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Supplementary Materials for

Copepods drive large-scale trait-mediated effects in marine plankton

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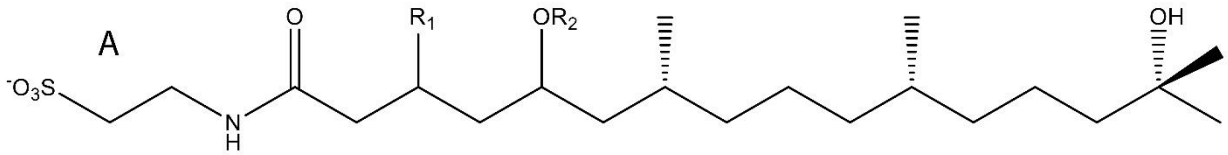
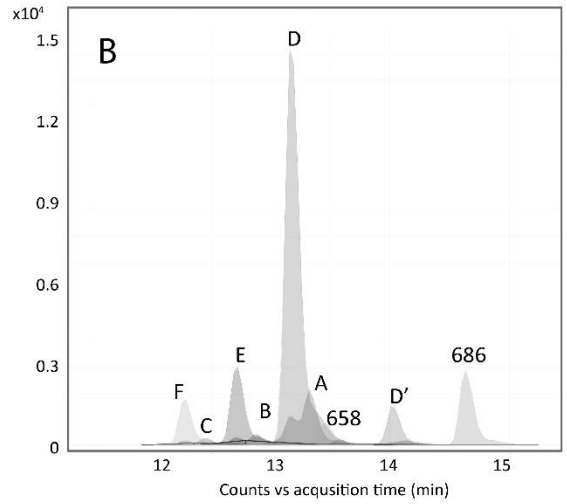
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/2/eaat5096/DC1)

Data file S1 (Microsoft Excel format). Zooplankton raw data.
Data file S2 (Microsoft Excel format). MS raw data.





C

Copepodamide	R_1	R_2
A	CH_3	Docosohexaenoic acid
B	CH_3	Eciosopentaenoic acid
C	CH_3	Stearidonic acid
D	CH	Docosohexaenoic acid
E	CH	Eciosopentaenoic acid
F	CH	Stearidonic acid
G	CH_3	H
H	CH	H
686	CH	Palmitic acid
658	CH	Myristic acid

Fig. S1. Composition of copepodamides from *C. finmarchicus* used in experiment. (**A**) Structure of the copepodamide scaffold. (**B**) Integrated peaks of copepodamides from LC-MS measurements of copepodamides from *Calanus finmarchicus* as described in materials and methods. Capital letters denote identified copepodamides, 658 and 686 the m/z values of two additional copepodamides (*34*). (**C**) Table showing the identity of the R_1 and R_2 groups for each of the copepodamides. R_1 is a methyl or methylene group, the R_2 is a fatty acid, except for the two inactive copepodamides G and H, which lack a fatty acid in this position. The relative concentration (% of total copepodamides) of each component was as follows: copepodamide D: 61 %,copepodamide E: 9 %, copepodamide with 686 m/z: 9 %, copepodamide A: 7 %, copepodamide F: 5 %, copepodamide D´: 4 %, copepodamide with 658 m/z: 3 %, copepodamide B: 1 % copepodamide C: 1 %. *Calanus finmarchicus* is one of the dominant copepod species in the geographical areas where the responding species were isolated and the blend of copepodamides consequently ecologically relevant.

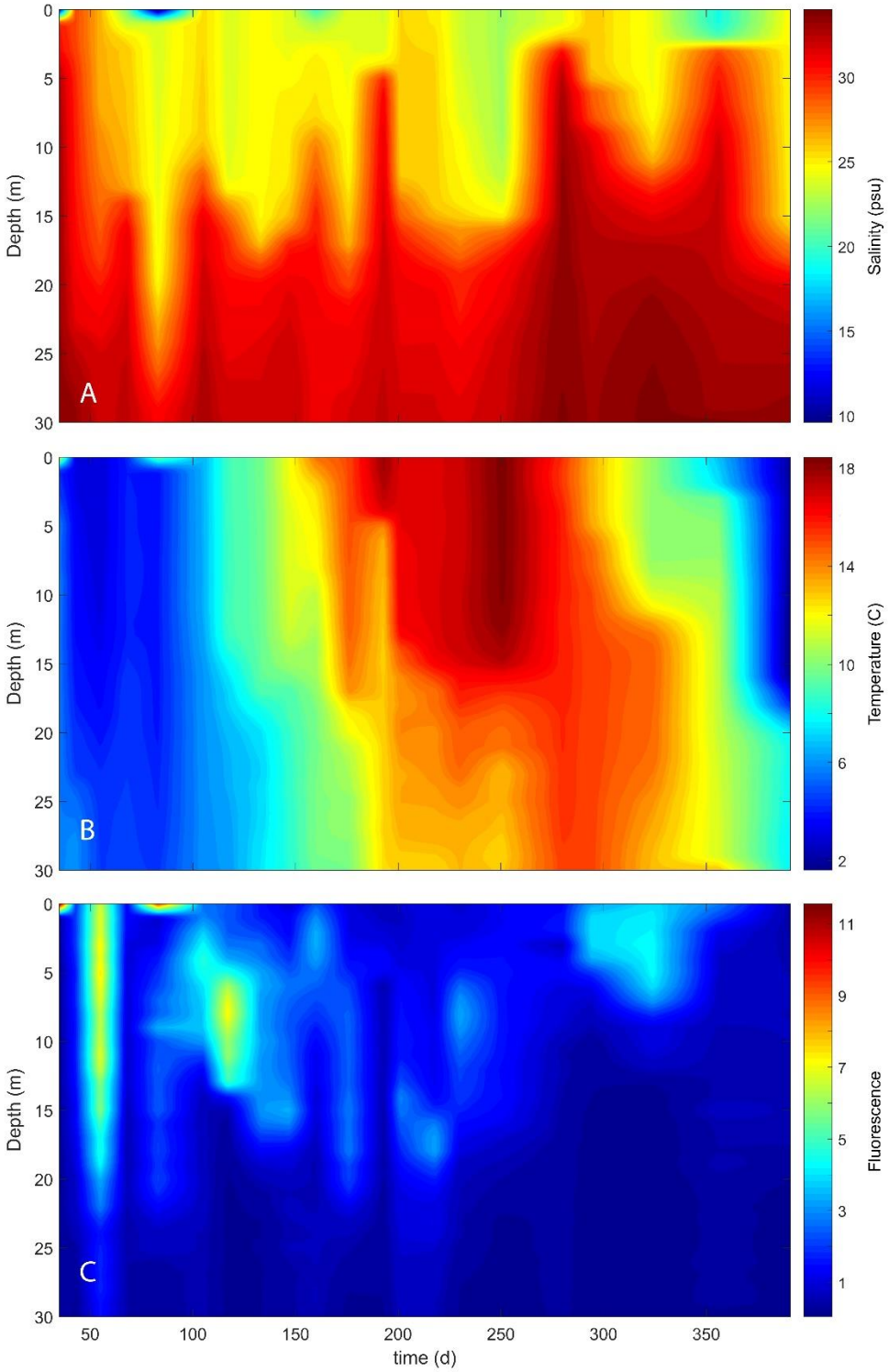


Fig. S2. Hydrographic data. We collected hydrographic data every second week when weather allowed, in total 21 sampling occasions during the one year long sampling effort. Conductivity, temperature and chlorophyll a was measured simultaneously with a CTD profiler (SeaBird/General Oceanic). The interpolated data is shown in panels A-C. (**A**) shows the influence of the Baltic surface current on salinity, with brackish water moving north above a more saline water mass. The halocline is usually found around 15 m depth. Temperature (**B**) increased from 6 ºC in the winter to 18 ºC in the surface water in late summer. Chlorophyll a

fluorescence (**C**) shows classical temperate seasonality with a spring bloom (end of February) and an autumn bloom (end of September).

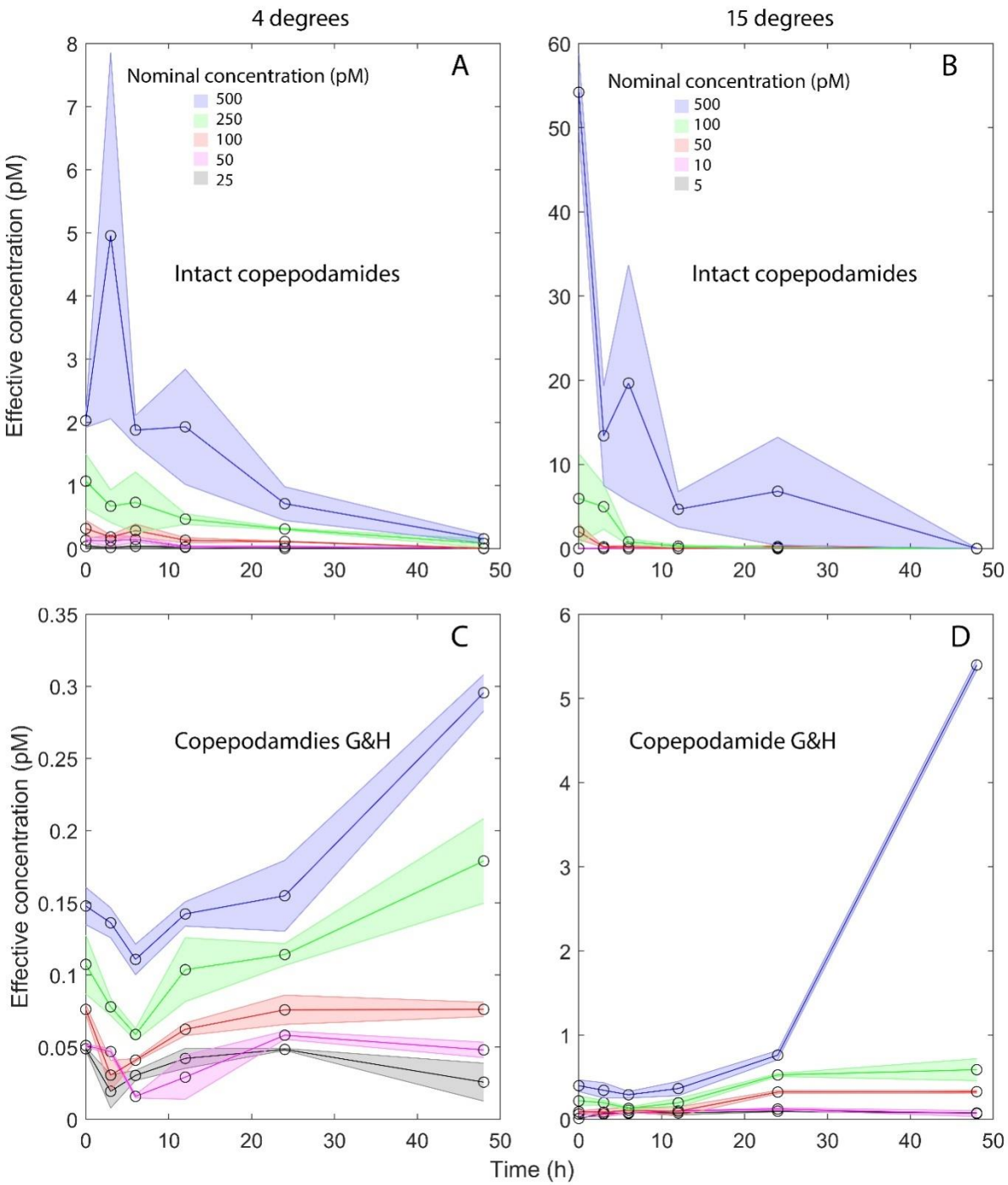


Fig. S3. Effective concentrations of copepodamides. To determine the concentration of copepodamides available to the algae in dose-response experiments we measured the effective concentrations of copepodamides at 4°C (panels **A**&**C**) and 15°C (panels **B**&**D**). Panels A and B show the summed effective concentrations of copepodamides A-F, 686, and 658. Panels C and D show the effective concentration of copepodamides G and H, these are de-acylated degradation products of intact copepodamides that are not bioactive at these concentrations (fig. S4). The average data points are shown by the open symbols, with shaded error interval (standard error) color-coded after the nominal concentration added and indicated in the legend. To calculate the average effective concentration for each nominal concentration we fitted the exponential decay function, $C = a * e^{bt}$. The average effective concentration was calculated as the mean of the fitted function over the duration of the experiment. To be conservative we doubled this concentration to predict the level of

induction in nature. Copepods migrate vertically to feed at the surface at night and hide in deeper water during day. Copepodamide concentration will consequently be pulsed in nature too.

The experiments were performed as in the main experiment but with larger containers to obtain measurable amounts of copepodamides. Glass bottles (132 ml) were coated with a blend of copepodamides from *Calanus finmarchicus* (fig. S1) corresponding to five of the concentrations in the dose response experiments (25, 50, 100, 250, and 500 pM for the 4°C treatment and 5, 10, 50, 100, and 500 pM for the 16°C treatment). We filled the flasks with 26 PSU silica enriched f/4 medium with 5×10^4 cells mL^{-1} *Skeletonema marinoi* culture and incubated at the same conditions as the respective dose response experiment. Three vials for each concentration and temperature were sampled at t=0, 3, 6, 12, 24, and 48 hours. Each sample was poured onto pre-conditioned (3 ml methanol followed by 3 ml milliQ water) solid phase extraction columns (Evolute, ABN 50 µm, 100 mg sorbent). Columns were de-salted with 4 ml milliQ water and retained copepodamides eluted with 3 ml methanol, evaporated (N2 flow, 30 °C), re-solved in 50 µl methanol and stored at -20 °C until analyses as described in the main text. The few concentrations not included in the five concentrations added for each experimental condition was inter, or extrapolated linearily from the experimental data. The proportion of de-acylated (in-active) copepodamides (G and H) increase with time, suggesting that de-acylation is an important route for de-activation of copepodamide signals in the sea.

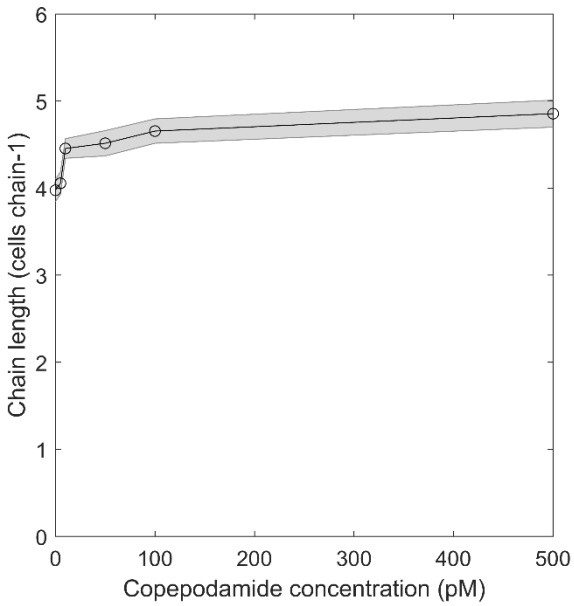


Fig. S4. Copepodamides G and H do not induce chain length shortening in *Skeletonema.* Cells were exposed the same way as in the main paper to a mixture of the copepodamide scaffolds G and H. The scaffolds trigger no chain length reduction in *Skeletonema.* In fact, exposed cultures grow slightly longer chains. The x-axis show the nominal concentration used in experiments. Each point represents the mean value of four independent replicates. From each replicates the number of cells per chain was counted for fifty chains. The shaded area shows the standard error of mean.

The supplementary data files S1 and S2 are provided in separate files