3 Collecting zooplankton

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3.1 INTRODUCTION

From the beginning of modern biological oceanography more than 100 years ago, remotely operated instruments have been fundamental to observing and collecting organisms. For most of the twentieth century, biological sampling of deep ocean has depended upon winches and steel cables to deploy a variety of instruments. The samplers developed over the years generally fall into three classes (Table 3.1):

- Water-bottle samplers that take discrete samples of relatively small volumes of water (a few liters)
- Pumping systems that sample intermediate volumes of water (tens of liters to tens of cubic meters)
- Nets of many different shapes and sizes that are towed vertically, horizontally, or obliquely and sample much larger volumes of water (tens to thousands of cubic meters).

Traps to collect animals in midwater or rising off the seafloor have been used less often to collect marine zooplankton.

Early depth-specific collecting nets opened or closed mechanically, either with weighted 'messengers' traveling down the towing cable by gravity to trigger a trip mechanism, or by a pressure- or flow-meter activated release. In the 1950s and 1960s, conducting cables and transistorized electronics were adapted for oceanographic use, and more sophisticated net systems began to do more than collect animals at specific depth intervals. Multiple net systems now routinely carry sensors to measure water properties such as temperature, pressure/depth, conductivity/salinity, phytoplankton fluorescence/biomass, and beam attenuation/total particulate matter. They also measure net properties such as volume of water filtered, net speed, and altitude from the bottom, as well as net function such as an alarm to tell when a net closes. In spite of their advanced features, all instruments deployed from cables to collect organisms are limited in their temporal and spacial coverage. This is not only because of the large amount of time it takes to collect a sample (tens of minutes to an hour or more, and many hours to complete an entire multiple net haul), but also because of the time required (hours to a day or more) to identify and count individuals by species under a microscope.

This chapter deals with improvements in mesozooplankton and macrozooplankton (in a size range $>200\,\mu$m to ~ 3 cm) net sampling as well as other sampling techniques for describing the vertical and geographic distribution and biomass estimation of these

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Table 3.1 Summary of zooplankton sampling gear types.

Sampling gear	Kind of sampling	Size fraction	Resolving scale		Typical operating range	
			Vertical	Horizontal	Vertical	Horizontal
A. CONVENTIONAL METHODS						
Water bottes	Discrete samples	Micro/Meso	0.1–1 m	–	4000 m	–
Small nets	Vertically integrating	Micro/Meso	5–100 m	–	500 m	–
Large nets	Vertical, obliquely, horizontally integrating	Meso/Macro	5–1000 m	50–5000 m	1000 m	10 km
High-speed samplers	Obliquely, horizontally integrating	Meso/Macro	5–200 m	500–5000 m	200 m	10 km
Pumps	Discrete samples	Micro/Meso	0.1–100 m	–	200 m	–
B. MULTIPLE NET SYSTEMS						
Continuous Plankton Recorder	Horizontally integrating	Meso	10–100 m	10–100 m	100 m	1000 km
Longhurst–Hardy Plankton Recorder	Obliquely, horizontally integrating	Meso	5–20 m	15–100 m	1000 m	10 km
MOCNESS	Obliquely, horizontally integrating	Meso/Macro	1–200 m	100–2000 m	5000 m	20 km
BIONESS	Obliquely, horizontally integrating	Meso/Macro	1–200 m	100–2000 m	5000 m	20 km
RMT	Obliquely, horizontally integrating	Meso/Macro	1–200 m	100–2000 m	5000 m	20 km
HYDRO-BIOs Multinet	Vertically, obliquely, horizontally	Micro/Meso	2–1000 m	100–2000 m	5000 m	5 km
C. ELECTRONIC OPTICAL OR ACOUSTICAL SYSTEMS						
Electronic Plankton Counter	High resolution in the horizontal/vertical plane	Meso	0.5–1 m	5–1000 m	300 m	100s km
In situ Silhouette Camera Net system	High resolution in the horizontal/vertical plane	Meso	0.5–1 m	5–1000 m	1000 m	10 km
Optical Plankton Counter	High resolution in the horizontal/vertical plane	Meso	0.5–1 m	5–1000 m	300 m	100s km
Video Plankton Recorder	High resolution in the horizontal/vertical plane	Meso	0.01–1 m	5–1000 m	200 m	100s km
Ichthyoplankton Recorder	High resolution in the horizontal/vertical plane	Meso	0.1–1 m	5–1000 m	200 m	10 km
Multifrequency Acoustic Profiler System	High resolution in the horizontal/vertical plane	Meso/Macro	0.5–1 m	5–1000 m	100 m	10 km
Dual-Beam Acoustic Profiler	High resolution in the horizontal/vertical plane	Meso/Macro	0.5–1 m	1–1000 m	800 m	100s km
Split-Beam Acoustic Profiler	High resolution in the horizontal/vertical plane	Meso/Macro	0.5–1 m	1–1000 m	1000 m	100 km
ADCP	High resolution in the horizontal/vertical plane	Meso/Macro	10 m	5–500 m	500 m	100s km

Notes: Most vertical nets are hauled at a speed of 0.5–1 m s^{-1}*. Normal speeds for horizontal tows are ~2 kn (1 m s*$^{-1}$*) and for high speed samplers ~5 kn (2.6 m s*$^{-1}$*). For further categorization of pumping systems which are used by a number of investigators see the review by Miller and Judkins (1981)* zooplankton. Substantial improvements have been achieved since the appearance of the UNESCO Manual (Tranter and Smith 1968).

Research requirements and the species of zooplankton of interest will dictate the sampling method used. In most cases some form of capture sampling will be needed to acquire specimens for taxonomic and/or experimental purposes. Nets are the most common method of capture, but the use of pumps and large water bottles has increased as a means of collecting zooplankton for biomass estimation and for collection of larval stages of zooplankton that nets do not sample effectively. Plankton traps are much less commonly used. Since 1968, there have been no radical advances in net sampling, but only a series of incremental improvements in the way nets are configured and integrated with instruments for the measurement of environmental parameters. Communication with instruments on net samplers through electrical, fiber optic conductor cable or by acoustic link are now commonly used to transmit data to and from the sampling device. These instrument packages allow accurate depth directed net sampling of zooplankton, while at the same time measuring other important physical and biological parameters when zooplankton are collected. This combination of instruments and nets has resulted in more efficient use of ship and sampling time as well as increased understanding of zooplankton ecology.

3.2 A SURVEY OF SAMPLING DEVICES

3.2.1 Pumps and traps

Pumps in various configurations have been used to sample plankton since at least the work of Hensen (1887). They offer advantages over towed nets, particularly in habitats with very high animal density. Volume filtered can be measured much more reliably, and interference from mesh clogging can be monitored when filtration is on deck. Depth of sampling is readily controlled with no contamination from surrounding levels and the desired parameter (T, chlorophyl, ammonia) can be measured in the same water inhabited by the animals collected plus serial sampling is simplified. However, volumes filtered with pumps of reasonable scale are small relative to towed nets. Limits to capacity are set by the power required to move large volumes and by hose friction, and depths that are commonly sampled with pumps are within 200 m of the surface. An exception is the MULVPS pumping system (Bishop *et al.* 1992) which has been used to sample to 1000 m and more.

Several pump configurations give satisfactory service, including centrifugal (Gibbons and Fraser 1937), diaphragm (Mullin and Brooks 1976) and vacuum, a large chamber on deck is evacuated, then opened to a hose with submerged intake (Lenz 1972). For systems delivering water to a ship's deck by hose, the main factors limiting transfer capacity are static lift from sea to deck and hose friction. If the pump is on deck, the actual motive force for pumped water is atmospheric pressure, which is limited to 10 m of static head. In most pumps, the available suction head is less, since some is required to prevent cavitation in the pump. If the pump is at the sampling depth, the available static head can be greater, but hose friction still limits transfer. Small bore hose has greater friction loss than large bore, so that large hoses improve flow more than expected from their larger transfer cross section. All joints, bends and elbows add friction, which must be accounted for in design. A set of design considerations and component selection guidelines for pump samplers involving hose with filtration on deck is given by Miller and Judkins (1981). Recent systems include a high capacity ($2.8\,m^3\,min^{-1}$) seagoing design by Harris *et al.* (1986), and a handy system for coastal boats from Omori and Jo (1989). A similar design, known as the Pacer Pump has been used by Durbin *et al.* (1987) in the US GLOBEC Georges Bank Program to sample naupliar stages of copepods as part of a population dynamics study on the Bank.

Another sampler configuration packages pump and filter together to be lowered by power cable (Mohlenberg 1987) eliminating the overside handling of hose and hose friction. The advantage of monitoring filtration on deck is lost, but can be partly replaced by telemetering flow meters in the pump stream. Clogging is indicated by reduced flow. Mohlenberg showed that his $0.42\,m^3\,min^{-1}$ net-pump was about equally as efficient as a WP-2 net, the zooplankton net described in the report of the Working Party 2 (Tranter 1968), except for capture of adult female copepods. Female *Calanus* and *Pseudocalanus* avoided the pump more effectively than the net when the pump was held stationary in the vertical. Another possibility not yet realized is to modify the Mohlenberg apparatus for multiple sampling by adding a filter carousel. The overall package would be about the size and shape of the CTD-sampling rosette.

Studies of the capture efficiency of pumps relative to nets are reviewed by Taggart and Leggett (1984). On the whole the comparisons are favorable enough to support use of pumps when their advantages are needed. Singarajah (1969) has studied the response of individual zooplankters to narrow suction intakes, they do leap clear when subjected to strong flow gradients. All but the smallest gelatinous zooplankton are badly damaged by impeller pumps, so they are mainly satisfactory for hard-bodied forms, particularly crustaceans. There is evidence that the smallest nauplii stages of *Calanus finmarchius* are damaged and lost in sampling with a centrifugal pump, but not with a diaphragm pump (Durbin, unpublished data).

3.2.2 Nets and serial samplers

SIMPLE NET SAMPLERS

A detailed description of simple plankton nets and their use was given by Tranter (1968), and these recommendations are still valid today. The recommended plankton net is still the WP-2 net with an open-mesh-filtering area to mouth area ratio of at least 6:1. A flow meter mounted in the mouth should be used whenever possible. The flow meter should not be located in the center of the net mouth opening, but in a position about halfway from the net mouth center to the net rim (Smith, Counts and Clutter 1968). The center position generally gives an over estimate of the flow into the net. The working group in 1968 recommended use of the TSK flowmeter (Tokyo Seimitsu Co. Ltd), but since then there have been a number of excellent new flow meters developed and manufactured, therefore we do not recommend any single brand of meter.

The main advantages of ring nets over multiple net samplers is their ease of use and low cost, they can be used with simple hydrographic cables, and can easily be towed from any type and size of vessel.

MULTIPLE SAMPLE INSTRUMENTS

There are a number of net samplers that collect multiple zooplankton samples and these fall into three main types. The first type is based on the principle of collecting animals on a continuous ribbon of netting and includes the Continuous Plankton Recorder (Hardy 1939), the Longhurst Hardy Continuous Plankton Recorder (Longhurst *et al.* 1966), the Autosampling and Recording Instrumented Environmental Sampling System (Dunn *et *al.* 1993a, 1993b) and the highspeed Gulf-III OCEAN sampler (Nellen and Hempel 1969).

The Continuous Hardy Plankton Recorder (CPR)

The Continuous Hardy Plankton Recorder (CPR) is a high speed zooplankton sampler designed to be towed in near surface waters over long distances from ships of opportunity (see Fig. 2.3). The original CPR was designed by Alister Hardy to be used to study patchiness of plankton in the Antarctic on the Discovery Expedition, 1925–1927 (Hardy 1926). During the 1930s, the CPR was deployed in the North Sea to monitor seasonal and annual changes in the plankton (Hardy 1935). Since that time, except for a break during the Second World War, the CPR has continued to be deployed on a monthly schedule in the North Sea and North Atlantic (Warner and Hays 1994). In addition to its plankton-sampling role, the CPR has the capability to carry environmental sensor packages under its box-section tail. Electromagnetic flow meters can be fitted to the exit apertures of the CPR to measure the volume of water sampled (which has a theoretical maximum of 3 m^3 per 10 nautical miles of towing).

CPR operation The CPR is designed to be towed at speeds up to 25 knots in the surface mixed layer (Hays and Warner 1993) by the non-scientific crews of commercial ships going about their regular business. The recorder is deployed from the ships mooring deck, off a davit or A-frame on a 10 mm steel-wire rope using the ship's winches. The CPR towing depth of approximately 10 m is maintained by an in-built diving plane and by regulation of the length of the towing wire. Water enters the CPR through an aperture of 1.27 cm^2, travels down a tunnel 5 cm × 10 cm in cross section where it passes through a graduated silk filter of mesh size 270 mm, and finally exits the machine via a tunnel and aperture (10 cm × 3 cm cross section) to the rear. As the CPR passes through the water, an impeller drives a take-up spool which moves the filter silk across the filtering aperture where it is covered by a second layer of silk and wound into a storage tank containing formaldehyde. The silk is wound across the aperture at a rate of approximately 10 cm per 10 nautical miles of tow. This rate can be controlled by adjusting the angle of the impeller blades. At the end of the tow, the CPR is retrieved, and the crew fill out a form detailing the times and locations of CPR deployment and retrieval, and any alterations in ship course. Upon docking, the CPR is unloaded and returned to the laboratory where the silks are processed using a standard procedure (Colebrook 1960).

Treatment of samples In the laboratory, the silk is removed from the storage tank and unwound. Using the tow data provided by the ship's crew, and assuming a constant tow speed, the silk is marked out and labeled in sections corresponding to 10 miles of towing. A visual estimate of the greenness of the silk is then made with reference to a standard color chart. The silk is then cut into the 10 mile sections and distributed for plankton analysis. Full details of analysis procedures are given by Rae (1952) and Colebrook (1960). The plankton is identified on the silk in three stages. The first examination, for phytoplankton, is of 20 fields of view along a traverse (a sub-sample of about one thousandth of the filter silk) under 450 × magnification. The species in each field are identified and the number of fields of view (out of 20) in which that species was present is recorded. The second examination is of both the filter and covering silk for selected zooplankton species at 48 × magnification (a sub-sample of about 1/40 of the silk). All the species identified are counted and recorded. Finally the whole sample is examined for large (generally >2 mm) organisms that are counted and recorded. The samples are then sprayed with borax-buffered 4% formalin, labeled, packaged and placed in an archive.

Longhurst Hardy Plankton Sampler (LHPR)

The most commonly used multiple cod-end sampler is the Longhurst Hardy Plankton Sampler (LHPR). The sampler is effective in collecting large numbers of samples and performs best in waters where net clogging due to phytoplankton or jellyfish is not a problem, as an accurate measure of the volume of water filtered is essential for accurate biomass or animal concentrations to be made. The LHPR performs best when samples are taken in a horizontal or upward oblique direction. The LHPR can be towed at speeds up to 6 knots and can take a series of samples on a single haul for studies of vertical or horizontal distribution (Coombs *et al.* 1985, 1992; Conway and Williams 1986; Haury and Wiebe 1982; Wiebe 1970; Williams and Conway 1988). The original single net system (e.g. 280 μm mesh aperture) described by Longhurst *et al.* (1966) has been superseded by an improved twin net system (e.g. 53 μm and 200 μm mesh) described by Williams (1983). More recent improvements incorporated in the LHPR system used by the Plymouth Marine Laboratory include real-time data display and deck control of sample acquisition.

Essentially the system consists of a modified high speed net frame (2.5 m in length and 76 cm diameter) in which is fitted a 200 μm mesh aperture conical net terminating in a cod-end unit. In the cod-end unit two rolls of filtering gauze are advanced by an electric motor to give a sequential series of samples. Volume filtered for each sample is recorded from a flowmeter mounted in a conical nose-cone on the front of the sampler. The nose-cone inlet aperture can vary between 20 cm to 40 cm diameter, depending on plankton concentration, typically 35 cm diameter giving samples from about 20 m^3 of water filtered during a 2 min advance interval.

A similar, but smaller, finer net system (35 to 100 μm mesh aperture, typically 53 μm) with a separate cod-end unit can be mounted on top of the main sampler frame to give a concurrent series of fine mesh samples for studies of smaller organisms (e.g. copepod nauplii and copepodite stages). Flow into the fine mesh system is via a small conical nose cone (inlet diameter 3 to 7 cm, depending on plankton concentration) with flow rate monitored by a flowmeter in an inlet tube assembly between the inlet cone and short tubular filtration net (typically giving samples each from about 400 l of water filtered on a 2 min sample advance).

Advantages of the use of the LHPR system are the large number of samples that can be taken (up to 100 for each of the fine and coarse mesh systems), the wide size range of organisms that can be sampled by the twin net arrangement and the extensive flow validation data available for similarly designed high-speed samples with conical nose-cones. Disadvantages of the single net/multiple cod-end configuration include net hang-up/residence time and inadequate discrimination between consecutive samples (Haury 1973; Fasham *et al.* 1974; Haury *et al.* 1976). Improvements in the more recent systems, including optimum net design, correct choice of inlet cone aperture and sampling interval have minimized the problems of filtration efficiency; similarly the inclusion of blank gauze between consecutive samples has removed any confusion between adjacent samples. However, a degree of operator experience is still required for optimum sampling integrity. Checks on sampling validity are available both from double oblique sampling, where samples on the ascent and descent profiles are compared (Pipe *et al.*

1981) and by analysis of the filtering net residue after a haul in comparison with the catches of the cod-end samples.

Recent developments of the LHPR system include the addition of chlorophyl fluorescence and conductivity (salinity) sensor for real time display with the existing temperature, depth, flow and gauze advance signals. Transmission is via a single-cored cable enabling its use on a wide range of research vessels.

Autosampling and Recording Instrumented Environmental Sampling System (ARIES)
The Autosampling and Recording Instrumented Environmental Sampling System (ARIES) is a highly modified LHPR developed for concurrent physical, biological and chemical sampling at sea and this is done by collecting serial plankton and water samples (Dunn *et al.* 1993).

Mechanics of ARIES The main frame of the sampler is constructed from sea-water resistant aluminum scaffolding tube. The principal sampling components are all inside the main frame and protected from any accidental damage. A major advantage of this frame is that any damage can be easily repaired in the field. The open design of the sampler means that a large number of sensor packages can be carried and they are not isolated from the flow of water.

Cable telemetry is not used, allowing deployment from a wide variety of vessels. It is normally deployed on 11–14 mm wire rope at a towing speed of 4–5 knots (2–2.5 $m\,s^{-1}$). Real-time depth data is required for control and safe operation of the sampler, therefore a commercially available acoustic telemetry system is used to transmit these data to the towing vessel.

Plankton sampler In the ARIES system, the ribbon of mesh layers of the LHPR is replaced with a single belt carrying a series of individual cod-end bags, each bag being placed over the tail of the conical collecting net for a selected sampling period. The belt, feed spool and take-up spool are housed in a simple rectangular cassette, bolted to a fixed base plate. The cassette is removable as a complete unit to allow a fast turn around of the sampling system. The take-up spool is driven directly by a DC motor and gearbox mounted in an underwater housing located below the baseplate; 110 cod-end bags, of 250 μm mesh, are spaced along the length of the belt and held in place by Velcro strips. Indexing holes, positioned directly above each bag, ensure exact alignment of each cod-end with the tail of the collecting net. The holes are detected by an optical sensor attached to the front of the cassette, which signals the control unit to start or stop and drive the motor.

Water sampler The design of the water sampler is similar to that of a conventional rosette sampler. Sixty free-flooding tubes with hinged sealing lids are placed in a double ring around a cylindrical carousel. The lids of each tube are connected together internally by a length of silicone elastic. Prior to sampling the lids are held open, against the tension of the elastics, by integral pins slotted into pivoting levers. At the end of each sampling period, the levers at each end of the next tube in the carousel are simultaneously turned by a rotating arm, thus releasing the lids and closing the tube. The rotating arm is driven by a DC motor and high-reduction gearbox, mounted in an underwater housing at one end of the carousel. The 6° angle of rotation required to close each tube is controlled accurately by the main control unit and a shaft encoded, with 1° resolution, mounted on the rotating arm.

Electronics The control unit consists of a commercially available single-board microcontroller and a custom interface card. On-board application software was also developed in-house. In addition to controlling both samplers, the control unit digitizes data from an integral pressure sensor and flowmeter, mounted in the mouth of the plankton net. All data are stored in solid-state memory, together with the date and any time information and status flags, indicating sampler operations. The control unit is programed by a host computer subsequent to deployment. User-selectable plankton and water sampling rates between 1 and 60 min are available, whilst depth and flow data can be logged at intervals ranging from 1 s to 60 min.

Gulf III OCEAN sampler

This type of sampler uses a carousel containing a number of nets that rotate and take sequential samples. The Gulf III high speed sampler has been used since the 1950s with various modifications for the purpose of primarily sampling fish larvae in European waters. The most recent modification of this type of sampler is the OCEAN sampler (Dunn *et al.* 1993). The body of the sampler is based on the Dutch version of the Gulf III plankton sampler. It is made of HE30 aluminum with a reinforced tow-point and depressor attachment point. It incorporates three tail fins to provide good stability and the fins are fixed by shear bolts and secured with lanyards. Instead of single sampling net and cod-end, the internal frame incorporates four 250 μm nets each with a mouth diameter of 150 mm and a length of 150 cm, each with a detachable 68 μm cod-end. The nets are arranged in a circle and attached to a fixed circular disk. The nets are tensioned at the rear of the frame using shock-cord loops. Attached to the disk is an underwater housing, containing a DC electric motor and reduction gearbox positioned immediately in front of the stationary disk. The rotating disk has eight, rather than four, sequential indent positions, allowing the sampler to be closed completely whilst maneuvering to the next sampling depth. The mating faces of both disks are covered with bondable PTFE material to provide a low friction water-tight seal.

In order to maintain a flow-rate into the four small nets, comparable to the flow in a standard Gulf III, the sampler is fitted with a reducing nose-cone, which also houses the flowmeter. This nose-cone is coupled to the sampling hole in the rotating disk by a flexible hose to ensure an adequate, non-turbulent flow of water into each sampling net.

Electronics and telemetry The electronic control unit is mounted on top of the main sampler body. This is a commercially available acoustic telemetry system, modified for use with the OCEAN sampler. Data such as date, time, ship's position and sea-bed depth are processed by a personal computer and displayed graphically.

Gulf V plankton sampler

The Gulf V sampler, which is a modified Gulf III sampler (Gehringer 1952) is an effective sampler for ichthyoplankton and macrozooplankton (Nellen and Hempel 1969). It can be towed at high speed (5 knots) and it was shown to filter more water and catch more plankton than the Gulf III. The increased performance of the Gulf V was due to the removal of the case of the Gulf III. Added advantages of this sampler were a lower weight, ease of handling and a lower cost.

MULTIPLE NET SAMPLERS

The second group of samplers, the multiple net samplers, is based on the principle of opening and closing a series of individual plankton nets in succession.

There are a number of commonly used multiple net samplers, the Multiple Plankton Sampler (MPS) (Bé 1962), the HYDRO-BIOS Multinet (Weikert and John 1981), the RMT 1+8 (Baker *et al.* 1973), MOCNESS (Wiebe *et al.* 1985), and the BIONESS (Sameoto *et al.* 1980). All use square mouth-opening nets and come in a variety of sizes. The BIONESS, MOCNESS, and RMT 1+8M are towed horizontally or obliquely while the MPS can be towed horizontally, vertically or obliquely. All of the samplers are similarly effective in collecting mesozooplankton. When the same mesh was used in the BIONESS and MOCNESS nets there was little difference in the biomass of mesozooplankton collected by the two different samplers. The BIONESS is generally towed at a speed of 3–4 knots and the MOCNESS at 1.5–2 kn. There was some indication that the higher speed of the BIONESS may increase the pressure inside the nets and result in more extrusion of zooplankton through the mesh of the nets than occurs in the slower MOCNESS. The BIONESS was more efficient in capturing the larger forms of zooplankton such as shrimp, krill, and juvenile fish, and this is attributed to the higher towing speed.

It is recommended that when using multi-net samplers a calibrated flowmeter be used, preferably in the mouth of the frame of the sampler. If this is not possible a flowmeter on the frame outside the net should be used. When no flowmeter is available the volume of water filtered can be estimated using the ship's speed and the accurate time of opening and closing each of the nets. If the nets are not clogged it can be assumed that approximately 85%–90% of the water in front of the net will be filtered. These multiple net samplers generally perform best when towed obliquely in an upward direction.

BIONESS

The BIONESS is a multiple net (10 nets) opening and closing zooplankton sampler that is made in two sizes, with either 1 m^2 or 0.25 m^2 mouth-opening nets. The 10 nets of the BIONESS are stacked horizontally one behind the other and open sequentially with one net opening as one is closed. The horizontal stacking reduces the frontal area of the frame to a minimum thereby reducing sampler visibility and the frontal pressure wave of the sampler. Each of the 10 horizontal dropping bars is lead filled and weighs approximately 25 kg. The entire sampler has a weight in air of approximately 800 kg. The nets and frame of the BIONESS are dyed and painted a dark gray color to reduce visibility. The BIONESS can be towed from a conductor cable, allowing continuous communication with the sampler, or from a non-conducting cable with the data stored in the BIONESS computer and retrieved when the sampler is on the deck of the ship. A cable with a minimum breaking strength of 6000 kg and a winch with a pulling load of at least 3000 kg is recommended.

The filtration area to mouth area ratio of the nets is normally 10:1 resulting in a filtration efficiency of close to 90% for a clean net towed at 1.5 m s^{-1}. The normal towing speed is 1.5 m s^{-1} but the BIONESS can be towed safely up to speeds of 3.0 m s^{-1}. However, there will be a drop in filtration efficiency at the higher speeds unless the R ratio of the nets is increased. The filtration efficiency is measured by comparing the difference in the flow between a flowmeter in the mouth of the net and the flow of a meter mounted on the outside of the BIONESS frame. The mouth angle of the BIONESS when towed at 1.5 m s^{-1} is near 0° from the vertical but it increases to about 15° from the vertical at speeds near 2.5 m s^{-1}.

The BIONESS may be towed at speeds as low as 0.5 m s^{-1}, but this may result in the zooplankton being captured only in the front part of the nets, making it difficult to wash the animals out of the net. A minimum speed of 1 m s^{-1} is recommended with the optimum speed being 1.5 m s^{-1}. Extrusion of zooplankton through the mesh of the nets increases with increasing speed, therefore the advantages of high speed (i.e. > 1.5 m s^{-1}) may be outweighed by the loss of the smaller zooplankton through the mesh.

Information obtained during a tow includes temperature, salinity, depth, speed through the water, pitch angle of the sampler, flow through the net, filtration efficiency, and net count indicating which of the 10 nets is open. An Optical Plankton Counter (OPC) fluorometer, and video camera can easily be added to the BIONESS. When the BIONESS is brought on to the ship's deck after a tow, the zooplankton are located near the cod-end of the net and require only a small amount of washing to collect the zooplankton in the cod-end bucket. However, if the BIONESS is held in the wash of the ship's propeller for any length of time during retrieval, the collected animals will be distributed throughout the net and this will require more washing of the nets to recollect the sample in the cod-end buckets. The buckets should all be numbered in sequence, as should the nets, to prevent confusion as to which net is associated with which bucket. The contents of each of the buckets are poured into numbered pails and processed during the next tow.

The handling methods of the BIONESS during launch and retrieval from the water will vary according to the capability of the ship's equipment. It is important that the ship be moving forward during both of these operations. The BIONESS is most easily handled with the use of an A-frame or large crane from the stern, but side towing is possible with a large crane. Because of the compact design of the BIONESS it can safely be handled in rough seas and moderately high winds.

LOCHNESS sampler

The LOCHNESS (Dunn *et al.* 1993b) is basically a large BIONESS sampler designed to capture fish larvae. The frame is 3 m high, 3 m wide and 2 m deep and houses five nets of 2 mm mesh each with a mouth opening of 2.3 m^2. The sampler is designed to be towed straight level at 4 kn (2 m s^{-1}). The control unit, motor housing, flowmeters and topside data processing system are identical to that used by the OCEAN sampler.

Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS)

The Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) is a family of net systems based on the Tucker Trawl principle. There are currently eight different versions of MOCNESS designed for capture of different size ranges of zooplankton and micronekton (Table 3.2; Wiebe *et al.* 1985). Each is designated according to the size of the net mouth opening and in two cases the number of nets it carries. The original (Wiebe *et al.* 1976) was a redesigned and improved version of a system described by Frost and McCrone (1974).

The MOCNESS-1/4 and the Double MOCNESS-1/4D carry nine and eighteen 1/4 m^2 nets respectively usually of 64 μm mesh and have been used to sample the larger microzooplankton. The MOCNESS-1 (Wiebe *et. al.* 1976) and the Double MOCNESS-1D carry nine and twenty 1 m^2 nets respectively usually of 335 μm mesh and are intended for macrozooplankton sampling. There are four mid-water systems: the MOCNESS-2 (with 2 m^2 nets), the MOCNESS-4 (with 4 m^2 nets), the MOCNESS-10 (with 10 m^2 nets) and the MOCNESS-20 (with 20 m^2 nets). These systems typically carry five or six nets of 3.0 mm circular mesh; however, the MOCNESS-2 was equipped with 505 μm mesh nets, and the MOCNESS-10 has been used with ten nets. All nets are dyed dark blue or black to reduce contrast with the background.

All MOCNESS systems use the same underwater and shipboard electronics. The nets are opened and closed sequentially by commands through a single conductor armored cable from the surface. The electronics has 16-bits of resolution and the basic data

Table 3.2 MOCNESS system dimensions and weights (Wiebe *et al.* 1985).

System	No. of nets	Width of frame	Height of frame	Net width	Mouth area @ 45° towing angle	Length of net	Approx. weight in air	Recommended wire diameter
MOCNESS-1/4	9	0.838 m	1.430 m	0.50 m	0.5 m^2	6.00 m	70 kg	6.4 mm
MOCNESS-1/4-Double	18/20	1.430 m	1.430 m	0.50 m	0.5 m^2	6.00 m	155 kg	7.4 mm
MOCNESS-1	9	1.240 m	2.870 m	1.00 m	1.0 m^2	6.00 m	150 kg	7.4 mm
MOCNESS-1-Double	18/20	2.560 m	2.870 m	1.00 m	1.0 m^2	6.00 m	320 kg	12.1 mm
MOCNESS-2	9	1.650 m	3.150 m	1.41 m	2.0 m^2	6.00 m	210 kg	11.8 mm
MOCNESS-4	6	2.140 m	4.080 m	2.00 m	4.0 m^2	8.44 m	460 kg	11.8 mm
MOCNESS-10	6	3.410 m	4.690 m	3.17 m	10.0 m^2	18.25 m	640 kg	11.8 mm
MOCNESS-20	6	5.500 m	7.300 m	4.47 m	20.0 m^2	14.50 m	940 kg	17.3 mm

stream consists of temperature, depth, conductivity, frame angle, flow counts, net number, and net response. An acquisition/controller computer retrieves data from the underwater unit at a rate of up to 2 times per second. Temperature (to approximately 0.01 °C) and conductivity are measured with SEABIRD sensors. A modified TSK-flowmeter (Tsurumi-Sikie-Kosakusho Co. Ltd; see Longhurst *et al.* 1966 for a description of the flowmeter modification) is normally used to measure flow past the net. A model 2031 General Oceanics flowmeter has been used less frequently. Both the temperature and salinity sensors and the flowmeter are attached to brackets which are mounted on the top portion of the frame so that they face directly into the flow when the frame is at a towing angle of 45 °. An electronic pendulum angle transducer (Humphrey) measures the angle of the towed net through the water. A GPS unit providing latitude and longitude can be integrated into the data stream. The electronics and mechanical frame can be modified to accommodate optional sensors, for example transmissometer, fluorometer, submarine photometer, and bottom finding transducer (altimeter). Furthermore, acoustical and video (Davis and Gallager 1993) systems have been adapted for use on MOCNESS-1.

The MOCNESS flowmeter should be calibrated before and after each cruise. This can be done in a flow-tank or in the field by mounting the flowmeter(s) on a frame that can be towed over a measured distance. For field calibrations, paired runs over the measured distance should be made in opposite directions and averaged to eliminate errors introduced by naturally occurring water movements.

A microcomputer (together with disk drive and printer) are the deck unit and permit shipboard real-time data acquisition and processing as well as net control. Salinity (to approximately 0.01%), net oblique velocity and vertical velocity, and volume filtered by each net is calculated after each string of data has been received by the computer. Raw and processed data are stored on disk (in separate files) and processed data can be printed out. Plots of net depth versus time, temperature and salinity versus depth, temperature versus salinity, and latitude versus longitude are made during a tow and displayed on the computer screen.

A motor/toggle release assembly is mounted on the top portion of the frame and stainless-steel cables with swaged fittings are used to attach the net bars to the toggle release. A stepping motor in a pressure-compensated case filled with oil turns the escapement crankshaft of the toggle release that sequentially releases the nets to an open then closed position on command from the surface.

All MOCNESS systems incorporate the same basic design, with the nets, the underwater electronics package, the environmental and net monitoring sensors, and electro/mechanical net release mechanism mounted on a rigid frame, and many of the components are interchangeable.

The MOCNESS systems are designed to be towed at a 45° angle which is usually obtained with a ship speed of approximately 2 kn when difference in vertical current shears are minimal. Higher angles indicate higher net speed and vice versa. An algorithm for the calculation of net speed is given in Wiebe *et al.* (1985). Current practice is to 'fly' the system so that it is moving through the water at 2 ± 0.5 kn. If net speed drops below 1.5 kn, ship speed should be increased by 1/2 to 2 kn. Although not precisely equivalent, the net can also be flown by maintaining the net angle between 55° and 35°. Both speed and angle should be monitored closely because most of the complications to tows have occurred during excessively low or high speeds.

Any single conducting armored cable (where the conductor is used to transmit the signal and the armor is used as the ground return) will serve for the sending of data between the underwater and deck units. The wire must be strong enough to withstand any realistically conceivable tension which might be experienced under tow. With the MOCNESS-1, wire tension on 0 to 1000 m tows of approximately 3000 lb (1360 kg) have been seen. Normal practice is to insist on a safety factor of at least 2 and preferably 3 in the breaking strength of the wire (e.g. 6000–9000 lb or 2730–4090 kg). For the larger MOCNESS-1D, -2, -10, and -20 trawls, a heavier conducting cable is required. A 0.68 inch (17.3 mm) diameter cable with a breaking strength more than 40 000 lb (18 200 kg) for these systems has been typically used in the USA. However, wire breaking strength is not the only factor that should be considered in choosing a wire diameter.

Multinet sampler

The Bé Multiple Plankton Sampler (Bé *et al.* 1959) and its improved HYDRO-BIOS multinet (Weikert and John 1981) are square-mouth samplers. The HYDRO-BIOS sampler contains five nets (0.25 m^2) that are closed on command from the deck via a conductor cable or by pressure release mechanisms that are preset to activate at predetermined depths. The sampler has three tow bridles in the mouth of the sampler which results in some degree of avoidance of the sampler by the larger mesozooplankton and macrozooplankton. This is a useful sampler for taking stratified depth samples of mesozooplankton during a vertical tow. Samplers such as the BIONESS, MOCNESS and RMT 1 + 8 are designed for horizontal or oblique towing.

RMT 1+8

The RMT 1 + 8 was the first opening and closing rectangular trawl to be widely used (Clarke 1969 and Baker *et al.* 1973). The RMT 1 + 8 was modified to have three 1 m^2 nets with a mesh size of 0.32 mm and three 8 m^2 mouth area nets with a mesh of 4.5 mm so that stratified sequential samples could be taken (Roe and Shale 1979). The different sized mesh nets allowed the capture of mesozooplankton, macrozooplankton and micronekton during the same tow resulting in considerable saving in ship's time.

Signals are sent to the sampler by acoustic communication to open and close the nets as desired. The RMT 1 + 8 and the multiple RMT mouth angles are sensitive to the towing speed, the greater the speed the greater the angle. At a speed of 2 kn the angle of the RMT 1 + 8 was 61° and 42° for the multiple RMT. The multiple RMT has successfully sampled to depths of 4500 m, deeper than most conductor cable systems. These depths were attained primarily because of the use of wire rope rather than expensive conductor cables, and because of the acoustic control.

3.3 FACTORS INFLUENCING MESOZOOPLANKTON SAMPLES

The variety of kinds of sampling devices for zooplankton is in part a reflection of the problems inherent with any particular sampler design. Factors which have been the subject of many studies include avoidance of the sampler by the organisms, clogging of the net meshes, and extrusion of animals through the mesh (escapement). These subjects and related topics are reviewed in this section.

3.3.1 Extrusion of zooplankton from nets

Animals which are captured by a net during the course of hauling it through the water can 'escape' the net by passing through the open area of netting mesh. In some cases, escapement is simply a case of individuals being smaller than the diameter of mesh opening. However, it is possible that water pressure associated with flow through the mesh will force or extrude an individual larger than the mesh opening through, thus enhancing escapement. The speed of the net directly influences the water pressure within the net and therefore the amount of animal extrusion through the mesh. Generally it is recommended that vertically towed nets be towed at speeds of $1\,\mathrm{m\,s^{-1}}$ or slower when sampling for mesozooplankton.

A fundamental parameter governing the performance of a net is R, the ratio of open area of net mesh to the area of the mouth opening of the net. The open area of a net mesh is determined by the total area of net mesh forming the net, α, and its porosity, $\boldsymbol{\beta}$, which is defined as the open area fraction of the mesh size. Thus:

$$R = \frac{\alpha \times \boldsymbol{\beta}}{A} \qquad (3.1)$$

Porosity values may be obtained from gauze manufacturers. Table 3.3 provides an example of the porosity of some selected mesh sizes taken from the Tetko Inc. General Catalogue No. 2000 for Nitex Swiss Nylon Monofilament. For a given net mesh opening, there can be more than one thread diameter. Generally as thread diameter increases, the strength of the netting increases, but the mesh porosity is reduced and more total mesh is required to achieve the same open mesh area to mouth opening ratio (R).

The 1968 joint working group (Tranter 1968) recommended that the WP-2 ring net has a filtration ratio of mesh area to mouth area of 6:1. This ratio is sufficient for nets of $>200\,\mu$m mesh vertically towed in waters that are not rich in phytoplankton. Sampling in phytoplankton bloom conditions will make it necessary to increase the R ratio or shorten the duration of the tow to overcome the clogging problem (additional information is provided in section 3.3.2). The R ratio for oblique and horizontally towed nets at speeds of about $1.5\,\mathrm{m\,s^{-1}}$ should be increased to at least 10:1 to compensate for the greater flow rate at these speeds and to reduce internal water pressure in the net.

The problem of zooplankton extrusion through the mesh of the net was examined by Nichols and Thompson (1991). They towed a series of high speed (9–$10\,\mathrm{m\,s^{-1}}$) nets of 61, 90, 124, 190, and 270 μm mesh sizes and an open net area to the mouth area of the nose cone ratio of between 23 and 40:1. They described a mathematical model that showed a mesh size of 75% of the copepod carapace width would capture about 95% of the animals present. They reported significant loss of *Calanus* copepodite stages 1 and 2 through mesh size 190 μm, with only about half of the stage 1 copepodites captured by this mesh. A 124 μm mesh was needed to capture all of the stage 1 copepodites of *Calanus* spp. The equation relating the number of copepods per $\mathrm{m^{-3}}$ retained by the net

Table 3.3 Porosity of Nitex mesh.

Product Number	Mesh opening (μm)	Open area as % of total area
HC 3-500	500	49
HC 3-400	400	47
HC 3-300	300	50
HC 3-202	202	47
HC 3-150	150	51

(N_{caught}) as a function of the total number per m^{-3} available to capture (N_{total}) to copepod width/mesh size ratio (R) was

$$\frac{N_{caught(i)}}{N_{total(j)}} = \frac{1}{1 + \exp[-8.9(R - 1.0)]} \tag{3.2}$$

or

$$R = 1 + \frac{\ln\left(\dfrac{N_{caught(i)}}{N_{total(j)}} - 1\right)}{-8.9} \tag{3.3}$$

To adequately sample early copepodite stages of *Pseudocalanus* and *Paracalanus* species required mesh sizes of 61 and 35 μm respectively. This study applied only to high speed nets that generate high internal water pressures in the nets increasing the amount of copepod extrusion. Conventional vertically towed nets at speeds of 1 m s^{-1} may or may not produce the same degree of copepod extrusion through a similar size mesh. It is recommended that the above mesh sizes be used as a guide for sampling at the lower speeds commonly used for vertical and oblique sampling.

Any obstruction in front of the net mouth may lead to avoidance of the sampler by the larger forms of zooplankton and may also create problems in recording an accurate water flow through the net (Tranter 1968). It is recommended that nets without frontal obstructions be used when sampling larger forms of mesozooplankton (> 5 mm). There are no published data dealing with the influence of net-bridles on catch rates of mesozooplankton, however, whenever possible it is best to use samplers without bridles.

Inaccurate measurement of water flow through the net is an important source of error in estimating concentrations of zooplankton, therefore it is important that flowmeters mounted in the mouth of the net be used if the sampler design will allow. The calibration of the flowmeter is a critical component in an accurate measure of flow. Many commercial meters are supplied with calibration tables or curves, however, through usage the calibration of a meter will change due to wear and therefore meters should be calibrated before each sampling program. Calibration after a sampling program is a risky procedure because of the possibility of net loss during the program, leaving the researcher with volume values that cannot be verified by calibration.

Calibration of the meters is best done in a tow tank through which the meters are towed at calibrated speeds and times to measure distance. If a tow tank is not available meters can be calibrated by towing them vertically on an open frame, without nets, over a known depth and speed. Towing the meters horizontally over a measured distance also can be used to calibrate meters at various speeds. Care must be taken to measure the speed of the vessel through the water and not over the bottom.

There is no convincing evidence that increasing the size of the mouth area of zooplankton nets increases the efficiency of the sampler (Pearcy 1983). The concentrations of animals per volume of water have not been shown to increase with larger mouth areas for the same type of net and mesh size. The larger mouth area only provides a larger volume of filtered water increasing the likelihood that rare animals will be caught. A 0.75 m diameter net is adequate for the common species of mesozooplankton in northern and temperate coastal zones. In oligotrophic waters the size of the net mouth area may have to be increased to filter larger volumes of water to collect a reasonable sample size of animals.

3.3.2 Clogging of net mesh

'Four places of decimals in a computed coefficient can hardly offer compensation for an error so fundamental as the variation in the straining capacity of the net' (Kofoid 1897). The design of a plankton net is a critical element in effective quantitative sampling. The amount of water that can be efficiently filtered depends upon net shape, mesh size, mesh area, netting porosity, filtering area, and the mesh area to mouth opening ratio. Smith, Counts, and Clutter (1968) conducted an extensive series of instrumented net tows using nets of different shapes (cone, cylinder-cone, and cylinder) and mesh sizes in what they defined as coastal or green water and oceanic or blue water. They measured percent efficiency of each net by using a flowmeter mounted in the net mouth and one mounted outside. They considered a net to begin to have significant clogging problems if the filtration efficiency during a tow fell below 85%. Smith, Counts and Clutter (1968) found that the clogging rate of a net was affected by four factors:

1) The composition and density of suspended material in the water. Coastal waters with generally higher particle loading than oceanic waters will cause clogging to occur more rapidly.
2) The mesh size – the smaller the mesh size, the faster the net clogged.
3) The ratio of filtering area to mouth area – the smaller the ratio the faster the clogging.
4) The form of the net – a cylinder cone resisted clogging the best, closely followed by the cylinder net.

There are important sampling implications which result from progressive net clogging since the water column will not be sampled uniformly. As the pressure difference between the inside and outside of the net increases, more organisms will be extruded through the mesh. Water will be pushed out of the way of the net and the disturbance (a bow wave) will provide a cue that could trigger an avoidance response by the animals in front of the net.

The results of this study provided a basis for two equations that are particularly useful in the design of nets.

$$\mathbf{Log_{10}(\mathit{R}) = 0.38 * Log_{10}(\mathit{V}/\mathit{A}) - 0.17} \qquad \textbf{Green Water} \qquad (3.4)$$

$$\mathbf{Log_{10}(\mathit{R}) = 0.37 * Log_{10}(\mathit{V}/\mathit{A}) - 0.49} \qquad \textbf{Blue Water} \qquad (3.5)$$

where R = filtering area/mouth area, A = mouth area (m^2), and V = volume of water to be filtered (m^3). These equations enable an investigator to develop a net design to meet the conditions that are likely to be met during the course of a study. Given a volume of water that needs to be filtered to catch sufficient individuals to provide a statistically valid sample and the mouth size of the net system, the mesh area required to prevent clogging can be computed.

3.3.3 Avoidance

Avoidance, the active swimming of zooplankton out of the capture path of a net, is the most serious bias affecting the catch of the larger meso- and macrozooplankton. While there have been numerous field, laboratory, and theoretical studies concerning avoidance effects, few solutions that effectively eliminate the problem exist.

The effects of avoidance are net-size dependent. McGowan and Fraundorf (1966) studied the relationship between the size of net used and estimates of zooplankton diversity. They took a series of net tows in an area off Baja, California using nets of 20, 40, 50, 80, 100, and 140 cm diameter. The tows were taken obliquely and the length of tow was regulated so that each net tow filtered the same amount of water. Two night series and one day series of tows were taken and the nets were used in random order to minimize the effects of patchiness. A total of 140 species of mollusks, euphausiids, larval fish, and larval squid were counted. The ability to catch species was $140 > 100 = 80 = 40 > 60 > 20$ cm diameter net. One conclusion was that the various species can avoid little nets better than large ones. When ordering the nets according to their ability to estimate abundances, the result was $140 > 100 > 80 > 60 > 40 > 20$. On a per unit volume, the larger the net, the larger the catch. By using the larger net, the avoidance error in this study was reduced. However, although the 140 cm net did the best job, it only took an average of 54.8 species/tow and cumulatively only captured 99 of the species out of the 140 caught by all of the tows. Thus, even the best net in this experiment seriously underestimated the number of species present in the area.

Fleminger and Clutter (1965) did an elaborate tank experiment to examine the effects of net size and lighting conditions on the avoidance of towed nets by a mysid and six copepod species. Net sizes used were 43, 32, and 22 cm diameter and they were towed about 30 cm s^{-1} through the tank in full light, reduced light, and darkness. The smaller nets caught significantly fewer mysids, changes in light resulted in significantly more caught in darkness, and avoidance tended to be less when population numbers were higher. For the copepods, smaller nets caught fewer individuals, but the level of avoidance differed among the species; changes in light had no effect on the abundance estimates; and avoidance tended to be less in denser populations. The differences in avoidance observed between these two taxa were ascribed to differences in visual acuity, mysids could 'see' in the lit medium, and tended to shun foreign objects and to aggregate more.

The theoretical aspects of zooplankton avoidance have been examined by Barkley (1964, 1972). In order to avoid a net, the required individual escape velocity increases proportionately with an increase in either towing speed or net radius and decreases in proportion to increases in the reaction distance or the initial offset of the individual from the center of the net mouth. Because minimum escape velocities decrease rapidly as the reaction distance decreases below optimum values, it is quite inefficient to reduce the net opening to low values. Thus, in the model runs and assumptions specified by Barkley, a net with a 300 cm radius was several times more effective than a 50 cm net. The basic problem that still exists for most zooplankton is that both the reaction distance to an approaching net and the escape velocities of individuals are poorly known. Wiebe *et. al.* (1982) applied the Barkley theory to a series of collections made with a 1 m^2 and a 10 m^2 MOCNESS from which an euphausiid, *Nematoscelis megalops* was counted and sized. There was significant differential day/night avoidance of both net systems, but there was no difference in estimates of catch rate between the nets. The results indicated that increasing the mouth area by a factor of 10 did not effectively reduce the avoidance of the net because the individuals apparently began their avoidance reaction further in front of the larger net. One conclusion of this study was that since vision was the likely sensory system used by *N. megalops* to detect the net approach, active measures to reduce net detection were needed to reduce the avoidance effect as described below in Effect of ambient light, and Mesh and frame color.

EFFECT OF AMBIENT LIGHT

The level of ambient light influences the degree of net avoidance by the larger forms of macrozooplankton (Sameoto *et al.* 1980; Wiebe *et al.* 1982; Sameoto 1983) and fish larvae (Heath and Dunn 1990) as evidenced by larger numbers of these animals captured per unit volume of water at night. There is no evidence that avoidance of samplers by copepods is affected by ambient light. When possible it is best to sample mesozooplankton at night, because this sampling will also provide a better estimate of macrozooplankton as well as an equally valid estimate of mesozooplankton in the same sample. It is important that the ship's deck lights are turned off when sampling at night. The bright lights from the ship will make the net visible to the macrozooplankton and at the same time may attract some species and repel others from the vicinity of the ship which will result in biased estimates of these species.

A study of the effect of artificial light on reducing net avoidance by euphausiids during daylight demonstrated an increase in catch of euphausiids of between 10–20 times that obtained when no light was used. It is believed that the light has a blinding effect on the euphausiids making it less likely that the animals will see the net. This effect was reduced at night to an increase of about 2–3 times as many caught with the light on, compared to no light (Sameoto *et al.* 1993). The light used in the experiments was a 125 W video light pointing straight ahead of the sampler. Recent studies using a flashing strobe light in the same manner as the above experiment showed a strobe light flashing at 10 s intervals had the same effect in reducing avoidance as the continuously shining video light (Sameoto, unpublished data).

MESH AND FRAME COLOR

The recommended mesh material for mesozooplankton nets is Nylon Nytal 7 P, with a mesh aperture width of 200 μm. Especially for sampling larger organisms (macrozooplankton), it is recommended to avoid bright colored nets. The color of the mesh should be one that makes the sampler less visible in the water such as a dull green, blue or gray, white nets should be avoided. The frame of the sampler should also be a dull dark color similar to the net with the bright metal rings or frames of the sampler painted to reduce light reflection. These color recommendations will help reduce macrozooplankton avoidance of the net.

3.4 HANDLING TOWED SAMPLERS

Precision towing of multiple net systems requires reasonably fine control of winch speed, especially in the range of 1–30 m min^{-1}. The net system is usually paid out at 30–40 m min^{-1}; occasionally when angle and speed are optimized and the system is well below the surface, a rate of 50 m min^{-1} may be used. Hauling speeds are generally between 10–20 m min^{-1}, although on shallow tows with finely spaced strata, a rate as low as 1 m min^{-1} may be required to evenly sample a stratum and at the same time filter adequate amounts of water. Under windy conditions the ship should steam into the wind during a haul. When winds exceed 10 kn, there is sufficient wind set so that the towing course should be chosen to put the wind and swell on the side of the bow which corresponds to the side of the ship where the net is to be launched and recovered. This should keep the wire out from under the ship. It will also give the bridge some advantage in keeping the ship moving ahead at a slow speed if under calm conditions the ship has trouble reducing its speed to that optimal for towing.

Under calm sea surface conditions, there is no preferred towing point, from the side or stern are equally good. In high winds or heavy swell and a ship's course into the wind and sea, towing from the side has the advantage of minimizing the effect of ship pitch on the wire and net. Severe pitch can seriously affect the quality of the catch (jerking of the nets up and down can damage the organisms) as well as stress the nets and frame (causing net blowouts) and damage the cod-end buckets. Towing from the ship's pitch pivot point will minimize this effect.

The handling of heavy samplers in a rough sea is dangerous and there is a high risk of losing the gear due to tow cable breakage, but there are ways of reducing this. The best means of reducing the sudden high peak load on the tow cable due to waves and swells causing the upward acceleration of the ship is to use a constant tension winch. Mitchell and Dessureault (1992) described a control unit with a pressure relief valve inserted in the hydraulic circuit of a winch that maintained a constant tension on a towing cable. This unit showed significant reduction in the cable tension peaks and marked improvement in the towed behavior of the BIONESS. A reduction in the total cable tension by a factor of 4 to 5 occurred when the pressure relief valve was used. Without the tension compensation there was significant relationship between the movement of the ship and the motion of the sampler, but no relationship existed when the winch was compensating for the ship's vertical motion. This not only makes the operation safer but it also provided better depth accuracy and control when sampling specific depth strata. The use of the controller made the towing, launching and retrieval of the BIONESS and other heavy gear much safer and easier in rough seas.

A less expensive but less effective method of keeping a load on the towing cable when the ship is pitching is to use a bungy cord attached to a roller block on the cable. The bungy cord applies a load to the cable at 90° from the cable. When the cable slackens in a swell the slack is taken up by the stretched bungy cord thereby reducing the snap in the cable when the tension returns to the cable and thereby reducing the likelihood of the cable parting. This method of loading the cable is not as effective as the constant tension winch in protecting against cable breakage.

The relationship between wire diameter and meters of wire out to get the net system to a given depth is also an important consideration. At any given tow speed, a given diameter cable will have an inherent angle which depends on its weight per unit length and its drag (a function of surface area). As a general rule, the larger the diameter cable, the larger the ratio of weight per unit length to drag and therefore the steeper the inherent angle at a given speed. Larger cable usually permits less wire to be paid out to get to a given depth and therefore cuts the time to shoot a net to depth. Larger cable also seems to tow straighter (with less catenary) thus reducing stalling of the net when hauling in.

When is it too rough to tow? The decision not to tow will depend on the stability of the ship in rough seas, the flexibility in adjusting the towing point between the side of the ship and the stern, and the ease of launch and recovery from the deck. MOCNESS systems have been towed in winds up to 40 kn and seas of 8–15 ft (2.4–4.6 m), but the collections under these conditions have not always been of the best quality. Perhaps the best advice is to be conservative until enough experience has been gained to judge the feasibility of towing under marginal conditions. This decision should be made with the safety of personnel during launch and recovery foremost in mind. While handling experience and ship's capability play a large part in the decision, personnel safety should always be the dominant factor.

3.5 CARE OF TOWING CABLES

It is important the all-metal towing cables whether conductor or wire rope type be properly cleaned and lubricated. The frequency of this maintenance will depend on the frequency and the conditions of use. Operations in tropical waters require much more frequent cable maintenance than those in colder regions due to the higher rate of corrosion at warmer temperatures.

3.6 HANDLING SAMPLES AND SAMPLE PRESERVATION

Quantitative work starts with careful rinsing of the plankton net, using an appropriate flow of sea water from the outside part of the gear, washing plankton quantitatively from the net material and concentrating it in the cod-end. The water jet should remove the organisms from net material but not damage them. Windows of the cod-end should be equipped with gauze of the same aperture as the net. Balachandran (1974) proposed closed plankton buckets, without side or bottom windows, especially to keep fragile organisms of tropical waters in good conditions. Closing nets like WP-2 net (UNESCO, 1968) should be rinsed only at the lower part after stratified sampling. Otherwise organisms from the abundant near surface plankton could stick in the part above the closing rope and contaminate the sample. This has to be taken into account especially for vertical stratified taxonomic studies. Finally the cod-end will be screwed off to pass the specimen on into a jar or in a bucket for further treatment. The use of filtered sea water for concentrating and passing the material on is essential to avoid any contamination. Take care that sampling containers or buckets are properly marked to avoid mistakes, especially if several catches will be performed at the same location.

Nets should be kept wet or damp between stations to avoid successive clogging of meshes by dried organic matter. The net material should always be checked to be sure not to use damaged gauze. Nets should be washed with a dilute alkali solution (detergent) after cruises, then rinsed with fresh water, dried and stored in bags.

It is necessary to know the amount of filtered sea water for calculation of abundance or of biomass concentration. The use of a calibrated flow-meter is best for that purpose. Calibration must be done at a speed which is in the range of the towing velocity of the gear, for example at 45 m min^{-1}. The variability of the mechanical TSK (Tsurumi-Seiki-Kosakusho Co., Ltd, Yokohama) flowmeter was about 4% during calibration conditions (Postel 1990). Be sure that the materials of the flowmeter do not react to temperature. Teflon-made instruments alter their revolution properties at low temperatures. The flowmeter should be mounted in that part of the net entrance where flow properties are optimal, for example in the center or in a quarter of the diameter. The latter version is needed because bridles in the center of the net opening area interfere with the flow (Tranter 1968). The revolutions correspond to a length of the water column (in meters) which has passed the net. The calibration factor determines the ratio of revolutions per meter. The amount of filtered water (m^3) is finally the product of flowmeter revolutions, calibration factor and net opening area (m^2). If the construction of the gear allows attachment of a second flowmeter outside the net, the ratio between both indicates the filtration efficiency. Samples from tows of less than 70% efficiency should be neglected (UNESCO, 1968). Electronic instruments permit on-line registrations through conductivity cable and allow stopping the tow in the worst conditions, or off-line readings by the memory probe for later consideration.

The estimation of the filtered water column without a flowmeter is generally not recommended. The use of the length of the released wire, multiplied by the net opening area, is restricted to vertical hauls. The calculation 'time × speed × net opening area' allows estimations independent of the towing direction (vertical, horizontal, oblique). The proper functioning of the flowmeter could be tested in calm weather, either by measuring the released wire versus revolution and given towing velocity, or by measuring the horizontal distance and ship speed if the ship is equipped with a precise navigation system, i.e. GPS (Geo-Positioning System). The reduction of the amount of filtered water by clogging effects in eutrophic waters on one hand or the influence of drifting ships during vertical towing on the other, cannot be considered without flowmeter. Particularly when sampling deeper levels, a drifting ship continues the filtration process, even when the winch is stopped for the time a messenger takes to reach a mechanical releasing mechanism. The ship drift depends on the size of ship and is proportional to the wind velocity. The effect became dominant in comparison to clogging (in shelf areas), at wind velocities larger than $6\,\mathrm{m\,s^{-1}}$ (wind force 4). It was assessed on R/V *A.V. Humboldt*, 1200 gross tons. The influence increased drastically at wind velocities of $12\,\mathrm{m\,s^{-1}}$ (wind force 6), especially if the closing depth of a vertical operating net (WP-2) was deeper than 50 m. The relationship

$$y = 0.64x_1 + 0.05x_2 - 3.9 \tag{3.5}$$

was statistically significant for x_1 and allowed an approximate correction of the underestimated amount of filtered water y ($\mathrm{m^3}$) from wind velocity x_1 ($\mathrm{m\,s^{-1}}$) and closing depth of the net x_2 (m).

Wire angles are another problem of drifting boats. The gear does not reach the intended depth for vertical hauls if no depth recorder is operating on-line, i.e. the depth is measured by the meter wheel only. In that case the wire angle correction must be performed by trigonometric rules. Then the final wire length z_f is the intended depth z divided by the cosine of the wire angle a, which is assessed by a clinometer, for example, to reach 200 m depth at a wire angle of 15°,

$$z_f = 200/\cos 15$$

$$z_f = 207\ \mathrm{m} \tag{3.6}$$

Important sources of error in the collection of zooplankton samples can occur after the net is retrieved from the water and taken on deck. It is important to wash the net properly so that all the contents are moved into the cod-end. When washing the net it is best to raise the net vertically and hold it in this position while washing with seawater from the outside of the net. Care should be taken not to get the washing water in the mouth of the net since this can introduce organisms from the washing hose and contaminate the sample. Special care should be given to the cod-end after the plankton bucket is removed to ensure that no animals remain in the seams of the net near the cod-end.

The type of preservative used to fix the zooplankton will depend on the purpose for which the samples were taken. An in-depth discussion of the various techniques for zooplankton fixation and preservation is given in *Zooplankton fixation and preservation* edited by Steedman (1976a). In general, a seawater formalin solution containing about 4% formaldehyde buffered with sodium borate and strontium chloride is recommended when animals are collected for taxonomic purposes. For long term preservation of calcareous shelled zooplankton, it is very important that the pH of the preserving fluid be maintained at about 8.2. With too little buffering, the calcareous shells will dissolve and with too much buffering the shell-binding protein may soften (Steedman, 1976b). Frequent monitoring and buffering of the pH of the preserving fluid should be done during the first days after the initial preservation of a sample and every few weeks thereafter for a period of 3 to 6 months (Turner 1976). It should be remembered that formaldehyde is a carcinogen and should be handled accordingly.

3.7 COLLECTION OF LIVE ZOOPLANKTON FOR EXPERIMENTAL STUDIES

Zooplankton for laboratory experiments must be collected with great care. Sampling procedures are designed to minimize physiological stress and physical damage to the organism during capture and during treatment immediately after the animals are brought on deck. In general, sampling protocols call for the capture and transfer of organisms to be performed quickly and gently. Direct sunlight or bright deck lights are avoided during transfer operations. Special care is taken to ensure that the seawater for reception of the animals is at ambient temperature and salinity and is free of contaminants that may be encountered on ships. One net system that has been widely used to collect animals for 'live work' was described by Reeve (1981). A net similar in design has a 1 m diameter ring net equipped with 333 μm mesh and a large 32 cm diameter by 46 cm tall cod-end bucket.

3.7.1 Copepods

To capture live copepods ring nets are typically towed either vertically or obliquely at low speed ($<0.5\,m\,s^{-1}$ forward and 0.1–$0.5\,m\,s^{-1}$ upward, depending on depth). A general rule of thumb is that the angle of the towing wire with the sea surface should be $>45°$. Some investigators pull in the net by hand or allow it to drift with the ship. This method is not feasible for larger taxa which may be residing deep in the water column, requiring tows of longer duration, 20–30 min or more depending on the depth of tow. To reduce damage to antennae and setae, the nets are preferably fine-mesh relative to the size of the animal (e.g. 150 μm mesh for female *Calanus finmarchicus*) although larger mesh sizes may be used, particularly during phytoplankton blooms or other conditions where net clogging or cod-end overcrowding due to the abundance of small organisms is a problem. The plankton is usually collected in a large-volume (5–20 l) cod-end. While some investigators use cod-ends with no drainage at all, others prefer that several screened holes are drilled near the top of the cod-end, in order to allow flow of water into the quiescent bottom part during towing.

Unless the cod-end volume is very large, the catch should be diluted immediately upon arrival on the ship's deck. Plastic, 4 l jars make excellent reception containers. They are filled with approximately 3 l of clean, ambient seawater and placed into seawater maintained at an appropriate temperature in thermally insulated coolers. Because there may be layering within the cod-end, it is prudent to pour a small portion of the catch into each jar to start, then return and add more according to a visual assessment of plankton density. When correctly diluted, copepods will resume normal swimming behaviors. With a large-bore pipette, copepods can be transferred directly from the jars to petri dishes for sorting. The reception containers should be scrubbed with hot water and rinsed with sea water between uses in order to reduce bacterial contamination.

3.8 OTHER ZOOPLANKTON INSTRUMENTS USED IN CONJUNCTION WITH NETS

To sample zooplankton efficiently the researcher should know as much as possible about the vertical distribution of the various components of the zooplankton community before the net samples are taken. There are relatively inexpensive instruments and methods that can provide this information. The OPC (discussed in detail in Section 7.3) provides *in situ* information on the concentration and size of zooplankton with depth when it is combined in a CTD profiling package. The size frequency data from the OPC can also be used to make an estimate of the wet biomass of the mesozooplankton (Heath 1995; Stockwell and Sprules 1995). The OPC data is presented during the vertical profile allowing the researcher to decide immediately which depth intervals are of interest to him for sampling with nets.

A commercial video camera in a pressure case can provide qualitative and quasi-quantitative data of zooplankton as it is lowered through the water column. The advantage the video camera has over the OPC is that larger organisms can be identified. There are a number of special video instruments that have been developed to identify and quantify zooplankton if only a few dominant species or developmental stages are present.

The use of acoustic sounders with multiple or single frequency will tell the researcher in which areas there are changes in patterns of organism distribution and this information will suggest regions of interest for other methods of sampling such as net sampling. In a zooplankton sampling program all types of information should be combined to create as much insight as possible into the distribution of the zooplankton before and during a net sampling program.

3.8.1 Optical plankton counter

This commercially available instrument provides *in situ* counts of zooplankton in the size range of 0.2–20 mm spherical diameter. The OPC is a non-video optical instrument for studying zooplankton distribution and abundance. Its initial design has been described by Herman (1988). The submersible version comprises a sampling tunnel with a 22 * 2 * 0.4 cm collimated light beam from a stack of 640 nm light emitting diodes (LEDs) to a photodiode receiver. The light attenuance of the water is monitored simultaneously to counting and sizing particles from 0.250 mm to approximately 20 mm equivalent spherical diameter (ESD). Data including time marks of 0.5 s intervals are reported to a deck unit via a single conductor cable. This time interval corresponds to a volume of water of 6.4 l and a spatial resolution of 1.3 m at a towing speed of 5 kn (2.6 m s^{-1}). A laboratory version having a shorter light beam can detect live and preserved plankton down to a lower size limit of about 0.1 mm. The maximum count rate (i.e. the product of flow velocity towing speed), and particle concentration, is 200 counts s^{-1} for both versions due to the response time of the sensor (see Herman 1992 for further technical details). The data are logged on to disk in a simple format accessible to subsequent processing, for example with standard spreadsheet software. Size discrimination of the particles allows the identification of species and stages of species.

The OPC can be towed horizontally at high speed (up to 12 kn), dropped vertically in a profiling mode and used as a bench sampler through which the samples are passed to get counts and sizes of zooplankton. The vertical profiling OPC can be used as a reconnaissance tool combined with a CTD to locate the depths at which concentrations of various sizes of zooplankton are found. The concentration profiles are used to guide the net sampling to particular depths of interest thus making the most efficient use of the net sampling and shiptime. By targeting certain depth strata, maximum information about the vertical distribution of zooplankton can be gained from the nets with the least amount of shiptime and sample analysis cost. A towed OPC has been used to carry out high speed surveys for zooplankton over large geographic areas (Herman *et al.* 1991; Huntley *et al.* 1995).

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