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Skeletal mechanical properties of Mediterranean corals along a wide latitudinal gradient

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Abstract This is the first study exploring skeletal mechanical properties in temperate corals. The variation of skeletal mechanical properties with distance from the aboral pole, coral age, and population was investigated in the corals *Balanophyllia europaea* (zooxanthellate) and *Leptopsammia pruvoti* (non-zooxanthellate), collected at three sites along a wide latitudinal gradient. Mechanical properties were measured by nanoindentation, a technique applied here in detail for the first time to a scleractinian. In both species, a reduction of Young's modulus was observed toward the oral pole, which is the youngest part of the skeleton. Skeletons of *B. europaea* increased their Young's modulus with age, unlike *L. pruvoti*. Only the zooxanthellate species showed reduced Young's modulus in southern populations, coherently with the observed

reduced skeletal bulk density and porosity with SST (decreasing latitude) in *B. europaea*, and with the lack of correlations with SST and latitude for skeletal bulk density and porosity in *L. pruvoti*. These results support previous hypotheses on differences in the skeletal mechanical properties of these two coral species in relation to the observed variations of skeletal bulk density and porosity with temperature/latitude, which may have consequences related to the envisaged seawater warming of next decades.

Keywords Dendrophylliidae · Elastic deformation · Global warming · Plastic deformation · Scleractinia · Skeletal resistance

Introduction

Sea surface temperature (SST) is one of the main factors that determine coral reef development (Kleypas et al. 1999), and it strictly depends on latitude (Kain 1989). The rate of climate change is accelerating, and the average surface temperature of the oceans is likely to increase by 1.1–6.4 °C during the current century (Solomon et al. 2007). Marine and terrestrial biodiversity and ecosystems are responding through variations in distribution, abundance, phenology, structure, and functioning (Stenseth 2002; Helmuth et al. 2006; Parmesan 2006). The magnitude of temperature change is expected to be greater in temperate areas than in tropical ones (Solomon et al. 2007). In the Mediterranean Sea, the current rate of warming averages 0.04 °C per yr (Díaz-Almela et al. 2007), twice than in the global ocean (Vargas-Yáñez et al. 2007). Recently, the coralligenous community, which is one of the most diverse in the Mediterranean Sea (~1,700 species) (Ballesteros 2006) and where suspension feeders are

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dominant, has been affected by several mass mortality events linked to excessively high temperatures (Cerrano et al. 2000; Perez et al. 2000; Garrabou et al. 2008). Engineer species, including gorgonians and sponges, have been the most affected taxa down to depths of 45 m (Cerrano et al. 2000; Perez et al. 2000; Garrabou et al. 2008). The Mediterranean region experienced large climate shifts in the past (Luterbacher et al. 2006), and it has been identified as one of the most prominent “hot spots” in climate change projections (Giorgi and Lionello 2006).

Environmental parameters such as light and temperature are fundamental for coral survival, but whereas their influence on tropical corals is widely studied, it is not so for temperate regions. In *Astrangia danae* and *Plesiastrea versipora* from temperate sites, calcification rate increases with temperature as occurs in tropical corals, albeit over a colder temperature range (Howe and Marshall 2002). Laboratory observations on calcification rates in *Cladocora caespitosa* and *Oculina patagonica* from the Mediterranean Sea and field observations on the Mediterranean endemic *Balanophyllia europaea* along a temperature gradient indicate negative correlations between temperature and calcification (Rodolfo-Metalpa et al. 2006; Goffredo et al. 2009). In the case of the latter species, the reduction of calcification with increasing temperature (decreasing latitude) results in skeletons which are less dense (Goffredo et al. 2007), due to an increase in skeletal porosity (Caroselli et al. 2011), and especially of pores with larger size (Fantazzini et al. 2013). It has also been hypothesized that this increase in porosity would lower the mechanical resistance of the skeletons (Fantazzini et al. 2013). Even if it is reasonable to expect that lower porosity and/or skeletal bulk density (Bucher et al. 1998) would result in a reduction of skeletal mechanical resistance, the studies on coral skeletal mechanical properties are almost absent in the literature. Another Mediterranean solitary coral, *Leptopsammia pruvoti*, has been studied along the same temperature/latitude gradient, but no effect of temperature on its calcification, skeletal bulk density, or porosity has been detected (Caroselli et al. 2011; Fantazzini et al. 2013).

The present study focuses on these two common scleractinian corals of the Mediterranean Sea: *B. europaea* (Risso, 1826) and *L. pruvoti* Lacaze-Duthiers, 1897, aiming to investigate the variations of coral skeletal mechanical properties. The two species differ for the presence/absence of photosynthetic symbionts, which differentiate the two taxa concerning both their biological processes (metabolism and growth), and the habitat they colonize. Studying the skeletal mechanical properties (Young's modulus and hardness) provides insights into the trend of these properties in relation to the environmental variables and reveals structural differences within and among individuals.

Balanophyllia europaea is a solitary and zooxanthellate (symbiotic with unicellular algae of the genus *Symbiodinium*) scleractinian coral, which is endemic to the Mediterranean Sea, where it colonizes rocky substrates (Zibrowius 1980, 1983). Because of its symbiosis with zooxanthellae, its bathymetric distribution is limited to the illuminated shallow waters, down to 50 m depth. In addition to the aforementioned negative effects of temperature on its calcification, skeletal bulk density, and porosity, also its population structure stability and abundance decrease with increasing SST (decreasing latitude), due to a progressive lack of young individuals (Goffredo et al. 2007, 2008). The negative correlations between SST and many biological parameters generate concern for the future of this endemic species with respect to global warming projections (Goffredo et al. 2008, 2009).

Leptopsammia pruvoti is a solitary, non-zooxanthellate scleractinian that is distributed in the Mediterranean basin and along the European Atlantic coastline, up to UK and Ireland (Zibrowius 1980). It is one of the most common species in sheltered rocky environments, where it colonizes the vaults of caves and crevices between 0 and 70 m deep, reaching densities that exceed 10,000 individuals per square meter (Goffredo et al. 2007). SST and latitude do not significantly influence its skeletal bulk density, porosity, population abundance and structure stability, and calcification rate along a latitudinal gradient spanning 850 km on the western coasts of Italy (Goffredo et al. 2007; Caroselli et al. 2012a, b).

The mechanical properties of the skeleton may be very important for coral survival. For example, skeletal strength may limit the viable coral size, influence the growth form, and determine the range of hydraulic conditions that corals can withstand, influencing the selection of suitable habitats (Chamberlain 1978). This latter aspect is strongly connected with the ongoing global climate change, which is expected to cause an increase in extreme meteorological events (Solomon et al. 2007) which could expose the corals to severe hydrodynamic stress. This is the first study exploring skeletal mechanical properties in temperate corals, with the aim of (1) defining their relationships with the distance from the aboral pole, coral age, and sampling site and (2) detecting differences in mechanical properties related to the decreasing skeletal porosity previously observed with increasing SST (decreasing latitude), thus highlighting possible differences in the mechanical resistance of their skeletons in face of global climate change. Analyzing different skeletal regions and corals of different age was expected to reveal differences related to the life stage of the coral, as most biological parameters of the two species have been found to vary with age, while analyzing different populations was expected to reveal differences related to the thermal regime characterizing the sites,

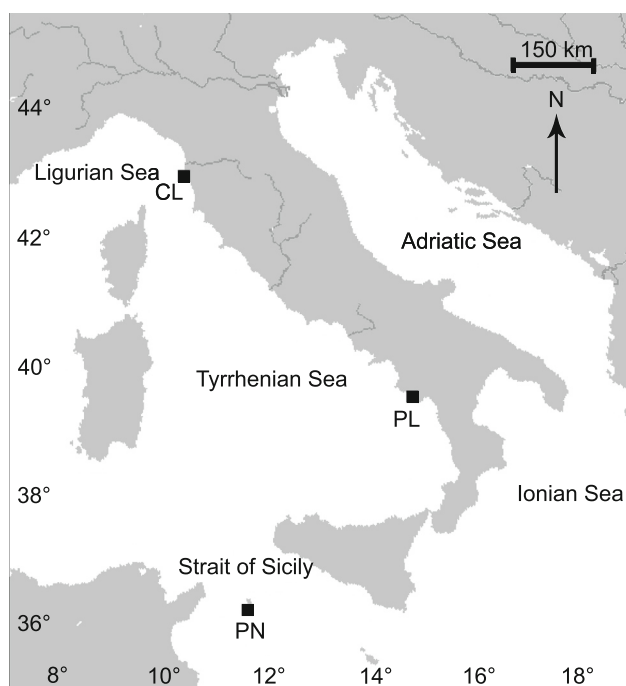


Fig. 1 Map of the Italian coastline indicating the sampling sites. Abbreviations and coordinates of the sites: CL Calafuria, 43°45'N, e 10°35'E; PL Palinuro, 40°03'N, e 15°26'E; PN Pantelleria Island, 36°75'N, e 11°95'E

which has been found to strongly influence biological parameters at least in *B. europaea* (Goffredo et al. 2007, 2008, 2009, 2012; Caroselli et al. 2011).

Materials and methods

Collection of specimens and age determination

Between July 1, 2010 and February 4, 2011, specimens of *B. europaea* and *L. pruvoti* were collected in three sites along a latitudinal temperature gradient from 43°45'N to 36°75'N (Fig. 1). In this study, 65 skeletons were analyzed: 33 specimens of *B. europaea* (11 from Calafuria, 12 from Palinuro, and 10 from Pantelleria) and 32 of *L. pruvoti* (11 from Calafuria, 10 from Palinuro, and 11 from Pantelleria). Corals were randomly collected at a depth of 5–7 m on reefs facing south for *B. europaea* and at 15–18 m on the vaults of caves and crevices for *L. pruvoti*. The sampling was performed at depths known to have high population densities and where the reproductive biology, biometry, growth and population dynamics of both species had been studied previously (Goffredo et al. 2002, 2004, 2005, 2006, 2007, 2008, 2009; Caroselli et al. 2012a, b).

At each site, corals of different size were sampled, to have a minimum of three individuals in each age class (Table 1). The three age classes represented three vital

stages with respect to sexual maturity (4–5 yr for *B. europaea*, 2–3 yr for *L. pruvoti*; Goffredo et al. 2002, 2006): the class of immature (*B. europaea* 0–5 yr; *L. pruvoti* 0–3 yr), mature (*B. europaea* 5–10 yr; *L. pruvoti* 3–6 yr), and old individuals (*B. europaea* >10 yr; *L. pruvoti* >6 yr; Table 1). The age of each sample was estimated from its size by using the growth functions previously obtained for each population (Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b).

Mechanical properties and specimen preparation

Mechanical properties were measured with nanoindentation techniques, which involve the application of a controlled force by an indenter, to produce a local deformation of the surface, with a typical penetration depth of 100–1,000 nm. The instrument used (Table Top Nanoindentation Tester, CSM Instruments, Massachusetts, USA) included a nanoindentation head equipped with a diamond Berkovich indenter, a microscope connected to a digital camera, a motorized X translation table, an anti-vibration table, and the software for control and data analysis. The hardness H and the Young's modulus E were obtained from the analysis of the load–displacement curves according to standard methods (Fig. 2; Oliver and Pharr 2004). The hardness is a measure of the resistance of the material to plastic deformation and is expressed as follows:

$$H = \frac{P_{\max}}{A}$$

where P_{\max} is the maximum applied load and A is the projected contact area between the indenter and the specimen under such load.

The Young's modulus E quantifies the material's stiffness, which is the resistance to reversible (elastic) deformations. This is derived from the initial slope dP/dh of the load–displacement curve during unloading (Fig. 2; Oliver and Pharr 2004).

To prepare the samples for nanoindentation measurements, coral skeletons were submerged in solutions of water and sodium hypochlorite commercial bleach (ratio 100:1) for 3 d, thus dissolving polyp tissue. After being dried at 50 °C for 3–4 d, corals were observed under a binocular microscope to remove fragments of sediment, rock, and encrusting organisms. To perform nanoindentations on coral skeletal surface, preliminary treatments were performed, which involved three processing phases: embedding, cutting, and polishing. Embedding was performed using an impregnating resin at room temperature under rotary pump vacuum (10^{-1} mbar) followed by 24 h hardening at room temperature. Coral skeleton was then cut using a diamond saw along the oral–aboral axis to obtain a longitudinal section that left two skeletal septa exposed,

Table 1 *Balanophyllia europaea* and *Leptopsammia pruvoti*

Pop.	Date of coral collection	Age (yr)								
		Species	<i>n</i>	Mean (SD)	<i>n</i>	Immature samples (SD)	<i>n</i>	Mature samples (SD)	<i>n</i>	Old samples (SD)
CL	February 4, 2011	<i>B. europaea</i>	11	7.0 (4.3)	5	3.7 (1.1)	3	6.6 (1.6)	3	12.9 (2.5)
		<i>L. pruvoti</i>	11	4.5 (1.7)	3	2.3 (0.3)	5	4.7 (1.1)	3	6.3 (0.2)
PL	July 1, 2010	<i>B. europaea</i>	12	9.4 (6.1)	3	4.3 (1.1)	6	7.5 (1.7)	3	18.2 (5.8)
		<i>L. pruvoti</i>	10	5.3 (2.7)	3	2.2 (0.4)	3	5.0 (0.7)	4	7.9 (1.8)
PN	September 8, 2010	<i>B. europaea</i>	10	8.5 (4.2)	3	4.1 (0.7)	3	7.0 (1.6)	4	12.9 (2.1)
		<i>L. pruvoti</i>	11	5.5 (3.1)	3	2.4 (0.5)	4	4.5 (0.8)	4	8.7 (2.4)

Mean age of the samples at each site and in each age class

Population codes: CL Calafuria, PL Palinuro, PN Pantelleria, *n* number of individuals, *SD* standard deviation

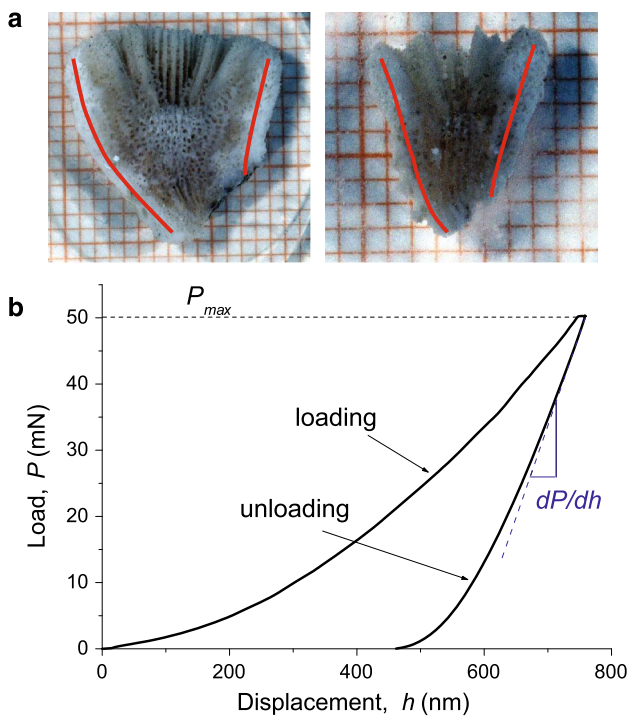


Fig. 2 **a** Pictures of one sample of *Balanophyllia europaea* (left) and one of *Leptopsammia pruvoti* (right) embedded in resin and cut in two. Nanoindentation measurements were taken along the red lines. Quadrats in background are 1 mm wide. **b** Typical load–displacement curve obtained on a *B. europaea* specimen at 50 mN maximum load. The hardness is expressed by the ratio between the maximum applied load (P_{max}) and the projected contact area A between the indenter and the specimen. The Young’s modulus E is derived from the initial slope dP/dh of the load–displacement curve during unloading

one on the right side of the section and one on the left. This section was first polished with glass paper (600 and 1,200 mesh) and finally using an alumina (Al_2O_3) colloidal suspension (3–1, 0.5–0.3, and 0.05 μm grain size). To check for differences in mechanical properties related to the

regions of the skeleton analyzed, the indentations, at a maximum force of 50 mN, were performed in three zones of the septa along the longitudinal section (Fig. 2), defined by dividing the height (oral–aboral axis) of each coral in three (oral zone, middle zone, and aboral zone). Because of data variability, measures were repeated on the right and left exposed septa (five indentations per side per zone), for a total of ten indentations in each zone. Because of irregularities in the samples, such as defects in the surface and problems due to thermal drift, indentations providing clearly erroneous measurements ($\sim 0.5\%$ of all indentations) were removed from the dataset. For this reason, the number of admitted indentations is lower than the number of effectuated indentations.

Statistical analyses

The nonparametric Kruskal–Wallis test was preferred to the analysis of variance to compare mean age among populations, because of the heteroskedastic nature of data. This test does not require normal distribution of the data, it is more reliable than its parametric counterpart in the case of non-Gaussian distribution of the data sample (Potvin and Roff 1993), and it was computed with SPSS 20.0.0.

Mechanical properties (E and H) did not meet the assumptions of normality and homogeneity and were thus analyzed using a permutational multivariate analysis of variance (PERMANOVA), which does not require homogeneity of variance or normal distributions (Anderson et al. 2008). Tests were run using Euclidean distances among samples and 999 permutations in the software Primer[®]. The model included effects of the factors skeletal zone (three levels: aboral, middle, and oral), age class (three levels: immature, mature, and old), population (three levels: Calafuria, Palinuro, and Panarea), and their interactions.

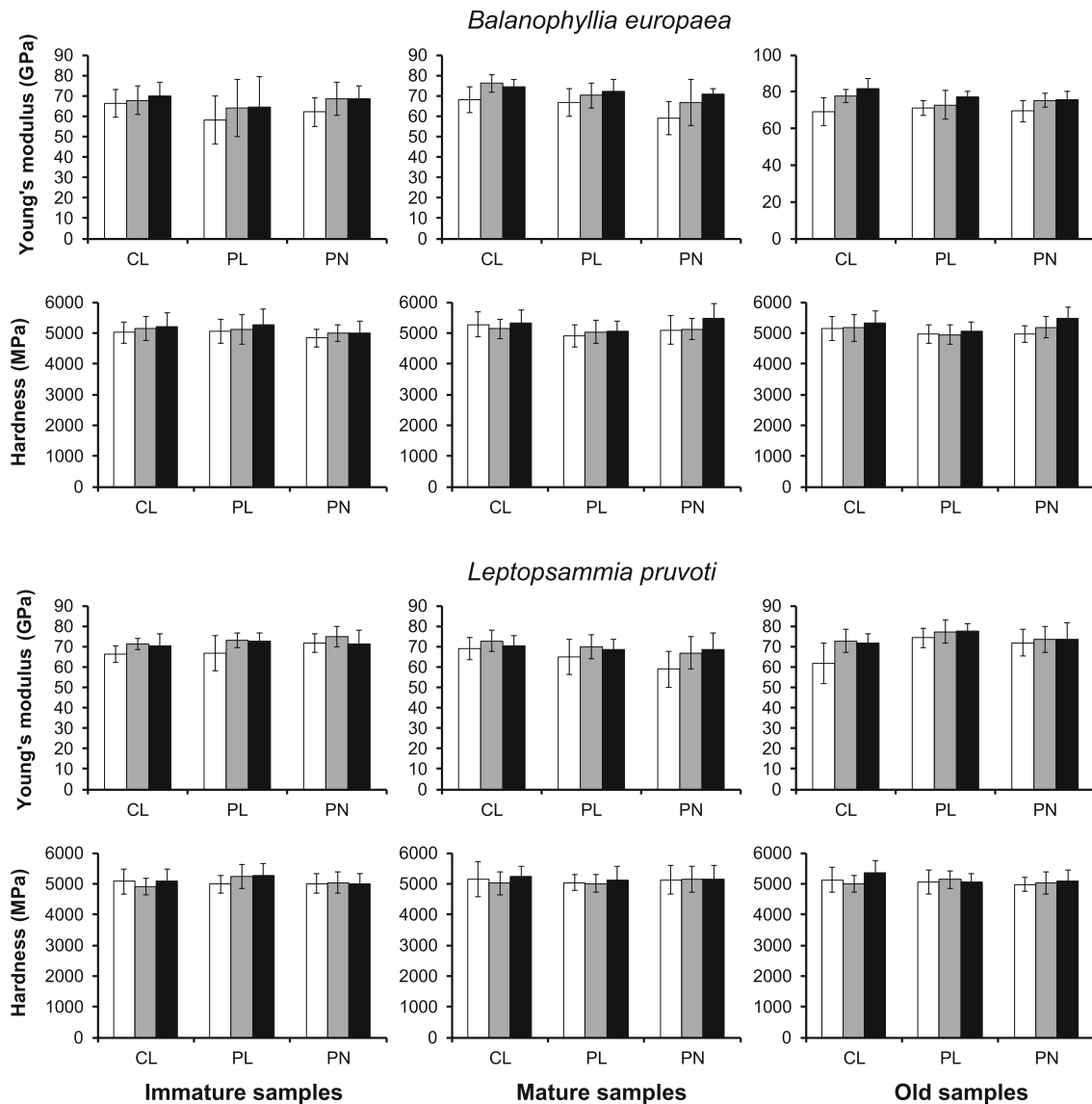


Fig. 3 Mean values of E and H in each coral zone and age class for the two studied species. Population codes: *CL* Calafuria, *PL* Palinuro, *PN* Pantelleria. *White bars* represent the mean values for the oral

zone, *gray bars* for the middle zone, and *black bars* for the aboral zone. *Error bars* represent one standard deviation

Results

Hardness and Young's modulus

Mean E and H values for each skeletal zone and age class, underlining the general means in the three populations and the total mean for the two species, were computed (Fig. 3; details in Tables 2, 3). The study of skeletal mechanical properties was divided into three analyses to assess the hardness and Young's modulus trends according to: (1) the skeletal zone (within the individual), (2) the age class of the individuals (within the population), and (3) the

population (among populations). In the PERMANOVA for the Young's modulus of both species, the interaction term population \times age \times zone was significant (Table 4), and then, the pair-wise comparisons among levels of each factor were computed using this term (Electronic Supplemental Material, ESM 1, 2, and 3 for *B. europaea*; ESM 4, 5, and 6 for *L. pruvoti*). For the hardness of *B. europaea*, the term zone was significant, without interactions (Table 4), and was used for pair-wise comparisons among skeletal zones (ESM 7). Also, the interaction term population \times age was significant (Table 4); then, this term was used for pair-wise comparisons among levels of the factors

Table 2 *Balanophyllia europaea*

Pop.	Zone	<i>n</i>	Immature samples (SD)	<i>n</i>	Mature samples (SD)	<i>n</i>	Old samples (SD)	<i>n</i>	Total zone (SD)
Young's modulus (GPa)									
CL	Oral	49	66.3 (6.9)	29	68.0 (6.2)	30	69.1 (7.3)	108	67.5 (6.9)
	Middle	43	67.8 (7.1)	24	76.0 (4.4)	30	77.7 (3.5)	97	72.9 (7.2)
	Aboral	32	69.7 (7.0)	29	74.6 (3.2)	28	81.6 (5.6)	89	75.0 (7.4)
	Total	124	67.7 (7.1)	82	72.6 (5.9)	88	76.0 (7.7)	294	71.6 (7.8)
PL	Oral	28	58.1 (11.7)	55	66.7 (6.8)	30	71.1 (3.8)	113	65.7 (9.1)
	Middle	27	64.1 (14.0)	58	70.2 (6.2)	27	72.7 (7.8)	112	69.3 (9.5)
	Aboral	28	64.5 (14.9)	58	72.3 (5.6)	28	77.1 (3.3)	114	71.5 (9.6)
	Total	83	62.2 (13.8)	171	69.8 (6.6)	85	73.6 (5.8)	339	68.9 (9.6)
PN	Oral	30	62.0 (7.1)	25	59.0 (8.3)	38	69.5 (5.8)	93	64.2 (8.2)
	Middle	30	68.5 (8.1)	24	66.6 (11.4)	38	75.2 (3.7)	92	70.8 (8.6)
	Aboral	29	68.7 (6.0)	24	70.6 (2.8)	37	75.5 (4.4)	90	72.0 (5.5)
	Total	89	66.4 (7.7)	73	65.3 (9.5)	113	73.4 (5.4)	275	69.0 (8.3)
Total indentations for <i>B. europaea</i>								908	69.8 (8.8)
Hardness (MPa)									
CL	Oral	49	5,011 (355)	29	5,280 (406)	30	5,155 (385)	108	5,123 (391)
	Middle	43	5,154 (395)	24	5,140 (312)	30	5,172 (432)	97	5,156 (385)
	Aboral	32	5,213 (438)	29	5,320 (423)	28	5,165 (409)	89	5,233 (424)
	Total	124	5,113 (398)	82	5,253 (390)	88	5,164 (404)	294	5,167 (400)
PL	Oral	28	5,046 (393)	55	4,909 (369)	30	4,961 (308)	113	4,957 (362)
	Middle	27	5,116 (478)	58	5,036 (366)	27	4,949 (304)	112	5,034 (384)
	Aboral	28	5,264 (497)	58	5,067 (325)	28	5,064 (290)	114	5,115 (374)
	Total	83	5,142 (461)	171	5,006 (358)	85	4,991 (302)	339	5,035 (378)
PN	Oral	30	4,841 (289)	25	5,092 (469)	38	4,972 (273)	93	4,962 (351)
	Middle	30	4,993 (276)	24	5,120 (347)	38	5,189 (336)	92	5,107 (328)
	Aboral	29	4,994 (403)	24	5,480 (474)	37	5,133 (356)	90	5,181 (444)
	Total	89	4,942 (331)	73	5,229 (464)	113	5,097 (334)	275	5,082 (386)
Total indentations for <i>B. europaea</i>								908	5,092 (391)

Mean values of *E* and *H* in each coral zone and age class

Population codes: *CL* Calafuria, *PL* Palinuro, *PN* Pantelleria, *n* number of indentations, *SD* standard deviation

population and age (ESM 8 and 9). For the hardness of *L. pruvoti*, the interaction terms population \times age and population \times zone were both significant (Table 4) and were used for pair-wise comparisons among levels of each factor (ESM 10, 11, and 12).

Analysis along the oral–aboral axis

In *B. europaea*, pair-wise comparisons showed that *E* consistently decreased from the aboral to the oral zone (ESM 1). The only exception was the subset of immature individuals at Palinuro, for which *E* was homogeneous among skeletal zones (ESM 1). The same trend of decreasing *E* from the aboral to the oral zone was highlighted for *L. pruvoti*, the only exceptions being the subset of immature individuals from Pantelleria, where *E* was higher in the middle zone, and the subsets of old individuals from

Pantelleria, having homogeneous *E* among skeletal zones (ESM 4).

In *B. europaea*, *H* decreased from the aboral to the oral zone (ESM 7). In *L. pruvoti*, significant differences in *H* among skeletal zones were found, but they did not follow any clear trend (ESM 10).

The reduction of *E* along the aboral–oral axis highlighted for both species represented a greater stiffness of the aboral pole with respect to the oral pole. The decreasing *H* along the aboral–oral axis in *B. europaea* represents a higher resistance to irreversible deformation (breakage) of the skeletal base with respect to the oral region.

Analysis according to age classes

In both species, mean age was homogeneous among the populations (Kruskal–Wallis test, $p > 0.05$; Table 1).

Table 3 *Leptosammia pruvoti*

Pop.	Zone	<i>n</i>	Immature samples (SD)	<i>n</i>	Mature samples (SD)	<i>n</i>	Old samples (SD)	<i>n</i>	Total zone (SD)
Young's modulus (GPa)									
CL	Oral	28	66.3 (4.1)	33	68.8 (5.4)	19	61.6 (10.0)	80	66.2 (7.0)
	Middle	29	71.3 (2.7)	48	72.7 (5.1)	14	72.8 (5.5)	91	72.3 (4.6)
	Aboral	30	70.2 (6.1)	37	70.3 (4.9)	29	71.9 (4.5)	96	71.6 (4.9)
	Total	87	69.3 (5.0)	118	71.6 (5.1)	62	68.9 (8.3)	267	70.2 (6.1)
PL	Oral	24	66.8 (8.6)	24	64.9 (8.6)	38	74.2 (4.7)	86	69.6 (8.2)
	Middle	24	73.0 (3.8)	24	69.8 (6.0)	39	77.3 (5.5)	87	74.1 (6.1)
	Aboral	25	72.5 (4.3)	28	68.5 (4.8)	38	77.5 (3.8)	91	73.4 (5.7)
	Total	73	70.8 (6.5)	76	67.8 (6.8)	115	76.4 (4.9)	264	72.4 (7.0)
PN	Oral	25	71.7 (4.4)	35	58.8 (8.9)	35	71.9 (6.4)	95	67.0 (9.4)
	Middle	30	74.7 (5.0)	34	66.8 (8.0)	40	73.5 (6.2)	104	71.6 (7.4)
	Aboral	25	71.3 (6.8)	38	68.3 (8.3)	40	73.4 (8.2)	103	71.0 (8.2)
	Total	80	72.7 (5.6)	107	64.7 (9.4)	115	72.9 (7.0)	302	70.0 (8.5)
Total indentations for <i>L. pruvoti</i>								833	70.8 (7.4)
Hardness (MPa)									
CL	Oral	28	5,074 (404)	33	5,135 (567)	19	5,130 (405)	80	5,112 (474)
	Middle	29	4,906 (267)	48	5,020 (370)	14	4,996 (275)	91	4,980 (327)
	Aboral	30	5,075 (410)	37	5,237 (322)	29	5,344 (412)	96	5,219 (390)
	Total	87	5,018 (371)	118	5,121 (428)	62	5,200 (404)	267	5,106 (409)
PL	Oral	24	4,983 (289)	24	5,040 (245)	38	5,057 (399)	86	5,031 (330)
	Middle	24	5,224 (390)	24	5,003 (280)	39	5,134 (283)	87	5,122 (322)
	Aboral	25	5,279 (390)	28	5,120 (432)	38	5,049 (290)	91	5,149 (371)
	Total	73	5,164 (378)	76	5,058 (335)	115	5,080 (327)	264	5,097 (345)
PN	Oral	25	5,003 (313)	35	5,129 (460)	35	4,980 (226)	95	5,041 (353)
	Middle	30	5,038 (333)	34	5,154 (419)	40	5,034 (358)	104	5,075 (373)
	Aboral	25	4,994 (341)	38	5,140 (455)	40	5,083 (377)	103	5,082 (400)
	Total	80	5,013 (326)	107	5,141 (441)	115	5,035 (331)	302	5,067 (376)
Total indentations for <i>L. pruvoti</i>								833	5,089 (378)

Mean values of *E* and *H* in each coral zone and age class

Population codes: *CL* Calafuria, *PL* Palinuro, *PN* Pantelleria, *n* number of indentations, *SD* standard deviation

In *B. europaea*, pair-wise comparisons showed that *E* consistently increased from immature to mature individuals, the only exception being the subset of oral zones from Palinuro, for which *E* was homogeneous among age classes (ESM 2). In *L. pruvoti*, even if significant differences were detected, no clear pattern was found for *E*, and its trend according to age classes varied depending on the particular subset of data analyzed (ESM 5).

In both species, *H* showed very different trends according to age classes depending on the subset of data analyzed, casting doubts on the actual presence, and/or biological significance of this pattern (ESM 8 and 11).

In *B. europaea*, the increase in *E* with increasing age class indicated that old individuals had stiffer skeletons than young ones.

Analysis among populations

Also in this analysis, *B. europaea* showed a clearer trend with respect to *L. pruvoti*. In *B. europaea*, in all the cases where *E* significantly differed among sites (67 %, six cases of nine; ESM 3), *E* decreased from Calafuria to Pantelleria following a southward latitudinal trend, indicating a decrease in skeletal stiffness (ESM 3). In *L. pruvoti*, instead, *E* showed cases of homogeneity among sites and both negative and positive trends from Calafuria to Pantelleria, without providing a clear pattern of interpretation (ESM 6).

In both species, hardness did not show any clear pattern of interpretation, as shown by the tables of pair-wise comparisons, which provided very different patterns depending on specific subset of data analyzed (ESM 9 and 12).

Table 4 *Balanophyllia europaea* and *Leptosammia pruvoti*

Source	<i>df</i>	<i>SS</i>	<i>MS</i>	Pseudo-F	P (perm)
<i>Balanophyllia europaea</i>					
Young's elastic modulus (stiffness)					
Pop	2	2,777.8	1,388.9	26.740	0.001*
Age	2	11,126	5,562.8	107.10	0.001*
Zone	2	8,113.1	4,056.6	78.101	0.001*
Pop × age	4	1,778.1	444.53	8.5584	0.001*
Pop × zone	4	246.91	61.727	1.1884	0.334
Age × zone	4	247.90	61.974	1.1932	0.304
Pop × age × zone	8	1,202.3	150.29	2.8936	0.008*
Residual	881	45,759	51.940		
Total	907	69,509			
Hardness					
Pop	2	2.6548 × 10 ⁶	1.3274 × 10 ⁶	9.4484	0.001*
Age	2	1.3330 × 10 ⁶	6.6651 × 10 ⁵	4.7441	0.008*
Zone	2	3.6341 × 10 ⁶	1.8171 × 10 ⁶	12.934	0.001*
Pop × age	4	4.5926 × 10 ⁶	1.1481 × 10 ⁶	8.1723	0.001*
Pop × zone	4	6.0631 × 10 ⁵	1.5158 × 10 ⁵	1.0789	0.371
Age × zone	4	9.1654 × 10 ⁵	2.2914 × 10 ⁵	1.6310	0.163
Pop × age × zone	8	1.8953 × 10 ⁶	2.3691 × 10 ⁵	1.6863	0.093
Residual	881	1.2377 × 10 ⁸	1.4049 × 10 ⁵		
Total	907	1.3888 × 10 ⁸			
<i>Leptosammia pruvoti</i>					
Young's elastic modulus (stiffness)					
Pop	2	501.86	250.93	6.6311	0.001*
Age	2	3125.9	1,563.0	41.303	0.001*
Zone	2	4,092.3	2,046.2	54.072	0.001*
Pop × age	4	4,645.2	1,161.3	30.688	0.001*
Pop × zone	4	189.51	47.378	1.2520	0.281
Age × zone	4	166.48	41.621	1.0999	0.365
Pop × age × zone	8	1,328.4	166.05	4.3880	0.001*
Residual	806	30,500	37.842		
Total	832	45,574			
Hardness					
Pop	2	2.7357 × 10 ⁵	1.3678 × 10 ⁵	0.99276	0.378
Age	2	2.6130 × 10 ⁵	1.3065 × 10 ⁵	0.94824	0.397
Zone	2	1.4265 × 10 ⁶	7.1324 × 10 ⁵	5.1765	0.006*
Pop × age	4	1.8748 × 10 ⁶	4.6870 × 10 ⁵	3.4017	0.010*
Pop × zone	4	1.9089 × 10 ⁶	4.7721 × 10 ⁵	3.4635	0.010*
Age × zone	4	1.5507 × 10 ⁵	0.3877 × 10 ⁵	0.28137	0.911
Pop × age × zone	8	1.4098 × 10 ⁶	1.7622 × 10 ⁵	1.2790	0.271
Residual	806	1.1105 × 10 ⁸	1.3778 × 10 ⁵		
Total	832	1.1857 × 10 ⁸			

Permutational multivariate analysis of variance (PERMANOVA) results for comparisons of stiffness and hardness by population (Pop), age class (Age), skeletal zone (Zone), and their interactions. Tests were run using Euclidean distances among samples and 999 permutations in the software Primer[®]. *df* Degrees of freedom, *SS* sum of squares, *MS* mean squares. Significant effects ($p < 0.05$) are indicated with an asterisk (*).

Discussion

To summarize the obtained results, the only clear pattern observed for the hardness was a decreasing trend from the aboral to the oral pole in *B. europaea*. Regarding stiffness, in both species, a general trend of reduction of *E* from the

aboral to the oral pole emerged. In *B. europaea*, also a positive trend of *E* with increasing individual age class and a negative trend of *E* when moving southwards were observed.

Mechanical properties were measured by nanoindentation, a technique applied here in detail for the first time to a

scleractinian, and applied to corals only in one previous study on the black coral (Juarez-de la Rosa et al. 2012). Furthermore, this is one of the first studies analyzing mechanical properties in scleractinian corals. One study performed on *Acropora* sp., *Goniopora* sp., and *Porites* sp. focuses on porosity and resistance to compression, which result strongly correlated with Young's modulus (Wu et al. 2009). A recent analysis of skeletal porosity, bulk density, and microdensity (Bucher et al. 1998) of *B. europaea* and *L. pruvoti* suggests a higher sensitivity to rising temperature of the former zooxanthellate species with respect to the latter azooxanthellate one (Caroselli et al. 2011). In fact, while skeletal porosity (especially macroporosity; Fantazzini et al. 2013) increases with increasing SST (decreasing latitude) in *B. europaea*, causing a consequent decrease in skeletal bulk density, in *L. pruvoti* bulk density and porosity do not vary with SST/latitude, while microdensity even seems to increase with increasing SST (Caroselli et al. 2011).

Skeletal stiffness (E) is inversely and closely related to skeletal porosity (Wainwright et al. 1976; Chamberlain 1978). In the case of nanoindentation, the type of porosity influencing the measured E values is a "fine" porosity with typical submicron sizes, because indentations are carried out far from coarser pores using the optical microscope coupled to the indenter. Mean E values did not reveal differences between the two species (Tables 2, 3). Although *L. pruvoti* has a lower skeletal bulk density and a higher mean porosity than *B. europaea* (Caroselli et al. 2011), their stiffness seemed to be the same (Tables 2, 3). Thus, *L. pruvoti* differs from *B. europaea* in its "coarse" porosity, which is higher in *L. pruvoti* (Fantazzini et al. 2013). Moreover, this is a new finding that was undetectable by buoyant weight measurements of porosity (Caroselli et al. 2011), but became measurable after the application of nanoindentation techniques.

The only clear pattern observed for the hardness was a decreasing trend from the aboral to the oral pole in *B. europaea*. This finding was quite unexpected, since the microdensity of this species (i.e., the density of calcium carbonate crystals, excluding the voids of skeletal porosity) has not been observed to follow any environmental trend (Caroselli et al. 2011). The observed presence of calcite in addition to aragonite, especially localized at the aboral pole, is unlikely to cause this pattern, since it has also been observed in *L. pruvoti* (Goffredo et al. 2012), whose hardness did not follow any clear trend in the present study. A possible explanation may be related to a different percentage of organic matrix embedded in the skeletal crystals along the oral–aboral axis, which could influence the hardness of skeletal material; however, this hypothesis requires further experiments to be properly tested. Apart this exception, the absence of any clear trend of hardness

for *L. pruvoti*, as well as for the factors age class and population in *B. europaea*, suggests that porosity does not influence hardness and Young's modulus in equal measures, even if possible trends may be masked by the natural high variability among individuals and study sites. Even if porous spaces should reduce the hardness of the material, they may also obstacle the plastic deformation, which is based on the mobility of crystalline defects (dislocation), which are blocked when there are no means of propagation. This effect might be responsible for the lack of clear trends of hardness, except the decrease from aboral to oral pole in *B. europaea*.

In both species, a general trend of reduction of E from the aboral to the oral pole emerged. Since the aboral pole is the oldest zone of the skeleton, the reduction of E along the oral–aboral axis might be due to the change of this property according to age, possibly in connection with changes in porosity. A reduction in porosity can occur due to the continued growth of the aragonite skeletal fibers, which fill the pores previously occupied by the gastrovascular channel and living tissue (Cohen and McConnaughey 2003). Since this process is dependent on age, it might explain the higher E values observed in the aboral pole. Gradations in microstructure and/or porosity are often encountered in biological structures such as bamboo, plant stems, and bone, where the strongest elements correspond to those regions that are subjected to the highest stresses (Suresh 2001). Artificial materials with porosity gradients have been used to realize isoelastic medical implants with good stiffness matching at the bone/implant interface (Traini et al. 2008). The porosity/Young's modulus gradient of coral skeletons may similarly favor the distribution of equal strain levels between skeletal parts that experience different stresses. In this way, by reducing the occurrence of localized strains higher than the elastic limit, the fatigue resistance of the structure as a whole is likely to be enhanced.

In *B. europaea*, a general positive trend of E with increasing individual age class was observed. The bigger mature corals are more prone to hydrodynamic stress; thus, an increase in stiffness and skeletal bulk density, resulting in a lower porosity, might increase the mechanical resistance to hydrodynamism. *B. europaea* could have an advantage at implementing this strategy with respect to *L. pruvoti*, because it lives at shallow depths, in unsheltered habitats, characterized by currents and strong waves. Indeed, whereas in *B. europaea* the increase in stiffness was rather clear, *L. pruvoti* showed an inconsistent and much more variable pattern. Several studies report reduced calcification and faster skeletal growth in sheltered habitats, leading to very porous skeletons, as occurs in *L. pruvoti* (Chamberlain 1978; Oliver et al. 1983). The lower porosity of *B. europaea* with respect to *L. pruvoti*

(Caroselli et al. 2011) might be an adaptation to resist the strong disturbances of the colonized habitat. Making a less porous skeleton, when not required, would waste a lot of energy in the non-zooxanthellate species, whose metabolism depends entirely on heterotrophic nutrition. In *B. europaea* and *L. pruvoti*, porosity and skeletal bulk density (Bucher et al. 1998) follow an inverse relationship according to the age of the individuals (Caroselli et al. 2011). An increase in bulk density is always accompanied by a reduction in porosity; when the former remains constant, also the latter does not vary (Caroselli et al. 2011). These two variables are closely connected to the Young's modulus (Wainwright et al. 1976; Chamberlain 1978): having a denser and less porous skeleton means higher stiffness. In fact, in this study, *B. europaea* showed a marked positive trend of E with age: old individuals had stiffer, denser, and less porous skeletons than young individuals. Conversely, a clear trend was not observed in *L. pruvoti*. These results highlighted a general strong relationship between stiffness and age. In fact, in both species, skeletal stiffness decreased from the aboral pole (the oldest part of the skeleton) to the oral pole (the newly deposited part of the skeleton), and in *B. europaea*, this trend is confirmed also according to the age of individuals: immature individuals had less stiff skeletons than mature and old individuals.

In *B. europaea*, a quite stable negative trend of E was observed when moving southwards (from Calafuria to Palinuro to Pantelleria). This trend in stiffness is consistent with the previously observed increase in skeletal porosity with increasing SST (decreasing latitude; Caroselli et al. 2011), especially the "coarse" porosity (Fantazzini et al. 2013). In fact, the three populations display higher average temperatures when moving southwards (Goffredo et al. 2007, 2008, 2009, 2012; Caroselli et al. 2011, 2012a, b). In *L. pruvoti*, a stable trend of E was not recorded, in agreement with the lack of correlations between porosity of this species and SST/latitude (Caroselli et al. 2011). Apart from temperature, also the nutrients might explain the reduction of E observed in *B. europaea* together with the increase in SST. In the Mediterranean Sea, the levels of nutrients and the availability of zooplankton are lower in summer (at higher temperatures) than in winter (at lower temperatures; Coma et al. 2000; Coma and Ribes 2003). Few nutrients and a low availability of zooplankton cause stress and starvation in *Cladocora caespitosa* (Peirano et al. 2005) and a summer quiescence in the metabolism of several benthic suspension feeders (Coma et al. 2000; Coma and Ribes 2003). In colonies of the tropical *Stylophora pistillata* and *Galaxea fascicularis*, calcification is significantly reduced in starved corals compared to fed ones (Houlbrèque et al. 2004). The reduction of E observed in *B. europaea* in the populations characterized by skeletons that are more porous might

therefore be explained because of the low energetic resources. However, if this were true, the inhibition of calcification and therefore the increase in porosity and decrease in Young's modulus should even be higher in *L. pruvoti*, which is completely heterotrophic, compared with *B. europaea*, which can also count on its symbionts. Instead, in the present study, the stiffness of *L. pruvoti* did not seem to change among populations in relation to temperature (southwards).

In conclusion, along the oral–aboral axis, in both species, significant changes were recorded in the Young's modulus, with higher values in the aboral zones with respect to oral ones. The analysis of age classes showed a trend of the Young's modulus in *B. europaea*, for which older corals had a stiffer skeleton. Conversely, in *L. pruvoti*, the skeletal stiffness did not vary consistently with age. Along the latitudinal gradient, the zooxanthellate species showed a southward trend of decreasing stiffness, while *L. pruvoti* did not show consistent trends. These results can be considered in the context of global climate change, as they confirm that the previously observed reductions in skeletal bulk density and porosity with increasing SST (decreasing latitude; Goffredo et al. 2007; Caroselli et al. 2011) translate in different mechanical properties of the skeleton, at least for the three populations investigated in the present study. An increase of 1.8–4 °C in sea surface temperature is expected by 2100, the highest increase being in the temperate areas of the northern hemisphere, compared with tropical ones (Solomon et al. 2007). The zooxanthellate species *B. europaea* is likely to be negatively affected in the stiffness of the skeletons of its polyps, due to the expected increase in skeletal porosity with rising temperatures, while *L. pruvoti* polyps are likely to be less affected, since the mechanical properties of their skeletons seem to show no particular influence along a wide latitudinal gradient, as well as most of their biological parameters investigated so far, including skeletal porosity (Fantazzini et al. 2013). The current study found that the zooxanthellate and the non-zooxanthellate representatives bear different mechanical properties. Since there is only one species represented in each coral group, any generalization to the broader groups of symbiotic and non-symbiotic corals should be made with caution. Nonetheless, the engineering meaning of reduced E value does not simply say that *B. europaea* skeletons will react worse mechanically toward global warming; it just mean that they will deform easier. This is the first study exploring mechanical properties of a scleractinian by nanoindentation, and the overall mechanical performance needs to be further tested in different species and environmental conditions, to have a clearer pattern of the possible consequences of climate change on skeletal structures.

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