14: Biomechanics

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I. Introduction

A. Use of biomechanics in ecological research

A major goal of ecology is to discover and to explain quantitatively the mechanisms responsible for the distribution and abundance of organisms in time and space. Both physical and biological factors determine the distribution of macroalgae. An important physical factor in the distribution and form of seaweeds is the degree to which the plants are exposed to wave action and water currents. Moving water can transport nutrients, wastes, and propagules to and from seaweeds, but it can also rip plants from the substratum.

Which features of an alga enable it to withstand moving water? The theory and practice of fluid and solid mechanical engineering allow us to study quantitatively how algal gross morphology affects the magnitude of the flow-induced forces plants must sustain in particular habitats, as well as the distribution of mechanical stresses within the thalli (stress is a force per cross-sectional area of tissue bearing the force). Mechanics also enable us to investigate how algal microscopic anatomy affects the deformation and possible breakage of plants in response to those stresses. A spoonful of mechanics can provide a rich, multilevel understanding of how seaweed structure contributes to survival in mechanically rigorous habitats.

Disturbance can have important effects on the diversity of macroalgal communities. Moving water that rips organisms off the substratum is a major agent of disturbance in many rocky shore communities. Using a biomechanical approach, one can assess the susceptibility of various members of such communities to physical disturbance.

Biomechanics is a tool, not a subject that is an end in itself. The tool allows ecologists to use simple physical principles and engineering practices to study the role of mechanical factors, such as wave action, in limiting the distribution of particular macroalgae. In this chapter we shall focus on some simple techniques for studying the mechanics of biological materials but shall first mention a few leads into the literature for those interested in the fluid mechanics of algae or in the effects of gross morphology on stress distributions within the bodies of organisms bearing forces.

B. Determining mechanical stresses in algae in nature

A number of techniques have been developed to work out forces on and stresses in organisms in nature; we shall mention a few, citing references that include more procedural details.

The main mechanical loads that macroalgae bear are probably due to moving water. Ways of measuring water-flow-induced forces [drag force, for plants in steady currents; acceleration reaction force and drag, for plants in waves] on organisms in the field are described by Koehl (1977a) and Denny (1982). Alternative ways of assessing flow-induced forces involve measuring water velocities and accelerations encountered by the organisms (e.g., Koehl 1977a,d; LaBarbera and Vogel 1976; Tunnicliffe 1980) and then calculating drag and acceleration reaction on the organisms for such flow conditions (e.g., Koehl 1977a; Vogel 1981). Alternatively, one can measure drag forces on the organisms at those velocities (e.g., Charters et al. 1969; Koehl 1977a) in a flow tank (e.g., Vogel and LaBarbera 1978; Charters and Anderson 1980).

When a benthic alga is subjected to a load, such as the force imposed on it by moving water, it can be deformed in a number of ways (Fig. 14–1). Note that, when an alga is pulled (Fig. 14–1B) or bent (Fig. 14–1C) by a load, some or all of the tissue in its thallus is stretched and thus experiences tensile stress. The magnitude and location of such stresses in stiff organisms subjected to flow-induced forces or other mechanical loads can be directly measured by means of strain gauges (William T. Bean; Measurements Group, Vishay) glued to the organisms in the field (e.g., Tunnicliffe 1980) or laboratory (e.g., Vosburgh 1977). Even if an organism is too deformable or lubricious, as most algae are, to have strain gauges glued to it, estimates of the magnitude of the stresses within the tissues can be calculated if the force on the plant is known. Descriptions of the types of stresses produced in structures of various shapes subjected to loads applied in different ways can be found in standard engineering texts (e.g., Roark and Young 1975; Faupel and Fisher 1981), as can formulas for calculating the magnitudes of those stresses. The biologist can find in Alexander (1968) or Wainwright et al. (1976) expressions for stresses in structures of certain regular shapes, such as cylinders, loaded in particular uncomplicated ways. For algae undergoing large deformations in flowing water, one should choose the stress equations derived for large deformations rather than the standard small-deformation formulas (e.g., see Charters et al. 1969).

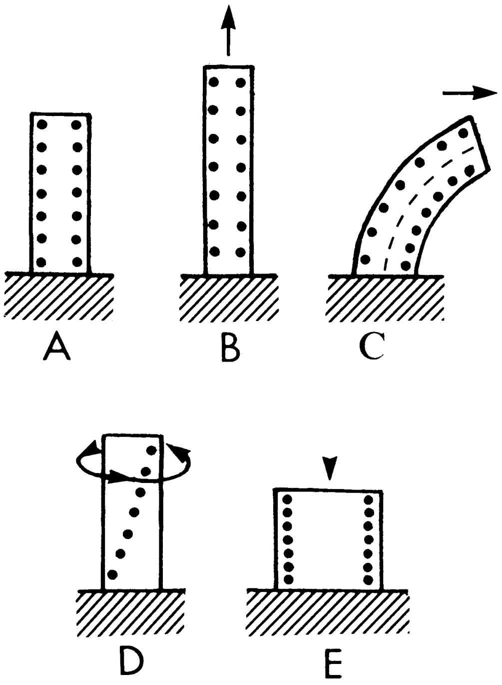

Fig. 14–1. Some ways in which a sessile organism can be deformed by a force. The arrows indicate the direction in which the organism is being deformed, the spots mark positions on the organism, and the hatched blocks represent the substratum. (A) Undeformed. (B) Pulled. (C) Bent. (Note that tissue on one side of the organism is stretched and on the other side is compressed. The dashed line indicates the neutral axis where tissue is neither stretched nor compressed.) (D) Twisted. (E) Compressed.

Analyses of flow-induced forces on, and stress distributions in, algae not only will enable one to design biologically relevant mechanical tests, but should also reveal ways in which the gross morphology of the plants affects their mechanical performance (e.g., see Charters et al. 1969; Neushul 1972; Koehl 1977a,b; Wainwright and Koehl 1976; Koehl and Wainwright 1977).

C. Deciding which mechanical tests to use

Mechanical tests can be used to measure basic mechanical properties of algal tisues (such as stiffness, resilience, strength, or toughness), to indicate the sorts of molecular mechanisms likely to be responsible for those properties, or to ascertain the response of algae to mechanical loading situations they would experience under various conditions in nature.

Which test(s) is chosen depends on the questions being asked. If one is interested in the deformability of an alga, the relevant tissues should be subjected to force–extension tests (see Sec. II) or the relevant structure to bending tests (see Sec. II.E). If the capacity of the tissues to recover their original shape after bearing a load is of interest, cyclic force–extension tests (Sec. III) or creep-recovery tests (Sec. IV) are appropriate. If one must know the breakability of the tissues, force–extension tests should be conducted until the specimens fracture (Sec. II). If one wants to study molecular adaptations of an alga to a mechanical habitat, long-term creep tests (Sec. IV) will point the way.

If the questions of concern are, for example, How do species A and B respond mechanically to wave action? Tidal currents? Rasping herbivores? then one should design algal loading regimes that simulate the situations of interest (Sec. V). In such simulations, the type (tensile, compressive, or shear; see Fig. 14–1) and magnitude of the stress applied to a specimen should mimic those in nature (see Sec. I.B). Furthermore, the rate at which the specimen is deformed during the experiment, as well as the duration of the deformation, should be the same as in the real-world situation. Such simulations should be specifically designed once these relevant variables have been measured or calculated for the algae in nature.

The types of specimens subjected to mechanical testing also depend on the questions asked. For example, if the behavior of whole stipes or blades is of interest, the relevant tests should be conducted on the entire structure. However, if the goal is to determine which tissues are the main load-bearing components of the structure (e.g., If a herbivore removes this tissue, what difference does it make mechanically?) or to work out the mechanical design of the alga (e.g., What morphological features of this alga render it so flexible?), the relevant tests should be conducted on isolated tissues from the plant (e.g., tissue just from the center and the periphery of a stipe or just from the proximal and distal ends of a stipe) and the results correlated with the microscopic structure of those tissues (Sec. I.D) and with their location in the plant. For an example of working out the mechanical design of an alga, see Koehl and Wainwright (1977).

Engineers have developed a large battery of mechanical tests to study the performance of materials. We describe here only a few basic types of tests that are relatively easy to perform and that yield a large amount of information relevant to ecological questions. For readers interested in conducting other types of tests, in more extensive discussions of molecular interpretation of test results, or in mathematical descriptions of mechanical behavior, we suggest consulting books on polymer mechanics, such as those of Nielsen (1965), Ferry (1970), or Aklonis et al. (1972). More information about the mechanics of biological materials can be found in Wainwright et al. (1976) or Vincent (1982).

Values should be expressed in SI units. For definitions of these units, as well as useful conversion tables, we recommend Mechtly (1973).

D. Importance of morphology in seaweed mechanics

The mechanical behavior of an alga depends on its gross and microscopic morphology. It is instructive for an ecologist to measure morphological correlates of the functional (in this case, mechanical) properties of algae and the physical environmental factors they encounter for several reasons. First, plant species are recognized by their morphology. Second, every function of an organism is permitted, controlled, and limited by its structure at lower levels of organization. Therefore, there is a high degree of interconnection among ecological, physiological, and developmental features of an organism via morphological parameters. Finally, morphological parameters can usually be readily measured with a high degree of accuracy and thus can be a sound basis for any analysis of a complex system.

A few mechanically important morphological parameters can be measured in a matter of seconds on fresh specimens that take less than 10 min to prepare. External linear measurements made with vernier calipers to $\pm$ 0.1 mm will allow accurate estimates to be made of the cross-sectional area of the blade, stipe, or holdfast of an alga. Alternatively, sections can be photographed with a Polaroid camera, attached to a microscope if necessary. The profiles in the photograph can be cut out and weighed and their weight compared with similarly prepared photographs of square millimeters.

Noncircular cross sections of an algal thallus prompt one to ask whether the wide or narrow side is aligned parallel to prevailing flow directions. This alignment is important in the consideration of bending of a seaweed thallus. When a structural element bends, the convex side is in tension, the concave side is in compression (Fig. 14–1C), and a plane somewhere between the two surfaces is called the "neutral axis" because there is no stress there. The resistance to bending of a structural element is called its "flexural stiffness" EI, where E is the stiffness of the tissue (see elastic modulus described in Sec. II) and I is the second moment of area of the cross section. Second moment of area measures the distribution of material around the neutral axis of the section and is given by $\int y^2\, dA$, where dA is an increment of area that is distance y from the neutral axis. For regular shapes, there are handy formulas for I that require the measurement of a radius and a face or two of the section. For a solid circular section, I is $\pi r^4/4$, where r is the radius, and for an oval shape is $\pi ab^3/4$, where a is the radius of the neutral axis and b is the radius perpendicular to a (Wainwright et al. 1976). Note that a small increase in the width of an alga in the direction of bending can lead to a large increase in its flexural stiffness.

A frozen section 10–20 μm thick of a tissue or organ can be made with a cryostat (an ordinary rotary microtome housed in a cooler that maintains a temperature around −20°C). Using the Polaroid photoprofile technique, one can determine the area percentage and then calculate the volume percentage of any tissue or cell wall component. By studying both cross and longitudinal sections, one can determine the orientations in an alga of continuous cell walls, which are often load-bearing structures.

By viewing these frozen sections between crossed Polaroid filters on a microscope, one can see the distribution of high concentrations of macromolecules that have a preferred orientation; such regions will shine brightly. With a first-order red interference compensator (available from Edmund Scientific Co.), one can readily determine the direction of this preferred orientation by determining the sign (+ or −) of birefringence by the simple methods described in Bennet (1961), Chamot and Mason (1958), and Wahlstrom (1960). One expects high strength and stiffness in places and directions where large molecules have preferred orientation. The location of these regions corresponds to the distribution of stress-resisting tissue components.

II. Force–extension tests

A. Purposes

Force–extension tests permit one to measure the stiffness, strength, and toughness of a piece of material. In such a test, a specimen is pulled until it breaks; the specimen's extension and the force with which it pulls back at each extension are measured throughout the test. So that results from different tests can be compared, extension is expressed as "extension ratio" λ, where

$$\lambda = L/L_o \tag{1}$$

Here, L is the extended length of the specimen and L_o is the original unstretched length of the specimen between the grips of the device used to pull it. Note that λ is a dimensionless number. Similarly, force is expressed as "stress" σ, where

$$\sigma = F/A \tag{2}$$

Here, F is the force (in newtons) with which the specimen pulls back against being pulled, and A is the cross-sectional area of the specimen (in square meters). Results of force–extension tests are plotted in Fig. 14–2. For examples of stress–extension ratio curves for kelp

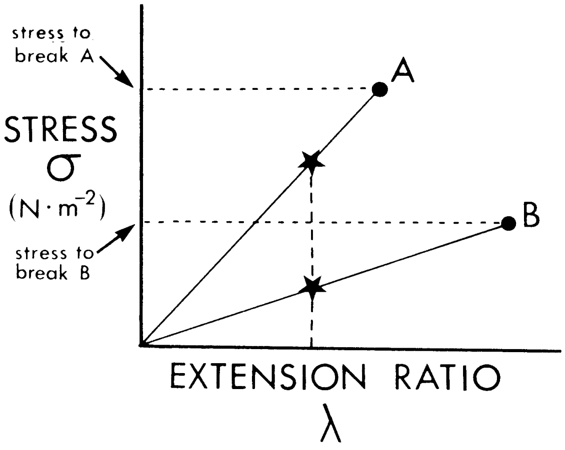

Fig. 14–2. Stress (σ)–extension ratio (λ) curves for pieces of tissue pulled until they broke. Note that, at a given extension, specimen A pulled back harder than specimen B (compare stars on the graphs). The slope (i.e., the elastic modulus, *E*) of line A is greater than that of line B; specimen A was stiffer than specimen B. Also note that the stress at which specimen A broke was greater than that at which specimen B broke (compare circles on the graph); specimen A was stronger than specimen B.

tissues, see Koehl and Wainwright (1977), or for various other plant and animal tissues, see Wainwright et al. (1976) or Vincent (1982).

One may encounter "stress–strain" curves in the literature. Some authors define strain ϵ in terms of the original length of the specimen (L_o),

$$\epsilon = \Delta L / L_o \tag{3}$$

where ΔL is an increment in length. Other authors define strain in terms of the length of a specimen at a given instant; such "true strain" (ϵ_T) is given by

$$\epsilon_T = \int_{L_0}^{L} dL/L = \ln L/L_o \tag{4}$$

where dL is a small increment in length, and L is the actual length of the specimen just before that increment is added. If one chooses to plot a σ–λ or a σ–ϵ curve, one calculates σ using the original cross-sectional area of the specimen. If one plots a σ_T–ϵ_T curve, one calculates "true stress" σ_T using the actual instantaneous cross-sectional area of the specimen (see the discussion of Poisson's ratio in Wainwright et al. 1976). Obviously, σ–λ curves are the easiest to do, which is why we have chosen to describe them here. However, before comparing results with those in the literature, one should make sure that all the curves being compared were calculated in the same way.

The "elastic modulus" E of a material is a measure of its stiffness,

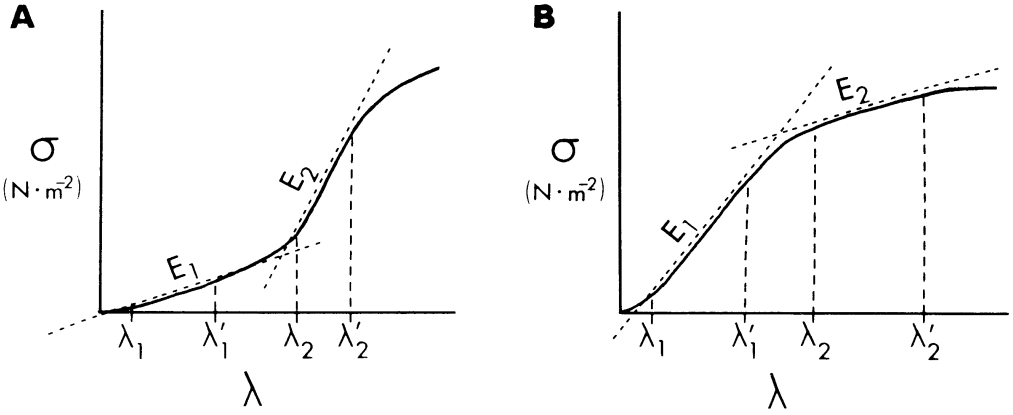

Fig. 14–3. Examples of nonlinear stress (σ)–extension ratio (λ) curves (solid lines). The slopes used to calculate elastic moduli (E_1 and E_2) for different portions of the curves (for extension ratios of λ_1 to λ'_1 and for λ_2 to λ'_2, respectively) are indicated by dashed lines.

(i.e., its resistance to deformation). Compare stress–extension curves A and B in Fig. 14–2. Note that, at a given extension, specimen A pulls back harder than specimen B; specimen A resists deformation more than specimen B. The slope of curve A is steeper than that of curve B. The elastic modulus is the slope of a σ–λ curve; the higher the E, the stiffer is the material. The E for a particular part of a σ–λ curve can be calculated using the formula

$$E = \Delta\sigma / \Delta\lambda \tag{5}$$

where $\Delta\sigma$ is the increment in stress accompanying a given increment in extension ratio $\Delta\lambda$. Since $\Delta\lambda$ is dimensionless, the units of E are the same as those for σ: newtons per square meter. Some materials (such as those in Fig. 14–2) have essentially one elastic modulus. Other materials are stiffer at some extensions than at others, (Fig. 14–3). In such cases, one elastic modulus can be calculated for a particular range of extension ratios, and another for a different range of extension ratios.

Many biological materials are stiffer when pulled quickly than when pulled slowly. Therefore, it is important to define the extension rate $\dot{\lambda}$ at which a test is conducted,

$$\dot{\lambda} = \Delta\lambda / \Delta t \tag{6}$$

where $\Delta\lambda$ is the increment in extension ratio made during a time interval, Δt. Since $\Delta\lambda$ is dimensionless, the units of extension rate are reciprocal seconds. Obviously, for stiffness measurements to be biologically relevant, tests should be conducted at $\dot{\lambda}$'s similar to those experienced by the tissues in the field, and E's should be calculated for realistic $\dot{\lambda}$'s, as discussed later.

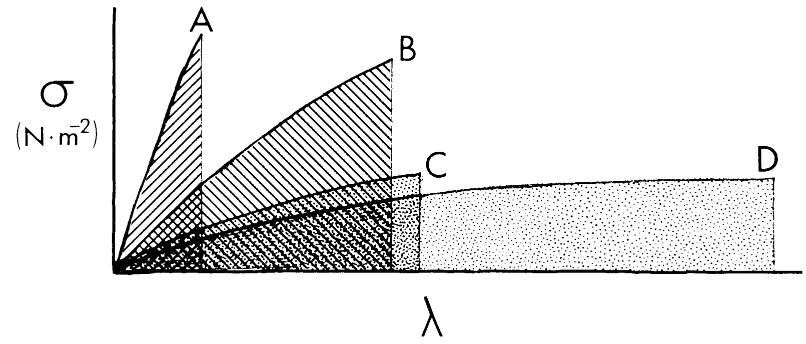

Fig. 14–4. Examples of stress (σ)–extension ratio (λ) curves for specimens of four different tissues pulled until they broke. The area under each curve (hatched or stippled) represents the work (joules) per volume (cubic meters) to break the specimen; the larger this area, the tougher is the specimen.

The strength of a material is defined as the stress required to break it (σ_{BRK}), given by

$$\sigma_{\mathrm{BRK}} = F_{\mathrm{BRK}}/A \tag{7}$$

where F_{BRK} is the force required to break the specimen and A is the cross-sectional area. Either the original cross-sectional area of the specimen or the instantaneous cross-sectional area may be used. We recommend the former because of ease of measurement. Which A has been used should be specified when σ_{BRK} is reported, or compared with σ_{BRK} in the literature. The greater the σ_{BRK} of a tissue, the stronger it is. Specimen A in Fig. 14–2 is stronger than specimen B.

Glass is stronger than leather, but it is generally more difficult to break a boot than a bottle because leather is tough whereas glass is brittle. The tougher a material, the greater the amount of work required to break it. The area under a σ–λ curve of a specimen pulled until it breaks is a measure of the work required to break the specimen. So that toughness values can be compared for different specimens, toughness should be expressed as work (in joules) per volume (in cubic meters) of tissue absorbing that work. Sometimes the toughness of a material appears to depend on the length of the specimen tested. Therefore, it is important to make toughness measurements on specimens of the same length if comparisons are to be made. Sometimes toughness is expressed in joules per square meter for specimens of a defined length rather than as joules per cubic meter.

In Fig. 14–4 specimens B and D are tougher than specimen A or C. By studying the relationship of strength (σ_{BRK}), stiffness (E), extensibility (λ_{BRK}), and toughness in Fig. 14–4, one will see that there are several different ways that a specimen can be tough. One easy way of measuring toughness is to cut out and weigh the paper area under a σ–λ curve; toughness w would then be calculated as

$$w = c\ (\text{work per volume}/s) \tag{8}$$

where c is the weight of the cutout area under the σ–λ curve, and s is the weight of a standard area of paper representing a known work (expressed in joules) per volume (expressed in cubic meters) for the scales used on the σ–λ graph. [Note that the units of stress are newtons per square meter and those of extension ratio are meters per meter; thus, the units of the area under such a curve are (newtons) (meters) per cubic meter, which equals joules per cubic meter.] Another easy way to measure toughness is to plot the σ–λ curve on very fine graph paper, to count the number of squares under the curve, and to multiply by the appropriate conversion factor to get joules per cubic meter. [If there is no need to calculate σ and λ, one can divide the area under the force (in newtons) versus extension (in meters) curve by the volume of the specimen between the grips (in cubic meters) to determine the toughness.]

B. Equipment

A number of instruments are available for conducting force–extension tests; we mention only a few examples that we have used. Mechanical testing instruments run the full range from huge machines equipped with electronic recording devices to small field-portable machines not requiring electricity. All of these devices consist of a pair of grips that hold opposite ends of a strip of material; these are pulled apart, the material thereby being stretched. The machines have some sort of mechanical or electronic means of measuring force and extension as the specimen is stretched. The instruments can generally be set up to bend, compress, or shear specimens, as necessary.

Generally, the large machines, such as Instron universal testing machines, are the best to use, if possible. Problems due to machine friction and vibration are minimized, a wide range of specimen types can be accommodated, specimens can be pulled over a wide range of steady rates, and a continuous record of force versus extension can be made for the duration of a test. Furthermore, Instrons are versatile and can be programmed to apply complex cyclic loading regimes. Instrons are usually available in mechanical engineering departments of universities.

At the opposite end of the spectrum of mechanical testing machines are small, portable, hand-operated devices, such as the Ametek LCTM, CTM, or RM universal testing machines. These small, inexpensive machines can be used in the field but have several disadvantages: Only a small range of specimen types can be tested (e.g., strong algae cannot be broken in such small machines, the force transducer may not be sensitive enough for flimsy algae, or the extension scale may not be sensitive enough for very stiff algae); machine friction and vibration can bias test results for delicate specimens; the range and steadiness of extension rates depend on the investigator's skill at turning cranks; and no time record is kept of force or extension (values are read on mechanical scales or dials). One way to eliminate the last problem is to film the dials during the course of a test.

A compromise between the Instron and Ametek machines is the Hounsfield tensometer (TecQuipment Inc.) This bench-top testing machine can be hand-operated and permits force and extension to be recorded mechanically but can also be set up to extend specimens and to record force and extension electronically.

C. Method

1. Preparation of specimens. Fresh algae should be used for mechanical testing. We have found no significant difference between the mechanical properties of freshly collected algal stipes, stipes from plants kept in running seawater for a day or two, and damp stipes kept chilled in coolers for a day or two while being shipped. Similar determinations should be made for algae if they will not be tested immediately after collection. If an intact stipe or frond is to be tested, use a razor blade to cut a length of the structure equal to the L_0 to be used plus the length on either end required for gripping. A specimen should never be wider than the grips. If a particular type of tissue is to be used, cut strip of it out of the alga; take care that the strip is of uniform width and thickness. Specimens should be cut just before testing and should be kept in seawater of the appropriate temperature. Any specimens with surface nicks or flaws should be discarded (unless, of course, the study includes the mechanical effects of particular types of flaws, such as grazer marks or epiphytes). The cross-sectional area of each specimen should be measured, as described earlier.

2. Gripping specimens. A range of grips are available, or can be machined, to hold specimens of different shapes in a testing machine. It is a challenge to clamp slippery algae so that they do not slide out of the grips when pulled. Unfortunately, if algal tissue is gripped too tightly, it can be damaged. We have found that, if mucilage is wiped off the parts of the specimen to be clamped and if rough paper toweling is glued (with cyanoacrylate contact cement) to the surfaces of the tissue that would otherwise come into contact with the grips, slippage and damage can be minimized. Alternatively, the grips can be padded with neoprene rubber and the ends of the specimen wrapped with dry paper toweling. Of course, specimens should be observed during the course of a test so that if grip problems occur, the test results can be discarded. Once a specimen is mounted in a testing machine, its length between the grips (L_o) should be measured with calipers.

3. Maintenance of specimens. If the duration of a test is only a few seconds, a specimen can be tested in air. If a test will last longer, the specimen should be bathed in seawater kept at the appropriate temperature (for some examples, see Gosline 1971 or Koehl 1977b,c).

D. Critical evaluation

It is important to watch a specimen carefully during a force–extension test and to discard results subject to the following artifacts. If a specimen slips in the grips, it will appear to be tougher and less stiff than it actually is. If a specimen breaks in or at a grip, the values for strength and toughness are likely to be too low. Furthermore, a nick in a specimen can cause fracture to occur at a lower stress and extension than it would in an unflawed specimen.

If the results of force–extension tests are used to interpret the functional morphology or ecological distribution of algae, the tests should be set up in biologically relevant ways: Is a tensile (i.e., pulling) force–extension test appropriate for the alga? When an alga is subjected to a load (such as the drag force imposed on it by moving water), it can be deformed in a number of ways (Fig. 14–1). Tensile tests are appropriate for plants that are bent in nature as well as for those that are pulled. One should observe the manner in which the algae under study are deformed in the field. If a tensile test is inappropriate, one can refer to one of the books cited at the end of Sec. I.C for advice on tests using other modes of deformation.

Are the main load-bearing tissues of the algae being tested? Are the values reported for stiffness E taken at a range of stresses likely to be encountered by the algae in nature? Do the algae break at stresses much greater than those they encounter in the field? To answer these questions, one should measure or calculate estimates of the loads the algae bear in nature and then calculate the magnitude of the stresses in their tissues when they bear such forces (see Sec. I.B).

Specimens should be pulled at rates similar to the extension rates they experience in nature. Such rates, for very deformable algae, can be determined from movies of plants in the field on which bright strings or spots have been affixed at known distances apart (another method for estimating extension rates is described by Koehl 1977b). If values for stiffness, strength, or toughness for different tissues are to be compared, all the tissues should be pulled at the same $\dot{\lambda}$, or all should be pulled at the $\dot{\lambda}$'s that are biologically appropriate for each.

E. Alternative techniques

An intact alga in the field can be pulled or bent with a spring scale and the distance apart of marked spots on the plant can be measured. For a description of how to determine E from bending a stipe, see White (1974: ch. 5). Similarly, an intact plant can be collected and hung overhead; weights can be added to a basket suspended from the free end of the alga, and the distance between spots measured. Both these techniques offer several advantages. Any artifacts introduced by disrupting the structural integrity of an alga by chopping it into pieces to be gripped in a testing machine can be avoided; the places in an alga where deformation and failure occur can be observed; and no fancy equipment is required to perform the force–extension tests, so that they can be conducted inexpensively and at remote field sites without electricity. The disadvantages of these techniques are that no continuous record of force and extension is obtained, and no good control or record of $\dot{\lambda}$ can be achieved. Another difficulty of the second type of test is that algae often continue to extend after a weight is added; hence, λ increases with time and is therefore difficult to measure in some repeatable and interpretable way. Of course, desiccation due to extended periods of atmospheric exposure must be avoided.

III. Cyclic force–extension tests

A. Purposes

A piece of algal tissue can be pulled and then returned to its original length by means of a testing device such as an Instron or a tensometer. Such cyclic tests permit the resilience of the tissue to be measured: The area under a force–extension curve represents the work required to stretch the specimen (hatched area in Fig. 14–5A); the area under the return curve (stippled area in Fig. 14–5A) represents the work stored in the tissue as "strain energy" that is used for elastic recoil. The greater the ratio of the area under the return curve to the area under the force–extension curve, the more resilient is the specimen. The specimen in Fig. 14–5A is more resilient than that in Fig. 14–5B. The more resilient an alga, the more likely it is to bounce back to resting shape before the next wave hits.

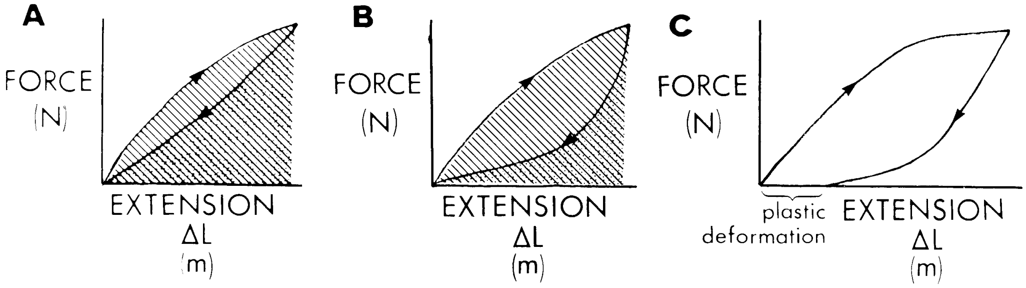

Fig. 14–5. Examples of force–extension (ΔL) curves for specimens pulled to a given extension and then returned at the same rate to their original length. Arrows pointing up and to the right indicate the curve for a specimen being pulled; arrows pointing down and to the left indicate the curve for a specimen being returned. The hatched area represents the work (joules) required to pull the specimen to that extension, and the stippled area represents the work stored elastically in the specimen and used for elastic recoil. Specimen A was more resilient than specimen B. Specimen C underwent plastic deformation and hung slack before it was returned to its original unstretched length.

Many materials undergo permanent ("plastic") deformation when pulled to extensions lower than those that break them. If a piece of an alga is subjected to a cyclic test and the return curve reaches zero stress before λ is back to 1 (i.e., force reaches zero before the grips of the testing machine have returned to their original distance apart of L_o), the specimen has undergone "plastic deformation" (Fig. 14–5C). Some tissues can recover from plastic deformation with time; a repertoire of repeated cyclic tests with different intervals of "rest" between tests can reveal such behavior. More importantly, cyclic tests can be designed to mimic biologically relevant extension regimes (such as an alga might encounter in the back-and-forth flow associated with waves) so that the resilience and plasticity of tissues can be measured and compared.

For a discussion of the use of cyclic force–extension tests to identify stress-softening behavior and the biological significance of such behavior, see Vincent (1982) or Koehl (1982).

B. Equipment, method, and critical evaluation

Cyclic tests should be done only on a testing machine, such as the Instron, the hysteresis of which is trivial compared with that of the specimen. The alga should be prepared, gripped, and maintained in the testing machine, as described earlier. The grips should be moved apart to a distance less than that which would break the specimen and then immediately moved back together at the same rate to their original position; the force and extension should be measured throughout this procedure. If more than one cycle of pulling is done, the time between successive cycles should be measured. The areas under the force–extension and return curves should be measured, as already described.

C. Alternative techniques

Resilience can also be measured by free-vibration tests (see Nielson 1965) or by forced-vibration tests (see Nielsen 1965; Gosline 1971). Plastic flow can be measured by creep-recovery tests, which are described in the next section.

IV. Creep tests

A. Purposes

Many deformable biological materials exhibit time-dependent mechanical behavior (i.e., their response to a load depends not only on the magnitude of the force, but also on the rate, duration, and history of force application). One sort of mechanical test that can be used to describe the time-dependent behavior of a tissue is a creep test. In this test a constant *stress* (force/area) is applied to a specimen and its extension with time is measured. The extensions of the specimen after measured time intervals are used to calculate the compliance D, a measure of extensibility of the specimen,

$$D(t) = \frac{\Delta L(t)/\mathrm{L_o}}{\sigma} \tag{9}$$

where $\Delta L(t)$ is the increase in length of the specimen at time t, L_o is the specimen's original length, and σ is the stress applied to the tissue. An example of how the compliance of tissues on different time scales is related to the habitat and behavior of organisms is given in Koehl (1977c).

Creep tests also can be used to indicate the sorts of macromolecular mechanisms that are likely to be responsible for the mechanical behavior of a tissue. Many pliable biological tissues are composed of fibers (e.g., cellulose and collagen) dispersed in an amorphous matrix of highly hydrated polymers (e.g., alginic acid and glycosaminoglycans). When such a tissue stretches in response to a load, the fibers tend to become aligned with the stress axis and to slide relative to each other as the polymer molecules in the matrix rearrange. The mechanical behavior of the matrix of such tissues has been likened to that of other polymeric materials (e.g., Gosline 1971; Wainwright et al. 1976).

Figure 14–6 shows examples of compliance $D(t)$ versus the log of time for different types of polymeric materials subjected to creep tests. Polymer solution A is more dilute than B; the molecules in A

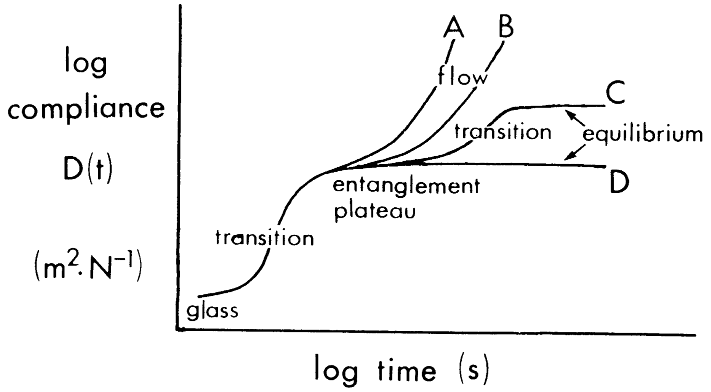

Fig. 14–6. Examples of plots of the log of compliance *D*(*t*) versus the log of time for different types of polymeric materials subjected to creep tests. For an explanation, see Sec. IV.A. Graph based on information given in Ferry (1970), Aklonis et al. (1972), and Wainwright et al. (1976).

and B are *not* covalently cross-linked to each other. Solution C is a lightly cross-linked polymer network, and D is a heavily cross-linked polymer network. When the materials stretch (i.e., when their compliance increases), the polymer molecules rearrange in response to the stress applied to the tissue. Such rearrangements occur when the molecules change their configurations as they undergo random thermal motion. Therefore, the higher the temperature, the more rapidly the events described in the following paragraphs take place.

At extremely short times after a stress has been applied, when the molecules have not yet rearranged, the material has a low compliance and is described as a "glass." Then, as the polymer molecules begin to straighten out in response to the stress, the compliance of the material rises (this is the first "transition" shown in Fig. 14–6). At some point, further stretching of the material becomes hindered by "molecular entanglements (physical tangles or other noncovalent attachments between molecules, such as hydrogen bonds.); the compliance does not continue to rise with time and the material shows an "entanglement plateau." The more concentrated the polymers in the material, or the more groups they contain that are capable of temporary attachment to other molecules, the longer is the entanglement plateau. Eventually, the molecules slip past each other and the compliance continues to rise ("flow" in A and B; "transition" in C). If the polymers are covalently bound to each other to form a network, these junctions between molecules prevent the material from extending indefinitely. Such polymer networks show an "equilibrium" plateau, where the compliance no longer rises with time. If the molecules in a network are very heavily cross-linked to each other, the transition between the entanglement and equilibrium plateaus may not be seen (as in D).

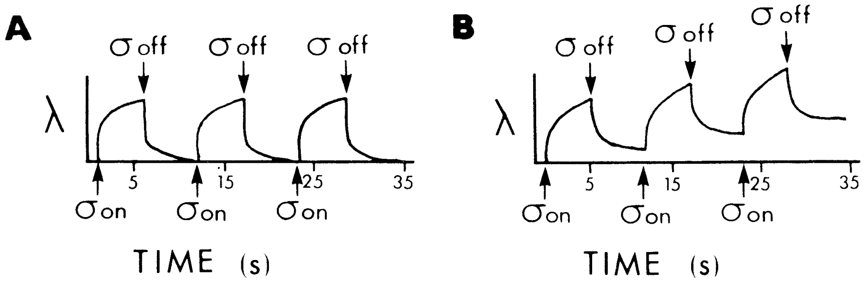

Fig. 14–7. Examples of results of cyclic creep-recovery tests designed to simulate wave-induced stresses in the tissues of two different marine organisms. Extension ratio λ is plotted versus time. Arrows pointing up indicate when stress σ was applied, and arrows pointing down indicate when the stress was removed. Tissue A bounced back to its resting length before "the next wave hit," whereas tissue B did not, and thus became stretched out as more and more "waves" pulled on it.

Thus, the results of creep tests can indicate which sorts of chemical and ultrastructural differences among species are likely to be responsible for differences in their mechanical behavior and thus point the way for further chemical or ultrastructural investigations. For examples of how creep tests or stress-relaxation tests have been used to point out mechanical adaptations or organisms at the molecular level of organization, see Gosline (1971) or Koehl (1977c).

Creep tests can also be designed to simulate the stress regime an alga might encounter in nature. After a specimen has been allowed to creep for a period of time, the stress can be removed so that the specimen is free to return to its original configuration. By means of such a creep-recovery test one can assess the ability of an alga to bounce back to resting shape. Results of creep-recovery tests designed to simulate wave-induced stresses on the tissues of two organisms are presented in Fig. 14–7.

B. Equipment and methods

A device for conducting creep tests is illustrated in Fig. 14–8. If one must work in the field with no electricity, the position of the tip of a long pointer, extending from one of the arms of the creep machine, can be traced onto a sheet of paper at timed intervals in order to calculate ΔL,

$$\Delta L(t) = x(t)c/d \qquad (10)$$

where $x(t)$ is the distance the pointer tip moved by time t, c the length of the lever arm between the knife edge and the chain of the specimen grip, and d the distance between the knife edge and the tip of the pointer (Fig. 14–8). A different simple design for a creep machine is given by Vogel and Papanicolaou (1983).

A specimen of algal tissue should be prepared and gripped as

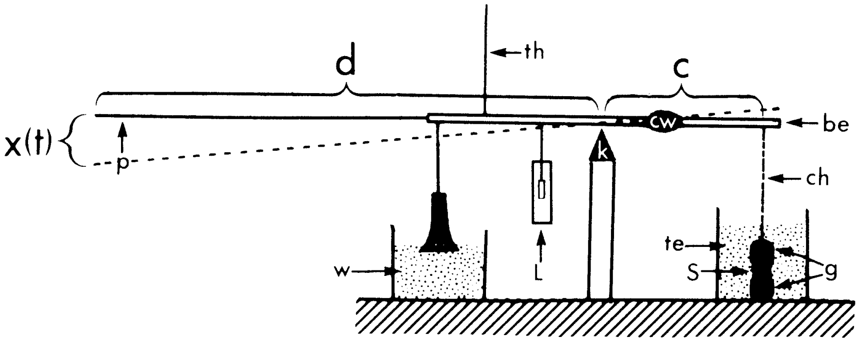

Fig. 14–8. Diagram of a device for conducting creep tests. The beam (be) sits on a knife edge (k) and is balanced (using the counterweight, cw) like a seesaw. The weight is then hung from the beam, and when the thread (th) is burned, the weight causes the tissue specimen (S) to stretch. The specimen is held between two grips (g), one of which is attached to the table and one of which is suspended by a light chain (ch) from the beam. The specimen is in a temperature-controlled bath of seawater (te). As the specimen stretches, its cross-sectional area decreases; since stress is force per cross-sectional area, the stress in the specimen would increase as it stretches. However, as the tissue stretches, the weight sinks into the water (w) and is bouyed up, thereby reducing the force on the specimen. The shape of the weight is such that the stress in the specimen is kept constant (for references on how to calculate the shape of the weight, see Koehl 1977c). Extension of the specimen can be measured electronically using a linear variable differential transformer (L) (available from Schaevitz Engineering) or visually using a pointer (p) (see Equation 10 in the text). The dashed line indicates the position of the beam and pointer at time *t* after the thread was burned.

described earlier. The specimen should be immersed in a bath of seawater held at the correct temperature; if the test is to run for a long time, an antibiotic should be added to the water to retard bacterial growth and decay of the tissue. Controls should be run on algal tissues to which no stress has been applied to measure how much elongation of the tissue is due to growth; controls should also be run in which the same weight applied to the alga is applied to a piece of very stiff material (such as metal or glass), so that any stretching of the creep machine can be measured.

C. Critical evaluation

As for other mechanical tests, one should ascertain whether the test is biologically relevant. Is the stress applied within the range encountered by the tissue in nature? Do the durations of stress application and recovery correspond to the durations of loading and unloading in the field? Are the load-bearing portions of the alga being tested? Is tension the appropriate mode of deformation?

Two other factors should also be considered when creep or creep-recovery test results are analyzed. One is that the tissue may have grown or degenerated during a long-term test. It is important not only to use the water baths and to run the controls already mentioned, but also to inspect the condition of the specimen carefully to make sure that it has not decayed. The other factor is that the shape of the hyperbolic weight (Fig. 14–8) or spiral pulley (Vogel and Papanicolaou 1983) used to apply the constant stress is calculated assuming that the specimens remain at a constant volume throughout the test. Therefore, the dimensions of the specimen at the beginning and end of the test should be carefully measured to make sure that no significant shrinking or swelling has occurred.

D. Alternative techniques

The creep test can be replaced by the stress-relaxation test, which can be performed using an Instron or tensometer. In such a test (described in Nielsen 1965; Gosline 1971; Wainwright et al. 1976) the specimen is "instantaneously" stretched to a given extension and held at that length; the stress with which it pulls on the grips is measured with time.

V. Design of mechanical tests

An advantage of using standard engineering tests is that results can be compared with a wide range of other results in the literature. However, a specific biomechanical question can sometimes be better answered by means of a new procedure designed for that question. For example, a question of interest to algal ecologists might be, How resistant are different types of seaweeds to rasping by gastropod radulae? Tissue hardness could be measured as an index of abrasion resistance (procedure described in Currey 1976). However, a more relevant measure could be made with a custom-built device that would permit a radula to be dragged across algal tissue at a defined rate and in a standardized fashion similar to the manner in which a gastropod rasps algae (Padilla 1982). A measure by which the "rasp resistance" of different algae could be compared should be defined (e.g., the minimum normal force that must be applied to the device to remove tissue when the radula is dragged across the alga or the cross-sectional area of the scrape made when a standard normal force is applied to the device as it is moved across the tissue). Similarly, a "gouge resistance" measure could be devised to compare the susceptibility of different algae to sea urchin teeth, or a "scour resistance" measure could be designed to compare the vulnerability of various tissues to abrasion by sand of defined grain sizes, and so on. The main points to keep in mind for all such custom-designed mechanical tests are that (1) the load should be applied in the same direction and at the same rate as in nature, (2) the procedure for load application should be standardized and repeatable, (3) the force used should be measurable [the specimen should be loaded with a known weight, or by a force transducer, e.g., Schaevitz Engineering; see Vogel (1981) for hints on how to build force transducers], and (4) the amount of deformation or damage produced should be measurable.

VI. Conclusions

The response of algae to mechanical loads, such as those imposed by water currents or crashing waves, is one important parameter affecting their distribution, their abundance, and the age structure of their populations. Thallus shape determines the distribution and magnitude of the stresses in an alga subjected to a load such as a flow-induced force. The parameters E, σ_{BRK}, and w of the tissues of an alga (all of which can be easily measured) determine the response of the plant to those stresses. Therefore, both gross and microscopic morphologies have an important influence on the way an alga is deformed and whether it will break in flowing water; mechanically important aspects of gross and microscopic structure can be easily measured. Mechanical tests can be designed to mimic stress conditions in the field, and similar tests can also be used to indicate molecular mechanisms responsible for the mechanical behavior of algal tissues. Thus, biomechanical analyses of algae can reveal how aspects of their structure are related to their distribution and behavior in the field.

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