Chapter 3. Methods for the Estimation of Zooplankton Abundance

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1 Introduction
This chapter is intended to outline and compare some of the most commonly employed methods for determining abundance of zooplankton organisms in lakes. I will emphasize the practical aspects of use of each of the different devices with respect to the specific environments and research questions. To some extent, the choice of examples given to illustrate and corroborate the discussion has been rather arbitrary in that specific examples chosen to illustrate a particular technique are usually those commonly available in the literature.

The census problem is usually the first difficulty encountered in studying zooplankton production. We must determine the sizes of the various zooplankton populations in a lake (or in a study area of a lake) and the manner in which these populations vary with time. This is problematic because the plankton do not constitute a closed population with respect to sampling possibilities but must be considered an open population in which immigration and emigration processes may occur, even in small environments. Further problems in zooplankton census arise from the fact that, in general, the zooplankton do not present a uniform horizontal or vertical distribution, but tend to be patchy, with a strong vertical gradient of abundance which is continuously changing due to circadial vertical migrations.

In addition, the size of zooplankton organisms range from a few μm to about 15 mm. Their ability to avoid different collection devices varies among species, and with the age (stage), size, shape, consistency, and behavior of different organisms.

In order to solve or minimize these problems with regard to research purposes or the organisms investigated many kinds of devices and

1. This chapter mainly treats equipment for sampling the zooplankton. Readers interested in an explanation of sampling design should consult Chapters 7 and 8.
Chapter 3

Experimental designs have been employed by planktologists. As a consequence, different researchers have sampled the plankton using such a variety of methods that in many cases a direct comparison of results is practically impossible.

Because the population density of zooplankton represents the number of individuals in a given volume of water, one of the first essentials in quantitative plankton sampling is to know the volume of water sampled. In many cases this is simple but in some cases it is not easy to measure this accurately, and only a rough value can be estimated (e.g. when net hauls without current meters are used). Furthermore, no single device exists that can make quantitative collections of the complete spectrum of pelagic zooplanktonic organisms. The smallest animals may pass through the nets, while larger, more motile organisms may avoid being captured by volumetric samplers. Accordingly, different devices and sampling procedures must be employed when studying different organisms, lakes, and problems. Criteria for selection depends upon the volume of water to be sampled, the depths of strata, the kinds of organisms and whether integrated or point samples are needed.

A researcher usually uses only one of the available devices. There are circumstances, however, in which the complete census of a population that is changing rapidly in size-stage structure and/or habits can be obtained only by using two or more different devices. This fact, commonly accepted in sampling other kinds of organisms (e.g. insects), seems to have attracted little or no attention from planktologists.

A standardized method cannot be recommended, because at present no single sampler is practicable on a large scale which would cover all water bodies. However, comparability of results can be achieved if, as discussed by Bottrell et al. (1976), the relative efficiency of the sampling procedure utilized is determined with regard to the most efficient one. This assumes, as did Bottrell et al., that 'the most efficient sampling (is) the one which catches the greatest number of individuals.'

The available devices for quantitative zooplankton collection can be conveniently divided into two basic categories:

1. Devices based on collection of water samples.
2. Devices based on the filtration of planktonic organisms from water directly in the field.

Gear of the first type includes bottles, pumps and tubes, while the second category includes plankton nets, towed plankton samplers, and plankton traps. Selection of one of the available devices will depend to a large extent upon the research purposes and characteristics of the environment. Some basic recommendations will be made in the following sections.
2 Descriptions of Sampling Gear

2.1 Sampling bottles

The most direct method for collecting plankton is to remove it from a known volume of water sample. Bottles can be used for collecting these samples. A variety of water-collection bottles can be used conveniently (Fig. 3.1), including those not designed specifically for this purpose.

A general characteristic of samples collected in this way is that a small amount of water is sampled. This implies that these methods, although generally efficient for small, less motile organisms, yield low collection efficiency when used to sample the larger, scarcer, and more active zooplankters. Furthermore, when a small amount of water is sampled, large

Fig. 3.1 Some examples of bottles used to sample zooplankton. (a) Ruttner bottle, (b) Friedinger bottle, and (c) Bernatowicz bottle (all redrawn from Chodorowski 1971).
numbers of replicates are needed in order to have a precise census of the organisms (including the rare forms) and to obtain representative figures. This is due to the spatial heterogeneity of plankton distribution both horizontally and vertically.¹

When this method is selected, one must choose among the different bottles available according to the research purposes, after carefully considering the drawbacks and advantages of each. As Smyly (1968) demonstrated, one of the criteria for the choice of a given bottle is that it must close as quickly as possible at the desired depth so that the avoidance response of the organisms is minimal. According to Smyly, the opacity and the direction of approach of the bottle had little or no influence on the numbers of rotifers collected. For other zooplankters, however, avoidance can have a great effect on the evaluation of their density (Schindler 1969). For these reasons, Hodgkiss (1977) suggests the use of a transparent bottle instead of an opaque one. Although theoretically preferable in some circumstances, small bottles (i.e. 1–2 litre capacity) require more replicates than larger bottles (> 10 litre).

Several methods have been used to remove organisms from a water sample. The most common are sedimentation, centrifugation, and filtration of the animals onto a net or glass filter. Filtration seems the most convenient. Bottrell et al. (1976), however, suggest that sedimentation is the 'only satisfactory way to concentrate planktonic protozoans' and the best method for rotifers, even though it is practical only at high densities. It must be stressed, however, that with sedimentation it is always laborious and difficult to determine whether all the organisms have settled. The complete settling of suspended material can take several days. In addition, removal of the supernatant liquid by siphon requires much care to ensure that sedimented animals are not disturbed. Centrifugation, on the other hand, has the disadvantage that the volume of water that can be handled in this way is very small.

1. Editor's Note. The spatial variability of the zooplankton will affect sampling programs using any type of sampler, not just water bottles (see Wiebe 1971). The number of samples necessary for a given precision can be calculated if an estimate of the variance ($s^2$) is known (see Chapter 8, Section 2.1.3). Unfortunately, no single work has summarized the published data on sampling variance for zooplankton taken with various samplers. Langeland & Rognerud (1974) and Evans & Sell (1983) have presented interesting summaries. For the present, one might seek other estimates of $s^2$ from single publications (e.g. Tonolli 1949a; Ragotzkie & Bryson 1953; Siebeck 1960; Langford & Jeromlajev 1966; Dumont 1967; Smyly 1968; Elster & Schwoerbel 1970; Burgis et al. 1973; Langeland & Rognerud 1974; Rey & Capblanq 1975; Wattiez 1978; Makarewicz & Likens 1979; Malone & McQueen 1983), bearing in mind that $s^2$ can vary markedly with population density and size of sampler (see e.g. Chapter 4, Sections 2.4.2 & 2.4.3).
Because it is rapid and easy to employ, filtration is the sorting method that is used most often. Some cautions are, however, necessary. The filtration must be done at low pressure and as gently as possible to avoid damage to the fragile organisms or their forced passage through the mesh. Rigid filters are preferable because their mesh size is invariable. Assuming that nets can theoretically select the lower size limit of organisms, but cannot discriminate for the upper size limit, which depends largely on the kind of plankton present, the mesh must be as small as the abundance of organisms permits without clogging. Likens & Gilbert (1970) suggest the use of nets with a mesh aperture of 35 \( \mu \text{m} \) which, according to the authors, permits an appropriate retention of even the smaller rotifers. Schindler (1969), however, indicates that nets with 28 or 10 \( \mu \text{m} \) mesh sizes are the most suitable for collecting the smallest forms from oligotrophic lakes. Similar results have been obtained by Ejsmont-Karabin (1978).

A very useful device that prevents high pressures and consequent damage to the organisms is described by Likens & Gilbert (1970). The device consists of a plexiglas funnel with three windows covered with nylon net (Fig. 3.2). The water sample is poured from the bottle into the funnel, which is partially submerged in a bucket of water. Lake water filtered through glass fiber filters is used to wash the plankton from funnels and screens. Special attention must be devoted to washing the net because, as shown by Ejsmont-Karabinova (cited by Bottrell et al. 1976), retention can greatly influence the estimation of

Fig. 3.2 Funnel for filtering water samples. All dimensions are in cm (after Likens & Gilbert 1970).
abundance, since it is directly affected by density of organisms and the adherence of certain species to the nylon net.

The use of bottle sampling to evaluate zooplankton density is not recommended in large, deep, oligotrophic lakes because too many samples would be necessary to obtain a realistic picture of population density and community composition. On the other hand, bottles can be usefully employed in shallow, eutrophic lakes, ponds, and pools, where the abundance of organisms and particulate matter might reduce the efficiency of other apparatus (e.g. clogging of nets), when spatial distribution on a fine scale must be estimated, or when littoral zone and interface layers between water and sediments must be sampled. Water collection bottles can also be employed to advantage for the study of small organisms, such as protozoans and rotifers, that do not usually exhibit significant avoidance reactions (Green 1977; Ruttner-Kolisko 1977).

Several authors have compared the efficiency of bottles with that of other devices in sampling different taxonomic groups of the zooplankton. There is no general agreement from these studies and no single picture emerges. Ferrari et al. (1973) have compared vertical net haul samples collected in a mountain lake with samples collected with a Ruttner bottle, and stated that the latter method seems to be much more effective in collecting Eudiaptomus intermedius and, in addition, yields information on its vertical distribution.1 Opposite results have been obtained by Hodgkiss (1977), who found that nets collected a greater number of Diaptomus gracilis (20X), Mesocyclops hyalinus (3X), Moina micrura (30X), and Diaphanosoma leuchtenbergianum (30X) than a Friedinger bottle. On the other hand, he found that the Friedinger bottle collected 30 times more Bosmina longirostris and 1.5 times as many Ceriodaphnia reticulata than the plankton net. Langeland & Rognerud (1974) compared a Schindler trap, a Clarke-Bumpus plankton sampler, and a Friedinger bottle. They pointed out that, on a statistical basis, the Friedinger bottle yields lower precision and probably does not collect rare species efficiently. In addition, the population density of crustaceans is greatly underestimated with this last method, confirming the similar results of Patalas (1954) who tested a Ruttner bottle.

Hrbáček (1966) describes a special use of bottles for obtaining plankton samples representative of small water bodies, where extreme patterns of variation in horizontal and vertical distribution of plankton can occur (ponds and pools). The method consists of collecting a composite sample, obtained by pouring many samples together, with sample volumes determined on the basis of volume distribution of different strata of the water body. It is summarized

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1. Editor’s Note. This can be very important in production studies, because egg development times are temperature dependent (see Prepas & Rigler 1978).
by the author (Hrbáček 1971) as follows: 'If the water volumes below various depths are expressed as fractions of the total volume, we obtain a volume depth curve that can be used to compare different kinds of waters. The curve of the depth distribution of volumes can be used to estimate the proper volume of water to be sampled for the establishment of an average figure, or, if the same volume from different depths is sampled and evaluated (separately), for proper calculation of the average. The average of values at different depths should be weighted in proportion to the different volume of these layers.' A treatment of both weighted and stratified sampling is given in Chapter 8.

2.2 Plankton traps

Plankton traps can be regarded as a particular kind of bottle specifically designed for plankton collection (Fig. 3.3). These quantitative samplers usually present some advantages compared to common bottles. They have very rapid closing systems and large mouths, which reduces the possibility of avoidance reactions, and they allow the simultaneous collection and filtration of a large volume of water. They can usually be handled easily by a single person even in a small boat. Some have remote closing systems (messengers) (Juday 1916; Clarke 1942; Acheson 1971), while others such as those of Patalas (1954) and Schindler (1969) are immediately self-closing at the desired depth.

Because plankton traps combine the characteristics of both bottles and nets, the same cautions apply. Larsson (cited by Bottrell et al. 1976) concluded that, in general, all types of volume samplers (e.g. Schindler trap, Friedinger bottle, Ruttner bottle) are very similar in the level of their sampling efficiency (Table 3.1). Authors who have compared the efficiency of plankton traps to other sampling devices, however, have shown that traps usually present one of the highest degrees of efficiency. This is especially true with regard to the species which usually display the strongest avoidance reactions such as *Daphnia* sp., *Leptodora* sp., and adult copepods (Table 3.2) (Patalas 1954; Schindler 1969). Because of their characteristics, the use of plankton traps is recommended as a useful alternative to water bottles in all cases.

2.3 Pumps and tubes

The use of pumps and tubes to collect zooplankton was introduced many years ago (Hensen 1887). Tubes can be regarded as a sort of long bottle, are usually flexible, and they collect an integrated sample when lowered through the water column. Their length must be equal to the maximum depth to be sampled. The internal cross-section of tubes should be as large as possible in order to minimize the escape of zooplankters that are rapid swimmers. Tubes
Fig. 3.3 Some plankton traps in common use for zooplankton collection. (a) Juday, (b & c) Akefors, (d) Schindler, and (e) Patalas.
Table 3.1 A comparison of the efficiency of various volume samplers. Values are the mean number $1^{-1} \pm$ one standard deviation (Larsson in prep.). After Bottrell et al. (1976).

<table>
<thead>
<tr>
<th>Species</th>
<th>Schindler trap</th>
<th>Hand pump</th>
<th>Friedinger bottle</th>
<th>Ruttner bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kellicottia longispina</td>
<td>4.33 ± 1.32</td>
<td>7.40 ± 2.96</td>
<td>10.23 ± 3.21</td>
<td>9.67 ± 4.08</td>
</tr>
<tr>
<td>Polyarthra vulgaris</td>
<td>11.30 ± 3.11</td>
<td>12.75 ± 5.15</td>
<td>18.29 ± 3.55</td>
<td>19.33 ± 4.37</td>
</tr>
<tr>
<td>Bosmina longispina</td>
<td>1.07 ± 0.50</td>
<td>3.05 ± 1.12</td>
<td>2.49 ± 1.26</td>
<td>2.40 ± 1.51</td>
</tr>
<tr>
<td>Holopedium gibberum</td>
<td>0.52 ± 0.26</td>
<td>0.80 ± 0.59</td>
<td>0.98 ± 0.59</td>
<td>0.79 ± 0.68</td>
</tr>
<tr>
<td>Cyclops nauplii</td>
<td>4.48 ± 0.71</td>
<td>2.25 ± 1.53</td>
<td>2.96 ± 1.13</td>
<td>3.33 ± 1.51</td>
</tr>
<tr>
<td>Cyclops copepodites</td>
<td>11.97 ± 2.50</td>
<td>6.50 ± 3.54</td>
<td>11.09 ± 2.28</td>
<td>9.60 ± 4.12</td>
</tr>
</tbody>
</table>

with a small diameter, however, can be closed more simply, and do not require special closing mechanisms. Flexible tubes are easily managed from small boats, but they must be weighted at the immersed end so that they remain vertical. If the weight is made of metal rings which are spaced at 10 cm intervals from one another (Tonolli 1971) and are connected to a small rope the same length as the tube, they can constitute an efficient system for closing the tube. The tube finds its greatest application in sampling an entire column of water in shallow environments or in littoral areas rich in vegetation (Pennak 1962). Transparent rigid tubes can also be employed for sample collection in

Table 3.2 Relative effectiveness of several zooplankton samplers. All figures are based upon an index of 100 for the transparent trap. Figures to the right of the ‘±’ sign represent approximate 95 % confidence limits for 10 replicates (after Schindler 1969).

<table>
<thead>
<tr>
<th>Species</th>
<th>28-l transparent trap</th>
<th>Opaque 9-l van Dorn</th>
<th>5-inch Clarke-Bumpus #15 net</th>
<th>12-inch metered townet #20 mesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holopedium gibberum</td>
<td>100 ± 18</td>
<td>65 ± 15</td>
<td>71 ± 18</td>
<td>65 ± 24</td>
</tr>
<tr>
<td>Daphnia sp.</td>
<td>100 ± 14</td>
<td>62 ± 18</td>
<td>74 ± 13</td>
<td>60 ± 30</td>
</tr>
<tr>
<td>Leptodora kindtii</td>
<td>100 ± 26</td>
<td>54 ± 41</td>
<td>35 ± 24</td>
<td>59 ± 33</td>
</tr>
<tr>
<td>Diaptomus leptopus</td>
<td>100 ± 21</td>
<td>60 ± 22</td>
<td>54 ± 23</td>
<td>49 ± 21</td>
</tr>
<tr>
<td>Diaptomus minutus</td>
<td>100 ± 18</td>
<td>105 ± 25</td>
<td>68 ± 12</td>
<td>86 ± 31</td>
</tr>
<tr>
<td>X</td>
<td>100 ± 19</td>
<td>69 ± 24</td>
<td>60 ± 18</td>
<td>64 ± 28</td>
</tr>
</tbody>
</table>
plastic bag enclosures where a representative sample of a small volume is needed, or in shallow littoral waters, ponds, and pools. One should remember that integrated tube samplers weight all strata sampled as if they contribute equally to the lake volume. In addition, all information regarding the depth distribution of zooplankton is lost. This can introduce important errors to production calculations (see Chapters 2 & 8).

Tube samplers seem to yield samples comparable to other methods of sample collection. George & Owen (1978) have illustrated the efficiency of their own water corer that has a pneumatic closing mechanism, similar to that used on a 5-l Friedinger bottle. They have suggested that tube samplers can be used when a 'detailed bottle cast profile is not required'.

Two types of pump samplers are presently used: hand pumps and motorized pumps. The latter are preferable because they guarantee a constant continuous water flow. Among volumetric samplers (bottles, tubes, pumps, and traps), pumps permit the collection of the largest volumes of water. In addition, they can be operated as a point or an integrating sampler. For this reason pumps can be employed as an alternative to bottles and tubes with some advantage if large water volumes are needed. When used as a point sampler, however, the definition of the sampling point is not very precise because the water currents that pumps produce can entrain waters from different layers.

The efficiency of pumps for plankton collection is, as yet, unclear. It must be stressed that some organisms seem to be negatively rheotactic. It must also be noted that different flow rates of pumps will select rheotactic organisms, with selectivity inversely related to the swimming abilities of the animals. More active zooplankters are usually captured less efficiently than with other methods (e.g. traps or nets). On the other hand, the collection of certain small organisms is favoured by the pump. Langford (1953) reported that pumps (both hand and motorized) collect more Cyclops and nauplii and less Diaptomus, Daphnia, and Diaphanosoma than a 45-l Juday trap. At present, however, a generalization of the efficiency of pumps with respect to the different zooplankton genera or species is still hazardous. The spatial patchiness of planktonic organisms probably contributes greatly to the confusion regarding the relative collection efficiency attained by pumps.

Patalas (1954) has compared different sampling devices (his own plankton trap, a Ruttner bottle, net, and pump), and found that the pump was more effective than the net and bottle, but less effective than the trap. For example, twice as many Eudiaptomus graciloides and Leptodora kindtii were caught

1. Editor's note. Tube samplers can rarely be used for production studies, since their valid use would require that all strata be at equal temperature, and contribute an equal proportion of the lake volume.
with the plankton trap as were obtained with the pump. Waite & O'Grady (1980) found that a filter pump collected an equal number of cladocerans but more rotifers and immature copepods and less adult copepods than a conventional plankton tow net. It should be noted, as Beers et al. (1967) and Ruttner-Kolisko (1977) have pointed out, that the results may vary greatly among seasons, even with regard to the sampling of a single species. Furthermore, Patalas (1954) has shown that the efficiency of various devices is different for day and night sampling. Pumps seem to have some advantages for sampling plankton in rivers (Bottrell et al. 1976; Waite & O'Grady 1980) even though the reason is not yet clear. They also seem to be the only practical method for the study of zooplankton entrained by the primary cooling water systems of power plants (Yocum et al. 1978; Waite & O'Grady 1980).

Tonolli (1971) has summarized some simple operational procedures and cautions that must be considered when using pumps to collect zooplankton samples: "If the pumps are of the submersible type, the pump itself can serve to weight down the lower end of the tube. If the pump is in the boat, as is the upper end of the tube, the bottom end must be weighted. The choice of type of pump will be determined primarily by tests to ascertain that planktonic organisms will not be damaged by the moving parts of the pump.

Before collecting the sample of water, it is necessary to let flow a volume of water which is at least three times the internal volume of the entire length of the immersed tube; this is necessary to avoid introducing into the sample water that had already entered the tube from lesser depths..." ... For the regular flow of water it is best that the exit tube also be immersed in the water, rather than coiled in the boat, thus preventing stopping the flow by kinks.

One should determine once and for all the capacity of the pump per unit of time so that one need not collect the water each time in graduated vessels.

The tube should have a relatively large diameter because of the great velocity of the flow of water which may damage the delicate organisms. However, flow should not be so slow that flushing takes more than a few minutes..."

Pumps can also be used in connection with a set of instruments that make possible a record of some of the most important physical and chemical parameters relevant for plankton studies such as chlorophyll, temperature, oxygen, etc. This equipment, such as that described by George (1976), although simple, is usually quite costly, and requires specialized assembly of the different components and continuous routine maintenance. These aspects render it impractical for many laboratories.

2.4 Plankton nets

Plankton nets have been used for the collection of zooplankton since the
beginning of the nineteenth century. Although several important improvements have been introduced, many of the oldest net designs are still in use. At present, we have at our disposal such a series of models that it is difficult to choose only one (see for example Omaly 1966, Lamotte & Bourlière 1971). Some were mainly designed for marine plankton collections, but they can be employed to advantage in freshwaters (Fig. 3.4). The simplest, and perhaps most widely used, is a simple conical net with a plankton bucket at its lower end. Others, such as Hensen, Apstein, or Juday nets, have a reducing cone forward of the mouth, and a simple closure system (see Fig. 3.4).

Shape and structure greatly affect the quantity of water that can pass

![Fig. 3.4 Examples of different plankton nets. (a) simple conical tow net, (b) Hensen net, (c) Apstein net, (d) Juday net, (e) Apstein net with semicircular closing lids, (f) Nansen closing net in open position, and (g) Hansen net in closed position (redrawn from Gehringer & Aron 1968).]
Fig. 3.5 The left panel indicates the water flow pattern established in front of plankton nets. Each line encloses 10% of the water entering a circular net. (1) a simple conical net, (2) a conical net with a porous collar, (3-4) conical nets with non-porous, mouth-reducing cones, (5) a conical net with a non-porous casing, (6) a conical net with non-porous mouth-reducing cone.

Patterns associated with some basic forms of plankton samplers: (1) a simple conical net which filters 12.5% of the water, (2) a conical net with a porous collar which filters 95%, and (6) a conical net with a non-porous casing and non-porous mouth-reducing cone.

(Reprinted from Tranter & Smith 1968).
through the mouth of a net (Tranter & Smith 1968). Plankton nets with a reducing cone (Fig. 3.5) and with a filtering area three times larger than the area of the mouth (Tranter & Heron 1965, 1967) are the most efficient. Net efficiency, however, is affected by a series of factors including characteristics of the fabric used to construct the net (gauze), mesh sizes, porosity, speed of sampling, avoidance by target organisms, escape of sampled organisms, and clogging. Some of these are difficult to evaluate and require further study. Others are considered below.

2.4.1 Characteristics of the gauze

The type of fabric used for the construction of a net has a marked effect on the selectivity of the net, filtration efficiency (i.e. the ratio of the volume of water filtered by a plankton net to the volume swept by the mouth), and clogging. To ensure that the population sampled is well defined the gauze should consist of uniform meshes that will not distort during operation and which will resist deterioration. Among the various fabrics usually available the two that have been used most often for the construction of plankton nets are bolting silk and monofilament (or multifilament) nylon cloth (Fig. 3.6). Monofilament nylon fabrics are the best because they assure more uniform mesh size (Fig. 3.7), less distortion, and are efficient in self-cleaning. The use of monofilament gauze, in addition, prevents clogging, because strong nylon filaments break less often than those of other fabrics.

Fig. 3.6 Major types of weave used in plankton net fabrics (i.e. gauzes).

2.4.2 Mesh size

In theory, organisms smaller than the mesh aperture size pass through the net, while organisms greater than the mesh aperture size are retained. In practice, retention of small organisms can occur due to progressive clogging of the net and the presence of body appendages. Larger organisms can avoid capture. As a result, it is not possible to predict exactly what the mesh selection will be for a particular organism, and it must be determined experimentally. A practice
Fig. 3.7 Nets of the same mesh width: (A) nylon, and (B) silk.
which permits the collection of a large spectrum of zooplankters is the simultaneous use of two nets with different mesh sizes: one for smaller (e.g. 50 \( \mu \text{m} \) aperture or less) and one for larger (e.g. 126 \( \mu \text{m} \) aperture) species. As pointed out by Ejsmont-Karabin (1978), however, nets with apertures as small as 10 \( \mu \text{m} \) seem unable to collect rotifers quantitatively, so that nets should be avoided for rotifer sampling whenever possible.

The use of the terminology 'meshes per inch' or 'meshes per cm' must be avoided, because this does not usually give a real measure of mesh aperture, which depends upon the relative size of filament and the characteristics of the gauze. In order to permit comparison of results, the mesh aperture size, and when possible, the filtering area of the net should be specified since these are much more useful.

**2.4.3 Volume of water filtered by plankton nets**

The volume of water filtered should be determined indirectly as a rough value assuming that the net filters the volume of the column of water traversed by the net:

\[
V = \pi r^2 d
\]  

(3.1)

where \( V \) is the volume of water filtered by the plankton net, \( r \) is the radius of the mouth of the net, and \( d \) is the distance through which the plankton net is towed. Clogging of the nets introduces an error in this calculation, however, and since clogging increases with the volume of water filtered the use of nets with flow meters is strongly recommended. As suggested by Gehringer & Aron (1968), the best position for the flow meter is not at the center of the mouth of the net, but in a position midway between the center and the net rim. A second flowmeter outside the net can give an estimate of net speed, and the two meters combined can yield an indication of filtration efficiency and clogging (Gehringer & Aron 1968).

**2.5 Towed plankton samplers**

Of the various instruments which have been devised for quantitative collection of zooplankton, the Clarke–Bumpus plankton sampler (Clarke & Bumpus 1940) is undoubtedly among the most versatile. Schematically, this instrument is simply a plankton net connected to a flowmeter which allows measurement of the volume of water filtered by the sampler. Its detailed structural characteristics are shown in Fig. 3.8.

The Clarke–Bumpus plankton sampler can be handled easily from a small craft, and can be equipped with nets of different mesh sizes to collect samples of organisms of very different body-size. It can be utilized advantageously for the collection of zooplankton samples along vertical, horizontal, or sinusoidal
Methods for the Estimation of Zooplankton Abundance

Fig. 3.8 Schematic representation of a Clarke-Bumpus plankton sampler: (A) tube, (B) net, (C) bayonet lock, (D) shutter, (E) pivot of shutter, (F) frame, (G) cable, (H) pivot for tube, (I) plane, (J) spring pin, (K) gate lock, (L) supporting clamp, (M) rod fixed to trigger, (N, N₂) arms in cap, (O₁) long finger lug, (O₂) short finger lug, (P) rod, (Q₁, Q₂) messengers, (R) trigger, (S) propeller, (T) counter, (U) semicircular bar, (V) escapement rod, (W) stop for messenger, and (1, 2, 3) are springs (after Tonolli 1971).
hulls within a selected layer of water. When it is used for vertical hauls it has the advantage over simple plankton nets of directly measuring the volume of water filtered, and consequently, has greater accuracy. Its most effective use, however, is for horizontal or sinusoidal hauls. Horizontal hauls make evaluation of the occurrence of irregularities and spatial discontinuities in the distribution of the planktonic organisms possible with precision and a high degree of resolution (Tonolli 1949a, b). Sinusoidal hauls, on the other hand, make possible the collection of representative samples from fairly extensive lacustrine areas (or water layers), and reduce the influence of the patchy distribution of plankton on the evaluation of its density.

The Clarke–Bumpus plankton sampler is equipped with a mechanism for opening and closing the mouth which can be operated at a distance by means of messengers. This means that even very deep layers can be sampled singly. In addition, a retaining device for a second pair of messengers makes possible the use and the simultaneous functioning of more than one sampler at different depths using a single cable. This has advantages when the vertical distribution of plankton in large and/or deep lakes is to be studied.

The basic model, conceived by Clarke & Bumpus in 1940, has, with the passage of time, undergone several improvements with regard to its efficiency and versatility. It has also been modified for the collection of plankton samples under specific conditions. Comita & Comita (1957) modified the mechanism which controls the volume of filtered water; Paquette & Frolander (1957) improved the closing system. Other modifications have increased the size of the instrument in order to collect more organisms in a single haul, especially in water bodies characterized by low zooplankton densities (Paquette et al. 1961; Yentsch et al. 1962).

At present there are two kinds of Clarke–Bumpus samplers on the market, one with a mouth opening of 5 inches (12.7 cm) and the other with a mouth opening of 12 inches (30.5 cm). Experiments on the comparative accuracy of these two samplers, equipped with nets of the same mesh size (No. 20: 76 μm mesh aperture), in the collection of crustacean zooplankton in a large subalpine lake (Lago Maggiore), have demonstrated some significant differences. The samples obtained from three replicates of each sampler exhibit apparent community structures which are very similar within the replicates taken using a single instrument, but which are very different between the two instruments (de Bernardi, unpublished data). In particular, as shown in Table 3.3, the sampler with the larger mouth collects larger, more motile, and faster swimming crustaceans more efficiently, while smaller organisms dominate the samples collected by the smaller sampler.

Comparisons of the efficiency of the Clarke–Bumpus sampler relative to other techniques often disagree, although they all suggest that the Clarke–Bumpus is a good piece of sampling gear. According to Currie & Foxton
Methods for the Estimation of Zooplankton Abundance

Table 3.3 Community structure as estimated by 3 replicate zooplankton samples collected with a 12 inch and a 5 inch Clarke-Bumpus sampler (net = No. 20: 76 μm pore size). Samples were taken in the upper 50 m of Lago Maggiore. [Editor's Note: A reviewer has suggested that percentage community structure figures may not reflect actual collection efficiencies well because the values for various species are not independent. Many researchers, however, use zooplankton samples for examining community composition using percentage composition measures. They should be warned that different samplers yield quite different pictures of the community.]

<table>
<thead>
<tr>
<th>Species</th>
<th>net 76 μm</th>
<th>Sampler 12&quot;</th>
<th>Sampler 5&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>s</td>
<td>%</td>
</tr>
<tr>
<td><strong>Diaphanosoma brachyurum</strong></td>
<td>57.9 ± 4.3</td>
<td>51.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td><strong>Daphnia hyalina</strong></td>
<td>4.4 ± 0.08</td>
<td>2.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td><strong>Bythotrephes longimanus</strong></td>
<td>0.15 ± 0.04</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Leptodora kindtii</strong></td>
<td>0.72 ± 0.11</td>
<td>0.21 ± 0.17</td>
<td></td>
</tr>
<tr>
<td><strong>Bosmina coregoni</strong></td>
<td>0.32 ± 0.14</td>
<td>0.74 ± 0.45</td>
<td></td>
</tr>
<tr>
<td><strong>Chydorus sphaericus</strong></td>
<td>0.17 ± 2.7</td>
<td>1.4 ± 3.9</td>
<td></td>
</tr>
<tr>
<td><strong>Mesocyclops leuckarti</strong></td>
<td>0.38 ± 0.16</td>
<td>0.46 ± 0.08</td>
<td>0.5 ± 0.26</td>
</tr>
<tr>
<td><strong>Cyclops abyssorum</strong></td>
<td>5.3 ± 0.33</td>
<td>2.1 ± 0.9</td>
<td>24.7 ± 5.6</td>
</tr>
<tr>
<td><strong>Eudiaptomus vulgaris</strong></td>
<td>3.0 ± 1.4</td>
<td>9.2 ± 2.0</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td><strong>Mixodiaptomus laciniatus</strong></td>
<td>0.6 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>copepodites</td>
<td>0.4 ± 0.1</td>
<td>1.5 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

(1957), the Clarke-Bumpus is one of the samplers which yield the highest filtration coefficient.\(^1\) Schindler (1969) reports that the sampling efficiency of the 5-inch Clarke-Bumpus plankton sampler is inferior to that of his own trap, but similar to that of other devices such as a 9-l Van Dorn bottle, and a simple plankton net with a 30 cm mouth. It must be emphasized, however, that the mesh size of the Clarke-Bumpus plankton sampler net was different from the mesh size of the sampler with the 12 inch mouth in this case, and this may have had a significant influence on the results. On the other hand, Langeland & Rognerud (1974) made a statistical comparison of the efficiency of three different devices for sample collection (the Clarke-Bumpus plankton

1. Editor's Note. 'Filtration coefficient' here means the ratio of the volume of water passed through the sampler to the volume of water through which the sampler would pass if there were no resistance to water flow. The implication is that the Clarke-Bumpus has little problem with clogging.
sampler, the Schindler trap, and the Friedinger bottle) in four lakes of different phytoplankton density. They found that the Clarke-Bumpus plankton sampler and the Schindler trap were of similar efficiency, while the Friedinger bottle was less efficient. Lewis & Saunders (1979) report that the efficiency of the Clarke-Bumpus sampler is very similar to that of a sampler of their own conception, which combines the features of the Van Dorn water bottle and the integrating tube sampler.

Because of its sampling characteristics, the Clarke-Bumpus plankton sampler is strongly recommended whenever the morphological features of the lake and the exigencies of the research allow it.\(^1\) It is a particularly useful device for research on large lakes characterized by low zooplankton densities, or when the object of study is the large scale spatial distribution (both vertical and horizontal) of the zooplankton. An appendix (Section 5) reproduces some particularly detailed suggestions for the use of the Clarke-Bumpus plankton sampler, given by Tonolli (1971).

Other samplers for the quantitative collection of plankton have been designed, such as the various types of high-speed samplers, the Hardy continuous recording sampler (Hardy 1926), and the Motoda multiple sampler (Motoda 1962). Since the use of these samplers from small boats is quite difficult they have been used primarily for the collection of marine plankton and will not be discussed further here.

3 Sample Manipulation: Killing and Preservation

Once the samples have been collected, the organisms must be killed and treated at once so as to preserve them whole until analysis can be completed.

The simplest method of killing and preserving the animals uses a 5\% formaldehyde solution. It must be remembered in this connection that formalin is sold as a 40\% solution of formaldehyde. While useful for the treatment of routine samples, this method has a distinct disadvantage when the morphological structure or the population dynamics of the different species are the object of study. This is especially true when rotifers and cladocerans are the organisms concerned. Since 5\% formaldehyde solution takes a relatively long time to kill the animals distortion of the body structure often takes place in the case of soft-bodied organisms. Where cladocerans are concerned (e.g. *Daphnia, Bosmina, Diaphanosoma*), carapaces may balloon and the eggs and embryos contained in them may be lost (see also Chapters 2, 4 & 6). In this case, it is advisable to kill the organisms using more rapid and efficient methods or to utilize special solutions and techniques.

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1. Editor's Note. K. Patalas suggests, however, that Clarke-Bumpus samplers should not be used with nets smaller than 70-80 \(\mu\)m because below this aperture, efficiency is very low.
A very quick and efficient method is to filter the collected material onto a nylon net of the appropriate mesh size which is then immersed in 95% ethanol. The organisms are killed quickly, largely avoiding the negative effects mentioned above, and for this reason, this method has been employed with success in the study of the dynamics of Daphnia populations (e.g. Hall 1964, de Bernardi 1974). The use of alcohol also avoids carapace ballooning, a fact of fundamental importance when it is necessary to know the body-size in order to estimate the size structure of the populations in production studies. The organisms killed in this way must then be transferred to a 5% formaldehyde solution in order to preserve them until analysis can be performed.

Recently, Haney & Hall (1973) have suggested using a solution of 40 g l\(^{-1}\) of sucrose and 4% formaldehyde to kill and preserve zooplankton samples. This method has been modified by Prepas (1978) who found it to be somewhat inefficient, especially with regard to the problem of egg loss from the carapace. Prepas suggests a method of her own which consists of concentrating the samples on a nylon filter and treating them with a solution of 60 g l\(^{-1}\) sucrose and 2% formaldehyde buffered with sodium borate and maintained at a low temperature (6°C).

### 4 The Choice of a Sampler

As has been emphasized in Section 1, the problem of the choice of a sampler for quantitative plankton collection is not an easy one. There are many factors involved which cannot be ignored, particularly the characteristics of the environment, the zooplankton species to be studied, and the aims of the research. Each single device, in fact, has its own exclusive features which determine its relative utility according to the specific use for which it is intended. In addition, once a sampler has been selected its efficiency must be tested in comparison to other samplers, and this comparison should be repeated more than once in the course of the year. The efficiency of a particular instrument is often related to the composition, structure, and density of the population to be sampled, and the density of other populations (e.g. phytoplankton); thus, one can see how apparent sampler efficiency might vary with the seasons.

Some schematic suggestions for the choice of particular devices for plankton collection in different experimental situations are presented in Table 3.4. This table is certainly not to be considered definitive or exhaustive, but is intended only to indicate some basic solutions to the complex problem of quantitative plankton collection. Further useful information has been published by Omaly (1966), Schwoerbel (1966), Gehringer & Aron (1968), Tonolli (1971), Lamotte & Boulière (1971), and Bottrell et al. (1976).
Table 3.4 Schematic recommendation for the choice of sampler to be used for the assessment of zooplankton population density under various conditions. [Editor's Note: K. Patalas suggests that the choice of sampling technique is not only reliant upon the nature of the water body and the spatial scale of the required data, but also upon the size of the target organisms. One should not, for example, use a small tube sampler for the collection of vertically integrated samples of mysids in deep, pelagic waters.]

<table>
<thead>
<tr>
<th></th>
<th>Deep and pelagic waters</th>
<th>Shallow and littoral waters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Point samples</td>
<td>Vertically integrated</td>
</tr>
<tr>
<td>Bottles</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Traps</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>Tubes</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Pumps</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Nets</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Plankton samplers (e.g. Clarke-Bumpus)</td>
<td>-</td>
<td>+ +</td>
</tr>
</tbody>
</table>
5 Appendix

Instructions for use of the Clarke–Bumpus plankton sampler given by Tonolli (1971).

The plankton sampler must be used from a moving boat, and it is therefore necessary to estimate the depth at which the instrument is towed. Knowing the angle which the cable coming off the winch forms from the vertical, measured with a clinometer, one can calculate the depth by applying the simple formula:

\[ D = L \cos \alpha \]

where \( D \) is the depth in metres of the instrument, 
\( L \) is the length in metres of the cable, and 
\( \alpha \) is the angle subtended by the cable to the vertical.

It is necessary that the angle not exceed a value greater than 50°, and it is therefore necessary to apply a weight to the lower end of the cable. This weight will be most efficient if it has the form of a cable depressor.

Actually the cable does not lie in the water in a straight line but rather as a reversed catenary, more or less pronounced according to the mass of the terminal weight, its more or less active hydrodynamic form, and the velocity with which the boat moves through the water.

When one is working in a small boat it may be inconvenient to measure the cable angle and calculate the true depth of the sampler. Advantage can be taken of the fact that an angle of 45° is rather easily estimated by eye and that the cosine is 0.7. Thus it is easy to calculate mentally the real depth under normal working conditions from a small boat when the cable will be nearly straight: one lets the cable out to a length 1.4 times the desired depth.

The collection of plankton should be carried out along as straight a course as possible, since each deviation from this involves a movement of the plankton sampler to a greater depth, but it may be convenient to move in the ark of a large circle to avoid towing the samples in the wake of the boat.

The velocity of the boat should not be less than 50 nor more than 125 m/min. Of course the velocity should remain constant over the whole collecting period. One may determine the velocity by measuring the distance travelled by the boat during a period of 60 seconds along a line of small floats placed at determined intervals, anchored to the bottom of a littoral zone, or by measuring the travel time between two points of known distance.

The plankton sampler may be used to collect horizontally, successively at different depths: this permits us quite exact knowledge of the populations of these depths, but requires interpolations that are not always acceptable, if from the data of horizontal collections we wish to obtain more general information about the total populations in a column of water.

One may sample a layer simply by raising the sampler from the bottom to
the top of the layer. With a little experience, one may make zig-zag collections in a vertical plane, in general limited by the surface of the lake above and below by that depth to which one wishes to sample. Thus one may combine the two motions, the uniform motion of the boat, with a vertical motion, also of uniform velocity, but alternating its direction of motion. This operation may be repeated with the same plankton sampler many times in such a manner that the apparatus will describe in the layer of water a series of ascending and descending passages.

The Clarke-Bumpus plankton sampler is designed for use with more than one instrument attached to the same cable, so that the arrival of the first and then of the second messenger at the plankton sampler nearest the surface will liberate messengers attached to the base of the metal frame which, running down the cable, serves to perform the same function successively on plankton samplers at greater depths along the same cable. One has thus the great advantage of exploring with zig-zag collections adjacent and overlapping superimposed strata layers of the same water mass, permitting the evaluation of the planktonic population of a given stratum in relation to the populations of overlying strata. Obviously a powerful winch and heavy cable are needed for use of more than one sampler.

This possibility may also be exploited by having two samplers function simultaneously attached at a short distance (even 1 m) from one another, supplied with nets of different mesh. One has thus the advantage of being able to ascertain the real relations of the presence of organisms of rather different sizes in the same layer of water.

The calibration value (litres/revolution) varies somewhat with velocity but is relatively constant over a range of velocity (see below). The minimum velocity (50 m/min) is imposed by the characteristics of the impeller, of the transmission, and of the counters. Only with the attainment of this velocity does the relation between the amount of water admitted and the number of revolutions become constant.

It may happen that the plankton sampler, towed behind the boat at the indicated rate, will have the interior of the net clogged by a film of algae. In this case the number of revolutions is not reliable, since during a more or less long fraction of the course there may have been admitted to filtration a volume of water per unit of time insufficient for proper functioning of the counter (Yentsch & Duxbury 1956).

Each plankton sampler is provided with a certificate giving the value of the factor $K$, by which it is necessary to multiply the number of revolutions $R$ registered by the counter, in order to obtain the volume of water filtered in litres. If the plankton sampler is used with care, and not too intensively, the value of $K$ will not change with use. By simple means one may however easily recalibrate it, for example, by running the sampler without a net several times,
in one direction and then the other, for the distance between two fixed buoys, making sure that the mouth remains normal to the direction of motion.

If the shutter fits imperfectly the capture of aliquots of small organisms may occur during the descent and recovery of the sampler through more highly populated layers. The remedy to this is very simple: apply to the shutter a disc of celluloid, or similar material, flexible, which conforms exactly to the interior dimensions of the tube: the disc of celluloid should be attached to the shutter by a single central screw, to keep the celluloid flexible and fitting accurately.

In reading the counter at the beginning and end of the collection, one must make sure that wind action does not add 'anemometric' revolutions to the count.

If the plankton sampler is not being used in horizontal tows or in oblique tows which terminate at the surface, it may become necessary to know the velocity of fall of the messenger along the cable. Because of the time it takes the messenger to fall to the depth of the sampler it may permit the sampler to rise above the desired level before it is closed.

One cannot furnish general information about the velocity of fall of the messengers, since it depends on the messenger itself (shape and weight), on the nature of the cable and of its angle of inclination, as also on the velocity with which the boat is moving. It is, however, not difficult to estimate the velocity of fall for small depths, since, holding one finger against the cable where it enters the water, one can feel the impact of the messenger when it strikes. Knowing the time of fall for a given depth, one can extrapolate arithmetically the times of fall for other depths, under the same conditions of those variables which can influence the velocity of fall.

The messengers may then be released far enough beforehand so that the plankton sampler in uniform motion can in the meantime reach the predetermined position.

The C-B sampler generally used has a mouth diameter of 25 cm but a larger model with 62 cm is now also available (Paquette et al. 1961). The use of this large model is recommended when one wishes to collect the planktonic predators which are large and fast-swimming, like Heterocope, Ponteporeia, Leptodora, Bythotrephes, etc.

6 References


Chapter 3


