METHODS & TECHNIQUES

The Construction of a Collecting Device for Small Aquatic Organisms and a Method for Rapid Weighing of Small Invertebrates [1]

BY

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(1 Text figure)

While studying the changes in populations of nudibranchs, I have frequently had to collect and obtain dry weights of many small animals. To facilitate this, I have devised two techniques which I feel may be helpful to others working with small invertebrates. One technique is the construction of a suction device used for the rapid collection of small aquatic organisms; the other is a method of rapidly weighing small objects.

I consider that nudibranch biologists have, in the past, placed too much reliance upon length as an indicator of size, probably because length is normally an easier measurement to obtain, as well as being non-destructive of the animal where only a few are available. It is my hope, therefore, that the techniques presented here, by enabling the collector to obtain greater numbers of animals, and lessening the labor involved in weighing, will encourage others to benefit from the greater precision resulting from the use of dry weight as a measurement.

The collecting device, intended primarily for use with SCUBA, utilizes suction to entrap clinging, floating, or swimming organisms. Its design was inspired by several other collection devices, including the "slurp gun" of tropical fish collectors and the "Acadian SOCK" (Bleakney, 1969). The most important feature of the collector is the use of two simple one-way valves, which create great efficiency and speed of action. The valves may be obtained as components of a polyethylene gasoline siphon pump, which costs about $1.25 and is available in most hardware, boating or automobile supply stores. The other materials required are a soft-walled 250 or 500 ml capacity polyethylene sample bottle with a 22 mm diameter threaded neck, obtainable from any biological or chemical supply department; plastic window screening or similar fine plastic mesh; a plastic "T" or "Y" tubing connector, or a "quick-disconnect" tubing connector (available from most laboratory supply houses); and an extremely sticky adhesive, "Touch'N'Glue," which is available at most hardware stores. This material is an adhesive used commercially to bond plasterboard and paneling to walls, and is the only substance I have found that will successfully bond polyethylene.

CONSTRUCTION

(Figure 1)

As shown in Figure 1A, the siphon may be disassembled into three sections: a rear part, connected to a length of ¼ inch polyethylene tubing, which may be discarded; the squeeze bulb, which contains one valve (A) where it is joined with the tubing connector; and the outlet or nozzle of the siphon, which contains the second valve (B). The nozzle may be unscrewed from the squeeze-bulb and is used essentially intact. A circle of plastic window screen, 22 mm in diameter, is inserted into the threaded end of the nozzle as far as the "shoulder" of the nozzle and cemented in place. When dry, this assembly will thread directly onto the neck of the bottle and will constitute the outlet valve of the collector bottle.

The rear section of the siphon may be pulled away from the squeeze-bulb to reveal valve "A", which is press-fitted into the squeeze-bulb and may easily be pried free. The assembly of this valve to the collector spout will depend upon the type of tubing connector used (see Figure 1B). The preferred type is one-half of a "quick-disconnect" snap-apart tubing connector which has a broad rim allowing the positioning and cementing of the intake nozzle assembly from the outside of the bottle. Alternatively, one arm of a "T" or "Y" tubing connector may be cut off and used as the spout. Either type of spout is cemented directly to the valve, making certain that no adhesive touches the flap of the valve and that the valve will open **inward** when assembly is completed. This intake nozzle assembly should be allowed to dry before assembly of the collector is completed.

To complete the collector, a hole of the appropriate size is drilled or cut one inch from the shoulder of the bottle. If the snap-apart type connector is used, adhesive is spread around the rim of the nozzle assembly and the valve portion pressed into the bottle. If the other type of connector is used, adhesive is added at the juncture of the valve and the nozzle, and the assembly is dropped through the neck of the bottle and pulled through the hole with

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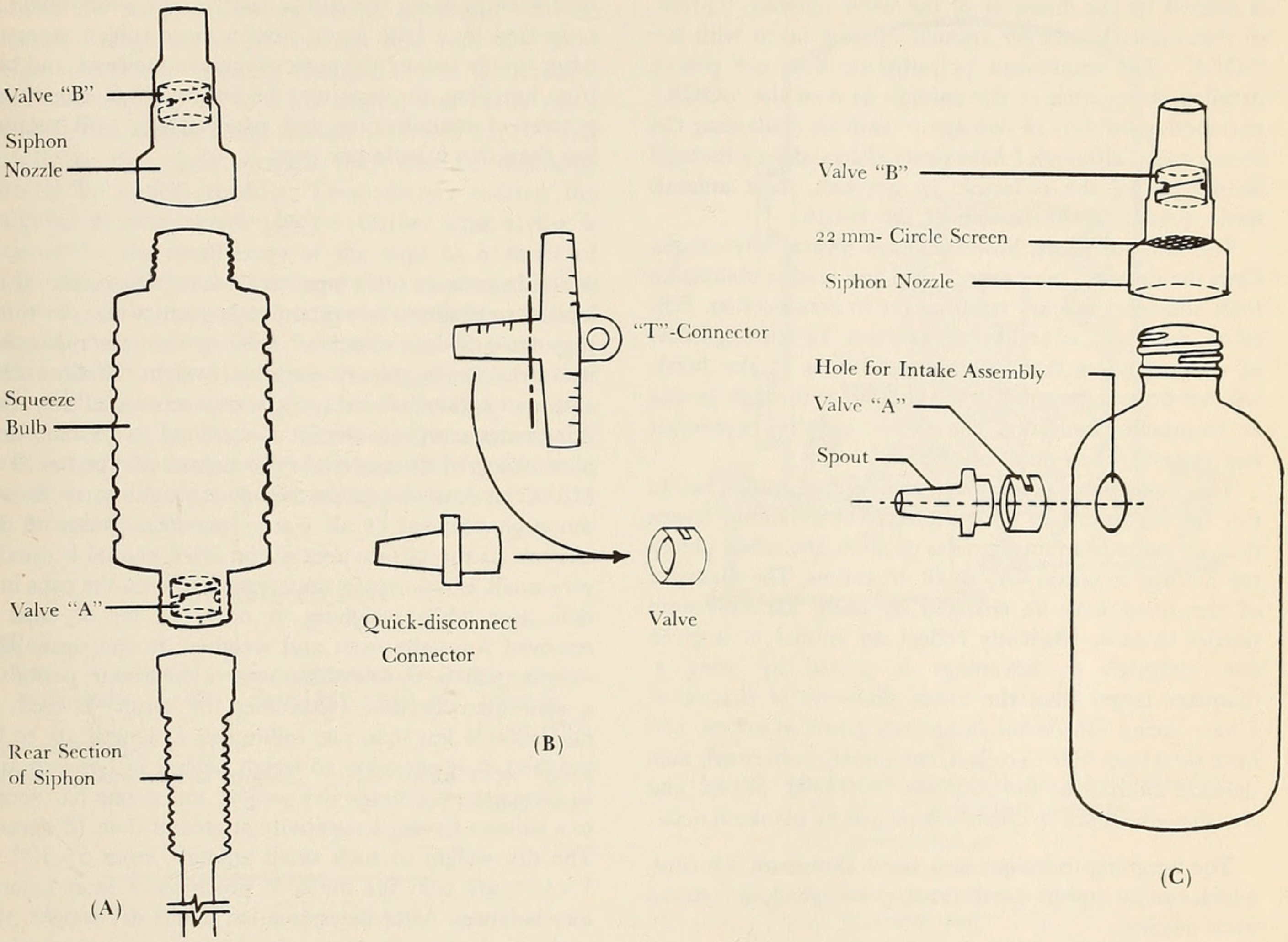

Figure 1

(A) View of three parts of gasoline siphon pump, showing location of one-way valves

(B) Assembly of intake nozzle from two types of tubing connectors
(C) Construction of completed collector

forceps. After drying overnight, the collector is ready for use.

USE

This device is used in essentially the same way as the 'Acadian SOCK', but one may suck animals into the bottle as rapidly as desired because of the one-way movement of water through the bottle. The walls of the bottle are squeezed together, forcing water through the top of the bottle, and when released, spring rapidly apart, pulling the animal harmlessly into the bottle along with the influx of water. As new animals are added to the bottle the retaining screen prevents expulsion of previously captured specimens.

DISCUSSION

The functions and utility of this device do not completely overlap those of the 'SOCK'. The use of the adhesive provides a structurally weaker bond than that of plexiglas joints, with the result that the collector is not quite as sturdy as the 'SOCK', though it is easily reparable. The maximum size of animals which may be collected is limited by the diameter of the valve opening, 11 mm, so that somewhat larger animals may be taken with the 'SOCK'. The translucent polyethylene does not permit detailed observation of the animals as does the 'SOCK', and some possibility of damage to animals contacting the screen exists, although I have never observed such damage during use of the collector. In practice, most animals settle quickly to the bottom of the bottle.

This collector does, however, have several advantages. Only the simplest, most economical and readily obtainable tools and materials are required for its construction. Efficiency and speed of action are maximal. The through-flow of water ensures that internal conditions of the bottle will not become harmful to the animal, as through anoxia or metabolite build-up. The device requires somewhat less dexterity than does the 'SOCK'.

This collection device may be modified somewhat to suit the organisms to be collected. The retaining screen may be made from finer grades of mesh, including plankton netting, to retain very small organisms. The diameter of the spout may be changed by using variously-sized nozzles to most efficiently collect an animal of a given size, although no advantage is gained by using a diameter larger than the inside diameter of the valve. I have found this device to be very gentle in action, and have even been able to collect, completely unharmed, such delicate animals as the medusae of *Obelia, Sarsia,* and *Aurelia,* which are frequently damaged by plankton nets.

The weighing technique uses small aluminum foil cups, which can be rapidly constructed, preweighed, and stored until needed.

CONSTRUCTION

An 8″ by 12″ sheet of aluminum foil is folded repeatedly, each time in half, to produce a block of foil about 1½″ square and containing 32 foil layers. This foil block is then trimmed with scissors to a circle 1″ in diameter, to yield a stack of 32 1″ foil disks. After peeling the layers apart, each disk is given a one- or two-digit identification number. The number should be scribed with a blunt probe or needle in the center of the dull side of the foil disk to ensure maximum legibility. This operation is best performed upon a firm, but not hard surface, such as a piece of cardboard, to prevent tearing the foil. Each disk is then molded into a small cup by centering the numbered portion of the foil, dull side up, over a piece of fire-polished ⅝″ glass tubing, and pressing the edges of the foil down around the walls of the tubing. The cup may then be removed from the tubing, and the identifying number should be readily legible on the bottom of the cup. The process of molding the cup is easier if the glass tubing is supported by a base made from a large rubber stopper. After briefly baking the cups to remove moisture and oils from handling, the cups may be pre-weighed. The entire process of manufacture and pre-weighing will require less than one minute per cup.

USE

A nudibranch, or other small marine organism, should be blotted to remove the accompanying salt water, as there may be sufficient dissolved salts in the accompanying water droplet to give an erroneous weight. Nudibranchs, and many other invertebrates, secrete mucus as they die; this mucus serves to cement the animal to the cup and prevent loss of the material. The cups should be baked at 110° C at least overnight before reweighing, to ensure thorough removal of all water from the tissues of the animal. As the surface area of the dried animal is usually very small, it is normally unnecessary to keep the cups in a desiccator while weighing, if only one set of cups is removed from the oven and weighed at one time. The weight gain is undetectable over a half-hour period if a semi-micro balance (sensitivity 10^{-5} gram) is used. If nudibranchs less than one millimeter in length are to be weighed, it is necessary to weigh several in one cup and to compute an average dry weight, unless one has access to a balance having a sensitivity of greater than 10^{-5} gram. The dry weight of such small animals, from 5×10^{-6} to 1×10^{-5}, are near the limits of precision of most laboratory balances. After determination of the dry weight, the nudibranch may be stored in the cup, and the radula and jaws may be later extracted by boiling in potassium hydroxide solution, as described by Fritchman (1960).

DISCUSSION

The use of small, lightweight, disposable cups provides several advantages. Cost is negligible, while minimal labor and skill are necessary for production. The small volume of the cups allows storage of many cups in a small space. For example, a set of cups may easily be stored in a 5″ finger bowl, and finger bowls stacked. In this way several hundred cups may be made and pre-weighed at one time, and stored dust-free until needed.

The mass of an individual cup should be between 0.01 and 0.05 g, depending upon the area of the foil disk and the brand of foil used. This small weight permits the use of the direct optical readout alone to weigh the cup and its contents, if such a balance is available. Even if an older, two-pan balance lacking optical readout must