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Linear measurements of growth of shells using laser diffraction

Abstract—An easy and rapid method of linear growth measurement of shells by laser diffraction is described that has a very high accuracy. Tested on *Mytilus edulis* it showed an average standard deviation of less than 3 μm. It has also been successfully applied to growth measurement of hydrocorals. The method is particularly useful for accurate measurements of fast growing species.

The growth of shells may be recorded as an increase in length along a defined axis. When a living shell is to be measured frequently, it is important to avoid mechanical damage or exposure to environmental conditions which would affect growth or survival. Mechanical measurements with sliding rules and micrometer screws may easily cause damage to the growth zones. Optical methods using microscopes or photo techniques thus seem preferable when precise measurements are required.

Here I describe a simple optical technique, laser diffraction, which permits linear growth measurements of living shells of *Mytilus edulis* with high accuracy (SD < 3 μm). The animals can be measured under water or in air and must tolerate being rigidly cemented to a frame. The length increase of the shell is measured as a decrease of a slit formed by a fixed silver edge and the growth edge of the shell.

When monochromatic light passes through a slit with an opening of the order of the wavelength of the light, a diffraction pattern is produced, consisting of dark and light spots (Fig. 1). Laser light is coherent and produces regular diffraction patterns (Borowitz and Beiser 1966). The laser used is a small Ne-He gas laser with a wavelength of 6.328×10^{-7} m and an output of 1.0 mW. At this low energy there is no health risk for the observer and harmful effects on the animal are assumed to be negligible.

The distance between two spots in the diffraction pattern (d) is inversely proportional to the width of the slit (a), which can be calculated from the formula $a = \lambda s/d$, where s is the vertical distance from the slit to the diffraction picture and λ is the wavelength of the light. The easiest way to measure d is to record the diffraction pattern on an ordinary panchromatic photographic plate and measure d or multiples of d from the negative under magnification. The best results were obtained with a shutter speed of 1/250 s and a document paper developer.

The slit is formed by a thin plate of an inert material, in this case silver, and the posterior edge of the shell of *Mytilus edulis*. Measuring at such high accuracy requires very rigid mounting. The shell is fastened on a steel plate (*see Fig. 2*) with Kerr Fastcure, a two component nontoxic and stable acrylate material used in dental work. The cement sets after 60–90 s in the air, and the shell may then be transferred to running water. During setting the shell edge is rinsed with water and then placed in running water. After hardening, the space between the lower shell half and the steel plate may be filled with aluminate cement to increase stability.

The silver plate is mounted with acrylate or is soldered to a small steel plate and can be brought into position by two adjusting screws and fixed tightly by a setscrew. The silver plate must be arranged in the same horizontal plane as the shell edge. The best diffraction pattern was obtained when the slit measured 100–600 μm.

Mytilus edulis, which is a littoral organism, was measured in air. If the organism has to be measured under water, optical glass must be used where the light has to pass and the water should be relatively free of particles; otherwise false diffraction patterns may occur. If absolute values for the slit opening are required, the reading must be corrected for refraction air/glass/water, but as we are concerned with relative val-

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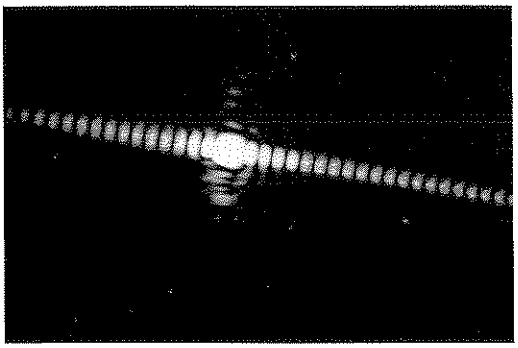

Fig. 1. Diffraction pattern.

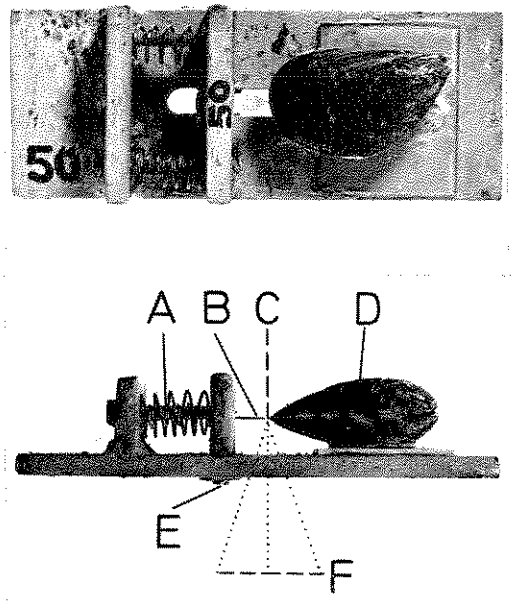

Fig. 2. Mounting of the shell, side and top view. A—Adjusting screws; B—silver edge; C—laser beam; D—shell; E—setscrew; F—diffraction pattern.

ues (i.e. length changes), this is not necessary.

The specimens of *M. edulis* used came from Trondheimsfjorden; 50 shells of good shape, 4–5 cm long, were used in testing the method. The experimental work was carried out between 15 April and 15 June under laboratory conditions. Water temperature varied from 7.3–8.3°C and salinity from 33.5–33.1‰. The water intake was at 30-m depth and the food content of the water was small.

Several factors, both physical and biological may induce errors. Repeated readings of the diffraction films of nonfiltering shells showed that the reading accuracy was high, with a standard deviation of 1.06 μm with very narrow 95% confidence limits (0.22) (Table 1).

Another problem is to ensure that exactly the same slit is measured each time. The silver edge is approximately linear, while the edge of the shell is curved. A good diffraction pattern was obtained only when the tangent to the shell edge was parallel to the silver edge, and refinding the same part of the slit was thus easy. To test the refinding of the slit, 10 separate and independent measurements were taken of each of 48 slits with nonfiltering shells. The standard deviation of all measurements combined was 1.23 μm (Table 1). Standard deviations were smallest when the slit was between 50 and 400 μm, while an increase above 600 μm also increased the standard deviation. The additional variability of measurements due to both reading accuracy and refinding of the slit gave an average SD of about 1.30 μm.

Table 1. Standard deviation and 95% confidence limits of measurements including different error sources.

	No. shells	No. measurements	Avg SD	95% confidence limits	Remarks
Reading accuracy	12	480	1.06	0.22	Nonfiltering shells
Refinding of the slit	48	480	1.23	1.24	Nonfiltering shells
Fouling of the slit	-	40	1.65	0.74	Two silver edges
Closing of the shell	42	168	2.88	-	Filtering shells
Closing of the shell	6	24	5.5–16.2	-	Filtering shells-rejected

Temperature changes may cause changes in length and volume of the apparatus, and the slit opening may thus be affected. This effect was tested in water baths from 8°C up to 22°C, in steps of 2°. There was a nearly linear increase of the slit of about 10 μm from the low to the high temperature. This factor may thus easily be controlled.

Mytilus edulis fixes itself in position with the foot and byssus threads. By tightening the byssus threads the shell can shift position. The drag of the byssus threads and of the foot was not sufficient to give a measurable response in the slit.

As the silver edge is mounted very near the shell edge it may come into contact with the mantle when the shell opens. During the 2-month observation period no morphological changes, damage, or harmful effects were observed. Nor was the short exposure to laser light observed to be injurious.

A substrate submerged in seawater will rapidly be covered by loose material and small organisms; this may reduce the opening of the slit or give a less exact diffraction pattern. The slit was gently rinsed with running water before measuring to remove such loose material. Measurements of two reference slits made by two silver edges during a period of 20 days showed a SD of 1.65 μm (Table 1), indicating that fouling is of minor importance. Biological activity by living mussels probably tends to reduce fouling even more.

Closing of the shell affected the size of the slit markedly. Firm contraction of the adductor muscle was obtained by a slight tapping on the shell. Repeated measurements of the same shell at intervals of 30 min, allowing the animal to open between measurements, gave more than 90% of the shells a SD ranging from 0.7 to 5.0 μm, with an average of 2.88 μm (Table 1). During this period daily growth was about 2.0 μm 24 h^{-1}; changes of the slit due to growth are thus negligible. The remaining 10% of the shells showed more variation (Table 1) and repeated testing showed that the same animals remained unstable; these were rejected. Feeding of starved animals (with cultures of *Thalassiosira pseudonana*) increased closing variability slightly.

Provided that the measuring procedure outlined above is followed, length growth measurements of *M. edulis* can be carried out with an average SD of less than 3 μm. The most important precautions to maintain accuracy are to make the slit as stable as possible and to ensure that the shells are firmly closed. Errors due to variable closing of the shells may be reduced by a test procedure and adequate irritation of the animal. It is one of the advantages of the diffraction method that inadequate stability of the closing can be detected by observing the diffraction pattern during such irritation. The measuring procedure is easy and during routine work one measurement takes less than 30 s.

For short periods no harmful effects on the animals due to the treatment were observed. It is, however, not known if long term exposure to the different artificial stimuli or the inability to move may injure the animal and influence its growth.

During maximal growth, *M. edulis* of the size used have in Trondheimsfjorden an average in situ growth rate of 50 μm of length per day at 18°C (Lande 1973). This corresponds to about 2 μm h^{-1}. By using laser diffraction, growth within very brief intervals may thus be recorded. An observed difference of 9 μm between two average measurements of the same shell, each based on four replicate readings ($df = 6$), is significant at the 0.05 level when SD = 5 μm. When SD = 2.88 μm the corresponding critical value for significant difference is about 5 μm.

Since this manuscript was submitted, I have finished three growth investigations based on the method: of the bivalves *M. edulis* (SD < 3 μm) and *Modiolus americanus* (SD < 4 μm) and the hydrocorals *Millepora complanata* and *Millepora alcicornis* (SD < 3 μm). I have also made preliminary measurements of two types of calcified red algae and various scleractinian corals.

Based on these experiences, I wish to emphasize three points.

1. What kind of animals can be measured? The procedure has to be adjusted to the biology of the particular organism. For sessile organisms it seems that the quality of the growing edge is most important for high accuracy. Secondly the animals should be rough enough to be kept in aquarium. I see no objection to using the method on a variety of organisms; foraminiferans, hydrocorals, scleractinians, calciferous polychaetes like serpulids, bivalves, brachiopods, and perhaps bryozoans, and other animals whenever a smooth and well defined growing edge can be matched with an artificial edge, adjustable or not, and the slit thus formed can be fixed immobile in an accurate position for 30–60 s. Leaves of plants have also given excellent diffraction patterns.

2. What can be measured by the method? During the three investigations mentioned above, I have recorded growth patterns which have been difficult or impossible to get from traditional methods: continuous (*Mytilus*) and discontinuous growth (*Millepora*); diurnal variation in growth (*Mytilus*, *Millepora*); circadian rhythms in growth (*Mytilus*); time lag for change of feeding conditions to corresponding growth response (*Mytilus*); rapid selection of species with similar growth rates (*Mytilus*).

As traditional measuring methods usually must be applied at intervals of several days or weeks, this method could possibly be used in studies of physiology and behavior.

3. Most feasible time scale? While this method will be most useful for short-interval measurements, general growth investigations of slow growing animals may also be made (*M. americanus*, winter growth rate <0.3 μm day^{-1}).

Tests for variability only have been made for the coralline algae *Lithophyllum congestum* (SD < 6.5 μm) and *Lithothamnion* sp. (SD < 6 μm).

The variability increases with the roughness of the growing edge, but the method should be suitable for accurate short-time measurements of fast growing species and growth investigations in general of slow growing species.

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