Current Biology

Supplemental Information

Unexpected Levels of Biological Activity during the Polar Night Offer New Perspectives on a Warming Arctic

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Supplemental Data

Supplemental figures and tables

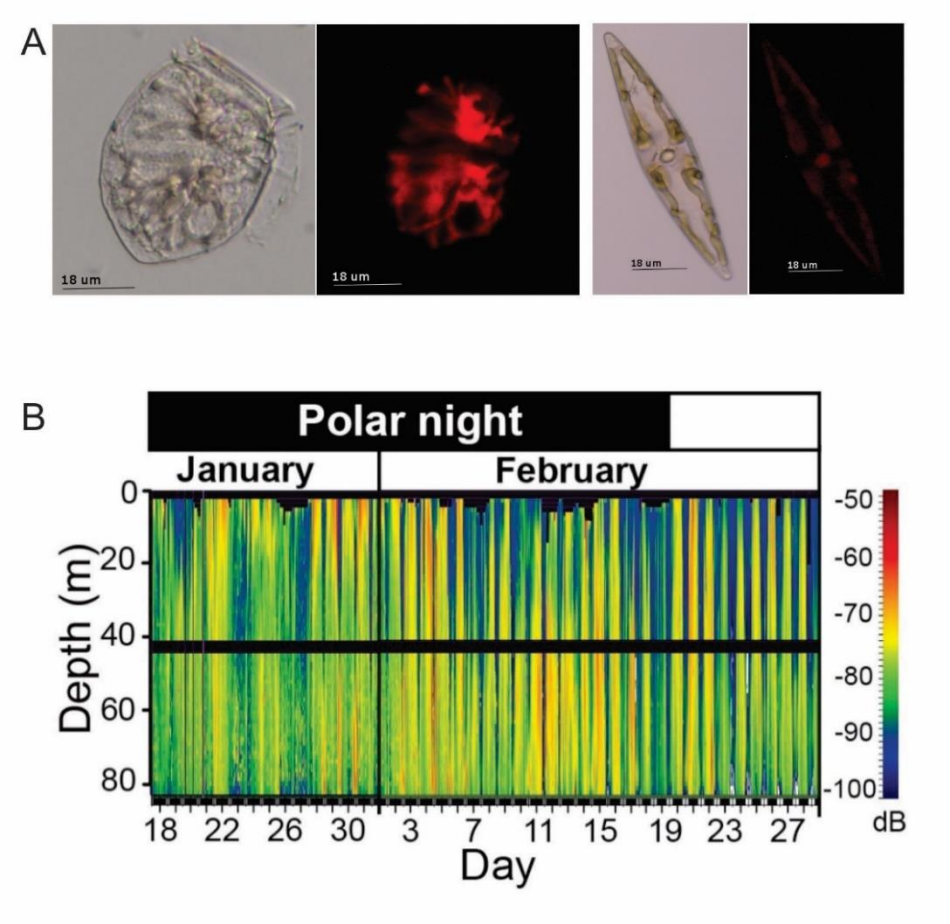

Figure S1: Microscopy and autofluorescence of (left) *Dinophysis* sp. and (right) *Pleurosigma* cf. *stuxbergii* (**a**). Time series of absolute volume backscatter (Sv) in decibel (dB) in the upper 80 m in Kongsfjorden from January-February 2014 (**b**); warmer colors indicate higher biomass (scale bar to the right). Vertical bands represent sound scattering layers. These bands become gradually stronger across the entire water column towards the end of the period, and represent migrating scattering layer. From January 27th the clear bands appear in the surface (0-30m), and from 4th of February they extend all the way down to 80m. Black horizontal line at about 40m depth represent a sediment trap (data deleted due to artificially high backscatter from the metal frame of the sediment traps). Related to Figure 2 and Table S1.

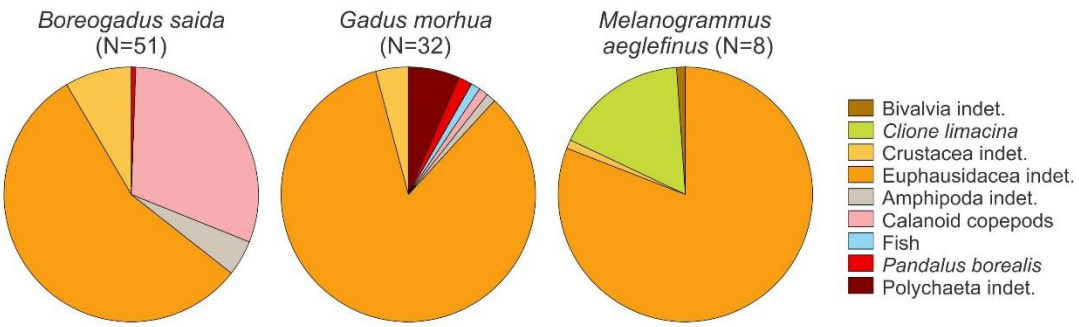

Figure S2: Prey species composition in the stomachs of three gadoid fish species caught in a single trawl in Kongsfjorden in January 2015. Data present relative contribution of prey items from each category. Related to Figure 3.

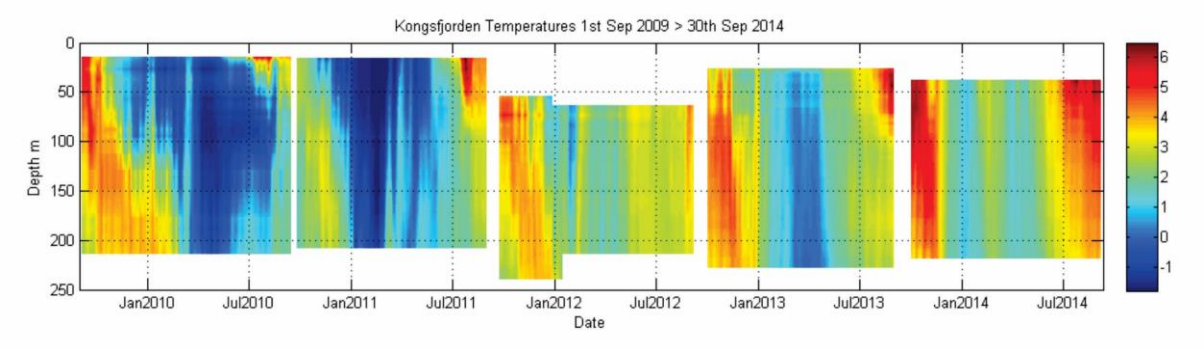

Figure S3: Temperature profile measured in Kongsfjorden from 2009-2014 by a mooring equipped with temperature loggers (37-SM MicroCAT, Sea-Bird Electronics and Vemco temperature mini loggers) spaced through the water column (for details on the mooring see [S1]). Related to Figure 1.

Table 1: Diel activity cycles in benthic bivalves (valve movements) and zooplankton during the polar night. Related to Figure 3.

Time period	Circadian period		P value
	Scallops	Zooplankton	
26th Aug - 21st Oct 2013	24.0 hrs	no data	p <0.0001
21st Oct - 17th Feb 2014	24.0 hrs	23.8 hrs	p <0.0001
17th Feb – 16th April 2014	23.9 hrs	23.8 hrs	p <0.0001

Supplemental information: Methods

Study area

Kongsfjorden is a glacial, west-facing fjord on the island of Spitsbergen in the Svalbard archipelago at 79ºN. Water flow in the fjord is under geostrophic control [S2], and there is no distinct shallow sill at the fjord's mouth. It is, therefore, directly linked to the shelf and slope along West Spitsbergen and as such, it provides a large "mesocosm environment" in which we may examine a variety of biotic parameters of relevance for larger oceanographic regions and processes. Consequently, Kongsfjorden is one of the most thoroughly studied marine ecosystems in the Arctic [S3, 4] including long-term observation of zooplankton, soft and rocky bottom benthos, and seabirds (e.g. [S3, 5, 6]). The majority of the research in Kongsfjorden, however, has been conducted during the Arctic light season (April-September). At 79°N the polar night is defined as *Nautical polar night* when the sun is at most 12.4° below the horizon. Levels of ambient downwelling irradiance (PAR, *E*) measured in air at noon when the sun is 9° below the horizon (medio January) is on the order of 10^{-5} µmol photon m^{-2} s^{-1}, rendering levels of ambient light at depth in the water several orders of magnitude lower [S7].

Primary producers and epifluorescence

Samples were collected with a plankton net (20 µm mesh size) close to the sea surface, and pre-screened through 63µm mesh to eliminate large grazers. Chl *a*-specific net primary production (NPP) was determined in 24h incubations both *in situ* as well as in the laboratory directly after sampling and after 2 days of exposure to low light (6 ±1 µmol photons m^{-2} s^{-1}; 14 hours daylength). After addition of a 20 µCi (0.37 MBq) spike of $NaH^{14}CO_3$ (PerkinElmer, 53.1 mCi $mmol^{-1}$ stock), duplicate samples (seawater pH_{NBS} of 8.04 at in situ temperature) were immediately placed in a culture room at 1.6 ±0.4 °C and 6 ±1 µmol photons m^{-2} s^{-1}. Another set of samples was incubated in the fjord at 0.5 m water depth. After 24h, samples were filtered

onto GF/F-filters, acidified with 6N HCl and left to degas overnight. Filters were then transferred back into scintillation vials, to which 10 mL of scintillation cocktail was added (Ultima Gold AB, PerkinElmer). After 2h, the samples were measured on a liquid scintillation counter (DPM_{sample}; Tri-Carb, PerkinElmer), using automatic quench correction and a maximum counting time of 5 minutes. For blank determination ($DPM_{0\%}$), one extra sample per treatment and measurement day was filtered and acidified immediately after addition of the ^{14}C spike. To determine the total amount of $NaH^{14}CO_3$ ($DPM_{100\%}$) added, filtered seawater was spiked with 1 µCi mL^{-1} $NaH^{14}CO_3$. From these samples, 10 different 0.5 mL aliquots were immediately removed and mixed with 10 mL of scintillation cocktail. These samples were directly measured after 2h of reaction time with the cocktail. Chlorophyll *a* concentrations at the start of the incubation period were measured fluorometrically after extraction overnight in 90% acetone (Trilogy Fluorometer, Turner Designs). The initial amount of dissolved inorganic carbon (DIC) of $HgCl_2$-fixed subsamples was measured colorimetrically using a QuAAtro autoanalyzer (Seal; (*46*)). NPP rates [µg C (µg Chl)$^{-1}$ d^{-1}] were calculated as:

$$NPP = ([DIC] * (DPM_{sample} - DPM_{0\%}) * 1.05)) / (DPM_{100\%} * t * [Chl])$$

where [DIC] and [Chl] denote the concentrations of dissolved inorganic carbon and Chl *a* in the sample, respectively. DPM_{sample} denotes the disintegrations per minute (DPM) in the samples, $DPM_{0\%}$ reflects the blank value, $DPM_{100\%}$ denotes the DPM of the total amount of $NaH^{14}CO_3$ added to the samples, and t is the duration of the incubation.

Phytoplankton autofluorescence was measured on samples fixed with buffered formalin and glutharaldehyde. Pictures were taken with a Nikon DS Camera (DS-5M & DS-L1) on a Nikon converted microscopy (Eclipse TE300) equipped with a Super High Pressure Mercury Lamp (C-SHG1).

Zooplankton sampling

Zooplankton were sampled by vertical hauls (towing speed 0.5 m s^{-1}) from close to the seafloor to the surface using a multiple opening/closing net (Multinet, Hydrobios, Kiel, mouth opening 0.25 m^2). *Calanus* spp. were sampled using a mesh size of 200 µm, smaller copepods and nauplii were collected using a mesh size of 64 µm. Samples were preserved in a 4% formaldehyde-in-seawater solution until analysis under a Leica stereomicroscope. Samples were examined by sub-sampling with aliquots obtained with 5 ml automatic pipette, with the pipette tip cut at 5 mm diameter to allow free collection of mesozooplankton. Large (total length>5 mm) organisms were removed before taking sub-samples. The number of subsamples

analyzed was chosen so that at least 150 *Calanus* and 300 small copepods were counted from each sample. Samples with low abundance were examined in their entirety.

Zooplankton respiration

Additional multinet casts were made to catch live mesozooplankton for respiration and biomass measurements at station KB3 (78°57N, 11°56E, 330 m) in January, May and September 2014. Each of the five nets of the sampler was fitted with a 2-L rigid cod-end with filtration apertures at the top of the cylinder to keep the animals in sufficient water until collection. Upon retrieval, each sample was diluted in cold filtered (0.2-0-7 µm GF/F) seawater (FSW) and the potential few large macrozooplankton (e.g. large amphipods, euphausiids, medusae) were removed. In a temperature-controlled room set at *in situ* temperature (1-4^{o}C), the live sample was poured into a funnel fitted with a 1-mm sieve inside and a gate valve to obtain two mesozooplankton size classes for incubation. The large size class was maintained in the top part of the device while the small zooplankton was gently evacuated through the sieve by adding cold oxygenated FSW and collected delicately in a container. Each size class was introduced in a separate airtight glass bottle (110-280 mL capacity) that was then filled to the brim with cold oxygenated FSW and capped. For each experimental setup, control bottles without zooplankton were prepared in triplicates. Oxygen-sensitive optical sensors (optodes) were glued on the inner-wall of the incubation bottles. Oxygen consumption was monitored by optode respirometry with a 10-channel respirometer (Oxy-10 Mini, PreSens Precision Sensing GmbH, Regensburg Germany) every 2 hours for 8-12 h. At termination of the experiment, the condition of the animals was verified before they were carefully blotted in absorbing material and preserved in cryovial at -20^{o}C. On land, the samples were transferred to pre-weighed plastic cups and dried in an oven at 60^{o}C for 48 h prior to weighing on a microbalance (±1µg). Respiration rates were calculated by determining the slope of the decrease of oxygen over time and subtracting the mean value for the controls. Oxygen consumption rates were transformed to respiratory carbon using a respiratory quotient of 0.75 in January, assuming a winter metabolism mainly by lipid reserves [S8], and 0.97 in May and September with a metabolism primarily based on proteins [S9].

Mooring with Acoustic Zooplankton Fish Profiler (AZFP) and sediment trap

A mooring with a sediment trap and a multifrequency AZFP (125, 200, 455, 769 kHz) was deployed at approximately 200 m depth in Kongsfjorden during 2014. The AZFP, installed at 84 m depth, sampled the 80 m between surface and the instrument. The sediment trap, illustrated by the dark thick strip below 40 m in Figure S2 collected sinking particles from the

top 40 m of the water column. The transducers of the AZFP transmit an acoustic pulse through the water column and record the echo. The intensity of the backscattered signal returning from each discrete depth interval (bins) is related to the zooplankton biomass present within each bin. From the start of the deployment on 16 January, the sampling rate was 1 ping per 10 seconds (0.1 Hz). At 09:42 on 22-Jan, the resolution reduced to 0.05 Hz until recovery on 9 September 2014. An AZFP software within the instrument processes the raw echo counts, using information from the manufacturer's calibration, to provide absolute volume backscatter (Sv, dB) as direct output. The 125 kHz frequency we used would detect zooplankton from large copepods (>5 mm) to macrozooplankton. The sampling bottles in the sequential carousel of the sediment trap were pre-programmed to sample 6 hrs each. The trap sample bottles were pre-filled with filtered seawater containing NaCl to provide a density discontinuity relative to ambient seawater, and 2% formalin buffered with sodium borate to preserve the deposited material. After samples were retrieved, the contents were gently sieved through a 60µm net. The contents in the sieve were further analysed for abundance of larger fecal pellets of larvaceans and euphausids. Cylindrical pellets produced by euphausids were not preserved intact. In order to compare the abundance of these fecal pellets among samples, the sample was concentrated to a known volume (20-100 ml). A 5 ml subsample was taken using a pipette with an enlarged opening. The subsample was placed in a petri dish with a pre-etched grid. The petri dish was placed under a stereomicroscope equipped with a mounted digital camera. 10-15 pictures were taken at 1.6x magnification, each picture captured fecal pellets within 2 squares of the grid (equal to 7 mm^2, petri dish size 2206 mm^2). If the concentration of fecal pellets was so dense that many pellets lay on top of each other, a second subsample of 5 ml was taken and the procedure repeated. The images were used to measure the length (l) and width (w) of each fecal pellet piece using ImageJ, an open source graphic program (http://rsb.info.nih.gov/ij/). Volume (V) of the fecal pellets was calculated assuming a cylindrical shape ($V = Pi\ (0.5w)^2\ h$). Total volume of fecal pellets was calculated from the number of measured squares, petri dish area, and subsample volume.

Growth of scallops

Fifteen Icelandic scallops *Chlamys islandica* (53.6 ± 1.2 cm length, 48.9 ± 1.1 cm width from 26/08/2013 to 16/04/2014 (233 days of data) were placed in a cage, with at least 3 m of overlying seawater, on the bottom under the Old Pier of Ny-Ålesund (78° 56' N, 11°56' E). The growth rate and the valve movement behaviour of the scallops were measured by high-frequency non-invasive valvometry [S10]. The technique involves gluing one lightweight

electromagnet on each valve, and then continuously measuring, at 0.6 Hz per the distance between the valves. In bivalve molluscs, calcification takes place in the mantle cavity, over the shell's internal surface. As a result, the consequence of daily growth is an increase in the minimal distance between electrodes when the valves close. To obtain a growth rate, we isolate these daily values and plot them as a function of time providing data on the stability, reduction, acceleration or cessation of growth.

Diel activity of scallops and zooplankton

To describe cyclic activity of valve movements we calculated the mean hourly valve opening amplitudes. Chronobiological analyses were made with the software Time Series Analysis Serial Cosinor 6.3 (http://www.euroestech.net/mainuk.php). Validating a rhythm in the scallop required a series of steps[S11, 12]. Briefly, to check the quality of the data set the absence of randomness using the autocorrelation diagram and the absence of a stationary character were determined by a Partial Autocorrelation Function (PACF) calculation[S13]. The periodicities in the recorded data were tested with the spectral method of the Lomb and Scargle periodogram [S14]. These methods give a threshold of probability ($p = 0.95$) defining the limit below which the signal can be regarded as "noise". We calculated the confidence interval of the period using Halberg's method [S15]. The cycle was modelled with the Cosinor model, which uses a cosine function calculated by regression [S16]. To validate the model two tests are necessary: the elliptic test [S16] must be rejected and the probability (p-value) for the null amplitude hypothesis must be lower than 0.05 (Table 2). Analysis of diel rhythms in zooplankton (Table 2) follow the methods outlined in Berge et al. [S17], and is based upon an acoustic dataset from Kongsfjorden, covering the same period as for the scallops (Table 2). Activity patterns are defined as sound scattering layers migrating in the water column, and are derived from acoustic (ADCP) data comparable to those used in Berge et al. [S17].

Benthic respiration and macrofauna density

Sediments were collected with a single corer at two stations in the middle of the fjord (Station B 78°56.902N, 11°55.691E 300 m and Station C 78°59.094N, 11°31.762E 300 m) in May 2012, August 2012 and 2014, October 2012 and January 2013 and 2015. For each station and each date, five replicate cores (12 cm diameter, 20-25 cm deep) containing sediments and overlying bottom water from the station were incubated in a cold room at 2°C, in the dark, for 24h, to measure total respiration by benthic community inhabiting sediment cores. Cores were sealed using tops that provided constant stirring of the overlying water [S18]. Parallel to sediment core incubation, control cores without sediment but with similar volume of bottom

water were incubated simultaneously to evaluate the effect of seawater microorganisms on oxygen and nutrient fluxes [S19, 20]. These values were used as blanks and were subtracted from the values obtained with sediment incubations. Oxygen concentrations were monitored every 2 hours using a microelectrode (Unisense A/S; Aarhus, Denmark) inserted into a small sampling port in the core top without introducing any air. Incubations were terminated after 24h when 15–20% of the oxygen had been consumed [S21] and sediment oxygen demand (SOD) was measured as the (negative) slope of the regression line between oxygen concentration and time, subtracting the mean control values[S22]. Oxygen consumption rates were converted into sediment carbon demand (SCD) by assuming a 1:1 stoichiometric relationship between oxygen and carbon consumption, and then applying a respiratory coefficient of 0.85 [S22]. Three van Veen grabs (of 0.1 m^2 sample area) were collected at each station/ each occasion alongside core sampling. The samples were sieved on 0.5 mm sieves and preserved with formalin. At the laboratory all individuals were identified to the lowest possible taxonomic level and enumerated.

Sampling of fauna associated with the macroalgae Saccharina latissima and fauna of sediments around the algae

In October 2013, January and May 2014 SCUBA divers collected 10 sample sets in Kongsfjorden (near Marine Lab in Ny-Ålesund) at 2 m depth. Each sample set included: (1) a whole individual of *Saccharina latissima* (together with the attached stone) which was packed underwater in a sampling net with 0.5 mm mesh to prevent loss of motile fauna, and (2) a sediment sample taken by cogged grab (areas sampled= 0.025 m^2) near *S. latissima* individuals. Sediment samples were washed through a sieve (0.5 mm mesh). All organisms retained on the sieve were identified to the lowest possible taxonomic level (except Nemertea), counted and wet-weighted with accuracy of 1 mg. To calculate densities of sessile species, we measured the area of each substrate type (blade and holdfast of *S. latissima* as well as stones). We also measured the holdfast's attachment area to calculate densities of motile fauna within a holdfast.

Scavenging fauna observed with time-lapse photography

To study the presence and composition of shallow-water scavenging fauna during the polar night, bait consisting of a 300g Atlantic Cod (*Gadus morhua*) was deployed by divers at 12 m depth in close vicinity of the Ny-Ålesund harbour. A custom-made time-lapse camera system fitted with a Canon 1100D (T3 Rebel) took photographs every 15 minutes for four days

(Supplemental data time-lapse video). The whole setup was additionally equipped with a bait-trap containing 30g of Atlantic Cod meat.

Fish and bird stomach content

Fishes were collected using a Campelen bottom trawl from R/V *Helmer Hanssen* inside Kongsfjorden during both 2014 and 2015. Trawling time was standardised to 10 min bottom time for all trawl hauls (>10 trawls). The opening of the trawl is 60 m across and the trawling speed was 2kt. The diet of four fish species caught in a single trawl are displayed in Figure S3. Although fish species diversity varied among trawls, the stomach fullness and content of dominating species were similar. All specimens in the haul were identified, counted, and weighed. Stomachs of all specimens of the dominant species (*Boreogadus saida, Gadus morhua*, *Clupea harengus* and *Melanogramus aeglefinus*) were dissected and weighed before being preserved in 70% Ethanol. Identification to the lowest taxonomic level possible of stomach contents was carried out in the lab under a Leica dissecting microscope (4-50x magnification). Prey species were weighed and each individual counted whenever possible. Birds were sampled from a small open boat and with permission from the local authorities. Stomachs were dissected and treated in a similar fashion to the fish stomachs (Table 1).

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