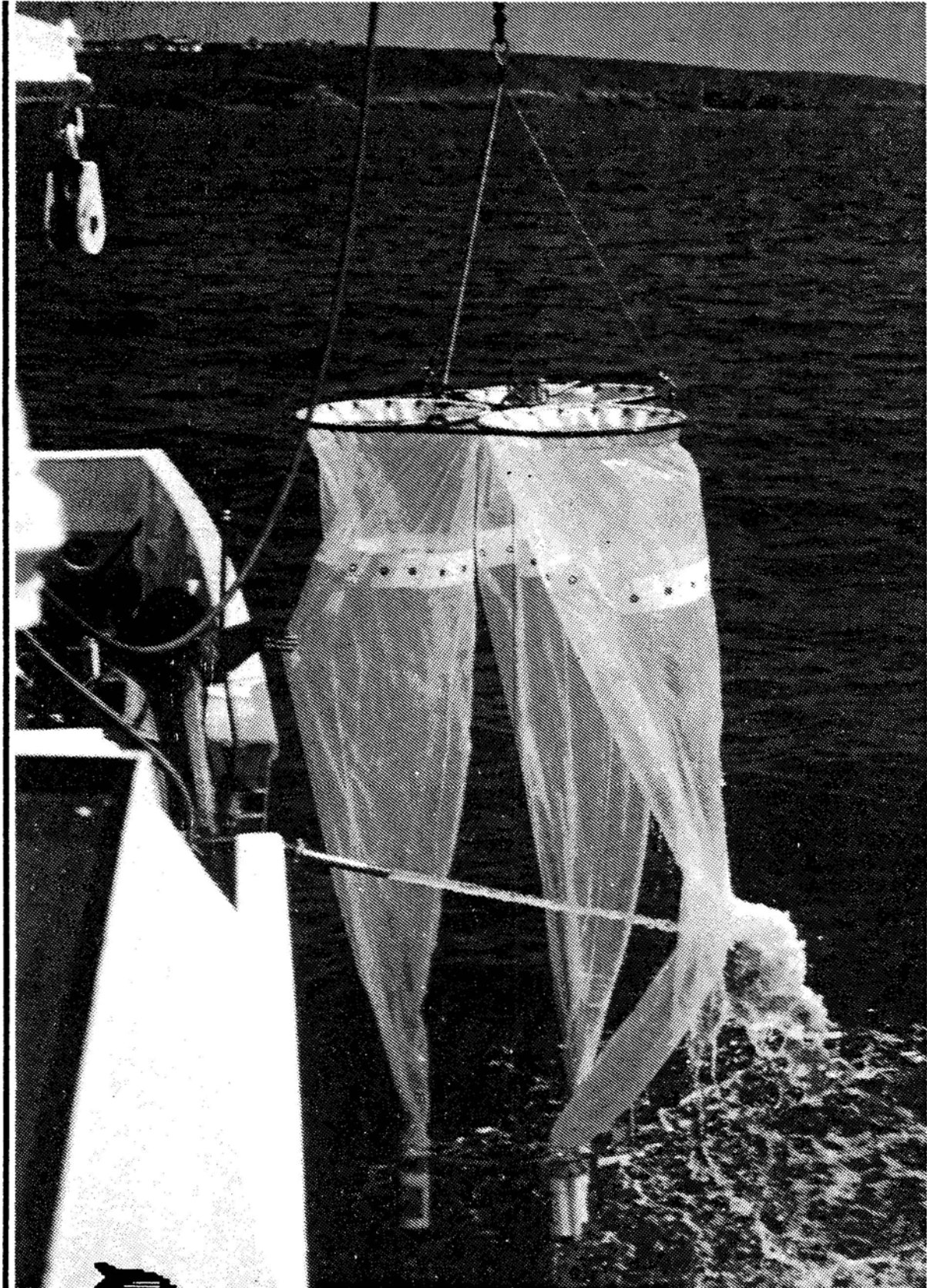


TECHNIQUES FOR ZOOPLANKTON STUDIES

AT NUCLEAR POWER STATION SITES ON THE
ENGLISH CHANNEL AND ATLANTIC COASTS OF FRANCE.

by

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AFREMER

CENTRE de BREST FRANCE

Techniques for zooplankton studies
at nuclear power station sites
on the
English Channel and Atlantic coasts
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Contents

	p.
Introduction	2
Sampling strategy	2
Tides	2
Currents	2
Spatial distribution	2
Seasonal variations	2
Microdistribution	2
Choice of sampling levels	3
Types of zooplankton samples collected	3
Sample collecting and preserving	3
Logsheet	5
Time	5
Point	5
Station	5
Wire angle	5
Sounding	5
Sampling depth	5
Flow-meter	5
Sampling duration	5
Sample numbering	5
Types of nets and their deployment	7
<i>Vertical haul, single net</i>	7
<i>Vertical haul, triple net</i>	9
<i>Horizontal tows</i>	9
Fishing procedure	11
Storage of samples	13
Laboratory processing	13
Overall quantitative data	13
<i>Dryweight biomass</i>	15
<i>Total organic matter</i>	15
<i>Organic carbon and nitrogen</i>	15
Taxonomical analysis	17
<i>Subsampling</i>	17
<i>Counting</i>	19
Dynamical study of copepodite stages	21
Data processing and recording	21
References	23

Introduction

Only those techniques and methods actually used in studies of nuclear power stations sites (Gravelines, Penly, Paluel, Englesqueville, Flamanville and Plogoff), in accordance to their specific requirements and working conditions, are dealt with here. Appropriate methodological choices, however, could be different in other circumstances, and more general accounts of zooplankton methodology (UNESCO, 1968; Bougis, 1974; Omori & Ikeda, 1984) should be consulted in this respect.

Sampling strategy

Sampling was defined according to the characteristics of the sites (large tides, strong currents) and the purpose of the studies, i.e. gaining a knowledge of the abundance and taxonomic composition of zooplankton, of its variations in the annual cycle and of its spatial distribution. The emphasis was put on mesozooplankton, mainly in the size range 200 - 1000 μm .

Tides: The tides are semi-diurnal over the northwest European continental shelf, where tidal range can be as large as 13 m on springs at Saint-Malo.

Currents: The existence of strong tidal currents is one major criterion for the choice of nuclear power station coastal sites, in order to favour dispersal of the thermal effluent. This characteristic, however, also results in sampling technical difficulties, as well as a high degree of variability in environmental conditions, especially with respect to the neaps-springs cycle. This variability must be somewhat filtered out in order to retrieve seasonal variations, which requires that the surveys be carried out under approximately constant tidal conditions, i.e. at fortnightly intervals corresponding to the periodicity of the semi-lunar cycle.

Sampling was therefore planned according to the following rules:

Spatial distributions: In order for spatial variations to be significant, especially between inshore and offshore locations, samples were taken as far as possible on either low water or high water slack, when tidal advection, and therefore space-time interference, is thought to be minimal.

Seasonal variations: Attempts were made at carrying out the surveys twice per month, once per month being the alternative when this proved impossible. The one major requirement that had to be met with was to operate at a nearly fixed phase of the neaps-springs cycle. The reference for this was provided by the tide coefficient, as available in French tide tables. This is the range of a given tide, expressed as percentage of that of an average equinoctial spring tide, the actual reference being provided by setting the value at 120 for the maximal astronomical tide (strictly speaking this is defined for the tide conditions in Brest, but can largely be applied to any place where the tide is semi-diurnal). The fixed phase condition was accordingly fulfilled by restricting surveys to the time when the tide coefficient was in the approximate range 60-70 (moderate neaps to average tide).

Microdistribution: Many authors (e.g. Cassie, 1963) have shown that plankton is not evenly distributed in the environment. The organisms are generally aggregated in swarms or patches of varying size (a few centimetres to a few metres), hence an apparently random increase in the variability of quantitative estimates. This contribution of microdistribution to the variations in plankton abundance has therefore to be estimated for a better assessment of the effects of environmental factors. Replicate samples were collected for

this purpose in as short a time as possible on certain occasions. At Flamanville, for instance, 5 replicate samples were taken within 5 minutes at high water slack, and 5 other ones at the subsequent low water slack, to provide microdistribution estimates to serve as confidence brackets in the comparison of plankton abundance between different points. The use of multiple nets (see below) also allowed replicate samples to be taken on a more routinely basis.

Choice of sampling levels: Discrete measurements or samples were taken at definite depths for physical parameters and phytoplankton; in the relevant areas, the water depth is usually less than 30 m, so that 3 sampling levels were most often thought sufficient. Zooplankton was collected using plankton nets and sampling was dealt with in two different ways, depending on the hydrographic structure. Where vertical mixing prevails, vertical hauls were performed from near-bottom to the surface. This allows for an average abundance estimate in the (largely homogeneous) water column (per unit volume), together with an estimate of the total plankton content in this water column (per unit area). The latter vertically integrated value is most convenient in comparing distant as well as nearby geographical areas for overall plankton standing stock, irrespective of the local height of the water column. Bottom to surface vertical sampling also offers the advantage of minimizing diel variations in plankton abundance due to vertical migrations, thus reducing the bias possibly associated with sampling at different times of the day, which can be significant even in shallow areas. Where stratification (either thermal or haline or both) prevails, horizontal tows are performed at different levels. Both vertical and horizontal sampling may be performed at the same point, as was done for instance at Plogoff, providing information on both the vertical structure of plankton communities and vertically integrated abundance values for use in general purpose comparisons.

Types of zooplankton samples collected: Different variables, requiring different types of sample collecting and subsequent analytical procedures, were used to characterize zooplankton with respect to overall abundance, taxonomic composition of the community and age structure of the population of some key species. The corresponding types of samples are respectively:

- Frozen samples for dryweight biomass measurement and subsequent evaluation in terms of organic carbon and nitrogen.
- Formalin preserved samples collected with a 200 μm mesh width net for the enumeration of mesoplankton (and occasionally macroplankton) organisms.
- Samples collected with a 80 μm mesh width net and preserved with alcohol or formalin, for the enumeration of shellfish larvae or developmental stages of key copepod species, respectively.

Sample collecting and preserving

The studies taken into consideration were carried out over a number of years, in various places, involved many persons and sometimes suffered from severe field working conditions, all characteristics which were expected from the beginning. It was therefore necessary to standardize as much as possible all steps in field work and laboratory processing and to set the rule that any change arising either from the introduction of a new type of equipment or from the need to deal with a specific problem should be first calibrated against the existing practice. All the participants to the field operations were accordingly instructed to strictly adhere to a working scheme, which may appear overly rigid, but was thought essential to maintain

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POINT:	STATION(N°):	HEURE (début de station):	HEURE (fin de station):	SECRETAIRE PONT	OBSERVATIONS:	
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Logsheet sample copy. One sheet is used per station occupied.

continuity and comparability in the results. This scheme can be summed up as follows:

Logsheets: All informations on measurements and sampling are recorded on a set of logsheets (see sample copy), one sheet being used per station. All logsheets individually bear the date and survey references.

Time: All indications are recorded in Greenwich Meridian Time (GMT), in order to avoid any confusion arising from changes in legal time in the year. In the area of interest, GMT also offers the advantage of always differing by less than half an hour from local solar time, which is the significant reference for biological diel cycles.

Points: This term, not to be confused with "station", stands for a fixed location, defined by its geographical coordinates, and where field work is normally carried out repeatedly. Points are generally referred to by conventional letters or numbers.

Stations: A complete series of operations carried out at a given point. For pelagic surveys, a station generally includes one or more hydrological cast(s) and plankton collecting; benthos sampling may also occasionally be included. Stations from a same survey are given numbers (1 to n) in chronological order. If time effects are being studied at a fixed location, there may be several stations at a single point.

Wire angle: This can produce a bias in estimating sampling depths and calculating plankton abundance from vertical hauls. Wire angle must therefore be recorded, especially if more than 30° from the vertical.

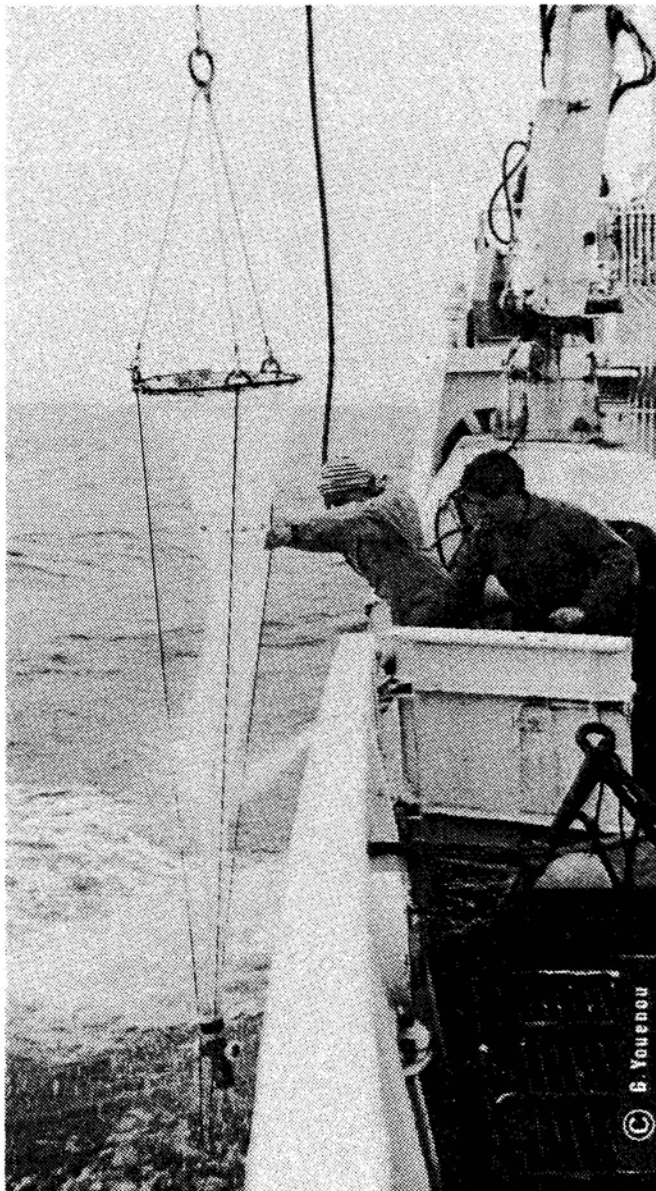
Sounding: This is the echosounder reading (in metres), i.e. the distance between the sounder's hydrophone and the bottom. This has to be corrected, especially in shallow areas, according to the draught and the hydrophone's position on the hull in order to obtain the actual water depth.

Sampling depth: For plankton net vertical hauls, this is a depth range, generally from the initial hauling depth to the surface. Initial hauling depth is the distance (in metres) between the mouth of the net and the sea-surface at the time when the hauls begins. If the wire is quite vertical, this is equal to the amount paid out, the meter wheel being set at zero with the net mouth at the surface. If not, it is obtained from amount paid out and wire angle, assuming no curvature of the wire. For horizontal tows, it is a definite depth, taken as that of the center of the net mouth.

Flow-meter: Ensure that flow-meter dials are set at zero before sampling begins and read when completed.

Sampling duration: This needs to be precisely known, together with the number of revolutions of the flow-meter's propeller, in order to calculate the volume of water filtered. A stopwatch is therefore used for proper precision and accuracy.

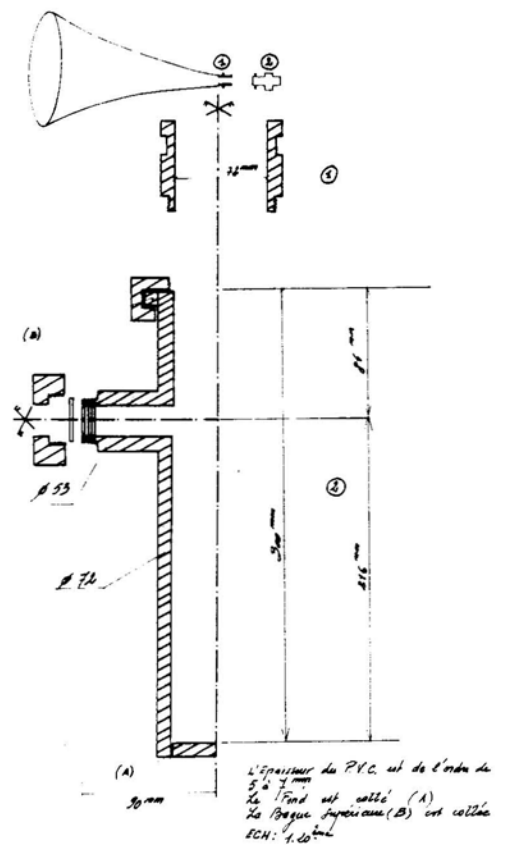
Sample numbering: Each sample is given a unique reference so that no confusion can arise. The reference consists of a prefix-letter characteristic of the site and a serial number (e.g. G.1509 for the 1509th sample taken at Gravelines); it is recorded both on the sample-containing jar (waterproof marker) and on the station logsheet (where place, date and time are referred to). If a sample happens to be too large for being contained in a single jar, then several jars are used, each given a unique reference consisting of the sample reference plus an index number (e.g. G.1509₁, G.1509₂, etc.).



Single WP₂ net

The net has been raised high above the sea-surface and is being copiously rinsed from top to bottom with a sea-water hose.

Schema of collecting bucket with protruding "ears".



The number of jars for a given sample must be recorded on the relevant logsheet to avoid any error when the sample is processed.

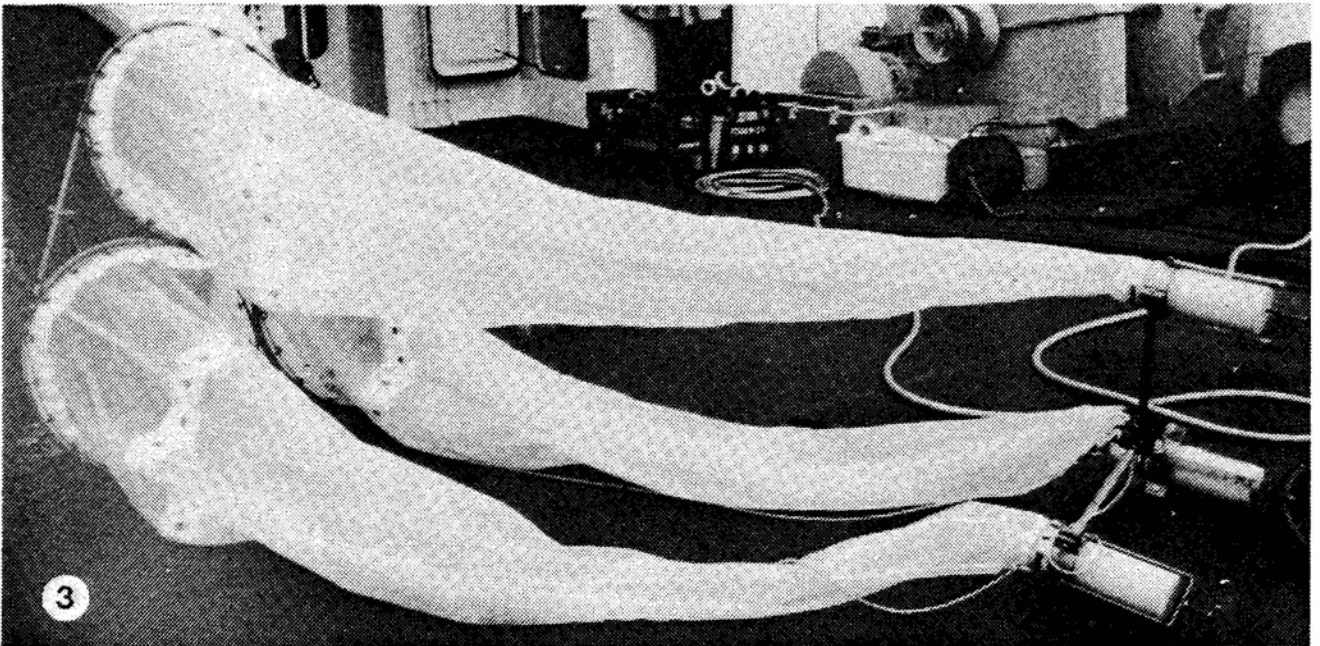
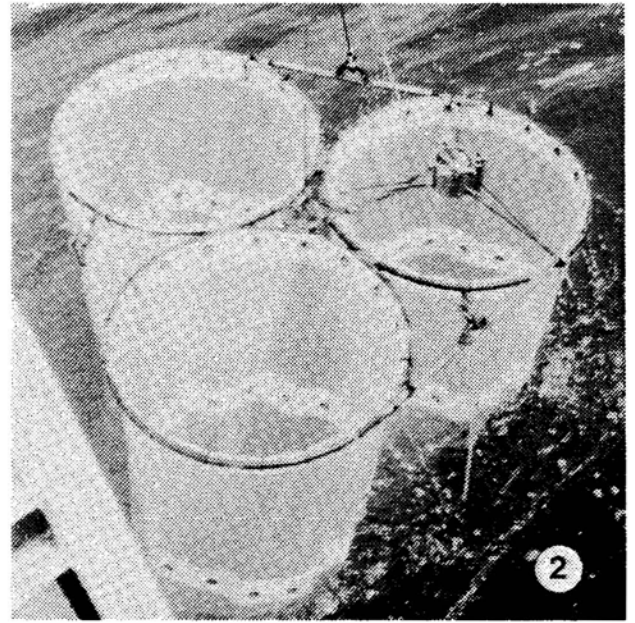
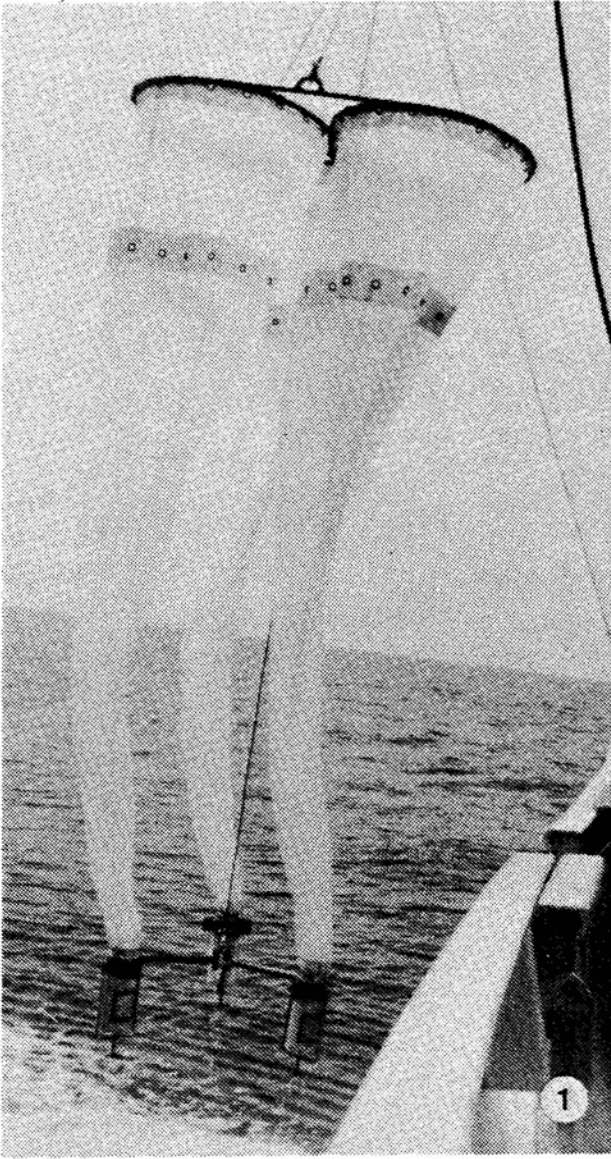
Types of nets and their deployment: For mesoplankton and small macroplankton (approximate size range 200 μm - 10 mm), the basic collecting device is the standard WP₂ net, as devised by D.J. Tranter in Sydney and described in UNESCO (1968). This net is a reliable quantitative tool, easy to handle and widely used in various parts of the world, especially in continental shelf regions. This is a cylindro-conical ring net with a mouth area of 0.25 m², i.e. an internal diameter of approximately 56.4 cm for the brass or stainless steel ring, which is fitted to the wire by three stainless steel bridles. The filtering material is monofilament nylon, plain gauze weaving, with a mesh width of 200 μm . When the net was designed, its hydrodynamic properties were adjusted to obtain a filtration coefficient exactly equal to unity (i.e. volume filtered equal to the product of mouth area by length of path in the water) for a towing or hauling speed of 1 m s⁻¹ and if no clogging takes place. These conditions were not always fulfilled with certainty under the various circumstances of the work considered here, but the use of flow-meters allowed a proper monitoring of the volume of water filtered. For this monitoring to be accurate, the flow-meter has to be set in a defined position, i.e. where the water velocity with respect to the net is equal to the integrated average velocity over the mouth area. This has also been determined when the net was designed (UNESCO, 1968) and the proper setting is to fit the center of the flow-meter at 14.25 cm from the ring. For both continued proper adjustment and easy handling, the flow-meter is attached to the ring with two fixed-length bridles and a rubber spring.

Smaller organisms (copepodite stages, mollusc larvae, etc.) are collected with a smaller-mesh (80 μm) cylindro-conical net, the size and shape of which are identical to those of the WP₂. This net clogs more readily than the original WP₂ and its filtration characteristics are less precisely known; under certain circumstances (e.g. phytoplankton bloom), the estimates of the volume of water filtered may therefore be less precise and less accurate. Both the WP₂ and the 80 μm net can be used for vertical hauls or horizontal tows, a single device being deployed at a time or three of them being mounted on a common frame.

Vertical haul, single net: Special features in the fitting of the net are that the collecting bucket is of a type with two protruding "ears" and that three suspension ropes are used for preventing the weight's traction to pull on the net itself. The "ears" end up on two circular filtering windows that allow partial draining of the bucket prior to the handling of the sample, in order to avoid any loss and subsequent quantitative error. The windows are closed by discs of the same filtering material (same mesh size) as the net; for easy handling and cleaning, these are glued on to a plastic flat ring of the proper diameter and held in position by a plastic collar that screws on to the end of the "ears". The bucket itself is screwed by a plastic collar on to a socket which is attached to the bottom of the net by a metal clamp collar. The suspension ropes are fixed on to the mouth ring at their upper end and joined together, in a loop that is used for attaching a 25 kg weight, 10 to 15 cm below the bottom of the bucket. They are tightly secured on the collector's socket by a second metal clamp collar. Their length between this point and the mouth ring must be adjusted for their bearing the weight rather than the net's, i.e. the distance must be slightly shorter than the length of the net. This is done by sliding the ropes in the collar before tightly screwing the clamp, the net being suspended in a vertical position in a suitable place.

Triple WP₂ net

1. Handling of the triple net after a vertical haul.
2. Mouth frame of the triple net. A TSK flow-meter is fitted in the mouth of one of the nets. The volume of water filtered is assumed to be identical for all three nets.
3. Triple net on the deck before a haul.

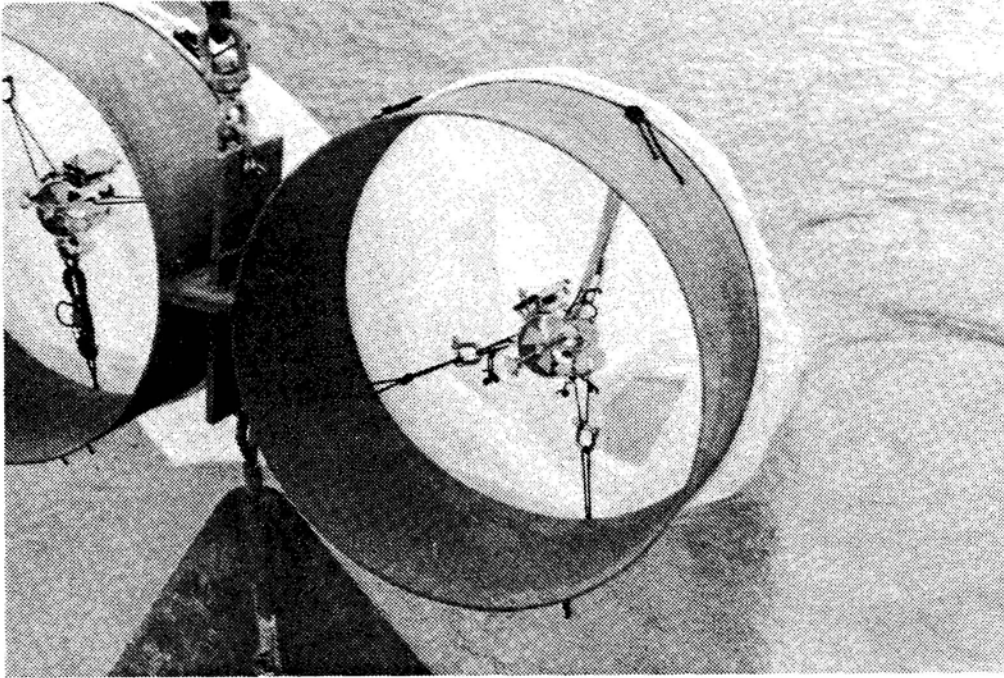


Vertical haul, triple nets: The idea of mounting three identical nets on a common frame was first put forward by Pr. A. Bourdillon at a meeting held in Roscoff in 1967. This was intended to solve the problem raised by the need of collecting two samples for biomass measurement, on the one hand, and species counting, on the other hand, while sample splitting devices do not work properly at sea and performing the sampling twice can be too time-consuming, especially for deep hauls in oceanic areas. With this purpose in mind, using three nets rather than two was simply a means of achieving a better-balanced behaviour of the gear in the water. The advantages of using a triple net are not restricted to deep sampling, however. It can provide a single type of sample in triplicate for the statistical analysis of variability, or two types of samples with one of them in duplicate, or three different samples for different purposes (e.g. biomass measurement, species counting and biochemical analyses). The triple net mounting consists of a mouth frame and a bottom frame. The mouth frame consists of three rings soldered together, the assembly being reinforced by straight bars the position of which corresponds to the external common tangent of adjacent rings, and which are soldered to the rings. The whole mouth frame is attached to the wire by a single crow's foot (3 bridles). Only one of the three nets is fitted with a flow-meter, identical filtering behaviour being assumed for the other two. The bottom frame is a metal (stainless steel) three-pointed star to which the collector's sockets are fastened by clamps. This rigid assembly allows an easier to handle collecting bucket system than in the case of the single net. The collecting buckets are cylindrical, with a simple square filtering window instead of the "ears". They just slide on to the cylindrical end of the sockets and are held in position by a stirrup-shaped clamp borne by the collector's frame. The latter also serves for attaching the weight (by means of a snap-hook, under the center of the star) and is linked to the mouth frame by a single piece of wire which serves the same purpose as the three suspension ropes used for single nets. As a result, although the three-net system is more bulky than a single net, it is less likely to get mixed up on handling. In shallow coastal waters with strong tidal streams, the handling of the triple net is made easier, especially on rinsing (see below), by mounting a swivel between the wire and the crow's foot. This, however, should not be done when the traction is expected to be important (deep haul or horizontal tow), otherwise the wire could untwist.

Horizontal tows: Single ring nets are used for this purpose. The difference with the vertical haul fitting is that neither a bottom weight nor suspension ropes are used. A depressor can be attached instead to the ring, to prevent the mouth from coming out of the water at high towing speeds.

Macroplankton (i.e. especially fish eggs and larvae) is collected using one of the versions of the Bongo net, which is a common standard for fishery institutions. This was first used as a standard instrument on the MARMAP programmes and the original description is given by Mac Gowan & Brown (1966). Modifications were subsequently introduced, as described by Smith & Richardson (1977). This sampler is used only for oblique hauls or horizontal tows. Its frame consists of two drums connected by a rigid bar; two nets are fastened on to the drums, from which they extend freely without a collector's frame, ropes or other structural pieces. Two versions are available, a large one with 61 cm diameter drums and a small one with 20 cm diameter drums; both of them are routinely equipped with 505 and/or 333 μm mesh nets. On the surveys considered here, only the large Bongo was used; depending upon the

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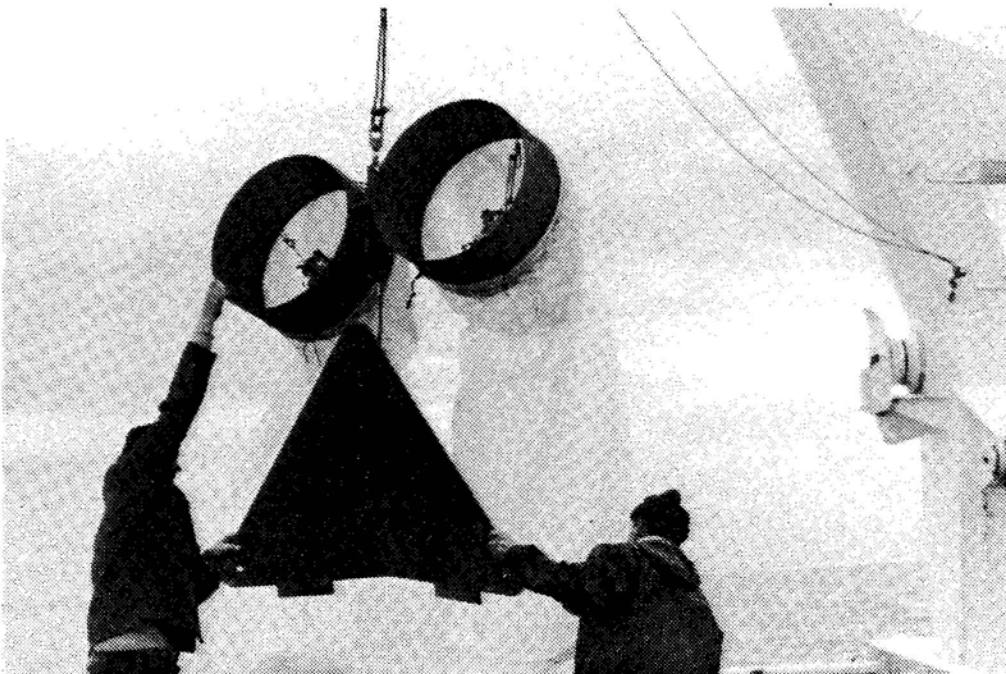


Above: Large Bongo net. Each of the two nets is fitted with a TSK flow-meter.

Bongo nets

Below: Paying out a Bongo net. A heavy depressor is fitted to the mouth frame, to perform a horizontal tow. The point where the depressor is attached is adjusted for the planned towing speed.

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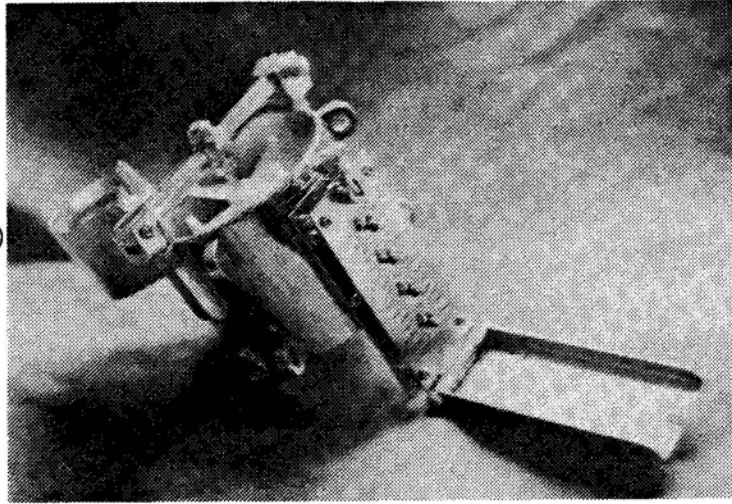


purpose of the sampling, it was fitted with either two 315 μm nets, or two 475 μm nets, or one of each. The size (and weight) of the depressor was chosen in accordance to the towing speed and weather conditions. The same flow-meter type was used as for the WF₂ net.

Fishing procedure: Vertical hauls and horizontal tows alike are part of the operations carried out at a station. Time and space references, accordingly, are normally recorded as part of the overall station routine. Some of the stations characteristics, however, such as the water depth, can change quickly, especially in inshore areas, owing to the tidal cycle or the drift of the ship due to tidal streaming. In addition to procedures which are special either to vertical hauling or horizontal towing, it is therefore essential to take the following steps, irrespective of the type of sampling being carried out:

- Check the echosounder reading and record it.
- Check that the flow-meter dials are set at zero.
- Check that the stopwatch is set at zero.
- Release the stopwatch at the instant when actual fishing begins and record the corresponding time (GMT).
- Stop the watch at the instant when fishing ends, i.e. when the mouth of the net passes through the sea surface, and record sampling duration.
- Raise the gear sufficiently high above sea-level so that the net(s) can be copiously rinsed from top to bottom with a seawater hose to drive all the plankton into the collector(s).
- Bring the net(s) back on board, keeping the collector(s) in upright position.
- Read the flow-meter dials and record number of revolutions.
- Allow the collector(s) to drain through the filtering windows and rinse the latter carefully to be sure to collect all the plankton. Take the collector(s) off the socket(s) and transfer the sample(s) into the jar(s). At this stage, rinsing the collector(s) and filtering windows one or several more time is generally necessary for a proper recovering of the sample. Biomass samples are filtered immediately (which can be done directly from the collecting bucket); other samples are transferred into jars and preserved. Most often, this is done by adding an amount of commercial formalin (30 - 40 % formaldehyde) equal to about 10 % of the volume of the sample (final formaldehyde concentration 3 - 4 %); in the other cases, the samples will be preserved in 70 % ethanol instead.
- Record sample references (letter and number, serial number of pre-weighed gauze disc used for biomass filtration, etc.).
- Rinse again copiously the net(s) and the collector(s). Although the steps described above normally guarantee that the quantitative recovery of the sample is satisfactory, a few specimens could still be stuck to the fishing equipment, resulting in a risk of qualitative contamination between samples taken in succession.

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TSK flow-meter

Four dials show the number of revolutions of the propeller (in units, tens, hundreds and thousands).

Filtration apparatus (from Millipore catalogue).



- Fit collecting device back to the fishing gear and reset flow-meter dials and stopwatch to zero, so that the equipment is ready for next operation.

Special steps to be taken for a vertical haul (WP₂ or 80 µm net) are setting the meter wheel at zero when the mouth is at the surface on paying the net out, lowering the net down to a safety distance (2-3 m) from the bottom and recording the corresponding meter wheel reading, hauling at a constant speed, as close as possible to 1 m s⁻¹ as recommended in UNESCO (1968). It is particularly essential not to stop hauling to prevent any loss of the catch; if this has to be done for safety reasons, then the sample obtained must be discarded and sampling carried out again from the beginning. Horizontal towing is carried out at 1.5 - 2 knots for the WP₂ and 2 - 3 knots for the Bongo. It is important to keep the towing speed as constant as possible; otherwise changes in fishing depth as well as loss of catch could take place. The weights or depressors at the front of the nets should also be properly balanced with respect to working conditions.

Storage of samples: No special procedures are required for preserving samples which have been pickled in formalin or alcohol. Biomass samples are stored in portable freezers whose power input can be switched to either 220 V AC or 12 V DC. Such freezers can therefore be operated continuously from the ship to the land based laboratory, through a road journey of any length.

Laboratory processing

Quantitative data are obtained in various forms, such as dryweight biomass, organic carbon and nitrogen content and number of individuals, for different size ranges of organisms corresponding to the different nets used. All of these quantities are expressed with respect to the unit volume or the unit area, which requires the estimation of the volume filtered. This is generally obtained from sampling time and flow-meter reading. The flow-meters used are of the TSK (Tsurumi-Seiki-Kosakusho) type and come with a rating certificate intended for the calculation of water velocity. The formula given in this certificate includes a term proportional to the number of revolutions of the propeller per second and a constant. For a volume estimation, this has to be multiplied by time and mouth area; the final formula can therefore be shown to be:

$$V = S (aN + kt), \text{ where:}$$

V is the volume filtered (m³),

S is the mouth area (m²), i.e. 0.25 for the WP₂ net,

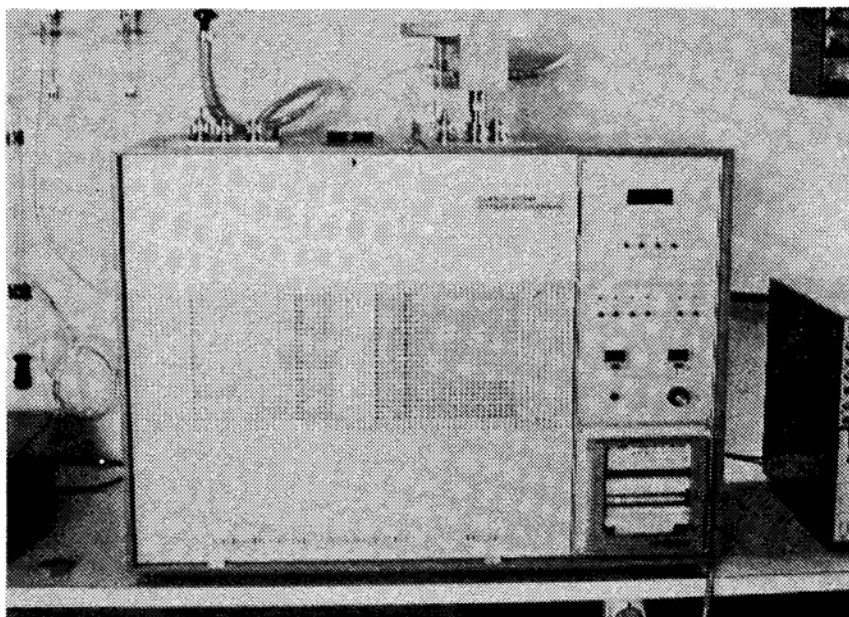
N is the number of revolutions,

t is sampling time (seconds),

a is the proportionality factor in the rating certificate,

k is the constant in the same certificate.

Overall quantitative data: These include dryweight biomass, total organic matter and organic carbon and nitrogen content. All of these variables are obtained from the same type of net (most often 200 µm mesh size) as the sample used for counting the individuals in taxonomic categories. The sample is filtered on board on a gauze disc which has been pre-weighed and is individually identified. To avoid any loss, the gauze disc is often finer-meshed than the net (e.g. a 100 µm disc for a sample collected with a 200 µm net), but, of course, the selectivity to be considered with respect to the



Carlo Erba CHN elemental analyzer model 1106 fitted with an automatic sampler for 50 samples.



C.E. data processor mod. 110

Photos :

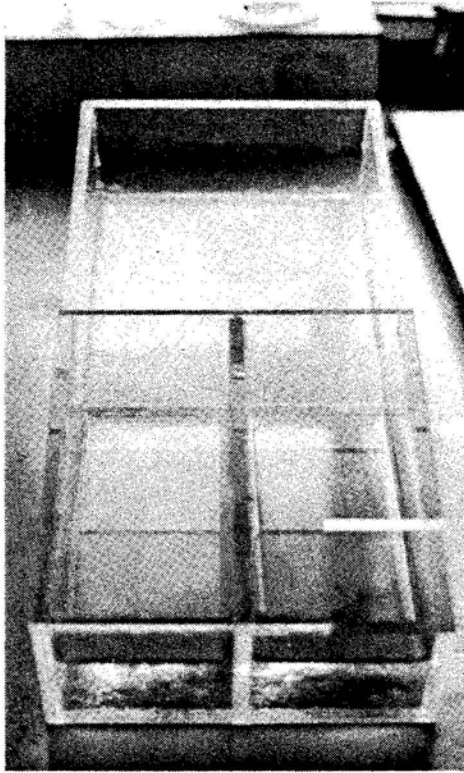
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representativity of the sample is that of the net, not of the disc. As mentioned above, the disc and its plankton load are immediately frozen on board and kept frozen until processing takes place. This includes several steps for the different possible expressions of plankton overall abundance. Depending upon the purposes and conditions of the survey, only some of the measurements may be made in some cases. Dryweight estimate is both the first step for all types of measurements and the kind of data obtained in all cases. Where the amount of particulate matter suspended in the water is large, this can include an important mineral fraction, hence a bias in the dryweight estimate; in such cases, it is better to obtain the biomass value as total organic matter. Organic carbon and nitrogen values are suitable for the study of the transfers along the food chains, because they are a common way to quantify all trophic levels. These cannot be estimated on the same material as total organic matter; it is therefore obtained either taking advantage of the collection of replicate samples (e.g. triple net) or from a fraction of the sample. In the latter case, dryweight values are used as a reference for calculating the correct amount of both total organic matter and organic carbon and nitrogen.

Dryweight biomass: The frozen sample is heat dried at 60 °C for 48 h, allowed to cool for half a day and weighed with a precision of ± 0.1 mg with a Mettler balance. The weight of the gauze disc is subtracted from the result to obtain the dryweight value.

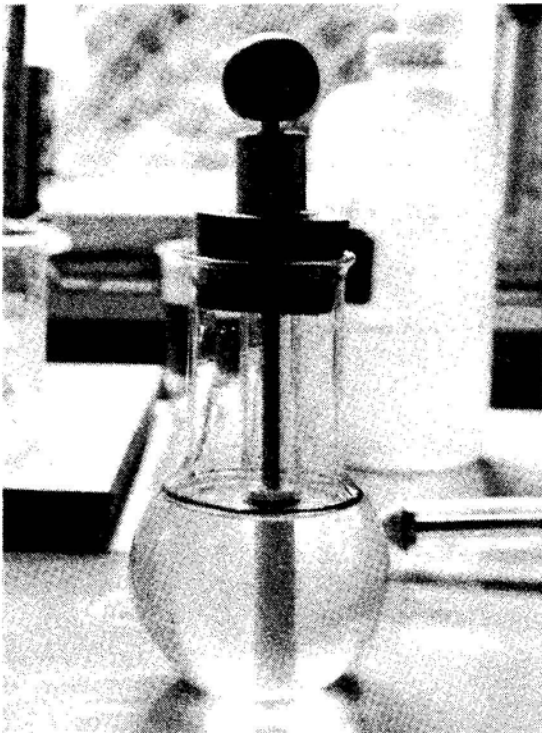
Total organic matters: After the dryweight value for the whole sample has been obtained, the load of the disc is removed and weighed again. If only the total organic value has to be obtained, all of the load is theoretically removed; in practice, however, removal can never be complete, so that the weight of the material actually removed, with respect to the dryweight value, provides a correction factor to be applied to the final value. The same type of correction is, of course, applied when only part of the sample is used for the organic matter estimate and the rest for carbon and nitrogen analyses. The material removed from the gauze disc is transferred to a crucible and combusted in a furnace whose temperature is increased by steps to 550 °C over 24 h. The furnace is then maintained at this temperature for 48 h, and cooled again by steps over 24 h. The weight of organic matter is the difference between initial dryweight and the weight of the material remaining after the combustion (ashes).

Organic carbon and nitrogens: This is obtained, for an amount of dried plankton of known dryweight, by using a CHN analyzer. This type of equipment is designed for the analysis of very small quantities of material. After being dried according to the procedure used for dryweight measurement, the sample is therefore homogenized by pulverization and a small fraction of it, weighed with a microbalance, is used for the analysis. This fraction is oxidized by combustion at high temperature (about 1000 °C), with the help of a catalyst. The resulting gases are subsequently reduced at lower temperature (400 to 700 °C) then separated (N_2 , CO_2 , H_2O) in a chromatographic column, at still lower temperature (100 °C or less). They are finally detected by a system sensitive to variations in thermal conductivity and the signal fed into a recorder, where it is visualized in the form of peaks; the quantitative values to be obtained are proportional to the area below these peaks, which is automatically calculated by an electronic integrator. The results obtained are, however, only relative and must be calibrated against the analysis of reference samples consisting of a precisely known quantity of a pure organic substance of known elemental composition

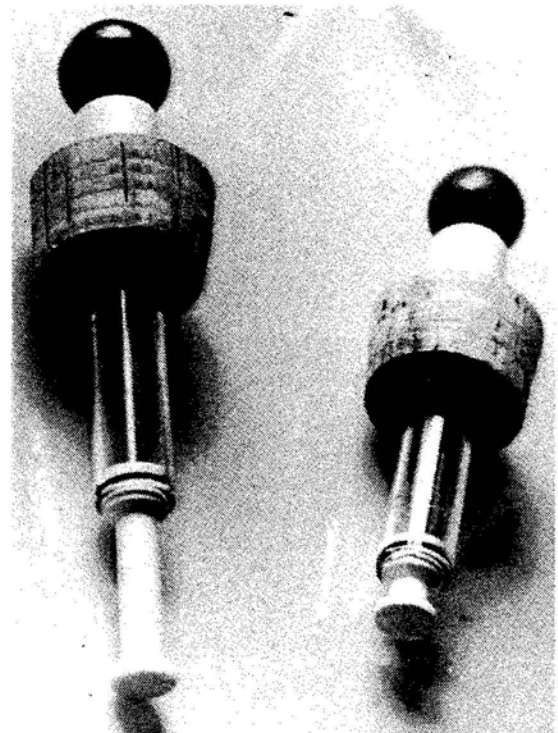


Motoda case

Stempel pipettes



Stempel pipette in its calibrated jar.



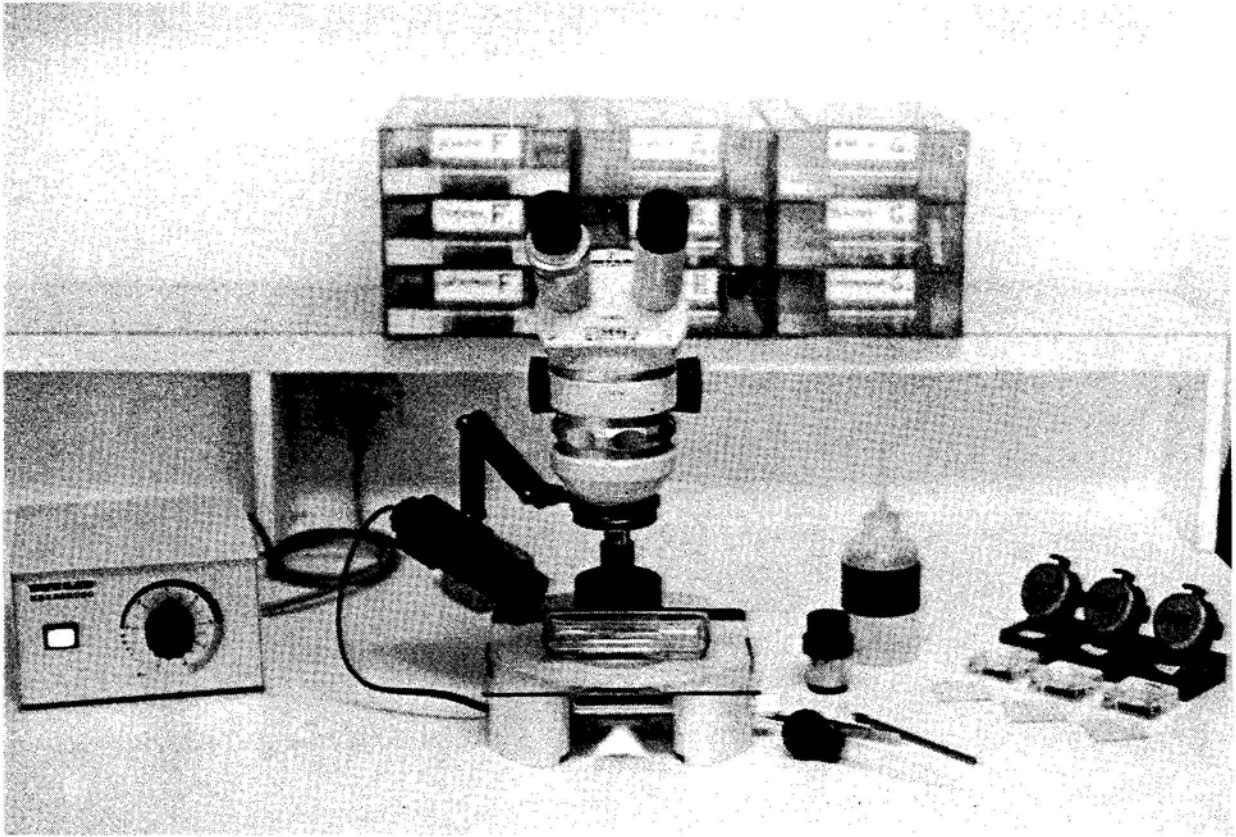
Two Stempel pipettes calibrated for 5 ml (left) and 1 ml (right).

(e.g. acetanilide). Two types of analyzers were used, a Hewlett Packard model 185 B prior to 1984 and a Carlo Erba model 1106 afterwards. Both of them use a flow of inert gas (helium) to convey the combustion gases through the analytical circuit, but they differ in a number of technical details. In the Hewlett Packard analyzer, for instance, the samples are deposited in small aluminium bowls which can be reused (although this is not recommended), together with the catalyst (MnO). In the Carlo Erba analyzer, the samples are introduced in capsules of oxidizable metal (tin) which are burnt with their content; the catalyst is different (Cr₂O₃) and stays in a part of the analytical circuit through which the hot gases are forced. In both machines standard samples have to be analyzed, but blanks, i.e. bowls containing only the catalyst, which should be nitrogen free, must also be run in the Hewlett Packard analyzer (in some cases, e.g. for phytoplankton introduced together with a piece of glass fibre filter, blanks are also necessary for the Carlo Erba; in the example given, running a blank is carrying out an analysis with a piece of filter alone). In all cases, drastic precautions must be taken not to contaminate the samples with any amount, however minute, of organic matter. This, for instance, means that the aluminium bowls must be pre-cleaned with ethanol and stored before use in a furnace at 400 °C. Identical measures must be taken with every piece of equipment (spatules, forceps) used for handling the samples, which must never come into contact with the hands. No smoking is, of course, an absolute requirement.

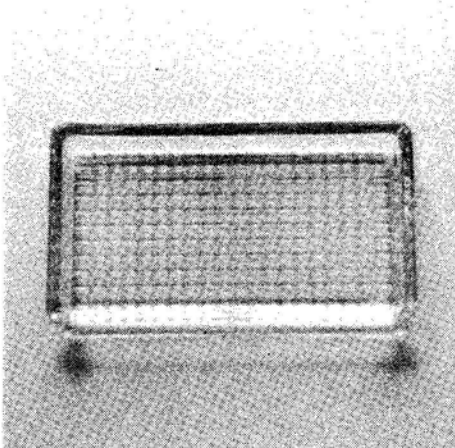
Taxonomical analysis: This consists of scanning the sample or an aliquot of it under a stereomicroscope for identifying the taxonomic categories (i.e. species if possible for the most important organisms) and counting the number of individuals in each category. This work is carried out on formalin preserved samples collected with various nets (80, 200, 315, 515 µm). Before the analysis is performed (from a few days to a few months after collection), examination of the samples with the naked eye can provide some useful information with respect to possible alterations of sampling strategy upon unexpected findings. This can even be done on board immediately after collection by a trained specialist. On board examination with a stereomicroscope is also useful, but the surveys considered here were carried out on board small ships which offered neither sufficient room nor sufficient stability for this task.

Subsampling: The size of the catch varies according to place and time of collection; most samples are too abundant for all the specimens to be counted. It is therefore necessary to take subsamples, corresponding to a known fraction of the whole sample. Several techniques are available for this purpose, between which the choice depends on the particularities of the relevant study. Subsampling problems can be avoided only if abundance is referred to a subjective scale (cf. Frontier, 1969); this speeds up considerably the analysis but is sufficiently reliable only if all samples to be compared are processed by the same well trained specialist. Because the studies considered here spread over a number of years and sample counting for the different sites was performed by different persons, this requirement is not met with and a standard counting procedure must be relied upon, which involves subsampling of large samples. The simplest subsampling devices are the Motoda case, the Folsom splitter and the Stempel pipette.

The Motoda case and the Folsom splitter both split the sample into halves; one of the halves can be further splitted, resulting in two subsamples corresponding to 1/4 of the whole sample, and so on,



Wild M5 stereomicroscope



Dollfus tray

The tray is divided into 200 squares,
each of them being 5 x 5 mm large.

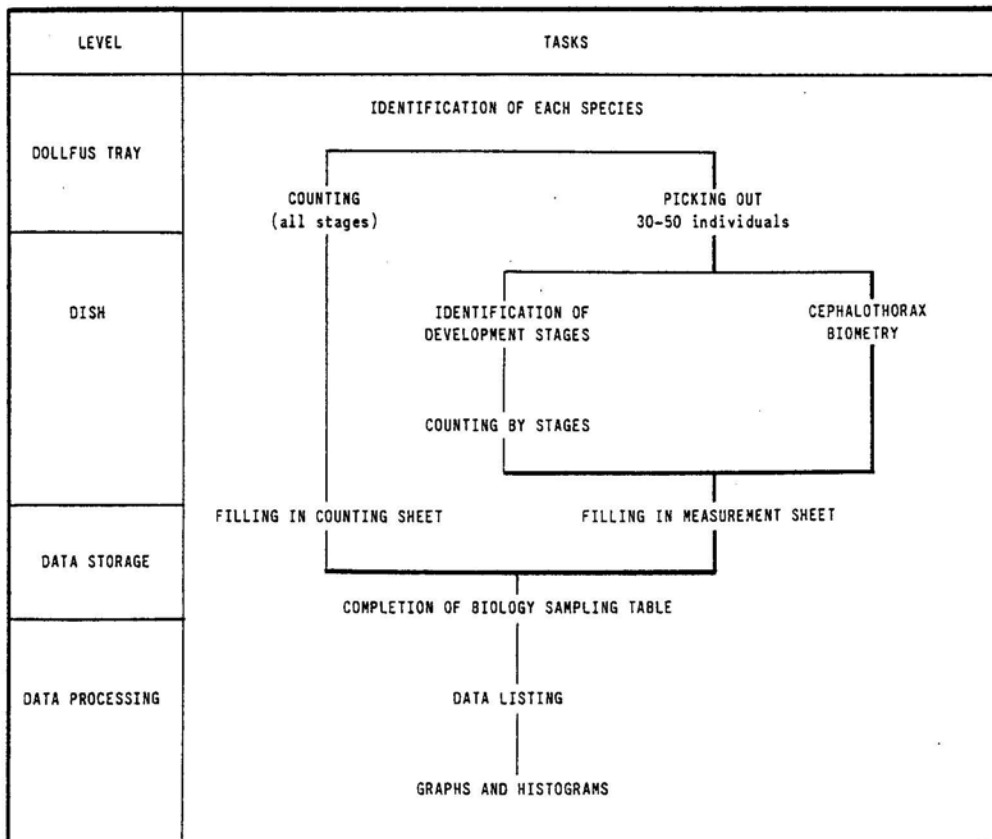
until n divisions are performed, resulting in a final subsampling factor equal to 2^n . The major disadvantage is that, because splitting never is done perfectly, there is an exponential growth of errors with each successive division. The technique is therefore acceptable only for small samples which will undergo few successive divisions. This generally holds for the samples dealt with in the studies considered here, since a vertical plankton haul in shallow waters most often corresponds to 2 to 10 m^3 of water filtered and does not require a high subsampling factor.

For using the Stempel pipette, the sample is first adjusted to a given volume in a calibrated jar; the pipette (which is actually a piston syringe) is then used to withdraw a fixed-volume fraction after the plankton suspension has been homogenized. A typical value is 5 ml for the volume of the syringe, which yields a subsampling factor equal to 50 if the volume of the sample is adjusted to 250 ml. The procedure can be repeated to obtain larger subsamples if necessary (by adding the content of elementary fractions), but becomes time-consuming if really large subsamples are required. The Stempel pipette was accordingly used only to deal with the largest samples, or to facilitate the counting of exceptionally numerous organisms, such as *Noctiluca*, which can exceed 10^5 individuals per m^3 in some cases in summer at Gravelines.

Counting: The fraction to be counted is placed in a Dollfus tray, which is a rectangular glass cuvette whose bottom is divided into 10 rows of 20 squares each, the squares being 5 x 5 mm large and separated by raised edges. These characteristics help to keep the organisms in a nearly fixed position in the tray and facilitate the scanning, which is performed row by row or column by column. The confidence bracket for subsampling errors becomes narrower as the number of individuals counted for a given species increases. A compromise has therefore to be found between the need for a precise estimate, which requires counting many specimens, and the time that can be devoted to this demanding task. Frontier (1972) has shown that a reasonable compromise is reached when about 100 individuals have been counted for a given taxonomic category. The subsample to be counted is, therefore, smaller as the species is more abundant. Small subsamples should therefore be counted first, then larger and larger ones; counting can be discontinued for a given taxonomic category as its number reaches the threshold, until only the rarer species are counted in the whole sample. Following this scheme rigorously is still too time-consuming, however, when, as is the case for the surveys considered here, a very large number of samples must be processed in a limited time. The most abundant species, which are also those with highest ecological significance, are therefore counted strictly applying Frontier's threshold and the other ones in a (2 to 8 times) larger fraction. The whole sample is examined with the naked eye to spot organisms which are rare but large (mysids, fish fry, chaetognaths) and could represent a large biomass or provide important qualitative information. The disadvantage of this procedure is that rare small species can be overlooked. When these are actually found in the largest subsample counted, the numbers are often too small for a quantitative estimate; presence versus absence (+ vs -) is recorded instead in the final data tables. For those organisms for which a quantitative estimate is actually possible, the number in sample is obtained by multiplying the number counted by the subsampling factor and the result is finally expressed with respect to the unit volume or unit area (see below).

	Paracalanus parvus	Pseudocalanus elongatus	Clausocalanus sp.	Ctenocalanus vanus
P ₁				
P ₂	Re ₃ B ₂ 	 	 	
P ₃	Re ₃ Comme P ₂ B ₂ Comme P ₂	Comme P ₂ Comme P ₂	Comme P ₂ Re ₁ Comme P ₂	 Comme P ₂
P ₄	Re ₃ Comme P ₂ & P ₃ B ₂ Comme P ₂ & P ₃	Comme P ₂ & P ₃ Comme P ₂ & P ₃	Comme P ₂ & P ₃ 	Comme P ₃ Comme P ₂ & P ₃
P ₅		♀: La présence d'une P ₅ (fig.3) est une anomalie. 	 ♂ figure d'après GIESBRECHT	

Key for the identification of Para-Pseudocalanidae showing, in table form, the most significant morphological details in the swimming legs (from Le Fèvre, 1971).



Steps in the dynamical study of copepodite stages

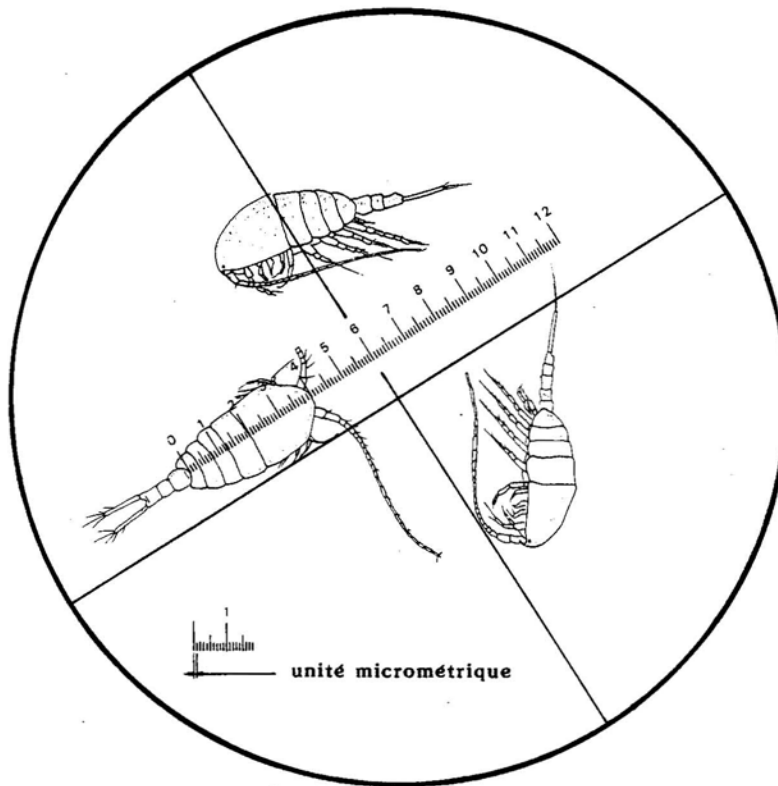
From time to time, an individual is encountered in the counting tray, which cannot be immediately identified under the stereomicroscope. All such specimens are picked out and kept aside until routine counting is completed, then individual examination is performed, with dissection and use of a microscope if necessary, using suitable taxonomic references. A special problem is that of the copepods of the families *Paracalanidae* and *Pseudocalanidae*, which are always very abundant in the samples but whose species (i.e. *Paracalanus parvus*, *Pseudocalanus minutus*, *Ctenocalanus vanus* and *Clausocalanus* sp. in the region considered, only the first two of them being really common) are almost impossible to distinguish under the stereomicroscope. Their precise identification is a long and tedious task which cannot be undertaken for all the individuals. Yet, however, some information must be obtained on the matter, because of the abundance of some of these species and their very different ecological behaviour, which would make their counting as a whole meaningless. The group is therefore dealt with as described by Le Fèvre (1971): as a first step, the heterogeneous category **Para-Pseudocalanidae** is counted as a whole according to usual subsampling rules; the first 30 or 40 individuals are picked out on the process (i.e. all specimens present in a number of squares to avoid any subjective selection bias) and subsequently examined with the microscope using a special mounting technique that allows the morphological details in the swimming legs to be seen without prior dissection, and the proportions found for the different species are applied to the global counting. Reference is also made to the sex and maturity stage of the specimens (e.g. ovigerous female). Finally, it must be emphasized that not all organisms can be identified to the species or even genus level. Some are referred to family or to a broader taxonomic category; these are often organisms thought not to play a major role in the ecosystem, but the main reason for this lack of precision is actually the lack of time to process the samples in ultimate details, i.e. still a compromise.

Dynamical study of copepodite stages: This is done using the samples collected with the 80 μm net. Three species thought to be of major ecological importance in coastal waters of the English Channel are considered: *Temora longicornis*, *Centropages hamatus* and *Acartia clausi*. As for the general taxonomic account, the samples are scanned under the stereomicroscope in a Dollfus tray, but the specimens are counted according to developmental stage as well as species. The first 30 to 50 individuals of each species are picked out on the process and their cephalothorax length is subsequently measured using an ocular micrometer fitted to the stereomicroscope. The results are extrapolated to the whole population in the relevant sample, following a proportionate rule, in very much the same way as for the taxonomical analysis of *Para-Pseudocalanidae*. The whole process yields data which allow for population dynamics studies, from the analysis of time variations in the age structure of the population of a given species, recognition of generations or cohorts and their turnover time, influence of environmental factors on size and moulting pace, etc.

Data processing and recording: All data (biomass, elemental content and species or stage count) are expressed per unit volume, and, for vertical hauls, per unit area in the water column. The reference unit volume is 10 m^3 for taxonomical analysis (to avoid unreasonably low figures for the rarer organisms) and 1 m^3 for other types of data. This, for instance, leads to the following formulas for a taxonomic count:

$$N_v = n_i \times f_i \times 10/V \quad , \text{ and:}$$

$$N_s = n_i \times f_i \times h/V \quad , \text{ where:}$$



Measuring cephalothorax length in *Temora longicornis*. The micrometric scale in the eyepiece is superimposed on the image of the specimen (dorsal view). The reading (in this case 40 units of the micrometric scale) is calibrated against an object-micrometer (drawing by J.Y. Quintin).

Development stages in three copepod species: *Temora longicornis*, *Centropages hamatus*, *Acartia clausi*

Stage	Number of pairs of legs	Number of abdominal segments	Test of selection	Remarks	Development		
					Example <i>Temora longicornis</i>		Description
					Abdomen ♀	P ₅ ♂	
1	2	2	a) number of pairs of legs				- Appendages of Cephalic region are present, - development of thorax by successive molts with a new pair of legs at each one.
2	3	2	b) number of thoracic segments				
3	4	2					
4♀	5	3	a) number of abdominal segments.	♀ uninflated genital segment,			♀ and ♂ - sexes are distinguished. formation of 5th legs ♀ beginning of swelling of genital segment. ♂ advent of asymmetry on A ₁ , right P ₅ .
4♂	5	3	b) maturity state of ♀ genital segment (first abdominal segment).	♂ slight asymmetry of 5th legs.			
5♀	5	3	c) morphological differentiation of P ₅ ♀ and of A ₁ ♂ (right A ₁).	♀ inflated genital segment, but genitalia incomplete,			Adult characteristics more pronounced.
5♂	5	4		♂ P ₅ asymmetry of 5th legs.			
Adult ♀	5	3		♀ genital segment very much inflated.			♀ genital segment much inflated. ♂ asymmetry of A ₁ and P ₅ . finished growth of body ♀ and ♂).
Adult ♂	5	4					

Drawing from SARS (1903) - KRAEFFT (1910) - GAUDY (1962)

Legend : ♀ = female ♂ = male

A₁ = first antennae

P₅ = 5th legs.

N_v is the number of individuals per unit volume (10 m^3),
 N_a is the number of individuals per unit area (m^2),
 n_i is the number of individuals counted for species i ,
 f_i is the relevant subsampling factor (see above),
 V is the volume filtered (m^3) calculated as shown above,
 h is the height of water column (echosounder reading, m).

Data are recorded on normalized forms, to be stored in a computerized file, which allows tables, graphs, etc., to be produced automatically. Taxonomic entries into the file are made through an alphanumeric code referring to genus and species (e.g. TEMO LON for *Temora longicornis*). This applies to the general account, where all stages are considered together. The code is slightly altered for archiving population dynamics data (e.g. TEMO 002 for copepodite stage 2 of *T. longicornis*) and space in the file is provided in this case for recording average cephalothorax length of the relevant stage. In this way, diagrams and tables can also be automatically produced with respect to population dynamics.

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