Bacterial epibionts of the deep-sea copepod *Calanus cristatus* Krøyer

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ABSTRACT

Bacterial attachment is reported for *Calanus cristatus*, the dominant, large, subarctic calanoid copepod in the Sea of Japan. Bacterial epibionts of this copepod whose fifth copepodites (CV) exhibit a broad vertical distribution (100-2 500 m) with animals moving to deeper layers as they grow older, occurred in CV obtained from 500-2 000 m. This suggests that somewhat older individuals of CV are more easily colonized by bacteria. Most bacteria attached to CV were slender rods, 2-3 µm. On an average 10% of CV suffered from bacterial colonization. No corpses of CV had bacteria, whereas the corpses of male and female were covered with a small number of bacteria which may be chitinoclastic.

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RÉSUMÉ

Colonisation bactérienne du copépode profond *Calanus cristatus* Krøyer

Ce travail a pour objet la colonisation par les bactéries de *Calanus cristatus*, grande espèce de copépode calanoide subarctique, dominante en mer du Japon. Les copépodites au stade V (CV) de cette espèce ont une grande distribution verticale (100-2 500 m), en raison du déplacement des animaux vers les couches profondes au cours de leur développement. Des bactéries épibiontes des copépodites ont été observées sur des échantillons récoltés entre 500 et 2 000 m. Les résultats suggèrent que les individus les plus âgés des CV sont plus facilement colonisés par les bactéries. La plupart des bactéries colonisatrices sont des bacilles fins, d'une longueur de 2 à 3 µm. En moyenne, 10% des CV sont colonisés par des bactéries. Aucun cadavre de CV n'est colonisé par les bactéries, tandis que les cadavres des mâles et des femelles sont couverts d'un petit nombre de bactéries pouvant être chitinoclastiques.

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INTRODUCTION

SEM examinations of copepods collected from aquatic environments have revealed bacterial attachment to copepods (Sochard *et al.*, 1979; Colwell *et al.*, 1980; Holland, Hergenrader, 1981; Huq *et al.*, 1983; Nagasawa *et al.*, 1985; Nagasawa, Nemoto, 1986 *a; b*: Nagasawa, 1986 *a; b*). Data on the association of bacteria with copepods reported to date pertain to coastal epipelagic species, particularly *Acartia*. Most bacteria attached to copepods were short, long, thick, slender or indented rods about 1 µm long (Nagasawa *et al.*, 1985; Nagasawa, Nemoto, 1986 *b*; Nagasawa, 1986 *b*), but chain-forming cocci have been found (Nagasawa, 1986 *a*). Bacteria capable of producing slime were also present; slime appears as filaments, interconnected fibres or film (Nagasawa, 1986 *a*). To date, no information on bacterial attachment to meso- and bathypelagic species of copepods has been reported. The copepod *Calanus cristatus* Krøyer is a large subarctic herbivorous calanid (Miller *et al.*, 1984). This copepod occurs abundantly in the Sea of Japan, from the thermocline to a depth of 3 850 m (Terazaki *et al.*, in press).

In general, bacteria which often appear to have settled on a substratum could be washed off by a stream of water, whereas firm attachment appears to occur only after a bacterium has settled for several hours (ZoBell, 1943). Attachment of bacteria by extracellular polymeric adhesives involves three stages: reversible adhesion, irreversible adhesion and microcolony formation (Fletcher, 1980).

Seki (1965) roughly estimated the rate of chitin decomposition; chitin may be mineralized within 370 days in the intermediate waters at 5°C and within 500 days in the deep waters at a few degrees Celsius.

Here we report bacterial atttachment to *C. cristatus* collected from depths below 200 m in the Sea of Japan. We also discuss the decomposition of copepod exoskeleton.

MATERIALS AND METHODS

Calanus cristatus were obtained from two stations in the Sea of Japan (Fig. 1) during the cruise of the Hakuhō Maru (KH-84-3) of the Ocean Research Institute, University of Tokyo. Zooplankton samples were taken horizontally with MTD nets (Motoda, 1969) of 330-μm mesh mounted on 56-cm rings, and preserved in 10% neutralized formalin seawater solution. Six and five layers were sampled simultaneously for 40 mn at stations 11 and 29, respectively.

In the laboratory, both male and female, fifth copepodites (designated CV) of *C. cristatus* and their corpses were sorted and then examined in a JSM-35 scanning electron microscope following the preparation procedure described by Nagasawa *et al.* (1985). Fifth copepodites are the last stage of copepodites and they will become adults after molting. The use of the term "corpse" is based on the appearance of the copepod with an obvious loss of muscle and internal tissue. The number of specimens examined per sample is listed in the Table together with details of sampling records.

The density of bacteria is defined as number of bacteria per unit surface area: several to several tens of bacteria are considered to be low concentrations; more than 100 bacteria per unit surface area are considered to be large concentrations.

In this study we use CWB (fifth copepodites with bacteria) and CCWB (corpses of fifth copepodites with bacteria) whose definition is basically the same as that of Nagasawa (1986*a*).
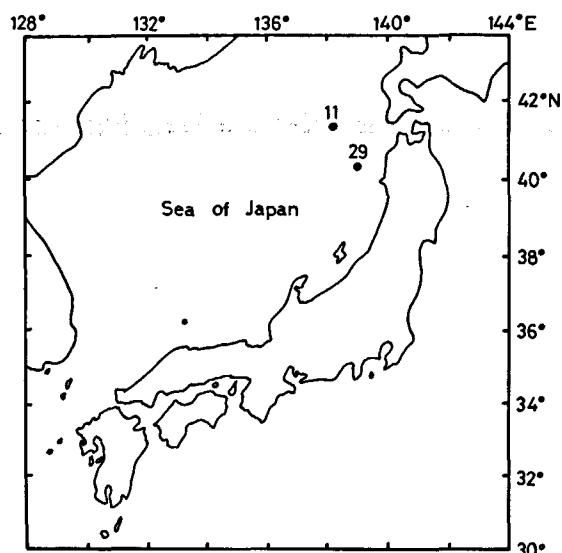

Figure 1

Plankton sampling stations in the Sea of Japan on 13-14 *September* 1984 (*station* 11) *and on* 27 *September* 1984 (*station* 29).

RESULTS

Live copepodites

Of the 11 samples examined, six contained CV colonized by bacteria. Such specimens were obtained from 1 000, 1 500 and 2 000 m at station 11 and from 500, 1 700 and 2 000 m at station 29 (Tab.). Bacterial attachment did not occur in CV collected at 200 m, probably because this CV is still young compared with CV in deeper layers.

Attachment of bacteria to CV was site selective; bacteria were found somewhere mostly on the ventral side of an animal. The density of CV-associated bacteria varied with depth; small numbers of bacteria were present on CV from the upper layers (Fig. 2), whereas large numbers of bacteria were attached to CV from deeper layers (Fig. 3). Most bacteria attached to CV

Table

Details of sampling records, the number of fifth copepodites (CV) *and corpses examined and percentage of these specimens with bacteria:* 1) *female;* 2) *male;* 3) *fifth copepodites with bacteria* (CWB); 4) *corpses of fifth copepodites with bacteria* (CCWB); 5) *zero means that corpses were not available;* (-) *indicates no data.*

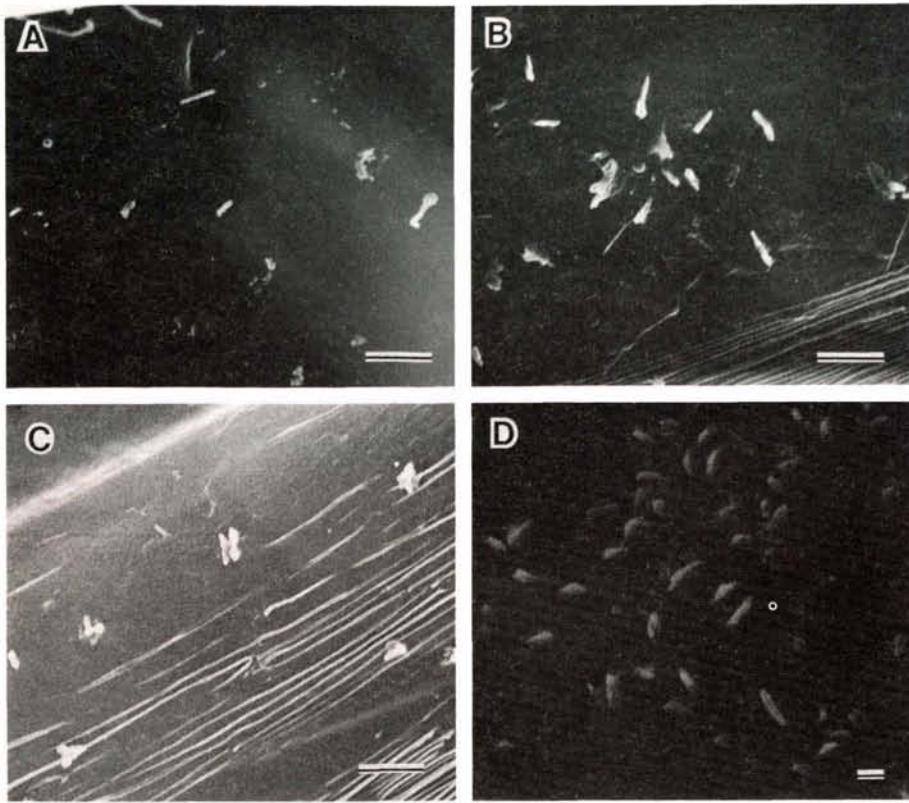| Station	Date (1984)	Depth (m)	No. of fifth copepodites examined	No. of corpses examined[5]	Incidence (%) of CWB[3]	Incidence (%) of CCWB[4]
11	13-14 September	200	9	0	0	–
		500	20	0	0	–
		700	20	0	0	–
		1000	20	0	5.0	–
		1500	20	20[2]	45.0	15.0[2]
		2000	20, 16[1]	12[1], 13[2]	10.0, 50.0[1]	8.3[1], 0[2]
29	27 September	200	5	10	0	0
		500	20	8	15.0	0
		700	20	0	0	–
		1700	20, 15[2]	0	20.0, 0[2]	–
		2000	20	10	10.0	0

Figure 2
Scanning electron micrographs of bacterial attachment of fifth copepodites (CV) obtained from 500 m at station 29. Three specimens (A, B-C and D) were used. Bacterial attachment occurs on the segment boundary of swimming leg (A), the base of mandible (B, C) and the lateral side of urosome (D). Scale bars indicate 1 (D) and 5 (A, B, C) μm.
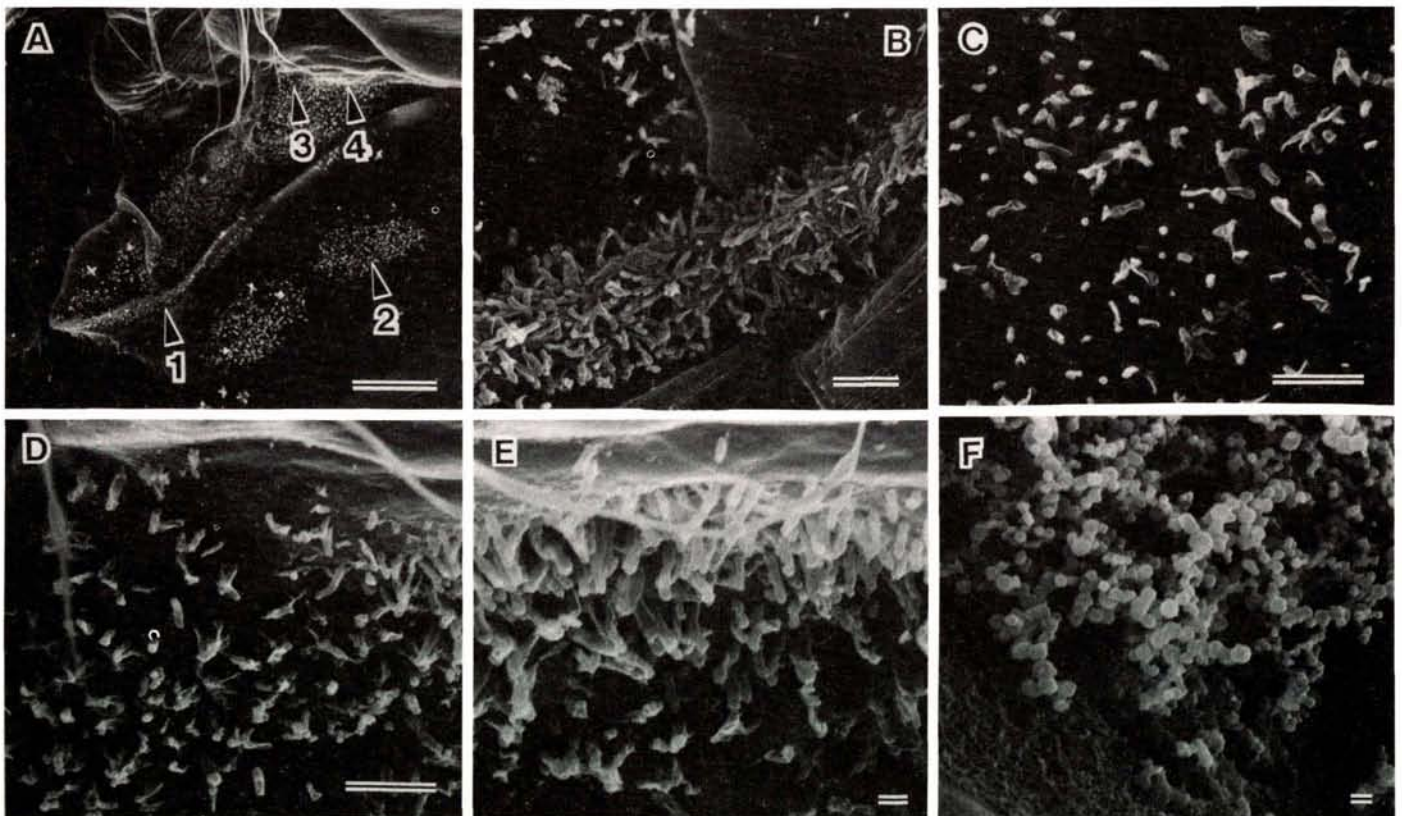

Figure 3
Scanning electron micrographs of bacterial colonization of fifth copepodites (CV) obtained from 2000 m at station 29. Two specimens (A-E and F) were used in the following six pictures. (A) Several colonies of rods are present at the base of the maxilliped. Higher magnification of the parts marked "1", "2", "3" and "4" in (A) is found in (B), (C), (D) and (E), respectively. (F) Slime and a large density of coccoid bacteria are observed close to antennule. Scale bars indicate 1 (E, F), 5 (B, C, D) and 50 (A) μm.

were extremely slender rods 2-3 μm in length. Along with these rods, coccoid bacteria associated with mucous material were observed (Fig. 3).

Two types of bacterial attachment were observed on CV: horizontal and polar attachments. Polar attachment was more frequent than horizontal attachment.

Although values of CWB ranged from 0 to 45.0%, mean values at stations 11 and 29 were similar; 10 and 9%, respectively. Values of CWB did not always vary with increasing depth, but copepodites from deeper layers were colonized by large numbers of bacteria. Bacterial colonization was observed on 50% of females from 2000 m at station 11, but each individual was colonized by a small number of bacteria. No bacteria were present on males obtained from 1700 m at station 29 (Tab.).

Corpses

At station 11 no corpses of CV were found above 1000 m, but corpses of females and males were obtained from deeper layers (Tab.) since adults inhabit deeper layers than most CV and may decay more rapidly than CV. Some corpses of females and males had a small number of bacteria (Fig. 4). Corpses of CV were obtained from 200, 500 and 2000 m at station 29 and they had no bacteria (Tab.).

DISCUSSION

Bacterial epibionts of C. cristatus are characterized by site selection, as has been reported by Nagasawa et al. (1985) and Nagasawa and Nemoto (1986 a; b) for other species, and by a higher frequency of polar attachment than horizontal attachment, which is the reverse for Acartia spp. from Tokyo Bay samples (Nagasawa, 1986 b).

Presence or absence of bacteria on the corpses may depend on the time elapsed after death. Bacteria attached to corpses differed from those attached to live copepodites at depths of 1700 and 2000 m (Fig. 3); however, attachment was rather similar to that on live copepodites at 500 m (Fig. 2) in terms of density and shape.

Individuals of C. cristatus undertake ontogenetic vertical migration during their life cycle (Heinrich, 1957; Sekiguchi, 1973), and fifth copepodites (CV) of C. cristatus which have a life span of about seven months (Heinrich, 1957; Nakai, 1969) have been reported from above 1600 m (Miller et al., 1984) to 2000-2500 m (Sekiguchi, 1973; Terazaki et al., in press). Individuals inhabiting bathypelagic layers are larger in size (Sekiguchi, 1973) and wet weight (Terazaki et al., in press) and contain a greater amount of lipid than those inhabiting upper layers (Ikeda, 1970). This suggests that animals from upper layers may have recently molted, whereas those from deeper layers are older CV. However, it is not certain how long individuals of CV stay at a certain depth or how quickly they descend.

In the present study, bacterial colonization was observed on animals from 500-2000 m, suggesting that somewhat older individuals of CV may be more easily colonized by bacteria. However, the ecological significance of bacterial attachment to CV's of older individuals or adults remains unclear. Nagasawa (1986 b) states that the relationship between Acartia and bacteria is due neither to mutualism nor to commensalism, indicating the presence of pores and slots which are scars of previous bacterial attachment taking place polarly and horizontally, and that the degree of bacterial infestation ($>10^5$ cells per copepod) may affect differences in respiratory rate for the same species of copepod. As for C. cristatus, such indications of bacterial colonization are not obtained. In addition, bacteria are not so abundant that they cannot affect oxygen consumption of Calanus, and the incidence of Calanus with bacteria is low, unlike Acartia (Nagasawa et al., 1987).

Rods of 2-3 μm attached to C. cristatus are somewhat larger than those attached to Acartia (Nagasawa, 1986 a; b). The former is extremely slender and the latter is plump, suggesting that this morphological difference depends on species and environmental factors.

Harding (1973) described progressive decomposition of Calanus finmarchicus under different conditions. At 4°C, by the second day, copepod exoskeletons were completely covered with bacteria, but by the eleventh day very few bacteria remained on the chitin. Internal tissues were indistinguishable beyond the sixth day and
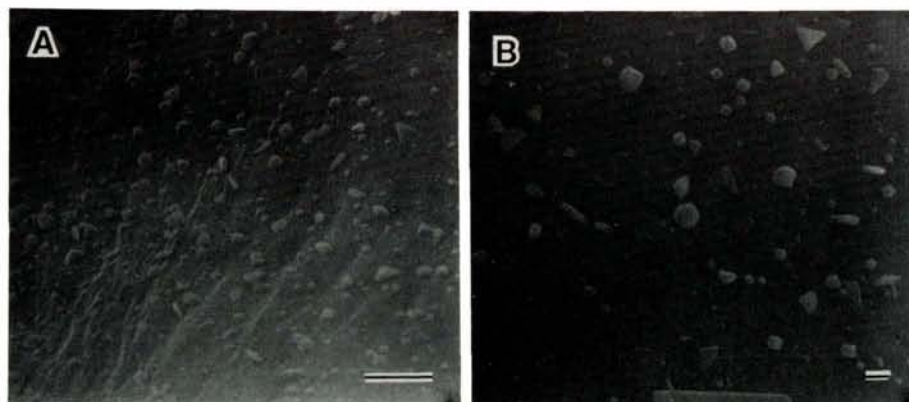

Figure 4
Scanning electron micrographs of corpses of males obtained from 1500 m (A, B) at station 11. A small number of bacteria are found close by antenna (A) and mandible (B) together with indeterminate objects. Scale bars indicate 1 (B) and 5 (A) μm.

on the eleventh day nothing remained of the copepod except the exoskeleton. We believe chitinoclastic bacteria would have appeared on the exoskeleton after 11 days soon after the disappearance of the labile internal tissues. Seki (1965) has shown that percentage of one type of chitinoclastic bacteria increases rapidly in proportion to the disappearance of easily decomposable organic matter of the plankton. These chitinoclastic bacteria are considered to be largely responsible for the decomposition of chitin in the sea. Corpses from station 29 may be exoskeleton which is not yet covered with chitinoclastic bacteria, whereas those from station 11 may be exoskeleton to which a small number of chitinoclastic bacteria have attached. It is not clear how long these corpses have been dead. Water temperature where corpses were obtained ranged from 0.15 to 0.72°C. If bacteria attached to corpses were chitinoclastic, complete mineralization of these corpses of *C. cristatus* would take far more than 500 days on the basis of Seki's (1965) estimation of chitin decomposition because of markedly low temperatures in the Sea of Japan.

Acknowledgements

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