Chemical bioactivity in common shallow-water antarctic marine invertebrates

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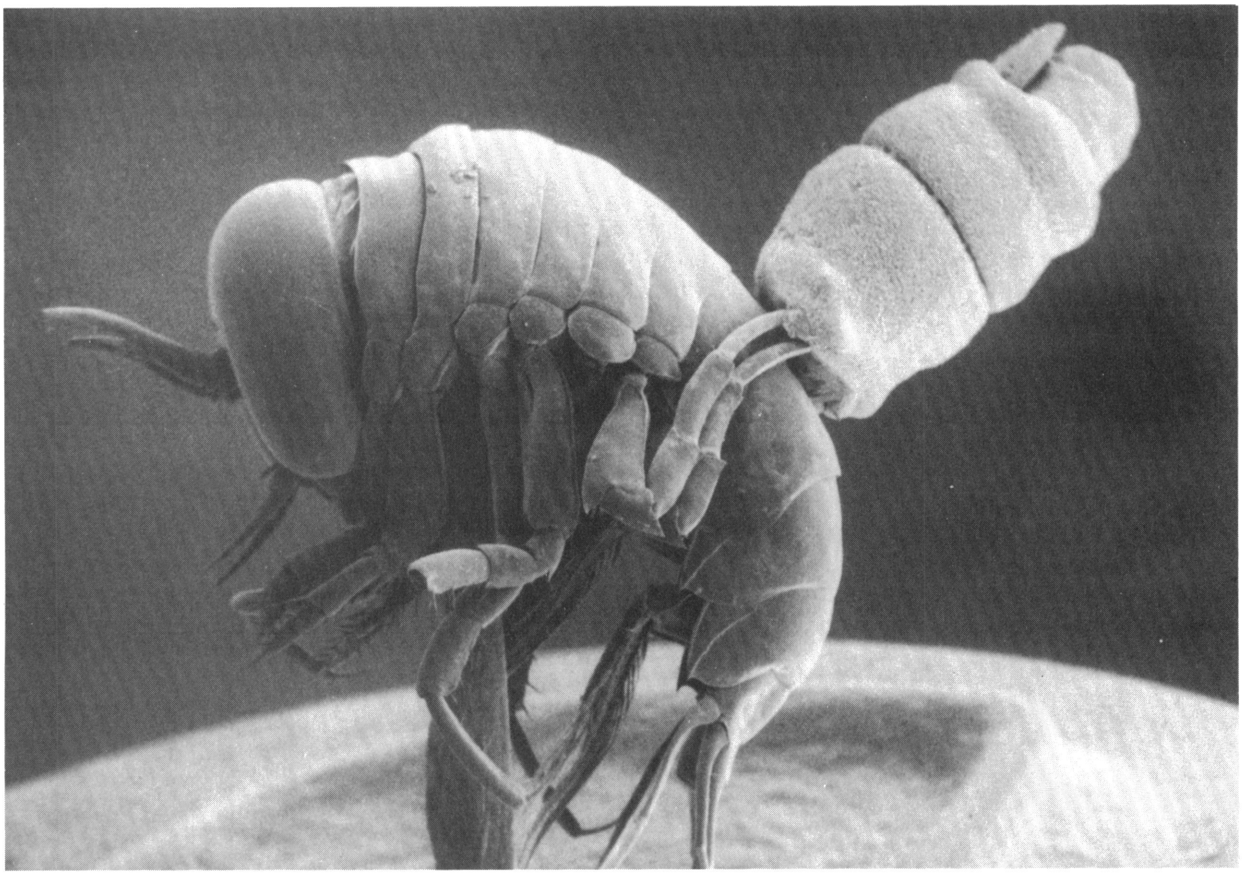
A number of studies have postulated that high-latitude marine invertebrate fauna should be characterized by low levels of chemical defense (Bakus 1974; Bakus and Green 1974; Green 1977; Vermeij 1978; Bakus 1981). The basis for this conclusion is that benthic and pelagic fish predation decreases with increasing latitude (Palmer 1979; Neudecker 1979), thereby releasing high-latitude species from predatory pressures and a mechanism to select for distasteful compounds. Studies of antarctic marine communities, however, have documented invertebrate predation and competition (Dayton et al. 1974; Dayton 1989). Moreover, extreme environmental stability and an extensive geological history suggest that opportunities exist for the evolution and co-evolution of predator-prey defense mechanisms such as the production of noxious or toxic chemicals. Our current program is examining chemical activity in common shallow-water marine invertebrates in McMurdo Sound. These studies allow an evaluation of the latitudinal hypothesis for chemical defense in marine invertebrates and provide important insights into the role of bioactive compounds in structuring antarctic marine communities.

Our program has concentrated on benthic sponges because they are abundant and conspicuous components of antarctic marine communities and many are known to possess chemical deterrents at lower latitudes (Bakus et al. 1986). During austral summer 1989–1990, we collected tissue samples of 18 species of sponges from depths of 20–40 meters at a variety of locations in McMurdo Sound including New Harbor, Cape Evans, Turtle Rock, Castle Rock, and Arrival Heights. Crude aqueous extracts were prepared from fresh sponge tissues and naturally occurring concentrations of extracts used to conduct a variety of bioassays. These included a cytotoxicity assay exposing the gametes of the sea urchin *Sterechinus neumayeri* to aqueous extracts and righting response times of the sea star *Odontaster validus* placed into sea water with aqueous extracts. In addition, aqueous extracts were tested for their ability to induce sensory tube-foot retraction (indicative of noxicity) in five species of sympatric sea stars, with feeding habits ranging from exclusive spongivores to those which do not include sponges in their diets.

We found that 11 of the 18 species of sponges examined had chemical activity (table). In most cases extracts of sponges with chemical activity had widespread effects on bioassay organisms, causing mortality in sea urchin gametes and a reduction in the capacity of sea stars to right themselves. Sea stars which do not feed on sponges or are feeding generalists generally showed sensory tube-foot retraction to aqueous sponge extracts from chemically active species. The exclusively spongivorous *Perknaster fuscus*, however, feeds primarily on *Mycale acerata* (Dayton et al. 1974), a chemically bioactive species (see table) (McClintock 1987). The body wall of *P. fuscus* is toxic (McClintock 1989), suggesting that it may sequester sponge

Bioassays examining the toxicity and noxicity of crude aqueous extracts of antarctic sponges. Crude aqueous extracts were prepared by homogenizing a known weight of sponge with an equal volume of sea water and filtering out particulate material. These were tested against *Sterechinus neumeyeri* gametes, *Odontaster validus* righting responses, and sea star tube-feet retraction. A plus sign (+) indicates a significant inhibitory effect at the 5 percent level when compared to controls (n = 10 trials/extract; Fisher's Exact Test). A minus sign (−) indicates no significant effect when compared to controls. O.v. = *Odontaster validus*, O.m. = *odontaster meridionalis*, D.b. = *Diplasterias brucei*, A.c. = *Acodontaster conspicuus*, P.k. = *Perknaster fuscus*.

Sponge species	Sea urchin sperm activity	Sea star righting response	Sea star tube-foot retraction				
			O.v.	O.m.	D.b.	A.c.	P.f.
Calyx acuarius	−	−	+	+	+	+	+
Cinachyra antarctica	−	−	−	−	−	−	−
Dendrilla membranosa	+	+	+	+	+	+	+
Gellius benedeni	+	+	+	+	+	+	+
Gellius tenella	−	−	−	−	+	−	−
Haliclona sp.	+	+	+	+	+	+	+
Haliclona dancoi	+	+	+	+	+	+	+
Homaxinella balfourensis	−	−	−	−	−	−	−
Inflatella belli	−	−	−	−	−	−	−
Isodictya erinacea	−	−	−	−	−	−	−
Kirkpatrickia variolosa	−	−	+	+	−	+	+
Latrunculia apicalis	+	+	+	+	+	+	−
Leucetta leptorhapsis	+	+	+	+	+	+	−
Mycale acerata	+	+	+	+	+	+	−
Polymastia invaginata	+	+	+	+	+	+	+
Rosella racoritzae	−	−	−	−	−	−	−
Scolymastia joubinia	−	−	−	−	−	−	−
Tetilla leptoderma	+	+	+	+	+	−	+



Scanning electron micrograph showing the hyperid amphipod *Hyperiella dilatata* carrying the chemically defended antarctic pteropod *Clione limacina* (McClintock and Janssen 1990). Amphipods carrying pteropods are chemically defended from predation by antarctic fish. Magnification is approximately × 50. (Micrograph by P. Oshel.)

toxins in its own tissues providing a means of chemical defense. There are no known predators on this sea star, while other antarctic sea stars are preyed upon (Dayton et al. 1974). Nonetheless, *P. fuscus* may possess saponins, a class of noxious compounds known to occur in the Echinodermata (Faulkner 1984).

Chemical compounds causing toxicity or noxicity in antarctic sponges are poorly known. *Dendrilla membranosa* has been shown to possess diterpenes (Molinski and Faulkner 1987), while *Latrunculia* sp. contains an active imino-quinone pigment (Perry et al. 1986). Sponges collected during our 1989–1990 summer season will be analyzed to isolate and identify bioactive natural products (Faulkner personal communication).

Additional benthic antarctic marine invertebrates were found to be noxic. Pieces of fresh mantle tissues of the nudibranches *Austrodoris mcmurdensis* and *Tritoniella eatoni* and the opisthobranch *Phylline antarctica* were rejected by fish (*Pagothenia borchgrevinki* and *Trematomus bernacchii*). Pieces of body wall of the nemertean *Parbolasia corrugatus* and the fleshy tissues of the soft coral *Alcyonium paessleri* were also rejected by fish. Although pieces of the body wall of the antarctic tunicate *Cnemidocarpa verrucosa* were readily consumed by fish, pieces of the tough outer tunic were not consumed and may be chemically defended and/or rejected due to their consistency. A pelagic antarctic marine invertebrate was also found to have noxious chemicals in its body tissues. The common pteropod *Clione limacina* was always violently rejected by *P. borchgrevinki*, as were whole animal homogenates imbedded in agar. Moreover, a unique symbiotic relationship was discovered. The pelagic hyperiid amphipod *Hyperiella dilatata* captures and carried the chemically defended *C. limacina*, thereby providing itself with chemical defense (see figure) (McClintock and Janssen 1990).

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Dissolved organic material in seawater as a source of nutrition for invertebrate larvae from McMurdo Sound, Antarctica

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As part of a study to determine the differences in energy requirements and feeding strategies of comparable larvae in two contrasting environments (polar—Antarctica; temperate—California), we investigated the role of dissolved organic matter as a food source for echinoderm larvae. Our approach was to:

- measure the concentrations of dissolved amino acids and sugars in samples of seawater from McMurdo Sound taken from areas where larvae have been found in net-tows,
- measure the rates of transport of specific organic substrates into larvae,
- measure the metabolic rates of larvae as a basis for a comparison of the contribution that the transport of dissolved organic matter could provide to larval energetics, and
- determine whether those stages of development lacking a digestive system can increase in biomass in the absence of particulate foods.

The concentrations of amino acids and sugars dissolved in seawater. The concentrations of amino acids and sugars dissolved in seawater from McMurdo Sound were measured using high-performance liquid chromatography. Figure 1 shows some representative data. The upper part (figure 1, block A) shows the composition of dissolved amino acids from three environments: the sea ice, water column, and the benthos. The samples were taken late in the austral winter (23 September 1989). When samples were taken from the platelet sea ice later in the season (austral spring, 17 November 1989), the diversity and concentrations of amino acids increased (see inset, figure 1, block A). The chemical spectrum of dissolved sugars in seawater from McMurdo Sound is shown in the inset in figure 1, block B. The spectrum is composed of three major sugars: glucose, fructose, and sucrose. The concentration of sugars

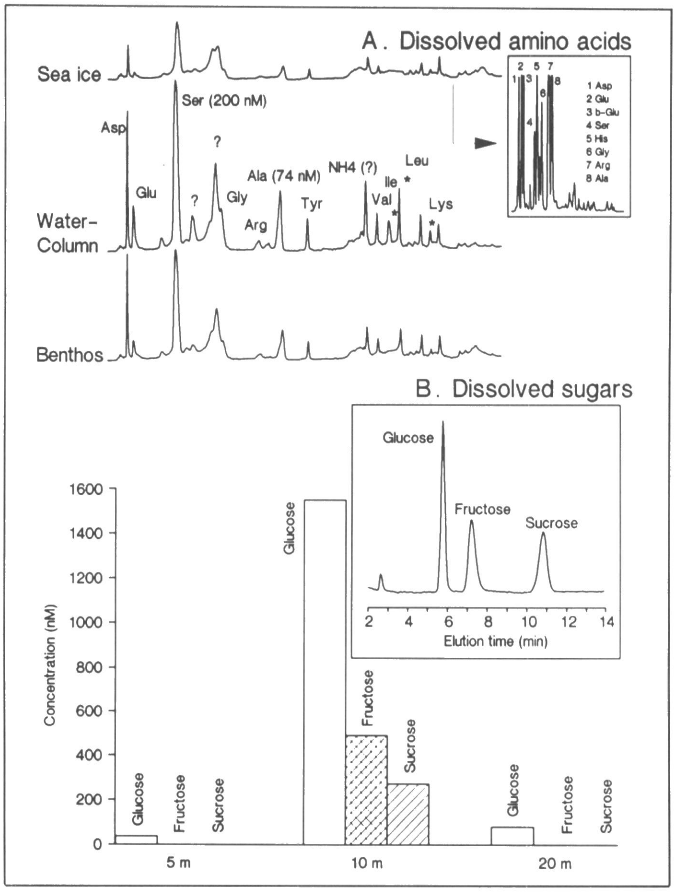

Figure 1. Dissolved amino acids and sugars in seawater from McMurdo Sound, Antarctica. A. Dissolved amino acids: samples were taken (23 September 1989) by scuba divers from under the sea ice (chromatogram labeled "Ice"), from the water-column at a depth of 10 meters, and from the seawater-sediment interface at the same location (depth of approximately 24 meters, chromatogram labeled "Benthos"). These three chromatograms are to the same scale (i.e., peak areas are proportional to substrate concentration). Peaks marked "?" are not identified because they did not have the same elution time as standards. Peaks marked "*" refer to the peaks for I1e, Leu, and Lys (in order). The NH_4+ peak is marked "?" to emphasize that the concentration of NH_4+ (ammonium) is uncertain because the sample was frozen for several (no more than 6) hours prior to analysis. Inset chromatogram (different scale) shows the dissolved amino acids in a sample of seawater taken later in the season (17 November 1989) from underneath the sea ice. B. Dissolved sugars: inset chromatogram shows the spectrum of dissolved sugars in seawater. Histogram gives a depth profile of dissolved sugars. Samples for this depth profile were taken (13 November 1989) with a Niskin bottle at depths of 5, 10, and 20 meters.