DANMARKS TEKNISKE UNIVERSITET

Investigating the Decline of Eelgrass, its Role in Marine Ecosystems and as Nurseries for Atlantic Cod

NATIONAL INSTITUTE OF AQUATIC RESOURCES - DTU AQUA

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Environmental Engineering

Bachelor Thesis

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1 Abstract

This project examines the historical reduction of eelgrass (*Zostera marina*) and cod (*Gadeus morhua*) in Danish waters and identifies their primary causes. Processes, consideration, outcomes and feasibility of eelgrass restoration are evaluated as well. Furthermore, it is evaluated how and why juvenile cod uses eelgrass habitats. Two field trips were conducted in Århusbugten to gather data about cod abundance, prey species, nutrient concentrations, and oxygen patterns in eelgrass, as opposed to sandy bottom, acting as the control area. It was hypothesised that more juvenile cods were present among eelgrass, that the ambient concentration of phosphorous and nitrogen were lower in waters surrounding eelgrass, and that dissolved oxygen saturation where more stable in eelgrass areas, as opposed to sandy bottom habitats. A General Linear Model estimated significance levels about the preference for eelgrass among juvenile cod, however the estimated catch were highly dependent on seasonality and the traps used for catching. Averaged values for N-NOx (*N-Nitrate* + *N-Nitrite*) and P-PO4 concentrations were lower in eelgrass, but were not statically significant due to high variability within the observations. Oxygen variability among eelgrass beds was higher when compared to sandy bottom, on one day and night, when conducting field trip 2. However, in the days that followed, the patterns of oxygen variation were similar in both the eelgrass and sandy bottom areas. The decline of both eelgrass meadows and the cod in Danish waters has been extensive for the past 40 years. While their decline both can be attributed to a serious deterioration in water quality, as a result of eutrophication and warmer sea temperatures, the decline cod population is also attributed to extensive overfishing. Eelgrass restorations efforts has proved its ability to sequester carbon, nutrients and providing habitat. However, restoration efforts are still limited to few areas, while the process is time consuming and expensive. The decline in cod stocks and eelgrass are mainly still limited by anthropogenic activities, hence eelgrass restoration efforts alone, can neither regain lost habitats or cod stocks.

2 Sammenfattende artikel Fiskeplejen

Balancen truet: Menneskets indflydelse på ålegræs og torsk

Havmiljøet er under pres. Stigende havtemperaturer stresser både torsk og ålegræs, og bundslæbende redskaber truer den naturlige genetablering af ålegræs. Det er også vigtigt at nævne den menneskeskabte forurening fra land til vand, både fra diffuse- og punktkilder. Samlet set har dette resulteret i, at vi i Danmark har mistet ca. 75 % af de danske ålegræsenge over de sidste hundrede år. Konsekvenserne heraf har været enorme. Mange sunde levesteder for torskeyngel er gået tabt, de gode fiskebestande er under maksimalt pres, millioner af tons CO2 er blevet frigivet fra havet til atmosfæren, og den økologiske tilstand i indre danske farvande har aldrig været værre.

Johan Hauser Jacobsen og Pernille Jacobsen fra DTU Aqua tog ud for at undersøge torskebestanden i ålegræs. Feltarbejdet blev udført i Kalø Vig i Århusbugten, hvor der blev indsamlet data i september og oktober med hjælp fra frivillige fra projektet Kysthjælper.

Hver måned blev der i én uge fisket på samme lokalitet på henholdsvis ålegræs og sandbund i 1 meters dybde. Sandbunden blev brugt som et kontrolforsøg for at sammenligne med, hvad der blev fanget i ålegræsset. Der blev fisket med forskelligt udstyr såsom kinaruser, åleruser og hummertejner. Ruserne blev sat ved solnedgang og tømt tidligt om morgenen dagen efter hver opsætning.

Resultaterne var klare. Der blev fanget næsten dobbelt så mange småtorsk i ålegræsbedene sammenlignet med den bare sandbund. Ligeledes var der et større fødegrundlag for torsk i ålegræsset, både i udvalg og antal. Temperaturforskellen mellem september og oktober spillede også en væsentlig rolle. I september, hvor vandtemperaturen var over 18 grader, blev der kun fanget meget få torsk, men da temperaturen faldt til 14 grader i oktober, blev der fanget ti gange så mange. I forsøget i Kalø Vig blev der også taget målinger af ilt, kvælstof og fosfor ved fiskelokaliteterne. Det blev observeret, at iltniveauerne i ålegræsset var højere om dagen og faldt tilsvarende om natten. Der blev også observeret en masse fedtemøg og snyltealger, som stjæler lyset fra ålegræsset. Overordnet set skal balancen i havmiljøet genoprettes, hvis ålegræsbedene skal udbredes.

Ålegræsset spiller en helt væsentlig rolle i havets økosystemer. Det binder enorme mængder CO2 og næringsstoffer. Med deres rødder stabiliserer ålegræsenge bundsedimentet og beskytter dermed vores kyster. Samtidig skaber ålegræsengene vigtige habitater for talrige arter. Alt fra bittesmå krebsdyr til større fisk som ål, havørred og torsk findes i ålegræsengene. Her er et stort fødegrundlag, og ålegræsengenes struktur giver gode gemmesteder, så de kan ligge godt skjult for rovdyr som skav og sæler. Især småtorsk, som ikke er kønsmodne endnu, bruger ålegræsset som en børnehave, hvor de kan vokse sig store, før de drager ud på dybere vand for at gyde.

Af Johan Hauser Jacobsen, bachelorstuderende ved DTU

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Contents

3 Introduction

The decline of eelgrass (*Zostera marina*) has significant ecological implications, especially for species like the Atlantic cod (*Gadus morhua*). Cod stocks in Denmark have declined to collapse, due to over fishing, rising sea temperatures, eutrophication, and habitat loss (Birgersson et al., 2022; ICES, 2023a,b,d). Especially in the juvenile stage, cod tend to use shallow eelgrass as a nursery habitat, before seeking deeper waters as they reach maturity to spawn and contribute to the next generation of cod. Therefore, eelgrass habitats also play an important role in sustaining healthy cod stocks (Lilley and Unsworth, 2014).

Eelgrass serves other critical function in the marine ecosystems as well, including, habitat provision, sequestering of carbon and nutrients, and protecting coastlines. However, eelgrass has been significantly impacted by anthropogenic factors, most notably eutrophication. In the past 50 years, substantial inputs of nitrogen and phosphor have led to a dramatic 75% reduction in the overall extent of eelgrass meadows in Denmark (Aertebjerg et al., 2003). The decline of eelgrass has resulted in the release of vast amounts of carbon and nutrients, and the loss of important habitats, furthermore contributing to an increasingly unbalanced marine ecosystem (Cole and Moksnes, 2016)

This unbalance has led to an increased focus, both in media, in legislation, politically and generally among the citizens. The increased political awareness has brought about initiated restorations of these important habitats. Also organizations and initiatives such as 'Ocean Institute' and the 'Kysthjælper,' funded by the Velux Foundation, have focused on investigating and facilitating eelgrass restoration efforts, involving citizens in the process. This point towards a wide willingness on acting on the issue. While action on the matter is crucial prior considerations are fundamentally important for the later results.

This thesis provides a comprehensive assessment of the crucial functions of eelgrass in marine ecosystems. Furthermore factors and mechanisms underlying the decline of both cod and eelgrass, is investigated. The thesis also evaluates the considerations, processes, and feasibility involved in re-establishing eelgrass. In pursuit of investigating some of these topics, two field trips were conducted in the bay of Århus. These trips were conducted in September and October 2023 and sought to collect empirical data on cod abundance, nutrient concentrations, and oxygen patterns in shallow eelgrass meadows, when compared to sandy bottom habitats.

A key aspect of this research involved quantifying the influence of eelgrass on cod catchment with a General Linear Model (*GLM*). This analysis also considered the impact of different fishing tools and the temporal variation between the field trips conducted in September and October. Nutrient concentrations were measured daily in both eelgrass and sand bottom habitats, and oxygen loggers were deployed overnight to monitor oxygen variations.

4 Research objectives for the field trips

For the field trips, three specific objectives were set up, which also create the framework of this research. The study of eelgrass as a habitat in relation to cod involved delving into the following questions for a better understanding of their association and the decline of both eelgrass and cod stock.

1. To what extent does the abundance of cod and their potential prey species differ between eelgrass habitats and sandy bottom ?

2. In what way does the stability in dissolved oxygen saturation differ within eelgrass- and sand-bottom marine habitats?

3. To what degree does nutrient concentrations in waters around eelgrass differ from those in sandy bottom areas?

5 Theory

5.1 Eelgrass (Zostera Marina)

5.1.1 Biological Background

Eelgrass is the most abundant flowering marine plant species in the Northern Hemisphere, and is mainly distributed as patchy settlements in shallow areas, such as bays, lagoons, and coastal regions, forming colony-like meadows. The plant is characterized by its leaves, which are around 1.2 cm wide and can grow up to 1 m long, as well as its thick, horizontally growing rhizome, anchored by clusters of roots. Since the roots require a substrate for anchorage, eelgrass typically prefers sandy and muddy bottoms (Berg, 2012). Eelgrass exhibits both sexual and vegetative reproduction mechanisms. Sexual reproduction involves seed dispersal and subsequent germination, while vegetative reproduction arises from new shoots within the rhizome (Johnson et al., 2020). Being a photosynthetic plant with roots, the growth, and distribution of eelgrass primarily depends on light availability. However, temperature, sediment conditions, oxygen conditions, wave and current exposure also play a huge role in the potential distribution (Flindt et al., 2023).

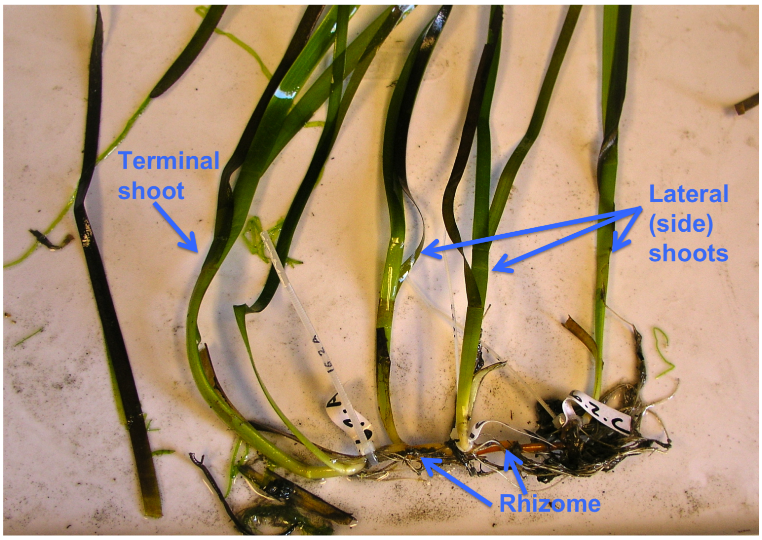

Figure 1: *Eelgrass shoot (Abbott, 2011)*

5.1.2 Important functions in the ecosystem

Eelgrass's structural characteristics not only offer protection from predators but also create a rich feeding ground for fish and birds (Kindeberg et al., 2022). Furthermore, eelgrass, being a rooted plant, plays a crucial role in stabilizing coastal sediments. The anchoring effect from roots helps to hold the sediment in place, reducing re-suspension (Bos and Katwijk, 2007). Lastly, eelgrass meadows dampen waves with their long, flexible leaves and dense root systems, which increase water friction and absorb wave energy (Walter et al., 2020). The combined effect of sediment stabilization and wave energy dissipation by eelgrass meadows is instrumental in protecting shorelines (Reidenbach and Thomas, 2018).

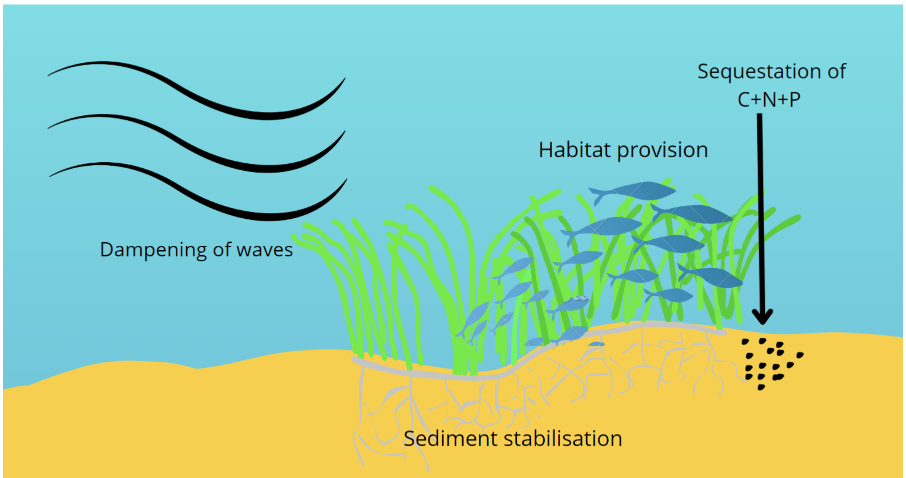

Figure 2: *Ecosystem functions in eelgrass. Inspiration from Ocean Institute (2022)*

5.1.3 Carbon and Nutrient Sequestering

Seagrasses, including eelgrass, are believed to possess a carbon sink capacity that accounts for approximately 20% of the ocean's total capability to sequester carbon (Röhr et al., 2016). As a phototropic organism, eelgrass requires carbon and nutrients to grow and thrive. The process of growth in eelgrass leads to the binding and immobilization of carbon and nutrients. The immobilization of carbon, nitrogen, and phosphor is firstly attributed to the current biomass of eelgrass meadows, constituted by living roots and leaves. Furthermore, eelgrass can bury these compounds, as during fall some of the leaves will shed. Most of these fallen eelgrass leaves will be decomposed, and carbon and nutrients will be biologically active again. However, a small part of the organic matter from shredded leaves will be buried in the seafloor, and thus permanently immobilized (Ocean Institute, 2022). The sink capacity of carbon and nutrients fixated by eelgrass meadows is therefore primarily defined by current biomass and the carbon and nutrients buried into the seafloor. Some estimates might even include the living biomass living in eelgrass meadows, hence numbers for the exact carbon and sequestration range a lot (Lange et al., 2022).

According to Cole and Moksnes (2016) 1 hectare of eelgrass can accumulate 12.3 kg of nitrogen, and 1664 kg of carbon per year, thus immobilizing this amount per year while the eelgrass bed is sustained. The ability to bury carbon is highly dependent on wave and current conditions, as this can affect how much of the carbon is buried within the seafloor (Billman et al., 2023; Ocean Institute, 2022). According to Röhr et al. (2016) average Danish carbon stocks in eelgrass meadows are 44.9t C per hectare. In comparison, the ability to bury carbon estimated is seven times lower in Finland, as a lot of the organic-bound carbon and nutrients is transported away from the burial site. On the other hand, a more conservative estimate made by the Ocean Institute (2022), suggests that current Danish eelgrass meadows immobilize binds between 23-97 t of CO2 per hectare, which is equivalent equal to 6.5-26t C per hectare. These estimates were based on the average content of carbon in sediments and living plant tissue. The estimation of total carbon stocks in Denmark varies a lot, yet it is still evident that the ecosystem function of carbon sequestration is highly valuable.

The numbers also explain the opposite negative effect when eelgrass habitats are lost. The immobilized nutrients and carbon both from plant biomass and accumulated compounds in the seabed from a lost eelgrass habitat will over time be degraded and remineralized, thus becoming biologically active again (Cole and Moksnes, 2016).

5.1.4 Historical Decline of Eelgrass Meadows in Denmark

Figure 3: *Map of eelgrass area distribution. The green color indicate areas with healthy eelgrass during the time of the research. The orange on the map for 1933 indicate the areas where eelgrass was affected by the wasting disease. The arrow is pointing at the location of Limfjorden. Source: Aertebjerg et al. (2003)*

A steep decline in eelgrass meadows has been observed since the first data on eelgrass distribution were available at the beginning of the 20th century. In 1901, Eelgrass accounted for one seventh of the areal coverage on the danish sea bottom, covering approximately 6700 km^2. I the late 1930's, the worldwide eelgrass wasting disease reduced danish eelgrass population with 93%, and only eelgrass in brackish waters were sustained. After the wasting disease most eelgrass population had recolonized to its former extent in the 1960's. Yet, in 1994, only 20 - 25% of eelgrass meadows sustained, when compared to the coverage 1901. The loss is mainly caused by the reduced colonisation depth of almost 50% (Aertebjerg et al., 2003).

Assuming that Denmark lost 75%, equal to approximately 5000km^2, of its eelgrass meadows between 1901 and 1994, and considering the organic carbon content in these meadows' sediments at 44.9 tons per hectare, the total CO2 released into the atmosphere would be approximately 83.8 million tons. This amount of CO2 is nearly equivalent to Denmark's total CO2 emissions for almost two years, based on its 2022 CO2 budget (Danmarks Statistik, 2022).

5.2 Antropogenic Threats Towards Eelgrass Meadows

The vast decline as seen of eelgrass meadows in Denmark can be attributed to several factors, however the most important reason for its historical decline is eutrophication.

5.2.1 Eutrophication and the Depth of Eelgrass

Eutrophication refers to the process where waters receive an excess of nutrients such as nitrogen and phosphorus. The main sources for an increased nutrient input in Danish waters are caused by leaching from agricultural land, atmospheric deposition, direct discharges, and inputs from adjacent waters (Thomsen et al., 2002). Thus, the input is strongly correlated to the amount of freshwater runoff from land (Aertebjerg et al., 2003). While eutrophication impacts more than just eelgrass meadows, there is a direct correlation between increased nutrient levels and the reduced depth range of these meadows, as illustrated in Figure 4.

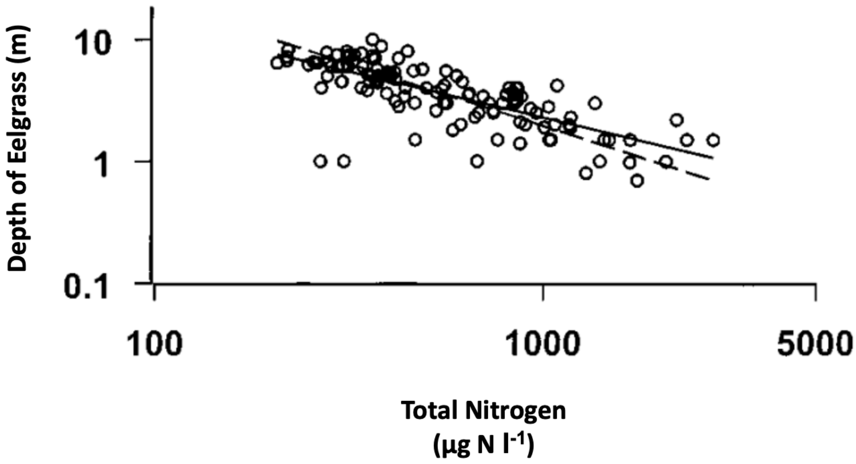

Figure 4: *Depth limits of eelgrass as a function of total nitrogen concentration. Legends are modified Nielsen et al. (2002)*

5.2.2 Primary Production and Reduced Light Availability

Primary production is constrained by the presence of light, temperature, and nutrients, mainly nitrogen and phosphate. Primary producers such as phytoplankton will typically use Dissolved Inorganic Phosphor (*DIP*) and Dissolved Inorganic Nitrogen (*DIN*) by the Redfield ratio. The optimal ratio for growth is 16 DIN/1DIP. Deviations from this ratio will indicate, whether the phytoplankton growth is limited by nitrogen or phosphor, assuming that all other macro and micronutrients are available. Kattegat and Belt Sea are mainly nitrogen-limited, and estuaries are nitrogen-limited during late summer and autumn (Aertebjerg et al., 2003).

Concentrations of nitrogen and phosphor in Danish waters display seasonal patterns. As temperatures and light conditions in early spring become more favorable, the spring bloom of primary production is initiated and explosive growth of primary producers occur, and thus the nutrient concentration will decrease. As the season progresses, the spring bloom typically collapses in early summer, due to nutrient depletion and increased grazing by higher trophic levels, such as zooplankton. Part of the accumulated biomass settles to the ocean floor, comprising both deceased plankton and feces from grazing organisms. In late summer the breakdown of thermal stratification allows the mixing of nutrient-rich deeper waters, and hence nutrients will be available again. As light and temperature still are favorable in late summer, a second bloom can occur. (Thomas and Bowers, 2021). Increased precipitation during fall will lead to elevated runoff from land-based nutrients, thus elevating concentrations of DIN and DIP during autumn and winter. During winter the light availability becomes lower, and the water masses are mixed, which explains the lower primary production, and hence higher nutrients concentrations, as illustrated in figure 5. (Thomsen et al., 2002; Aertebjerg et al., 2003).

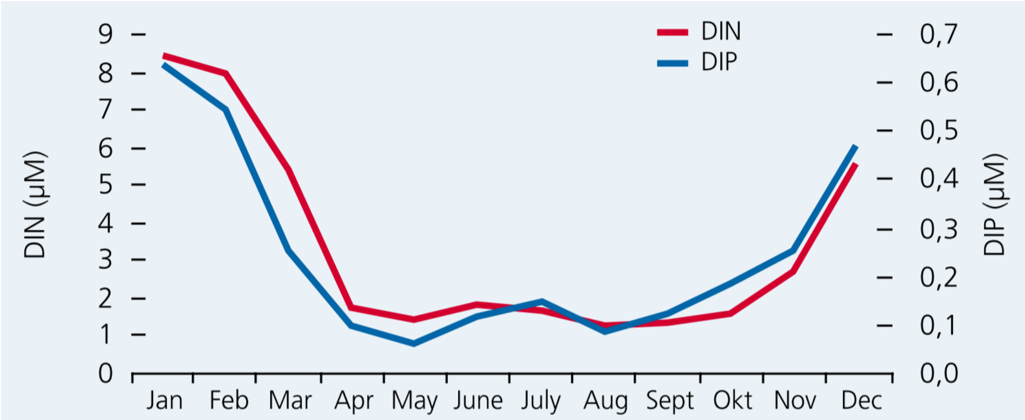

Figure 5: *Dissolved inorganic Nitrogen (DIN) Dissolved inorganic Phosphor (DIP), throughout a year. Monthly averages from 1998-2001, in the southwestern Kattegat. Source: (Aertebjerg et al., 2003)*

While eelgrass needs nutrients for growth an increased input of nutrients might suggest better growth conditions. However, an excess amount of nutrients has the direct opposite effect. The first and most obvious problem with the explosive growth of primary production is the increase in turbidity of water, reducing the sunlight's penetration through the water column. A shift towards opportunistic fast-growing microalgae growing directly on eelgrass leaves is also found in eutrophic waters, shading leaves directly (Flindt et al., 2023; Ocean Institute, 2022; Aertebjerg et al., 2003).

5.2.3 Oxygen Depletion and Hydrogen Sulfide Production

The high production of primary producers exceeds the amount that can be grazed and transported further up the food chain. When primary producers die they sink to the bottom and get decomposed by microorganisms. The decomposition of organic material on the seafloor requires a vast amount of oxygen. If the rate of oxygen consumption exceeds the rate at which new oxygen is supplied, the seabed becomes depleted of oxygen. Oxygen depletion is defined by concentrations below 4 mg/l. In extremer cases when oxygen concentration falls below 2 mg/l, it would be defined as acute. In worst case, when the seabed, becomes completely deficient of oxygen, the seabed would be considered anoxic.

Danish waters are exceptionally vulnerable to this phenomena, due to periodic thermal and salinity-driven stratification. The stratified waters hinder the oxygenation of deeper waters as the oxygenated surface water, does not mix with the heavier bottom water. When no oxygen is available fish and benthic fauna might escape or die. As these hypoxic zones can spread over vast areas this might result in mass mortality for benthic organisms. The cascading effect of when a seabed becomes anoxic is further enhanced by anaerobic sulfate reducers releasing toxic hydrogen sulfide gas (Aertebjerg et al., 2003). Oxygen depletion has a direct effect on eelgrass, especially when combined with higher temperatures. Short-term events, with the high die-off of eelgrass meadows, have been observed in late summer, with high water temperatures, and low oxygen saturation. As water temperatures increase, the eelgrass respiratory demand for oxygen exceeds the oxygen available in the water. If oxygen demand does not respond to the oxygen available, eelgrass has to adapt by switching to an anaerobic metabolism, resulting in a lower energy output. The anaerobic metabolism can only be utilized for short time spans as switching to an anaerobic metabolism accumulates toxins, which damage and eventually kill plant tissue (Raun and Borum, 2013). In addition, high concentrations of H_2S can depress the photosynthetic processes of eelgrass, and kill seedlings, potentially limiting the seed bank buried in the seafloor (Dooley et al., 2013). The worsened oxygen conditions enhance a shift in the species composition in the seafloor towards low oxygen-tolerant species, such as lug worms (Thomsen et al., 2002). Lugworm tends to bury eelgrass seeds, to depths, where they can't germinate, and their bioturbation loosens newly established shoots (Flindt et al., 2023). Decreased oxygen levels also hinder the predation on green crabs, as predatory fish such as cod, escape from hypoxic zones. High green crab densities are associated with damage to eelgrass, due to the shedding of eelgrass leaves (Howard et al., 2019).

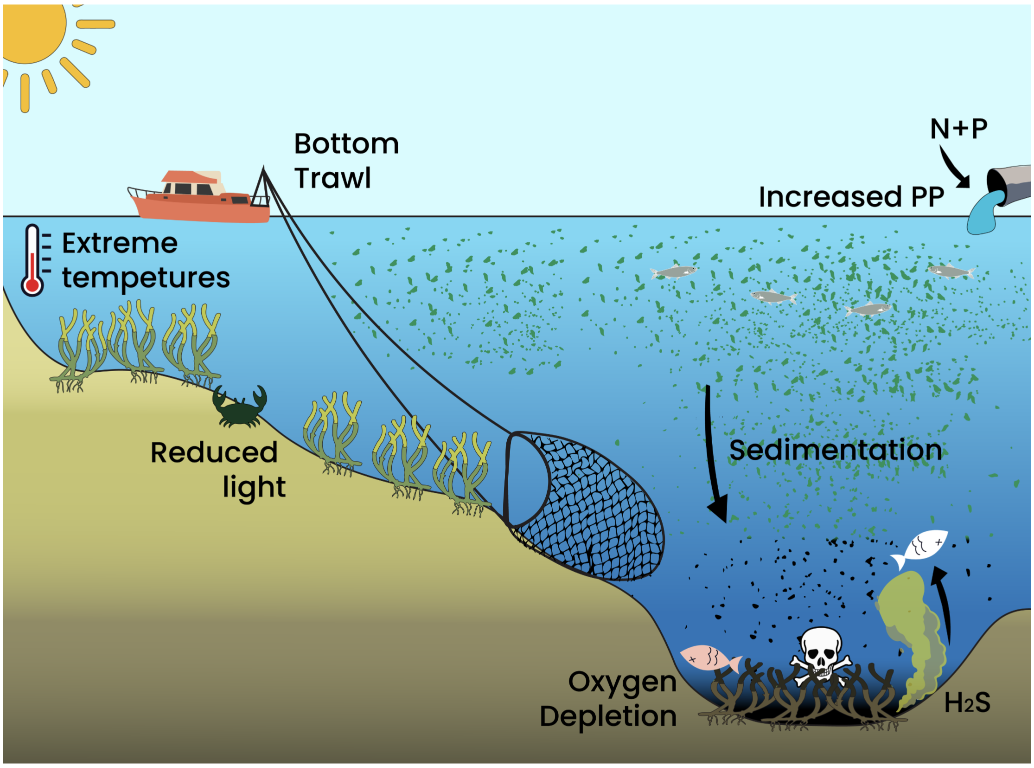

Figure 6: *Some of the risk factors for eelgrass. Illustration: Johan Hauser and Christian Bavnhøj*

5.2.4 Other Stressors

While eutrophication significantly impacts the decline of eelgrass beds, other stressors also play a role in this issue. Even after a substantial decline in nutrient inputs to Danish waters, the recolonization of eelgrass has been slower than expected. Bottom trawling, a fishing method that involves dragging heavy nets across the sea floor, can physically damage eelgrass meadows. (Krause-Jensen et al., 2021). Additionally, sediment conditions, mainly due to the sedimentation of organic material, may have deteriorated in areas where eelgrass was once abundant. As a result, the recolonization of these areas might be hindered (Flindt et al., 2023).

5.3 Reestablishment of Eelgrass Meadows

The substantial decline in eelgrass habitats has also led to a loss of the vital ecological functions that these meadows previously provided. For the past two decades, the replanting of eelgrass meadows has been widely researched. Recently, the only existing national guideline on this matter comes from Sweden (Andersson, 2021), while other guidelines have been developed primarily at the university research level. A summary is given based on several guidelines and research experiences from mainly Denmark and Sweden. A common thread in most recommendations is the division of the eelgrass meadow establishment process into the following five phases:

5.3.1 Step 1 - Site Selection

Choosing the right site involves evaluating sediment quality, wave exposure, light availability, nutrient input, temperature, oxygen, and salinity conditions, as these factors influence the survival rate of eelgrass. The site selection procedure can be based on simple field measurements, and mathematical modeling, based on environmental data (Staehr et al., 2019; Short et al., 2002).

Figure 7 describes suitable habitats for eelgrass based on several environmental conditions. GIS models as in Figure 7 could be great tools for overview when evaluating suitable habitats. However, the resolution might be very low, as local environmental conditions could deviate from this model approach. The modeling approach should be supplied with actual field measurements.

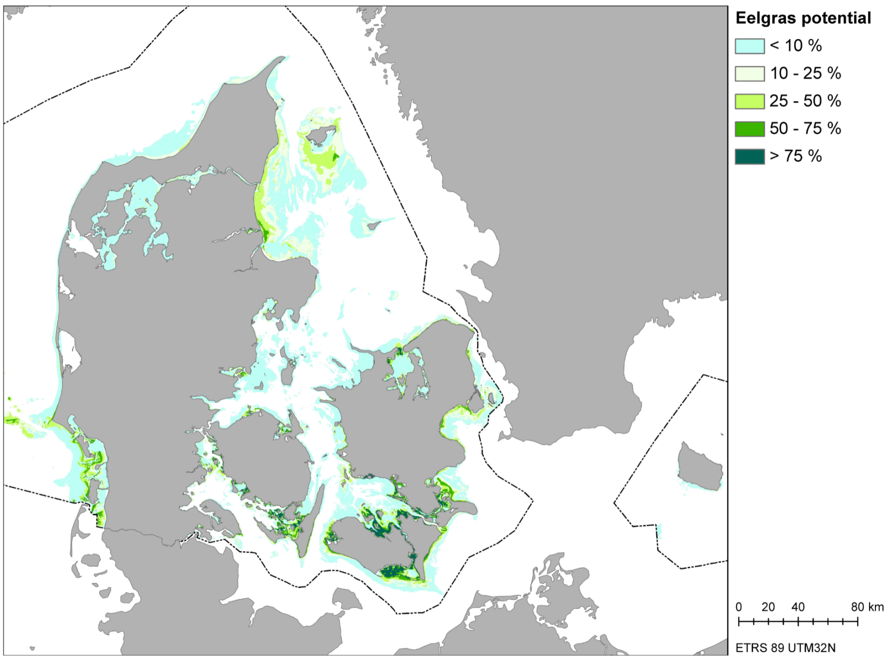

Figure 7: *Eelgrass potential and habitat suitability of eelgrass in Denmark. Model is made by Staehr et al. (2019). Eelgrass potential is based on bathmetry, light conditions, temperature, salinity, oxygen, physical exposure and sediment conditions*

When selecting a location for eelgrass replanting, a key consideration is to choose an area where the natural increase in eelgrass density is unlikely. This ensures the replanting efforts are targeted where they are most needed and do not interfere with areas where eelgrass density is expected to increase naturally (Eriander et al., 2016).

5.3.2 Step 2 - Choosing Replanting Methods

Once a suitable site has been found based on the site selection process, replanting methods should be considered. The distribution of eelgrass can either be facilitated by replanting shoots or dispersing seeds. When applying the method of seed dispersal, it can be done by placing seeds or reproductive shoots containing seeds into a mesh bag. The idea is, that the mesh bag should distribute seeds evenly across the seafloor, either facilitated by hand or passively. A study conducted by Pickerell et al. (2005) found an innovative way to facilitate this method. Reproductive shoots containing seeds, were placed into a mesh bag. The mesh bag was then attached to a bouy, which on the seafloor was bound to a stone. Once the seeds ripened they would naturally fall down, in an arc shaped pattern. Despite having a low establishment rate Eriander et al. (2016) also suggests that seed planting from a mesh bag is most effective when planting eelgrass in deeper areas (3 - 4 m), as planting shoots per hand in deeper waters is more troublesome.

Dispersal from a mesh bag is generally not adviced on shallower waters. The germinated seedlings tend to have very low establishment rates (< 1 %).

Within methods for replanting eelgrass two methods are generally used; root planting and anchoring. The bare root planting technique involves transplanting single eelgrass shoots with rhizomes into the sediments to a depth of between 25-50 mm (Orth et al., 1999). On the other hand, anchoring roots uses mussels, iron nails, or bamboo stems to stabilize the plant and prevent detachment. This will give the plants a higher survival chance, especially, when eelgrass beds are exposed (Zhou et al., 2014; Lange et al., 2022). In Sweden the bare root planting technique is recommended (Andersson, 2021). In Denmark, it is recommended to anchor the shoots. Survival of bare-root transplanted seedlings, and seed germinated seedlings, where almost zero in pilot studies in Odense fjord (Lange et al., 2020).

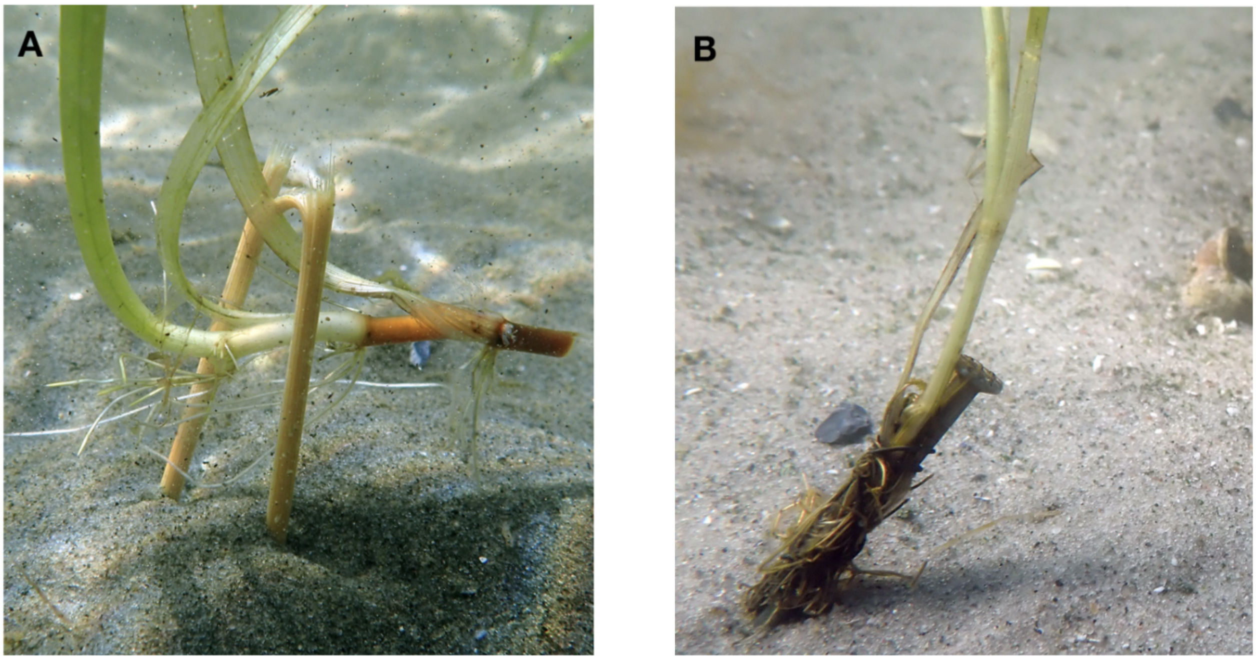

Figure 8: *Bamboo anchoring method (left), and iron nail anchoring method (right). A replanting study in Horsens by Lange et al. (2022)*

To lower the cost of replanting eelgrass, there has been different tests and developments of implementing machines within the procedure. Machinery has been utilized to replant shoots in Chesapeake Bay in Virginia, however, it was still deemed less efficient than manual transplanting due to high resource costs and poorer initial planting success (Fishman et al., 2004). In both cases, seeds and shoots have to be collected by divers manually from donor sites. As this development progresses, it becomes evident that there are various methods available for application, and no one-size-fits-all solution exists. Therefore, it is important to have a thorough understanding of the dynamics within the replanting site, in order to choose the most appropriate method for replanting eelgrass.

5.3.3 Step 3 - Pilot Experiment

A small-scale experiment should evaluate the suitability of the proposed region before using large amounts of resources and money on a large-scale restoration. Ideally, the shoots should be harvested and planted in May or June, as the damage of harvesting replanting is minimal in this period. The tested plants should be from the same mother population in a nearby eelgrass meadow. The small-scale planting could occur in small grids of 0.25 - 3 m^2 (Andersson, 2021; Lange et al., 2020).

The optimal shoot density should be evaluated in this phase as well, as higher shoot density can increase the survival rate of shoots, especially in exposed areas with high wave exposure and frequent storms. On the other hand, high shoot densities might limit growth due to internal competition among the shoots. The cost is almost proportional to the shoot density when establishing a full-scale operation, which makes this consideration very important. Andersson (2021) suggests a shoot density of a minimum of 4 shoots per m^2. Trials in Horsens fjord were planted with a shoot density of 21 shoots per m^2.

The effect of different environmental gradients should be tested as well. In the trial phase conducted in Horsens Fjord, the experiment highlighted the significance of eelgrass growth to a eutrophication gradient. This insight narrowed down the feasible areas for a full-restoration project effort downstream of Horsens Fjord (Lange et al., 2020).

The shoot growth and loss, from newly planted shoots, should be monitored closely within frequent intervals. Furthermore, environmental data such as light intensity, oxygen concentration, temperature, and salinity should be obtained as well. The environmental data associated with the decline or increase of eelgrass shoots during the testing phase provides insight into the causes of potential failures or successes (Flindt et al., 2023).

5.3.4 Step 4 - Large Scale Restoration

When a suitable habitat and optimal shoot density have been found based on the test plantings and environmental data, the large-scale operation can begin. An appropriate donor meadow has to be selected, from where adult vegetative shoots are carefully selected by divers. Environmental conditions in the donor meadow should not differ much from those at the selected site. To minimize impact, not more than one third of the vegetative shoots from the donor meadow should be taken. Donor shoots can either be transported directly from the donor meadow by boat to the restoration sites or from storage facilities on land (Andersson, 2021). However, it is advised that donor shoots are planted as quickly as possible to reduce stress on donor shoots. Whereas in Sweden, it is recommended that all shoots should be planted equally spaced from each other in Denmark, a chess pattern planting technique has been utilized switching between grids of planted eelgrass and sand (Andersson, 2021; Lange et al., 2022).

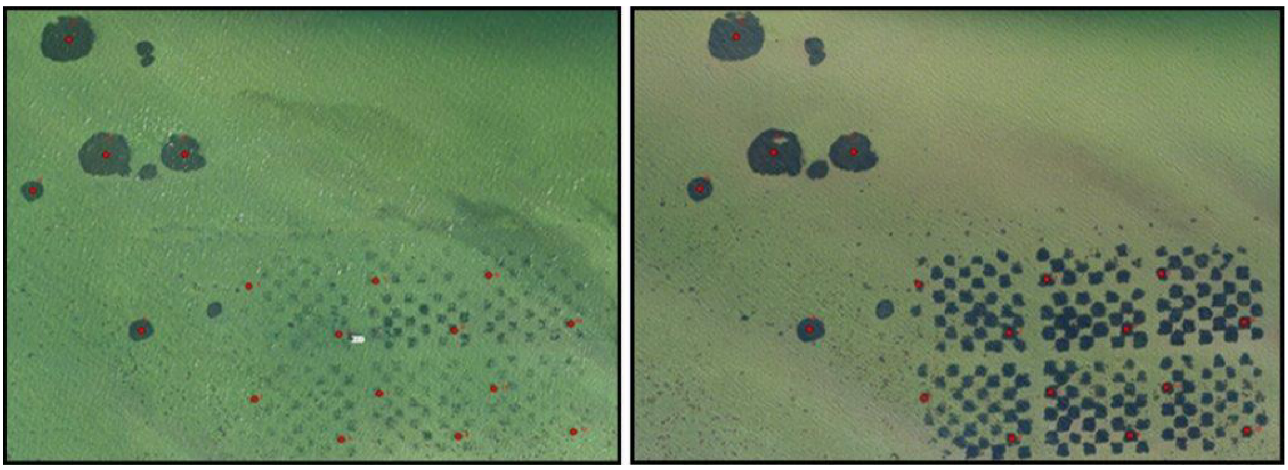

Figure 9: *Photo of large-scale restoration in Horsens fjord in 2018 (left) and in 2019 (right). Eelgrass shoots were initially planted by nails and bamboo stems to the restoration side in 2017 from mother populations (upper left corner)*

5.3.5 Step 5 - Evaluation

An assessment should be conducted to understand the ecosystem services provided by the large-scale established eelgrass beds. Parameters assessing ecosystem services could include an increase in biomass, carbon, and nutrient sequestering, as well as an improvement in water quality, fauna, and sediment conditions (Andersson, 2021). An assessment of the newly planted eelgrass bed in Horsens over two years showed a total immobilization of 3895 kg C, 283 kg N, and 58 kg P per hectare/year. These numbers stem from the immobilization of plant biomass, roots, seabed burial, and biomass in the increased fauna (Lange et al., 2020). However, eelgrass meadow restoration is an expensive and time-consuming process. The restoration process, estimated to span over 10 years, is expected to incur expenses ranging from 0.8 mio DKK to 4.8 mio DKK per hectare (Andersson, 2021). Ocean Institute (2022) estimates the restoration efforts in Denmark to cost 'between 53.000 DKK to 350.000 DKK per hectare depending on the shoot density. The majority of these costs are attributed to labor expenses. Incorporating citizen science projects like "Kysthjælper" leads to a substantial reduction in these costs. This is mainly because tasks such as harvesting and planting could be carried out by volunteers, thereby reducing labor expenses (Bech, 2023).

5.4 The Atlantic Cod

5.4.1 An Introduction to the Atlantic Cod

The Atlantic cod belongs to the family of Gadiee and has been one of the most important commercial fish species in the whole North Atlantic. Spawning happens in the open-water column in cold and deep waters. It takes approximately 3-4 weeks for eggs to hatch. At this stage they are still more or less unable to swim long distances themselves and are carried by ocean currents, drifting them far away from their spawning locations (Frausing et al., 2023). During the larval stage, they mainly feed on copepod and copepod eggs. However, at the length of 7 cm, they adopt a demersal lifestyle seeking shallower waters (ICES, 2006). When becoming demersal, their diet becomes highly omnivorous, consisting mainly of crustaceans, smaller fish, annelids, and mussels (ICES, 2006; Hoffmann et al., 2021). As a rule of thumb cod tends to eat prey that is 20-40% of its length or 2% of its weight. (van Deurs et al., 2016; ICES, 2006). Generally, as the size of cod increases, it seeks towards deeper waters, and a dietary shift occurs (Freitas et al., 2015; ICES, 2006). When getting older, cod may also feed on pelagic fish such as herring and sprat (Hoffmann et al., 2021; van Deurs et al., 2016).

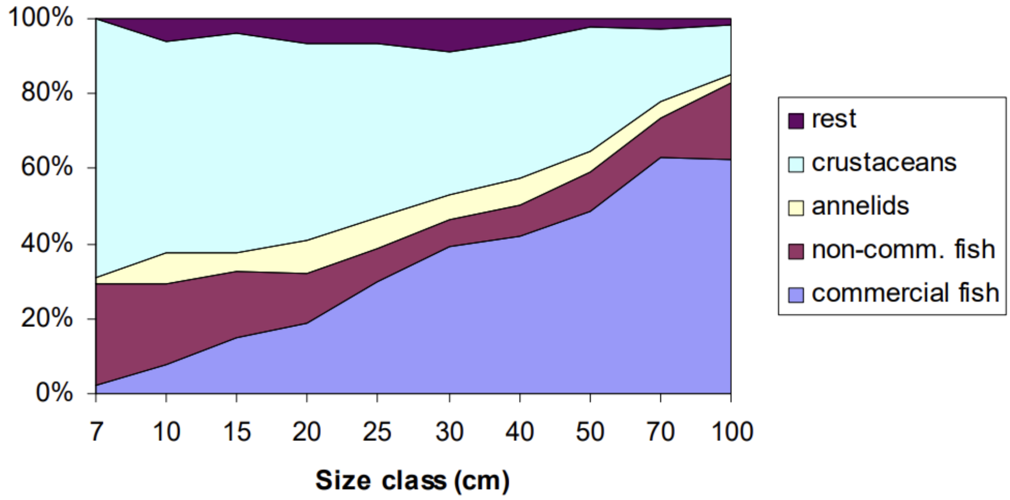

Figure 10: *Size-dependent intake of cod. Source: ICES (2006)*

5.4.2 Cod and Eelgrass

At the juvenile stage, eelgrass becomes an important habitat for the cod. The motivation behind using vegetated habitats are mainly based on adequate protection and the vast range of species found within eelgrass, which offers small cod a great food source. The abundance and preference for eelgrass among juvenile cod has been tested in several field and lab studies. A comprehensive meta-study by Lilley and Unsworth (2014), states that juvenile cod was generally found more frequently among eelgrass meadows and that both growth and viability gains were higher for cod among eelgrass when compared to other bottom habitats. Lab experiments have also shown a preference for eelgrass meadows when small cods were exposed to predatory threats (Gotceitas et al., 1997). Furthermore, small cod tend to utilize a schooling behavior, when exposed to bare sand bottom, illuminating the lack of protection (Anderson et al., 2007). However, other habitats offering the same structural complexity and feeding ground as eelgrass habitats, such as kelp forests and stone reefs also act as an important habitat for smaller cod (Hoffmann et al., 2021). This leads to the assumption that cod may have habitat preferences, but they are not solely dependent on these preferences and can shift and adapt to different habitats.

5.4.3 Salinity

Cod tolerates a broad range of salinity levels. The range includes oceanic waters with a salinity of around 35 ppt, extending into the Baltic Sea's eastern part, where salinity can be as low as 5 ppt. The salinity gradient ranging from the North Sea to the Baltic Sea has several implications for the distribution and adaptation of Atlantic cod. Studies have shown that cod in areas with lower salinity, such as the eastern Baltic, exhibit genetic differences from those in higher salinity areas like the western Baltic and the North Sea. The difference in salinity is one of the reasons for the reproductive isolation between cod populations in Danish waters. Cod adapted to lower salinity levels may not be as successful in reproducing in higher salinity environments, and vice versa (Kijewska et al., 2016).

5.4.4 Temperature and Oxygen Levels

The temperature preference of cod varies and depends on their momentary life stage, food abundance, and oxygen levels. Thus defining exact temperature ranges can be a trade-off between food availability, oxygen levels, and temperature. Being a cold-blooded animal (*ectothermic*) a higher temperature results in faster metabolic activity. This is simply caused by the faster enzymatic activity in their body. A higher metabolic rate increases oxygen consumption. Hence, if temperature increases so does the oxygen demand. To compensate for the increased oxygen consumption at higher temperatures cod either have to ventilate their gills at a faster rate, thus consuming more energy, or seek habitats where oxygen is sufficient, or into colder waters. Notably, less oxygen can be dissolved in warmer waters as in colder waters, which further makes warmer temperatures unviable. While cod can tolerate temperature between 0-20 °C, their thermal preference is 3-15 °C(Behrens, 2023; Freitas et al., 2016).

The study by Plante et al. (1998) on hypoxia in cod reveals that high mortality rates are observed when oxygen saturation falls to 16% for prolonged periods. However, at 40% oxygen saturation, there was no observed mortality. However, these numbers are not deterministic, as cod oxygen demands can be flexible. In the Baltic Sea for example cod are observed to swim into demersal hypoxic zones for short periods, to feed. While residing in lower depths they switch to an anaerobic metabolism. After feeding in hypoxic waters, cod migrate back to more oxygenated waters to digest and recover (Behrens et al., 2018).

5.4.5 Depth

Cod can be found in various depths, depending on their momentary age. However, normally their depth range is between 0-300 m (Hoffmann et al., 2021). The depth distribution of coastal cod is dependent on season and temperature as reported by Freitas et al. (2016), and illustrated in Figure 11.

During summer where sea surface temperatures are high cod retreat to to deeper waters under the thermocline. Being in warmer waters, above cod's thermal preference of 15 °C, disrupts the balance between oxygen requirements and available oxygen as stated by Freitas et al. (2016). Hence, they can not utilize the high production of prey found in shallower habitats such as eelgrass. During fall when temperatures become equally favorable in shallower waters cod tend to utilize shallower habitats more.

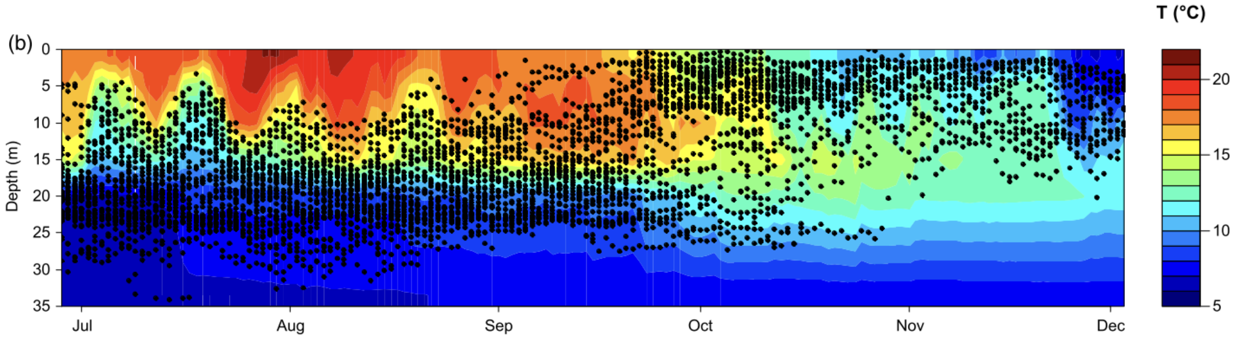

Figure 11: *Average depth distribution (night) of 48 acoustic tagged cod and the temperature in the southern part of Norway (Tvedestrand) (Freitas et al., 2016)*

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5.4.6 The Decline in Cod Stocks

Since the 1980s, extensive overfishing has led to a substantial reduction in cod populations in all waters surrounding Denmark. While the total landings in the North Sea was at its peak in 1977 with 600.000 tons landed, in 2022 this has been reduced to less than 20.000 tons (ICES, 2023a). The same tendency in decline has also been observed in Kattegat and the Baltic Sea, merging stocks into an almost collapse (ICES, 2023a,b,d,c).

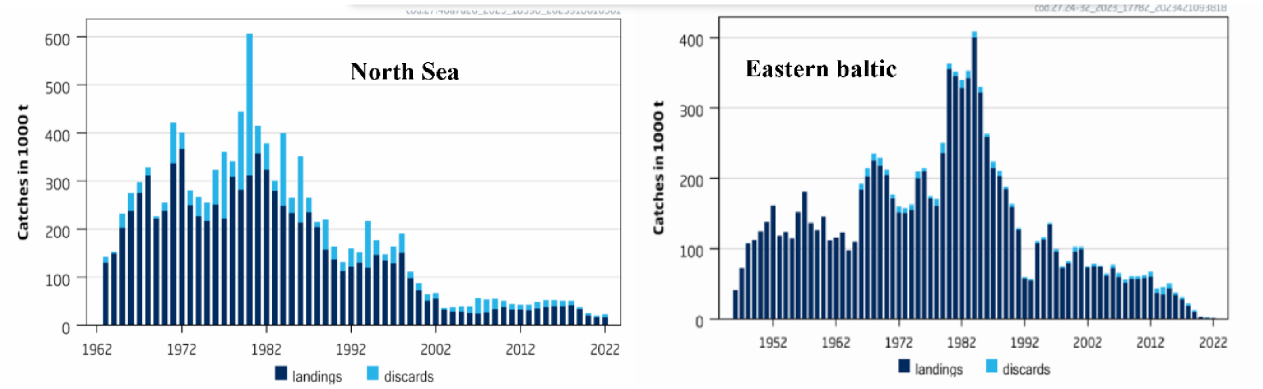

Figure 12: *Total landings in the North Sea (left) (ICES, 2023a), and in the Baltic Sea (right) (ICES, 2023d)*

The reasons behind this development are very complex and can be attributed human activities, such as overfishing, rising temperatures, and deterioration of water quality. Firstly, especially bigger fish were targeted for human consumption, and mature females were removed from cod populations. This has resulted in a significant decrease in the spawning stock biomass (SSB) and, consequently, the reproductive capacity of the cod populations (ICES, 2006; Birgersson et al., 2022).

In addition, another consequence of targeting larger fish from cod populations is that a human-induced genetic selection has occurred among cod in Danish waters. This phenomenon is also referred to as fisheries-induced evolution, resulting in a decline in the age of maturation (Johnson, 2023; Andersen et al., 2007). The quantity of eggs a female can produce is directly linked to the size. Consequently, females that reach maturity at a younger age, and thus are smaller in size, tend to produce fewer and smaller eggs (Hoffmann et al., 2021).

Another significant factor is the increase in sea temperatures. As Danish waters exhibit the southern part of cod's range, higher temperatures have been linked with lower recruitment rates and a northward migration of cod (O'Brien et al., 2000). This effect is further enhanced when SSB is low (Lindegren and Eero, 2013).

Rising temperatures also mean that less oxygen can be dissolved in water. Waters such as the Danish straits and the Baltic Sea, are already prone to hypoxia, due to the permanently stratified halocline layer. This, combined with increased nutrient input, creates vast areas of hypoxic zones at the bottom. Bottom water can only be oxygenated by heavier and more saline waters entering from the North Sea. The freshwater outflow from the Baltic Sea defines the amount of inflow from the North Sea, affecting the extent to which bottom waters are oxygenated (Lehmann et al., 2022). The expansion of hypoxic bottom areas is related to a decline in the maximum length of cod as well as decreased survival of cod eggs (Hoffmann et al., 2021; Orio et al., 2022).

The weakening state of cod has also left cod stocks more prone to both diseases and predation. An increasing incidence of seal-worm (*Contracaecum osculatum*) infection in Baltic cod has been documented over the past decade, resulting in chronic liver disease and further weakening the physical condition of the cod (Ryberg et al., 2020). Furthermore, an estimate by Jepsen (2022) suggests that at least one third of the yearly recruitment of the western Baltic cod stocks is predated by cormorants, further reducing the reproductive capability.

The International Council for the Exploration of the Sea (ICES) recommends a complete halt to commercial fishing in the eastern Baltic Sea and Kattegat in 2024 (ICES, 2023b,d). Although some quotas still exist for the North Sea and western Baltic cod, they are at historically low levels (ICES, 2023a,c). For the Baltic cod stock it remains uncertain whether merely reducing fishing pressure will be sufficient to return cod populations to self-sustaining levels. In the case of the Baltic Sea, despite a decrease in fishing activity, cod stocks continue to be beneath a self sustaining levels (Eero et al., 2020).

6 Method

6.1 Field Work

Two field trips were conducted in Kalø Vig. The first survey was done from 15-23 September 2023 and the other one from 20-29 October 2023. The surveys aimed to collect data regarding cod abundance, prey species, oxygen and nitrogen, and phosphor concentration in eel grass-covered areas, as opposed to areas with a sandy bottom. Originally, the intention was to survey on deeper areas as well, which could only accommodated by boat. This was partly done in field trip 1, with the help of volunteers. However, as going by boat was limited by weather conditions and volunteers, surveying on deeper stations were only done for one day at field trip 1. As half of the gear was intended to be used at deeper stations in field trip 1, the amount of gear utilized on shallower habitats were half as low as in field trip 2. In field trip 2 it was decided to use all of the fishing gear on shallow habitats, hence two additional stations were made, surving as a replica.

6.1.1 Selecting Area for Study - Field trip 1 in September

The first step was to choose an area where eelgrass was present. This was done as by inspecting the bottom habitat from the surface with an Aqua scope. The weather was relatively calm on the first day of field trip 1, which made this part relatively easy. When the suitable eelgrass habitat was found, 4 buoys were set up to mark the area, and GPS coordinates were marked on Google Maps. The depth of the test area habitat site was measured and used later as a baseline for the control area. The average depth was 0.8m, however during low and high tide the depths could range from 0.2m to 0.9m. The two chosen stations were approximately 500-600 meters away from the nearest public parking spot at the public school of Kalø Vig. The gear for the survey had to be transported either in wheel borrows, or with a dinghy that we tugged by hand.

Figure 13: *Eelgrass A and Sand bottom B*

6.1.2 Selecting Area for Study - Field trip 2 in October

For the second field trip, two additional stations were chosen, each serving as a replica for Eelgrass A, and Sandbottom B. Nevertheless, the initial Sandbottom area (B) had to be relocated due to its proximity to 'Havskov bæk,' which had been designated as a conservation zone by the time of the second fieldwork. Fishing in conservation zones is prohibited, and a new area with the same depth and bottom habitat was found.

Figure 14: *Control and Treatment Area for field trip 2*

6.2 Data Collection on Fieldwork

6.2.1 Fishing with Traps

Three different types of passive nets/ traps were used to collect data for night fishing. The eel fyke and the elongated lobster trap were both strung out with a brick in each end. To each brick, a flag buoy where bound marking the west- and east ends of each net. The lobster trap was bound to a brick. On the surface, the brick for the lobster trap was marked with a handball-sized plastic buoy.

The fishing tools were set in the evening before sunset. To standardize the experiment every location was fished with the same tools on a given night. However, strong onshore winds and waves made it difficult to fish with non-rigid tools for two nights at field trip 1, and for one night at field trip 2. Nets were placed in the same formation, and towards the wind direction, as it would minimize the surface area towards incoming waves and current, ensuring stability throughout the night.

Figure 15: *Gear used for fishing. Left: Netted lobster trap. Middle: Rigid lobster trap. Right: Eel fyke*

6.2.2 Shrimp net Survey

An 8x8m grid was marked with bouyes on selected locations on eelgrass and sand bottom, with the same depth as the stations with passive fishing gear. The shrimp net survey was intentionally not conducted at the selected stations (A, B, C and D). The net was pushed by hand with a 45-degree angle towards the bottom. A steady "walking" speed was maintained when pushing the shrimp rush. It was assured that the whole area was covered, without interfering with non-fished and already surveyed transects.

6.2.3 Length Measurements

Each individual was identified by species and measured with a length board. Dead individuals were noted as well. In some cases, the carcasses were molested by crabs, and the length was therefore estimated.

Figure 16: *Length measurement of Cod during field trip 1*

6.2.4 Monitoring of Oxygen Concentration

Oxygen measurements were done with the minidotPE Oxygen logger. To ensure accurate and stable readings over longer periods, custom holders were fabricated for this project. These holders were designed to keep the oxygen logger steady and unaffected by waves, and current. To each leg, 1 kg of diver lead was attached to keep it with plastic strips, to ensure a low center of gravity.

Figure 17: *The miniDot PE Oxygenlogger*

Figure 18: *Custom-made tripod in Aluminium for the MinidotPE. Made in DTU-skylab*

6.2.5 Nitrate and Phosphor Measurements

Two daily samples for nitrate and phosphorus measurements were taken at each station using a syringe. For eelgrass areas, it was ensured that the samples were taken as close to eelgrass leaves as possible. The samples were subsequently passed through a 0.02 μm nylon filter and transferred into sample containers. The sample containers were then placed in a cooled box immediately afterward, and by the arrival to the field trip summerhouse, the samples were stored in a freezer. The samples were taken to the environmental lab at DTU-sustain for further analysis.

6.3 Statistical Analysis

6.3.1 General Linear Model - Number of Cod per Night

Question 1 aimed to investigate, whether cod was more abundant among eelgrass. To test this relation a general linear model explaining the number of cod caught per night per tool per gear employment. The model compares the effect of eelgrass versus sand bottom habitat, the gear used which was either netted lobster trap, eel fyke, or rigid lobster trap, and whether the fishing was conducted on field trip 1 in September or in field trip 2 in October. A negative binomial distribution was used to account for the large proportion of zero observations. The GLM was linked with a log function and was conducted with the GLmmTMB package in R.

The models were specified as follows

$$\log(\text{N}) = \beta_0 + H_{(\text{Eelgrass, Sandbottom})} + G_{(\text{Netted lobstertrap, Eel fyke, Rigid lobster trap})} + M_{(\text{September, October})} \quad (1)$$

N describes the number of cod per night per gear employment. H represents the habitat, which is either eelgrass or sandbottom. G represents the type of gear used, whereas M represents the month. Each of these discrete values will have a set of coefficients that describe the effect on the logarithmic transformed number of cod when compared to a reference category. If the reference category is chosen it would display an effect of zero, in that category. To estimate the actual number of cod from this model following equation should be used.

$$\text{Nr of Cod/night} = \exp(\log(\text{N})) \quad (2)$$

6.3.2 Nitrate and Phosphate Levels

Question 3 aimed to investigate whether the average concentration of nutrients was lower in eelgrass areas, due to eelgrass' ability to sequester nutrients. As already stated in the theory section nutrient concentrations in water might fluctuate a lot, as nutrient concentration depends on various factors, such as light, temperatures, runoff, time of the year etc. Hence, a two-sample t-test was used to test whether mean N-NOx concentrations and P-PO4 differed in eelgrass meadows compared to sand bottom areas. It compares the means of those values, based on the assumption that the concentrations of samples are normally distributed. In some cases where the input data is not normally distributed, the data can be logarithmic transformed and thus resembling a normal distribution.

Results from the lab measured the concentration of phosphorus bound in phosphate (P-PO4), and the total concentration of nitrogen bound in nitrate (N-NO3), and nitrate (N-NO2). NOx is the sum of nitrogen bound in nitrate and nitrite, hence NOx = N-NO2 + N-NO3.

To investigate whether there is an actual difference in the means of N-NOx and P-PO4 concentrations, two null hypothesis were formulated as follows:

For N-NOx:

$$H_{0,\text{N-NOx}} : \mu_{\log(\text{N-NOx, eelgrass})} = \mu_{\log(\text{N-NOx, sand bottom})} \tag{3}$$

For P-PO4:

$$H_{0,\text{P-PO4}} : \mu_{\log(\text{P-PO4, eelgrass})} = \mu_{\log(\text{P-PO4, sandbottom})} \tag{4}$$

7 Results - Objective 1 - Cod in Eelgrass

The overall presentation of results will follow the chronology of the research objectives from page 2. In the following section, findings related to the first objective are provided, which aimed to investigate whether eelgrass habitats in Århusbugten attract cod.

7.1 Total Catch of Cod

The total catch of cod in Areas A, B, C, and D is displayed below in Figure 19. For both field trips, the abundance of cod in low-depth eelgrass was almost twice as high as on the sandy bottom. However, the total amount of cod caught on field trip 1 in low-depth eelgrass was very low compared to the total cod caught a month later on field trip 2. The low abundance of cod on field trip 1 can be partly explained by lower fishing intensity. Two stations (C and D) were not fished during field trip 1. In addition, due to stormy weather conditions on field trip 1, fishing with eel fyke and elongated lobster traps were put on hold for two nights. During these two nights, the eel fyke and the elongated lobster traps were each substituted by a rigid lobster trap. On field trip 2, fishing with eel fyke and netted lobster traps was only put on hold for one night. Due to logistical complications, they were not substituted by a rigid lobster trap, as in field trip 1. The average sea surface temperature when conducting field Trip 1 was 18.9 °C, whereas in October, the average temperature was 10.15 °C, which could play a significant factor and will be discussed later.

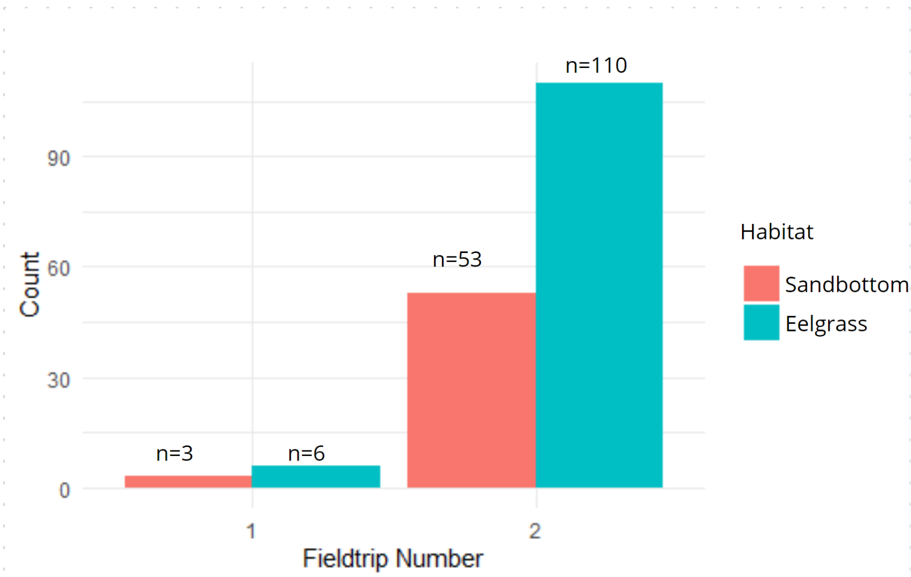

Figure 19: *Total catch of Cod on field trip 1 (September) and field trip 2 (October), on Eelgrass meadows (treatment) and Sandbottom habitat (control). Note that the fishing intensity was dobbelt doing field trip 2.*

7.2 Age and Size Distribution of Cod

The size distribution of cod for both field trips is plotted below, distinguishing between the eelgrass area (*blue*) and the sand bottom area (*red*). The biggest range of cod size was found within the eelgrass with the smallest individual measuring 10 cm and the biggest 37 cm. The size range of cod within sand bottom habitat was smaller ranging from 11cm to 29 cm. Notably, several larger individuals over 29 cm were only caught in eelgrass, otherwise, the size range is quite similar in eelgrass and sand bottom. Hence, it is not assumed that two distinct populations co-exist in eelgrass and sand bottom.

Precise age determination typically involves examining otoliths, which would involve killing the collected cod. All cods where under the legal limit, hence direct age reading was not possible. Age categories were approximated using the size of the cod and information from previous studies. Cods ranging from 10-20 cm would be less than one year old, belonging to the 0+group. Cods ranging from 20cm to 35 cm would presumably belong to 1+ year group (Dunlop et al., 2022). As the age and size of cod increased, much fewer individuals were observed.

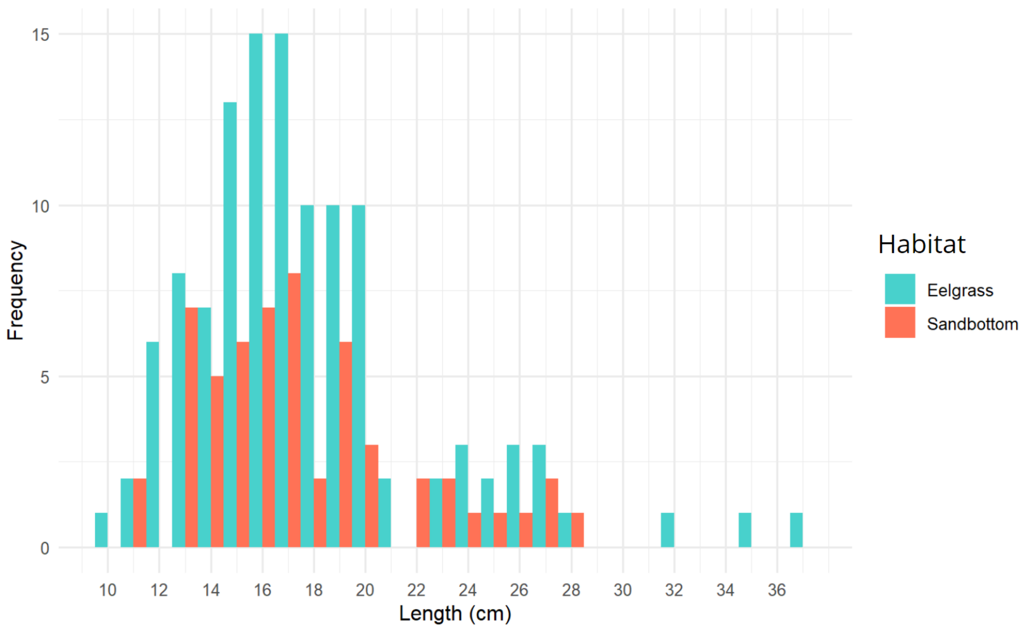

Figure 20: *Size distribution of total catch on field trip 1 and 2*

7.3 Prey Species - Night Fishing

The abundance of prey species is evaluated from catch overnight with eel fyke, rigid- and netted lobster trap. The largest cod caught was 37 cm. Assuming that a cod of that size is capable of eating 40% of its body weight potential prey would be 15 cm and less. The catch data in figure 21, displays the species composition of species, with a length under 15 cm. The abundance of corkwing wrasse (*Symphodus melops*), flounder (*Platichthys flesus*) , and sculpin (*Cottoidea*) where higher when fishing in eelgrass meadows. The total number of eelpouts (*Zoarcidae*) and gobies (*Gobius*), where higher when fishing in the sand bottom areas D and B. Notably, all species where caught within the each habitat, suggesting that prey species could migrate back in forth in between sandbottom and eelgrass. Furthermore, larger cods could potentially prey on smaller cod.

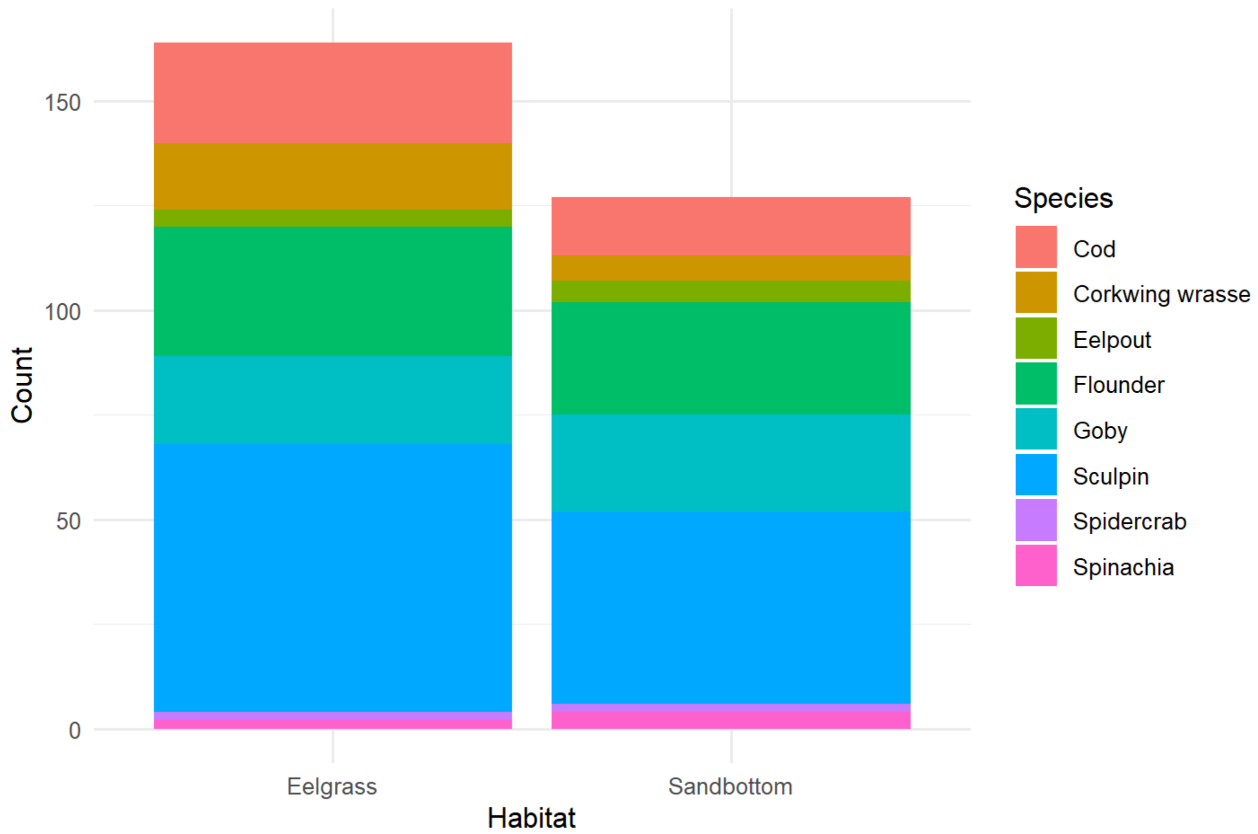

Figure 21: *Catch of prey species below 15 cm of length during night fishing with Eel fyke, rigid- and netted lobster trap at eelgrass and sand bottom during both field trips*

7.4 Prey Species - Shrimp Rush Survey

Due to the smaller mesh size of the shrimp rush, this type of survey was able to catch much smaller species and individuals. While the passive fishing gear required that potential prey actively had to swim into the fishing gear, the shrimp net survey were able to catch stationary species as well. A vast majority of specimen caught in the shrimp net were under 3-7 cm in length. Hence, it assumed almost all cod caught could feed of these.

A broader variety of potential prey species were found in the eelgrass, including green crab (*Carcinus maenas*), baltic prawn (*Palaemon adspersus*), flounder, pipe fish (*Syngnathinae*), sculpin, spider crabs (*Macropodia rostrata*), and spinachia (*Spinacia oleracea*).

Only brown shrimp (*Farfantepenaeus aztecus*) and flounder were caught in the sand bottom, however, the abundance of brown shrimp where higher when surveying on sand bottom.

Summarized, the shrimp rush survey displays a higher diversity and total numbers of potential prey in the eelgrass, when compared to sandy bottom.

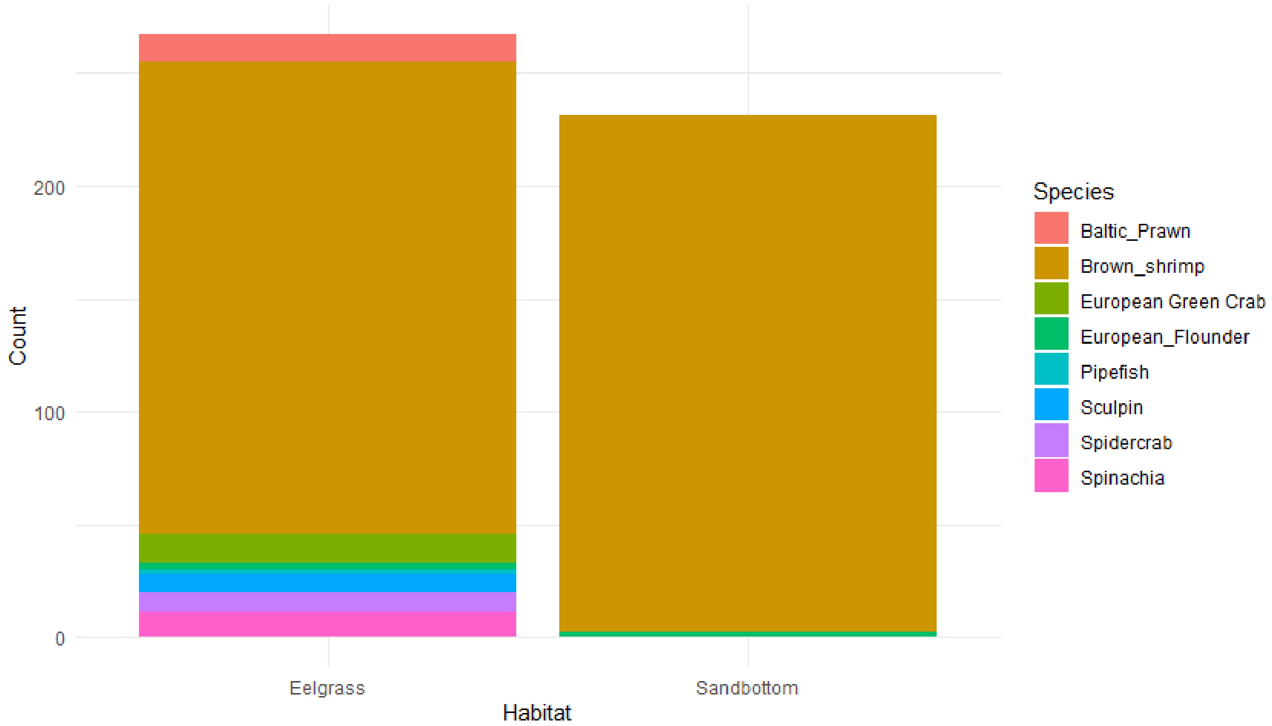

Figure 22: *Shrimp rush survey field trip 2, species below 15 cm in length*

7.5 Generalized Linear Model

7.5.1 Summary of Model

Cod catch for each gear deployment per night for both field trips was noted, and fitted into the model. The result was as follows:

$$\log(\mathrm{N}) = \beta_0 + H_{(\text{Eelgrass, sand bottom})} + G_{(\text{Netted lobster trap, eel fyke, rigid lobster trap})} + H_{(\text{September, October})} \quad (5)$$

Table 1: *Summary fit 1 - Intercept, eelgrass vs sand bottom, netted lobster trap and eel fyke vs rigid lobster trap and September vs October*

	Estimate	Std. Error	p-value	Validation
Intercept (β_1)	-4.01	0.74	4*e^{-}8	AIC: 241
H(Eelgrass)	0.84	0.38	0.025	logLik: -114.5
G(Netted lobster trap)	3.4	0.57	5*e*-10	Deviance: 229
G(Eel fyke)	1.68	0.60	0.005	
M(October)	1.92	0.54	0.0003	

The model assesses the effect of various explanatory variables on predicting the number of cod caught per night per gear employment. The explanatory variables include habitat types which are eelgrass and sand bottom, three types of designated tools, namely netted lobster trap, eel rush, and rigid lobster trap, and whether a cod was caught in September or October. Each effect estimate is compared to a reference category; for habitat (H), the reference is sandy bottom. For gear type (G), the rigid lobster trap serves as the reference category, as it was the least efficient. The variable M (Month) explains the effect of fishing in October, compared to the reference month, September. All effects were significant at a 5% significance level, as all p-values were below 0.05.

The most significant impact on the expected number of cod per night per gear employment was gear type, followed by field trip number and habitat type, respectively.

7.5.2 Residuals

The plot shows the observed residuals against simulated residuals from fit. The residual test was conducted with the Dharma package in R and can be used for validating the model.

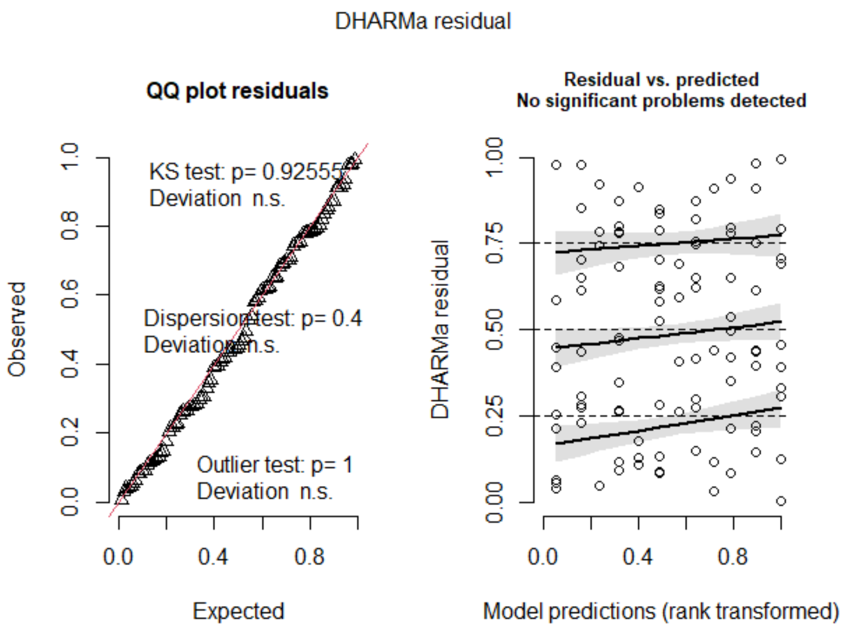

Figure 23: *Residuals based validation on the fit.*

The points of the QQ plot lie close to the 45-degree line, suggesting that the residuals have a distribution similar to the theoretical distribution. The KS-test gives a p-value, well above a significance level of 0.05, indicating no significant deviation from the expected distribution. The residuals are likely well distributed from the model's assumptions. The scatter plot of residuals vs predicted values does show a small skewed pattern, however, the confidence intervals suggest that it is within an acceptable range.

7.6 Summary - Objective 1

Cod and potential prey species were more abundant among eelgrass compared to sand bottom habitats. However, the catch of cod was highly depending on whether we were conducting the survey in

September or October. In addition, the tool used had a vast influence on the catch of cod. The netted lobster trap was the most efficient tool, followed by the eel fyke, and rigid lobster trap, respectively.

8 Results - Objective 2 - Dissolved Oxygen

The next part will present results from the study of Objective 2 which aimed to investigate whether eelgrass areas could resemble more stable oxygen patterns compared with the control area with sandy bottom.

8.1 Dissolved Oxygen Saturation

The dissolved oxygen was calculated from Minidot's software, and data were extracted and plotted with R. The data were plotted displaying the whole week during the field trip conducted in October in figure 24. Data from field trip 1 have been excluded, do to the fact that the dissolved oxygen saturation was merely measured doing night. Data from field trip 2 provides a more comprehensive understanding of the development over time.

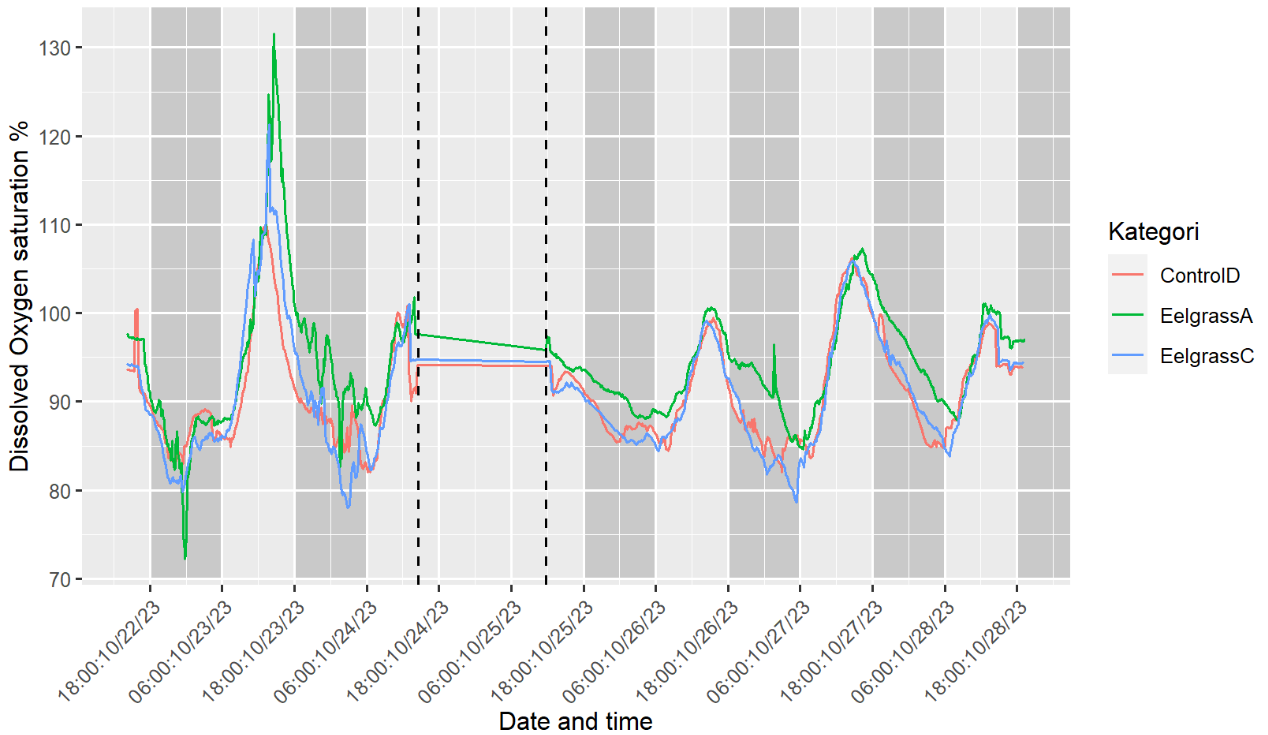

Figure 24: *Dissolved Oxygen Saturation % i different habitats during field trip 2 . Dark-shaded areas resemble night*

Notably, daily fluctuations were observed during the night and day in all areas. During the day the dissolved oxygen saturation would rise, presumably due to photosynthetic activity from phototropic organisms, whereas in the night only respiration using oxygen would occur. On the first night (22/10-2023) dissolved oxygen saturation was lower in eelgrass areas A and C when compared to the sandy bottom habitat (Control D). The following day the eelgrass areas (A, and C) displayed a significantly higher dissolved oxygen saturation during the daytime when compared to the sand bottom D. Due to a storm on the third night, oxygen-loggers were taken out of the water. Time out of the water is marked with two vertical lines in Figure 24. After redeployment, the peaks of dissolved oxygen saturation for each day in every area were almost equal.

To further investigate differences in oxygen patterns a summary of all three oxygen measurements is made. Measurements, where Minidot-oxygen loggers were out of the water, were not included in the summary.

Table 2: *Summarizing table of dissolved oxygen (%) during field trip 2*

DO (%)	Min	Mean	Max	Sd
Control D	82	91.2	109.8	5.98
Eelgrass A	72.2	94.3	131.6	7.01
Eelgrass C	77.9	90.9	121.3	7.06

From the table, it is evident that eelgrass A and eelgrass C had the widest range of dissolved oxygen saturation, indicating slightly more variability in oxygen concentration in this area. Eelgrass A had the highest average dissolved oxygen concentration, whereas Control D showed the lowest variability. Summarized, the eelgrass A and C displayed more variability in dissolved oxygen saturation, when compared to the control area D.

8.2 Summary Objective 2

On the first day and night of field trip 2 dissolved oxygen saturation actually showed a higher variability, and would in that matter be less stable. However, when dropping to a lower level in the eelgrass area at night, it would be equally higher during the day. Hence, the mean dissolved oxygen saturation would almost be equal in eelgrass areas and sand bottom.

9 Results - Objective 3 - Nutrients Concentrations

The third research objective stated that there might be a difference in the nutrient concentrations among eelgrass meadows and sand bottom habitats. This was based on the assumption, that eelgrass can uptake nutrients through leaves and roots, thus lowering the nutrient concentrations in the ambient waters among eelgrass.

9.1 Nutrient measurements

Notably, almost no nitrogen compounds could be measured during field trip 1 as displayed in figure 4. The mean values of N-NOx are based on very few measurements, as many measurements were zero. Hence a two.sample t.test will only be made for field trip 2.

Table 3: *Results from water samples - Field trip 1. n = 12 for each row*

Sample mean	$\overline{P-PO_4^{3-}}$	$\overline{N-NO_x}$	$\overline{N-NO_2^-}$	$\overline{N-NO_3^-}$	**Unit**
Sandbottom (B)	55.5	3.72	NA	NA	$\mu g/L$
Eelgrass (A)	45.5	11.73	NA	NA	$\mu g/L$

9.1.1 Field trip 2

The concentrations of nitrogen compounds increased dramatically in October when compared to September. Concentration of phosphor remains almost equal in in between the two field trips.

Table 4: *Results from water samples - Field trip 2. n = 24 for each row*

Sample mean	$\overline{P-PO_4^{3-}}$	$\overline{N-NO_x}$	$\overline{N-NO_2^-}$	$\overline{N-NO_3^-}$	**Unit**
Sandbottom (B, D)	55.9	432.5	27.1	405.4	$\mu g/L$
Eelgrass (A,C)	45.8	361.2	26.4	334.8	$\mu g/L$

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9.2 Results from Two Sample Significance Test

The mean concentration of N-NOx and P-PO4 for samples made in October were compared and tested with a two-sample t-test. The t-test evaluates whether there is a significant difference between P-PO4 and N-NOx concentrations among eelgrass meadows and sandy bottom habitats. This test assumes that the concentrations, when log-transformed, follow a normal distribution. The p-value indicates the probability that the observed difference in the means occurred by chance. Therefore, a low p-value suggests that the difference is statistically significant and not caused by random variation.

Table 5: *Result of two-sample t.test, assuming that concentrations are log-normally distributed when log-transformed*

Results from two.sample t-test	**mean concentration sandbottom**	**mean concentration Eelgrass**	**p-value**	**95% Confidens interval**
logPO4 Control / logPO4 treatment	3.82	3.68	0.33	(-0.14; 0.41)
logNOx Control / logNOx treatment	5.5	5.41	0.78	(-0.6 ; 0.8)

Even though an actual mean difference was observed in both P-PO4 and N-NOx the difference was not statistically significant on a 5 % significance level, due to a very high p-value. The zero hypothesis stating that there were no differences among the concentrations cannot be rejected.

Especially concerning N-NOx, samples showed a very high variance, and an actual distribution was hard to determine. The concentrations of P-PO4 samples, however, more closely followed a log-normal distribution as displayed in figure 25. Hence, the two sample test method of testing whether N-NOx concentrations differ from each other among eelgrass meadows and sand bottom areas might not be sufficient.

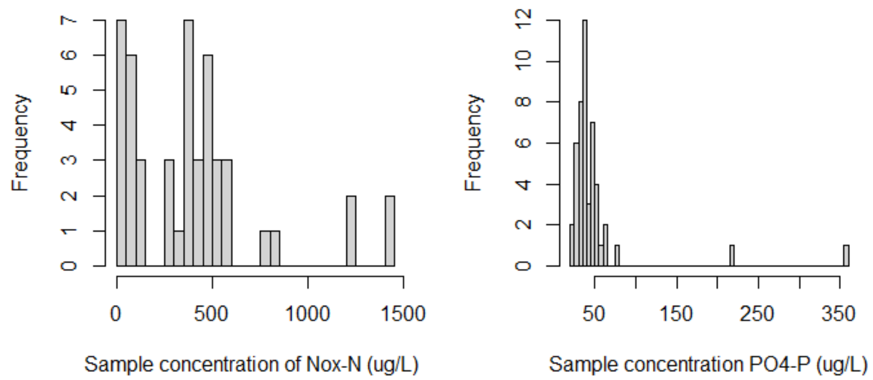

Figure 25: *Sample concentrations from field trip 2 in October. Especially NOx showed high variance.*

9.2.1 N:P ratio

The ratio of TotalN and TotalP for field trip 2, based on mean values from table 4 was calculated to 3.6 indicating that our field location was nitrogen-limited. The calculations were based on the mean molar concentration of N-NOx divided by the mean molar concentration of P-PO4.

9.3 Summary Objective 3

A two-sample t-test was unable to demonstrate a significant difference in the concentrations of NOx and phosphates (PO4) between waters surrounded by eelgrass and those with sandy bottom habitats. This can possibly attributed to other environmental effects which had higher effect on the concentrations of nutrients. Furthermore, the our observations confined that this area were nitrogen limited.

10 Other Important Observations

10.1 Algae Mats and Washed-up Eelgrass

During the first field trip, all eelgrass meadows observed were covered heavily with algal mats. Heavy onshore winds seemed to drift a lot of the algae mats into the shore, and when fishing, into the fishing equipment. The algal mats were not present to the same degree when surveying the eelgrass bed a month later in October. When arriving one month later in October, huge quantities of eelgrass leaves, and whole shoots detached from the sediment were drifted onshore, showing that natural mortality of eelgrass indeed is present. Presumably due to the storms and insufficient anchorage.

Figure 26: *Algael mats and epiphyte growth during on eelgrass at field trip 1. Small bubbles suspended between algae might indicate the production of oxygen*

Figure 27: *Fresh eelgrass washed onshore when arriving one month later in October (field trip 2)*

10.2 Green Crabs

Green crabs were the most abundant species caught, both in mass and numbers. Quite often they were a problem, as they would either kill fish trapped in nets or be a hurdle when emptying the nets. Approximately 11.000 crabs were caught while fishing. For each fish during night fishing, 20 crabs had to be emptied from the net. As a result 8.3% of all fish were killed by crabs in the net.

Figure 28: *Dead fish as a result of being trapped with green crabs*

10.3 Daily Catches on Field Trip 2 in October

An interesting pattern was observed when collecting data in October. On the first night of fishing, the relative abundance of cod was at its highest with 62 cod in eelgrass habitats, and 36 in the sand. The abundance afterward was stagnating, with fewer catches for every day.

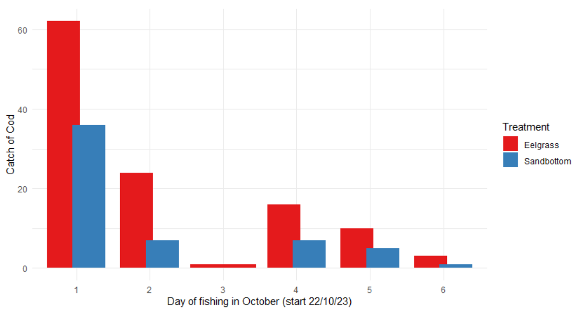

Figure 29: *Day-to-day catch of cod of field trip 2, on Eel grass habitat areas (A,B), and Sand bottom (C,D)*

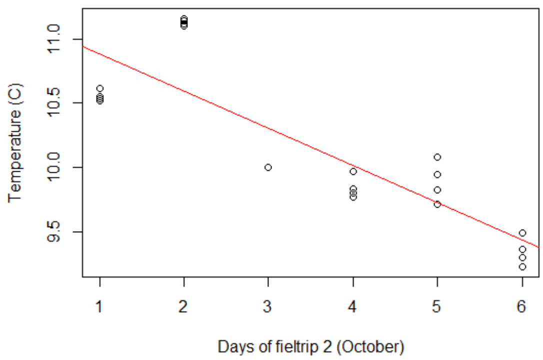

Figure 30: *Averaged temperature from Oxygen loggers during the night while fishing. R squared value of = 0.73 indicating a relationship between temperature and days during field trip 2*

11 Discussion

11.1 Cod during fieldtrips

Both from the literature and our field observations, it was evident that juvenile cod utilize eelgrass habitats, rather than sandy bottom habitats. This preference could be attributed to the high availability of prey, both in terms of quantity and the diverse fauna found in eelgrass habitats compared to sandy bottom.

Unfortunately, it was not possible to take samples from cod stomachs, as harvesting cods under the legal minimum size requires a special license granted by the Fisheries Agency in Denmark. Few cod were over the legal minimum size, which is less than 35 cm, and in all cases they were already dead, with their stomachs consumed by crabs. Therefore, potential prey species were identified based on the literature and the size relationship between prey and cod.

Two different fishing intensities were used on field trip 1 and 2, and would partly explain the lower amount of catch in September. The low proportion of cod caught in September, compared to October can however not only explain the vast difference. As outlined in the theory section, cod have a thermal preference with an upper limit of 15°C. During our surveys in September, the average sea surface temperature was 18.9°C, indicating that the warmer, low-depth eelgrass environments may not be ideal for cod to feed in. In such warmer periods, juvenile cod may utilize cooler, deeper waters, benefiting from the lower temperatures found in these zones due to thermal layering, during summer and early Autumn. The areas studied were relatively shallow with frequent water mixing, leading to a uniform temperature distribution from top to bottom which was closely linked with the air temperature. The average water temperature in October where 10 °C, and hence the temperatures were more favorable for cod when conducting field trip 2.

Cods were only caught during the night on our field trips. Occasionally, we fished during the day, but not a single cod was caught. Research by Freitas et al. (2016) found that during daylight hours, smaller cod remained at depths greater than 2.5 meters, avoiding very shallow waters. Together with our study, this behavior indicates that cod only use very shallow habitats at night. This could be due to a higher predation risk during the day from day active hunters, such as cormorants. Occasionally we did see cormorants patrolling our stations in daytime.

Another pattern was observed during field trip 2 in October, where cod catches stagnated with each day of fishing (Figure 29). Interestingly, the sea surface temperature also declined steadily during this period by almost 2°C, reaching a minimum of 9.5°C. Our observations could align with those from Freitas et al. (2016) (Figure 11), showing that cod tend to utilize extremely shallow habitats (below 1 meter in depth) until mid-October when temperatures are around 13-14°C. A slight shift towards deeper habitats (minimum 3 m) is observed as temperatures decrease further. As very shallow waters become more affected by air temperatures, deeper waters may again become more favorable in late fall. However, the decline in cod catches during the field trip in October could also be explained by the setup of the experiment, where all areas were intensely fished. Caught fish might flee the area after release, and 8% of the fish died when trapped, and were not available for recapture. This could also explain the stagnating catches for each day.

11.2 Comparing Different Tools

The generalized linear model indicated the highest effectiveness with the netted lobster trap and the lowest with the rigid lobster trap. This was expected, as the rigid lobster trap is 10 times smaller than the netted lobster trap. Both traps work by having narrowing inlets on each side. However, the netted lobster trap makes it more difficult for fish to escape once trapped. Most fish were observed migrating along the netted lobster trap, passing through narrow constrictions, and ending up at each end of the net. The spacious structure of the rigid- and netted lobster trap ensured that cod were able to avoid crabs, when compared to the eel fyke. The eel fyke had the highest mortality, due to its confined space and rigid structure when deployed, making it very hard for fish to avoid crabs. The eel fyke had some advantages as well, as it was the lightest and most handy tool. The netted lobster trap, very effective at catching cod, was also the most challenging to handle in terms of weight and when emptying and cleaning. Some fish would get trapped in the cells near the inlets. To ensure that every fish was counted, the netted lobster trap was brought ashore, strung up to a stone or tree, and each cell was carefully emptied. This was not the case with the eelfyke and rigid lobster trap. Both of these could easily be emptied while in the water into buckets placed in the dinghy. Furthermore, one day in September, approximately 30 kg of algae were washed into the netted lobster trap. The rigid structure of the lobster trap, while limiting in terms of size for transport, was very manageable in the field. While not optimal for catching cod, its small mesh size made it an excellent tool for catching smaller prey.

11.3 Oxygen Measurements

Measurements of dissolved oxygen showed greater variability in eelgrass meadows for the first day and night. This means that when dissolved oxygen became higher during the day when respiring and photosynthesizing, it became equally less during the night when only respiration occurred. Hence, the mean values of dissolved oxygen were approximately the same in eelgrass and sand bottom.

While it was impossible to find eelgrass which were not overgrown by algae and epiphytes the production and consumption of oxygen were also probably heavily affected by these. In eelgrass areas, these algae were more present, as the eelgrass would act as an anchorage. Benthic fauna would probably also affect respiration both day and night.

Since oxygen saturation was never under 70% the oxygen conditions were sufficient for cod, and no measurements were under any critical levels. In general, a high dissolved oxygen saturation where measured, and slightly often it was over-saturated. This could be due to its proximity to the atmosphere where a big part of the high abundance of dissolved oxygen was caused by mechanical mixing from atmospheric oxygen. To mitigate the noise from high atmospheric mixing the oxygen sensor could be deployed in deeper waters, preferably under the halocline and/or thermocline layers, as these environments are more isolated in terms of atmospheric diffusion and mechanical mixing. Furthermore, hypoxia is more common in deeper stratified waters and the background levels could therefore be lower.

11.4 Nutrients concentrations

When comparing the eelgrass habitat to a sandy bottom a difference in mean NOx and P-PO4 levels was present. However, a significant difference could not be proven, when conducting a t.test in R, assuming a log-normal distribution. P-PO4 concentrations does resemble more to a log-normal distribution than N-NOx, as N-NOx concentrations showed a variability, from which no regular distribution could be found. Hence, a comparison between the means of N-NOx concentrations in eelgrass and sand bottom, might not be representative, as few outliers would have a very high effect on the means value. It was almost impossible to predict, whether lower mean concentrations of nitrogen and phosphor compounds were due to the eelgrass itself, the algal mats growing on it, or other local environmental variables, such as wind, currents, precipitation, etc. which decreases the overall reliability of the data collection, and could be improved for later research. The waters were quite often turbulent due to wind and waves. An eventual nutrient uptake from an eelgrass area might quickly be mixed with other water masses.

It is known that eelgrass meadows bind and bury significant amounts of nutrients, however, whether the uptake directly affects the concentrations of nutrients in ambient water could not be proven with this experiment. This would require a more controlled environment, either in the lab or in more closed systems where the waters are not turbid.

However, the measurements of N-NOx and P-PO4 might still indicate some dynamics of the nutrients in small coastal ecosystems. All the nitrogen compounds were probably bound in organic material as the summer generally displays high production, due to high light availability and more favorable temperatures for primary producers and eelgrass. When arriving one month later in October during field trip 2, the higher nitrogen levels measured could originate from several sources, such as land-based runoff due to increased precipitation during fall, and from degradation of organic material, where remineralized nitrogen would be available again. Due to decreased light availability during fall and hence reduced primary production, nitrogen compounds were probably not uptaken as fast as in spring and summer. N-NOx concentrations resembled some daily patterns, hence the daily concentrations between areas very roughly following each other. This could indicate that other environmental parameters played a role, and explained the high variability among N-NOx concentrations.

When modelling suitable habitats for eelgrass restoration, threshold concentration for DIN ($N - NO_3 + N - NO_2 + N - NH_4$), were set at 40-75 ug/L , which our measurements very often exceeded (Flindt et al., 2023). However, as nitrogen concentrations fluctuate a lot, and our measurements were a snapshot, our results alone would not be sufficient when evaluating whether the habitat is suitable for replanting eelgrass or not. Other eutrophication parameters were evident, especially during field trip 1, as most eelgrass was covered in algae and epiphytes. Sometimes to an extent where eelgrass was not visible due to heavy coverage.

It was evident, that our fieldtrip location was nitrogen limited. This was displayed by the Redfield ratio of 3.6. Furthermore, when compared to phosphate, almost no nitrogen concentration could be measured when measuring in September. A further increase in nitrogen inputs, would probably increase the frequency of algael blooms during late summer, and hence worsening the state of the estuary. Likewise, it is not assumed that an increasing input in phosphor would have a significant impact.

11.5 Limitations of Promoting Eelgrass Meadows as a Universal Solution

There have been significant ecological improvements after large-scale restoration efforts, with the sequestration of carbon and nutrients, and increased fauna as a result (Lange et al., 2022). However, even large-scale restoration efforts are still constricted to very small areas when compared to potentially feasible areas in Denmark. Due to high labor costs, eelgrass restorations might not be economically feasible in terms of mitigating climate change, as costs are very high when compared to other mitigation strategies (Ocean Institute, 2022). This makes it rather unfeasible when integrating this method over very large areas, purely in terms of climate mitigation.

Several Danish marine scientists illuminate the importance of not relying on measures such as eelgrass, restoration efforts as well as natural expansion, when mitigating climate change. There are still high uncertainties associated with the actual amount of carbon that is buried, and even if the eelgrass were regrown in feasible areas, the yearly burial of carbon would only contribute to a maximum of 0.7 %, when compared to Denmark's annual CO2 emissions (Christensen et al., 2024). Although restoration efforts, particularly those involving citizen participation, may not be directly economically feasible in terms of CO2 reduction, they yield other positive outcomes. Engaging citizens in these restoration activities creates a heightened awareness of the deteriorating condition of Danish waters. Spreading this knowledge among citizens as well may enhance political action. Likewise, eelgrass reestablishment also has other ecological benefits, which cannot be translated directly into economic value.

In some years the recruitment of small cod has been relatively high, as seen with the Kattegat cod in 2013, and as observed within eelgrass meadows in Øresund in 2019 (ICES, 2023b; Nørgaard, 2019). Even when recruitment is high, cod populations still have difficulties sustaining stable populations. Thus, only enhancing nursery areas for juvenile cod, which often are found among eelgrass, might not be adequate if the cod population should have a chance of recovery. Furthermore, despite the recommendation by ICES to completely stop cod fishing in Kattegat and the eastern Baltic Sea, substantial quotas for cod by-catch are still being allocated. In the eastern Baltic Sea, for instance, the allowance for cod by-catch in 2024 is set at 596 tons in the eastern part of the Baltic Sea. In Kattegat, the by-catch quotas of cod in 2024 are 84 tons (Fiskeristyrelsen, 2023). This creates a conflicting situation where efforts to protect the cod are undermined by the will to sustain other industrial fishing activities.

Altogether, given the strong correlation between human pressures, from overfishing, nutrient input, etc., and the decline in both cod stocks and eelgrass, it is essential to address the issue at its root. A more holistic approach should be taken. This includes implementing more sustainable fishing practices and reducing nutrient runoff. This would support the full life cycle of the cod and would allow eelgrass to recolonize again in a more balanced ecosystem.

12 Conclusion

The role of eelgrass in supporting juvenile cod was highlighted during the two field trips, as more cod and prey were caught in eelgrass when compared to sandy bottom habitats. However, the catch of cod were highly dependent on temperature. The most effective tool for catching cod in this survey was the netted lobster trap, followed by the eel fyke, and rigid lobster trap. During the initial night and day of our second field trip, we observed significantly higher oxygen fluctuations in the eelgrass habitat compared to later observations. For the remainder of the trip, oxygen fluctuation in eelgrass areas showed similar variations to those in the sandy bottom habitat. Mean concentrations of N-Nox and P-PO4 were lower in eelgrass meadows, however it was not statistically significant when using a two sample t.test, due to high variations.

The reestablishment of eelgrass meadows has on several occasions proven to be successful in Denmark, as a result of thorough preparatory work. However, replanting of eelgrass itself is not deemed viable when regaining historically lost habitats, and as a climate mitigation strategy alone. The main cause for the decline in eelgrass is identified as eutrophication, whereas overfishing is primarily responsible for the significant decrease in cod stocks. Nevertheless, both eelgrass and cod stocks are threatened by factors such as eutrophication and rising temperatures, which can be traced back to anthropogenic activities. Addressing these underlying causes is essential to halt the decline of both cod stocks and eelgrass. A further reduction in nutrients inputs and the implementation of more sustainable fisheries are advised.

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