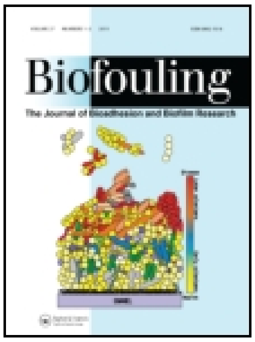

Biofouling

ISSN: 0892-7014 (Print) 1029-2454 (Online) Journal homepage: http://www.tandfonline.com/loi/gbif20

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To cite this article: David B. Ramsay , Gary H. Dickinson , Beatriz Orihuela , Daniel Rittschof & Kathryn J. Wahl (2008) Base plate mechanics of the barnacle Balanus amphitrite (=Amphibalanus amphitrite), Biofouling, 24:2, 109-118, DOI: 10.1080/08927010701882112

To link to this article: http://dx.doi.org/10.1080/08927010701882112

Published online: 23 Feb 2011.

Biofouling
Vol. 24, No. 2, January 2008, 109–118

Base plate mechanics of the barnacle *Balanus amphitrite* (=*Amphibalanus* amphitrite)

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(*Received 14 October 2007; final version received 20 December 2007*)

The mechanical properties of barnacle base plates were measured using a punch test apparatus, with the purpose of examining the effect that the base plate flexural rigidity may have on adhesion mechanics. Base plate compliance was measured for 43 *Balanus amphitrite* (= *Amphibalanus amphitrite*) barnacles. Compliance measurements were used to determine flexural rigidity (assuming a fixed-edge circular plate approximation) and composite modulus of the base plates. The barnacles were categorized by age and cement type (hard or gummy) for statistical analyses. Barnacles that were 'hard' ($\geq$70% of the base plate thin, rigid cement) and 'gummy' ($>$30% of the base plate covered in compliant, tacky cement) showed statistically different composite moduli but did not show a difference in base plate flexural rigidity. The average flexural rigidity for all barnacles was 0.0020 Nm (SEM $\pm$ 0.0003). Flexural rigidity and composite modulus did not differ significantly between 3-month and 14-month-old barnacles. The relatively low flexural rigidity measured for barnacles suggests that a rigid punch approximation is not sufficient to account for the contributions to adhesion mechanics due to flexing of real barnacles during release.

Keywords: mechanics of adhesion; adhesion of plates; fouling release; barnacle; release coatings; *Balanus amphitrite*

Introduction

Biofouling of ship hulls significantly increases fuel consumption because of increased drag (Kan et al. 1958; Champ 2000; Yebra et al. 2004). Although traditional toxic surface treatments eliminate fouling organisms effectively, they have unacceptable environmental impacts, and therefore research has targeted fouling release coatings. These fouling release coatings are designed to facilitate weak adhesion of fouling organisms, thereby allowing them to be removed by hydrodynamic or biotic forces (Schultz et al. 1999; Schultz 2007).

To date, the most effective fouling release coatings have been silicone-based (Swain et al. 1992; Swain and Schultz 1996; Wendt et al. 2006). Some barnacles produce thick, opaque, compliant cement when adhered to these silicone coatings. This is in contrast to their characteristic thin, hard adhesive observed when grown on glass or polystyrene (Berglin and Gatenholm 2003; Wiegemann and Watermann 2003; Holm et al. 2005). For *Balanus amphitrite* (= *Amphibalanus amphitrite*) (Pitombo 2004; Clare and Høeg 2008), Holm et al. (2005) showed that the propensity to produce the thick, compliant cement is heritable, with h^2 of 0.46–0.59 depending on the silicone substratum. Production of a thicker, more compliant cement (referred to hereafter as 'gummy') may result in significant changes to both surface chemistry and barnacle base plate mechanics as compared with the usual hard, thin cement (which will be referred to as 'hard').

Most experiments and models of barnacle release have used rigid punch adhesion mechanics (Kendall 1971), where the punch is assumed to be perfectly rigid and the adhesion mechanics are governed by a balance of energy terms between stored elastic energy of the applied load, the energy from elastic deformation of the substratum, and the surface energy released during fracture of the punch/substratum interface. This model, when applied to evaluate the adhesion stresses observed upon removing barnacles from thin elastomer release coatings, assumes both good adhesion between the barnacle and substratum and that no flexure occurs in the barnacle during the release process. Studies to date have thus focused extensively on deriving relations between release stress and elastomer coating mechanical properties and thickness (Kohl and Singer 1999; Brady and Singer 2000; Kim et al. 2007). Experimental data have shown a discrepancy between the pull-off stress predicted by Kendall's model and actual pull-off stress values for barnacles (Sun et al. 2004); others have led to questions about the predictive capabilities of experiments with pseudobarnacles rather than barnacle foulants (Swain

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ISSN 0892-7014 print/ISSN 1029-2454 online

DOI: 10.1080/08927010701882112
http://www.informaworld.com

et al. 1997). It can be speculated that either adhesion chemistry or base plate mechanics could be responsible; neither is well understood at this time.

Recent studies by Chung and Chaudhury (2005) have shown that base plate mechanics may indeed play a significant role in the release properties of hard foulants such as barnacles. They have modified the rigid punch approximation to account for flexural rigidity of the punch. Both their analytical model and experiments suggest a strong dependency of pull-off stress with punch flexural rigidity and compliance. Although that model uses a simplified punch geometry (flexible plate on a compliant backing), the trends suggest that the flexural rigidity of real barnacles may contribute to reducing release forces and may account for some of the differences between barnacle release forces and pseudobarnacle release forces (Swain et al. 1997).

The goal of this study was to improve understanding of barnacle adhesion mechanics by determining the flexural rigidity of barnacle base plates. Ultimately, the flexural rigidity will determine the validity of applying Kendall's rigid punch model without modification. If base plate flexure is not significant, the difference in model and real values can be solely attributed to cement chemistry. On the other hand, if base plate flexure is significant, rigidity values can be used to establish more accurate barnacle-release models that take into account actual barnacle mechanics.

In this article, flexural rigidity of mature barnacle base plates were measured as a function of age and cement type (fractional coverage of 'hard' or 'gummy' cement on the base plate) using a custom-built punch test apparatus. Measurements of the base plate flexural rigidity, combined with the thickness and radial dimension were then used to determine the composite elastic modulus of the material comprising the base plate.

Materials and methods

Barnacle larval culture, settlement and maintenance

The barnacle *B. amphitrite* (= *A. amphitrite*) was used for this study. Barnacle larval culture and settlement was conducted at the Duke University Marine Laboratory in Beaufort, North Carolina, following Rittschof et al. (1984). Barnacle larvae were settled on 7.6 cm × 15.2 cm × 0.64 cm glass panels coated with silicone (Dow Corning Silastic T2 or International Veridian) and maintained in the laboratory as described by Holm et al. (2005). After 5 weeks of growth, barnacles on silicone-coated panels were transported to the Naval Research Laboratory (NRL) in Washington, DC, where they were kept in individual plastic containers filled with artificial seawater (32 ppt, Instant Ocean in doubly distilled water, aerated overnight before use). The artificial seawater was changed twice a week. At NRL, barnacles were fed with 10 ml dense *Artemia* sp. (supplied by Sanders, Morgan UT, hatched from approximately 1 teaspoon cysts in 1 l seawater) every day for 10 weeks and then every other day thereafter. Mortality rates for barnacles kept at NRL for these studies were low; >90% of the barnacles other than those used and cross-sectioned after penetration procedures were still alive after 18 months in the laboratory.

Punch test apparatus

The punch test apparatus was a 1000-g load cell (GSO, Transducer Techniques) attached to a vertically mounted linear positioning stage (Aerotech ATS50). A cylindrical punch mounted on the load cell was lowered at 0.005 mm s^{-1} toward an inverted, rigidly mounted barnacle (Figure 1). Two barnacle supports were fabricated and sized to accommodate the geometry of the majority of barnacles grown on release panels. Each support consisted of two steel plates 1.6 mm in thickness (Kimball Physics, Inc.) glued to a partially hollowed brass cylinder (~25 mm diameter, 30 mm height) using cyanoacrylate adhesive. The steel plates of the larger support had a central hole of 10 mm diameter, whereas the smaller design had a 6.5 mm diameter opening. Two punches were used in conjunction with the different supports: a 5.10-mm diameter load stem (ALS-08, Transducer Techniques) for larger barnacles, and a 4.10-mm diameter punch head for the smaller. Labview (National Instruments) data acquisition program was used to monitor load cell voltage readings and simultaneously control stage motion.

The punch apparatus was configured to maximize the rigidity of the system. Two steel pseudo-barnacles, one large and one small, were created from screw heads and used to measure the total compliance of the optimized system. The instrument compliance was accounted for in all calculations. Indenting

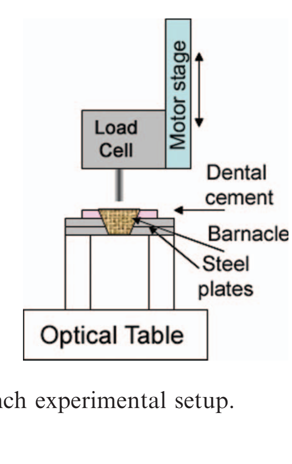

Figure 1. Punch experimental setup.

rates of 0.001 mm s^{-1}, 0.005 mm s^{-1}, 0.010 mm s^{-1}, 0.025 mm s^{-1} and 0.050 mm s^{-1} were examined using these pseudo-barnacles; compliance was independent of rate in this range. A loading rate of 0.005 mm s^{-1} was selected for experiments, balancing the need for rapid measurements with high data resolution. Measurements were taken at 0.001 mm s^{-1} intervals.

Barnacle punch testing

Compliance measurements were made on barnacles of two different age groups, 3 months old (Set #1) and 14 months old (Set #2), from barnacles settled at the same time (same larval cohort). Barnacles were affixed securely to the steel supports using a methyl methacrylate resin (Teets Denture Material) around the barnacle that cured for 20–60 min before testing. Experiments were initiated with the punch out of contact, and data were collected as the punch came into contact, pushed into the barnacle and pulled back out of contact. The majority of the recorded force curves included a short nonlinear regime upon contact. In every case, as the punch pushed further into contact with the barnacle base plate, a linear regime developed. The slope of this linear regime, in mm N^{-1}, corrected for instrument compliance, provided a measure of the barnacle base plate compliance.

Before testing, the amount of compliant, 'gummy' cement was empirically approximated and recorded as a percentage of the total base plate area. For this study, barnacles were qualitatively categorized as 'hard' if $\geq$70% of the base plate was covered in thin, rigid cement and 'gummy' $>$30% of the base plate was covered in compliant, tacky cement. The base plate diameter was measured using calipers along parallel and perpendicular axes to the opercular opening. After testing, the barnacle base was fractured by hand, and a section from the center (where the punch contact was made) was removed and measured for thickness with digital calipers to the nearest 10 μm. Care was taken not to compress the 'gummy' adhesive during the thickness measurement.

The experimental procedure was modified slightly between measurements on a group of 18 3-month-old barnacles (Set #1) and another group of 25 14-month-old barnacles (Set #2). The 3-month-old barnacles (Set #1) were released from their silicone substrata before mounting in the dental cement, exposing the base plate to air during the curing process before testing. It has been suggested that the thick, compliant cement that some barnacles produce on silicone is highly hydrated (Saroyan et al. 1970; Wiegemann and Watermann 2003). Because of a concern that some dehydration of the base plate might affect the measurements, the 14-month-old barnacles were not released from the substratum until immediately prior to testing. Instead, barnacles were coated in methyl methacrylate although still attached to silicone panels, and the substratum was inverted and clamped to a manual stage. The coated barnacle was then lowered onto the support using the stage, cured in air for 25 min, removed from the silicone, and immediately tested. This technique reduced the chance for mechanical modification of gummy cement by exposure to air.

Analysis of cement water content

To determine if mechanical data obtained using the two different procedures explained above could be directly compared, the change in water content of gummy barnacle cement over time was analyzed. Barnacles were removed from their silicone substratum and the base plate was inspected for compliant, 'gummy' cement. For barnacles with 100% of their base plate covered in compliant, 'gummy' cement, the cement layer was immediately cut from the base plate using a scalpel and placed into two roughly 1 cm^2, clean aluminium foil dishes. The cement was allowed to sit in air at room temperature and humidity (21°C, 70% humidity). The mass of the cement was recorded at 0, 5, 10, 15, 20, 30, 45, 60 and 960 min. To assess water vapour deposition during the course of the experiment, empty aluminium foil dishes were weighed on the same time intervals as cement samples.

Mechanical model

The physio-chemical geometry of barnacles tested in this study is an overlapping series of six $CaCO_3$-rich shell plates in the shape of a truncated cone, with a base plate composed of $CaCO_3$ and cement. All plates contain radial canals, which are lined with epithelial tissue. The plates grow at the region nearest the base plate (Bourget 1987). The living barnacle inside is physically compliant. For the purpose of this article, the contribution of the animal to the overall mechanics is neglected and only the properties of the base plate are considered for calculations. Because the base plate is supported around its entire circumference by the vertical shell plates, the base plate can be approximated as a circular plate supported around its perimeter. The fixed edges version of this model was selected because the barnacle base plate is supported securely by the shell plates, and is attached to the shell plates by muscle tissue (Bourget 1987).

The flexural rigidity (D) of a circular plate is defined as:

$$D = Et^3/12(1 - v^2) \qquad (1)$$

where E is the modulus of elasticity, t is the thickness of the plate (measured in this study with digital calipers to the nearest 10 μm) and v is Poisson's ratio (Timoshenko and Goodier 1970). To calculate the flexural rigidity of each barnacle based on compliance (C) measurements, a mechanical model of a plate with fixed edges and uniform load over a concentric circular area (in this instance a cone) was assumed (Kutz 1998):

$$1/C = [3(m^2 - 1)/16E\pi m^2 t^3] \times [4a^2 - 4r^2 \ln(a/r) - 3r^2] \quad (2)$$

where $m = 1/v$ or the reciprocal of Poisson's ratio, a is the radius of the plate, and r is the radius of the punch (Figure 2). Thus, to determine the flexural rigidity, the equation can be written:

$$D = [1/C] \times [1/64\pi] \times [4a^2 - 4r^2 \ln(a/r) - 3r^2] \quad (3)$$

The radius of the barnacle for this calculation was estimated using the average of the longest and shortest diameters (parallel and perpendicular to the opercular opening). The modulus of the base plate was then calculated by solving Equation (1) for E:

$$E = 12D(1 - v^2)/t^3 \quad (4)$$

where D is taken from the previous calculation. Poisson's ratio is estimated at 0.3, because calcium carbonate is the primary constituent of calcerous base plates such as those in *B. amphitrite* (Bourget 1987; Rodriguez-Navarro et al. 2006). The ratio of 0.3 is an average taken from 95% pure calcium carbonate samples (Bell 1981), but may differ from this value because of the complexity of the combination of cement and cement channels in and on the calcerous base plate. Deviation from 0.3 is only expected to modify the resulting modulus values slightly.

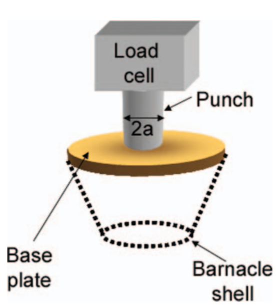

Figure 2. Detail of experimental setup, showing the physical relationship of the radius of the punch to the radius of the barnacle base plate, as described in Equations 1 and 3.

During retraction of the punch, some force curves showed adhesion between the punch and the barnacle base plate adhesive. For these tests, adhesion values, in N mm^{-2}, were calculated by taking the peak adhesion force and dividing it by plate area. This calculation is reported for fully hydrated barnacles.

Data analysis

Compliance data, in mm N^{-1}, were determined from the slope of regressions fit to the final linear regime of each force curve. Only linear fits with an R^2 value greater than 0.997 were considered. The compliance of the system, measured before each set of trials, was then subtracted to determine the compliance of each barnacle. Raw data yielding negative compliance or yielding composite plate moduli greater than an order of magnitude from the average were determined to have misaligned punch contact and were removed. Of the remaining data, one 'gummy' value from the 14-month-old group of 25 barnacles (Set #2) was greater than three times the interquartile range above the mean and removed. Pull-off stresses for gummy barnacles were calculated using the maximum adhesion force divided by the contact area (barnacle base plate area). Critical fracture forces for base plates that fractured were determined by the force to achieve the first fracture event, indicated by a sudden discontinuity (drop) in the force–displacement curve. Statistical analyses were conducted using SigmaStat V3.1 (Systat, San Jose CA). Pull-off stress values for adhesion between the punch and gummy barnacle base plates were calculated using the maximum pull-off force divided by the punch area.

Results

Typical force-displacement curves are shown in Figure 3 for hard (Figure 3a) and gummy (Figure 3b) barnacles. The recorded force curves exhibited non-linear behaviour upon contact followed by a linear regime until fracture as shown in Figure 3a. The nonlinear behaviour is attributed to imperfectly aligned punch to base plate contact; the slope in Figure 3a is consistent with a 0.5° cant. Specimens exhibiting nearly perfect alignment (Figure 4) between punch and base plate indicate the authors mechanical assumption of the base plate securely supported by the shell plates is reasonable. In several cases, multiple fracture events were visible as discontinuities in the curves (eg Figure 3b). All of the hydrated gummy barnacles (except for one) displayed measurable adhesion between the punch and base plate in the force curves, because of the tackiness of the gummy cement. The hydrated gummy barnacle group provided

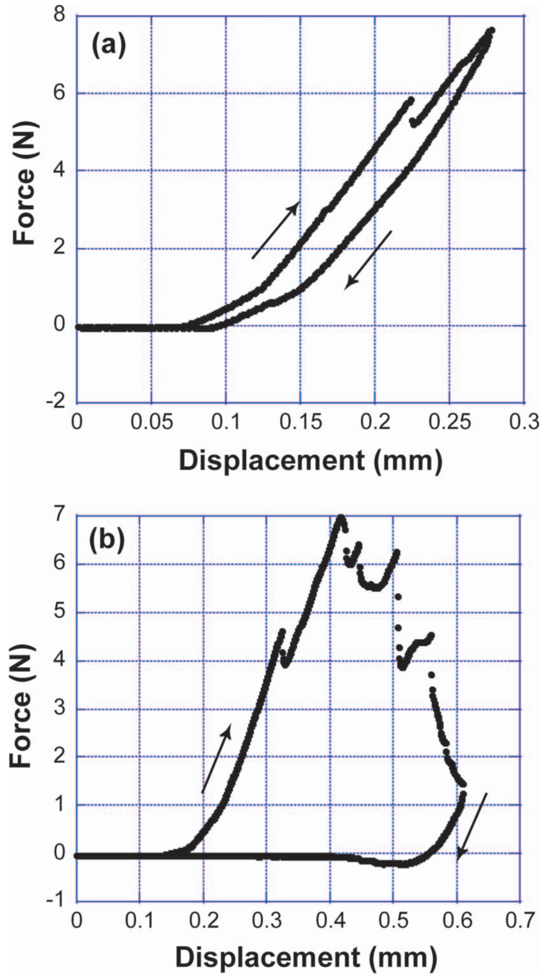

Figure 3. Representative barnacle force curves for both (a) 'hard' barnacle with 0% gummy cement and (b) a 'gummy' barnacle (90% gummy cement). Both enter linear regimes before fracture. The 'gummy' barnacles often exhibited adhesion as the punch was withdrawn.

an average pull-off stress value of 0.004 N mm^{-2} (SEM $\pm$ 0.002). Hydrated hard barnacles with less than 20% of their base plate covered in gummy cement showed no adhesion. The hydrated hard barnacles with greater than 20% of their base plate covered in gummy cement displayed adhesion and pull-off stress values similar to the gummy group. Regression of pull-off stress on percent of base plate covered in compliant cement was significant (one-way ANOVA: $p = 0.003$), with barnacles having more gummy cement tending to have higher pull-off stress.

Flexural rigidity of the two different sets of barnacles was determined using Equation (3) and is reported in Table 1. Of the 18 barnacles in Set #1, the average diameter was 9.5 mm and of these, 12 had 'hard' base plates ($\geq$70% of the base plate thin, rigid cement) and 6 were nominally 'gummy', having $>$30% of the base plate area covered in opaque, compliant cement. Set #2 (25 barnacles from the same larval cohort tested 11 months later) had an average diameter of 8.7 mm, and were 14 'hard' and 11 'gummy.' Note that the average diameter given is only for barnacles fitting supports and used in experiments; it does not represent a measure of all barnacles on the release panels.

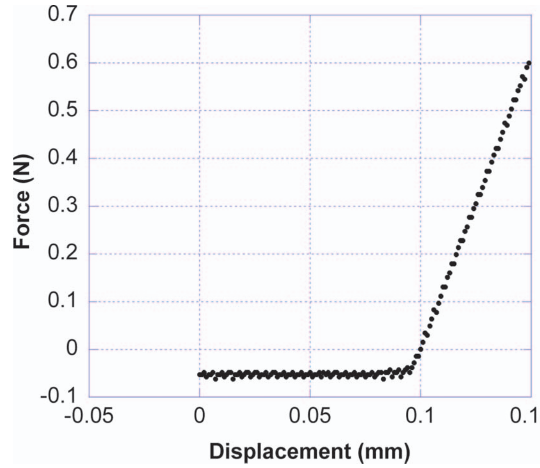

Figure 4. Portion of a force curve as the punch comes into contact with the barnacle base plate. The interaction immediately entered a linear regime, where it remained until fracture. This is consistent with the base plate being rigidly fixed in relation to the rest of the shell plates.

The change in the mass of cement over time in air, presumably because of water loss, for gummy barnacles is shown in Figure 5. The majority of water loss occurred after the time scale relevant to mechanical testing. Cement on barnacles in Set #1 was exposed to air for about 20 min, at which time mass loss was no more than 18%. Water loss over 960 min was considerable (up to 87.5%). The mass change of empty aluminium dishes was negligible over the full course of the experiment.

Table 2 lists flexural rigidity least squared means for the hard and gummy barnacles from both age groups. The average flexural rigidity of all barnacles was 0.0020 Nm (SEM $\pm$ 0.0003). A two-way ANOVA statistical analysis was used to analyse flexural rigidity data, with age (3 or 14 months) and cement type (hard or gummy) as factors. Neither age nor cement type showed a significant difference between test groups (Table 2, Figure 6a). There was no interaction between factors (year $\times$ cement; Table 2).

Modulus values between hard and gummy barnacles were significantly different as shown by two-way ANOVA (p < 0.001) with age and cement type as factors (Table 3, Figure 6b). Barnacle age was not significant, and there was no interaction between year and cement type (age $\times$ cement). The hydrated barnacle data (14-month-old barnacles) resulted in an

Table 1. Barnacle geometric and mechanical data.

Barnacle compliance, C (mm N^{-1})	Base plate area (mm^2)	Base plate thickness (μm)	Flexural rigidity, D (Nm)	Base plate modulus, E (GPa)	Gummy fraction (%)	Barnacle age (mo)
Set #1						
Hard						
0.0271	57.0	150	0.00088	3.1	0	3
0.0077	86.7	220	0.00468	5.3	0	3
0.0087	58.9	170	0.00285	7.0	0	3
0.0117	80.3	150	0.00275	9.8	0	3
0.0215	86.0	180	0.00166	3.4	5	3
0.0169	104.3	220	0.00271	3.1	5	3
0.0176	40.2	160	0.00083	2.4	5	3
0.0178	69.2	165	0.00172	4.6	10	3
0.0312	41.4	170	0.00049	1.2	10	3
0.0300	98.2	135	0.00141	6.9	20	3
0.0072	82.2	200	0.00466	7.0	20	3
0.0179	74.1	180	0.00162	3.3	20	3
Gummy						
0.0466	62.9	190	0.00058	1.0	40	3
0.0197	38.4	160	0.00069	2.0	50	3
0.0377	70.3	230	0.00072	0.7	70	3
0.0136	59.7	180	0.00186	3.8	90	3
0.0087	110.3	260	0.00571	3.9	95	3
0.0118	70.4	190	0.00228	4.0	100	3
Set #2						
Hard						
0.0043	83.8	200	0.00800	12.0	0	14
0.0191	61.0	150	0.00136	4.8	0	14
0.0075	79.9	180	0.00492	10.1	0	14
0.0160	39.1	110	0.00087	7.9	5	14
0.0830	60.8	110	0.00031	2.8	5	14
0.0695	70.6	120	0.00045	3.1	5	14
0.0199	65.5	120	0.00143	10.0	10	14
0.0096	67.6	170	0.00267	6.5	15	14
0.0649	49.0	100	0.00030	3.6	15	14
0.0109	63.0	160	0.00250	7.3	15	14
0.0091	50.6	170	0.00224	5.5	20	14
0.0259	47.2	120	0.00071	5.0	25	14
0.0068	45.9	180	0.00257	5.3	30	14
0.0055	53.8	190	0.00398	7.0	30	14
Gummy						
0.0222	43.2	140	0.00073	3.2	35	14
0.0899	62.8	150	0.00030	1.1	45	14
0.0783	49.8	110	0.00025	2.3	50	14
0.0255	61.4	160	0.00103	3.0	60	14
0.0066	45.9	170	0.00267	6.5	65	14
0.0714	49.8	110	0.00028	2.5	80	14
0.0121	68.5	230	0.00251	2.5	90	14
0.0123	67.2	230	0.00242	2.4	100	14
0.0136	65.3	240	0.00209	1.8	100	14
0.0149	69.6	170	0.00209	5.1	100	14
0.0324	61.1	190	0.00080	1.4	100	14

average modulus of 2.9 GPa (SEM $\pm$ 0.7) for gummy barnacles, and an average modulus of 6.5 GPa (SEM $\pm$ 0.6) for hard barnacles (Figure 6b). (It was important to account for the punch radius as was done in Equations (3) and (4) relative to the barnacle radius. When compliance was estimated assuming a point load, modulus values showed a more than 50% deviation from values determined with punch radius taken into account.)

Statistically significant relationships were observed between the load to initiate base plate fracture and measured barnacle properties including: (i) base plate thickness, (ii) flexural rigidity and (iii) thickness/(base plate radius) ratio. For the 14-month-old set of barnacles (Set #2: $n = 25$), one-way ANOVA of the regression for each of these provided a highly significant increasing linear relationship ($p < 0.001$). Figure 7 shows plots of critical fracture force as a

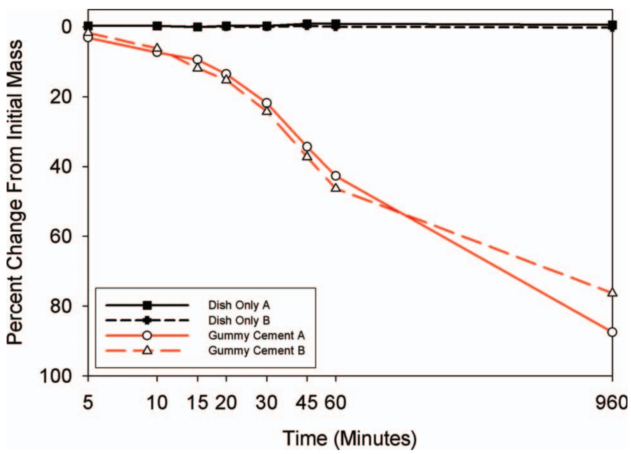

Figure 5. Gummy barnacle cement mass change over time, in air at room temperature and humidity. The cement layer was removed with a scalpel and placed in clean, 1 cm^2 aluminium dishes. Empty dishes were weighed as control.

Table 2. Two-way ANOVA for flexural rigidity.

Source of variation	df	SS	MS	F	p
Age	1	5.50 E^{-7}	5.50 E^{-7}	0.195	0.661
Cement	1	3.16 E^{-6}	3.16 E^{-6}	1.122	0.296
Age × cement	1	1.25 E^{-6}	1.25 E^{-6}	0.444	0.500
Residual	39	2.82 E^{-6}	2.82 E^{-6}		
Total	42	2.76 E^{-6}	2.76 E^{-6}		

Least squared means ANOVA table.

Group	Size	Mean (Nm)	SEM (Nm)
Hard, 3 month old	12	0.0022	0.0005
Gummy, 3 month old	6	0.0020	0.0007
Hard, 14 month old	14	0.0023	0.0004
Gummy, 14 month old	11	0.0014	0.0005

function of flexural rigidity (Figure 7a) and base plate thickness/radius (Figure 7b) ratio, t/a, for these specimens; both show increasing trends in the force to initiate fracture. Although ANOVA of the regression indicates a significant linear relationship between flexural rigidity and critical fracture force, a portion of the data deviates from linear (Figure 7a). Given the complexity of the base plate structure, and the probability of defects controlling fracture initiation, a more thorough examination of these properties for a larger sample set would be warranted to derive a clearer trend. Accounting for the size of the punch to derive critical fracture stress (force/area) did not significantly change the relationships or statistics. The complexity of interpreting fracture mechanics for the composite base plate prevents further speculation on the significance of this observation.

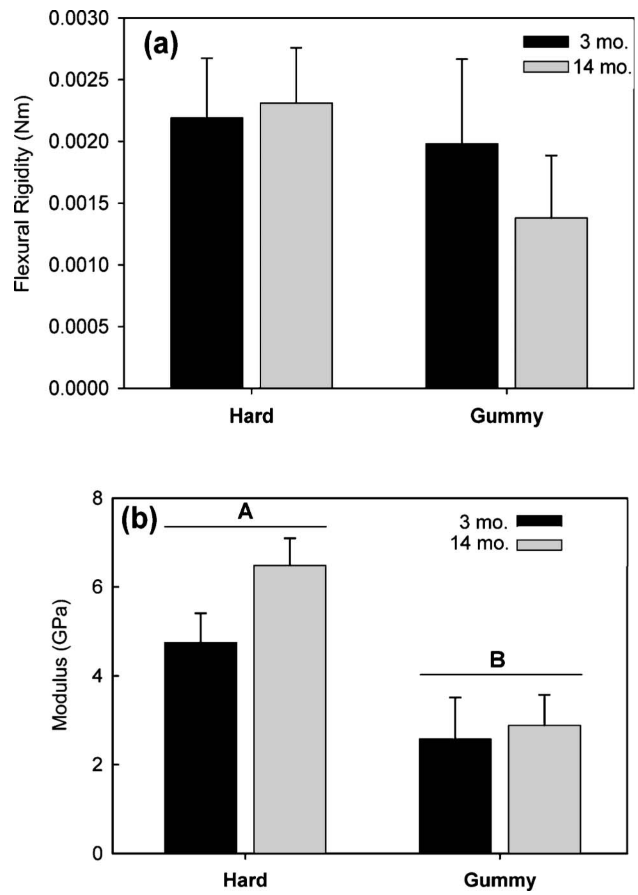

Figure 6. Flexural rigidity and modulus for (a) 3- and (b) 14-month-old gummy and hard barnacles. 'Gummy' is defined as $>30\%$ of the base plate covered in compliant, opaque cement, whereas 'hard' is defined as $\leq 30\%$ of the base plate covered in compliant, opaque cement. Groups marked with A and B are significantly different (Holm–Sidak multiple comparisons, $p < 0.05$).

Finally, the possibility of a relationship between the fraction of base plate that exhibited 'gummy' cement and base plate thickness, flexural rigidity and modulus was examined. Barnacles with more of their base plate covered in gummy cement tended to have a thicker base plate. Regression of base plate thickness on percent of base plate covered in gummy cement was significant (one-way ANOVA: $p = 0.006$; Figure 8). Regression of modulus on percent gummy cement was also significant (one-way ANOVA: $p = 0.003$). Neither regression of flexural rigidity on percentage gummy nor modulus on base plate thickness were significant at $p < 0.05$.

Discussion

The punch experiments show that *B. amphitrite* do not exhibit significant mechanical change as they age between 3 and 14 months. Data from both hard and

Table 3. Two-way ANOVA for modulus.

Source of Variation	df	SS	MS	*F*	*P*
Age	1	10.100	10.100	1.932	0.172
Cement	1	80.737	80.737	15.448	<0.001*
Age × cement	1	4.948	4.948	0.947	0.337
Residual	39	5.226	5.226		
Total	42	7.392	7.392		

*The two-way ANOVA revealed a significant effect for cement type (hard vs. gummy) at $p < 0.001$.

Least squared means ANOVA table.

Group	Size	Mean (GPa)	SEM (GPa)
Hard, 3 month old	12	4.7	0.7
Gummy, 3 month old	6	2.6	0.9
Hard, 14 month old	14	6.5	0.6
Gummy, 14 month old	11	2.9	0.7

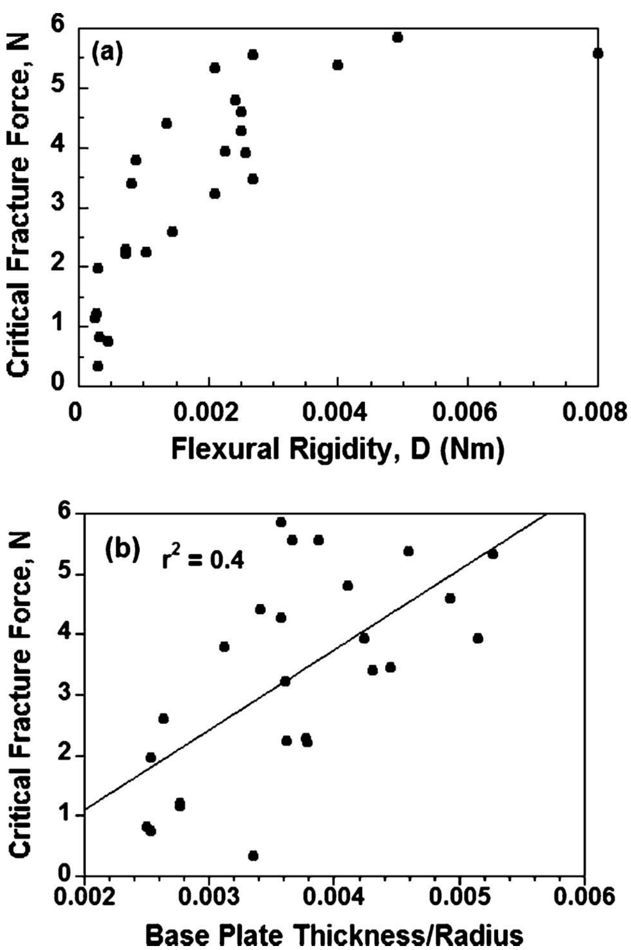

Figure 7. (a) Force required to fracture a hydrated barnacle base plate (N) plotted against its flexural rigidity (Nm) and (b) base plate thickness/radius ratio, t/a, (one-way ANOVA: $p < 0.001$).

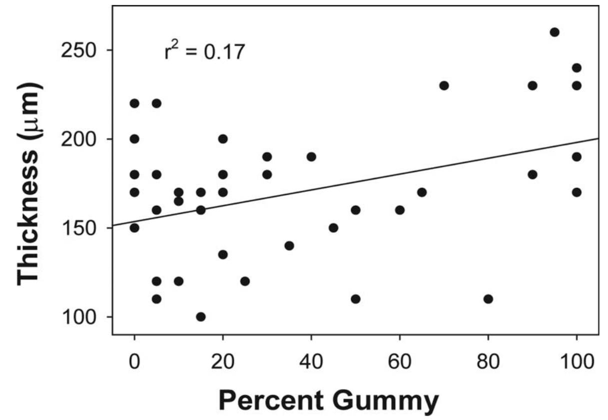

Figure 8. Thickness of the base plate (μm) versus the percentage of the base plate covered in opaque, 'gummy' cement. This linear regression is statistically significant (one-way ANOVA: $p = 0.006$).

gummy barnacles support the idea that age is not a major mechanical factor. Base plate flexural rigidity of hard barnacles remained constant through both age groups. Despite differences in both age and hydration level, the gummy barnacles also showed statistically similar flexural rigidity values. Although 3- and 14-month-old barnacles did differ in hydration level owing to differences in our testing procedure, the majority of cement water loss occurred after the time scale (~20 min) relevant to mechanical testing. It is interesting to note that despite the higher percentage of gummy barnacles measured in the 14-month-old barnacle group (which had a larger sample size), and the higher hydration level expected in 14-month tests, the gummy barnacles did not show statistically different flexural rigidity than hard barnacles.

In contrast, modulus values for hard and gummy cement barnacle base plates differed significantly. Hard barnacles showed a higher modulus than gummy barnacles. Data for 14-month-old barnacles provided the best estimation, as they most closely reflect the hydrated gummy cement in its natural state and the sample size was larger. From these experiments, the most reliable values for base plate modulus are 6.5 GPa (SEM ± 0.6) for hard and 2.9 GPa (SEM ± 0.7) for gummy cement. The lower modulus values for the gummy base plates are likely because of a combination of incomplete curing of the cement (resulting in greater water content) and possibly reduced mineralization of the cement, because crystallization of $CaCO_3$, if present, would require a supersaturated solution for crystallites to grow (see eg Dove and Hochella 1993). The lower modulus for gummy barnacle base plates is consistent with other mechanical measurements made at different scales, including near-surface mechanical response by atomic force microscopy (AFM), which showed lower modulus for gummy barnacle cement than hard barnacle cement (Sun et al. 2004). The measurements of barnacle base plate moduli from the present punch mechanics studies resulted in 2–3 orders of magnitude higher overall moduli than the measurements of Sun et al. (2004). This is not surprising, as the measurements assess the flexibility of the entire base plate, and not simply the near surface regions consisting primarily of proteinaceous cement. As such, the modulus measurements should be most appropriately referred to as composite moduli of barnacle base plates.

The results show that despite the lower modulus of gummy barnacle base plates, the flexural rigidity of gummy barnacles was not statistically different from hard barnacles. Chung and Chaudhury's (2005) model predicts that a reduction in the flexural rigidity of a flexible punch will reduce critical pull-off stress. Studies of release stresses as a function of base plate type for *B. amphitrite* barnacles (Wendt et al. 2006) showed that the 'gummy' base plate barnacles had lower removal forces than 'hard' barnacles. It is therefore likely that differences in adhesion between the gummy cement and silicone are responsible for the lower removal stresses, because base plate compliances were statistically similar for all barnacles. In accord with Kavanagh et al. (2005), it was observed that upon release of gummy barnacles from their silicone panel some water is often left behind. This is consistent with poor adhesion between gummy cement and the silicone, and consistent with others' observations of concave base plate geometry for some gummy barnacles (Wiegemann and Watermann 2003; Wendt et al. 2006).

It is interesting to consider the possibility of base plate thickness influencing the mechanical properties and flexural response of barnacle base plates. For gummy barnacles that are well-adhered to the substratum, it would be advantageous to have a flexural rigidity similar to that of hard barnacles to maintain as high as possible release stress. Although dislodgement of the base plate because of hydrodynamic forces is unlikely under natural conditions (Denny 1995), maintenance of high release stress may reduce the risk of dislodgement by neighbouring barnacles or removal by predators (such as fish; Swain et al. 1998). Analysis of base plate gummy cement fraction vs base plate thickness showed a weak, but significant ($p = 0.006$), increasing trend as shown in Figure 8. Certainly, the increasing thickness appears to compensate for the overall lower modulus of 'gummy' barnacle base plates.

Finally, by comparing the flexural rigidity data presented here to the model of Chung and Chaudhury (2005) it can be inferred that that flexural rigidity of barnacle base plates can contribute to the release forces for real barnacles. Flexural rigidity values were of order 0.002 Nm, which is considerably lower than what would be expected for a steel punch. For example, a thin steel plate (100 μm thick) yields a flexural rigidity of $\sim$0.02 Nm; increasing the thickness to 0.001 m results in a flexural rigidity of $\sim$20 Nm. Based on this simple estimate, it is plausible that the flexibility of barnacle base plates contributes to release stresses. How flexural rigidity of barnacle base plates relates to the mechanics for specific coating thicknesses and moduli will be explored in future studies.

Conclusions

The flexible rigidity of barnacle base plates was measured and found to be statistically similar for all barnacles studied, independent of age or base plate type (gummy or hard). Barnacles with $>30\%$ gummy cement (by area fraction of base plate) showed significantly lower composite modulus of the base plate than 'hard' barnacles ($\leq 30\%$ gummy area fraction). The similarities in flexural rigidity despite the differing modulus between hard and gummy base plates was attributed to the increase in thickness of the base plate found in 'gummy' barnacles.

The flexural rigidity of barnacle base plates was found to be of order 0.002 Nm. This is considerably lower than the flexural rigidity of a hollow steel punch as thin as 0.0001 m. These results suggest that the rigid punch approximation (Kendall 1971) often applied to model barnacle release mechanics is not sufficient to account for the contributions to the adhesion mechanics because of flexing of real barnacles during release.

Acknowledgements

The authors gratefully acknowledge Manoj Chaudhury of Lehigh University and Eric Holm of the Naval Surface Warfare Center, Carderock Division for helpful discussions. DBR and GHD were supported through the Naval Research Enterprise Internship Program at NRL. This research was funded by the US Office of Naval Research both at NRL and at Duke University (Grants N000140-510469, -710170 and -510580).

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