

Feeding behaviour and diet of *Corophium volutator* in an estuary in southeastern England

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ABSTRACT: Experiments with radioactively labelled diatoms confirmed the ability of *Corophium volutator* (Pallas) to suspension feed and deposit feed. Using either method individual amphipods consumed a mean of approximately 4000 small diatoms in 1 h. However, no diatoms were visible in the guts, probably because they were crushed by the mandibles before ingestion. There was no consistent difference in the amounts of material in the guts of amphipods collected from the mudflats at 4 different states of the tide nor in 4 different seasons. *C. volutator* were immersed for approximately 3 h in each tidal cycle and as most feeding occurred when they were emersed they must have been deposit feeding on the surface sediment. The chlorophyll *a* concentrations in the guts of the amphipods collected from the estuary were similar to those from amphipods that had fed on large numbers of diatoms in the laboratory. Despite the absence of intact diatoms in the guts of the amphipods it was concluded that benthic diatoms form a significant part of their diet and that *C. volutator* populations have the potential to reduce significantly the densities of benthic diatoms by feeding.

KEY WORDS: *Corophium volutator* · Feeding · Benthic diatoms

INTRODUCTION

Corophium volutator (Pallas) is common in intertidal mudflats of the North Atlantic. In southeastern England it is often abundant in the mud immediately below the vegetated saltmarshes and in the creeks that dissect these marshes. *C. volutator* can feed in 3 ways. When deposit feeding the amphipods rake surface sediment into the burrow using the second antennae (Hart 1930, Meadows & Reid 1966, Icery & Nott 1985). The water current generated by the beating pleopods draws this material to the setose second gnathopods, where particles are retained and passed to the mouthparts. In suspension feeding, particles in suspension are drawn to the gnathopods in a similar way (Hart 1930, Fenchel et al. 1975). These 2 methods of feeding may not be independent since capture of particles in suspension may occur incidentally during deposit feeding and deposited material may be swept into a suspension feeding current. In epipsammic browsing

the amphipods scrape organic material off the surface of sediment particles, which are not consumed, using teeth-like structures on the first pair of gnathopods (Nielsen & Kofoed 1982, Stuart et al. 1985).

The evidence concerning the natural diet of *Corophium volutator* is indirect and inconclusive (Murdoch et al. 1986). *C. volutator* ingest particles within the size range 4 to 63 µm (Fenchel et al. 1975, Hawkins 1985, Icery & Nott 1985), although bacteria smaller than this may be consumed if attached to larger particles (Fenchel et al. 1975, Murdoch et al. 1986). Several authors have considered that benthic and pelagic diatoms are consumed, but only on indirect evidence. Most of the evidence is a decline in, or negative correlation with, diatom abundance (Mossman 1977, Coles 1979, Hargrave et al. 1983, Hawkins 1985) but this could also be due to bioturbation rather than feeding (Mossman 1977, Murdoch et al. 1986). Lopez & Levinton (1978) reported an efficient digestion of diatoms, compared to bacteria, and Stuart et al. (1985) related seasonal changes in enzyme complement to a presumed dependence on diatoms, in summer particularly. Schwinghamer et al. (1983) studied stable ¹³C

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values and concluded that diatoms could be the main diet, but their data could not exclude plant detritus as the food source (Murdoch et al. 1986). A high survivorship of *C. volutator* in laboratory experiments when diatoms were the only offered food (Stuart et al. 1985) was evidence that the amphipods can eat diatoms and not that they do so under natural circumstances. The problem with accepting the hypothesis that diatoms are the major food source is that they are rarely seen in the guts. This led to the conclusion that bacteria must be the major food source (Fenchel et al. 1975, Murdoch et al. 1986), possibly through the ingestion of faeces (Icely & Nott 1985). Murdoch et al. (1986) concluded that living diatoms contributed little to the nutrition of *C. volutator*, and the question of what was the major food source remained unanswered.

This study was prompted by the lack of conclusive evidence on how and when *Corophium volutator* feed and what forms the major part of their diet under natural conditions, and aimed to investigate whether the low densities of benthic diatoms in the presence of *C. volutator* (Gerdol & Hughes 1994, this issue) could be caused by their feeding activities or by disturbance. To this end the contents and the chlorophyll *a* (chl *a*) content of the guts were examined from amphipods collected at different times in the tidal cycle and in 4 different seasons. These data were related to laboratory data on the consumption of radioactively labelled diatoms, both in suspension and in sediment.

METHODS

Laboratory experiments. Radioactively labelled diatoms were used to investigate the possibility that *Corophium volutator* feed on diatoms by both suspension feeding and deposit feeding, but perhaps crush them so that the cells are not visible in the gut. The diatom species chosen was *Cyclotella cryptica* Reinmann, Lewin & Guillard, as their size (5 to 10 μm) is at the lower end of the size range of particles ingested by *C. volutator*, and being a pelagic diatom it would better remain in suspension when required than a benthic species. *C. cryptica* was cultured in Tropic Marine Neu (TMN) (30 ppt) enriched with nutrients and micronutrients according to Harrison et al. (1980) as modified by Santillo (1993). The diatom culture was labelled with ^{14}C -bicarbonate (10 μCi per 250 ml) at 20°C under constant lighting for 48 h. Immediately before the experiment, 2 ml of the culture was fixed with Lugol's iodine solution for estimation of diatom density. The radioactivity of 2 samples (4 ml and 2 ml) of the diatom culture was determined. The diatoms were filtered onto 0.8 μm Nucleopore filters which were acidified by addition of 2.5 ml acidified methanol, dried using an

infrared lamp, placed in scintillation cocktail and their radioactivity counted. The mean radioactivity of 1 diatom cell was calculated using the known density of diatom cells in the culture, in order to estimate the number of cells ingested by the amphipods.

Three beakers were partially filled with 200 ml of TMN (suspension feeding experiment) and 3 beakers with 2 cm depth of mud, previously sieved to remove *Corophium volutator* (deposit feeding experiment). In the suspension feeding experiment 25 ml of the labelled diatom culture was added to each of the 3 beakers. In the deposit feeding experiment 25 ml of diatom culture was mixed with the surface of the sediment and left to settle before 200 ml of TMN was added with care to avoid suspension of any diatoms. Ten *C. volutator*, previously sieved from the sediment and kept without food in aerated TMN at 20°C for 24 h, were added to each of the beakers. In the suspension feeding experiment *C. volutator* fed on *Cyclotella cryptica* kept in suspension by their natural buoyancy and by the turbulence generated by the beating pleopods of the amphipods. In the deposit feeding experiment the major food source available was *C. cryptica* in the surface sediment, together with any bacteria and benthic diatoms. To control for any radioactivity which may have been taken up by the amphipods from leachate in the water, the water from one of the suspension feeding experiments was filtered through a 0.8 μm filter into a clean beaker. Ten *C. volutator* were added to this water and treated in the same way as the experimental amphipods. After 1 h all the amphipods were sieved, rinsed in fresh TMN, acidified with acidified methanol and left to dry under an infrared lamp. Each amphipod was weighed individually and homogenized in a drop of methanol in an Eppendorf tube (1.5 ml). The contents of each tube were rinsed into a scintillation vial with 1 ml of methanol which was evaporated before 2 ml of scintillation cocktail was added for counting of radioactivity.

Concurrently, a similar experiment was performed using unlabelled diatoms and with 4 replicates for each treatment. At the end of this experiment, the amphipods were sieved, rinsed in fresh TMN, fixed in 4% formaldehyde in seawater, frozen and kept in the dark until used. These amphipods were dissected and the fullness of each gut noted. The amount of chl *a* in the guts of amphipods that had been suspension feeding was measured (as described below). The values were corrected by subtracting the mean value for the chl *a* concentration of 20 empty guts which were obtained from amphipods starved for 24 h. Ten guts from each treatment were dissected for examination of the gut contents by use of a fluorescence microscope (as described below).

Field observations. *Corophium volutator* were collected from below mean high water neap tide level at Wallasea Island, UK, (see Gerdol & Hughes 1993) on 4 dates approximately 3 mo apart: in autumn (2 September 1992), winter (5 December 1992), spring (17 March 1993) and summer (8 June 1993). On each date samples were taken at 4 different times in the tidal cycle: at the time of low tide (substratum emerged for ca 5 h), as the flood tide reached the site (substratum emerged for ca 10 h), at the time of high tide (substratum immersed for ca 1.5 h) and as the ebb tide exposed the site (substratum immersed for ca 3 h). Each sample consisted of approximately 100 amphipods which were sieved from the sediment on a 354 μm mesh and immediately fixed in 4% formaldehyde in seawater and frozen using solid CO_2 . These samples were kept frozen and in darkness until used. In the laboratory only amphipods longer than 5 mm were dissected, the gut removed and the degree of fullness of each gut scored as: 0% (empty), 30% full, 60% full or 100% full. The 100% full guts of 50 amphipods were used for measurement of chl *a* concentration using fluorometry (Parsons et al. 1985). Ten dissected guts were placed in each of 5 Eppendorf tubes (1.5 ml) and freeze-dried overnight. One ml of 90% acetone was added to each tube and these were left in a refrigerator (4°C), in the dark, for 24 h. The tubes were centrifuged for 5 min at 7000 $\times g$ and the chl *a* concentration of the supernatant measured with the fluorometer. The concentration of chl *a* in 10 to 70 empty guts was measured and this mean control value was subtracted from the mean values obtained for the full guts.

A further 10 full guts from each collection were dissected and the contents mounted on a microscope slide with polyvinyl lactophenol. The gut contents were examined in UV light using a fluorescence microscope at 315 \times magnification. Chlorophyll fluoresces red in UV light, which made any diatoms and other chlorophyll-containing structures easily visible.

RESULTS

Laboratory experiments

There was no significant difference in the radioactivity of amphipods that had fed on labelled diatoms by deposit feeding and by suspension feeding (unpaired *t*-test, $t = 0.45$) (Fig. 1). The amount of radioactivity ingested in 1 h was equivalent to approximately 4000 diatom cells per amphipod. In the controls the amount of radioactivity leached into the water by the diatoms and subsequently taken up by the amphipods was insignificant.

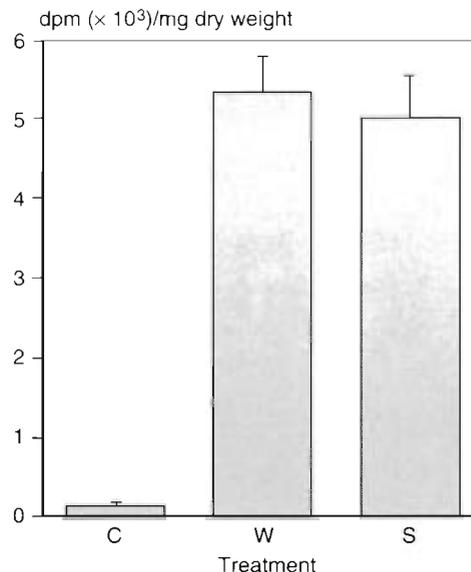


Fig. 1. *Corophium volutator*. Mean (+ SE) disintegrations $\text{min}^{-1} \text{g}^{-1}$ dry wt of amphipod in control (C), water only (W) and sediment (S) treatments. The values in the water and sediment treatments are means of 30 replicates (each a single amphipod) while the control value is a mean of 10 replicates

Almost all of the amphipods dissected had full guts but no diatom cells or distinct chloroplasts were observed under the fluorescence microscope in the amphipods that had been suspension feeding. The mean chlorophyll content of the guts of amphipods that had been suspension feeding for 1 h was 1.5 ng gut^{-1} . The amphipods that had been deposit feeding also had full guts. Most of the identifiable particles were sediment particles, but a few diatoms other than *Cyclotella cryptica* were observed, including *Diploneis* sp. and *Stephanopyxis* sp. These were presumed to have been in the sediment.

Field observations

The mean fullness of the guts of *Corophium volutator* at the 4 different states of the tide, in the 4 different seasons, shows no consistent pattern with time of immersion or emersion, nor with season (Fig. 2). Some amphipods fed at all stages of the tidal cycle, and in any one collection some individuals had full guts while others were empty. In winter most of the amphipods had full guts at low tide while at other times most had empty guts. In spring most amphipods had full guts at all states of the tide, but in summer most had empty or nearly empty guts, except those collected during the ebb tide.

The mean amounts of chl *a* per full gut varied from 0.067 to 3.87 ng and showed no consistent trend within

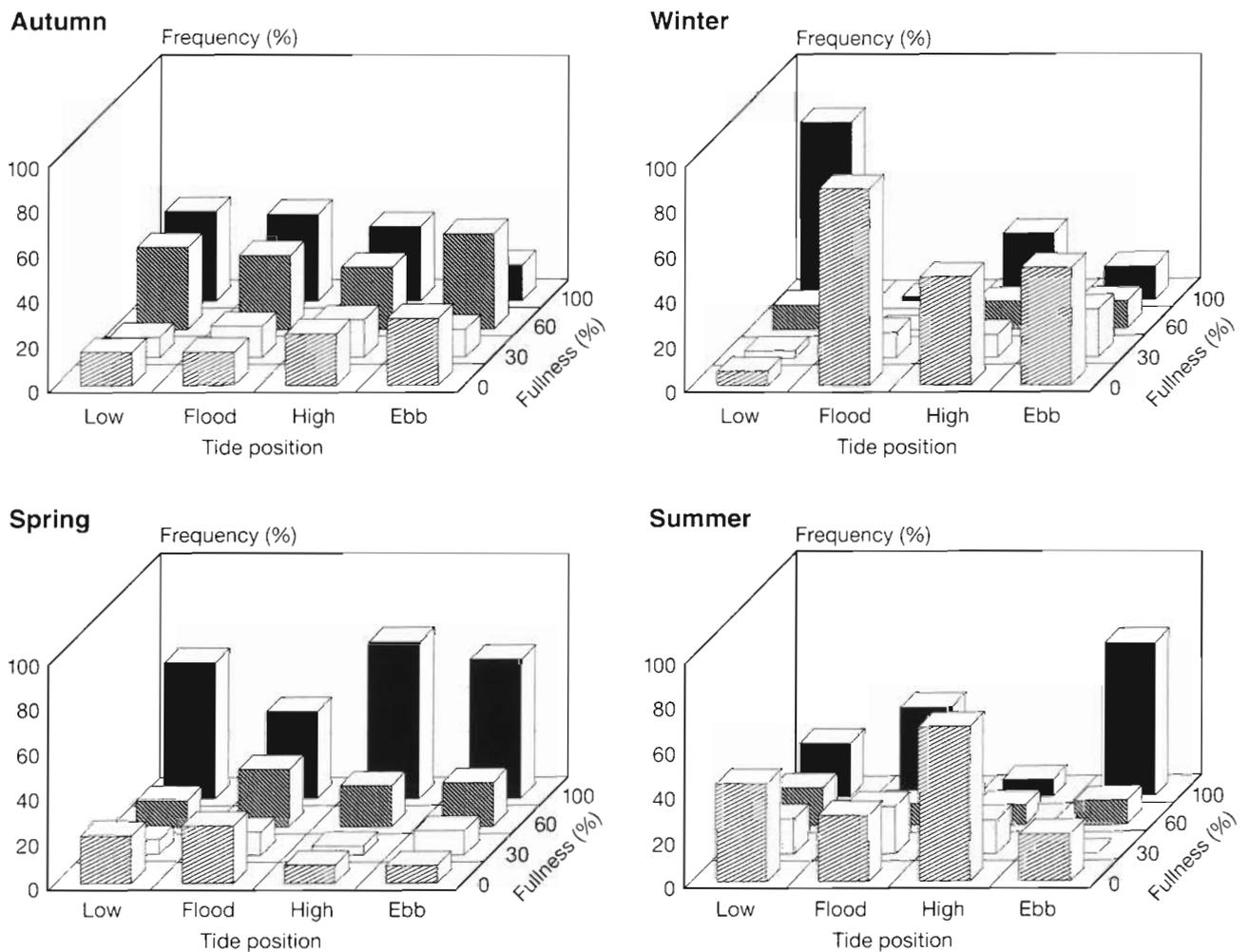


Fig. 2. *Corophium volutator*. Frequency of various gut fullness levels (0, 30, 60 and 100% full) of amphipods in 4 different seasons and at 4 different states of the tide (see text for full explanation)

the tidal cycle (Fig. 3). The amphipods collected in the autumn had the highest amounts of chl *a* in their guts during high tide, those collected in the winter and spring had the highest amounts at low tide and those collected in the summer had the highest amounts during the ebb tide. The seasonal differences in the mean amounts of chl *a* in the guts, which were 0.31 ng in autumn, 1.09 ng in winter, 0.82 ng in spring and 2.17 ng in summer, were significant (Kruskal-Wallis test, $H = 30.2$, $p < 0.01$).

Most of the gut contents observed under the fluorescence microscope were inorganic particles and particles of organic detritus of unknown origin. Only a few intact diatom cells were seen, and never more than 2 in one gut. Red fluorescent chloroplasts were observed, but mostly these were not contained in recognisable cells and their origin was not identifiable. Often the whole field of view under the microscope was fluorescent red.

DISCUSSION

The laboratory experiments confirmed that *Corophium volutator* were able to feed on diatoms by suspension feeding and by deposit feeding, and in approximately equal quantities. Although large numbers of diatoms were consumed, no intact *Cyclotella cryptica* were present in the guts but red fluorescent masses, caused by the presence of chl *a*, were observed. Murdoch et al. (1986), in discussing the absence of diatom cells in the guts, pointed out that *C. volutator* could not digest the siliceous frustules, because of the short residence time of food in the gut where the pH is approximately 6.7. Amphipods of the genus *Corophium* have large molars on the mandibles (Lincoln 1979) and Hart (1930) observed that most of the food of *C. volutator* was ground by these molar processes. It seems that mandibular grinding of the

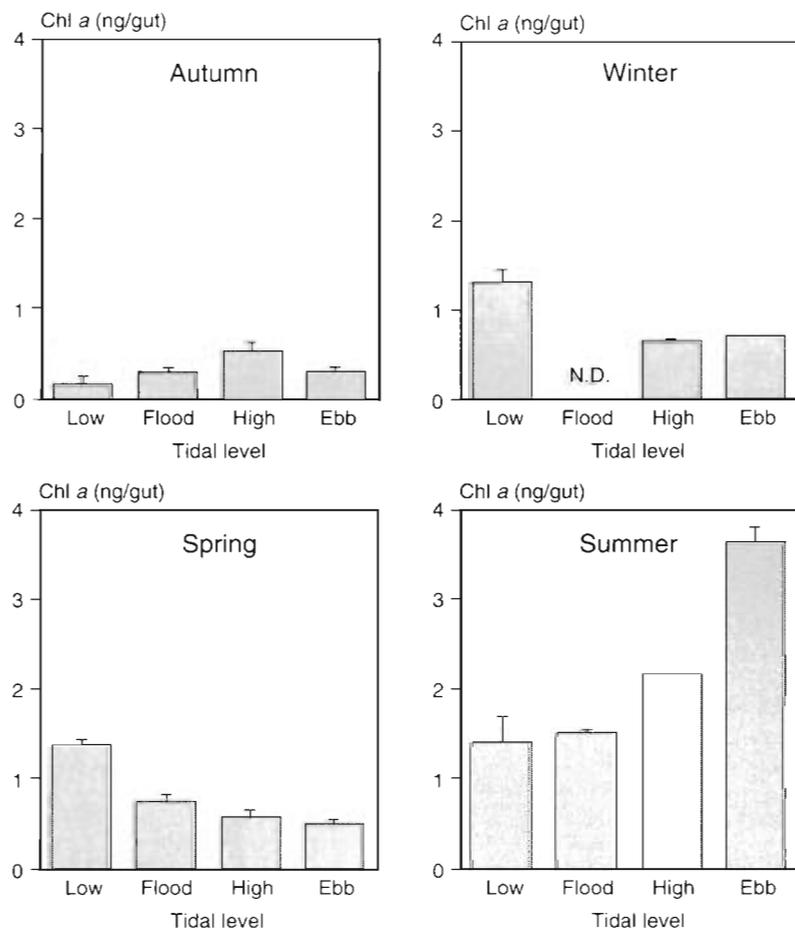


Fig. 3. *Corophium volutator*. Mean chlorophyll *a* concentrations (+ SE) in the full guts of amphipods in different seasons and at different states of the tide. Each value represents a mean of between 7 and 10 full guts. N.D. = not enough full guts to perform the analysis. Where no SE is indicated, no replication was performed due to lack of full guts

diatoms probably explains the often observed lack of intact cells in the guts, which therefore cannot be used to indicate that diatoms do not form a significant part of the diet.

The field results of this study support the conclusion of Icelly (1981) that *Corophium volutator* feed at all stages of the tidal cycle, given that the residence time of food in the gut is about an hour (Fenchel et al. 1975, Hawkins 1985, Icelly & Nott 1985). There was no relationship between chl *a* concentrations and state of tide, indicating the absence of any feeding rhythm. There was individual variation in the times of feeding and the proportion of amphipods with empty guts varied between 6 and 87%, in contrast to the observations of Mossman (1977) who found a constant proportion (about 35%) with empty guts. Mossman (1977) and Icelly (1981) suggested that individuals probably do not feed when moulting.

Corophium volutator could deposit feed at any time but could suspension feed when emersed only if sufficient water remained on the surface of the mud (Icelly 1981). The mud occupied by *C. volutator* remained wetter than the surrounding mud, perhaps because of compaction and by the retention of water by the lining of the burrows. However, this thin film of water would seem insufficient for sustained suspension feeding, by animals whose densities ranged from 30 000 to over 100 000 m⁻², for the approximately 10 h period of emersion in each tidal cycle. The inevitable conclusion that *C. volutator* were predominantly deposit feeding, at least when emersed, is supported by the observation that the gut contents of the amphipods at all stages of the tide were mostly inorganic sediment particles.

The mean concentrations of chl *a* in the guts of the amphipods collected from the mudflats were similar to the mean concentration in individuals which had recently consumed large numbers (ca 4000) of diatoms in the laboratory.

The results of this study indicate that *Corophium volutator* is an unselective deposit feeder that recognises and crushes most individual diatom cells, even relatively small ones, prior to ingestion, probably to gain access to

the cell contents. Relatively large organic detritus particles are ingested, as are inorganic particles, probably for the digestion of the epipsammic bacteria and organic films. Gerdol & Hughes (1994) showed that removal of *C. volutator* led to an increase in bacterial densities. Evidence of digestion of bacteria was provided by Fenchel (1972) and Murdoch et al. (1986) but Cammen (1989) considered that no deposit feeding organism had been shown to use bacteria as a major fraction of its nutritional requirements. Although numerous studies demonstrated that microbes associated with particulate matter were efficiently removed when ingested by deposit feeding macrofauna, calculations of the actual energy or carbon obtained through assimilation of microbes showed that bacterial densities in sediments are too low to meet the energy demands of macrofauna (Kemp 1990). Kemp (1990) suggested that most, if not all, sediment macrofauna depended on microalgae and non-living detritus.

Agrawal (1963) and Bärlocher et al. (1988) concluded that *C. volutator* lacked cellulase, and although cellulase activity was recorded by Stuart et al. (1985) it was equivalent to only 1% of the activity of other enzymes and was regarded as not playing a major role in digestion. Thus *C. volutator* may not be able to feed efficiently on plant detritus, and Murdoch et al. (1986) concluded that organic detritus supplied less than 5% of the amphipod's nutritional requirements. Extracellular mucopolysaccharides produced by bacteria and microalgae may make up a significant portion of the organic matter in sediments (Hobbie & Lee 1980) and, therefore, may be important in the diet of macrofauna. Bärlocher et al. (1988) considered that *C. volutator* could feed on the organic material in seafoam adsorbed onto sediments as the tide receded. The ability of *C. volutator* to digest the non-cellular organic material in estuarine sediments has not been investigated but it would be difficult to separate the direct effects of feeding from the indirect effects caused by reductions in diatom and bacteria abundance. It is concluded that benthic diatoms are the major food of *C. volutator* and feeding, not disturbance, can explain the significant impact these high density populations have on benthic diatom and bacteria abundance and hence on sediment stability (Gerdol & Hughes 1994).

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