

synthetic characteristics is about 3 times greater than predicted from a single 6-hour mid-day incubation extrapolated to 24 hours. Similarly, bacterial growth and grazing by microzooplankton exhibit distinct diel periodicities, thus extrapolating results of short incubations to daily rates may be misleading. For example, extrapolating mid-day rates to 24 hours would overestimate bacterial growth and production by a factor of 2 to 5 and underestimate grazing rates of some microzooplankton by two- to fourfold.

This study demonstrates, for the first time, diel periodicities in polar microbial populations. It also shows that the physiological characteristics of populations in polar and temperate regions can differ significantly, thus models developed for temperate species may not be appropriate in polar regions. This work represents part of our continuing study of the metabolism of polar microbial populations. We are currently examining the coupling and trophic interaction among phytoplankton, bacteria, and microzooplankton.

This research was supported by National Science Foundation grant DPP 83-14607 and DPP 85-20278 to R.B. Rivkin.

### References

- Ducklow, H.W., and S.M. Hill. 1985. Tritiated thymidine incorporation and growth of heterotrophic bacteria in warm core rings. *Limnology and Oceanography*, 30, 260-272.
- Fuhrman, J.A., and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Applied and Environmental Microbiology*, 39, 1085-1095.
- Harding, L.W., B.B. Prézelin, B.M. Sweeney, and J.L. Cox. 1982a. Diel oscillations of the photosynthesis-irradiance (P-I) relationship in natural assemblages of marine phytoplankton. *Marine Biology*, 67, 167-178.
- Harding, L.W., B.B. Prézelin, B.M. Sweeney, and J.L. Cox. 1982b. Primary production as influenced by diel periodicity of phytoplankton photosynthesis. *Marine Biology*, 67, 179-186.
- Holm-Hansen, O., S. El-Sayed, G.A. Franceschini, and R.L. Cuhel. 1977. Primary production and factors controlling phytoplankton growth in the Southern Ocean. In G.A. Llano (Ed.), *Adaptations within antarctic ecosystems*. Houston: Gulf Publishing.
- Lessard, E.J., and E. Swift. 1985. Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters measured with a dual-label radioisotope technique. *Marine Biology*, 87, 289-296.
- Lessard, E.J., and R.B. Rivkin. 1986. Nutrition of microzooplankton and macrozooplankton from McMurdo Sound. *Antarctic Journal of the U.S.*, 21(5), 187-188.
- Nelson, D.M., and W.O. Smith. 1986. Phytoplankton bloom dynamics of the western Ross Sea ice edge. II. Mesoscale cycling of nitrogen and silicon. *Deep-Sea Research*, 33, 1389-1412.
- Prézelin, B.B., B.W. Meeson, and B.M. Sweeney. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates. I. Pigmentation, photosynthetic capacity and respiration. *Plant Physiology*, 60, 384-387.
- Putt, M., and B.B. Prézelin. 1985. Diurnal patterns of photosynthesis in cyanobacteria and nanoplankton in California coastal waters during "el Nino." *Journal of Plankton Research*, 7, 779-790.
- Rivkin, R.B., I. Bosch, J.S. Pearse, and E.J. Lessard. 1986. Bacterivory: A novel feeding mode for asteroid larvae. *Science*, 233, 1311-1314.
- Rivkin, R.B., and M. P putt. 1987. Diel periodicity of photosynthesis in polar phytoplankton: Influence on primary production. *Science*, 238, 1285-1288.
- Sournia, A. 1974. Circadian periodicity in natural populations of marine phytoplankton. *Advances in Marine Biology*, 12, 325-389.
- Sweeney, B.M. 1983. Circadian timekeeping in eucaryotic cells, models and hypothesis. *Progress in Phycological Research*, 2, 189-225.

## Chitin degradation during the austral summer in Antarctica

RUSSELL P. HERWIG and JAMES T. STALEY

*Department of Microbiology  
University of Washington  
Seattle, Washington 98195*

Chitin, a biopolymer composed of subunits of the amino sugar N-acetyl-D-glucosamine, is the primary constituent of the exoskeleton of zooplankton. The dominant zooplankton species in the Antarctic is *Euphausia superba*, commonly called krill. This crustacean serves as the primary item in the diet for many different kinds of animals, including seals, whales, birds, and fish. Approximately 4-10 percent of the dry weight of krill is chitin (Clarke 1980; Raymont, Srinivasagam, and Raymont 1971). The degradation of chitin requires the action of a specific enzyme called chitinase which is produced by a small group of microorganisms (Clarke and Tracey 1956) and is found in the tissues of some vertebrates (Jeuniaux and Cornelius 1978). Millions of tons of chitin are produced each year in the antarctic ecosystem, and our research project focuses on finding the routes of chitin degradation in Antarctica. An objective of our study was to determine if krill-feeding animals in the Antarctic can degrade a significant portion of the chitin that they con-

sume. A second objective was to examine the chitin degradation rates in the environment and characterize the microorganisms that are responsible for this activity. If chitin is not degraded, large quantities of carbon and nitrogen would become lost in the Antarctic. During the 1986-1987 austral summer our research group examined the rates of chitin degradation and the presence of chitinolytic bacteria in an Adélie penguin rookery, and in marine sediments and water. In addition, the chitin degradation ability of crabeater seals (*Lobodon carcinophagus*) was investigated.

*Crabeater seal studies.* Crabeater seals are the most numerous seal species in Antarctica and in the world with a population estimated to be about 30 million (Laws 1985). These marine mammals feed almost exclusively on krill. With the cooperation of scientists from the National Marine Mammal Laboratory (Seattle, Washington) a small number of seals was sacrificed and the digestive contents removed for chitin degradation studies. Digestive tract tissues were also examined for the presence of chitinase.

*Chitin degradation in an Adélie penguin rookery.* During the austral summer Adélie penguins (*Pygoscelis adeliae*) breed and raise chicks in rookeries (figure). Studies were performed in an Adélie penguin rookery located on Torgersen Island, a site near Palmer Station, Anvers Island. This rookery serves as the home for over 15,000 adult penguins during the austral summer (Heimark and Heimark 1984), and large quantities of guano, which contains chitin, are deposited in the rookery. For deter-



Adélie penguins, adults, and chicks, on Torgersen Island.

mination of the rates of chitin degradation, radioactive carbon-14-chitin was synthesized in our Seattle laboratory using a procedure similar to that used for the production of hydrogen-3-chitin (Molano, Duran, and Cabib 1977) but in which carbon-14-acetic anhydride was allowed to react with chitosan (deacetylated chitin). Soil samples were collected from different depths at two separate sites and were incubated with the radioactive chitin within closed flasks at 1°C. After a 48-hour incubation, the samples were acidified and the released carbon-14 dioxide was collected within the flask on filter paper, soaked with base that was suspended above the sample. Using the radionuclide tracer microbial degradation of chitin was measured by determining the quantity of carbon-14 dioxide released by microbial respiration of the carbon-14-chitin. Bacteria capable of degrading chitin in the penguin rookery were enumerated on an agar medium containing a rookery soil extract and chitin that was incubated at 5°C for a minimum of 4 weeks. The chitinolytic bacterial isolates and their chitinases will be characterized.

*Chitin degradation in marine waters and sediments.* The R/V *Polar Duke* and the U.S. Coast Guard *Arctic Survey Boat* were used to collect sediment and water samples from selected sites along the Antarctic Peninsula. Samples were collected in the Palmer Station vicinity, Wilhelmina Bay, Bransfield Strait, Bismark Strait, and Penola Strait. Chitin degradation activity was measured using two different approaches. First, a method similar to that described above for the rookery soil was used. Sediment slurries and water samples were incubated at 1°C with carbon-14-chitin for 48 hours and the release of carbon-14 dioxide was collected and measured. Second, a fluorogenic substrate, methylumbelliferyl chitotriose, was used to determine the activity of chitinase in the marine environment. This substrate has been used for the measurement of lysozyme activity (Delmotte,

Privat, and Monsigny 1975; Yang and Hamaguchi 1980). Samples were incubated at 1°C for 48 hours with methylumbelliferyl chitotriose, and chitinase activity was determined by measuring the quantity of methylumbelliferyl that was cleaved from the fluorogenic substrate by using a fluorometer. Chitinolytic bacteria from the marine environment were enumerated on an agar medium containing chitin that was incubated at 5°C for a period greater than 4 weeks. Bacterial isolates were purified and isolated for taxonomic studies. Chitinases from selected marine isolates will be purified and characterized.

During the course of our investigation of chitin degradation in the Antarctic, we have developed new procedures for the determination of chitinase activity in environmental samples. In particular, the uses of carbon-14-chitin synthesized from chitosan and carbon-14-acetic anhydride, and a fluorogenic substrate for chitinase assays have not been previously reported. These techniques developed for our antarctic investigations would be applicable to the study of chitin degradation in other regions of the world.

This work was supported in part by National Science Foundation grant DPP 84-15069. Nancy Pellerin, Roar Irgens, and James Maki were members of the 1986-1987 field team. We would like to acknowledge the excellent cooperation that we received from the support personnel of ITT/Antarctic Services, Inc., the captain and crew of the R/V *Polar Duke*, and the U.S. Coast Guard crew of the *Arctic Survey Boat*.

## References

- Clarke, A. 1980. The biochemical composition of krill, *Euphausia superba* Dana, from south Georgia. *Journal of Experimental Marine Biology and Ecology*, 43, 221-236.
- Clarke, P.H., and M.V. Tracey. 1956. The occurrence of chitinase in some bacteria. *Journal of General Microbiology*, 14, 188-196.
- Delmotte, F.M., J.-P.D.J. Privat, and M.L.P. Monsigny. 1975. Glycan-protein interactions. Synthesis of 4-methylumbelliferyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside, di-N-acetyl- $\beta$ -chitobioside, and tri-N-acetyl- $\beta$ -chitotrioside. Interaction of these compounds with lysozyme. *Carbohydrate Research*, 40, 353-364. (In French)
- Heimark, G.M., and R.J. Heimark. 1984. Birds and marine mammals in the Palmer Station area. *Antarctic Journal of the U.S.*, 19(4), 3-8.
- Jeuniaux, C., and C. Cornelius. 1978. Distribution and activity of chitinolytic enzymes in the digestive tract of birds and mammals. In R.A.A. Muzzarelli (Ed.), *Proceedings, First International Conference on Chitin/Chitosan*. MIT Sea Grant Program.
- Laws, R.W. 1985. The ecology of the Southern Oceans. *American Scientist*, 73, 26-40.
- Molano, J., A. Duran, and E. Cabib. 1977. A rapid and sensitive assay for chitinase using tritiated chitin. *Analytical Biochemistry*, 83, 648-656.
- Raymont, J.E.G., R.T. Srinivasagam, and J.K.B. Raymont. 1971. Biochemical studies on marine zooplankton. IX. The biochemical composition of *Euphausia superba*. *Journal of the Marine Biological Association of the United Kingdom*, 51, 581-588.
- Yang, Y., and K. Hamaguchi. 1980. Hydrolysis of 4-methylumbelliferyl-N-acetyl-chitotrioside catalyzed by hen and turkey lysozymes. pH dependence of the kinetic constants. *Journal of Biochemistry*, 87, 1003-1014.