Structural integrity of *Ellisolandia elongata* reef: a mechanical approach to compare tensile strengths in natural and controlled environments

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Abstract

Geniculate coralline algae are oases of biodiversity, providing nursery areas and shelter for the species that live among their fronds.

The key of their success in the intertidal is the ability to withstand hydrodynamic forces. Under culturing conditions most of the physical and ecological stressors such as intense hydrodynamic forces and grazing are extremely reduced, thus affecting species mechanical properties and their response to external threats.

The aim of the present study is to investigate tensile mechanical properties of *Ellisolandia elongata* cluster of fronds from natural (sheltered and exposed reef) and culturing conditions (after one month of culturing). The tensile test showed that the first failure stress (σ_I) was not significantly different between the natural and culturing conditions indicating that the two reefs were characterized by the same distribution of pre-existing, inherent structural flaws. Interestingly the σ_{max} (maximum stress before rupture) was significantly different between the two conditions, with the culturing condition being more resistant to average load compared to the natural conditions. The maximum stress before rupture (σ_{max}) showed the influence of the environment in reducing strength and elasticity of the fronds.

Introduction

In the marine realm, intertidal environments present some of the most demanding conditions on the planet: large temperature fluctuation, desiccation, exposure to solar radiation, waves and currents (Morris and Taylor, 1983; Larcher, 2003; Raffaelli and Hawkins 2012). Despite these physical limits, intertidal environments host diverse and productive assemblages of organisms, mainly dominated by algae. The success of seaweeds in this mechanically very demanding environment is due to the strength in their attachment to the substrates and the ability to reduce hydrodynamic forces by either passively bending or changing shape and size when subject to flow (Vogel, 1994; Harder et at., 2004).

Wave swept habitats are susceptible to physical disturbance which results in major changes of their community structure and diversity. While active rapid adaptive processes to flow conditions are common in animals, algae and plants have to rely on passive means to cope with various flow regimes (Harder et al., 2004). Thus, the various structural units of a plant body have to be flexible enough to allow rapid adjustments to the shape of the organism (Vogel, 1984). The overall morphology of intertidal algae subsequently is adapted to survive in flow dominated habitats, thereby hosting a rich assemblage of associated organisms.

In the Mediterranean Sea, both geniculate and non-geniculate coralline algae create intertidal underwater architectures which include the association of *Lithophyllum cystosirae* (former *Lithophyllum papillosum* var. cystosirae (Hauck) Lemoine and *Polysiphonia* spp., the 'encorbellement' of *Lithophyllum byssoides* (Lamarck) Foslie (Laborel et al., 1994) and *Lithophyllum tortuosum* (Esper) Foslie, concretions of *Neogoniolithon brassica-florida* (Harvey) Setchell & L.R.Mason and the 'bourrelet' or 'corniche' of *Ellisolandia elongata* (J. Ellis & Solander) K.R.Hind & G.W. Saunders (Laborel et al., 1994; Nannini et al., 2015).

Approximately 100 million years ago crustose coralline algae developed flexible joint (genicula) which are primary responsible for bending in flowing water (Aguirre et al., 2010). This evolutionary step was fundamental for some of the rigid calcified algae since flexibility is essential to survive in exposed rocky shores with intense hydrodynamic forces. Some genera such as *Calliarthron* proved to have a near optimal morphology achieved by having the basal genicula longer and more resistant than the apical ones which maximize bending and minimize amplification of stress contributing to the survival of the fronds under breaking waves (Martone et al., 2010; Martone and Denny, 2008a). This strategy has been successful and allows erect coralline algae to be the dominant competitors for space in the intertidal zone at many wave-exposed sites around the globe (Denny et al., 2013).

E. elongata (Rhodophyta, order Corallinales, family Corallinaceae) is a geniculate (i.e. articulated) alga, originating from a crustose base with flexible feather-like fronds (up to 200 mm long). Fronds, which typically branch in one plane, are characterized by dense and simple lateral pinnate branchlets separated by inconspicuous gaps resulting from narrow branch-angles combined with short intergenicula in the main axes (Brodie et al., 2013). In the Mediterranean Sea, species' distribution range from the North-West Mediterranean Sea (from Southern coast to the Spain to Greece) to the South-East Mediterranean Sea (Cabioch et al., 1992) (from Lebanon to Algeria, with the highest concentration in Tunisia) (Bressan and Babbini, 2003). By favouring life in highly exposed sites, *E. elongata* represent a 'model' species being characterized by distinct morphological and mechanical properties that, like other articulated coralline algae, maximise flexibility and reduce the risk of breakage.

This coralline alga creates an important carbonate structure, hereinafter termed as 'reef', which comprises the physical structure provided by the algae but also the structural organization of the community itself, the composition and relative proportions of the hosted species (Hiscock, 2014).

E. elongata 'reef' is a physical structure which is essential in maintaining species richness and influencing ecosystem processes; it provides microhabitats and refuges from predation, including grazing, and protection from adverse conditions such as current and waves.

In the last decade, there has been an increase in long term culturing experiments, mainly due to the threat of climate change. However, under culturing conditions most of the physical challenges such as intense hydrodynamic forces, grazing, abrading sediments and air exposure are extremely reduced, and their potential in influencing the growth of organisms is not extensively considered. These physical and ecological stressors can cause damage to the organisms through cuts, holes and scars in the thallus (De Bettignies et al., 2012) making it more prone to structural failure and crack propagation leading to loss in structural integrity. In this context, mechanical properties are a key point for understanding species response to environmental forces, and need to be taken into consideration during the lab experiments.

The aim of the present study is to investigate tensile mechanical properties of *E. elongata* reefs grown under natural (sheltered and exposed sites) and culturing conditions. Differently from previous studies that considered the mechanical properties of a single frond, our approach was to investigate macroscopic tensile strength of cluster of fronds (i.e. simulating the frond clusters composing the reef) in order to understand how structural properties of the geniculate algae could potentially affect the reef structure. In detail, the objectives of the present study were 1) to design a new experimental set-up for testing tensile strength by simulating natural environmental forces (e.g. waves) experienced by *E. elongata* reef; 2) to estimate fundamental quantities as tensile stress and elastic modulus of *E. elongata* frond clusters living under natural and culturing conditions.

Materials and methods

Sample collection and experimental set-up

Ellisolandia elongata was collected from two different reefs: in April-May 2015 from floating pontoons (site 1) in Santa Teresa bay (44°04′54.3″ N; 9°52′54.5″ E) and in October-November 2015 from a vertical cliff in Palmaria Island (site 2) (44°02′19.3″ N; 9°50′30.3″ E) (Gulf of La Spezia, N-W Mediterranean Sea). In both sites, 16 bushes (5cm x5cm, including base and substratum) of *E. elongata* were collected using hammer and chisel. After both collections, *E. elongata* bushes were put in plastic bags with seawater and brought to the lab using a refrigerated trolley.

While samples collected from both sites in May and November were transported to the lab and the cluster of fronds were directly tested for changes in the mechanical properties (F1 and F2, Table 1), samples collected from both sites in April and October were placed in the experimental system for 1 month (L1 and L2, Table 1). At the end of each experiment, May and November respectively, clusters of fronds were tested for changes in the mechanical properties.

The experimental set-up consisted of a recirculating closed system composed of 4 experimental glass tanks (size: 50 x 35 x 35 cm; capacity: 50 L), a fibreglass sump (capacity: 170 L) pumping 430 L/h (Pump: NewaJet 2300 L/h, valve CALABER with 4 exits for water distribution) of water in each tank; a chiller BOYU (model: L-075, Voltage: 240 V - 50 Hz, Power: 1/8 HP, Aquarium Size: 80-400 L, Flow Rate: 600-2000 L/h) provided with a NewaJet 3000 L/h pump for temperature control and skimmer created *ad-hoc* for the system (cylinder: ø 5 cm, height: 50 cm; pump Newjet 400 L/h pumping 200 L/h; pump NewaJet 3000 L/h and aerator (Wave Aerator Mouse 54 L/min)).

Each experimental tank was provided with one pump for circulation and wave (Hydor Koralia Circulation & Wave Pump 2200 L/h) and one surface pump (SUNSUN HJ-311 300 L/h). Each aquarium, containing *E. elongata* reef (25 x 25 cm) was exposed to 2 ceiling lights (Radior TS 150 NDL/230V) provided with 2 bulbs (HQI Metal-Halide Lamp; HITLITE 150 W, 10.000 K). Photoperiod and light intensity were kept constant (10:14 dark light cycle; light intensity of 1000 - 1200 µmol s^{-1} m^{-2}) (LI-COR LI-250A Light Meter).

Seawater was collected weekly in the bay next to the lab by using an industrial pump from the mussel farm Headquarter (Cooperativa Mitilicoltori Spezzini, IT) and transported in the laboratory by using 20 L and 30 L tanks. Once in the lab the water was processed using Mechanical (0.1 µm) and UV filters (Vecton V2 600). Renewal rate was 50% of water *per* week in the entire system (200 L/week) allowing salinity and nutrients to follow the seasonal trend of natural conditions (see Table 2a, b). Temperatures in the system were set according to in-field temperature (Table 2) in the Gulf of La Spezia (March- April 2015: min- max= 13-15 °C; end of September-October 2015 min-max= 20-24 °C; frequency of collection: one-day campaigns with 4H PocketFerry Box- JENA engineering Gmbh, temperature probe SBE 45).

Environmental variables in the laboratory were measured daily: pH (Mettler Toledo SevenGo pH meter with electrode Mettler Toledo inLAB® 413 SG/2m), salinity (Hach HQ30d Flexi + Hach

Conductivity Probe), Oxigen (Hach HQ30d Flexi + Hach LDO Probe), temperature (Hanna HI 935005 K-Thermocouple Thermometer). Nutrients were randomly sampled weekly and measured by means of the auto-analyser (3 Bran+ Lu Ebbe).

Differences in environmental parameters (pH, temperature, salinity) for both experiments (April and October) were analysed by using ANOVA (Underwood, 1997). The Student Newman Keuls test (SNK) was performed *a posteriori* whenever a significant difference was found. Prior to analysis, a Cochran's C test was employed to assess the homogeneity of variance. These statistical analyses have been performed by using Statistica 8®.

Sample preparation

E. elongata fronds were detached from their natural bases in order to remove the ‘substrate effect’ since different substrates can determine different strength of the reef (Madin, 2005). For the algae cultured in the lab, only fronds that grew more than 1 cm were used for the tensile tests. A total of 400 fronds (40 samples, 10 fronds each) have been tested in this experiment. In order to determine the mean diameter of the algae populations, a total of 170 fronds were photographed with a USB stereo-microscope (Dyno-Lite) and measured (5 replicates for each frond) with ImageJ ® software. Chi–square test and Gaussian-fit were used to assess the normal distribution of thallus diameters. Each sample consisted of 10 fronds of the same length mounted between two empty aluminum cylinders, with a base of epoxy resin each (HoldFast, USA) (Fig. 1). The aluminum cylinders aimed to ensure a proper mechanical coupling to the testing machine. All of the fronds composing each sample were oriented in the same direction: the distal and proximal parts of fronds were inserted into the cylinders and held with a cyanoacrylate gel-type glue (Loctite SuperGlue, Henkel, USA). In order to avoid any damage due to frond deterioration, samples were prepared and tested in few days, and kept in the aquaria before being tested. The length of each sample (L_{ength} = internal distance between the two cylinders used for the tensile tests) was measured (i.e. three replicated measures for each sample) by using a 0.05 mm resolution caliper.

Experimental apparatus and procedure

A mechanical setup (Fig. 1) was designed (by using Autodesk Inventor Professional 2015) and built to coupling samples to MTS electro-hydraulic machine used for the tensile tests in the thermo-mechanical Research Laboratories at ENEA-Faenza.

The testing machine consisted mainly of a 100 kN two-column frame, a 5000/500 N strain-gauge load-cell and a 200 mm stroke piston, whose displacements were measured by high-sensitivity inductive-type transducer. All sensors are periodically calibrated so that the metrological traceability of force and displacement outputs is guaranteed according to international standards. Because of the sample characteristics (very low forces to be applied the tensile test), the load cell accuracy was preventively and successfully verified in the range up to 15 N by means of a proper set of calibrated masses (Fig. 2). Piston speed of a tensile test is directly connected to the duration of the test itself, i.e. to the time necessary to pull to break the sample. This duration was actually an unknown parameter, so it was set under the following hypothesis: in natural environment, the frond clusters composing the reef will be exposed to several fatigue cycles until a ‘critical wave’ will cause the rupture. Due to the limit to measure the real wave period of such ‘critical wave’, this period has been estimated by using the mean wave period in the study area. All wave parameters were extracted from time-series data (from 1989 to 2001) provided by the altimeter wave buoy closest to sampling sites, where *E. elongata* were sampled (Fig. 3). The mean wave period calculated from the time series was approximately 4 s. Thus, piston speed for the tensile test was set according to the criterion that the sample should be pulled to break under the mean wave period experienced in natural conditions. From a preliminary test, we estimated that in order to break the samples under this condition, the mean piston speed needed to be equal to 0.5 mm/s. Once the piston speed was determined, the tests were performed automatically by acquiring the signals of time [s], load [N] and displacement [mm] by means of the proper software that manages the testing machine. Data acquisition was carried out with a frequency of 500 samples/s. Data were successively elaborated by a custom-made software (LabVIEW ®).

Physical quantities measured by the tensile test

Pull-to-break tensile test generated three main parameters: the first failure stress (σ_I), the maximum stress before rupture (σ_{max}) and the modulus of elasticity (*E*). The modulus of elasticity was calculated using the best estimate of the strain compatibly with the testing conditions (ΔL_{ength} / L_{ength}, were ΔL_{ength} was the displacement measured while pulling the sample and L_{ength} was the initial length of the sample). A typical stress *vs* strain diagram obtained during this experimental test (Fig. 4) shows the zone of linearity selected for the calculation of *E* by linear regression and the stress at the first failure of the sample. Mean stress failures were calculated by taking into account the mean values measured for the two forces (first and maximum failures) and resistant sections. The dispersion of measured values was used to estimate the standard combined uncertainty associated with the analyzed parameters, as indicated by the current standards on the uncertainty evaluation (JCGM 100:2008 - Evaluation of measurement data – Guide to the expression of uncertainty in measurement).

Results

Preparatory phase

In order to determine the cross section of the samples, it was assumed that the section of the thallus of each frond was circular. The total resistance of the section of each sample was given by the sum of the resistance sections of each frond (total number of fronds per cluster = 10). The diameter of the frond thallus was determined by an a-priori characterization (as mean diameter) of the original algal population from which the fronds under test were sampled (Table 1). The overall thallus diameter resulted 0.51±0.01 mm and 0.58±0.01 mm (mean ± s.e.m.) for group F_1 (Field, first sampling) and L_1 (Lab, first sampling) fronds; values of 0.54±0.01 mm and 0.56±0.02 mm were similarly obtained for group F_2 and L_2 fronds. The experimental distribution of thallus diameter values was verified to be reasonably comparable to a normal distribution by means of both Chi-square test (positive outcome) and Gaussian fit (values of the coefficient of determination R^2 approximately equal to 1), thus excluding systematic bias due to both samples and measurement processes. In figure 5, experimental distributions of the stem diameters are shown for group F_1 and L_1 algae. Furthermore, mean value of each sample “L_{ength}” was measured and obtained values were 2.14±0.62 cm and 1.61±0.37 cm (mean ± s.d.) for group F_1 and L_1 samples, respectively; values of 1.46±0.12 cm and 0.89±0.38 cm (mean ± s.d.) were similarly obtained for group F_2 and L_2 samples.

Experiment

The comparison between groups F_1, F_2 and L_1, L_2 fronds was performed by analysing three mechanical parameters, whose average values were determined experimentally by means of pull-to-break tests: i) the tensile stress at first failure (σ_I), ii) the maximum stress before rupture (σ_{max}) and iii) the estimated elastic (or Young’s) modulus (E).

For σ_I, values of 2.7±0.4 MPa and 3.4±0.4 MPa (mean ± s.e.m.) were measured for group F_1 and L_1 samples, respectively. The overlapping of the uncertainty bars suggests that F_1 and L_1 fronds do not show any significant difference on the stress in correspondence of the first failure (Figure 6a). For σ_{max}, values of 3.4±0.5 MPa and 5.4±0.5 MPa (mean ± s.e.m.) were measured for group F_1 and L_1 samples, respectively; in this case, the difference between the two mean values seems to be significant as indicated by the lacking of overlap between the uncertainty bars. The same conclusion can be drawn for the mean values measured for the E quantity (35±6 MPa and 48±7 MPa (mean ± s.e.m.) for group F_1 and L_1 samples, respectively).

In order to assess the differences among mean values of F_1 and L_1 parameters, a two-tailed t-test was performed. The first failure stress did not show any significant difference of the sample means; differently, both the maximum stress before rupture and the estimated elastic modulus shown significant differences of the sample means (Table 3).

The results from the reef collected in October and November, even if based on a less significant statistical basis, confirm this trend: L_2 samples showed more performing values of mechanical parameters than F_2 ones. In particular, for σ_I, values of 1.6±0.3 MPa and 2.8±0.9 MPa (mean ± s.e.m.) were measured for group F_2 and L_2 samples; for σ_{max}, values of 2.1±0.4 MPa and 3.5±1.0 MPa (mean ± s.e.m.) were measured for group F_2 and L_2 samples; finally, for *E*, values of 17±4 MPa and 36±12 MPa were measured for group F_2 and L_2 samples, respectively.

The comparison among groups (F_1, L_1 and F_2, L_2) revealed that L/F ratios of all mechanical parameters (First failure stress (σ_I), Max stress before rupture (σ_{max},), elastic modulus (*E*)) were comparable (Tab. 4). The overlapping of uncertainty bars suggests that reefs tested in both sites, although characterized by some differences, maintain the same intrinsic contents for what concerns mechanical properties (Figure 6b).

Environmental data of the system (pH, temperature and salinity) during the experiments (Months: April and October) revealed differences between months for temperature (Two-way ANOVA: $F_1 = 182.8$, $p < 0.01$) and salinity ($F_1 = 230.96$, $p < 0.01$). No differences were found among tanks within each month and for the combination of month*tank.

Discussion

Growth reactions as an adaptation or response to physical loads are widespread in plants, typically taking place on a time scale of hours, days or even years (Wainwright et al., 1976; Ennos, 1999). *E. elongata* living in habitats dominated by high flow velocities may have adaptive mechanisms involving growth reactions that maximise flexibility and reduce the risk of breakage. Most of the experiments in the laboratory (Martin and Gattuso, 2009; Form and Riebesell, 2012; Ragazzola et al., 2012; Ragazzola et al., 2013; Kato et al., 2014; Nannini et al., 2015) used pumps to recreate water motions however the algae are not exposed to the full range of oscillatory motion with changes in forces due to different waves heights and periods that natural populations would experience and important for mechanical studies. High flow velocities, grazing and abrading sediment are very difficult to be recreated in the aquaria during the experiments, however it's important to determine the growth reaction and their structural properties. Our experiment showed that *E. elongata* reefs growing in the lab and growing in the field withstand mechanical stress in slightly different ways.

First failure stress (σ_I) proved to be not significantly different meaning that probably the clusters of fronds coming from the two reefs are characterized by the same distribution of pre-existing, inherent structural flaws. A possible explanation is that even if the samples growing in the lab were cultured in a controlled environment, without any mechanical stress, this wasn't sufficient to change

σ_I showing that the overall reef structure has more weight than the environment for the point of breakage.

Flexible thalli bend, reorient and move with the flow by making the species able to withstand under wave action and bioerosion, while maintaining a structurally and functionally complex habitat. The maximum stress before rupture (σ_{max}) shows the fundamental role played by the environment. The σ_{max} is significantly different between the two groups, with the cultured cluster of fronds being more resistant to average load compared to the clusters from the natural reefs. Studies from Mach and coauthors (Mach et al., 2007) showed the importance of notches (cracks or different type of discontinuities) in reducing strength. The stress in the material at the crack tip exceeds the applied stress in the entire thallus. In this case, the breakage can happen even if the applied force is not considered to be sufficient to cause the breakage. While in the natural environment we have conditions that can damage the algae, in the laboratory all these conditions are buffered. Together with the crack, another factor that could possibly influence the σ_{max} is the rupture of the genicula. The genicula don’t usually break abruptly (Martone, 2007) but the cell frayed sequentially with increasing force. The culturing condition could have modified the speed of the rupture. The tensile moduli (E) of samples group F_1, F_2 decrease in respect to those group L_1, L_2 implying an increased flexibility and reduce tissue stress under culturing conditions (Martone and Denny, 2008b).

The different exposure of the reefs (sheltered and exposed sites) and the different stage of algal development do not seem to affect the mechanical properties of the fronds cluster. In both sites (sheltered and exposed) analysed in May and November respectively, the lack of physical and ecological stressors under culturing conditions are the key factors in determining the difference in flexibility and tissue stresses in the cluster of fronds. Further experiments need to be performed in order to confirm our preliminary observations and investigate the reef forming algae though the entire life cycle in different exposed environments.

Previous bio-mechanical analyses on corallinales have been focusing on single fronds, with particular emphasis to the genicula (Telewiski et al., 1986) in order to elucidate the mechanisms behind the resistance to breaking waves and other forces (Martone and Denny, 2008b). Martone showed the mitigating effect of neighbouring fronds on breakage and within dense stands, streamlining of individuals probably plays a minor role, as neighbouring fronds may interact and thus form a drag-reducing aerodynamic unit with higher wind velocities (Harder et al., 2004). Our studies integrate these previous findings by investigating the tensile properties of the cluster instead of the single frond in order to simulate the neighbouring effect within the reef.

Coastal irregular topography produces exceptionally complicated flows which are hard to define (Gaylord, 1999), the fluid trajectories under breaking waves become energetically disorganized due to the degeneration of the waveform. Wave’s velocities in the intertidal routinely exceed 5 m/s

(Gaylord, 1999; Koehl, 1982, 1984) but the level of variation in velocity through a wave change substantially with time. All the information we have regarding flow data on the intertidal refers to temporal variation of velocity and acceleration in one single point in space, therefore we still do not have information on the overall spatial structure of the flow fields under breaking waves. In our experiment, we simulated the stress conditions experienced by the algae in natural environment by inducing the breakage in a temporal frame comparable to the mean wave period recorded in the Gulf of La Spezia.

In this study, we used a single application of force, equivalent to a single wave rushing past an alga. A previous study (Mach et al., 2007) highlighted that single application of force might predict lower rates of breakage and dislodgment than those actually observed. Repeated loadings imposed by waves and cracks in the algae thallus could break/dislodge them even when individual forces are not sufficient to cause complete fracture. However, even if the absolute number of the applied force leading to the breakage should be interpreted with caution, we proved that the culturing set up have an influence on the structural integrity of the organism.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

FR, GR, and CL designed the study, did experimental work and drafted the manuscript; GR did statistical analyses; PF and MS did all mechanical tests and revised the manuscript; MF and MN did the experimental work and sample preparation; all authors gave final approval for publication.

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Tables and captions

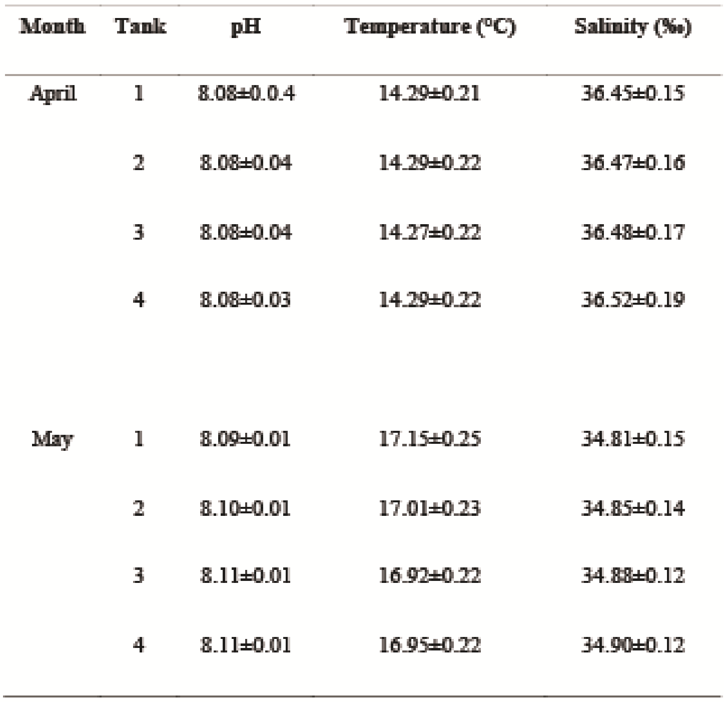
Table 1. Table summarizing the characteristic of the samples at the different site. Site 1: Santa Teresa bay (44°04′54.3″ N; 9°52′54.5″ E); site 2: Palmaria Island (44°02′19.3″ N; 9°50′30.3″ E).

Sample group: L_1 and L_2 are the samples used in one-month experiment in the Laboratory, while F_1 and F_2 refers to the samples collected in the Field and directly tested for the material properties without prior culturing. Number of fronds per sample: 10. Sample length and frond diameter shown as mean ± s.d and mean ± s.e.m., respectively.

Sampling site	**Month**	**Sample group**	**Number of samples**	**Sample length (cm)**	**Frond diameter (mm)**
1	April	L_1	15	2.14 ±0.62	0.51±0.01
	May	F_1	15	1.61±0.37	0.58±0.01
2	October	L_2	5	1.46±0.12	0.54±0.01
	November	F_2	5	0.89±0.38	0.56±0.02

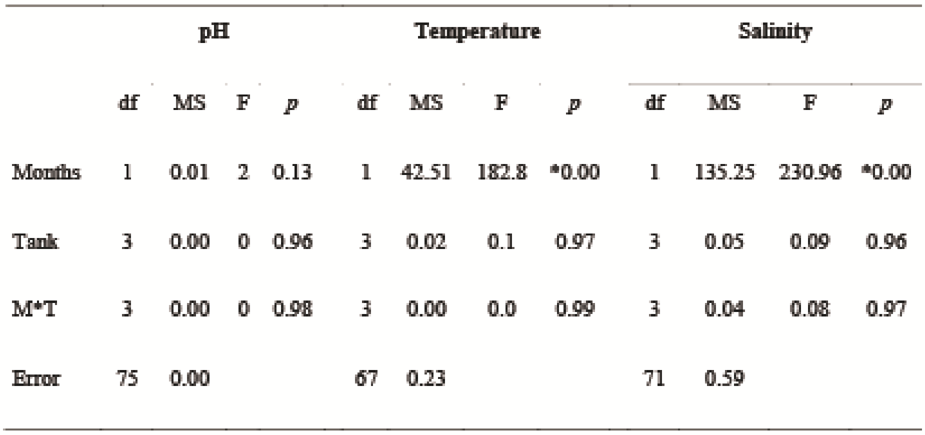
Table 2.a, Environmental parameters of the experimental system. PH, temperature and salinity in the experimental setup (April and October). Data (mean ± s.e.m.) are reported *per* tank. **b**, Nutrients monitored in the experimental system. NO_3, PO_4, $Si(OH)_4$ and NO_2 (mean ± s.e.m.) in the experimental treatments for April and October, respectively

a



Month	Tank	pH	Temperature (°C)	Salinity (‰)
April	1	8.08±0.0.4	14.29±0.21	36.45±0.15
	2	8.08±0.04	14.29±0.22	36.47±0.16
	3	8.08±0.04	14.27±0.22	36.48±0.17
	4	8.08±0.03	14.29±0.22	36.52±0.19
May	1	8.09±0.01	17.15±0.25	34.81±0.15
	2	8.10±0.01	17.01±0.23	34.85±0.14
	3	8.11±0.01	16.92±0.22	34.88±0.12
	4	8.11±0.01	16.95±0.22	34.90±0.12

b



	pH				Temperature				Salinity			
	df	MS	F	*p*	df	MS	F	*p*	df	MS	F	*p*
Months	1	0.01	2	0.13	1	42.51	182.8	*0.00	1	135.25	230.96	*0.00
Tank	3	0.00	0	0.96	3	0.02	0.1	0.97	3	0.05	0.09	0.96
M*T	3	0.00	0	0.98	3	0.00	0.0	0.99	3	0.04	0.08	0.97
Error	75	0.00			67	0.23			71	0.59		

Table 3. Two-tailed t-test results between the mean values of F_1 and L_1 parameters (Site1, May 2015). Significance level: 0.05 - F_1 and L_1 populations considered as independent - null hypothesis: mean (F_1) = mean (L_1).

Parameter under test	**Null hypothesis**	**d.o.f.**	**Student's $\|t\|$**	**p**	**Results**
First failure stress (σ_I)	$\sigma_{I\_F} = \sigma_{I\_L}$	28	1.697	0.101	null hypothesis: **accepted**
Max stress before rupture (σ_{max})	$\sigma_{max\_F} = \sigma_{max\_L}$	28	3.916	< 0.001	null hypothesis: **rejected**
Elastic modulus (E)	$E_F = E_L$	28	2.609	0.014	null hypothesis: **rejected**

Table 4. Ratio L/F for mechanical parameters (first failure stress (σ_I), max stress before rupture (σ_{max},), elastic modulus (E)) of *Ellisolandia elongata* reefs estimated by means of tensile tests for Site 1 (May) and Site 2 (November), respectively. u_r (L/F): relative standard uncertainty, u (L/F): absolute standard uncertainty.

	σ_I			**σ_{max}**			**E**		
	L/F	**u_r(L/F) (%)**	**u(L/F)**	**L/F**	**u_r (L/F)(%)**	**u(L/F)**	**L/F**	**u_r (L/F) (%)**	**u(L/F)**
Site 1	1.28	18	0.23	1.61	17	0.27	1.37	22	0.30
Site 2	1.79	38	0.68	1.71	33	0.56	2.19	40	0.87

Figures Legend

Figure 1. Views of the experimental apparatus (figures not in scale). First row: design of the mechanical grips used to mount the sample on the testing machine. Second row (from left to right): testing machine, mounted sample (cylinder dimensions are: ϕ_{ext} = 10 mm, ϕ_{int} = 8 mm, height = 15 mm) before and after the test.

Figure 2. A) Metrological tests with a load-cell of 5 kN verified in the range of 15 N force. B) Calibration curve: mean difference of load-cell from the reference values was approximately of 4%.

Figure 3. a, Positions of F_1 and F_2 (arrowed) sites and of the buoy (circle) in the Gulf of La Spezia (Coordinates: 43° 55' 41.99" N, 9° 49' 36.01" E).**b,** Distribution of the mean wave period.

Figure 4. Example of a stress *vs* strain diagram obtained performing a tensile test. The two sliders identify the zone of linearity selected for the calculation of *E* by linear regression. The cross pointer identifies the stress at the first failure of the sample.

Figure 5. Experimental distribution of the stem diameters (mm) for group F_1 and L_1 algae (Site 1, May 2015).

Figure 6. a, Mechanical parameters (first failure stress (σ_I), max stress before rupture (σ_{max},), elastic modulus (*E*)) of *Ellisolandia elongata* reefs estimated by means of tensile tests (Error bars = s.e.m). F_1 and L_1: site 1, May 2015, n =15. F_2 and L_2: Site 2, November 2015, n = 5. **b**, Ratios L/F of all mechanical parameters (first failure stress (σ_I), max stress before rupture (σ_{max},), elastic modulus (*E*)) measured for site 1 (May 2015, n =15) and 2 (November 2015, n=5), respectively (error bars = s.e.m).

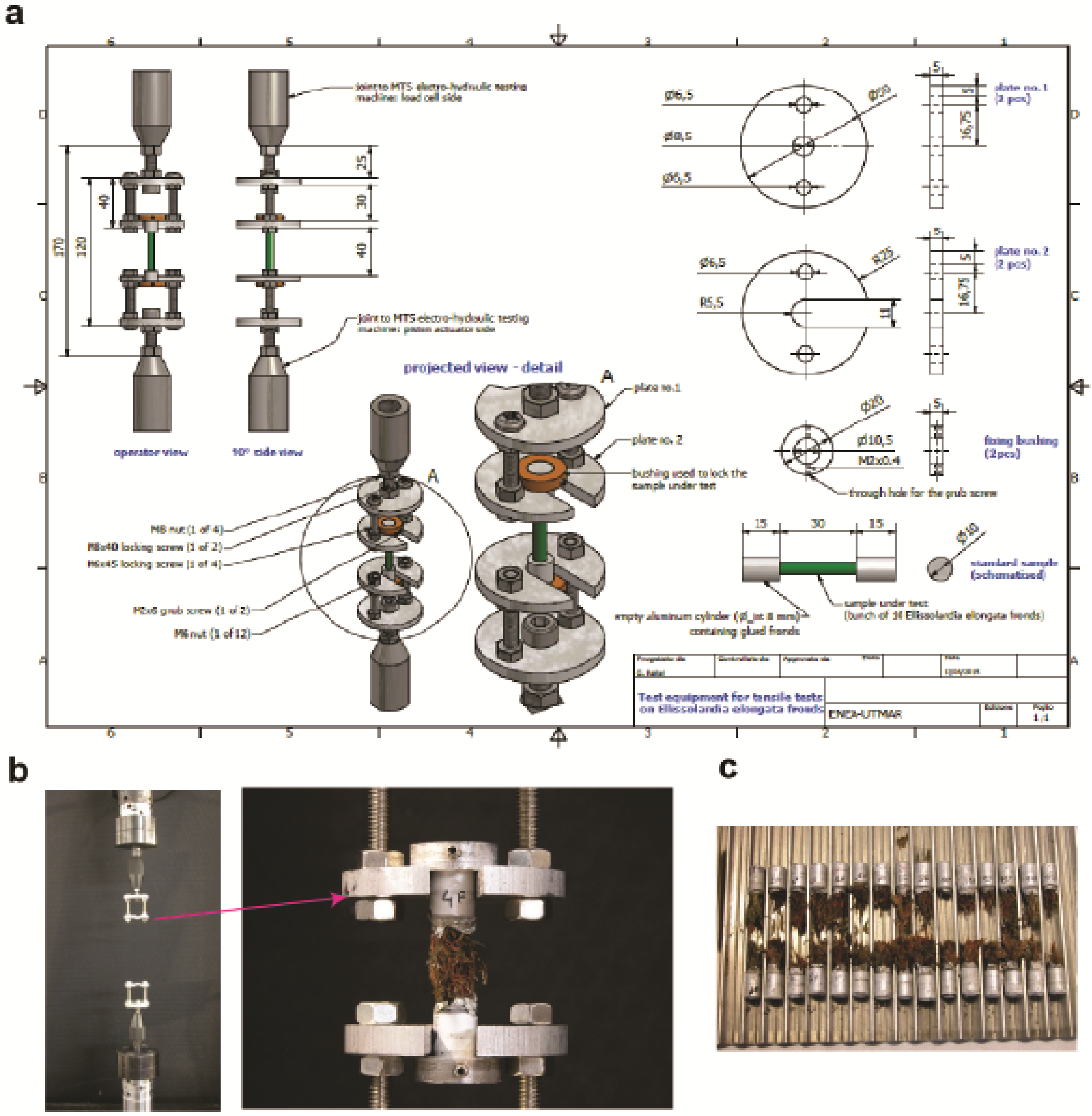

a
joint to MTS electro-hydraulic testing machine: load cell side
170
120
40
25
30
40
joint to MTS electro-hydraulic testing machine: piston actuator side
operator view
90° side view
projected view - detail
A
M8 nut (1 of 4)
M8x40 locking screw (1 of 2)
M6x45 locking screw (1 of 4)
M2x6 grub screw (1 of 2)
M6 nut (1 of 12)
plate no.1
plate no. 2
bushing used to lock the sample under test
ø6,5
ø8,5
ø6,5
ø25
5
16,75
plate no. 1 (2 pcs)
ø6,5
R5,5
R25
11
plate no. 2 (2 pcs)
ø20
ø10,5
M2x0.4
fixing bushing (2pcs)
through hole for the grub screw
15
30
15
ø10
standard sample (schematized)
empty aluminum cylinder (ø_int 8 mm) containing glued fronds
sample under test (bunch of 10 Ellissolardia elongata fronds)
Test equipment for tensile tests on Ellissolardia elongata fronds
ENEA-UTMAR
1/1
b
4F
c

Fig 1

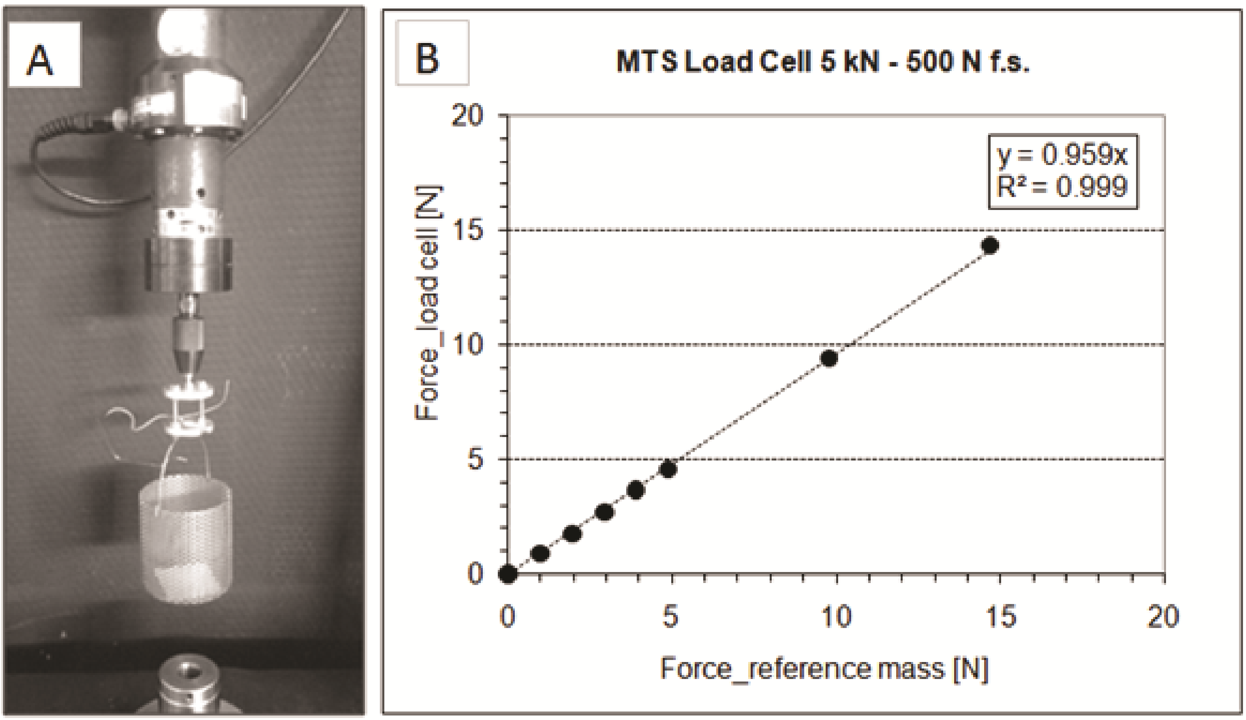

A
B
MTS Load Cell 5 kN - 500 N f.s.
y = 0.959x
R² = 0.999
Force_load cell [N]
Force_reference mass [N]
0
5
10
15
20

Fig 2

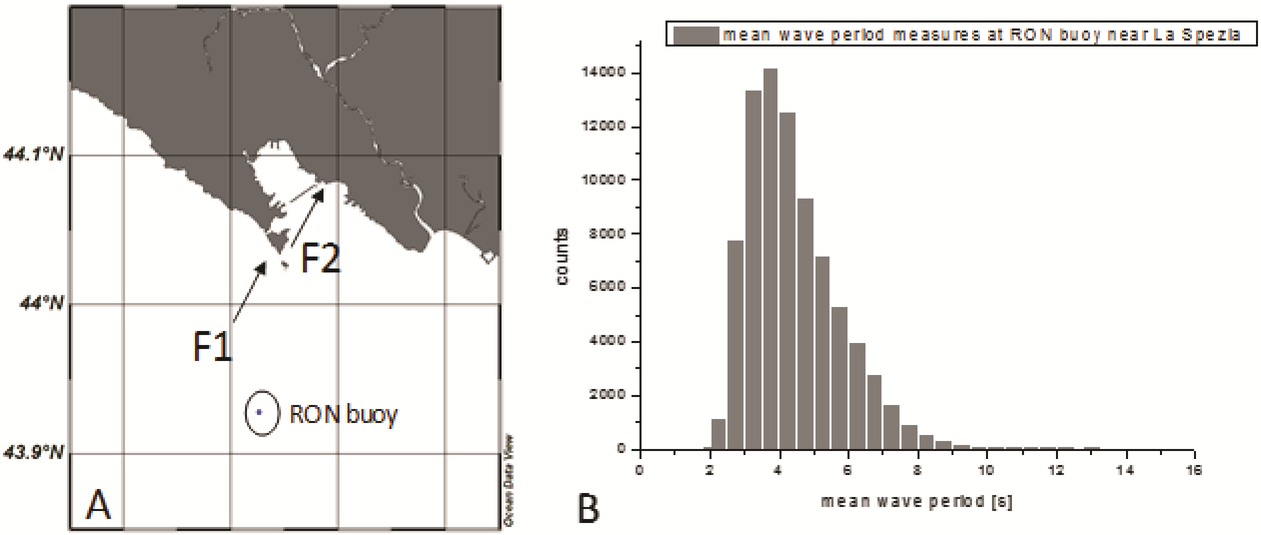

A
44.1°N
44°N
43.9°N
9.7°E
9.8°E
9.9°E
10°E
F1
F2
RON buoy
Ocean Data View
B
mean wave period measures at RON buoy near La Spezia
counts
mean wave period [s]
0
2000
4000
6000
8000
10000
12000
14000
0
2
4
6
8
10
12
14
16

Fig 3

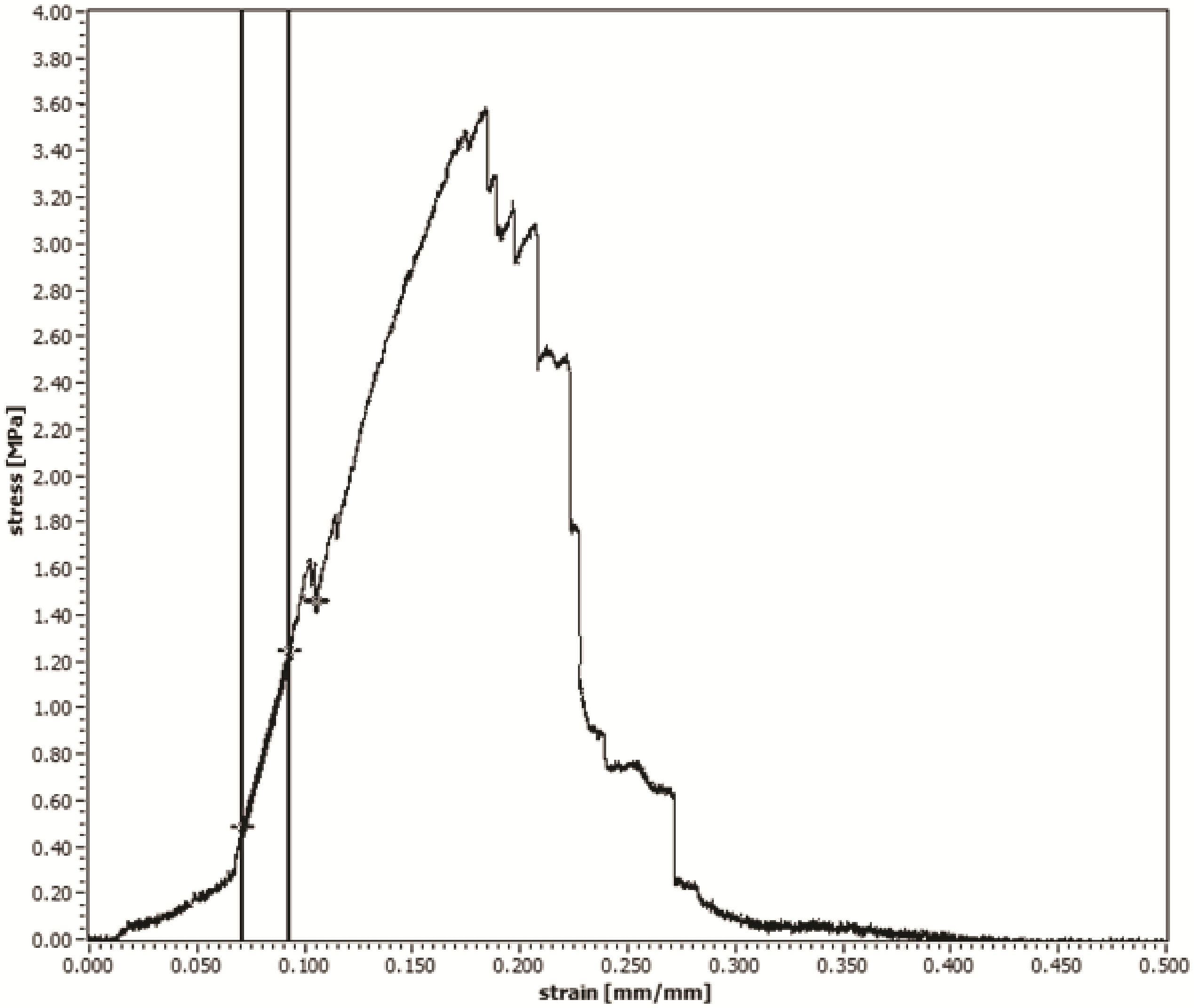
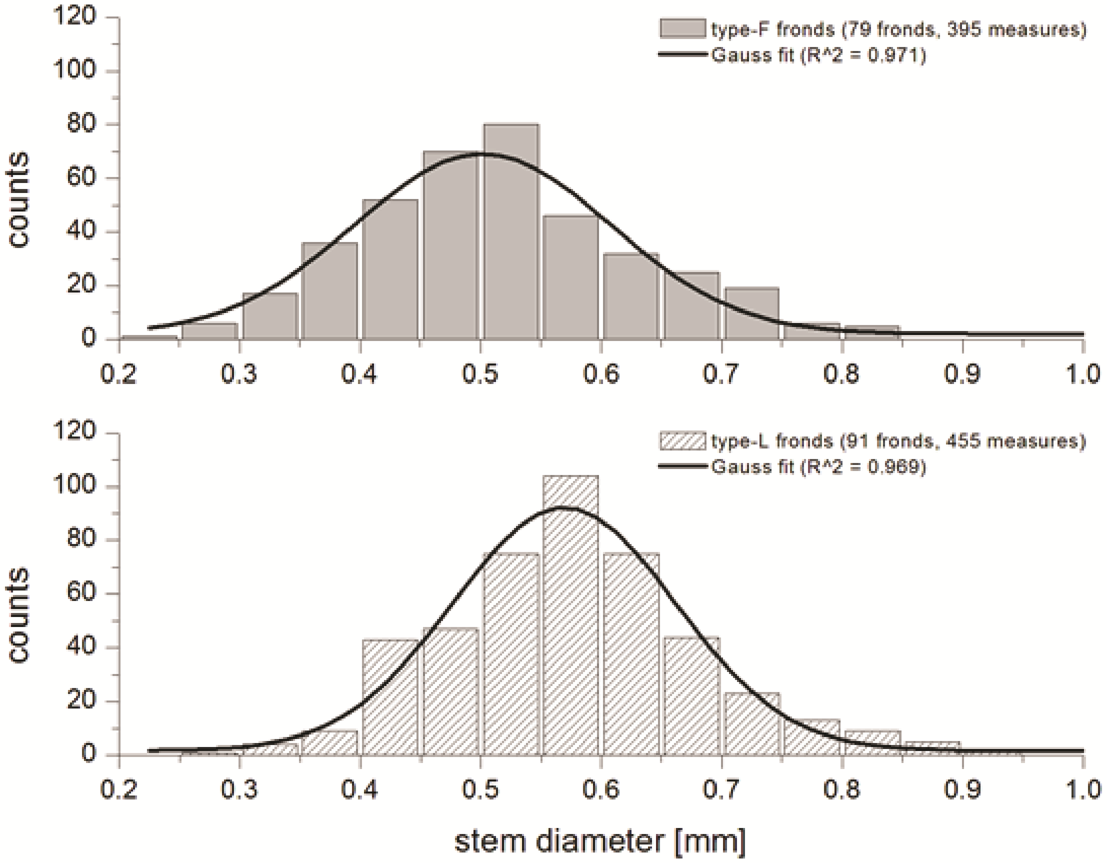

4.00
3.80
3.60
3.40
3.20
3.00
2.80
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
stress [MPa]
0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
0.400
0.450
0.500
strain [mm/mm]

Fig 4



120
100
80
60
40
20
0
counts
type-F fronds (79 fronds, 395 measures)
Gauss fit (R^2 = 0.971)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
type-L fronds (91 fronds, 455 measures)
Gauss fit (R^2 = 0.969)
stem diameter [mm]

Fig 5