A new sensitive tracer for the determination of zooplankton grazing activity

Rik L.J. Kwint & Kees J.M. Kramer

Publication granted with the kind permission of Kluwer Academic Publishers
A new tracer compound is presented for determining zooplankton grazing activity. The gut content in zooplankton is measured as β-dimethylsulphoniopropionate (DMSP), which can be measured even in individual copepods. Species specific DMSP/chl a ratios allow applications in, for instance, prey selectivity studies.

Zooplankton grazing activity can be determined by several methods based on measuring the variation of a tracer in the food as a function of time. The incubation method is based on the decrease with time in the medium of phytoplankton numbers (or POC, chlorophyll a), and is thus essentially an indirect method (Tackx & Van de Vrie 1985, Harris 1994). Another indirect method is based on the production of faecal pellets. The gut fluorescence method (Dagg 1983, Helling & Baars 1985, Peterson et al. 1990) is based on the calculation of the grazing rate from the increase or decrease of chlorophyll and phaeopigments in the gut of, for example, copepods. The 14C method uses the uptake of this radio-tracer by the zooplankton after incubation with 14C labelled phytoplankton (Daro 1978, Daro & Baars 1985). This method is so sensitive that relatively few zooplankton individuals are required (10-20 individuals, Gulati 1985). The use of radio-labelled material is a disadvantage. Since the measurements in the gut fluorescence and 14C methods are performed in the zooplankton itself, and are therefore, direct methods, they can also be used when food is available in surplus. A disadvantage of both the incubation method and the gut-fluorescence method is that a considerable number of copepods is necessary for each measurement in time (up to 200 for copepods < 500 µm), as the sensitivity of these methods is relatively low (Kleppel & Pieper 1984, Morales et al. 1991, Bautista & Harris 1992, Rodriguez & Durbin 1992). Furthermore, pigments are susceptible to denaturation due to light and temperature effects (Morales et al. 1991). Methods have been compared by Peterson et al. (1990). Only the gut fluorescence method can be applied in exposure experiments under field conditions.

The tracer compound we propose here combines a high sensitivity, detection in the zooplankton gut and possible field applications. Both the gut filling rate and the gut clearance rate can be estimated. Determination is based on the analysis of a sulphur compound, β-dimethylsulphoniopropionate (DMSP). DMSP is present in most marine phytoplankton species (a main food source for zooplankton), in which it probably has the function of an osmolyte (Gröne & Kirst 1992). DMSP is mainly present in living cells, as it is rapidly degraded in the water column by physical or microbiological action. DMSP can be analyzed as dimethylsulphide (DMS) by gas chromatography, after hydrolysation with NaOH. The DMS formed by this hydrolysis step is only related to DMSP (Kiene & Gerard 1994).

DMS was analyzed on a ‘Varian 3500’ gas chromatograph equipped with a capillary linear plot column and a photo-ionization detector (PID) of 10.2 eV, with hydrogen as the carrier gas. Calibration was performed using DMSP.HCl salt standards, that were
hydrolysed to DMS. After hydrolysation, the DMS was purged from the vial with high grade Helium through hypodermic syringes and trapped cryogenically on Tenax-ta (Chrompack). The detection limit for DMS and DMSP was 1.5 pmol. If the sampled copepods are immediately frozen in liquid nitrogen, they can be stored in the freezer at -18 °C for at least a few weeks (Kwint et al. 1996b). After hydrolysis the samples can be stored for months in a refrigerator at 4 °C until analysis. Details for the analysis of DMS(P) is presented elsewhere (Kwint & Kramer 1995).

The calculation of the clearance rate is based on the decrease of DMSP in the zooplankton itself as a function of time (e.g. eight points or more). For each measurement only five individuals of similar age and size were selected by sieving from a stock culture. They were captured from a petri dish by means of a small glass pipet and counted on a microscopy slide, that was backlit with a cold light source. From the slide, each batch of five was transferred to a 100 ml dark incubation bottle filled with a phytoplankton suspension of known concentration. The zooplankton was incubated for 24 hours. This procedure limits the time during which the organisms can be disturbed by later handling, which may interfere with the analysis. After incubation, each batch was sieved (55 µm mesh), rinsed with artificial seawater and transferred to a 20 ml vial filled with artificial seawater (essentially to ensure that the DMS(P) concentration was below the detection limit). Care should be taken to use the same salinities throughout as the zooplankton may be sensitive to large changes in salinity and the possible osmolytic function of DMSP.

The zooplankton were allowed to (partially) defecate in this filtered seawater. The moment the zooplankton was put into the vials is defined as t = 0. Zooplankton was subsampled as a function of time, taking one batch at each sampling time. They were rinsed, and immediately transferred into a new 20 ml crimp-cap vial, filled with 10 ml 1 M NaOH, after which the vial was immediately sealed in order to prevent loss of DMS. The handling procedure can easily be performed within 5 minutes, enabling 12-15 measurements per hour. Some defecation may occur during handling, but this effect will be very small if the copepods are frozen in liquid nitrogen first. The first sample (t = 0) was transferred directly into the hydroxide, where the DMSP was hydrolysed to DMS. After analysis, the gut passage time and ingestion rate were calculated from the concentration vs time graph (Helling & Baars 1985).

In the experiments described below, we used the following conditions. A stock culture of the copepod Eurytemora affinis was grown on a mixture of the diatom Phaeodactylum tricornutum and the red flagellate Rhodomonas baltica. The copepods were sorted into size classes by means of sieves (>300 µm, >180 µm and > 55 µm) weekly. Individuals of the largest size class were used for the experiments (adults only). At the start of an experiment, the copepods were incubated with Phaeodactylum only (13,500 cells ml⁻¹, a chlorophyll a content of about 275 µg l⁻¹ and a particulate DMSP content of about
800 nM), at 17.5 °C, a salinity of 28.5 ‰ and a light/dark cycle of 14/10 h. Water samples were analyzed for chlorophyll \( a \), phytoplankton cell numbers (Elzone particle counter), particulate and dissolved DMSP (DMSP\(_p\) & DMSP\(_d\)) and DMS.

The results of an example of a gut clearing experiment are shown in Figure 1. Each data point consists of one observation with 5 copepods. The observation at \( t = 2 \) hours is a duplicate measurement, however. The gut clearance rate was calculated from \( G_t = G_0 e^{-gt} \), with the variables: \( G_t \) (gut content of DMSP in pmol ind\(^{-1}\) at sampling time \( t \)), \( t \) (sampling time) and \( g \) (gut clearance rate in min\(^{-1}\)). The curve was fitted using the ‘least square’ method. The gut passage time \( GPT = 1/g \) and the ingestion rate \( I = G_0 60/GPT \) (in pmol ind \(-1\) h\(^{-1}\)). Since the GPT was in the order of half an hour, the calculation was performed over the first hour only in order to avoid the negative effects of food depletion. This resulted in a GPT of 36 min, a value which compares well with observations by others (Baars & Helling 1985). An ingestion rate of 48 pmol ind \(-1\) h\(^{-1}\) (DMSP) was calculated. The ingestion rate is food source specific as the DMSP content (per cell) is dependent upon the phytoplankton species (Keller et al. 1989), as is the case with e.g. chlorophyll \( a \).

In order to optimize the number of copepods used, we investigated the effect of the number of copepods taken per batch. In Figure 2, the number of individuals per batch is plotted against the total gut content DMSP (triplicate observations). It should be noted that the gut content of only one individual could be measured significantly as the average of 18 pmol is about one order of magnitude above the detection limit, with a coefficient of variation (C.V.) of about 8%. Similar results were obtained for 2, 4 and 8 individuals per batch, resulting in a linear relation between copepod number and DMSP content of the sample. Surprisingly, the larger the batch size, the higher the C.V. (from 8% for 1 individual to 28% for 16 individuals). This is due to the time effect (see below) and the higher risk of counting errors. Natural variability of the individuals and avoidance of the problems with higher batch sizes had to be balanced. Therefore all other experiments were performed using an appropriate batch size of 5 individuals.

When the procedure was simplified so that the preparation of batches was carried out just before the transplantation into the 20 ml seawater vials (thus without a separate 24 h incubation), we observed a decrease in the average gut content within only minutes of \( t=0 \) (Figure 3). The time \( t=0 \) indicates the first batch that was selected from a freshly sieved stock culture. The time between observations in this figure represents the time needed to sub-sample a new batch of 5 individuals. The decreasing tendency was attributed to the diminution of the food availability while excretion
continued, and to stress caused by handling during experimentation. This implies that collection of larger batches will lead to reduced gut contents of DMSP and, that probably, the linear relation in Figure 2 should be slightly steeper. This demonstrates that the time needed for the sampling of batches needs to be standardized. The time dependency of the gut content is the reason why we propose to include an extra incubation step after the preparation of the batch. Validation of the incubation step revealed that when each separate batch was treated without time delay, no large variations in DMSP in the gut were found (n = 7, mean 19.3 ± 3.03 pmol ind⁻¹).

We conclude that this new tracer compound for the determination of grazing activity offers several advantages. Its sensitivity is extremely high, perhaps even sensitive enough for experiments using nauplii. The DMSP content of an individual copepod can be measured easily. Batches of 5 are proposed in order to assure rapid handling. Samples can be stored for longer periods without deterioration, and no toxic and/or radioactive compounds are involved in the analytical procedure.

DMSP is a compound that is specific for (living) phytoplankton. As DMSP is present in the phytoplankton cells in dissolved form, the effect of sloppy feeding can easily be measured by analyzing the increase of dissolved DMSP in the medium during grazing. Since the method provides direct information on the gut content and incubation is part of the methodology, the technique also allows field exposure of ‘cages’ with preselected batches of zooplankton.

The availability of DMSP as an extra parameter, e.g. in addition to chlorophyll a, can be used advantageously. The fact that the DMSP/chl a ratio is different for different phytoplankton species (and possibly also for different physiological states of the phytoplankton), offers the opportunity to study in carefully designed experiments the selectivity of the copepods for certain phytoplankton species in mixed communities, or in mixed phytoplankton/detritus assemblages.

Figure 3. Decrease in gut DMSP content in batches of 5 copepods without the incubation step. Curve equation: \( G_t = G_0 \cdot e^{-gt} \). Gut Passage Time: 21 min