the oyster boxes which may have an adverse effect on the respiration and feeding of the oysters similar to what high quantities of sediment may cause.

O. edulis was selected not only for their nutrient removal and cycling capacity but also as a potential restoration tool for the flat oyster populations.[Norrie et al., 2020] found that bivalve aquaculture conducted in areas with degraded populations could be considered in restoration programs, and [Helmer, 2019] proved the effectiveness of using broodstock cage systems as a starting point in the early stages of *O. edulis* restoration plans. Nevertheless, in this study there was a high mortality rate which means the environmental conditions were not suitable for their growth and survival.

6 Conclusion

In this research, a Constructed floating wetland (CFW) consisting of two species of halophytes (*A. tripolium* & *S. europaea*) and two species of filter-feeding bivalves (*M. edulis* & *O. edulis*) was established in the Limfjord to assess the viability of applying it as a bioremediation tool.

Based on the results previously discussed, *M. edulis* positive growth rate and condition index showed that the conditions were ideal for their growth which means they provide a constant removal and cycling of nutrients,*O. edulis* with a mortality rate of 100% after 74 days proved that it can not be used as a bioremediation organism or with repopulation purposes. The halophytes' low growth and low survival rate also showed that the environmental conditions were not ideal for their development and therefore it can be concluded that CFWs do not provide a sufficient removal and cycling of nutrients to be considered as a viable bio-engineering tool for improving the environmental state of the Limfjord in the specific study location and mainly due to the constant high salinity levels, nevertheless the halophytes and mussels showed potential to be applied in some other location at the Limfjord with lower salinity levels that allow the halophytes reach its full growing potential.

The experimental floating wetland showed resistance to harsh environmental conditions. However, some adjustments for buoyancy could improve the system and should be taken into consideration. The selection of a location with lower salinity levels and higher amounts of nutrients like the river mouths could improve the growth of the halophytes and increase the nutrient removal and a longer monitoring period would be important to evaluate the entire growing season of the halophytes.

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