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Structural resistance of algae to herbivores

A biomechanical approach

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Abstract

Experiments were conducted to determine the forces required for two limpet species to remove tissue from three different types of algae. This was done by using the radulae of the limpets, mimicking the way they use this feeding apparatus. For both *Acmaea mitra* Rathke (a specialist herbivore) and *Notoacmea scutum* (Rathke) (a generalist herbivore), *Iridaea cordata* (Turn.) Bory (a thin, bladed red alga) required the most force; *Hedophyllum sessile* (C.Ag.) Setch. (a thick, bladed brown alga) the next; and *Pseudolithophyllum whidbeyensi* (a crustose, calcified alga) required the least amount of force for tissue removal. These results are the inverse of the predictions made by current functional form and functional group models which use the thallus form as a predictor of herbivore resistance. Thallus form alone is a poor predictor of structural resistance to molluscan feeding; the material properties of the alga, the feeding apparatus of the herbivore, and the mode of feeding all must be considered.

Introduction

Algal form and herbivore mode of feeding have been considered to be major factors that influence plant susceptibility to herbivore damage. Several researchers have placed algae into functional groups based on thallus form, and predicted that these groups would exhibit differential susceptibility to herbivores (Littler and Littler, 1980; Steneck and Watling, 1982).

Limpets are common intertidal herbivores. They have a docoglossan type radula (the major feeding structure of herbivorous gastropods that consists of a long chitinous ribbon of tissue with numerous rows of teeth). Radular tooth number and shape differ greatly among limpets (Fretter and Graham, 1962). Their diets also vary substantially. Some limpets are specialists and eat only a few

species of algae, while others are generalists and have broad diets (Branch, 1981).

A docoglossan radula is characterized by few teeth per row, with the teeth rigidly fixed to the radular ribbon rather than articulating with it (Fretter and Graham, 1962). Teeth are constantly being produced posteriorly and shed anteriorly (Fig. 1). This radular type functions differently than those of other herbivorous gastropods (Fretter and Graham, 1962). In the docoglossa (families Patellidae, Acmaeidae, and Lepetidae), the buccal mass and the teeth move together, without any pivoting of one on the other. Numerous rows of teeth are applied to the substrate simultaneously and the whole structure is moved as a unit. This action scores the substratum with a number of parallel lines, indicating the absence of tooth rotation (for a more complete description of gastropod feeding see Fretter and Graham, 1962).

The radular teeth of limpets are generally mineralized with iron and/or silica. These minerals are very hard (5.0 to 6.5 on Mohs scale of hardness; Runham *et al.*, 1969), and therefore allow the limpets to eat endolithic or

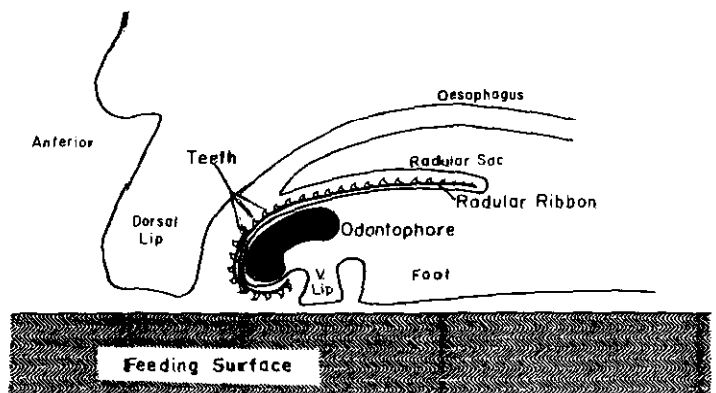


Fig. 1. Generalized longitudinal section through the anterior portion of a limpet, showing the position of the radula relative to the mouth and oesophagus (adapted from Barnes, 1980 and Fretter and Graham, 1962)

calcified algae whose hardness is less than this (the hardness of calcite is 3.0).

This study involved several aspects of feeding in two limpet species: *Acmaea mitra*, a specialist herbivore which preys only on encrusting, calcified algae in the family Corallinaceae, and *Notoacmea scutum*, a generalist herbivore. These species are common in the mid and low intertidal region (+1.3 m to -1.0 m; 0 = mean lower low water) of the Pacific Northwest coast of North America. Both limpets have docoglossan radulae and therefore feed in a similar fashion. Both have teeth mineralized with iron and silica (Lowenstam, 1962, 1981; Padilla, unpublished data).

Initially, the feeding mode was observed to confirm the description of docoglossan feeding by Fretter and Graham (1962). I then determined the forces required for radulae of these limpets to remove tissue from three algal species with different thallus forms. The algae tested were: (1) *Hedophyllum sessile*, a leathery, brown alga, (2) *Iridaea cordata*, a thin, bladed red alga, and (3) *Pseudolithophyllum whidbeyensi*, an encrusting, calcified red alga. Finally, I attempted to determine some of the parameters of tooth morphology that are important in removing plant tissue. In particular, the role of the cutting angles of teeth was examined by the use of scale models, comparing this biological system to what is known about abrasive tools in machining.

This information was used to test some of the predictions made by functional form (Littler and Littler, 1980) and functional group (Steneck and Watling, 1982) models which predict algal structural resistance to herbivores on the basis of thallus form. These two models predict that the encrusting coralline alga would be the most resistant to herbivores, and that the bladed red alga the least resistant.

Materials and methods

I observed feeding with a video camera focused through a dissecting microscope. A limpet was placed on a glass slide that had been lightly coated with diatoms to induce feeding. The slide was then inverted over a dish filled with sea water, completely submerging the limpet.

The forces required to remove tissue from the algae were determined as follows. Radulae from freshly collected limpets were cleaned to tissue and kept moist until used. Isolated radulae were attached to a Plexiglas support (2.2 mm wide) that placed the radulae in feeding position. The anterior-most portion of each radula was attached with a cyanoacrylate adhesive, positioning four to six rows of teeth on the working surface of the support. The support was then attached to a beam, placing the radular ribbon parallel to an alga placed beneath it (Fig. 2A). The beam was counterbalanced in such a way that the radula and support placed no force on the alga. Various loads (= normal forces) were applied to the radula by attaching weights with dual adhesive tape to the beam directly above the radula.

The alga being tested was attached at one end with a cyanoacrylate glue to a glass slide. The glass slide rode on tracks of ball bearings, thus minimizing friction (Fig. 2B). The slide was attached to a force transducer mounted on a micromanipulator. Thus, when the alga was pulled past the radula with the micromanipulator, the force required to do so (= lateral force) was determined.

A linearly variable differential transformer (LVDT, Pickering and Co., Inc. model 7307 W3-AO) was mounted on the micromanipulator to determine the velocity at which the alga was moved. The LVDT measures the

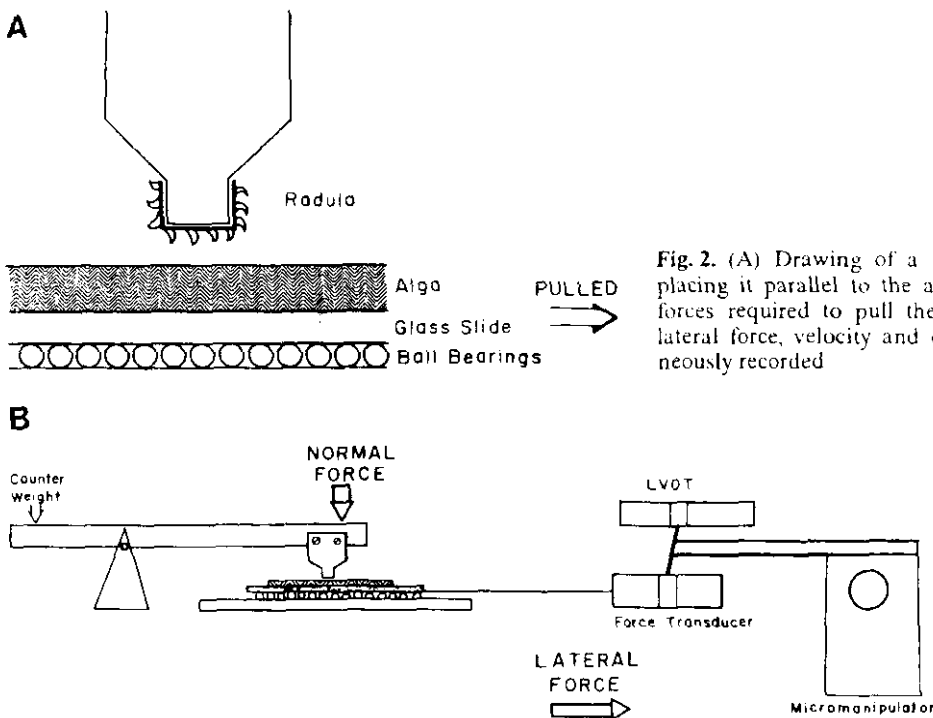


Fig. 2. (A) Drawing of a radula attached to the Plexiglas support, placing it parallel to the alga. (B) Apparatus used to determine the forces required to pull the alga past the radula. The normal force, lateral force, velocity and distance the radula traveled were simultaneously recorded

distance the alga travels, and using the chart speed of the recorder as a time base one can calculate the velocity as the slope of the charted displacement curve. These velocities were matched to those of radulae in live limpets when feeding. At least four replicate trials each were made for a range of normal forces (0.049 N to 0.400 N) with radulae from both limpet species on all three algal species.

Algae and radulae were kept wet, but not submerged during all trials (they were removed from the water immediately before each trial). At the end of each test, pieces of the bladed algae were frozen and sectioned with a

cryostat. Cross sections were examined to determine if any tissue had been removed by the radulae. The crustose coralline algae were air dried and examined with a scanning electron microscope (SEM) to determine if any tissue had been removed by the radulae. These procedures also allowed the size and characteristics of the marks made by the radulae to be determined.

Size, tooth angles and relative proportions of the radular teeth of both *Acmaea mitra* and *Notoacmea scutum* were determined from SEM micrographs. Camera lucida drawings were used to obtain profiles of the teeth. This information was used to construct large scale models of radular teeth from Plexiglas. Models were of equal width, so that only tooth shape varied. The models were then scraped across a smooth plaster of Paris surface to determine the effect of varying tooth angle.

Finally, the radula of *Acmaea mitra* was attached in an orientation backwards from the normal feeding position, and the algae pulled past the radula in the same fashion as described above. This manipulation maintained all of the properties of this radula constant except for the tooth angles. Comparing these results with those of the radula in feeding position is an additional test of the importance of tooth angles.

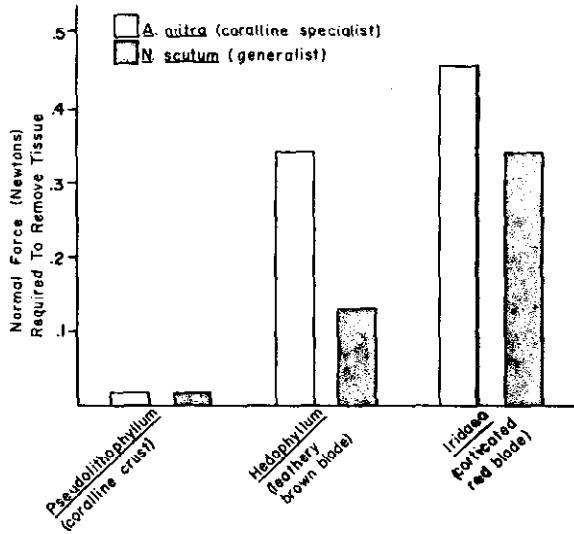


Fig. 3. Minimum normal force required for *Acmaea mitra* and *Notoacmea scutum* to remove tissue from *Iridaea cordata*, *Hedophyllum sessile*, and *Pseudolithophyllum whidbeyensi*. Although the absolute values were not always equal, the rank order of vulnerability of the algae for the two limpets was the same. (In all cases the variance is zero, therefore these differences are significant)

Results

Force required to remove tissue

Video observations of feeding in *Notoacmea scutum* confirmed the reports of feeding behavior in the literature (Fretter and Graham, 1962). *Acmaea mitra* would not feed under these conditions. However, the marks made by the radulae of *A. mitra* in the force experiments closely resem-

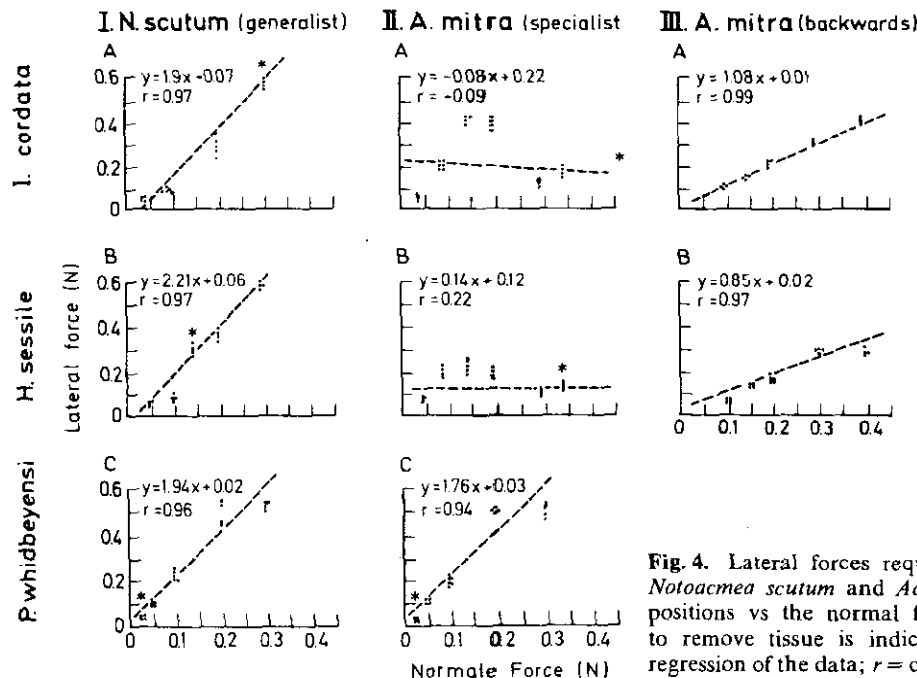


Fig. 4. Lateral forces required to pull each alga past the radulae of *Notoacmea scutum* and *Acmaea mitra* in normal and reversed feeding positions vs the normal force applied. The minimum force required to remove tissue is indicated (*). (Lines represent the least-squares regression of the data; r = correlation coefficient)



Fig. 5. *Acmaea mitra*. Side (A) and front (B) views of the radula of *A. mitra*. The scale bar = 100 μm

bled the marks made by these limpets when feeding in the field and under laboratory conditions (Padilla, personal observation).

For all algae except the crustose coralline, the radula of *Acmaea mitra* required more normal force to remove tissue than that of *Notoacmea scutum*. However, for both limpets, the rank order of vulnerability of the algae was the same (Figs. 3, 4). The crustose coralline required the least amount of normal force to remove tissue, the leathery brown blade required the second most, and the thin red blade required the most force.

Tooth Structure

Acmaea mitra has six similarly-shaped teeth per row (Fig. 5), and each tooth has a width-to-length ratio of approximately 1:2. These teeth have a rake angle (the angle of the face of the tooth from normal to the working surface; Fig. 6) near 50° , and a clearance angle (the angle from the working surface to the back of the tooth; Fig. 6) of 0° to 10° , depending on the amount of wear on the teeth. The total amount of tooth area in contact with an alga was $97 \mu\text{m}^2/\mu\text{m}$ length of radula, or $1.2 \times 10^6 \mu\text{m}^2/5$ rows of teeth (the average number of rows in contact with the surface during a single motion of the radula). *Notoacmea scutum* has four effective teeth per row, with the first lateral teeth being longer than the second (Fig. 7). The third lateral teeth are greatly reduced and fused to the second lateral teeth. As the first lateral teeth are longer and in the same path as the second lateral teeth, they were used for the scale model. Compared to the teeth of *A. mitra*, they are proportionally longer and thinner, with a width-to-length ratio of 1:3 or 1:4, depending on the amount of wear. They have a rake angle of approximately 20° (it was impossible to measure this angle exactly from the profiles as these teeth are concave), and a clearance angle of about 50° . The surface area in contact with the substrate was $13 \mu\text{m}^2/\mu\text{m}$ length of radula, or $1.6 \times 10^5 \mu\text{m}^2/5$ rows of teeth; one-eighth of the tooth area was in contact with the substratum of *A. mitra*.

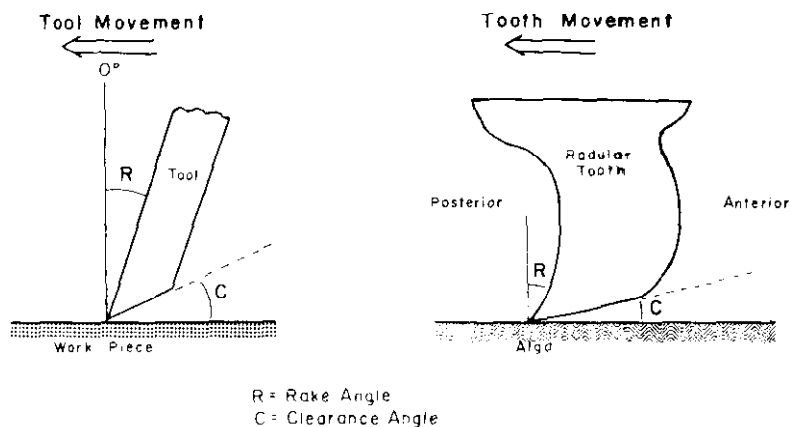


Fig. 6. Rake angle (measured from normal to the work piece) and clearance angle (measured from the surface of the work piece) for an abrasive tool. The corresponding angles of a radular tooth are also indicated.

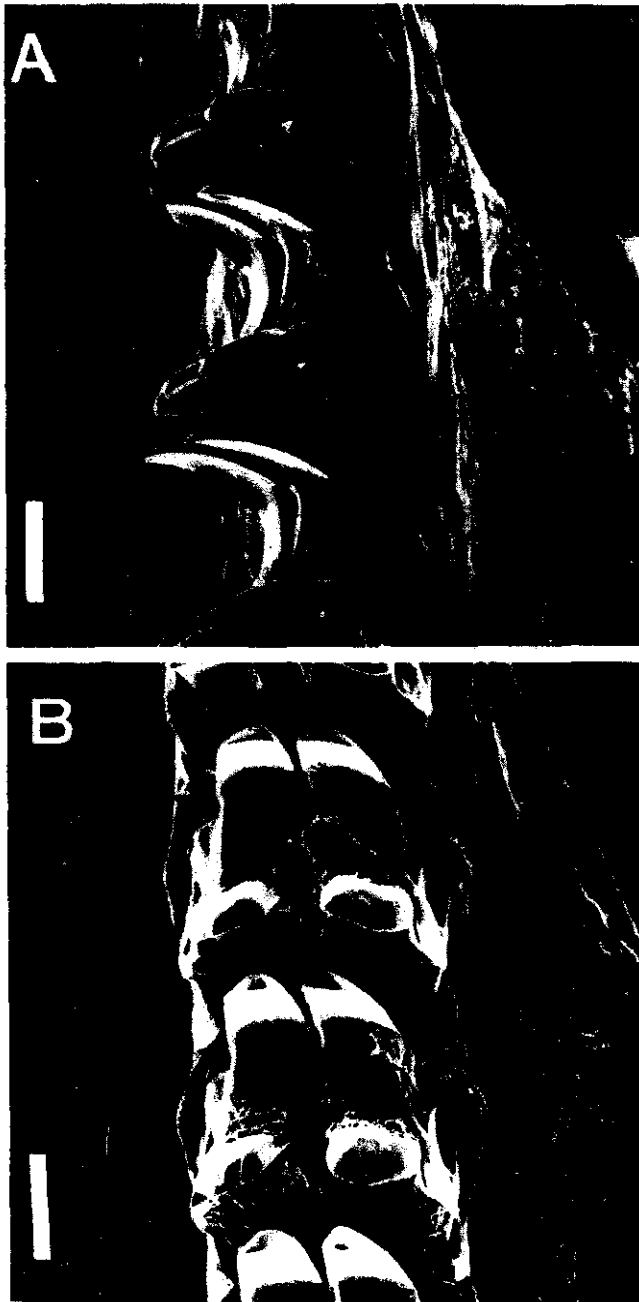


Fig. 7. *Notoacmea scutum*. Side (A) and front (B) views of a worn radula of *N. scutum*. The teeth are more worn anteriorly and less worn posteriorly (anterior: top A and B). The scale bar = 100 μm

Effect of tooth orientation

When the model teeth were scraped across plaster of Paris, the *Notoacmea scutum* model was more effective at a wider range of tooth angles (-45° to $+45^\circ$) than the *Acmaea mitra* model. The *A. mitra* model was effective at the normal position (0°) and negative angles, but less effective at positive angles where friction caused by the low clearance angle resulted in the tooth sliding along the surface rather than cutting it.

When the radula of *Acmaea mitra* was reversed to test the importance of tooth angles, the rake angle changed from $+50^\circ$ to -90° and the clearance angle from $+10^\circ$ to $+120^\circ$. The forces required to pull the algae past the radula were quite different than for the radula in the natural feeding position (Fig. 4).

Discussion

Researchers have placed algae into groups based on thallus form and have claimed that these groups exhibit differential resistance to herbivores (Littler and Littler, 1980; Steneck and Watling, 1982). These models predict that the thin blade would be the least resistant and the crustose coralline would be the most resistant to herbivores. The results of the above experiments were the inverse of the predictions of these models. This discrepancy could be a result of the experimental methods that were used by Littler and Littler (1980) and Steneck and Watling (1982) to predict structural resistance. The experiments reported here used actual radulae of herbivorous gastropods and mimicked the manner in which these limpets use their radulae. Littler and Littler (1980) used a penetrometer (a plunger-like apparatus) and measured the force required to push this plunger through the algae, referring to this as "toughness". This plunger had rounded edges, a surface area four to five orders of magnitude greater than the area of the feeding apparatus of a limpet, and did not mimic the feeding behavior of any marine herbivore examined to date (fishes, crabs, sea urchins or molluscs; personal observation). Steneck and Watling (1982) determined the "scratchability" of algae using a stylette (of unmentioned material), referring to this as "toughness". Such scratch tests, however, are a measure of hardness, and do not take into account the importance of tooth composition and the consequences of different degrees of normal force applied, both of which were shown to be important in this study.

The ability of plants to resist tissue loss to herbivores depends on the properties of the algae as well as the feeding apparatus of the herbivore. The more energy required to remove tissue from a plant, the more difficult that plant will be to eat.

Materials may have different mechanical properties when forces are applied to them in different ways (i.e. a material may be more resistant to breakage in compression than in tension, or more resistant to forces applied in one direction than another; Wainwright *et al.*, 1975). Thus, to determine a plant's ability to resist tissue loss to a particular herbivore, it is important to mimic the manner in which the herbivore feeds.

The hardness of a material is generally measured as the ability of one material to scratch another; hardness is poorly correlated with the amount of energy that is required to break that material. If the feeding apparatus of the herbivore is not as hard as the algal material, the herbivore will not be able to scratch the plant. However,

limpets and many other herbivorous molluscs have radulae enriched with iron and silica (Lowenstam, 1962, 1981; Runham *et al.*, 1969), both of which are much harder than calcified algae. Although the teeth of limpets are capable of scratching all types of algal tissue, it still requires different amounts of force (and therefore energy) to remove tissue from different types of algae.

In the coralline algae, calcification occurs in the form of minute crystals of calcite, which form an integral part of the cell walls (there is no intercellular calcification; Borowitzka, 1982). Coralline algal tissue, like many biological ceramics, is very brittle and breaks rather than bends under applied forces (Wainwright *et al.*, 1975). The fleshy algae, on the other hand, are flexible, and initially stretch under applied forces, therefore requiring more energy to break them.

An alga that is very resistant to tissue loss against one consumer may not be resistant to another that feeds in a different manner or that has a feeding apparatus with different mechanical properties. For the consumer to be an effective feeder on a particular type of alga, its teeth must be minimally harder than the algal materials. In addition, the numbers of teeth and the surface area of the teeth in contact with the alga influence the tip stress of the teeth (force per unit area of tooth in contact). The fewer the number of teeth and the smaller the surface area in contact with the alga, the greater the applied force (and stress) at each tooth for a given force applied to the entire radula. For example, the total surface area of the teeth in contact with an alga is eight times greater for *Acmaea mitra* than it is for *Notoacmea scutum*. Therefore, for any force applied to the entire radula, the actual force at each tooth tip will be eight times greater for *N. scutum* than for *A. mitra*. This difference may explain why the normal force required to remove tissue from noncalcified algae was much greater for *A. mitra* than for *N. scutum*.

Acmaea mitra and *Notoacmea scutum* differ in the shapes of their teeth, particularly in the clearance angles. In machining, the rake and clearance angles are critical aspects of abrasive tools (Fig. 5). Mulhearn and Samules (1962) and Samules (1978) determined that there is some critical value for the rake angle of an abrasive tool to be effective at removing material; the critical angle is dependent on the material being abraded. If the tool is held at a rake angle more positive than the critical angle, it will cut the material (cleanly remove material); otherwise, the tool tends to plow (push material to the side). The actual magnitude of the rake angle, if it is greater than the critical angle, is unimportant until it becomes so positive that the clearance angle is decreased to the point where friction occurs between the cutting surface and the substratum (Samules, 1978).

For soft tissues, such as bladed algae, a 0° clearance angle may result in the tooth sliding across the surface rather than cutting it (e.g. *Acmaea mitra* teeth on *Hedophyllum sessile* and *Iridaea cordata*). On a material that is harder, such as a calcified alga, a 0° clearance angle would result in rapid wear of the tooth, but this wear may in fact

make the teeth more effective tools. Runham and Thornton (1967) found, when studying the mechanical wear of the mineralized teeth of the limpet *Patella vulgata*, that initially the tips of the teeth break off and then the teeth wear to an effective chisel shape. Runham *et al.* (1969) found that for *P. vulgata* there was differential wear on different portions of the tooth that maintained a sharpened edge on the tooth, essentially like a self-sharpening knife. This worn configuration could therefore be the most effective shape for these teeth (Hickman, 1980).

Although the thallus form may be important in influencing the impact of herbivores (or other disturbance agents) on algae, it may act more by influencing availability of the plant or causing difficulty in handling, rather than by influencing structural resistance to tissue loss. In any case, the material properties of the alga, the properties of the feeding apparatus and mode of use of the feeding apparatus of the herbivores are important components in determining algal susceptibility to herbivores.

Both Littler and Littler (1980) and Steneck and Watling (1982) chose to describe physical resistance to herbivores as "toughness", although they were measuring different properties in different ways. Engineers generally use the term toughness to refer to the ability of a material to resist the propagation of critical cracks (cracks or breaks in the material that result in structural failure), but even this is not totally agreed on (Gordon, 1968; Wainwright *et al.*, 1975; Vincent, 1982). Terms such as toughness carry both technical and colloquial meanings and connotations, and consequently result in much confusion. I therefore suggest that terms such as toughness should not be used unless carefully and explicitly defined.

Present functional group and functional form models do not appear to be good predictors of algal resistance to herbivores. Rather, one must consider other material properties of the plant, the feeding apparatus of the herbivore, and mode of feeding.

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