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Technical note

A new technique for simultaneous collection of macroalgal propagules in the water column

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Abstract

A Simultaneous Collection Module (SCM) was designed to concurrently collect multiple samples of macroalgal propagules, and to evaluate their abundance and spatial distribution in the water column. The basic sampling units are water collectors (tubes), held together to form an evenly spaced grid forming a spatial array with three dimensions. Each collector in the module possesses a simultaneous closing mechanism. The water collected in each tube is then filtered and the propagules retained on each filter cultivated under controlled conditions. The sampling system was deployed in the field in central Chile. Results suggest the system is an efficient collector, able to provide data on time–space changes of macroalgal propagules in the water column.
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1. Introduction

Little is known about the scales of variation in macroalgal propagules in nearshore environments, much less where in the water column propagules reside. Basic, descriptive studies are scarce due to difficulties of obtaining multiple, simultaneous samples of microscopic organisms in a dynamic fluid, the microscopic size of the propagules, their variability in production and release and the difficulties involved in identifying them to generic or specific levels (see Santelices, 1990 for review). Although various techniques

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for sampling and culturing seaweed propagules are available (see Table 1), none has solved the problem of simultaneously sampling different points in the water column. The techniques relying on water samples (Table 1) are generally limited to the number of samples the collector could gather, either manually, by Niskin bottles, or by means of suction pumps and they do not allow discrimination among several sampling points in the water. On the other hand, the techniques for sampling propagules on new substratum (Table 1) are in fact measuring recruitment, and results depend on successful settlement and post-settlement survivorship.

Here we present an inexpensive, easily constructed sampling device that can be used to collect water samples simultaneously over local scales of varied spatial extent, depending on individual studies. After describing the sampling system, we report data of a test trial run at a shallow rocky channel on the coast of central Chile and discuss the utility of the device.

Table 1
Main techniques used to sample seaweed propagules

Techniques		
(1) Water samples	Sampling techniques	Reference
	Collected manually	Hruby and Norton, 1979[a] Hoffmann and Ugarte, 1985[a] Zechmann and Mathieson, 1985[b] Santelices et al., 1995[a]
	Collected by suction pumps	Kendrick and Walker, 1991, 1995[a]
	Collected by Niskin bottles	Fredriksen et al., 1995[a]
	Samples fixed directly	Graham, 1999[c]
(2) New substratum	Type of substratum used	Reference
	Cement blocks	Foster, 1975[d]
	Plastic slides covered with sand grains	Harlin and Lindbergh, 1977[d]
	Glass and plastic slides	Hruby and Norton, 1979[d] Amsler and Searles, 1980[d] Reed et al., 1988[d]
	Pieces of rock	Kennelly and Larkum, 1983[d] Kennelly, 1983[d]
	Clay plates	Vadas et al., 1990[d]
	Ceramic plates	McCook and Chapman, 1993[d]
	Autoclaved stones	Santelices et al., 1995[d]
	Plexi-glass plate	Fredriksen et al., 1995[d]
	Plate of epoxy resin	Johnson and Brawley, 1998[d]
	Glass slides covered with gastropod mucus	Santelices and Bobadilla, 1996[d]
	Glass slides covered with synthetic adhesives	Santelices and Aedo, 1999[d]
	Ropes	Forrest et al., 2000[e]

[a] Water cultured for 3–5 weeks.
[b] Filter cultured for 3–5 weeks.
[c] Light absorption spectra patterns.
[d] Substratum incubated in the lab for 3–5 weeks.
[e] Ropes exposed in the field for 4 months.

2. Materials, methods and example

2.1. The sampling device

The sampling instrument is a negatively buoyant aluminum grid (0.95 × 0.55 m) with 25 regularly positioned, cylindrical PVC tubes (Fig. 1A). Each 2.0-cm diameter tube is 13 cm long, corresponding to an interior volume of 20 ml. Positioned at the ends of each tube are rubber balls (2.0 cm diameter, 50 shore of hardness) that are connected to one another by a stainless steel spring placed inside the tube. Each ball is also attached to a releasing mechanism (see below) by a nylon string and 9.0 mm galvanized steel washer. Upon release,

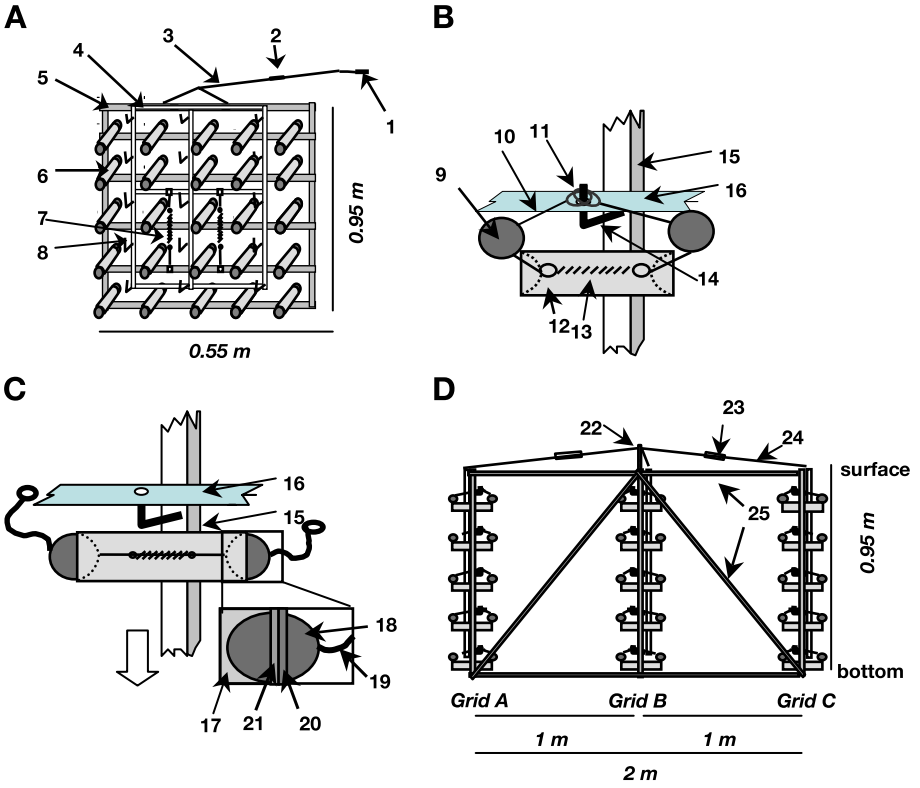

Fig. 1. (A) Schematic representation of sampling grid: (1) anchor rod for releasing mechanism; (2) tension device; (3) steel wire covered with plastic; (4) wood frame (give frame dimensions), 10 × 10 mm in cross section; (5) aluminum frame; (6) water collector (tubes); (7) stainless steel spring; (8) L-shaped screw (4.0 cm in length). (B) Design of individual water collectors: (9) rubber balls; (10) nylon string; (11) galvanized steel flat washers (9.0 mm in diameter); (12) PVC tube 13 cm in length; (13) stainless steel spring; (14) L-shaped screw (4.0 cm in length); (15) wood frame; (16) aluminum bar (20.0 × 3.0 mm). (C) View and detail of closed water collector: (15) wooden frame; (16) aluminum bar (20.0 × 3.0 mm); (17) PVC tube; (18) rubber ball; (19) Nylon string; (20) rubber O-ring; (21) internal PVC ring. White arrow shows the wooden frame move to release the rubber balls. (D) Lateral view of the Simultaneous Collection Module (SCM): (22) triggering system of SCM; (23) tension device; (24) steel wire covered with plastic; (25) aluminum frame 2.0 × 2.0 cm.

the internal spring pulls the balls together, closing each end of the tube (Fig. 1C), and forming a hermetic seal as the rubber balls contact petroleum jelly-covered, rubber O-rings attached at each end of the tube (Fig. 1C).

To simultaneously open and close all sampling tubes, we incorporated a mobile wooden frame with regularly spaced, L-shaped brass screws, each 4.0 cm long. The frame is able to move vertically "up" and "down" with the aid of a stainless steel spring connected to the larger, aluminum grid. The L-shaped screws act as hooks to hand the washers and fix the position of the balls. The system is "open" when the mobile frame is in "up" position, pulling the balls above the tube ends and allowing water to enter the tube. Lowering the mobile frame releases the balls, closing all tubes simultaneously (Figs. 1B,C).

To expand the spatial extent of sampling, several grids can be placed in parallel alignment (Fig. 1D), forming a Simultaneous Collection Module (SMC). Here, we used three grids separated by a fixed distance of 1.0 m each (Fig. 1D). Each grid contained 25 water collectors in a 5 × 5 spatial arrangement, leaving a margin of 5.0 cm along the borders of the frame.

The system was tested at Maitencillo (31°28′ S; 71°28′ W) in central Chile on February 2, 2001. We ran a trial at high tide; the SCM was deployed in a shallow (1–1.5 m deep), open channel along the rocky shore and remained open for 2 min to permit an equal water replacement in all collectors. Water samples were filtered using 13 mm diam., 1.2 μm nitrocellulose filters, as previous testing indicated this size avoided saturation and deterioration of the filter during the filtration process. To avoid potential bias caused by spore settlement on internal walls of sampling tubes, the sequential order in which tubes were filtered was completely randomized within each grid. Filtration time for each collector was 1–2 min. Handling time of each grid therefore is 30–35 min. We had three people working to filter samples (1 per grid) so that all samples were filtered within 35 min.

After filtration, individual filters were placed in labeled 1 × 1cm cells of Sterilin© cultivation plates. Each cell received 3 ml of SFC culture medium (Correa and McLachlan, 1991). Filters were then incubated in the lab under 12 h of daily light at 15 ± 1 °C and irradiance of 30–50 μmol m^{-2} s^{-1}. Culture medium was replaced weekly and incubation continued until all individuals growing on the filter could be identified to division level (20–30 days). The juvenile stages growing on each filter were identified and counted with the aid of a Zeiss inverted light microscope.

As mentioned, seaweed propagules tend to settle on the internal walls of the collecting tubes and their settlement rates vary among species. Based on previous experiments estimating settlement under laboratory conditions, (Bobadilla, unpublished data), we calculated a correction factor for those species frequently found in our sampling sites (*Mazzaella laminarioides* (Bory) Fredericq and Hommersand, *Enteromorpha intestinalis* (Linnaeus) Link, *Ulva rigida* C. Agardh and *Lessonia nigrescens* Bory. Experimental procedures used were similar to those described in Santelices and Bobadilla (1996) and Santelices and Aedo (1999).

Results are shown as total density of green (Chlorophyta) and brown (Pheophyta) macroalgal propagules per tube. Few red algal propagules were found. Spatial patterns of propagule abundance are shown as separate Kriging's maps (Legendre and Fortín, 1989) for each grid using Surfer 6.04 software (Surfer (win32), 1996).

3. Example

In the field-testing, green algal propagules were more abundant that brown algal propagules (Fig. 2). Green algal propagules conformed high-density patches which magnitude and position varied among grids and depths (Fig. 2A). Brown algal propagules were less abundant than green algal propagules and conformed small patches that varied in position among the grids (Fig. 2B).

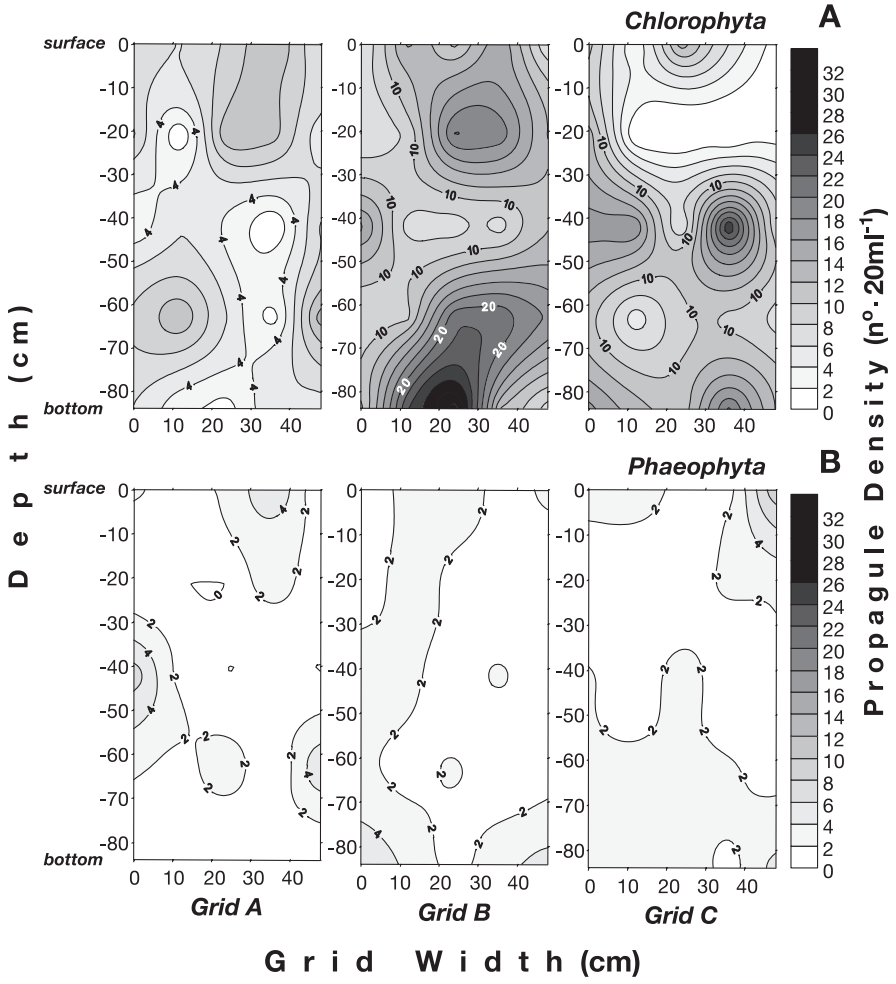

Fig. 2. Kriging maps showing total densities of propagules in each grid during high tide. The isolines connect equal propagule densities at different points in the water column. The scale on the right indicates propagule density. (A) Chlorophyta and (B) Phaeophyta.

4. Discussion

The technique described in this note provides a new tool to study the distribution of algal propagules in the field and is the first to allow simultaneous, in situ sampling of algal propagules at multiple locations in the water column. Our samples have a greater water volume (1.5 l) than those used in previous reports (e.g. Hruby and Norton, 1979; Zechmann and Mathieson, 1985; Hoffmann and Ugarte, 1985; Santelices et al., 1995), and have the additional advantage that the spatial extent and volume sampled can be varied to meet necessities of individual studies. This can be done by increasing the number of collectors per grid, the total number of grids, or the volume of each collector.

The maximum propagule density encountered using the SCM was 1.3 propagules ml^{-1}, a value comparable to other recent studies (e.g. 1.36 zoospores ml^{-1}; Graham, 1999). The most important advantage of the simultaneous collecting device is the possibility of detecting patches of aggregated propagules at different spatial scales. The spatial distributions inside and between grids were found to be different, and the patterns of green and brown algal propagules were notoriously dissimilar. Ongoing studies extensively using this sampling technique suggest that various factors (e.g. wind, currents, and distance from a propagule source) may modify the distribution patterns of macroalgal propagules in the water column.

The principal problem that we encountered with our method was the time spent processing water samples after collection. Propagules tend to settle on the internal walls of the water collectors. To solve this problem, we have to increase the number of people processing samples in order to reduce the time that the water in the tubes remain unfiltered or to standardize the results against known settlement rates of seaweed propagules. These alternatives are not mutually exclusive and both can be combined, as we have done in this study. Previous test in still water (Bobadilla, unpublished data), showed that within 30 min, 13–15% of small-sized propagules (<15 μm as in *Enteromorpha*, *Ulva* and *Lessonia*), and 33–38% of large-sized propagules (>15 μm as in *Mazzaella*) are lost because they settle in the sampling tubes. This factor should be considered in future uses of the method with other species.

The simultaneous sampling device could constitute a general solution to problems related to spatial structure of planktonic propagules, particularly those dispersing over short distances. The versatile design composed of modules provides added flexibility that can be custom-fitted to specific studies. Moreover, the sampling device is simple, inexpensive, and easy to transport and operate. Future applications will determine whether the system could also be applied to other types of particles, including invertebrate's larvae, pollutants, sediments and nutrients.

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