

## Can domoic acid affect escape response in copepods?

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### ARTICLE INFO

#### Keywords:

Pseudo-nitzschia  
Domoic acid  
Calanus  
Escape jumps

### ABSTRACT

Copepods are important grazers on toxic phytoplankton and serve as vectors for algal toxins up the marine food web. Success of phytoplankton depends among other factors on protection against grazers like copepods, and same way copepod survival and population resilience relies on their ability to escape predators. Little is, however, known about the effect of toxins on the escape response of copepods. In this study we experimentally tested the hypothesis that the neurotoxin domoic acid (DA) produced by the diatom *Pseudo-nitzschia* affects escape responses of planktonic copepods. We found that the arctic copepods *Calanus hyperboreus* and *C. glacialis* reduced their escape response after feeding on a DA-producing diatom. The two species were not affected the same way; *C. hyperboreus* was affected after shorter exposure and less intake of DA. The negative effect on escape response was not related to the amount of DA accumulated in the copepods. Our results suggest that further research on the effects of DA on copepod behavior and DA toxicity mechanisms is required to evaluate the anti-grazing function of DA.

Predation is a major cause of copepod mortality, and processes that negatively affect the sensory system of copepods or their mobility may affect predation (Kerfoot, 1978; Hirst and Kiørboe, 2002; Almeda et al., 2017; van Someren Gréve et al., 2017). Copepods detect predators by sensing hydromechanical disturbances (Kerfoot, 1978; Haury et al., 1980; Kiørboe and Visser, 1999) and their escape response is a forceful jump away from that stimulus (Ohman, 1988; Fields and Yen, 1997; Wohlrab et al., 2010; Bradley et al., 2013). Hence a successful escape response in copepods requires both detecting the disturbance in the water and the muscle power to perform a jump.

Diatoms of the genera *Nitzschia* and *Pseudo-nitzschia* produce a neurotoxin, domoic acid (DA), which can be transferred via copepods to higher food web levels (Leandro et al., 2010a; Tammilehto et al., 2012; D'Agostino et al., 2017) and intoxicate organisms such as seabirds, whales and seals (Fritz et al., 1992; Leandro et al., 2010b; Jensen et al., 2015). Domoic acid can also cause amnesic shellfish poisoning in humans (Quilliam and Wright, 1989; Landsberg, 2002). Presently, two species of *Pseudo-nitzschia*, *P. seriata* and *P. obtusa*, are known to induce DA production in the presence of copepods (Harðardóttir et al., 2015; Tammilehto et al., 2015). *Pseudo-nitzschia seriata* is known to elevate DA production as a respond to predator cues from various copepods

species with high DA levels in correlation to high concentration of predator exoduses (Lundholm et al., 2018). Suggesting that DA production is a chemical defense mechanism against predation. Copepods, one of main grazers of diatoms in marine ecosystems, seem to be resistant to DA, making the role of the grazer-induced DA speculative. Copepod species of *Calanus* and *Acartia* are known to feed on toxic *Pseudo-nitzschia* without any grazing deterrence and they do not select alternative non-toxic prey when given a choice (Windust, 1992; Tester and Douchette, 2001; Maneiro et al., 2005; Leandro et al., 2010a). A single report exists of a changed temporal feeding pattern in *Calanus* spp. after consumption of DA (Tammilehto et al., 2012). Egg production and hatching is not reduced in *Calanus* spp. by feeding on toxic *Pseudo-nitzschia* (Miesner et al., 2016). Only one report of increased mortality, due to DA has been published until now, for ecologically relevant DA levels (Lundholm et al., 2018).

Domoic acid is an excitatory amino acid and an analogue to glutamate (Wright et al., 1989). Crustaceans use glutamate as a neurotransmitter in both the peripheral and central nervous system (Eckert and Randall, 1983). We hypothesize that DA may interfere with neurotransmission regulation and consequently affect escape responses in copepods. In this study we tested if the escape response is reduced in

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<https://doi.org/10.1016/j.hal.2018.08.009>

**Table 1**

Average concentration of toxic and nontoxic diet, saturation level for copepods is  $400 \mu\text{g C L}^{-1}$ . Domoic acid (DA) levels of *Pseudo-nitzschia*, and accumulated DA in the copepod tissue after either 12 or 72 h incubation, in either copepod affected by ingested DA or unaffected, each replicate ( $n = 3$ ) contained 5 animals. Values are given as mean and standard deviation.

Copepod Treatment Time	Start diet concentration: ( $\mu\text{g C L}^{-1}$ )	End diet concentration: ( $\mu\text{g C L}^{-1}$ )	Start DA content of <i>Pseudo-nitzschia</i> ( $\text{pg DA cell}^{-1}$ )	End DA content of <i>Pseudo-nitzschia</i> ( $\text{pg DA cell}^{-1}$ )	Accumulated DA in copepods unaffected / affected ( $\text{ng DA mgC}^{-1}$ )
<b><i>Calanus hyperboreus</i></b>					
Treatment with toxic <i>Pseudo-nitzschia</i>					
12 h:	1628 $\pm$ 602	696 $\pm$ 445	16.3 $\pm$ 5.5	20.9 $\pm$ 5.9	120.7 $\pm$ 47.5 / 62.5 $\pm$ 16.5
72 h:	3661 $\pm$ 108	1034 $\pm$ 151	6.6 $\pm$ 0.4	14.4 $\pm$ 2.3	103.1 $\pm$ 87.8 / 45.5 $\pm$ 22.2
Control with non-toxic <i>Thalassiosira</i>					
12 h:	2875 $\pm$ 460	1981 $\pm$ 238			
72 h:	3300 $\pm$ 841	2620 $\pm$ 445			
<b><i>Calanus glacialis</i></b>					
Treatment with toxic <i>Pseudo-nitzschia</i>					
12 h:	1628 $\pm$ 602	762 $\pm$ 130	16.3 $\pm$ 5.5	18.1 $\pm$ 3.4	1047.5 $\pm$ 351 / 676.4 $\pm$ 182.4
72 h:	3661 $\pm$ 108	1083 $\pm$ 180	6.6 $\pm$ 0.4	10.5 $\pm$ 1.6	514.6 $\pm$ 192.6 / 353.7 $\pm$ 102.5
Control with non-toxic <i>Thalassiosira</i>					
12 h:	2875 $\pm$ 460	2620 $\pm$ 445			
72 h:	3300 $\pm$ 841	1300 $\pm$ 929			

copepods after feeding on toxic *Pseudo-nitzschia*. Prior to incubations, the animals were starved for > 24 h. The incubations were carried out in the dark at  $\sim 4 \text{ C}^\circ$ . Two arctic *Calanus* copepod species *C. hyperboreus* and *C. glacialis*, were exposed to diets of either the toxic *Pseudo-nitzschia seriata* or a non-toxic *Thalassiosira gravida* for 12 and 72 h in 5 L buckets, one bucket per treatment, with saturating food conditions of  $> 400 \mu\text{g C L}^{-1}$ , (Hansen et al., 1996). Food concentration was above saturation levels for both diets at the end of the experiment (Table 1). The two diatoms differ in size, shape and carbon content but both diatom species are in the size range that both *Calanus* species are known to feed on.

A siphon experiment was conducted to trigger escape jumps. From the incubation buckets, single copepods were carefully placed in a petri dish filled with filtered sea water. A pasteur pipette was used to create a hydromechanical signal by placing the tip in the liquid and using the suction to create a this signal, similar to theory in Titelman, (2001) and Kjørboe and Visser (1999), and the set up in Wohlrab et al., (2010). When a copepod showed at least one escape response, by jumping when perceiving the stimuli, it was classified as “unaffected” by DA. Only when copepods did not respond after three stimuli, they were classified as “affected” copepods. Each assay was tested on 100 copepods for each treatment variable; diet, exposure time and copepod species. The data were statistically analyzed by applying a generalized linear model (glm) (McCullagh, 1984) to interpret the proportion of success with numerical vector values (0 or 1). This was done with the statistical package MASS (Venables and Ripley, 2002) using the programme R (R Core Team, 2018) and SigmaPlot (2018).

Domoic acid content in the affected and unaffected copepods was measured in triplicates of five copepods to assess if accumulation of DA explain the changes in escape responses (for method details see suppl. Material).

The species *C. hyperboreus* was affected after incubation with DA-producing *Pseudo-nitzschia*; the escape responses were reduced from 66 and 60% in the control treatments to 40 and 37% after 12 h and 72 h of incubation, respectively (Fig. 1, top). In contrast, the escape response of *C. glacialis* was not affected after 12 h, but after 72 h a reduction in the escape response became evident, from 67% in the control to 25% in the treatment (Fig. 1, bottom). Both diet and exposure time significantly affected escape responses of the copepods (gls,  $P < 0.0001$  for both variables), and the response of the two species was significantly different (gls,  $P < 0.05$ ), with a more pronounced response in *C. glacialis* after 72 h. Domoic acid content in the copepods differed between

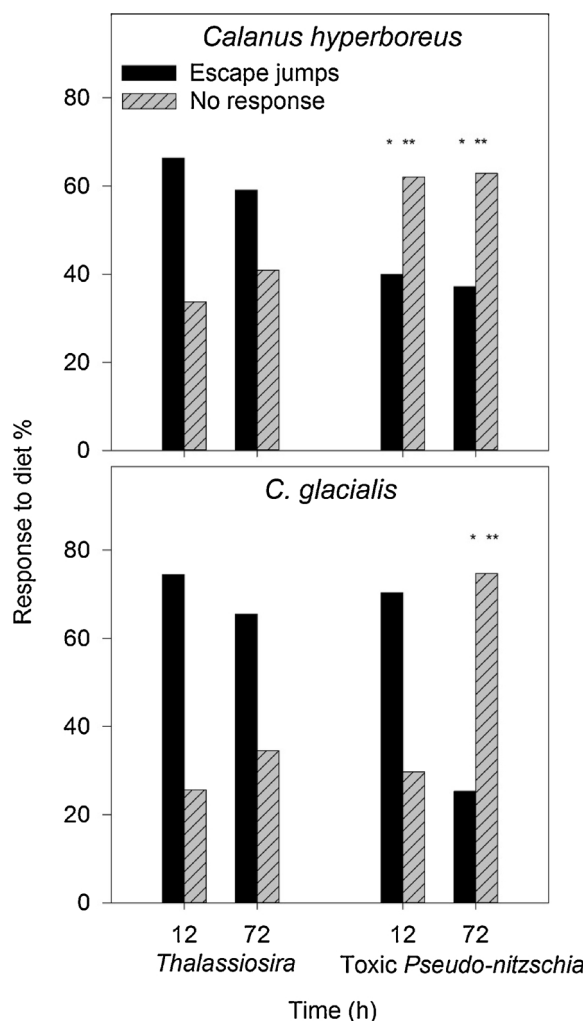
affected or unaffected but the differences were not significantly different (One-Way ANOVA,  $P > 0.05$ ) (Table 1). Of the two species studied, *C. glacialis* accumulated more DA  $\text{mg C}^{-1}$  than *C. hyperboreus* after both 12 and 72 h of incubation (t-test,  $P < 0.05$ ) (Table 1). The negative effect of DA was only observed after 72 h in *C. glacialis*.

Our results suggest that escape responses of both copepod species are affected by ingestion of DA-producing diatoms, however not in the same way. The copepod *C. hyperboreus* is affected both sooner and after having accumulated less DA than *C. glacialis* indicating that *C. hyperboreus* is more vulnerable to the toxin. The feeding strategy of the two species is the same; selective suspension feeders that graze on phytoplankton and heterotrophic protists (Mullin, 1963). The two copepods are different in size (carbon body weight 0.11 mg C for *C. glacialis* and 1.31 mg C for *C. hyperboreus* Swalethorp et al., 2011), have different life strategies (Madsen et al., 2001; Swalethorp et al., 2011), and hence behavior and escape capabilities vary between species which can affect the risk of predation (Almeda et al., 2017). The threshold for an escape response is variable between different species of copepods, and between different stages and sex within a single species (Fields & Yen, 1997; Buskey et al., 2002). There was not a difference between the *Calanus* species in the control, suggesting they have similar initial response to the stimuli. The amount of accumulated toxins is variable between the time of exposure and affected or unaffected, but this difference is not significant for either species. We used five animals in each replicate but the standard variation is high (Table 1) indicating a large individual variation and may explain the lack of statistical difference.

The results of this study thus indicate a sub-lethal affect. The reduced escape response observed here might be caused by DA interfering with neurotransmission regulation in the copepods as hypothesized above. A parallel mechanism has previously been suggested for saxitoxin production in the dinoflagellate *Alexandrium* (Wohlrab et al., 2010). The reduction in escape response of copepods will increase copepod mortality by predation and thereby reduce grazing pressure on the toxic diatoms and consequently promote the transfer of DA to higher trophic levels of the food web. In conclusion, the results suggest that DA has a role in the defense mechanism to reduce grazing on toxic diatoms.

## Acknowledgements

We wish to thank the crew on Arctic Station and local hunters in



**Fig. 1.** Copepod escape jumps in *Calanus hyperboreus* and *C. glacialis* after feeding on toxic *Pseudo-nitzschia seriata* or control diet of non-toxic *Thalassiosira gravida* after 12 or 72 h.  $n = 100$ . Each treatment results are given in % of total. The data was statistically analyzed by applying a generalized linear model and found a difference between the species  $p < 0.05$  (results not presented), diet and time result presented as \* = difference to control and \*\* = difference over time  $p < 0.001$  for both.

Qeqertarsuaq. We thank Lumi Haraguchi for valuable help with the statistical analysis and Ditte Marie Hjort for assistance with experiments. The project is funded by Independent Research Fund Denmark, grant DFF-1323-00258 and partially financed by the Helmholtz-Gemeinschaft Deutscher Forschungszentren through the research programme “Polar regions And Coasts in the changing Earth System” (PACES II) of the Alfred Wegener Institut-Helmholtz Zentrum für Polar- und Meeresforschung. [CG]

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.hal.2018.08.009>.

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