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An inexpensive automatic counter for cultures of aquatic organisms

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Introduction

A serious handicap in experimental culturing of invertebrates exists in counting and distributing definite and statistically representative numbers of organisms among many replicates.

Manual pipetting of the test animals, one by one, is not only time consuming and tedious, but also requires the necessary skill to transfer the organisms without mechanical damage or error in counting.

Although automatic counting seems to be the solution for this particular problem, most of the equip-

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ment presently available does not fulfill the requirements. Indeed the particle counters based on a resistivity detection (Coulter-Counter, TOA, Celloscope, etc. ...) have not been designed to recuperate the organisms after counting.

Only a few optical counters have been described in the literature for the dual purpose of counting and recuperating and they have either been designed for distributing high numbers of *Artemia* nauplii (Mitson, 1963), for counting fingerlings (Jensen, 1969) or for sizing and counting zooplankton samples (Cooke et al., 1970).

Because our experiments had to be carried out with a large number of parallel samples each containing from 20 to several hundreds of invertebrate larvae we constructed a simple optical counter. Presently it is used in daily routine sampling from batches of larvae with a counting error of less than 2%.

Construction and mechanism

A schematic diagram of the counter is given in Figure 1. Light from a 15 Watt-lamp L is focused on a capillary tube C by the objective lens M_1. The light beam passing through the capillary tube is transmitted to the phototransistor of an amplifier P by another objective lens M_2.

Focusing of the light beam on the capillary tube with the aid of an objective gives an amplification of the light intensity and ensures a more accurate counting of the larvae compared to ordinary light transmission in the counter described by Mitson (1963).

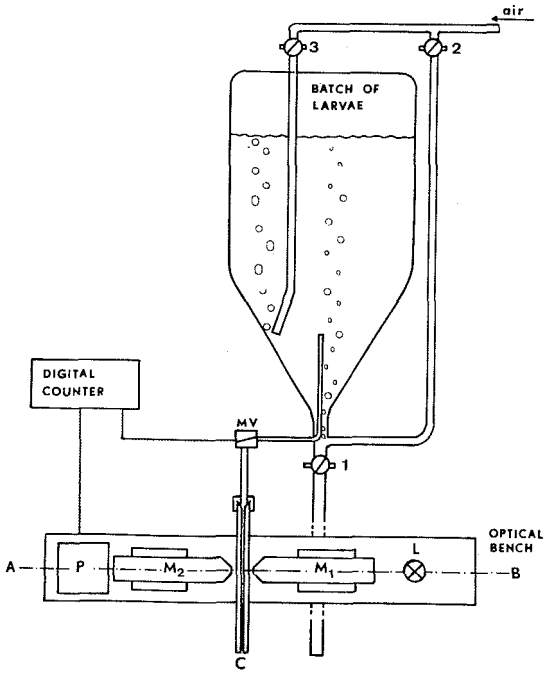

Figure 1. Schematic diagram of the counting apparatus.

The detection accessories L, M_1, M_2, C and P are mounted in an optical axis A–B on an U-shaped aluminium profile. In order to count batches of organisms of different sizes the counter can be used with capillary tubes of different internal diameters. As each change requires an adjustment of the objective focus, the lenses M_1 and M_2 are best mounted on two movable microscopic tubes. For the same reason the amplifier P and the lamp L must be movable in the optical axis.

In front of the detecting light spot, the diameter of the capillary tube must be only slightly wider than the dimensions of the larvae to be counted in order to prevent two organisms passing through together.

The batch of organisms to be counted is poured in a funnel-shaped 1 litre glass cylinder and kept in suspension by a gentle air-bubbling, which can be adjusted with the control valves 2 and 3.

Larvae are drained from the cylinder into the capillary tube C through a piece of plastic tubing which can be closed by a magnetic valve MV. In order to allow an undisturbed draining of the larvae into the capillary tube, the top of the latter is drilled out conically.

The animals passing through the small illuminated section of the capillary tube modulate the light beam, which is projected on the phototransistor, and induce a change of the collector current. These variations are amplified with the aid of a pulse amplifier to a sufficient level to trigger the electronic counter. The circuit diagram of the pulse amplifier, which consists of two PNP-NPN stages, is shown in Figure 2. The phototransistor OCP 71 is a part of the first stage. The temperature drift of this phototransistor is of no importance as only fast light changes are to be detected.

The digital counter which we use has a mechanical preset from 1 to 99999 and commands the magnetic valve MV which is normally closed. When the zero-reset-circuit of the digital counter is activated, MV is energized and larvae are drained into and through the capillary tube. When the preset number is attained, MV is closed. Although extremely short, there is a certain time-lapse between the moment of attainment of the preset number and the drain-stop. As a result, a small volume of water, containing some larvae, flows "extra" into the receiving vial, and the preset number of organisms is exceeded. Thus the larger the density of the batch, the larger the number of organisms in this "extra-flow". We therefore keep

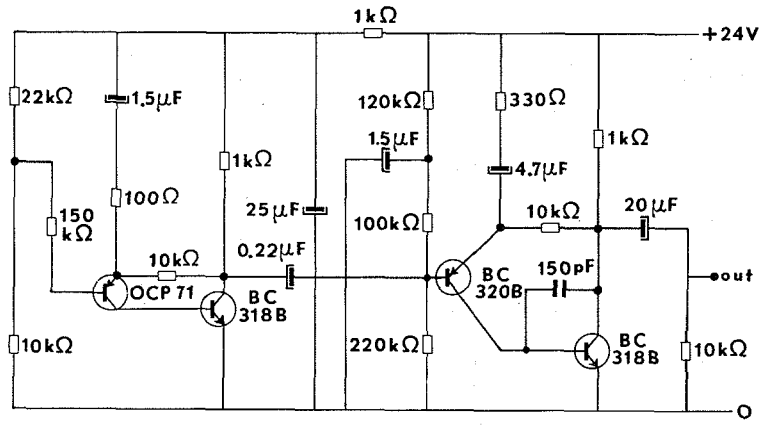

Figure 2. Circuit diagram of the pulse-amplifier P.

the density in the cylinder quite low, especially when presetting a small number.

Experience has shown that the counting error in replicates of 100 *Artemia* larvae per vial is less than 2%, each vial being filled in about 20 seconds, sampled from a batch with approximately 5 nauplii/ml.

The counter can also be used to determine the density of organisms in a batch, simply by presetting the counter at a certain number and determining the volume poured through the system in calibrated cylinders.

Acknowledgements

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