

## RESEARCH ARTICLE

# Will the Antarctic tardigrade *Acutuncus antarcticus* be able to withstand environmental stresses related to global climate change?

Ilaria Giovannini<sup>1,\*</sup>, Tiziana Altiero<sup>2,\*</sup>, Roberto Guidetti<sup>1</sup> and Lorena Rebecchi<sup>1,‡</sup>**ABSTRACT**

Because conditions in continental Antarctica are highly selective and extremely hostile to life, its biota is depauperate, but well adapted to live in this region. Global climate change has the potential to impact continental Antarctic organisms because of increasing temperatures and ultraviolet radiation. This research evaluates how ongoing climate changes will affect Antarctic species, and whether Antarctic organisms will be able to adapt to the new environmental conditions. Tardigrades represent one of the main terrestrial components of Antarctic meiofauna; therefore, the pan-Antarctic tardigrade *Acutuncus antarcticus* was used as model to predict the fate of Antarctic meiofauna threatened by climate change. *Acutuncus antarcticus* individuals tolerate events of desiccation, increased temperature and UV radiation. Both hydrated and desiccated animals tolerate increases in UV radiation, even though the desiccated animals are more resistant. Nevertheless, the survivorship of hydrated and desiccated animals is negatively affected by the combination of temperature and UV radiation, with the hydrated animals being more tolerant than desiccated animals. Finally, UV radiation has a negative impact on the life history traits of successive generations of *A. antarcticus*, causing an increase in egg reabsorption and teratological events. In the long run, *A. antarcticus* could be at risk of population reductions or even extinction. Nevertheless, because the changes in global climate will proceed gradually and an overlapping of temperature and UV increase could be limited in time, *A. antarcticus*, as well as many other Antarctic organisms, could have the potential to overcome global warming stresses, and/or the time and capability to adapt to the new environmental conditions.

**KEY WORDS:** Meiofauna, Life history traits, Desiccation, Thermo-tolerance, Ultraviolet radiation, Antarctica

**INTRODUCTION**

Antarctica is the coldest and windiest continent, with the highest average altitude and often with very dry environmental conditions. In continental Antarctica, liquid water availability varies widely on seasonal and geographical scales, although it may be abundant locally during the summer months (Convey, 1996). Most of

continental Antarctica is permanently covered by snow or ice, with the only exception being ice-free terrestrial habitats restricted to coasts and inland nunataks. The snow covers terrestrial habitats for much of the year, and the frequency of daily freeze–thaw events on land is often unpredictable, sometimes occurring over hours, minutes or even more frequently (Convey, 1997; Wall, 2007; Convey et al., 2014). Moreover, the temperature is normally close to 0°C, with a narrow daily high temperature range, so temperatures suitable for life cycle activities are restricted to only a few weeks during the Antarctic summer (Convey et al., 2014; Everatt et al., 2014). As a consequence of these peculiar environmental conditions, the biota of continental Antarctica is depauperate. Antarctic organisms can be at constant risk of freezing or loss of body fluids; therefore, they must possess specific adaptive strategies to cope with Antarctic environmental conditions. This risk is generally bypassed by adopting strategies of desiccation tolerance and freeze tolerance or avoidance (Convey, 1996; Everatt et al., 2014). Indeed, in continental Antarctica, animal communities are almost entirely invertebrates, dominated by meiofaunal organisms, such as tardigrades, rotifers and nematodes, and a few arthropods, which have evolved different kinds of survival strategies (Convey, 1997; Convey and McInnes, 2005; Pugh and Convey, 2008; Yeates et al., 2009; Everatt et al., 2014; Altiero et al., 2015).

For organisms adapted for life in the Antarctic region, global climate change has become an important factor for survival. Atmospheric CO<sub>2</sub> levels are rising as a result of human activity and are leading to warming on a global scale. Indeed, environmental temperatures have increased 0.34°C per decade across Antarctica, and the frequency of localized short-term extreme temperature values is also predicted to increase (Krinner et al., 2007; Bracegirdle et al., 2008; Convey et al., 2009; Everatt et al., 2014). Another important environmental change is due to the widespread distribution of synthetic organic compounds such as chlorofluorocarbons (CFCs; Vitousek, 1994), which catalyze the stratospheric ozone depletion with a consequent increase of ultraviolet (UV) radiation. These environmental changes can affect the survivability and life history traits of Antarctic organisms and could be particularly effective during the Antarctic spring/summer season, when the animals are reproducing and growing. In view of these ongoing climate changes, we considered the possible consequences of their synergistic and/or cumulative effects on the health of Antarctic organisms and ecosystems. Could these changes lead to global/local decrease, or extinction of species/populations? Could Antarctic organisms be able to adapt to the new environmental conditions?

In order to obtain information about the fate of Antarctic organisms in view of ongoing climate changes, tardigrades were used as the animal model, because they, together with rotifers and nematodes, represent the main terrestrial components of Antarctic meiofauna in

<sup>1</sup>Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy. <sup>2</sup>Department of Education and Humanities, University of Modena and Reggio Emilia, 42121 Reggio Emilia, Italy.

\*These authors contributed equally to this work

‡Author for correspondence ([lorena.rebecchi@unimore.it](mailto:lorena.rebecchi@unimore.it))

 T.A., 0000-0001-6863-8754; R.G., 0000-0001-6079-2538; L.R., 0000-0002-0702-1846

terms of distribution, number of specimens and colonized substrates. Tardigrades survive dehydration or freezing of their habitat by temporally suspending their metabolism through entering anhydrobiosis or cryobiosis, respectively (for reviews, see Rebecchi et al., 2007; Welmicz et al., 2011; Guidetti et al., 2011b; Møbjerg et al., 2011). These animals are known to tolerate, both in the dormant and hydrated state, a number of extreme chemical and physical stressors, including very high and low temperatures and high doses of UV radiation (Li and Wang, 2005; Rebecchi et al., 2007, 2009b; Altiero et al., 2011; Guidetti et al., 2011a; Horikawa et al., 2006, 2013).

In this study, the tardigrade *Acutuncus antarcticus* (Richters 1904) was used as the animal model/target, because it is a pan-Antarctic species and the most widespread and common tardigrade in Antarctica (Cesari et al., 2016). It colonizes different kinds of substrates (e.g. algal or bacterial mats in lakes and temporary ponds, soil and mosses), although it is also very common and abundant in freshwater ponds (Dougherty, 1964; Everitt, 1981; Dastyk, 1991; McInnes, 1995; Kagoshima et al., 2013; Tsujimoto et al., 2014; Cesari et al., 2016). Specimens used in this study belong to a population of *A. antarcticus* that comes from a temporary freshwater pond at Victoria Land. This population has a short life cycle and reproduces by automictic thelytokous parthenogenesis, a reproductive strategy that allows a rapid increase in the population size, capitalizing on the short Antarctic summer, during which environmental conditions are favourable for active life, growth and reproduction (Altiero et al., 2015). Because the natural habitat of *A. antarcticus* is subject to unpredictable dehydration/rehydration events, its anhydrobiotic capability was evaluated in this study on both animals and eggs. As a consequence of global warming, the water temperature of the pond colonized by *A. antarcticus* could increase, so hydrated animals were exposed to increasing temperature values in order to analyze their thermo-tolerance. Moreover, their tolerance to UV radiation was tested on hydrated and desiccated animals and on eggs at different developmental stages, evaluating the sensitivity of both hydrated and desiccated animals to the cumulative effects of UV radiation and increasing temperature. Finally, because the growing season of *A. antarcticus* occurs in the spring period of ozone depletion, the possible effects of increasing UV radiation on embryonic development and successive generations, and on the life history traits of the offspring hatched from irradiated eggs, were analyzed and compared with previous data obtained from untreated specimens (Altiero et al., 2015).

## MATERIALS AND METHODS

The eutardigrade *Acutuncus antarcticus* (Parachela, Hypsibioidae, Hypsibiidae) was collected from bottom sediments of a temporary freshwater pond at Victoria Land (125 m a.s.l., 74°42.580'S, 164°06.086'E, Terranova Bay, Antarctica), close to the 'Mario Zucchelli' Italian base. At the time of the sampling, the temperature of the water pond was 9.5°C (Altiero et al., 2015). Several specimens were extracted from the freshwater sediment of the pond and cultured according to Altiero et al. (2015) in order to obtain culture microcosms. Stress experiments were carried out using animals and eggs collected from these microcosms. Animals were starved for 24 h in water at 14°C to standardize and perform stress experiments.

To analyze the stress response of eggs at a specific developmental stage, we used eggs differing only in the development time at which they were exposed to stresses (desiccation or irradiation). On the basis of development time, eggs 1–2 days after oviposition (called early development eggs) and eggs 5–6 days after oviposition (called

late development eggs) were used. These two kinds of eggs were obtained from animals collected from the microcosms and individually reared according to Altiero et al. (2015) until oviposition. Newly laid eggs were kept in mineral water at 14°C until experiments began.

## Desiccation stress of animals and eggs

To analyze the capability of *A. antarcticus* to tolerate desiccation, six replicates of animals, and six replicates of both early and late development eggs were used. Replicates were made up of 10 specimens each. Animals and eggs were desiccated under laboratory conditions, using the slightly modified protocol of Rebecchi et al. (2009a). Specifically, a group of animals or eggs was placed on Whatman filter paper with a few drops of natural mineral water and maintained in a climatic chamber at the following temperature and relative humidity (RH): 15°C at 80% RH for 4 h; 15°C at 50% RH for 4 h; and room temperature (about 20°C) at 0–3% RH for 2 days. After this time, both animals and eggs were rehydrated by adding water drops to each filter paper every 10 min for a total of 60 min. After rehydration, coordinated movements of the animal body (locomotion performance) constituted the criterion to confirm animal viability (Rebecchi et al., 2009a). Locomotion performance was evaluated after 1 h ( $t_1$ ) and 24 h ( $t_{24}$ ) from the end of the rehydration process. During this time, animals were kept individually in plastic boxes containing natural mineral water at 14°C with a 12 h:12 h light:dark photoperiod. The term 'final survival' refers to the survival rate of animals recorded at  $t_{24}$ .

The survivorship of the eggs was evaluated by analyzing their hatching capability. Prior to hatching, eggs were kept individually in plastic boxes containing natural mineral water at 14°C and a 12 h:12 h light:dark photoperiod. The eggs were monitored three times a week, and a partial water change was carried out during each control. Data on hatching time and hatching success were recorded. The hatching time of eggs was determined without including the 2 days spent by the eggs in the desiccated state. As controls, we used data on hatching time and hatching success recorded from eggs maintained hydrated until their hatching from three successive generations studied by Altiero and coworkers (2015).

A *t*-test was used to compare the percentage of viable animals between  $t_1$  and  $t_{24}$  (final survival). This test was also used to compare the hatchability and the hatching time between early and late development eggs after the desiccation stress followed by a successive rehydration. All statistical analyses were carried out using SPSS 20 (SPSS Inc., Chicago, IL, USA). As a general rule, parametric tests were used when data showed a normal distribution evaluated with the Levene test, whereas non-parametric tests were used when data did not show a normal distribution.

## Heat stress

To analyze the tolerance of *A. antarcticus* to high temperatures, active and hydrated animals were exposed to high temperatures for a short time period (1 h), producing an acute heat stress. The tested temperatures were: 21.5, 28, 30, 33, 35, 37, 39 and 41°C. Six replicates each of 10 animals were used for each tested temperature. As a control, six replicates of 10 animals were maintained at 14°C (rearing laboratory temperature; Altiero et al., 2015).

According to the protocol by Rebecchi et al. (2009b), each group of animals was placed in a covered glass capsule (4 cm in diameter) containing 4 ml of mineral water pre-heated at the specific stress temperature, and then exposed to the same stress temperature in a climatic chamber. After heat stress, tardigrades were transferred

into new natural mineral water and kept at 14°C and a 12 h:12 h light:dark photoperiod to verify their viability. Coordinated movements of the animal body (locomotion performance) constituted the criterion to confirm animal viability (Rebecchi et al., 2009a). Locomotion performance was evaluated immediately after the end of heat stress ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ , final survival).

The non-parametric Jonckheere–Terpstra test for ordered alternatives was used to test the hypothesis that the survival rate of animals declines with an increase in stress temperature. Data on final survivorship at  $t_{24}$  were used to statistically quantify the thermal tolerance by calculating the temperature that caused 50% of animal mortality (lethal thermal temperature,  $LT_{50}$ ; Mora and Maya, 2006).  $LT_{50}$  was calculated with Probit analysis. The first temperature causing 100% of mortality (lethal thermal maximum,  $LT_{max}$ ) was also determined. To compare the viability trends recorded at  $t_0$ ,  $t_1$  and  $t_{24}$ , the Kruskal–Wallis test was used.

### UV radiation stress

The UV radiation tolerance of *A. antarcticus* was evaluated on animals and on eggs at early and late developmental stages. Specimens were exposed to a wide spectrum of UV radiation with a peak of emission at 312 nm (UV-B). The UV source consisted of a transilluminator 15 W bulb (Sigma-Aldrich, St Louis, MO, USA) 40 cm in length. Midrange UV fluorescence  $\lambda_{em}$  312 nm yielded a spectral output extending from the UV-C through the UV-A spectra with an emission peak at 312 nm (see Altiero et al., 2011). The irradiance, as measured with a spectroradiometer (Macam SR9910, Macam Photometrics, Livingstone, UK), was  $0.26 \text{ kJ m}^{-2} \text{ min}^{-1}$ . The lamp was positioned 30 cm above the samples. In all experiments, the lamp and the samples were located in a climatic chamber in dark conditions (see Altiero et al., 2011), while the temperature value depended on the experiment (see below).

### UV radiation stress on animals

To evaluate the response of *A. antarcticus* to UV radiation, both hydrated and desiccated animals were tested. Animals were exposed to nine UV doses by increasing their exposure time period:  $2.58 \text{ kJ m}^{-2}$  (equivalent to an exposure of 10 min),  $5.16 \text{ kJ m}^{-2}$  (20 min),  $10.32 \text{ kJ m}^{-2}$  (40 min),  $23.22 \text{ kJ m}^{-2}$  (90 min),  $36.12 \text{ kJ m}^{-2}$  (140 min),  $49.02 \text{ kJ m}^{-2}$  (190 min),  $61.92 \text{ kJ m}^{-2}$  (240 min),  $74.82 \text{ kJ m}^{-2}$  (290 min) and  $87.72 \text{ kJ m}^{-2}$  (340 min). During the irradiation, the temperature was kept at 8°C. In these irradiation experiments, two replicates of 20 animals each were used for each physiological condition and for each UV dose.

Hydrated animals were exposed to UV radiation within a thin layer of natural mineral water (350  $\mu\text{l}$ ) in a small plastic Petri dish (diameter=1 cm, height=0.7 cm) without a cover, whereas desiccated tardigrades were irradiated on the Whatman filter paper on which they were desiccated. The desiccation protocol was as described above.

For non-irradiated controls, two replicates each of 20 hydrated or desiccated animals were kept in an open plastic box within the climatic chamber (at 8°C and in dark conditions) containing the UV source, but not exposed to the UV radiation.

The criterion of locomotion performance was used to evaluate tardigrade viability after irradiation. The locomotion performance of hydrated animals was recorded immediately after the end of irradiation ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ; final survival). Desiccated tardigrades were rehydrated immediately after irradiation (see above), and their locomotion performance was recorded at the end of rehydration ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h

( $t_{24}$ ; final survival) from the end of rehydration. During these periods, tardigrades were kept in the dark at 8°C.

The non-parametric Jonckheere–Terpstra test for ordered alternatives was used to analyze the hypothesis that the survival rate of the animals at  $t_{24}$  declines with the increase of the UV radiation doses for both hydrated and desiccated animals. The non-parametric Kruskal–Wallis was used to compare the viability trends among  $t_0$ ,  $t_1$  and  $t_{24}$ . Probit analysis was used to statistically calculate the lethal doses at  $t_{24}$  that cause 50% mortality of the specimens ( $LD_{50}$ ) in hydrated and desiccated animals.

### UV radiation stress in combination with temperature

The effects of UV radiation in combination with temperature were assessed in hydrated and desiccated animals using two different temperatures (8 and 15°C). Hydrated animals were exposed to a UV dose of  $28.67 \text{ kJ m}^{-2}$ , which corresponds to the  $LD_{50}$  calculated on the basis of the survival rate at  $t_{24}$  of hydrated animals exposed to nine increasing UV doses (see previous section). Similarly, desiccated animals were exposed to a UV dose of  $30.02 \text{ kJ m}^{-2}$ , which corresponds to the  $LD_{50}$  calculated on the basis of the survival rate at  $t_{24}$  of desiccated animals exposed to nine increasing UV doses (see previous section).

In irradiation experiments, six replicates of 10 animals each were used for each physiological condition and combination of temperature and UV dose. Irradiation was performed within a climate chamber in dark conditions (see previous experiment). As a control, two replicates each of 10 hydrated or desiccated animals were kept in dark conditions in an open plastic box within the climate chamber containing the UV source, but they were not exposed to the UV radiation.

The locomotion performance and the final survival of hydrated and desiccated animals were recorded as described in the previous section.

At each temperature, for both hydrated and desiccated animals, the percentages of viable animals recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  (final survival) were statistically compared by ANOVA after arcsine transformation of the data to meet the assumption of normality. Pairwise comparisons between percentages of viable animals recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  at the two temperatures were carried out for both hydrated and desiccated states using a *t*-test. A *t*-test was also used to compare the percentages of viable animals recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  at the two different physiological conditions at both 8 and 15°C.

### UV radiation stress on eggs

The effects of UV radiation on egg hatching capability were assessed on hydrated eggs of *A. antarcticus* at both early and late developmental stages. Three different UV doses were tested:  $28.67 \text{ kJ m}^{-2}$  ( $LD_{50}$  calculated on hydrated animals),  $1.29 \text{ kJ m}^{-2}$  (equivalent to an exposure for 5 min) and  $2.58 \text{ kJ m}^{-2}$  (10 min of exposure). Six replicates of 10 hydrated eggs were used for each egg developmental stage and for each tested UV dose. The eggs were always individually exposed to UV radiation (at 8°C, in dark conditions) within a thin layer of natural mineral water (350  $\mu\text{l}$ ) in a small plastic Petri dish (diameter=1 cm, height=0.7 cm) without a cover.

After irradiation, the survivorship of both early and late development eggs was evaluated by analyzing their hatching capability (hatching success). Prior to hatching, the eggs were kept individually in plastic boxes containing natural mineral water at 14°C and a 12 h:12 h light:dark photoperiod. The eggs were monitored three times a week, and a partial water change was carried out during each period.



The hatching time and hatching success of early and late development eggs after irradiation were recorded and compared using a *t*-test. As a control, data on hatching time and hatching success of eggs obtained by a previous study on the life cycle of *A. antarcticus* were used (see Altiero et al., 2015).

### Effects of UV radiation on life history traits

Newborns that hatched from each irradiated egg (generation 1) and their offspring (generation 2) were individually reared from birth to death according to Altiero et al. (2015). Data on life history traits of animals belonging to both generations (1 and 2) were collected in order to evaluate how the UV radiation affects the life cycle of *A. antarcticus*. Specifically, data on active life span, number of moults, age at first and last oviposition, number of ovipositions per life span, interval of time among ovipositions, number of laid eggs per life span (fecundity), number of eggs per clutch (fertility), egg hatching time and egg hatching percentage were determined. Pairwise comparisons of life history traits within and between generations were performed using the *t*-test or the Mann–Whitney *U*-test.

Unhatched irradiated eggs and animals that died during rearing were mounted on slides in a drop of Faure-Berlese's mounting medium to analyze their morphology. Observations of these samples were carried out using a Leica Leitz DM RB microscope (Leitz, Wetzlar, Germany) with phase contrast or differential interference contrast. Images were captured using a Nikon Digital Sight DS-Fi1 camera (Nikon Corporation, Tokyo, Japan).

## RESULTS

### Desiccation tolerance

Specimens of *A. antarcticus* were able to survive desiccation. The mean (all presented  $\pm$ s.d.) percentage of viable animals at  $t_1$  was  $87.4\pm 4.8\%$ , and the final survival ( $t_{24}$ ) was  $92.8\pm 9.0\%$  (Fig. 1A). No significant differences between the percentage of viable animals at  $t_1$  and  $t_{24}$  were found.

After desiccation and rehydration, the hatching success of the early development eggs was  $11.7\pm 7.5\%$ , and the success of late development eggs was  $33.3\pm 28.1\%$  (Fig. 1B). The hatching times of early and late development eggs were  $9.3\pm 2.6$  and  $10.0\pm 1.4$  days, respectively. No statistical differences were observed comparing the hatching success and the hatching times of eggs at the two different stages of development.

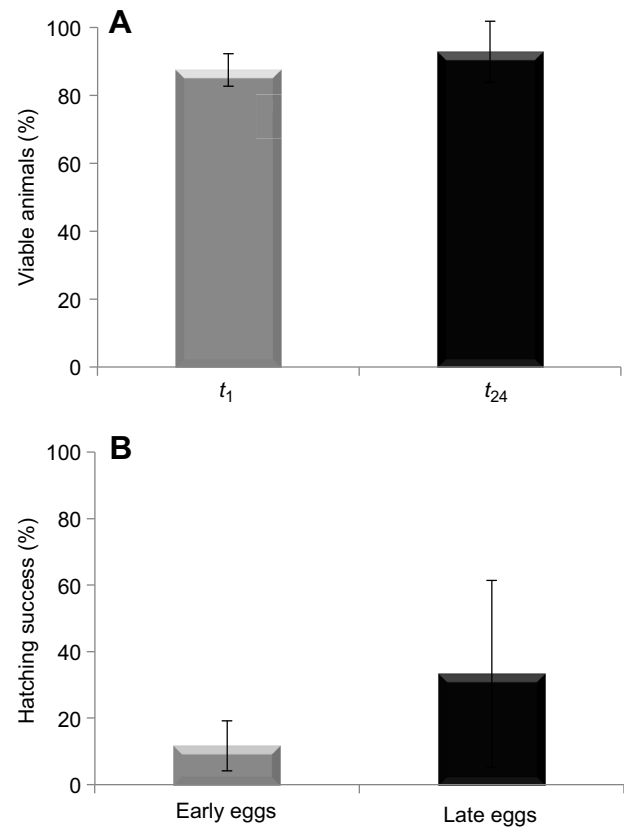
### Thermo-tolerance of animals

In the hydrated state, specimens of *A. antarcticus* were able to tolerate increasing temperature values for a short period of time (Fig. 2). The trends of animal viability recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  from the end of thermal stress were similar, and no statistical differences were detected. The non-parametric Jonckheere–Terpstra test for ordered alternatives showed a statistically significant decrease of median survivorship of animals with increasing temperature values ( $t_{24}$ :  $T_{JT}=215.50$ ,  $z=-7.234$ ,  $P<0.0005$ ). The mean survival rate at  $t_{24}$  was 100% up to  $33^\circ\text{C}$ . Above this temperature, there was a dramatic decline in survivorship, which dropped to  $35.0\pm 34.5\%$  at  $37^\circ\text{C}$ , and no animals survived at  $39^\circ\text{C}$  (corresponding to the  $LT_{max}$ ; Fig. 2). The  $LT_{50}$  value at  $t_{24}$  was  $36.2^\circ\text{C}$ . The survival of control specimens maintained at  $14^\circ\text{C}$  was 100%.

### UV radiation tolerance

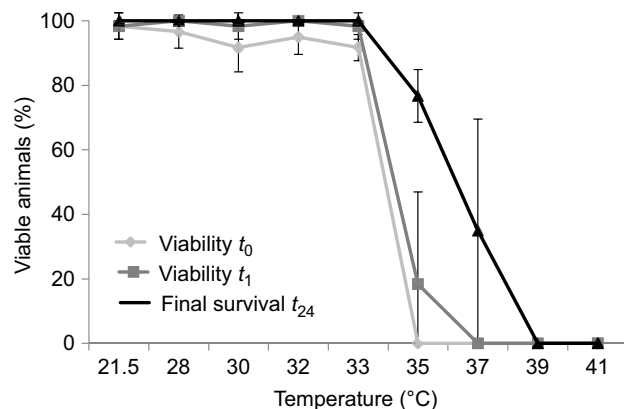
#### UV radiation tolerance of animals

Hydrated and desiccated animals of *A. antarcticus* showed tolerance to UV radiation at  $8^\circ\text{C}$ . At each UV dose, in both hydrated (Fig. 3A) and desiccated (Fig. 3B) animals, the comparison of viability trends

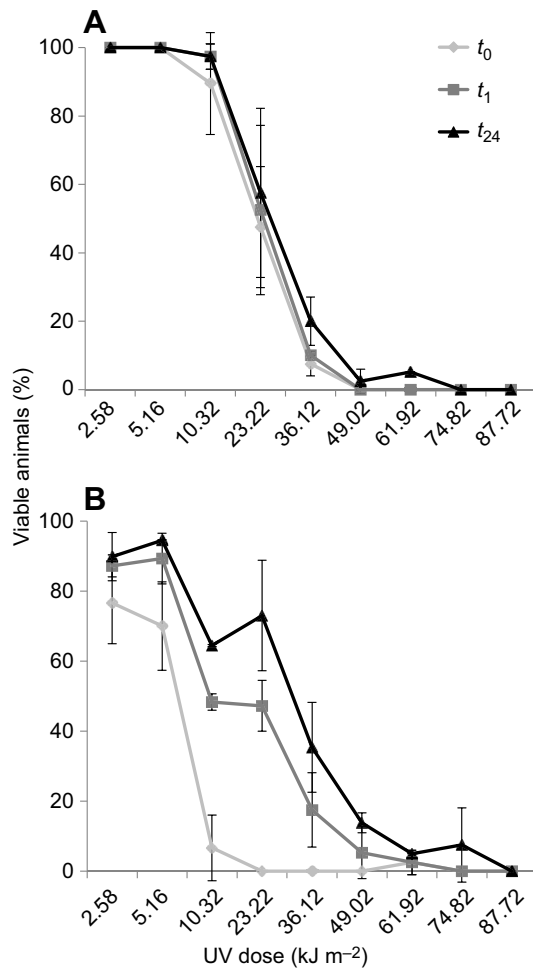


**Fig. 1. Desiccation tolerance of *Acutuncus antarcticus*.** (A) Percentage of animal viability 1 h ( $t_1$ ) and 24 h ( $t_{24}$ ; final survival) after rehydration. (B) Hatching success of both early and late development eggs after desiccation and rehydration. Data are means  $\pm$  s.d.

recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  did not show differences. The non-parametric Jonckheere–Terpstra test for ordered alternatives showed a statistically significant decrease of median survivorship of animals with the increasing of UV dose in both active ( $t_{24}$ :  $T_{JT}=16.50$ ,  $z=-4.956$ ,  $P<0.0005$ ) and desiccated specimens ( $t_{24}$ :  $T_{JT}=9.50$ ,  $z=-5.274$ ,  $P<0.0005$ ). At  $t_{24}$ , hydrated animals survived up to the UV dose of  $61.92 \text{ kJ m}^{-2}$ , although the mean final survival rate was  $5.1\pm 0.2\%$  (Fig. 3A). On the other hand, at



**Fig. 2. Thermo-tolerance of *A. antarcticus*: viability trends of animals under heat stress.** Viability evaluated immediately after the end of thermal stress ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ) is reported. For each tested temperature value, the mean percentage of animal viability and its standard deviation are reported.

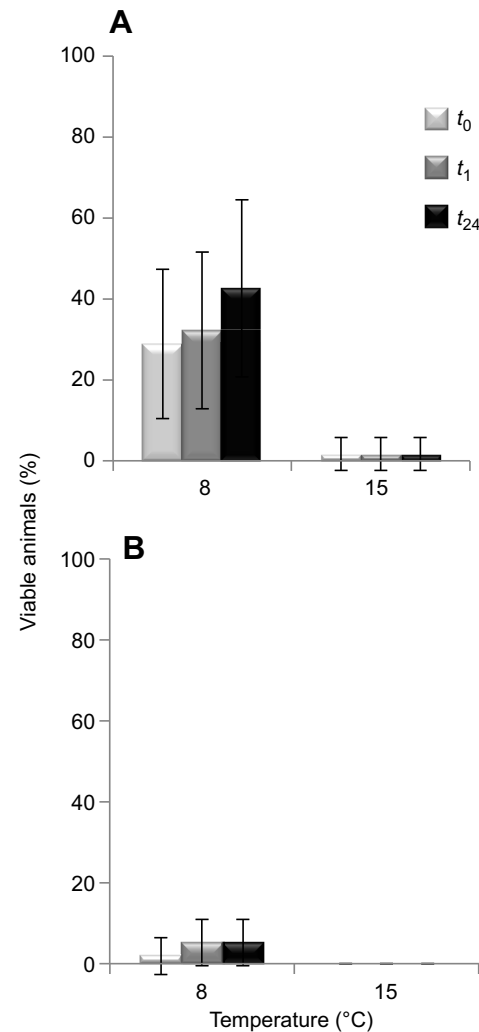


**Fig. 3. Radiation tolerance of *A. antarcticus*: viability trends of animals after exposure to increasing doses of UV radiation.** (A) Hydrated animals. Viability evaluated immediately after the end of the stress ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ) is reported. (B) Desiccated animals. Viability evaluated immediately after the end of rehydration ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ) is reported. For each tested UV dose, the mean percentage of animal viability and its standard deviation are shown.

$t_{24}$  desiccated specimens survived up to a dose of 74.82 kJ m<sup>-2</sup>, showing a final survivorship of 7.5±10.5% (Fig. 3B). The LD<sub>50</sub> UV doses (evaluated at  $t_{24}$ ) were 28.67 kJ m<sup>-2</sup> in hydrated specimens and 30.02 kJ m<sup>-2</sup> in desiccated specimens. The survival of the hydrated and desiccated animals used as controls was 100%.

#### UV radiation stress in combination with temperature

A negative cumulative effect on animal viability ( $t_0$  and  $t_1$ ) and final survival ( $t_{24}$ ) of *A. antarcticus* was found after the simultaneous exposure of both hydrated and desiccated animals to their respective LD<sub>50</sub> UV doses at two temperature values (8 and 15°C; Fig. 4). At each temperature value, no statistical differences were found among the percentages of viable animals and the final survival of both hydrated and desiccated animals. The survival of all hydrated and desiccated animals used as controls was 100%. Hydrated animals exposed to the UV dose of 28.67 kJ m<sup>-2</sup> (LD<sub>50</sub>) at 8°C showed a final survival of 42.6±21.9%, whereas at 15°C their final survival dropped to 1.7±4.1% (Fig. 4A). Based on the final survival of hydrated animals, highly significant differences were present between 8 and 15°C ( $t=6.01$ ,  $P<0.001$ ). Unexpectedly, the



**Fig. 4. Percentage of viable animals of *A. antarcticus* after exposure to the LD<sub>50</sub> UV doses under two different temperature values.** (A) Hydrated animals. Viability evaluated immediately after the end of the combined stresses ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ) is reported. (B) Desiccated animals. Viability evaluated immediately after the end of rehydration ( $t_0$ ), after 1 h ( $t_1$ ) and after 24 h ( $t_{24}$ ) is reported. Data are means±s.d.

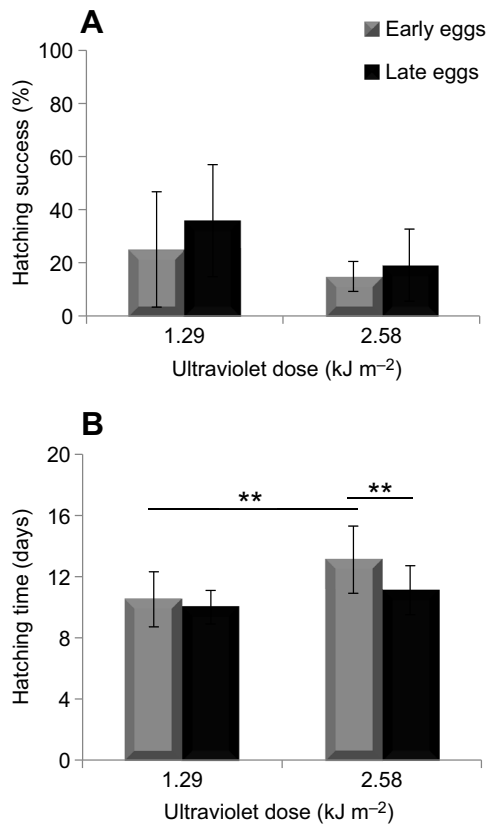
desiccated animals exposed to the UV dose of 30.02 kJ m<sup>-2</sup> (LD<sub>50</sub>) at 8°C showed a final survival of only 5.2±5.7%, whereas no animals survived at 15°C (Fig. 4B).

At 8°C, the final survival of irradiated hydrated animals was higher than the survival of animals irradiated in the desiccated state ( $t=4.37$ ,  $P=0.001$ ).

#### UV radiation tolerance of eggs

No hydrated eggs in the early and late developmental stages of *A. antarcticus* exposed to the UV dose of 28.67 kJ m<sup>-2</sup> (LD<sub>50</sub> of the hydrated animals) hatched. At the 1.29 kJ m<sup>-2</sup> dose, the hatching success of early development eggs was 25.0±21.7% and the success of late development eggs was 35.9±21.1% (Fig. 5A). At this dose, the mean hatching times were 10.5±1.8 days for early development eggs and 10.0±2.2 days for late development eggs (Fig. 5B).

At the 2.58 kJ m<sup>-2</sup> dose, the hatching success of eggs irradiated at the early developmental stage was 14.9±5.7%, whereas that of late development eggs was 19.2±13.6% (Fig. 5A). At this dose, the mean hatching times were 13.1±1.1 days for the early development eggs and 11.1±1.6 days for the late development eggs (Fig. 5B).



**Fig. 5. Hatching features of both early and late development eggs of *A. antarcticus* after UV irradiation at two different doses.** (A) Hatching success. (B) Hatching time. Data are means $\pm$ s.d. \*\* $P$ <0.01.

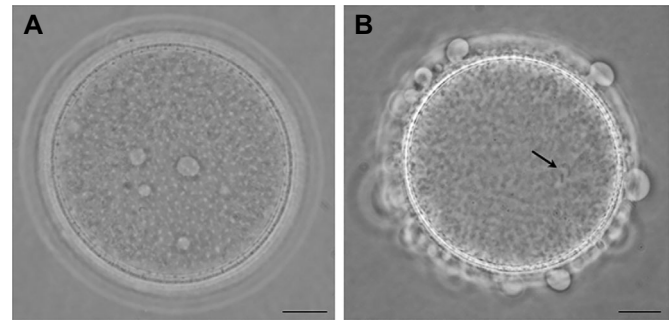
At each UV dose (1.29 or 2.58 kJ m<sup>-2</sup>), no statistical differences were present between the hatching success of early and late development eggs. Similarly, at each stage of development (early or late), no statistical differences were found between the hatching successes recorded after the exposure at the two UV doses. In contrast, a significant difference between the hatching time of early and late development eggs irradiated at the 2.58 kJ m<sup>-2</sup> UV dose was found ( $t=3.26$ ,  $P$ <0.01; Fig. 5B). In particular, late development eggs hatched earlier than early development eggs. Moreover, early development eggs irradiated at the 2.58 kJ m<sup>-2</sup> UV dose hatched significantly later than those irradiated at the 1.29 kJ m<sup>-2</sup> UV dose ( $t=-3.88$ ,  $P$ <0.01; Fig. 5B).

Regardless of irradiation dose and egg developmental stage, the majority of unhatched eggs did not show the presence of an embryo (Fig. 6A), and the outlines of claws were visible only in a few eggs (Fig. 6B).

#### Life history traits of *A. antarcticus* after exposure of eggs to UV radiation

The life history traits of 47 animals hatched from irradiated eggs (generation 1) and those of their offspring (29 specimens belonging to generation 2) are presented in Tables 1 and 2, respectively.

In generation 1, pairwise comparisons of each life history trait between animals hatched from early and late development eggs exposed to 1.29 or 2.58 kJ m<sup>-2</sup> UV doses did not show significant differences. In addition, no significant differences were found in each life history trait comparing animals hatched from eggs at the same developmental stage, but irradiated at the two tested UV doses. The mean hatching time of late development eggs irradiated at the



**Fig. 6. Features of unhatched eggs of *A. antarcticus* after UV irradiation.** (A) Egg without the presence of an embryo. (B) Egg with the outlines of the embryonic claws (arrow). Scale bars: 10  $\mu$ m (phase contrast).

UV dose of 1.29 kJ m<sup>-2</sup> was significantly higher (10.0 $\pm$ 2.2 days) than the hatching time (8.4 $\pm$ 2.1 days) of eggs laid by the animals hatched from these irradiated eggs (generation 1;  $t=2.28$ ,  $P$ <0.05; Table 1).

The pairwise comparisons of the analyzed life history traits within generation 2 (made up of offspring of generation 1) did not show any significant differences.

Pairwise comparisons of each life history trait between generations 1 and 2 showed only one significant difference. The fertility (number of eggs per clutch) was higher in animals hatched from eggs irradiated at 2.58 kJ m<sup>-2</sup> in the late developmental stage (generation 1) with respect to the fertility of their offspring (generation 2;  $U=27.00$ ,  $P$ <0.05).

#### Morphology of animals belonging to generations 1 and 2

Animals with morphological defects were observed in both generations (generation 1: 5 out of 47 animals; generation 2: 1 out of 29 animals; Fig. 7). In particular, these morphological defects included the absence of an eyespot (Fig. 7A), the presence of swellings on several parts of the animal body (Fig. 7B,C), deformities in the bucco-pharyngeal apparatus, supernumerary claws (Fig. 7C,D), and missing and/or malformations of branches of the claws (Fig. 7C–E).

Furthermore, some egg reabsorption events were observed in animals belonging to both generations (10 animals in generation 1; 7 animals in generation 2). A maximum of three successive egg reabsorption events per animal was observed.

## DISCUSSION

### Desiccation tolerance

The tardigrade *A. antarcticus* entered anhydrobiosis in every stage of its life cycle. This high capability is noteworthy for a tardigrade species that generally colonizes aquatic environments, as freshwater tardigrades either are not able to withstand desiccation or they have a very low survivorship (Guidetti et al., 2011b). The higher hatching success of the desiccated eggs at the late developmental stage (33%), with respect to those desiccated at the beginning (~12%) of development, is in line with the results obtained in another eutardigrade species, *Milnesium tardigradum* (see Schill and Fritz, 2008). The eggs of *A. antarcticus* desiccated at the early developmental stage showed a lower hatching success (12%) than that of eggs laid by females of the P, F1 and F2 generations (72%, 67% and 22%, respectively) and maintained always in water (see Altiero et al., 2015). The eggs of *A. antarcticus* desiccated at the late developmental stage had a hatching success (33%) lower than that of eggs laid by females of both the P and F1 generations, but higher

**Table 1. Life history traits of generation 1 *Acutuncus antarcticus* hatched from early and late development eggs irradiated at 1.29 or 2.58 kJ m<sup>-2</sup> UV doses**

Life history trait	UV dose: 1.29 kJ m <sup>-2</sup>		UV dose: 2.58 kJ m <sup>-2</sup>	
	Early eggs	Late eggs	Early eggs	Late eggs
Life span (days)				
<i>N</i>	11	16	8	12
Mean±s.d.	63.5±44.5	87.5±50.8	52.6±56.0	50.5±38.9
Median	71.0 (11.0; 106.0)	96.5 (32.3; 109.5)	20.0 (9.5; 104.0)	48.5 (13.5; 85.3)
Number of moults				
<i>N</i>	11	16	8	12
Mean±s.d.	4.7±4.1	8.2±6.1	5.8±8.2	4.9±5.3
Median	5.0 (0; 8.0)	8.0 (2.3; 11.8)	0.5 (0; 12.0)	3.5 (0; 10.0)
Age at first oviposition (days)				
<i>N</i>	6	10	3	3
Mean±s.d.	33.2±12.0	24.9±13.1	34.0±9.8	24.3±4.7
Median	33.0 (21.5; 43.3)	20.0 (16.5; 28.8)	37.0 (23.0; n.p.)	26.0 (19.0; n.p.)
Age at last oviposition (days)				
<i>N</i>	6	10	3	3
Mean±s.d.	65.0±11.8	66.9±40.8	66.0±27.0	66.3±5.5
Median	70.0 (52.8; 73.8)	51.5 (46.5; 83.0)	66.0 (39.0; n.p.)	66.0 (61.0; n.p.)
Oviposition number per life span				
<i>N</i>	11	16	8	12
Mean±s.d.	2.4±2.8	3.1±3.8	1.5±2.3	1.0±1.8
Median	2.0 (0; 5.0)	2.5 (0; 4.8)	0 (0; 3.0)	0 (0; 3.0)
Interval of time among ovipositions (days)				
<i>N</i>	25	40	9	9
Mean±s.d.	9.0±4.6	10.5±7.4	10.7±8.9	14.0±12.1
Median	7.0 (7.0; 10.0)	7.5 (7.0; 13.3)	7.0 (5.0; 15.0)	7.0 (6.0; 25.5)
Number of eggs per female per life span (fecundity)				
<i>N</i>	11	16	8	12
Mean±s.d.	2.7±3.6	4.3±5.6	1.9±2.8	1.6±2.9
Median	2.0 (0; 6.0)	2.5 (0; 6.5)	0 (0; 4.5)	0 (0; 3.8)
Number of eggs per clutch (fertility)				
<i>N</i>	32	50	12	12
Mean±s.d.	1.2±0.4	1.4±0.5	1.3±0.5	1.6±0.7
Median	1.0 (1.0; 1.0)	1.0 (1.0; 2.0)	1.0 (1.0; 1.8)	1.5 (1.0; 2.0)
Hatching time (days)				
<i>N</i>	10	16	2	5
Mean±s.d.	9.7±2.5	8.4±2.1	7.0±0.0	11.2±5.0
Median	9.0 (7.0; 12.3)	9.0 (7.0; 10.0)	7.0 (7.0; 7.0)	9.0 (7.0; 16.5)
Hatching success				
<i>N</i>	6	10	3	3
Mean±s.d.	16.9±18.3	14.5±22.0	15.9±16.7	27.6±12.9
Median	16.7 (0.0; 26.1)	0 (0; 31.0)	14.3 (0; n.p.)	28.6 (14.3; n.p.)

Medians are presented with 25th and 75th percentiles in parentheses. *N*, sample size; n.p., not present.

than that of eggs laid by females of the F2 generation (Altiero et al., 2015). These comparisons suggest that the capability of tardigrade eggs (embryos) to survive desiccation increases with developmental time and, consequently, age of the embryos. The beginning of development represents a sensitive phase during which undifferentiated cells are involved in a rapid proliferation process that makes up the embryos. Moreover, the cells of *A. antarcticus* could have a limited or null capability to activate the synthesis of molecules working as bioprotectants under desiccation, as occurred in newborn and adult specimens of other tardigrade species (for reviews, see Rebecchi et al., 2007; Guidetti et al., 2011b). Nevertheless, the low hatchability of eggs after desiccation does not represent a limit to the persistence of *A. antarcticus* in space and time, because the eggs may exploit additional strategies to survive extreme environmental conditions, such as cryobiosis and bet-hedging strategies, as occurs in other tardigrades or micrometazoans (Crean and Marshal, 2009; Altiero et al., 2015). In contrast, the developmental time of the eggs of *A. antarcticus* was not affected by the desiccation event. Indeed, after a period of desiccation, the early and late development eggs showed mean hatching times (9 days)

similar to those of eggs constantly maintained in water (7–9 days), as has been demonstrated in a previous study on the life history traits of *A. antarcticus* (Altiero et al., 2015). These data also suggest that egg development stopped during the period of desiccation and then restarted after rehydration. Indeed, during anhydrobiosis, the absence of water does not allow metabolism to occur, similarly to what happens in adult tardigrades (Hengherr et al., 2008).

Differently from the developing eggs, *A. antarcticus* animals showed a high tolerance to desiccation, as indicated by their high final survival (about 93%) recorded in this study. This pattern might be due to their capability of having functional biochemical pathways in order to synthesize molecules assumed to play a role in mediating desiccation tolerance, such as trehalose, heat shock proteins, Late Embryogenesis Abundant proteins, DNA associated protein (Dsup), Cytoplasmic (CASH), Secretory (SASH) and Mitochondrial (MAHS) Abundant Heat Soluble proteins, and other intrinsically disordered proteins (IDPs), if any (Yamaguchi et al., 2012; Tanaka et al., 2015; Hashimoto et al., 2016; Boothby et al., 2017). These molecules have been recently identified in anhydrobiotic tardigrades and are involved in their desiccation



**Table 2. Life history traits of generation 2 *Acutuncus antarcticus* hatched from early and late development eggs irradiated at 1.29 or 2.58 kJ m<sup>-2</sup> UV doses**

Life history trait	UV dose: 1.29 kJ m <sup>-2</sup>		UV dose: 2.58 kJ m <sup>-2</sup>	
	Early eggs	Late eggs	Early eggs	Late eggs
Life span (days)				
<i>N</i>	9	13	2	5
Mean±s.d.	32.6±42.0	57.6±35.5	52.5±50.2	41.0±34.7
Median	17.0 (7.0; 51.5)	58.0 (22.5; 86.5)	52.5 (17.0; n.p.)	37.0 (8.5; 75.5)
Number of moults				
<i>N</i>	9	13	2	5
Mean±s.d.	1.9±3.3	3.9±3.7	4.5±5.0	2.6±4.0
Median	0 (0; 4.5)	3.0 (0; 8.0)	4.5 (1.0; n.p.)	0 (0; 6.5)
Age at first oviposition (days)				
<i>N</i>	2	5	1	2
Mean±s.d.	14.0±0	35.8±23.0	16.0±n.p.	27.5±14.8
Median	14.0 (14.0; 14.0)	35.0 (13.5; 58.5)	16.0 (16.0; 16.0)	27.5 (17.0; n.p.)
Age at last oviposition (days)				
<i>N</i>	2	5	1	2
Mean±s.d.	86.0±43.8	69.8±29.8	42.0±n.p.	68.0±22.6
Median	86.0 (55.0; n.p.)	79.0 (44.5; 90.5)	42.0 (42.0; 42.0)	68.0 (52.0; n.p.)
Oviposition number per life span				
<i>N</i>	9	13	2	5
Mean±s.d.	0.7±1.3	1.3±2.0	2.0±2.8	1.8±2.7
Median	0 (0; 1.5)	0 (0; 2.5)	2.0 (0; n.p.)	0 (0; 4.5)
Interval of time among ovipositions (days)				
<i>N</i>	4	11	3	7
Mean±s.d.	36.0±40.0	15.0±12.4	8.7±2.9	11.6±7.4
Median	20.5 (10.0; 77.5)	9.0 (7.0; 18.0)	7.0 (7.0; n.p.)	8.0 (7.0; 16.0)
Number of eggs per female per life span (fecundity)				
<i>N</i>	9	13	2	5
Mean±s.d.	0.7±1.3	1.6±2.4	2.5±3.5	1.8±2.7
Median	0 (0; 1.5)	0 (0; 3.5)	2.5 (0; n.p.)	0 (0; 4.5)
Number of eggs per clutch (fertility)				
<i>N</i>	6	17	4	9
Mean±s.d.	1.0±0.0	1.2±0.6	1.3±0.5	1.0±0.0
Median	1.0 (1.0; 1.0)	1.0 (1.0; 1.0)	1.0 (1.0; 1.8)	1.0 (1.0; 1.0)

Medians are presented with 25th and 75th percentiles in parentheses. *N*, sample size; n.p., not present.

tolerance (Yamaguchi et al., 2012; Tanaka et al., 2015; Hashimoto et al., 2016; Boothby et al., 2017).

In addition to its capability of entering anhydrobiosis, *A. antarcticus* is also able to tolerate freezing of its habitat through cryobiosis (Altiero et al., 2015), as also demonstrated by the survival of specimens after more than 30 years of storage at -20°C within a frozen moss sample (Tsujiimoto et al., 2016). These two cryptobiotic capabilities, together with a short life cycle and reproduction via thelytokous parthenogenesis (Altiero et al., 2015), allow *A. antarcticus* to synchronize growth and reproduction with favourable environmental conditions, and to persist in habitats where conditions can change unpredictably even within the same day, such as the Antarctic ponds in which it lives. These features also play an important role in its dispersal, which occurs by a passive transport via avian vectors or wind, as supported by the genetic similarity of *A. antarcticus* collected across continental Antarctica (Cesari et al., 2016).

### Tolerance to temperature and UV radiation

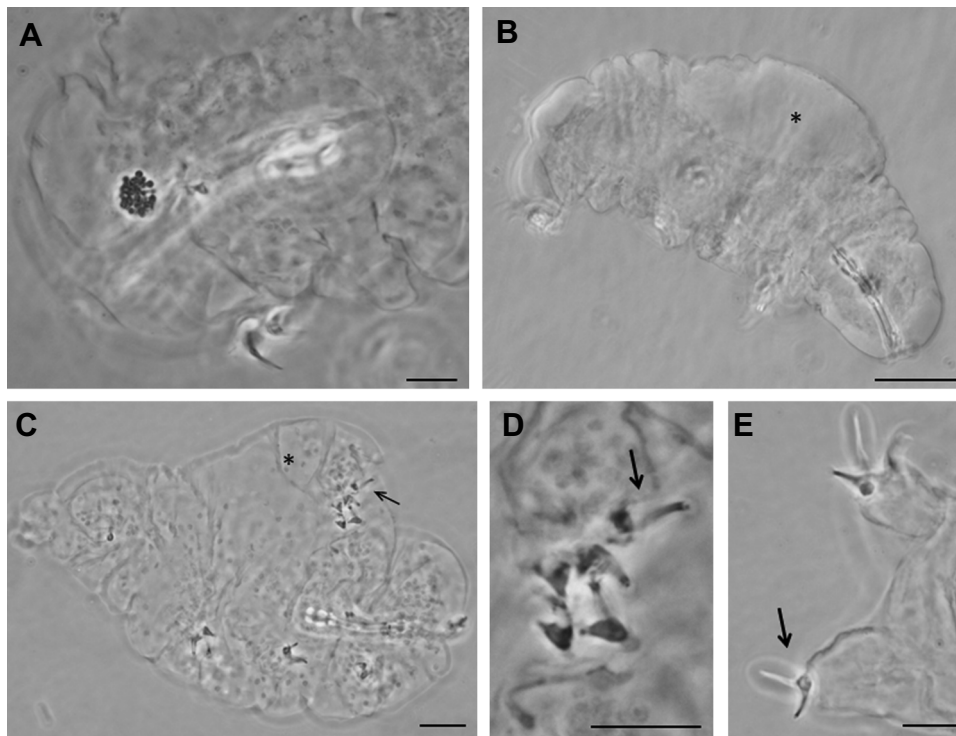
As a consequence of global warming, *A. antarcticus* could be exposed to increasing temperatures in the water ponds in which it lives. In this study, we demonstrated that hydrated animals show a high ability to withstand an acute heat shock (up to 33°C). This ability is unexpected and noteworthy, but it represents a valuable physiological flexibility that allows *A. antarcticus* to acquire thermo-tolerance; this capability could represent an adaptation to 'high temperature' (22°C; Convey, 1996) for Antarctica. Therefore,

increasing temperature per se should not be the limiting factor for survivorship and persistence of *A. antarcticus* in continental Antarctica.

The heat stress resistance of *A. antarcticus* could be related to its anhydrobiotic ability as the molecules and biochemical mechanisms involved in desiccation tolerance could also be involved in thermo-tolerance (for reviews, see Rebecchi et al., 2007; Rebecchi, 2013). A similar heat stress resistance was found in other eutardigrades, such as the limnic boreo-alpine *Borealibius zetlandicus* (LT<sub>max</sub>=37°C; Rebecchi et al., 2009b), *Mesobiotus harmsworthi* from temperate regions (LT<sub>max</sub>=38°C; Li and Wang, 2005) and the marine *Halobiotus crispae* from the North Sea and from Arctic and Sub-Arctic regions (LT<sub>max</sub>=36°C; Halberg et al., 2013). These data may be somehow surprising, as these species colonize different kinds of habitats at different latitudes or altitudes, but all of them experience unpredictable fluctuations in the temperature values of their habitat.

In the future, organisms living in Antarctica could be exposed to an increase in UV radiation owing to ozone depletion (Solomon et al., 2014). Experiments carried out in our study showed that both hydrated and desiccated *A. antarcticus* exhibited a good tolerance to the increase in UV radiation. Nevertheless, animals in both physiological conditions showed a decrease in final survival inversely proportional to the increase in UV exposure. In addition, desiccated specimens of *A. antarcticus* survived better at high doses of UV radiation than hydrated specimens, as found in another tardigrade species, *Ramazzottius varieornatus* (Horikawa





**Fig. 7. Morphological defects in *A. antarcticus* specimens born from irradiated eggs and in their offspring.** (A) Anterior region of an animal (generation 1) with the right eyespot only. (B) *In toto* specimen (generation 1) showing a swelling on the back of its body (asterisk). (C) *In toto* specimen (generation 1) with a ventral swelling (asterisk), and the supernumerary claws (arrow). (D) Magnification of the supernumerary claws. (E) The fourth pair of legs (generation 2), showing a half of the double claw missing on the right leg (arrow). Scale bars: (A, C–E) 10  $\mu\text{m}$  (phase contrast); (B) 50  $\mu\text{m}$  [differential interference contrast (Nomarski)].

et al., 2013). Because anhydrobiotic tardigrades have very low water content with consequently undetectable metabolism and high levels of bioprotectants and antioxidant molecules (Mali et al., 2010; Rizzo et al., 2010; Guidetti et al., 2011a; Bonifacio et al., 2012; Horikawa et al., 2013; Rebecchi, 2013; Boothby et al., 2017), they were expected to be less damaged than hydrated tardigrades after UV radiation. The UV radiation per se should not represent a limiting factor for the survivorship of *A. antarcticus*, as the decline in survival of animals in both physiological states occurred when specimens were exposed to the highest tested UV doses, which should not be reached in the natural environment (unfortunately, data on UV radiation at ground level of Victoria Land are not available). Nevertheless, a negative synergic effect on survivorship of *A. antarcticus* was found when hydrated and desiccated specimens were simultaneously exposed to UV radiation (i.e. the LD<sub>50</sub> doses of hydrated and desiccated animals) and relatively high temperature, indicating that temperature has a strong impact on tardigrade survival during UV exposure. A similar negative synergic effect has been recorded in other organisms such as nematodes and cladocerans (Jagdale and Grewal, 2007; Connelly et al., 2009), and in other tardigrade species from temperate regions such as *Paramacrobrotus richtersi* and *Ramazzottius oberhaeuseri* (see Altiero et al., 2011). Moreover, as in the latter two species, hydrated specimens of *A. antarcticus* tolerated the combination of UV radiation and temperature similarly or even better than desiccated specimens. A possible explanation of the higher resistance of the hydrated tardigrades with respect to the desiccated ones could be related to the fact that they are metabolically active during the irradiation and they can immediately and continuously repair damaged biological molecules or destroy harmful molecules (e.g. reactive oxygen species) produced during the desiccation stress. This explanation is also supported by the similar viability trends recorded at  $t_0$ ,  $t_1$  and  $t_{24}$  in hydrated specimens of *A. antarcticus* after UV irradiation. As regards the desiccated animals, we detected a very low survival

when they were exposed to UV (LD<sub>50</sub>) and relatively high temperature. The LD<sub>50</sub> UV dose used might not be appropriate because it came from viability data of animals with very large standard deviations (Fig. 3B), and the animal survival at 8°C varied far from 50% as expected. Nevertheless, the viability trends indicate that desiccated animals have a higher sensitivity to the simultaneous exposition to UV radiation and temperature. This probably occurs because desiccated animals are metabolically and/or biochemically inactive and, consequently, they accumulate damaged molecules, the amount of which is positively affected by the synergistic effect of temperature, dose of irradiation and time spent in an anhydrobiotic state. For this reason, animals need recovery time to repair molecular damage after coming back to active life after rehydration, as found in previous stress experiments (Rebecchi et al., 2006, 2009a,b; Altiero et al., 2011; Guidetti et al., 2011a).

These results strengthen the assumption that the combination of several stresses could drastically reduce the survivability of an organism. Therefore, increasing temperature together with increasing UV radiation owing to global climate change could be factors limiting the capability of *A. antarcticus* to survive and persist in Antarctica in the long term.

#### Life cycle of *A. antarcticus* under UV radiation stress

The negative effects of UV radiation in different stages of the *A. antarcticus* life cycle (e.g. embryonic development eggs and offspring) have been demonstrated. In particular, a lower hatching success and a delay in the hatching time of irradiated eggs with respect to the non-irradiated eggs (for data on non-irradiated eggs, see Altiero et al., 2015) were observed. The early and late development eggs in the hydrated state were more sensitive to UV radiation than hydrated animals, as no irradiated eggs were able to hatch when exposed to the LD<sub>50</sub> of hydrated animals. A similar pattern was observed in the eutardigrade *Richtersius coronifer* after irradiation with gamma rays (Jönsson et al., 2013). Moreover, the early development eggs appear to be more sensitive to UV radiation

than the late development eggs, as the increase of UV dose causes a delay in the hatching time of early development eggs. Similar results were obtained in the eutardigrades *Hypsibius dujardini* and *Milnesium cf. tardigradum* after irradiation with gamma rays (Beltrán-Pardo et al., 2013, 2015). All these data are fully in line with the common knowledge that the radiosensitivity of a cell is directly proportional to its mitotic activity and, therefore, that developing eggs and tissues are the most harmed by radiation (see Jönsson et al., 2013). In addition, radiation tolerance of tardigrades may not be a feature present from the start of the embryonic development, but may be acquired during embryonic development, by biochemical mechanisms to repair damaged molecules that are completed or activated only toward the end of development. As it is known that tardigrades have an efficient set of molecules working as antioxidants (Neumann et al., 2009; Mali et al., 2010; Rizzo et al., 2010; Bonifacio et al., 2012) or acting as DNA protectants (Horikawa et al., 2013; Hashimoto et al., 2016), the increase in the synthesis and activity of these molecules during the egg development of *A. antarcticus* could be responsible for the different tolerance to UV radiation, similarly to that shown in the larvae of the chironomid *Polypedilum vanderplanki* (see Gusev et al., 2010).

A negative effect of UV radiation on the life cycle of *A. antarcticus* was also elucidated by alterations in the life history traits of newborns hatched from UV irradiated eggs and their offspring. Indeed, animals able to complete their life cycle and to produce eggs reached sexual maturity later, laid fewer eggs and fewer times during their life span, and with a lower frequency of oviposition, compared with the animals born from non-irradiated eggs (for data on non-irradiated eggs, see Altiero et al., 2015). Similar results were found in the eutardigrade *H. dujardini*, in which animals hatched from irradiated eggs laid a reduced number of eggs (Beltrán-Pardo et al., 2015). Similarly, in the eutardigrades *R. coronifer* and *M. cf. tardigradum*, gamma radiation affected the embryonic development, as the eggs laid by irradiated animals did not hatch (Jönsson et al., 2005; Horikawa et al., 2006). Finally, egg reabsorption and morphological deformities observed in some newborns from irradiated eggs of *A. antarcticus* and in their offspring further confirm that UV radiation affects the life cycle of this species. Egg reabsorption events are relatively common in tardigrades and have been observed in another population of *A. antarcticus* (Tsujimoto et al., 2015), in *P. richtersi* (T.A., unpublished observation) and in *Isohypsibius dastychi* (Bingemer et al., 2016), although eggs or animals were not irradiated. In *P. richtersi*, egg reabsorptions were sometimes observed in old animals, whereas in *I. dastychi*, reabsorptions occurred when the mating did not occur. In a previous study of the life cycle of *A. antarcticus*, egg reabsorption events were not observed (Altiero et al., 2015); therefore, we believe that the remarkable increase of egg reabsorptions observed in the present research was due to UV irradiation.

Furthermore, this is the first study that shows teratological events in tardigrades exposed to radiation. The higher number of morphological defects in generation 1 with respect to generation 2, and in animals hatched from eggs irradiated at the highest UV dose (2.58 kJ m<sup>-2</sup>), indicates that UV radiation affects the cells of embryo that will differentiate as germinal cells in the adult animal, with the consequent transmission of damages to the following generations. The negative impact of UV radiation on life history traits of *A. antarcticus* could lead this species to be at risk of population reductions or even extinction. To counteract this risk, and persist in space and time, *A. antarcticus* has to adapt to the new environmental conditions that will gradually occur as a consequence

of global climate change, even evolving its reproductive strategy (Altiero et al., 2015).

## Conclusions

*Acutuncus antarcticus* has been proven to well tolerate individual stress events, such as desiccation, temperature increase and UV radiation. Nevertheless, UV radiation has a negative impact on its life history traits, and the survivorship of *A. antarcticus* is strongly affected by the synergistic action of increasing temperature and exposure to UV radiation. In the long run, the deleterious effects of global climate change could lead *A. antarcticus* to be at risk of population reductions or even extinction. Nevertheless, assuming that global climate change will proceed gradually and that an overlapping of temperature and UV increase could be limited in time (and in value), *A. antarcticus*, as well as many other Antarctic organisms, could have the potential to overcome stress conditions owing to global warming, and/or the time and capability to adapt to the new environmental conditions.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: I.G., T.A., R.G., L.R.; Methodology: I.G., T.A., R.G., L.R.; Formal analysis: I.G., T.A.; Resources: R.G., L.R.; Writing - original draft: I.G., T.A.; Writing - review & editing: R.G., L.R.; Supervision: L.R.; Funding acquisition: R.G., L.R.

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