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# Morphometry and mechanical design of tube foot stems in sea urchins: a comparative study

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## Abstract

To withstand hydrodynamic forces, sea urchins rely on their adoral tube feet, which are specialized for attachment. Although it has been often suggested that the degree of development of these tube feet is intimately related to the maximum environmental energy a species can withstand, it has never been demonstrated by mechanical testing. To address this subject, we studied the mechanical properties of the stem of adoral tube feet from three species of sea urchins, *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis*, which have distinct taxonomic, ecological and morphological characteristics. The tube feet of the three species have a very similar morphology. When a tensile force is applied to the tube foot stem, the connective tissue is the only tissue layer bearing the load. The mechanical properties of this tissue give the tube feet an ideal balance of extensibility (139–166%), strength (23–29 MPa) and stiffness (152–328 MPa), which together produce a material with adequate toughness (2.5–2.9 MJ/m<sup>3</sup>) to absorb the impact of waves and currents, and thus to resist the environmental challenges of the habitats in which sea urchins live. Extended stems of *P. lividus* were significantly stiffer (328 MPa) than those of the other two species (152 and 183 MPa, for *A. lixula* and *S. granularis*, respectively). No interspecific difference was found in terms of extensibility, strength, initial stiffness and toughness between the tube feet from the three species. The difference in local distribution between the species investigated is therefore not only explained by the mechanical properties of their tube feet, but may involve other factors such as tube foot number and arrangement, tube foot disc tenacity or sea urchin size.

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## 1. Introduction

Echinoids are exclusively benthic animals. Among the different sea urchin appendages, both the primary spines and the coronal tube feet participate in several activities in relation to the substratum. These activities

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are locomotion and maintenance of position (involving mostly adoral appendages), and righting and covering reactions (involving mostly aboral appendages) (Lawrence, 1987). Coronal tube feet are located in the five ambulacra, from the edge of the apical system to the edge of the peristomeal membrane. They consist of an enlarged and flattened distal extremity, the disc, which makes contact with the substratum and a proximal extensible cylinder, the stem, which connects the disc to the test. Both the stem and the disc consist of four tissue layers: an inner myomesothelium, a connective tissue layer, a nerve plexus, and an outer epidermis (Kawaguti, 1964; Nichols, 1966; Florey and Cahill, 1977; Flammang and Jangoux, 1993).

In every echinoid species investigated, the tube feet on the adoral surface have always a thicker stem wall and a wider disc compared with those of the aboral surface (Smith, 1978). These morphological differences are accompanied by mechanical differences: adoral tube feet are significantly more extensible and stronger than aboral tube feet (Leddy and Johnson, 2000) and may also have more adhesive power (Smith, 1978, Flammang, 1996).

Regular echinoids generally inhabit rocky or other types of hard bottoms exposed to wave action, although some species are found on soft bottoms in sheltered areas. It has been reported that echinoid species belonging to different taxa and inhabiting different environments possess different types of tube feet, and that a general correlation might exist between the degree of development of the tube feet and the maximum environmental energy that a species can withstand (Sharp and Gray, 1962; Smith, 1978). To withstand hydrodynamic forces, sea urchins are able to adhere strongly but temporarily to the substratum with their adoral tube feet. The tenacity with which an individual can anchor to a surface is determined partly by the number of tube feet which are involved. In addition, the strength of attachment depends on the strength of the tube foot itself, which is determined by the tensile strength of the stem and the adhesive power of the disc (Sharp and Gray, 1962; Smith, 1978). However, when a sea urchin is subjected to a constant pull, the majority of tube feet rupture before the terminal disc is detached from the substratum (Smith, 1978; Santos et al., unpublished observation), and therefore, the strength

of the stem could limit tube foot attachment strength.

The aim of the present study was to characterize the biomechanics of tube feet in different species of regular echinoids and see if there is a correlation between mechanical properties and habitat. To address this question, we studied the material properties of the stem of adoral tube feet from three common European species, *Arbacia lixula* (Linné, 1758), *Paracentrotus lividus* (Lamarck, 1816) and *Sphaerechinus granularis* (Lamarck, 1816), which have distinct taxonomic, ecological and morphological characteristics.

## 2. Materials and methods

Specimens of the three species were collected in the Mediterranean Sea (Banyuls-sur-mer, France) in a semi-sheltered rocky area with sandy bottoms. The arbacioid *A. lixula* and the echinid *P. lividus* were collected between 1 and 3 m in depth but, although the two species co-occurred in the same rocky area, individuals of the former were usually observed deeper than individuals of the latter. The temnopleuroid *S. granularis* was found at about 10 m in depth on a sandy bottom area. After collection, the animals were kept in re-circulating aquariums at 14–15 °C and 33‰.

### 2.1. Morphometric and ultrastructural analysis of tube foot stem

The mean values of the cross-sectional areas of each tissue layer of the tube foot stem from each sea urchin specimen were obtained using tube feet dissected after the mechanical tests (see Section 2.2.). These tube feet were fixed in Bouin's fluid for 24 h, subsequently dehydrated in a sequence of graded ethanol and embedded in paraffin wax. They were then cut transversely into 7- $\mu$ m-thick sections with a Microm HM 340 E microtome. The sections were mounted on clean glass slides and stained with Masson's Trichrome. Measurements were made with a Leica Laborlux light microscope equipped with a graduated eyepiece on sections taken halfway between the base and the disc of the tube foot.

For transmission electron microscopy (TEM), tube foot stems from the three echinoid species were fixed for 3 h at 4 °C in 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8; adjusted to 1030 mOsm with NaCl). Then they were rinsed in cacodylate buffer, post-fixed for 1 h in 1% OsO<sub>4</sub> in the same buffer, dehydrated in graded ethanol, and embedded in Spurr's resin. Transverse ultrathin sections (about 80 nm in thickness) were cut with a Leica UCT ultramicrotome equipped with a diamond knife, collected on copper grids, and stained with uranyl acetate and lead citrate before observation with a Zeiss LEO 906E transmission electron microscope.

## 2.2. Mechanical properties of the tube foot stem

The material properties of the tube foot stem were measured by tensile tests performed on three individuals per species. For each individual, 10 tube feet were tested in seawater at room temperature. Then the animal was anaesthetized in an isotonic solution of 7.5% MgCl<sub>2</sub>·6H<sub>2</sub>O at 10 °C for 10 min, and 10 other tube feet were tested in the MgCl<sub>2</sub> solution. Finally, a last set of 10 tube feet was dissected and preserved for the measurement of the stem tissue cross-sectional area in this particular individual (see Section 2.1.). The anaesthetizing solution was used to measure only the passive mechanical properties of tube foot stem.

Measurements were carried out with a Mecmesin Versa Test motorized stand, fitted with an

electronic force gauge (Mecmesin AFG 2.5 N) connected to a computer collecting the data. The precision of the tensile force measurements was 0.0005 N. Sea urchins were placed upside down and a small surgical clip was attached to one adoral tube foot in the portion of the stem just under the disc and then pulled perpendicularly to the test of the sea urchin (i.e., in the direction of the natural extension) at a constant extension rate of 25 mm/min, until failure. Before pulling the tube foot, the initial length of the stem (distance between the base of the tube foot and the clip) was measured at the point at which the force started to increase and reached 0.0015 N. This initial length of the stem together with the time required to break the tube foot, at constant extension rate, were subsequently used to calculate the stem final length at failure. During the mechanical test, data were continuously recorded as force–extension curves. Then these curves were converted into stress–strain curves (Fig. 1). True values of strain and stress were used instead of nominal values because of the high extensions observed for coronal tube foot stem of sea urchins (Shadwick, 1992).

The *true strain* ( $\epsilon$ ), a unitless parameter, was calculated as the Napierian logarithm of the ratio between each incremental value of length ( $L$ ) by the initial length ( $L_0$ ) of the tube foot (extension ratio). Strain expresses the deformation of the tube foot in response to a certain force and, at the point at which

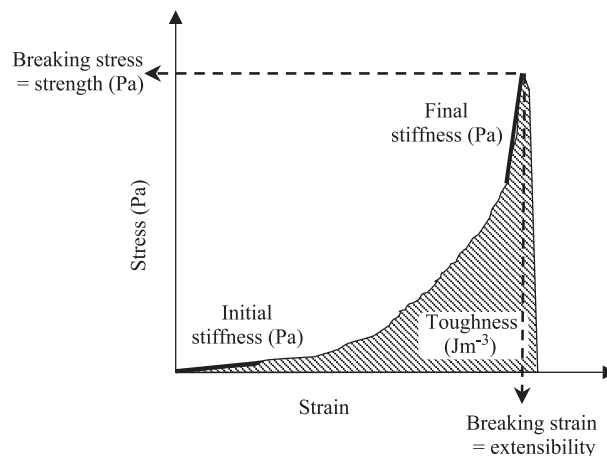


Fig. 1. Typical J-shaped stress–strain curve of the stem of a regular echinoid adoral tube foot showing the different material properties measured.

the stem fails (at final length), it is a measure of the material's *extensibility* ( $\epsilon_{\max}$ ).

$$\epsilon = \ln(L/L_0) \quad (1)$$

The *true stress* ( $\sigma$ ) results from the product of two ratios: the ratio of each incremental value of tensile force ( $F$ ) by the mean cross-sectional area of stem connective tissue ( $S$ ; see Section 2.1.), and the extension ratio. It is expressed in  $\text{N/m}^2$  or Pa (Pascal). The maximum value of stress (at breaking force) is an indicator of the *strength* ( $\sigma_{\max}$ ) of the tube foot.

$$\sigma = (F/S) \times (L/L_0) \quad (2)$$

The *modulus of elasticity* ( $E$ ) was calculated as the tangent to the slope of the stress–strain curve. It is a measure of the *stiffness* of the tube foot which is also expressed in Pa.

$$E = (\sigma_2 - \sigma_1)/(\epsilon_2 - \epsilon_1) \quad (3)$$

The stress–strain curve obtained for the tube feet of the three species studied was typically J-shaped (Fig. 1), and thus, *initial* and *final stiffness* were calculated, corresponding, respectively, to the stiffness of the tube foot at the beginning and at the end of extension.

Finally, we calculated the *breaking energy density* as the integral of force times extension (i.e., the area under a force–extension curve) divided by the volume of stem connective tissue (calculated as the product of tube foot initial length by mean connective tissue cross-sectional area). This parameter is an indicator of the energy needed to extend and break the tube foot. It is also referred to as the *toughness* and is expressed in  $\text{J/m}^3$ .

The results were then analyzed in order to look for significant differences between the species and the two solutions. Sea urchin specimens were used as the replicate, each value resulting from the average of ten tube feet per specimen. When necessary, logarithmic transformation was used to achieve homoscedasticity, followed by one-way analysis of variance (ANOVA) and Tukey test for multiple comparisons.

### 2.3. Strain rate

The influence of strain rate on the mechanical properties of the tube feet was investigated in *P.*

*lividus*. Fifteen individuals were anaesthetized for 10 min in  $\text{MgCl}_2$  and, for each of them, 10 tube feet were tested at one of the three following extension rates: 25, 75 and 200 mm/min. The different mechanical properties were calculated as described above and *strain rate* was calculated as the ratio of the extension rate to the initial length of the tube foot. Simple linear regression analysis was used to search for significant relationships between the mechanical properties measured and the strain rate.

## 3. Results

### 3.1. Tube foot stem morphometry and ultrastructure

Table 1 summarizes the morphometric measurements performed on three individuals of the three species of sea urchins as well as on their tube feet. We observed that *S. granularis* had on average a test two times bigger in diameter and height than the two other species, the latter being quite similar in size. In terms of the tube foot stem external diameter, there was a significant variation between the three species. The tube feet of *S. granularis* and *A. lixula* had similar external diameters, both larger than the stems of *P. lividus*. When tube foot stem diameter is expressed relative to test diameter, however, both *A. lixula* and *P. lividus* had larger tube feet for their size than *S. granularis*. In terms of tube foot mean wall thickness, no significant difference was found between the three species, not even when expressed as a percentage of stem diameter. Regarding stem wall cross-sectional area, both *A. lixula* and *S. granularis* had tube feet with a larger area than those of *P. lividus*. However, if the tissue cross-sectional area is expressed as a percentage of stem total cross-sectional area, the differences between the three species disappear.

In all the echinoid species, the stem wall consists of four tissue layers: an inner myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a nerve plexus, and an outer epidermis covered by a cuticle (Fig. 2A). Since the stem tissue cross-sectional areas varied significantly among the species (Table 1), the cross-sectional area of each tissue layer was expressed as a percentage of the total cross-sectional area (Fig. 2B). In the three

Table 1

Mean morphometric values ( $\pm$ S.D.,  $n=3$ ) of the test and adoral tube feet in the three echinoid species considered

	Species			$P_{ANOVA}$
	<i>Arbacia lixula</i>	<i>Paracentrotus lividus</i>	<i>Sphaerechinus granularis</i>	
<i>(A) Test measurements</i>				
Diameter (mm)	45.97 $\pm$ 7.47 <sup>a</sup>	43.27 $\pm$ 5.22 <sup>a</sup>	91.29 $\pm$ 8.58 <sup>b</sup>	0.008
Height (mm)	23.72 $\pm$ 5.06 <sup>a</sup>	22.74 $\pm$ 2.26 <sup>a</sup>	55.76 $\pm$ 4.57 <sup>b</sup>	0.004
<i>(B) Tube foot measurements (in MgCl<sub>2</sub>)</i>				
Stem diameter (mm)	0.444 $\pm$ 0.022 <sup>b</sup>	0.309 $\pm$ 0.028 <sup>a</sup>	0.424 $\pm$ 0.020 <sup>b</sup>	0.003
Stem diameter relative to test diameter (%)	0.99 $\pm$ 0.21 <sup>b</sup>	0.72 $\pm$ 0.03 <sup>b</sup>	0.47 $\pm$ 0.02 <sup>a</sup>	0.003
Stem initial length (mm)	12.39 $\pm$ 2.86 <sup>a</sup>	8.13 $\pm$ 1.40 <sup>a</sup>	9.10 $\pm$ 1.33 <sup>a</sup>	0.087
<i>(C) Tissue thicknesses</i>				
Stem wall (mm)	0.088 $\pm$ 0.007 <sup>a</sup>	0.061 $\pm$ 0.024 <sup>a</sup>	0.081 $\pm$ 0.004 <sup>a</sup>	0.130
Stem wall thickness relative to stem diameter (%)	20.11 $\pm$ 1.02 <sup>a</sup>	19.73 $\pm$ 6.36 <sup>a</sup>	19.24 $\pm$ 0.59 <sup>a</sup>	0.961
Epidermis and nerve plexus (mm)	0.033 $\pm$ 0.003 <sup>b</sup>	0.020 $\pm$ 0.007 <sup>a</sup>	0.027 $\pm$ 0.002 <sup>b</sup>	0.036
Connective tissue (mm)	0.034 $\pm$ 0.007 <sup>a</sup>	0.024 $\pm$ 0.010 <sup>a</sup>	0.036 $\pm$ 0.005 <sup>a</sup>	0.181
Myomesothelium (mm)	0.021 $\pm$ 0.003 <sup>a</sup>	0.017 $\pm$ 0.007 <sup>a</sup>	0.019 $\pm$ 0.001 <sup>a</sup>	0.583
<i>(D) Tissue cross-sectional areas</i>				
Stem wall (mm <sup>2</sup> )	0.100 $\pm$ 0.011 <sup>b</sup>	0.047 $\pm$ 0.019 <sup>a</sup>	0.088 $\pm$ 0.008 <sup>b</sup>	0.007
Stem wall cross-sectional area relative to stem cross-sectional area (%)	63.95 $\pm$ 2.29 <sup>a</sup>	61.64 $\pm$ 16.03 <sup>a</sup>	61.92 $\pm$ 1.45 <sup>a</sup>	0.954
Epidermis and nerve plexus (mm <sup>2</sup> )	0.043 $\pm$ 0.002 <sup>b</sup>	0.018 $\pm$ 0.007 <sup>a</sup>	0.033 $\pm$ 0.002 <sup>b</sup>	0.002
Connective tissue (mm <sup>2</sup> )	0.037 $\pm$ 0.009 <sup>ab</sup>	0.019 $\pm$ 0.008 <sup>a</sup>	0.038 $\pm$ 0.006 <sup>b</sup>	0.033
Myomesothelium (mm <sup>2</sup> )	0.019 $\pm$ 0.004 <sup>b</sup>	0.010 $\pm$ 0.004 <sup>a</sup>	0.016 $\pm$ 0.002 <sup>ab</sup>	0.035

Percentages were arcsine transformed before comparisons with ANOVAs and Tukey tests. Significant differences between means for a given parameter are indicated by letters in superscripts; means sharing at least one letter are not significantly different ( $p_{Tukey} \geq 0.05$ ).

echinoid species, the epidermis (including the basiepithelial nerve plexus) and the connective tissue were the dominant layers of the stem wall.

They were almost equally represented, contributing each to about 40% of the stem cross-sectional area, whereas the myomesothelium represented about

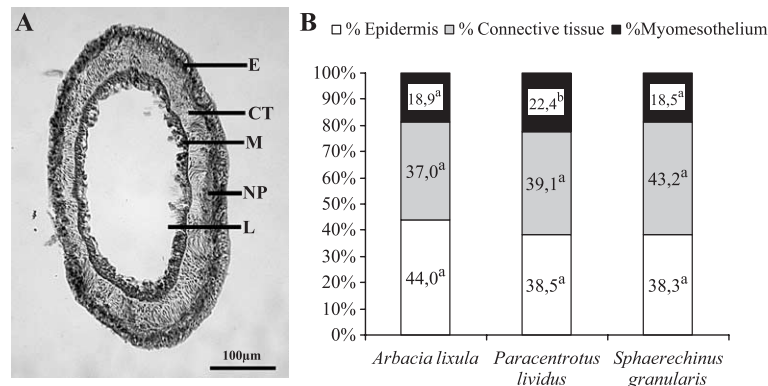


Fig. 2. (A) Transverse section through the stem of an adoral tube foot of *P. lividus*. (B) Mean percentages of stem wall tissue cross-sectional areas for the tube feet of *A. lixula*, *P. lividus* and *S. granularis*. Significant differences between means for a given tissue are indicated by letters in superscripts; means sharing at least one letter are not significantly different ( $p > 0.05$ , multiple comparison test of Tukey; percentages were arcsine transformed). Abbreviations: CT, connective tissue layer; E, epidermis; L, lumen; M, myomesothelium; NP, nerve plexus.

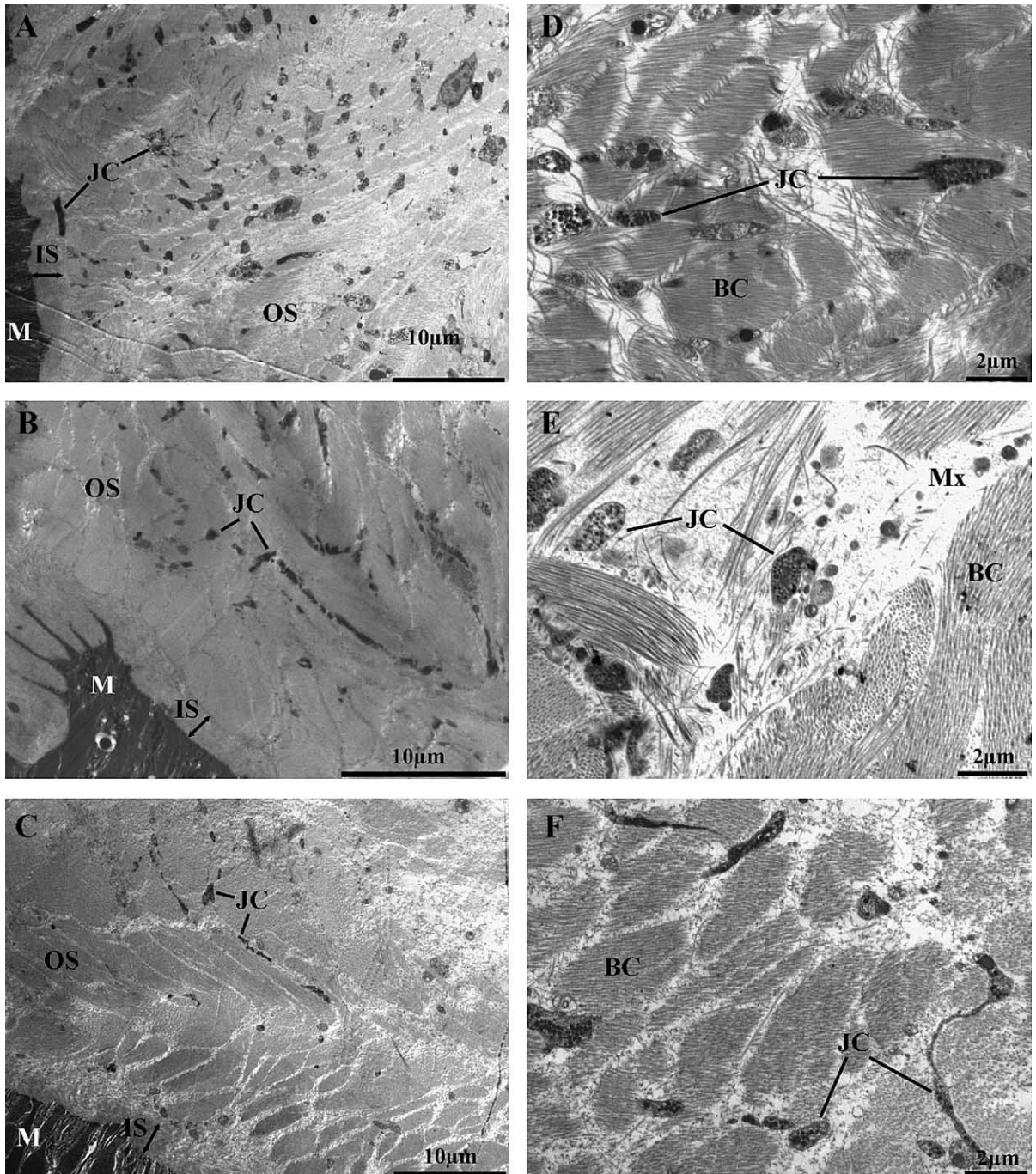


Fig. 3. Ultrastructure of the connective tissue layer of the adoral tube feet in the three species studied. (A–C) General view of transverse sections through the stem connective tissue of the tube feet of *A. lixula*, *P. lividus* and *S. granularis*, respectively; and (D–F) corresponding details showing the bundles of collagen fibrils and the juxtaligamental-like cell processes. Abbreviations: BC, bundle of collagen fibrils; IS, connective tissue inner sheath; JC, juxtaligamental-like cell processes; M, myomesothelium; Mx, matrix; OS, connective tissue outer sheath.

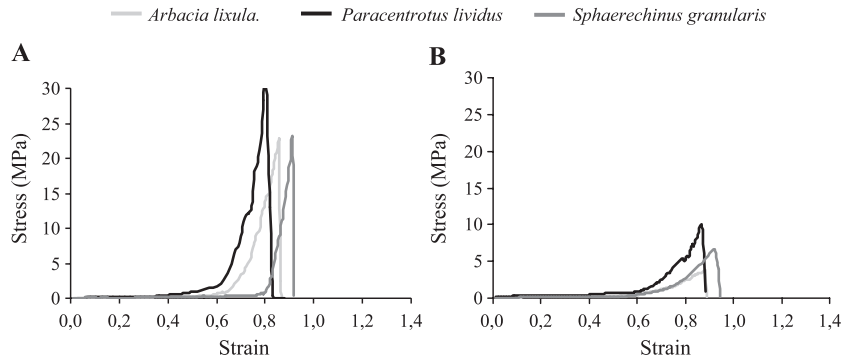


Fig. 4. Typical stress–strain curves for the tube feet of *A. lixula*, *P. lividus* and *S. granularis*. Tests performed (A) in seawater and (B) in an anaesthetizing magnesium chloride solution, both using a constant extension rate of 25 mm/min.

20%. The myomesothelium layer was significantly more developed in *P. lividus* (Fig. 2B).

The TEM observations revealed that in the three species, the connective tissue layer is subdivided into an inner sheath of helically orientated fibers and an outer sheath of longitudinally orientated fibers (Fig. 3A–C). The former is markedly thinner than the latter: in *A. lixula* and *P. lividus*, this proportion was 1/20 while in *S. granularis* it was 1/10. In the three species, the connective tissue layer consisted of bundles of collagen fibrils disposed in a matrix, and regularly interspersed cell processes

containing large, electron dense granules (Fig. 3D–F), which resemble the juxtaligamental cells associated with echinoderm mutable collagenous tissue (Wilkie, 1996). In cross-section, the collagen fibrils presented various thicknesses ranging from 50 to 120 nm in diameter, suggesting the possibility that they are spindle-shaped as in holothuroid dermis (Trotter et al., 2000). The juxtaligamental-like cells processes were seemingly more numerous in *A. lixula* than in the other two species. These processes were filled with granules whose diameters ranged from 90 to 150 nm in *P. lividus*, from 100

Table 2

Mean values ( $\pm$ S.D.,  $n=3$ ) of the material properties measured for tube foot stem in the three echinoid species considered

	Species			<i>P</i> ANOVA
	<i>Arbacia lixula</i>	<i>Paracentrotus lividus</i>	<i>Sphaerechinus granularis</i>	
<i>(A) In seawater</i>				
Breaking force (N)	$0.36 \pm 0.11^a$	$0.21 \pm 0.08^a$	$0.34 \pm 0.07^a$	0.173
Extensibility ( $\epsilon_{\max}$ )	$0.83 \pm 0.16^a$	$0.87 \pm 0.06^a$	$0.93 \pm 0.16^a$	0.595
Strength ( $\sigma_{\max}$ ; MPa)	$23.1 \pm 6.4^a$	$29.1 \pm 1.3^a$	$24.0 \pm 5.3^a$	0.342
Initial stiffness ( $E_{\text{initial}}$ ; MPa)	$1.1 \pm 0.9^a$	$1.3 \pm 0.7^a$	$0.8 \pm 0.2^a$	0.703
Final stiffness ( $E_{\text{final}}$ ; MPa)	$152 \pm 25^a$	$328 \pm 24^b$	$183 \pm 36^a$	0.001
Toughness ( $\text{MJ/m}^3$ )	$2.9 \pm 0.9^a$	$2.5 \pm 0.2^a$	$2.9 \pm 0.1^a$	0.777
<i>(B) In MgCl<sub>2</sub></i>				
Breaking force (N)	$0.058 \pm 0.023^a$	$0.072 \pm 0.019^a$	$0.102 \pm 0.003^a$	0.051
Extensibility ( $\epsilon_{\max}$ )	$0.93 \pm 0.06^a$	$0.87 \pm 0.11^a$	$1.06 \pm 0.06^a$	0.075
Strength ( $\sigma_{\max}$ ; MPa)	$3.9 \pm 0.9^a$	$10.1 \pm 2.7^b$	$7.9 \pm 0.8^b$	0.003
Initial stiffness ( $E_{\text{initial}}$ ; MPa)	$0.33 \pm 0.09^a$	$0.80 \pm 0.38^a$	$0.37 \pm 0.11^a$	0.052
Final stiffness ( $E_{\text{final}}$ ; MPa)	$16 \pm 5^a$	$63 \pm 22^b$	$41 \pm 5^b$	0.003
Toughness ( $\text{MJ/m}^3$ )	$0.67 \pm 0.08^a$	$1.35 \pm 0.39^b$	$1.11 \pm 0.12^{ab}$	0.041

Significant differences between means for a given property are indicated by letters in superscripts; means sharing at least one letter are not significantly different ( $p_{\text{Tukey}} \geq 0.05$ ).

to 180 nm in *S. granularis*, and from 80 to 200 nm in *A. lixula* (Fig. 3D–F).

### 3.2. Mechanical properties of the stem

When a tensile force is exerted on a tube foot stem, the connective tissue is most likely the only tissue layer bearing the load. Florey (1974, cited in Florey and Cahill, 1977) reported that the connective tissue resists extensions with forces bigger by one or two orders of magnitude than those developed by the muscle when stimulated to maximal contraction. The ultrastructure of the epidermis and nerve plexus suggests that they are even weaker. Therefore, stress values were calculated using only the connective tissue cross-sectional

area, and toughness values using the connective tissue volume. Typical stress–strain curves obtained for each species during the mechanical tests are presented in Fig. 4A and B. Characteristically, each curve shows an initial region of low resistance to the applied force followed by a region presenting a sudden increase in stress until rupture. These two regions correspond to two different moduli or stiffnesses ( $E_{\text{initial}}$  and  $E_{\text{final}}$ ).

Mean values of the material properties measured for tube foot stems in the three sea urchin species are presented in Table 2 and in Fig. 5. The analysis of the anaesthetizing effect at the intraspecific level showed that treatment with  $\text{MgCl}_2$  did not affect significantly the extensibility nor the initial stiffness of the tube feet of the three species (Fig. 5A and C). Yet, in all three

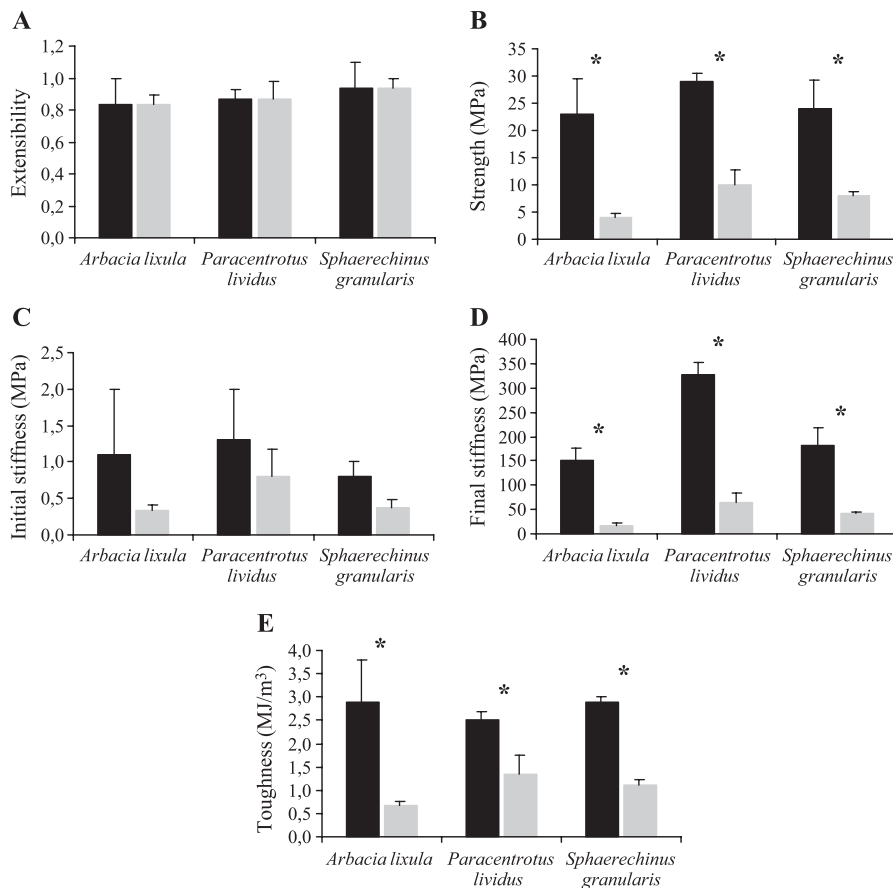


Fig. 5. Mean mechanical properties ( $\pm$ S.D.,  $n=3$ ) of the tube foot stems in *A. lixula*, *P. lividus* and *S. granularis* measured in seawater (black bars) and in an anaesthetizing solution of magnesium chloride (gray bars), with an extension rate of 25 mm/min. Asterisks indicate significant differences between the two solutions ( $p_{t\text{-test}} < 0.05$ ).



species, there was a considerable decrease in stem strength, final stiffness and toughness in the anaesthetizing solution compared with seawater (Fig. 5B,D,E). This decrease appears to be twice as important in *A. lixula* as in the other species (Table 2).

In seawater, the three species had similar extensibility, strength, initial stiffness and toughness. Nevertheless, the tube foot final stiffness was markedly higher in *P. lividus* (328 MPa) than in the other two species (152 and 183 MPa, for *A. lixula* and *S. granularis*, respectively) (Table 2).

In the anaesthetizing solution, the extensibility and the initial stiffness were again similar between the tube feet of the three species. However, the tube feet of *P. lividus* and *S. granularis* were both stronger and stiffer than those of *A. lixula*. In terms of toughness, only the tube feet of *P. lividus* were significantly tougher than those of *A. lixula*.

### 3.3. Influence of strain rate

The analysis of strain-rate dependence of the tube foot mechanical properties using different extension rates demonstrated that extensibility, strength and final stiffness were positively depend-

ent on strain rate (Fig. 6A,B,D). Strength and final stiffness (Fig. 6B,D), however, were less affected by strain rate than extensibility (Fig. 6A). Tube foot initial stiffness was not significantly related with strain rate (Fig. 6C).

## 4. Discussion

### 4.1. Tube feet as tough tethers

Most regular echinoids use their adoral tube feet to anchor to the substratum in order to resist to the action of waves and currents. If the tube foot disc is important because it produces the adhesive secretion that fastens the sea urchin to the substratum (Flammang and Jangoux, 1993), the stem is equally important because it must bear the tensions placed on the animal by hydrodynamics. Two tissues are candidates for this load-bearing function: the connective tissue and the muscles (myomesothelium). Florey (1974, cited in Florey and Cahill, 1977) reported that tube foot muscle is very weak compared to the connective tissue. Smith (1978), on the other hand, suggested that the quantity of muscle fibers in the tube foot stem would determine its

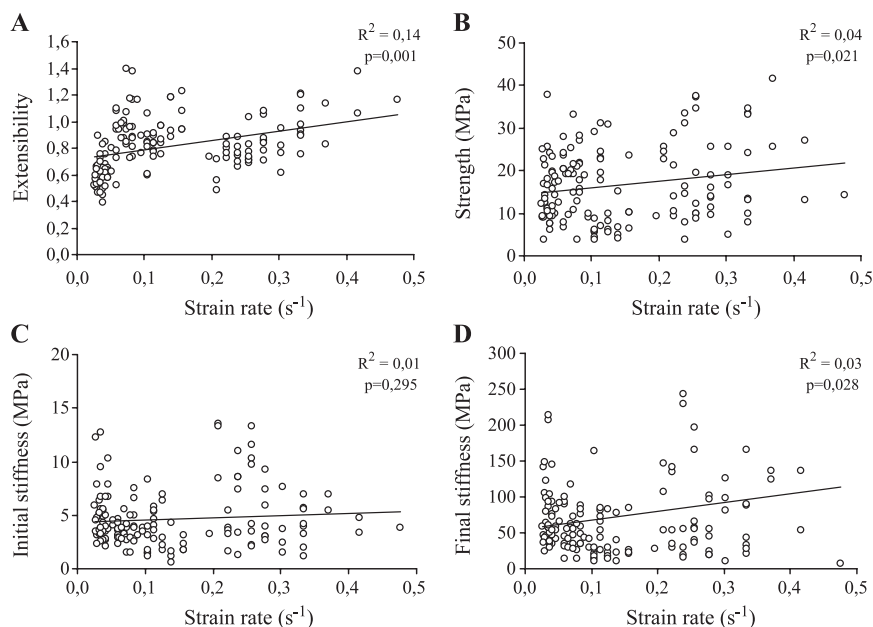


Fig. 6. Relationships between the mechanical properties of tube foot stems in *P. lividus* and strain rate (lines are linear regressions,  $p_{t\text{-test}} < 0.05$  indicates that the slope is significantly different from zero).

tensile strength. To settle this contradiction, we performed our measurements of the mechanical properties of the tube foot stem both in seawater and in an isotonic solution of magnesium chloride. In the latter, muscle contraction is prevented and only passive properties of the tube foot are measured. Using a breaking stress value of 0.05 MPa for relaxed muscle (Magid and Law, 1985), we can calculate that, according to the sea urchin species considered, the myomesothelium accounts for only 0.7% to 1.7% of the tube foot breaking force. To the best of our knowledge, no data are available on the breaking stress of fully contracted echinoderm muscle. The strongest known muscle is the anterior byssus retractor muscle of *Mytilus edulis* Linné, 1758. Its breaking strength while generating a maximal isometric force is approximately 10 MPa (Wilkie, 2002). Therefore, assuming a similar strength for tube foot muscle (which is highly unlikely), the myomesothelium would account for only about 50% of the tube foot breaking force in seawater. It is apparent therefore that, when a tensile force is exerted on a tube foot stem, the connective tissue is the only tissue layer bearing the load. The significant decrease in strength, final stiffness and toughness observed when tube feet are changed from seawater to  $MgCl_2$  is then explained by the mutable character of the connective tissue. The so-called mutable collagenous tissue has the capacity to undergo rapid changes in passive mechanical properties under the control of juxtaligamental-like cell processes, and is a characteristic of echinoderms (Trotter et al., 2000; Wilkie, 1996; 2002). It has been described from various echinoderm

organs, including sea urchin tube feet (Florey and Cahill, 1977; Santos et al., unpublished observation). In our anaesthetizing solution, the tube foot connective tissue is in the relaxed (“soft”) state (Santos et al., unpublished observation), hence the differences observed in comparison with seawater. The stronger effect of  $MgCl_2$  on the tube feet of *A. lixula* could then be correlated with the higher number of juxtaligamental-like cell processes observed in their connective tissue layer, suggesting a more important involvement of the mutable collagenous tissue in their functioning.

Regular echinoid tube foot stems present the stress–strain behavior of a typical pliant composite made up of a fibrous phase of collagen and a matrix phase of other proteins and proteoglycans (Vincent, 1990; Vogel, 2003). All biomaterials of this kind show a J-shaped stress–strain curve in which the first, low modulus, region is usually attributed to the stretching of the elastomeric matrix and/or the progressive reorientation and recruitment of the collagen fibers in the direction of pull; whereas the second, high modulus, region is attributed to the transfer of load to the aligned collagen fibers (Vincent, 1990). Such curves, in which high extensibility is combined with progressive stiffening to produce high toughness, are characteristic of shock-absorbing materials like, e.g., mammalian skin and artery (Vincent, 1990; Vogel, 2003). Compared with other biological materials, the echinoid tube foot stem has an extensibility (140–170%) similar to the protein rubber elastin, but it is stronger and stiffer (Table 3). This higher strength and stiffness is presumably accounted for by the fibrous

Table 3  
Tensile mechanical properties of sea urchin tube foot stems and other biomaterials

Material	Extensibility	Strength (MPa)	Stiffness (MPa)	Toughness (MJ/m <sup>3</sup> )	Reference
Bovine nuchal ligament (80% elastin)	1.50	2	1.1	1.6	Gosline et al., 2002
Mammalian tendon (80% collagen)	0.13	120	1200	6	Gosline et al., 2002
Coral skeleton	0.0003	40	60000	1	Wainright et al., 1976
Mussel shell	0.002	56	31000	0.0005	Wainright et al., 1976
Mussel byssal thread					
( <i>Mytilus edulis</i> )	0.44	21	85	12.5	Smeathers and Vincent, 1979
( <i>Mytilus californianus</i> )	0.97	29	44	14.9	Bell and Gosline, 1996
Sea urchins tube foot stem					
( <i>Arbacia lixula</i> )	1.39	10	26	2.9	Present study
( <i>Paracentrotus lividus</i> )	1.50	12	32	2.5	Present study
( <i>Sphaerechinus granularis</i> )	1.66	9	27	2.9	Present study

In order to make valid comparisons with values from the literature, our data had to be recalculated using the engineer’s definitions of strain (extensibility) and stress (strength):  $\epsilon=(L-L_0)/L_0$  and  $\sigma=(F/S)$ .

phase of collagen. Yet, the tube foot stem is far from reaching the strength and stiffness values of mammalian tendons. However, tendons have a low extensibility and are designed to store energy (Gosline et al., 2002) while tube feet must dissipate the energy produced by breaking waves. So it is not surprising that their mechanical properties are so different.

The mussel byssus is a structure that is functionally comparable to echinoid adoral tube feet. Like tube feet, byssal threads act as tethers that fasten the mussel to the substratum and function as shock absorbers (Bell and Gosline, 1996; Waite et al., 2002; Carrington and Gosline, 2004). Byssal threads are quite extensible, although not as much as tube feet, but they are stronger, stiffer and tougher (Table 3). However, byssal threads are constructed entirely of non-cellular, extraorganismic material and their unique function is to attach the mussel to rocks in the wave-swept marine intertidal zone. Tube feet, on the other hand, are cellular organs that are used as a holdfast, but that can also perform complex movements through a combination of protraction, retraction and bending (Flammang, 1996).

Two additional factors may further enhance the mechanical properties of echinoid tube foot stem, and bring them closer to those of mussel byssal threads. The first one is the mutable nature of the connective tissue. In its stiff state, the stem connective tissue of *P. lividus* may reach mean values as high as 1.16, 100 MPa, 1000 MPa and 11 MJ/m<sup>3</sup> for extensibility (true strain), strength (true stress), final stiffness and toughness, respectively (Santos et al., unpublished observation; compare with Table 2). The second factor is the positive strain-rate dependence of tube foot mechanical properties we observed in *P. lividus*: extensibility, strength, and final stiffness increased as strain rate increased. A similar dependence was reported for the tube feet of *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) by Leddy and Johnson (2000). In both studies, about 10% of the variation in extensibility was attributed to variation in strain rate. However, our correlations for the other mechanical properties were weaker than those described in *S. droebachiensis*, for which nearly half of the variation of strength and final stiffness was explained by the variation in strain rate. This strain-rate dependence might be an indication that the performance of sea urchins tube feet may be improved

by increasing strain rate. This would correspond in the natural environment to a higher stem resistance to rapid loads (such as waves) than to slower, self-imposed loads (such as natural extension). The strain rates used in our study and in the one of Leddy and Johnson (2000) are closer to natural extension rates (0.2 s<sup>-1</sup> for the adoral tube feet of *S. droebachiensis*) than to strain rates applied by waves ( $\gg 1$  s<sup>-1</sup>; Denny et al., 1998). However, in other biomaterials, the positive strain-rate dependence extends to strain rates as high as 0.7 s<sup>-1</sup> for mussel byssal thread (Carrington and Gosline, 2004) and 30 s<sup>-1</sup> for spider silk (Gosline et al., 2002), and this could also hold true for echinoid tube feet.

Our study of the mechanical properties of regular echinoid adoral tube feet therefore seems to indicate that the tube foot stem possesses an ideal balance of extensibility, strength and stiffness, which together produce a material with adequate toughness to absorb the impact of waves and currents, and thus to resist the environmental challenges of the habitats in which sea urchins live.

#### 4.2. Are tube foot morphology and mechanics correlated with species habitat?

Bell and Gosline (1996) studied the mechanical design of mussel byssus threads produced by wave-exposed species versus calm water species, and showed that the threads from the former were more extensible, stronger and stiffer than those from the latter. In regular echinoids, a similar relationship between adoral tube foot mechanical properties and the maximum environmental energy that a species can withstand has been proposed (Sharp and Gray, 1962; Smith, 1978), but has never been demonstrated. Smith (1978) categorized regular echinoid coronal tube feet in four types based on their structures. Type 1 tube feet lack discs and are thin-walled. The apex of the tube foot lacks any skeletal framework and is developed into a small sensory pad. The connective tissue layer and the retractor muscles of the stem are very thin. Type 4 tube feet bear a large and well-developed disc. The connective tissue layer of the stem is thick and the retractor muscles are developed to such an extent that the lower one-third of the tube foot appears when contracted to have no lumen. Types 2 and 3 tube feet are intermediates between the

structures of types 1 and 4 tube feet. This author showed that sea urchin species possessing type 2 adoral tube feet are restricted to soft or firm sediment bottoms in low to moderate energy environments, and that those with type 4 tube feet inhabit steep rocky bottoms or coral reefs in exposed high energy environments. Type 3 tube feet are found in species living on rocks in intermediate environments and being restricted to burrows and crevices when exposed in high energy environments. In a recent study, Leddy and Johnson (2000) showed that the tube feet of *S. droebachiensis* achieve functional specialization along the adoral–aboral axis through the differentiation of distinct mechanical properties, adoral type 3 tube feet being significantly more extensible and stronger than aboral type 2 tube feet. Their work confirms the correlation between the morphological type of tube foot and the stem mechanical properties.

The three species investigated in the present study were chosen because, although they can be found in the same area, they live in habitats with distinct hydrodynamic characteristics. *A. lixula* and *P. lividus* occur in a shallow rocky area subjected to wave action, whereas *S. granularis* lives deeper on a sandy bottom. Moreover, according to the classification of Smith (1978), *A. lixula*, *P. lividus* and *S. granularis* possess type 4, 3 and 2 adoral tube feet, respectively. Our morphometric results, however, do not support this classification. Indeed, although at scaled test size *A. lixula* and *P. lividus* have significantly larger tube foot stems than *S. granularis*, at scaled tube foot size the three species possess identical stem wall thicknesses and cross-sectional areas. According to the definition of Smith (1978), they should therefore all be classified within the same tube foot type.

As far as the mechanical properties of the adoral tube feet are concerned, the stems of *P. lividus* have the highest final stiffness in seawater. Märkel and Titschack (1965, quoted in Smith, 1978) carried out experiments in which a spring balance was used to measure the maximum pull individuals of *A. lixula*, *P. lividus* and *S. granularis* could withstand. They reported that *P. lividus* is able to withstand much greater pull than the other two species. Although they proposed that this superiority was due to a greater number of adoral tube feet involved, the high stiffness of the stems in *P. lividus* may also be an

explanation. Similarly, in our sampling site, *P. lividus* was the most exposed of the three species. Nevertheless, in terms of extensibility, strength and toughness, there are no difference between the adoral tube feet from the three species in seawater. This also indicates that adoral tube feet in these species could belong to the same tube foot type. The difference in local distribution between species is therefore not completely reflected in the mechanical properties of their adoral tube feet, but may also involve other factors such as tube foot number and arrangement, tube foot disc tenacity or, more importantly, size of the sea urchin. Indeed, the larger an echinoid, the stronger the drag forces it experiences (Denny, 1988). To test further the hypothesis of Smith (1978) regarding tube foot mechanics, there needs to be an investigation of other species that possess larger differences in tube foot morphology and that live in more contrasted environments.

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