

First report of *Laternula elliptica* in the Antarctic intertidal zone

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Abstract Many Antarctic marine invertebrates are considered to be highly stenothermal, subjected to loss of functionality at increased temperatures and so at high risk of mortality in a rapidly warming environment. The bivalve *Laternula elliptica* is often used as a model taxon to test these theories. Here, we report the first instance *L. elliptica* from an intertidal site. Genetic analysis of the tissue confirms the species identity. A total of seven animals ranging in length from 6 to 85 mm were collected from $3 \times 0.25 \text{ m}^2$ quadrats of intertidal sediments at St Martha Cove on James Ross Island, Eastern Antarctic Peninsula. Ambient temperatures of 7.5 °C within the sediment and 10 °C (air) were recorded. This raises questions as to the current perception that “many Antarctic marine invertebrates cannot adapt to higher temperatures”.

Keywords Ecophysiology · Temperature · Stenothermal · Climate change · Bivalve

Introduction

Much experimental research has been conducted over the last few decades on Antarctic marine invertebrates physiological responses to increases in temperature. These studies attempt to quantify the effects of predicted environmental change in the region on a variety of marine organisms (see Peck 2015 for a review). Many laboratory-based studies have used model taxa and examined their response to short-term manipulated environmental parameters. As a result of unfavourable changes to their habitat, species have been shown to lose functional abilities necessary for survival (e.g. Peck et al. 2004; Pörtner et al. 2007; Peck 2011; Bylenga et al. 2015).

One of the most commonly used taxa in these studies is the soft-shelled burrowing bivalve *Laternula elliptica*. Its widespread distribution, high abundances, ease of collection and ability to survive under experimental conditions have resulted in its inclusion in over 240 papers (Web of Science search on “*Laternula elliptica*”), including 153 papers that include the term “physiology”. Publications peaked in 2009 with 19 papers and in the last 20 years averaged at around 11 papers per year. A search using the term “temperature” returned 95 relevant documents, most of which examined ecophysiology.

Laternula elliptica has been reported from depths ranging from the shallow sublittoral <10 m to the continental slope ~700 m (Fig. 1a). Prior to this study, there have been no reports of animals surviving the more variable environmental conditions of the littoral zone south of the Antarctic Circumpolar Current (ACC) and only one intertidal record from sub-Antarctic Kerguelen Island, (Johnston 1937). Here, we report the presence of *L. elliptica* within intertidal sediments at locations on James Ross Island, East Antarctic Peninsula, (EAP) during the austral summer of 2007.

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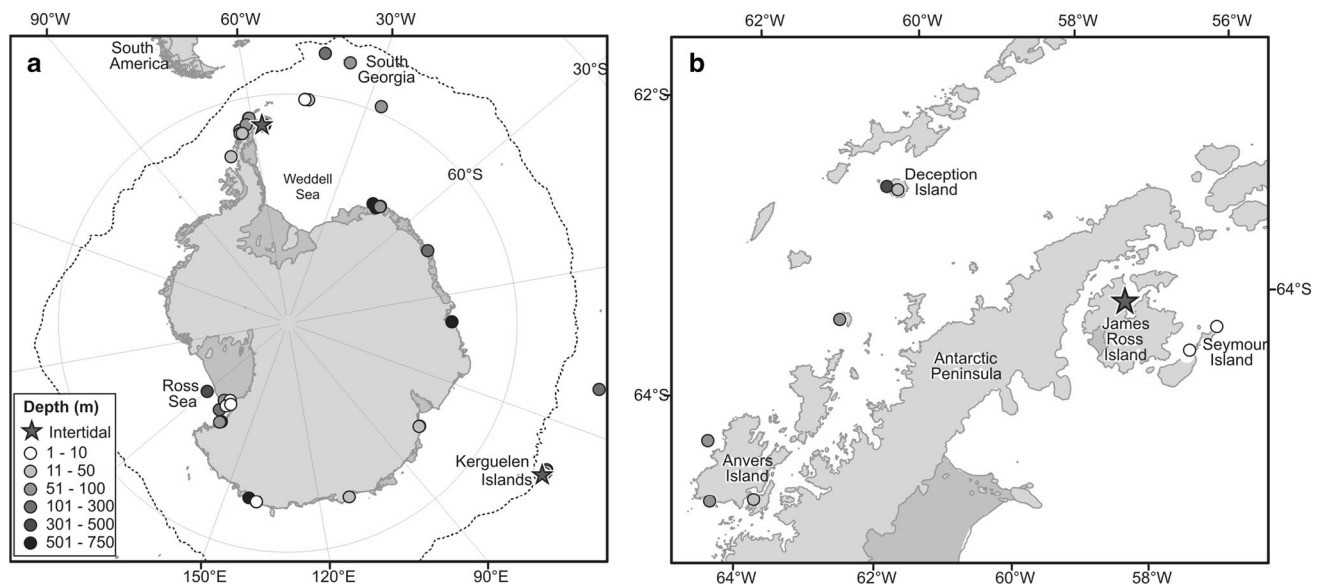


Fig. 1 Recorded locations of *Laternula elliptica* in **a** the southern ocean and **b** the northern Antarctic Peninsula

Methods

Study sites and sampling protocol

Samples were collected from St Martha Cove on James Ross Island, EAP (Figs. 1b, 2). The sediment was medium to coarse sand within a pebble matrix (as defined by the Wentworth scale). Animals were collected by hand from three haphazardly placed 0.25 m² quadrats (approximately 10 m apart) in the mid-shore at low water spring tide. They were located by the presence of their syphons at the surface or after sieving the sediment for smaller individuals. Four specimens were preserved in 96 % ethanol for molecular identification.

DNA extraction, PCR and sequencing

Samples were washed in distilled water, and total DNA was extracted from approximately 1 mm³ of foot tissue using the DNeasy Tissue Kit (Qiagen) and manufacturer's protocol. Up to 625 bp of the 18S rRNA gene was amplified using the 18A1 forward primer 5'-CCT ACC TGG TTG ATC CTG CCA G-3' and the 600r reverse primer 5'-CCG AGA TCC AAC TAC GAG CT-3' (Dreyer et al. 2003). PCR was carried out in a final volume of 25 mL containing 1' PCR buffer [Invitrogen 10' (100 mM Tris-HCl (pH 8.4), 500 mM KCl)]; each dNTP (0.2 mM); MgCl₂ (1.5 mM); each primer (0.2 mM); 1 unit Taq polymerase (Invitrogen Platinum[®]) and 10 ng DNA template. PCR cycles were as follows: initial denature of 94 °C for 2 min; 35 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min; and final primer extension of 72 °C

for 10 min. PCR products were run out on 2 % agarose gels stained with ethidium bromide. Bands were excised from the gels and cleaned up using QIAquick gel extraction kit (Qiagen) following manufacturer's protocol. DNA was sequenced on an ABI 3730xl at Beckman Coulter Genomics, UK. Sequences were aligned using CLUSTALX 2.0.11 (Larkin et al. 2007) against reference *L. elliptica* sequence (GenBank: AY192687.1; AY820759.1) and identified using similarity scores from BLASTN 2.2.22 (Zhang et al. 2000) searches of nucleotide databases with default settings.

Results

A total of 7 *L. elliptica* were found from the total area of 0.75 m² of sediment. They ranged in length from 6 to 85 mm (Table 1). Within sediment, temperatures were consistent across sites at 7.5 °C with an air temperature of +10 °C at time of sampling (midday local time, at low tide).

All four 18A1/600r sequences aligned to *Laternula* sequences obtained from the GenBank database. Using the sequence between positions 34 and 669 (taking 18A1 to start from position -20 (Dreyer et al. 2003)), BLASTn results identified *Laternula elliptica* (GenBank: AY192687.1; AY820759.1) as the best hits finding 100 % similarity with AY192687.1. *Laternula marilina* (AM774487.1) and *Laternula creccina* (AY192694.1) were identified as the next best hits each with 96 % similarity. Based on these results, we assigned the intertidal Eastern Antarctic Peninsula specimens to *L. elliptica*.

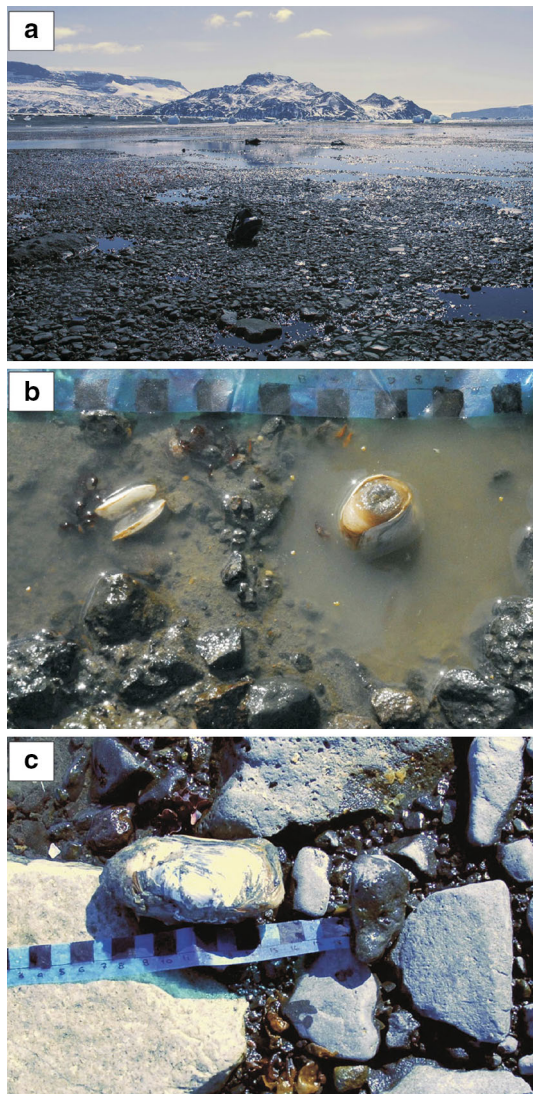


Fig. 2 Photographs of **a** the beach at St Martha Cove, James Ross Island, **b** partially exposed *Laternula elliptica* in sediments on the beach and **c** an adult *Laternula elliptica* after removal from the sediment

Discussion

While the depth range of *L. elliptica* is relatively broad (from 3 to 732 m), 75 % of the recorded specimens are from localities shallower than 100 m [data taken from the Southern Ocean Mollusc Database (SOMBASE)]. These sublittoral populations are exposed to low and stable water temperatures in the range -1.9 to $+1.8$ °C (depending on geographic location and season).

Collection of *L. elliptica* is usually done by SCUBA diving in close proximity to research bases. This environment usually represents a relatively stenothermic habitat, which is then replicated in laboratory aquaria (e.g. Peck 2011). Environmental conditions are then manipulated over

Table 1 Length (mm) of intertidal *Laternula elliptica* collected from sediments at St Martha Cove, James Ross Island

Specimen	Length (mm)
1	6
2	18
3	25
4	25
5	45
6	60
7	85

a period of days or weeks and organism responses observed. Some authors have noted that these experimental, short-term, rapid temperature rises are not necessarily representative of real-world responses (Peck 2011). However, animals living in the Antarctic intertidal zone can be subjected to rapidly changing temperatures and environmental conditions over daily and seasonal timescales (Griffiths and Waller 2016).

During the duration of the expedition (approximately 4 weeks), there had been no extreme weather events that may have washed the specimens into the intertidal. The individuals appeared healthy and undisturbed and were all well buried with only the syphons visible. Ralph and Maxwell (1977) found that the annual growth ring (i.e. max size) at one year old was ~ 24 mm and that 8-year-old individuals had a mean size of ~ 80 mm suggesting that individuals collected ranged from under 1 to 8 years of age. The densities of individuals (~ 10 per m^2) in the intertidal were similar to many subtidal locations (Ahn 1994; Urban and Mercuri 1998) but far lower than the >250 individuals per m^2 reported by Philipp et al. (2011). The exact abundances found at this intertidal location are unknown due to the qualitative nature of the sampling and the restricted nature of the opportunistic fieldwork.

Subtidal, cold, stenothermic conditions contrast sharply with the conditions experienced by the intertidal population found on James Ross Island, where temperatures at approximately 4 cm depth in the sediment reached 7.5 °C, and an air temperature of 10 °C was recorded at the time of sampling. These temperature data for the sample location represent temperatures on one of the warmer summer days. Between 2003 and 2010, the maximum recorded air temperature at the nearby Marambio Base weather station (Seymour Island) varied between 10.2 and 12.6 °C, similar to those recorded at the time of sample collection. Minimum air temperatures at Marambio for the same time period were between -35.5 and -28.2 °C, and mean annual temperatures were between -6.5 and -10.8 °C. If intertidal conditions mirror those of air temperature, then it can be concluded that the sampled habitat experiences a wide temperature regime with both daily and seasonal fluctuations.

The presence of this species in intertidal sediments raises questions about their physiological tolerances and capacity to cope with warming sea temperatures. Under experimental conditions, *L. elliptica* has been shown to suffer 50 % failure in essential biological activities at 2–3 °C and complete loss of function at 5 °C, although there is some anecdotal evidence that individuals can survive for over a month at 3 °C (Peck et al. 2004; Peck et al. 2009). Given the age range of specimens found in this study, it is likely that the individuals sampled had survived in situ through the temperature extremes experienced in this area as recorded by the weather station at Marambio Base. This has major implications for our understanding and interpretation of the physiological tolerances of Antarctic shallow water marine organisms. If one of the best-studied model species can be found surviving far beyond its predicted environmental envelope, then our use laboratory-based experimental results may underestimate the ability of polar organisms to cope with environmental change.

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