

This document includes:

Biology/Zooplankton/Zooplankton taxonomy-related abundance per unit volume of the water column

Biology/Zooplankton/Copepod egg production, particularly *Calanus helgolandicus*

SAMPLE COLLECTION

Zooplankton abundance

Normally two vertical tows for zooplankton abundance are taken with the 200micron WP-2 net.

Net hauled from the bottom (bottom depth recorded) to the surface at a slow towing speed (10-20 metres per minute, so 3-5 minutes for a 55 metre tow)

Nets washed down with non toxic supply and the cod-end contents concentrated.

The two duplicate samples are placed in the two polythene sample bottles provided (which contain formalin to preserve the organisms) along with waterproof label recording sample number, date, and time. Sample bottles kept cool and out of the sun in the “grey box”

Live zooplankton

Normally as part of the L4 sampling additional nets are towed to collect zooplankton for experimentation (for example, *Calanus* egg production)

Empty bottles with yellow lids are kept in the PML white cool-box, and these will be labelled with the sampling requirements. (Usually either fine, medium or coarse tow nets or WP-2 nets for a vertical haul)

Samples collected either with WP-2 nets or “tow-nets” depending on the need and the crew operation/advice on the day. Tow-nets can be streamed at the surface while other sampling operations are carried out.

Samples placed in the yellow lidded bottles in the white cool-box provided and diluted with seawater.

VERY IMPORTANT that these samples are kept as cool as possible and out of the direct sun at all times

The non-toxic supply can be connected to the coolbox to circulate cool water around the sample bottles, to prevent organisms overheating while on deck.

Take live samples just before leaving station if possible

Transport back to the lab as rapidly as possible and **KEEP COOL**

Keep cool and shaded and out of direct sunlight.

Copepod egg production

Samples collected using a 'coarse' plankton net 500 micron mesh size using a horizontal tow at the surface for a duration of 10 minutes. Sample transferred and diluted with surface seawater from a hose into a cool box and kept cold until arrival in lab.

Seawater sample for particulates

A 5-litre sample volume is collected for chlorophyll a and CHN analyses from a depth of 10 m (surface sample) and placed in rectangular Nalgene carboys fitted with taps. Samples are kept cool and in the dark in a large plastic coolbox.

CALCULATIONS AND ANALYSIS

Zooplankton abundance

Samples are collected by vertical net hauls (WP2 net, mesh 200 μm ; UNESCO 1968) from the sea floor (approximately 55m) to the surface and stored in 5% formalin. Much of the zooplankton microscopic analysis has been to the level of "major taxonomic groups" only, and a number of different analysts have participated over the years. The level of expertise has generally been consistent, but the user should be aware that levels of taxonomic discrimination may vary during the course of the data-set.

Copepod egg production

Five replicates of 5 female *Calanus helgolandicus* are incubated in filtered seawater for 24 hours. The eggs produced are counted and collected. The incubator temperature is adjusted to sea surface temperature value at L4. Egg collectors are set up in 2 litre beakers and filled with ~1500 ml 0.2 micrometer filtered seawater. To each egg collector 5 female *Calanus* are added, picked out from the 'coarse' zooplankton sample. Animals from the buckets are filtered onto a small 500 micron mesh and females picked out under a WILD microscope with stork bill forceps straight into the egg collectors. The egg collectors are placed into a cold room. The set up time for each beaker is noted on a provided sheet. After the experiment has incubated for ~24 hours, egg collectors are removed from beakers and eggs filtered onto a small 53 micron mesh for each replicate. Contents of the mesh are washed into a gridded 60 mm petri dish and the eggs counted under a WILD microscope. Time and number of eggs produced are noted on the provided sheet.

DATA STORAGE

Data are added to the Pangea database (www.pangea.de) at regular intervals (3-6 months).