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AESOPS (Antarctic Environment Southern Ocean Process Study) is a multidisciplinary program designed to address three broad objectives: to quantify the net flux of carbon dioxide between the ocean and atmosphere and its seasonal variability, to evaluate the factors that lead to phytoplankton blooms in both the Ross Sea and the antarctic polar front, and to understand the controls on production and fate of biogenic material in these regions. To complete these objectives, a series of four process studies on the R/V Nathaniel B. Palmer were conducted on the Ross Sea continental shelf (as well as a benthic cruise). The cruises were completed during the winter-spring transition, the spring bloom, summer conditions, and the autumn-winter transition. This article describes preliminary results from the first process study.

The Ross Sea polynya has been identified as the region supporting the southern oceans’ most spatially extensive and...
earliest phytoplankton bloom (Arrigo and McClain 1994; Smith and Gordon 1997). The causes of the bloom's initiation remain elusive, however. We hypothesized that deep convective mixing in the polynya is reduced by both low-density water from melting ice as well as decreased winds, which allow the phytoplankton to grow at near maximal rates. We reached the study area (figure 1) on 18 October 1996. Ice cover was near 100 percent, although the ice thickness was about 30–50 centimeters. Mixed layers at station Orca were initially about 154 meters (m) (figure 2) and chlorophyll levels were uniformly low [less than 0.1 microgram per liter (µg L–1); figure 2]. The phytoplankton consisted of single-celled *Phaeocystis* sp. cells. At the end of the cruise the mixed layer at the same site was about 67 m (figure 3), and chlorophyll levels had increased to 0.5 µg L–1 (figure 3). *Phaeocystis* sp. continued to dominate and began to form small colonies.

We also conducted sampling at a number of stations situated along an east-west transect at 76°30'S. Mixed layers along this transect ranged from 48 m to more than 600 m during our first occupation and from 24 m to 600 m during our last occupation of this line. Chlorophyll levels ranged from 0.001–0.380 µg L–1 during mid-October to 0.021–2.371 µg L–1 during early November. Nitrate concentrations had decreased minimally [approximately 1 micromolar (µM) reduction] despite

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Figure 2. Density (σt, or sigma-t) and chlorophyll concentrations at station 103 (site Orca) on 18 October 1996. Mixed layer depths are calculated as the depth at which an increase of 0.05 unit relative to the surface value occurred.

Figure 3. Density (σt, or sigma-t) and chlorophyll concentrations at station 117 (site Orca) on 3 November 1996.
the fact that the photoperiod extended to 24 hours by 28 October and maximal irradiances approached 2,000 micromole quanta per square meter per second on clear days. Winds were modest for much of the time in the Ross Sea.

We believe that we observed the initiation of the seasonal phytoplankton bloom in the Ross Sea (chlorophyll concentrations exceeded more than 10 µg L⁻¹ in December). Phytoplankton growth appeared to be restricted by deep vertical mixing, which was driven by surface cooling and convective overturn. As the mixed layer shoaled, the mean irradiance encountered by the phytoplankton assemblage increased (Nelson and Smith 1991). Given that the phytoplankton were nutrient saturated (with both macronutrients such as nitrate and micronutrients such as iron) during this period, and that the assemblages were adapted to extremely low photon flux densities (5 micromole quanta per square meter per second or less), phytoplankton growth began early in the year, under ice, and under extremely low irradiance conditions. Furthermore, we believe that as maximum growth was attained, biomass increased rapidly until growth became limited by trace metals in late spring and early summer. A complete assessment of the temporal changes of the controls of phytoplankton growth awaits the synthesis of all data from all process cruises.

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References


Bacterivory and herbivory play key roles in fate of Ross Sea production

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The Ross Sea polynya is a site of an extensive, albeit seasonally restricted, phytoplankton bloom (Smith and Gordon 1997). It is also presently an active study site for the Antarctic Environment Southern Ocean Process Study (AESOPS), a research program within the U.S. Joint Global Ocean Flux Study (JGOFS). A major goal of this initiative is to characterize the production and fate of primary productivity in this coastal ecosystem and the factors affecting these processes. Within the context of this multi-investigator JGOFS program, we have been examining the trophic activities of herbivorous and bacterivorous microbial assemblages during a series of four cruises spanning October 1996 to December 1997.

Heterotrophic microbial processes play a pivotal role in determining the fate of pelagic production (figure 1). Microbial consumers, predominantly protozoa, are important consumers of primary producers in ocean plankton communities as well as bacterial assemblages that are supported by the utilization of dissolved and particulate organic material (Caron and Finlay 1994; Sherr and Sherr 1994). Conventional wisdom suggests that much of the organic material consumed by protozoa is remineralized and recycled in the water column because of the small size of these consumers and the waste.

Figure 1. Diagrammatic representation of the major trophic interactions involving microbial assemblages at the base of the pelagic food web in the Ross Sea. (DOM denotes dissolved organic matter. POM denotes particulate organic matter.)
material that they release. In contrast, consumption by metazoan grazing may contribute significantly to the removal of compounds of considerable biogeochemical significance (e.g., carbon dioxide) from the water column, whereas microbial grazing in surface waters may promote the retention of these materials in the upper ocean and atmosphere (Michaels and Silver 1988).

Relatively few investigators report the grazing activities of protozoa in antarctic ecosystems (Burkill, Edwards, and Sleigh 1995; Archer et al. 1996; Froneman and Perissinotto 1996). Nevertheless, heterotrophic protists have been reported to constitute a significant, and sometimes dominant, component of the total protistan abundance and biomass in the southern oceans, their coastal seas, and the sea-ice microbial communities associated with these water masses (Gowing and Garrison 1992; Stoecker, Buck, and Putt 1993). Quantification of the rates of growth and trophic activity of these microbial consumers is, therefore, imperative to understanding how these food webs function.

Our component of the JGOFS process study is designed to obtain information on the abundances and trophic activity of microbial assemblages within the water column of the Ross Sea proper, mostly along a transect line at 76°30'S 168°E to 178°W (Anderson 1993). We have completed three cruises at this time (October and November 1996; January and February 1997; April and May 1997) with one remaining (November and December 1997) to provide seasonal coverage from austral spring through fall. Our ongoing research program entails

- characterization of the standing stocks of nanoplankton and microplankton (photosynthetic and heterotrophic microorganisms 2–200 micrometers in size) by direct microscopy (Kemp et al. 1993) and
- experimental investigations of the rates of herbivory and bacterivory by the microbial consumers within these size classes using the dilution technique for estimating herbivory (Landry, Kirshtein, and Constantinou 1995) and the disappearance of fluorescently labeled bacteria (FLB) as an indicator of the activity of bacterivorous protozoa (Marrasé, Lim, and Caron 1992).

We observed standing stocks of chlorophyll during mid-to-late austral summer (January and February 1997) that were nearly three orders of magnitude greater than chlorophyll concentrations observed during cruises conducted in the spring or fall. Surprisingly, rates of herbivory during all three cruises were exceedingly low, even during periods of high chlorophyll concentrations (table). The validity of these experimental results was corroborated by independent methods of examining herbivory. These ancillary measurements indicated that microorganisms smaller than 200 micrometers had only a minor impact on the standing stock of phytoplankton. Herbivory by larger zooplankton was not demonstrable. Herbivorous microplankton were present during austral summer (as evidenced by microscopical examination), but their grazing activities did not affect significantly the phytoplankton assemblages that were composed largely of colonies of Phaeocystis spp. in the central polynya and diatoms at the periphery of the sea.

Figure 2. A colonial choanoflagellate from the euphotic zone of the Ross Sea. Approximately 30 individual cells are visible by light photomicrograph (A) among a tangle of costae that compose the vasilike loricae of this species. An epifluorescence micrograph (B) of the same colony shows the presence of numerous ingested fluorescently labeled bacteria inside most of the protozoan cells (examples shown by arrows in B). These ingested prey indicate an active bacterivorous assemblage.
Samples from bacterivory experiments are being analyzed at this time, but preliminary conclusions based on available data indicate that bacterivores were abundant and active during the period of the phytoplankton bloom and subsequent decrease during the austral summer cruise. Up to 30 percent of the bacterial assemblages were removed daily by bacterivores at specific depths and locales during that cruise. High abundances of colonial choanoflagellates in surface waters at that time accounted for much of the grazing pressure on bacteria (figure 2). Our preliminary analyses of microbial grazing during the AESOPS cruises revealed the surprising finding that microbial trophic activities did not result in high rates of mortality of phytoplankton even during periods of peak phytoplankton biomass. We speculate that other processes (e.g., sinking) must be responsible for the removal of much of this primary production. On the other hand, a bacterivorous protozoan fauna was abundant and active during austral summer 1997, and bacteria were consumed at significant rates in some of our experiments. We speculate that much of the energy entering the “microbial loop” in this coastal sea at that time may be a consequence of release of dissolved organic material by the abundant phytoplankton assemblage with subsequent uptake and growth by the heterotrophic bacterial assemblage.

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Quantitative estimation of the phlorotannin content of three antarctic brown macroalgae

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Brown macroalgae similar to those that dominate communities along the Antarctic Peninsula are often chemically defended against herbivory via sequestration of phlorotannins (polyphenolics) (reviewed by Steinberg 1992). Physodes that may contain phlorotannins are produced by antarctic members of the Phaeophyceae (Anderson 1985; Moe and Silva 1989), and Iken (1994, 1995) has detected phlorotannins and suggested that they may be involved in deterring grazing, but we are aware of no published reports that provide quantitative data on the concentrations of these defensive compounds in antarctic macroalgae. The goal of this article is to provide preliminary, quantitative estimations of phlorotannin levels in three large and ecologically important antarctic brown macroalgae.

Individual plants of the brown macroalgae Desmarestia menziesii, Himantothallus grandifolius, and Cystosphaera jacquinotii were collected near Palmer Station on Anvers Island, Antarctica (64°S 64°W). Triplicate samples of each plant were analyzed for phlorotannins following the method of Arnold, Tanner, and Hatch (1995). C. jacquinotii was subdivided into reproductive receptacles and into individual vegetative components (blade, air bladders, and lower stipe for analysis). Levels detected in vegetative tissues of D. menziesii and H. grandifolius and in the reproductive tissues (receptacles) of C. jacquinotii (figure) were very high relative to levels known to deter feeding by herbivores (cf. Steinberg 1992). Conversely, vegetative tissues of C. jacquinotii contained levels of phloro-
tannins that were very low and that would be unlikely to prevent herbivory.

These results indicate that antarctic brown macroalgae produce phlorotannins at concentrations that would provide defense against a wide variety of herbivores in lower latitude communities (cf. Steinberg 1992). We postulate that the phlorotannins are likely to play this same role in antarctic brown macroalgae. Our observation that C. jacquinotii differentially allocates phlorotannins to its stalked (and, therefore, very exposed) reproductive structures (figure) is consistent with the “optimal defense theory” of plant chemical defense (Rhoades 1979). This species appears to invest in defenses of its vulnerable and, presumably, high energy content reproductive structures even though it does not seem to defend its vegetative tissues.

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References


Iken, K. 1995. Food relations between antarctic macroalgae and herbivores. *Berichte zur Polarforschung*, 155, 21–23. [In German]


Detection of ultraviolet radiation induced DNA damage in microbial communities of the Gerlache Strait

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There is now strong evidence that ultraviolet radiation (UVR) is increasing over certain locations on the Earth’s surface. Of primary concern has been the annual pattern of ozone depletion over Antarctica and the southern oceans where ozone levels have declined as much as 74 percent compared to pre-ozone-hole events. Reduction of ozone concentration selectively limits stratospheric adsorption of ultraviolet-B (UV-B) radiation [280–320 nanometers (nm)], resulting in higher irradiance on the Earth’s surface. As a result, studies of the impact of natural UVR on marine microorganisms have received much attention. The impact of increased UV-B due to ozone depletion on phytoplankton and
primary production has attracted extensive interest. The effects of UV-B on bacteria, in contrast, have been largely overlooked. It is apparent from previous studies in the southern ocean and elsewhere that bacteria play a vital role in mineralization of nutrients and provide a trophic link to higher organisms. The objectives of our study have been to identify the effects of UVR and ozone depletion on bacterioplankton in the southern ocean. Our approach has been to combine state-of-the-art molecular approaches with more traditional microbial ecology methodologies. We have examined the extent of DNA damage in bacterioplankton resulting from UVR and as a function of ozone depletion with the ultimate goal of estimating the effect of the stress on carbon fluxes through bacterial assemblages.

Cyclobutane pyrimidine dimers (CPDs) are one of the unique photoproducts created by UV-B and these DNA lesions may be identified using radioimmunoassays (Mitchell 1996). If these photolysis are not repaired, they may affect bacterial DNA and mRNA synthesis resulting in gene mutations, altered physiological activities, or lethality. Because they are induced by UV-B, quantification of these photoproducts is a direct means by which UVR effects may be monitored.

Our primary objectives were to determine the distribution of UV-B–induced DNA damage as a function of depth in the water column, time of day (diel studies), and how these may change as a function of ozone conditions and sea state (i.e., surface-water mixing). Results from representative depth profiles are presented herein. Depth profiles of damage were determined by collecting water at discrete depths at sunrise and again near sunset. The bacterioplankton fraction was separated from the larger organisms and concentrated by filtration onto 0.2-micrometer (µm) pore filters. DNA damage was determined upon return to our laboratories (Jeffrey et al. 1996). Potential damage with depth was estimated by deploying DNA dosimeters (solutions of calf thymus DNA in quartz tubes; Jeffrey et al. 1996) at discrete depths during sunlight hours. By comparing DNA damage collected from in situ dosimeters with water column bacterioplankton depth samples, we have been able to identify the role of mixing in distribution of UV-B effects.
such as the southern oceans may result in reduced impact of UVR. These results demonstrate the difficulty in predicting in situ UVR effects based on physical measurements. Further, although these effects have been observed for DNA damage in bacterioplankton, other reports have suggested that surface-water mixing may intensify the distribution of phytoplankton production inhibition (Cullen et al. 1994). These results suggest that different trophic levels and processes may be affected differently by changes in the physical environment, further complicating predictive modeling of UVR effects.

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References


Epipelagic communities in the northwestern Weddell Sea: Results from high-resolution trawl surveys

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The southern ocean is one of the most productive areas of the marine environment, supporting an abundant and diverse pelagic community (e.g., Lancraft, Torres, and Hopkins 1991; Hopkins et al. 1993; Voronina, Kosobokova, and Pakhomov 1994). One of the most important features structuring communities in this part of the ocean is the antarctic ice sheet, which covers up to 20 million square kilometers during the austral winter but contracts during the summer to less than 4 million square kilometers (Zwally et al. 1983; Laws 1985). The magnitude of this seasonal variation, arguably the most dramatic seasonal process in the ocean, may have substantial effects on the biota inhabiting the underlying water column (Eicken 1992; Loeb et al. 1997; Nicol and Allison 1997). In particular, the presence/absence of pack ice may substantially affect the trophic coupling between surface predators (e.g., seabirds, marine mammals) and their pelagic prey (Ainley et al. 1986). Until recently, little was known about the community living beneath seasonal sea ice in the southern ocean, due in part to the logistic difficulty of sampling in areas covered by pack ice (cf. Kaufmann et al. 1995). As a result of these logistical constraints, previous studies have been confined to brief periods of time that had broad spatial coverage but poor temporal coverage and low temporal resolution.

As part of a study to sample the pelagic community beneath seasonal pack ice with high temporal resolution over a complete annual cycle, we conducted a series of trawls during September and October 1995 in an ice-covered area of the northwestern Weddell Sea near 63°S 46°W. The primary goal of this effort was to sample the epipelagic community with high temporal resolution during a period when our study area was covered by seasonal pack ice, for later comparison with samples taken during seasons characterized by different degrees of ice coverage. Samples were collected using a multiple opening-closing trawl with six nets [MOCNESS, with a 10-square-meter mouth opening (Wiebe et al., 1985), 4-millimeter circular mesh in the main body, and 505-micrometer (μm) mesh cod ends]. Sampling periods lasted 1 hour and covered a 50-meter (m) deep portion of the epipelagic zone, defined here as the region of the ocean between the surface and 100 m depth. Volumes of water sampled during the 1-hour sampling periods ranged from 24,274 to 46,832 cubic meters.

The most abundant species collected with the MOCNESS trawl were the euphausiids Euphausia superba and Thysanoessa macrura and the salp Salpa thompsoni. Also abundant was the siphonophore Diphys antarctica, and lesser numbers of hyperiid amphipods (Cyllopus lucasii, Hyperiella dilatata, and Primno macropa), polychaetes (Vanadis antarctica and Tomopteris carpenteri), pteropods (Clio sp.), and chaetognaths were collected as well. A noteworthy component of our collections was a number of individuals of the pelagic medusa Periphylla periphyla. Although this species typically occurs at greater depths (e.g., Lancraft et al. 1991), we collected five medusae in the epipelagic zone. The largest of these had a fresh wet weight in excess of 4 kilograms, and all but one of the others were larger than 2 kilograms, by far the most massive organisms collected in our nets.

A temporal pattern in biomass was observed at this site; reduced values were recorded in the upper 50 m of the water column between 0800 and 1800 hours and maximum values between 2100 and 0100 hours (figure 1). During the period covered by this study, sunrise occurred between 0430 and 0530 hours (ship time) and sunset between 1700 and 1800 hours. The temporal pattern observed between the surface and 50 m depth was less evident between 50 and 100 m depth; however, a slight elevation in biomass was observed 3–4 hours after sunset (figure 1). It should be noted that trawl-based biomass from 0 to 50 m was consistently lower than between 50 and 100 m (figure 1), possibly as a result of surface perturbations due to the passage of the ship ahead of the trawl.

A portion of this temporal pattern may be explained by diel variation in the depth distribution of the abundant euphausiid Euphausia superba. Although virtually absent from surface waters during the day, substantial numbers of E. superba were collected in the epipelagic zone between 1800 and 0200 hours (figure 2). A similar pattern was observed for Salpa thompsoni, although estimated salp biomass was substantially lower than estimated krill biomass. Thysanoessa macrura exhibited an opposite pattern: elevated biomass in surface waters during the day and a reduced presence at night. Total biomass fluctuations were strongly influenced by T. macrura, as exemplified by the correspondence between total biomass (figure 1) and T. macrura biomass (figure 2) peaks at 1000 and 1400 hours between the surface and 50 m depth.

Our data indicate that biomass distribution within the epipelagic zone in the presence of seasonal pack ice was influenced primarily by krill species, specifically Euphausia superba and Thysanoessa macrura. The results thus far agree well with data collected to the northwest of our sampling site, near Elephant Island, that show a predominance of krill, compared to salps, during the austral spring and summer of 1995–1996 (Loeb et al. 1997). Our sampling program included trawl collections in the same area during April and May 1996 and
November and December 1996. Results from these operations indicate substantial differences in community composition among seasons: trawls from April and May 1996 contained up to 100 times more biomass than the samples from September and October 1995. Collections from November and December 1996 were intermediate in size between the other two sampling periods. We will continue to analyze the data from these three cruises and compare the results among periods characterized by different degrees of ice cover, to generate a greater understanding of the role of seasonal ice cover in structuring antarctic epipelagic communities.

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Figure 1. Hourly total biomass of animals (in grams per 1,000 cubic meters) collected with the MOCNESS net system in the upper 100 m of the water column in the northwestern Weddell Sea. Data are presented for hourly time periods beginning at the time indicated by the symbols. Symbols indicate values from individual trawls, and solid lines represent mean values for trawl samples from a given time period. Biomass estimates are based on measurements of preserved wet weight, uncorrected for the dehydrating effects of the preservation process, and should be regarded as underestimates of the true values.

Figure 2. Hourly total biomass (in grams per 1,000 cubic meters) of krill, Euphausia superba; salps, Salpa thompsoni; and krill, Thysanoessa macrura, collected with the MOCNESS net system in the upper 100 m of the water column in the northwestern Weddell Sea. Data are presented for hourly time periods beginning at the time indicated by the symbols. Symbols indicate mean values for individual time periods. Closed circles connected by a solid line indicate data from 0 to 50 m and open squares connected by a dashed line indicate data from 50 to 100 m depth. Biomass values were generated as in figure 1 and should be regarded as underestimates.

References


Evaluation of the functional role of suberitenones A and B from the sponge *Suberites* sp. found in McMurdo Sound, Antarctica

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Biological interactions of invertebrates on the benthos of McMurdo Sound, Antarctica, have received considerable attention over the last three decades. Beginning with seminal studies of Dayton, Robilliard, and Paine (1970) and Dayton et al. (1974) characterizing the community structure, subsequent studies have focused on chemical ecological interactions (reviewed by McClintock and Baker 1997). The field of chemical ecology seeks to understand biological interactions that are chemically mediated.

The chemical basis for such ecological interactions lies in secondary metabolic pathways. Metabolites derived from the secondary metabolic pathways differ from primary metabolites in that they are not involved in life-sustaining processes such as energy transport and storage, respiration, and reproduction. Such compounds generally are not ubiquitous. Secondary metabolites, also known as natural products, were once regarded as superfluous, evolutionary dead ends but are now widely regarded as playing a role in ecological interactions of the producing organism (Williams et al. 1989; Eisner and Meinwald 1995).

Our interest in the secondary metabolites present in McMurdo Sound organisms has been driven by an interest in their functional role. Although we have investigated a number of marine phyla, sponges have proven to be a rich source of natural products suitable for an evaluation of their functional role. During our 1997 field season, we had the opportunity to collect specimens of the sponge *Suberites* sp., and we report here our preliminary chemical ecological evaluation of this common benthic invertebrate.

Lipophilic and hydrophilic extracts of several pooled *Suberites* sp. were prepared by exhaustive extraction of the freeze-dried specimens with methanol/dichloromethane (1:1) and methanol/water (1:1). The extracts were examined by proton (1H) nuclear magnetic resonance (NMR) spectroscopy, which revealed the presence of a relatively high concentration of terpenoid components in the lipophilic extract. The lipophilic extract was then fractionated by normal phase gradient flash chromatography, producing a fraction eluting in 8:2 hexane/ethyl acetate that contained the terpenoid NMR signals. Further purification of the terpenes was achieved by reversed phase high-performance liquid chromatography (HPLC), yielding two compounds (1 and 2, figure) in a ratio of 3 to 1.

The major component (1) displayed 1H NMR signals characteristic of several functional groups, including two olefinic protons, two protons on carbons bearing oxygen, an acetoxyl methyl, a vinyl methyl, and four aliphatic methyl groups. The carbon-13 (13C) NMR spectrum contained 27 carbon signals including four olefinic, one ester/amide/acid, and one ketone/aldehyde carbon. The DEPT (distortionless enhancement by polarization transfer) spectrum indicated two olefinic carbons were protonated and two quaternary; the remaining carbons included six methyl, seven methylene, five methine, and five quaternary signals.

Structure elucidation of this isolate was achieved by analysis of two-dimensional NMR spectra including correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence spectroscopy (HMQC). Partial structures representing ring D and parts of rings A and C were generated from analysis of the COSY spectrum. These partial structures were sufficiently unique to narrow the focus of our analysis to two compounds previously reported from an antarctic *Suberites* species collected from King George Island, suberitenone A (Shin et al. 1995). Comparison of the NMR spectral data of suberitenone A with our isolate 1 established their identity. Similar comparison of our isolate 2 confirmed its identity with suberitenone B.

We investigated the functional role of the suberitenones in two ecological bioassays. The tube-foot retraction assay (McClintock et al. 1994) is designed to detect the ability of a sponge to deter predation by production of toxic or noxious...
secondary metabolites. The spongivorous sea star *Perknaster fuscus*, the major sponge predator in McMurdo Sound (Dayton et al. 1974), was used as the test animal in the tube-foot retraction bioassay. In the disk diffusion assay, which provides data to evaluate the ability of sponge chemistry to deter microbial fouling or infection, antibiotic tester strains (ATSs) were employed as a general indicator of microbial cytotoxicity and microbes isolated from McMurdo Sound benthic invertebrates and substratum as indicators of ecologically relevant antibi-otic activity. The eight ATS strains included Gram-positive *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, and *Micrococcus luteus*; Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*; yeast *Saccharomyces cerevisiae*; and fungi *Aspergillus niger*. The invertebrate-associated microorganisms, which are currently undergoing identification, include one isolate each from the homogenized tissues of the hydroid *Halecium arboreum*; the sponges *Latrunculia apicalis*, *Calyx arcuarius*, and *Dendrilla membranosa*; and the sea star *Acodontaster conspicus*, as well as three microbes from sub-stratum rocks. The original report of the suberitenones failed to find bioactivity in these unusual sesterterpenes.

Results of the bioassays suggest the suberitenones are mediators of chemical defense in *Suberites sp.* in McMurdo Sound. Suberitenone A (1) caused a mean 33.6-second (standard deviation (SD)=14.8, number (n)=10) sustained tube-foot retraction and suberitenone B (2), a 29.4-second retraction (SD=18.2, n=10). Control experiments (n=20 each) were consistently shorter in duration: mechanical, 3.6-second retraction (SD=9.0); and silicone matrix, 4.1-second retraction (SD=11.3). A feeding stimulant control used in our prior study (McClintock et al. 1994) was shown to elicit a response indistinguishable from the mechanical and silicone matrix controls; thus, such tube-foot retraction behavior as seen with suberitenones A and B is indicative of feeding deterrence (McClintock et al. 1994).

Suberitenone A (1) displayed modest activity against two of the ecologically relevant microorganisms. Isolate 28.2, from *Halecium arboreum*, showed a 3-millimeter (mm) zone of inhibition at 500 micrograms per disk (µg/disk) and isolate 22.3, from *Acodontaster conspicuus*, a 1-mm zone at 1,000 µg/disk. Suberitenone B (2) was less active against the same two isolates, displaying a 1-mm zone of inhibition against both at 1,000 µg/disk. No antibiotic activity was found in ATS strains. Because the suberitenones have activity against sympatric bacteria, they have the potential to mediate fouling and infection in *Suberites sp.*

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References


Effects of feeding conditions on sodium pump (Na⁺,K⁺-ATPase) activity during larval development of the antarctic sea urchin *Sterechinus neumayeri*

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The seawater of McMurdo Sound is characterized by low and constant temperatures and by high seasonal fluctuations in the availability of phytoplankton food sources. The sea urchin *Sterechinus neumayeri*, which is widely distributed and abundant in McMurdo Sound, has feeding larval forms that have to survive for long periods (weeks to months) in the water column in the near absence of algal foods. The lifespan of these larval forms without food is set by their maternally endowed energy reserves and their rate of utilization of these reserves (i.e., their metabolic rate) (Shilling and Manahan 1994). The aim of this study is to understand the major biochemical processes that establish the metabolic rates for these larval forms. In particular, we have focused on the role of the sodium pump (Na⁺,K⁺-ATPase). This enzyme is an important transmembrane protein responsible for maintaining ion gradients in animal cells. Previously, we have shown that the physiological activity of this single enzyme could account for over 40 percent of the metabolic rate of larvae of temperate species of sea urchin (Leong and Manahan 1997). In this study, we quantify the changes in activity of the sodium pump during development of the antarctic sea urchin *S. neumayeri*. In addition, we present the developmental changes in the activity of Na⁺,K⁺-ATPase for fed and starved larvae.

Adult sea urchins were collected from McMurdo Sound (off Cape Evans) by scuba divers in October 1996. Males and females were induced to spawn by standard methods (injection of 0.5 molar potassium chloride) and fertilized eggs were placed in 200-liter culture vessels at a concentration of 7 per milliliter. All subsequent culturing over a 2-month period was done using ambient seawater from McMurdo Sound (–1.5°C) that had been passed through a 0.2-micrometer pore-size filter prior to use. The culture water was changed every 3 to 4 days by gently sieving the animals onto mesh screens. Under these rearing conditions, the embryos developed to the first larval feeding stage (early pluteus) after 22 days. Once the feeding larval stage was reached, two experimental treatments were set up for which larvae were either

- fed *ad libitum* on a mixture of algal cells (*Rhodomonas* sp. and *Dunalieilla tertiolecta*, each at a concentration of 7,500 cells per milliliter of culture water) or
- kept in filtered seawater under starvation conditions.

The total (potential) Na⁺,K⁺-ATPase activity and the physiologically active fraction of the total were measured in both the fed and starved larvae (figure 1A and B). The *in vivo* Na⁺,K⁺-ATPase activity of the enzyme (physiological activity at –1.0°C) in larvae was measured as the difference in transport rates of potassium ion (K⁺) from seawater [rubidium-86 ion transport].

![Figure 1](image-url)

**Figure 1.** A. Total Na⁺,K⁺-ATPase activity in *Sterechinus neumayeri* during development of prefeeding stages (open circles) and in fed (solid circles) and starved larvae (after day 22, open circles). Measurements were made at 15°C by an *in vitro* enzyme assay and are presented as equivalents of ATPase activity at –1.0°C (see text for details on rate conversions using measured Q₁₀ value). A final concentration of 14 millimolar of ouabain was used for the assay. Each data point is the mean of 3 to 5 replicates. Error bars are ± standard error of the mean; where not shown, bar is within the graphical representation of the data point. B. Physiologically active (*in vivo*) Na⁺,K⁺-ATPase in prefeeding stages (before day 22) and in fed (solid circles) and starved larvae (open circles). Each data point represents the rate of K⁺ transport calculated from a time course transport experiment performed at –1.0°C with 8–9 individual data points. [K⁺ transport was measured as ⁸⁶Rb⁺ flux with correction for specific activity of K⁺ in seawater, see Leong and Manahan (1997) for details.] Each error bar is ± standard error of the slope of each individual linear regression line. [pmol Pi (indiv.)–1 (h)–1 denotes picomoles of inorganic phosphate per individual per hour.]
(86Rb+) used as a radiotracer] in the presence and absence of ouabain, a specific inhibitor of Na+,K+-ATPase. The total Na+,K+-ATPase activity was measured in vitro using tissue homogenates of larvae (with and without ouabain). The method of Esmann (1988) was used for the in vitro measurements, where the rate of release by Na+,K+-ATPase of inorganic phosphate (P) from ATP was measured. All in vitro assays were conducted at 15°C. The activities so obtained were converted to their corresponding values at physiological temperature (–1.0°C) using a Q10 of 2.9. This value was previously determined for the effect of temperature on the activity of Na+,K+-ATPase in S. neumayeri (data not shown). The protein contents of prefeeding stages and of fed and starved larvae were determined using a modified Bradford method (Leong and Manahan 1997) to allow for calculations of developmental changes in the protein-specific activity of the enzyme.

Fed and starved larvae had increases during development in total (figure 1A) and in vivo (figure 1B) Na+,K+-ATPase activities; the fed larvae had higher activities for both sets of measurements. The protein content of the fed larvae increased with feeding at a rate of 8.1 nanograms per larva per day (figure 2A). The starved larvae continuously lost protein at –1.27 nanograms per larva per day (negative slope of regression for loss of protein in starved larvae is statistically significant: F(0.05,1,90) = 5.2, variance ratio = 71.34, P<0.001). Due to this loss of protein, the starved larvae had higher protein-specific total Na+,K+-ATPase activities compared to the fed larvae (figure 2B). This finding, which shows that the Na+,K+-ATPase protein was retained in starved larvae relative to the loss of total protein, resulting in higher specific activities of this enzyme in starved larvae, is further illustrated in figure 2C, where the relationship is shown for enzyme activity as a function of the change in protein content per larva. This figure shows that the data set for the starved larvae was higher than would be predicted from the regression shown for the fed larvae. These data suggest that a critical amount of Na+,K+-ATPase activity has to be maintained in larvae of S. neumayeri. The consequence of this for starved larvae is a higher metabolic rate which will affect the lifespan of these antarctic sea urchin larvae.

We extend our thanks to Tracy Hamilton (University of Southern California) and Robert Robbins (Antarctic Support Associates) for diving operations necessary to collect adult sea urchins and to Adam Marsh and Tracy Hamilton for assistance with larval culturing. This research was supported by National Science Foundation grant OPP 94-20803 to D.T. Manahan.

References

Feeding and energetic costs of larval development in the antarctic sea urchin *Stereochinus neumayeri*

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Currently, a debate is underway concerning why antarctic invertebrate embryos and larvae have such protracted developmental times in comparison to their temperate counterparts. Thorson (1950) suggests that cold polar temperatures slow metabolism and, hence, developmental rates in antarctic invertebrates whereas Clark (1983) argues that the limited nutritional resources in polar environments restrict metabolic rates of developing embryos and larvae. Despite almost a half century of debate, we still know very little about how larvae survive and metamorphose in extreme polar environments. As a first step toward empirically determining the relative importance of temperature or nutritional controls, our research measures the specific costs of early development and the relative impact of feeding on those energetic costs in the larvae of a polar invertebrate, the antarctic sea urchin *Stereochinus neumayeri*.

The energetic cost of early development in *S. neumayeri* was measured by culturing embryos to the four-arm pluteus larval stage (day 22 of development at –1.5°C) and then raising the larvae to the beginning of the six-arm pluteus stage (day 60). For this study, larvae were then raised in two treatments: one group was fed *ad libitum* (a mixture of the red alga *Rhodomonas* sp. and the green alga *Dunaliella tertiolecta*) and the other group was starved (all culturing details described in Leong and Manahan, *Antarctic Journal*, in this issue).

Over the course of development, the biochemical composition of different embryonic and larval stages was measured in terms of:
- organic mass by the analysis of total elemental carbon and nitrogen,
- protein content as determined by a standard Bradford assay, and
- DNA content as determined using a quantitative fluorescent dye technique (Hoescht’s stain) in a fluorometer equipped to hold small (10-microliter) capillary tubes.

Oxygen (O₂) consumption rates were quantified using small (<1-milliliter volume) biological oxygen demand vials (µBOD), a new method developed in our laboratory. Between 50 and 500 individual embryos or larvae are incubated in a µBOD vial for 8–10 hours (at –1.5°C). A gas-tight syringe is used to inject an aliquot of the µBOD seawater into a polarographic oxygen sensor (POS), providing a direct measure of the O₂ concentration in the µBOD vial.

During early development, the biomass of embryos does not decline as expected due to the oxidation of energy reserves (figure 1A). The lack of a significant decline in mass during early development has been previously documented for *S. neumayeri* (Marsh and Manahan 1996) and for another antarctic echinoderm, the asteroid *Odontaster validus* (Shilling and Manahan 1994). From figure 1A, embryos of *S. neumayeri* reach the four-arm pluteus stage (day 22) with approximately the same total mass as the initial eggs. Early development, however, does have a total metabolic cost of 2.2 millijoules per individual (mJ individual–1) for the first 22 days of development (figure 2B). With no detectable change in mass, the energy source fueling development must be derived from an external origin. These findings suggest that embryos of *S. neumayeri* may exhibit a far greater ability for nutrient uptake than has been found in temperate sea urchin embryos.

Once larvae begin feeding at day 22, individual biomass increases steadily relative to starved larvae. At day 50, the third pair of larval arms begins to form, and larval biomass in the fed treatment increases rapidly between days 50 and 60. Figure 1B shows that the cellular protein content does not change during development between the fed and starved treatments, indicating the change in biomass does not occur as a change in cell volume or size. This finding is substantiated in figure 1C where the individual DNA content is higher in fed than starved larvae indicating that fed larvae have a higher cell number count. Thus, changes in larval biomass occur at the level of changes in cell number and not in terms of changes in cell size.

During embryonic development, respiration rates steadily rise to a maximum value of 16 picomoles of oxygen per embryo per hour (pmol O₂ embryo–1 h–1) at day 22 (figure 2A). From day 22 to day 32, respiration rates are equivalent between fed and starved treatments. After day 32, fed plutei evidence a large increase in respiration rates, which continue to increase until day 60. By day 60, respiration in the fed plutei has more than doubled to 37 pmol O₂ larva–1 h–1 from the initial pre-feeding level.

When the four-arm plutei begin to feed *ad libitum* at day 22, the total metabolic costs from fertilization to day 60 increase from 7.42 mJ individual–1 in the starved group to 12.67 mJ individual–1 in the fed group (figure 2B). Feeding results in a 71 percent increase in metabolic energy expenditure. A regression of the cumulative cost of development against time (between day 30 and day 60) results in a daily energy expenditure for fed larvae of 299.1 microjoules per individual per day (µJ individual–1 d–1) (r²=0.9980) while starved larvae expended only 135.7 µJ individual–1 d–1 (r²=0.9986) (figure 2B). The absolute difference in cumulative energy expended between fed and starved larvae at day 60 was 5.3 mJ individual–1 (assuming 484 kilojoules per mole of O₂), whereas the mass difference was 24.0 mJ individual–1 [in energy equivalents, 31.75 kilojoules per gram for 50 percent...
protein and 50 percent lipid (Shilling and Manahan 1994)]. For fed *S. neumayeri* plutei, the energy acquired relative to energy expended during feeding increased by 457 percent between day 22 and day 60. This comparison indicates that feeding by this echinoplutei is highly energy efficient under the culturing conditions used here.

Feeding by *S. neumayeri* plutei did not alter the timing of development for the progression from the four-arm to the six-arm stage. The morphometric events associated with the formation of the third pair of larval arms evidenced an identical time of appearance between fed and starved plutei despite the large biochemical differences between them. In these experiments, the rate of early larval development was not limited by

Figure 1. Biochemical components during development of *Sterechinus neumayeri*. A. Organic mass. B. Cellular protein content. C. Individual DNA content. Symbols are plotted as means (± standard error of the mean; n=3); embryos = open circles; fed plutei = closed squares; starved plutei = shaded triangles. (ng individual$^{-1}$ denotes nanograms per individual, µg prot pg$^{-1}$ DNA denotes microgram of protein per picogram of DNA.)

Figure 2. Energy metabolism during development of *Sterechinus neumayeri*. A. Respiration rates. B. Cumulative cost of development. Symbols are plotted as means (± standard error of the mean; n=3); embryos = open circles; fed plutei = closed squares; starved plutei = shaded triangles. (µJ d$^{-1}$ denotes microjoules per day, mJ individual$^{-1}$ denotes millijoules per individual, pmol O$_2$ h$^{-1}$ ind$^{-1}$ denotes picomoles of oxygen per hour per individual.)
endogenous resources and the availability of exogenous food did not affect the timing of these events. In summary, the net effect of feeding was an increase in cell number but not in apparent cell size or function. Fed and starved larvae possessed similar energetic and developmental rate processes; the primary difference in metabolic rate between the two treatments was likely the total number of cells performing those activities.

We thank Patrick Leong and Tracy Hamilton (University of Southern California) for help with culturing and feeding the larvae; Rob Robbins (Antarctic Support Associates) assisted in diving operations required to collect adult sea urchins. This research was supported by National Science Foundation grant OPP 94-20803 to D.T. Manahan.

References


Fishes from the southern and western Ross Sea

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The fish fauna of the antarctic shelf is unique among marine habitats. The fauna is dominated by a single group of perciform fishes known as notothenioids. In the absence of competition from other fish groups, notothenioids underwent a depth-related diversification involving the alteration of density. Although lacking swim bladders, notothenioids occupy most water column and benthic niches and constitute 90–95 percent of the fish biomass on the antarctic shelf (Eastman 1993). The six families and 95 antarctic species of this endemic suborder may be the marine equivalent of a lacustrine adaptive radiation and the only known species flock of marine fishes (Eastman and Clarke in press). The notothenioid antifreeze glycopeptides, originating from trypsinogen, are a uniquely derived key innovation that may have allowed notothenioid diversification in subzero waters (Chen, DeVries, and Cheng 1997). Thus, the antarctic shelf is an insular evolutionary site, with an endemic fish fauna equally as interesting, but less well known, as those in Lake Baikal in Siberia or the African Great Lakes.

Although fishes were first collected in the southern Ross Sea nearly 100 years ago, the fauna is still incompletely characterized. This was demonstrated during cruise 96-6 (18 December 1996 to 8 January 1997) of the R/V Nathaniel B. Palmer when this vessel was first employed for fishing in the Ross Sea. Although time for fishing was limited, a small but important collection was obtained, including one new species. The effectiveness of the Palmer in towing relatively large commercial bottom trawls was also established. The fishes will be utilized in a continuing study of the evolution and adaptation of the antarctic fish fauna (Eastman 1993).

Fishing was conducted at 12 stations, with additional surface collecting in a tide crack at station 108 (table 1). A variety of gear was used, but a 10-meter-long Marinovich Gulf Coast style flat trawl, a type of otter trawl, was most effective in obtaining maximum familial and species diversity. A summary of the catch is presented in table 2. Taxonomic nomenclature for fishes is that used by Gon and Heemstra (1990), with the exception that the nominal species Cryodraco atkinsoni was recognized as distinct from Cryodraco antarcticus. To ensure accuracy, experts were enlisted to verify identifications of some groups. Artedidraconoids were identified by R.R. Eakin, University of New England–Westbrook College Campus, Portland, Maine; zoarcids by M.E. Anderson, J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa; and rajids by J.D. McEachran, Texas A&M University, College Station, Texas. Voucher specimens were deposited in the museums associated with these institutions (table 2).

The catch yielded 326 specimens representing 8 families and 32 species (table 2). Among the specimens were one new species, seven rare species, two new locality records for the Ross Sea, three records for most southerly occurrences in the southern ocean, and eight new depth records. Notothenioids dominated the catch in terms of number of specimens (76.1 percent) and species richness (84.4 percent). Notothenioids accounted for 77.3 percent of fish biomass at station 93 (75°S) and 91.2 percent at station 119 (77°S).

The new species, a large (250 millimeters standard length) and distinctive artedidraconid of the genus Pogonophryne, will be formally described by R.R. Eakin and J.T. Eastman. It has been deposited in the Smithsonian Institution, National
Table 1. Data for fishing stations during cruise 96-6 of R/V Nathaniel B. Palmer in the southern and western Ross Sea

<table>
<thead>
<tr>
<th>Station number</th>
<th>Position</th>
<th>Date</th>
<th>Local time</th>
<th>Sampling depth (m)</th>
<th>Bottom depth (m)</th>
<th>Gear</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>72°59.8’S 175°08.6’E</td>
<td>18 Dec 1996</td>
<td>2345–0128</td>
<td>360</td>
<td>360</td>
<td>Blake trawl</td>
</tr>
<tr>
<td>11</td>
<td>74°59.7’S 172°33.9’E</td>
<td>20 Dec 1996</td>
<td>0134–0351</td>
<td>560</td>
<td>560</td>
<td>Blake trawl</td>
</tr>
<tr>
<td>15</td>
<td>75°02.3’S 166°16.1’E</td>
<td>21 Dec 1996</td>
<td>0100–0319</td>
<td>939</td>
<td>939</td>
<td>Blake trawl</td>
</tr>
<tr>
<td>30</td>
<td>76°30.0’S 175°00.2’E</td>
<td>23 Dec 1996</td>
<td>2343–0045</td>
<td>469</td>
<td>469</td>
<td>Blake trawl</td>
</tr>
<tr>
<td>34/42</td>
<td>77°58.7’S 178°03.6’W</td>
<td>24–26 Dec 1996</td>
<td>(48-hour set)</td>
<td>690</td>
<td>690</td>
<td>Set line</td>
</tr>
<tr>
<td>34/42</td>
<td>77°59.0’S 170°21.7’E</td>
<td>29 Dec 1996</td>
<td>2012–2346</td>
<td>?</td>
<td>830</td>
<td>I-K midwater</td>
</tr>
<tr>
<td>74</td>
<td>77°11.1’S 164°45.4’E</td>
<td>31 Dec 1996</td>
<td>1055–1257</td>
<td>0–200</td>
<td>471</td>
<td>I-K midwater</td>
</tr>
<tr>
<td>87</td>
<td>75°59.9’S 171°00.4’E</td>
<td>2 Jan 1997</td>
<td>1908–2203</td>
<td>?</td>
<td>428</td>
<td>I-K midwater</td>
</tr>
<tr>
<td>93</td>
<td>75°30.1’S 174°56.8’E</td>
<td>3 Jan 1997</td>
<td>2213–0058</td>
<td>300</td>
<td>300</td>
<td>Flat bottom trawl</td>
</tr>
<tr>
<td>108</td>
<td>74°39.6’S 165°21.0’E</td>
<td>5 Jan 1997</td>
<td>1941–2102</td>
<td>Surface</td>
<td>200?</td>
<td>Tide crack</td>
</tr>
<tr>
<td>119</td>
<td>77°19.7’S 165°41.3’E</td>
<td>8 Jan 1997</td>
<td>0931–1125</td>
<td>874</td>
<td>874</td>
<td>Flat bottom trawl</td>
</tr>
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Table 2. Summary species list for fishes obtained on cruise 96-6 of R/V Nathaniel B. Palmer in the southern and western Ross Sea

<table>
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<tr>
<th>Family and species</th>
<th>Number</th>
<th>Comments&lt;sup&gt;a&lt;/sup&gt; (museum abbreviations&lt;sup&gt;b&lt;/sup&gt; and catalog numbers)</th>
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<tbody>
<tr>
<td>Rajidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bathyraja eatonii</td>
<td>5</td>
<td>Most southerly record for a chondrichthyan in the Ross Sea (75°30.1’S) Rare; second record for this species in Ross Sea (TCWC 8909.01)</td>
</tr>
<tr>
<td>Muraenolepididae</td>
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<td></td>
</tr>
<tr>
<td>Muraenolepis microps</td>
<td>3</td>
<td>Most southerly record for family and species (75°30.1’S)</td>
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<td>Liparidae</td>
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<td></td>
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<tr>
<td>Paraliparis antarcticus</td>
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</tr>
<tr>
<td>Zoarcidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ophthalomolycus amherensis</td>
<td>22</td>
<td>New depth record (939 m); RUSI 54820, 54822, 54823, 54824</td>
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<tr>
<td>Pachycara brachycaphalum</td>
<td>46</td>
<td>RUSI 54819, 54821, 54825</td>
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<tr>
<td>Nototheniidae</td>
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<td>Pleuragramma antarcticum</td>
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<td>Trematomus eulepidotus</td>
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<td>Trematomus lepidorhinus</td>
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<tr>
<td>Trematomus loennbergii</td>
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<td>New depth record (874 m)</td>
</tr>
<tr>
<td>Trematomus scotti</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Artedidraconidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artedidraco orinae</td>
<td>1</td>
<td>Most southerly record for this species (72°59.8’S)</td>
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<td>Dolioidecrabs longedoralis</td>
<td>23</td>
<td></td>
</tr>
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<td>Histiodraco velifer</td>
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<td>Rare; new depth record (874 m)</td>
</tr>
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<td>Pogonophryne phylopon</td>
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<td></td>
</tr>
<tr>
<td>Pogonophryne scotti</td>
<td>1</td>
<td>New species (USNM 345594) to be described by R. Eakin and J. Eastman</td>
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<tr>
<td>Pogonophyrone sp</td>
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<tr>
<td>Bathymichthyidae</td>
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<td></td>
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<tr>
<td>Akarotaxis nudiceps</td>
<td>15</td>
<td>Rare; new depth record (939 m)</td>
</tr>
<tr>
<td>Bathymichthys macrolepis</td>
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<td>Rare</td>
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<td>Bathymichthys marin</td>
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<td>Cygnodraco mawsoni</td>
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<td>New locality record for Ross Sea</td>
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<td>Gerlachea australis</td>
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<td>Rare</td>
</tr>
<tr>
<td>Gymnodraco acuticeps</td>
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<td>Priodraco evansi</td>
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<td>New depth record (874 m)</td>
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<td>Racovitza glacialis</td>
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<td>Vomeridens infuscipinnis</td>
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<td>Channichthyidae</td>
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<td>Chionodraco hamatus</td>
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<td>New depth record (874m)</td>
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<td>Chionodraco myersi</td>
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<tr>
<td>Cryodraco antarcticus</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Cryodraco atkinsoni</td>
<td>4</td>
<td>Rare; new locality and depth (874 m) records for Ross Sea</td>
</tr>
<tr>
<td>Dacodraco hunteri</td>
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<tr>
<td>Pagetopsis macropterus</td>
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<tr>
<td>Pagetopsis maculatus</td>
<td>1</td>
<td>New depth record (874 m)</td>
</tr>
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</table>

<sup>a</sup>New occurrence and depth records established relative to information in *Fishes of the Southern Ocean* (Gon and Heemstra 1990).  
<sup>b</sup>Museum abbreviations: TCWC, Texas Cooperative Wildlife Collection, Texas A&M University, College Station, Texas; RUSI, J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa; USNM, Smithsonian Institution, National Museum of Natural History, Washington, D.C.
The loss of hemoglobin and/or myoglobin affects cardiac ultrastructure in antarctic fishes

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Channichthyidae, a family of antarctic fishes, are unique among all vertebrates because they lack the oxygen-carrying protein, hemoglobin. Much is known about the cardiovascular adaptations of these fish that allow them to survive despite the loss of hemoglobin:

- their heart-to-body mass ratio is three times greater than red-blooded notothenioids and allows for a cardiac output four to five times greater than red-blooded teleosts,
- capillary diameters are two to three times larger, and
- blood volume is two to four times greater than red-blooded teleosts (Hemmingsen 1991).

These adjustments in the cardiovascular system allow a large volume of blood to be pumped through the circulatory system at high flow against only a small peripheral resistance; this configuration maintains oxygen delivery to working muscles.

Not only do Channichthyids (icefishes) lack hemoglobin, but some species within this family also lack myoglobin in their oxidative muscle tissue (Sidell 1997). Myoglobin is the intracellular oxygen-binding protein that is important for the storage and transport of oxygen within muscle cells (Wittenberg and Wittenberg 1989). Johnston and Harrison (1987) studied hearts from icefish and determined that modifications in the architecture of cardiac myocytes may enhance oxygen delivery to the mitochondria in those species that lack myoglobin. They compared the ultrastructure of ventricles

References

between the hemoglobinless Chaenocephalus aceratus and the red-blooded Notothenia neglecta. Hearts of C. aceratus had greater mitochondrial surface and volume densities compared to the hearts of N. neglecta. Johnston and Harrison concluded that elevated mitochondrial density in myocytes compensated for the loss of myoglobin, because increased mitochondrial surface and volume densities decrease the diffusion distance of oxygen between the lumen of the heart and the mitochondrial membrane. However, these two species also differ in the presence and absence of hemoglobin, and therefore, the observed increase in mitochondrial volume and surface densities could also reflect the loss of hemoglobin in C. aceratus.

The purpose of our study was to differentiate between cardiac myocyte modifications that result from the loss of hemoglobin from those that result from the loss of myoglobin. Three species of notothenioids were examined in this study:

- **C. aceratus**, which lack both hemoglobin and ventricular myoglobin;
- **Chionodraco rastrospinosus**, which lack hemoglobin, but have myoglobin in their ventricle; and
- **Gobionotothen gibberifrons**, which have both hemoglobin and myoglobin.

We examined differences in the architecture of the heart that correlate with the loss of myoglobin by comparing hearts of C. aceratus and C. rastrospinosus. We determined differences in ultrastructure that correlate with the loss of hemoglobin by comparing hearts of C. rastrospinosus and G. gibberifrons. All three species are closely related and are sedentary, benthic fishes, so any differences observed in the hearts are likely due to differences in the expression of hemoproteins.

Fishes were collected with an otter trawl in Dallman Bay and transported to Palmer Station where they were maintained in running sea water. Fishes were killed by a sharp blow to the head and heart ventricles were excised and perfused with an ice-cold ringer solution. Ventricles were then slowly perfused with an ice-cold fixative solution of 3 percent glutaraldehyde, 0.1 mol (mol) sodium cacodylate, 0.11 mol sucrose and 2 millimole (mmol) calcium chloride. Ventricles were stored in fixative at 4°C for 8 hours and then transferred to a fixative solution of 1 percent glutaraldehyde, 4 percent formaldehyde, 0.1 mol sodium cacodylate, 0.11 mol sucrose, and 2 mmol calcium chloride. Tissues were transported to our laboratory at the University of Maine, postfixed in 1 percent osmium tetroxide, and embedded in resin. Tissue blocks were thin-sectioned (approximately 80 nanometers) and placed on 400 mesh copper grids. Sections were stained with 2 percent uranyl acetate followed by 0.5 percent lead citrate. Tissue sections were viewed and photographed using a Phillips CM 10 transmission electron microscope. Micrographs were projected onto a digitizing tablet and ultrastructural parameters were quantified using point-counting methods (Weibel 1979). Parameters measured included mitochondrial volume density, mitochondrial surface density, mitochondrial cristae surface density, and myofibril volume density.

Mitochondrial volume density in hearts of C. rastrospinosus is greater than that found for G. gibberifrons (p=0.06) (figure). There is also a significant difference in the mitochondrial surface density between the two species (table). Thus, loss of hemoglobin correlates with an increase in the number and volume of mitochondria per myocyte. This increase, however, is not as great as that which results from the loss of myoglobin.

Mitochondria occupy nearly 37 percent of cell volume in the hearts of C. aceratus. This volume is significantly greater than the mitochondrial volume density in hearts of C. rastrospinosus.
pinosus or G. gibberifrons (table) and suggests that loss of myoglobin accounts for the large increase in mitochondrial volume density, not the loss of hemoglobin.

The electron transport machinery is located on the inner mitochondrial membrane, and thus, density of inner mitochondrial membrane reflects capacity for oxidative phosphorylation. Among the species of antarctic fishes that we examined, surface density of inner mitochondrial membrane per mitochondrial volume varied inversely with the percentage of cell volume displaced by mitochondria. The result is that mitochondrial cristae density per volume tissue remains fairly constant. This finding is consistent with our measurements of the activity of cytochrome oxidase (CO), the terminal electron acceptor in the electron transport chain. The activity of CO per gram wet weight of ventricle tissue is equal among the three species (O’Brien and Sidell unpublished data). Thus, aerobic metabolic capacity per gram heart muscle may be equal between the three species, despite differential expression of hemoproteins.

Increases in mitochondrial surface and volume densities decrease the oxygen diffusion distance between the lumen of the heart and the mitochondrial membrane. This may enhance oxygen delivery and maintain aerobic metabolic capacity when oxygen-binding proteins are absent. These ultrastructural modifications are more pronounced in species that lack both hemoglobin and myoglobin, compared to species that lack only hemoglobin.

We greatly appreciate the support from the staff at Palmer Station and the masters and crew of the R/V Polar Duke. This research was supported by National Science Foundation grant OPP 94-21657 to Bruce D. Sidell.

## References


Quantification of myoglobin and myoglobin mRNA in heart ventricle of antarctic fishes

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The six families of the perciform suborder Notothenioidei dominate the fish fauna of the southern ocean. Our work has focused primarily on the Channichthyidae, commonly referred to as icefishes, which are largely endemic to the waters surrounding Antarctica (Dewitt 1971). The 15 known species of the icefishes are unique among vertebrates in their complete lack of hemoglobin expression (Ruud 1954). In contrast, the presence of the intracellular oxygen-binding protein myoglobin in these fishes has been a source of some debate. A report by Douglas et al. (1985) found myoglobin in heart ventricles of icefishes, whereas others (Hamoir 1988; Eastman 1990) suggest that myoglobin is absent in icefishes. Using a combined immunological and molecular approach, we have shown that there is extremely variable expression of both myoglobin protein and the mRNA coding for this protein among the Channichthyidae (Sidell et al. 1997). One of the next logical steps was to determine the intracellular concentrations of myoglobin protein and myoglobin mRNA in heart ventricle of these fishes, the primary objective of this study.

Intracellular concentrations of myoglobin in heart ventricles were determined by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblot analysis. After electrophoresis, gels were stained to reveal protein bands. To identify the myoglobin-associated band, protein from a duplicate gel was immediately electroblotted to a membrane and detected with both monoclonal and polyclonal antihuman myoglobin antibodies as previously described (Sidell et al. 1997). Each gel also contained a standard curve generated by loading known amounts of myoglobin purified from heart ventricle of the nototheniid fish Notothenia coriiceps. Myoglobin concentrations in sample lanes were calculated from the linear relationship between milligram (mg) purified myoglobin standard and integrated area of densitometrically scanned peaks.

This study reports myoglobin protein concentrations in heart ventricles in eight species of icefishes (Chionodraco rastrinus, hamatus, and myersi, Pseudoachaenichthys georgianus, Cryodraco antarcticus, Chionobathyscus dewitti, Neopagetopsis ionah, and Chaenodraco wilsoni) and in two nototheniid species (Gobionotothen gibberifrons, Trematomus newnesi). Five additional icefish species lacked detectable myoglobin protein (Chenocephalus aceratus, Pagetopsis macropterus and maculatus, Champsocephalus gunnari, and Dacodraco hunteri), (table). Myoglobin expression was extremely tissue-specific in both icefishes and related red-blooded nototheniid species examined (G. gibberifrons and T. newnesi). Myoglobin protein was found only in heart ventricle and was absent from other tissues including the primary oxidative skeletal muscle in these labriform swimmers, the pectoral adductor profundus. Estimates of myoglobin concentration in hearts of channichthyid and nototheniid species are comparable in both families (table) and are similar to values reported for sedentary, benthic fishes from temperate zones (Driedzic and Stewart 1982).

To confirm the pattern of myoglobin protein expression revealed by immunoblot analysis, a myoglobin-specific cDNA probe was used to test definitively for the presence of a messenger RNA (mRNA) coding for myoglobin protein. Total RNA was extracted from heart muscle, separated electrophoretically on agarose gels and transferred to membrane and incubated with a myoglobin-specific cDNA probe (Sidell et al. 1997). As expected, an mRNA of the appropriate size did hybridize to the probe in RNA isolated from heart ventricle of the eight species expressing myoglobin protein. Of the five icefish species lacking myoglobin protein expression, four (P. macropterus, P. maculatus, D. hunteri, and C. aceratus) also lack detectable myoglobin mRNA. In the fifth species (C. gunnari), an mRNA of the same size as myoglobin mRNA (0.9 kilobases) did hybridize to the myoglobin probe, in low but consistently detectable amounts (Sidell et al. 1997). Thus, for C. gunnari the mechanism(s) responsible for loss of myoglobin protein expression appears to differ from that occurring in the four non-mRNA expressing species.

Slot blot analysis was used to quantify the amount of myoglobin mRNA present in heart ventricle and pectoral adductor tissues. Denatured N. coriiceps myoglobin cDNA insert served as an internal positive control and was used to generate a standard curve within each blot. Myoglobin mRNA concentrations in slots were calculated from the linear relationship between picogram (pg) purified N. coriiceps cDNA standard loaded versus integrated signal-area of densitometrically scanned peaks. Myoglobin mRNA concentrations in heart ventricle for channichthyid species expressing myoglobin protein ranged from 16.22±2.17 to 0.78±0.02 picograms of myoglobin mRNA per milligram of total RNA [pg Mb mRNA (mg total RNA)−1]. The lowest myoglobin mRNA concentrations were found in C. gunnari [0.33±0.09 pg Mb mRNA (mg total RNA)−1], the only icefish species that lacked detectable myoglobin protein while still expressing message. Myoglobin mRNA concentrations for the two red-blooded nototheniid species fell between the high and low values determined for the Channichthyidae (table).

Comparing levels of myoglobin protein versus the corresponding mRNA pool for a given species reveals that the steady-state concentration of myoglobin protein does not parallel the steady-state concentration of myoglobin mRNA (table). The greatest range in myoglobin mRNA levels found in icefishes is the approximately 20-fold difference observed in...
the congeneric species *C. rastrospinosus* and *C. hamatus*. This observed difference in myoglobin mRNA pool is not reflected in the standing stock of myoglobin protein, which is equivalent in hearts of these two species. This result suggests that synthesis and/or turnover of the protein is not coordinately regulated with the mRNA pool. The lack of correlation between myoglobin protein and myoglobin mRNA concentrations in notothenioid fishes suggests protein concentrations are not regulated at the mRNA level.

Phylogenetic analysis possible at this time does not suggest the Channichthyidae are undergoing selective pressure toward a myoglobinless state (Sidell et al. 1997). Indeed, given the seemingly random loss of myoglobin expression in this family, we cannot determine if myoglobin (+) or myoglobin (−) is the most derived state within the Channichthyidae.

We gratefully acknowledge the generous contribution of samples by A.L. DeVries (University of Illinois), G. di Prisco and R. Acierno (Italian National Antarctic Program), T. Iwami (Tokyo Kasei Gakuin University), and H.W. Detrich (Northeastern University). Personnel at the U.S. Antarctic Program’s Palmer Station and the masters and crew of R/V Polar Duke provided invaluable support during the course of our work. This work was supported by National Science Foundation grants OPP 92-20775 and OPP 94-21657 to Bruce D. Sidell.

### Concentrations of myoglobin (mb) protein and myoglobin mRNA in heart ventricles of antarctic nototheniid fishes

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Mb concentration [mg Mb·(g wet weight)⁻¹]</th>
<th>Mb mRNA concentration [pg Mb mRNA·(µg total RNA)⁻¹]</th>
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<tr>
<td><em>Channichthyidae</em></td>
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<td></td>
</tr>
<tr>
<td><em>Chionodraco rastrospinosus</em></td>
<td>(n=6) 0.64±0.07</td>
<td>(n=6) 16.22±2.17</td>
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<tr>
<td><em>C. hamatus</em></td>
<td>(n=6) 0.62±0.04</td>
<td>(n=4) 0.78±0.02</td>
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<td><em>C. myersi</em></td>
<td>(n=4) 0.71±0.08</td>
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<tr>
<td><em>Pseudochoenichthys georgianus</em></td>
<td>(n=6) 0.46±0.04</td>
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<td>(n=1) 5.31</td>
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<td><em>Neopagellopsis ionah</em></td>
<td>(n=1) 0.70</td>
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<td><em>Chaenocephalus gunnari</em></td>
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<td>(n=6) N.D.</td>
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<td>(n=2) N.D.</td>
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<td>(n=6) 0.85±0.11</td>
<td>(n=6) 7.27±1.94</td>
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<td><em>Trematomus newnesi</em></td>
<td>(n=6) 1.12±0.07</td>
<td>(n=3) 7.11±1.04</td>
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</table>

<sup>a</sup> Concentration data are presented as mean ± standard error.

<sup>b</sup> N.D. = not detected.

### References


Patterns of nest attendance and relief in Adélie penguins, *Pygoscelis adeliae*

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The Adélie penguin breeds at rookeries distributed along the coast of Antarctica and on islands south of 60°S latitude. Numerous investigators have documented reproductive success and causes of breeding failure of banded birds at various latitudes, most often in colonies on Ross Island, Antarctica, all located south of 77°S latitude (see Ainley, LeResche, and Sladen 1983) but also at King George Island (62°10′S 58°30′W) (Trivelpiece et al. 1990) and Signy Island in the South Orkney Islands (60°43′S 45°36′W) (Croxall et al. 1988). We studied the breeding biology of a colony of Adélie penguins on Torgersen Island, west of the Antarctic Peninsula (64°46′S 64°05′W). We documented the pattern of nest attendance and reliefs of banded birds and here compare the pattern we found with that described for colonies at higher and lower latitudes.

A fairly consistent pattern of nest attendance and reliefs has been described and strongly linked to breeding success or failure in the most southerly populations studied on Ross Island (Davis 1982, 1988; Davis and Miller 1990). After females lay their second eggs, they go to sea to forage. Males take the first and longest incubation bout; females take the second bout while the males forage at sea; males take the third bout; and the females return from sea at approximately the time of hatching of the first egg. Therefore, there are usually three attendance switches before hatching, and the total time of the first three incubation bouts approximately equals the incubation period (table). After hatching, the parents shorten the length of their alternate foraging bouts to feed the chicks regularly. Incubation from the laying of the second (last) egg until the first egg hatches usually is between 32 and 34 days.

In contrast to the Ross Island birds, Trivelpiece et al. (1990) reported a different pattern for birds nesting at Admiralty Bay on King George Island (table). Both male first bouts and female second bouts were shorter than the bouts reported for Ross Island, and the average number of attendance switches until hatching ranged from 3.4 to 6.7 over five different seasons. The year with the lowest average number of switches also had the lowest number of chicks hatched per pair and the lowest number of chicks fledged per pair.

At the most northerly latitude on Signy Island, South Orkney Islands, Croxall et al. (1988) reported first and second bout lengths of 13.7 and 12.8 days, respectively, for an “average” year between 1976 and 1987. This first bout length is similar to those reported at Admiralty Bay, but the second bout length is similar to those reported for the more southerly Ross Island colonies. The total number of days for the first two bouts suggests that there are probably more than three attendance switches during incubation until hatching.

At the midlatitude site on Torgersen Island, we found that the mean number of attendance switches varied significantly between years [ANOVA, F=94.2(3,533)]. It averaged from five to nine in different years and ranged from as few as one to as many as 19 among nests within different years (table). First incubation bouts of males and second bouts of females were shorter on Torgersen than at any of the other sites.

There is not a single pattern of coordination of incubation bouts and nest reliefs that leads to successful hatching either within colonies or across colonies in widely separated locations. However, there are consistent differences between locations. For example, Ross Island colonies consistently have longer bouts and fewer switches than do Torgersen Island colonies.

Davis and Miller (1990) suggest that food per se does not determine the length of foraging trips, at least during the incubation period. Ice conditions, including heavy ice and late breakup and lack of easy access to open water, affect the length of the first foraging trip and this change in turn affects the length of the second and third foraging trips because of the change in the length of time available until hatching (Davis 1988). Desertions are more prevalent (Ainley and LeResche 1973), and reproductive success is lowest in the years when ice persists the longest (Croxall et al. 1988).

Although social behavior within a species has traditionally been viewed as rather static, recently this view has changed. Social interactions, as well as life history, mating systems, and morphology, vary intraspecifically and correlate with environmental heterogeneity. Such a situation has been reported by Waas (1990) in cave- and burrow-dwelling little blue penguins (*Eudyptula minor*). Incubation behavior may vary with environmental heterogeneity as well. In Adélie penguins, the length of foraging trips during incubation may be related to the distance to open water and/or to the extent and type of ice near the colony, both of which will vary temporally and spatially and may affect the presence and accessibility of food.

We thank our field assistants, Asrun Kristmundsdottir, Wendy Reed, David Vleck, Lori Ross, and David Lott for their able assistance with all aspects of this research, and the support staff at Palmer Station. This research was supported by National Science Foundation grant OPP 93-17356 to C.M. Vleck.
### Incubation bout lengths and number of attendance switches of Adélie penguins

<table>
<thead>
<tr>
<th>Year</th>
<th>First incubation bout males, days (range)</th>
<th>Second incubation bout females, days (range)</th>
<th>Third incubation bout males, days (range)</th>
<th>Number of switches</th>
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<td>1984–1985</td>
<td>19.8 (10.7–24.7)</td>
<td>12.6 (6.5–16.1)</td>
<td>3.1 (1.4–7.1)</td>
<td>3</td>
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<tr>
<td>1986–1987</td>
<td>14.2 (10.5–18.5)</td>
<td>12.0 (9.5–15)</td>
<td>4.3 (1.5–7)</td>
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<td>1987–1988</td>
<td>15.8 (11.5–20.5)</td>
<td>9.7 (7.5–13.5)</td>
<td>4.3 (2–7)</td>
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<td>1982–1983</td>
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<td>1995–1996</td>
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<td>1996–1997</td>
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<td>5.3 (1–20)</td>
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<td>8.8</td>
<td>Torgersen Island (3–19) Bucher and Vleck</td>
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### References


Reproductive endocrinology of free-living Adélie penguins at Torgersen Island, Antarctica

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The population dynamics of penguins in Antarctica have been the focus of several studies (Ainley, LeResche, and Sladen 1983; Trivelpiece et al. 1990). Our research focuses on the physiological underpinnings of individual differences in reproductive behavior that contribute to population-level performance. We concentrate on the reproductive hormones that influence reproductive behavior. Adélie penguins are a particularly tractable species in which to study the hormonal control of reproductive behavior and individual variance. Birds can be caught easily and repeatedly in the breeding colonies to take blood samples for hormone analysis, and it is easy to observe their behavior and reproductive success.

One of our first objectives was to determine the hormonal and body-mass changes that accompany the reproductive cycle of penguins on Torgersen Island near Palmer Station (64°46'S 64°04'W), Antarctic Peninsula. Adélie penguins begin to arrive at the breeding colonies in mid-October. After arrival they generally do not leave until their two-egg clutch is complete. Pairs defend a small nest site within the colony and construct a nest of rocks. Egg laying peaks in mid-November; chicks hatch in mid to late December. Males take the first shift of incubation. Females return to the sea to forage after egg laying is complete. On Torgersen Island, females return approximately 8–10 days later, and males leave the colony for the first time to forage. During mid to late incubation and once the eggs have hatched, males and females trade off in attendance at the nest every few days. Adélie chicks are brooded or guarded nearly continuously until they are about 3 weeks old. After reaching thermal independence, the chicks join crèches while both parents forage simultaneously, returning every day or so to feed the chicks. By mid-February, some chicks begin leaving the colony.

Figure 1. The level of plasma testosterone, estradiol, and prolactin in focal Adélie penguins nesting during the 1995–1996 breeding season at Torgersen Island. Data for males (A) and females (B) are shown relative to the day on which the first egg was laid in the nest. A smoothing spline fit (lambda=10,000) was fit to the data for each hormone. The dashed lines demarcate incubation of the eggs. (ng/ml denotes nanograms per milliliter, pg/ml denotes picograms per milliliter.)
During the 1995–1996 field season, we banded 62 focal pairs of Adélie penguins as they arrived in the colony and set up territories. These birds were followed through the course of the breeding season. Birds were weighed and a small blood sample taken by jugular venipuncture every 10–15 days. Hormone levels were later assayed by radioimmunoassay at our home institution. We surveyed the colonies daily, weather permitting, to determine which birds were present and their reproductive stage. Of the 62 focal pairs, 56 laid eggs, nine lost their eggs during incubation, three lost the chicks after hatching, and 44 raised at least one chick to the crèche stage. The mean date on which the first eggs were laid for these pairs was 14 November 1995, and the mean date of the first hatching was 20 December 1995. The mean interval from first egg to first hatching was 36 days (standard deviation (SD)=4), from lay of the second egg to its hatching was 33 days (SD=4), and between laying of the first and second eggs was 3 days (SD=1).

Plasma testosterone was elevated in both males and females during the courtship (pre-egg laying) stage, although the levels of testosterone were approximately an order of magnitude higher in males than in females, and there was substantial variability between individuals (figure 1). A distinct drop in testosterone occurred prior to the day the first egg was laid. This decrease in testosterone may be necessary for the birds to shift their behavioral focus from courtship and nest-site defense to incubation of the eggs (Wingfield et al. 1990). Likewise, estradiol was elevated in both sexes during courtship but was about threefold higher in females than in males. Prolactin is the hormone most strongly associated with parental care in birds (Buntin 1996). Plasma prolactin was low when the birds arrived in the colony and rose during the courtship period. It reached its highest levels by mid-incubation and thereafter remained elevated through the brooding stage. During the parental phase, prolactin levels were significantly higher in females than in males (F=78.2, P<0.001), even though the two sexes share equally in parental duties.

Adélie penguins arrive on the breeding colony with large fat reserves. Body mass decreases as the birds fast through the courtship phase (figure 2). The body mass of focal males in October after arrival from the winter foraging grounds was 5.01 kilograms (kg) (SD=0.34, sample size (n)=44). Females were slightly smaller; the body mass of focal females arriving in October was 4.55 kg (SD=0.32, n=43). The body mass of those males that did not successfully acquire a mate who laid eggs was not different from the body mass of males that mated with females that did lay eggs (figure 2A). Those females that never laid eggs were smaller on average than females that laid eggs (figure 2B). Of birds weighed in October, two female nonlayers weighed 0.5 kg less than those females that did lay eggs during the season. These nonlaying females may be unsuccessful because they do not have sufficient fat stores to undergo reproduction and/or they may be young, pre-breeding birds that have returned to the colony and keep company with another bird but do not lay eggs (Ainley et al. 1983). During the courtship fast, focal females lost approximately 38 grams (g) of body mass per day or about 25 percent of the body mass over a 1-month fast. Males lost approximately 43 g of body mass per day or approximately 39 percent of their body mass over an approximate 45-day fast. When the birds were alternating between foraging at sea and tending eggs or chicks, their mean body masses were 4.20 kilograms (SD=0.43) for males and 3.85 kilograms (SD=0.36) for females (figure 2).

We thank our field assistants, Asrun Kristmundsdottir and Wendy Reed, for their able assistance with all aspects of this research, and the support staff at Palmer Station. This research was supported by National Science Foundation grant OPP 93-17356 to C.M. Vleck.
Factors regulating population size and colony formation of Adélie penguins in the Ross Sea

DAVID G. AINLEY, H.T. Harvey and Associates, Alviso, California 95002

Collaborating with biologists from LandCare Research New Zealand, Ltd. (LCRNZ), we conducted the third year of an investigation into the factors responsible for dramatic growth of Adélie penguin (Pygoscelis adeliae) colonies in the Ross Sea during the past two decades (Taylor and Wilson 1990; Blackburn, Taylor, and Wilson 1991). The LCRNZ biologists had collected certain key data during the past two seasons, anticipating our joining the effort in 1996–1997. With our arrival into the project, we increased the effort to work simultaneously at three penguin breeding colonies collecting the same suite of data at each. We hope to continue this project for at least 5 more years.

We are testing the hypothesis that population growth, which has been greater at the smallest colonies (Taylor and Wilson 1990), has resulted from a lessening of pack-ice cover. The smaller pack-ice cover has made food resources more available in a region characterized by concentrated pack ice well through the spring and into the summer; concentrated ice cover increases the effort that penguins must expend during foraging (because they have to walk rather than swim), which, in effect, is a reduction of food availability. In turn, the lessened effort required to find food has led to increased reproductive success and juvenile and adult survival (or so we hypothesize). We ask: Why has growth disproportionately favored the smaller colonies, given that ample space for expansion exists at all colonies? Using the Adélie penguin as a model, this question is of increasing relevance as managers more and more attempt to restore seabird colonies decimated by disasters such as oil spills. Which colonies should be restored: The small ones, which may owe their existence to over-flow of breeders (emigrants) from the larger ones, which have suffered the greatest impact, or the large colonies, where growth is slow and size presents logistical problems and, likely, little in the way of dramatic results to keep the purse managers interested?

In our research, we are comparing the colonies in the Ross/Beaufort Island cluster, in ascending order of size: Cape Royds (about 4,000 pairs), Beaufort Island (45,000), Cape Bird (45,000), and Cape Crozier (180,000). On Beaufort Island and at the three colonies on Ross Island, we are examining rates of colony growth determined from annual counts of breeding pairs in aerial photos. These photos have been taken by LCRNZ biologists beginning in 1981. Soon, we will inspect archived satellite imagery of pack-ice cover during the penguin breeding season to look for correspondence in colony growth and ice cover. In addition to the colony counts, we also
• estimated reproductive success by comparing counts of chicks in mid-January to number of breeding adults on 1 December;
• used radio telemetry to determine overlap in the foraging areas of breeding adults from each colony;
• collected food samples using stomach lavage;
• estimated parental foraging effort by determining feeding frequency and food load size brought to chicks; and
• calculated a chick growth index.

We also banded a sample of chicks and adults to estimate postbreeding survival and emigration when we find these birds in subsequent years. To complement the diet samples, we also collected tissues from chicks killed by south polar skuas (Catharacta maccormicki); these will be analyzed for the stable isotopes of nitrogen and carbon to determine the trophic level at which their parents were feeding (low = krill, high = fish). A diet composed of fish is much more energetically rich than one of krill.

The telemetry was conducted by taping small radios to the backs of 15 adults at the three Ross Island colonies in mid-December. Three times daily until mid-January, we then triangulated positions of radios by using directional antennas and receivers to search simultaneously the waters in sight from the colonies from vantage points 15–200 meters (m) above sea

References

level. For 4 days, we tracked also from Hanson Ridge (600 m) on the west side of McMurdo Sound opposite Capes Royds and Bird, for 2 days from the top of Beaufort Island (650 m); and once from Mount Bird (900 m), we assessed overlap in foraging area of birds from Capes Bird and Crozier; and on a 2-day excursion of USCGC *Polar Sea*, we assessed overlap among all colonies. Owing to the high altitudes and relatively interference-free air waves, our receiving range was on the order of 35 kilometers (km) (as also experienced by Sadleir and Lay 1990); from sea level (ship), range was 5–10 km.

Parental foraging effort was assessed by implanting radio frequency identification (RFID) tags under the skin of 60–70 breeding birds in a breeding group at each colony, encircling the colony with a plastic fence and, thus, channeling the birds to come and go over a computerized scale/RFID tag reader. The weights of birds and tag readings were downloaded daily into a laptop computer for analysis.

Highest chick growth rates and greatest breeding success were experienced by penguins at the smallest colony. These birds also put least effort into procuring food (shortest feeding trips) and ate food of highest caloric content (highest percentage of fish in the diet). Data collected during breeding seasons previous to 1996–1997 indicated similar trends more often than not. The big question, then, is: If breeding conditions are so wonderful at the smallest colony, why does it remain small? We will attempt to answer this question with additional years of research.

Collaborators in the project were my coworkers, Grant Ballard, Sophie Webb, Stephani Zador, and Ian Gaffney; Peter Wilson, Kerry Barton, and Brian Karl, LCRNZ; Mike Beigel and Nathaniel Polish, Beigel Technology Corp.; Christine Ribic, U.S. Geological Survey–Biological Research, University of Wisconsin; Greg Rau, University of California–Santa Cruz; and Nadav Nur, Point Reyes Bird Observatory. We are grateful for the expert logistical support provided by personnel of the National Science Foundation, Antarctic Support Associates and their subcontractors, the New Zealand Antarctic Program, and the USCGC *Polar Sea*. Our portion of the project was funded by National Science Foundation grant OPP 95-26865.

**References**


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**Population ecology of Weddell seals (Leptonychotes weddellii) in McMurdo Sound, Antarctica, 1995–1997**

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Long-term examinations of the demographic processes acting on the dynamics of a large mammalian vertebrate are rare throughout the world. It is often necessary to document these factors over a period of time at least as long as a single generation in order to recognize any real differences in the most important members of the population: the breeding adults. An extensive program of tagging and re-sighting Weddell seals in Erebus Bay, near McMurdo Sound, Antarctica, carried out by the Universities of Minnesota and Alaska–Fairbanks since the early 1970s, has permitted in-depth analyses of these processes. This work continued in the 1995–1996 and 1996–1997 field seasons with the primary objectives of updating the extensive database by marking all of the newborn pups and performing weekly censuses within the study area extending from Scott Base to Cape Evans, McMurdo Sound. In addition, the continued monitoring of the survival of individual seals sampled in the early 1990s was requested to permit follow-up investigations by researchers at the University of Alaska.

We used Jolly–Seber mark-recapture functions to estimate the Weddell population in the area by comparing the ratios of marked seals seen in consecutive censuses. These censuses have revealed that the population has remained essentially stable since the last thorough analyses in the mid-1980s (Testa and Siniff 1987). Likewise, the number of pups born annually appears to have leveled out over the previous 14 years.

Our seasons extended from 14 October to 3 December and 10 October to 10 January in 1995–1996 and 1996–1997, respectively. The first pup was tagged on 18 October in both seasons, and in total, 386 pups were tagged in 1995 and 380 in 1996. Two helicopter flights were used in each season to visit the isolated population of seals at White Island approximately
15 kilometers southeast of McMurdo Station. This population is surrounded by the Ross Ice Shelf and recent molecular genetic work suggests that some inbreeding may be occurring (Fleischer, Perry, and Testa 1995, unpublished data). Eight tagged adult females were seen in each season; four pups were tagged in 1995 and eight in 1996. One pup in 1995 and three in 1996 appeared to have died soon after birth. Tissue samples were collected for further genetic analyses and an entire carcase was collected in 1995 for examination at the University of Alaska. Blood samples were collected from pups of various ages, within the study area, in both seasons, for baseline investigations of trace metal and vitamin concentrations.

Blood and tissue samples were also collected in 1996 from all seals at the Big Razorback colony for preliminary work examining the mating system of Weddell seals. We are utilizing recent advances in molecular genetics, which now permit individual identification. Information obtained from the long-term database has shown that Weddell seals have a relatively high fidelity for returning to the study area in subsequent years to breed and give birth. All breeding occurs under the ice and has been observed only once via remote cameras (Cline and Siniff 1971). By sampling mother-pup pairs soon after the pups’ birth, however, and comparing these to the males in the same area the previous year, we hope to shed some light on the breeding system and obtain good estimates of male reproductive success as estimated by number of pups produced. The database provides information on maternal lines from the tagging histories of known-age breeding females. Using a combination of molecular genetics, behavioral observations, radio telemetry, and paternity analyses, we have initiated a project to

- develop techniques to extract DNA from blood and tissue samples,
- confirm mother-pup relationships using microsatellite primers, and
- measure male reproductive success from paternity analyses and correlate success with territorial activity.

Preliminary analyses have confirmed the first two objectives. Research is continuing on the third.

In January 1997, we collaborated with scientists from Hubbs Sea World Research Institute to collect blood samples for blood parasite and chemistry analyses. Additionally, we attached four satellite-linked radio transmitters to weaned pups to gather information on the movements of these individuals after the pupping season. Annual census records on individual seals tagged as pups in the study area have shown that nonreproductive animals tend to leave the study area, only to return again when they are reproductively active. Our objective was to track some seals in their first year to compare their movements to the annual ice break-up and to the known range of adult seals. Two of the four transmitters were still collecting locations in September of 1997, and one continued to transmit until early November.

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References

