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Mapping of a seagrass habitat in Hopavågen, Sør-Trøndelag, with the use of an Autonomous Surface Vehicle combined with optical techniques

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Marine Coastal Development	
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Front page: *Aequipecten opercularis* (Harpeskjell) living in the seagrass habitat of Hopavågen.

Photo: Geir Johnsen

Abbreviations

AUR-LAB	Applied Underwater Robotics Laboratory, NTNU
AUV	Autonomous underwater vehicle
ASV	Autonomous surface vehicle
CDOM	Colored dissolved organic matter
IOP	Inherent optical properties
HPLC	High-Performance Liquid Chromatography
NTNU	Norwegian University of Science and Technology
OOI	Object(s) of interest
PAM	Pulse Amplitude Modulated fluorometer
ROV	Remotely operated vehicle
TSM	Total suspended matter
WHOI	Woods Hole Oceanographic Institution

Abstract

Mapping and monitoring of marine habitats are important for proper management and decision making. However, shallow coastal habitats (0 – 5 meters depth) are largely under-sampled in time and space. Knowledge about morphology, ecology and biodiversity regarding these areas is therefore strongly needed. Advances in technology have provided a large range of instrument bearing platforms that are useful for mapping and monitoring purposes. This includes underwater robotics, such as remotely operated vehicles (ROV's) and autonomous underwater vehicles (AUV's). As operation of these platforms may be both expensive and impractical for shallow-water mapping, the use of an autonomous surface vehicle (ASV) is a new and promising approach.

This thesis aims at mapping a seagrass habitat by combining non-destructive optical techniques with new technology, in order to evaluate the potential of new mapping approaches, revealing the challenges that may occur and suggesting performance improvements that is required for optimal results in the future.

A shallow seagrass habitat located in a semi-enclosed lagoon (Hopavågen, Sør-Trøndelag) was mapped by combining several optical techniques with the use of an ASV, including both qualitative and quantitative measurements. The study consisted of habitat description (underwater photography), seagrass distribution (video transect, ASV), coverage estimates (square analysis), physiological measurements (PAM and HPLC, see Abbreviations), and studies of morphology and epigrowth (physical measurements and species identification). The findings indicated a patchy and biodiverse eelgrass (*Zostera marina*) meadow. The overall physiological status of the plants was found to be relatively high, but differences in maximum photosynthetic quantum yield of photosystem II (Φ_{PSII}) and pigment content were observed for different months and leaf locations. Changes in light availability as a result of organisms growing on the leaves (epigrowth) are likely to be the main cause of variation.

The imagery provided by the ASV ("JetYak" developed by WHOI and NTNU, see Abbreviations) was combined into a photo mosaic, indicating the seagrass distribution in the area. It was possible to discriminate between the seagrass and the seafloor, but the level of detail was restricted by the image quality. For increased data quality in future mapping, improved speed and stability control (pitch, roll and yaw) of the ASV, weather conditions, availability of light for image illumination, depth and camera specifications should be taken into consideration. Overall, the ASV seems to be a suitable platform for mapping of shallow areas, but further developments indicated above are required for optimal use in the future.

Sammendrag

Kartlegging og overvåking av marine habitater er viktig for ordentlig forvaltning og beslutningstaking. Grunne habitater langs kysten (0 – 5 meter dyp) er imidlertid lite undersøkt i tid og rom. Det er derfor et økt behov for kunnskap om morfologi, økologi og biodiversitet knyttet til disse områdene. Fremskritt innenfor teknologi har bidratt til utvikling av en rekke instrumentbærende plattformer som har vist seg å være nyttige for kartlegging- og overvåkningsformål. Dette inkluderer undervannsrobotikk, slik som fjernstyrte- (ROV'er) og autonome undervannsfarkoster (AUV'er). Ettersom drift av slike plattformer kan være både dyrt og upraktisk for kartlegging av grunne områder, er bruken av et autonomt overflatefartøy (ASV) en ny og lovende tilnærming.

Denne oppgaven tar sikte på å kartlegge et sjøgresshabitat ved å kombinere ikke-destruktive optiske teknikker med ny teknologi for å evaluere potensialet til nye kartleggingstilnærminger, avdekke eventuelle utfordringer som kan oppstå og foreslå utbedringer som kreves for optimale resultater i fremtiden.

Et grunt sjøgresshabitat i en semi-lukket lagune (Hopavågen, Sør-Trøndelag) ble kartlagt ved å kombinere en rekke optiske teknikker med bruk av en ASV, inkludert både kvalitative og kvantitative undersøkelser. Studien bestod av habitatbeskrivelse (undervannsfotografi), utbredelse av sjøgress (videotransekt, ASV), estimering av dekningsgrad (firkantanalyse), fysiologiske målinger (PAM og HPLC, se Abbreviations) og undersøkelser av morfologi og epivekst (fysiske målinger og artsidentifikasjon). Funnene indikerte en flekkvis og artsmangfoldig ålegraseng (*Zostera marina).* Den generelle fysiologiske tilstanden til plantene var relativt høy, men forskjeller i maksimalt fotosyntetisk kvanteutbytte fra fotosystem II (Φ_{PSII}) og pigmentsammensetning ble observert for ulike måneder og deler av bladene. Endringer i tilgjengeligheten av lys som et resultat av organismer som vokser på bladene (epivekst) er sannsynligvis hovedårsaken til denne variasjonen.

Bildene fra ASV'en ("JetYak" utviklet av WHOI og NTNU, se Abbreviations) ble kombinert til en fotomosaikk for å indikere utbredelsen av sjøgresset i området. Det var mulig å skille mellom sjøgresset og havbunnen, men detaljnivået var begrenset av bildekvaliteten. For økt datakvalitet i fremtidig kartlegging, burde forbedret fart- og stabilitetskontroll (pitch, roll og yaw) utvikles for fartøyet. Værforhold, tilgjengeligheten av lys, dybde og kameraspesifikasjoner bør også tas i betraktning. Alt i alt virker ASV'en som en passende plattform for kartlegging av grunne områder, men videre utvikling som nevnt ovenfor, kreves for optimal bruk i fremtiden.

1 INTRODUCTION

Norway has a long and convoluted coastline, comprising over 100 000 km of littoral zone in total (Statistisk Sentralbyrå 2013). This includes a large number of fjords, bays and islands, providing suitable living conditions and potential habitats for many different organisms. Mapping of the Norwegian coastal zone has been done to some extent through collaborative projects such as “National Program for Mapping and Monitoring of Biodiversity” and “Marine AREAdatabase for Norwegian coast and sea areas” (MAREANO), led by the Norwegian Environment Agency and various governmental departments (Oug and Naustvoll 2008). However, further systematic mapping of the coastal zone is strongly needed for proper management, especially regarding shallow habitats ranging from surface to 5 meters depth.

Seagrass habitats located in shallow coastal areas are considered highly valuable ecosystems that provide a large range of important ecosystem services. This includes regulation of water quality, prevention of coastal erosion and their function as shelter, nursery- and feeding ground for many different species (Moy 2012). Seagrass habitats are threatened and declining worldwide, mainly as a result of their shallow distribution in areas largely affected by human impact (Borum et al. 2004, Bodvin et al. 2011). In order to prevent further damage and preserve these areas in the future, increased knowledge about their morphology, ecology and biodiversity is strongly needed.

In Norway, the distribution of seagrasses has been modeled and partly mapped for the Skagerrak region, Hordaland, Trøndelag and Troms through the “National Program for Mapping and Monitoring of Biodiversity”. The remaining coastal areas will likely be mapped in the continuation of this program, but the current mapping status is considered “intermediate” (Bergan 2001, Christie et al. 2011).

Commonly used mapping techniques for seagrass habitats includes interview surveys (e.g. local fishermen), modelling based on bathymetric data from nautical charts, aerial photography and field registrations by boat with the use of water binoculars (Bergan 2001). New enabling technology is however likely to phase out or supplement traditional methods in the future, for new applications in marine sciences. Remotely operated vehicles (ROV’s) and autonomous underwater vehicles (AUV’s) have previously been widely used for mapping purposes, especially for deep water habitats (Moline et al. 2005, Ødegård 2014). Autonomous and remotely operated platforms are however expensive in operation and may not be suitable for shallow-water mapping. The use of an autonomous surface vehicle (ASV) is therefore a new and promising approach. One of the main advantages of using an ASV is that it can be preprogrammed to map bigger areas continuously, which is both cheaper and more practical than traditional methods (Kimball et al. 2014).

1.1 Meadows of the sea

1.1.1 Seagrasses

Seagrasses confines an ecological and functional group of higher plants, more specifically angiosperms (flowering plants). Despite having terrestrial ancestors, seagrasses have adapted to a life submerged in seawater, with great success (Hemminga and Duarte 2000, Borum et al. 2004, Papenbrock 2012). Contrary to algae, they possess true roots and an internal vascular system consisting of veins (Seagrass-Watch HQ 2009). However, the pigment composition of seagrasses is very similar to green algae. This includes both light harvesting and photo protective pigments such as chlorophyll *a* and *b*, lutein, neoxanthin β,β-carotene and carotenoids involved in the xanthophyll cycle (Casazza 2002, Ralph et al. 2002, Johnsen and Sakshaug 2007). The level of photosynthetic pigments present in the seagrass leaves may indicate the photosynthetic capacity and growth status of the plants (Palta 1990, Liu et al. 2011).

Generally, seagrasses are found in shallow, sheltered areas near land, at depths normally ranging from 0 – 15 meters, depending on water clarity (Borum et al. 2004, Bodvin et al. 2011). Currently, there is no precise estimate of the global seagrass area, as many shallow coastal areas remains unmapped and –monitored (Borum et al. 2004). However, the World Atlas of Seagrasses states an estimated global seagrass area of minimum 177 000 km^2, based on existing knowledge (Green and Short 2003). Around 60 different species of seagrasses exists worldwide, whereof four are native to Europe (Borum et al. 2004, Seagrass-Watch HQ 2009). Seagrass species commonly found in Norway includes *Zostera marina*, *Zostera noltii* and *Zostera angustifolia* (Borum et al. 2004, Tullrot 2009, Christie et al. 2011).

Some consider additional aquatic plants as seagrasses, such as the genus *Ruppia*, occurring in marine environments of low to moderate salinity. However, in order to qualify as “true seagrasses” the plants need to occur in oceanic water with consistently high salinity (den Hartog 1970, Borum et al. 2004, Hopley 2011). Species within this genus should therefore not be referred to as seagrasses.

Zostera marina

Zostera marina, also known as common eelgrass, is considered the most widely distributed seagrass species in the world. It is found along the entire Norwegian coast, and extends into the White Sea and southward into the Mediterranean. It is especially abundant in the Baltic Sea, the North Sea and along the Atlantic coast, south to northern Spain (Figure 1) (Borum et al. 2004, Tullrot 2009).

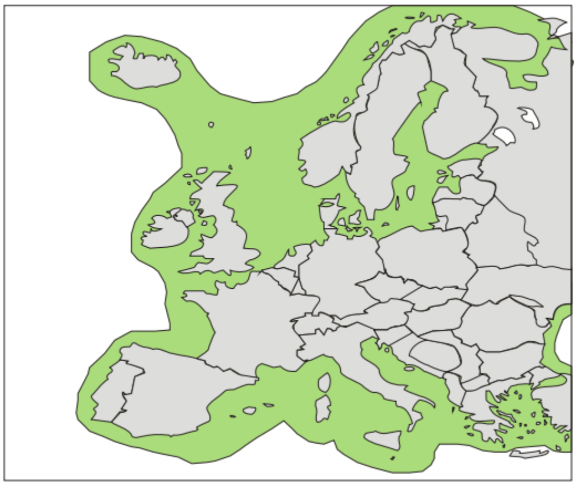


Figure 1. Geographical distribution of *Zostera marina* in European coastal waters (Borum et al. 2004).

Figure 2. Subtidal patch of *Zostera marina*. Photo: P. B. Christensen

Z. marina is a perennial, occasionally annual (Wadden Sea), seagrass species that forms isolated populations on shallow exposed and sheltered sandy bottoms. It is mainly subtidal, typically ranging from 0 – 10 meters depth (Figure 2). The species is generally adapted to relatively cold habitats, with temperatures ranging from -1 °C in winter and 25 °C in summer. As *Z. marina* is often found in estuaries, it is also adapted to a large variation and rapid changes in salinity (Borum et al. 2004).

Species characteristics include leaf lengths typically ranging from 15 - 100 cm and leaf widths of 3 – 10 mm, depending on the age and stage of the plants. Normally each plant consists of 3 – 7 leaves, each with 5 nerves and a rounded leaf apex. The shoots do however change morphology during flowering (early spring to fall), with production of more leaf bundles (Borum et al. 2004, Lid and Lid 2005).

Subtidal *Z. marina* beds are considered threatened in some countries, but according to the Norwegian red list *Z. marina* is classified as “Least Concern” (LC) (Tullrot 2009, Artsdatabanken 2015). However, *Z. marina* are known to have a relatively rapid recovery rate and declines are considered to be reversible (Borum et al. 2004).

Zostera noltii

Zostera noltii, also known as dwarf eelgrass, is distributed from the southern coasts of Norway to the Mediterranean, the Black Sea and the Canary Islands, and have been recorded as far south as the Mauritanian coast (Figure 3) (Borum et al. 2004). In Norway, it occurs in three areas: Oslofjorden, Jæren and Sunnhordaland (Tullrot 2009).

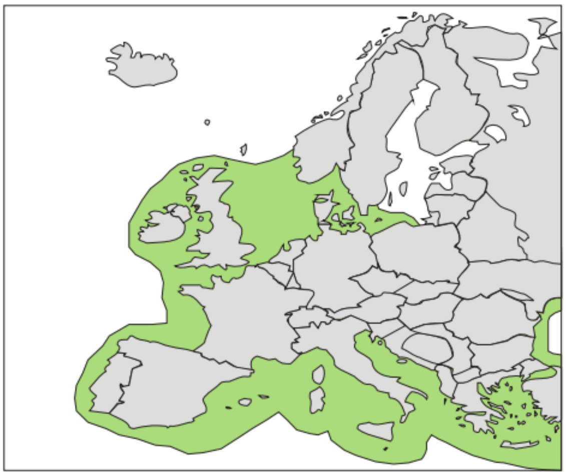


Figure 3. Geographical distribution of *Zostera noltii* in European coastal waters (Borum et al. 2004).

Figure 4. Intertidal patch of *Zostera noltii*. Photo: J. Borum

Z. noltii is mainly found on muddy sand in intertidal areas, often fully exposed to air (Figure 4). The thin film of water on muddy sediments does however keep the plants moist. The species is sometimes found subtidal, but is often outcompeted by other seagrasses. *Z.noltii* generally endures higher temperatures than *Z. marina* (Borum et al. 2004).

Species characteristics include leaf lengths typically ranging from 5 – 20 cm and leaf widths of 0.5 – 1.5 mm. Normally each plant consists of 2 – 5 leaves, with one clear central nerve and an emarginated leaf apex (Borum et al. 2004, Lid and Lid 2005, Christie et al. 2011, Lundberg 2013).

Z. noltii is classified as "Endangered" (EN) according to the Norwegian red list (Lundberg 2013, Artsdatabanken 2015).

Zostera angustifolia

The global distribution of *Zostera angustifolia* is uncertain, but in Norway it is relatively common north to Troms (Lid and Lid 2005). The species is often considered a narrow-leaved intertidal variation of *Z. marina*, as the species characteristics are not very clear (Borum et al. 2004). In World Atlas of Seagrasses, *Z. angustifolia* is not considered a separate species; hence it is not included in the 4 European seagrass species (Green and Short 2003, Christie et al. 2011).

Species characteristics include leaf lengths typically ranging from 15 - 40 cm and leaf widths of 1 – 3 mm. The leaves normally have three nerves (occasionally 5), and a rounded or slightly emarginated leaf apex (Borum et al. 2004, Lid and Lid 2005).

1.1.2 Patches & Meadows

A seagrass meadow or bed can be defined as plants covering an area larger than 2 x 2 meters. If patchy distributed, it is considered the same meadow as long as the distance between patches is no longer than 10 meters (Tullrot 2009). When describing an area of seagrasses, the coverage is one of the most important parameters. It is usually given as a percentage and may be divided into different classes: 1 = single findings, single plants, 2 = scattered plants, sparse meadow, 3 = dense patchy meadow and 4 = dense continuous meadow (Vannportalen 2015). To qualify as a *Zostera* bed, the plant density should provide at least 5% coverage. However, the coverage is usually larger than 30 %, and normally increases with depth as a result of increased leaf lengths. Cover and density estimates should generally be monitored during peak vegetation period, as they are highly seasonal (Tullrot 2009).

Since most seagrasses are completely submerged in seawater, they both grow and reproduce under water. Seed dispersal is usually carried out by water currents, but some species also have their seeds spread by birds. However, the survival rate of seeds and flowering probabilities of seagrasses are generally low. *Zostera* species are hermaphrodites, each individual plant being both male and female. Generally, new patches are made by sexual reproduction, further expanding by clones through rhizome elongation. Growth of patches through clones is the most important way of colonization, and may persist over centuries. The time required for a patch to become a meadow is species dependent, ranging from months to a year for *Zostera noltii*, to less than a decade for *Zostera marina* (Borum et al. 2004).

1.1.3 Distribution

Seagrass distribution is dependent on different environmental factors, including both biotic and abiotic factors. For photosynthesis, light and inorganic carbon is especially important. The seagrass also require a suitable substratum and a generally low degree of physical exposure by waves, currents and tides. Additionally, the distribution is controlled by abiotic factors such as temperature, salinity, nutrient and oxygen levels, as well as biotic factors like competition between species and herbivory. Seagrass distribution cannot be determined by one factor alone, as multiple factors are often tightly related. They do however show a varying degree of importance (Borum et al. 2004).

Light

Light is considered one of the most important factors determining seagrass distribution, as it regulates the maximum growing depth. In contrast to land, light is a limiting resource in the ocean as a result of attenuation, absorption and reflection of light by the inherent optical properties (IOP's) of the water itself and particles such as phytoplankton, TSM (total suspended matter) and cDOM (colored dissolved organic matter). Water turbidity may also affect the amount of light reaching the seafloor. Light is generally being attenuated exponentially with depth. Since most seagrass species require at least 10 % of the surface irradiance to grow, they are usually distributed at shallow depths, typically ranging from 0 – 15 meters. Many species are however able to acclimate to low light conditions, for instance by prolonging their leaves or through thinning of shoot density for more efficient light harvesting. *Zostera marina* often shows this type of acclimation response (Borum et al. 2004, Sakshaug et al. 2009).

Exposure

Physical exposure by currents, waves and tides is the most important factor controlling the upper depth limit of seagrass distribution. Currents and waves prevent growth and distribution by causing resuspension and transport of sediment. In addition to affecting the light climate by reducing water clarity, erosion can expose the roots and rhizomes of the seagrass, causing it to detach from the sediment. Strong currents and wave action may also tear up plants or prevent new shoots from establishing in an area. Additionally, settling sediment may cause burial of plants (Borum et al. 2004).

Nutrients

The nutrient requirements (nitrogen and phosphorous) of seagrasses are generally low, especially compared to macro algae and phytoplankton. It is however important to note that seagrasses are not only dependent on the nutrient levels of the water column, but also in the sediments. Contrary to algae, seagrasses are able to take up nutrients from the sediment, which most often are rich in nutrients because of mineralization of organic matter. As a result, seagrass meadows are often found in areas with low nutrient levels in the water column (Borum et al. 2004).

Oxygen

Oxygen is needed for metabolism, and is hence another important variable determining the distribution of seagrasses. Seagrass leaves are usually getting enough oxygen from the water column, but the rhizomes and roots are often buried in anoxic sediments. To cope with oxygen deficiency, oxygen produced by photosynthesis is transported to the roots from the leaves, by diffusion through a system of air tubes (lacunae) running through the plant. In periods of high degradation of organic matter in the sediments, coupled with a stratified water column, there may be a problem with anoxia. Anoxia may result in poor energy availability and production of toxic metabolites, negatively affecting growth and survival of the seagrass. Anoxic conditions may also cause invasion of sulphide (a plant toxin) from the sediment, inhibiting respiration. It is typically present in sediments rich in organic matter and poor in iron, and is toxic when entering the plant through the roots, into the lacunae. If it reaches the meristem, it might be fatal for the plant (Borum et al. 2004).

Competition

High nutrient levels may cause epiphytes and filamentous algae to develop in high densities on the seagrass leaves, affecting the light climate in the water column due to high phytoplankton biomass, and in turn the depth distribution of the seagrass. Epiphytes may also reduce the uptake of oxygen, inorganic carbon and nutrients through the leaves. Filamentous algae, forming dense mats at the seafloor might additionally reduce the water flow around the leaves, lowering the oxygen content of the water column when they are degraded (Borum et al. 2004).

Competition may also occur between different species of seagrass, where some may have an advantage over another. Seagrasses may also compete for occupation of space with other organisms, e.g. mussels (Borum et al. 2004).

Herbivory

Grazing by birds and invertebrates may also affect the distribution of seagrasses locally, but is not considered a controlling factor. Birds like mute swan (*Cygnus olor*), brent goose (*Branta bernicla*), pintail (*Anas acuta*) and mallard (*Anas platyrhynchos*), all graze on *Zostera* species. Some fish species, such as the sparid fish (*Sarpa salpa*), as well as the crustacean *Idotea chelipes* and the purple sea urchin *Paracentrotus lividus* have also been found to graze on seagrasses. Grazing effects are however considered relatively small in Europe (Borum et al. 2004).

1.1.4 Flora & Fauna

The presence of seagrass plants provides a three dimensional system to otherwise plain soft bottom areas. Their leaves and rhizomes provide substrates for attachment and reduce water movements and incoming irradiance, while their roots are stabilizing and transporting oxygen to the sediment, creating many small microhabitats with favorable living conditions. As a result, biodiversity is generally higher in areas with seagrass, compared to adjacent bare sand (Borum et al. 2004, Fredriksen et al. 2010). The biodiversity of a seagrass meadow is dependent on different environmental factors such the degree of exposure (e.g. to human activity or wave action) and the density of microhabitats. As a general rule the biodiversity is deemed to be highest in perennial, fully marine, subtidal communities; and lowest in intertidal, estuarine, annual beds (Tullrot 2009).

Seagrass meadows are key habitats in the life cycle of many organisms. They do for instance provide shelter against predators, and often function as nursery- and feeding grounds for many different species, including commercially targeted species of fish and crustaceans (Borum et al. 2004, Tullrot 2009, Moy 2012). The leaves are often colonized by macro- and micro algae, and sometimes stalked jellyfish and anemones. Gastropods are frequently found grazing on the epiphytic algae. The infauna is typically dominated by amphipods, polychaetes, bivalves and echinoderms, and is often more abundant within the bed than outside (Tullrot 2009, Fredriksen et al. 2010). The seagrass itself can also function as a food source for different animals, including wildfowl, dugongs, manatees and sea turtles (Borum et al. 2004, Seagrass-Watch HQ 2009). It is however quite rare to exclusively feed on seagrass leaves. Most species therefore have a secondary food source such as epiphytes or small invertebrates living in the habitat. Intertidal seagrass meadows are also an important feeding ground for many shorebirds, and are often used by migrating birds as a resting area (Borum et al. 2004).

1.1.5 Ecosystem services

Seagrass habitats are known to provide us with many different ecosystem services, including both direct and indirect benefits for humans and the environment. The value of these services has in the past been appraised to a minimum of 15 837 € per hectare per year, two orders of magnitude higher than for croplands and three times larger than for coral reefs. As a result, seagrass meadows are considered one of the world’s most productive coastal habitats (Borum et al. 2004, Seagrass-Watch HQ 2009).

The most important ecosystem services provided by seagrass habitats includes the provision of harvestable goods (e.g. fish and shellfish), regulation of water quality (filtering and absorption of nutrients and pollutants), prevention of coastal erosion (stabilizing sediments and reducing water movements) and overall primary productivity (oxygen production and carbon cycling). Since many seagrasses are perennial, they are often used as indicator organisms for changes in the environment over time. This makes them especially important for management and monitoring. However, further development of methods and managing strategies are required for future work (Borum et al. 2004).

1.1.6 Threats

Globally, over a billion people live within a 50 km distance to a seagrass meadow, making them especially vulnerable to human impact. As a result, seagrass habitats are threatened and declining worldwide. Losses of certain seagrass species are considered irreversible, but many species show fast recolonization rates and their decline can be reversed. Over the last 20 years, the losses of seagrasses have been substantial and accelerating. Globally, the estimated losses caused by human impact corresponds to 33 000 km^2, equivalent to 18 % of the documented seagrass area. According to reports, the documented global losses since 1980 are equivalent to two football fields per hour. Since there is no long-term record of seagrass distribution for many areas and a general lack of mapping and reporting, the loss estimates are expected to be much higher in reality. Increased knowledge about seagrass habitats is therefore essential in order to reverse and prevent further damage (Borum et al. 2004, Seagrass-Watch HQ 2009).

The causes of decline in seagrass distribution are manifold, including both natural (e.g. climate change) and anthropogenic factors. The primary cause is likely to be reduction in water clarity, resulting from increased nutrient loading, turbidity and suspension of sediments and particles in the water, mainly connected to run-off from human activities. Physical disturbances such as dredging, trawling and coastal construction are other important factors. Seagrasses are especially vulnerable this type of disturbance as they are not physically robust and can easily be dislodged from the sediment (Borum et al. 2004).

Awareness of the need to monitor seagrasses has rapidly grown over the past two decades, and is essential in order to conserve and stop any further decline of these habitats. Volunteer and scientific monitoring programs, such as Seagrass-Watch, increase awareness of the threats to the sustainability of coastal ecosystems, help citizens understand environmental problems and issues, and encourage people to become involved in solving them (Borum et al. 2004, Seagrass-Watch HQ 2009). Currently, seagrasses are being prioritized internationally, e.g. through the Rio Convention and EU’s Habitats Directive. The member states of the Water Framework Directive have also agreed to ensure a “good ecological status” of seagrass meadows (Borum et al. 2004). In Norway, eelgrass meadows are considered so-called “selected nature types”, meaning that they are being prioritized to get increased protection and that any use of the areas should be sustainable (Bodvin et al. 2011, Miljødirektoratet 2012).

1.2 Underwater photography

The development and use of photographic techniques in mapping and monitoring of marine habitats has drastically increased during the last decades (Solan et al. 2003). Underwater photography can be used for a wide range of studies, including species identification, population dynamics and estimates of area coverage and biomass. One of the main advantages of underwater photography is that it is non-destructive, unlike traditional methods such as grab sampling and trawling (Boyd et al. 2006). This may allow researchers to re-visit and sample an area several times, without confounding effects that traditional methods may cause. Additionally, photography is an overall well-known and highly available method, as well as being relatively cheap (Ludvigsen 2011). The possibility of long-term storage of digital data is also an advantage, as stored images can be re-analyzed many times if needed, without losing their quality.

1.2.1 The imaging process

A camera produces an image by recording the light (natural or artificial) reflected by an object of interest (OOI). When considering underwater photography, the amount of reflected light received by the camera sensor depends on how much of the light is being transmitted, scattered and attenuated in the water column (Figure 5). The seawater itself attenuates a portion of the light, in addition to the components of the water such as cDOM (colored dissolved organic matter), TSM (total suspended matter) and phytoplankton. Seawater in particular attenuates the red part of the light spectrum (600 – 700 nm) (Funk et al. 1972, Sakshaug et al. 2009, Ludvigsen 2011).

When a photon hits a particle in the water, the direction of the photon is either changed back towards the camera or out of its field of view. This reduces the amount of light that actively creates the image. The overall quality of an image is reduced by scattering and absorption of light (both to and from the OOI), lowering the contrast and blurring the image (Funk et al. 1972, Ludvigsen 2011). Image quality may also be affected by other factors such as distance from the OOI and spatial resolution (Andersen 2011). It is additionally important to keep in mind that the marine environment is dynamic and in motion, which also may affect the image quality.

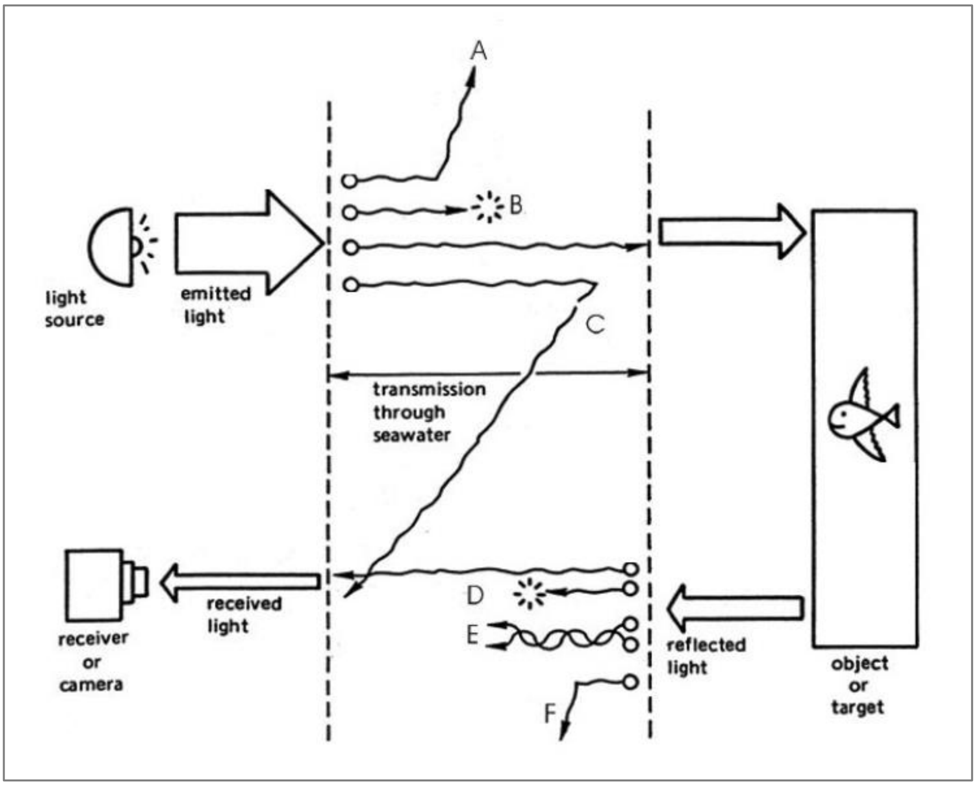


Figure 5. The underwater imaging process illustrating losses of light to an image in an underwater imaging system. A: Projected light outward scattered. B: Projected light attenuated. C: Projected light backscattered. D: Reflected light attenuated. E: Reflected light small angle forward scattered. F: Reflected light outward scattered (Funk et al. 1972).

1.3 Goals

The main goal of this thesis is to increase the ecological knowledge about seagrasses and mapping of such habitats, by combining traditional methods with new technology. The information gathered will be important for further mapping and monitoring, and may be used for an enhanced nature management of seagrass habitats in the future.

Research questions:

What are the general characteristics of the seagrass habitat in Hopavågen?

- How is the seagrass distributed?
- Which species are present in the habitat?

What is the physiological status of the seagrass?

- Are the plants photosynthetically active throughout the year?
- Do physiological characteristics change over time and between different parts of the plants?

Does the use of an ASV have a future potential for mapping and monitoring of shallow habitats, such as seagrass beds?

- Can underwater photography be used to provide information about area distribution of seagrasses?
- Is it possible to discriminate between seagrass and the seafloor?
- What are the limitations concerning this method?

2 MATERIAL AND METHODS

2.1 Study site and sampling period

Sampling and mapping of seagrass was done in Hopavågen, Agdenes, Sør-Trøndelag (63°59’28.49”N, 9°54’54.50”E) in March, April, October and November 2016 (Table 1). Weather, temperature and tidal conditions for each sampling date are given in Table 2.

Hopavågen is a sheltered, virtually unpolluted, semi-enclosed lagoon, connected to the main fjord through a narrow channel known as Straumen. It covers an area of ~ 370 000 m^2, where seagrass is mainly found in the subtidal zone on the south-western side, following the shoreline (Figure 6). Temperature and salinity differences are generally low in the area (van Marion 1996).

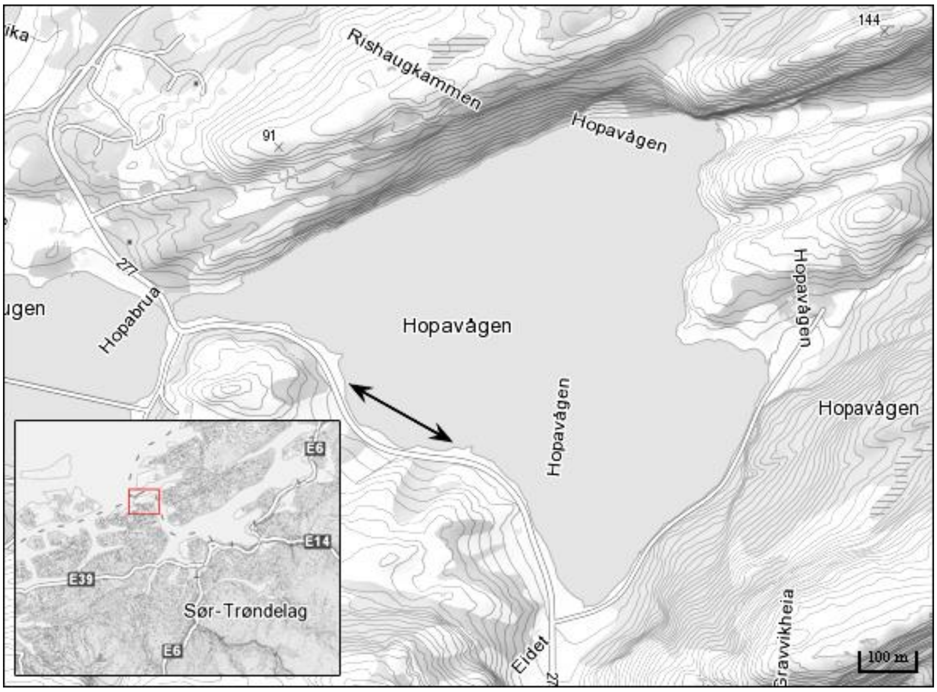


Figure 6. Map showing the study area: Hopavågen. The black arrow indicates the sampling site. Figure: Modified from Kartverket (www.kartverket.no).

Table 1. Overview of fieldwork dates and the type of techniques used on the respective dates. Physical sampling includes sampling of seagrass with epigrowth.

Date	Physical sampling	Habitat images	JetYak transects	Square analysis	Video
07.03.16	x	x			
25.04.16	x	x	x		
16.10.16	x			x	
07.11.16	x		x		x

Table 2. Weather and tidal data for the different fieldwork dates. The weather information is based on data from Ørland Air Base observation station, the closest official weather station (12.6 km away) (www.yr.no).

Date	**Weather**	**Wind**	**Tides**
07.03.16	☁ Cloudy 1.9 °C	Light breeze	Low tide, rising
25.04.16	🌤 Fair 5.2 °C	Gentle breeze	Low tide, rising
16.10.16	⛅ Partly cloudy 9.1 °C	Fresh breeze	High tide, falling
07.11.16	☀ Clear sky - 3.3 °C	Moderate breeze	Low tide, rising

2.2 Imaging techniques

A range of different imaging techniques were used during the field work. For overview images of the habitat, a DSLR camera (Canon EOS 5D Mark II) was used and equipped with a macro lens for details. For mapping purposes, a goPro camera (Hero 4) was used. An overview of the different camera specifications is given in Table 3.

Table 3. Overview of camera specifications and settings used for the different types of measurements.

Type of sample	**Habitat** (overview)	**Habitat** (details)	**JetYak transects** (mapping)		**Square analysis** (coverage)
Camera	Canon EOS 5D Mark II	Canon EOS 5D Mark II	goPro Hero 4 Black	goPro Hero 4 Black	goPro Hero 4 Black
Sensor type	CMOS	CMOS	CMOS	CMOS	CMOS
Sensor size	36 x 24 mm (Full-frame)	36 x 24 mm (Full-frame)	6,17 x 4,55 mm (CF = 5.64)[1]	6,17 x 4,55 mm (CF = 5.64)[1]	6,17 x 4,55 mm (CF = 5.64)[1]
Focal length	14 mm	50 mm	3 mm (15 mm)[2]	3 mm (15 mm)[2]	3 mm (15 mm)[2]
Field of View	Wide	Close-up/Macro	Wide	Wide	Wide
Mode	Manual	Manual	Time-lapse	Video	Multi-shot
Adjustable focus	Yes	Yes	No	No	No
Flash	Yes (Subtronic)	Yes (Subtronic)	No	No	No
White balance	Daylight	Daylight	Auto	Auto	Auto
Aperture	f/2.8 - f/22	f/29	f/2.8	f/2.8	f/2.8
Shutter speed	1/30 - 1/60 sec[3]	1/40 sec	1/120 sec[3,4]	N/A	1/60 sec
Frames per second	N/A	N/A	2	24	10
ISO limit	200	200	800 (default)	1600 (default)	800 (default)
Spatial resolution	5616 x 3744 (21.1 MP)	5616 x 3744 (21.1 MP)	4000 x 3000 (12 MP)	1920 x 1080 (1080p)[5]	4000 x 3000 (12 MP)

---

[1] Crop factor | [2] 35 mm equivalent | [3] Most frequently used shutter speeds | [4] Frame interval: 0.5 seconds |
[5] Video resolution (Full HD)

2.2.1 Habitat description

To provide an overview of the seagrass habitat, a DSLR camera (Canon EOS 5D Mark II) with underwater housing (Subal, Switzerland) was used to take images of the seafloor, both within and outside different patches of seagrass in the study area. The investigations were done by snorkeling and SCUBA-diving, providing information about important features such as bottom type/substrate and key species. The images were later analyzed on a computer and used for species identification.

2.2.2 Autonomous surface vehicle (ASV)

An autonomous surface vehicle called "JetYak" was used for seagrass mapping in Hopavågen, in April and November 2016 (Figure 7). The JetYak (originally made by WHOI, USA) is a kayak made of polyethylene (Kimball et al. 2014). New electronics for autonomous tracking of sea floor and various modifications have been made by NTNU AUR-Lab (Ludvigsen et al., submitted).

The JetYak is fitted with a petrol engine (Subaru EX21D, 5 hp) driving a water jet unit at the aft. The vehicle is 3 meters long and has a mass of 160 kg. It is equipped with two 12V, 70 Ah batteries connected in parallel to power up the electronics on-board, for navigation and scientific instruments (Ludvigsen et al., submitted).

The mission in April was a trial run. A goPro camera (Hero 4) was mounted on a metal rod sticking 5 cm into the water column beneath the JetYak (Figure 8). The camera was pointing downwards (bird's eye view) and set to take 2 images per second. The JetYak was programmed (auto mode) to follow a predefined transect pattern in an area selected by divers (2 – 3 meters depth). The ground speed of the JetYak was ~3 knots.

Figure 7. Preparation of the JetYak and sensors before transect. Photo: D. M. Alvsvåg

Figure 8. GoPro camera mounted on a metal rod. Photo: D. M. Alvsvåg

In November, the goPro camera (Hero 4) was mounted on a metal frame directly underneath the hull of the JetYak. The camera was pointing downwards (bird's eye view) and set to video mode. The transect area (approx. 100 x 7 m) was selected by a diver, and the edges marked with buoys (1 – 2 meters depth). Nine squared frames (50 x 50 cm) were randomly placed within the transect area. The JetYak performed 3 transects on total; the first one in auto mode and the latter ones in manual mode. Only the last transect was used (Figure 9). The ground speed of the JetYak was ~1 knot.

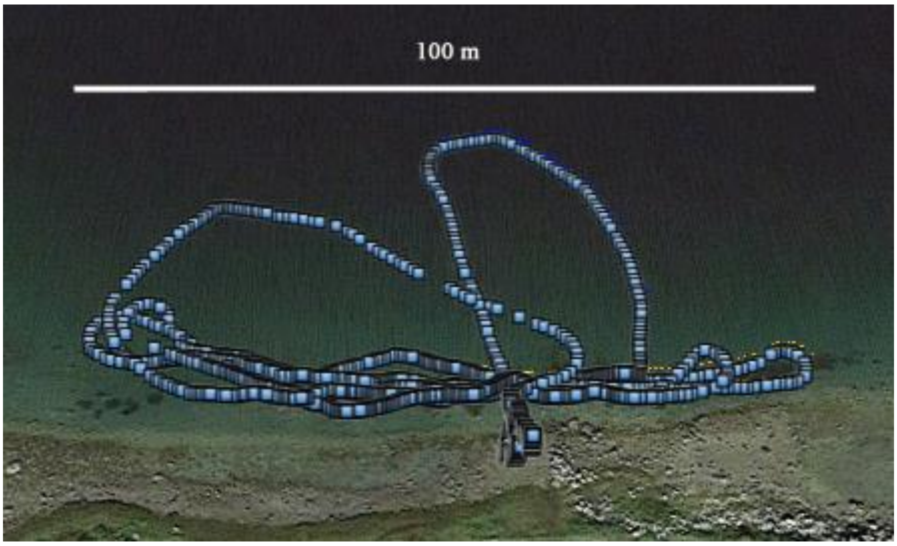


Figure 9. Map showing the path of the JetYak during the last transect in November 2016.

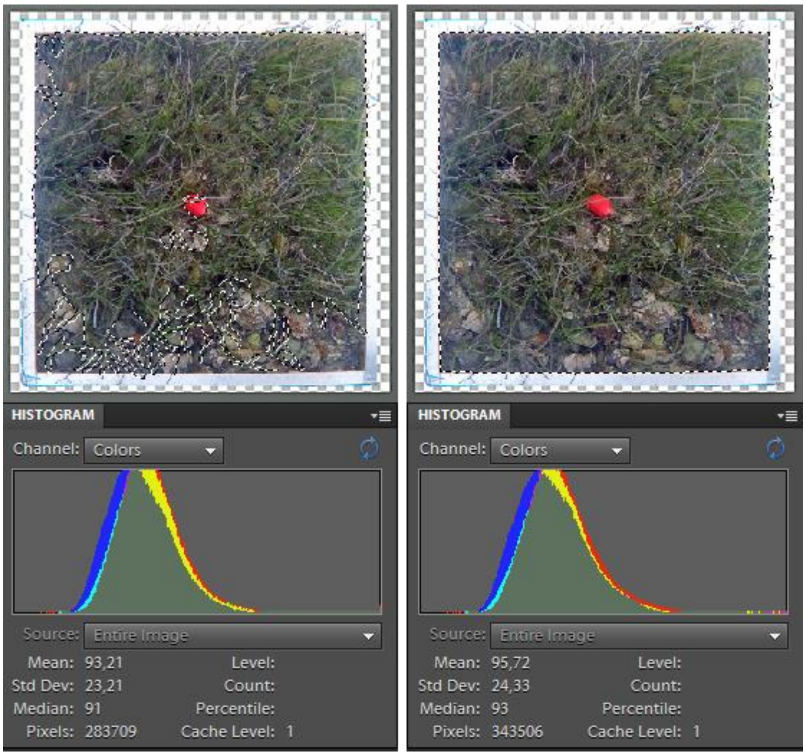
The video footage provided by the JetYak was used to make a photo mosaic of the seagrass distribution in the study area. Still frames were extracted from the video per second. Four images were selected for the photomosaic, using the "Photomerge Panorama" command in Photoshop Elements 10.

2.2.3 Square analysis (50 x 50 cm)

To estimate seagrass coverage (%), square analyses were performed within randomly selected patches of seagrass (Figure A, Appendix). A square metal frame (50 x 50 cm) was placed at each location (10 in total) with a marker in the center, and images were taken from a bird's eye view (facing downwards) with a goPro camera (Hero 4) mounted on a handheld stick.

The depth at each location was measured using a measuring stick (no tidal correction).

The images were processed on a computer, using Photoshop Elements 10. Coverage (%) was estimated using the histogram function, based on the number of pixels (manually selected) containing seagrass inside each frame, relative to the number of pixels comprising the total inner frame area (Figure 10). The values were multiplied by 100 to get percentages (Table A, Appendix).



HISTOGRAM
Channel: Colors
Source: Entire Image
Mean: 93,21 Level:
Std Dev: 23,21 Count:
Median: 91 Percentile:
Pixels: 283709 Cache Level: 1

HISTOGRAM
Channel: Colors
Source: Entire Image
Mean: 95,72 Level:
Std Dev: 24,33 Count:
Median: 93 Percentile:
Pixels: 343506 Cache Level: 1

Figure 10. Example of pixel selection in Photoshop Elements 10 used for coverage estimation. Photo: D. M. Alvsvåg.

2.3 Physical sampling

Seagrass was sampled during each fieldwork (4 times) by hand, snorkeling and/or SCUBA-diving (Figure 11, Figure 12). The samples were brought to TBS (Trondhjem Biological Station, Dept. of Biology, NTNU) for further analysis, including physical measurements, morphology and epigrowth studies, species identification, PAM-measurements and HPLC (Table 3).

Figure 11. Photographing and sampling of seagrass by snorkeling. Photo: D. M. Alvsvåg

Figure 12. Bucket with sampled seagrass. Photo: D. M. Alvsvåg

Table 3. Overview of the lab work dates and the different analyses and methods used on the sampled seagrass.

Date	Analysis	Method	Sample state
08.03.16	Physiological status	PAM	Living, 1 night in fridge
25.04.16	Physiological status	PAM	Living, fresh
17.10.16	Physiological status	PAM	Living, 1 night in fridge
08.11.16	Physiological status	PAM	Living, 1 night in fridge
Nov. – Dec. 2016	Morphology and epigrowth	Stereo microscope	Dead, frozen
March 2017	Pigments and epigrowth	HPLC	Dead, frozen

2.4 Diving-PAM

A Pulse Amplitude Modulated fluorometer (Diving-PAM, Walz, Germany) was used to measure photosynthetic activity (maximum quantum yield of photosystem II, Φ_{PSII}) in dark acclimated seagrass leaves, indicating physiological status (Wägele and Johnsen 2001). Prior to the measurements (and when changing measuring light intensity), the PAM was set to auto-zero: FL (fluorescence) between ~ 0 and 1. Living seagrass was placed in a plastic container with seawater (~ 4 °C), in low light conditions (irradiance (E) < 1 µmol photons m^{-2} s^{-1}). Measurements were performed at the base, middle and top part of the leaves, approximately 0.5 cm away from the tissue. The procedure was done for 10 individuals per sampling date (n = 40), whereas 3 (per date) were selected for additional HPLC analysis. The data were tested for statistical significance in R (Tukey multiple comparisons of means).

The different PAM settings used are listed in Figure 13.

Diving-PAM settings (M = menu)

M3: *Measuring light ON (red LED, emission maximum of 650 nm))*
M4: *Measuring light flash LOW (LED pulsed at 0.6 kHz)*
M5: *Measuring light burst OFF*
M46: *Flash duration/Saturation width 0.8 sec (white halogen lamp)*
M47: *Saturation flash intensity 12 (4000 µmol $m^{-2}s^{-1}$, white halogen lamp)*
M50: *Measuring light intensity 8 - 12*

Figure 13. PAM-settings used during measurements of maximum quantum yield of photosystem II (Φ_{PSII}) in seagrass from Hopavågen.

2.5 Morphology & Epigrowth

Morphology studies were performed on frozen samples, using three individuals per sampling date (n = 12). The samples were placed in a plastic container with water and analyzed using a stereo microscope (Leica MZ 6, magnification 0.63x – 4.0x). The number of leaves per shoot was counted, and the length and width of the longest leaves were measured using a measuring stick. The shape of the leaf apex and the number of nerves were also noted in order to identify the seagrass species. Numeric values were tested for statistical significance in R (Tukey multiple comparisons of means).

The samples were additionally used for epigrowth studies. The different taxa present on the leaves were photographed (Iphone SE) through the oculars of the stereo microscope, and later identified. Variation in epigrowth for different seasons and locations on the leaves were also noted.

2.6 Chemotaxonomy & HPLC

High-Performance Liquid Chromatography (HPLC) was performed in order to investigate the physiological status (amount and state of pigments) and chemotaxonomy (including epigrowth) of the seagrass (Rodríguez et al. 2006). From each sampling date, 3 samples of seagrass were selected (previously used for PAM-measurements) and measurements were performed at the base, middle and top part of the leaves for each sample (n = 36).

Frozen samples of seagrass were dried with paper to remove excess water. Pieces (2 - 4) of approx. 2 - 3 cm were cut from each part of the plant and weighed on a scale (SAUTER AR 1014) to estimate the amount of pigments per wet weight. The pieces were then placed in test tubes with methanol (100 %, 3 mL). For easier pigment extraction, the tissue was grinded using a glass rod. The test tubes were centrifuged and placed in the fridge for approx. 24 hours. The extracted samples were then re-filtered using a syringe with a 0.2 µm filter to avoid debris and scattering particles, and poured into smaller glass containers (2 mL). The re-filtered pigment extracts were injected into the HPLC (Figure 14, Figure 15). The data was later analyzed and tested for statistical significance in Excel and R (Tukey multiple comparisons of means).

Figure 14. Preparation of samples for the HPLC.
Photo: D. M. Alvsvåg

Figure 15. HPLC.
Photo: D. M. Alvsvåg

3 RESULTS

3.1 Habitat description

The seagrass in Hopavågen was generally patchy distributed, and the size and number of patches seemed to increase with depth. Seagrass was observed from the intertidal zone, occasionally exposed to air at low tide; to the subtidal zone down to approximately 4 meters depth (Figure 16).

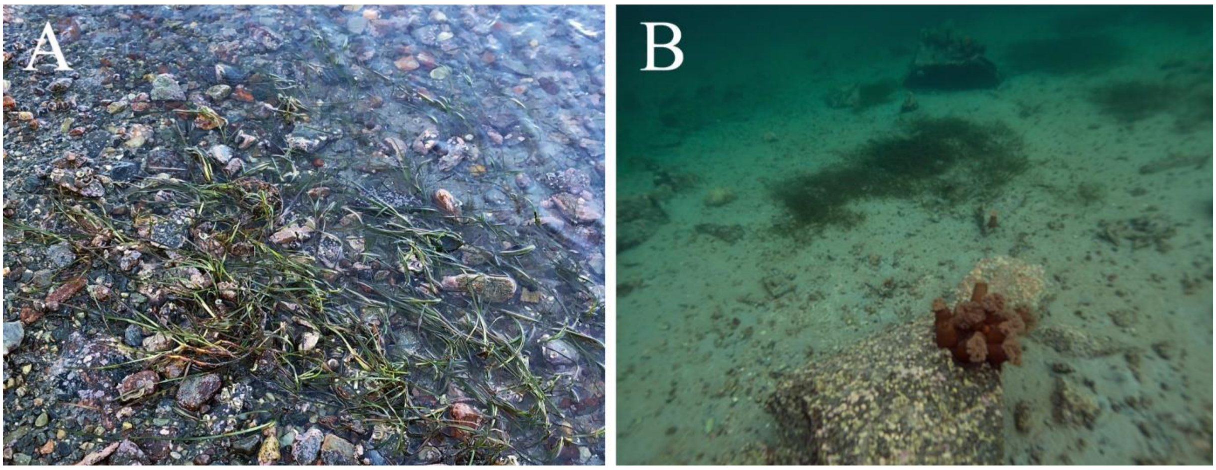


Figure 16. Distribution of seagrass in Hopavågen. A: Intertidal patch of seagrass exposed to air at low tide. B: Subtidal patch of seagrass fully submerged. Photo: D. M. Alvsvåg & G. Johnsen

The shallowest parts (~ 0 – 1.5 m depth) of the study area were mainly dominated by cobbles and gravel, with patches of sand and silt in-between. At greater depths (> 1.5 m), sandy sediments and boulders were dominating. Seagrass was typically growing wherever the substrate was soft enough, often in-between cobbles and gravel. The plants were often more frequently present in mixed substrates compared to bare sand (Figure 17).

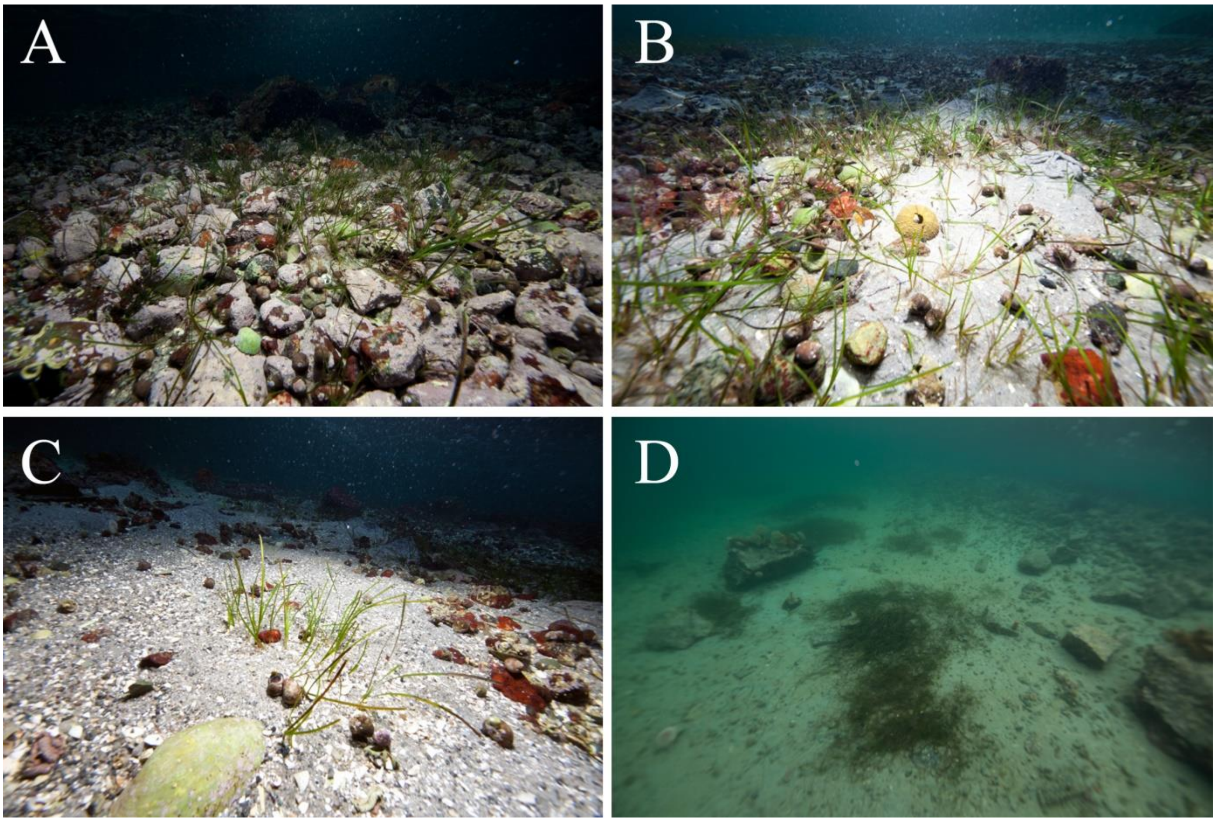


Figure 17. Habitat overview. A: Shallow area dominated by cobbles covered with red calcareous algae. B: Mixed substrate of sand, cobbles and gravel. C: Area dominated by shell-sand. D: Deep area dominated by sand and boulders. Photo: G. Johnsen

Encrusting organisms, mainly red calcareous algae, typically covered the cobbles and boulders in the area. The faunal composition of the habitat mainly consisted of sessile filter feeders, predatory and scavenging organisms, and burrowing infauna such as the sea anemone *Cerianthus lloydii* (Figure 18). A total of 66 taxa from 9 different phyla (excluding the seagrass itself) were identified for the study area based on image analysis and field observations (Table 4). The most abundant taxa included polychaetes, decapods, gastropods and bivalves, while the highest species diversity was found for mollusks (Figure 19).

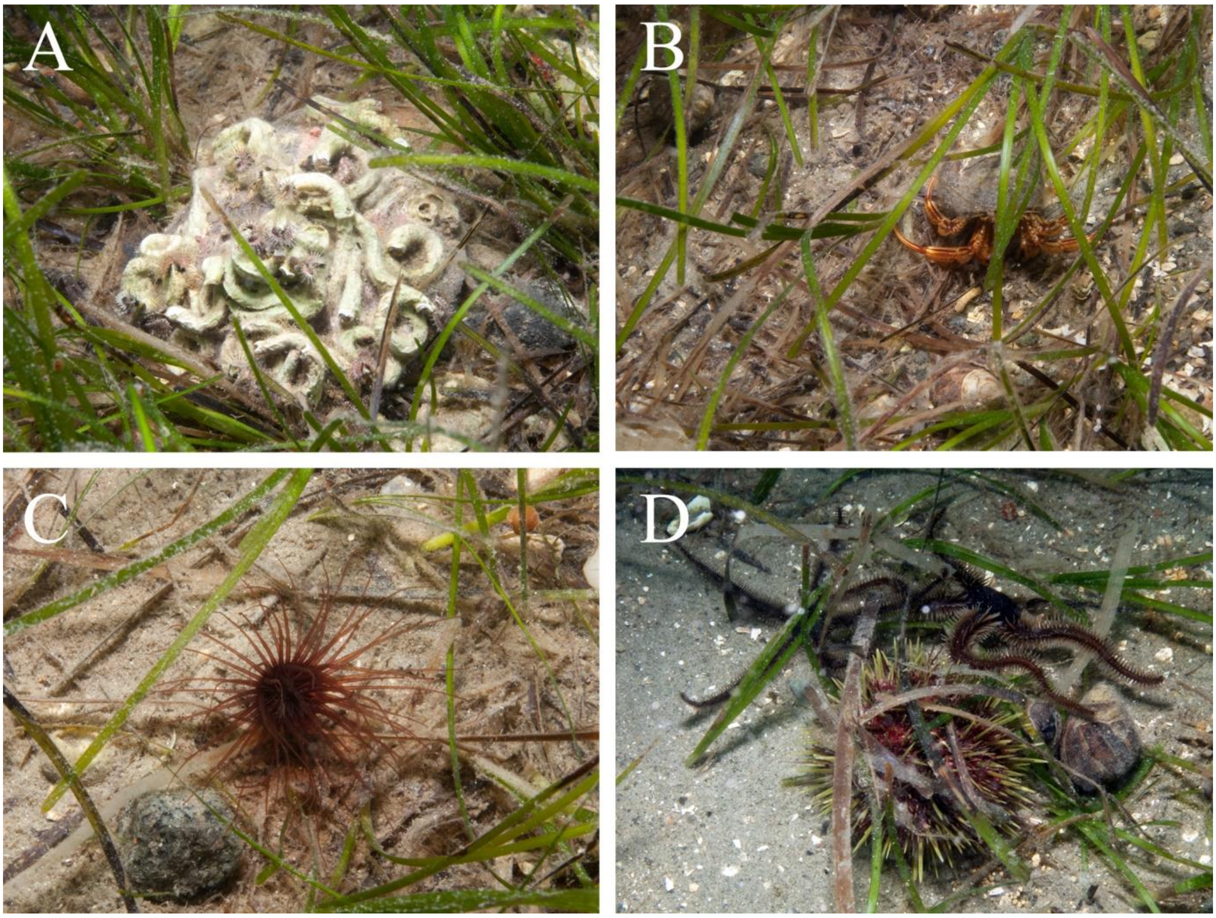


Figure 18. A selection of benthic organisms observed in the seagrass habitat of Hopavågen. A: *Pomatoceros triqueter*. B: *Pagurus bernhardus* and *Littorina littorea*. C: *Cerianthus lloydii*. D: *Strongylocentrotus droebachiensis*, *Ophiocomina nigra* and *Littorina littorea*. Photo: G. Johnsen

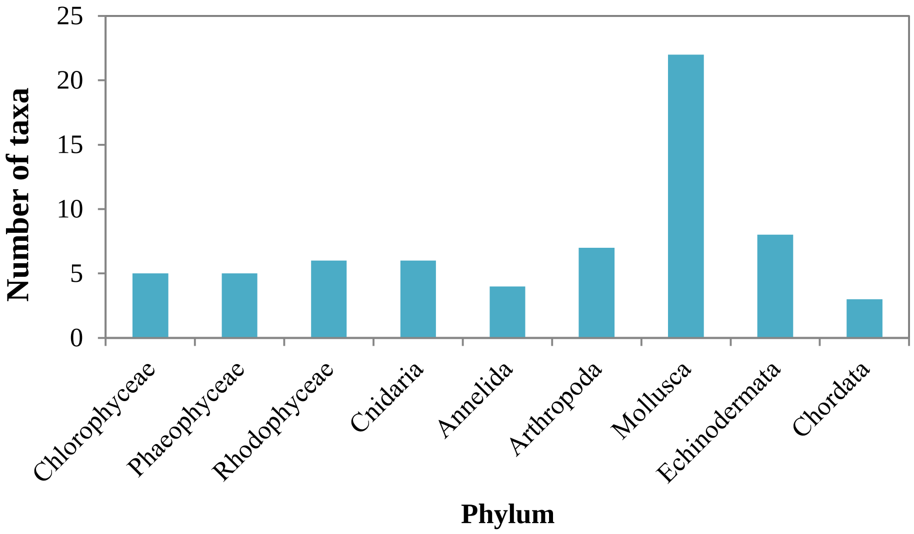


Figure 19. Number of taxa per phylum observed in the seagrass habitat.

Table 4. Overview of taxa identified based on image analysis and field observations. Norwegian names and authors are based on data from the official taxonomic thesaurus “Artsnavnebasen” hosted by the Norwegian Biodiversity Information Centre (www.artsdatabanken.no).

Phylum	Scientific name	Author	Norwegian name
Algae			
CHLOROPHYCEAE			
	Chlorophyceae indet.[1]		
	Codium fragile	(Suringar) Hariot	pollpryd
PHAEOPHYCEAE			
	Chordaria flagelliformis	(Müller) Agardh	strandtagl
	Colpomenia peregrina	Sauvageau, 1927	østerstyv
	Fucus serratus	Linnaeus	sagtang
	Fucus sp.		
	Phaeophyceae indet.		brunalger
RHODOPHYCEAE			
	Chondrus crispus	Stackhouse	krusflik
	Corallina officinalis	Linnaeus	krasing
	Corallinales indet.		
	Hildenbrandia rubra	(Sommerfelt) Meneghini	fjæreblod
	Lithothamnion glaciale	Kjellman	vorterugl
	Phymatolithon lenormandii	(Areschoug) Adey	slettrugl
Animals			
CNIDARIA			
	Actiniaria indet.[2]		
	Beröe cucumis	Fabricius, 1780	agurkkammanet
	Cerianthus lloydii	Gosse, 1859	
	Metridium senile	(Linnaeus, 1761)	
ANNELIDA			
	Arenicola marina	(Linnaeus, 1758)	fjæremark
	Pectinaria sp.		
	Spirobranchus triqueter	(Linnaeus, 1767)	trekantmark
	Spirorbis sp.		
ARTHROPODA			
	Balanus balanus	(Linnaeus, 1758)	steinrur
	Carcinus maenas	(Linnaeus, 1758)	strandkrabbe
	Galathea sp.		
	Hyas sp.		pyntekrabbe
	Mysida indet.		mysider
	Pagurus bernhardus	(Linnaeus, 1758)	bernakeremittkreps
	Semibalanus balanoides	(Linnaeus, 1758)	fjærerur

MOLLUSCA			
	Aequipecten opercularis	(Linnaeus, 1758)	harpeskjell
	Ansates pellucida	(Linnaeus, 1758)	blåsnegl
	Aporrhais pespelecani	(Linnaeus, 1758)	pelikanfotsnegl
	Buccinum undatum	Linnaeus, 1758	kongsnegl
	Cerastoderma edule	(Linnaeus, 1758)	saueskjell
	Chlamys varia	(Linnaeus, 1758)	urskjell
	Gibbula sp.		
	Leptochiton asellus	(Gmelin, 1791)	
	Littorina littorea	(Linnaeus, 1758)	storstrandsnegl
	Littorinidae indet.[2]		
	Mya arenaria	Linnaeus, 1758	
	Mya truncata	Linnaeus, 1758	
	Mytilidae indet.		
	Mytilus edulis	Linnaeus, 1758	blåskjell
	Patella vulgata	Linnaeus, 1758	albusnegl
	Tectura testudinalis	(Müller, 1776)	skilpaddesnegl
	Turritella communis	Risso, 1826	tårnsnegl
	Veneroida indet.[2]		
ECHINODERMATA			
	Asterias rubens	Linnaeus, 1758	vanlig korstroll
	Crossaster papposus	Linnaeus, 1767	piggsolstjerne
	Echinocardium cordatum	(Pennant, 1777)	sandsjømus
	Echinus esculentus	Linnaeus, 1758	svabergsjøpiggsvin
	Marthasterias glacialis	(Linnaeus, 1758)	piggkorstroll
	Ophiocomina nigra	(Abildgaard, 1789)	svartslangestjerne
	Ophiopholis aculeata	(Linnaeus, 1767)	kameleonslangestjerne
	Strongylocentrotus droebachiensis	(Müller, 1776)	drøbaksjøpiggsvin
CHORDATA			
	Ascidiacea indet.		sekkdyr
	Ciona intestinalis	(Linnaeus, 1767)	grønnsekkdyr
	Pomatoschistus sp.		

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[1] Three different species | [2] Two different species

3.2 Autonomous surface vehicle (ASV)

The transect lines from the manual control of the JetYak were generally not very straight (Figure 20). Additionally, the degree of overlap was low at several points inside the selected area, leading to incomplete data (lower area coverage).

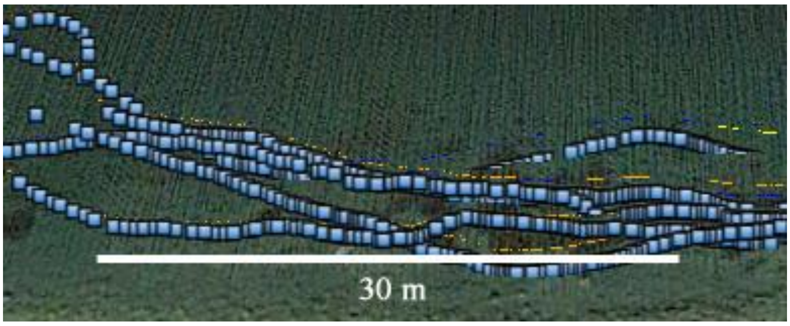

Figure 20. Close-up of transect lines from manual control of the JetYak, compared to a straight line (white bar).

The goPro images from the trial with the JetYak in April were generally blurry and dominated by different shades of green (Figure 21). No seagrass was possible to detect or identify.

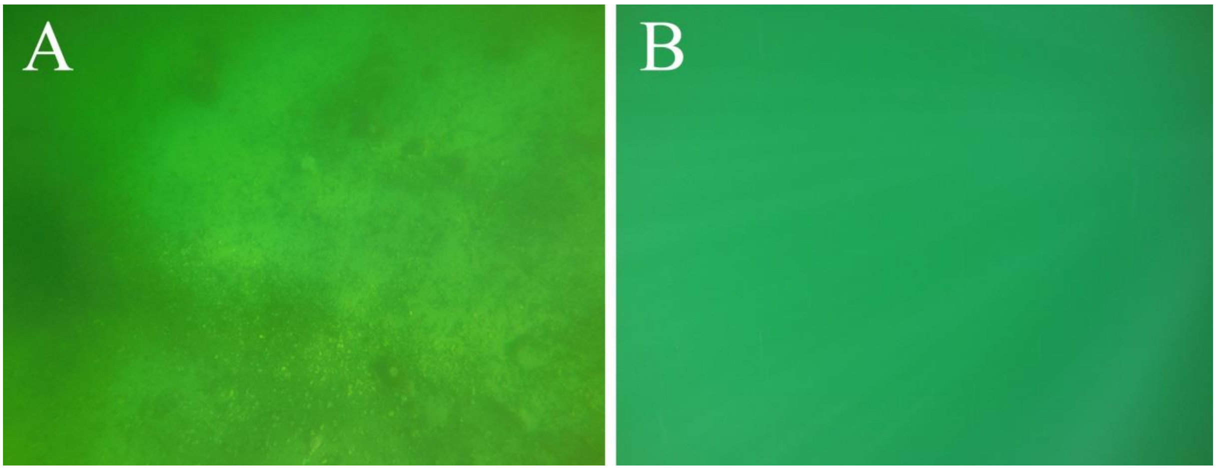

Figure 21. goPro images from the first trial with the JetYak in Hopavågen, April 2016. A-B: Blurry images with green hue at different depths.

Based on still images from the video transect in November it was possible to discriminate between the seagrass and the seafloor (e.g. sand and boulders). The images were somewhat blurry, but had a higher resolution than the images from April. By joining several images together into a photo mosaic, it was possible to look at a larger area at once (Figure 22).

The overall seagrass coverage (%) in the transect area seemed to be relatively high.

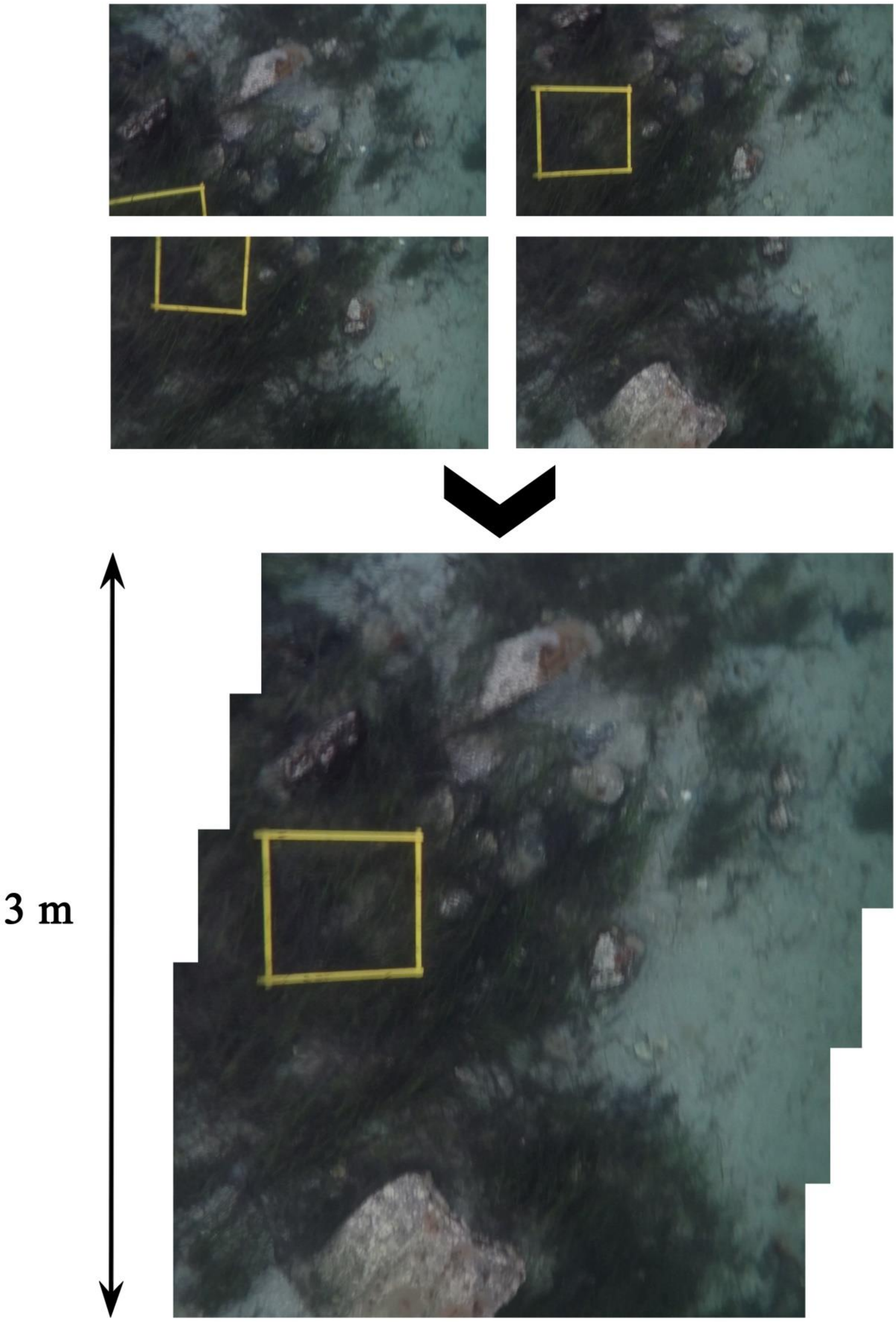

Figure 22. Example of photomosaic of the seagrass habitat in Hopavågen, based on video footage provided by the JetYak. Four consecutive images (upper) were joined together into a photomosaic (lower) by using the "Photomerge Panorama" command in Photoshop Elements 10.

3.3 Square analysis of seagrass coverage (50 x 50 cm)

Overall, the seagrass coverage in the study area ranged from 19.8 – 74.2 %, while the mean coverage was estimated to 46.9±6.3 % (Table A, Appendix). A general trend of higher coverage at greater depths can be suggested from Figure 23. The correlation between depth and coverage was however not significant ($P > 0.05$).

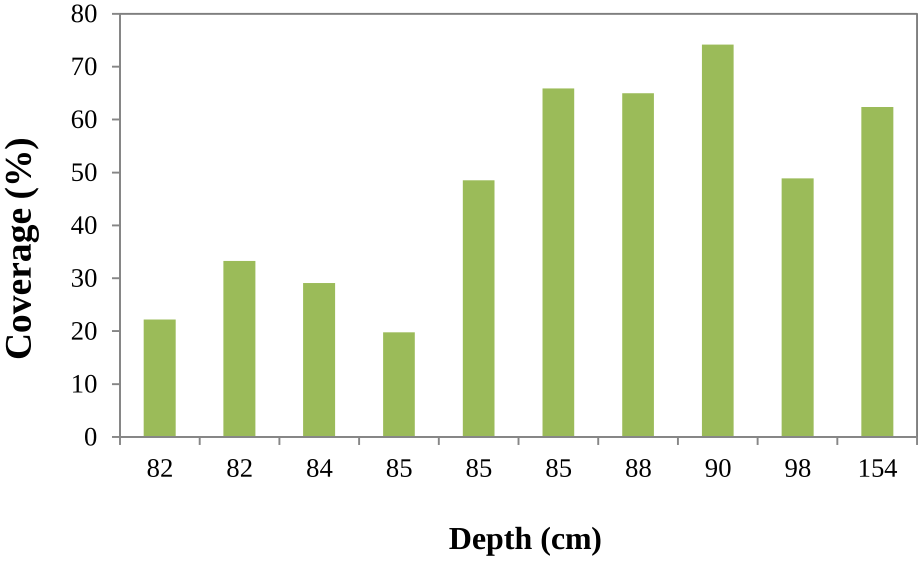

Figure 23. Estimated seagrass coverage (% of total frame area) and depth measurements (cm) for 10 randomly selected locations. Depths do not include tidal corrections.

3.4 Morphology & Epigrowth

The sampled seagrass was identified as *Zostera marina*, based on leaf shape and number of nerves. All investigated leaves had 5 nerves and a smoothly curved apex (leaf tip) (Figure 24).

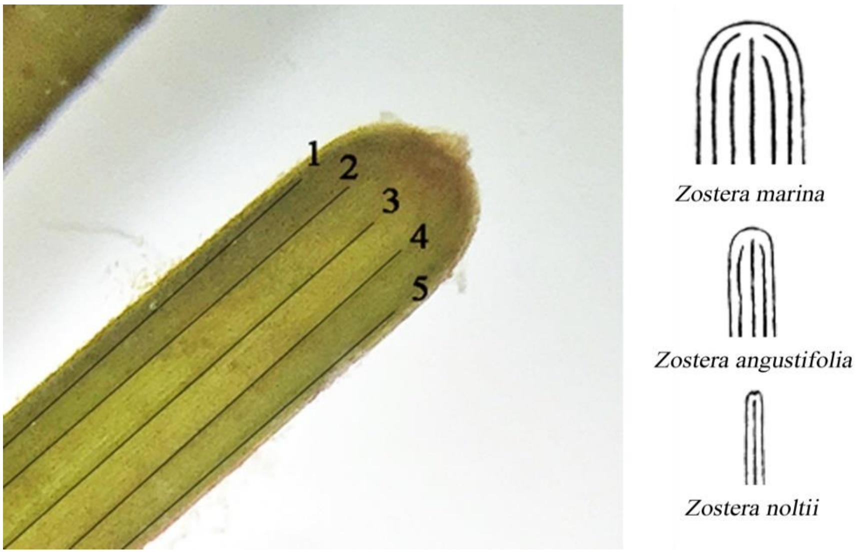

Figure 24. Leaf characteristics of seagrass found in Hopavågen (left) and different species of seagrass found in Norway (right). Photo: D. M. Alvsvåg | Drawing: Modified from Mossberg and Stenberg (2012).

The mean leaf length and width of the seagrass was found to be 16.1±2.0 cm and 1.8±0.2 mm, respectively, while the mean number of leafs was 3.8±0.6. The number of leaves per shoot generally seemed to increase from March to April, towards the summer period. A similar trend was also observed from October to November. Leaf length followed a similar pattern, being shorter in March and October and longer in April and November. The longest leaves were observed in November, while April showed the highest number of leaves per shoot. The shortest leaves were observed in October, while March showed the lowest number of leaves per shoot. Leaf width was relatively constant for all four months, but narrowest in March (Table 5, Figure 25).

No significant relationships were found between the different months and variables (length, width and number of leaves) (P > 0.05).

Table 5. Morphological measurements of seagrass, including length (cm), width (mm) and number of leaves per shoot. Values are presented as means with corresponding standard errors.

Date	**Length (cm)**	**Width (mm)**	**No. of leaves (#)**
March	14.1±1.1	1.5±0.2	2.7±0.5
April	17.2±3.5	1.8±0.1	4.7±0.7
October	13.2±0.3	1.9±0.2	3.7±0.3
November	19.6±0.5	2.1±0.2	4.0±0.5
Mean	16.1±2.0	1.8±0.2	3.8±0.6

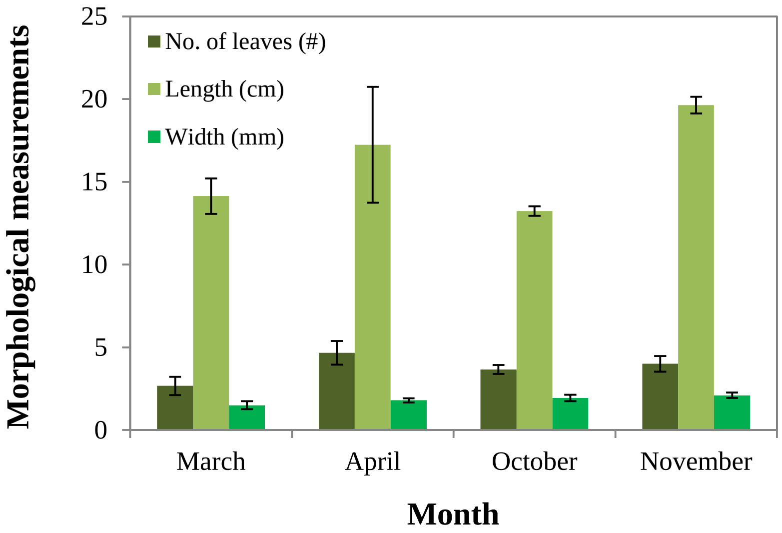

Figure 25. Morphological measurements of the seagrass, including length (cm), width (mm) and number of leaves per shoot. Values are presented as means with corresponding standard errors.

A total of 15 taxa from 7 different phyla were identified as epigrowth on the seagrass leaves, based on microscopy and image analysis (Table 6). Red algae seemed to be the most abundant type of epigrowth, especially in March and April (Figure 26). Bryozoans were also covering relatively large portions of the leaves. A gastropod egg capsule was observed on one of the leaves in March (Figure 27). The amount of epigrowth was generally low in March, increasing towards summer and peaking in October/November. The top of the leaves were generally more covered than the base, and old leaves seemed to have more epigrowth than young ones.

Table 6. Overview of epigrowth taxa identified based on microscopy and image analysis. Norwegian names are based on data from the official taxonomic thesaurus "Artsnavnebasen" hosted by the Norwegian Biodiversity Information Centre (www.artsdatabanken.no).

Phylum	Scientific name	No. of taxa	Norwegian name
Algae			
CHLOROPHYCEAE	Chlorophyceae indet.	2	
PHAEOPHYCEAE	Phaeophyceae indet.	2	brunalger
RHODOPHYCEAE	Rhodophyceae indet.	5	
Animals			
CNIDARIA	Hydrozoa indet.	1	hydrozoer
BRYOZOA	Bryozoa indet.	2	mosdyr
ANNELIDA	*Spirorbis* sp.	1	
MOLLUSCA	Littorinidae indet.	2	

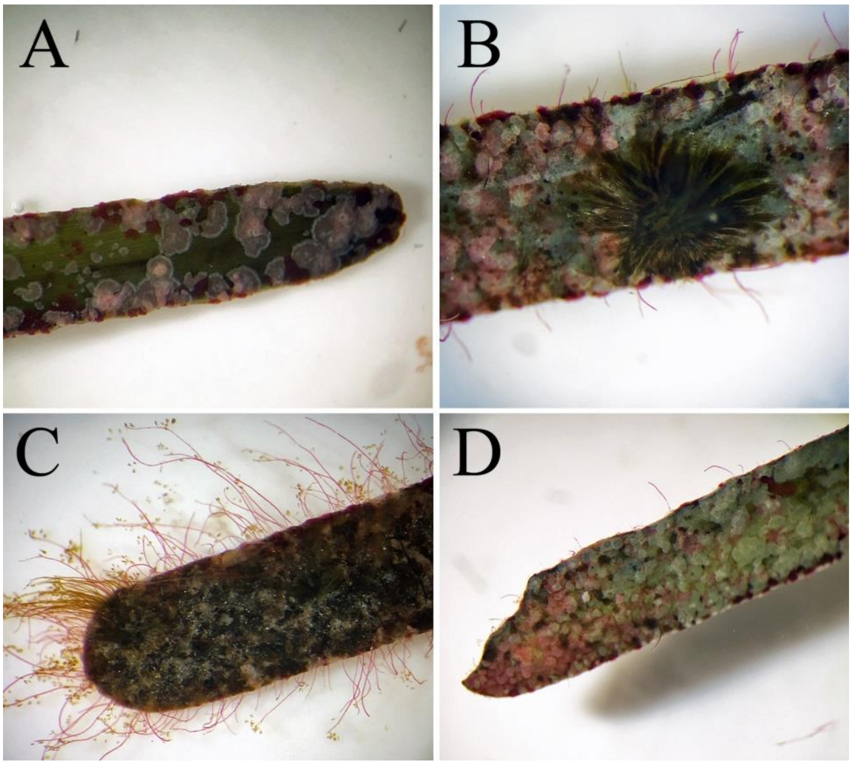

Figure 26. Epigrowth on seagrass leaves. A-D: Leaves almost completely covered by red algae, brown algae and bryozoans in October and November. Photo: D. M. Alvsvåg

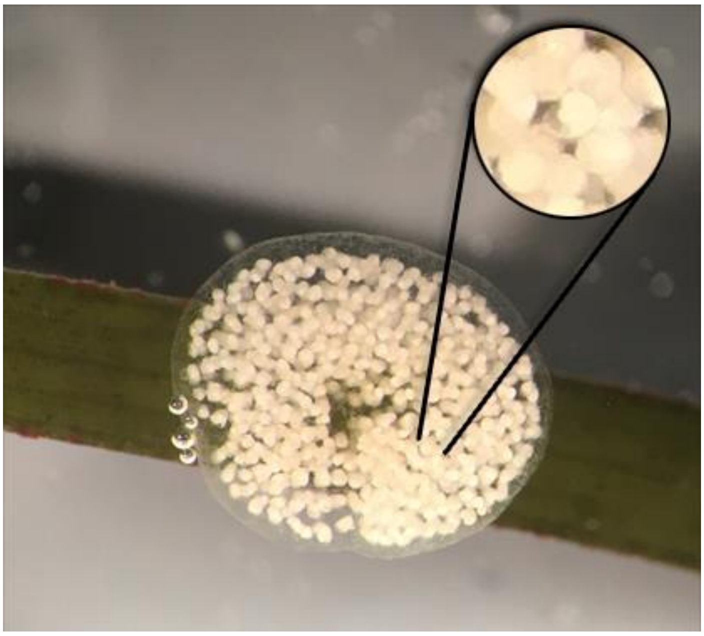

Figure 27. Gastropod egg capsule (possibly *Lacuna vincta* or *Littorina obtusata*) found on a seagrass leaf in March. Photo: D. M. Alvsvåg

3.5 Diving-PAM

The maximum photosynthetic quantum yield of PSII, Φ_{PSII}, from the seagrass for different months and leaf locations are shown in Figure 28. The overall activity was relatively high for all four months, with a Φ_{PSII} of 0.71±0.01 in April and Φ_{PSII} of 0.59±0.03 in October. Considering seasonal variation, the yield differences between April and October were the only ones found to be statistically significant ($P < 0.05$) (Figure B, Appendix).

A general trend showing the highest photosynthetic activity (indicated by Φ_{PSII}) at the base of the leaves (youngest tissue), decreasing towards the top (oldest tissue) is shown in Figure 28. The difference between the base and the top of the leaves were found to be statistically significant ($P < 0.05$) (Figure C, Appendix). The biggest Φ_{PSII} differences considering leaf location were found in autumn/early winter. During spring, the yield was seemingly more evenly distributed throughout the leaves.

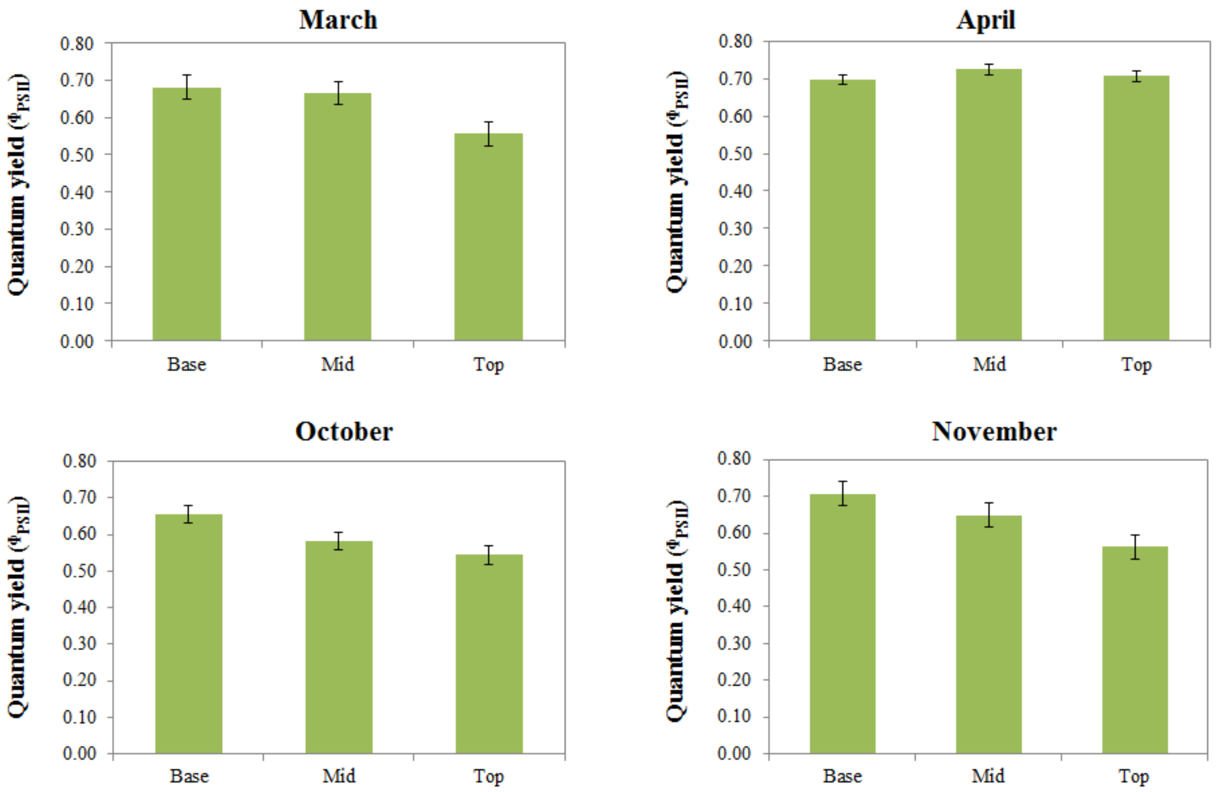

Figure 28. Maximum photosynthetic quantum yield of photosystem II (φ_{PSII}) for dark-acclimated cells of seagrass in Hopavågen, for March, April, October and November at different leaf locations. Values are presented as means with corresponding standard errors.

3.6 Chemotaxonomy & HPLC

The overall pigment composition (main peaks) of the seagrass, revealed by HPLC, is shown in Table 7. Chlorophyll a, chlorophyll b and lutein were the most abundant pigments. Degradation products of chlorophylls were not common in the samples. The carotenoids neoxanthin, violaxanthin, antheraxanthin and β,β-carotene were also present in significant amounts. The photo protective carotenoid zeaxanthin was only found in one sample.

Additionally, trace amounts of fucoxanthin was found in several samples which is a marker pigment for phototrophic algae growing on the leaves.

Table 7. Pigment content (mg/g wet weight) of seagrass (*Zostera marina*) as revealed by HPLC. Seasonal variation (whole plant per month) and pigment content based on leaf location (all months per leaf location) are presented as mean values with corresponding standard errors.

Pigment (mg/g)	**MARCH**	**APRIL**	**OCTOBER**	**NOVEMBER**	**BASE**	**MID**	**TOP**
CHLOROPHYLLS	0.92±0.28	1.75±0.76	0.89±0.42	1.41±0.66	0.35±0.09	1.85±0.57	1.53±0.50
Chl a	0.78±0.09	1.45±0.25	0.76±0.14	1.19±0.22	0.29±0.04	1.16±0.23	1.29±0.20
Chl b	0.15±0.02	0.29±0.07	0.14±0.04	0.22±0.06	0.06±0.01	0.30±0.05	0.24±0.04
CAROTENOIDS	0.27±0.07	0.45±0.12	0.26±0.07	0.34±0.08	0.10±0.01	0.49±0.06	0.39±0.06
Lutein	0.14±0.02	0.24±0.05	0.14±0.03	0.16±0.03	0.05±0.01	0.23±0.03	0.24±0.03
Neoxanthin	0.01±0.004	0.05±0.02	0.02±0.01	0.03±0.01	0.01±0.001	0.05±0.01	0.04±0.02
Violaxanthin	0.02±0.01	0.07±0.02	0.05±0.01	0.05±0.01	0.01±0.002	0.08±0.01	0.06±0.01
Antheraxanthin	0.02±0.01	0.01±0.004	0.01±0.00	0.01±0.00	0.01±0.003	0.05±0.02	0.00±0.001
β,β-carotene	0.03±0.01	0.07±0.02	0.05±0.01	0.06±0.01	0.01±0.002	0.08±0.01	0.07±0.01

The maximum total chlorophyll content (chl *a* + chl *b*) was found in April, containing 1.75±0.76 mg/g wet weight (whole plant). It was highest in the middle part of the leaves, containing 1.85±0.57 mg/g wet weight (all months). The minimum total chlorophyll content was found in October, containing 0.89±0.42 mg /g wet weight (whole plant). It was lowest in the base part of the leaves, containing 0.35±0.09 mg/g wet weight (all months) (Table 7). The total chlorophyll content increased from spring towards summer, with a peak in April. In autumn, the chlorophyll content was low again, but higher concentrations were found in November (Figure 29).

The maximum total carotenoid content (neoxanthin, violaxanthin, antheraxanthin, lutein, β,β-carotene) was found in April, containing 0.45±0.12 mg/g wet weight (whole plant). It was highest in the middle part of the leaves, containing 0.49±0.06 mg/g wet weight (all months). The minimum total carotenoid content was found in October, containing 0.26±0.07 mg/g wet weight (whole plant). It was lowest in the base part of the leaves, containing 0.10±0.01 mg/g wet weight (all months) (Table 7). The total carotenoid content increased from spring towards summer, with a peak in April. In October, the carotenoid content was low followed by higher values in November (Figure 29).

Chlorophylls and carotenoids showed similar trends for pigment content, both considering seasonal variation and leaf location (Figure 29, Figure 30). Differences between months were however found to be not statistically significant (P > 0.05) (Figure D, Figure F, Appendix). Leaf location showed significant differences between the base of the leaves and the middle and top (P < 0.05). No significant differences were found between the middle and top part of the leaves (P > 0.05) (Figure E, Figure G, Appendix).

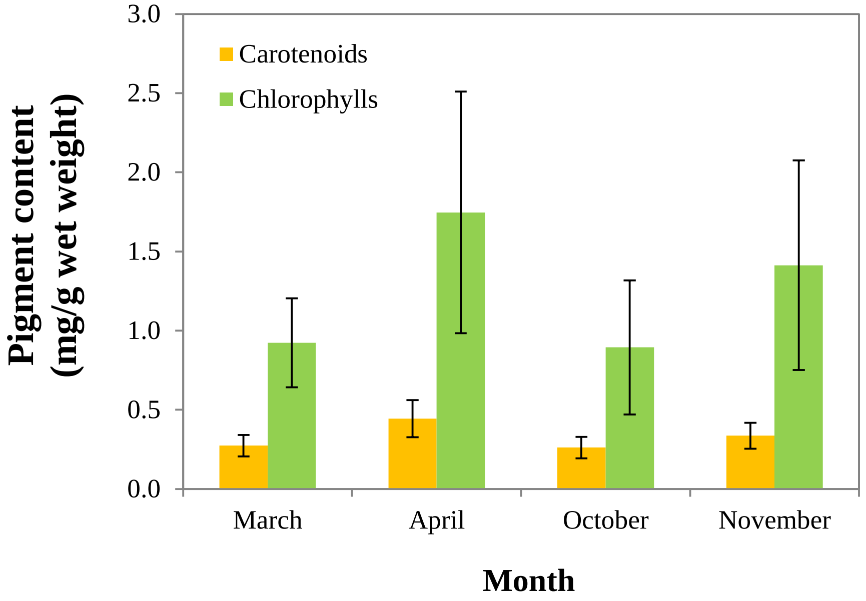

Figure 29. Total chlorophyll and carotenoid content (mg pigment/g wet weight) in the seagrass leaves for different months (n = 36). Values are presented as means for the whole plant per month with corresponding standard errors.

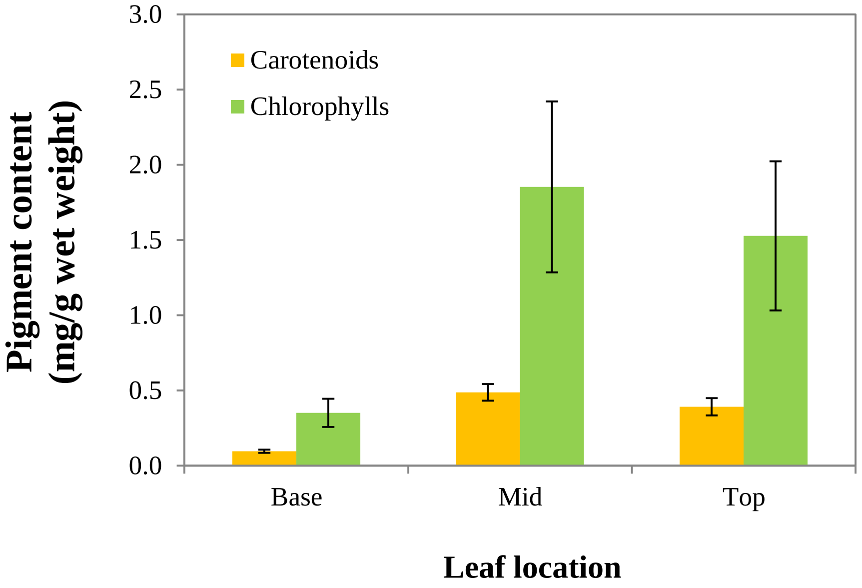

Figure 30. Total chlorophyll and carotenoid content (mg pigment/g wet weight) in the seagrass leaves based on leaf location (n = 36). Values are presented as means for all months per leaf location with corresponding standard errors.

4 DISCUSSION

The main findings of this study include increased knowledge about species, distribution and physiological characteristics of the seagrass habitat in Hopavågen. The meadow was patchy distributed, consisting of *Zostera marina*, with an area coverage tending to increase with depth. The plants were photosynthetically active throughout the sampling period, but differences in photosynthetic activity and pigment content were observed between months and different parts of the plants, possibly related to epigrowth and changes in light availability. Additionally, the ASV showed promising results regarding its use for mapping purposes, but limitations concerning this method were also observed. The main findings will be further elaborated and discussed in the following sections.

4.1 General characteristics of the seagrass habitat in Hopavågen

4.1.1 Species characteristics

The seagrass species present in Hopavågen was identified as *Zostera marina*, characterized by a rounded leaf apex and 5 leaf nerves (Figure 24) (Lid and Lid 2005). However, the mean length and width of the leaves were lower than what is normally expected for this species. *Zostera angustifolia* are known to have shorter and narrower leaves than *Z. marina*, which may suggest a potential misidentification of the seagrass species. However, 5 leaf nerves are generally rare for *Z. angustifolia* (Borum et al. 2004, Lid and Lid 2005, Christie et al. 2011). Previous studies have shown that local variations in morphology exist, and that intertidal, shallow-growing individuals of *Z. marina* often have short narrow leaves, compared to individuals from greater depths (Christie et al. 2011, Park et al. 2016). Since most of the sampled seagrass were taken from relatively shallow locations (0 – 2 meters depth), smaller individuals are justified.

The pigment composition of the seagrass leaves consisted of chlorophylls and carotenoids, mainly dominated by chlorophyll *a* and *b*. Lutein was found to be the most abundant carotenoid, while the remaining pigments included neoxanthin, carotenoids involved in the xanthophyll cycle (viola- and antheraxanthin) and β,β-carotene (Table 7). These findings are supported by previous work regarding the pigment composition of marine angiosperms (Casazza 2002, Ralph et al. 2002).

4.1.2 Distribution

The seagrass in Hopavågen did not form a continuous meadow, but was patchy distributed from the intertidal zone down to approximately 4 meters depth, typically growing in soft sandy sediments and in-between cobbles and gravel. A shallow distribution of seagrasses is characteristic for sheltered locations such as Hopavågen, as the upper depth limit is mainly controlled by the degree of physical exposure (e.g waves and currents). Additionally, the shallow distribution may be favored by the small fluctuations in temperature and salinity previously observed in the area (van Marion 1996, Borum et al. 2004).

The overall seagrass coverage was relatively high (mean of ~ 47 %) and the habitat may be classified as a locally dense, patchy meadow. The patches generally increased in size and number with depth. The coverage inside each patch seemed to follow a similar trend, supported by observations based on comparison between the square analysis (shallow location) and the photo mosaic (deep location) (Figure 22, Figure 23). The trend can be explained by a general increase in leaf length at greater depths, as a result of reduced light availability (Borum et al. 2004). Longer leaves will cover a bigger area, thereby increasing the coverage.

4.1.3 Flora & Fauna

The biodiversity of the seagrass habitat in Hopavågen was found to be relatively high, with a total of 66 taxa from 9 different phyla observed in the area (excluding epigrowth) (Table 4). The number of taxa is likely to be higher in reality, as a result of seasonal variation in species composition and limitations concerning the identification method used. The observed organisms included burrowing infauna, filter feeders, grazers, as well as predatory organisms, scavengers and detrivores. This suggests that the habitat allows many different feeding strategies, providing suitable living conditions for a large range of species.

The most abundant taxa observed in the area included polychaetes, decapods, gastropods and bivalves. This is consistent with previous findings, as invertebrates belonging to these groups are known to be common residents in similar seagrass habitats. Additionally, former studies have shown that vegetated areas generally support a higher density of infauna as a result of increased oxygen influx to the sediment, thereby preventing anoxia (Fredriksen et al. 2010, Christie et al. 2011). Feces piles from *Arenicola marina* were relatively common in the area, indicating suitable living conditions in the sediment, possibly facilitated by the root system of the seagrass plants.

The egg capsule found on one of the seagrass leaves in March indicates that some gastropod species uses the leaves as a substrate for their reproduction, and that the habitat as a result plays an important role in their life cycle (Figure 27). These findings are supported by previous studies such as Woods and Podolsky (2007) and Rueda et al. (2008), reporting the presence of egg capsules from *Lacuna* sp. and *Jujubinus striatus* on the leaves of *Zostera marina* in the US and Southern Spain, respectively (Rueda et al. 2008).

A total of 15 taxa from 7 different phyla were observed as epigrowth growing on the seagrass leaves (Table 6). Red algae were the most abundant group, followed by bryozoans; while the highest species diversity was found for algae in general. Fredriksen et al. (2005) have reported similar findings. Additionally, the HPLC revealed the presence of fucoxanthin in some of the leaves. This is a known marker pigment for several chromophytes that usually occurs together with chlorophyll *c*’s (1 + 2). However, since no chlorophyll *c*’s (1 + 2) were observed in the samples, it is likely that these pigments, if present, were below the detection limit of this survey (Johnsen and Sakshaug 2007, Roy et al. 2011).

4.1.4 Challenges and future perspectives

The seasonal differences observed in leaf morphology were found to be not significant ($P > 0.05$). However, literature states that the leaf length tends to increase towards the summer, while being somewhat reduced during winter time (Christie et al. 2011, Zhang et al. 2016). A low number of replicates ($n = 3$ per month) are likely to be responsible for the low statistical significance, and should be increased as well as supplemented with samples from the missing months, in order to properly evaluate the seasonal variation.

Coverage estimates are recommended to be carried out during peak vegetation period (mid of July to mid of September) (Tullrot 2009). Since square analyses were only performed in October, the estimated seagrass coverage is likely to be lower than its overall potential. Standardized methods for coverage estimation should also be used in order to reduce variability between locations. A coverage estimate based on the edge of a patch, will naturally be lower than for the middle part. As the selection of locations for square analyses were random and not very consistent, this should be taken into consideration when analyzing the results of this study. Additionally, the correlation between depth and coverage were found to be not statistically significant. This is likely due to a low variation in depth between squares. Performing additional measurements at gradually increasing depths would therefore be required in order to verify this relationship.

Underwater photography is a non-destructive and widely used mapping technique with many advantages. However, when used for species identification, this method has some limitations. The quality of an image is generally dependent on water transparency, distance from OOI, camera specifications and light exposure, restricting the level of detail available. Larger species are generally easy to detect and identify, but the identification process becomes harder as the size of a species decreases. Individuals less than 0.5 mm are generally hard to detect and identify, unless their species characteristics are very conspicuous. This may lead to an underestimation of the species diversity in an area. Additionally, the 2-dimensional view provided by an image is restricting the identification process, as key characteristics may be hidden from the viewer (Andersen 2011). For verification and increased reliability, photographic analysis should therefore be combined with physical sampling.

The low amount of marker pigments for epiphytic algae present in the samples, revealed by HPLC, may be somewhat misleading. Since the method does not separate between the pigments from the seagrass itself and the organisms growing on it, pigments such as violaxanthin and lutein that have an overlapping distribution, will be assumed to originate solely from the seagrass (Roy et al. 2011). In order to avoid this, separate techniques should be used to investigate the pigment composition of the leaves and the epigrowth, e.g. by scraping off the algal layer and perform HPLC on each part separately.

4.2 Physiological status and variation in time and space

The overall physiological status of the seagrass in Hopavågen was found to be relatively high, both considering photosynthetic activity (mean Φ_{PSII} ranging from 0.59 - 0.71) and pigment composition (few degradation products). The plants were present and photosynthetically active throughout the sampling period, and most likely perennials. However, seasonal variation in photosynthetic activity and pigment content was observed, as well as spatial variation based on leaf location (base, mid, top).

The maximum quantum yield of photosystem II (Φ_{PSII}) was generally found to be higher in April (spring) and lower in October (autumn) (Figure 28). This might be explained by the observed increase in epigrowth at the end of the year, possibly reducing the photosynthetic efficiency. Organisms growing on the seagrass leaves generally lowers the quality and quantity of light reaching the chloroplasts, as a result of direct blockage (shading) or by competitive photosynthetic organisms (micro- and macroalgae) utilizing the incoming light themselves (Brodersen et al. 2015).

The amount of epigrowth covering the seagrass leaves generally increased towards the top, decreasing the Φ_{PSII} accordingly. Since seagrasses are known to elongate from the basal part, where the leaf meristem is located, the oldest tissue is found at the top of the leaves (Fredriksen and Christie 2003, Borum et al. 2004). The amount of epigrowth present is therefore expected to increase from the base towards the top, and are usually most abundant and species diverse at the leaf apexes (Larkum et al. 2006). This is presumably a result of the plant’s reduced defenses with age, and that the epiphytic organisms have had a longer time to grow. This may also explain why older leaves seemed to be more heavily fouled than younger ones.

The total chlorophyll content of the seagrass leaves was also found to be high in April and low in October, reflecting the Φ_{PSII} results (Figure 29). Since chlorophyll content is known to indicate photosynthetic capacity, the base of the leaves would be expected to have a higher content of chlorophyll, as the Φ_{PSII} was highest at this location (Palta 1990). However, the chlorophyll content was found to be lowest at the base (indicating low chloroplast density per area), compared to the mid and top (Figure 30). This is supported by literature, as a general decrease in chlorophyll *a* and accessory pigments from the top to the base have been observed for *Zostera marina* (Vernberg and Vernberg 2013). An explanation might be that the tissue near the root was generally pale. Since chlorophyll is known to be the pigment responsible for giving leaves their green color, the paleness suggests a lower content of this pigment at the base (Palta 1990). The high Φ_{PSII} observed at this location, despite the low amount of chlorophyll, implies that the chloroplasts present are highly active and photosynthetically efficient.

The highest chlorophyll content was found at the mid and top part of the leaves (Figure 30). Since the age of the leaf tissue generally increases towards the top, the physiological status of this area would be expected to be worse than at the base (Borum et al. 2004). The high chlorophyll content in the upper part of the leaves, combined with an increased amount of carotenoids, may be an acclimation response to reduced light availability connected to the high degree of epigrowth observed at this location. An increase in photosynthetic pigments will ideally enhance the light absorption efficiency (Park et al. 2016). Since the degree of epigrowth is generally low at the base, the plants are likely to manage with a lower content of chlorophylls and carotenoids present at this location.

Since carotenoids also function as photo protective pigments, a higher content at the top of the leaves would be expected, as seen in this study (Johnsen and Sakshaug 2007). The upper part of the leaves is generally closer to the surface and exposed to stronger light illumination, thus increasing the need for photo protection. A higher carotenoid content in April, compared to October, might therefore be explained by the increased light availability in spring (Figure 29). However, the high content of violaxanthin and low content of anthera- and zeaxanthin, indicates that the seagrass habitat in Hopavågen can be characterized as a low light environment (Table 7) (Ralph et al. 2002).

4.2.1 Challenges and future perspectives

The seasonal variation in pigment content (chlorophylls and carotenoids) and maximum quantum yield of PSII (except for April and October) was found to be not significant ($P > 0.05$). This is likely due to a low number of replicates, but can also result from a low variation between the measured variables (months). This study only considered 4 different months, excluding the winter (December, January, and February) and the summer period (May, June, July and August). However, seagrasses are known to grow most vigorously during summer, and previous studies have shown that the chlorophyll content tends to decrease with increased temperatures (Liu et al. 2011). Additionally, seagrass leaves are usually less covered in epigrowth in the summer, as a result of a high leaf production rate (Fredriksen and Christie 2003). It is therefore likely that the photosynthetic efficiency will be high during this time. Whether this is the case for Hopavågen is hard to tell without doing further measurements, but it is important to keep in mind that variations in physiological characteristics may differ between geographic areas.

For a more complete and reliable study, additional sampling should be performed, scattered throughout the year.

4.3 The use of an ASV in shallow water mapping

The images provided by the ASV in April could not be used for mapping purposes, as the image quality and level of detail were too low to provide any useful information (Figure 21). The selected transect area seemed to be too deep for the camera, resulting in blurry images solely containing signals from the water. The green color observed in most of the images is likely a result of attenuation of red (400 – 700 nm) and blue (450 – 480 nm) light, combined with effects from the inherent optical properties of the water (e.g. phytoplankton, cDOM and TSM). These optically active components also affect the image quality, by reducing the water transparency (Sakshaug et al. 2009).

The still frames extracted from the video transect in November, had a higher quality than the images from April, likely resulting from a shallower transect area. It was possible to discriminate between the seagrass and the seafloor with a high degree of certainty, which may favor the use of an ASV over aerial photography for mapping of shallow areas (Bergan 2001). By joining still frames together into a photo mosaic, a larger area of the seagrass habitat could be observed at the same time (Figure 22). As photo mosaics generally require a high degree of image overlap, it was however not possible to combine all the still frames in order to present the total seagrass distribution of the area (Ludvigsen et al. 2007). The low degree of overlap is likely a result of the manual control of the ASV. The transect map shows that the lines were generally not very straight, resulting in undersampling of some parts of the transect area, lowering the area coverage (Figure 20). This is a partly a result of human error. Automatic mode would therefore be preferred, as the ASV would follow a predefined path with a high degree of overlap, assuming ideal conditions (Kimball et al. 2014).

4.3.1 Challenges and future perspectives

Automatic and manual mode

For mapping of larger areas, the ability of the ASV to follow a predefined path would be essential. However, the manual mode was found to be most reliable during this study, despite the drawback of human error. When preprogrammed, the ASV had a tendency to not fully execute its given commands. Further technological developments are therefore needed in order to solve such issues, making the vehicle more reliable in the future.

Speed

The speed of the ASV was found to have a great impact on the image quality, as a result of lower stability at greater speeds, thereby increasing camera movements. To prevent this, the speed should be as low as possible (< 1 knot), in order to secure high quality footage. Overall stability improvements of the vehicle would also be preferred. Additionally, it is important to note that the stability and movements of the ASV can be affected by the weather. Strong winds and waves may prevent the ASV from following a specific transect pattern or moving in a straight line, and will decrease the overall stability of the vehicle.

Depth

The image quality was also found to be dependent on depth. The ideal distance to the OOI depends on water transparency and the IOP's of the water. This should be considered before defining a transect area, as it will vary between locations. Camera specifications should also be considered when defining the optimal survey depth (Andersen 2011). The goPro camera used in this study did for instance have a fixed focus. As a result, the camera was not able to adapt to changing conditions (e.g. depth), resulting in blurry images. Finding the ideal depth for the built-in camera focus is therefore important. However, since most areas do not have a uniform depth distribution, a better solution might be to choose a camera with adjustable focus, in order to increase the depth range.

The sharpness and contrast of the images generally seemed to increase with decreasing depth. This suggests that shallow areas should be preferred when using an ASV for mapping purposes. However, if the area is too shallow, the vehicle may hit the seafloor and damage the sensors attached. As a result, a trade-off between depths needs to be made. Additionally, it is important to consider tides when selecting a transect area. Mapping should generally be performed at low tide, for increased data quality.

Light

Light conditions also affect the quality of the images. In this study, the sun was the only light source used. As light is known to be heavily attenuated in water, the amount of light reaching the OOI is generally not very high (Sakshaug et al. 2009). A solution to this issue might be to use artificial light sources, mounted on the ASV. Additionally, variation in natural light availability should be considered. For optimal results, mapping should be done in sunny weather, during daytime, at the time of year with the longest day length.

Data processing

In order to use the images provided by the ASV for mapping purposes, the data needs to be further processed. Creating a photo mosaic of the transect area may for instance give an indication of the overall distribution of the OOI (e.g. relative to the total bottom area). If combined with proper geo-referencing and details about pixel size relative to the seafloor, the distribution may be presented in a map. As data processing often tend to be very time consuming, development of an algorithm that combines the time-stamps of the images with corresponding GPS-coordinates, would be desirable.

5 CONCLUDING REMARKS

- The seagrass habitat in Hopavågen consisted of a patchy bed of *Zostera marina*, present from the intertidal zone down to approximately 4 meters depth. The plants were characterized by short, narrow leaves, with a rounded leaf apex and 5 leaf nerves. The area covered (%) by the seagrass leaves was relatively high and seemed to increase with depth. The habitat was biodiverse and used by many different organisms as shelter and substrate for attachment and reproductive processes; by supplying the sediments with oxygen and functioning as shelter and feeding ground for many different species. The fauna was dominated by benthic invertebrates such as polychaetes and decapods, while mollusks confined the most species diverse group.

- The seagrasses in Hopavågen were photosynthetically active throughout the sampling period, with a mean Φ_{PSII} ranging from 0.59 - 0.71. As the pigment data additionally showed low amounts of degradation products, the overall physiological status of the plants seemed to be relatively high. Differences in Φ_{PSII} and pigment content were observed between months and leaf locations. The Φ_{PSII} was generally low in autumn and high in spring, decreasing from the base towards the top of the leaves. This is likely a result of increased epigrowth towards the top and at the end of the year, reducing the quantity and quality of light available for photosynthesis. The observed chlorophyll content supported the Φ_{PSII} measurements regarding seasonal variation. The trend was opposite for leaf location, showing a lower chlorophyll content at the base of the leaves and higher at the top. Higher chlorophyll content is possibly an acclimation response to lower light availability. The carotenoid content showed a similar trend, likely increasing the light absorption efficiency.

- The use of an ASV for mapping purposes in shallow areas is likely to have a great potential, but as seen in this study it will require further developments in order to increase the reliability of this type of instrument bearing platform, as well as the overall data quality. Proper planning is strongly needed in order to get desirable data, and environmental conditions such as weather, tides and light availability should all be considered in the planning process. The speed and stability of the ASV, in addition to depth and factors regarding the imaging process, are also affecting the data quality. Based on the findings of this study, an ASV equipped with a camera are able to provide an overview of the seagrass distribution in an area, as it is possible to discriminate between the seagrass and the seafloor with a high degree of certainty. However, this method should be combined with other mapping techniques, if further details are required. If advances in technology are able to overcome the current challenges regarding this method, the use of an ASV has a potential to become a standard technique for shallow water mapping and monitoring, as it is both cheaper and more efficient than already existing methods.

- Increased consistency in sampling procedures and methods used, in addition to a higher number of replicates, scattered throughout the year, should be considered in the future in order to increase the statistical significance of the data. It is however important to note that despite a low significance for several variables discussed in this study, the results were generally in accordance with each other, supporting the observed trends.

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7 APPENDIX

7.1 Tables

Table A. Pixel values used to calculate seagrass coverage (%) based on data from square analyses (50 x 50 cm).

Frame (#)	Sel. Pixels	Tot. Pixels	Ratio	Coverage (%)
1	224 764	359 927	0.6	62.4
2	121 569	248 443	0.5	48.9
3	166 136	223 773	0.7	74.2
4	165 836	255 005	0.7	65.0
5	70 681	145 877	0.5	48.5
6	28 917	146 185	0.2	19.8
7	43 480	195 701	0.2	22.2
8	95 289	144 551	0.7	65.9
9	106 146	318 805	0.3	33.3
10	52 370	179 754	0.3	29.1

7.2 Figures

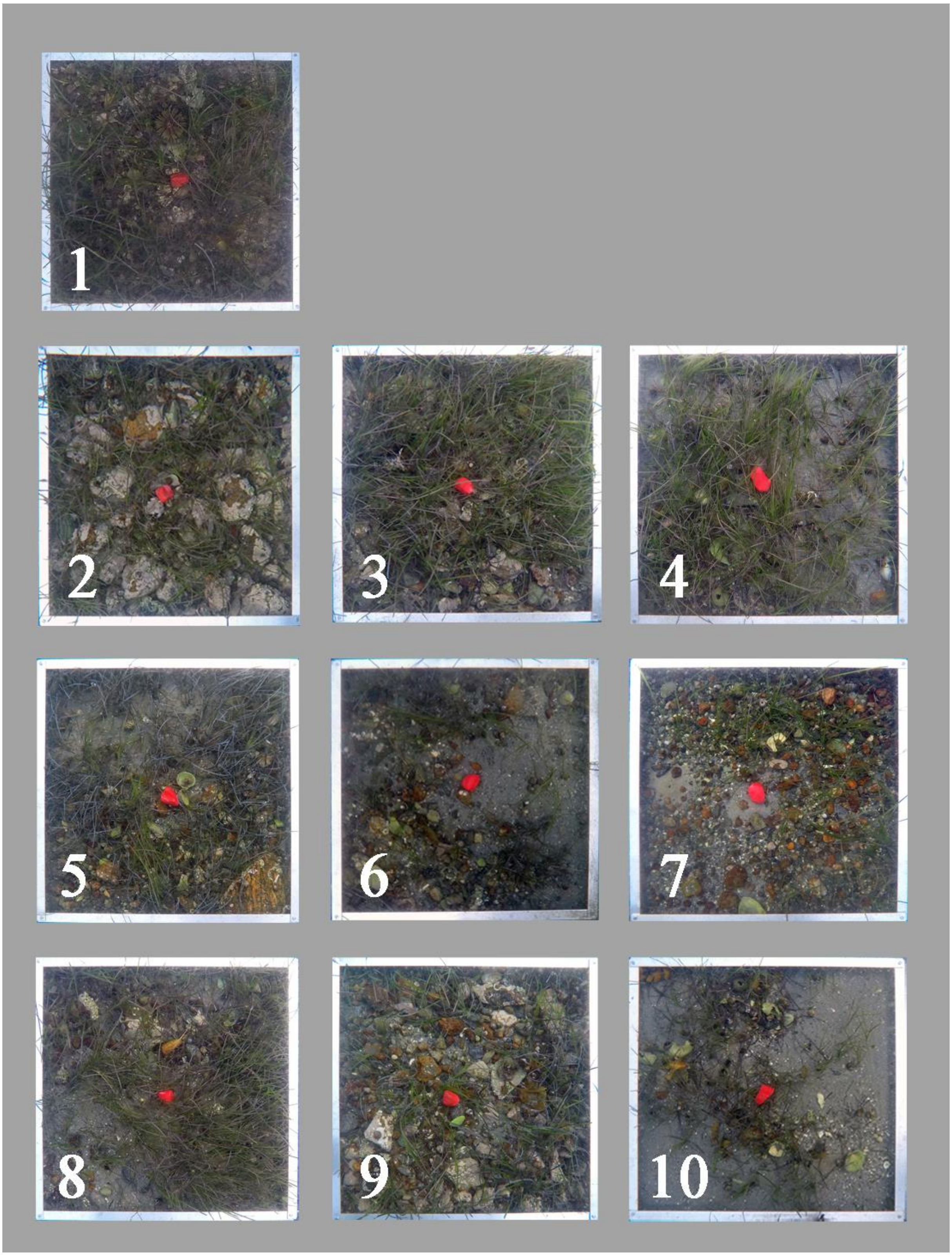
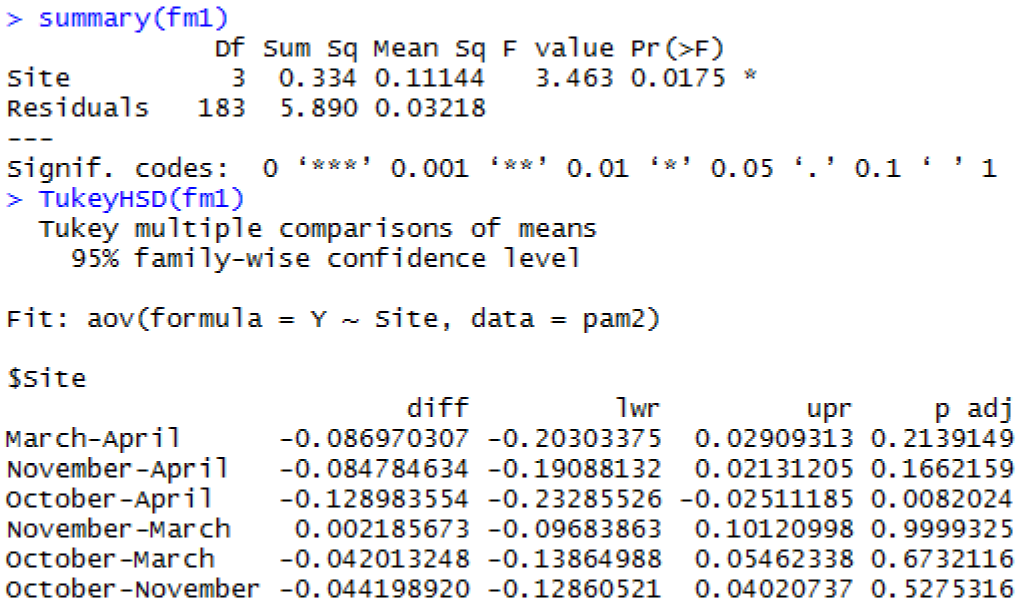


Figure A. Overview of the 10 frames used for square analysis and estimation of seagrass coverage (%).

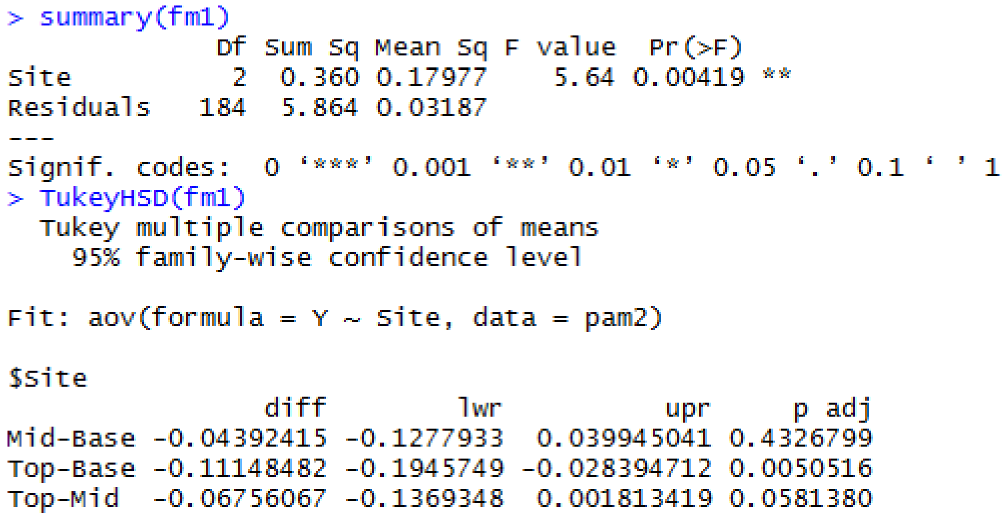


```
> summary(fm1)
             Df Sum Sq Mean Sq F value Pr(>F)
Site          3  0.334 0.11144   3.463 0.0175 *
Residuals   183  5.890 0.03218
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(fm1)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Y ~ Site, data = pam2)

$Site
                         diff         lwr         upr     p adj
March-April      -0.086970307 -0.20303375  0.02909313 0.2139149
November-April   -0.084784634 -0.19088132  0.02131205 0.1662159
October-April    -0.128983554 -0.23285526 -0.02511185 0.0082024
November-March    0.002185673 -0.09683863  0.10120998 0.9999325
October-March    -0.042013248 -0.13864988  0.05462338 0.6732116
October-November -0.044198920 -0.12860521  0.04020737 0.5275316
```

Figure B. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in maximum photosynthetic quantum yield (Φ_{PSII}) between different months.

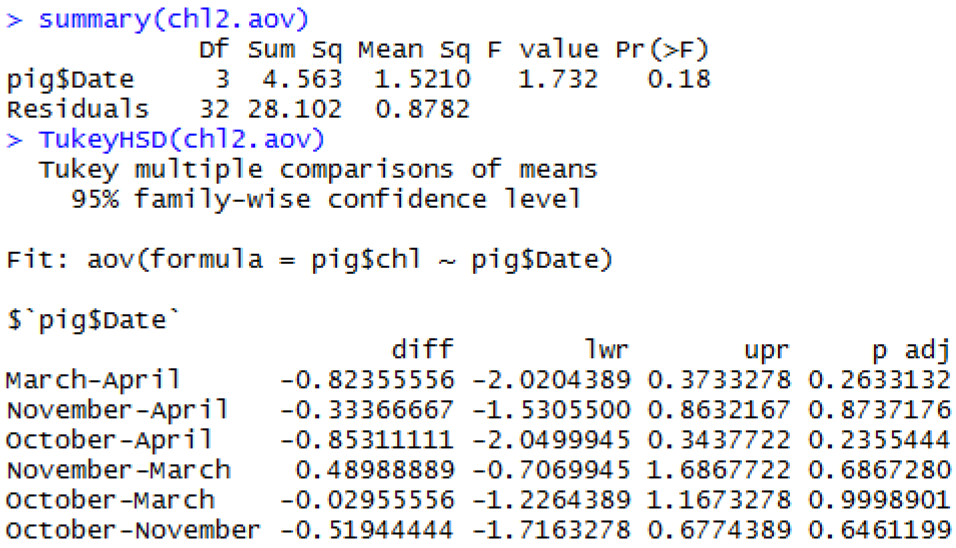


```
> summary(fm1)
             Df Sum Sq Mean Sq F value  Pr(>F)
Site          2  0.360 0.17977    5.64 0.00419 **
Residuals   184  5.864 0.03187
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(fm1)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Y ~ Site, data = pam2)

$Site
                diff        lwr          upr     p adj
Mid-Base -0.04392415 -0.1277933  0.039945041 0.4326799
Top-Base -0.11148482 -0.1945749 -0.028394712 0.0050516
Top-Mid  -0.06756067 -0.1369348  0.001813419 0.0581380
```

Figure C. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in maximum photosynthetic quantum yield (Φ_{PSII}) between different leaf locations.

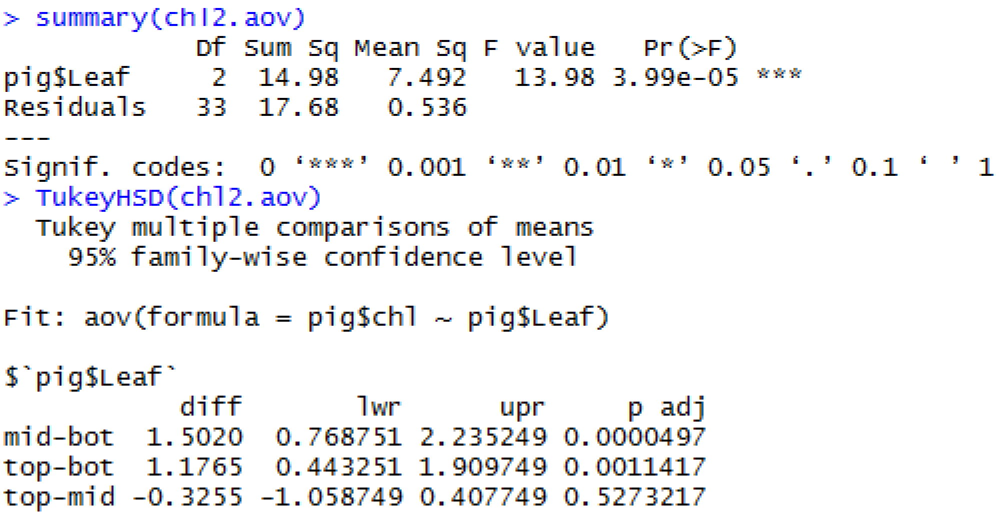


```
> summary(chl2.aov)
            Df Sum Sq Mean Sq F value Pr(>F)
pig$Date     3  4.563  1.5210   1.732   0.18
Residuals   32 28.102  0.8782
> TukeyHSD(chl2.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pig$chl ~ pig$Date)

$`pig$Date`
                       diff        lwr       upr     p adj
March-April      -0.82355556 -2.0204389 0.3733278 0.2633132
November-April   -0.33366667 -1.5305500 0.8632167 0.8737176
October-April    -0.85311111 -2.0499945 0.3437722 0.2355444
November-March    0.48988889 -0.7069945 1.6867722 0.6867280
October-March    -0.02955556 -1.2264389 1.1673278 0.9998901
October-November -0.51944444 -1.7163278 0.6774389 0.6461199
```

Figure D. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in total chlorophyll content between different months.

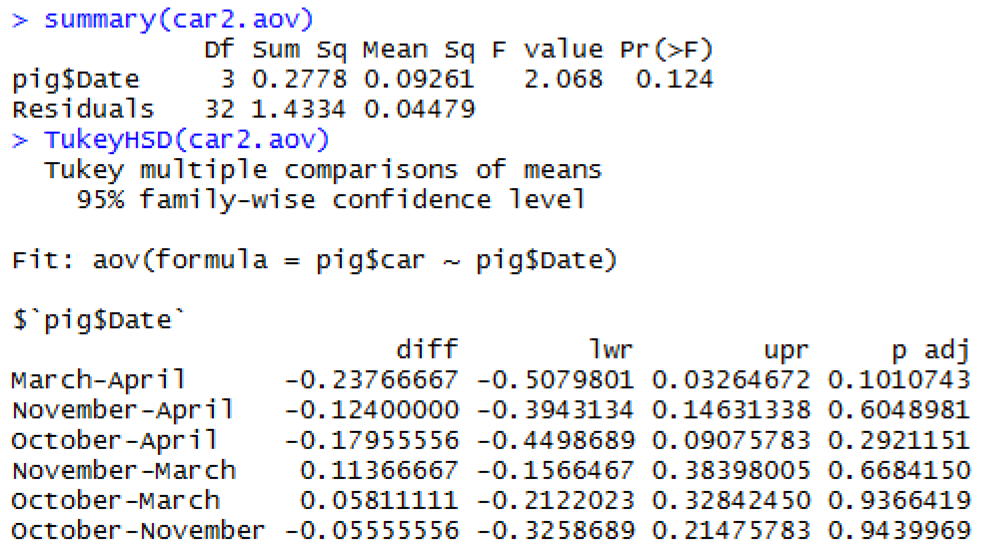


```
> summary(chl2.aov)
            Df Sum Sq Mean Sq F value   Pr(>F)
pig$Leaf     2  14.98   7.492   13.98 3.99e-05 ***
Residuals   33  17.68   0.536
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(chl2.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pig$chl ~ pig$Leaf)

$`pig$Leaf`
           diff       lwr      upr     p adj
mid-bot  1.5020  0.768751 2.235249 0.0000497
top-bot  1.1765  0.443251 1.909749 0.0011417
top-mid -0.3255 -1.058749 0.407749 0.5273217
```

Figure E. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in total chlorophyll content between different leaf locations.

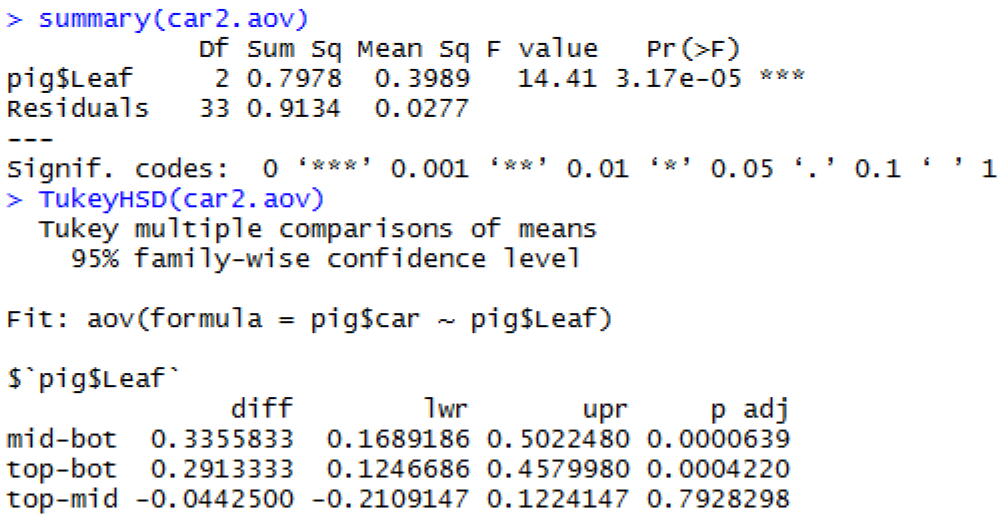


```
> summary(car2.aov)
            Df Sum Sq Mean Sq F value Pr(>F)
pig$Date     3 0.2778 0.09261   2.068  0.124
Residuals   32 1.4334 0.04479
> TukeyHSD(car2.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pig$car ~ pig$Date)

$`pig$Date`
                        diff        lwr        upr     p adj
March-April      -0.23766667 -0.5079801 0.03264672 0.1010743
November-April   -0.12400000 -0.3943134 0.14631338 0.6048981
October-April    -0.17955556 -0.4498689 0.09075783 0.2921151
November-March    0.11366667 -0.1566467 0.38398005 0.6684150
October-March     0.05811111 -0.2122023 0.32842450 0.9366419
October-November -0.05555556 -0.3258689 0.21475783 0.9439969
```

Figure F. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in total carotenoid content between different months.



```
> summary(car2.aov)
            Df Sum Sq Mean Sq F value   Pr(>F)
pig$Leaf     2 0.7978  0.3989   14.41 3.17e-05 ***
Residuals   33 0.9134  0.0277
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(car2.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pig$car ~ pig$Leaf)

$`pig$Leaf`
              diff        lwr       upr     p adj
mid-bot  0.3355833  0.1689186 0.5022480 0.0000639
top-bot  0.2913333  0.1246686 0.4579980 0.0004220
top-mid -0.0442500 -0.2109147 0.1224147 0.7928298
```

Figure G. Output from R based on Tukey multiple comparisons of means, indicating the statistical significance of differences in total carotenoid content between different leaf locations.