

Evaluation of the different forces brought into play during tube foot activities in sea stars

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SUMMARY

Sea star tube feet consist of an enlarged and flattened distal extremity (the disc), which makes contact with the substratum, and a proximal contractile cylinder (the stem), which acts as a tether. In this study, the different forces brought into play during tube foot functioning were investigated in two related species. The tube feet of *Asterias rubens* and *Marthasterias glacialis* attach to glass with a similar mean tenacity (0.24 and 0.43 MPa, respectively), corresponding to an estimated maximal attachment force of 0.15 and 0.35 N. The contraction force of their retractor muscle averages 0.017 N. The variation of the retractor muscle contraction with its extension ratio follows a typical bell-shaped length–tension curve in which a maximal contraction of approximately 0.04 N is obtained for an extension ratio of approximately 2.3 in both sea star species. The tensile strength of the tube foot stem was investigated considering the two tissues that could assume a load-bearing function, i.e. the retractor muscle and the connective tissue. The latter is a mutable collagenous tissue presenting a fivefold difference in tensile strength between its soft and stiff state. In our experiments, stiffening was induced by disrupting cell membranes or by modifying the ionic composition of the bathing solution. Finally, the force needed to break the tube foot retractor muscle was found to account for 18–25% of the tube foot total breaking force, showing that, although the connective tissue is the tissue layer that supports most of the load exerted on the stem, the contribution of the retractor muscle cannot be neglected in sea stars. All these forces appear well-balanced for proper functioning of the tube feet during the activities of the sea star. They are discussed in the context of two essential activities: the opening of bivalve shells and the maintenance of position in exposed habitats.

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Key words: Echinodermata, podia, biomechanics, mutable collagenous tissue, muscle contraction, adhesion.

INTRODUCTION

Forcipulid sea stars, under the normal circumstances of their life in tide pools or in subtidal waters, display a range of activities that include locomotion, the capture of food and the maintenance of position. These and other organismal activities require a harmonious integration of movement of the different parts and organs of the body, chief among which are the tube feet (Smith, 1947; Lawrence, 1987). These organs typically consist of a proximal extensible cylinder (the stem), topped apically by an enlarged and flattened structure (the disc) (Flammang, 1996). These two parts act together to allow tube feet to take part cooperatively in rather elaborate tasks (e.g. locomotion, righting, bivalve shell opening), which all require the formation of an adhesive bond between the tube foot and the substratum and a great mobility of the foot (Lawrence, 1987). Attachment is mediated by the disc, which makes contact with the substratum, adapts to its surface profile and produces the adhesive secretion that fastens the tube foot to the substratum (Flammang et al., 1994; Flammang et al., 1998; Santos et al., 2005a; Hennebert et al., 2008). The mobility of the tube foot, on the other hand, is permitted by the stem, more precisely by the action of its muscular layer, the so-called retractor muscle (Flammang, 1996). When this muscle contracts, it initiates movements of the foot by pulling on its wall and exerting pressure on the ambulacral fluid contained within the tube foot lumen (Smith, 1947). This results in tube foot bending or retraction when the disc is not attached, or traction force generation when the disc is attached to a substratum. The stem

contraction force, combined with the disc adhesion force, allows the sea star to climb vertical surfaces, to right itself, to pull bivalve molluscs open or to clamp its body against the substratum, a behaviour that may play an important role in maintenance of position because friction generated in this way decreases the risk of dislodgement by shear forces (Kerkut, 1953; Lawrence, 1987). There are scattered reports on tube foot adhesion and contraction forces in the literature (for reviews, see Hyman, 1955; Lawrence, 1987; Santos et al., 2009). However, they may be confusing because both types of forces are described as pull forces, and one is sometimes misinterpreted for the other.

The tube foot stem also acts as a tough tether connecting the adhesive disc to the animal's body. In asteroid species living in the intertidal or in the shallow subtidal, one important role of the tube foot stem is to bear the tensions placed on the animal by hydrodynamic forces (Santos et al., 2005b). This load-bearing function may be critical. Indeed, when sea stars are subjected to a constant pull, a significant number of their tube feet sometimes rupture before they are detached from the substratum (Berger and Naumov, 1996; Flammang and Walker, 1997). The tensile strength of the stem can thus limit the capacity of sea stars to be strongly anchored to the substratum. Among the stem tissues, only the connective tissue and the retractor muscle contain fibrillar elements (i.e. collagen fibres, microfibrils, myofilaments) oriented in parallel to the tube foot axis, in the direction of the tensile stress (Flammang, 1996; Santos et al., 2005b). The comprehension of their respective

mechanical properties is therefore important to understand how sea star tube feet resist environmental forces. This is difficult, however, because of the close association of these two tissues in the stem, which precludes separate measurements of their respective contributions. Moreover, it has been demonstrated recently that the stem connective tissue of the sea star *Marthasterias glacialis* is a 'mutable collagenous tissue' (MCT) (Santos et al., 2005b). Such tissues, which are characteristic of echinoderms, can undergo rapid changes in their mechanical properties under nervous control via a specialized cell type, the juxtaglamental cells (Trotter et al., 2000; Wilkie, 1996; Wilkie, 2005). The presence of a MCT in asteroid tube feet makes the study of the respective roles of connective tissue and retractor muscle in the resistance of the stem to hydrodynamic forces even more difficult because solutions that could have been used to specifically relax the muscle, such as MgCl_2 , also have an effect on the connective tissue mechanical properties (Santos and Flammang, 2005; Santos et al., 2005b).

The aim of the present study was to evaluate the different forces brought into play during the different activities of the tube feet of two sea star species, *Asterias rubens* and *Marthasterias glacialis*. Adhesion strength of the tube foot disc and isometric contraction of the retractor muscle were measured and compared between the two species. The mechanical properties of the tube foot stem were also tested under different conditions and were combined with results obtained on another echinoderm muscle, the holothuroid longitudinal body wall muscle, to estimate the respective contribution of the connective tissue and retractor muscle to the tube foot tensile strength.

MATERIALS AND METHODS

Collection and maintenance of specimens

Adult individuals of the asteroids *Asterias rubens* Linné 1758 and *Marthasterias glacialis* (Linné 1758) were collected at low tide in Audresselles (Pas-de-Calais, France) and Morgat (Finistère, France), respectively. Adult individuals of the holothuroid *Holothuria tubulosa* Gmelin 1788 were purchased from the biological station of Banyuls-sur-Mer (Pyrénées-Orientales, France). The animals were kept in re-circulating aquariums at 14–15°C and 33‰ salinity.

Preparation of specimens and bathing solutions

All animals were dissected under water to prevent tissue desiccation. For experiments on tube feet, the five arms of the asteroids were separated from each other. Tube feet were used while still attached basally to the arm or, alternatively, the ambulacra were dissected and tube foot-ampulla units were isolated. Tube feet were always chosen from the middle area of the arms. Holothuroids were dissected and their longitudinal muscles were carefully separated from the body wall dermis. The longitudinal body wall muscles (LBWM) were used as references as they lack dense connective tissue and are thus a good model for studying the mechanical properties of isolated echinoderm muscles (Hill, 2004).

Nine bathing solutions were used in the different experiments: (1) ASW (artificial seawater made up of 445 mmol l^{-1} NaCl, 60 mmol l^{-1} MgCl_2 , 10 mmol l^{-1} KCl, 2.4 mmol l^{-1} NaHCO_3 , 10 mmol l^{-1} Hepes and 10 mmol l^{-1} CaCl_2 ; pH ~8); (2) ASW-EGTA [in which CaCl_2 was replaced by 2.5 mmol l^{-1} ethylene-bis-(oxyethylenetriolo)-tetraacetic acid]; (3) ASW-EGTA-TX (in which 1% Triton-X100 was added to the ASW-EGTA solution); (4) ASW-TX (ASW solution with 1% Triton-X100); (5) DW (deionised water); (6) High $[\text{Ca}^{2+}]$ (ASW with 100 mmol l^{-1} CaCl_2); (7) High $[\text{K}^+]$ (ASW with 100 mmol l^{-1} KCl and 355 mmol l^{-1} NaCl); (8) High $[\text{K}^+]$ -EGTA (High $[\text{K}^+]$ solution in which CaCl_2 was

replaced by 2.5 mmol l^{-1} EGTA); and (9) High $[\text{Mg}^{2+}]$ (ASW in which CaCl_2 was replaced by 10 mmol l^{-1} MgCl_2).

Traction tests

Traction tests were performed on tube feet of the two asteroid species to establish their tensile properties under various conditions (i.e. in eight different solutions). Each of the five arms of one individual was incubated for 1 h at room temperature in one of the bathing solutions, and the tests were performed and repeated with at least three different individuals for each species. These tests were arranged in two different experiments since each sea star only possesses five arms (two solutions were common to both experiments).

Measurements of the mechanical properties were carried out according to the protocol described in Santos et al. (Santos et al., 2005b). Mechanical traction tests were performed with a Mecmesin Versa test motorized stand fitted with an electronic force gauge that measures forces up to 10 N (Mecmesin AFG 10N, Horsham, UK), connected to a computer collecting the data. The precision of the tensile measurements was 0.002 N. The arms were placed upside down and maintained by hand. A small surgical clip was attached to one tube foot in the portion of the stem just under the disc and then pulled perpendicular to the specimen (in the direction of the natural extension) at a constant rate until failure. Failure never occurred at the clip. Before pulling the tube foot, the initial length of the stem (i.e. the distance between the base of the tube foot and the clip) was measured to the nearest mm with a ruler at the point at which the force started to increase and reached 0.003 N. The initial length of the tube foot, together with the time required to break the tube foot at a constant extension rate, was subsequently used to calculate the stem final length at failure.

During the mechanical tests, data were continuously recorded as force–extension curves, which were then converted into stress–strain curves (see Santos et al., 2005b). True values of strain and stress were used instead of nominal values because of the high extensions observed for tube feet of both sea star species (Shadwick, 1992). The connective tissue cross-sectional area was used for the calculation of the true stress because this tissue appears to be the layer bearing most of the load exerted on a tube foot (Santos et al., 2005b). Indeed, in echinoid tube feet, Santos and Flammang reported that the connective tissue resists extensions with forces bigger by about two orders of magnitude than those contributed by the muscle alone (Santos and Flammang, 2005). True strain expresses the deformation of the tube foot in response to a certain force and, at the point at which the stem fails (at final length), is a measure of the stem extensibility. Similarly, the maximum value of true stress (i.e. at breaking force) is an indicator of the stem tensile strength. Two other mechanical properties were calculated: the modulus of elasticity, the highest slope of the stress–strain curve, and the breaking energy density, the energy needed to extend and break the tube foot per unit of volume (product of the initial length of the tube foot and the connective tissue cross-sectional area). These are measures of the stem stiffness and toughness, respectively (for a review, see Vogel, 2003).

Unless indicated, tractions tests were performed at a constant extension rate of 25 mm min^{-1} , which corresponds to the range of natural extension rates measured for asteroid (Van Veldhuizen and Phillips, 1978) and echinoid tube feet (Leddy and Johnson, 2000). On the other hand, we were not able to reproduce the extremes in extension rate that wave-exposed sea stars are likely subjected to in nature ($\geq 1\text{ cm s}^{-1}$) (Denny et al., 1998) due to limitations of the motorized stand. Nevertheless, the influence of strain rate on the

mechanical properties of the tube feet was investigated in *A. rubens* and *M. glacialis*. Three individuals from each species were anaesthetized for 20 min in the High[Mg²⁺] solution and, for each of them, 10 tube feet were tested in this same solution at one of the following three extension rates: 25, 75 and 200 mm min⁻¹. The strain rate was calculated as the ratio of the extension rate to the initial length of the tube foot.

Similar traction tests were performed on the LBWM from three individuals of *H. tubulosa*. In this case, the muscle was connected to the stand and force gauge by two cotton threads tied around its ends. Mechanical properties were measured for LBWM that had been incubated for 1 h either in the ASW-EGTA solution or in the High[K⁺] solution. Before pulling the muscle, its initial length was measured to the nearest 0.01 mm using a digital calliper. In the case of LBWM, nominal values of strain and stress were used instead of true values (Shadwick, 1992). The LBWM cross-sectional area was calculated as the product of muscle width and thickness measured with the digital calliper before immersion in the solutions.

Contraction tests

Muscle contraction tests were performed with the same mechanical device as the one used in traction experiments, except that the gauge was replaced by a Mecmesin AFG 2.5 N (precision of 0.0005 N). For each of three sea stars from the two species, 20–30 tube foot-ampulla units were dissected in the middle area of the arms and were relaxed in the High[Mg²⁺] solution for 30 min. Two cotton threads were tied around the extremities of the tube foot: one was placed around the base of the stem, just above the ambulacral plates, and was connected to the stand; the other was placed at the apex of the stem, just below the disc, and connected to the gauge. Once the threads were tautened, the initial length of the tube foot was measured using a digital caliper. For most tube feet, the stem was then extended (by up to three times the initial length), and the elongated length was measured again. The High[Mg²⁺] solution was then replaced by the High[K⁺] solution, and the isometric muscle contraction force was recorded. When this force was stabilized (i.e. when a plateau was reached on the force–time curve), the motorized stand was turned on and the stem was extended to failure, as in a classical traction test.

Similar experiments were conducted on the LBWM from three holothuroids. In this case, the High[Mg²⁺] solution was replaced by the High[K⁺] solution directly after the measurement of the muscle initial length, width and thickness (no preliminary extension).

Adhesion tests

For both asteroid species, the force needed to detach a single tube foot from a glass substratum was measured with the same electronic force gauge as the one used for the measurement of stem mechanical properties (see 'Traction tests'), but this time the gauge was handheld. These experiments were conducted in natural (33‰ salinity) seawater with fresh living sea stars. A piece of microscope slide (~1 cm²), attached to the force gauge by a surgical thread, was presented to the tube feet of a sea star lying upside down in a container filled with seawater (Flammang and Walker, 1997). When a single tube foot attached to the substratum for at least 10 s, the gauge was manually moved upwards at approximately constant speed in order to apply a force normal to the disc. After tube foot detachment, the maximal force, or detachment force, was recorded. Then, the glass substratum was immersed for 1 min in a 0.05% (w/v) aqueous solution of the cationic dye crystal violet to stain the adhesive material (footprint) left by the tube foot after detachment (Flammang and Walker, 1997). In order to calculate the disc surface

area, the diameter of the footprint was measured using a light microscope equipped with a graduated eyepiece. A digital picture of the footprint was taken and its surface area was measured using the SemAfore 3.0 Pro[®] software (JEOL, Tokyo, Japan). The tenacity (expressed in Nm⁻² or Pascal) was then calculated by dividing the measured detachment force by the corresponding footprint surface area. Measurements were carried out on about 30 tube feet from five different individuals in *A. rubens* and on about 40 tube feet from 11 different individuals in *M. glacialis*. After each measurement, the piece of substratum was either replaced by a new piece or carefully cleaned.

Morphological analyses

The mean values of the cross-sectional area of each tissue layer of the tube foot stem from both sea star species were obtained using relaxed tube feet. For *M. glacialis*, tube feet were dissected from the arm placed for 1 h in the ASW-EGTA solution during the study of the stem mechanical properties. For *A. rubens*, tube feet were dissected from six individuals (different from those used in the mechanical tests) with arm lengths overlapping the size range for which tube foot mechanical properties were measured. These sea stars were previously anaesthetized by incubation in ASW containing 0.1% propylene phenoxetol for 1 h at room temperature. All tube feet were fixed in Bouin's fluid for 24 h. They were subsequently dehydrated in a sequence of graded ethanols, embedded in paraffin wax using a routine method and cut transversely into 10 µm-thick sections with a Microm HM 340 E microtome. The sections were mounted on clean glass slides and stained with Heidenhain's azan trichrome (Gabe, 1968). 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 feet.

For transmission electron microscopy (TEM), each arm of one individual of both asteroid species was placed for 1 h in one of the following solutions: (1) ASW; (2) ASW-TX; (3) High[K⁺]; (4) ASW-EGTA; and (5) DW. Several tube feet were dissected from the middle area of these arms and fixed for 3 h at 4°C in 3% glutaraldehyde in cacodylate buffer (0.1 mol l⁻¹, pH 7.8; adjusted to 1030 mOsm l⁻¹ with NaCl). They were then rinsed in the cacodylate buffer, post-fixed for 1 h in 1% OsO₄ in the same buffer, dehydrated in graded ethanols and embedded in Spurr resin. Transverse ultra-thin sections (70 nm in thickness) were cut with a Leica Ultracut UCT ultramicrotome equipped with a diamond knife, collected on copper grids and stained with uranyl acetate and lead citrate. Ultra-thin sections were observed with a Zeiss LEO 906E transmission electron microscope, and morphometric measurements were obtained using SemAfore 3.0 Pro[®] Software (JEOL).

Statistical analyses

All statistical tests were performed with the software Systat 9[®] (Tulsa, OK, USA). For traction tests, the results were analysed in order to look for significant intraspecific differences in the mechanical properties of the tube feet between the different bathing solutions within a single experiment (i.e. five solutions, one arm of each individual being tested in each solution). Data were analysed by a randomized block analysis of variance (ANOVA) followed by the multiple comparison test of Tukey (starfish individual was the blocking factor, and tested solution was the independent variable). The results were also analyzed in order to look for significant interspecific differences in terms of tube foot morphometry, mechanical properties and attachment strength by one- or two-way ANOVAs or by *t*-tests. For these tests, sea star individuals were

always used as the replicate (i.e. when more than one measurement was made on one individual, the mean value for this individual was used in the tests). Regression analysis was also used to search for significant relationships between asteroid arm length and tube foot tissue cross-sectional area, between the stem mechanical properties and strain rate and between tube foot extension ratio and muscle contraction force. The level of significance was set at $\alpha=0.05$.

RESULTS

Morphology of the tube foot stem

Histology and morphometry

The tube feet of the two asteroid species have a similar histological structure, the stem wall consisting of an outer epidermis, a basiepidermal nerve plexus, a connective tissue layer organized into an outer sheath and an inner sheath, and an inner myomesothelium (the so-called retractor muscle) that surrounds the water-vascular lumen (Fig. 1). Table 1 summarizes the morphometric measurements made on individuals of the two species and their tube feet. Regarding the stem external diameter, the tube feet of *M. glacialis* were significantly larger than those of *A. rubens*. However, this variation disappeared when the stem diameter was expressed relative to the length of the longest arm. In terms of the thickness of the stem tissue layers, no significant difference was found between the two species, except for the epidermis (including basiepidermal nerve plexus and cuticle), which was more developed in *M. glacialis*. The stem wall cross-sectional area was also larger in this species. But when this area was expressed relative to the total cross-sectional area of the stem (including the lumen area), the difference between *A. rubens* and *M. glacialis* disappeared for this parameter. In the two species, the retractor muscle and the epidermis were the dominant layers of the stem wall. The former was significantly more developed in *A. rubens* than in *M. glacialis*, and the latter was more developed in *M. glacialis*.

In *A. rubens*, a significant linear relationship ($R^2=0.70$, $N=6$) was found between the logarithm of the stem connective tissue cross-sectional area (S_{CT} ; μm^2) and the length of the longest arm of the sea star (AL ; cm) (Table S1 in supplementary material). This surface area can therefore be estimated according to the following equation:

$$S_{CT} = 10^{0.181AL+3.295} \quad (1)$$

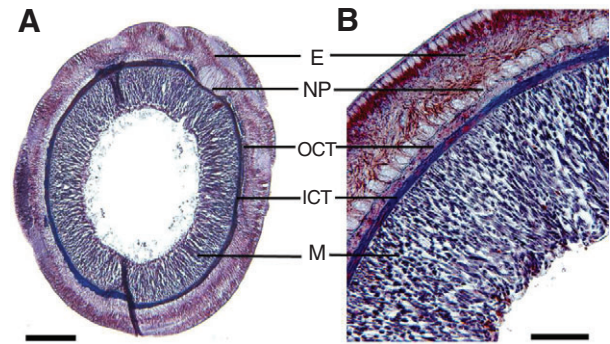


Fig. 1. Transverse sections (light microscopy) through the tube foot stem of *Marthasterias glacialis* (A; general view) and *Asterias rubens* (B; detail) illustrating the different tissue layers. E, epidermis; ICT, inner connective tissue layer; M, myomesothelium (retractor muscle); NP, nerve plexus; OCT, outer connective tissue layer. Scale bars: 200 μm (A), 50 μm (B).

A similar relationship ($R^2=0.63$; $N=6$) was observed between the stem retractor muscle cross-sectional area (S_{RM} , μm^2) and the length of the longest arm (Table S1 in supplementary material).

$$S_{RM} = 10^{0.140AL+4.368} \quad (2)$$

The cross-sectional areas calculated with these equations were used in the calculation of the tube foot mechanical properties.

In *M. glacialis*, no significant relationship was found between arm length and tissue cross-sectional areas. For this species, therefore, the tube foot mechanical properties for each individual were calculated using the tissue cross-sectional areas measured on tube feet dissected from the same individual.

Ultrastructure of the connective tissue layer

In both sea star species, the connective tissue layer was organized into two sheaths of equivalent thickness: an outer sheath made up of longitudinally oriented collagen fibres and an inner sheath of helicoidally oriented fibres (see also Flammang, 1996). Within the outer sheath, the collagen fibres were embedded in an electron-dense microfibrillar network. Cells containing electron-dense granules

Table 1. Mean morphometric values for individuals of two sea star species and for their tube feet

	Species		P
	<i>Asterias rubens</i>	<i>Marthasterias glacialis</i>	
Length of the longest arm (cm)	6.5 \pm 1.6	9.4 \pm 3.0	0.048
Tube foot measurements			
Stem diameter (mm)	0.795 \pm 0.150	1.018 \pm 0.104	0.005
Stem diameter relative to arm length (%)	1.261 \pm 0.216	1.149 \pm 0.235	0.361
Tissue thicknesses			
Stem wall (mm)	0.219 \pm 0.103	0.271 \pm 0.034	0.174
Epidermis (includes cuticle and nerve plexus) (mm)	0.065 \pm 0.026	0.112 \pm 0.015	0.001
Connective tissue (mm)	0.017 \pm 0.009	0.022 \pm 0.005	0.191
Retractor muscle (mm)	0.137 \pm 0.068	0.137 \pm 0.023	0.988
Tissue cross-sectional areas			
Stem wall (mm^2)	0.405 \pm 0.212	0.642 \pm 0.134	0.019
Stem wall cross-sectional area relative to stem cross-sectional area (%)	75.12 \pm 15.24	77.87 \pm 4.05	0.829
Epidermis (mm^2)	0.157 \pm 0.086	0.323 \pm 0.073	0.001
Epidermis cross-sectional area relative to the stem wall cross-sectional area (%)	38.30 \pm 1.79	50.10 \pm 1.95	<0.001
Connective tissue (mm^2)	0.036 \pm 0.024	0.053 \pm 0.013	0.100
Connective tissue cross-sectional area relative to the stem wall cross-sectional area (%)	8.38 \pm 1.44	8.40 \pm 2.32	0.972
Retractor muscle (mm^2)	0.212 \pm 0.103	0.267 \pm 0.059	0.213
Retractor muscle cross-sectional area relative to the stem wall cross-sectional area (%)	53.32 \pm 2.70	41.49 \pm 2.64	<0.001

Values are means \pm s.d. ($N=6$ for *A. rubens* and $N=9$ for *M. glacialis*). Percentages were arcsine transformed before their comparison with the *t*-test.

were observed in the outer sheath of the connective tissue layer of both species. These cells are similar in appearance to the juxtaligamental cells present in echinoderm MCTs (for a review, see Wilkie, 1996). They were interspersed between the collagen fibres and generally appeared as bundles of cell processes with occasional cell bodies (Fig. 2A,D). In the two species, two populations of cell processes were distinguished on the basis of the size of their electron-dense granules: one with granules of about 150 nm in diameter (type 1 granules) and one with granules of about 250 nm in diameter (type 2 granules) (Fig. 2B,D).

The ultrastructure of the connective tissue layer of tube foot stems that had been incubated in ASW-EGTA, High[K⁺], ASW-TX and DW was investigated (Fig. 2). The first two solutions did not seem to affect the ultrastructure of the juxtaligamental-like cells although, in High[K⁺], their secretory granules appeared slightly less numerous (no quantification was done; results not illustrated). Triton-X100 had no influence on these cells in *A. rubens* while it caused cell disruption in *M. glacialis* (Fig. 2B,E). Finally, events of cell lysis were observed in the two species for tube feet that had been incubated in DW (Fig. 2C,F). Cell fragments were observed, and the number of apparent granules was much lower in this solution than in seawater. However, the lytic effect seemed to be more important in *A. rubens* than in *M. glacialis*, in which some juxtaligamental-like cells still contained intact granules.

Mechanical properties of tube foot stem

Whole stem

When a tensile force was exerted on the tube feet of *A. rubens* and *M. glacialis*, their stem reacted in a similar way. The stress increased with the strain, first slightly and then more rapidly until stem rupture (Fig. 3). A J-shaped stress–strain curve was thus obtained, from which four mechanical properties (extensibility, strength, stiffness and toughness) were calculated. Mean values of the material properties measured in ASW for tube foot stems in the two sea star species are presented in Table 2. In this solution, the two species had similar extensibility, strength, stiffness and toughness.

The effect of eight solutions on the tube foot mechanical properties was tested in two different experiments. In both experiments, ASW was used as a standard solution and ASW-EGTA as a calcium-free medium (EGTA is a calcium chelator that removes the endogenous calcium from the tissues). In the first experiment, the other three solutions were treatments that disrupted cellular membranes: two made use of the non-ionic detergent Triton-X100, either in the presence (ASW-TX) or absence (ASW-EGTA-TX) of calcium, and the third – deionised water (DW) – worked by osmotic shock. In the second experiment, two solutions, High[K⁺] and High[Ca²⁺], were known to influence the physiological state and hence mechanical properties of different MCTs (Motokawa, 1981; Hayashi and Motokawa, 1986; Byrne 1985; Noskor et al., 2008).

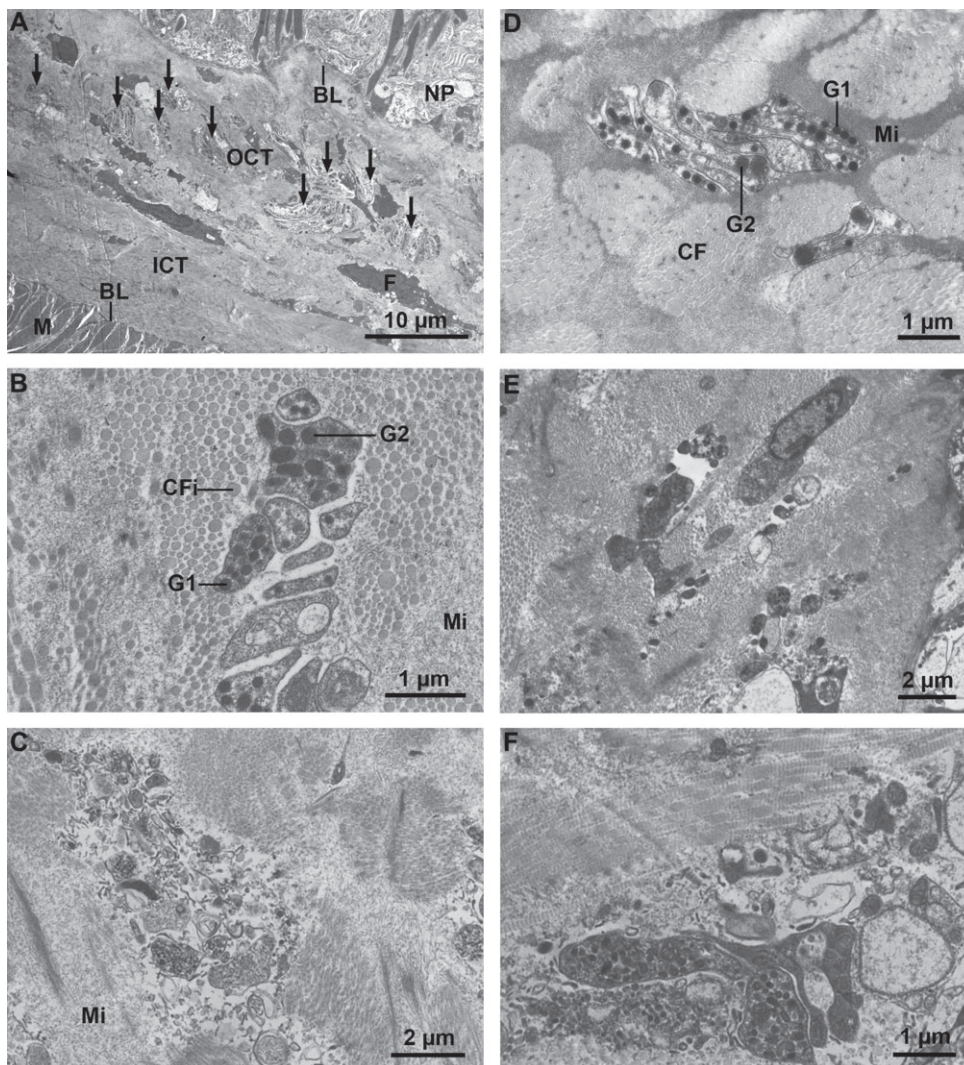


Fig. 2. Ultrastructure (TEM) of the juxtaligamental-like cells of the tube foot connective tissue layer in *Asterias rubens* (A–C) and *Marthasterias glacialis* (D–F) after incubation in ASW (A,D), ASW-TX (B,E) and DW (C,F). Arrows in A indicate the juxtaligamental-like cell processes. Abbreviations: BL, basal lamina; CF, collagen fibre; CFi, collagen fibril; F, fibrocyte-like cell; G1, type 1 granule; G2, type 2 granule; ICT, inner connective tissue sheath; M, myomesothelium (retractor muscle); Mi, microfibrillar network; NP, nerve plexus; OCT, outer connective tissue sheath.

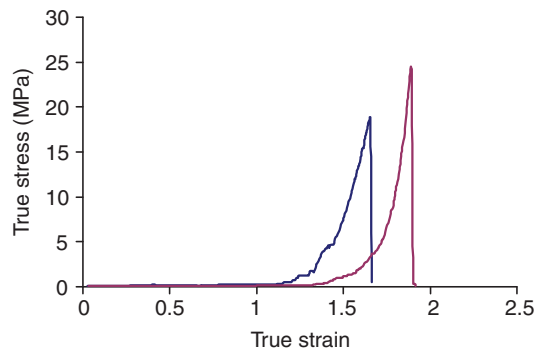


Fig. 3. Typical J-shaped stress–strain curves obtained for the tube feet of *Asterias rubens* (blue) and *Marthasterias glacialis* (red) in ASW.

Finally, High[K⁺]-EGTA was tested to look for possible combined effects of the previous solutions. The whole data set for material properties of the tube feet was analysed by a randomized block ANOVA (Table S2 in supplementary material), and the detailed results are presented in Fig. 4. The analysis showed significant differences between the bathing solutions for all the parameters in the two species, except for stem extensibility in *A. rubens* in the first experiment. In general, the treatment with ASW-EGTA induced a significant decrease of all tube foot mechanical properties, except extensibility, in comparison with ASW (Fig. 4). In the first experiment, addition of Triton-X100 in ASW significantly decreased the tube foot strength and stiffness in *A. rubens* in comparison with ASW (Fig. 4A). However, this treatment did not affect their toughness. A different response was observed for the tube feet of *M. glacialis*: the strength and stiffness were not influenced by the ASW-TX solution in comparison with ASW while the extensibility decreased and the toughness significantly increased. Addition of Triton-X100 in ASW-EGTA solution did not influence the strength and stiffness of tube feet in *A. rubens* but decreased their toughness in comparison with ASW-EGTA (Fig. 4A). On the other hand, the ASW-EGTA-TX solution caused a significant increase of all the parameters in the tube feet of *M. glacialis* in comparison with ASW-EGTA, except for the extensibility. In *A. rubens*, DW significantly increased tube foot strength, stiffness and toughness in comparison with the four other solutions tested (Fig. 4A). In *M. glacialis*, this solution caused an increase of tube foot mechanical properties in comparison with ASW-EGTA, except for the extensibility, but not in comparison with the other bathing solutions (Fig. 4A). In the second experiment, for both sea star species, the High[Ca²⁺] solution increased all tube foot parameters except extensibility in comparison with ASW-EGTA but also in comparison with ASW (Fig. 4B). A similar effect was observed for the High[K⁺] solution, though only in *A. rubens* (Fig. 4B). In *A. rubens*, the High[K⁺]-EGTA medium caused a decrease of all material properties' parameters in

comparison with the High[K⁺] solution. On the other hand, in both species, tube feet bathed in the High[K⁺]-EGTA solution showed a higher strength and stiffness than tube feet bathed in ASW-EGTA (Fig. 4B).

The analysis of strain-rate dependence of the tube foot mechanical properties in the High[Mg²⁺] solution using different extension rates demonstrated that, for both species, extensibility, breaking force, strength and final stiffness were positively dependent on strain rate (Fig. 5), the variation in strain rate accounting for 14–68% of the variation in the different parameters (Table S3 in supplementary material).

Retractor muscle

Because it is not possible to isolate the tube foot retractor muscle from the other tissue layers, traction experiments were performed on other echinoderm muscles, the holothuroid longitudinal body wall muscles (LBWM), which lack dense connective tissue. The results obtained for these muscles can then be used to extrapolate the mechanical properties of tube foot retractor muscle in sea star. We measured the force needed to break LBWM of *H. tubulosa* in a relaxed state (i.e. incubated for 1 h in the ASW-EGTA solution) and in a contracted state (i.e. incubated for 1 h in the High[K⁺] solution). This force was then converted to a nominal breaking stress value (σ_{LBWM} , MPa) by dividing by the cross-sectional area of the muscle, giving values of 0.059 ± 0.023 MPa and 0.206 ± 0.080 MPa for relaxed and contracted LBWM, respectively (mean \pm s.d., $N=3$). These breaking stresses are significantly different ($P=0.038$; t -test).

The reverse calculation was used to deduce the force (F_{RM} , N) needed to break the tube foot retractor muscle in each of these two solutions. This force was calculated according to the following equation:

$$F_{\text{RM}} = \sigma_{\text{LBWM}} \times S_{\text{RM}}, \quad (3)$$

where S_{RM} is the cross-sectional area of the tube foot retractor muscle expressed in m². It was then compared to the total force required to break the tube feet, which was measured in the traction tests performed in the ASW-EGTA and High[K⁺] solutions (Fig. 6). In order to estimate the contribution of the retractor muscle to the tube foot tensile strength, a ratio of the two forces was calculated. The percentages obtained range between 17 and 25% (Fig. 6). There was no significant difference in the contribution of the retractor muscle to the total tube foot breaking force between the two tested solutions, but a difference was detected between the two species (Table S4 in supplementary material).

Muscle contraction

The isometric contraction of the LBWM of *H. tubulosa* and of the tube foot retractor muscle of the two sea star species was measured after the application of the High[K⁺] solution. Indeed, potassium is a depolarising factor known to cause muscle contraction (Hill, 2004; Takemae and Motokawa, 2005). In *A. rubens* and *M. glacialis*, only

Table 2. Comparison between the mechanical properties of the tube foot stem for two sea star species in artificial sea water (ASW)

	Species		<i>t</i>	d.f.	<i>P</i>
	<i>Asterias rubens</i>	<i>Marthasterias glacialis</i>			
Extensibility	1.62 \pm 0.16	1.80 \pm 0.16	–2.00	12	0.069
Strength (MPa)	20.71 \pm 12.79	23.06 \pm 6.28	–0.41	12	0.688
Stiffness (MPa)	121.62 \pm 89.55	169.65 \pm 88.42	–1.00	12	0.338
Toughness (MJ m ^{–3})	3.49 \pm 1.85	3.66 \pm 0.87	–0.20	12	0.842

Values are means \pm s.d. ($N=8$ for *A. rubens* and $N=6$ for *M. glacialis*).

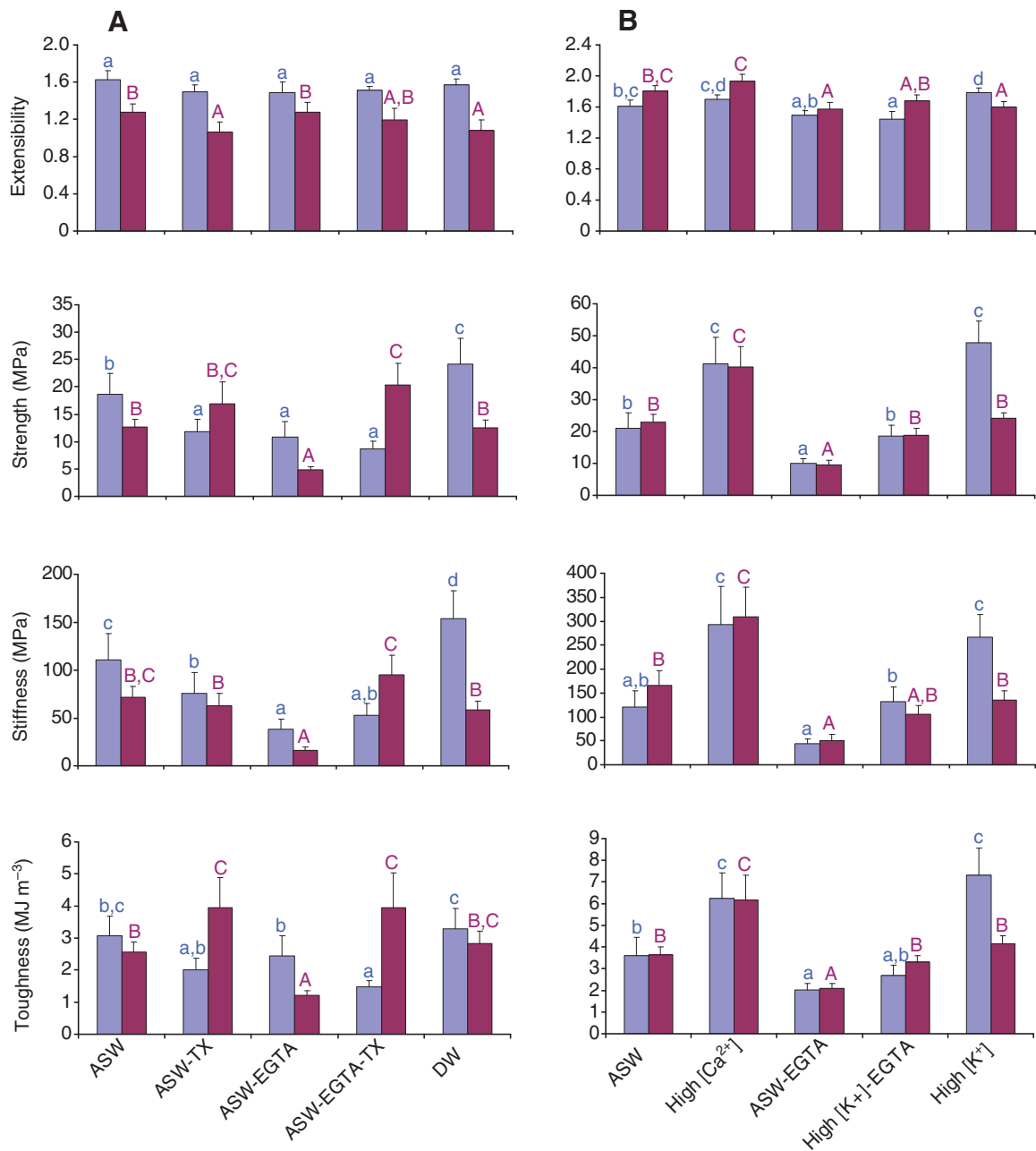


Fig. 4. Variation of the mechanical properties of the tube foot stems of *Asterias rubens* (in blue) and *Marthasterias glacialis* (in red) with different bathing solutions. Values are means \pm 95% C.I. for at least three individuals. (A) Experiment 1 ($N=3$ for the two species); (B) Experiment 2 ($N=5$ for *A. rubens* and $N=6$ for *M. glacialis*). Data analysed using randomized block ANOVAs; for each species and experiment, means sharing the same superscripts are not significantly different ($P \geq 0.05$; Tukey). Lowercase letters are used for *A. rubens*, and uppercase letters for *M. glacialis*. See text for abbreviations.

a weak contraction force was recorded for the tube feet at resting length (i.e. non-extended tube feet). The isometric contraction force of the retractor muscle was thus measured for different extensions of the tube feet. A polynomial relationship was found between this force and the tube foot extension ratio (i.e. the ratio between stem extended length, measured before the application of the High[K⁺] solution, and stem resting length) (Fig. 7). To avoid interindividual differences, contraction forces measured for the tube feet of one individual were expressed as percentages of the highest force measured for this individual. The determination coefficients (R^2) of the fitting curves are 0.5 for *A. rubens* and 0.22 for *M. glacialis*. In the two species, the contraction of the retractor muscle is maximal

for an extension ratio of approximately 2.3 and is weaker for smaller or higher extensions. The mean maximal contraction of the retractor muscle was therefore calculated for extension ratios ranging between 1.7 and 2.9, and is approximately 0.018 N in *A. rubens* and 0.016 N in *M. glacialis* (i.e. ~ 1.7 g for one tube foot). Table 3 summarizes these mean muscle contraction values together with those collected for LBWM of *H. tubulosa*. The mean contraction forces obtained were also expressed relative to the muscle cross-sectional area in order to allow comparisons between the holothuroid LBWM and asteroid tube foot muscles. No significant difference was found between the contraction force per unit cross-sectional area among the three muscles investigated ($P=0.372$; ANOVA).

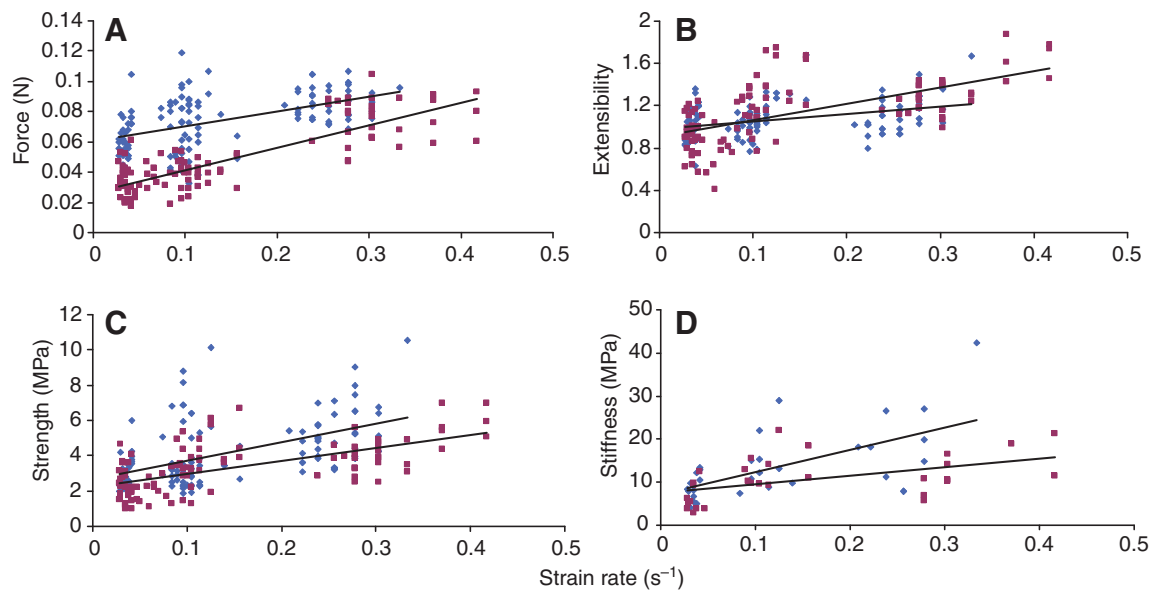


Fig. 5. Relationship between tube foot stem mechanical properties (A, breaking force; B, extensibility; C, tensile strength and D, final stiffness) and strain rate in *Asterias rubens* (blue) and *Marthasterias glacialis* (red). Lines are linear regressions ($P < 0.05$).

Adhesion strength of tube foot disc

For this experiment, two groups of sea stars of approximately the same sizes were chosen (Table 4). The mean force required to detach the tube feet from a glass substratum was similar for the two species (i.e. ~ 0.1 N; Table 4). In both *A. rubens* and *M. glacialis*, the footprints left on the substratum by the tube feet were not always complete, i.e. circular and homogeneous. Incomplete 'patchy' footprints were frequent and presumably left by tube feet that had adhered with only a fraction of their disc surface. Therefore, footprint surface area was measured as the surface area of stained adhesive material, which corresponds to the surface of the disc that effectively contacted the substratum (Table 4). Total adhesive surface area of the disc, on the other hand, was considered equivalent to footprint maximal surface area calculated from footprint diameter (Table 4). In terms of tenacity, no significant difference was observed between the strength of *M. glacialis* and that of *A. rubens* (0.43 and 0.24 MPa, respectively; Table 4). The tube foot maximum attachment

force was estimated by multiplying the tenacity by the total adhesive surface area of the disc. Like tenacity, this force did not differ significantly between the two species (Table 4).

DISCUSSION

Three tube foot morphotypes were distinguished in asteroids by Santos et al. (Santos et al., 2005c), based on the morphology of their distal extremity: knob-ending, simple disc-ending and reinforced disc-ending tube feet. The tube feet of the sea stars *A. rubens* and *M. glacialis* belong to the third morphotype. In this morphotype, the morphology of the distal extremity, the disc, is well adapted to strong attachment, which is necessary in turbulent environments. These two species belong to the family of Asteroiidae and are thus relatively close. This phylogenetic proximity comes with a high similarity in the histological structure of the tube feet. Indeed, the tissue organisation of the stem wall was very similar and the ultrastructure of the connective tissue layer was also constant between the two species. Morphological similarities were also reported for the disc (Flammang et al., 1994).

The importance of the tube feet in asteroiid biology has been emphasized by several workers (Smith, 1947; Nichols, 1966; Lawrence, 1987; Flammang, 1996). Indeed, these organs may be involved in many different activities such as locomotion, feeding or strong fixation to the substratum. These activities all rely on the mobility and tensile strength of the tube foot stem as well as on the secretion of an adhesive material by the tube foot disc (Flammang, 1996). Several different forces (i.e. disc adhesion forces, retractor muscle contraction forces and stem tensile forces) are therefore brought into play during these tube foot activities; forces which must be balanced for a proper functioning of the tube feet.

Disc adhesion

The disc is the component of the tube foot that secretes the adhesive that fastens the sea star to the substratum. Disc tenacity, measured as the force needed to detach the foot relative to the surface area in contact with the substratum, reflects the adhesive power of the tube

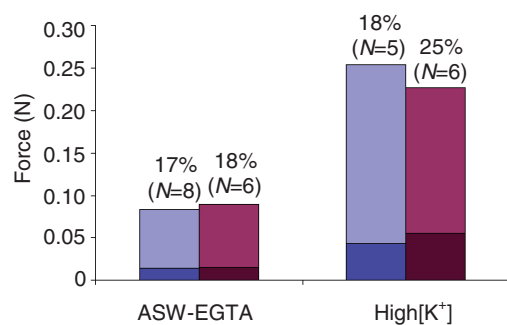


Fig. 6. Mean values of the forces needed to break whole stems (light colours) and of the estimated forces required to break the stem retractor muscle (dark colours) for tube feet of *Asterias rubens* (blue) and *Marthasterias glacialis* (red) bathed in different solutions. Percentages above the bars indicate the contribution of the retractor muscle to the total breaking force. See text for abbreviations.

Table 3. Mean isometric contraction of the retractor muscle of sea star tube feet and of the longitudinal body wall muscle of holothuroids

	Contraction force (N)	Contraction force/muscle cross-sectional area (MN/m ²)
<i>Asterias rubens</i>	0.018±0.007	0.048±0.010
<i>Marthasterias glacialis</i>	0.016±0.003	0.062±0.017
<i>Holothuria tubulosa</i>	0.266±0.056	0.063±0.013

Values are means ± s.d. (N=3).

foot. The mean tenacities measured in *A. rubens* and in *M. glacialis* (0.24 and 0.43 MPa, respectively) did not differ significantly and were also very close to data obtained by other authors [0.17 MPa in *Asterias vulgaris* (Paine, 1926); 0.20 MPa in *A. rubens* (Flammang and Walker, 1997)]. These values are in the same range as those observed in other marine invertebrates using non-permanent adhesion (0.1–0.5 MPa) and approach the adhesive strength of permanent adhesives, which is typically 0.5–1 MPa (Smith, 2006). From tenacity and disc surface area, mean maximal attachment forces were estimated: 0.15 N in *A. rubens* and 0.35 N in *M. glacialis*. Once again, these values are similar to the few maximal forces reported in the literature for single sea star tube feet [0.25 N in *M. glacialis* (Preyer, 1886); 0.29 N in *A. vulgaris* (Paine, 1926)]. However, these adhesion measurements were all done on smooth glass substrata. Thus, they cannot be extrapolated easily to what occurs in the natural conditions. Indeed, many studies have shown that several factors may profoundly influence the adhesion strength of invertebrates. Among these, the physico-chemical characteristics (e.g. roughness, hydrophobicity, surface charges) of the substratum are known to change the adhesion of organisms by up to an order of magnitude (Young and Crisp, 1982; Yule and Walker, 1987; Santos et al., 2005a). Such large differences have not been observed in asteroids but it was shown that the force required to detach a whole sea star was 20% higher on rock than on glass (Berger and Naumov, 1996) and that, on the contrary, tenacity of single tube feet on a mussel shell amounted only to about one-third of tenacity on glass (Flammang and Walker, 1997).

Retractor muscle contraction

The isometric contraction force of the tube foot retractor muscle averaged 18 mN and 16 mN for one tube foot in *A. rubens* and *M. glacialis*, respectively, with a maximum of 40 mN in both the species. Reports on contraction and pulling forces for asteroid tube feet are scarce in the literature. In *Asterina pectinifera*, Saha et al. measured a tube foot contraction of ~5 mN after application of acetylcholine (Saha et al., 2006). Kerkut measured a pulling force of 3 mN for a single tube foot of an individual of *A. rubens* climbing

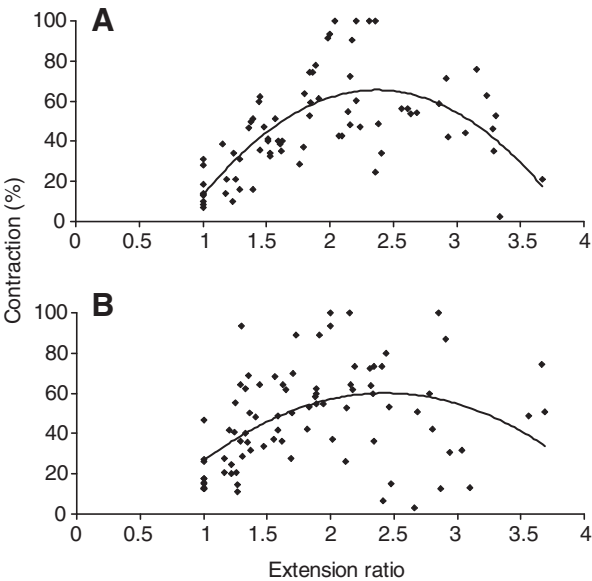


Fig. 7. Relationship between the isometric contraction of the retractor muscle and the stem extension ratio in the tube feet of (A) *Asterias rubens* ($y = -28.12x^2 + 133.13x - 91.98$, $R^2 = 0.5$, $P < 0.001$) and (B) *Marthasterias glacialis* ($y = -16.48x^2 + 80.14x - 37.30$, $R^2 = 0.22$, $P < 0.001$). The muscular contraction is expressed as a percentage in order to pool the results obtained for three different individuals. The contraction force measured for each tube foot of one individual was expressed as a percentage of the highest contraction force measured for this individual.

a vertical surface (Kerkut, 1953). In terms of isometric stress, the retractor muscle tensions measured were 48 and 62 kPa for *A. rubens* and *M. glacialis*, respectively. This stress was also measured in the LBWM of the holothuroid *H. tubulosa*, in which it was ~63 kPa. The contraction values from these three muscles were not significantly different. They were also in the range of what was observed for muscles of another sea cucumber, *Actinopyga mauritiana* (20 kPa) (Takemae et al., 2009).

The variation of the retractor muscle contraction with its extension ratio follows a typical bell-shaped length–tension curve that was identical in the two sea star species. This kind of curve was first described for vertebrate skeletal muscles and is a direct result of the degree of interdigitation of the thick and thin filaments within individual sarcomeres (Apon, 2003; Vogel, 2003). Although its underlying cellular basis is uncertain, smooth muscle has a length–tension curve similar to that of skeletal muscle but with a greater range of optimal lengths (Apon, 2003). It is generally

Table 4. Mean size of individuals of two sea star species and adhesion strength measurements on their tube feet

	Species		P
	<i>Asterias rubens</i>	<i>Marthasterias glacialis</i>	
Arm length (cm)	8.44±0.89	8.06±0.97	0.464
Detachment force (N)	0.13±0.04	0.12±0.07	0.947
Adhesive surface area (mm ²)			
Footprints	0.70±0.16	0.60±0.45	0.650
Disc	0.82±0.20	0.94±0.43	0.600
Tenacity (MPa)	0.24±0.12	0.43±0.38	0.294
Estimated maximum attachment force* (N)	0.15±0.07	0.35±0.27	0.139

Values are means ± s.d. (N=5 for *A. rubens* and N=11 for *M. glacialis*). *Calculated as the product of disc surface area and tenacity.

admitted that contraction is maximal when the muscle length is near its normal resting length (Apkon, 2003). Regarding the tube foot retractor muscle, the maximal contraction is obtained for an extension ratio of ~ 2.3 in both sea star species. This ratio, which corresponds to a nominal strain of 130%, is close to that observed for tube feet protracted during natural activities of sea stars, such as locomotion (McCurley and Kier, 1995; E.H., unpublished observations). On the other hand, contraction is minimal when the tube foot is unstrained (relaxed), suggesting that the retractor muscle is then shorter than its normal resting length. For the correct functioning of the tube foot, the relaxed length of the stem and the resting length of retractor muscle must therefore be different. If these lengths were identical, the maximal contraction of the muscle could only occur before the tube foot protraction, which would not provide any advantage to the animal. The elastic network of microfibrils present in the stem connective tissue could be responsible for the maintenance of the retractor muscle in a short state, since it presumably determines the tube foot resting length (Thurmond and Trotter, 1996).

Whole stem mechanical properties

Another important function of the tube foot stem is to resist the tensions imposed on the animal by hydrodynamics. It is usually admitted that the connective tissue layer is the stem tissue layer bearing most of the load exerted on a tube foot (Florey and Cahill, 1977; Santos and Flammang, 2005; Santos et al., 2005b). Recently, it was demonstrated that the connective tissue layer of the tube feet of the echinoid *Paracentrotus lividus* and of the asteroid *M. glacialis* has mutable properties (Santos et al., 2005b). The presence of juxtaligamental-like cells in the outer sheath of the stem connective tissue and the changes observed in the mechanical properties of tube feet bathed in different solutions also confirm the occurrence of a MCT in the tube foot stem in the species *A. rubens*. The presence of a MCT in their tube feet gives sea stars an obvious adaptive advantage. In its soft state, the MCT may facilitate the action of the ampulla muscle in tube foot protraction and of the retractor muscle in bending and retraction; in its stiff state, it could play a role in the energy-saving maintenance of position, for example during strong attachment to the substratum to resist external loads (Santos et al., 2005b). In *M. glacialis*, contrary to what occurred in sea urchin tube feet as well as in two other well-studied MCTs [the spine ligament of sea urchins (Szulgit and Shadwick, 1994) and the dermis of sea cucumbers (Trotter and Koob, 1995)], treatments causing cell lysis did not induce a significant stiffening of the stem connective tissue (Santos et al., 2005b). Although this was interpreted as a functional difference between echinoid and asteroid tube foot MCTs (Santos et al., 2005b), it is also possible that the solutions used were not effective on this species. In the present study, more solutions were therefore tested and the effect of some of them on the ultrastructure of the juxtaligamental-like cells was investigated.

The manipulation of calcium concentration in the bathing medium strongly influenced stem mechanical properties, as has been observed for several other MCTs (e.g. Byrne, 1985; Hayashi and Motokawa, 1986; Trotter and Koob, 1995; Szulgit and Shadwick, 2000; Santos et al., 2005b; Noskor et al., 2008). In both species, the tube feet became softer in ASW-EGTA in comparison with ASW. Conversely, the High[Ca²⁺] solution induced an increase in tube foot strength, stiffness and toughness in comparison with the ASW and ASW-EGTA solutions for both sea star species. Extracellular calcium probably affects tissue viscosity indirectly, by acting on cell secretion (Szulgit and Shadwick, 1994; Trotter

and Koob, 1995). Indeed, exocytosis depends on a pulse of calcium in the cytoplasm of the secretory cell (Alberts et al., 2002). An ionic gradient such as that artificially created with the High[Ca²⁺] solution could sufficiently increase the intracellular calcium concentration to trigger off the exocytosis of the contents of the granules enclosed in juxtaligamental-like cells, leading to the stiffening of the connective tissue. In the same way, the High[Ca²⁺] medium could cause the contraction of the retractor muscle. Alternatively, this solution could act on the nervous system, either at the level of the secretion of neurotransmitters, as explained above, or directly on the action potentials, which, in echinoderms, require calcium (Berrios et al., 1985; Cobb, 1987). Conversely, in the absence of calcium, these different cellular processes cannot take place, which could explain the relaxed state of the tube feet.

The High[K⁺] solution acted differently according to the species studied, stiffening the tube feet of *A. rubens* but not those of *M. glacialis* (compared with ASW). Potassium may have different effects (stiffening or softening) according to the MCT considered (e.g. Takahashi, 1967; Motokawa, 1981; Hayashi and Motokawa, 1986; Takemae and Motokawa, 2005). This solution, by its depolarising effect, stimulates excitable cells such as muscle cells and neurones, the latter being able to act indirectly on MCT *via* the juxtaligamental-like cells (e.g. Motokawa, 1984; Byrne, 1985; Wilkie, 1984; Wilkie et al., 1990; Takemae and Motokawa, 2005). It is not clear, however, why this solution had no effect on the tube feet of *M. glacialis* despite the relatedness between the two species. In *A. rubens*, the values of strength and stiffness of tube feet incubated in the High[K⁺]-EGTA solution appear to be intermediate between the values of these parameters in the ASW-EGTA and High[K⁺] solutions, which suggests antagonist effects.

As far as treatments causing cell lysis are concerned, tube feet were weaker and softer in ASW-TX than in ASW in *A. rubens*; conversely, this solution did not affect the strength and stiffness of the tube feet in *M. glacialis* (see also Santos et al., 2005b). Cell-disrupting solutions depleted of calcium (ASW-EGTA-TX and DW) have to be compared with ASW-EGTA rather than with ASW. In *A. rubens*, ASW-EGTA-TX had no effect, while DW increased all the parameters except extensibility. In *M. glacialis*, the situation was reversed: both solutions had an effect but ASW-EGTA-TX was more potent. This complex pattern of responses can be explained by the observation of the ultrastructure of the juxtaligamental-like cells after incubation in these solutions. Indeed, Triton-X100 did not lyse juxtaligamental-like cells in *A. rubens* while it did in *M. glacialis*. Regarding DW, although it caused the rupture of the juxtaligamental cells in the two species, its effect appeared to be more pronounced in *A. rubens*, in which the lysis seemed complete. Thus, in the stem of sea star tube feet, a complete disruption of the juxtaligamental-like cells in the absence of calcium leads to a stiffening of the MCT, as in the case of sea urchin tube feet (Santos et al., 2005b), with values of tensile strength higher than in ASW.

Contribution of the retractor muscle to the stem mechanical properties

In sea urchins, Florey and Cahill reported that the retractor muscle of the tube feet is very weak in comparison with the connective tissue and would be only weakly involved in the tube foot tensile strength (Florey and Cahill, 1977). This was confirmed by Santos and Flammang (Santos and Flammang, 2005), who calculated the possible contribution of the retractor muscle to the stem tensile strength using data collected from literature for muscle mechanical properties (Table S5 in supplementary material). They reported that the connective tissue resists extensions with forces bigger by about

two orders of magnitude than those contributed by the muscle alone. However, their muscle models (the frog skeletal muscle and the mussel anterior byssus retractor muscle) were not echinoderm muscles for which no data were available. Moreover, although the retractor muscle represents only about 20% of the stem cross-sectional area in echinoids (Santos and Flammang, 2005), it represents up to 50% of the stem wall in *A. rubens* and *M. glacialis*. Because the tube foot retractor muscle cannot be isolated from the other stem tissue layers, its contribution to the tube foot tensile strength was estimated using data collected for relaxed or contracted LBWM. Indeed, these muscles are considered as a good experimental model to study mechanical properties of echinoderm muscles (Hill, 2004), and the ultrastructure of their cells is very similar to that of the cells from the tube foot retractor muscle in sea stars (Wood and Cavey, 1981; Hill, 1993). Moreover, the similarity between the contraction stress of LBWM and that of the retractor muscles of *A. rubens* and *M. glacialis* (see above) confirms that data collected from holothuroid muscles could be applied to model tube foot muscles. After calculation, the force needed to break the tube foot retractor muscle was found to be 18% of the tube foot total breaking force in *A. rubens*, for both relaxed and contracted tube feet, while it accounted for 18 and 25% of the total breaking force for relaxed and contracted tube feet of *M. glacialis*, respectively. The latter higher value could be explained by the fact that in this species the High[K^+] solution did not seem to stiffen the MCT, thus indirectly increasing the contribution of the retractor muscle. In sea urchin tube feet, a similar calculation gives a muscle contribution of only 1% for both relaxed and contracted muscles. These results are not different from those calculated by Santos and Flammang (Santos and Flammang, 2005) using data from a frog relaxed skeletal muscle (Magid and Law, 1985) but do differ from those calculated using data from a mussel contracted muscle (Wilkie, 2002), which highly overestimated the contribution of the retractor muscle in the tube foot tensile strength (Table S5 in supplementary material). Therefore, in both echinoid and asteroid tube feet, the connective tissue is the tissue layer that supports most of the load exerted on the stem. However, for structures such as sea star tube feet, in which the relative muscle content is significant, the contribution of the retractor muscle to the stem tensile strength cannot be neglected.

Biomechanics of tube foot activities

A comparison of the distribution of forces brought into play during tube foot activities in *A. rubens* and *M. glacialis* (Fig. 8) gives a clue to how sea stars use their tube feet. For valid comparisons, only maximal forces recorded in all relevant experiments in this investigation were plotted, i.e. estimated maximal disc attachment forces, maximal stem breaking forces (measured in the High[Ca^{2+}] solution) and maximal retractor muscle contraction forces (measured on tube feet stretched from 2 to 2.5 times their original length). Forces appear well-balanced (retractor muscle contraction force \leq disc attachment force \leq stem breaking force) for proper functioning of the tube feet during the activities of the sea star.

Asteriids feed by inserting their stomach into thin slits occurring between the two valves of a closed mollusc shell (for reviews, see Jangoux, 1982; Lawrence, 1987). Force is usually used to produce the gap into which the stomach can be inserted. In this behaviour, numerous tube feet attach to the valves of the mollusc and then contract strongly (Christensen, 1957). Several workers have measured the total pull exerted by the tube feet during prey opening and have reported forces from 7 to 60 N (Feder, 1955; Christensen, 1957; Lavoie, 1956; Norberg and Tedengren, 1995). Taking into

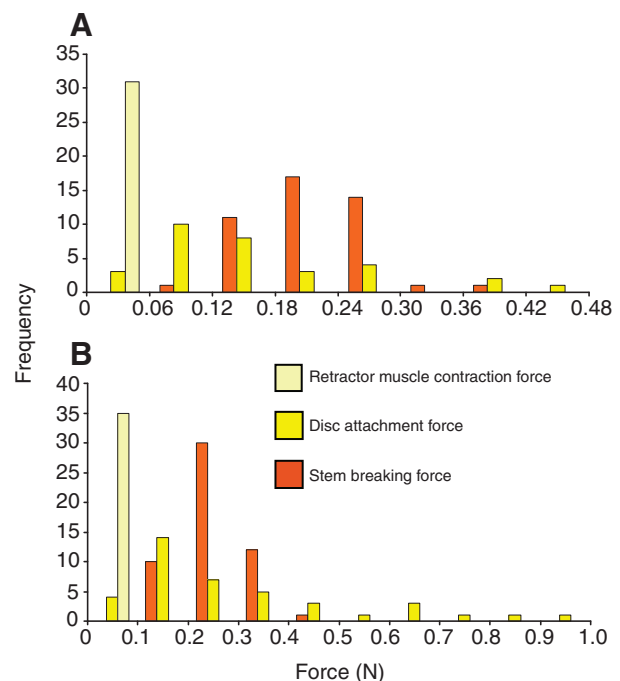


Fig. 8. Histogram of the maximal forces involved in the tube foot activities of (A) *Asterias rubens* and (B) *Marthasterias glacialis*. Forces recorded or estimated in all relevant experiments in this investigation: retractor muscle contraction forces measured at extension ratios around 2.3, maximal disc attachment forces calculated from tenacity and disc surface, and stem breaking forces measured in the High[Ca^{2+}] solution (see text for details).

account a maximal retractor muscle contraction force of ~ 0.04 N, these data indicate that hundreds of tube feet are used cooperatively in the feeding behaviour. An individual of *A. rubens* with arms ~ 5 cm long producing a 60 N force (Norberg and Tedengren, 1995) would therefore require all of its tube feet (a middle-sized sea star of this species possesses about 1500 tube feet; E.H. and G. Dewille, unpublished observations). However, during the entire opening process, the sea star also establishes itself firmly on the substratum using its distal tube feet (Christensen, 1957; Eylers, 1976; Jangoux, 1982; E.H., unpublished observations). It then humps up over the bivalve, thus stretching greatly the proximal tube feet attached to the mussel. This stretching places a passive tension on the stems and allows higher contraction forces to be produced (see above). Therefore, it appears that the total pulling force depends on both the stem active and passive tensions, the latter being produced by the body wall (Lawrence, 1987). It is also important that discs do not detach when stems contract. Disc attachment force is usually much higher than retractor muscle contraction force (Fig. 6), although the difference between the two forces would be smaller if, like tenacity, attachment force on mussel shell is three times lower than on glass (Flammang and Walker, 1997).

Tube feet can also function as strong holdfasts. This capacity of tube feet to adhere strongly to the substratum and act as tough tethers confers on the sea star a great resistance to wave-induced dislodgement (Siddon and Witman, 2003). In the field, a mean attachment force of 34 N has been measured for *Asterias forbesi*, largely exceeding the hydrodynamic force imposed by the flow regime on this sea star during calm days (i.e. ~ 3 N) (Siddon and Witman, 2003). If disc adhesion forces in this species are similar to those in *A. rubens* and *M. glacialis*, 100–200 tube feet would be

necessary to produce this overall attachment force. To respond to increased water velocities, sea urchins use more tube feet, thereby increasing their attachment force (Santos and Flammang, 2007). Sea stars probably rely on the same behaviour, and maximal attachment forces reaching several hundred Newtons can therefore be expected for asteriids. Such forces would allow sea stars to resist dislodgement even during storms [storm-generated hydrodynamic forces of 60 N have been calculated for *A. forbesi* (Siddon and Witman, 2003)]. Fig. 6 shows that, although the maximal forces needed to detach the disc are lower than the maximal stem breaking forces, the two distributions overlap considerably. This explains why, when manipulating sea stars in their natural environment or in an aquarium, some of their tube feet sometimes break at the level of the stem, leaving their disc attached on the substratum (Berger and Naumov, 1996; Flammang and Walker, 1997). It seems evident that this apparent 'weakness' of the stems is probably not the rule under natural conditions, as the sea star cannot afford to lose tens of tube feet each time it is subjected to an important load. A possible explanation for this paradox is that the strain rates used in our study ($0.02\text{--}0.42\text{ s}^{-1}$) are very far from the strain rates applied by waves ($\geq 1\text{ s}^{-1}$) (Denny et al., 1998). If the positive strain rate dependence of stem mechanical properties extends up to high strain rates, as has been demonstrated for other biomaterials [up to 0.7 s^{-1} for mussel byssal thread (Carrington and Gosline, 2004) and 30 s^{-1} for spider silk (Gosline et al., 2002)], rapid loads would enhance their capacity to withstand traction forces. Therefore, forces would also be well-balanced for proper functioning of the tube feet as holdfasts to secure the organism, and no tube foot component would appear as 'overdesigned'.

LIST OF SYMBOLS AND ABBREVIATIONS

AL	arm length
ASW	artificial seawater
C.I.	confidence interval
DW	deionised water
EGTA	ethylene-bis-(oxyethylenenitrilo)-tetraacetic acid
F_{RM}	force needed to break the tube foot retractor muscle
LBWM	longitudinal body wall muscle
MCT	mutable collagenous tissue
TEM	transmission electron microscopy
TX	Triton-X100
S_{CT}	stem connective tissue cross-sectional area
S_{RM}	stem retractor muscle cross-sectional area
σ_{LBWM}	nominal breaking stress of LBWM

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