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ELECTRON MICROSCOPY OF GUT CONTENTS AND FAECES OF *EUPHAUSIA SUPERBA* DANA

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Abstract: Research on the diet and feeding of krill can broadly be divided into three main approaches.

a) Investigations of the feeding apparatus, including anatomy and hydrodynamics.
b) Grazing experiments.
c) Investigation of gut contents and faecal material.

Studies on the feeding apparatus indicate that *E. superba* is able to ingest food particles spanning a wide size range, including nanoplanktonic organisms (2–20 μm in diameter). Organisms of this size are known to comparise a large proportion of the phytoplankton of the Southern Ocean throughout the year and represent an important potential food stock available for krill. The main components of the nanoplankton are diatoms and flagellates. Whilst most small diatoms can be identified by optical microscopy, flagellates are fragile and readily disrupted. They are difficult, if not impossible, to identify by optical microscopy in gut and faecal material. To better understand the diet and feeding preferences of krill we are undertaking electron microscopical analysis of the gut contents and faeces of wild krill. Further, we have been feeding various species of flagellates to krill in laboratory culture to investigate which structures survive digestion. These studies have shown that the organic external scales of the prasinophyte *Pyramimonas gelidicola* and the scales and thread-like material produced by the prymnesiophyte *Phaeocystis pouchetii* are not digested by *E. superba*. These have been found in gut and faecal material of wild krill. Large numbers of nanoplanktonic organisms occur in gut and faecal material from krill. The small diatom *Nitzschia curta* is especially abundant. Choanoflagellates and lightly silicified diatoms including *Dactyliosolen antarcticus* also occur. These studies support morphological evidence that krill feed extensively on nanoplanktonic organisms.

1. Introduction

The Antarctic krill, *Euphausia superba* DANA is receiving increased attention both because of its potential as a resource and also because of its apparent key role in the Antarctic marine food web where it forms an important link between the primary producers and many of the top predators including the baleen whales, crabeater seals and penguins.

Considering the importance of krill, its diet and mechanism of feeding are not well understood. To date, the three main approaches used to investigate the feeding and diet of krill are hydrodynamic and anatomical studies on the feeding apparatus, (BARKLEY, 1940; NEMOTO, 1967; McCLATCHIE and BOYD, 1983; MORRIS, 1984), grazing experiments (KATO *et al.*, 1979; MEYER and EL-SAYED, 1983; BOYD *et al.*, 1984; IKEDA and DIXON, 1984; MORRIS, 1984) and investigations of gut contents and faecal material (BARKLEY, 1940; MARR, 1962; NEMOTO, 1968, 1972; PAVLOV, 1969, 1971). Until recently, identification of organisms in gut contents and faeces have been undertaken by optical microscopy. This technique, however, only permits the identification of the larger and more robust organisms, such as diatoms and silicoflagellates.

Estimates of the contribution of nanoplankton (organisms in the size range 2–20 μm in diameter) to the biomass of phytoplankton in Antarctic waters are of the order of 60–70% and responsible for 48–90% of the primary productivity (EL-SAYED and TAGUCHI, 1981; VON BRÖCKEL, 1981; HOLM-HANSEN and HUNTLEY, 1984; YAMAGUCHI and SHIBATA, 1982; PERRIN *et al.*, 1986). Recent evidenec suggests that picoplankton (organisms in the size range 0.2–2 μm in diameter) may also constitute a large proportion of what has been ascribed to the nanoplankton (PLATT *et al.*, 1983). Very many of these smaller organisms cannot be identified by light microscopy and require transmission electron microscopy for their identification which is based on the morphology of extracellular scales and spines and cellular ultrastructure (JOHNSON and SIEBURTH, 1982). Here we report scanning and transmission electron microscopical studies on the gut contents and faecal material collected from wild populations as well as krill maintained in laboratory culture and fed on nanoplanktonic flagellates.

2. Materials and Methods

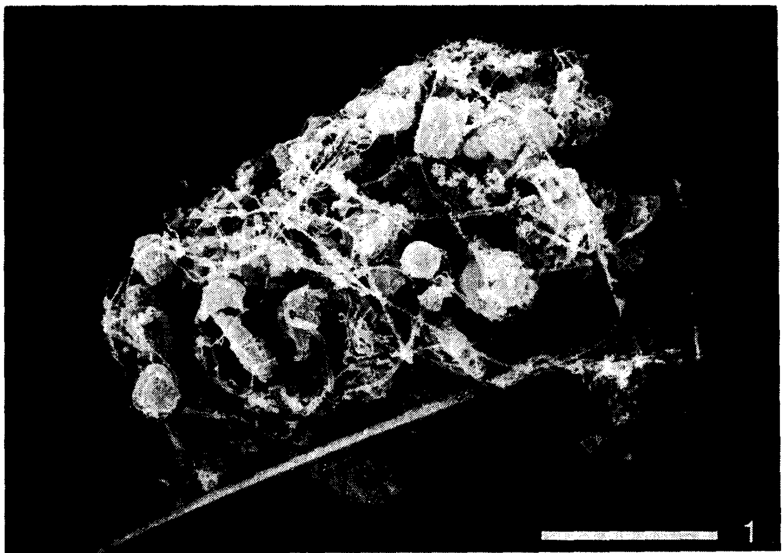
The Antarctic krill, *Euphausia superba* DANA, were caught using an RMT 8 net during the austral summer of 1980–81 during the First International BIOMASS Experiment (FIBEX), the Antarctic Division BIOMASS Experiment (ADBEX) in 1983 and during the second phase of the Second International BIOMASS Experiment (SIBEX II) in January 1985. The stomach and hind gut were dissected from living animals and immediately fixed in 1% glutaraldehyde made up in seawater filtered through a 0.22 μm pore size Millipore filter. Faeces were collected from krill caught on these cruises and also preserved in 1% glutaraldehyde.

In the laboratory krill were maintained at 0°C. They were fed, with or without a period of starvation, on pure cultures of the flagellates *Pyramimonas gelidicola* and *Phaeocystis pouchetii* for periods up to one week before their faeces were collected.

For the examination of gut contents and faecal material by scanning electron microscopy, the glutaraldehyde fixed material was macerated in filtered sea water and the slurry allowed to settle on polylysine treated glass coverslips for 10–30 minutes (MARCHANT and THOMAS, 1983). Coverslips with material attached were dehydrated through a graded acetone series, dried by the critical point method and sputter coated with approximately 30 nm thickness of gold. For transmission electron microscopy, the slurry was applied to polylysine treated formvar coated electron microscope grids, negative stained with uranyl acetate or shadow-cast with platinum-palladium.

3. Results

Scanning electron microscopy reveals that a wide diversity of phytoplankton and protozooplankton are eaten by krill. Readily apparent in gut contents and faecal material examined in this way are diatoms of various sizes (Figs. 1–3, 5, 6), including large numbers of very small cells (Figs. 3, 6), siliceous cysts of indeterminate taxonomic position (Figs. 3, 4), silicoflagellates (Fig. 7) and choanoflagellates (Figs. 8–11). Less conspicuous are flagellates and their extracellular scales (Figs. 7, 12–14). Diatoms and siliceous cysts that are recovered from krill faeces are almost invariably well "cleared" (Figs. 2–6), with much of the organic material removed. In other organisms, however,



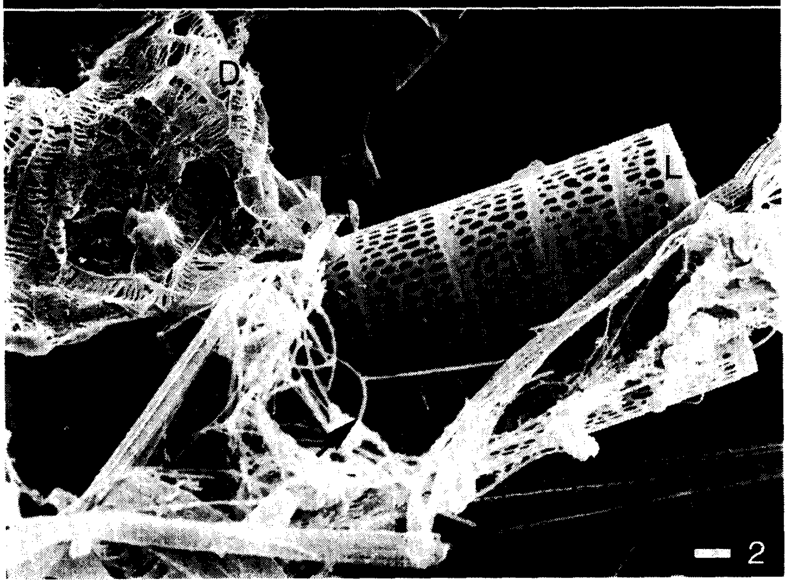


Fig. 1. Part of a krill faecal pellet showing a diversity of remains of organisms. SEM. Scale marker = 10 μm.

Fig. 2. Frustules of the diatoms Dactyliosolen tenuijunctus *(D),* Leptocylindrus mediterraneus *(L) and* Chaetoceros *sp. (C) together with other organisms including choanoflagellates. SEM. Scale marker = 1 μm.*

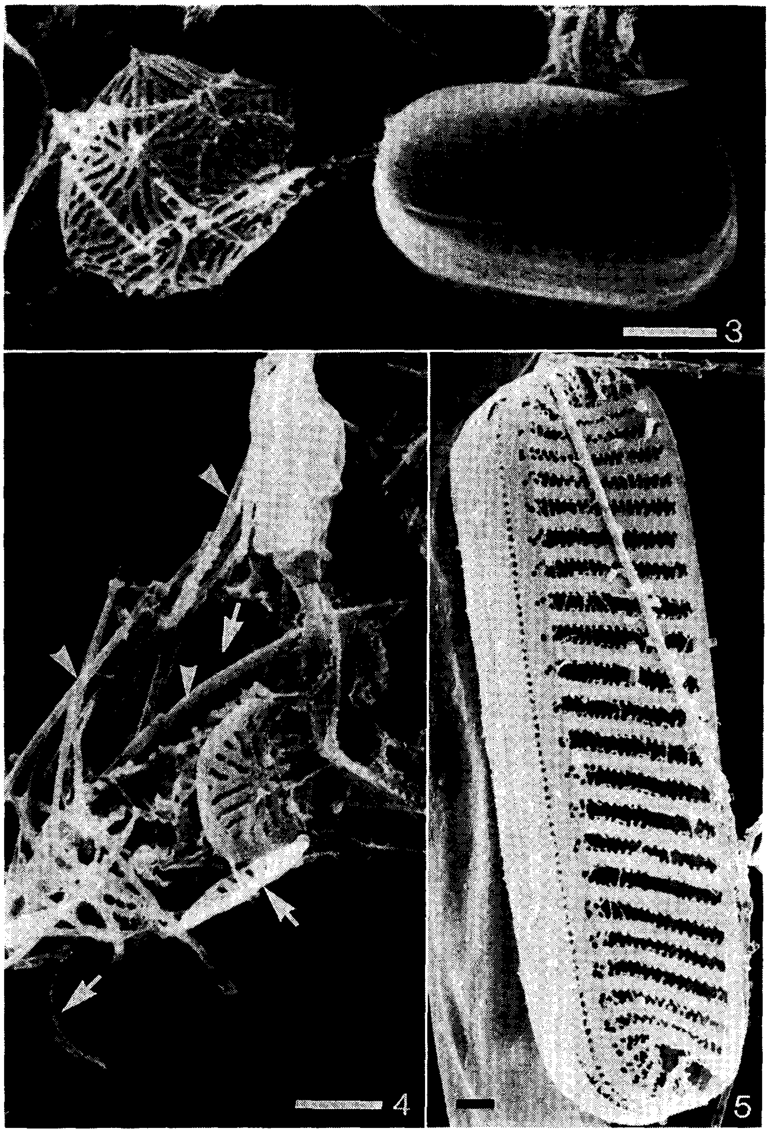


Fig. 3. The diatom Nitzschia curta *and a siliceous cyst from gut contents. SEM. Scale marker = 1 μm.*

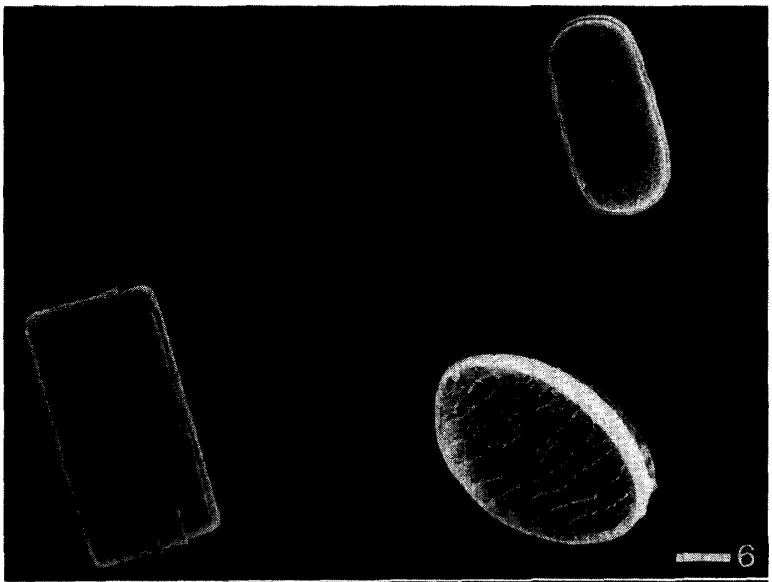
Fig. 4. Fragments of the wall of a siliceous cyst (arrows) and costal strips of a choanoflagellate (arrowheads). SEM. Scale marker = 1 μm.

Fig. 5. Nitzschia curta *from krill faeces showing some evidence of solubilization of the silica around the bands of areolae. SEM. Scale marker = 1 μm.*

organic material remains. Costal strips of the loricae of choanoflagellates are often seen attached to one another and the veil which lies on the inside of the lorica of some species (Fig. 8) are apparently unaffected by passage through the krill gut.

The extracellular scales and flagellar hairs of *Pyramimonas* (Figs. 12–15) survive digestion both in the wild as well as in laboratory culture as do the scales and thread-like material produced by *Phaeocystis* in culture (Figs. 16, 17).

The small diatom *Nitzschia curta* occurs frequently in high numbers in both gut contents and faecal material (Figs. 3, 5, 6).



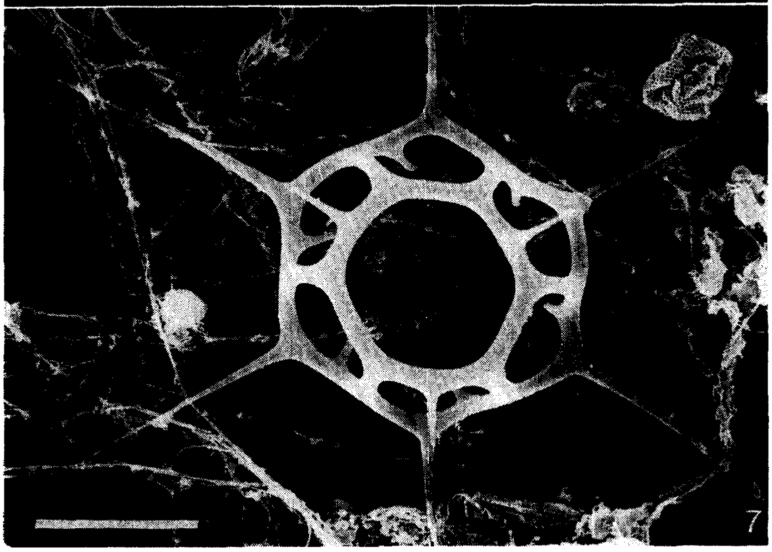
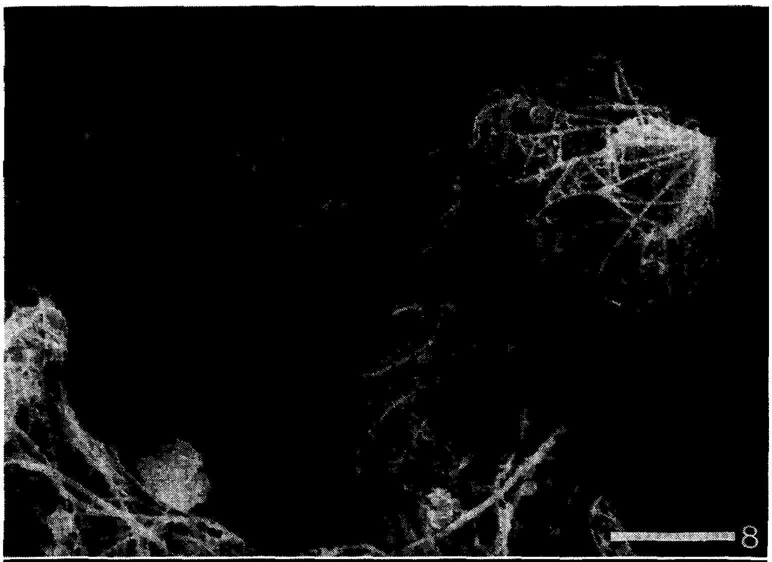


Fig. 6. Small diatoms Nitzschia curta *and* N. angulata. *SEM. Scale marker = 1 μm.*

Fig. 7. The intact skelecton of the silicoflagellate Distephaunus speculum *and a* Pyramimonas *sp. cell (P) the external box scales of which remain attached. SEM. Scale marker = 10 μm.*

4. Discussion

Euphausia superba concentrates food particles from seawater by the movement of a filter basket composed of the thoracic legs, the thoracopods. Whether the accumulation of food is solely by filtration by the thoracopods acting as sieves or whether the surface chemistry and the charge on the food particles influence of its ingestion remains unclear (GERRITSEN and PORTER, 1982; CLARKE and MORRIS, 1983). Recent studies indicate that krill are, to some extent, selective in their feeding indicating that it is likely a variety of feeding techniques are employed (MEYER and EL-SAYED, 1983; IKEDA and DIXON, 1984).



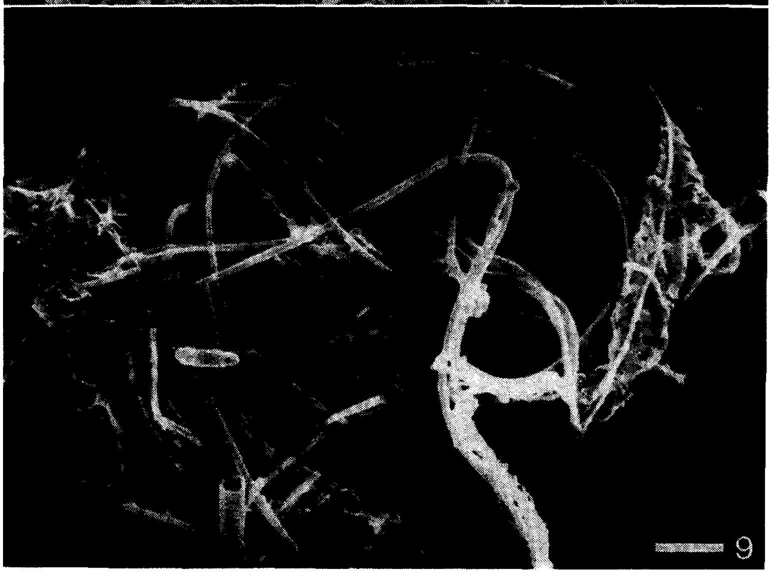


Fig. 8. Choanoflagellates, Diaphanoeca *sp. showing the relatively intact lorica and the fibrillar veil-like material both at the anterior end and around the posterior part where the protoplast is usually situated. SEM. Scale marker =5μm.*

Fig. 9. Highly distorted lorica of a choanoflagellate, the costal strips of which remain substantially attached to one another. SEM. Scale marker = 1μm.

Krill feed predominantly on phytoplankton during the summer (MAUCHLINE, 1980). This evidence comes from both the examination of gut contents and the biochemical analysis of lipids and pigments (CLARKE, 1980; NEMOTO, 1968). The extent to which krill are carnivorous or cannibalistic remains to be clarified. Reports exist of finding copepod remains in krill gut contents (MARR, 1962) and observing cannibalism in krill maintained in laboratory conditions (McWHINNIE and MARCINIAK, 1964).

Relatively little attention has been paid to the grazing of krill on nanoplaknton. Krill have been reported to be absent from areas dominated by such large spiny diatom genera as *Chaetoceros* and *Corethron* and to favour regions where smaller diatoms occur

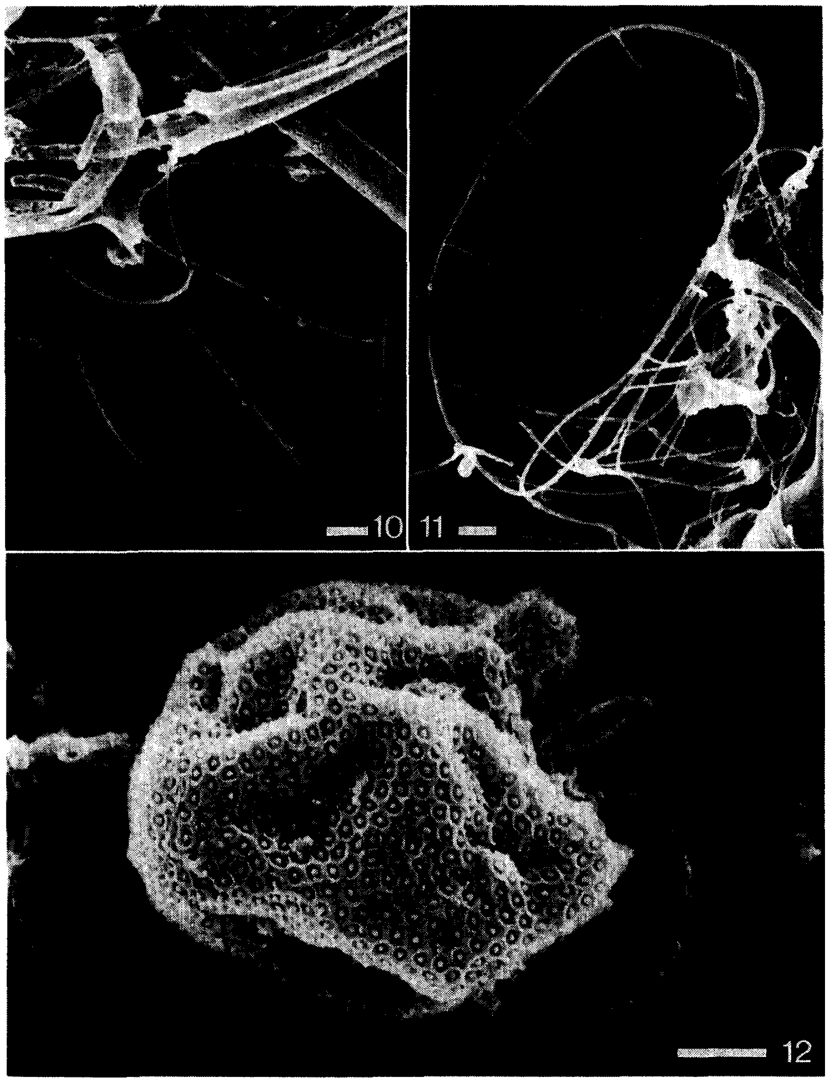


Fig. 10. *Diatoms and the essentially intact lorica of a choanoflagellate. SEM. Scale marker = 1 μm.*

Fig. 11. *The lorica of the choanoflagellate* Parvicorbicula socialis. *SEM. Scale marker = 1 μm.*

Fig. 12. Pyraminonas obovata *from faeces showing the distinctive scales. SEM. Scale marker = 1 μm.*

(KAWAMURA, 1973). However, other studies (*e.g.*, MARR, 1962), including our own, indicate that krill do ingest large diatoms and the silicoflagellate *Distephanus speculum* and a considerable number of these organisms have been found in faecal samples. A number of recent studies have demonstrated that nanoplankton are a major component of the phytoplankton (EL-SAYED and TAGUCHI, 1981; VON BRÖCKEL, 1981; MARCHANT, 1985). In Prydz Bay we have found that the nanoplankton is composed mainly of flagellates, pennate diatoms and the so called siliceous cysts (MARCHANT, 1985). In contrast, the nanoplankton in the vicinity of South Georgia was composed mostly of both pennate and centric diatoms, flagellates and dinoflagellates. Recent studies on the feeding apparatus of krill indicate that they are capable of ingesting organisms smaller than

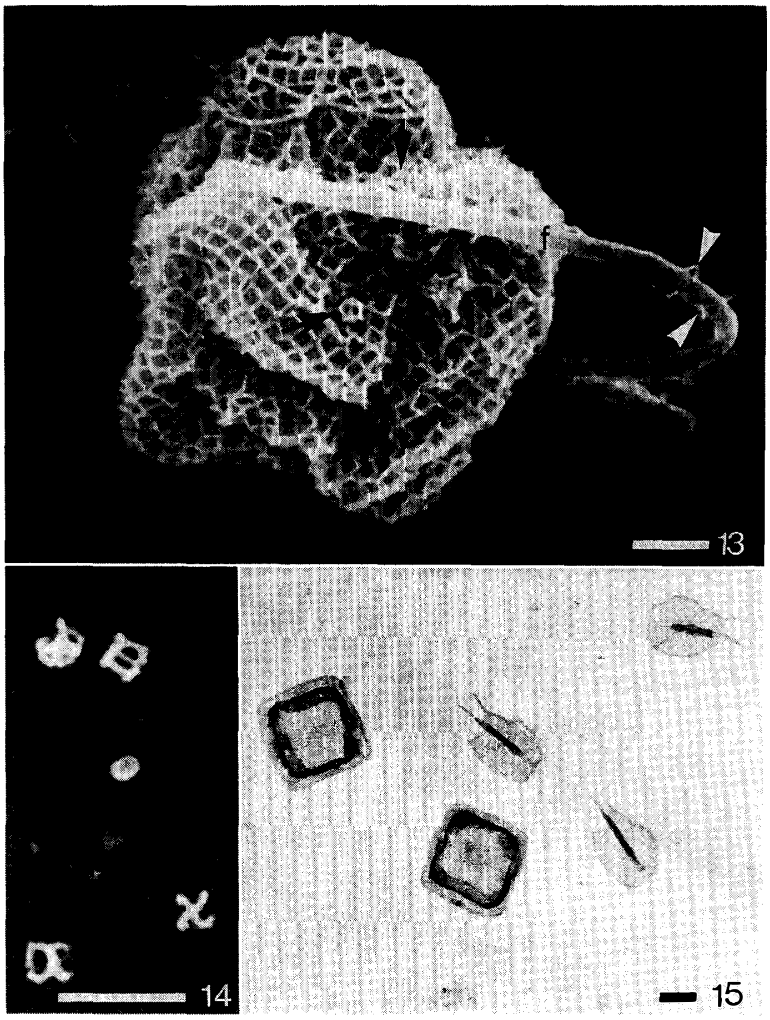


Fig. 13. Pyramimonas gelidicola *recovered from krill faeces with the flagella (F) partially intact and some flagellar hairs attached (arrowhead). The body of the alga is covered with a layer of box scales. Very few of the outer layer of crown scales (arrows) remain. SEM. Scale marker = 1 μm.*

Fig. 14. *Crown scales of* Pyramimonas *recovered from krill gut contents. SEM. Scale marker = 1 μm.*

Fig. 15. *Box scales from the cell body and limulus scales from the flagella of* Pyramimonas gelidicola *recovered from faeces of krill fed on this alga. TEM. Scale marker = 100 nm.*

3 μm in diameter but retain organisms of 10–20 μm with greatest efficiency (McClatchie and Boyd, 1983). Our finding of the smallest nanoplanktonic organisms in the gut and faeces of krill provides direct evidence that krill are utilizing this most abundant component of the phytoplankton. Studies on gut contents and faecal material in the past have indicated that krill consume diatoms, silicoflagellates and dinoflagellates, organisms with robust and recognizable walls or skeletons large enough to be identified by optical microscopy. Transmission electron microscopy has revealed that krill eat scale-covered flagellates in both the laboratory and in the wild.

Diatoms and the silicoflagellate *D. speculum* found in the hind gut and faeces of

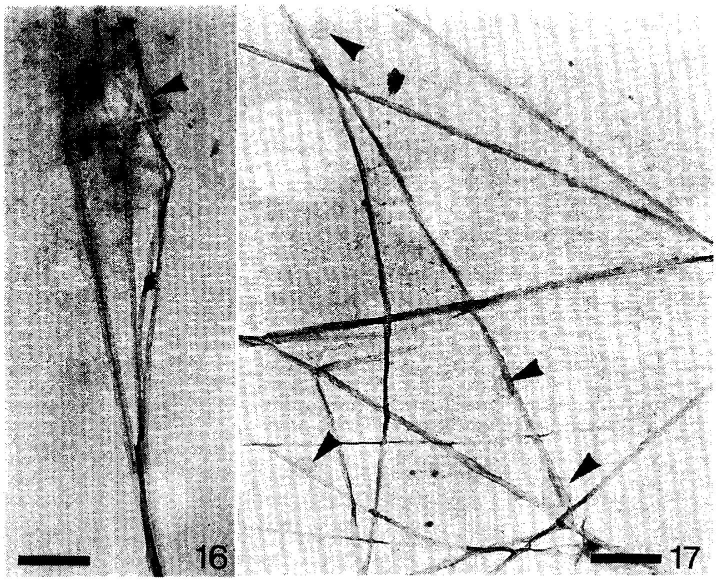


Fig. 16. Thread-like material with disrupted pentagonal array (arrowhead) that is produced by Phaeocystis pouchetii. *This specimen was found in krill faeces. TEM. Scale marker = 500 nm.*

Fig. 17. Thread-like material and scales (arrowheads) characteristic of Phaeocystis pouchetii, *found in the faeces of krill that had been fed on this alga. TEM. Scale marker = 500 nm.*

krill are most often devoid of their plasma membrane and cellular components other than the siliceous structures. However, other organic materials, including the substance that interconnects the costal strips in the lorica of choanoflagellates (LEADBEATER, 1981) and the scales of *Pyramimonas* and *Phaeocystis* are apparently unaffected by the digestive enzymes of krill. Recent preliminary investigations have shown that the scales of *Pyramimonas pseudoparkeae* are predominantly carbohydrate in composition with about 4% protein. The scale polysaccharide is pectinaceous, containing polygalacturonic acid with neutral sugars and is sulphated (AKEN and PIENAAR, unpublished data). Thus krill may lack an α 1–4 hydrolase.

Little published information exists on the digestive enzymology of krill. Finding that some lightly silicified parts of diatom frustules appear eroded indicates the possibility of the dissolution of the silica. This is more likely to occur at high pH.

Acknowledgments

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