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Salinity tolerance of alien copepods *Acartia tonsa* and *Oithona davisae* in the Black Sea

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ABSTRACT

Acartia tonsa and *Oithona davisae* are typically near-shore species which were transferred to the Black Sea with ship ballast water and established self-sustaining populations in estuarine ecosystems. In order to estimate the salinity tolerance range and osmotic response in *A. tonsa* and *O. davisae* from Sevastopol Bay (a salinity of 18), the effect of salinity changes on mortality and body mass density of females of both species was studied. More than 50% of *A. tonsa* survived for 3–7 days after the gradual salinity decrease and increase at a rate of 2–3 h^{-1} within a salinity range from 3 to 30 while separate individuals withstood the salinity decrease down to 0.5 and salinity increase of up to 70 at a rate of 2–5 day^{-1}. In accordance with the LD_{50} index, the salinity tolerance range of *O. davisae* acclimated to a salinity of 18 amounted to 3–40. *A. tonsa* females maintained constant body mass density within the whole salinity tolerance range but hyper-regulated at the salinities higher than 30. In *O. davisae* females we observed a hysteretic response of body mass density within the salinity range of 12–40, probably, due to low osmotic water permeability of their integuments. At the lowest salinities (about 3) *O. davisae* hypo-regulated with body swelling, however, this species was iso-osmotic at the salinities higher than 40. Consequently, estuarine *A. tonsa* showed osmoregulatory capacities at low and intermediate salinities while salinity tolerance range of *O. davisae* was shifted to high salinities indicating marine origin of that species. Probably, an invasive success of *A. tonsa* and *O. davisae* in the brackish Black Sea is due to their osmoregulatory abilities.

1. Introduction

Among the great number of alien copepods permanently penetrating through the Bosphorus Strait into the Black Sea (Kovalev et al., 1998) or being transported there with ship ballast water (Selifonova, 2011), only *Acartia tonsa* Dana, 1849 and *Oithona davisae* Ferrari and Orsi, 1984 are the species which have established viable self-sustaining populations (Gubanova et al., 2014).

A. tonsa was found in the Black Sea for the first time in 1990 (Belmonte et al., 1994), however, the examination of historical zooplankton samples showed that this species had appeared there in the early 70s (Gubanova, 2000). Due to the fact that *A. tonsa* was not reported for the Mediterranean basin earlier than 1985, Gubanova et al. (2014) suggested that the species was introduced to the Black Sea with ship ballast water from another region of the World Ocean, probably, from the North American Atlantic coastal regions considered to be a native area of this species (McAlice, 1981).

O. davisae was found in Sevastopol Bay in December 2001 and at first identified as *Oithona brevicornis* (Zagorodnyaya, 2002). After the first appearance in Sevastopol Bay this new species was reported only four years later. During the period from October 2005 till 2009 the number of the species increased dramatically along the Crimean (Altukhov, 2010; Gubanova and Altukhov, 2007) and North-western Black Sea coast (Selifonova, 2009). Meanwhile, the specimens identified as *O. brevicornis* were re-examined from the Black Sea samples in 2010 and proved to be *O. davisae* (Temnykh and Nishida, 2012). *O. davisae* is a representative of the Indo-West Pacific Oithonidae which is widely distributed all over the World due to the synanthropic introduction, mainly in ship ballast waters, probably, from Asian estuaries (Ferrari and Orsi, 1984). This species is broadly distributed in Japanese coastal waters (Turner, 2004; Uye and Sano, 1995).

The organisms inhabiting the coastal zones have to adapt to unstable salinity regime. Marine holoplanktonic copepods are considered as principally osmoconformers (Mauchline, 1998), their internal osmolarity, body volume and density follow the salinity changes (Knutsen et al., 2001; Lance, 1965; McAllen et al., 1998; Svetlichny et al., 2012). Nevertheless, the level of osmotic tolerance in osmoconformers turned out to be as high as that in osmoregulators. According to Svetlichny et al. (2012), *Calanipeda aquaedulcis* survived within the salinity tolerance range of 0.1–50 and body mass density of this species altered strictly in accordance with the seawater salinity changes. On the contrary, sinking speed in both species was stable allowing them to avoid buoyancy problems with

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changing salinity. Some estuarine and benthic copepods are capable to regulate inorganic ion concentrations (Battaglia and Bryan, 1964; Sartoris et al., 2010) or organic osmolyte content in response to salinity alterations (Goolish and Burton, 1989; Roddie et al., 1984; Tang et al., 2000). The ability to keep body fluid homeostasis allows species-osmoregulators to overcome abrupt salinity changes at rain, evaporation, river flow, tides. Jeffries (1962) suggested that *A. tonsa* had developed an efficient osmoregulatory mechanism. Nevertheless, Lance (1965) found no clear evidence that *A. tonsa* could effectively control its water balance although in the laboratory experiments the body fluid in *A. tonsa* was hyper-osmotic to the external medium salinities ranging from 90% to 15% sea water for 12 h.

In spite of the great interest to the biology of *O. davisae*, the data about the salinity tolerance of the species are scarce. According to Uye and Sano (1998), *O. davisae* inhabited Fukuyama Harbor (Japan) with the salinities of 28.6–32.3, however, this species was found in Sacramento-San Joaquin Estuary (California) at a salinity of 12 (Ferrari and Orsi, 1984). Lougee et al. (2002) studied the behavior of *O. davisae* collected in the San Francisco Bay estuary at ambient salinity near 17.3–18.9 in experimental haloclines with the magnitude varying between 1.4 and 4.7.

Since both *A. tonsa* and *O. davisae* were transferred into the brackish Black Sea from high-saline oceanic waters, we suggest that the invasive success of these species was due to their broad salinity tolerance. The present study provides information on the salinity tolerance of the Black Sea laboratory-reared *A. tonsa* and collected from the sea *O. davisae* and the effects of gradual changes in salinity on survival, body volume and body mass density in order to determine the maximum salinity tolerance range and understand the type of osmotic stress response in these species. We hypothesize that the characteristics of changes in body volume and body mass density of copepods at the salinity variations can be evidence of being osmoconformers or osmoregulators. The second objective of this study was to determine the effect of salinity on sinking speed and swimming activity of *A. tonsa* and *O. davisae* in order to evaluate the ability to maintain themselves in the water column at the extreme salinities.

2. Materials and methods

2.1. Laboratory experiments

A. tonsa were cultivated in 3 l flasks in the laboratory in 0.45 μl filtered Black Sea water (FSW) with the salinity of 18 at room temperature of 22–25 °C and fed *ad libitum* the microalgae *Monochrysis lutheri*. The culture of *A. tonsa* was originally generated from the specimens captured in the Black Sea in autumn 2011. *O. davisae* was collected in Sevastopol Bay using a plankton net (opening diameter 30 cm, mesh size 100 μm) by horizontal hauls in the near-surface layer. Laboratory studies of the effect of salinity on mortality, sinking speed, body mass density and swimming behavior of *A. tonsa* and *O. davisae* were conducted during winter–spring 2012. We separated about 100 females of *A. tonsa* (prosome length of 0.068 ± 0.005 cm) or *O. davisae* (prosome length of 0.0309 ± 0.0012 cm) from the laboratory culture and samples collected before the experiments, respectively, and placed the copepods into 100 ml aquaria with aerated FSW at room temperature. During the experiments *O. davisae* was fed *ad libitum* with dinoflagellate *Oxyrrhis* sp. (IBSS, Department of Ecological Physiology of Algae).

2.2. Effect of gradual salinity change on mortality

20–30 active females of *A. tonsa* or *O. davisae* were placed using a pipette into the separate beakers of 50 ml containing FSW and then subjected to four treatments in 3 to 5 replicates:

(M1) Salinity was increased gradually over the periods ranging from 6 to 8 h (2–4 h^{-1}) from 18 to the final salinities of 30 and 35 in *A. tonsa*, and from 18 to the final salinities of 30, 35, 40, 45 and 50 in *O. davisae*;

(M2) Salinity was decreased gradually over the periods from 6 to 8 h at a rate of about 2 h^{-1} from 18 to the final salinities of 10, 6, 4, 3 and 2;

(M3) In *A. tonsa* and *O. davisae* acclimated for 1 week to salinities of 30 and 40, respectively, salinity was uniformly (due to the evaporation at a room temperature) increased to 70 at a rate of 4 ± 1 day^{-1}; and

(M4) *A. tonsa* were cultivated for 1 month at a salinity of 8 (from eggs produced by females acclimated to 8) and then acclimated for 1 week to 4, after that the salinities were changed in a stepwise fashion from 4 to 2 and from 2 to 1 and 0.5 at 24 h intervals. The purpose was to reduce the salinity progressively to minimum values.

In control experiments both species were monitored at a salinity of 18 for 10 days.

Throughout the periods of acclimation to low or high salinity, *A. tonsa* was fed with freshwater microalgae *Haemotococcus pluvialis* or marine microalgae *M. lutheri*, respectively, while *O. davisae* was fed with dinoflagellate *Oxyrrhis* sp. at different salinities. Marine microalgae were acclimated to the salinities of 18 and 40 and used as a food for experimental copepods at the salinities lower and higher than 30, respectively. During long-term experiments the final salinity was adjusted with food additions.

The number of live and dead individuals was counted every day for 5 days from the 3rd till the 7th day of the maintenance at the final salinities. Copepod mortality (m, %) was calculated as: $m = 100d / (d + s)$, where d is the number of dead individuals and s is the number of survived individuals. In the experiments with the evaporation the mortality was calculated as a mean number (%) of dead individuals registered every day for each 3 days of continuous salinity increase.

Salinity tolerance ranges for females of both species were estimated taking into account the lethal salinity values affecting more than 50% of the initial number (LS_{50}) of individuals. Low- and high-salinity water was prepared by stepwise addition and steady interfusion of distilled water or artificial sea salt to FSW with the salinity of 18. Water salinity (S, practical salinity units) was measured by a temperature-compensated handheld refractometer RHS-10ATC (accuracy of ± 1.0).

2.3. Effect of gradual salinity change on body mass density

Three treatments were used for *A. tonsa*:

(DA1) Salinity was gradually increased from 18.5 to 28.3 for 6 h and the copepods were kept at this salinity for 18 h. Then salinity was increased gradually from 28.3 to 39.7 and from 39.7 to 50 at 24 h interval;

(DA2) Salinity was decreased in the same way from 18.5 to 9 and 3; and

(DA3) In *A. tonsa* acclimated gradually (similar to M4) to a salinity of 3 and kept at this salinity for 2 weeks, salinity was increased to 11 and then from 11 to 18.3, and decreased from 3 to 1 and from 1 to 0.5 at 24 h intervals.

Three treatments were used for *O. davisae*:

(DO1) Salinity was gradually increased from 18.4 to 30, 35.1, 39.9, 50 and 60 in the same way as in the treatment DA1;

(DO2) Salinity was decreased in the same way from 18.4 to 12.1, 6.2 and 3; and

(DO3) In *O. davisae* acclimated to a salinity of 41 and kept at this salinity for 8 days, salinity was gradually decreased to 36, 25.7, 18.3 and 12 at 24 h intervals.

In all cases the measurements of body density were made in 18 h after the salinity changes. A Hach conductivity meter (SensIon 5) was used in these experiments to measure precisely water salinity (accuracy of ± 0.1) and temperature (accuracy of ± 0.1 °C).