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Microbial colonization of copepod body surfaces and chitin degradation in the sea

M. Kirchner

Biologische Anstalt Helgoland (Meeresstation); D-27498 Helgoland, Germany

ABSTRACT: Next to cellulose, chitin (composed of N-acetyl-D-glucosamine sugar units) is the most frequently occurring biopolymer in nature. Among the most common sources of chitin in the marine environment are copepods and the casings of their fecal pellets. During the mineralization of chitin by microorganisms, which occurs chiefly by means of exoenzymes, nitrogen and carbon are returned to the nutrient cycle. In this study, the microbial colonization of the moults (exuviae), carcasses and fecal pellets of *Tisbe holothuriae* Humes (Copepoda: Harpacticoida) was examined in the laboratory. Results obtained with DAPI staining indicated that a succession of microorganisms from rodshaped bacteria and cocci to starlike aggregates took place, followed by the yeastlike fungus *Aureobasidium pullulans* (de Bary) Arnaud. No differences were noted between moults from various developmental stages, from nauplius to adult. The ventral sides and extremities of exuviae and carcasses were more rapidly colonized than other parts of the bodies. The casings of fecal pellets were frequently surrounded by bacteria with fimbriae or slime threads. In situ studies of chitin degradation (practical grade chitin from crustacean shells) with the mesh bag technique showed that about 90 % of the original substance was lost after 3 months exposure in seawater at temperatures between 10 and 18 °C. Chitinase activity was measured in the water at two stations near Helgoland, an island in the North Sea. A higher exoenzymatic activity was found in the rocky intertidal zone, compared to the Station Cable Buoy located between the main and Düne island. These values correspond to the higher bacteria numbers (cfu ml^{-1}) found in the rocky intertidal: 10 to 100× greater than those found at the Cable Buoy Station.

INTRODUCTION

Since chitin, next to cellulose, is one of the most frequently occurring biopolymers in the sea, the fate of chitinaceous materials is of great importance in the recycling of nutrients. Apart from the shells of crustaceans, sources of chitin also include molluscs and fungi, the egg shells of nematodes and rotifers, cuttlefish bone, squid pen, coelenterates, diatoms and protozoans (ZoBell & Rittenberg, 1937; Dunn & Liberman, 1983; Smucker & Dawson, 1986; Gooday, 1990; Greco et al., 1990). According to Johnstone (1908), the production of chitin by copepods alone is estimated at several million tons annually. In addition to their exoskeletons and exuviae, the casings of copepod fecal pellets may also be an important source of chitin (Yoshikoshi & Kô, 1988).

Bacteria and fungi are the main agents in the mineralization of organic substances, including chitin. In this paper, the colonization of chitinaceous substances such as the exuviae, carcasses and fecal pellets of *Tisbe holothuriae* by microorganisms, and the

occurrence of bacteria and chitinase activity in seawater were studied, as well as the degradation rate of crustacean chitin in the field.

MATERIALS AND METHODS

Microbial colonization of chitinaceous substances: copepod moults, carcasses and fecal pellets

Two healthy ovigerous females from a laboratory culture of *Tisbe holothuriae* (culture conditions as described in Rieper, 1978) were washed 2× with sterile seawater, then placed into separate glass vessels with 40 ml sterile seawater. Each animal received about 1 mg dried, sterile fish food mixture. The vessels were then left standing overnight in the laboratory at room temperature (20 °C); the adult females were removed as soon as the eggs hatched and the nauplius larvae appeared. Initially the vessels were examined daily, then at intervals of several days. After each examination, all exuviae present were removed. Some were fixed immediately in 0.2 µm filtered 3.7 % formalin-seawater mixture. The remainder were allowed to age in vessels with about 5 ml sterile seawater for 7–21 days at 20 °C before they were also fixed in formalin. Fixed samples were stored in the refrigerator at 2 °C until examination. Exuviae from the progeny of ♀ 1 were investigated under a light microscope (Zeiss Standard, Nomarski differential-interference contrast optics); those from ♀ 2 progeny were stained with DAPI (Porter & Feig, 1980; Coleman, 1980) and examined with epifluorescence microscopy. From ♀ 1, 54 nauplius (N) and 78 copepodid (C) exuviae were recovered, from which 32 N and 47 C exuviae were microscopically evaluated. From ♀ 2, 84 N and 85 C exuviae were recovered, from which 46 N and 34 C exuviae were DAPI-stained and examined.

The carcasses of 2 adult *T. holothuriae* from laboratory stock cultures, which were moribund and then died of apparently natural causes, were washed 2× with 50 ml sterile seawater and transferred to separate glass vessels with 40 ml sterile seawater. The vessels were left standing at about 20 °C and examined regularly under a Wild dissecting microscope for a total duration of 43 days.

Fecal pellets were obtained from a healthy stock culture of *T. holothuriae*; 22 adult and copepodid ♂♂ and ♀♀ were washed 2× with 50 ml sterile water, then transferred to a glass vessel with 40 ml sterile seawater. About 3 mg dried sterile fish food mixture were added. After 29 h at 20 °C, the animals were removed with a sterile pipette. Fecal pellets present were carefully separated from food remains and transferred to 6 small watch glasses, each containing 5 ml sterile seawater. About 15 pellets were placed into each glass. After aging for periods of 1, 2, 4, 7, 14 and 21 days at 20 °C, the contents of the glasses were fixed with 5 ml 3.7 % formalin-seawater and stored in the refrigerator at 2 °C. Fecal pellets were later examined unstained with light microscopy, and then with epifluorescence microscopy after DAPI staining.

Bacterial numbers and chitinase activity

From May to September and November to December in 1991 and from April to August in 1992, samples of water were taken at regular intervals from two sites near the island of Helgoland in the southern North Sea. Station 1 is located in the rocky intertidal

zone on the north side of the island. By means of a sterile glass bottle, a 100-ml sample was taken by hand near the surf zone at low tide; sampling here was done on a bi-monthly basis. The second station, Cable Buoy, is located between the dune and main island. Samples were obtained weekly from 1 m below the surface, where the total depth is 6–9 m. Bacterial numbers were determined after suitable dilution by the spread plate method on ZoBell 2216E medium for marine bacteria. (Both stations were already the object of earlier microbiological investigations employing similar methods described in Rieper-Kirchner, 1989).

Chitinase activity in the water from both stations was determined by means of the fluorimetric test method. Since chitin is a biopolymer composed of N-acetyl-glucosamine units, the substrate used was 4-methylumbellilferyl-N-acetyl-β-D-glucosaminide. 20 mg of this substrate (Sigma 8756) were dissolved in 5 ml dimethylformamide and kept frozen at −18 °C until use (O'Brien & Colwell, 1987, modified). From this stock solution, 50 µl were added to each of 3 replicates of 3 ml seawater sample, and incubated 4 h at 18 °C in the dark on a vibrating table (93 vib. min^{-1}). Blanks consisting of 3 ml deionized autoclaved water were treated in the same manner as the samples. After incubation, 750 µl of 0.1 M glycine buffer pH 10 were added to each sample and blank, which were then immediately measured with a spectrofluorimeter (Jobin Yvon JY3, France) in 10 mm optical glass cuvettes (364 nm excitation, 445 nm emission). A standard calibration series of methylumbelliferylferon (Serva 29660) in ethyleneglycolmonomethylether (Serva 11310), final concentration of the working solution 1 µg ml^{-1} (Obst & Holzapfel-Pschorn, 1988), was run for each series of measurements; 10, 20, 50 and 100 µl of the working solution were added to 3 ml autoclaved deionized water, then 750 µl glycine buffer as above. The amount of methylumbelliferylferon released from the substrate is a measure of exoenzymatic activity, expressed in relative fluorescence units on the fluorimeter.

Chitin degradation rate in the field

For these studies, two different brands of chitin were used: Serva (Heidelberg) 16625 "Chitin from crustacean shells, pract.", and Sigma (St. Louis, MO, USA) C-4666 "Chitin (Poly-N-acetylglucosamine) practical grade, from crab shells". The chitin flakes from both companies varied in size from powderlike particles to large flakes about 1 cm long. Prior to use, all flakes were shaken over a 1 mm mesh sieve, to remove particles less than 1 mm. Portions of 2 g each were then sealed into 180 µm nylon (Nytal) mesh bags (6 × 12 cm). Pairs of bags, representing one from each company, were then placed in PVC open flow-through cylindrical chambers designed by Minas et al. (1986). The ends of each chamber were covered with 1 mm mesh size nylon gauze, to allow water exchange. The chambers were fastened by SCUBA divers to concrete blocks 50 cm above the sea bottom at 6 m depth near the southern part of Helgoland. After time intervals of 11, 24, 43, 70, 116 and 179 days, two chambers with 2 bags each were recovered by divers. The bags were rinsed briefly with tap water followed by distilled water to remove debris, air-dried and weighed. These field studies took place between 27th May and 22nd November, 1991. Water temperature data for this period are included in the following section of this paper.

RESULTS

Microbial colonization of chitinaceous substances: copepod moults, carcasses and fecal pellets

After 13 days generation time, the progeny of *T. holothuriae* ♀ No. 1 consisted of 9 adult ♂♂ and 6 adult ♀♀. From the exuviae recovered, 32 N and 47 C were examined without prior staining. The progeny of ♀ No. 2 consisted of 9 adult ♂♂, 7 adult ♀♀ and 1 copepodid. From the exuviae recovered, 46 N and 34 C were DAPI-stained for epifluorescence microscopy. The results may be summarized as follows: exuviae from both N and C stages which were fixed immediately after removal from the vessels always exhibited a rich bacterial flora. This was composed primarily of single rods and cocci, distributed over the entire surface of the exuviae (Fig. 1 a). Filaments were rare, if observed at all. Slender rods 1.9–2.3 µm long were common; occasionally large stout curved rods 5–10 µm long with granular inclusions were also observed. The bacteria cells were chiefly horizontally attached; vertical attachment was less frequent, occurring occasionally on the extremities of the exuviae (swimming legs, antennae, mouth parts).

Exuviae which had been transferred after removal into vessels with sterile seawater and then allowed to age were characterized by a different microbial flora than that described above. Both N and C exuviae aged for 7, 10 and 14 days were increasingly colonized by the yeastlike fungus *Aureobasidium pullulans*. Slender rodshaped bacteria 2–3 µm long were also observed. Generally the numbers of bacteria tended to decrease with longer aging periods, while cells of *A. pullulans* increased. After aging 21 days, the exuviae were densely colonized by the fungal cells as well as with colonies of small coccoid cells (Fig. 1 b). Fungal colonies and particles of unidentified debris occurred most frequently on the ventral sides of the exuviae and on the bases of the extremities. Unattached bacteria became increasingly numerous with aging time; after 21 days, these consisted mainly of bacteria with fimbriae or slime threads. Various filamentous forms were also observed.

The 2 carcasses of *T. holothuriae*, examined over a period of 6 weeks, were colonized more heavily on the ventral sides and also on the bases of the extremities, similar to the exuviae. Colonization apparently proceeded more rapidly in the presence of body tissue than with the exuviae alone. After 2 weeks, the carcasses were completely covered with various epibionts, including fungal hyphae and unidentified debris.

Fecal pellets aged 1–4 days were only sparsely colonized by some rods and coccoid bacteria. After 7–14 days, bacterial attachment was denser; different rods, cocci and an occasional *Vibrio* sp. were observed. After 21 days, pellets were heavily colonized chiefly by rods, the majority of which appeared to have fimbriae-like projections. Spirillae were also observed on the pellet surfaces. Bacteria-free zones appearing on slides of DAPI-stained pellets may or may not be an artifact.

Bacterial numbers and chitinase activity

The numbers of heterotrophic bacteria in seawater expressed as colony-forming units on ZoBell medium follow a seasonal pattern with maxima in the late summer of 1991 and again in the spring of 1992 (Fig. 2). The numbers found in rocky intertidal water

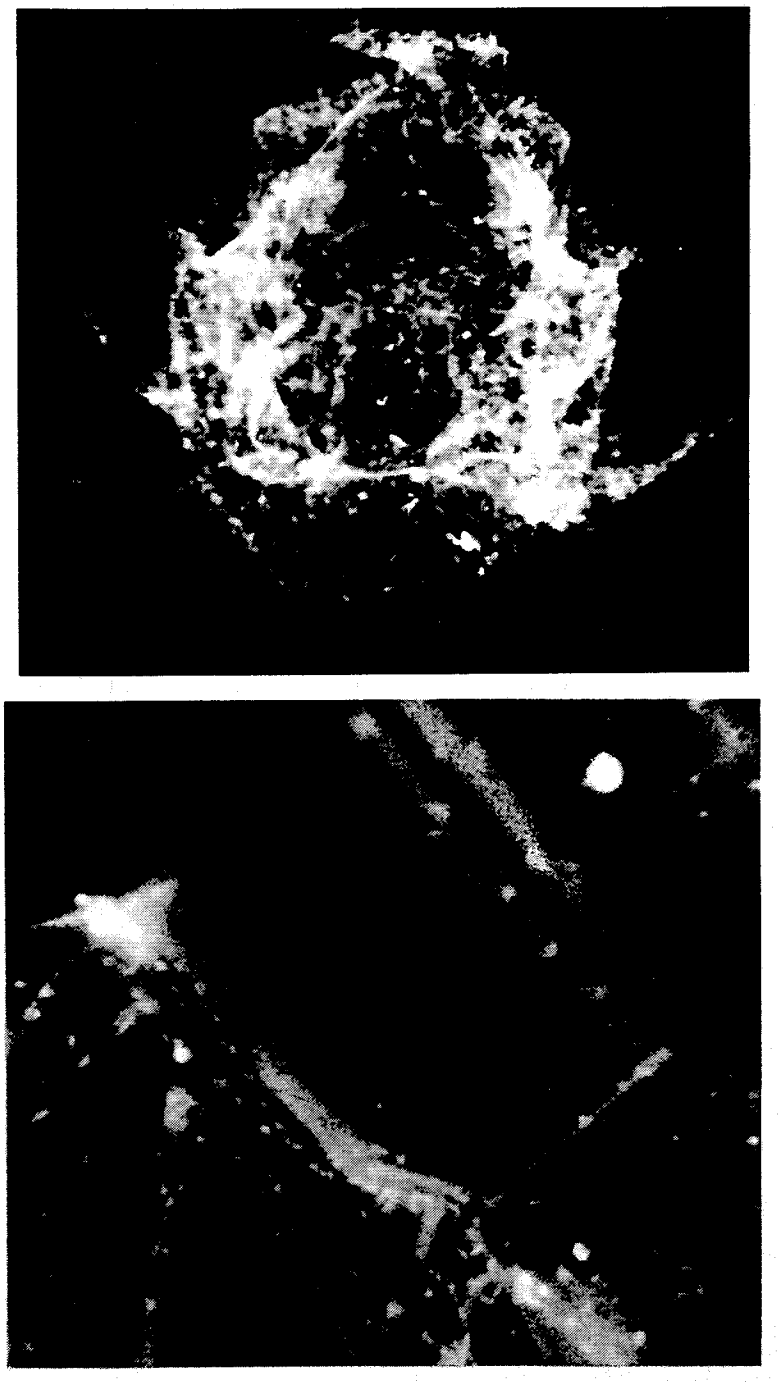
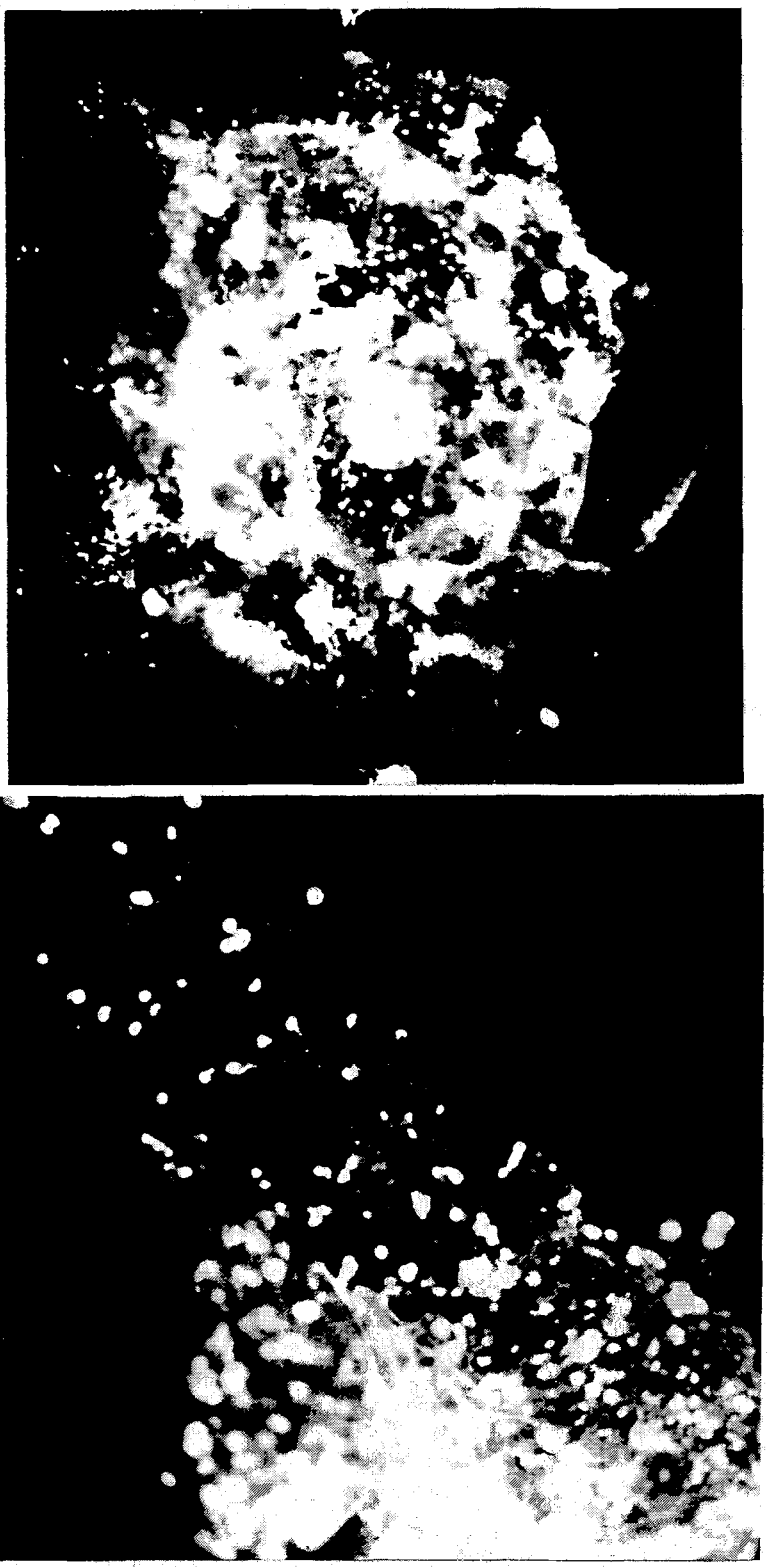


Fig. 1a. *Tisbe holothuriae* exuviae, DAPI-stained to show microbial colonization on freshly moulted nauplius (top figure; 400 ×), and on copepodid (bottom figure; 1000 ×); both with rodshaped bacteria 2 μm long



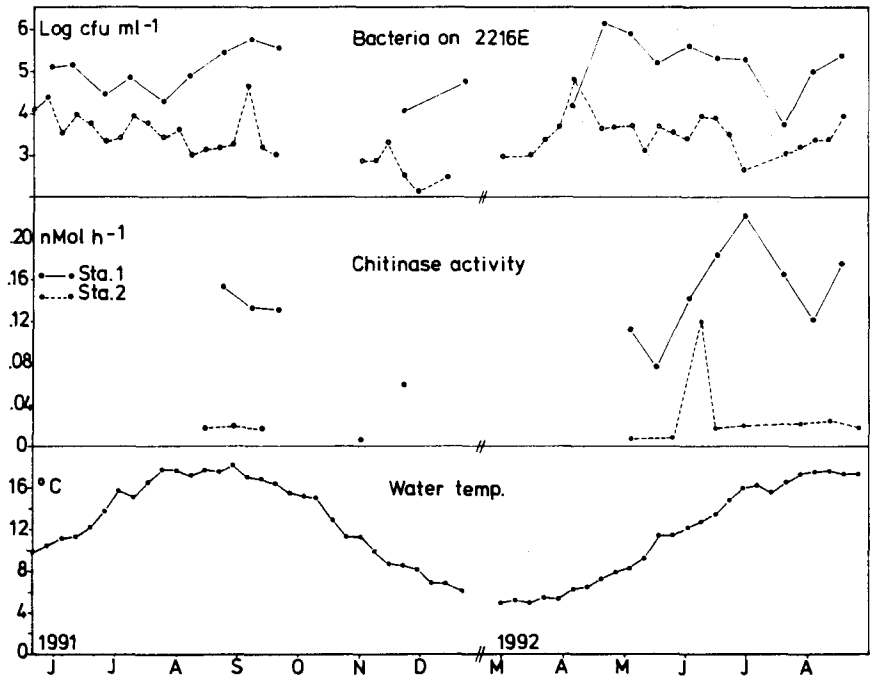


Fig. 2. Bacteria (cfu) on ZoBell 2216E medium and chitinase activity in seawater samples from the rocky intertidal (Sta. 1 ●—●) and the Cable Buoy (Sta. 2 ○--○) with the water temperature at Helgoland

samples generally were 1–2 orders of magnitude higher than those in samples from the Cable Buoy.

Chitinase activity in the samples from both stations was measured only sporadically in 1991 (August–September and November), so an annual pattern could not be established. The results from May to August in 1992 indicate a relatively high activity in the rocky intertidal, particularly in June–July. The higher chitinase activity at this site corresponds to the higher bacteria counts, compared to those for Cable Buoy water samples.

The water temperature data for 1991 (Fig. 2) were provided by Treutner and Mangelsdorf (Hickel et al., in Jahresbericht Biologische Anstalt Helgoland, 1991; p. 41) and Mangelsdorf & Treutner (unpubl.).

Chitin degradation rate in the field

Results of field studies on chitin degradation in mesh bags exposed in the sea are shown in Figure 3. For both brands of crustacean chitin used, the loss of substance

Fig. 1b. *Tisbe holothuriae* exuviae, DAPI-stained to show microbial colonization on nauplius, aged 21 days (top figure; 400 ×), and on copepodid, aged 14 days (bottom figure; 250 ×). Both are heavily colonized by the yeastlike fungus *Aureobasidium pullulans*

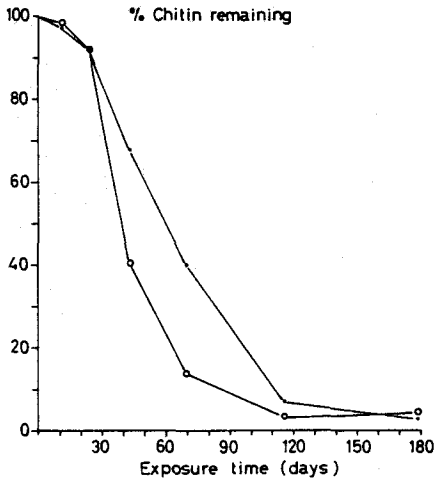


Fig. 3. Percentage of original chitin dry weight remaining after exposure in seawater at Helgoland; (●–●) Sigma chitin; (○–○) Serva chitin. Values represent the average of duplicate determinations, with a variation range of ± 0.1–1.9 percentage points

proceeded very slowly during the first 4 weeks of exposure. After 3 months, a dry weight loss of 75 % or more was determined. After nearly 4 months, less than 10 % of the initial dry weight was still present.

Although the degradation rate was not linear, the average loss of chitin over 116 days is calculated at 16 mg day^{-1} for Sigma and 17 mg day^{-1} for Serva chitin. The Sigma chitin was more resistant to breakdown than the Serva chitin under the given conditions. On one of the two flow-through chambers retrieved on 9th September 1991 (116 days), both outer gauzes had been torn off and the mesh bags lost (one from each brand). Thus for this date, the dry weight values in Figure 3 are single determinations only, in contrast to all others, which represent the average of two replicates for each brand.

The water temperature at time zero on 27th May 1991 was 9.8 °C. The temperature increased to a maximum of 18.6 °C at the end of July and fell to 7.9 °C at the end of November 1991 when the last mesh bags were retrieved.

DISCUSSION

The bacteria most commonly associated with freshly moulted exuviae from healthy, laboratory-reared *T. holothuriae* were slender rods about 2 µm long. All exuviae from the different N and D developmental stages which were examined were colonized by bacteria, nearly all of which were attached horizontally. Since there was no evidence of chitinolytic activity such as scarring, pitting or other damage, these bacteria may be regarded as commensals on the copepod body surfaces. Microbial association with the body surfaces of marine organisms is not uncommon (Sieburth, 1975). Nagasawa et al. (1985) found that 9–30 % of the copepods in Tokyo Bay were colonized by bacteria; among others, "short stout rods" were frequently found on *Acartia clausi* Giesbrecht. In two seawater samples collected from Helgoland in September 1983, 28.8 % and 37.4 % of

Acartia longiremis were associated with bacteria (Nagasawa & Nemoto, 1986). Among attached bacteria, members of the genus *Vibrio* are often observed (Kaneko & Colwell, 1978; Sochard et al., 1979; Huq et al., 1983; Lavilla-Pitogo et al., 1990; Tamplin et al., 1990). According to Simidu et al. (1971), the bacteria associated with marine planktonic organisms are dominated by *Vibrio* and *Aeromonas* spp. Not all of these forms are commensals, however; in particular the genus *Vibrio* includes many chitinoclastic and/or pathogenic forms such as *V. cholerae* and *V. parahaemolyticus*. Sochard et al. (1979) found that *Acartia tonsa* Dana from pelagic samples were colonized mostly by *Vibrio* spp., but laboratory-reared copepods harboured more *Pseudomonas* spp.; the authors suggest that the differences may be due to the artificial conditions of the laboratory environment, imposing restrictions on the expression of the normal flora and selective pressures. This may also apply to the bacteria attached to both freshly moulted and aged exuviae from *T. holothuriae*.

N and C exuviae as well as carcasses of *T. holothuriae* aged in seawater were colonized most heavily on the ventral sides, in depressed areas between segments and on the bases of the extremities. This agrees with the observations made on other copepods and exuviae where epibionts were also site-selective, occurring mostly on the ventral depressed surfaces (Nagasawa & Terazaki, 1987; Nagasawa, 1989).

A succession of bacteria on decomposing copepods *Tigriopus japonicus* Mori was noted by Fukami et al. (1985): during the first few days of decomposition, the count of free-living bacteria was higher than that of attached bacteria. Then the number of free-living bacteria decreased rapidly, while that of attached bacteria remained high up to 3 weeks. The succession occurred in the following order: from *Vibrio* to *Pseudomonas, Acinetobacter-Moraxella,* and others. This was not the case, however, with the qualitative examination of exuviae, carcasses and fecal pellets of *T. holothuriae*. The slender rods initially observed on fresh moults were replaced in time by diverse other forms – rods, cocci and starlike rosettes. With increasing aging time, the attached bacteria generally decreased, while unattached forms – filaments and especially bacteria with fimbriae – increased. Carcasses and exuviae aged 21 days were generally heavily colonized by the yeastlike fungus *Aureobasidium pullulans* (Hyphomycetes). When more fungal colonies were present, fewer attached bacteria were observed, and vice versa. The role of *Aureobasidium pullulans* here is not clear. The fungus is of widespread occurrence, capable of existing in terrestrial, marine and freshwater environments (Roth et al., 1964). It has been found on marine organisms such as clams, shrimp, starfish and various kinds of fish (Capriotti, 1962; Kohlmeyer & Kohlmeyer, 1979) in the water column and in sediment, and was common in seawater samples from the Cable Buoy station near Helgoland in earlier studies (Meyers et al., 1967). There is no record of *A. pullulans* in association with chitin degradation. On substrates, the fungal cells may compete with bacteria for space and nutrients, or they may aid the work of other bacteria by, for example, secreting metabolites or breaking down compounds which bacteria may use. Bacterial utilization of fungal degradation intermediates has been described by Rüttimann et al. (1991) for lignin degradation processes. The fungus may profit from products arising from the decomposition activities of other bacterial species or consortia. Hermanides-Nijhof (1977) describes *A. pullulans* as a saprophyte on a variety of substrates, including human skin and nails, which contain keratin. Higher marine fungi may be involved in the degradation of the chitinous exoskeletons of Hydrozoa and the

keratinous-like tubes of annelids (Kohlmeyer, 1972). The rapid and dense growth of *A. pullulans* on chitinaceous substrates in this study calls for further attention to be given to the role of this fungus in marine degradation processes.

During previous microbiological investigations on the two sites described here, results obtained with acridine orange direct count methods showed neither a strong seasonality nor clear differences in the bacteria numbers from the rocky intertidal or Cable Buoy stations. On the other hand, specialized bacteria enumerated on different media demonstrated maxima during the months from April to October at both stations, and were 1–2 orders of magnitude higher in the rocky intertidal zone than at the Cable Buoy. This pattern of distribution has been reconfirmed in this study. Higher chitinase activity in the rocky intertidal zone also corresponds to the higher bacteria numbers observed at this site. Here the samples were taken in shallow water in the immediate vicinity of macroalgal vegetation (*Fucus* and *Laminaria* spp.). Since algal cover provides food and shelter for many organisms such as crustaceans, it is possible that there was a greater chitin input at this station, in the form of moults and carcasses, and thus a higher exoenzymatic potential. The higher numbers of bacteria are also a reflection of the greater amount of nutrients arising from the macroalgae and their breakdown products (Rieper-Kirchner, 1989). Bacteria are primarily responsible for the exoenzymatic activity, as has been shown in size-fractionated water samples (Hoppe, 1983). Chitinolytic bacteria in seawater are estimated at 0.1 to 1.0 % of the total numbers (ZoBell & Rittenberg, 1937; Seki & Taga, 1963). High numbers have been found associated with macroalgae from the surf zone (Wolter & Rheinheimer, 1977) and particularly in the sediment (Rheinheimer, 1991).

The rate of chitin degradation in the field depends on the site (whether it takes place in the water or in sediments) on the temperature, available organic matter, the microbes present, the source of the chitin and the particle size (Hood & Meyers, 1977). Copepod exuviae may be more rapidly degraded than the heavy carapace of a large crab. The data obtained in this study for crustacean chitin with a degradation rate of 16–17 mg day^{-1} may be compared to that from other environments: 4.5–18.8 mg chitin day^{-1} in sediments, 87 mg day^{-1} g^{-1} in a salt marsh, and 118 mg day^{-1} g^{-1} chitin in shrimp nursery grounds at 30 °C (in Hood & Meyers, 1973). A very high degradation rate of 207 mg day^{-1} g^{-1} at 20 °C was found by Boyer & Kator (1985) in batch cultures with estuarine river water.

In the North Sea at Helgoland, less than 10 % of the chitin particle dry weight remained after 116 days exposure at 10–18 °C. Similar results have been found for lobster shells with 60–78 % dry weight loss after 114 days exposure in the deep ocean, but the loss from squid pen chitin was only 13–33 % under the same conditions (Gardner et al., 1983). Employing mesh bags with a finer gauze exposed in muddy sediment, Hillman et al. (1989) found that one year was required to achieve 80 % loss of squid pen dry weight. Seki (1965) generalizes that "Chitin in the sea is roughly calculated to be mineralized within 50–140 days in surface waters at 25 °C and 15 °C respectively, 370 days in intermediate waters at 5 °C and 500 days in deep waters at temperatures below this". This has been confirmed for chitin exposed in Antarctic waters at depths to nearly 4000 m, whereby one year was required for a weight loss of 70–96 % (Weyland & Helmke, 1989). In contrast, Zdanowski (1988) examined the decomposition rate of whole killed samples of krill (*Euphausia superba* Dana) exposed 10–32 days at 25 m depth at tem-

peratures from −1.8 °C to +1.4 °C in Antarctic waters, and found only 3 % of the initial dry weight was still present after 32 days.

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LITERATURE CITED

Boyer, J. N. & Kator, H. I., 1985. Method for measuring microbial degradation and mineralization of ^{14}C-labeled chitin obtained from the blue crab, *Callinectes sapidus*. – Microb. Ecol. *11*, 185–192.

Capriotti, A., 1962. Yeasts of the Miami, Florida, area. III. From sea water, marine animals and decaying materials. – Arch. Microbiol. *42*, 407–414.

Coleman, A. W., 1980. Enhanced detection of bacteria in natural environments by fluorochrome staining of DNA. – Limnol. Oceanogr. *25*, 948–951.

Dunn, D. F. & Liberman, M. H., 1983. Chitin in sea anemone shells. – Science, N.Y. *221*, 157–159.

Fukami, K., Simidu, U. & Taga, N., 1985. Microbial decomposition of phyto- and zooplankton in seawater. II: Changes in the bacterial community. – Mar. Ecol. Prog. Ser. *21*, 7–13.

Gardner, W. D., Hinga, K. R. & Marra, J., 1983. Observations on the degradation of biogenic material in the deep ocean with implications on accuracy of sediment trap fluxes. – J. mar. Res. *41*, 195–214.

Gooday, G. W., 1990. The ecology of chitin degradation. In: Advances in microbial ecology. Ed. by K. C. Marshall. Plenum Press, New York, 387–430.

Greco, N., Bussers, J.-C., Daele, Y. van & Goffinet, G., 1990. Ultrastructural localization of chitin in the cystic wall of *Euplotes muscicola* Kahl (Ciliata, Hypotrichia). – Eur. J. Protistol. *26*, 75–80.

Hermanides-Nijhof, E. J., 1977. *Aureobasidium* and allied genera. – Stud. Mycol. *15*, 141–177.

Hickel, W., Mangelsdorf, P., Hagmeier, E. & Treutner, K., 1991. 30 Jahre Helgoland-Reede-Zeitreihe. – Jber. Biol. Anst. Helgoland *1991*, 40–44.

Hillman, K., Gooday, G. W. & Prosser, J. I., 1989. The mineralization of chitin in the sediments of the Ythan estuary, Aberdeenshire, Scotland. – Estuar. coast. Shelf Sci. *29*, 601–612.

Hood, M. A. & Meyers, S. P., 1973. The biology of aquatic chitinoclastic bacteria and their chitinolytic activities. – La Mer *11*, 213–229.

Hood, M. A. & Meyers, S. P., 1977. Rates of chitin degradation in an estuarine environment. – J. oceanogr. Soc. Japan *33*, 328–334.

Hoppe, H.-G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. – Mar. Ecol. Prog. Ser. *11*, 299–308.

Huq, A., Small, E. B., West, P. A., Huq, M. I., Rahman, R. & Colwell, R. R., 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. – Appl. environ. Microbiol. *45*, 275–283.

Johnstone, J., 1908. Conditions of life in the sea. Cambridge Univ. Press, Cambridge, 332 pp.

Kaneko, T. & Colwell, R. R., 1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. – Microb. Ecol. *4*, 135–155.

Kohlmeyer, J., 1972. Marine fungi deteriorating chitin of hydrozoa and keratin-like annelid tubes. – Mar. Biol. *12*, 277–284.

Kohlmeyer, J. & Kohlmeyer, E., 1979. Marine mycology. Acad. Press, New York, 690 pp.

Lavilla-Pitogo, C. R., Baticados, M. C. L., Cruz-Lacierda, E. R. & Pena, L. D. de la, 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. – Aquaculture *91*, 1–13.

Meyers, S. P., Ahearn, D. G., Gunkel, W. & Roth, F. J., 1967. Yeasts from the North Sea. – Mar. Biol. *1*, 118–123.

Minas, W., Gunkel, W. & Tadday, G., 1986. An open flow-through chamber system – a new tool for experimental ecological investigations in the marine sublittoral. – Mar. environ. Res. *20*, 299–305.

Nagasawa, S., 1989. Bacterial epibionts of copepods. – Sci. Prog. *73*, 169–176.
Nagasawa, S. & Nemoto, T., 1986. The widespread occurrence of copepod – bacterial associations in coastal waters. – Syllogeus *58*, 379–384.
Nagasawa, S. & Terazaki, M., 1987. Bacterial epibionts of the deep-sea copepod *Calanus cristatus* Krøyer. – Oceanol. Acta *10*, 475–479.
Nagasawa, S., Simidu, U. & Nemoto, T., 1985. Scanning electron microscopy investigation of bacterial colonization of the marine copepod *Acartia clausi*. – Mar. Biol. *87*, 61–66.
O'Brien, M. & Colwell, R. R., 1987. A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide. – Appl. environ. Microbiol. *53*, 1718–1720.
Obst, U. & Holzapfel-Pschorn, A., 1988. Enzymatische Tests für die Wasseranalytik. Oldenbourg, München, 86 pp.
Porter, K. G. & Feig, Y. S., 1980. The use of DAPI for identifying and counting aquatic microflora. – Limnol. Oceanogr. *25*, 943–948.
Rheinheimer, G., 1991. Mikrobiologie der Gewässer. Fischer, Jena, 294 pp.
Rieper, M., 1978. Bacteria as food for marine harpacticoid copepods. – Mar. Biol. *45*, 337–345.
Rieper-Kirchner, M., 1989. Microbial degradation of North Sea macroalgae: field and laboratory studies. – Botanica mar. *32*, 241–252.
Roth, F. J., Orpurt, P. A. & Ahearn, D. G., 1964. Occurrence and distribution of fungi in a subtropical marine environment. – Can. J. Bot. *42*, 375–383.
Rüttimann, C., Vicuña, R., Mozuch, M. D. & Kirk, T. K., 1991. Limited bacterial mineralization of fungal degradation intermediates from synthetic lignin. – Appl. environ. Microbiol. *57*, 3652–3655.
Seki, H., 1965. Microbiological studies on the decomposition of chitin in marine environment. IX: Rough estimation on chitin decomposition in the ocean. – J. oceanogr. Soc. Japan *21*, 253–260.
Seki, H. & Taga, N., 1963. Microbiological studies on the decomposition of chitin in marine environment. I: Occurrence of chitinoclastic bacteria in the neritic region. – J. oceanogr. Soc. Japan *19*, 101–108.
Sieburth, J. McN., 1975. Microbial seascapes. Univ. Park Press, Baltimore, plates.
Simidu, U., Ashino, K. & Kaneko, E., 1971. Bacterial flora of phyto- and zoo-plankton in the inshore water of Japan. – Can. J. Microbiol. *17*, 1157–1160.
Smucker, R. A. & Dawson, R., 1986. Products of photosynthesis by marine phytoplankton: chitin in TCA "protein" precipitates. – J. exp. mar. Biol. Ecol. *104*, 143–152.
Sochard, M. R., Wilson, D. F., Austin, B. & Colwell, R. R., 1979. Bacteria associated with the surface and gut of marine copepods. – Appl. environ. Microbiol. *37*, 750–759.
Tamplin, M. L., Gauzens, A. L., Huq, A., Sack, D. A. & Colwell, R. R., 1990. Attachment of *Vibrio cholerae* Serogroup 01 to zooplankton and phytoplankton of Bangladesh waters. – Appl. environ. Microbiol. *56*, 1977–1980.
Weyland, H. & Helmke, E., 1989. Barophilic and psychrophilic bacteria in the Antarctic Ocean. In: Recent advances in microbial ecology. Ed. by T. Hattori, Y. Ishida, Y. Maruyama, R. Y. Morita & A. Uchida. Japan Scientific Societies Press, Tokyo, 43–47.
Wolter, K. & Rheinheimer, G., 1977. Bakteriologische Untersuchungen an in der Brandungszone angetriebenem Algenmaterial. – Botanica mar. *20*, 171–181.
Yoshikoshi, K. & Kô, Y., 1988. Structure and function of the peritrophic membranes of copepods. – Bull. Jap. Soc. scient. Fish. *54*, 1077–1082.
Zdanowski, M. K., 1988. Matter conversion in the course of krill (*Euphausia superba* Dana) decomposition in the Antarctic ecosystem. – Polskie Arch. Hydrobiol. *35*, 65–96.
ZoBell, C. E. & Rittenberg, S. C., 1937. The occurrence and characteristics of chitinoclastic bacteria in the sea. – J. Bact. *35*, 275–287.