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A Major Ecosystem Shift in the Northern Bering Sea

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Until recently, northern Bering Sea ecosystems were characterized by extensive seasonal sea ice cover, high water column and sediment carbon production, and tight pelagic-benthic coupling of organic production. Here, we show that these ecosystems are shifting away from these characteristics. Changes in biological communities are contemporaneous with shifts in regional atmospheric and hydrographic forcing. In the past decade, geographic displacement of marine mammal population distributions has coincided with a reduction of benthic prey populations, an increase in pelagic fish, a reduction in sea ice, and an increase in air and ocean temperatures. These changes now observed on the shallow shelf of the northern Bering Sea should be expected to affect a much broader portion of the Pacific-influenced sector of the Arctic Ocean.

The northern Bering Sea, despite its seasonal ice cover (Fig. 1A), supports some of the highest benthic faunal biomass densities in the world's oceans (*1*, *2*). Over most of the Bering Sea shelf, zooplankton grazing and microbial processing in the water column have a small impact on pelagic retention, and therefore a large fraction of usable carbon settles to the benthos (*3*). However, in the southern (subarctic) region of the Bering Sea, the resulting benthic biomass is largely consumed by upper trophic level demersal fish and epifaunal invertebrates, whose northern distribution is limited by the presence of near-freezing bottom temperatures (*4*, *5*). The location of this temperature-determined subarctic-Arctic boundary is thus the primary agent determining ecosystem variability. In the absence of demersal fish and predatory invertebrates, benthic-feeding seabirds and marine mammals become the primary consumers in the northern (Arctic) region of the Bering Sea (*6*, *7*).

A change from arctic to subarctic conditions is under way in the northern Bering Sea, with an attendant northward shift of the pelagic-dominated marine ecosystem that was previously limited to the southeastern Bering Sea (*8*). The ice-dominated, shallow ecosystem favoring benthic communities and bottom-feeding sea ducks, such as spectacled eiders (*Somatera fuscgeri*), and marine mammals, including walrus (*Odobenus rosmarus*) and gray whales (*Eschrichtius robustus*), is being replaced by one dominated more by pelagic fish. Such shifts clearly affect both subsistence harvests and commercial fisheries. Yupik hunters of St. Lawrence Island, for example, have observed an increase in warm winds in winter and the replacement of stable pan and pack ice with brash and thin ice, changes that affect their ability to

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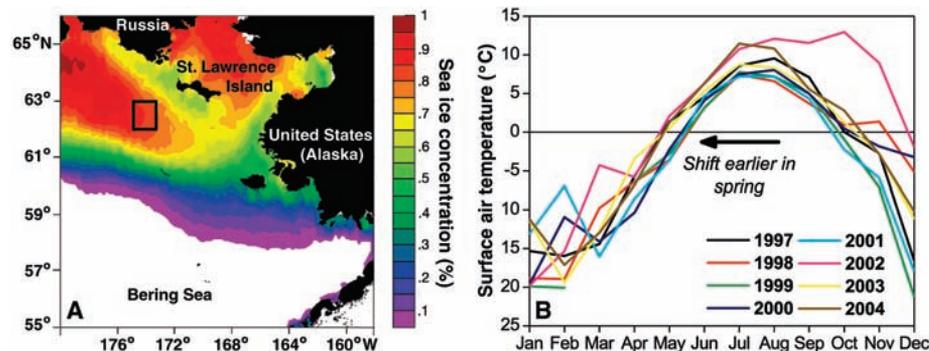*To whom correspondence should be addressed. E-mail: jgrebmei@utk.edu

Fig. 1. (**A**) Location map (box indicates location of time-series biological sites) and average April sea ice concentration (1 corresponds to 100%, and 0.1 corresponds to 10%) in the northern Bering Sea from 2000 to 2004. Ice concentrations are based on microwave satellite instruments, Defense Meteorological Satellites Program SSM/I (*12*, *16*). (**B**) Monthly averaged surface air temperature measured at Savoonga (63.68°N, 170.5°W) on St. Lawrence Island over the years 1997 to 2004. Note the interannual variability in the timing of melt onset (~3 weeks) based on date air temperature rises above 0°C (*13*).

hunt and fish along with fundamental changes in animal behavior (9).

A critical feature that affects benthic predators in the northern Bering Sea is a recurrent subsurface cold pool (<0°C) extending southward of St. Lawrence Island. This feature forms as a result of ice formation in the winter and subsequent trapping of this cold water in place as sea ice melts and surface water warms in spring and summer (10) (Fig. 2). Higher air temperatures and lower winter ice cover, however, result in a contraction of the summertime extent of the cold pool and an associated increase in mean seawater temperature (4). In the northern Bering Sea, our measurements of temperatures within the cold pool obtained over 12 years (1988 to 2005) suggest, after factoring out seasonal variation, that bottom water temperatures are increasing (Fig. 2, bottom, and tables S1 and S2) (11, 12). This warming trend is comparable to increases in atmosphere and ocean temperatures that were observed in the southeastern Bering Sea after 1977, changes that led to an ecosystem reorganization that favored pelagic over benthic predators (8). Likewise, data collected on St. Lawrence Island from 1997 to 2004 show an increase in air temperatures over time with the onset of ice melt occurring earlier (~3 weeks) (Fig. 1B) (13). These temperature trends directly affect the ecosystem changes we report here.

Clement et al. (14) compared late winter sea ice extent and duration in 1999 and in 2001 and found that ice was thinner and retreated earlier in 2001, which supports the Yupik observations of declining ice conditions (9). The Clement et al. study was an initial indication of what a recent time-series sea ice data analysis showed to be a

continued decline from 2002 to the present for Arctic sea ice in the western Amerasian Arctic (15). Similarly, sea ice concentrations for the northern Bering Sea in April, averaged for the years 2000 to 2004 by using satellite-derived passive microwave measurements, show ice concentrations below 0.7 (70%) in the region between the Alaska coastline and St. Lawrence Island (Fig. 1A) (12, 16). These data support an increasing trend in open water documented by an analysis of the 24-year (1979 to 2002) satellite-derived time series of ice cover in the Chirikov Basin north of St. Lawrence Island (17). Thus, satellite, field, and Yupik traditional ecological observations support the conclusion that the northern Bering Sea has had lower ice concentrations in the past decade, with declines similar to those observed in the southeastern portion of the Bering Sea (8).

Sediment oxygen uptake is an indicator of carbon supply to the benthos, and time series data from 1988 to 2004 collected in March to September southwest of St. Lawrence Island show a decline from ~40 mmol O_2 m^{-2} day^{-1} in 1988 to sustained values of ~12 mmol O_2 m^{-2} day^{-1} for 1998 to 2004 (Fig. 3A and table S2) (5, 11, 18). Measurements of benthic standing stock show a decrease from about 40 g C m^{-2} to 20 g C m^{-2} over the same time period (Fig. 3B and table S2) (5, 11). In the past, benthic trophic efficiencies in the northern Bering Sea have been high (up to 50%) (19), reflecting tight pelagic-benthic coupling, and therefore support high benthic standing stocks (20). However, these data suggest that the prey base for benthic-feeding gray whales, walrus, and sea ducks is declining in the northern Bering Sea. One possible explanation for this is that these

apex predators have exceeded the carrying capacity for this system. An alternative hypothesis is that ecosystem change and declining productivity are reducing food supply to benthic prey, thus affecting apex predators (3, 5, 21). A comparison of surveys from 2002 with those from 1981 to 1985 shows that gray whales have responded by relocating their primary foraging area northward, from the Chirikov Basin adjacent to the north shore of St. Lawrence Island to north of Bering Strait (7). Additional evidence that gray whales are traveling north and extending their residence times in Arctic waters includes the surprising detection of gray whale calls during an over-winter (2003 and 2004) deployment of autonomous hydrophones near Barrow, Alaska (22). These data come from the first ever deployment of acoustic recorders mounted on moorings deployed during the Western Arctic Shelf-Basin Interactions (SBI) global change project in 2003 and 2004 (22, 23). Local subsistence hunters in Barrow, Alaska, continue to report more numerous gray whales than in any time previously. Bivalve populations, used as prey items by diving sea duck populations, including the threatened spectacled eider, are also in decline (6, 21).

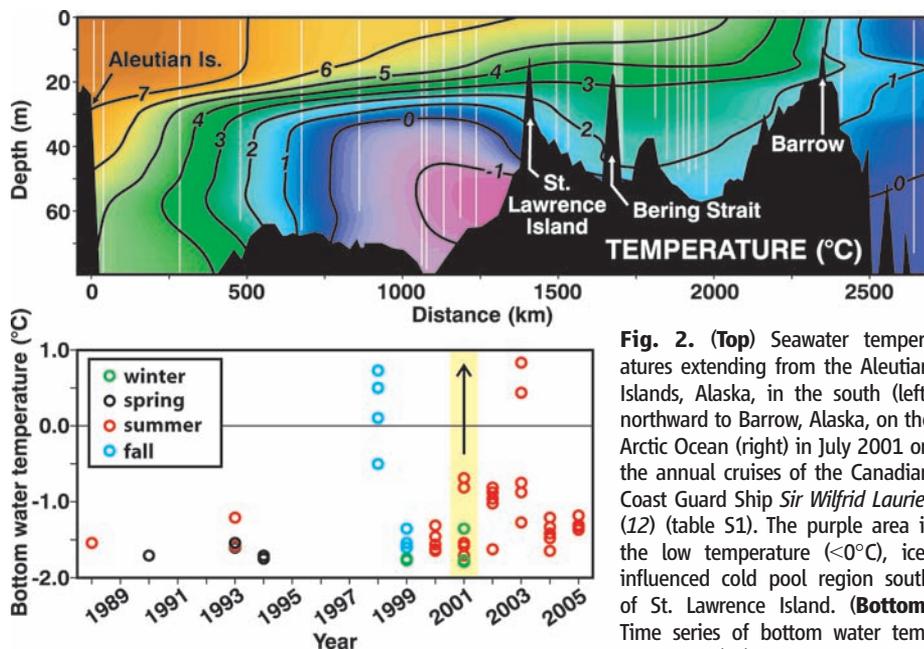Native hunters have observed changes in walrus behavior in response to changes in sea

Fig. 3. Time series measurements (1988 to 2004) of (**A**) total sediment oxygen uptake (mmol O_2 m^{-2} day^{-1}) and (**B**) benthic macrofaunal biomass (g C m^{-2}) at stations occupied southwest of St. Lawrence Island in the northern Bering Sea over four seasons (see small box in Fig. 1A for station locations). Color coding indicates the seasonal measurements: winter (March and April), spring (May and June), summer (July and August), and fall (September). Each point represents the mean of two sediment cores (133 cm²) for sediment oxygen uptake and four 0.1 m² van Veen grabs for the benthic macrofaunal biomass (12) (table S2). Trend lines are drawn through the mean of these station values for each year.

Fig. 2. (**Top**) Seawater temperatures extending from the Aleutian Islands, Alaska, in the south (left) northward to Barrow, Alaska, on the Arctic Ocean (right) in July 2001 on the annual cruises of the Canadian Coast Guard Ship *Sir Wilfrid Laurier* (12) (table S1). The purple area is the low temperature (<0°C), ice-influenced cold pool region south of St. Lawrence Island. (**Bottom**) Time series of bottom water temperatures (°C) in the cold pool south of St. Lawrence Island occupied at different seasons from 1988 to 2005 (12) (table S2). The yellow highlighted area indicates data collected in 2001 that corresponds to the top graph.
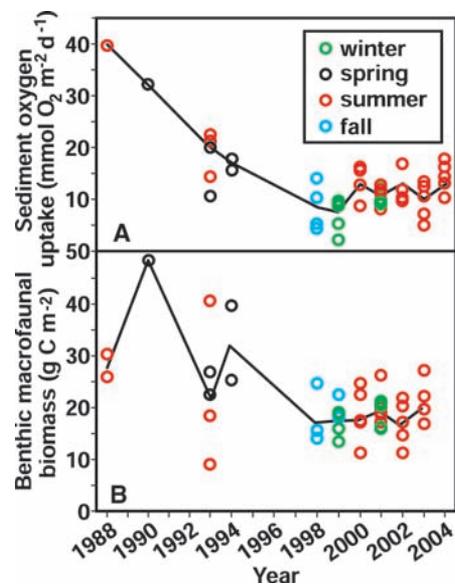
ice cover (24). Walrus prefer broken ice floes, and when the ice melts or moves away early walrus go with it, taking them too far away to hunt (25). Reduced sea ice in the late 1990s caused walrus to swim farther between feeding areas; when the ice retreated far to the north in the Chukchi Sea, the animals suffered (24). Field observations during the SBI field program in 2004 documented separated walrus pups coincident with unusually warm surface water being advected northward into the Arctic Basin (water temperature > 10°C) (26).

Other examples of ecosystem change are becoming evident. Fish population surveys of the eastern Bering Sea shelf by the Alaska Fisheries Science Center in late summer and early fall from 2002 to 2004 found large numbers of juvenile pollock (*Theragra chalcogramma*) in the northern Bering Sea just south of St. Lawrence Island in 2004, possibly in response to warm ocean temperatures. Northward range extensions of pelagic fish species include major increases in the number of juvenile pink salmon (*Oncorhynchus gorbusha*) in the northern Bering Sea, which feed on pollock (27). Local observations indicate that pink salmon are now colonizing rivers that drain into the Arctic Ocean north of Bering Strait (28).

A proximate cause for the shift in the climate of the Bering Sea is the decrease in the strong positive phase of the Arctic Oscillation (AO), beginning in 1996 (29), and the subsequent establishment of a multiyear period of weaker northerly winds over the Pacific Arctic (30). A positive AO (also referred to as a northern annular mode) reflects an increase in the strength of the polar vortex winds and a decrease in sea-level pressure over the central Arctic. A strong polar vortex generally acts as a barrier to northward propagation of storms in the Pacific sector. In contrast to the AO pattern, the composite 1000-hPa geopotential height anomaly field for March to May during 2000 to 2005 (Fig. 4A) (31) shows low height anomalies over the Siberian Peninsula and high height anomalies over Alaska, with a strong gradient between

these centers. This structure corresponds to wind reversals, including stronger southerly winds over the northern Bering Sea, and thus to a weakening of climatologically dominant northerly wind regime. Each of the years individually is similar to the composite, as are the height anomaly fields in 1996 and 1997. Near-surface air temperatures for 2000 to 2005 had warm anomalies (~3°C) over eastern Siberia and the northern Bering Sea (Fig. 4B) (31); direct observations from coastal weather stations show anomalies of the same magnitude. The region of warm temperatures is associated with minimum ice cover north of eastern Siberia in these years (15), warming of permafrost (32), as well as increases in the 1000- to 500-hPa thickness field over the region. Although the reason for the year-to-year persistence of the weak northerly wind field over the northern Bering Sea in spring is unknown and includes influences from the atmospheric general circulation, some of the multiyear persistence might be associated with regional feedbacks from the surface to the atmosphere from the loss in Arctic sea ice in the marine environment (15) and tundra on land (33, 34) over the past decade. Recent warming in the Arctic has allowed shrubs and the treeline to move northward into regions previously occupied by Arctic tundra (33). As the terrestrial plant type changes, warmer temperatures interact with the land, resulting in more exposure to sunlight and a loss of snow cover, which reduces the albedo effect and provides a positive feedback for continued land warming (34). Similarly, a loss of sea ice reduces surface ocean reflectivity and allows for surface ocean warming, which acts as a positive feedback for continued sea ice melt.

Although a shift back to cold climate conditions is possible because of large climate variability in high latitudes, the northern Bering Sea is part of a larger climate system (30) and thus likely to follow global patterns of diminished sea ice cover. It is difficult to envision rapid removal of the heat already gained by the ocean

in recent years from diminished sea ice. These observations support a continued trend toward more subarctic ecosystem conditions in the northern Bering Sea, which may have profound impacts on Arctic marine mammal and diving seabird populations as well as commercial and subsistence fisheries.

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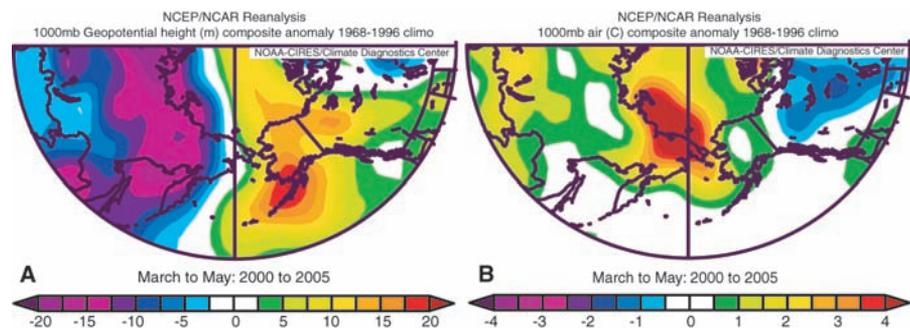29. D. W. J. Thompson, J. M. Wallace, *Geophys. Res. Lett.* **25**, 1297 (1998).

Fig. 4. (A) Anomalies of low geopotential heights over the eastern Siberian Peninsula and high heights over Alaska with a strong gradient between these centers for 2000 to 2005, which corresponds to strong southerly wind anomalies over the northern Bering Sea. **(B)** The same multiyear spring period shows a major near-surface temperature anomaly over eastern Siberia and the northern Bering Sea on the order of 3°C (31).

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Structure and Mechanism of the Lantibiotic Cyclase Involved in Nisin Biosynthesis

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Nisin is a posttranslationally modified antimicrobial peptide that is widely used as a food preservative. It contains five cyclic thioethers of varying sizes that are installed by a single enzyme, NisC. Reported here are the in vitro reconstitution of the cyclization process and the x-ray crystal structure of the NisC enzyme. The structure reveals similarities in fold and substrate activation with mammalian farnesyl transferases, suggesting that human homologs of NisC posttranslationally modify a cysteine of a protein substrate.

The accelerated appearance of pathogenic bacteria that are resistant to the existing battery of antibiotics is a growing global health concern. This problem has prompted efforts toward the development of effective antibiotics with limited avenues for bacterial drug resistance. Attractive candidates are compounds with multiple modes of action, making resistance more difficult to develop. One example is nisin, a ribosomally synthesized and posttranslationally modified antimicrobial peptide that, despite worldwide use for decades in the food industry, has not induced widespread resistance (*1*). Nisin is highly effective [minimum inhibitory concentrations (MICs) on nM scales] against a range of Gram-positive bacteria, including food-borne pathogens. It exerts its biocidal activity through both pore formation in the membrane and the disruption of cell wall biosynthesis by binding to lipid II, which is an essential intermediate in peptidoglycan biogenesis (*2, 3*). The targeted affinity for the bacterial cell wall increases the efficacy of nisin by several orders of magnitude compared with other pore-forming molecules, such

as the eukaryotic peptide magainin. In a third independent biological activity, nisin inhibits the outgrowth of bacterial spores (*4*). Although the biosynthetic gene cluster of nisin was sequenced in 1989 (*5*), to date, in vitro reconstitution of its biosynthesis and that of other class I lantibiotics has been unsuccessful. Furthermore, no structural information is available to provide insights into the molecular mechanism of biosynthesis. We present here the in vitro enzymatic synthesis of nisin A and the crystal structure of the cyclase enzyme that introduces the characteristic thioether rings that are essential for biological activity. The bacterial enzyme bears structural similarities with the β subunit of mammalian farnesyl transferases, and it appears to use an unusual integral protein module, similar in motif to Src homology 2 (SH2) and Gyf domains, for substrate binding.

Nisin contains five thioether cross-links formed by the addition of cysteine residues to dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues that originate from serine and threonine, respectively (Fig. 1A). These thioether structures are called lanthionines and methyllanthionines and are found in lantibiotics, a group of posttranslationally modified peptide antibiotics (*6*). The size of these rings in nisin varies from four amino acids for the B, D, and E rings to seven amino acids for the C ring. The cyclase enzyme NisC is believed to be responsible for the formation of all rings, but its activity or that of its homologs has not been demonstrated, and these proteins have no

sequence homology with any other functionally characterized proteins in the Swiss-Prot database.

We cloned *nisC* from *Lactococcus lactis* [American type culture collection (ATCC) accession number 11454] and developed a high-level heterologous expression system in *Escherichia coli*. The substrate was obtained from an engineered *L. lactis* strain that secretes dehydrated prenisin peptide (*7, 8*). This strain contains a plasmid that harbors the *nisA* gene, which codes for the precursor peptide (prepeptide); the *nisB* gene, which produces the dehydratase that acts on the serine and threonine residues; and the *nisT* gene, which encodes the transporter that secretes the modified peptide (*6, 8*). Cells were grown on minimal media, and the secreted dehydrated prepeptide was precipitated from the spent medium with trichloroacetic acid and desalted. Analysis with mass spectrometry and gel electrophoresis shows the expected molecular mass (Fig. 1B) and an estimated purity of >85%. Treatment of the dehydrated prenisin with the thiol modifying agent p-hydroxymercuribenzoic acid (PHMB) (*9*) after reduction of any disulfides with triscarboxyethyl phosphine (TCEP) demonstrates the presence of five free thiols (Fig. 1C). This treatment indicates that nisin is not present in this sample, because it would not have free thiols and should remain unmodified: No such peak is observed. Conversely, when the dehydrated peptide was treated with NisC followed by reduction with TCEP and reaction with PHMB, the product mixture showed a clear peak at 5715 daltons, which is associated with a peptide that is devoid of free cysteines as a result of five cyclizations (Fig. 1D). The removal of the leader peptide was achieved by treatment of the NisC product with substoichiometric concentrations of trypsin, resulting in a bioactive compound as shown by a clear zone of inhibition when spotted on a lawn of bacteria of the indicator strain *L. lactis* NZ9000 (fig. S1). When either NisC or trypsin treatment was omitted, no bioactivity was observed, corroborating the conclusion from Fig. 1, C and D, that cyclization is catalyzed by NisC.

Mass spectrometric analysis of the product of successive NisC and trypsin action showed the expected peak for mature nisin (Fig. 1E). No peaks were observed for proteolysis after Lys[12]

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