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Preparing random distributions of diatom valves on microscope slides

Abstract—Diatom cells are mounted on a microscope slide in a statistically random distribution for subsequent population counting. The cells are allowed to settle from aqueous suspension onto a cover slip suspended in a plastic well. By placing the well in a desiccator, the water is evaporated off with minimal disturbance to the layer of settled valves, and most of the residual salts are left in the base of the well after the water level drops below the cover slip surface. When dry the cover slip is mounted on a microscope slide using a medium of high refractive index.

The traditional method of mounting cleared diatoms on microscope slides by allowing a drop of suspension to evaporate on a cover slip under gentle heating (Schrader 1974) produces an unevenly distributed layer of settled valves with clumping and size sorting due to convection currents which develop in the water and cohesion of the water moving particles as it dries quickly. Besides introducing counting errors, such a slide is awkward to examine, and the extra time spent in preparing a slide containing a convenient density of valves for counting (generally 6–10 per field of view) is amply repaid by time saved at the microscope.

Previously proposed procedures for preparing random distributions for microscope examination allow the cells to settle from suspension onto a cover slip, then either examine the cells from underneath with an inverted microscope (Evans 1972; Lund et al. 1958; Schoeman 1979) or dry the cells with limited disturbance and mount the cover slip on a slide (Battarbee 1973, 1986;

Dickman 1968; Sanford et al. 1969). The first approach is of limited use, since small or lightly silicified diatoms will easily be overlooked unless the cells are fixed in a medium of a refractive index higher than the refractive index of silica (1.2). The second approach has hitherto been limited by techniques that are cumbersome or prone to displace settled valves near the edge of the cover slip. The procedure described here is simple to use and reliably produces a layer of randomly distributed valves on the cover slip. If absolute counts of cell numbers are required, marker microspheres can be added to the suspension (Battarbee and Kneen 1982; Kaland and Stabell 1981).

The diatoms are cleared of organic material by leaving the sample overnight in at least 10 times its own volume of concentrated nitric acid and then increasing the temperature to boiling point for 5 min. The solids are allowed to settle for at least 12 h, then the supernatant liquid is withdrawn with a Pasteur pipet attached to a water suction pump. Deionized water is added to the solids and the mixture briefly swirled. The valves are allowed to settle for 12 h and the liquid is then carefully withdrawn with a Pasteur pipet. This rinsing stage is repeated five times to remove electrolytes.

Before use, the slides and cover slips are washed in hot detergent solution and then stored in alcohol until needed. The procedure begins by diluting an aliquot of the diatom valve suspension to 10 ml with deionized water, then adding ammonium

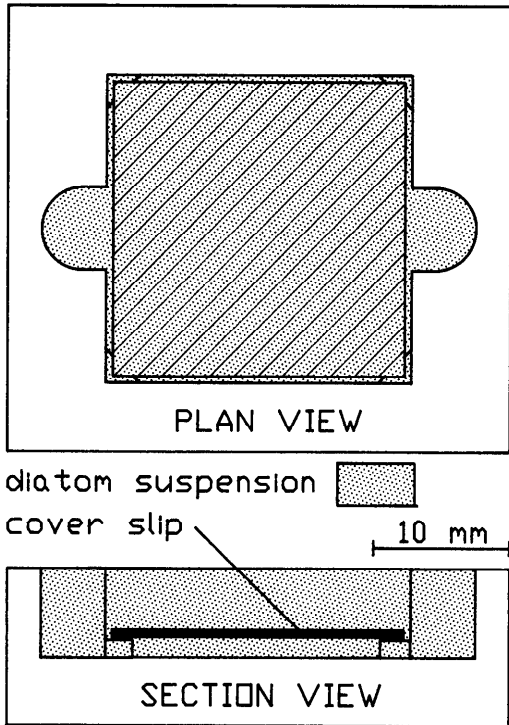


Fig. 1. The Perspex well used to settle diatom valves from aqueous suspension onto a cover slip. Dissolved solids remain mostly with the liquid as the level falls below the cover slip, reducing deposition of salts on the cover slip. The internal cutouts assist in lifting the edge of the cover slip during removal.

chloride (1 drop of 10% solution) to neutralize electrostatic charges on the suspended particles and reduce aggregation. Next, a cover slip (22 mm²) is placed in a specially designed Perspex well (Fig. 1) so that it rests 2 mm above the base; the diatom valve suspension is then added to the well with a Pasteur pipet until the water surface is level with the edges of the well (corresponding to a height of 5 mm above the cover slip). The fixed height of water column above the cover slip allows accurate determination of the concentration of valves in the suspension. The well is then placed in a desiccator over silica gel (to avoid disturbance by air currents and hasten drying) until the water evaporates (about 2 d). The cover slip is removed from the well and placed diatom side up on a hot plate at ~350°C for 2 min to sublime the residual ammonium chloride

Table 1. The number of valves of the most abundant species, *Eunotia rhomboidea*, and the total number of valves observed in three separate north-south and three separate east-west transects across the area of the cover slip on a slide prepared by the method described here. For 5 df the expected value of χ^2 lies between 1.15 and 11.07 at the 95% probability level.

Transect	<i>E. rhomboidea</i> valves	Total valves
E-W 1	308	447
E-W 2	341	487
E-W 3	325	483
N-S 1	310	488
N-S 2	330	527
N-S 3	297	475
χ^2 value	4.16	6.86

deposited with the diatom valves. When the cover slip is cool, 2 drops of a 10% solution of suitable mountant such as Naphrax (R.I. 1.7, Northern Biological Supplies, Ipswich, England) are added in solvent (toluene for Naphrax) to displace air bubbles from within whole frustules (Stidolf 1982). The cover slip is placed, diatom side down, onto a drop of mountant on a glass microscope slide. A light spring clamp is fitted over the slide to compress the mountant layer under the cover slip to minimal thickness and the slide is placed in an oven at 105°C for 2 h to harden the mountant.

Frustules of genera with wide girdle bands (especially *Eunotia*) usually settle from suspension in girdle view. To display a majority in valve views, the valves are separated, before preparation of the slide, by sonication of the diatom suspension (Unisonics model FX12 sonic bath; 100 W, 20 kHz) for 2 min.

Diatom valves on a slide prepared by this procedure were counted in a series of 50 evenly spaced fields of view along three east-west and three north-south traverses of the cover slip, excluding a 2-mm border around the edge. The distribution of valves on the cover slip was tested for randomness by comparing the actual χ^2 values with those expected for total counts and counts of the most abundant species (Lund et al. 1958). The results (Table 1) show that the assumption of a random distribution of diatom valves on the slide is not disproved at the 95% probability level. The distribution

was neither excessively regular (χ^2 below lower limit) nor unevenly clumped (χ^2 above upper limit).

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Sex size ratios and their influence on mating success in a calanoid copepod

Abstract—Mating experiments with 182 individual pairs of *Diaptomus birgei* Marsh were videotaped in the laboratory. The following were measured and scored: attempted capture of the female by the male, time to successful capture and mounting, duration of copulation, spermatophore placement, time to clutch extrusion, metasomal lengths of all individuals, and sex size ratios (female : male lengths) of all pairs. The percentages of successful copulation and clutch formation were highest between sex size ratios of

1.15 and 1.23. The average sex size ratio of 1.20 is apparently selected for, since it was relatively constant over time in the observed natural populations.

Sexual size dimorphism and the co-occurrence of copepod species are topics which have generated a great deal of research in the past decade (e.g. Bayly 1978; Gilbert and Williamson 1983; Chow-Fraser and Maly 1988). Bayly (1978), studying Australian calanoids, postulated that high degrees of sexual size dimorphism found in species only inhabiting temporary ponds could be attributed to lack of predation. Selection for increased female size presumably decreases

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