Variability and similitudes in the structural properties of two related *Laminaria* kelp species

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Acknowledgments

Graphical abstract

Abstract

Kelps of the genus *Laminaria* have long been studied and shown to exhibit a seasonal shift in growth and morphology, as nutrients and light levels change during the year. However, the variation of kelp biomechanical properties has been little explored despite the importance of these properties for the interaction of kelp with the flow. Previous research showed that aging does influence the algae biomechanical properties, so this study further investigates the variability of kelp biomechanical properties and morphological characteristics at a given site as a function of the season (growth phase), species, and different kelp parts. Mechanical parameters and morphological characteristics were measured on kelps sampled in winter and summer, and DNA sequencing was used to identify the two related species, *L. digitata* and *L. hyperborea*. Descriptive statistics and statistical analysis were used to detect trends in the modulation of kelp mechanical design. Although two distinct species were identified, only minor structural differences were observed between them. The biomechanical properties varied significantly along the kelp, and significant seasonal shifts occurred at the blade level, in relation to the different morphological changes during blade renewal. In general, the variations of the structural properties were mostly linked to the blade growth activity. The absence of significant variation in the mechanical design of the two species highlights the significance of the adaptation to the same local environmental conditions, this adaptation being a key process in vegetation-flow interactions and having implications on the interaction between kelp and hydrodynamics.

Key words

INTRODUCTION

Vegetation is present in most aquatic environments, affecting many physical, chemical, and biological processes across a wide range of spatial and temporal scales (Nikora, 2010; Nepf, 2012; Nikora et al., 2012; Reed et al., 2015). Benthic assemblages of marine macroalgae and seagrasses are good examples, as they can be a net source of dissolved organic carbon vital for the microbial food web in the nearshore water column (Barrón et al., 2004; Wada and Hama, 2013). Kelp forests are also known to be among the most productive marine macrophyte communities (Reed and Brzezinski, 2009; Smale et al., 2013). Due to their nutritional and ecological properties, macroalgae are cultivated world-wide for various applications, such as food production (Gutierrez et al., 2006), biofuel production (Camus et al., 2016), agriculture purposes (Seth and Shanmugam, 2016) and they are increasingly used as a water quality regulator in Integrated Multi-Trophic Aquaculture systems (Lüning and Pang, 2003; Kim et al., 2017). Simultaneously, vegetated canopies generate and regulate turbulent processes, playing a major role in the natural environment (e.g. wave dampening, Løvås and Tørum, 2001; Möller et al., 2014; Carus et al., 2016). As a consequence, aquatic vegetation, macroalgae and seaweeds in particular, is increasingly considered in engineering applications and bio-inspired coastal management strategies to face the future changes of hydrodynamic regimes triggered by global changes (Temmerman et al., 2013).

Flexible vegetation reconfigures under energetic flow conditions (high current or wave-induced velocities) to reduce their exposure to the prevailing flow (de Langre et al., 2012; Miller et al., 2012; Albayrak et al., 2013). This reconfiguration depends on the mechanical and morphological properties of the plants and defines the organism survival strategy in high-energy flows (Biedka et al., 1987; Johnson and Koehl, 1994; Gaylord and Denny, 1997; Koehl, 1999; Stewart, 2004, 2006; Boller and Carrington, 2007; Martone and Denny, 2008; Demes et al., 2011; Miller et al., 2012). Thus, the knowledge of the mechanical properties is a

prerequisite for a proper characterization of the mechanical interactions between aquatic vegetation and its environment (Nikora, 2010), but only few studies have focused on this issue (Biedka et al., 1987; Hale, 2001; Harder et al., 2006; Boller and Carrington, 2007; Paul et al., 2014; Vettori and Nikora, 2017). Most importantly, the mechanical properties of vegetation elements are characterized by a high variability as they depend on species, scales and habitat conditions (Miler et al., 2014; Krumhansl et al., 2015). For example, many marine macroalgae are able to adjust their growth rates and morphology over time as a plastic response to environmental conditions (Fowler-Walker et al., 2006). Several kelps in the genus *Laminaria* have been studied for a long time and are known to exhibit a seasonal shift in growth and morphology, which can be related to changing levels of nutrients and light during the year (Davison et al., 1984; Schaffelke and Lüning, 1994). Additionally, Krumhansl et al. (2015) recently provided evidence of the impact of tissue aging on the mechanical properties of the blades of two distinct macroalgae groups (kelp and red algae). The growth cycle and aging processes are generally described by seasonal cycles, implying that the mechanical properties of vegetation vary with the different seasons. As aging and seasonal shifts affects the vegetation flexibility and thus the reconfiguration process, it can in turn lead to major changes in the physical and ecological environment. Seasonality and the process of aging of biological tissues has mostly been neglected in current numerical or physical representation of aquatic vegetation despite the fact that it may be relevant for many applications.

To remedy this knowledge gap and contribute to the development of a larger database on the biomechanical properties of aquatic vegetation, this paper describes and identifies the variability and similarities in the structural properties (mechanics and morphology) of two related species of marine brown macroalgae from a medium-exposed site. The hypothesis that kelp morphological and mechanical properties vary with the season and/or the species was tested by measuring and analyzing kelps collected from a single site at different times of the

year (winter/summer). Although DNA sequencing allowed the identification of the two distinct species *L. digitata* and *L. hyperborea*, only minor interspecific structural differences were observed, suggesting a similar adaptation strategy to the local hydrodynamic conditions. On the other hand, significant seasonal shifts have been observed on the biomechanics, confirming the need to consider and further characterize these temporal variations in the description of vegetation-flow interactions.

MATERIAL AND METHODS

Kelp collection and site description – The kelps analyzed in this paper were collected by free-divers at the spring tide mean low water level (MLWS) on a pebble beach adjacent to the city of Trondheim in Norway (63.448 N, 10.329 E), on 28 February 2013 for the winter season and 02 September 2013 for the summer season. Healthy specimens were collected about every meter along the vegetation belt (parallel to the shoreline), regardless of their size and shape, and preferably with their holdfast. After collection, the kelps were transported to the Trondheim Biological Station and temporarily stored in open basins with natural light and running water pumped in front of the station directly from the fjord, from a depth of 80-90m. As the site is only 1.5 km away from the station, light exposition and water properties were the same as at the sampling site, minimizing the chances of variations of the water composition and temperature (around 7°C in winter and 8.5°C in summer). The kelps were analyzed within a week of sampling. The algal community on this site is organized in a clear zonation typical for a low/medium exposed shore of the Eastern North-Atlantic region (Lüning et al., 1990), with a dense belt of kelps, identified later as *Laminaria digitata/hyperborea*. The average density of this belt was estimated to correspond to around 35 kelps per square meter (by counting specimens in a quadrat frame). The bathymetry of the site is rather steep, with the 50/100 m isobaths being located at about 160/300 m away from the shore, respectively (NHS Nautical Chart NHS458 - Trondheim Havn). Although the tidal

currents along the coastline are oriented from west to east during ebb and flood (Jacobson, 1983), the site is sheltered between two headlands and drifter observations suggested that there was no major tidal current at the shoreline. The highest astronomical tide at this location is 3.5m. The fjord of Trondheim is a closed basin so the waves reaching the shoreline are essentially wind-generated, and the abundance of natural pebbles on the beach confirms the presence of a wave activity. Although no direct wave measurements were taken prior to, nor during the field sampling, an estimation of wind generated waves following the engineering approach of Tucker and Pitt (2001) was realized from wind data recorded at the closest measuring station (63.411 N, 10.454 E - Norwegian Meteorological Institute), for the last 5 years before the first sampling. The most frequent wave height during the 5 years before sampling was estimated to correspond to approx. 7 cm and the significant wave height of the "once per year event" as defined by Mangor et al. (2017) to ca. 74 cm. The wave periods were most commonly between 1s and 2s. These conditions describe a low exposure of this site to the wave activity (Mangor et al., 2017).

__Morphology analysis__ – The specimens were picked up one by one to describe their morphological characteristics, such as the *Blade width* (maximum width of the blade), the *Blade ratio* (ratio of the blade width and the blade length) and the *Stipe length*. Minimum and maximum blade thicknesses (noted *tmin blade* and *tmax blade*) and stipe diameters were measured with a caliper gauge (+/- 0.005mm). In case of ellipsoidal stipe cross sections, major and minor diameters were measured, and averaged aspect ratios were reported for the cross-sections of the different stipe parts (noted *Ratio Sb* and *Ratio St* for the bottom and the top of the stipe, respectively). To determine the density of the different kelp parts, all excess water was removed from the surface of the sample cut from the kelp (see the mechanical analysis paragraph below) and weighed on a balance with a precision of ±0.05 g. The volume of the blade strips could be deduced from the previous blade morphology measurements, as

the longitudinal cross-section of the samples was found to be trapezoidal (the thickness varied linearly along the sample). The volume of the stipe samples was determined by measuring their displacement in unfiltered fresh water at room-temperature (20 °C). Based on the precision of the scale and the caliper gauge, the relative uncertainty on the estimation of the density varied typically between 3% and 7%. As illustrated in Fig. 1, the angle of the connection between the blade and stipe was measured and categorized in three groups (Lund, 2014): angles >90°, angles approx. 90° and angles <90°. Every specimen was photographed on a light table at a distance of about 1.8m (Lund, 2014), with a special attention given to some details, such as the stipe-lamina angles (Fig. 1). Tissue samples (1 × 1 cm) for DNA sequencing were taken from the meristem.
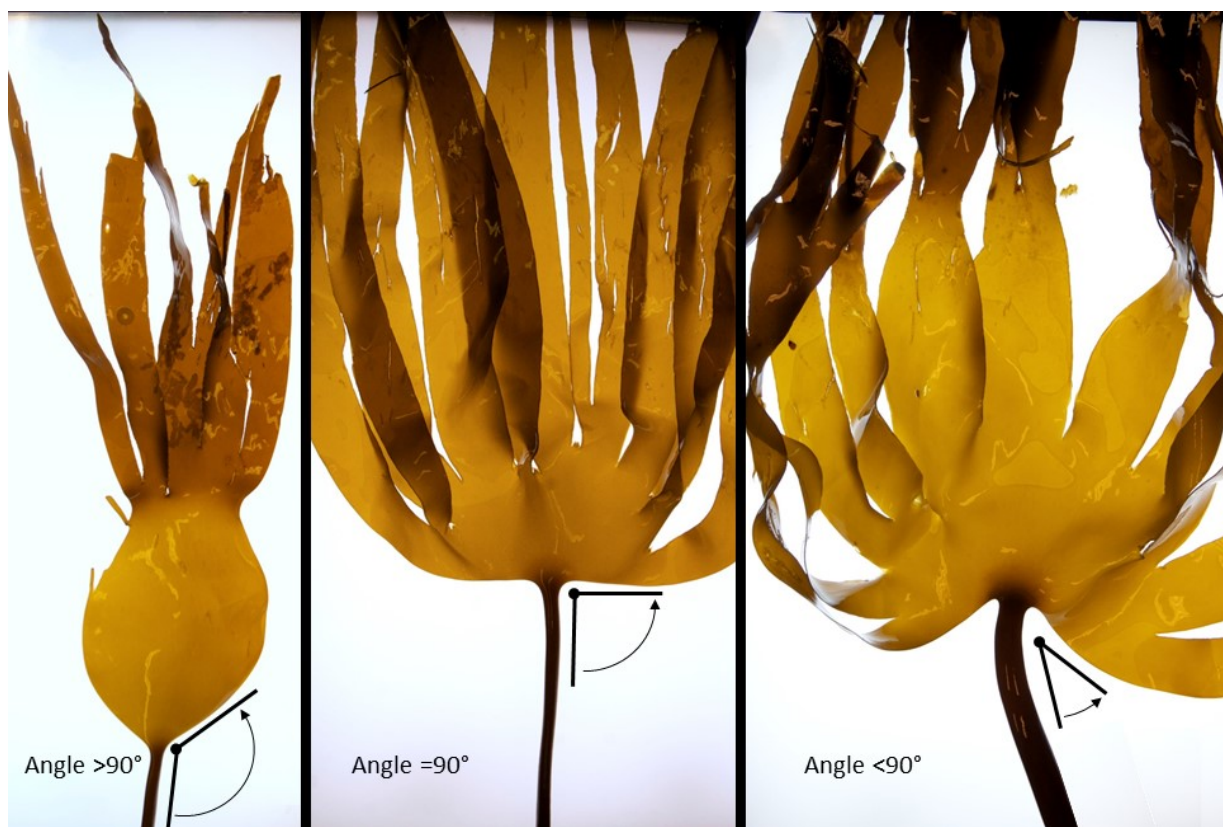

Fig. 1: Example of three different kelps sampled. The photos are focussing on the connection between the blade and stipe (adapted from Lund (2014)). The angle between the blade and the stipe were measured and categorised in three groups (superior to 90°, equal to 90° and inferior to 90°).

    DNA analysis – The DNA analysis was carried out by Lund (2014) in coordination with the other measurements presented in this study. The tissue samples (1 × 1 cm) were taken from the meristem of each collected specimen, frozen and later dried onto silica gel. The genomic DNA from the dried tissue samples was then extracted using a modified CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987) based on the protocol given by Gabrielsen et al. (2003). In short, the material was ground at room temperature using steel beads prior to adding the CTAB-buffer. The lysis step was performed at room temperature and no ribonuclease (RNase) was added to the samples. The gene cytochrome c oxidase I (COI) is a standardized marker for identification of species (Hebert et al., 2003) and was targeted to differentiate between species of Phaeophyceae (McDevit and Saunders, 2009). The combination of forward primer GazF2 (5′ CCAACCAYAAAGATATWGGTAC 3′) and reverse primer GazR2 (5′ GGATGACCAAARAACCAAAA 3′) was used for amplification and sequencing of the coxI-5' barcoding region, as previously described by Lane et al. (2007).

    Each 25 µL Polymerase Chain Reaction (PCR) contained 1× DreamTaq Green Master Mix (Thermo Scientific, Germany), 0.4 µM of each primer and 2 µL diluted DNA (1:10). The thermal cycling conditions were: 3 min at 94 °C, 35 cycles of 94 °C for 30 s, 49 °C for 45 s and 72 °C for 60 s, and 10 min at 72 °C. PCRs were run with Eppendorf Mastercycler ep gradient S (Hamburg, Germany) and GeneAmp PCR Systems 9700 (Foster City, USA). PCR products were purified by using E. Z.N.A. Cycle-Pure kit (Omega Bio-Tek Inc. Georgia, USA) following the E. Z.N.A. Cycle-Pure Spin Protocol (Omega Bio-Tek Inc. 2009) and sent to GATC Biotech (https://www.gatc-biotech.com/en/index.html) for Sanger sequencing. The sequence data were processed using Geneious R7 (version 7.1.7) (Biomatters, New Zealand). After quality trimming, the species were identified by blasting the sequences with Megablast (Altschul et al., 1990) which is optimized for very similar sequences. This method allowed for

the identification of two different species (*L. digitata* and *L. hyperborea*), but some of the specimens remained unidentified (see Table 1).

Table 1. Sample size as a function of the different species identified, the different kelp parts tested and the sampling season. The kelp samples tested were coming from the Blade top and bottom (*Bt* and *Bb*) and the Stipe's top, middle and/or bottom (noted *St*, *Sm* and *Sb*).

Season	Species identified			All species	Kelp parts tested				
	L. digitata	*L. hyperborea*	Unidentified		*Bt*	*Bb*	*St*	*Sm*	*Sb*
Feb.	18	2	1	**21**	20	20	14	6	17
Sept.	15	6	2	**23**	23	22	13	9	13
Total	**33**	**8**	**3**	**44**	**43**	**42**	**27**	**15**	**30**

Mechanical analysis – To describe the kelp mechanics, tensile, flexural, and torsion tests were carried out adapting standard engineering methods on the different parts of the kelps. For the blades, rectangular stripes of 14 cm by 2 cm were cut close to the meristem and from the top of one of the blade finger (parts referred as blade top – *Bt*, and blade bottom – *Bb*, respectively). Parts of the blades without obvious nicks, flaws nor epigrowth were preferably chosen. For the stipes, the samples consisted of sections of 14 cm long taken from the upper and the lower parts (referred as stipe top – *St*, and stipe bottom – *Sb*). In case a stipe curvature was too pronounced to carry out a test properly, the stipe sample was discarded. In case of a stipe being too short, one single sample was taken from either the top (*St*), the bottom (*Sb*), or the middle (*Sm*). Thus, the total number of measurements differed for each kelp part. The final sample size as a function of the kelp part or the species detected for the two sampling seasons is summarized in Table 1. The samples were tested using a portable custom-built test bench, designed to carry out simple mechanical tests with a minimum logistic requirement (e.g. in the field or wet laboratories). As illustrated in Fig. 2, this test bench is based on a drill column press equipped with a horizontal rounded bar (1 cm diameter) attached to a 3 kg PW4M load cell from HBM (resolution of 1 part in 3000, tested accuracy of ± 3 mN) and a WS10 displacement sensor from ASM GmbH (tested accuracy below ± 0.005 mm). Outputs

were amplified with a Quantum X MX840 (HBM GmbH) and acquired with a recording frequency of 10Hz. For the uniaxial tension tests, both sides of the samples were clamped to the head of the column (clamp attached to the bar, see Fig. 2) and to the base of the column, and force displacement curves were recorded. For bending tests, the clamp on the rounded bar was removed (head of the column) and side supports were installed to perform a 3-point bending test. The force was directly applied on the sample via the rounded bar. For torsion tests, one of the side support was changed to a support equipped with a rotating clamp mounted on a ball bearing (friction resistance below the accuracy of the load cell). The rotating clamp was attached to the head of the column via a thin chain so that the rotation and the moment applied on the clamp during the test would be directly converted into a displacement and force on the bar. For all these tests, the length between the different clamps was constant and set to 10 cm. The clamps were covered with a rough cork material to ensure a good and uniform grip without damaging the tissues, and tightened with two wing-nuts per clamps. The strain rates were kept constant by manually operating the drill column at a speed of about 70 mm/min. Variations caused by the manual operation of the test bench may have introduced some uncertainty into the measured material properties, which is considered in the discussion of the results.

Following the definitions of the classical beam theory applied to plant mechanics (Niklas, 1992; Vettori and Nikora, 2017) the Young's modulus in tension and flexion (*E* and *B*, respectively), and the shear modulus (*G*, deduced from the torsional rigidity) were all estimated from the slope a of the first linear part of the force-displacement curves, and thus derived as:

$$E = \frac{aL}{A} \tag{1}$$

$$B = \frac{aL^3}{192I} \qquad (2)$$

$$G = \frac{aLr^2}{J_T} \qquad (3)$$

where L is the span of the sample tested, A the nominal cross-section, I the second moment of inertia of the sample cross-section, r the radius of the rotating clamp for torsion tests and J_T polar moment of inertia (Young et al., 2011). The test-bench described above was used to carry out tensile, bending and torsion tests of all stipe samples, as well as for the tensile tests of the blade samples. However, for the blade samples which were too thin to meet the requirements of a 3 point bending test, the bending Young's moduli were estimated from the cantilever test described and discussed by Henry (2014). No torsion test was performed on the blades.
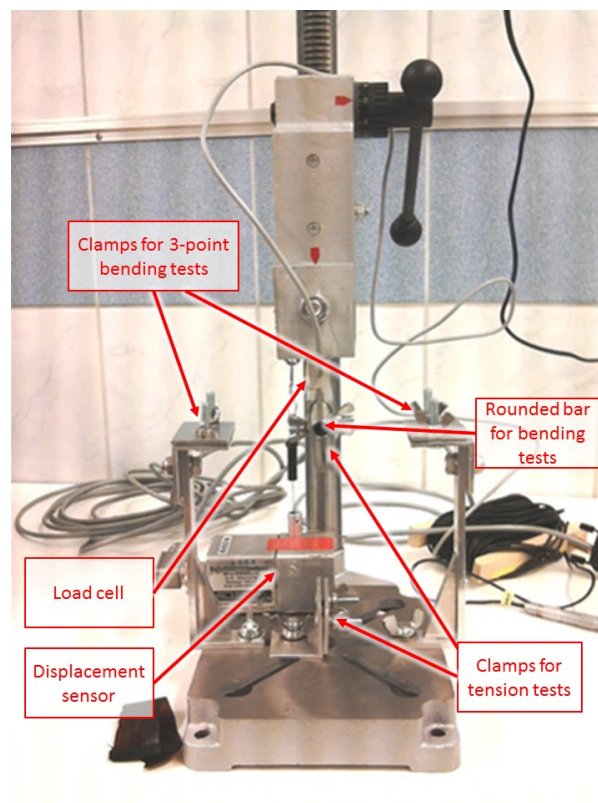

Fig. 2: Testing apparatus equipped with the ASM-WS10 displacement sensor and a HBM-PW4M load cell. The clamps are removable/adjustable so that the apparatus can be used for tensile tests (clamps on the bar and on the base), 3-point bending tests (clamps on the side) and torsion tests (with one of the side clamp mounted on ball bearings, not shown here).

Statistical analysis – To test the hypothesis that the kelp morphological and mechanical properties vary with the season and/or the species detected, two-way analyses of variance (ANOVA, see e.g. Fujikoshi (1993); Motulsky (2013)) regarding the species (*L. digitata*, *L. hyperborea*) and season (Feb. for winter and Sept. for summer) was conducted based on the measured morphological and mechanical parameters. Each parameter was analyzed in a two-way ANOVA with a between-subject factorial design, with the species and the season as the two independent variables. To test the hypothesis that seasonal variation of the structural parameters varies depending on the species (interactions), a 2×2 factorial between-subject ANOVA was conducted on each mechanical or morphological parameter. Further tests characterizing potential interactions between each factor were carried out using post-hoc comparison procedures (Šidák, 1967; Holm, 1979). The equal variance assumption (Brown and Forsythe, 1974) and the normality assumption (Shapiro and Wilk, 1965) were additionally tested for each comparison. If one of these assumptions is violated, the result of the ANOVA testing procedure was not considered, as a significant difference may be caused by a non-Gaussian distribution of the dataset or an unequal variance between the groups (Motulsky, 2013). All statistical tests were performed within the SigmaPlot software and the results were considered significant when the P-value was below a significance level set to 0.05. Following its common definition in statistical analysis, the P-value is defined here as the smallest level of significance at which the original hypothesis is rejected. As a result of the ANOVA testing procedure, the P-values are reported with the associated F-ratios, subscripted with the degrees of freedom between groups/within groups (For example $F_{1, 24} = 5.859$, $P = 0.023$; see e.g. (Motulsky, 2013)). The statistical analysis was complemented by descriptive statistical analyses (calculation of the means, standard deviations and some modified forest plot analyses) of each parameter as a function of the species variable (*L. digitata, L. hyperborea*) or the season variable (*Feb.* and *Sept.*).

RESULTS

The species identification via DNA sequencing analysis revealed that the collected specimens consisted mainly of the two species *L. digitata* and *L. hyperborea*. In total, 33 specimens were identified as *L. digitata* and 8 specimens as *L. hyperborea*. For 3 other specimens, the DNA sequencing did not allow for the species characterization (Lund, 2014). L. digitata and *L. hyperborea* are closely related from a genetical point of view, leading to a higher probability to observe hybrid specimens and potentially making the species identification more difficult (McDevit and Saunders, 2009). Thus, the three unidentified specimens may belong to the *L. digitata* species, the *L. hyperborea* species or may be hybrids of both. Since this aspect is not in the scope of this work, the unidentified specimens were not considered further in the statistical analysis of the measured parameters.

The detailed samples size as a function of the species and the season variables is given in Table 1. In addition, a summary of descriptive statistics of the measured morphological and mechanical parameters is presented in Table 2. The sample size used to calculate the mean values and standard deviations correspond to the numbers presented in Table 1. The diameter ratio at the top of the stipe, *Ratio St*, was the only parameter calculated with a slightly different number of kelps. For this parameter, the sample size is indicated by the bold numbers in the last column of the Table 2. For the different morphological parameters, there is no clear difference between the two identified species as the mean values for each species are in the same range (±the standard deviation). This observation is confirmed by the results of the Two-way ANOVA as only the diameter of the bottom of the stipe (*Diam. Sb*) appears to vary significantly from one species to another ($F_{1, 37} = 17.570$, $P < 0.001$).

Table 2. Mean values and standard deviations of the morphological (upper table) and mechanical (lower table) parameters characterised as function of the detected species. For the mechanical parameters such as density, tensile and bending Young's moduli, and shear modulus in torsion (ρ, E, B and G, respectively), the values are presented as average over the blade (top and bottom) or the stipe (top, middle and bottom). Here *tmin* and *tmax* relate to the minimum and maximum thicknesses measured on the blades, respectively. The corresponding sample size for the different kelp parts is presented in Table 1. Only the diameter ratio at the top of the stipe, *Ratio St*, was calculated with a slightly different number of kelps (sample size indicated in bold).

	Blade width (mm)	*Blade ratio* (-)	*tmin blade* (mm)	*tmax blade* (mm)	*Diam. Sb* (mm)	*Stipe length* (mm)	*Ratio Sb* (-)	*Ratio St* (-)
L. digitata	372 ± 186	0.68 ± 0.32	0.59 ± 0.12	1.21 ± 0.31	10.3 ± 2.52	408 ± 120	1.22 ± 0.15	1.97 ± 0.55 **(22)**
L. hyperborea	403 ± 153	0.76 ± 0.30	0.57 ± 0.10	1.36 ± 0.23	13.7 ± 2.65	489 ± 126	1.08 ± 0.05	1.19 ± 0.11 **(6)**
Unidentified	228 ± 95	0.39 ± 0.13	0.50 ± 0.07	1.40 ± 0.07	10.6 ± 0.53	365 ± 11	1.20 ± 0.14	# **(0)**
All species	368 ± 180	0.67 ± 0.32	0.58 ± 0.11	1.25 ± 0.38	10.9 ± 3.11	420 ± 129	1.19 ± 0.15	1.80 ± 0.59 **(28)**

	Density ρ (kg/m3)		Tensile Young's mod. *E* (Mpa)		Bending Young's mod. *B* (Mpa)		Shear mod. *G* (Mpa)
	Blades	Stipes	Blades	Stipes	Blades	Stipes	Stipes
L. digitata	1067 ± 96	1063 ± 58	8.44 ± 3.49	2.66 ± 1.07	1.10 ± 0.56	7.08 ± 1.86	1.30 ± 0.51
L. hyperborea	1086 ± 79	1070 ± 35	10.8 ± 2.69	1.97 ± 0.66	1.20 ± 0.43	6.88 ± 0.65	1.27 ± 0.27
Unidentified	1297 ± 264	1102 ± 54	10.4 ± 1.25	2.59 ± 0.43	1.12 ± 0.03	7.43 ± 3.63	1.13 ± 0.88
All species	1086 ± 118	1067 ± 59	9.01 ± 3.37	2.532 ± 1.04	1.12 ± 0.51	7.00 ± 1.73	1.28 ± 0.47

Although the aspect ratio of the top and the bottom of the stipe (*Ratio St* and *Ratio Sb*, respectively) seems to vary from one species to another according to Table 2 ($F_{1, 24} = 5.859$, $P = 0.023$ for *Ratio St*, and $F_{1, 37} = 8.831$, $P = 0.005$ for *Ratio Sb$), it is not possible to conclude on the statistical significance of this result as the Brown-Forsythe tests (equal variance) failed both for *Ratio St* ($P < 0.05$) and *Ratio Sb* ($P < 0.05$), which may be a consequence of the different sample size (Table 1). The collected data revealed that the blade thickness of the two species was about 2 times thinner at the top (*tmin*) than at the bottom (*tmax*) blade, while the ratio between the length and the width of the blade remained the same (*Blade ratio*). Based on the *Blade width*, *Blade ratio* and *Stipe length* parameters, the blade-to-stipe length ratio of the collected kelps was just above 1 for all the species (Not shown in Table 2: average blade-to-stipe length ratio of 1.56 ± 0.94 for *L. digitata*, 1.17 ± 0.25 for *L. hyperborea* and 1.52 ± 0.86 for all the specimens collected). For both species, the change of the stipe aspect ratio between the bottom and the top indicates that the cross section of the stipe gradually deviates from an almost circular section at the bottom to a strongly ellipsoidal section at the top of the stipe. Similarly, for the different mechanical parameters, there is no clear difference between the two species as the mean values of the different species are in the same range ($\pm$the standard deviation). The analysis of variance confirms this result since none of the mechanical parameters presented in Table 2 were found to vary significantly from one species to another ($P > 0.05$). However, considering the mechanical parameters for different kelp parts (and not averaging them over the blade/stipe as in Table 2), the tensile Young's modulus of the top of the stipe was found to vary significantly between the two species ($F_{1, 23} = 6.528$, $P = 0.018$), with a mean of 2.32 MPa $\pm$ 0.73 MPa for *L. digitata*, and 1.76 MPa $\pm$ 0.78 MPa for *L. hyperborea*. For both species, there is a clear difference between the tensile and bending Young's moduli of the blade compared to the moduli of the stipe. The tensile Young's modulus of the blade is approximately 9 MPa (averaged over all the kelps) while the

tensile Young's modulus of the stipe is about four times lower. On the other hand, the bending Young's modulus of the blade corresponds to approximately 1 MPa (averaged over all the kelps) while the tensile Young's modulus of the stipe is about seven time higher. However, the density of the blade and the stipe was the same. The shear modulus in torsion as well as the density of the blades and stipes of the unidentified species appear to be different from the two detected species, but the limited sample size (three specimens) does not allow the determination of the significance of this result.
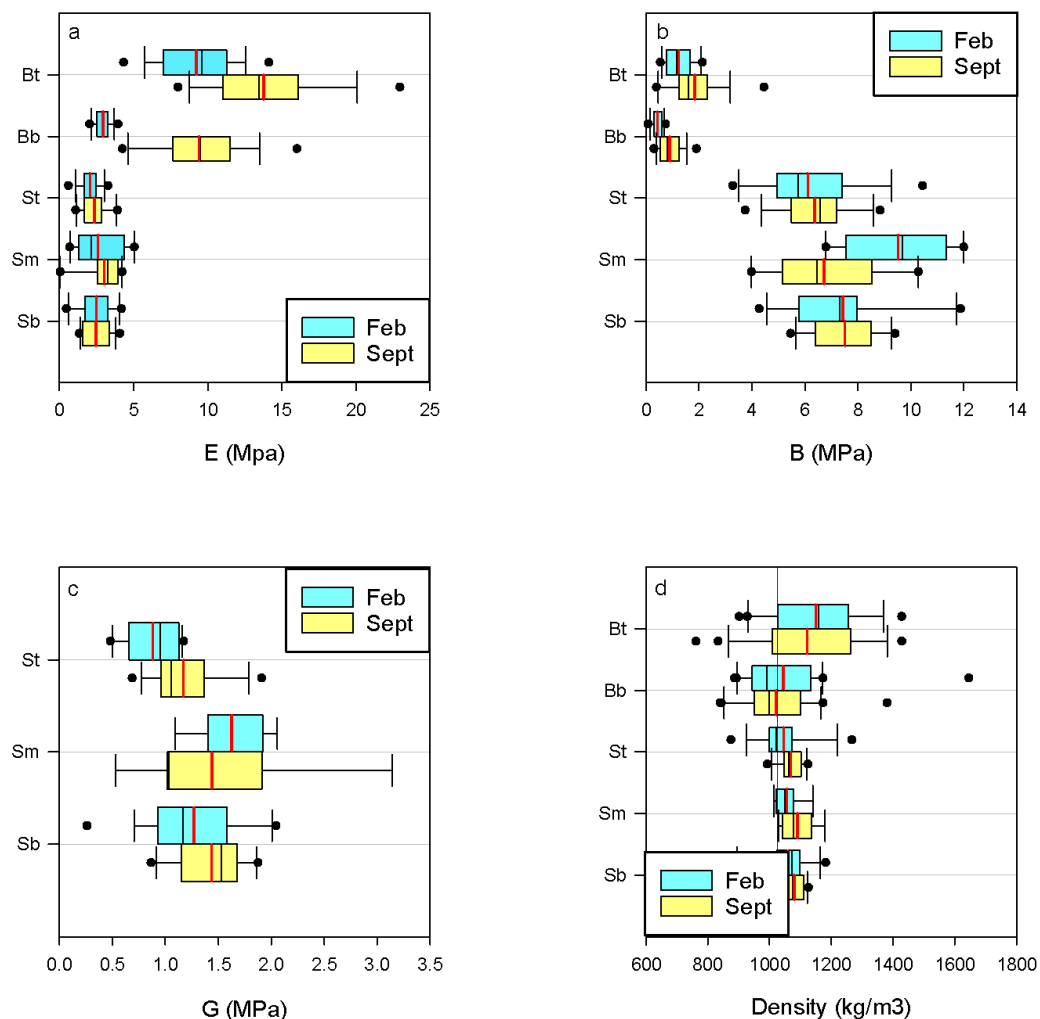

Fig. 3 Forest plot detailing the variation along the kelps (kelp parts in ordinate) of the tensile and bending Young's moduli E and B (a and b), the torsional shear modulus G (c), and the density ρ (d) as a function of the sampling season (upper boxes for February, lower boxes for September). For a given box, the lines in the box represent the median (black) and the mean (red) while the box edges define the upper and lower quartiles. The error bars define the 10th and 90th percentiles and the dots indicate the data points falling outside of that range. The blue vertical line in Fig. 3d represent an averaged density of sea water of 1025 kg/m3 (Sharqawy et al., 2010).

Since little or no dependency related to the species was identified, the results of the different parameters were pooled among the two species and the dependency on the factor season was characterized with regard to themorphological and mechanical parameters. The number of independent measurements for each kelp part are the same as described in Table 1. For the morphology, this analysis revealed no significant difference between the seasons (P > 0.05, and therefore not detailed here), except for the blade width for which the seasonal dependency is significant (F1, 37 = 17,954, P < 0.001). In fact, the mean blade width is 259 mm ± 105 mm for the February data set and 491 mm ± 167 mm for the September data set. Some of the mechanical parameters appear to vary with the season (Fig. 3). The tensile and bending Young's moduli E and B, and the shear modulus in torsion G do not vary significantly (P > 0.05) neither along the stipe (Sb, Sm and St) nor as a function of the season. Along the stipe, E, B and G correspond to approximately 2.5 MPa, 7 MPa and 1 MPa, respectively, confirming the values reported in Table 2. However, Fig. 3a and b suggest a variation of E and B for both blade parts as a function of the season. This variation is significant for E at the blade top (9.72 MPa ± 1.27 MPa in February against 13.56 MPa ± 0.82 MPa in September, F1, 36 = 6.436, P = 0.016), and potentially for E at the blade bottom (2.79 MPa ± 0.66 MPa in February against 9.92 MPa ± 0.43 MPa in September, F1, 36 = 82.152, P < 0.001) although the normality and equal variance tests failed for the latter (P < 0.05). Regardless of the potential seasonal variation, Fig. 3a and b indicate that E and B are smaller at the blade bottom than at the blade top. As suggested in Table 1, Fig. 3d shows that the density of the kelps is slightly larger than the density of water and relatively constant along the kelp except for the density of the blade bottom (*Bb*) which is corresponds roughly to the density of water. Note also that the scatter of the measured density increases for the upper parts of the kelp (the stipe top *St*, and the blade bottom and top *Bb* and *Bt*).

Within the two-way ANOVA analysis, potential interactions between the factors species ×
season (i.e. season dependency within species and reciprocally) were systematically tested for
all the considered parameters. The only significant variation of the species × season
interaction was found for the tensile Young's modulus E of the blade bottom (F1, 34 = 8.735,
P = 0.006). A post-hoc analysis confirmed the season dependency within each species group
(t = 7.426 and P < 0.001 for *L. digitata*, and t = 6.593 and P < 0.001 for *L. hyperborea*), and
suggests significant differences between species for the summer period (t = 4.83 and P <
0.001). For *L. digitata*, the mean values of the tensile Young's modulus E of the blade bottom
were 3.02 MPa and 7.83 MPa for February and September, respectively, while for *L.
hyperborea* the mean values were 2.56 MPa and 12.0 MPa for February and September,
respectively. As stated previously, the normality and equal variance tests were not met for this
parameter (P < 0.05) so that these results cannot be regarded as conclusive. However, the
analysis of the different mean values suggests that the supposed species × season interaction
does not affect the dependence on the tensile Young's modulus E of the blade bottom to the
season factor. Instead, it suggests that the season dependency is more pronounced for *L.
hyperborea*. The significant seasonal variations previously reported were independent of the
factor species for all the other morphological and mechanical parameters characterized.

Table 3. Detailed samples size as a function of the angle category (column to the right) and
the season and species factors (top row). For each species, the sample sizes are also given in
bracket as a percentage of all the samples collected for that species.

	Total	Feb.	Sept.	*L. digitata*	*L. hyperborea*	Undefined
Angle > 90°	**8**	8	0	6 (18%)	2 (25%)	0
Angle = 90°	**14**	6	8	10 (30%)	1 (13%)	3
Angle < 90°	**22**	7	15	17 (52%)	5 (62%)	0
Total	**44**	**21**	**23**	**33**	**8**	**3**

Three different groups categorise the angle of attachment between the blade base and the
stipe (Fig. 1), and the analysis of the dependency of this angle as a function of the
species/season factors is investigated separately (Table 3). As the different species were not

recognizable during collection, the total amount of *L. digitata* collected in February and in September is different from the collected amount of *L. hyperborea*. The number of kelp collected as a function of the species and angle categories is reported in the columns 4 and 5 of Table 3 together with the percentage relative to the total amount of kelp collected for a given species (in brackets). For the two different species, the counts distribution has a similar pattern. More than 50% of the kelps collected had an angle <90° (52% for *L. digitata* and 62% for *L. hyperborea*) with the rest being distributed over the two other angle categories. Thus, for all the mechanical and morphological parameters considered in this study, there is no evidence of a species-specific phenotypic trait, suggesting that the two species can be pooled together to describe further potential seasonal variations.
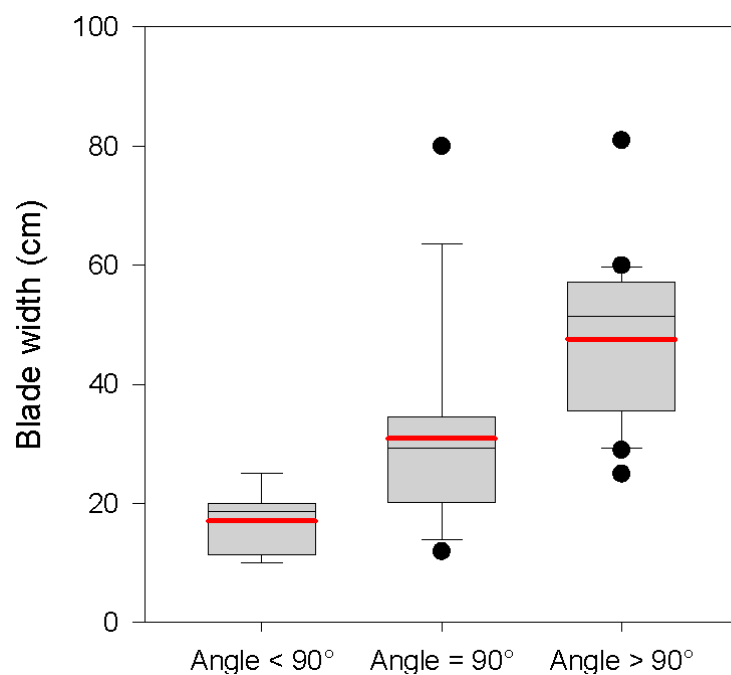

Fig. 4 Relation between the blade width (linked to the growth phase) and the different angle categories. For a given box, the lines in the box represent the median (black) and the mean (red) while the box edges define the upper and lower quartiles. The error bars define the 10th and 90th percentiles and the dots indicate the data points falling outside of that range.

For the kelp sampled in February (column 2 in Table 3), a similar number is found in each angle sub-category. In September (column 3 in Table 3), however, 65% of the kelps had an angle <90°, 35% had an angle approx. 90° and none had an angle >90°. Thus, the angle between the blade base and the stipe appears to be influenced by the season. Fig. 4 deepens

this analysis by characterizing the link between the angle and the blade width, the blade width being considered as an indicator of the growth phase of the blade (see e.g. (Sjøtun et al., 1998; Buck and Buchholz, 2005; Klochkova et al., 2012). The angle at which the blade is attached to the stipe appears to be inversely correlated to the blade width since it decreases as the blade width increases. All the blades with a large angle have a smaller width (17.1 ± 5.2 cm), while the blades with an angle < 90° are characterized by a wider range of larger blade width (47.6 ± 13.6 cm). The blades with an angle = 90° occupy an intermediate position with a typical width around 31.0 ± 16.8 cm.

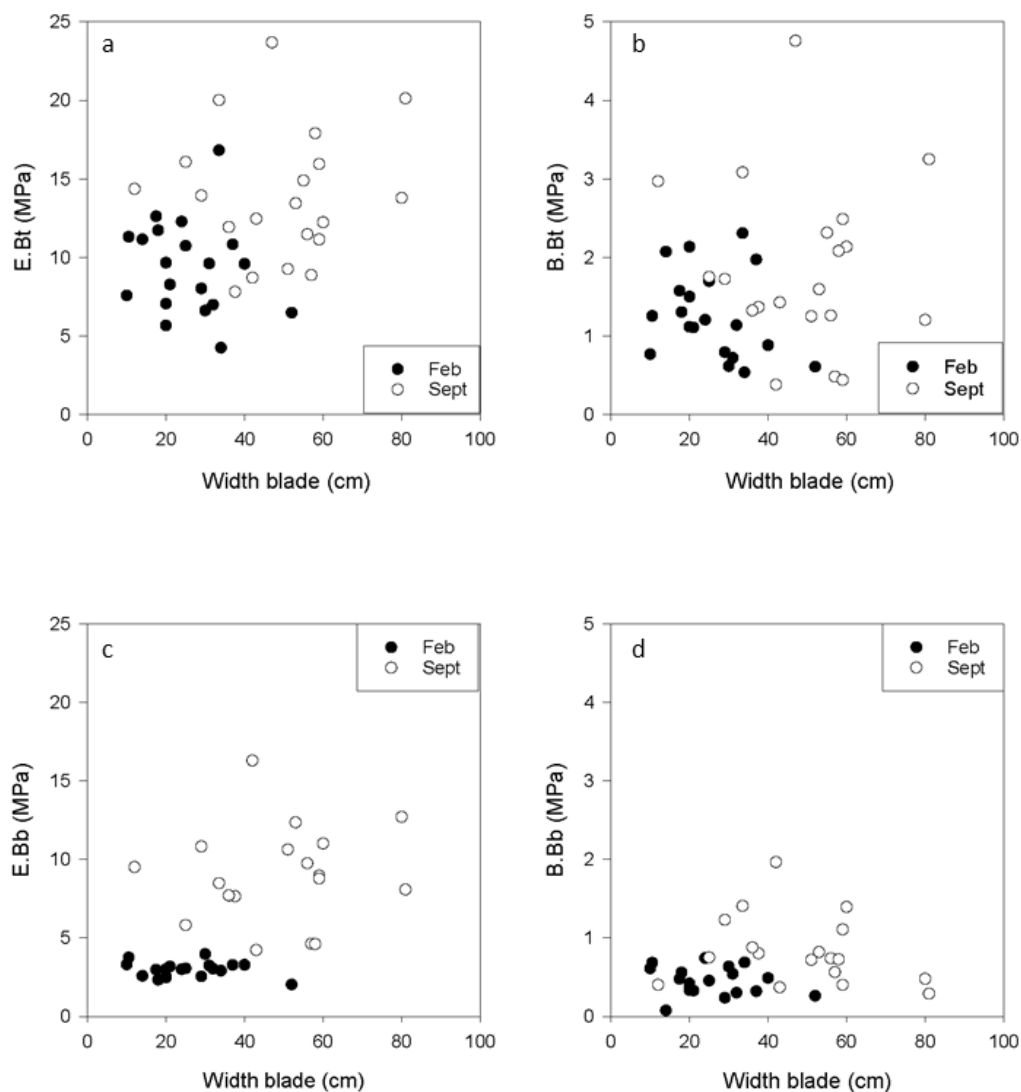**Fig. 5** Scatter plots of the tensile and bending Young's moduli (*E* and *B*) of the blade top (*Bt*) and blade bottom (*Bb*) against the parameter '*Width blade*'. The black/white dots correspond to the kelps collected in February/September.

The seasonal dependency of the mechanical parameters described previously (Fig. 3) was further investigated using the blade width as an indicator of the growth phase of the blades (Fig. 5). These results tend to agree with the general increase of the tensile and bending Young's moduli for the top and the bottom of the blade, as well as the increase of the average blade width between February and September (Fig. 3). Most importantly, the spreading of the tensile and bending Young's moduli of the blade bottom (*E.Bb* and *B. Bb*) appear to be limited and constant around the mean value in February than in September, indicating that these two moduli are independent from the blade width in in February. For the tensile and bending Young's moduli of the bottom of the blades (*E.Bb* and *B. Bb*) collected in September and for the tensile and bending Young's moduli of the top of the blades (*E.Bt* and *B.Bt*) collected in February and September, there is no clear correlation with the width of the blade.

DISCUSSION

Interspecific and geographic variability - The general morphology of the sampled kelps agrees well with the typical morphology of these two species (see e.g. Sjøtun and Fredriksen (1995); Harder et al. (2006)). The blade-to-stipe length ratio of the kelp described in this study is slightly larger than 1, which is in accordance with Sjøtun and Fredriksen (1995) on L. hyperborea from wave-exposed sites. Although the site of collection is not exposed to offshore waves, the observed blade digitation of the kelps confirms the importance of the wave tearing action (Koehl et al., 2008). Traditionally, *L. digitata* is known to have a shorter, narrower and more flexible stipe in comparison to *L. hyperborea* (Lamouroux, 1813; Guiry, 2001). The stipe cross-section is also typically oval for *L. digitata* and round for *L. hyperborea* (Lamouroux, 1813; Guiry, 2001). The stipes of the *L. digitata* were slightly more oval than the ones of *L. hyperborea*, but the difference was not significant. As the average diameter of the bottom of the stipe appeared to be the only significant interspecific morphological difference, it can be concluded that the two species present a very similar

morphological phenotype, which may result from the adaptation to the same environmental conditions. Within the family Laminariaceae, the great phenotypic (morphologic) plasticity resulting from the adaptation to local environmental factors is known to make the species identification based on morphological criteria rather uncertain (Kain, 1979; McDevit and Saunders, 2009). Another difference usually observed between the two species is that *L. digitata* is present on rather sheltered sites while *L. hyperborea* dominates the fully exposed sites (Lüning et al., 1990; Harder et al., 2006), thus explaining why *L. digitata* was originally thought to dominate the inner parts of the Trondheimsfjord (Lund, 2014). As 20% of the kelps belong to the *L. hyperborea* species, this study and the work from Lund (2014) show that geographical distribution cannot be a criterion to distinguish *L. hyperborea* and *L. digitata*. The fjord steep bathymetry leads to a narrow distribution width of the kelp belts, which suggests that a strong genetic differentiation process among a same species is taking place along the steep Norwegian coastline (Robuchon et al., 2014). This may have also contributed to a local convergence of the phenotypes. Other varieties of L. hyperborea, such as *Laminaria hyperborea f. cucullate*, have been reported in wave sheltered areas in Scandinavia (Athanasiadis, 1996), while Sundene (1958) characterized a phenotype of *L. digitata* in the Trondheimsfjord which would closely describe a phenotype traditionally attributed to *L. hyperborea*. By conducting a transplantation experiment where specimens of *L. digitata* were moved from their normal habitat, Sundene (1964) confirmed the capacity of adaptation of the kelps to a sudden change of wave exposure by rapidly growing wider and digitated blade in exposed sites.

From a mechanical point of view, the phenotype of the two species is also very similar as no significant interspecific difference was detected over the full blade or stipe of the kelps (Table 2). This varies from the conclusions of Harder et al. (2006), who found some significant differences between some *L. digitata* and *L. hyperborea* collected near a

breakwater in Helgoland, Germany (fully-exposed site). Harder et al. (2006) also showed that these species typically had a changing elastic behavior (two different tensile Young's moduli) at low/high strain. In the present study, the maximum nominal strain during the tension tests was kept lower, about 0.1 for the stipe samples and 0.2 for the blade samples (ratio of the maximum deformation over the tested sample length). Similarly, the maximum torsional deflection was 120°. Thus, the tensile Young's moduli and torsional shear moduli characterized correspond to low strain/stress conditions. For these conditions, the tensile and bending Young's moduli and the shear modulus in torsion characterized by Harder et al. (2006) for the stipes of *L. digitata* were 13.2 ± 8.6 MPa, 83.8 ± 84.1 MPa and 0.7 ± 0.5 MPa for E, B and G, respectively; while these moduli for *L. hyperborea* were 28.2 ± 10.5 MPa, 109.4 ± 54.9 MPa, and 9.7 ± 4.8 MPa for E, B and G, respectively. Although the tensile and bending Young's moduli (E and B) are 1–1.5 order of magnitude larger than in this study, Harder et al. (2006) also found that the bending Young's modulus B is in general higher than the tensile Young's modulus E. On the other hand, Paul et al. (2014) characterized the stipe bending Young's modulus of some *L. digitata* from a wave-sheltered tidal inlet at the entrance of the Trondheimsfjord. Although this site is close to the one of the present study, the hydrodynamic conditions were significantly different. The bending modulus of the stipes was found to be 28.67 ± 13.22 MPa, which falls in the range defined by the results described here and by Harder et al. (2006). The mean kelp density described by Paul et al. (2014) is similar to the values detailed in the present work.

The differences between the moduli may be affected by the different strain rates used in the different studies. Harder et al. (2006) reported strain rates between 0.1 and 0.4 min-1, while this information was not given by Paul et al. (2014). In the current study, a strain rate of about 0.07 min-1 was applied in tension (for the stipe and blade samples) and a flexural strain rate of about 0.42 min-1 was generated during the 3-point bending tests of the stipes. In

general, the use of higher strain rates generates a higher Young's modulus, as reported by Niklas (1992) on parenchymous tissues. However, the effect of the strain rate variation between a tension test and a flexural test remains unexplored on algae tissues. The tensile Young's moduli and the shear modulus in torsion moduli of the of *L. digitata* and the *L. hyperborea* are close to the values of the moduli reported by Harder et al. (2006) for *L. digitata*. The bending Young's moduli of *L. digitata* and *L. hyperborea*, however, are significantly lower than the ones reported by Harder et al. (2006), suggesting a greater flexibility of the algal tissues found in this study. Harder et al. (2006) showed that the bending Young's moduli of the two species was higher than their tensile Young's moduli, while the present study suggests the opposite. Extending this comparison to another species within the same genus, Vettori and Nikora (2017) found that values for the tensile and bending Young's moduli were similar for *L. saccharina* (approaching isotropy). These results suggest that the degree of mechanical anisotropy in kelp tissue is varying significantly from one site to another, as well as from species to species, thus calling for further investigations.

For the organism to survive, algae tissues must be stronger than the forces they experience. Thus, some species favor the strengthening of their tissues (e.g. Martone (2007)), while other species favor more flexibility to enable their reconfiguration under hydrodynamic forcing (e.g. Martone et al. (2012)). Although exposed to smaller waves or current intensities than in the two other studies, the Laminaria from the studied site emerge occasionally from the water when the tidal amplitude is sufficiently large. This suggests that the species found here may have adapted to a regular exposure to broken waves by increasing their flexibility, thus increasing their survival (Demes et al., 2013).

Seasonal variability – Seasonal growth patterns in Laminariacae are fairly well documented and growth cycles have been shown to follow day length variations (Dieck, 1991; Lüning and Kadel, 1993; Schaffelke and Lüning, 1994) and may reflect seasonal

fluctuations in nutrient availability (Gagné et al., 1982; Davison et al., 1984). In return, these cycles are known to affect the chemical constitution of the Kelp through the year (Black, 1948; Sjøtun et al., 1996; Abdullah and Fredriksen, 2004). The morphological characterization of the kelps collected in this study showed that only the blade width and the angle of attachment between the stipe and the blade are varying significantly in time. This result is consistent with the fact that kelp species adopt a basal growth strategy, and new tissues are produced during the growing season at the blade base, from the meristem (Kogame and Kawai, 1996). The most rapid growth usually takes place from March until May (in the Northern hemisphere) and the old blade is torn away from April and replaced by a new blade that has grown out since January (Kain, 1979). Thus, the blade size increases during the growing season and remains stable over the summer and autumn seasons (Sjøtun et al., 1998). For this reason, the blade width is directly correlated to the growth of the blade and is thus an indicator of the blade maturity. This was for example characterized for *L. saccharina* by Buck and Buchholz (2005). Klochkova et al. (2012) also found that, after the stage of linear growth of the blades of *L. digitata* and *L. appressirhiza*, the subdivisions of the blades (digitates) were spreading radially, thus widening the blade width and affecting the angle between the blade and the stipe. Here, the angle of attachment between the blade and the stipe is correlated to the width of the blade, thus linked to the growth phase of the blade (Fig. 4).

The impact of seasonal variations on the kelp and vegetation mechanics has been little investigated (see e.g. Krumhansl et al. (2015)). As presented in Fig. 3d, no seasonal variations are observed for the density of the different kelp parts. Density adjustments are vital to kelp biology and physiology (Schiel and Foster, 2006) and it is thus important for the kelps to maintain the buoyancy constant through the year and the growth phases to ensure an optimum position in calm conditions. This conclusion is, however, different for the other mechanical parameters. As described in the result section and presented in Fig. 3, a seasonal variation is

observed only for the tensile and bending Young's moduli of the blade top and bottom. Since the thickness of the blade top and the blade bottom were found constant throughout the year, this implies that the blade material is more flexible and extensible in February than in September (i.e. lower flexural rigidity, see Henry (2014)). The variations appear to be stronger at the blade base than at the top, which indicates that the young blade tissues growing from the base of the blade at the beginning of the growth season are more flexible and elastic than the rest of the blade. This conclusion agrees with the fact that older tissues are less flexible and extensible than younger tissues in red algae and kelps species, as shown by Krumhansl et al. (2015). In addition, Fig. 5 suggest that the mechanical properties of the young blade tissues are relatively constant from one kelp to another, while the aging process brings more variability from a mechanical point of view. As a variability of the tissue mechanical properties has consequences on the survival of the kelp (Demes et al., 2013), the positioning of the younger/older tissues on the blade may have a major consequence on the capacity of the kelp to survive high mechanical stress from its environment. For *L. digitata* and *L. hyperborea*, this impact is even more important as the youngest tissues are located at the meristem, which transfers the forces experienced by the blade onto the stipe. The results from the characterization of the angle of attachment of the blade showed additionally that the shape of the meristem varies through the growing season. This suggests that the flexibility of the meristem, connecting the blade and the stipe, is strongly affected by the growth phase of the blade. As the meristem is a key structural organ transferring the flow-induced drag stresses from the blade to the stipe attached to the sea bottom, this may have a major impact on the mechanical behavior of the kelp in its environment.

__Variability along the kelp structure -__ This study highlights that flexural rigidity variations between the different kelp parts are not only due to changes in the shape and cross-sections of the vegetation (Boller and Carrington, 2006), but are also induced by the modulation of the

apparent mechanical properties along the kelp, and more precisely between the stipe and the blade (Fig. 3). The blade material is significantly more flexible (lower bending Young's modulus B) and less elastic in tension (higher tensile Young's modulus E) than the material of the stipe. These lower B values contribute to a more flexible blade, favoring undulations and exchange processes at the blade scale (Huang et al., 2011), while higher E values improve the stiffness properties of the blade. As discussed in the previous paragraph, the blade base is more flexible and elastic than the rest of the blade as the new blade tissues are growing from the meristem. When these tissues are ageing and shifting towards the blade top, the blade top becomes less flexible and extensible than the blade base (Krumhansl et al., 2015). The modulation of the mechanical properties along the kelp is a result of the variation of the spatial distributions of tissues differing in their function and mechanical properties (Niklas, 1992; Charrier et al., 2012). The tensile Young's modulus of the blade base (Bb) towards the beginning of the growing season (February) appears to be the same as the tensile Young's modulus from the stipe top (St) which suggest a continuity of the rigidity between the tissue structure of the young blade and stipe (Sahoo and Seckbach, 2015). However this is not observed for the bending Young's modulus of the young blade material ($B.Bb$ in February). The mean density of the different kelp parts is varying between the seawater density and 1150 kg/m3, so essentially neutrally buoyant, with an increased scatter towards the upper parts of the kelp (blade and upper part of the blade). This increased scatter may be the result of the change of cell characteristics in older tissues and can be influenced by an increased occurrence of epibionts (organisms living on the surface of the blade) on older tissues.

General implications on hydrodynamics and survival strategy - As for terrestrial plants, aquatic vegetation changes its characteristics during the year to accomplish vital functions such as reproduction and tissue renewal (see Niklas (1992)). In the context of marine macroalgae like *L. digiata* and *L. hyperborea*, this study showed that such vital changes imply

wider blades in summer and more flexible tissues at the base of the blade. As these two parameters influence both the projected area of the algae and its capacity to streamline under increased flow speed (see e.g. de Bettignies et al. (2013)), which control the drag forces experienced by the vegetation, the mechanical response to a same hydrodynamic event (i.e. storm) will vary depending on the season. Reciprocally, as the drag forces are expected to vary, the capacity that kelp belts have to dissipate the energy contained in the flow (by reduced mean flow velocities or dampening waves) is expected to be affected, with a potential influence on the ecosystem services provided (Smale et al., 2013). However, these processes remain mostly unknown and are difficult to quantify, so more research is needed in this direction.

When the hydraulic condition changes or when a species colonizes a new site with different hydraulic conditions, a plastic response of the vegetation structural properties is triggered for the organism to survive to the new environmental constrain (Niklas, 1992), which in the case of *L. digiata* and *L. hyperborea*, may lead to significant changes in morphology/mechanics, such that it becomes difficult to identify different species. For example, Buck and Buchholz (2005) showed for *L. saccharina* that drag forces were considerably higher on algae from sheltered areas (with ruffled margin and wider blade) than on flat and narrow *L. saccharina* farmed in exposed locations despite comparable blade areas. However, such successful adaptations to a local hydrodynamic stress factor may be at the expense of one of its vital function, such as reproduction Puijalon et al. (2008).

CONCLUSION

The present study investigates aquatic vegetation biomechanics and its implications on the physical environment. More precisely, this work is the first attempt to describe and identify the causes of similarity and variability of mechanical and morphological properties of two related marine species from the same site. The two species *L. digitata* and *L. hyperborea* were characterized by very similar morphological and mechanical properties. These two species usually have two very distinct phenotypes, suggesting that the absence of interspecific variability for the surveyed site is a result of an adaptation process to the same environmental conditions (phenotypic plasticity). For both species, morphological and mechanical parameters of the blades are characterized by a seasonal variation (blade width and shape angle, tensile and bending Young's moduli). These variations appeared to be a consequence of the growth activity and ageing of the blade tissue, but no such seasonal variation was observed for any of the parameters of the stipes. For both species, the mechanics of the tissue varies significantly between the blade and the stipe, ensuring the different structural functions of the stipe and blade in the wave-swept environment. Such seasonal changes and variations along the vegetation structure are also observed in fresh water environments (Miler et al., 2014). Thus, this study confirms and highlights that the collection and analysis of biomechanical and morphological datasets is a prerequisite to improve the general understanding of the interaction between aquatic vegetation and its environment. In fact, site-specific and seasonal mechanical variability may play a significant role in aquatic environments and this variability should be considered in numerical or physical representation of aquatic vegetation in addition to, and in complement to potential differences between species.

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