North Sea seaweeds: DIP and DIN uptake kinetics and management strategies
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Citation for published version (APA):
Chapter 1

1. General introduction

1.1 Seaweed

Seaweeds are found throughout the world’s oceans and seas. They generally grow attached to rock or other hard substrate (with a few exceptions, i.e. Sargassum natans (Linnaeus) Gaillon, Avrainvillea erecta (Berkeley) A. Gepp & E.S. Gepp and Halimeda macroloba Decaisne) in the intertidal and subtidal zones of coastal areas, down to depths where light levels attenuate to 0.05% of the surface irradiation (Lüning 1990). They differ substantially in many microstructural and biochemical features, including the photosynthetic pigmentation. Based on their pigmentation, seaweeds are classified into three main groups: green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta). Typically, a seaweed consists of holdfast, stipe, and frond forming the thallus, but more complex structures can develop (Figure 1-1). Many seaweeds have specialized tissues, for example Fucus vesiculosus Linnaeus (bladderwrack), which develops air-filled vesicles (air bladders) along the frond for its buoyancy and thus optimizes the surface position for photosynthesis.

Figure 1-1. Diagram of a typical seaweed with a frond consisting of holdfast, stipe and blades. Some species also develop gas filled bladders (pneumatocysts) for float.
Seaweeds serve many important functions in ecosystems. As so-called ecosystem engineers, they can influence the availability of resources by influencing sedimentation and erosion, thus shaping their habitat and barren rocky sites can be transformed into areas of high structural complexity and diversity (Jones et al. 1994, Bouma et al. 2005). Seaweeds also provide a food source for primary consumers, offer protection (i.e. shelter) from predators and can serve as a nursery for many animal species (Lalli & Parson 1997, McClelland & Valiela 1998). Furthermore, seaweeds play a considerable role in the world’s carbon cycle (global primary production). Approximately 6 % of the global net primary production (NPP) of 104.9 Pg \(104.9 \times 10^{15}\) g carbon per year is ascribed to seaweeds, although seaweeds only colonize 0.1 % of the seafloor (Lieth 1974, Smith 1981). Seaweeds not only grow on saltwater, which covers 70 % of the surface of the planet, they also take up excess nutrients from coastal waters, thus can also reduce eutrophication. Like all photo-autotrophic organisms, seaweeds fix CO\(_2\), a major greenhouse gas.

There is a growing interest in seaweed cultivation in Western Europe (including The Netherlands), as seaweeds are an attractive marine source of biomass and its cultivation offers great possibilities. Unlike terrestrial crops, seaweeds do not require agricultural land for cultivation and many species grow in saltwater (or brackish waters) avoiding competition for land and freshwater. Furthermore, they grow on nutrients available from the sea and do not need pesticides for crop-protection during growth. This thesis adds fundamental knowledge on sustainable seaweed cultivation of ecologically and economically relevant representatives of the marine flora in North-West Europe, all native to the North Sea area.

1.2 The North Sea

The North Sea, a biologically productive sea on the north-western European continental shelf, belongs to one of the world’s most productive marine areas (Figure 1-2). Its topography ranges from muddy lowland coastline in the south towards rocky upland coasts in the north.
A dominant feature of the North Sea is the tidal motion, which contributes to horizontal and vertical mixing of the water properties (Otto et al. 1990). The overall nutrient budget of the North Sea ecosystem is widely influenced by oceanic inflow from the north-east Atlantic Ocean (Reid & Edwards 2001, Edwards et al. 2002) and nutrient concentrations show a seasonal highly heterogeneous distribution, with nutrient accumulation during the winter (Figure 1-3) and depletion in the summer. Spatial differences in nutrient concentrations exist in coastal areas, largely affected by the run-off waters of several rivers, including Rhine, Elbe, and Thames. These run-off waters often contain considerable amounts of inorganic phosphorus (P) and nitrogen (N) from anthropogenic land-based activities (Sharpley et al. 1992, Rabalais et al. 2009). Besides natural fluctuations, the anthropogenic discharge of nutrients can generate concentration gradients and limitations, which are often observed along coastal zones of the North

Sea (Brockmann et al. 1990), sometimes causing eutrophication. Eutrophication can be a problem, for example when opportunistic seaweeds like species belonging to the genus *Ulva* (Chlorophyta), can vastly increase their growth and can build up a large biomass in extensive blooms (Teichberg et al. 2008, 2010). These massive blooms are known as ‘green tides’, when beached and rotting piles of biomass hinder shore-based activities. Green tides were reported along the coastline of southern North Sea since the 1970s, including The Netherlands (Malta & Verschuure 1997), The United Kingdom (Scanlan et al. 2007), and Denmark (Lyngby et al. 1999). Moreover, the sinking and degrading seaweed biomass can cause hypoxia in the water and the development of hydrogen sulphide (H$_2$S) by microbial decomposition processes, as reported for the German Bight and Danish waters in August 1982 with dramatic consequences for the pelagic and the benthic communities (Westernhagen & Dethlefsen 1983). Measures against eutrophication were installed, when these dramatic effects became evident. Recently it was shown, that the de-eutrophication
efforts have led to a large imbalance in the N:P stoichiometry of coastal waters of the North Sea in north-western Europe (Burson et al. 2016). Increasing N:P ratios, which outpace the Redfield ratio of 16:1 were observed (Radach & Pätsch 2007, Grizzetti et al. 2012) and a pronounced P-limitation can be effective in coastal regions of the southern North Sea.

1.3 Seaweed farming in Europe

A wide variety of seaweeds have been harvested for domestic use, for example as food, feed, and fertilizer by coastal populations in Europe for millennia (Hallsson 1964, Grall & Hall-Spencer 2003). The commercial utilization of seaweed started as early as the 17th century around Europe, with France, Ireland, and Norway being the biggest contributors. Harvested seaweeds were burned to produce potash, which was used in the glass and soap industry. While historically seaweeds were harvested for their crude biomass, the on-going research and development of refinement techniques allowed the extraction of valuable biochemical components, and in the 19th century the main utilization of seaweeds shifted towards the production of iodine. Nowadays, seaweeds in Europe are mainly exploited for their hydrocolloids and especially alginic acid (Porse & Rudolph 2017). Efforts to establish a sustainable seaweed farming in Europe are rising, as seaweeds are increasingly seen as an alternative to land-grown products for food, feed and energy, as well as others (extracts, clothes) and new seaweed product innovation in Europe is at a record level. Seaweeds are known to be an excellent source of polysaccharides, proteins, lipids (including high amounts of poly-unsaturated fatty acids), anti-bacterial compounds, minerals, fibers, photopigments and vitamins (Lüning 1990, Lobban & Harrison, 1994, Holdt & Kraan 2011). Their biomass is also recognized as a sustainable source for the utilization to biofuels (Wei et al. 2013, Bikker et al. 2016, Fernand et al. 2016) and bioplastics (Rajendran et al. 2012, Gade et al. 2013) amongst other applications, for example biofiltration purposes (Cahill et al. 2010). While cultivation and use of seaweeds is very common in Asia, it is developing in Europe, but it is still at its infancy. The European seaweed industry is dominantly based on the harvesting of wild seaweed stocks (FAO, 2014). This has raised concern regarding an over-harvesting of natural resources and
the deterioration of ecosystems by harvesting-techniques, as documented for some regions in North- and South America (Ugarte & Sharp 2001, Buschmann et al. 2014). Cultivation of seaweed is the only solution to adequately meet the increasing commercial demand. The high productivity of the North Sea, linked to nutrient availability, is promising for the economical (large scale) cultivation of seaweeds (van der Molen et al. 2018). Currently, seaweed is not farmed at large scale in the North Sea and wild harvests are limited to the very northern region of the North Sea area, around the coastlines of Norway (Vea & Ask 2011), Ireland and Scotland (Kenicer et al. 2000). A considerable number of investigations have been conducted to investigate parameters of seaweed production in the North Sea under environmental and laboratory conditions (i.e. Buck & Buchholz 2004, 2005, Sanderson et al. 2012, Tørring & Oddershede-Nielsen 2014). Available environmental and physiological parameters were used to establish models to calculate yields of seaweed production at different locations in the North Sea, for example for *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (Broch & Slagstad 2012). The overall productivity of the open North Sea related to seaweed biomass was estimated at approx. 20 tons drymatter per hectare and year without the addition of nutrients (Reith et al. 2005). It had been concluded that the productivity could substantially be increased through the addition of nutrients and/or layered cultivation (Reith et al. 2005). A recent study investigated the potential production and ecological impacts of seaweed farms by including seven experimental farms and one hypothetical farm site in Dutch and United Kingdom coastal waters into a 3-D numerical model of hydrodynamics and biogeochemistry (van der Molen et al. 2018). This model could not detect significant changes in biogeochemistry and plankton dynamics at any of the farm sites averaged over the farming season. Results also showed that seaweed production depended on prevailing nutrient concentrations and light conditions, with higher levels of both resulting in higher production.

When nutrients are added to a cultivation site, a precise dosage is essential for optimal growth and to prevent eutrophication. On the other hand, large scale cultivation of seaweed can lead to spatial nutrient limitations and depletions, which can mitigate, shift, or change composition
of phytoplankton blooms, thus affecting the whole food web of an ecosystem (Burson et al. 2016).

The uptake of nutrients by seaweeds is not always a negative or undesired effect. Seaweed farms could act as a last resort/recycling place, before a scarce nutrient as phosphate is diluted into the deep sea. Phosphate is deemed the first compound to limit agricultural production, hence essential to recycle (Ashley et al. 2011). In a similar context, seaweed cultures can conceivably be used for bioremediation purposes and, for example, minimize the impact of nutrients released by fish farms and areas of eutrophication, as well as in land-based bio-filtration facilities (i.e. Neori et al. 2003, 2004, Cahill et al. 2010). In order to assess the efficiency for bioremediation purposes and to avoid unacceptable damage to the ecosystem by large scale operations, it is necessary to understand the nutrient uptake kinetics and (management) dynamics of the seaweed used. This thesis can, based on its ecophysiological data on different species of seaweeds, help in finding the balance between preserving the marine ecosystem and unlocking its potential for sustainable production of food, feed and fuel.

Each seaweed species has its own growth characteristics and internal composition related to nutrient availability. Some species need large amounts of nitrogen and can handle low concentration of phosphorus, while others require larger quantities of phosphorus, and can cope with relatively low nitrogen concentration. Therefore knowledge on the nutrient management of the different seaweed species is essential, whether it concerns mono-cultures, layered cultures, integrated aquaculture systems, or the integration of multiple approaches. In addition, knowledge on the internal storage capacity in respect to growth rates and the time before nutrient limitations cause significant losses of yield are important physiological factors for a sustainable mariculture. The North Sea seaweed species with the most potential for cultivation were identified to be *Ulva lactuca* Linnaeus, *Saccharina latissima* Linnaeus J.V. Lamouroux, *Laminaria digitata* Hudson J.V. Lamouroux, and *Palmaria palmata* Linnaeus F. Weber & D. Mohr (Reith et al. 2005) (Figure 1-4). These 4 seaweeds are the protagonists in this thesis.
1.4. Thesis outline

Seaweeds offer interesting options for fundamental research. The North Sea seaweeds identified as the ones with the most potential for cultivation (see above) were the species that I worked with during my PhD at NIOZ (Royal Netherlands Institute for Sea Research). An important infrastructure for seaweed research in Europe is the NIOZ Seaweed Research Centre (https://www.nioz.nl/en/expertise/seaweed-research-centre) consisting of 26 fully insulated, heated/cooled, and aerated cultivation tanks (1600 L each) specially designed for seaweed research. Here, seaweed fundamental scientific work on physiology and ecology can be combined with clear applied options. For example, uptake of nutrients, growth, and their role as crop in a seaweed farm can be studied. The fundamental scientific knowledge gained can be used for sustainable production for food, feed, and chemical extraction for energy in the future, and be a guide how to preserve (coastal) marine ecosystem services.

In my experimental work at the NIOZ Seaweed Research Centre and in the laboratory, I studied the eco-physiology of *Ulva lactuca*, *Saccharina latissima*, *Laminaria digitata*, and *Palmaria palmata* related to nutrient availability with a focus on long term (i.e. up to several weeks) nutrient uptake kinetics. A
considerable amount of scientific literature is available on uptake kinetics and growth of several seaweed species in relation to dissolved inorganic nitrogen (DIN) (e.g. Thomas & Harrison 1987, Ahn et al. 1998, Naldi & Viaroli 2002, Pérez-Mayorga et al. 2011, Benes & Bracken 2016). Fewer studies refer to uptake kinetics of dissolved inorganic phosphorus (DIP) in seaweeds (e.g. Hurd & Dring 1990, Chopin et al. 1997, Gordillo et al. 2002, Pederson et al. 2010). Often DIN and DIP uptake kinetics were tested independently in short term experiments, in the range of hours (e.g. Runcie et al. 2003, Martínez & Rico 2004, Luo et al. 2012) or provide a momentary insight under field conditions (e.g. Neori et al. 2003, Naldi & Viaroli 2002). Knowledge of long term (weeks) effects on uptake kinetics under DIN- and DIP-replete, limited, or deplete conditions is essential for a proper ecological understanding and a sustainable production and is a key factor in this PhD thesis.

A full factorial design was used to determine DIN and DIP uptake kinetics and management strategies of *U. lactuca, S. latissima, L. digitata,* and *P. palmata* under laboratory conditions, controlling for temperature, light and hydrodynamics. Prior the experiments the specimen were gently cleaned (potential epiphytes removed and detritus rinsed off) and kept under laboratory conditions for adaptation. They were maintained in filtered, nutrient depleted seawater to ensure nutrient starvation. All experimental conditions were tested on individual specimen in a range of 5 to 7 replicates. During the experiments it was made sure that ample nutrients were available by daily refreshment of the seawater medium, so that constant nutrient levels were pulsed on a daily basis. The removal of nutrients from the seawater medium was followed (chased) in time, assuming the nutrients had been removed by the seaweed (Figure 1-5).
This ‘pulse-and-chase’ approach ensured sporophytes to be exposed to nutrient concentrations as intended during the experiment up to several weeks, and as neither random nor large shifts in DIP and DIN concentrations were observed between days during the experimental period, I am confident about the validity of values for DIP and DIN concentrations, respectively uptake rates. By this daily “pulse-and-chase” of nutrient concentration in the seawater medium, surge uptake rates ($V_S$), maintenance uptake rates ($V_M$), correlations and ratios of DIN/DIP uptake rates, and internal storage capacity (ISC) for DIN and DIP of the 4 seaweeds were calculated, all standardized for surface area (SA). A standardization of uptake kinetics to SA was chosen, as it enables an intra- and interspecific comparison of the seaweeds over time, while uptake kinetics expressed as a function of dry weight (DW) define the end of the living biomass. The non-destructive method of standardized determination of fresh weight (FW) was not regarded as a useful parameter of biomass, as little variations in the amount of water attached to the living (and growing) seaweed can lead to huge differences in its weight, not only between different samples and over time, but also amongst different experimentators.

**Figure 1-5.** Example of daily removal (referred to uptake) of DIN ($\mu$mol·L$^{-1}$) from the seawater medium by nutrient-starved *Ulva lactuca* in a ‘pulse-and-chase’ approach with daily refreshment of the seawater medium (30 and 60 $\mu$mol·L$^{-1}$) over 5 days.
It can be envisioned that nutrient limitations/depletion will not only determine growth, but also affect internal composition (e.g. carbohydrate and protein concentration), colour appearance and texture. The combined effects of DIN/DIP availability on growth, photosynthetic efficiency (Fv/Fm), and total dissolvable carbohydrate- and protein concentration were assessed for *P. palmata* and *U. lactuca*. This assessment included the introduction of a novel method to evaluate the total dissolvable protein concentration (nutritional value) in *U. lactuca* by digital imaging of its frond colour. This colorimetric method was integrated into the free smartphone application ‘EyeOnUlva’ for Android and IOS systems (www.eyeonwater.org/ulva) and enables citizen scientist to participate in ecological studies (Figure 1-6).

**Figure 1-6.** The smartphone application ‘EyeOnUlva’ for Android and IOS systems (www.eyeonwater.org/ulva) records the frond colour of the green seaweed *Ulva lactuca* and provides a fast quantification of its total dissolvable protein concentration in % dry weight. The data can be send to a data base, which is part of the CITCLOPS project (www.citclops.eu).

Furthermore, a new approach that allows for standardised methods to study morphological effects on seaweed individuals in response to varying hydrodynamic forces based on the example of *L. digitata*, is proposed. This methodology allows the determination of the total strain deformation (Ɛ) and breaking points by means of applying tensile and compression forces, using
an industrial texture analyser mounted with customized clamps for attaching seaweed samples (Figure 1-7).

Figure 1-7. A) Front view on fittings of the texture analyser (CT3, Brookfield Engineering, USA) with attached seaweed sample, B) customized clamps, sample attachments and sample stamp for toughness measurements on seaweed (bottom to top) and C) punched out sample of *Laminaria digitata* frond, ready for toughness analysis.

The studies presented on the 4 native North Sea seaweed species are divided into the following chapters:

Figure 1-8. Infographics of results on dissolved inorganic phosphate- and dissolved inorganic nitrate uptake dynamics ($V_s$ under starvation, $V_M$ for maintenance, and storage capacity) in Ulva lactuca (Chlorophyta).

Chapter 2 is devoted to uptake kinetics and internal storage capacity (ISC) of DIP and corresponding N:P dynamics in U. lactuca (Figure 1-8). DIP-uptake kinetics of U. lactuca exposed to a range of nominal DIP concentrations (1 – 50 µmol·L$^{-1}$) and a non-limiting DIN concentration (5000 µmol·L$^{-1}$) under fully controlled laboratory conditions in a ‘pulse-and-chase’ assay over 10 days are presented. DIP-uptake kinetics and storage capacity were quantified, as well as N:P-uptake dynamics, and all were standardized for surface area (SA). In order to make comparisons possible with other standardizations, factors for conversion to fresh weight (FW) and dry weight (DW) were presented. The results contribute to the understanding of ecological aspects of nutrient uptake kinetics in U. lactuca and quantitatively evaluates its potential for bioremediation and/or biomass production for food, feed and energy. High DIP- and DIN uptake under $V_s$ in saturating
concentrations quickly filled the ISC (within 1 day) and demonstrated the fast response of *U. lactuca* to nutrient pulses, typical for an opportunistic species. After ISC had been filled, uptake rates rapidly declined by approximately 90 \% for DIP and 80 \% for DIN and reached $V_M$. In turn, the ISC for DIP and DIN in relation to $V_M$ was depleted after 10 days of external nutrient depletion. This information is indispensable in order to predict the efficiency and sustainability of *U. lactuca* in bio-filtration systems and can help to efficiently clean control effluent streams, as well as monitor productivity. Furthermore, the data allows an estimation of ecological effects on nutrient availability and can contribute to development, modification and preservation of marine ecosystem services at cultivation sites. The conversion factor can assist to assess the total SA from its biomass (FW) and by that can help to select a precise dosage of nutrient additions to (large scale) production sites and/or estimate the efficiency of bio-filtration facilities, as FW is a more practical measure for procedures on a large scale.

![Figure 1-9. Two shades of green. *Ulva lactuca* cultivated under A) nutrient depletion conditions for 2 weeks and B) with addition of non-limiting nutrient concentration for 5 days.](image)

Chapter 3 is dedicated to evaluate the total dissolvable protein content in the seaweed *Ulva lactuca* (Chlorophyta) by digital imaging of its frond colour, applying a combination of spectro-radiometry and colorimetric techniques. In my eco-physiological work with *U. lactuca* (Chapter 2), I observed remarkable (green) colour differences in their fronds (Figure 1-9). These colour differences appeared to be related to their total dissolvable protein concentration. This led to this study, as described in chapter 3, in which we examined the possibility to deploy spectro-radiometry and colorimetric techniques to evaluate the total dissolvable protein concentration in the green seaweed *U. lactuca* based on its frond colour with the clear objective to integrate our results into a smartphone app. The fundamental work and feasibility of a new analytical method to evaluate the nutritional value of a seaweed by colorimetric analysis, on the example of *U. lactuca* is presented.
Furthermore, the sensitivity of *U. lactuca* to nutrient (nitrate, phosphate) availability by means of frond colour is demonstrated. The apparent optical property (frond colour) provided information on the nutritional status of the seaweed, as a correlation between frond colour and the total dissolvable protein concentration was found. The nutritional status of seaweed can give information on the levels of nutrients available in a certain habitat, integrating this over prolonged periods of time (which cannot be monitored with discrete samples taken every now and then). On the other hand, detailed information on nutrient concentrations in the seawater can be used to infer to the nutritional history of a seaweed and hence allow an approximate estimate on its nutritional status in relation to the ISC. This can be very useful for tank cultivations, where effluents are fully controlled.

Based on the data, we developed a smartphone application (EyeOnUlva) for Android and IOS systems, which records the frond colour and provides an inexpensive, reliable, safe and easy-to-use method to give a fast evaluation on the total dissolvable protein concentrations in *U. lactuca*. ‘EyeOnUlva’ has been tested successfully by a selected group of international university students to verify performance, reliability and ease of use of the application. The ‘EyeOnUlva’ application not only represents a useful tool to the aquaculture industry to assess the nutritional value of their seaweed crop and determine its feeding quality in a cost-effective way, but also may function as a bio-indicator, giving insight in the nutritional background of a coastal habitat or cultivation site. This makes it also applicable in environmental surveys, including citizen science programs.

**Figure 1-10.** Infographics of results on dissolved inorganic phosphate- and dissolved inorganic nitrate uptake dynamics (*V_S* under starvation, *V_M* for maintenance, and storage capacity) in *Saccharina latissima* (Phaeophyta).
Chapter 4 is devoted to the uptake kinetics and internal storage capacity of DIP and corresponding DIN uptake in *S. latissima* and *L. digitata* (Figure 1-10 and 1-11). In this study young sporophytes of both species were exposed to a range of nominal DIP concentrations (0 - 6 μmol·L\(^{-1}\)) and non-limiting DIN concentration (50 μmol·L\(^{-1}\)) under laboratory conditions in a ‘pulse-and-chase’ approach over 3 weeks. In an additional ‘pulse-and-chase’ approach, sporophytes of both species were exposed to DIP-depleted, DIN-depleted, DIP and DIN-depleted, as well as DIP and DIN-enriched seawater and the photosynthetic efficiency \(F_v/F_m\) as a measure of plant stress was followed over 9 weeks. Based on the data, the DIP and DIN-uptake kinetics, as well as the internal storage capacity of DIP and DIN in *S. latissima* and *L. digitata* were quantified and standardized to SA (Figure 1-10 and 1-11).

The results open opportunities to project impacts of nutrient limitation and shifts in limitation from one element to another, and shed light on possible competitive advantages of *S. latissima* versus *L. digitata* in relation to nutrient availability. Uptake kinetics as a function of SA
allow for a direct comparison of the net nutrient removal from the seawater. *S. latissima* in comparison to *L. digitata* exhibited higher growth rates, as well as a higher $V_S$ and $V_M$ for DIP and DIN and would outcompete *L. digitata* in terms of nutrients. Similarly, *S. latissima* has a larger ISC for DIP than *L. digitata* and can survive longer in limiting DIP conditions. Both species are efficient users of P, as the N:P uptake ratio during $V_M$ indicates. Conclusively, the results support *S. latissima* to be superior competitor for nutrients (as compared to *L. digitata*) and an effective candidate for bioremediation with similar uptake kinetics as *U. lactuca* (Chapter 2). *S. latissima* (Phaeophyceae) typically can be regarded a winter species, while *U. lactuca* (Chlorophyceae) flourishes at relative high temperatures and light intensities. This allows for crop rotation in seagriculture. Analogue to the physiological data on *U. lactuca* (Chapter 2), the information on DIP and DIN uptake kinetics of *S. latissima* and *L. digitata* can help to identify potential locations and modification for commercial cultivation, allows modelling studies to project yields of seaweed biomass at different locations, evaluate the efficiency for bioremediation, estimate ecological effects on nutrient availability, and select nutrient concentration in correspondence to SA and duration of the experimental time related to seaweed research.

Chapter 5 describes the first standardized data on physical properties in L. digitata (Phaeophyceae) thalli by texture analysis. Texture analysis is a method to test physical properties of a material by compression and tension (Figure 1-12), which are important parameters for the selection and survival of stationary organisms, exposed to steady turbulent flow and its varying drag-forces. Breaking points by means of tensile and compression forces, as well as total elongation and thickness of the tissue were evaluated and discussed in an ecological, physiological and morphological context.

Although the method described in this chapter was not aimed for marketing reasons in the first place, it can be transferred to the perception and acceptance of consumers, and also to marine herbivores that graze on seaweeds. The phenotypic plasticity not only serves as a mechanical defence against herbivores and other consumers as tissue toughness is the first physical barrier to overcome (Mauricio 1998), but it is also a key factor to endure mechanical stress caused by hydrodynamic forces (e.g. Koehl 1984, Denny 1994, Harder et al. 2006). Furthermore, knowledge

Figure 1-12. Exemplary graphs of (A) ultimate tensile strength (UTS) and (B) ultimate piercing load (UPL) during texture analysis (analyzer: CT3, Brookfield Engineering, USA) of a Laminaria digitata frond.
on the phenotypic plasticity and physical trade interaction, also on cellular level, is essential to understand morphological, ecological and physiological responses of seaweed and seaweed communities to changing environmental terms (Berg & Ellers 2010, Young et al. 2011, Coumou & Rahmsdorf 2012). Laminaria digitata is known to be a leathery and tough seaweed and is among the largest seaweeds that thrive in the wave-dominated intertidal zone of the NE Atlantic, including the North Sea area. The occurrence of L. digitata in wave-exposed areas can stimulate the settlement of other seaweeds and organisms, and by that opens opportunities to enhance diversity in its habitat. For example, the spatial distribution of epiphytic P. palmata population attached to the stipes of Laminaria populations (Whittick 1983). A destruction and reduction of ‘habitat-engineers’ like L. digitata for example by an increase of extreme weather conditions, such as intense hydrodynamic forces caused by rough storms, would result in the loss of associated marine flora and fauna. In the North Sea, for example, rough weather conditions can prevail during grow season in the winter months (Grabemann & Weisse 2008) and in cultivation an adequate selection of seaweeds with regard to phenotypic plasticity seems useful, especially in future offshore procedures. In addition, data on physical properties allow to develop and modify mechanical structures for manipulation in seaweed cultivation. Information on physical properties can be very relevant for the design of seaweed supporting structures. In a seaweed cultivation set-up, optimisation should be achieved in ensuring optimal nutrient availability, also in large cultivation farms in combination with structural support elements. When, for example, seaweed is cultivated for carbohydrates, a flexible cultivation set-up should allow for multiple hydrodynamic forcing on a flexible seaweed, in order for the seaweed to invest in structural elements of the cell wall. Laminaria digitata and other brown seaweeds are known for their high content in alginates (e.g. Kloareg & Quatrano 1988, Fertah et al. 2017). Alginates, a family of complex polysaccharides, occupy major sectors of global commerce and are widely used in various fields of industry, for example, food and feed, paper, textiles, cosmetics, and pharmaceuticals (McLachlan 1985, Pérez et al. 1992).
The data on the textural properties of seaweed, as gained by the methodology presented, can help to develop adequate seaweed supporting structures for cultivation, as well as help to select and adjust adequate pre-treatment to reduce size of raw material prior bio-refining processes in an energy- and cost efficient way (Zhu and Pan 2010). Many seaweeds have a tough and strong physical structure, including high contents of cellulose, hemicellulose, and lignin (e.g. Martone et al. 2009) that make them very defiant to microbial destruction, similar to woody biomass (Zhu and Pan 2010). An adequate size reduction (in turn increase of SA) of seaweed biomass is an important aspect for pre-treatment in bio-refinery activities.

In this study, results on *L. digitata* showed a positive toughness gradient of 75% from young to old tissue by means of tensile strength. Reciprocal responses to compression and tension along the lamina and along the gradient from old to newly formed biomass indicated a twined structural alignment to optimise constituent tissue toughness and flexibility. Similar principles of a twined alignment can be found in e.g. historical manufacturing processes of ropes, but also in modern nanotechnology. This experimental approach similarly allows for standardised methods of inferring the effects on nutrient availability and varying hydrodynamic forces on seaweed individuals.
Chapter 6 – Lubsch, A. & Timmermans, K.R. *in revision*. Dissolved inorganic phosphate uptake and corresponding dissolved inorganic nitrate uptake in the seaweed *Palmaria palmata* (Rhodophyceae): ecological and physiological aspects of nutrient availability.

![Diagram](image)

**Figure 1-13.** Infographics of results on dissolved inorganic phosphate- and dissolved inorganic nitrate uptake dynamics (V<sub>S</sub> under starvation, V<sub>M</sub> for maintenance, and storage capacity) in *Palmaria palmata* (Rhodophyta).

Chapter 6 gives insight into the DIP and DIN uptake dynamics, as well as internal storage capacities of DIP and DIN in the red seaweed *P. palmata* (Figure 1-13). Young sporophytes were exposed to a range of nominal DIP concentrations (0 - 6 µmol·L<sup>-1</sup>) and non-limiting DIN concentration (50 µmol·L<sup>-1</sup>) in a ‘pulse-and-chase’ approach over 20 days to quantify DIP and DIN uptake kinetics, all described as a function of SA for comparability. The photosynthetic efficiency F<sub>v</sub>/F<sub>m</sub> was followed for an additional 2 weeks, and based on the response in F<sub>v</sub>/F<sub>m</sub> to nutritional stress, the internal storage capacity for DIP and DIN was estimated. Finally, the total...
dissolved protein- and total dissolved carbohydrate concentration in the sporophytes, exposed to different DIP concentrations were determined after 5 weeks exposure.

The results add to the physiological and ecological understanding of the red seaweed *P. palmata* and opens further insight into ecological aspects of nutrient availability and interspecific competition. In addition, ecological effects on nutrient availability and shifts in limitations from one element to another can be estimated. The observed nutrient management strategies by *P. palmata* differed substantially from those of *U. lactuca* (Chapter 2), *S. latissima* and *L. digitata* (Chapter 4). An elevated DIN uptake in *P. palmata* was coupled to the availability of DIP, which consequently was mirrored by the total dissolved protein concentration, thus the nutritional value of the seaweed. Furthermore, the study showed an oscillating or rhythmic DIP and DIN uptake in weekly intervals, when sporophytes were exposed to saturating nutrient concentration. This rhythmic DIP and DIN uptake management can be related to a niche separation, which transferred to the interspecific competition for nutrients, can secure the coexistence of different seaweed populations competing for the same resources and by that species diversity in an ecosystem can be enhanced. To our knowledge, this nutrient uptake strategy was only been described for microalgae so far. Our findings on uptake dynamics and growth rates support *P. palmata* to be a potent species for bioremediation purposes in layered poly-cultures. Although there is a strong dependency on the availability of DIP for an efficient DIN uptake, the oscillating uptake of *P. palmata* can be used to complement the DIP and DIN removal from the seawater by other seaweeds in bioremediation activities, for example, when integrated into *S. latissima* cultivation in close proximity to fish farms, where high concentrations of N and P compounds can be found. DIP uptake rates in *P. palmata* under V_M outcompete those of the brown seaweed *S. latissima*, while DIN uptake rates are comparable in both species (Chapter 4). Thus our results on uptake kinetics in *P. palmata* not only allow an optimal modification and manipulation for a viable mariculture, but also help to evaluate the efficiency for bioremediation. Analogue to the physiological data on *U. lactuca* (Chapter 2), *S. latissima*, and *L. digitata* (Chapter 4) and related to fundamental seaweed
research, our data on uptake kinetics can serve as a measure to select nutrient concentration in correspondence to SA and duration of the experimental time.

Chapter 7 is devoted to practical implications of the main findings in this thesis. Examples on the implementation of results on DIP and DIN uptake kinetics and strategies in *U. lactuca*, *S. latissima*, *L. digitata*, and *P. palmata* into seaweed related operations, such as offshore cultivation, integrated multi-trophic aquaculture (IMTA), tank cultivation, and bio-filtration are given in the form of a ‘manual for nutrient uptake kinetics in seaweed cultivation’. The examples given in this chapter include eco-physiological data on DIP and DIN uptake kinetics, uptake ratios, uptake strategy, as well as DIP and DIN management related to ISC.

Chapter 8 gives a synthesis of general findings and the innovative aspects/highlights of this thesis and includes an overview of the main results (Table 8-1). Conclusions from results and experimental work are provided and an outlook for future research on seaweed is proposed.