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Calanus spp.—Vectors for the biotoxin, domoic acid, in the Arctic marine ecosystem?

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ABSTRACT

Three *Calanus* species, *Calanus glacialis*, *Calanus finmarchicus* and *Calanus hyperboreus*, which are the most important zooplankton herbivores in Western Greenland, were fed with unialgal cultures of toxic *Pseudo-nitzschia seriata* and non-toxic *Pseudo-nitzschia delicatissima*. All three copepod species grazed on toxic *P. seriata* and also accumulated domoic acid during the grazing. There were no differences in ingestion rates between toxic and non-toxic *Pseudo-nitzschia* species in any of the copepods. *C. finmarchicus* and *C. hyperboreus* grazed on toxic *P. seriata* during the first 6 h of the experiment but seemed to stop grazing during the last 6 h of the experiment suggesting that the copepods may have suffered some kind of physiological incapacitation due to ingestion of domoic acid. *C. glacialis* grazed on toxic *P. seriata* continuously during the whole experiment, probably due to the lower domoic acid cell quota of *P. seriata* during the experiment on *C. glacialis* than on the other two copepod species. The depuration experiment on *C. glacialis* indicated that the copepods still retained domoic acid after 10 h of depuration in filtered sea water. The results show that the three *Calanus* species are potential vectors for domoic acid to higher trophic levels in the Arctic.

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1. Introduction

Twelve out of ~37 described species of the diatom genus *Pseudo-nitzschia* Peragallo are known to produce the neurotoxin, domoic acid (Trainer et al., 2012 and references therein). Domoic acid has been observed to accumulate in consumers grazing on the toxic *Pseudo-nitzschia*, e.g. copepods (Lincoln et al., 2001), krill (Bargu et al., 2002), blue mussels and scallops (Wohlgeschaffen et al., 1992), and fish (Costa and Garrido, 2004). The grazers then may transfer domoic acid to higher levels in food webs. As a consequence, adverse effects of domoic acid have been recorded e.g. in birds (Fritz et al., 1992), sea lions (Scholin et al., 2000), whales (Fire et al., 2010) and humans (Perl et al., 1990) which have suffered from different kinds of neurological disorders or even mortality caused by domoic acid intoxication. The first toxin producing diatom, *Pseudo-nitzschia multiseries*, was discovered after an extensive intoxication incident (Amnesic Shellfish Poisoning, ASP) in Prince Edward Island, Canada, in 1987. The intoxication affected more than 100 humans and was caused by blue mussels contaminated with domoic acid (Bates et al., 1989). Monitoring of *Pseudo-nitzschia* spp. in North America, northern and western Europe, Japan, Australia and New Zealand seems to have been effective in preventing acute domoic acid intoxications in humans (Lefebvre and Robertson, 2010), but knowledge of putative chronic effects of domoic acid on humans and wildlife is almost non-existing. Also, very little is known of possible acute impacts of domoic acid on zooplankton and other phytoplankton grazers. In fact, only five grazing experiments on toxic diatoms and copepods have been reported to date (Windust, 1992; Lincoln et al., 2001; Tester et al., 2001; Maneiro et al., 2005; Leandro et al., 2010b), whereas grazing of copepods on toxic dinoflagellates have been studied intensively (reviewed by Turner and Tester, 1997; Turner et al., 1998; Turner, 2006). As far as phycotoxin dynamics is concerned, very few copepod studies have fully addressed the topic with respect to any algal group (see Dam and Haley, 2011 and references therein).

The first record of domoic acid producing species in Arctic waters is from western Greenland (Hansen et al., 2011). The species in question is *Pseudo-nitzschia seriata* (P.T. Cleve) Peragallo, a cold-water species (Hasle, 2002), which is distributed in cold

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temperate Atlantic areas, such as Canadian, Irish, Scottish and Danish waters (Lundholm et al., 1994; Gallacher et al., 2001; Bates et al., 2002; Cusack et al., 2002) and is now also known from the Pacific Ocean (Stonik et al., 2011). The records of *P. seriata* occurrence from Greenlandic and adjacent waters are numerous (e.g. Cleve, 1896; Grøntved and Seidenfaden, 1938; von Quillfeldt, 1997, 2000; Lovejoy et al., 2002; Hansen et al., 2011, this study), but cell concentrations have rarely been recorded (Braarud, 1935). Yet, the earliest records of *P. seriata* from polar waters likely include the congeneric *Pseudo-nitzschia obtusa*, which is morphologically very similar to *P. seriata*, but has been grouped into separate species (Hasle and Lundholm, 2005). *P. obtusa* has so far not been shown to produce domoic acid (Hasle and Lundholm, 2005).

The copepod *Calanus finmarchicus* (Gunnerus), with its congeners *Calanus glacialis* Jaschnov and *Calanus hyperboreus* Krøyer, are the most important metazoan grazers in Arctic waters (Conover and Huntley, 1991; Hirche and Mumm, 1992; Nielsen and Hansen, 1995). *Calanus* spp. are essential prey especially for shrimps (Hopkins et al., 1993), and the northern shrimp (*Pandalus borealis*) is nowadays the most important export product for Greenland (Buch et al., 2004). For example, in 2009, northern shrimp accounted for 54% of the total annual export value in Greenland (Michelsen, 2011). Naturally, seafood is consumed to a great extent by the local population in Greenland as well. It has been shown in a single study from temperate waters that domoic acid may accumulate in *C. finmarchicus* when feeding on toxic *Pseudo-nitzschia* sp. (Leandro et al., 2010b). Thus, *Calanus* spp. may be potential vectors for domoic acid to the higher trophic levels also in the Arctic waters where knowledge of the effects and transfer of phycotoxins is practically non-existent.

Here, we explore the potential risk for transfer of domoic acid up the Arctic food web through the three dominating *Calanus* species in the area. We addressed the following questions:

(1) Do the three *Calanus* spp. accumulate domoic acid when fed with unialgal toxic cultures?
(2) Are the copepods affected by the toxin measured as changes in grazing rates and/or mortality?
(3) Do the three copepods differ in grazing rate and toxin accumulation?
(4) Do the copepods ingest toxic and non-toxic *Pseudo-nitzschia* at the same rate?

2. Materials and methods

2.1. Copepods

Specimens of *C. glacialis*, *C. finmarchicus* and *C. hyperboreus* for experiments were collected from Disko Bay (69°14′N, 53°23′W) at a 300-m-deep monitoring station (Madsen et al., 2001; Hansen et al., 2012), Western Greenland, in April 2011 prior to and during the spring bloom. The copepods were collected in the upper 100 m using a WP-2 net (200 μm), kept dark and cold for a maximum of two weeks in 0.45 μm filtered sea water and fed with *Thalassiosira* sp. The copepods were starved 24 h prior to the experiments.

2.2. Phytoplankton cultures

Toxic *Pseudo-nitzschia seriata* strain (P5G3) and non-toxic *Pseudo-nitzschia delicatissima* (P.T. Cleve) Heiden strain (P2F2) were isolated into clonal culture from Disko Bay, April 2010 by isolating single cells or chains into micro well plates and later transferring the cultures to Nunclon flasks. Prior to the grazing experiments, clonal strains of both species were grown in batch cultures at 4 °C in 50% silica-reduced L1-medium (Guillard and Hargraves, 1993) under a light intensity of 100 μmol photons m^{-2} s^{-1} at a 19:5 light:dark cycle using cool white fluorescent bulbs. The silica concentration of the L1-medium was reduced in order to trigger domoic acid production in *P. seriata* as Si-limitation is known to enhance domoic acid production this species (e.g. Fehling et al., 2004).

2.3. Grazing experiments

Each *Calanus* species was fed with both the non-toxic *P. delicatissima* and the toxic *P. seriata*. The nominal initial cell concentration of the two species was 20,000 cells ml^{-1} and 4000 cells ml^{-1}, respectively, in order to provide copepods with saturating carbon supply (>400 μg C l^{-1}, Parsons et al., 1969; Hansen et al., 1997). Carbon content per *Pseudo-nitzschia* cell was calculated using measured carbon content and size of *P. multiseries* (0.12 pg C μm^{-3}) (Bargu et al., 2003; Sibel Bargu, pers. comm.) and relating this to the volumes of *P. seriata* and *P. delicatissima* (Table 1). No specific carbon-to-volume formula was used for estimating the carbon content per algal cell because the formulas (Montagnes et al., 1994; Menden-Deuer and Lessard, 2000) underestimated the carbon content in *P. seriata*. The cell volume of *Pseudo-nitzschia* spp. was calculated as follows (Lundholm et al., 2004):

$$\text{volume} = (0.6 \times L \times W^2) + (0.4 \times 0.5 \times L \times W^2)$$

where L is the cell length and W is the width of the cell. *P. delicatissima* occurred as single cells in all experiments, whereas *P. seriata* formed also chains. In the experiment with *C. glacialis* the *P. seriata* cells were divided as follows: single cells (42%), 2-cell chains (33%), 3-cell chains (16%), 4-cell chains (8%) and 5-cell chains (1%). In the experiment with *C. finmarchicus* and *C. hyperboreus* there were single cells (73%), 2-cell chains (23%), 3-cell chains (5%) and 4-cell chains (1%).

All experiments were conducted in 1.55-l polycarbonate flasks mounted on a plankton wheel (speed 1.3 rpm) in the dark, at 4–6 °C and using 0.45 μm filtered sea water with a salinity of 35. The experiments were run in parallel using toxic and non-toxic diatom cultures. In the grazing experiments, copepods were grazing on the diatoms for 12 h and controls without grazers were incubated simultaneously. The first batch of grazing experiments was done on *C. glacialis* grazing on both *Pseudo-nitzschia* species using triplicate controls and grazing treatments. The second batch of experiments was done in parallel on *C. finmarchicus* and *C. hyperboreus* grazing on both *Pseudo-nitzschia* species with four replicate controls and grazing treatments. The same controls were used for *C. finmarchicus* and *C. hyperboreus*. Due to the different sizes of the copepods, the nominal initial number of copepods per 1.55-liter flask was 6 for *C. glacialis*, 10 for *C. finmarchicus* and 2 for *C. hyperboreus*.

Table 1
Cell length (mean ± sd), cell width (mean ± sd), cell volume (mean ± sd), and cell carbon content (mean) for *Pseudo-nitzschia delicatissima* and *P. seriata*.

	Cell length (μm)	Cell width (μm)	Cell volume (μm^3)	Carbon (pg C $cell^{-1}$)
P. delicatissima	49 ± 6.9	1.9 ± 0.01	147 ± 21	18
P. seriata	73 ± 14.9	5.7 ± 0.10	1877 ± 384	231

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