Intra- and interspecific variation of attachment strength in sea urchins

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ABSTRACT: To withstand hydrodynamic forces, sea urchins rely on their oral tube feet, which are specialised for attachment. It has been proposed that the degree of development of these tube feet is intimately related to the maximum wave force a species can withstand. To address this, the variation of scaled attachment force and tenacity among and within echinoid species, and with environmental conditions, was investigated. Three populations of *Paracentrotus lividus* from different habitats and geographical regions were compared. There were few significant intraspecific variations in tenacity, but those that were detected were found to be positively correlated with the seawater temperature. For one *P. lividus* population, the influence of environmental parameters on the temporal variation of the attachment strength measured under laboratory and field conditions was analyzed. Strong significant correlations were found with wave height at the time of collection, but only when sea urchins were tested directly in their natural habitat, where they appear to respond to increased wave height by using more tube feet, thereby increasing their attachment force. Among species, *P. lividus* attached with a significantly higher tenacity (adhesion force per unit adhesive surface area) (0.37 MPa) than *Sphaerechinus granularis* (0.19 MPa) and *Arbacia lixula* (0.12 MPa). However, when the safety factor (which accounts for animal shape, size and number of adoral tube feet) was calculated, the larger *S. granularis* from calm deep subtidal habitats was predicted to be the first species to be dislodged at water speeds above 4.6 m s^{-1}, whereas the smaller *A. lixula* and *P. lividus*, which typically occur in shallow areas subjected to stronger hydrodynamic forces, were able to remain attached up to water velocities of 5.5 and 8.2 m s^{-1}, respectively.

KEY WORDS: Regular echinoids · Adhesion · Hydrodynamic disturbance · Tube feet · *Arbacia lixula* · *Paracentrotus lividus* · *Sphaerechinus granularis*

INTRODUCTION

Wave-swept shores are among the most stressful environments on earth. Near the shore, ocean waves create high water velocities and accelerations (Denny 1988, Bell & Denny 1994), imposing substantial hydrodynamic forces on intertidal and shallow subtidal marine organisms (Denny et al. 1985, Denny 1999). Most of the studies on hydrodynamic disturbance have focused on permanently attached organisms, such as macroalgae (Carrington 1990, Dudgeon & Johnson 1992, Friedland & Denny 1995, Gaylord & Denny 1997,

Milligan & DeWreede 2000, Kawamata 2001, Denny & Gaylord 2002, Pratt & Johnson 2002, Duggins et al. 2003) or mussels (Price 1980, 1982, Bell & Gosline 1997, Hunt & Scheibling 2001a,b, Carrington 2002a,b, Schneider et al. 2005). Mobile organisms face many of the same hydrodynamic challenges, but have received relatively little attention, although studies have been carried out on limpets (Branch & Marsh 1978, Denny 2000, Denny & Banchette 2000), whelks (Rilov et al. 2004), echinoderms (Gallien 1986, Denny & Gaylord 1996, Kawamata 1998, Siddon & Witman 2003), and crabs (Martinez 2001, Lau & Martinez 2003).

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In a wave-swept environment, the survival of an organism depends on its ability to withstand the hydrodynamic forces generated by breaking waves (Bell & Gosline 1997, Milligan & DeWreede 2000). To assess risk of dislodgment of an organism, the nature of the water flow, the resultant hydrodynamic forces and the organism's ability to resist those forces must be characterized (Lau & Martinez 2003). Sea urchins are common inhabitants of the rocky intertidal environment, being able to withstand mechanical stress by a combination of strong adhesion to the substratum and ability to move, through voluntary attachment–detachment cycles of their tube feet (Thomas & Hermans 1985, Flammang 1996, Flammang et al. 2005). Attachment is achieved by means of a multitude of independent adhesive organs, the tube feet. Each tube foot consists of an enlarged and flattened distal extremity, the disc, which makes contact with and attaches to the substratum, and a proximal extensible cylinder, the stem, which connects the disc to the animal's body. The epidermis of the disc encloses a duo-gland adhesive system comprising 2 types of cells: cells releasing adhesive secretions and cells releasing de-adhesive secretions, allowing sea urchins to attach and detach repeatedly from the substratum (Flammang 1996).

The strength with which individual sea urchins attach to a surface is determined by the tube foot tenacity and the number of tube feet involved. These factors, together with the size and shape of the test with spines, determine the maximum wave force that each species can withstand and, hence, the distribution of species (Smith 1978). In general, species that inhabit high-energy environments are reported to have a higher ability to remain attached for longer periods of time and to withstand much greater pull than species from calmer environments (Sharp & Gray 1962, Märkel & Titschack 1965). Their superiority is generally attributed to a higher degree of development of their tube feet, i.e. bigger discs and stronger and thicker stems (Sharp & Gray 1962, Smith 1978), combined with a higher number of tube feet involved in attachment (Märkel & Titschack 1965). Only a few experimental studies have integrated species attachment strength with animal body size and shape in order to estimate the probability of dislodgment in relation to hydrodynamic conditions (Gallien 1986, Denny & Gaylord 1996, Siddon & Witman 2003). In these studies, attachment forces were measured and reported as scaled forces (the force is normalized to the organism size and expressed either in N or in N m^{-2}). Other studies reported echinoid attachment strength as a tenacity (force per unit adhesive surface area expressed in N m^{-2} [=Pa]; Flammang et al. 2005, Santos & Flammang 2006). Contrary to tenacity which corresponds to a material property (i.e. the strength of the holdfast), scaled attachment force, even when expressed in N m^{-2}, should not be interpreted as a material property, but rather as a size-independent force (Bell & Gosline 1997). For organisms possessing a simple holdfast (e.g. barnacles or gastropod molluscs), the scaled attachment force correlates with the tenacity, as the size of the adhesive organ is generally proportional to the size of the animal (Grenon & Walker 1981, Rilov et al. 2004). This is, however, not necessarily the case for organisms possessing a multi-tethered holdfast (e.g. mussels or echinoderms) because they can use a variable number of individual tethers. Therefore, scaled attachment force is proportional to the number of tethers used, and is not necessarily correlated with tenacity, which is independent of the number of tethers involved. We evaluated the attachment strength of sea urchins by measuring their scaled attachment force (size-independent attachment force) and their tenacity (force per unit adhesive surface area). The variation of these 2 parameters among and within species, and with environmental conditions, was then investigated.

MATERIALS AND METHODS

Study sites and specimen collection. The 3 species of sea urchins were collected in the Mediterranean Sea (November 2002), in a subtidal rocky area, located at the south-western end of France (Banyuls-sur-mer, 42° 29' N, 03° 08' E). The arbacioid *Arbacia lixula* (Linné, 1758) and the echinoid *Paracentrotus lividus* (Lamarck, 1816) were collected on vertical rocky boulders of considerable size, in a shallow area (1 to 3 m) exposed to wave action. Although the populations of the 2 species overlap, individuals of *A. lixula* were usually observed slightly deeper than those of *P. lividus*. The temnopleuroid *Sphaerechinus granularis* (Lamarck, 1816) was found in deeper areas (~10 m) with lower hydrodynamism, on gravel seabed or amongst *Posidonia* seagrasses. Two other populations of *P. lividus* were sampled: one from an aquaculture facility and one from an intertidal rocky area exposed to wave action. The aquaculture facility was located in the north of France (Luc-sur-mer, 49° 31' N, 00° 35' E). Individuals from this population have been bred in aquaria for several generations. The other population of *P. lividus* was sampled on the central west coast of Portugal (Cabo Raso, 38° 42' N, 09° 29' W) on a gently sloping rocky platform. After collection, sea urchins were kept in aquaria and placed inside net bags to prevent attachment to the aquarium walls.

Sea urchin tenacity, scaled attachment force and morphometry. Tenacity measurements of individual sea urchins were performed with an electronic force gauge (Mecmesin AFG 250 N) that measures forces up

to 250 N with a precision of 0.01 N. Experiments were performed with sea urchins totally immersed in small aquaria filled with seawater, the walls of which were covered internally by removable glass plates. Animals were allowed 15 min to attach, after which the area where each sea urchin was sitting was delimited on the external side of the aquarium for later identification of the attachment zone. Then, attached sea urchins were clamped by the test with a metal grab (test tube holder) connected by a hook to the electronic force gauge, and manually pulled at approximate constant speed. After detachment, the maximum force required to dislodge the sea urchin was recorded, as well as the angle of pull (0, 45 or 90° from the plane of the substratum), which varied according to the animal's position in the aquarium (on the side wall, in the corner or on the bottom, respectively). The glass plate(s) to which the animal was attached were removed from the aquarium, the broken tube feet were counted and discarded, and the plate(s) immediately immersed in a 0.05% aqueous solution of the cationic dye crystal violet for 1 min, in

order to stain the footprints left by the tube feet (Flammang et al. 1994, Santos & Flammang 2006). Digital photographs of the plate(s) bearing footprints were taken, each picture being calibrated with a piece of millimetric paper (Fig. 1A). Then, these pictures were used to measure the total surface area of the footprints with the software Scion Image® 4.0.2 (Fig. 1B). The total number of footprints was also counted, as well as the number of circular footprints (tube foot attached with the entire disc; Fig. 1C) and irregular footprints (tube foot attached with part of the disc; Fig. 1D). The tenacity of individual sea urchins (T) was then calculated as the ratio of attachment force (F, expressed in N) to footprint surface area (S, expressed in m^2). T is expressed in $N\ m^{-2}$ or Pascal (Pa):

$$T = F/S \qquad (1)$$

In some experiments, sea urchin adhesion on rock was quantified. As, in this case, it was not possible to visualize the footprints and measure their surface area, scaled attachment force was used instead of tenacity.
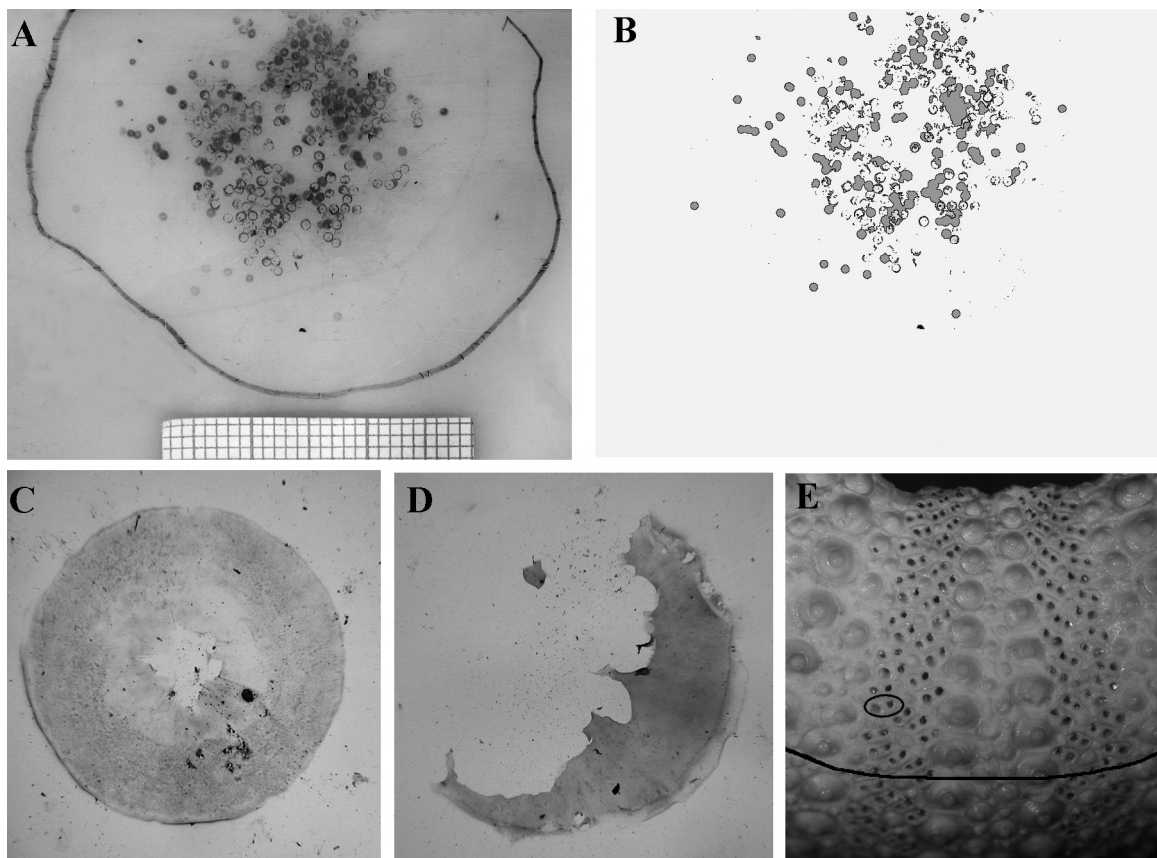

Fig. 1. (A) Stained footprints of *Sphaerechinus granularis* tube feet. (B) The same footprints outlined by the software Scion Image® 4.0.2 and used in the calculation of footprints total surface area. (C) Circular footprint left by a tube foot of *Paracentrotus lividus*. (D) Irregular footprint left by a tube foot of *P. lividus*. (E) Cleaned test (oral side up) of *P. lividus* showing the adhesive adoral surface (limited by the line) and the pores (each pair of pores represents one tube foot, as indicated by the oval)

The scaled force (F_{scaled}) was calculated by multiplying each individual attachment force (F) by the ratio of mean to individual test diameter (D_{m} and D_{i}, respectively); it is expressed in N:

$$F_{\mathrm{scaled}} = F (D_{\mathrm{m}}/D_{\mathrm{i}}) \qquad (2)$$

Immediately after each experiment, the animals' ambital diameter and height of the test (excluding spines) were measured to the nearest 0.01 mm using a digital caliper and the immersed weight was measured using a net basket connected to an electronic dynamometer (Mecmesin AFG 10 N) that measures forces up to 10 N with a precision of 0.001 N. Moreover, 5 animals (with sizes falling within those ranges for which attachment strength was measured) of each species were cleaned to remove their tube feet and spines in order to quantify the total number of adoral tube feet. The adhesive adoral area (containing adoral tube feet responsible for attachment) was defined as the area corresponding to 80% of the adoral area of the test (black line in Fig. 1E). The total number of adoral tube feet was obtained by counting the number of pores per row (2 pores correspond to 1 tube foot; black circle in Fig. 1E) in each ambulacrum within the adhesive adoral area.

All statistical tests were performed with the software Statistica®. When necessary, data were log-transformed to achieve homoscedasticity and percentages were arcsine transformed to achieve normality. The null hypothesis was rejected at a significance level (p) of 0.05. When analysis of variance (ANOVA) indicated significant effects, the Tukey test was used for pairwise multiple comparisons. Simple linear regression analysis was also used to search for a significant relationship between the number of adoral tube feet and the test diameter for the 3 species studied.

Safety factor. In order to quantify the adhesion strength of sea urchins relative to the impact of the waves on them, a safety factor (SF) was calculated as the ratio of mean sea urchin attachment force (F) to mean maximum hydrodynamic force ($F_{\mathrm{environment}}$) (Lowell 1985):

$$SF = F/F_{\mathrm{environment}} \qquad (3)$$

When this index is ≤1, it indicates that the animal is dislodged. The maximum hydrodynamic force exerted on sea urchins by waves or currents is due to both the water's velocity and its acceleration (Denny 1988). However, Siddon & Witman (2003) showed that forces imposed on sea urchins from acceleration are minimal and, therefore, they will not be considered in this study. The maximum hydrodynamic force was then obtained using the following equation (Denny 1988):

$$F_{\mathrm{environment}} = \tfrac{1}{2} \rho \, u^2 \sqrt{[(C_{\mathrm{drag}} S_{\mathrm{profile}})^2 + (C_{\mathrm{lift}} S_{\mathrm{planform}})^2]} \qquad (4)$$

in which ρ, seawater density (1024 kg m^{-3}); u, the water velocity in m s^{-1}; C_{drag}, the drag coefficient; S_{profile}, the surface area (expressed in m^2) of the sea urchin on which the drag force is exerted (force in the direction of the flow); C_{lift}, the lift coefficient; S_{planform}, the surface area (expressed in m^2) of the urchin on which the lift force is exerted (force perpendicular to the direction of the flow). No drag and lift coefficients for the 3 species considered in this study were available in the literature, so the coefficients proposed by Denny & Gaylord (1996) for *Strongylocentrotus purpuratus* were used:

$$C_{\mathrm{drag}} = 0.922 - 0.057 \log Re \qquad (5)$$

$$C_{\mathrm{lift}} = -0.165 + 0.047 \log Re \qquad (6)$$

in which Re, Reynolds number, was calculated as:

$$Re = \rho \, u \, L/\mu \qquad (7)$$

where L is sea urchin length in the direction of the flow in m, and μ is the dynamic viscosity of seawater in kg m^{-1} s^{-1}. The latter was deduced from Vogel's (1994) measurements of seawater viscosity at different water temperature (t in °C) which was shown to vary negatively and exponentially according to the following equation ($r^2 = 0.99$, p = 0.01):

$$\mu = 0.0018 \exp(-0.0259 \, t) \qquad (8)$$

Mean projected profile and planform areas were estimated using 3 animals of each species whose sizes fell in the range of sizes of the animals for which attachment strength was measured. Digital photographs of each individual were taken laterally (i.e. in the direction of the flow, Fig. 2A–C) and from above (i.e. in the direction perpendicular to the flow, Fig. 2D–F). The surface areas (S_{profile} and S_{planform}, respectively) were then calculated with the software
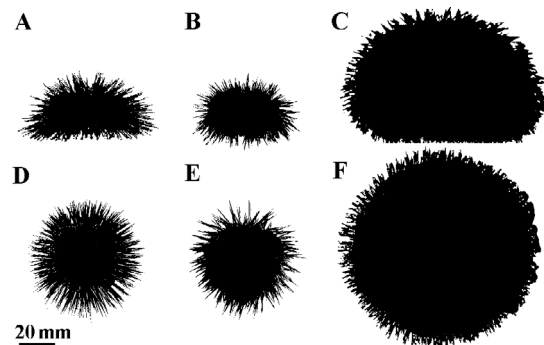

Fig. 2. (A,D) *Arbacia lixula*, (B,E) *Paracentrotus lividus* and (C,F) *Sphaerechinus granularis* (Banyuls-sur-mer, France). Projected surface areas of sea urchins outlined and shaded with the software Scion Image® 4.0.2. (A–C) Silhouettes of the sea urchins in the direction of the flow (S_{profile}); and (D–F) in the direction perpendicular to the flow (S_{planform})

Scion Image® 4.0.2, the pictures being calibrated with millimetric paper.

Two safety factors were calculated: one using the mean attachment force measured during experimental dislodgment and another using an estimation of the maximum attachment force that each species could produce if attached with all their adoral tube feet. This maximum force (F_{max}) was calculated by multiplying the tenacity (T) by the total number of adoral tube feet (TF) and by the surface area of a single tube foot disc (S_d):

$$F_{max} = T \, \text{TF} \, S_d \qquad (9)$$

The surface area of a single tube foot was obtained from measurements of the largest circular footprints left on glass substratum by each species (Santos & Flammang 2006).

Influence of temporal fluctuations in hydrodynamism. A possible effect of the temporal changes in the wave climate on sea urchin attachment was examined on the population of *Paracentrotus lividus* from Cabo Raso. At 6 different periods over 2 yr (May, July and December 2002, and March, July and October 2003), adhesion was measured in sea urchins in their natural rocky area, and in sea urchins collected from the same area and then tested on glass substratum in laboratory conditions. For field experiments, performed at low tide, 30 randomly chosen sea urchins (avoiding those located in crevices or depressions) were clamped by the test and dislodged. The force of attachment was recorded as well as the number of broken tube feet, test dimensions and immersed weight. The scaled attachment force was then calculated as described above. For laboratory experiments, 30 other randomly chosen sea urchins were collected and transferred to open-circuit aquaria, where they were kept in net bags to prevent attachment to the aquarium walls. Tenacity was then measured for individuals attached to glass plates, according to the procedure previously described.

Values of 6 hydrodynamic parameters were obtained from the nearest oceanographic buoy located approximately at 90 km south of Cabo Raso (Sines 35° 55′ 22″ N, 08° 55′ 48″ W). The parameters considered were the significant wave height (the average height of the highest one-third of waves recorded [m]); the maximum wave height (m); the mean wave period (s^{-1}); the maximum wave period (s^{-1}); the period of the highest wave (s^{-1}) and the water temperature (°C). Mean values of these parameters over various periods of time (i.e. 12 h, 1 d, 3 d and 7 d) before sea urchin collection were calculated from the hourly measurements from the buoy. Correlation analysis was used to look for significant relationships between the scaled attachment force measured under field conditions or the tenacity measured under laboratory conditions and the hydrodynamic parameters. Furthermore, for each sampling period, the data of maximum wave height at the moment of sampling were used to calculate maximum water velocity (u in m s^{-1}) according to Denny's (1988) formula for gently sloping shores, as is the case at Cabo Raso:

$$u = 0.3 + \sqrt{[g\,(H + d)]} \qquad (10)$$

where g is the acceleration due to gravity (9.81 m s^{-2}), H the mean maximum wave height (m) for the first 12 h on the day of sampling and d the water height during sampling (≈ 0.25 m at low tide). This calculated maximum water velocity allowed us to estimate the safety factor for the sea urchins on the day of sampling, using the mean scaled attachment force measured at that moment. A curve was fitted to the relationship between the safety factor and water velocity.

RESULTS

Intraspecific analysis

Influence of type of substratum

To evaluate the influence of the type of substratum on the attachment of individual sea urchins, specimens of *Paracentrotus lividus* from the population of Banyuls-sur-mer were tested on glass and rock substrata under laboratory conditions. Since footprint surface area cannot be measured on rock substratum because stained footprints are indistinguishable on this dark, non-transparent substratum, only scaled attachment force was calculated. No significant difference was found between the scaled force required to dislodge individuals of *P. lividus* on glass (11.70 ± 7.35 N) and rock (10.61 ± 4.19 N) substrata ($p_{t\text{-test}} = 0.48$).

Influence of temporal fluctuations in hydrodynamism

Temporal variation in sea urchin attachment was investigated in the population of *Paracentrotus lividus* from Cabo Raso at 6 different periods over 2 yr. During one of the periods (December 2002), bad weather precluded any direct measurement in the field and only laboratory tests were done. Linear regression analysis showed that both scaled attachment force and tenacity were always size and and weight independent (p > 0.05) during all the sampling periods in both field and laboratory experiments, thus allowing comparisons among the different periods. In field experiments, there were significant differences between the scaled attachment forces ($p_{\text{ANOVA}} = 0.02$), with the highest
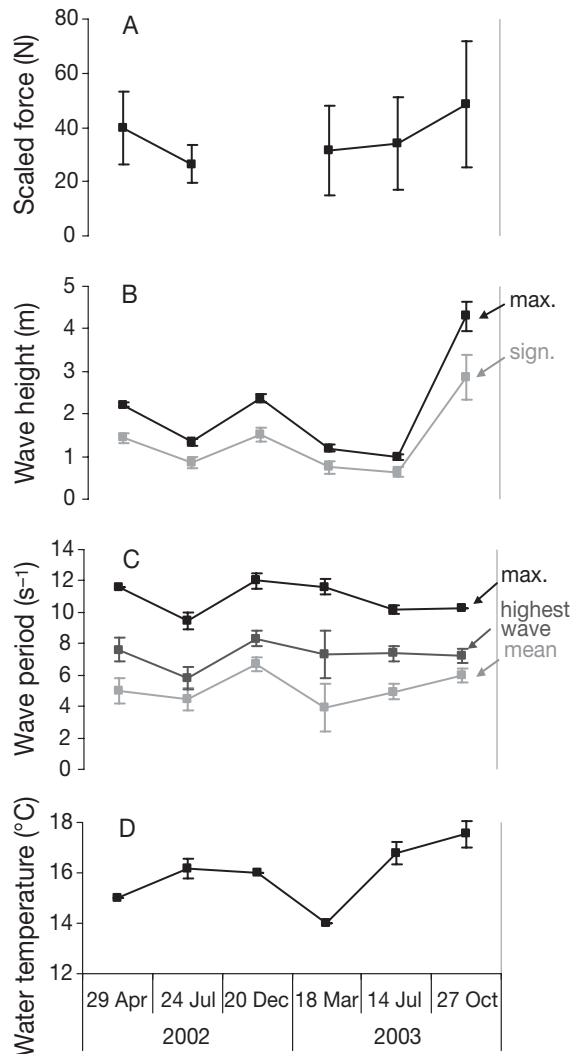
Fig. 3. *Paracentrotus lividus* (Cabo Raso, Portugal). Temporal variation of scaled detachment force of sea urchins (mean ± SD, n = 30) measured in the field (A), and of various hydrodynamic parameters (mean ± SD, n = 72); namely wave height (B), wave period (C) and water temperature (D) (see text for explanations). sign.: significant; max.: maximum. For (A) no sample was taken in December. NB: the data points are connected by lines to facilitate comparison, but the lines do not imply interpolation between periods

Table 1. *Paracentrotus lividus* (Cabo Raso, Portugal). Correlations between the mean scaled attachment forces measured in the field and the mean hydrodynamic parameters during 1, 3 and 7 d before sampling. Values are coefficients of correlation; asterisks indicate significant correlations

	Days before sampling		
	1	3	7
Significant wave height (m)	0.91*	0.98*	0.67
Maximum wave height (m)	0.91*	0.98*	0.68
Mean wave period (s^{-1})	0.88*	0.75	−0.15
Maximum wave period (s^{-1})	0.32	−0.43	−0.30
Period of the highest wave (s^{-1})	0.62	−0.18	−0.29
Water temperature (°C)	0.50	0.45	0.46

lated with the variation in the significant and maximum wave heights (r = 0.90, p = 0.03) (Fig. 3A,B). Conversely, no significant correlation was found with the other hydrodynamic parameters (Fig. 3A,C,D). The hydrodynamic parameters were also averaged over periods covering 1, 3 and 7 d before sampling. Once again, strong and positive correlations were observed between the variation in scaled attachment force and that of wave height, up to 3 d before sampling (Table 1). However, for 7 d, no correlation was found between scaled force and any of the hydrodynamic parameters (Table 1).

The scaled attachment forces measured in the laboratory were up to 4 times lower than those measured in the field, and their respective fluctuations over the study period were not correlated (r = 0.46, p = 0.43). The tenacity calculated for the laboratory experiments with Cabo Raso sea urchins differed significantly among sampling periods, with the lowest value obtained in April 2002 (0.14 MPa) and the highest one in July 2003 (0.33 MPa) (Fig. 4A). Tenacity was not significantly correlated with any of the hydrodynamic parameters (p > 0.1), though a coefficient of correlation superior to 0.5 was found with water temperature.

Mean scaled attachment forces measured in the field and water velocity at the moment of sampling were used to calculate safety factors, which ranged from 1.20 to 2.85 (Fig. 5). A regression analysis was used to model the data using Eqs. (3) & (4) (Fig. 5, solid line). The relationships between the safety factor (SF) and water velocity (u, m s^{-1}) was found to be:

$$SF = 50 \, u^{-2} \qquad (11)$$

According to this relationship, *Paracentrotus lividus* from Cabo Raso would be predicted to be dislodged at a water velocity of about 7.0 m s^{-1} (Fig. 5). Another curve was calculated according to Eq. (9) using the average tenacity over the 6 sampling periods (i.e. 0.25 MPa) and the estimated total adhesive area (i.e.

mean value measured in October 2003 (48.5 N) and the lowest in July 2002 (26.6 N) (Fig. 3A). In terms of the hydrodynamic parameters, some seasonal differences could be identified. The fall and winter seasons (October to December) were generally characterized by highest waves with highest mean wave periods. Spring (March to April) had moderately high waves but always the lowest water temperatures, while summer (July) was the season with the lowest hydrodynamism. The fluctuations in the scaled attachment forces measured in the field were positively and strongly corre-
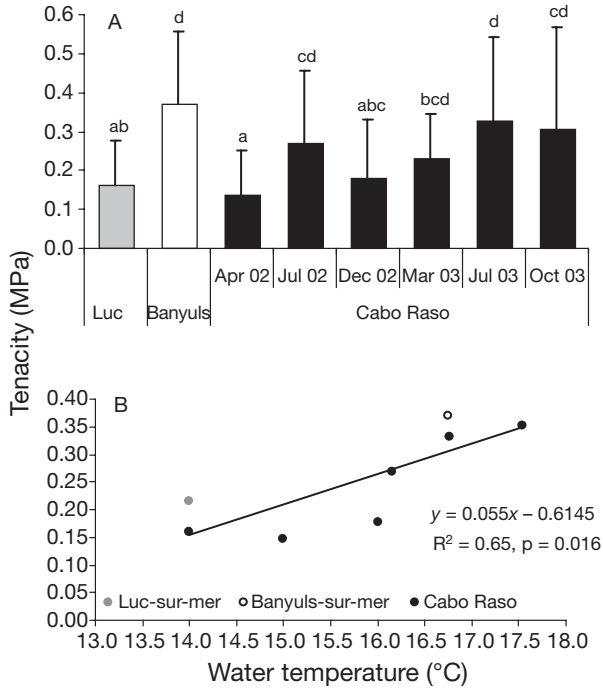
Fig. 4. *Paracentrotus lividus* from Luc-sur-mer (aquaculture facility, France), Banyuls-sur-mer (France) and Cabo Raso (Portugal). (A) Mean values (+SD, n = 30) of tenacity measured on individual sea urchins attached to glass substratum. Significant differences between the means are indicated by letters; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$). (B) Relationship between the mean tenacity of individual sea urchins attached to glass substratum and water temperature

$125 \ mm^2$). Using this second curve, sea urchins from the same population are predicted to be dislodged at a water velocity of about 6.1 m s^{-1} (Fig. 5, dashed line).

Variations in tenacity among populations

The tenacity of individuals of *Paracentrotus lividus* from the population of Luc-sur-mer (the aquaculture population, in which tenacity measurements were also size and weight independent) and Banyuls-sur-mer (see below) were also compared with each other and with different values measured for the sea urchins at Cabo Raso (Fig. 4A). The tenacity of sea urchins from Luc-sur-mer (0.16 MPa) was less than half that of sea urchins from Banyuls-sur-mer (0.37 MPa), but they both fell within the range of tenacity observed at different periods in Portugal, the former lying among the lowest and the latter among the highest values (Fig. 4A). A significant positive linear relationship ($r^2 = 0.65$, $p = 0.016$) between sea urchin tenacity and seawater temperature existed when the 8 values of tenacity were taken into account (Fig. 4B). Indeed, aside

from the location of origin, the main difference between the tenacity measurements performed in Luc-sur-mer and in Banyuls-sur-mer was seawater temperature: 14.00 and 16.75°C, respectively.

Interspecific analysis

Sea urchin morphometry and tenacity

Table 2 summarizes the morphometric and tenacity measurements of individual sea urchins of the 3 species studied. In terms of test dimensions, there were significant differences among the 3 species. *Arbacia lixula* and *Paracentrotus lividus* had tests measuring half the size of those of *Sphaerechinus granularis*; they were also 3 times lighter in terms of immersed weight. *A. lixula* presented considerably smaller test diameter and height than *P. lividus*, but both species had similar test height:diameter ratios (H:D = 0.52 to 0.57, tests with the same shape) and immersed weight. If spines are taken into consideration, *A. lixula* and *P. lividus* are no longer different. However, the longest spines of *A. lixula* significantly decreased their height:diameter ratio (H:D = 0.66) giving them a more flattened form than *P. lividus* (H:D = 0.77). *S. granularis* had the roundest test shape (H:D = 0.73) of the 3 species, but with spines it had approximately the same shape as *P. lividus* (H:D = 0.76). In addition, *S. granularis* was also the species which had the highest number of adoral tube feet ($\approx$570), followed by *P. lividus* ($\approx$400) and *A. lixula* ($\approx$250). In all 3 species, significant linear relation-
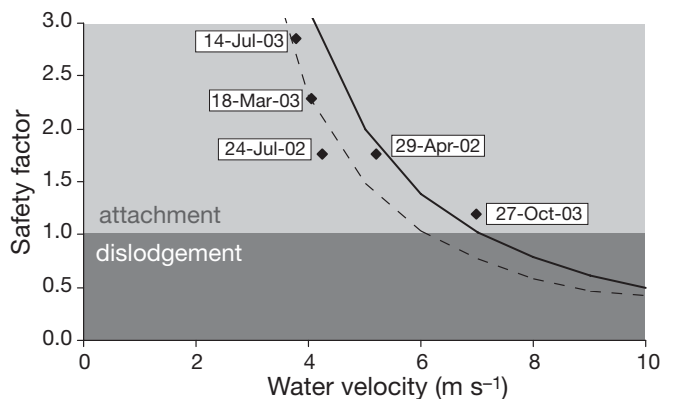

Fig. 5. *Paracentrotus lividus* (Cabo Raso, Portugal). Variation of the safety factor in relation to water velocity. Model curve (solid line) deduced from factors calculated with the mean scaled attachment force and the mean maximum water velocity measured in the field for each sampling period (black diamonds); and curve calculated with the mean estimated maximum attachment force deduced from laboratory-measured tenacity (dashed line). Light and dark grey areas indicate the index range of values corresponding to sea urchin attachment or dislodgement, respectively

ships ($r^2 > 0.8$, $p < 0.04$) existed between the number of adoral tube feet (TF) and the diameter of the test (TD, expressed in mm). Therefore, the following equations were used to estimate the number of adoral tube feet of the 30 sea urchins of each species used in the experiments:

$$\textit{Arbacia lixula} \ \text{TF} \ = \ 4.90 \ \text{TD} + 35.95 \tag{12}$$

$$\textit{Paracentrotus lividus} \ \text{TF} \ = \ 6.06 \ \text{TD} + 78.70 \tag{13}$$

$$\textit{Sphaerechinus granularis} \ \text{TF} \ = \ 4.10 \ \text{TD} + 203.79 \tag{14}$$

Tenacity was always size and weight independent ($p > 0.05$ for linear regressions of tenacity with test diameter, height and immersed weight), permitting comparisons between species. Tenacity measurements were first analyzed to search for significant intraspecific differences with the 3 angles of pull (0, 45 and 90°) tested. One-way analyses of variance did not detect any significant difference in the attachment force, adhesive surface area or tenacity obtained with the 3 angles of traction ($p_{ANOVA} > 0.05$). Therefore, data were pooled within each species and a one-way analysis of variance was performed, which revealed significant interspecific differences in tenacity. The tenacity of *P. lividus* was 2 to 3 times higher than those of *S. granularis* and *A. lixula* (Table 2, Fig. 6A). Among these last 2 species, the tenacity of *S. granularis* was significantly greater than that of *A. lixula* (Table 2, Fig. 6A). The number of adoral tube feet involved in attachment varied significantly among the 3 species ($p_{ANOVA} = 0.001$). On average, *S. granularis* employed 50% (≈280) of its adoral tube feet while *P. lividus* and *A. lixula* attached with 33% (≈130) and 12% (≈30), respectively (Fig. 6B). Furthermore, the percentage of attached tube feet that failed at the level of the stem before disc detachment was significantly higher in *P.*

Table 2. *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis* (Banyuls-sur-mer, France). Mean values (±SD) of morphometry, tenacity and estimates of maximum attachment forces and adhesive surfaces of individual sea urchins tested in laboratory conditions. Significant interspecific differences between means are indicated by letters in superscript; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$). †From Santos & Flammang (2006)

	n	*Arbacia lixula*	*Paracentrotus lividus*	*Sphaerechinus granularis*	p_{ANOVA}
Morphometric measurements					
Test diameter (TD; mm)	30	43.39 ± 5.84[a]	52.95 ± 7.97[b]	89.05 ± 6.30[c]	0.001
Test diameter with spines (mm)	3	92.03 ± 14.09[a]	78.87 ± 12.64[a]	134.50 ± 10.83[b]	0.004
Test height (mm)	30	22.26 ± 4.17[a]	30.00 ± 5.14[b]	64.80 ± 6.48[c]	0.001
Test height with spines (mm)	3	60.93 ± 15.63[a]	59.85 ± 5.19[a]	101.58 ± 5.20[b]	0.003
Profile surface area (mm²)	3	3570 ± 1263[a]	3704 ± 380[a]	11 098 ± 325[b]	0.001
Planform surface area (mm²)	3	3839 ± 1214[a]	2583 ± 829[a]	10 954 ± 295[b]	0.001
Immersed weight (g)	30	10.76 ± 3.32[a]	12.78 ± 3.48[a]	40.14 ± 7.92[b]	0.001
Number of adoral tube feet	5	248 ± 29[a]	399 ± 48[b]	569 ± 26[c]	0.001
Tenacity measurements	30				
Attachment force (N)		3.45 ± 2.09[a]	13.21 ± 9.14[b]	33.81 ± 17.94[c]	0.001
Adhesive surface area (mm²)		35.63 ± 25.58[a]	45.13 ± 34.53[b]	205.23 ± 121.93[c]	0.001
Tenacity (MPa)		0.12 ± 0.08[a]	0.37 ± 0.19[c]	0.19 ± 0.06[b]	0.001
Estimates of maximum forces and surfaces					
Single disc surface area (mm²) †		1.07 ± 0.26[c]	0.37 ± 0.09[a]	0.62 ± 0.16[b]	0.001
Maximum adhesive surface area (mm²)		266.0 ± 30.6[b]	147.2 ± 17.8[a]	352.6 ± 16.0[c]	0.001
Maximum attachment force (N)		32.3 ± 22.0[a]	53.8 ± 27.2[b]	65.6 ± 24.4[b]	0.001

Table 3. *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis* (Banyuls-sur-mer, France). Correlations between the attachment forces, the adhesive surface area and the number of attached and broken adoral tube feet in sea urchins tested in laboratory conditions. r: correlation coefficient

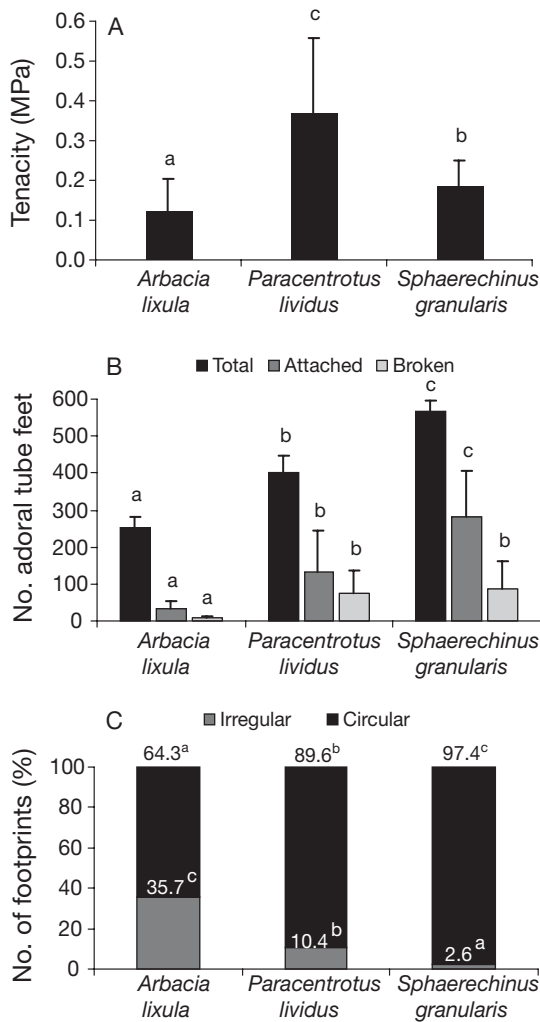| Variables	*Arbacia lixula*		*Paracentrotus lividus*		*Sphaerechinus granularis*	
	r	p	r	p	r	p
Attachment force × Adhesive surface area	0.57	0.002	0.74	0.001	0.76	0.001
Attachment force × No. attached tube feet	0.46	0.015	0.73	0.001	0.58	0.001
Attachment force × No. broken tube feet	0.72	0.001	0.83	0.001	0.76	0.001
No. attached tube feet × No. broken tube feet	0.56	0.001	0.48	0.001	0.75	0.003

Fig. 6. *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis* (Banyuls-sur-mer, France). (A) Mean values (+SD, n = 30) of tenacity measured on individual sea urchins attached to glass substratum. (B) Mean number of total, attached and broken adoral tube feet. (C) Mean percentages of circular and irregular footprints left by detached tube feet. Significant differences between the means are indicated by letters in superscript; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$)

lividus (60%) than in the other 2 species (30 and 24% in *S. granularis* and *A. lixula*, respectively) (p_{ANOVA} = 0.001). The number of tube feet that failed during dislodgment was found to be positively correlated with the number of attached tube feet as well as with the attachment force, correlations being generally stronger in *P. lividus* than in the other 2 species (Table 3). In all 3 species, attached tube feet left a higher percentage of circular footprints than irregular ones (Fig. 6C). However, the percentage of circular and irregular footprints varied significantly among species (p_{ANOVA} < 0.001). *S. granularis* showed an aver-

age percentage of circular/irregular footprints of 97/3, followed by *P. lividus* with 90/10 and finally *A. lixula* with 64/36. Attachment force was always positively correlated with the adhesive surface area, as well as, with the number of attached tube feet (Table 3).

Safety factor

Sphaerechinus granularis had the largest projected profile ($S_{profile}$) and planform ($S_{planform}$) surface areas exposed to drag and lift forces, respectively. As for *Arbacia lixula* and *Paracentrotus lividus*, the areas subjected to drag ($S_{profile}$) and lift ($S_{planform}$) forces were not statistically different. During our laboratory experiments, *A. lixula* attached with a strength 4 and 10 times less than that of *P. lividus* and *S. granularis*, respectively (Table 2). When the safety factor is calculated using these attachment forces and plotted as a function of water velocity (dashed lines in Fig. 7), it predicted that individuals of *A. lixula* would be the first to be dislodged, at water velocities of 2.0 m s^{-1}, followed by individuals of *S. granularis* and *P. lividus*, which would resist dislodgement up to 3.6 and 4.1 m s^{-1}, respectively. However, these forces were produced by individuals attached with only a fraction of their adoral tube feet (see Fig. 6B). If they had been attached with all their adoral tube feet, individuals of *A. lixula*, *P. lividus* and *S. granularis* would have been able to resist dislodgment with forces respectively 10, 4 and 2 times higher than the experimentally measured attachment force (Table 2). Under these conditions, *A. lixula* would have attained maximum attachment forces
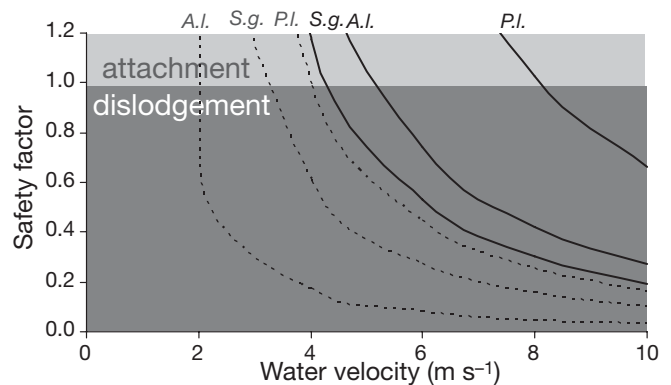

Fig. 7. *Arbacia lixula* (*A.l.*), *Paracentrotus lividus* (*P.l.*) and *Sphaerechinus granularis* (*S.g.*) (Banyuls-sur-mer, France). Variation of the safety factor as a function of water velocity. Factor calculated with the mean attachment force measured on glass substratum in the laboratory (dashed lines) and mean estimated maximum attachment force (solid lines). Light and dark grey areas indicate index values corresponding to sea urchin attachment or dislodgement, respectively

closer to those of *P. lividus*. When the safety factor was recalculated using maximum attachment forces (solid lines in Fig. 7), *S. granularis* was then predicted to be dislodged at water speeds around 4.6 m s^{-1}, whereas individuals of *A. lixula* and *P. lividus* would have resisted water velocities close to 5.5 and 8.2 m s^{-1}, respectively.

DISCUSSION

Intraspecific analysis

The attachment forces of *Paracentrotus lividus* measured in the field at Cabo Raso ranged from 9 to 87 N, and are similar to those measured by Märkel & Titschak (1965) for a Mediterranean population of the same species (up to 82 N). Attachment forces measured in the laboratory, on the other hand, ranged from 1 to 45 N, again corresponding to forces measured for a Mediterranean population (1 to 15 N; Guidetti & Mori 2005). Forces measured in the field and in the laboratory were converted into scaled attachment force and tenacity, respectively.

In their natural habitat, scaled attachment force of sea urchins was not constant over the sampling period. Similarly, the tenacity measured in laboratory experiments differed significantly among sampling dates. However, the variations among the 2 parameters were different and no correlation was found between scaled attachment force and tenacity. Scaled attachment force measured in the field was positively and strongly correlated with wave height at the time of sampling. Moreover, this correlation was still significant when wave heights were averaged over up to 3 d before sea urchin collection, but no longer when they were averaged over 7 d. No other significant correlation was found with the other hydrodynamic parameters (wave period and water temperature). The lack of correlation between scaled attachment force and tenacity suggests that variations in the former can be accounted for by the number of tube feet involved in attachment to the substratum. If this is the case, then sea urchins appear to respond to increased wave height by using more tube feet, hence increasing their attachment force. A similar phenomenon has been observed in mussels for both spatial and temporal variations of wave exposure. In the blue mussel *Mytilus edulis*, Witman & Shuchanek (1984) showed that attachment force was significantly higher in populations from exposed habitats than from protected habitats, suggesting that mussels subjected to large forces from wave action might be able to decrease the risk of dislodgment by producing a greater number of threads. Mussel scaled attachment force also varies with sam-

pling period, in direct relationship with wave height (Price 1982, Carrington 2002a). Attachment force was found to be significantly correlated with the number of byssal threads tethering the mussel to the substratum, more threads being produced under increased hydrodynamic loading (Carrington 2002a). There is, however, a major difference between mussels and sea urchins as far as the influence of wave exposure on scaled attachment force is concerned: the time lag between the stress and the response. In the mussel *M. edulis*, this lag is about 1 mo (i.e. the changes in attachment force follow the changes in wave heights by 1 mo; Carrington 2002a); whereas in the sea urchin *Paracentrotus lividus*, the change in attachment is immediate persisting during 3 d. This is not surprising because, as temporarily attached organisms, sea urchins probably adjust the number of attached tube feet very quickly, and thus adapt continuously their attachment force to the current hydrodynamic stress. Indeed, transplanted sea urchins *Strongylocentrotus droebachiensis* were reported to react to the new hydrodynamic conditions and fully attach in a matter of minutes (Siddon & Witman 2003). On the contrary, the production of new byssal threads by mussels is a longer and, above all, energy-consuming process (Carrington 2002b).

Mean scaled attachment forces measured in the field and water velocity at the moment of sampling were used to calculate safety factors, which ranged from 1.20 to 2.85 according to the period considered. The only other safety factors reported for sea urchins were measured for *Strongylocentrotus droebachiensis* and amounted to about 10 (Siddon & Wittman 2003). The large difference between the 2 studies may come from the fact that Siddon & Wittman (2003) measured water velocity directly onsite, while our values were extrapolated from wave heights, and there is sometimes a poor correlation between significant wave height and onshore water velocity (Helmuth & Denny 2003). On the other hand, the population of *Paracentrotus lividus* from Cabo Raso was intertidal and thus fully exposed, whereas the population of *S. droebachiensis* was subtidal (Siddon & Wittman 2003). In mussels, Carrington (2002) followed the variation of safety factor over a 3 yr period, and observed that it spanned a broad range from 9 down to about 1.

Tenacity measured in the laboratory is a size-independent parameter and does not depend on the angle of pull either. To allow measurement of footprint areas, tests have to be performed on glass, a transparent substratum, and not on rock as in the field. However, our experiments on individuals of *Paracentrotus lividus* from the population of Banyuls-sur-mer showed that scaled attachment forces measured on glass and rock are not significantly different. These results strengthen

the case for using a glass substratum for the measurement of tenacity, at least in the case of *P. lividus*. For the sea urchin population of Cabo Raso, contrary to the scaled attachment force measured in the field, tenacity measured in the laboratory did not correlate with any of the hydrodynamic parameters. Tenacity measured under laboratory conditions thus appears to be independent of the environmental conditions the animal has experienced before collection. This has also been demonstrated in the gastropod mollusc *Littorina obtusata* (Trussel 1997). Snails collected from different habitats with contrasted wave climate all possessed the same tenacity in the laboratory, although their scaled attachment forces in the field were different and correlated with the hydrodynamic stress. Nevertheless, in our study, the values of tenacity measured for *P. lividus* at various periods and places were different. In the intertidal population of Cabo Raso, sea urchin tenacity was higher in summer and autumn than in spring and winter. Tenacity was also higher in sea urchins from the subtidal population of Banyuls-sur-mer than in those from the aquaculture population of Luc-sur-mer. The 2 values fitted, however, within the range of tenacity measured in Portugal, but were among the extremes. As measurements in Banyuls-sur-mer were done in autumn when the seawater was still warm and the measurements in Luc-sur-mer were done indoor but in a cold aquarium, the whole tenacity dataset was re-analyzed and a positive relationship between seawater temperature and tenacity was found, the former explaining about 65% of the variation in the latter. Variation of tenacity with temperature has already been reported in various marine invertebrates (limpets, Grenon & Walker 1981; mussels, Crisp et al. 1985; sea cucumbers, Flammang et al. 2002). This phenomenon should not be overlooked, as it may partly explain differences measured within or between species (Grenon & Walker 1981).

Although laboratory-measured tenacity is independent of wave exposure, it may be used to estimate the maximum hydrodynamic stress a species can withstand. Indeed, using the estimated maximal adhesive area of the sea urchin (i.e. from all adoral tube feet), a maximum attachment force can be calculated. Using this force, *Paracentrotus lividus* individuals from Cabo Raso are predicted to be dislodged at a water velocity of 6.1 m s^{-1}. This is close to the value of 7.0 m s^{-1} obtained with scaled attachment forces and hydrodynamic parameters measured in the field (see Fig. 7). Therefore, in addition to providing useful information on the adhesion mechanism, the measurement of tenacity can give a good estimation of the resistance of a species to hydrodynamic stress when field measurements are impracticable or when hydrodynamic parameters are not available.

Interspecific analysis

We used laboratory-measured tenacity (see above) to compare the attachment strength and resistance to hydrodynamic stress among 3 sympatric species of sea urchins. During experimental dislodgment in aquaria, mean attachment forces recorded were 3.4, 13.2 and 33.8 N for *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis*, respectively. When the adhesive surface area was taken into account, however, *P. lividus* attached to the substratum with significantly higher mean tenacity (0.37 MPa) than *S. granularis* (0.19 MPa) or *A. lixula* (0.12 MPa). Within each species, tenacity was shown to be independent of the size and weight of sea urchins as well as from the angle at which they were dislodged.

Accounts of attachment strengths of sea urchins are scarce in the literature, and are usually reported as attachment forces. Few measurements have been performed in the field (Märkel & Titschak 1965, Siddon & Witman 2003), most data coming from aquarium measurements (Sharp & Gray 1962, Yamasaki et al. 1993, Guidetti & Mori 2005). Like in our study, measurements made in the field (up to 82 N in *Paracentrotus lividus* and up to 45 N for *Arbacia lixula*, Märkel & Titschak 1965; 42.4 ± 1.8 N [mean ± SE] for *Strongylocentrotus droebachiensis*, Siddon & Witman 2003) are usually higher than those made in the laboratory (3 to 28 N for *Strongylocentrotus intermedius* and 2 to 24 N for *Strongylocentrotus nudus*, Yamasaki et al. 1993; 1.1 to 14.5 N for *P. lividus* and 2.8 to 33 N for *A. lixula*, Guidetti & Mori 2005). Contrary to our observations, Guidetti & Mori (2005) measured higher attachment forces for *A. lixula* than for *P. lividus*, which is more in accordance with their usual distribution in the field. Indeed, where they coexist, *A. lixula* and *P. lividus* adopt a different vertical zonation; the former usually dominating the upper levels and the latter the deeper ones (Régis 1979, Chelazzi et al. 1997, Bulleri et al. 1999). However, at our study site, *P. lividus* occurred above *A. lixula*, though they partially overlapped. Differences may also stem from the substrata used in both studies. Guidetti & Mori (2005) made their measurements on rock, while we used glass and found that individuals of *A. lixula* were reluctant to attach to this substratum. The influence of substratum on sea urchin attachment has only been investigated once on 2 species: *Arbacia punctulata* and *Lytechinus variegatus*. Sharp & Gray (1962) measured attachment time on glass and rock substrata when these sea urchins were subjected to a constant pull of approximately 2.5 N. Individuals of *A. punctulata* remained attached for longer periods on rock than on glass while the reverse was observed for *L. variegatus*. Ability to attach thus appears to vary among substrata from species to spe-

cies. We found no difference between rock and glass for *P. lividus* (see above), but the other 2 species were not tested for this factor.

Measurements of tenacity on glass have the advantage of standardising the substratum and generating information on the number of tube feet involved in adhesion and the adhesive surface area of the tube foot disc. In our study, *Sphaerechinus granularis* was the species that employed significantly more tube feet for attachment, followed by *Paracentrotus lividus* and *Arbacia lixula*. Those tube feet involved in adhesion represented about 50, 30 and 10% of the total number of tube feet of each species, respectively, indicating that *A. lixula* can potentially attach much more strongly than was measured in our conditions. To calculate the maximal attachment force of each species, the maximum adhesive surface area is first estimated by multiplying the mean adhesive surface area of single tube foot discs by the total number of tube feet (Santos & Flammang 2006). Thus, *P. lividus* was estimated to be able to attach with an adhesive surface area ($147~mm^2$) lower than the ones of *S. granularis* ($352~mm^2$) or *A. lixula* ($266~mm^2$). Since tenacity was size independent, the attachment force associated with the mentioned maximum adhesive surface areas can be deduced and amounted to about 66 N in *S. granularis*, 54 N in *P. lividus*, and 32 N in *A. lixula*. It is noteworthy that *A. lixula* increases its total adhesive surface area by using a small number of tube feet with very large discs, while *P. lividus* increases this surface area by using a large number of tube feet with relatively small discs.

Sea urchin size and shape also influence the hydrodynamic forces imposed on them by water velocities, bigger and taller animals experiencing stronger drag and lift forces (Denny et al. 1985, Denny 1988). The 3 species studied presented significantly different morphometric characteristics. *Arbacia lixula* and *Paracentrotus lividus* had much smaller tests than *Sphaerechinus granularis*. *A. lixula* had smaller test dimensions than *P. lividus* but, possessing longer spines, its surface area exposed to flow was identical to that of *P. lividus*. The combination of these morphometric differences had the important consequence of drastically increasing the drag (projected profile area) and lift (projected planform area) on *S. granularis* relative to the other 2 species. Therefore, the calculation of the safety factor showed that, despite its larger maximum attachment force, *S. granularis* was predicted to be the first species dislodged at water speeds of above $4.6~m~s^{-1}$, whereas *A. lixula* and *P. lividus* should be able to remain attached up to water velocities of 5.5 and $8.2~m~s^{-1}$, respectively. These predictions are in accordance with species distribution in the Mediterranean Sea, where the bigger *S. granularis* typically inhabits calm and rel-

atively deep areas while the smaller *A. lixula* and *P. lividus* are found in shallower areas subjected to stronger hydrodynamic forces. This size-related constraint on the distribution of sea urchins in shallow habitats has already been reported in other studies (Denny et al. 1985, Siddon & Witman 2003). For these organisms, therefore, tenacity alone is not an indicator of the capacity of a species to withstand hydrodynamic stresses. This is similar to the situation described for whelks, but different from the case of limpets. Rilov et al. (2004) studied 2 Mediterranean whelks, *Stramonita haemastoma* from a wave-exposed habitat and *Hexaplex trunculus* found only subtidally in more calm littoral environments. The 2 species possess the same tenacity, but the former is less prone to dislodgment by waves due to a more streamlined shell and a larger foot. In limpets, on the contrary, Branch & March (1978) studied 6 species of the genus *Patella* with different shell shapes and sizes and from habitats with different wave climate. They observed that the species subjected to strong wave action possessed higher tenacities than the species from sheltered to moderately exposed areas, and this independently of shell shape and size. Therefore, there is no unique rule in marine invertebrates as to the relationship between tenacity and species distribution in wave-swept and tidal environments but, taken in combination, tenacity and body shape and size are adapted to prevailing wave forces.

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