

Shell clamping behaviour in the limpet *Cellana tramoserica*

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Accepted 5 December 2001

Summary

The behaviour of clamping the shell against the substratum may play an important role in the limpet adhesion mechanism because friction generated by this behaviour resists dislodgement by shear forces. This paper describes the development of an apparatus to analyse limpet clamping activity in relation to known forces, including simulated wave activity and predator attack. The results show that *Cellana tramoserica* clamps its shell in a closely regulated manner consistent with an active role in the limpet adhesion mechanism. Limpets clamped sharply for several seconds in response to single disturbances such as tapping the shell. In response to more continuous disturbance simulating a concerted

predator attack, limpets clamped tightly for several minutes. In response to lifting forces applied to the shell, limpets clamped at a set proportion of the lifting force, even if the lift force was a highly dynamic wave profile. This behaviour has implications for numerical models that attempt to describe limpet adhesion because it shows that limpets cannot be represented by a simple mechanical analogue and that the clamping behaviour must be accounted for if useful predictions are to be drawn.

Key words: limpet, *Cellana tramoserica*, shell clamping, behaviour, adhesion, defence, force, wave action.

Introduction

It is widely acknowledged that limpets ‘clamp’ or ‘hunker down’ when disturbed in an effort to prevent dislodgement (Cook et al., 1969; McAlister and Fisher, 1968). Until now, however, there has been no attempt to quantify the clamping response or to investigate its role in the limpet adherence mechanism. This lack of information regarding clamping has meant that models of limpet adhesion/shell shape have been unable to include clamping and, hence, treat limpets as mechanical structures rather than biological organisms that may be able to respond to applied forces (Denny, 2000). This arguably limits the accuracy of these models, particularly their ability to provide insight into the selection of limpet structural characteristics and the metabolic costs of adhesion (Santini et al., 1995).

Shell clamping brings the lower rim of the conical shell of a limpet into direct contact with the substratum. This creates friction between the shell and substratum that provides increased resistance to horizontal shear and prevents dislodgement. Most studies that observed clamping involved the role of clamping as a defence against predators (Branch and Cherry, 1985; Thompson et al., 2000), although Denny (1988) suggested that clamping has a role in resisting hydrodynamic drag.

Smith (1992) found that limpets use suction while foraging at high tide. This is a problem for limpets because, although

suction provides good resistance to hydrodynamic lift, it provides poor resistance to shear forces. In this paper, we propose that limpets clamp their shell against the substratum to generate a frictional force that resists horizontal shear. Shell clamping against the substratum uses the vertical adherence strength of the suction mechanism to create a frictional force that resists the shear force of hydrodynamic drag. Fig. 1 outlines the forces exerted on the limpet while exposed to fluid flow.

Smith (1991b) showed that the force with which limpets adhere to the substratum is often greater than the force applied to the shell during detachment. Although this result may have been indicative of shell clamping behaviour, the apparatus used by Smith (1991b) (which was designed to investigate the role of suction in the adherence of limpets) was not able to isolate clamping from other mechanisms that may lower the pressure beneath the foot. A complete analysis of clamping behaviour requires an ability to differentiate between these mechanisms so that the role of each in adhesion may be understood.

The ideal experiment to verify the use of clamping by limpets in resisting drag would be to measure the forces of lift and drag induced by wave action on a number of limpets while simultaneously measuring the force of shell clamping for the same limpets. Thus, in an ideal experiment, the force of clamping could be directly related to hydrodynamic forces, and

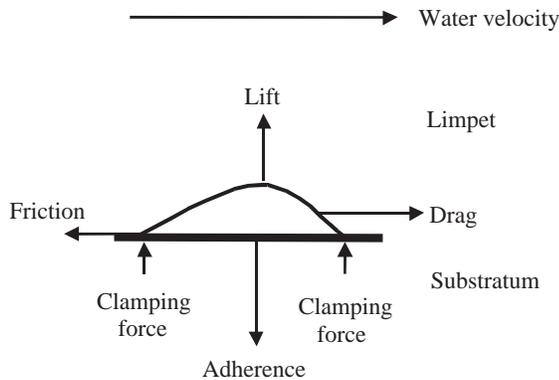


Fig. 1. A force diagram describing the forces acting during wave action. The model assumes that the limpet clamps its shell against the substratum in order to generate friction to resist horizontal shear.

the behavioural mechanics of limpet shell clamping would be known.

Unfortunately, both these tasks are difficult. It is impossible to measure directly the hydrodynamic forces that are placed on the limpet without resorting to placing the limpet on some sort of force plate. Another technique may be to predict hydrodynamic forces on the basis of flow measurements and shell shape parameters; however, the highly turbulent flow of the intertidal zone limits the precision with which the actual forces may be measured, and the magnitude of these errors is likely to dominate any evidence of behavioural regulation. It is also difficult to measure the degree of shell clamping without inserting something under the shell and, in doing so, changing the flow characteristics of the organism. It is not, therefore, feasible to measure either the hydrodynamic forces placed on the organism *in vivo* or the behavioural response of the limpets to these forces *in vivo*.

We decided to construct an apparatus that removed the vagaries of force measurement inherent in trying to conduct experiments *in vivo*. The apparatus was able to apply lifting forces to the limpet shell that could be accurately controlled and measured, allowing simulation of wave impact or other force profiles, as required. The apparatus was also able to measure the force of shell clamping directly and independently of the pressure beneath the limpet foot and therefore could, unlike the apparatus of Smith (1991b), specifically identify and quantify clamping for the first time.

While a number of previous researchers have developed apparatus to measure the adherence of intertidal organisms (Branch and Marsh, 1978; Denny, 2000; Grenon and Walker, 1981; Miller, 1974; Smith, 1991b; Thomas, 1948), none has attempted to measure limpet clamping responses or, with the exception of Smith (Smith, 1991b), addressed behavioural responses. The results presented here suggest that shell clamping behaviour is an active component of the limpet adherence mechanism of resistance to wave action. This has implications not only for biomechanical models of adhesion but also in identifying the pressures of natural selection on shell design. Recognition that limpets have biologically active

responses to wave action suggests that energy efficiency, not resistance to maximum wave forces, may be the primary selective force for limpet shell design.

Materials and methods

The method developed for the measurement of shell clamping involved removing limpets from their natural environment and performing measurements in a laboratory under controlled conditions. While this technique had the obvious disadvantage of placing the limpets in a foreign environment that may have confounded their behaviour, it had the essential advantage that accurate measurements could be performed. Accurate measurement was required because the results showed that the clamping force was often an order of magnitude lower than the adherence force, and therefore difficult to detect in the absence of accurate measurements.

Limpet collection and maintenance

Cellana tramoserica (Holten 1802) longer than 27 mm were collected from exposed, sandstone platforms near Terrigal (NSW, Australia). Limpets were removed from the substratum by placing the flattened tip of a diving knife under the leading edge of the shell and twisting the knife slowly. The majority of limpets collected in this way showed no sign of injury; however, less than 5% of the limpets, mainly those collected while exposed at low tide, showed signs of damage to the underside of the foot or musculature. It is thought that these limpets were using a glue-type adherence mechanism, as described Smith (1992), and thus suffered greater injury because of their high adherence. Damaged limpets were discarded at the collection site.

The limpets were placed in a bucket of freshly collected sea water. The bucket was lined with a netting bag to prevent the limpets adhering to the side of the container. They were then transported to a constant-temperature laboratory (18 °C), where an air hose was inserted into the water to ensure oxygenation. Limpets remained in the bucket and were used within 12 h of collection.

Development of the apparatus

We developed an apparatus that was able to measure directly the forces acting during adhesion. Newton's first law states that, if the limpet remains adhered to the apparatus and does not undergo acceleration, then the sum of the force acting in each direction must be zero. Hence, for forces acting in the vertical direction during adhesion:

$$F_{\text{Adherence}} = F_{\text{Clamping}} + F_{\text{Lift}}, \quad (1)$$

where $F_{\text{Adherence}}$ is the tensile force placed by the foot on the substratum, F_{Clamping} is the force with which the covering shell is clamped against the substratum and F_{Lift} is the simulated lift force applied by the apparatus to the limpet shell. $F_{\text{Adherence}}$ is not to be confused with the maximum force of adhesion, or $F_{\text{Adherence}}$ at adhesive failure, used in previous studies.

The apparatus was designed so that it could accurately

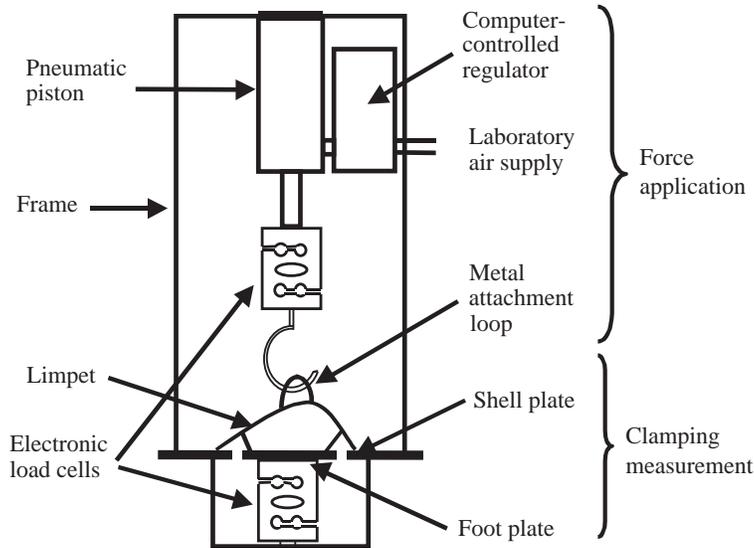


Fig. 2. Clamping measurement apparatus. The upper load cell was used to measure the simulated lift force applied to the limpet by the piston; the lower load cell measured the force of pedal adhesion.

measure $F_{\text{Adherence}}$ and F_{Lift} using electronic load cells; hence, F_{Clamping} could be calculated directly from these two measurements using equation 1.

Hardware

The apparatus consisted of a mechanical component and an electrical component. The mechanical component was used to apply, direct and measure forces (Fig. 2), while the electrical component was used for apparatus control, data acquisition, signal processing, storage and analysis.

Mechanical component

The mechanical component of the apparatus fulfilled two main tasks. The first of these was to generate and apply a lifting force to the limpet shell; the second was to measure the force of adherence of the foot.

Lift forces were generated using a pneumatic piston that connected to the limpet shell *via* a load cell to a hook and a metal loop that had been glued previously to the centre of the shell. The force exerted by a piston is dependent upon the cross-sectional area of the piston and the pressure within the cylinder. The force output is therefore independent of the extension of the piston shaft from the cylinder. This allowed a controlled force to be applied to the limpet in a similar way to hydrodynamic lift because the limpet was allowed to select its own preferred position of adhesion during the application of the force. This was different from the force application apparatus of Grenon and Walker (Grenon et al., 1979; Grenon and Walker, 1981), which raised the limpet shell from the substratum at a set rate, causing extension of the foot and hence applied force in a way that was very different from natural phenomena such as wave action in which lift is independent of shell height.

Measurement of the force with which the foot adhered to the

substratum required the construction of a force cell that isolated the tensile attachment of the foot. The force cell (Fig. 2) consisted of a central foot plate surrounded by a peripheral shell plate. The limpet was placed on the cell such that the foot adhered to the central plate while the shell contacted the outer plate. The underside of the central plate was connected to the outer plate *via* a load cell and a rigid metal frame. This design allowed the measurement of the forces described in equation 1. The force cell measured the force of adhesion ($F_{\text{Adherence}}$), and a separate load cell measured the forces acting on the shell (F_{Lift} and F_{Clamping}).

Electrical component

A PC-based data-acquisition and control system was developed that used a commercially available data-acquisition and control card and associated software (Computer Boards Inc.). The card contained a number of analogue inputs and outputs and supplied 12-bit analogue-to-digital conversion for a maximum digitising error of $\pm 0.03\text{ N}$ at a logging frequency of 1 kHz. The relatively high degree of digitisation accuracy was needed to reduce digital noise in later comparative analyses of applied and clamping forces. The high logging frequency was needed to ensure accuracy of measurement when applied and clamping forces were changing rapidly.

Load cells were a commercially available S-beam design using a full Wheatstone bridge configuration, hermetically sealed, and a natural frequency of 11 kHz (Cooper Instruments). Bridge power, and resultant signal amplification, used a strain gauge amplifier integrated circuit (RS components) configured to provide three different gain settings for different force measurement ranges.

The cylinder pressure for the pneumatic piston was controlled by an electronic pressure regulator controlled directly *via* an analogue output of the data-acquisition and control card. Power for the regulator came from a 12 V laboratory supply.

Calibration

The system was calibrated using 500 g metal blocks at the beginning and end of each data collection period. Calibration variation was less than 0.01 % of full-scale deflection during the entire experimental period. Calibration consisted of measuring the output of the load cell in the absence of load and then repeating the procedure with incrementing numbers of metal blocks of known mass attached. The data collection system was calibrated to give force readings in newtons.

Performance

A trial was conducted to determine the ability of the mechanical section of the apparatus to follow controlling electronic inputs, specifically in relation the rate of change in the force (dF/dt) supplied by the pneumatic piston. A model limpet (suction cap) and live *Cellana tramoserica* were

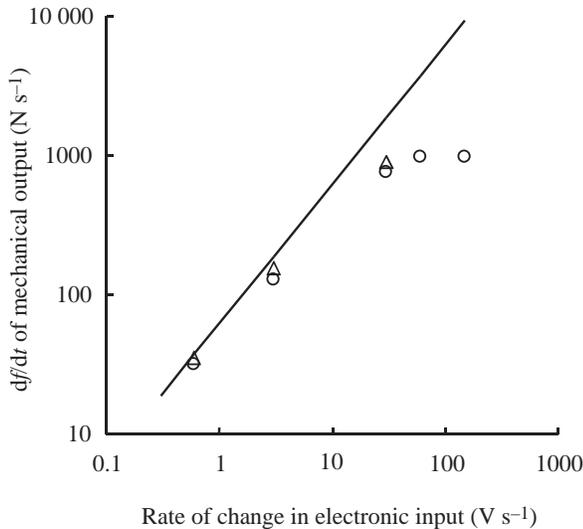


Fig. 3. Performance curve for the force application apparatus. The mechanical output matched that predicted by the electronic input for force application rates up to 1000 N s^{-1} . The line is theoretical. Δ , *Cellana tramoserica*; \circ , suction cap.

detached using the apparatus at a number of different electronic control rates. The output performance of the mechanical section of the apparatus was then compared with that predicted from the electronic controlling input (Fig. 3).

Rates of force increase achieved by the apparatus were very similar to that predicted by the electronic input up to a rate of force change of 1000 N s^{-1} (Fig. 3). A value of $dF/dt > 400 \text{ N s}^{-1}$ was considered sufficient for the experiment since this exceeded the rate of change for hydrodynamic forces associated with a limpet undergoing extreme wave action, based on the rate of change for an average limpet of basal area 12.5 cm^2 undergoing a change in water velocity from 0 to 20 m s^{-1} in less than 50 ms .

A number of different force profiles were used during the investigation. Simulated wave force profiles were adapted from wave force data collected by Gaylord (1999). Wave profiles were used so that the regulation of clamping could be studied under conditions that simulated the dynamic force regime of the exposed intertidal zone. Linearly increasing force profiles of 40 N s^{-1} were also used so that the lifting force could be increased to the point of detachment for the limpet. This allowed us to observe the behavioural characteristics of limpet clamping across the entire range of limpet adhesion and provided a stable stimulus so that clamping could be studied at the population level. Force was also applied by hand; this replicated the linearly increasing force profiles, although with less precision.

Determination of the degree of clamping

Limpets were placed on the force cell so that the foot of the limpet attached to the central foot-plate and the shell rested on the peripheral shell-plate (see Fig. 2). Small barriers were placed around the limpet to limit movement of the foot relative

to the adherence plate. Studies of the re-attachment of the foot showed that the anterior and peripheral sections sealed against the substratum early in the re-attachment process, often resulting in the limpet trapping a bubble of air or water under the foot. Forward motion causes this bubble to be ejected from the rear of the foot; however, since motion was restricted in this study by the need to position the foot accurately on the sensor, significant bubbles remained beneath the feet of limpets used in the study. The size of the bubbles did not appear to influence behaviour in any way, but a drop of up to an order of magnitude was observed in maximum adhesion compared with limpets that were able to re-attach normally.

A lifting force was applied to the re-attached limpets using one of the two techniques mentioned previously (20 simulated wave profiles; 38 linearly increasing profiles). The lift and adherence forces were measured simultaneously, and the force due to limpet clamping was calculated by subtracting the lifting force from the adherence force for each simultaneous measurement. A linear regression was performed to determine the response of clamping to lift for each individual.

Results

Cellana tramoserica placed on the sensor showed a number of small clamping movements of less than 1 N within the first 2 min . This was consistent with the limpet attempting to orient properly and attach its foot to the sensor. After this initial re-attachment period, no clamping activity or attempted movement was observed without the application of a stimulus.

The relaxed state of the limpets after the initial settling period was taken as evidence that the relocation process did not stress the limpets. The limpets responded with sudden clamping actions to individual stimuli such as tapping the shell or the apparatus. This clamping rose sharply to a maximum force of $2\text{--}5 \text{ N}$ before decaying back to a relaxed state within $1\text{--}2 \text{ s}$. This response was thought to be analogous to the clamping or 'hunkering down' of limpets when disturbed in the intertidal zone.

Prolonged 'harassment' of the limpet (continuous tapping) induced a more concerted response in which the force of clamping increased to approximately 25 N and remained at that level until the harassment stopped. When this stimulus was stopped, the degree of clamping decayed slowly over the next 5 min to less than 5 N , often persisting for up to 15 min at $1\text{--}2 \text{ N}$. Any repeat of the harassment stimulus during the decay period induced an immediate return to higher levels of clamping and a similar decay period on cessation of the harassment. This behaviour illustrates the clamping behaviour of *Cellana tramoserica* when under attack from predators such as crabs, birds or fish or, of course, repeated attempts to remove the limpet with a flat-tipped diving knife.

The relaxed state of the limpets in the absence of the above stimuli and the responses of the limpets to the different types of stimuli provided evidence that the limpets were behaving in manner similar to what would be expected in their natural

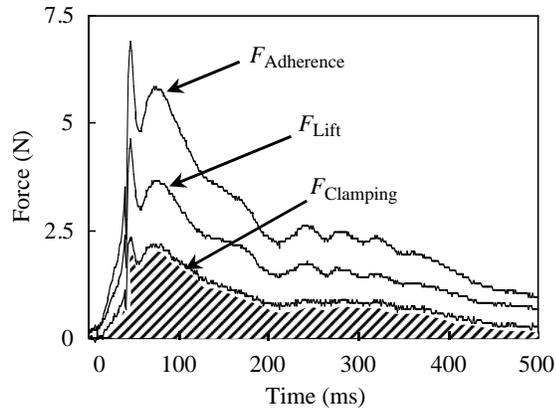


Fig. 4. A typical experiment for the limpet *Cellana tramoserica* exposed to a simulated wave force. The adherence force ($F_{\text{Adherence}}$) of the foot was consistently greater than the lift force (F_{Lift}). The force difference between the adherence and lift force was generated by the action of shell clamping by the limpet (F_{Clamping}) (hatched area).

environment. This observation provides a degree of confidence that the observations collected during later experiments involving the application of lift forces were effective representations of the behaviour of the limpets in response to lift in their natural environments.

Detection of clamping in response to lift forces

The adherence force for each limpet placed on the force cell was greater than the lifting force when exposed to a simulated wave profile (Fig. 4). In each case, the limpet remained attached to the force cell during the experiment and did not undergo acceleration, implying the existence of a third force such that the net force on the limpet was zero.

The difference between the adherence force and the lift force was highly dynamic and closely followed the variation in the lift force. Analysis of cross covariance showed there was no time lag between adherence and lift forces within the 1 ms detection limit of the apparatus. This raised the possibility that the force difference was a result of inaccurate calibration between the load cells rather than evidence of limpet shell clamping activity.

The experiment was repeated in the absence of the limpet, with the two load cells stuck directly together using double-sided tape. This showed that, in the absence of *Cellana tramoserica*, the difference between the lift and adherence force was zero (Fig. 5). In addition, the apparatus was sensitive enough to detect the elasticity in the double-sided tape as it stretched during the first 25 ms of force application. In the absence of elasticity, the error between the two load cells was 0 ± 0.08 N. We therefore interpreted any sustained difference between the adherence and lift of more than 0.16 N as evidence of shell clamping activity.

These tests allowed us to discount calibration errors in the force measurement and confirmed the existence of a third force generated by the limpet clamping its shell against the outer shell plate. This clamping force rose to almost 2.5 N, which

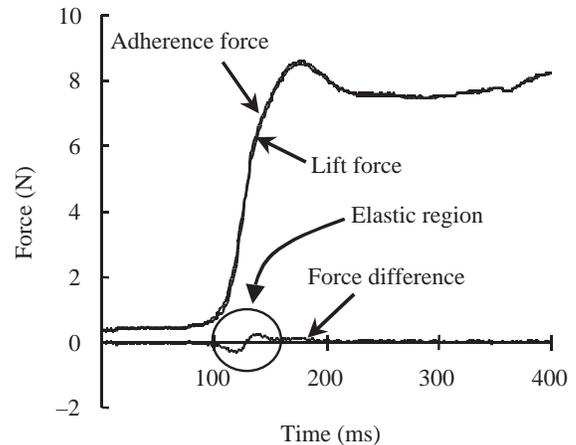


Fig. 5. Force/time curve in the absence of the limpet *Cellana tramoserica*. The transient force difference seen at the onset of the lift force (circled) was a result of elasticity in the double-sided tape connecting the two force sensors.

was an order of magnitude higher than the 0.16 N threshold set for the detection of clamping.

Clamping in response to simulated wave forces

Further analysis was performed on the data presented in Fig. 4 to determine the relationship between the degree of clamping and the lift force (Fig. 6). The force of shell clamping was found to be proportional to the lift force ($y=0.38x-0.09$, $r^2=0.99$). The limpet was able to maintain this response despite the dynamic nature of the lift/time profile applied during the experiment (see Fig. 4). The dynamic range of lift used in the experiment was comparable with that observed in natural wave conditions. The limpet therefore rapidly increased and decreased the tension within the pedal musculature in order to follow the wave-like lift profile closely.

Taken together, Figs 4 and 6 showed that limpet shell clamping behaviour has a role in the adhesion mechanism used by limpets to resist wave forces. Clamping was observed to be an active process regulated by the limpet in response to changes in the lift force.

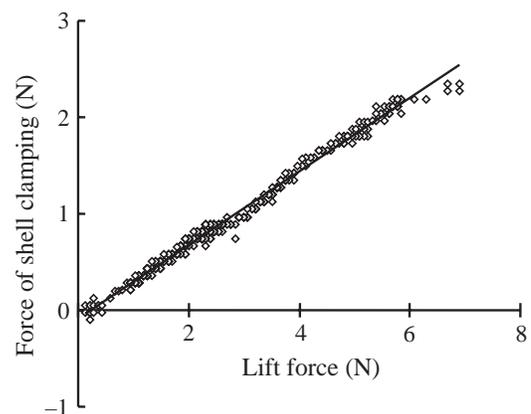


Fig. 6. The force of shell clamping as a function of the lift force for the limpet undergoing the simulated wave profile shown in Fig. 4.

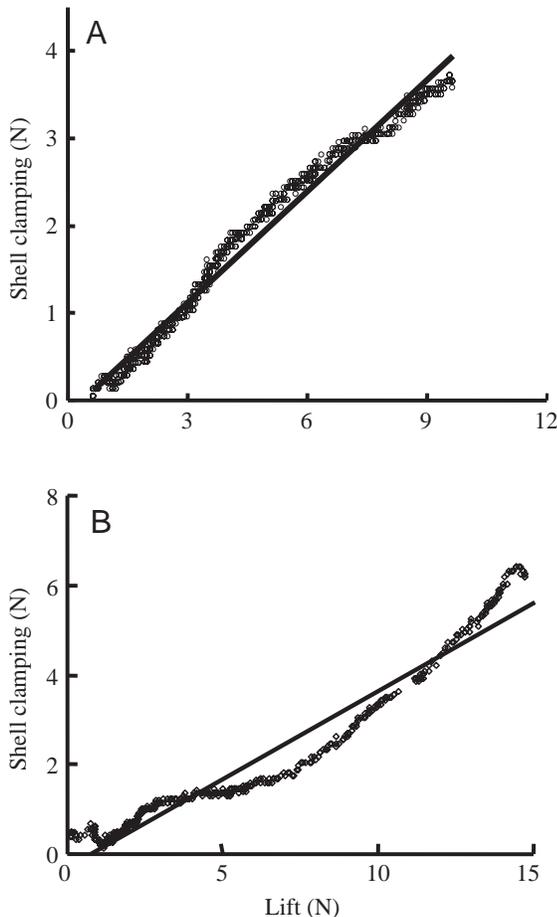


Fig. 7. The degree of shell clamping in response to applied lift for *Cellana tramoserica* exposed to linearly increasing lift forces. Limpet clamping displayed a linear trend (A, $y = 0.43x - 0.16$, $r^2 = 0.98$; B, $y = 0.39x - 0.27$, $r^2 = 0.95$) with respect to lift; however, differences could be observed between limpets (see A and B) both in the slope of the trend and in the degree of variation about the trend line.

Clamping in response to linearly increasing tensile loads

Observation of clamping in response to simulated wave action showed that limpets were able to clamp in an active manner consistent with the use of clamping as part of their adhesion mechanism. It was impossible, however, to predict the maximum force that could be applied to the limpet without detaching it from the sensor. The study of responses to simulated wave profiles did not, therefore, allow the clamping behaviour of the limpet at the limits of pedal adhesion to be examined. An examination of the response of the limpets at the limits of adhesion required a new set of force profiles that allowed lift to be applied to the limpets in a uniform and comparable way.

The clamping response of *Cellana tramoserica* was observed in response to linearly increasing lift profiles. The force of shell clamping displayed a linear trend in response to the lift force (Fig. 7), and this trend persisted while the limpet was attached to the sensor. The force of clamping displayed some variation around the linear fit for each limpet; however,

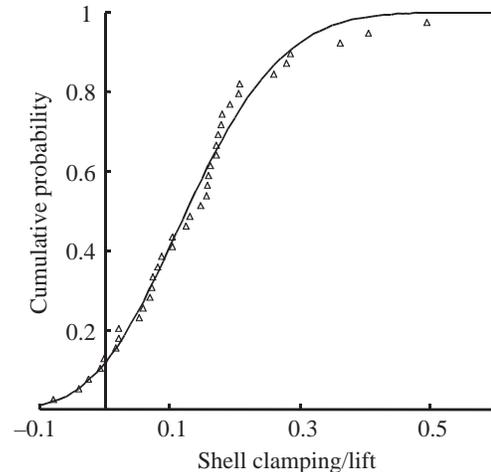


Fig. 8. The cumulative probability distribution of shell clamping as a proportion of the lift force for 38 individuals of the limpet *Cellana tramoserica*. Each limpet consistently clamped at a given proportion of the lift force. The figure shows the variation in the proportional response among individuals. The curve is described by $P(x) = \exp\{-[(a-bx)/(a-bc)]^{1/b}\}$, where $a = 0.1241345$, $b = 0.2015748$ and $c = 0.08828305$.

no patterns in this variation were discernible among individuals (Fig. 7A,B). Variation was also observed among limpets in the gradient of the clamping trend in response to lift.

Measurements were made of the degree of clamping for 38 individuals, and the gradient of the clamping trend with respect to lift was calculated. Experience showed that the clamping response of each limpet was unchanged between force applications, providing that the limpet was not detached from the apparatus. However, variation in the gradient of the clamping response existed among the 38 individuals tested, showing a range of clamping responses from -0.08 to $+0.5$ times the applied lift force (see Fig. 8).

The degree to which the observed variation in clamping was due to measurement error, rather than to variation in the limpet population, could not be determined. Clearly, negative values for the degree of clamping as a proportion of the applied lift reported in Fig. 8 would be impossible if the limpets were to remain attached to the substratum. If the lift force is greater than the adherence force of the foot, then immediate detachment should result. These individuals remained attached to the apparatus, indicating that, in these cases at least, the apparatus may have significantly underestimated the magnitude of pedal adhesion. Checks revealed that the apparatus was correctly calibrated in each of these cases.

It appears that the location of attachment of the foot may influence the measurement of pedal adhesion. Despite attempts to hold the limpet in place during attachment to the apparatus, the limpet could move its foot within the shell in some cases, so that some of the foot adhered to the peripheral shell plate rather than to the central foot plate. The force sensor was therefore able to measure only the adherence force generated by the section of the foot adhered to the central plate and, hence, underestimated the adherence force. In

some cases, presumably when individual clamping activity was already low, this underestimation caused the measured adherence to be lower than the applied force. This resulted in the negative value observed for the degree of clamping as a proportion of the applied lift. It was impossible to determine the degree to which clamping was reduced for each individual; hence, it was impossible to correct for this error in the data. Therefore, while strong clamping was detected in limpets during the experiment, the actual degree of clamping would be marginally higher than the evidence presented here suggests.

It therefore seems clear that limpets actively clamped their shells against the substratum as part of their adherence mechanism. This clamping was regulated and was related to the potential threat to adherence faced by the individual. When no threat to adherence was evident, clamping was absent. In response to predator-like stimuli, *Cellana tramoserica* engaged in continuous defensive clamping; in response to wave-like action, a linear response to lift was observed that varied among individuals.

Discussion

The experimental data presented here report the first quantified evidence of shell clamping by limpets. The actively regulated nature of the clamping observed suggests a role in the overall limpet adhesion mechanism. The possible use of clamping in adhesion by limpets has been suggested previously; Denny (1988) suggested that it was a mechanism involved in countering hydrodynamic drag. This is especially important for limpets using a suction adherence mechanism because, although the foot is able to generate an adherence force several times atmospheric pressure in the vertical direction, it is relatively weak in shear (Smith, 1992). Indeed, it was poor resistance to shear that Smith (1992) used as a distinguishing feature between limpets using suction adherence and those using glue-type adhesion for individuals adhered to Perspex sheets.

Clamping of the shell against the substratum causes friction between the shell and substratum that resists horizontal motion (shear). It is therefore not surprising that limpets may use shell clamping as an integral part of their adhesion strategy since this allows the limpet to resist both hydrodynamic lift and drag with a pedal suction attachment that is only able to generate significant resistance to vertical detachment. The present study shows that *Cellana tramoserica* use clamping to aid in resistance to hydrodynamic shear and predator attack.

Hydrodynamic forces generated by wave action are highly dynamic and may vary from zero to maximal values in as little as 50 ms for impinging flows on hard-bodied organisms (Gaylord, 2000). The present study demonstrated that limpets were able to respond in a regulated manner to rates of change of force of this magnitude. It was also evident that the muscular strength of the limpets was such that the degree of clamping could be maintained up to the limits of adhesion of the foot. It is therefore likely that clamping adhesion plays an integral part

in the limpet adhesion mechanism at all times when a suction-type adhesion mechanism is being used by the limpet.

No limpets were observed to be using a glue-type adherence mechanism during the trials (Smith, 1992; Smith et al., 1999), so it is impossible to predict whether clamping behaviour is active when limpets are using this adhesion method. Our observation that limpets 'hunkered down' when disturbed during collecting at low tide provides anecdotal evidence that the clamping mechanism was still active during glue-type adhesion; however, nothing is known of the behavioural regulation of clamping in this situation.

Suction and clamping

Smith (1991b) performed an experiment that positively identified suction as an adhesion mechanism in limpets. In the process of this experiment, he developed an apparatus that was able to measure the pressure under the foot of the limpet in comparison with a vertically applied force. This apparatus was roughly comparable with the one developed here in that it was able to measure independently an applied lift force and the force of adhesion of the foot (providing that the area of the attached foot could be accurately determined). The results from this apparatus showed that the force of adhesion due to suction was greater than that required to resist the applied force. Indeed, this was the evidence used by Smith (1991b) to suggest suction as a viable adherence mechanism.

The findings of Smith (1991b) and the results presented here support the idea that limpets cannot be approximated by the simple mechanical analogue of a suction cap. A suction cap is a passive device in which the pressure drop beneath the cap is a direct result of the detachment force placed on the cap. The force of adherence in this instance is therefore equal to the applied force. The individuals of *Cellana tramoserica* observed in this experiment used the muscular tissue of the foot actively to develop pedal adhesion in excess of the force applied to them. This process of active adhesion is a conceptual departure from previous adhesion models that treat limpets as mechanical devices and, as such, exclude consideration of muscular activity and associated metabolic costs (Denny, 2000).

There are two possible mechanisms by which limpets could reduce the pressure beneath the foot independently of the application of a detachment force. The first has been demonstrated in the present experiment. Clamping the shell against the substratum requires additional adhesive force for the maintenance of translational force equilibrium. This places additional tension on the fluid enclosed by the foot and results in a reduction in the under-foot pressure.

It is also apparent that limpets remain adhered whilst moving over the substratum, and this suggests that another attachment mechanism also operates by which limpets are able to achieve adhesion through suction in the absence of shell clamping. A model for this mechanism was described by Jones and Trueman (Jones, 1973; Jones and Trueman, 1970; Trueman, 1969) and involves the foot acting simultaneously in both tension and compression. Sections of the foot contract upwards

to produce tension in the fluid enclosed beneath the foot fluid, creating zones of reduced pressure; these are balanced by normal forces from the substratum placing other sections of the foot under compression. The limpet is able to move across the rock surface as these zones of reduced pressure migrate across the foot in pedal waves. Hence, the limpet can maintain suction adhesion and translational movement in the absence of both an applied or clamping-derived force.

This proposed non-clamping adhesion mechanism is analogous to suction adhesion produced by cephalopod suckers (Smith, 1991a, 1996). In the case of cephalopods, a central spindle is retracted to generate a reduced pressure beneath the sucker, while the outer edges of the sucker act under compression against the surface. In the limpet, however, this process occurs in several sections of the foot simultaneously and involves a complex interaction between muscular activity and fluid partitioning within the pedal haemocoel (Jones, 1968, 1970, 1973; Jones and Trueman, 1970; Trueman, 1969).

The clamping and non-clamping suction adherence techniques are both supported by experimental evidence (Jones, 1968, 1970, 1973; Smith, 1991b, 1992; Smith et al., 1993; Trueman, 1969). The behavioural regulation of these mechanisms with respect to each other has, however, yet to be investigated. This regulation is important because limpets must maintain adherence across a range of movement requirements within a dynamic wave force environment. How they do this has consequences for energy usage and exposure to detachment so should be an important factor in describing limpet physiology. Investigation of this interaction awaits the construction of an apparatus that is able to measure under-foot pressure and the force of clamping simultaneously.

Substratum-dependence of shear resistance

Grenon and Walker (1981) examined the resistance of limpets to horizontal shear on a number of substrata and found that resistance was substratum-dependent, in contrast to their findings for resistance to vertically applied forces, which was independent of substratum. This is consistent with the role of shell clamping behaviour in the resistance to horizontal shear because shell clamping generates frictional forces that are substratum-dependent in the direction of shear, but are substratum-independent for lift. For surfaces to be equivalent in resisting lift, they need only have a similar surface energy and, hence, provide equal adherence for pedal mucus in the event that negative pressures are generated beneath the foot (Smith, 1991a, 1996).

Given the complex nature of the rocky substratum, it is likely that the coefficient of friction between the limpet shell and the substratum varies greatly within small spatial scales as a result of mechanical interactions between the shell and small deformations in the surface. Measurement of the coefficient of friction between the shell and substratum is therefore not a simple task and has not been included in this paper. Evaluation of the frictional environment of limpet shells is important if the evidence of clamping presented here is to be included in the biomechanics of limpet adhesion.

Clamping and limpet adhesion models

Denny (2000) has developed an integrated model of the limpet adhesion mechanism that includes adhesion, shell shape and resistance to hydrodynamic forces. This model determined maximal hydrodynamic forces placed on the foot by wave action on the limpet shell. It then compared these forces with the empirical ability of limpets to resist these forces independently in the directions of lift and horizontal shear, and used this to calculate orthogonal stress indices. The model was then used to determine the optimum value of a shape parameter that minimised stress on the foot.

Unfortunately, when the optimum value of the shape parameter was compared with empirical values, it was found that a large variation in limpet shell shapes existed, and most of these were different from that derived from the adhesion/shell shape model. In response to this, a number of non-hydrodynamic selective forces were identified that may have influenced shell shape and thus limited the application of the model to real situations (Denny and Blanchette, 2000).

The present quantification of the limpet clamping mechanism facilitates the development of adhesion/shell shape models that involve active suction adhesion and proportional shell clamping in the resistance to horizontal shear. The results indicate that a consideration of energy expenditure should be added to the list of influences on shell shape (Denny and Blanchette, 2000).

Selection pressure due to exposure to maximum wave forces is limited in its ability to shape limpet shells because limpet adherence is so high that non-streamlined shapes may persist in all but the most severe of wave conditions. Once shells have even a small degree of streamlining, they are able to withstand even the largest waves; hence, the selective mechanism becomes inoperative (Denny and Blanchette, 2000). This is in contrast to the shape of limpet shells, which have a cross section associated with low drag, and may include fine-scale adaptations such as ribs that induce boundary layer separation for early transition to lower drag coefficients (Denny, 1989). This suggests that hydrodynamics is an important factor in the selection of shell shape; however, the critical hydrodynamic parameter is likely to be something other than the maximum fluid velocity.

Thanks go to Mr Barry Lees and Mr Bruce Ellem who aided in the construction of the apparatus. We would also like to thank Dr Brian Gaylord for his then unpublished wave data. Professor Mark Denny and Dr Andrew Smith, together with the two referees, are acknowledged for their comments and feedback, which were most helpful. Lastly, we would like to thank Dr Paul Dastoor for his enthusiastic encouragement and constructive criticism

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