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BIOLOGY OF THE ROSS SEA

Life history traits and reproductive mode of the tardigrade *Acutuncus antarcticus* under laboratory conditions: strategies to colonize the Antarctic environment

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Abstract Global climate change has become an important issue, particularly for organisms living in the Antarctic region, as the predicted temperature increase can affect their life history traits. The reproductive mode and life history traits of one of the most widespread species of tardigrades in Antarctica were analyzed. Specimens of the eutardigrade *Acutuncus antarcticus* from a temporary freshwater pond at Victoria Land (Antarctica) were individually cultured. This species reproduced continuously by thelytokous meiotic parthenogenesis. Its life cycle was short (60–90 days) and the reproductive output was low, with a short generation time (25–26 days). The maternal effect can be responsible of the phenotypic plasticity observed in life history traits of the three analyzed generations that may be seen as a bet-hedging strategy, as also observed in other animals inhabiting stochastic environments. These traits, along with the cryptobiotic capability of *A. antarcticus*, are advantageous for exploiting the conditions suitable for growth and reproduction during the short Antarctic summer, and can explain its wide distribution on the Antarctic continent. These results open new avenues of research for determining the role of bet-hedging strategy in organisms living in unpredictable environments.

Keywords Karyotype · Antarctica · Life history traits · Reproduction · Parthenogenesis · Stochastic habitats · Adaptive strategies

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Introduction

Invertebrates living in extreme environments (e.g., polar regions, deserts, and high elevations), as well as those living also at temperate latitudes in stochastic habitats (e.g., mosses, lichens, and temporary ponds), are subject to desiccation or freezing and must be able to withstand strong environmental stresses. Organisms can respond to these stresses using regulative, acclimation, developmental, and evolutionary responses that are linked to different levels of hierarchical organization (Willmer et al., 2000; Everatt et al., 2014). In addition, environmental stresses can produce substantial modifications in the structure of biological communities, even leading to modifications of the functional integrity of the ecosystems (Irons et al., 1993).

Global climate change has strong repercussions on all organisms. In particular, the increase of mean and maximum air temperature values has a higher impact on organisms living in areas in which the temperature is normally close to 0°C, such as polar and alpine regions. In these areas even small changes can have remarkable consequences on the survivability of organisms. Therefore, organisms must be able to adapt to the new environmental conditions in order to avoid the risk of extinction or the risk of dramatic reduction in population numbers, with a subsequent reduction in biodiversity (Wall, 2007).

Continental Antarctica has the coldest, windiest, and driest environmental conditions of all other continents. Most of the continental Antarctic areas are permanently covered by snow or ice, with ice-free terrestrial habitats restricted to coasts and inland nunataks, forming less than the 0.5% of the total surface area. Therefore, the biota of terrestrial habitats is depauperate. Cryptogams form the dominant vegetation, whilst animal communities are almost entirely invertebrates, dominated by meiofaunal organisms such as nematodes, rotifers, and tardigrades, and a few arthropods. These communities experience temperatures well below zero in all months of the year, and the duration of temperatures suitable for life cycle activities may be restricted to only some weeks (Convey, 1997; Everatt et al., 2014). The predicted temperature increase of 0.34°C per decade across Antarctica (Bracegirdle et al., 2008), and an increase in the frequency of localized short-term extreme temperature values and precipitation events (e.g., in Victoria Land; Krinner et al., 2007) due to global climate change, can affect the life history traits of Antarctic organisms. These events can lead to global/local increase or extinction of species/populations able or unable to adapt to the new environmental conditions.

Relatively few studies have been done on life history traits of terrestrial Antarctic species and on the role of phenotypic plasticity in their ability to cope with environmental changes. These studies were mainly devoted to arthropods (collembolans, insects, mites; Burn, 1981, 1984; Sugg et al., 1983; Lee Jr., 1989; Block & Convey, 1995) and nematodes (Overhoff et al., 1993; Moorhead et al., 2002; Yeates et al., 2009). Tardigrades are an important element of the continental Antarctic meiofauna and are one of the few animal groups highly abundant in Antarctica, but only preliminary data have been collected on life history traits of a population of the eutardigrade *Acutuncus antarcticus* (Richters, 1904) (previously reported as *Hypsibius arcticus*) collected at Ross Island (Dougherty, 1964). Continental Antarctic tardigrades colonize both terrestrial (lichens, mosses, detritus, organic material) and freshwater (sediment, algae, aquatic plants, and biofilm) habitats of the coastal snow-free areas, nunataks, or even cryoconite holes (Velasco-Castrillón et al., 2014). Tardigrades are always aquatic animals, and are able to survive the absence of "free" water due to their ability to temporarily suspend metabolism and consequently active life. This process, generically named cryptobiosis, involves a series of adaptive strategies to withstand adverse environmental conditions such as freezing (cryobiosis) or dehydration (anhydrobiosis). These phenomena are immediately reversed by removal of the adverse external stimuli (for review see Guidetti et al., 2011; Møbjerg et al., 2011; Wełnicz et al., 2011). *Acutuncus antarcticus* is one of the most widespread species in continental and maritime Antarctica where it is endemic, although it was once reported from Argentina (Claps et al., 2008). Even though it colonizes different kinds of substrates (e.g., algal or bacterial mats in lakes and temporary freshwater ponds, mosses), it is very common and abundant in freshwater ponds (Everitt, 1981; Dastych, 1991; McInnes, 1995; Kagoshima et al., 2013). The capability of *A. antarcticus* specimens to enter anhydrobiosis and cryobiosis (Dougherty, 1964; Giovannini et al., 2013) allows them to survive desiccation and freezing of their habitats. Nevertheless, to understand how this species persists and colonizes Antarctic habitats it is important to have information on its reproductive mode and on the features of its life history traits. Considering that thelytokous parthenogenesis is the most widespread mode of reproduction among tardigrades in terrestrial and freshwater habitats (Bertolani, 1982; Rebecchi et al., 2003) and that parthenogenesis allows a rapid increase of the population size, we suggest that *A. antarcticus* reproduces by thelytokous parthenogenesis to better exploit the conditions suitable for growth and reproduction during the short Antarctic summer. To take full advantage of the short period of time during which the water is available at liquid phase, we hypothesize that *A. antarcticus* has a short life span, an early age at first oviposition, a short interval of time between two successive ovipositions, a short hatching time and therefore a short generation time, similarly to what occurs in other Antarctic

invertebrate species (see Goddard, 1979; West, 1982; Convey, 1996; Yeates et al., 2009). To verify these hypotheses, we identified the reproductive mode of *A. antarcticus* and analyzed its life history traits. In particular, active life span, number of molts, age at first and last oviposition, number of ovipositions per life span, number of laid eggs per life span, number of eggs per clutch, time interval between ovipositions, egg hatching time and egg hatching percentage were determined and compared among generations of *A. antarcticus*. These comparisons allow to evaluate the possible presence of phenotypic plasticity at population level and whether it changes among generations as a strategy to cope with extreme environmental conditions which can vary even within the same day, therefore assuring survival and fitness in space and time.

Materials and methods

Species characteristics and sampling procedure

The eutardigrade *A. antarcticus* (Fig. 1a, b) belongs to the Hypsibiidae (Parachela, Hypsibioidea) (Bertolani et al., 2014) was used. This eutardigrade is an herbivorous and bacteriophagous species (Dougherty, 1964; Kagoshima et al., 2013; Guidetti, personal observation).

The substrate containing specimens of *A. antarcticus* utilized in this study was collected from a temporary freshwater pond (sample code C3402) close to the Italian Antarctic base at Victoria Land (125 m a.s.l., 74°42.580′S, 164°06.086′E, Terranova Bay, Antarctica) at the beginning of January 2011. In this area the species was in practice present and abundant only in freshwater ponds (Guidetti, personal communication).

At the time of sampling, the pond contained several centimeters of water and its temperature was 9.5°C. By the end of January this pond was completely dried out. The collected substrate was a reddish bacterial mat mixed with small gravel that covered all the bottom surface. Specimens of *A. antarcticus* dominated the substrate, but other tardigrade species were present: *Macrobiotus polaris* Murray, 1910, *Milnesium antarcticum* Tumanov, 2006, and *Diphascon* cf. *sanae*. The substrate was maintained frozen (−20°C) until it was processed in the laboratory of Evolutionary Zoology at the University of Modena and Reggio Emilia.

Tardigrade rearing method

The availability of tardigrades in culture is very useful to obtain information on their reproductive mode and life history traits (Altiero & Rebecchi, 2001; Kagoshima et al., 2013; Schill, 2013). To create a culture pool (microcosm) of *A. antarcticus*, several specimens were removed from the thawed substrate collected at Victoria Land and placed in a flask with algal culture medium in spring mineral water (volume ratio 1:3) and food. Once a week, the culture medium was partly changed and food was added.

To collect data on life history traits of *A. antarcticus*, animals of the parental generations were collected from the microcosm. Animals were individually cultured in small plastic boxes (15 mm in diameter and 7 mm in height) with spring mineral water. The uncovered culture boxes were placed in a wet chamber consisting of a covered larger glass Petri dish containing a film of water to avoid desiccation of the culture boxes. The Petri dishes with culture boxes were kept at 14°C with a photoperiod 12 h/12 h (L/D). Animals were monitored three times a week under a stereomicroscope, with a gap of two days as a maximum. During each check, a part of water contained in each plastic culture box was changed, food was added, and the presence of laid eggs was monitored. The microcosm and boxes with a single tardigrade in each were kept at 14°C with a photoperiod 12 h/12 h (L/D). The unicellular alga *Chlorococcum* sp. was used as source of food ad libitum.

The laid eggs were isolated in individual plastic boxes containing only water until they hatched. The eggs were kept at the same laboratory conditions as the adult animals. Eggs were monitored three times a week under a stereomicroscope, with a gap of two days as a maximum, and water was partly changed at every check. The newborns were transferred into new plastic boxes, and reared individually from birth to death at the same laboratory conditions described above.

Collection of data on life history traits

To analyze the life history traits of *A. antarcticus*, animals belonging to three successive generations (*P*,

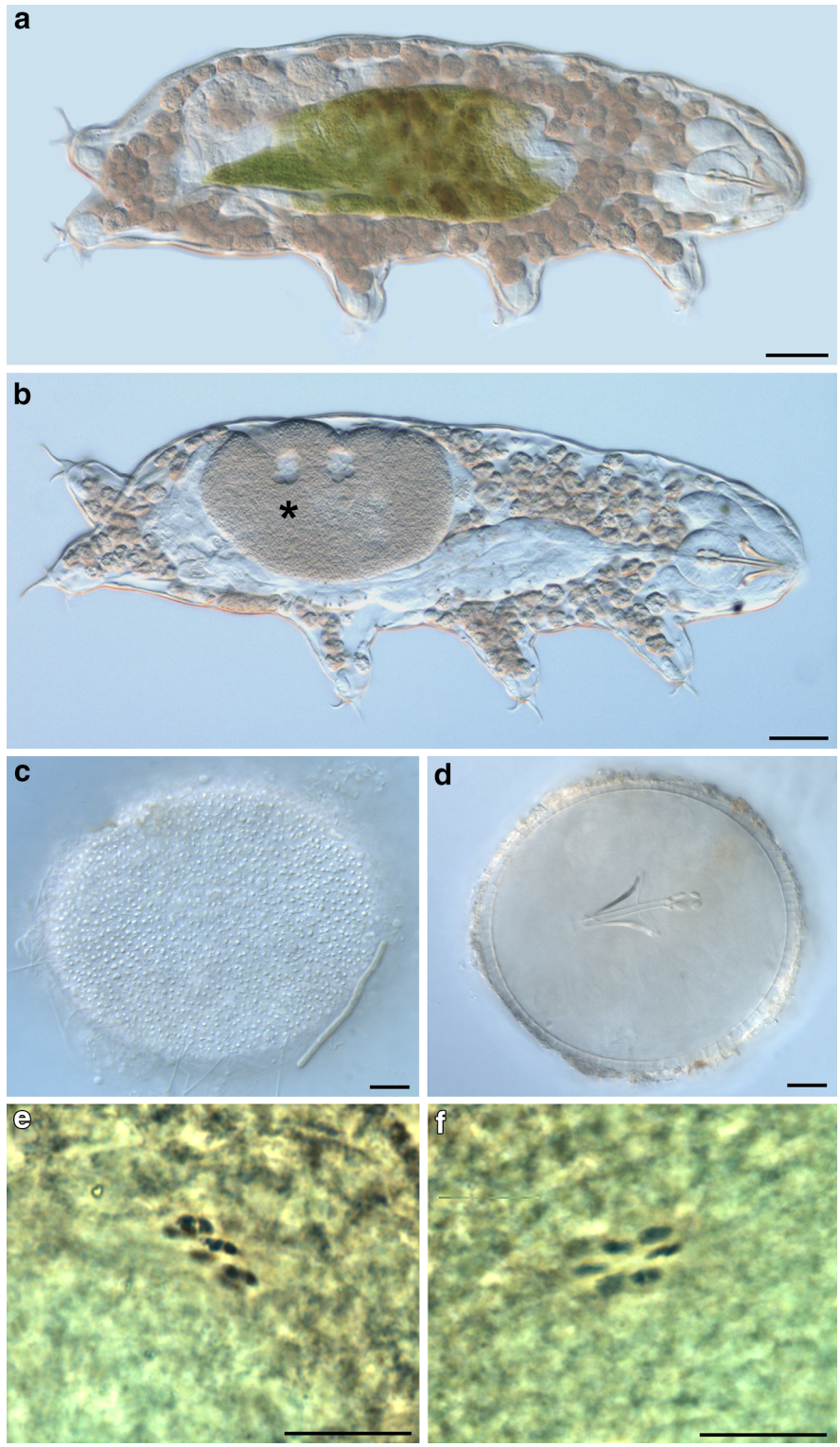


Fig. 1 *Acutuncus antarcticus.* **a** In vivo adult specimen with the body cavity almost filled by a large midgut full of food (LM, DIC). **b** In vivo adult specimen with an ovary containing large oocytes (*asterisk*) and a flat and empty midgut (LM, DIC). **c** In toto egg showing the characteristic ornamentation of the shell surface (LM, DIC). **d** In toto egg showing the shell made up by two thin layers separated by short and rod-like processes and the buccal-pharyngeal apparatus of a late embryo (LM, DIC). **e**, **f** Six bivalents in the oocytes (Orcein staining, LM, PhC). **a**–**f**: *Scale bars* 10 µm

F_1, and F_2) were used. The parental generation (P) was represented by 12 adult females with oocytes in their gonad collected from the culture microcosm, and individually reared for a month. The F_1 and F_2 generations were composed of all offspring generated from two females belonging to P generation. Data on life history traits of F_1 and F_2 generations were collected on the following biological and reproductive characteristics: active life span, number of molts, age at first and last oviposition, number of ovipositions per life span, number of laid eggs per life span (fecundity), number of eggs per clutch (fertility), interval of time between ovipositions, egg hatching time and egg hatching percentage. For the P generation, only the interval of time between ovipositions, egg hatching time, and hatching percentage were determined.

Statistical analysis

To evaluate the presence of phenotypic plasticity at the population level and whether it changes among generations of *A. antarcticus*, a statistical comparison of each life history traits among generations was made. First, the Shapiro–Wilk normality test was carried out to evaluate if the data collected for each life history traits of each generation (P, F_1, F_2) do or do not show a normal distribution. Since life span, age at first and last oviposition, fecundity and time interval between ovipositions exhibited a normal distribution in all analyzed generations, comparison of each trait between F_1 and F_2 generations was made using the parametric t test. Number of molts, number of ovipositions, fertility, egg hatching time, and egg hatching percentage did not exhibit a normal distribution, therefore the non-parametric U Mann–Whitney test was used to compare these traits between the F_1 and F_2 generations. Egg hatching time among all studied generations (P, F_1, F_2) was compared with the non-parametric Kruskal–Wallis test. To evaluate the relationships between the age of the mother and the number of eggs per oviposition in each of the considered generations (F_1 and F_2) the non-parametric Spearman correlation test was used. All statistical analysis was carried out using the SPSS 20 program.

Evaluation of the reproductive mode

The reproductive mode in tardigrades is defined by evaluating the sex ratio through the analyses of the type of germinal cells found within the gonad, the type of maturation of oocytes (meiotic or ameiotic) and the karyotype (Rebecchi et al., 2003). To define the reproductive mode of the population of *A. antarcticus* from Victoria Land, 67 specimens were collected from microcosm, fixed *in toto* in Carnoy fluid (methanol: acetic acid, 3:1), individually mounted on slides, and stained with a drop of acetic-lactic orcein. All slides were examined with a Leitz DM RB light microscope (LM) equipped with phase contrast (PhC), and differential interference contrast (DIC) objectives. Micrographs of in vivo adult animals, eggs, and chromosomes were produced with a Polaroid DMC Ie LowLight Kit camera.

Results

Analysis of the gonad content of the population of *A. antarcticus* from Victoria Land allowed us to recognize cells belonging to female germinal cell line in only 41 out of 67 animals stained with acetic-lactic orcein. Several of them showed an ovarium containing large oocytes ready to be laid (Fig. 1b). The gonad of the other 26 animals contained only undifferentiated germinal cells. Male germinal cells were never observed. Karyological analysis showed six bivalents ($n = 6$) in the oocytes in metaphase I and at late vitellogenesis. These chromosomes were morphologically very similar to each other (Fig. 1e, f). Chiasmata were not observed in prophase I of the oocytes. The active life span of *A. antarcticus* reached a maximum value of 130 days. In general, adult females laid eggs once a week, and they molted before every egg oviposition. Each female laid eggs freely (Fig. 1c, d) or, rarely, into the old cuticle (exuvium). The number of eggs per oviposition (1–4 eggs) increased significantly with the age of the mother in both considered generations (Spearman correlation test: F_1, $n = 139$, $P < 0.01$; F_2, $n = 91$, $P < 0.05$). Throughout her lifespan, each female laid up to 20 eggs. Old females stopped laying eggs, in general, at the age of about 30–50 days. Newborns molted once, rarely twice, before their first oviposition at the age of about 17 days. The offspring of *A. antarcticus* were all females; newborns were reared individually and were able to reach sexual maturity and lay eggs capable of hatching.

Table 1 Life history traits of *Acutuncus antarcticus*

Life history traits	Generation		
	P	F_1	F_2
Life span (days)			
Sample size		22	43
Mean ± SD		88.8 ± 20.0	49.5 ± 26.4
Median (percentiles 25; 75)		91.0 (76.3; 105.8)	49.0 (32.0; 71.0)
Number of molts			
Sample size		22	33
Mean ± SD		7.3 ± 1.0	3.9 ± 2.4
Median (percentiles 25; 75)		7.0 (7.0; 8.0)	4.0 (2.0; 5.0)
Age at first oviposition (days)			
Sample size		22	30
Mean ± SD		17.1 ± 3.6	16.9 ± 3.5
Median (percentiles 25; 75)		17.0 (14.0; 18.3)	16.0 (14.0; 19.3)
Age at last oviposition (days)			
Sample size		22	30
Mean ± SD		54.3 ± 10.6	30.6 ± 12.5
Median (percentiles 25; 75)		53.0 (49.0; 60.5)	27.0 (21.0; 40.0)
Oviposition number per life span			
Sample size		22	30
Mean ± SD		6.4 ± 1.0	3.0 ± 1.9
Median (percentiles 25; 75)		7.0 (5.8; 7.0)	2.5 (2.0; 4.3)
Interval of time among ovipositions (days)			
Sample size	44	182	61
Mean ± SD	6.7 ± 2.3	6.3 ± 2.1	6.9 ± 3.5
Median (percentiles 25; 75)	6.0 (5.0; 7.0)	7.0 (5.0; 7.0)	7.0 (4.0; 8.0)
Number of eggs per female per life span (fecundity)			
Sample size		22	30
Mean ± SD		13.0 ± 3.7	5.3 ± 4.4
Median (percentiles 25; 75)		13.0 (10.0; 16.0)	4.0 (2.0; 8.0)
Number of eggs per clutch (fertility)			
Sample size		251	92
Mean ± SD		1.8 ± 0.8	1.7 ± 0.8
Median (percentiles 25; 75)		2.0 (1.0; 2.0)	2.0 (1.0; 2.0)
Hatching time (days)			
Sample size	88	266	50
Mean ± SD	8.9 ± 1.9	8.8 ± 2.6	7.7 ± 1.6
Median (percentiles 25; 75)	9.0 (8.0; 10.0)	9.0 (7.0; 10.0)	7.0 (7.0; 9.0)
Hatching percentage			
Sample size	12	22	30
Mean ± SD	76.1 ± 12.2	67.1 ± 22.7	22.4 ± 27.1
Median (percentiles 25; 75)	74.3 (64.9; 84.7)	73.2 (57.4; 83.7)	16.7 (0.0; 37.5)

SD standard deviation

Table 2 Distribution analysis of the life history traits of the three generations (P, F_1, F_2) of *Acutuncus antarcticus*

Life history traits	Skewness	Kurtosis	Normality test (Shapiro–Wilk)		
			W	*df*	*P*
Life span					
P	–	–	–	–	–
F_1	−0.19	−0.54	0.98	22	n. s.
F_2	0.16	−0.75	0.97	30	n. s.
Number of molts					
P	–	–	–	–	–
F_1	−0.16	−0.07	0.92	22	n. s.
F_2	0.98	0.57	0.90	30	<0.01
Age at first oviposition					
P	–	–	–	–	
F_1	0.95	0.25	0.89	22	<0.05
F_2	0.32	−0.30	0.96	30	n. s.
Age at last oviposition					
P	–	–	–	–	
F_1	1.44	4.66	0.87	22	<0.01
F_2	0.72	−0.67	0.89	30	<0.01
Oviposition number per life span					
P	–	–	–	–	
F_1	–0.56	–0.28	0.87	22	<0.05
F_2	1.08	0.45	0.86	30	<0.01
Interval of time among ovipositions					
P	1.76	3.18	0.79	44	<0.001
F_1	1.16	3.11	0.88	182	<0.001
F_2	1.14	0.83	0.87	61	<0.001
Number of eggs per female per life span (fecundity)					
P	–	–	–	–	
F_1	−0.18	−0.42	0.97	22	n. s.
F_2	1.20	0.53	0.85	30	<0.01
Number of eggs per clutch (fertility)					
P	–	–	–	–	
F_1	0.51	−0.35	0.82	251	<0.001
F_2	1.00	0.53	0.78	92	<0.001
Hatching time					
P	1.69	4.90	0.83	88	<0.001
F_1	2.73	14.52	0.76	266	<0.001
F_2	1.21	5.05	0.77	50	<0.001
Hatching percentage					
P	0.63	−0.52	0.92	12	n. s.
F_1	−0.88	0.60	0.94	22	n. s.
F_2	1.29	1.20	0.82	30	<0.001

P parental generation, F_1 first generation, F_2 second generation, *n.s.* not significant

A detailed analysis of life history traits of P, F_1, and F_2 generations is presented in Table 1 and Figs. 2, 3, 4, 5, 6 and 7. As previously stated, for the P generation only data on the time interval between ovipositions, egg hatching time, and hatching percentage were collected.

Analysis of the P generation life history traits showed a normal distribution in the egg hatching percentage, whereas significant differences from normality with a leptokurtic distribution were noted in the egg hatching time and time interval between ovipositions (Table 2; Figs. 4a, 6a, 7a). The distribution of the F_1 generation life history traits exhibited significant differences from normality with a leptokurtic distribution in the age at first and last oviposition, the time interval between ovipositions, and egg hatching time, and with a platykurtic distribution in fertility and number of ovipositions (Table 2; Figs. 3a, c, e, 4b, 5c, 7b). Life span, number of molts, fecundity, and egg hatching percentage had a normal distribution (Table 2; Figs. 2a, c, 5a, 6b). In the F_2 generation, significant differences in the distribution of the life history traits from normality with a leptokurtic distribution were found in the number of molts, number of ovipositions, time interval between ovipositions, fecundity, fertility, hatching time, and hatching percentage of eggs; whereas a platykurtic distribution was shown in the age at last oviposition (Table 2; Figs. 2d, 3d, f, 4c, 5b, d, 6c, 7c). Only the life span and the age at first oviposition showed a normal distribution (Table 2; Figs. 2b, 3b).

Comparisons between the F_1 and F_2 generations showed significant differences in life span (t test: $t = 6.13$, $P < 0.001$), number of molts (U Mann–Whitney test: $U = 87.50$, $P < 0.001$), age at last oviposition

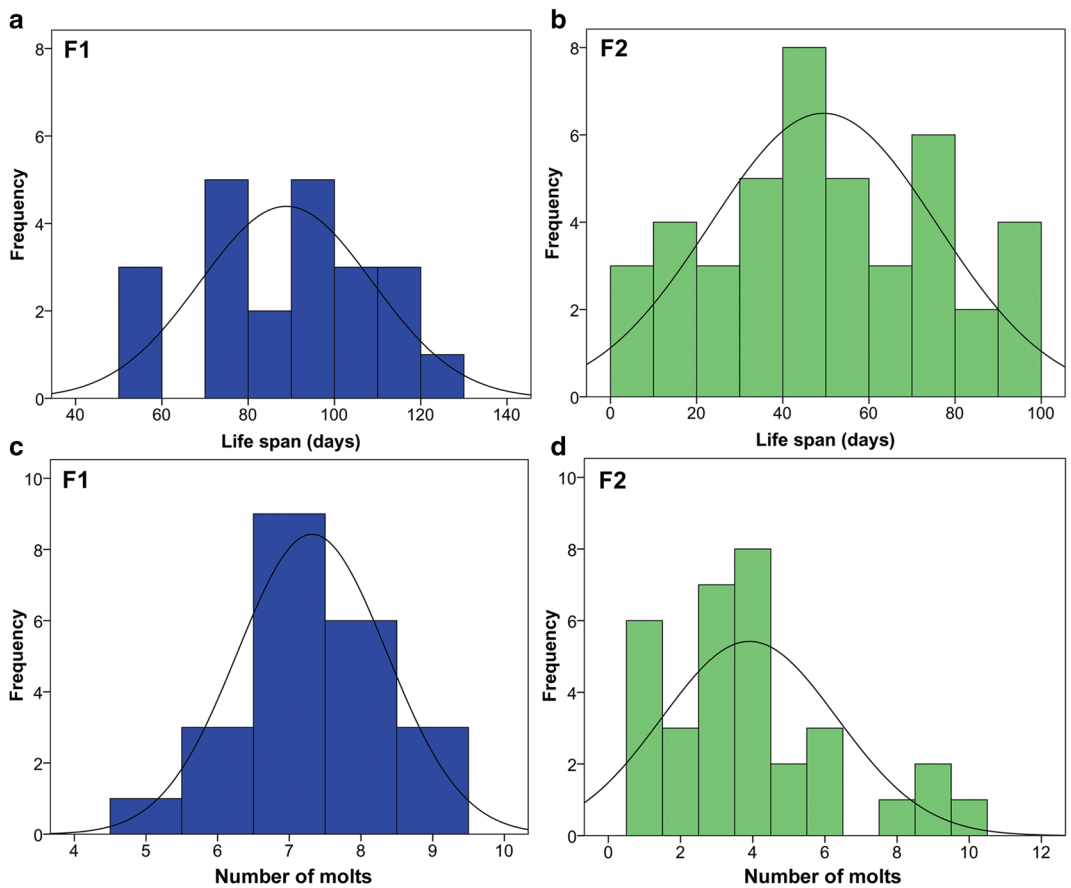


Fig. 2 Distribution of life span and number of molts in F_1 (**a**, **c**) and F_2 (**b**, **d**) generations of the eutardigrade *Acutuncus antarcticus* under rearing conditions

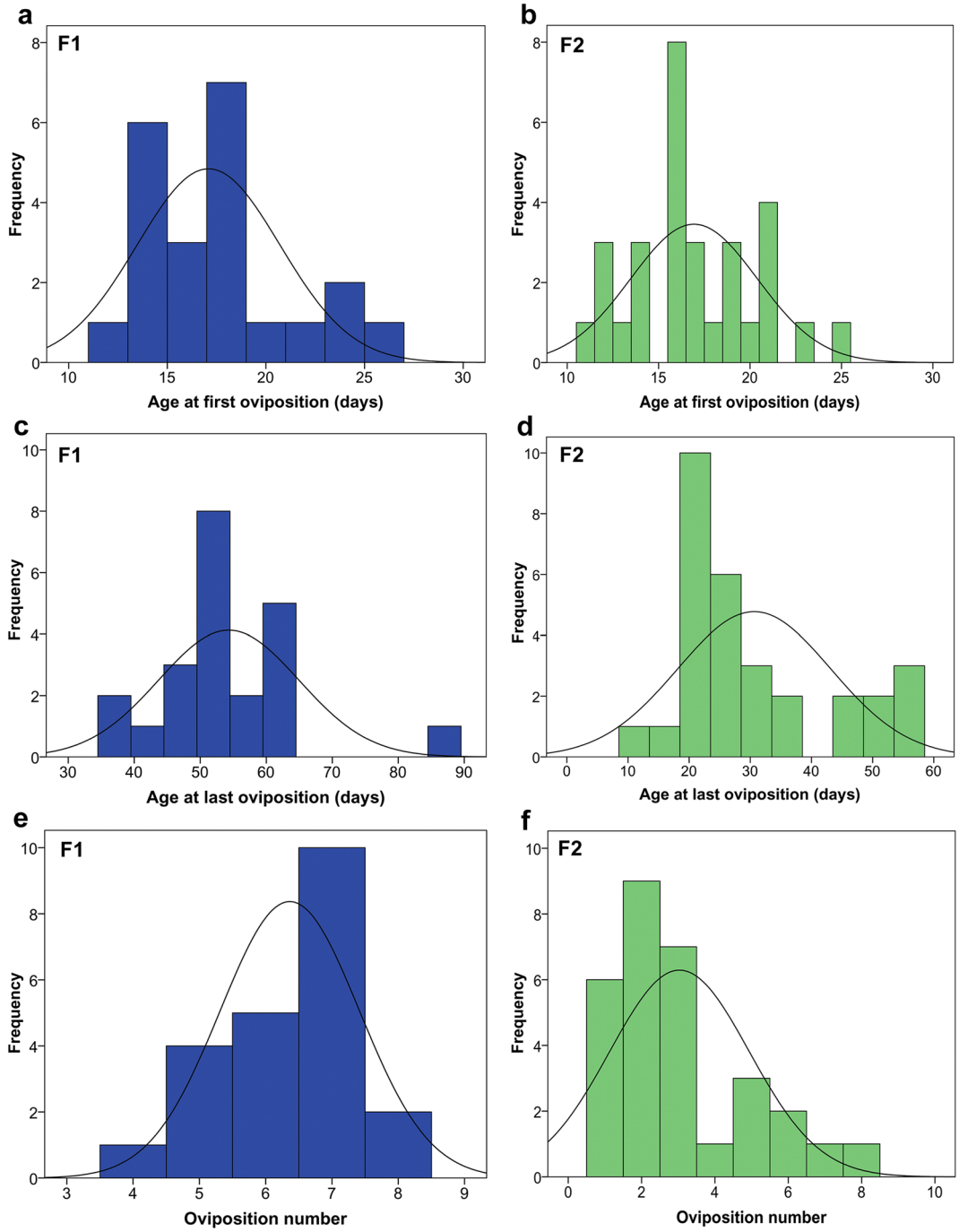


Fig. 3 Distribution of age at first oviposition, age at last oviposition and number of ovipositions in F_1 (**a**, **c**, **e**) and F_2 (**b**, **d**, **f**) generations of the eutardigrade *Acutuncus antarcticus* under rearing conditions

(U Mann–Whitney test: $U = 58.00, P < 0.001$), number of ovipositions (U Mann–Whitney test: $U = 60.50$, $P < 0.001$), fecundity (t test: t = 6.75, $P < 0.001$), egg hatching percentage (U Mann–Whitney test: $U = 76.00$, $P < 0.001$) and egg hatching time [U Mann–Whitney test: U (F_1–F_2) = 63.57, $P < 0.001$]. No significant differences between F_1 and F_2 generations were observed in the age at first oviposition or in fertility.

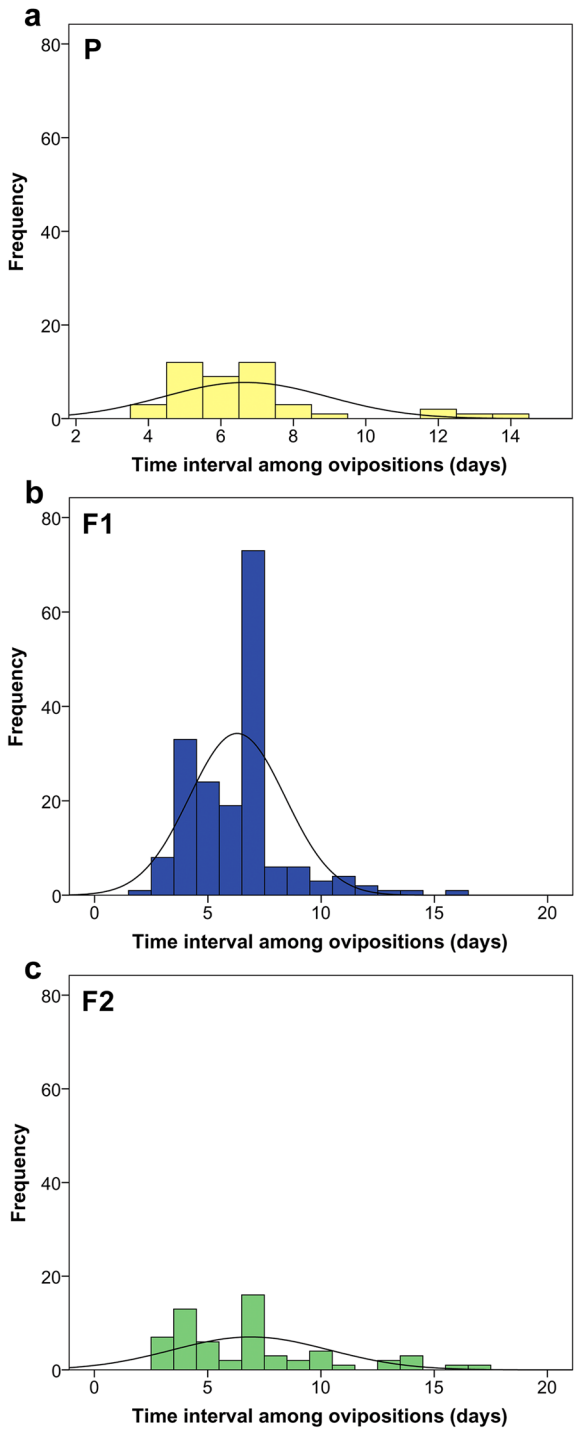


Fig. 4 Distribution of time intervals among ovipositions in *P* (**a**), F_1 (**b**) and F_2 (**c**) generations of the eutardigrade *Acutuncus antarcticus* under rearing conditions

Comparison among the three generations (*P*, F_1, and F_2) did not evidence significant differences in the interval of time among ovipositions. Significant differences among *P*, F_1, and F_2 generations were detected in egg hatching time [Kruskal–Wallis test: $K = 15.73$, $df = 2$; $P < 0.001$; *U* Mann–Whitney test: $U\ (F_1–F_2) = 63.57$, $P < 0.001$; *U* Mann–Whitney test: $U\ (P–F_2) = 74.48$, $P < 0.001$]. The hatching time of F_2 was significantly shorter than those observed in *P* and F_1 generations (Fig. 7d).

Discussion

The identification of an appropriate culture system for *A. antarcticus* allowed us to develop microcosms for rearing individuals of this species. Consequently, we were able to obtain information on its life history traits and on its reproductive mode. In *A. antarcticus* from Victoria Land reproduction occurs by thelytokous parthenogenesis, as only females were present in the cultured population. Chromosome pairing showed evidence of meiotic maturation of the oocytes, and therefore automictic parthenogenesis. In tardigrades, this kind of parthenogenesis is less frequent than apomixis (Bertolani, 1982; Rebecchi et al., 2003), and it can occur with or without recombination. In *A. antarcticus* recombination does not occur, since chiasmata have not been observed in the oocytes. Another population of the same species collected in the East Antarctica (Kagoshima et al., 2013) also reproduced by parthenogenesis although the type of parthenogenesis (automixis or apomixis) was not specified. The reproductive strategy of *A. antarcticus* allows rapid increase in the population size, capitalizing on the short period of time during which environmental conditions are favorable for active life.

Analysis of the life cycle of the population of *A. antarcticus* from Victoria Land showed that females laid eggs freely but rarely within the old cuticle (exuvium). A similar pattern has been described also by Utsugi & Ohyama (1989) and by McInnes (1995) in other two Antarctic populations of the same species collected at Syowa Station and Signy Island, respectively. According to Kagoshima et al. (2013), there might be two different strains of *A. antarcticus* each

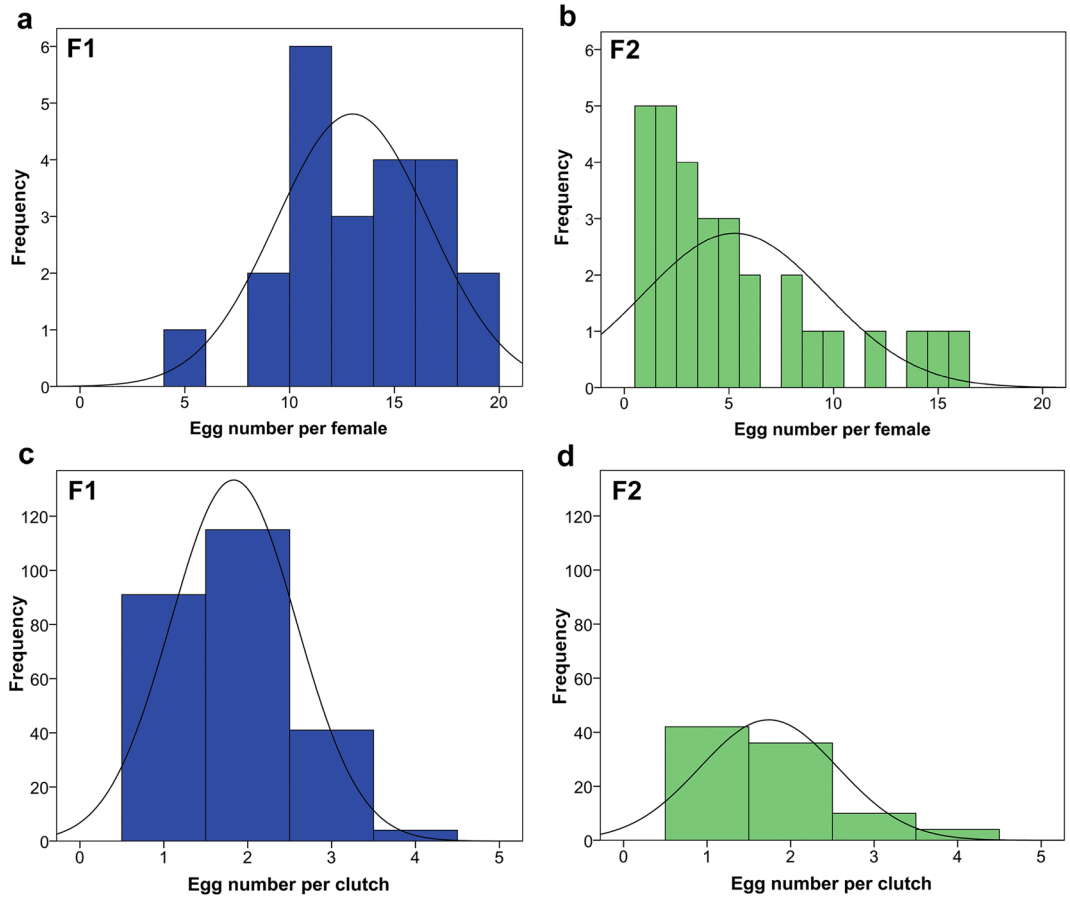
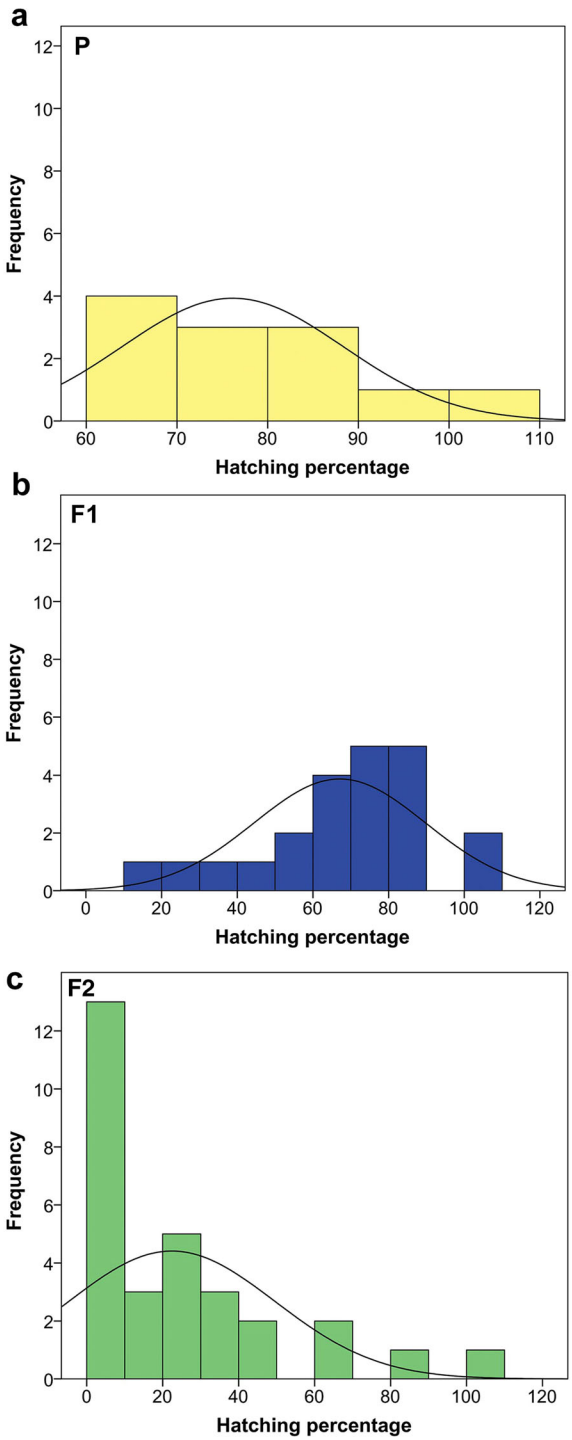


Fig. 5 Distribution of number of eggs per female (fecundity) and number of eggs per clutch (fertility) in F_1 (**a**, **c**) and F_2 (**b**, **d**) generations in the eutardigrade *Acutuncus antarcticus* under rearing conditions

with different egg-laying strategies (freely or within the exuvium). Alternatively, they suggested the presence of the two egg-laying strategies in the same parthenogenetic strain (Kagoshima et al., 2013). Our observations of the same female laying eggs both freely and rarely within the old cuticle are in agreement with the latter hypothesis by Kagoshima et al. (2013).

We evidenced that the life cycle of the population of *A. antarcticus* from Victoria Land was very short (about 60–90 days) compared with that of tardigrade species from temperate regions (about 137–195 days, Altiero & Rebecchi, 2001; Altiero et al., 2006; Schill, 2013). This short life cycle is in accordance with data reported for other Antarctic invertebrate species as springtails, oribatid mites, and nematodes, which are characterized by a short life cycle compared with their temperate relatives, even though short life cycles are not ubiquitous amongst Antarctic invertebrates (see Goddard, 1979; West, 1982; Convey, 1996; Yeates et al., 2009). We also evidenced an early age at first oviposition, a short interval of time between two successive ovipositions, and a short hatching time that allow *A. antarcticus* to produce more than one generation in a brief time. In addition, peculiar fitness traits such as a relatively high fecundity and fertility and high hatching percentage allow *A. antarcticus* to quick increase the population size to better exploit the conditions suitable for growth during the short Antarctic summer.

In Antarctica the environmental conditions can change unpredictably even within the same day, for example, the exact timing or frequency of freeze–thaw events, and periods of drought cannot be predicted in advance. The Antarctic invertebrate communities can respond differently to withstand these unpredictable



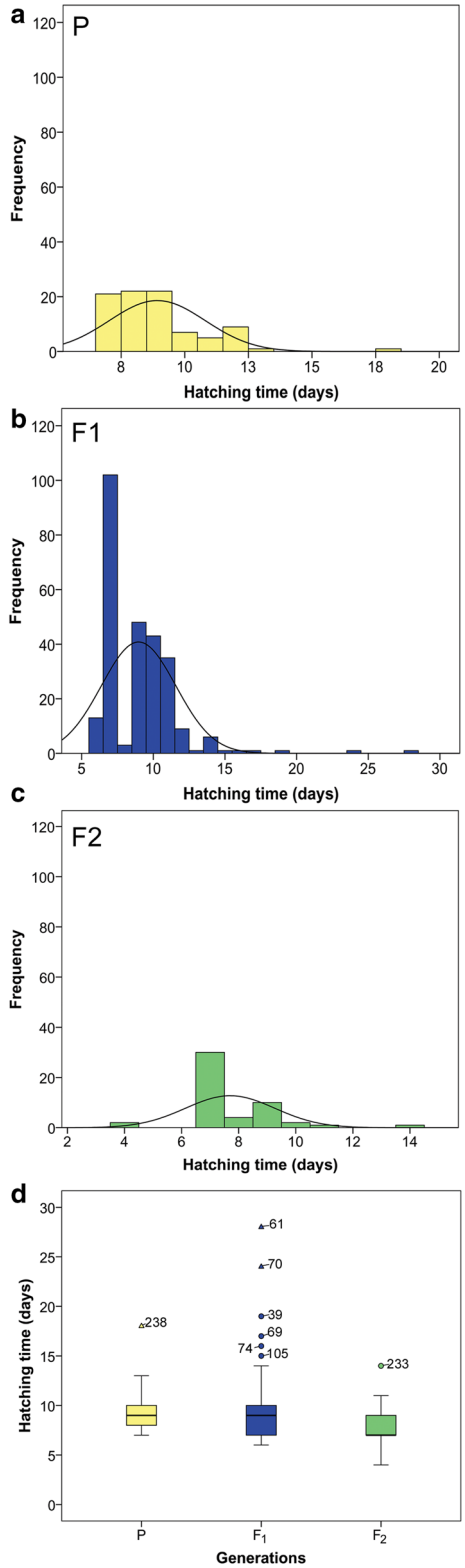


Fig. 6 Distribution of hatching percentage in P (**a**), F_1 (**b**) and F_2 (**c**) generations in the eutardigrade *Acutuncus antarcticus* under rearing conditions

◀**Fig. 7** Distribution of hatching time in P (**a**), F_1 (**b**), and F_2 (**c**) generations of the eutardigrade *Acutuncus antarcticus* under rearing conditions. **d** Box plots comparing the hatching time among the three generations of *A. antarcticus*

environmental conditions that are hostile to life. Phenotypic plasticity of life history traits, and particularly of those traits associated with reproduction, is one of the possible survival strategies, as it occurs in many invertebrates (e.g., ostracods, zooplankton) colonizing habitats subjected to stochastic environmental conditions (Seger & Brockmann, 1987; Altiero et al., 2006, 2010; Crean & Marshal, 2009; Simons, 2009). The phenotypic variability evidenced in the life history traits of the population of *A. antarcticus* from Victoria land has to be interpreted as a strategy to cope with the extreme and stochastic environmental conditions of the Antarctica, assuring survival and fitness in space and time. The phenotypic plasticity observed within and among generations in life history traits of *A. antarcticus* could be due to genetic or maternal effects. The genetic factors are limited because of the continuously parthenogenetic reproduction of *A. antarcticus* and because of the phenotypic variability evidenced also within generation. Therefore, the maternal effect can be responsible of the phenotypic variability detected in *A. antarcticus* from Victoria land; in practice, the environmental conditions experienced by the mother could indirectly affect the phenotype of her offspring (Marshall & Uller, 2007). When mothers face uncertainty regarding their offspring's environment, they could diversify their response, by increasing the variability of their offspring, as it occurs in a range of taxa (see Crean & Marshal, 2009). In *A. antarcticus* the mothers invest in eggs having different hatching time. The eggs showing short hatching time (F_2: mean = 7.7 days) are able to hatch early with respect to those laid by previous generations (F_1: mean = 9.0 days) in order to allow offspring to complete their life cycle before environmental conditions become unfavorable. The adaptive response is also evidenced considering the hatching percentage of eggs of *A. antarcticus* since in F_1 generation the hatching percentage (67.1%) was higher than that of the F_2 generation (22.4%). In both generations, the unhatched eggs could be able to withstand the long Antarctic winter as resting eggs and hatching the next summer, when the conditions will be newly advantageous for growth and reproduction, as occurs in another tardigrade species (Altiero et al., 2010). In *A. antarcticus*, the increase of unhatched eggs across generations may be explained by the fact that environmental conditions may be changing (e.g., winter season is approaching) and a strategy involving resting eggs may be successful. The phenotypic variation observed in the life history traits of the three generations of *A. antarcticus* may be seen as a bet-hedging strategy, which offers insurance against risks associated with reproduction by expressing a range of diversified phenotypes, as in the adage that one should "not put all your eggs in one basket" (Seger & Brockmann, 1987; Crean & Marshal, 2009; Simons, 2009).

The phenotypic plasticity of life history traits of *A. antarcticus,* along with its reproduction by thelytokous parthenogenesis and its anhydrobiotic and cryobiotic abilities (present work; Dougherty, 1964; Giovannini et al., 2013), are advantageous for exploiting fully the conditions suitable for growth and reproduction during the short Antarctic summer. In addition, they can explain its wide distribution on the Antarctic continent, as single parthenogenetic and anhydrobiotic specimens could colonize new territories due to relatively long-range dispersal as shown for other tardigrade species (Janiec, 1996; McInnes & Pugh, 1998; Rebecchi et al., 2003; Jørgensen et al., 2007). Finally, *A. antarcticus* has the potential capabilities to cope with global climate change as adult specimens in both physiological states (hydrated and desiccated) showed the capability to resist increasing values of temperature, even for a short time (Giovannini et al., 2013).

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