Response of planktonic copepods of the White Sea to the water salinity changes in acute and chronic experiments

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In the White Sea, planktonic copepods *Calanus glacialis*, *Pseudocalanus* spp., *Triconia borealis*, and *Metridia longa* are usually not considered as tolerant to the water salinity decrease, and *Oithona similis* is generally attributed to the euryhaline species. However, the life cycle of the first two genera includes active feeding during the spring ice melting, when a large part of their food sources are concentrated in the upper water layer characterized by salinity less than 18‰. *M. longa* and *T. borealis* are considered to be deep-dwelling species. The first one reproduces in autumn and thus relies on the spring phytoplankton bloom the least, the second spawns in spring in deep water layers; however, they are both found in the productive (photic) layer during this season. In order to study if these copepods can use food-rich freshened water layers to get enough energy for growth and reproduction, a series of experiments were performed in July 2019 and March 2020, including chronic and acute ones. The copepod mortality was analyzed at 25‰ or 26‰ (control), 18‰ and 15‰ (experiment). Surprisingly, *C. glacialis* and *Pseudocalanus* spp. stood the salinity decrease with minor mortality rates ranging from 0 up to $1.1 \pm 0.2\%$ in short-term 24-h experiments. The mortality rate in these species was the highest after five days of gradual water freshening down to 15‰ ($12.1 \pm 1.8\%$), while the mortality rate was half as much in control vials at 25/26‰ and at 18‰ ($6.4 \pm 0.5\%$ and $6.9 \pm 0.6\%$, respectively). *T. borealis* was the most stenohaline species, exhibiting the 100% mortality rate already at 18‰ after 2 hours of exposure. *M. longa* did not tolerate the salinity stress at 15‰, reaching 100% mortality in 24 h. *O. similis* tolerated the salinity decrease well and thus exhibited euryhaline features as reported for this species in different areas of its range.

Keywords: salinity tolerance, epipelagic copepods, mesopelagic copepods, *Calanus*, *Pseudocalanus*, *Metridia*, *Triconia*, *Oithona*

Introduction

Salinity tolerance plays an important role in biology and life cycles of many copepod species. Generally, the fauna in the White Sea is historically adapted to lower salinity of 24–28‰ comparing to the oceanic values of 32–35‰, on average (Berger et al., 2001). Tolerant *Acartia biflosa*, *A. longiremis*, and *Oithona similis* stand wide range of salts' concentration, which promotes their populations' success in various habitats, from brackish to oceanic waters (Dutz and Christensen, 2018; Hubareva and Svetlichny, 2016; Lance, 1963); these three species are common for the White Sea. Meantime, the representatives of the group of stenohaline species are usually considered to be inhibited by low salinity. In the White Sea, there are four copepod species characterized as true marine (stenohaline): *Calanus glacialis*, *Metridia longa*, *Triconia borealis*, and *Pseudocalanus minutus* (Berger et al., 2003; Kosobokova and Pertzova, 2012; OBIS, 2020). Recently, another representative of *Pseudocalanus* genus, *P. acuspes*, was found in the White Sea (Markhaseva et al., 2012). Most of the biogeographical databases describe this one as a «true» marine (stenohaline) species (Razouls et al., 2005–2020; OBIS, 2020; WoRMS..., 2020). However, in the Baltic Sea, this copepod is able to tolerate the salinity decrease down to 7‰ (Renz and Hirche, 2006; Renz et al., 2007).

Coping with low salinity may be essential for some epipelagic neritic copepods inhabiting high latitudes, since their reproduction period is timed to the phytoplankton bloom in spring (Nicolajsen et al., 1983), which, in turn, occurs during the ice melt. One may suggest a high food concentration in the under-ice thin water layer, resulting by the release of the ice algae, on one hand (Gradinger, 2009; Hirche and Bohrer, 1987). On another hand, this food-rich layer is also freshened due to the same process of the ice melt (Runge and Ingram, 1991; Durbin and Casas, 2014). In the White Sea, this may be a limiting factor for *C. glacialis* and *Pseudocalanus* sp. species, which start to form the gonads and even to reproduce as early as in March (Prygunkova, 1974), having the highest grazing rates during that period (Conover et al., 1986), when the peak of reproduction falls to the short period lasting about a month and coinciding with the ice melt (Tourangeau and Runge, 1991). The fecundity and reproduction success in copepods usually depends on the food supply during pre-spawning and spawning periods (Daase et al., 2011; Hirche and Bohrer, 1987; Lischka and Hagen, 2005). In order to use as much of the food sources as possible, epipelagic species inhabiting the ice-covered seas may evolve the features helping them tolerate short-term exposure to the freshened water.

Our goal was to test the ability of the White Sea copepods characterized by different ecology to stand the osmotic stress.

Materials and methods

The sampling was performed in the Chupa Inlet, Kandalaksha Bay, the White Sea (N 66°19'50", E 33°40'06") in July 2019 (from vessel) and March 2020 (from ice). The water temperature and salinity profiles at the sampling site were obtained by *MIDAS* CTD + multiparameter profiler (Valeport Ltd., UK), the chlorophyll *a* profile, by *Cyclops-6K* submersible sensor (Turner Designs, USA) before the zooplankton sampling. The profiles were downloaded immediately on PC for tracking the vertical profile of the main parameters using the C-soft and Valeport Terminal X2 programs. Then the zooplankton samples were obtained by standard Juday plankton net (opening diameter of 36 cm, 100-µm mesh) at the standard layer of 0–10 m, 10–25 m, and 25–50 m; these samples were checked quickly for the rough estimation of the most abundant species. These data were referred to the water profiles. The seawater was taken from the appropriate depth by Niskin water bottles and poured with care in order to avoid stress for phyto- and microzooplankton (the food source for copepod) into the 2.0-L Pyrex glass bottles (1.8 L of seawater in each). The live mesozooplankton (e.g. copepod) samples were taken only after all the preparations were performed. The live copepod samples were obtained by standard Juday plankton net (opening diameter of 36 cm, 100-µm mesh) at the appropriate water layer (see above) and poured with the maximal precaution into the pre-filled Pyrex glass bottles, so the animals had enough water volume to avoid oxygen stress until they have got to the laboratory. The bottles were kept at dark and cool place in order to avoid heat stress (Rahlff et al., 2017) and light stress (Ludvigsen et al., 2018) all the way to laboratory.

The experiments were aimed on studying the salinity stress, so all the other stress factors were minimized. This was accomplished by the precautions taken during the sampling and transporting to the laboratory, as well as at the stage of experiment launching. The live samples were handled with the most care in the cold laboratory (0–4 °C) at 1% photosynthetically active radiation (PAR). The freshly taken samples were diluted by 5 times with the seawater of the same temperature and obtained from the same water layer immediately after reaching the laboratory. They were left for 24 hours in order to level down the capture stress, then all the dead copepods (if any) were removed cautiously from the bottom with the pipette. The primary (native) sample in the bottle was then used for the experiment as a whole, without primary sorting the copepods by species and stages in order to avoid the light stress and capture stress again. The primary sample was divided into 150-mL aliquots and carefully placed into the 2.0-L Pyrex glass bottles filled with 400 mL of natural seawater.

The experiments were performed at 2 °C (Arctic species *C. glacialis*, *M. longa*, *T. borealis* were considered) and 4 °C (eurybiont and Arctic-boreal species: *O. similis*, *Pseudocalanus* spp.) in July 2019 and at 0 °C in March 2020 (*C. glacialis*, *M. longa*), and 1% PAR (twilight zone). The temperature in the experiment was set in accordance to the water temperature of the layer where the peak of the studied species was observed at the sampling date. Two seasons were chosen to search for possible differences in osmotic stress tolerance related to the copepod developmental stage. The copepod mortality was analyzed at 25.0‰ (control, July 2019) and 26.0‰ (March 2020), 18.0‰ and 15.0‰ (experiment, both seasons). According to preliminary data (Smurov et al., unpubl.), in most species of the studied invertebrates so far in the White Sea, the lower limit of salinity tolerance is about 18‰, a serious inhibition of vital functions is observed at 15‰.

Three different experimental schemes were applied in July 2019: (no. 1) acute dilution from 25‰ down to 18/15‰ at 0 h, checking at 0 h, 1 h, 3 h, 6 h, 12 h, and 24 h; (no. 2) gradual dilution from 25‰ down to 18/15‰ at 0 h, 1 h, 3 h, 6 h, and 12 h (checking at 0 h, 1 h, 3 h, 6 h, 12 h, and 24 h); and (no. 3) gradual dilution from 25‰ down to 18/15‰ during five days, checking every 24 hours (Tables 1, 2).

In March 2020, due to small amount of live material, one chronic experiment (no. 3) was performed with some modifications, when the dilution down to 15‰ and 18‰ was applied at the begin-

ning of the experiment, and the observations were performed for 5 days. In all the experiments, a freshly distilled water (0‰) of the same temperature was used for diluting. In order to follow the stress of “mechanical dilution” and to avoid the additional negative factor of restricted volume, similar volume of the seawater was added to the control vials, so the copepods had the same certain water volume in each bottle at each stage of experiment. Each salinity factor was tested in three replicates per observation; for example, the total number of vials used in experiment no. 3 was $45 = 3$ (factor of salinity) $\times 5$ (days, checking daily) $\times 3$ (replicates).

The checking procedure was organized in two steps. Firstly, the excess water was gently removed from the vial using the silicon hose, the tip placed into the vial was closed by 100-μm mesh in order to avoid losing the copepods with the water flow. After the initial volume was decreased down to about 100 mL, the residual sample was checked under the stereomicroscope. The second step was to count the copepods in regard to the species and stages in a round plate with the bottom area of 9.4 cm² with grid crossing lines every 0.5 cm. The animals were checked for five random squares 0.5 $\times$ 0.5 cm, then their number was averaged and recalculated for the total area. This procedure was applied for *Pseudocalanus* spp., *T. borealis*, and *O. similis*. Large copepod species (*C. glacialis* and *M. longa*) were checked totally. At this step, the copepod species, developmental stage, and

Table 1. Dilution scheme in the acute experiments. Values in italics indicate the water salinity (‰) in the experimental vials at given time.

Treatment	Time, h					
	0	1	3	6	12	24
Acute no. 1						
15‰	15	15	15	15	15	15
18‰	18	18	18	18	18	18
Acute no. 2						
15‰	23	21	19	17	15	15
18‰	24	22.5	21	19.5	18	18

Table 2. Dilution scheme in the chronic experiment. Values in italics indicate the water salinity (‰) in the experimental vials at given time.

Treatment	Time, h					
	0	24	48	72	96	120
Chronic, no. 3						
15‰	23	21	19	17	15	15
18‰	24	22.5	21	19.5	18	18

status (dead, alive, stressed) was checked. Dead animals were sunk and not moving, even if touched by pipette. Alive animals were actively swimming and avoiding the water inflow to the pipette. Stressed animals were either moving too slowly in comparison to the other copepods of the same species / stage or were laying on the bottom with antennas folded along the body, but were moving if touched by pipette. The number of specimens of particular species / stage per vial considered for analysis was not less than 10 ind. for large copepod species *C. glacialis* and *M. longa*, and exceeded 30 ind. for small species (*Pseudocalanus* spp., *T. borealis*, and *O. similis*).

The differences between the control and experimental groups of each species were tested by Student *t*-test, the significance level was set as $p = 0.05$. The values are presented as mean $\pm$ standard deviation ($M \pm SD$).

Commonly accepted abbreviations of copepodite developmental stages are used in the text: CI – CV are the immature copepodite stages I–V, CF are females, CM, males.

Results

Hydrological conditions in July 2019 and March 2020

In July 2019 and March 2020, the water temperature and salinity profiles at the sampling site (Fig. 1) were usual for the season (Usov et al., 2013). Generally, water salinity varied as 25.0–27.8‰ in July 2019, and in March 2020, most of the water column referred to the range of 26.0–27.1‰, except the very upper surface layer of about 1-m thickness, where it dropped down to 4.9‰, gradually increasing along this layer up to normal values. The chlorophyll *a* concentration in this particular layer in March 2020 reached very high values exceeding 9 µg/L, but this parameter did not exceed 0.5 µg/L in the deeper layers (Fig. 1 C). Thus, there was obvious food-rich layer confined exactly to the freshened water layer, and the deeper layers were characterized by a low food concentration. In July 2019, the highest concentration of Chl *a* (4.3 µg/L) was observed in 0–15-m water layer, which was usual pattern for summer (Usov et al., 2013).

Copepod response to the water salinity change in the experiment

Pseudocalanus spp.

The experiments were performed in July 2019 with CIII – CV copepodites very abundant in plankton community (552–1644 ind. per vial). The data on CIII, CIV, and CV in July have been pooled for the analysis since the ratio of these stages in the vials was nearly the same ($31.4 \pm 2.4\%$, $34.7 \pm 1.1\%$, and $33.4 \pm 2.2\%$, respectively).

In acute experiment no. 1, 70.1% of animals were deeply stressed after the first hour of acute exposure to 15‰. However, most of them have returned to the “alive” status in the next two hours (77.8%), and after 6 hours, all of survived were moving normally. The mortality rate was low (2.2%) but significant at 15‰ after the first hour of exposure; in 6-h and 24-h periods, the mortality rate of less than 2% were observed in both freshened treatments (Fig. 2 A). Interestingly, no dead animals have been found after 3-h and 12-h periods. In acute experiment no. 2, zero mortality was observed for all the stages in all the treatments through the experiment.

The most pronounced dynamics of mortality rate was tracked in chronic experiment no. 3. Gradual and slow dilution of rate of about 2‰ per day, comparing to rapid dilution of 2‰ per hour (acute experiment no. 2), resulted as zero mortality for the first 72 hours of experiment (Fig. 2 B). After the water salinity has reached 19‰ (15‰ treatment) and 21‰ (18‰ treatment), the mortality rate of 2–3% was observed in both treatments after 24 hours of exposure to these salinities. As the salinity decreased, the mortality rate increased, reaching about 4% at 17‰/19.5‰ (day 3 to day 4) and exceeding 14% as the salinity dropped down to 15‰ (day 4 to day 5). Regard must be paid to the mortality rates in the control vials at day 4 and day 5, which was similar to that in 18‰ treatment at the same days, averaging $3.8 \pm 0.1\%$ and $6.9 \pm 0.6\%$ (18‰, day 4 and day 5) and $2.3 \pm 0.3\%$ and $6.4 \pm 0.5\%$ (control, day 4 and day 5).

In March 2020, this copepod species was represented at the sampling site by a low number of CV and CF insufficient for statistics.

Calanus glacialis

In July 2019, CIII copepodites comprised most of *C. glacialis* population. The number of copepodites per vial ranged from 66 up to 143. In acute experiment no. 1, 29.8% of animals were deeply stressed after the first hour of acute exposure to 15‰. However, most of them have returned to the “alive” status in the next two hours (97.3%), and after 6 hours, all of them were moving normally. Zero mortality was observed for 6 hours in all the treatments (both control and experimental vials), but after 12 h/24 h the pattern changed abruptly, when up to 23.5% of CIII were dead at 15‰, and 4.0%, at 18‰ (Fig. 3 A). In acute experiment no. 2, the survival rate was 100% through the whole experiment, i.e., gradual decrease of salinity down to 15‰ during 12 hours did not affect this stage at all. In chronic experiment no. 3, the mortality rate after 5 days did not exceed 6.6% at 18‰ and 7.6% at 15‰, this parameter was as low as 1.1% in control (Fig. 3 B).

In March 2020, only females of this species were considered in the chronic experiment no. 3 with modifications (13–18 animals per vial),

Fig. 1. Water temperature (A), water salinity (B), and chlorophyll a concentration (C) in July 2019 (solid line) and March 2020 (dashed line) at the study site.

Fig. 2. Mortality rate of *Pseudocalanus* spp. in acute experiment no. 1 (A) and chronic experiment no. 3 (B) in July 2019. Asterisk (*) indicates significant differences (Student *t*-test, *p* < 0.05).

since CV copepodites were rare, and other stages were either absent (CI – CIII) in the plankton community or represented by single specimens (CIV and CM). The studied cohort (CF) tolerated well the acute decrease in the 18‰ treatment: no dead specimens were found after 48 h of exposure for this salinity, and the mortality rate was 42.9% after 72 h. At 15‰, the mortality rate was $44.4 \pm 11.0\%$ and $57.1 \pm 17.1\%$ after 24 h and 48 h, respectively; after 3 days of experiment, the females were all dead.

Metridia longa

In July 2019, there were single specimens in the samples, so they were not considered for the experiment. In March 2020, this copepod species was represented by a wide range of different developmental stages, from copepodites II (CII) to females (CF), there were 14 to 30 animals of each stage per vial (chronic experiment no. 3 with modification). The youngest copepodites (CII) were the most vulnerable to the salinity decrease down to 15‰, their mortality rate was 100% after 24 hours of exposure (Fig. 4 A). Surprisingly, the elder copepodites (CIII, CIV, and CV) and females were more tolerant to osmotic stress, their mortality rate after the first day of exposure to the same salinity averaged $65.5 \pm 11.9\%$ and ranged from 50.0% (females) up to 83.3% (CIV), never reaching 100% for this period. The other surprise was zero mortality of all the stages at 18‰ for the first 24 h of exposure. After 48 hours of exposure to 18‰, CV and CF were all alive, but the mortality in younger copepodites (CII – CIV) was clearly expressed (Fig. 4 B). The 100% mortality was observed after 72 h of exposure to 18‰ in all the stages.

Triconia borealis

Only immature copepodite stages have been considered in July 2019. These stages were nearly absent in March 2020; the number of CF and CM in the samples both in July 2019 and in March 2020 was also insufficient for statistics. There were from 32 up to 67 immature copepodites in each vial (July 2019). All the specimens were dead (100% mortality) in acute experiment no. 1 after two hours of exposure both to 15‰ and 18‰. In chronic experiment no. 3 and acute experiment no. 2, all the animals were stressed after the gradual decrease of the water salinity brought to the values less than 20‰; the 100% mortality was registered as the water salinity has decreased down to 18‰ and lower.

Oithona similis

The experiments were performed in July 2019 with immature copepodite stages; there were a few of them in March 2020. The number of immature copepodites per vial ranged 40–171 ind. (July 2019). In all the performed experiments (three schemes applied), the survival rate of *O. similis* was 100%, except single specimens dead after five days of gradual decrease down to 15‰ in July 2019 (< 1% mortality).

Discussion

Salinity tolerance was described for many marine copepod species of genera *Acartia* (Cervetto et al., 1999; Dutz and Christensen, 2018; Lance, 1963), *Tigriopus* (Damgaard and Davenport, 1994), *Eurytemora* (Karlsson et al., 2018; Lee and Petersen, 2002), and *Oithona* (Hansen et al., 2004) inhabiting the estuarine or coastal biotopes. However, there is a scarce data on the species that are usually not attributed to the areas characterized by low salinity. Our study aims at searching for the particular response (mortality rate) of certain marine copepod species received minor attention so far in regard to the specific and short-term environmental change (osmotic stress). The threshold of 15‰ appears to be critical for the marine mesopelagic copepods, which is a higher value comparing to 12‰ reported as a critical level for many marine species (Kinne, 1964). On the other hand, a salinity range of 15–32‰ forms a natural border in the Baltic Sea for distribution of many marine fauna representatives (Feistel et al., 2008). Generally, our data are also congruent with those obtained for other invertebrates of the White Sea adapted to life at lower salinity of 25–28‰ and exposed to the salinity of 15‰ and 18‰ (Smurov et al., unpublished: pers. comm.).

Salinity tolerance may play an important role in copepod species biology. The phenomenon of a salinity tolerance to a wide range of salts' concentration is well-known for *Oithona similis*. The response of this species to desalination within our study perfectly fits its euryhaline characteristics (OBIS, 2020). In the White Sea, there is another evidence of its wide range of salinity tolerance (Dvoretiskii and Dvoretiskii, 2011), supported by a number of studies performed in other areas of its geographical range (Fransz, et al., 1991; Hansen et al., 2004; Hubareva and Svetlichny, 2016). Ecologically, euryhaline crustacean species have more advantage comparing to stenohaline species of the same genera, when expanding their geographical ranges (Marie et al., 2017; Svetlichny and Hubareva, 2014), so high tolerance to osmotic stress is biologically feasible for the population success in some cases.

The copepods of genera *Calanus* and *Pseudocalanus* reproduce during the period when a significant amount of food (such as ice algae) may be unavailable due to its accumulation in the under-ice freshened layer (Fig. 1); however, they ultimately need to utilize this food source. There are still two ways to reach it for the copepods if one assumes their tolerance to short-term salinity decrease. The first one is a "targeted migration", when the copepods migrate actively to this layer to feed despite the salinity gradient. Another way is that they occur in this water layer by chance, when performing the "jumps" that may cover up to 0.5 m in *Calanus* (Kjørboe et al., 2010). *Metridia* moves more evenly

Fig. 3. Mortality rates of *Calanus glacialis* CIII in July 2019 in acute experiment no. 1 (A) and chronic experiment no. 3 (B). Asterisk (*) indicates significant differences (Student *t*-test, $p < 0.05$).

Fig. 4. Mortality rates of different stages (CII – CF) of *Metridia longa* in modified chronic experiment no. 3 in March 2020 at 15‰ (A) and 18‰ (B).

even at mechanical disturbance (pers. data), so this species may perceive the salinity gradient long before it reaches critical level and thus avoids it more successfully than the species performing quick jumps. In other words, *Metridia* has no need to develop salinity tolerance mechanisms since it may easily avoid the water layer of unfavorable salt concentration. The last is also supported by the species biology, since most *Metridia* species tend to occupy greater depths even during the spring phytoplankton bloom (Darnis and Fortier, 2014) and lead the life in mesopelagic (Padmavati et al., 2004). *Triconia borealis* is another species that does not stand the salinity decrease (Auel and Hagen, 2002); this species is also confined to mesopelagic in the White Sea (Usov et al., 2013). Looking back to the difference between the responses of epipelagic (*Calanus* and *Pseudocalanus*) and mesopelagic (*Metridia* and *Triconia*) species to salinity decrease, one will find obvious pattern, when the last group is more sensitive to such changes than the first one.

Searching for the biochemical and genetic nature of such salinity tolerance observed in “true marine” species lies beyond the scope of the present study. However, one should consider a period of the White Sea evolution lasting for about 6,000 years. This means at least 6,000 generations for the species characterized by one-year life cycle, which is enough for developing genetic grounds (Barrera-Morena et al., 2015; Peijnenburg and Goetze, 2013) for living at the lower salinity than in the donor region, the Barents Sea, known by oceanic salinity range (Lind et al., 2016; Smedsrud et al., 2010). It is supposed that the modern White Sea was initially formed as a two-layered basin (Naumov, 2006), which gives us two completely different ways of developing the zooplankton species composition in this sea. Apparently, mesopelagic species (*Metridia longa* and *Triconia borealis*) could be initially brought by the Deryugin Current predecessor, which promoted the income of some Arctic fauna from the Barents Sea (Naumov, 2006) and thus primarily occupied the cold waters at a greater depth, which, in turn, could slow developing of genetic adaptations for life at lower salinity. There is also a question now if not only the White Sea epipelagic marine copepods may cope with salinity decrease successfully, but the other species of these genera in the ice-covered seas.

Regard must be paid to rapid acclimation of copepods to the dramatic salinity decrease in acute experiments, for example, as observed for *Pseudocalanus* spp., when it took two hours for most of the copepods to recover. This period corresponds well to that observed in different groups of crustaceans exposed to acute osmotic stress. In particular, a significant elevation in heat-shock protein (HSP) mRNA expression is reported after 0.5 hour of osmotic stress in the American

lobster *Homarus americanus* (Chang, 2005) and 1-h exposure to low salinity in the Pacific white shrimp *Litopenaeus vannamei* (Huang et al., 2018). The highest expression rate of the stress proteins is found after 3–6-h period in the mud crab *Scylla paramamosain* exposed to low salinity (Huang et al., 2019). The large amount of differential gene expression is observed in shrimps *Palaemon longirostris* and *P. macrodactylus* under osmotic stress (Marie et al., 2017); unfortunately, the authors have not included the data on the exposure period. Nevertheless, a certain tolerance to the salinity decrease supported by rapid transcriptomic response seems to be a feature of many crustaceans.

Another issue arising since two sibling *Pseudocalanus* species were identified in the White Sea (and for the study site, in particular), is the absence of reliable criteria for separating these two species at immature stages (Markhaseva et al., 2012). This, in fact, opens two possible ways for discussing the results on salinity tolerance obtained for *Pseudocalanus* spp. in this study. The first explanation is that *P. minutus* does not stand the freshening, so all the dead specimens within the *Pseudocalanus* spp. pool belong to this species, and thus the mortality rates differ for the two species. It seems to be very attractive conclusion, however, no data still exist on the species ratio at the study site. The second way to explain our data is that both species have evolved historically a certain tolerance for low salinity, since both species are epipelagic and inhabiting a large area of coastal waters in the White Sea, which is a coastal sea itself (Berger et al., 2001). The last suggestion is supported by different ranges of salinity tolerance rates found for the estuarine, coastal, and marine species of phytoplankton (Brand, 1984). Particularly, the salinity tolerances of estuarine and oceanic species were appropriate for their environments, but most of the coastal species could tolerate much lower salinity than their ambient range (Brand, 1984). We suggest that epipelagic copepods may also have a certain degree of “cryptic” salinity tolerance, which may evolve quite rapidly when it becomes necessary for the species success. Rapid selection under chronic osmotic stress was found in the intertidal copepod *Tigriopus californicus* already after five generations exposed to high / low salinity (Kelly et al., 2016). In fish, net diversification rates were higher in freshwater than marine environments (Bloom et al., 2013). Finally, a significant genotype-by-environment interaction for development time was reported for copepod *Eurytemora affinis* inhabiting brackish to hypersaline environments (Lee and Petersen, 2002). These examples together with our results may serve as a starting point for the subsequent studies of the genetic nature of salinity tolerance in the copepods of different ecological groups inhabiting the same realm.

The scarcity of data on the salinity tolerance in different developmental (copepodite) stages of the same species, as it is observed for *M. longa* in our study, also requires attention. Up to date, the studies are focused either on copepod embryos (Charmantier and Charmantier-Daures, 2001) or adult stages (Calliari et al., 2019; Dutz and Christensen, 2018) with the only exception for *Eurytemora affinis* from the Baltic Sea (Karlsson et al., 2018). Different response to the osmotic stress in ontogeny is reported for isopods (Charmantier and Charmantier-Daures, 1994), shrimps (Torres et al., 2011; Vázquez et al., 2016), and crabs (Charmantier et al., 1998; de Jesus de Brito Smith et al., 2012). It is obvious that this problem needs a complex approach in regard to Copepoda as applied so far for other crustaceans in order to assess possible adaptations through the species lifespan.

Conclusions

Generally, our study considers the species life cycle strategy to use the available resources with the highest efficiency, especially when the food maximum is limited in time and is indispensable for the population success. Epipelagic marine copepod species, relying on the seasonal peak of food, such as *C. glacialis* and *Pseudocalanus* spp. (Conover et al., 1986; Durbin and Casas, 2014), seem to tolerate short- and even long-term significant decrease of salinity quite successfully. The species attributed to mesopelagic, such as *T. borealis* and *M. longa*, which life cycles are not tightened to spring / summer phytoplankton blooms followed by microplankton abundance peak (Kosobokova and Pertsova, 2012; Padmavati et al., 2004), exhibit the least tolerance to the salinity decrease.

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Compliance with ethical standards

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References

- Auel, H., Hagen, W., 2002. Mesozooplankton community structure, abundance and biomass in the central Arctic Ocean. *Marine Biology* **140**, 1013–1021. <https://doi.org/10.1007/s00227-001-0775-4>
- Barrera-Moreno, O.A., Ciro-Pérez, J., Ortega-Maya-goitia, E., Alcántara-Rodríguez, J.A., Piedra-Ibarra, E., 2015. From local adaptation to ecological speciation in copepod populations from neighboring lakes. *PloS One* **10** (4), e0125524. <https://doi.org/10.1371/journal.pone.0125524>
- Berger, V., Dahle, S., Galaktionov, K., Kosobokova, X., Naumov, A., Rat'kova, T., Savinov, V., Savinova, T., 2001. White Sea. Ecology and Environment. Derzhavets Publisher, St-Petersburg – Tromso, Russia – Norway, 157 p.
- Berger, V., Naumov, A., Zubaha, M., Usov, N., Smolyar, I., Tatusko, R., Levitus, S., 2003. 36-Year Time Series (1963–1998) of Zooplankton, Temperature and Salinity in the White Sea. *International Ocean Atlas and Information Series. V. 7*. St. Petersburg – Washington, Silver Springs, 362 p.
- Bloom, D., Weir, J., Piller, K., Lovejoy, N., 2013. Do freshwater fishes diversify faster than marine fishes? A test using state-dependent diversification analyses and molecular phylogenetics of New World silversides (Atherinopsidae). *Evolution* **67** (7), 2040–2057. <https://doi.org/10.1111/evo.12074>
- Brand, L., 1984. The salinity tolerance of forty-six marine phytoplankton isolates. *Estuarine, Coastal and Shelf Science* **18** (5), 543–556. [https://doi.org/10.1016/0272-7714\(84\)90089-1](https://doi.org/10.1016/0272-7714(84)90089-1)
- Calliari, D.L., Espinosa, N., Martínez, M., Rodríguez-Graña, L.M., 2019. Salinity regulation of copepod egg production in a large microtidal estuary. *Brazilian Journal of Oceanography* **67**, e19267. Epub October 03, 2019. <https://dx.doi.org/10.1590/s1679-87592019026706708>
- Cervetto, G., Gaudy, R., Pagano, M., 1999. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). *Journal of Experimental Marine Biology and Ecology* **239** (1), 33–45. [https://doi.org/10.1016/S0022-0981\(99\)00023-4](https://doi.org/10.1016/S0022-0981(99)00023-4)
- Chang, E.S., 2005. Stressed-out lobsters: Crustacean hyperglycemic hormone and stress proteins. *Integrative and Comparative Biology* **45** (1), 43–50. <https://doi.org/10.1093/icb/45.1.43>

- Charmantier, G., Charmantier-Daures, M., 1994. Ontogeny of osmoregulation and salinity tolerance in the isopod crustacean *Sphaeroma serratum*. *Marine Ecology Progress Series* **114**, 93–102. <https://www.int-res.com/articles/meps/114/m114p093.pdf>
- Charmantier, G., Charmantier-Daures, M., 2001. Ontogeny of osmoregulation in crustaceans: the embryonic phase. *American Zoologist* **41** (5), 1078–1089. <https://doi.org/10.1093/icb/41.5.1078>
- Charmantier, G., Charmantier-Daures, M., Anger, K., 1998. Ontogeny of osmoregulation in the grapsid crab *Armases miersii* (Crustacea, Decapoda). *Marine Ecology Progress Series* **164**, 285–292.
- Conover, R.J., Herman, A.W., Prinsenberg, S.J., Harris L.R., 1986. Distribution of and feeding by the copepod *Pseudocalanus* under fast ice during the Arctic spring. *Science* **232** (4755), 1245–1247. <https://doi.org/10.1126/science.232.4755.1245>
- Daase, M., Søreide, J., Martynova, D., 2011. Effects of food quality and food concentration on naupliar development of *Calanus glacialis* at sub-zero temperatures. *Marine Ecology Progress Series* **429**, 111–124. <https://doi.org/10.3354/meps09075>
- Damgaard, R.M., Davenport, J., 1994. Salinity tolerance, salinity preference and temperature tolerance in the high-shore harpacticoid copepod *Tigriopus brevicornis*. *Marine Biology* **118**, 443–449. <https://doi.org/10.1007/BF00350301>
- Darnis, G., Fortier, L., 2014. Temperature, food and the seasonal vertical migration of key arctic copepods in the thermally stratified Amundsen Gulf (Beaufort Sea, Arctic Ocean). *Journal of Plankton Research* **36** (4), 1092–1108. <https://doi.org/10.1093/plankt/fbu035>
- Durbin, E.G., Casas, M.C., 2014. Early reproduction by *Calanus glacialis* in the Northern Bering Sea: the role of ice algae as revealed by molecular analysis. *Journal of Plankton Research* **36** (2), 523–541. <https://doi.org/10.1093/plankt/fbt121>
- Dutz, J., Christensen, A.M., 2018. Broad plasticity in the salinity tolerance of a marine copepod species, *Acartia longiremis*, in the Baltic Sea. *Journal of Plankton Research* **40** (3), 342–355. <https://doi.org/10.1093/plankt/fby013>
- Dvoretskii, V.G., Dvoretskii, A.G., 2011. Biologiya i rol' *Oithona similis* v zooplanktone arkticheskikh morey [Biology and role of *Oithona similis* in zooplankton of the Arctic seas]. Kola Scientific Center, Russian Academy of Sciences, Apatity, Russia, 349 p. (In Russian).
- Feistel, R., Feistel, S., Nausch, G., Szaron, J., Łysiak-Pastuszek, E., Ertebjerg, G., 2008. BALTIC: Monthly Time Series 1900–2005. In: Feistel, R., Nausch, G., Wasmund, N. (eds.), *State and Evolution of the Baltic Sea, 1952–2005: A Detailed 50-Year Survey of Meteorology and Climate, Physics, Chemistry, Biology, and Marine Environment*. John Wiley & Sons, Inc., Hoboken, USA, 311–336. <https://doi.org/10.1002/9780470283134.ch11>
- Franz, H.G., Colebrook, J.M., Gamble, J.C., Krause, M., 1991. The zooplankton of the North Sea. *Netherlands Journal of Sea Research* **28** (1–2), 1–52. [https://doi.org/10.1016/0077-7579\(91\)90003-J](https://doi.org/10.1016/0077-7579(91)90003-J)
- Gradinger, R., 2009. Sea-ice algae: Major contributors to primary production and algal biomass in the Chukchi and Beaufort Seas during May / June 2002. *Deep Sea Research Part II: Topical Studies in Oceanography* **56** (17), 1201–1212. <https://doi.org/10.1016/j.dsr2.2008.10.016>
- Hansen, F., Möllmann, C., Schütz U., Hinrichsen, H.-H., 2004. Spatio-temporal distribution of *Oithona similis* in the Bornholm Basin (Central Baltic Sea). *Journal of Plankton Research* **26** (6), 659–668. <https://doi.org/10.1093/plankt/fbh061>
- Hirche, H.-J., Bohrer, R.N., 1987. Reproduction of the Arctic copepod *Calanus glacialis* in Fram Strait. *Marine Biology* **94**, 11–17. <https://doi.org/10.1007/BF00392894>
- Huang, W., Li, H., Cheng, C., Ren, C., Chen, T., Jiang, X., Cheng, K., Luo, P., Hu, C., 2018. Analysis of the transcriptome data in *Litopenaeus vannamei* reveals the immune basis and predicts the hub regulation-genes in response to high-pH stress. *PLOS One* **13** (12): e0207771. <https://doi.org/10.1371/journal.pone.0207771>
- Huang, H., Huang, C., Guo, L., Zeng, C., Ye, H., 2019. Profiles of calreticulin and Ca²⁺ concentration under low temperature and salinity stress in the mud crab, *Scylla paramamosain*. *PLOS One* **14** (7): e0220405. <https://doi.org/10.1371/journal.pone.0220405>
- Hubareva, E., Svetlichny, L., 2016. Copepods *Oithona similis* and *Oithona davisae*: Two strategies of ecological–physiological adaptation in the Black Sea. *Oceanology* **56**, 241–247. <https://doi.org/10.1134/S0001437016020089>

- de Jesus de Brito Smith, D., de Souza, A.S., Maciel, C.R., Abrunhosa, F.A., Diele, K., 2012. Influence of salinity on the larval development of the fiddler crab *Uca vocator* (Ocypodidae) as an indicator of ontogenetic migration towards offshore waters. *Helgoland Marine Research* **66**, 77–85. <https://doi.org/10.1007/s10152-011-0249-0>
- Karlsson, K., Puiaç, S., Winder, M., 2018. Life-history responses to changing temperature and salinity of the Baltic Sea copepod *Eurytemora affinis*. *Marine Biology* **165** (2), 30. <https://doi.org/10.1007/s00227-017-3279-6>
- Kelly, W.M., DeBiasse, M.B., Villela, V.A., Roberts, H.L., Cecola, C.F., 2016. Adaptation to climate change: trade-offs among responses to multiple stressors in an intertidal crustacean. *Evolutionary Applications* **9** (9), 1147–1155. <https://doi.org/10.1111/eva.12394>
- Kinne, O., 1964. Non-genetic adaptation to temperature and salinity. *Helgoländer wissenschaftliche Meeresuntersuchungen* **9**, 433–458. <https://doi.org/10.1007/BF01610056>
- Kjørboe, T., Andersen, A., Langlois, V., Jakobsen, H., 2010. Unsteady motion: escape jumps in planktonic copepods, their kinematics and energetics. *Journal of the Royal Society Interface* **7** (52), 1591–1602. <https://doi.org/10.1098/rsif.2010.0176>
- Kosobokova, K.N., Pertsova, N.M., 2012. Zooplankton Belogo morya: structura, dinamika i ekologiya soobshchestv [Zooplankton of the White Sea: structure, dynamics and ecology of communities]. In: Lisitzyn, A.P. (ed.), *Sistema Belogo morya. T. II. Vodnaya tolshcha i vzaimodeistviyushchie s nei atmosfera, kriosfera, rechnoi stok i biosfera [System of the White Sea. Vol. II. Water column and interacting atmosphere, cryosphere, river flow, and biosphere]*. Nauchnyi Mir, Moscow, Russia, 640–674. (In Russian).
- Lance, J., 1963. The salinity tolerance of some estuarine planktonic copepods. *Limnology and Oceanography* **8** (4), 440–449. <https://doi.org/10.4319/lo.1963.8.4.0440>
- Lee, C., Petersen, H.C., 2002. Genotype-by-environment interaction for salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology* **75** (4), 335–344. <https://doi.org/10.1086/343138>
- Lind, S., Ingvaldsen, R.B., Furevik, T., 2016. Arctic layer salinity controls heat loss from deep Atlantic layer in seasonally ice-covered areas of the Barents Sea. *Geophysical Research Letters* **43**, 5233–5242. <https://doi.org/10.1002/2016GL068421>
- Lischka, S., Hagen, W., 2005. Life histories of the copepods *Pseudocalanus minutus*, *P. acuspes* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard). *Polar Biology* **28**, 910–921. <https://doi.org/10.1007/s00300-005-0017-1>
- Ludvigsen, M., Berge, J., Geoffroy, M., Cohen, J.H., De La Torre, P.R., Nornes, S.M., Singh, H., Sørensen, A.J., Daase, M., Johnsen, G., 2018. Use of an autonomous surface vehicle reveals small-scale diel vertical migrations of zooplankton and susceptibility to light pollution under low solar irradiance. *Science Advances* **4** (1), eaap9887. <https://doi.org/10.1126/sciadv.aap9887>
- Marie, A.D., Smith, S., Green, A.J., Rico, C., Lejeune, C., 2017. Transcriptomic response to thermal and salinity stress in introduced and native sympatric *Palaemon* caridean shrimps. *Scientific Reports* **7**, 13980. <https://doi.org/10.1038/s41598-017-13631-6>
- Markhaseva, E.L., Abramova, A.A., Mingazov, N.D., 2012. *Pseudocalanus acuspes* (Crustacea: Copepoda) from the White Sea. *Proceedings of the Zoological Institute RAS* **316** (1), 57–70.
- Naumov, A.D., 2006. Molluski Belogo morya. Ekologicheskii i faunisticheskii analiz [Clams of the White Sea. Ecological and faunistic analysis]. Zoological institute, Russian Academy of Sciences, St. Petersburg, Russia, 367 p. (In Russian).
- Nicolajsen, H., Møhlenberg, F., Kjørboe, T., 1983. Algal grazing by the planktonic copepods *Centropages hamatus* and *Pseudocalanus* sp.: Diurnal and seasonal variation during the spring phytoplankton bloom in the Øresund. *Ophelia* **22**, 15–31. <https://doi.org/10.1080/00785326.1983.10427222>
- OBIS, 2020. Ocean Biogeographic Information System. Intergovernmental Oceanographic Commission of UNESCO. Web page. URL: www.iobis.org (accessed: 05.04.2020).
- Padmavati, G., Ikeda, T., Yamaguchi, A., 2004. Life cycle, population structure and vertical distribution of *Metridia* spp. (Copepoda: Calanoida) in the Oyashio region (NW Pacific Ocean). *Marine Ecology Progress Series* **270**, 181–198. <https://doi.org/10.3354/meps270181>
- Peijnenburg, K.T., Goetze, E., 2013. High evolutionary potential of marine zooplankton. *Ecology and*

- evolution* **3** (8), 2765–2781. <https://doi.org/10.1002/ece3.644>
- Prygunkova, R.V., 1974. Nekotorye osobennosti sezonnogo razvitiya zooplanktona guby Chupa Belogo morya [Some patterns of seasonal succession of zooplankton in Chupa Inlet, the White Sea]. In: Skarlato, O.A. (ed.), *Sezonnye yavleniya v zhizni Belogo i Barentseva morey* [Seasonal Developments in White and Barents Seas]. Nauka, Leningrad, USSR, 4–55. (In Russian).
- Rahlf, J., Peters, J., Moyano, M., Pless, O., Clausen, C., Peck, M.A., 2017. Short-term molecular and physiological responses to heat stress in neritic copepods *Acartia tonsa* and *Eurytemora affinis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **203**, 348–358. <https://doi.org/10.1016/j.cbpa.2016.11.001>
- Razouls, C., de Bovée F., Kouwenberg J., Desreumaux, N., 2005–2020. Diversity and Geographic Distribution of Marine Planktonic Copepods. Sorbonne University, CNRS. Web page. URL: <http://copepodes.obs-banyuls.fr/en> (accessed: 23.04.2020).
- Renz, J., Hirche, H.-J., 2006. Life cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: I. Seasonal and spatial distribution. *Marine Biology* **148**, 567–580. <https://doi.org/10.1007/s00227-005-0103-5>
- Renz, J., Peters, J., Hirche, H.-J., 2007. Life cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: II. Reproduction, growth and secondary production. *Marine Biology* **151**, 515–527. <https://doi.org/10.1093/plankt/fbn016>
- Runge, J.A., Ingram, R.G., 1991. Under-ice feeding and diel migration by the planktonic copepods *Calanus glacialis* and *Pseudocalanus minutus* in relation to the ice algal production cycle in southeastern Hudson Bay, Canada. *Marine Biology* **108**, 217–225. <https://doi.org/10.1007/BF01344336>
- Smedsrud, L.H., Ingvaldsen, R., Nilsen, J.E.Ø., Skagseth, Ø., 2010. Heat in the Barents Sea: transport, storage, and surface fluxes. *Ocean Science* **6**, 219–234. <https://doi.org/10.5194/os-6-219-2010>
- Svetlichny, L., Hubareva, E., 2014. Salinity tolerance of alien copepods *Acartia tonsa* and *Oithona davisae* in the Black Sea. *Journal of Experimental Marine Biology and Ecology* **461**, 201–208. <https://doi.org/10.1016/j.jembe.2014.08.012>
- Torres, G., Giménez, L., Anger, K., 2011. Growth, tolerance to low salinity, and osmoregulation in decapod crustacean larvae. *Aquatic Biology* **12** (3), 249–260. <https://www.int-res.com/articles/ab2011/12/b012p249.pdf>
- Tourangeau, S., Runge, J.A., 1991. Reproduction of *Calanus glacialis* under ice in spring in southeastern Hudson Bay, Canada. *Marine Biology* **108**, 227–233. <https://doi.org/10.1007/BF01344337>
- Usov, N., Kutcheva, I., Primakov, I., Martynova, D., 2013. Every species is good in its season: Do the shifts in the annual temperature dynamics affect the phenology of the zooplankton species in the White Sea? *Hydrobiologia* **706**, 11–33. <https://doi.org/10.1007/s10750-012-1435-z>
- Vázquez, M.G., Bas, C.C., Spivak, E.D., 2016. Ontogeny of salinity tolerance in the invasive shrimp *Palaemon macrodactylus* (Caridea: Palaemonidae). *Journal of Crustacean Biology* **36** (2), 214–219. <https://doi.org/10.1163/1937240X-00002410>
- WoRMS, 2020. World Register of Marine Species. Web page. URL: <http://www.marinespecies.org> (accessed: 23.04.2020). <https://doi.org/10.14284/170>