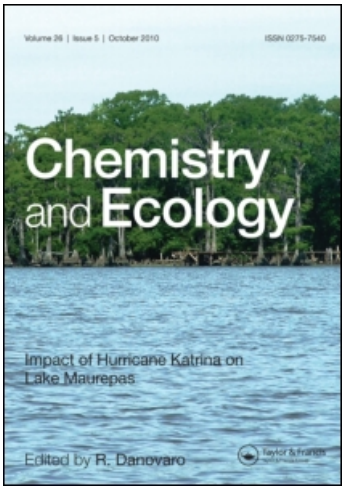

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The potential effects of sucralose on the Arctic copepods *Calanus finmarchicus* and *Calanus glacialis* were studied in Disko Bay, Greenland. Sucralose is a non-calorie sweetener and chlorine derivate of sucrose containing three chlorine atoms. Scandinavian screening studies of sucralose in 2007, revealed sucralose in all effluent samples. To investigate whether sucralose is harmful to the Arctic aquatic ecosystems, possible short-term effects were investigated on egg production, hatching rate, food intake and mortality of two species of Arctic copepods. The copepods were exposed to six different concentrations (0–50,000 ng $\cdot$ L^{-1}) of sucralose, which spans the range of concentrations found in the screening studies. Exposure led to no mortality among the copepods. Food intake by *C. glacialis* increased with increasing concentrations of sucralose. In *C. finmarchicus*, food intake did not differ with increasing concentrations. No effect of sucralose was observed on egg production of *C. finmarchicus*. Despite increased food intake with increasing concentrations of sucralose, *C. glacialis* did not increase its egg production. The results show that both species responded weakly to sucralose, but with *C. glacialis* being possibly slightly more sensitive to sucralose than *C. finmarchicus*.

Keywords: sucralose; sublethal effects; *Calanus*; copepods; Arctic; Greenland

1. Introduction

A new artificial sweetener has been introduced during recent years and today it is added to >4000 food and beverage products. It is known as sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-β/α-D-galactopyranoside) and is considered to be 600 times more sweet than ordinary table sugar (sucrose) [1]. Sucralose was permitted for commercial use during the 1990s in Canada, Australia and USA and from 2004 in the European Union also [1]. Sucralose is a chemically-manufactured derivate of sucrose with three attached chlorine atoms. The compound has been given some attention in the media [2], partly because its chemical structure makes it resilient to microbial degradation and this resilience in the environment may be harmful, as has been the case for other organo-halogenic compounds. Furthermore, sucralose has been accused of being carcinogenic [2]. Studies in rats have shown that sucralose intake may cause

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reduced food ingestion, reduced body weight increase and weight change in certain organs [3]. The Joint FAO/WHO Expert Group on Food Additives has decided on an acceptable daily intake (ADI) of sucralose of 15 mg · kg body weight^{-1} [4].

A Swedish screening study determined concentration levels of sucralose in the influent/effluent waters of sewage-treatment plants and in neighbouring water bodies [1]. Less than 10% of the incoming sucralose was removed by the plants and water concentrations up to 1 μg · L^{-1} have been observed in several European waters [5,6]. Sucralose only degrades slowly in aquatic environments, where it can accumulate and it has even been found in oceanic current systems [7]. Degradation rates are temperature dependent with lower rates in colder temperatures. A combination of accumulating abilities, slow degradation rates and potential negative effects may present a risk to aquatic environments. Experiments have shown that sucralose is not absorbed by rodents, dogs and humans, which excrete it, unchanged through faeces and urine [3]. When sucralose is not absorbed or degraded by humans or detained in waste water treatment plants, there is a risk of increasing additions to the water environment if consumption of sucralose-containing products increases. Plant studies have shown decreased sucrose transport in grasses, when exposed to high concentrations of sucralose [8].

In marine waters, copepods represent >70% of the zooplankton biomass [9]. In Arctic marine waters such as the Davis Strait between Canada and Greenland, three species of *Calanus* dominate the plankton during the spring bloom, where almost all of the season's biological production occurs. The three species are *Calanus hyperboreus*, *Calanus glacialis* and *Calanus finmarchicus*, of which the latter is the smallest and constitutes <20% of the total biomass [10,11]. *C. hyperboreus* and *C. glacialis* are species of Arctic origin, while *C. finmarchicus* is a temperate species from the Atlantic Ocean [9,12]. All three species are adapted to the Arctic environment, for example, by overwintering in deep waters with no food uptake and low metabolic rates. In early spring, copepod larvae and adults ascend to the euphotic zone to feed, spawn and build up lipid stores [12,13,14]. Copepods are vital to the Arctic marine food webs as efficient accumulators of lipid energy storages by feeding on phytoplankton [14,15]. Via copepods, lipids are transferred through the food chain to higher trophic levels such as fish, birds and marine mammals where they constitute an important energy source [16]. As a temperate species, *C. finmarchicus* contains lower amounts of lipid and is more dependent on food uptake for successful completion of maturation and spawning. There is a direct link between egg production of *C. finmarchicus* and the concentration of phytoplankton during the spring bloom in the Arctic [13]. Food uptake is also linked to maximum egg production in *C. glacialis* [17], but the species do not need food to initiate spawning as is the case for *C. finmarchicus* [18].

The true Arctic *C. glacialis* is 2.8–4.6 mm in length and has a life span of 1–3 years [19]. The species begins reproduction a few weeks earlier than *C. finmarchicus* and spawns its eggs simultaneously with the beginning of the spring bloom, but it can begin several weeks before. Maximum egg production occurs during the spring bloom [13]. The species grows fast and produces lipid droplets to function as energy storages. After the spring bloom has expired, *C. glacialis* descend to 200–300 m depth, where overwintering takes place.

The smallest of the three dominating *Calanus* species in the Arctic, *C. finmarchicus* is 2.2–3.2 mm in length and it also has the shortest life span of one year [19], and a northern limit in Disko Bay [13]. Under favourable conditions, it can develop from eggs to overwintering stages in 6–10 weeks. Like *C. glacialis*, this species uses lipids as energy storage and overwinters in deep waters [19]. Successful reproduction is dependent on a perfect timing with the spring bloom. If there is a mismatch between spawning and phytoplankton availability, young stages of the copepod may be food limited [20]. Species of *Calanus* are of great importance to marine ecosystems in the Arctic. The copepods are the primary food source for many fish, sea birds and mammals, and are important for the energy transfer from primary production to higher trophic levels. They also play a role in the flux of energy and carbon to the benthic environment through their sinking

faecal pellets, which are a significant food source to many benthic organisms. The grazing on phytoplankton by copepods partly controls phytoplankton biomasses [21,22]. In the process of grazing on phytoplankton, copepods quickly assimilate low-energy carbohydrates and proteins into specialised lipids with high energy content [23].

The objective of this study was to investigate possible short-term effects of sucralose exposure on *C. glacialis* and *C. finmarchicus*. During daily pulse exposures to a range of sucralose concentrations, egg production, hatching percent, prey uptake and mortality were investigated in both species in ambient conditions immediately after collection. The choice of parameters makes it possible to determine whther sucralose has negative effects on copepods both on an individual and population level. If sucralose can decrease egg production rates and/or hatching percentages, it may have implications not only for the next generations of copepods, but also on a longer term for the productivity of the marine ecosystem. Lower abundances of copepods can lead to larger biomasses of phytoplankton and a decrease in lipid accumulation in the food web. The choice of exposure concentrations was made on the basis of screenings made by the Swedish Environmental Research Institute (IVL) and the Norwegian Institute for Air Research (NILU), who estimated a median concentration of sucralose in water from waste water treatment plants of 4900 $ng \cdot L^{-1}$ [1]. The chosen range in this study covers concentrations one magnitude higher and three magnitudes lower in order to anticipate environmentally realistic concentrations of sucralose. This investigation was part of a larger screening project of the effects of sucralose in Arctic ecosystems which included studies of other invertebrates. The choice of Disko Bay as a study site was based upon the assumption of Disko Bay as an environment with no previous record of sucralose exposure. This assumption builds upon the fact that major sources of sucralose are limited to sugar-free consumer products containing sucralose [1], which are not available in the scarcely populated Disko area. Furthermore, the mesozooplankton system in this region is well known. We chose to investigate two of the dominant copepod species to assess any possible change in response between them as they coexist in the area.

2. Material and methods

Individual females of the selected species were sampled in April 2009 on two occasions, one week apart, in Disko Bay on the west coast of Greenland, ~300 km north of the Arctic circle (Figure 1). The bay is 300–600 m deep and bordered by Disko Island towards the Davis Strait. On the eastern side of the bay is the mouth of the most productive glacier in the northern hemisphere [24]. Samples were taken from the research vessel $R/V\,Porsild$ (Arctic Station, University of Copenhagen) on a permanent station (69° 15′ N, 53° 33′ W), with a depth of 300 m, located where the surface water from the bay flows out into the Davis Strait. On the first sampling event, specimens of *C. finmarchicus* were targeted; *C. glacialis* were collected on the second sampling. Sampling was timed to coincide with the break-up of sea ice and the start of the spring bloom. Tows were done with WP-2 nets (200 μm mesh size) with a non-filtering cod end from 250 m to the surface. The samples were transported to the laboratory diluted in surface water and kept at 0 °C. On arrival, females were selected for exposure experiments under ice-chilled conditions with the aid of dissecting microscopes.

2.1. *Phytoplankton culture*

Exposure experiments were carried out on copepods with and without food to evaluate variations in exposure pathways. Food was added as monocultures of the diatom species *Thalassiosira weissflogii*. The algae were cultured in 15 L plastic bags containing 0.2 μm filtered seawater, at room temperature. Growth media was added every second day in the form of B1 medium (1 $mL \cdot L^{-1}$)

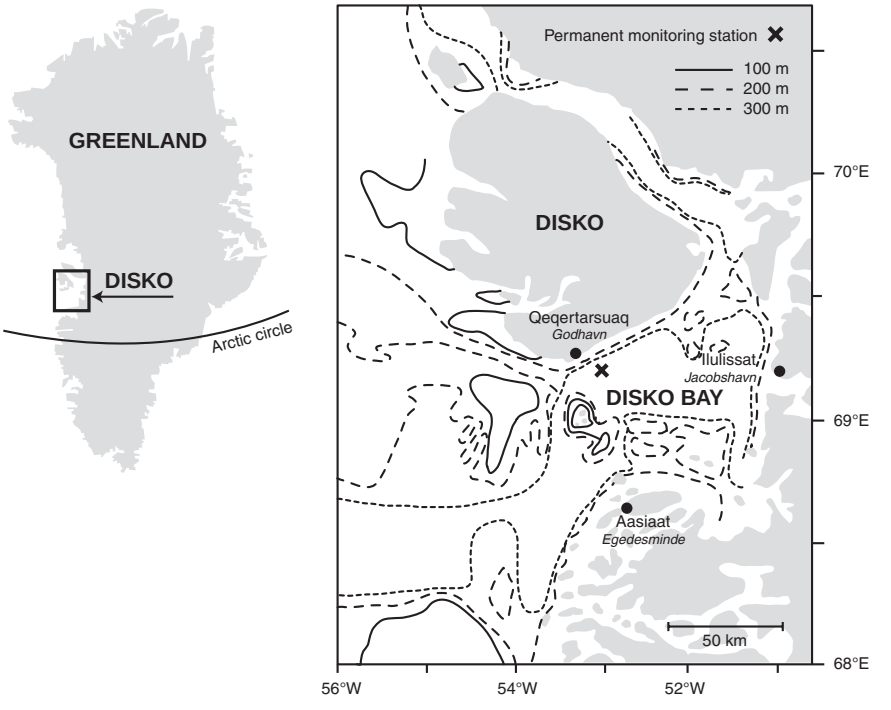

Figure 1. Location of sampling site in Disko Bay on the west coast of Greenland.

[25], silicate ($0.9\,\mathrm{mL}\cdot\mathrm{L}^{-1}$) and vitamins ($0.5\,\mathrm{mL}\cdot\mathrm{L}^{-1}$). The bags were constantly aerated and kept on a 12 h dark and 12 h light cycle with a light source (2 pcs., Osram L, 36 W/840, Lumilux Cold white) placed 40 cm from the culture. Concentrations of the culture were monitored by daily fluorescence measurements (Turner Design 700, USA).

2.2. *Exposure experiments*

Exposure experiments were carried out for both species over 96 h with pulse exposures of sucralose every 24 h. During all handling of animals, temperatures were kept at 0 °C. Four individual females were carefully transferred to plastic bottles (volume 200 mL) containing 0.2 μm filtered surface water from the sampling site. Replicate samples ($n = 3$) were made for each sucralose concentration in the range 0, 5, 50, 500, 5000 and 50,000 $\mathrm{ng}\cdot\mathrm{L}^{-1}$ prepared from a stock solution of sucralose (Sigma–Aldrich, Germany) dissolved in 0.2 μm filtered seawater. The treatment of $0\,\mathrm{ng}\cdot\mathrm{L}^{-1}$ received only filtered seawater and acted as a control treatment. After acclimatisation of the copepods for 24 h, experiments were started with additions of sucralose solution. Food was added for half the samples as cells of *T. weissflogii* in final concentrations of 15 μg chlorophyll $a\cdot\mathrm{L}^{-1}$, which corresponds to the maximum chlorophyll *a* concentration measured *in situ* during the spring bloom and thereby ensures food-saturated conditions for the copepods. Every 24 h, all bottles were filtered through a 40 μm mesh and live copepods were returned to the bottles with fresh seawater. The remaining filtrate containing faecal pellets, eggs and nauplii were conserved in lugol (final concentration of 2%) for later quantification. Faecal pellet production is a measure of food uptake by copepods, and eggs and nauplii are estimates of the total production by the copepods [26,27]. Eggs from samples taken after 48 and 96 h of sucralose exposure were not conserved, but transferred to multi-wells containing fresh filtered seawater and kept for 72 h at 5 °C to estimate hatching success.

Numbers of female copepods, eggs and faecal pellets were converted to carbon units using conversion factors estimated on specimens from Disko Bay [28]. It was therefore possible to calculate specific rates of food uptake and egg production. The rates were compared using linear regression and sucralose effects on the parameters were analysed by ANOVA followed by Dunnett's *t*-test, where sucralose treatments were compared to control treatments. All calculations were performed in SAS statistical software.

3. Results

The water column at the sampling site was characterised by a spring bloom in progress and the salinity was more or less constant throughout the water column. At the time of sampling, most of the sea ice had broken up and allowed sunlight to penetrate the water column.

Exposure to sucralose in the chosen range of concentrations (0, 5, 50, 500, 5000, 50,000 ng $\cdot$ L^{-1}) did not cause any mortality in either *C. finmarchicus* or *C. glacialis*. The production of faecal pellets by *C. finmarchicus* ranged from 23 to 38 pellets per individual after four days of exposure with addition of food. The copepods without food produced between 1 and 7 pellets per individual. The faecal pellet production by *C. glacialis* ranged from 3 to 6 pellets per individual with food and 1 to 3 pellets per individual without food. There were no significant negative effects on faecal pellet production from exposure to sucralose in any of the two investigated species.

Linear regression analyses were performed on cumulated specific faecal pellet production (SPP) to compare rates of *C. finmarchicus* and *C. glacialis* when exposed to sucralose. In the experiments without food, there were no significant effects of sucralose exposure to SPP in *C. finmarchicus*

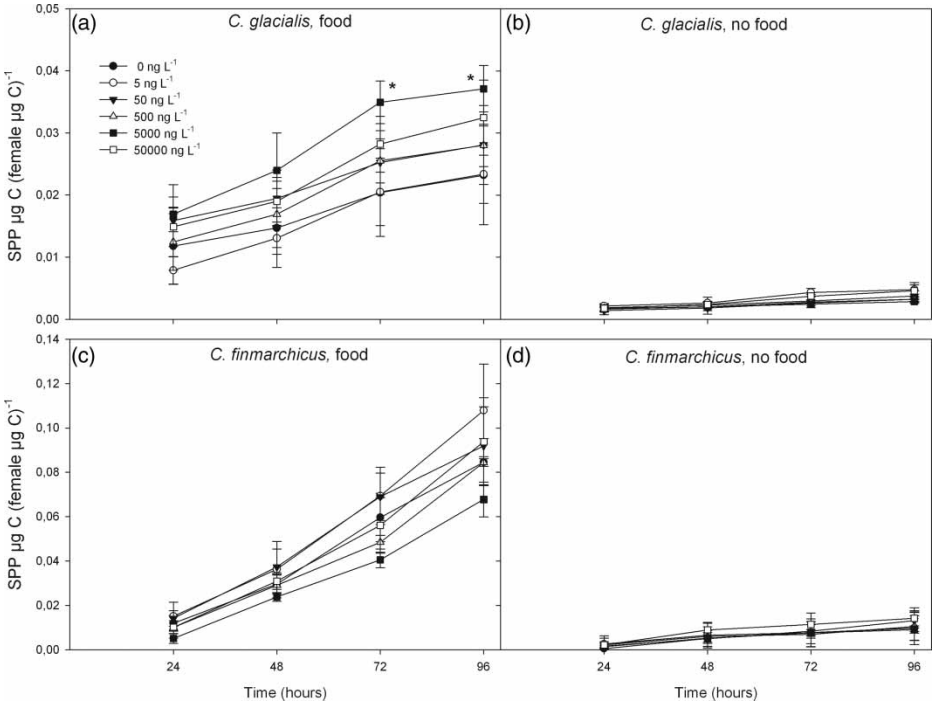

Figure 2. Cumulated specific faecal pellet production (SPP) in *Calanus glacialis* and *Calanus finmarchicus* during exposure to six concentrations of sucralose with and without food. Asterisks denote a significantly different ($p < 0.05$) development from control.

(Figure 2(d)). There was a decreasing tendency of SPP with increasing sucralose exposure in *C. glacialis*, although values were low (Figure 2(b)). By contrast, the rate of SPP increased in *C. glacialis* with food available when sucralose concentrations increased (Figure 2(a)). In all treatments, SPP were at least ten times higher among fed copepods compared to starved copepods and ranged between 0 and 0.12 μg carbon per μg adult female carbon for *C. finmarchicus* and up to 0.04 μg carbon per μg adult female carbon for *C. glacialis*. Cumulated SPP in *C. glacialis* exposed to 5000 $ng \cdot L^{-1}$ sucralose was significant higher than control after 72 h of exposure (Figure 2(a)).

After 96 h of exposure to sucralose, *C. finmarchicus* produced 3–12 eggs per individual when fed and 0–3 eggs per individual when not fed. Egg production for *C. glacialis* was 2–7 eggs per individual when fed and 1–8 eggs per individual when not fed.

Cumulated specific egg production (SEP) was not affected by sucralose in any of the two species in the absence of food (Figure 3(b),(d)). Egg production by *C. finmarchicus* was markedly higher with food, whereas egg production by *C. glacialis* was uncoupled from food availability. Specific egg production was 0–0.09 μg carbon per μg adult female carbon in *C. glacialis* and 0–0.07 μg carbon per μg adult female carbon in *C. finmarchicus*, which is comparable with other studies of the species in the region [28,29]. After exposure to sucralose concentrations of 500 and 50,000 $ng \cdot L^{-1}$ for 48 h, egg production in *C. glacialis* decreased significantly when compared with control, and exposure to 50 $ng \cdot L^{-1}$ significantly decreased egg production after 72 h (Figure 3(a)). Cumulated SEP by *C. finmarchicus* was not affected by sucralose (Figure 3(c),(d)).

Egg hatching success was monitored from eggs produced by females after 48 and 96 h exposure (Figure 4). Collected eggs were kept at 5 °C for three days and the percentage of hatched eggs was determined. There was no significant difference in percentage of eggs hatching from *C. finmarchicus* exposed to increasing sucralose concentrations, and food availability did not

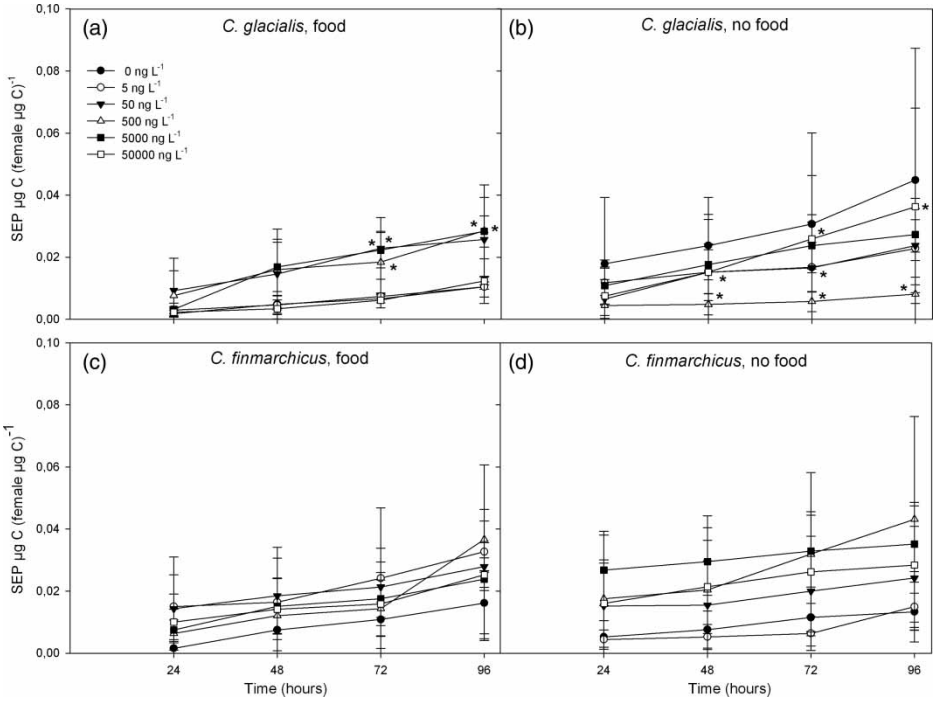

Figure 3. Cumulated specific egg production (SEP) in *Calanus glacialis* and *Calanus finmarchicus* during exposure to six concentrations of sucralose with and without food. Asterisks denote a significantly different ($p < 0.05$) development from control.

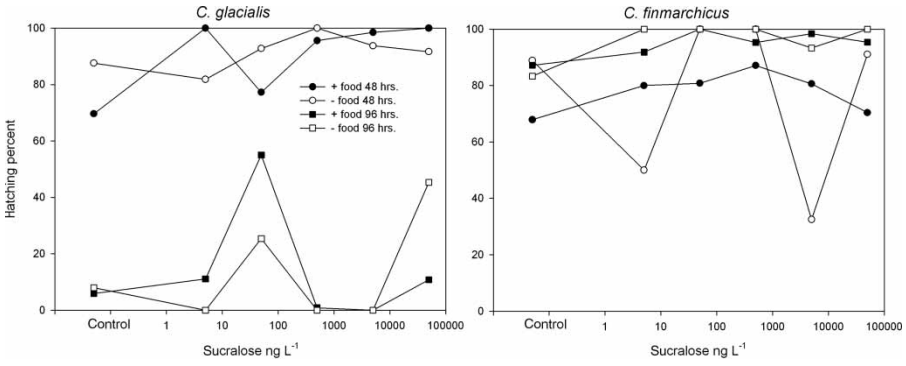

Figure 4. Hatching percent of eggs from females of *Calanus glacialis* and *Calanus finmarchicus* exposed to sucralose over 48 and 96 h with and without food.

affect hatching either. The same results were found for hatching success in *C. glacialis*, although fewer eggs hatched from females exposed to sucralose for 96 h than those exposed for 48 h.

4. Discussion

Based on the present study of two Arctic species of copepods in a 96 h exposure to a range of concentrations of sucralose, the sweetener does not seem to affect food uptake by *C. finmarchicus* negatively. The larger species, *C. glacialis*, showed a tendency towards increased food uptake with increasing exposure concentrations of sucralose. No effects were observed in individuals of either species without food access, which may suggest that the mode of uptake is predominantly via food rather than by passive uptake. Data on specific faecal pellet production are comparable with previous investigations in Disko Bay on the two species [28–30].

Egg production by *C. finmarchicus* was not affected by sucralose exposure, regardless of food availability, but access to food increased egg production in *C. finmarchicus*. Egg production is not coupled to food availability in *C. glacialis* [20], which is also reflected in the fact that the total number of eggs produced with or without food is the same for *C. glacialis*, whereas starved *C. finmarchicus* only produce 24% of the eggs produced when they have access to food. However, *C. glacialis* seemed to be more sensitive to exposure to sucralose because egg production decreased.

Some negative effects of sucralose on copepods were expected on the basis of the compound's chemical structure, which closely resembles sucrose and contains chlorine. Sucrose has been documented to be a significant chemosensory compound in crustaceans and plays a role in prey search and identification [31], and if sucralose may interfere with these sensory mechanisms, it could have negative effects on food uptake. Decreased food uptake after sucralose exposure has been the case in rat studies [3]. However, studies on other aquatic crustaceans performed as part of the same project programme as this study, revealed no negative effects. Short-term as well as long-term exposures of *Daphnia magna* Straus to sucralose up to $1\,g \cdot L^{-1}$ had no acute or chronic effects on survival or reproduction (S.V. Kholodkevich, pers. comm.). In the same study, exposure of crayfish to sucralose in concentrations of $1\,g \cdot L^{-1}$ for one week did not evoke any significant changes in their physiological state, as determined by cardiac activity characteristics. The positive effects on food uptake seen in *C. glacialis* exposed to sucralose, seems contradictory to the abovementioned findings, but it may still be a reflection of interference with the chemosensory apparatus of the organisms.

Standard toxicity tests can be performed as 96 h exposure studies on laboratory reared organisms where mortality rates are evaluated. Conditions surrounding such a test are kept under strict control

to ensure a simple and straight relationship between cause and effect and to avoid interference from variations due to natural variability in test organisms. Such tests may fail in recognising sublethal effects and/or effects on functional parameters in the tested organisms. Furthermore, indirect effects, which can cause harm to other organisms closely connected to the tested organism in the ecosystem may not be detected either. This study did investigate short-term sublethal effects on the functional parameters food uptake and egg production and it was carried out with organisms sampled from the field immediately prior to exposure, which was performed under conditions as close to ambient conditions as possible. No mortality was observed and we found only a slight negative response in egg production by *C. glacialis*, which may have implications for population growth and future grazing pressure on phytoplankton. A difference in sensitivity to contaminants between the investigated species may have consequences for competition between them. *C. finmarchicus* is the one with the smallest number of lipids [14], and its nutritional value to higher trophic levels in the Arctic is not as big as *C. glacialis*. A shift in dominance between the species due to pressure from contaminants may have ecological consequences in terms of less energy transfer. In this study, we did not find any significant detrimental effects of sucralose, but as this was only a short-term exposure there is a need to address long-term exposures.

It cannot be ruled out that further effects might be detected, if a longer exposure time was applied. There is a potential for long-term exposure to sucralose in the aquatic environment, because if sucralose containing products are consumed, inevitably the compound will be found in the aquatic environment [1,7], and more research is needed to address this issue.

5. Conclusion

The artificial sweetener sucralose is found in marine waters in areas where sucralose-containing products are consumed and the compound degrades slowly in cold, marine waters, which consequently leads to a risk of long-term exposure to marine ecosystems. This study focused on possible short-term effects on two ecologically important species of the calanoid copepods of the genus *Calanus* in Arctic areas. No mortality was observed after 96 h of exposure to concentrations in the range 0–50,000 $ng \cdot L^{-1}$. No effects were observed on any parameters in any of the species, when exposed to sucralose without food access. Food uptake was not negatively affected by sucralose exposure, and for *C. glacialis* exposure led to increased food uptake. Exposure to sucralose decreased egg production in the same species although not consistently, in contrast to no response in *C. finmarchicus*. Whether this implies a smaller sensitivity of *C. glacialis* to sucralose cannot be concluded based only on 96 h exposures. More studies are needed to investigate the long-term effects of artificial sweeteners on aquatic ecosystems.

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